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Phylogeny, biogeography and evolution of clutch size in South American lizards of the genus *Kentropyx* (Squamata: Teiidae)

FERNANDA DE P. WERNECK,* LILIAN G. GIUGLIANO,† ROSANE G. COLLEVATTI‡ and GUARINO R. COLLI*

**Departamento de Zoologia, Universidade de Brasília, 70910-900, Brasília, DF, Brazil, †Programa de Pós-Graduação em Biologia Animal, Universidade de Brasília, 70910-900, Brasília, DF, Brazil, ‡Programa de Pós-Graduação em Ciências Genômicas e Biotecnologia, Universidade Católica de Brasília, 70790-160, Brasília, DF, Brazil*

Abstract

The lizard genus *Kentropyx* (Squamata: Teiidae) comprises nine species, which have been placed in three species groups (*calcarata* group, associated to forests ecosystems; *paulensis* and *striata* groups, associated to open ecosystems). We reconstructed phylogenetic relationships of *Kentropyx* based on morphology (pholidosis and coloration) and mitochondrial DNA data (12S and 16S), using maximum parsimony and Bayesian methods, and evaluated biogeographic scenarios based on ancestral areas analyses and molecular dating by Bayesian methods. Additionally, we tested the life-history hypothesis that species of *Kentropyx* inhabiting open ecosystems (under seasonal environments) produce larger clutches with smaller eggs and that species inhabiting forest ecosystems (under aseasonal conditions) produce clutches with fewer and larger eggs, using Stearns' phylogenetic-subtraction method and canonical phylogenetic ordination to take in to account the effects of phylogeny. Our results showed that *Kentropyx* comprises three monophyletic groups, with *K. striata* occupying a basal position in opposition to previous suggestions of relationships. Additionally, Bayesian analysis of divergence time showed that *Kentropyx* may have originated at the Tertiary (Eocene/Oligocene) and the 'Pleistocene Refuge Hypothesis' may not explain the species diversification. Based on ancestral reconstruction and molecular dating, we argued that a savanna ancestor is more likely and that historical events during the Tertiary of South America promoted the differentiation of the genus, coupled with recent Quaternary events that were important as dispersion routes and for the diversification at populational levels. Clutch size and egg volume were not significantly different between major clades and ecosystems of occurrence, even accounting for the phylogenetic effects. Finally, we argue that phylogenetic constraints and phylogenetic inertia might be playing essential roles in life history evolution of *Kentropyx*.

Keywords: biogeography, *Kentropyx*, life-history evolution, phylogenetic ordination, phylogenetic subtraction, phylogeny

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Introduction

A great deal of biotic and abiotic factors may influence lizards' reproductive cycles (Fitch 1970). Food and water availability are often considered the main constraints on reproduction (Magnusson 1987; De Marco 1989). Due to this influence, local populations tend to adapt their reproductive cycles to the environment, being under continuous selective pressure (Roff 1992). As a result, populations and species from different localities, under distinct environmental conditions, may exhibit variation in life-history traits, such as reproductive frequency, and number and size of offspring (Fitch 1982, 1985; Brown & Shine 2006).

Correspondence: Fernanda de P. Werneck, Department of Biology, WIDB, Brigham Young University, Provo, UT84602, USA. Fax: 801-422-0090; Email: fewerneck@gmail.com

Quantitative characteristics, such as clutch size and egg volume, are essential to the study of life history because they can elucidate how energy is allocated to reproduction. The amount of energy available for reproduction and limiting factors, such as body size and shape, foraging mode and habitat specificity, may determine the number and size of offspring (Vitt 1981; Zug *et al*. 2001). Thus, based on hypotheses of trade-offs in life-history evolution, an offspring should represent an optimal compromise between number and size of eggs that results in maximum survival of juveniles and gravid females (Stearns 1989; Shine & Schwarzkopf 1992; Pough *et al*. 1998). As an adjustment to different selective pressures, species of nonseasonal and seasonal environments usually have distinct reproductive strategies. Fitch (1982) hypothesized that species in tropical forest (often aseasonal) ecosystems should temporally spread out their reproductive investments, thus producing more clutches with fewer and larger eggs. Conversely, species in open (often seasonal) ecosystems should concentrate reproductive investment during the favourable (rainy) period, and thus produce larger clutches with smaller eggs (Fitch 1982). This hypothesis has never been adequately tested within monophyletic groups that have species in both seasonal and aseasonal environments.

A problem of most comparative studies is that they do not consider the phylogeny of species under study. In such a case, it is difficult to determine whether the option for one reproductive strategy was determined by ecological relations of the population or by the inheritance of ancestral adaptations. Species belong to hierarchical phylogenies, and thus cannot be treated as independent observations for the study of covariation among life-history traits (Felsenstein 1985b; Harvey & Pagel 1991). Dunham & Miles (1985) suggested that phylogenetic constraints have a central importance in reproductive patterns of lizards and snakes and cannot be ignored in analyses of the life-history evolution.

The lizard genus *Kentropyx* (Squamata: Teiidae) is distributed in South America, east of the Andes (Gallagher & Dixon 1992). The genus was described by Spix in 1825 and is distinguished from all other teiid genera by the presence of keeled ventral scales (Gallagher 1979). The systematics of *Kentropyx* had been problematic, with 19 nominal taxa already proposed, most of which were later considered as junior synonyms. Gallagher & Dixon (1992) recognized eight species in three species groups, based on qualitative characteristics of dorsal scales: (i) the *calcarata* group (*K. calcarata, K. pelviceps* and *K. altamazonica*), with small granular dorsal and lateral scales, and a clear distinction between the dorsals and the keeled plate-like supracaudals; (ii) the *paulensis* group (*K. paulensis, K. viridistriga* and *K. vanzoi*), with granular dorsals and lateral scales gradually enlarging towards the tail, where dorsals and supracaudals are almost indistinct; and (iii) the *striata* group (*K. striata* and *K. borckiana*) with rows of enlarged plate-like dorsals and granular lateral scales. This arrangement, however, was based solely on total similarity without assessing phylogenetic relationships among species or the monophyly of the groups proposed. It should be noted that *K. borckiana* is parthenogenetic and its hybrid origin between *K. calcarata* and *K. striata* has been supported (Cole *et al*. 1995; Reeder *et al*. 2002). Through a similarity analysis of mitochondrial DNA, Reeder *et al*. (2002) observed that the maternal ancestor of *K. borckiana* was *K. striata*. More recently, we collected an undescribed species from the Jalapão region in central Brazil, one of the largest remaining tracts of undisturbed Cerrado, the largest Neotropical savanna biome (Oliveira & Marquis 2002). This species seemingly belongs to the *paulensis* group and is hereafter referred to as *Kentropyx* sp.

Species of the *calcarata* group occur mostly in forests of the Amazon Basin, including forest edges, clearings caused by fallen trees, secondary growth, river margins and plantation sites; however, some isolated populations of *K. calcarata* exist in the Atlantic forest of Brazil (Gallagher & Dixon 1992; Ávila-Pires 1995). On the other hand, species of the *paulensis* group inhabit open ecosystems of the Brazilian Shield, with *K. vanzoi* being endemic to the Cerrado, particularly in areas with sandy soils (Nogueira 2006; Vitt & Caldwell 1993), and *K. viridistriga* being endemic to the flooded savannas of the Chaco-Paraná Basin, in the Pantanal and Guaporé depressions. Finally, species of the *striata* group occur in open ecosystems of the Guiana Shield, northern Amazon Basin, and in some Caribbean islands. Gallagher & Dixon (1992) identified some isolated populations of *K. striata* in northeastern Brazil (Gallagher & Dixon 1992).

Within Teiidae, *Kentropyx* forms a monophyletic group with *Ameiva*, *Cnemidophorus* and *Aspidoscelis* ('cnemidophorines'; Vanzolini & Valencia 1965; Gorman 1970; Presch 1974; Reeder *et al*. 2002; Teixeira 2003; Giugliano *et al*. 2007). Gallagher & Dixon (1992) proposed that dorsal scales increased in size and femoral pores decreased in number during the evolution of *Kentropyx*, with the *calcarata*, *paulensis*, and *striata* groups, in this order, being arranged in a linear progression of increasing size of dorsal scales (and consequent decreasing number) and decreasing number of femoral pores. This progression was interpreted as being related to thermoregulation, such that large numbers of femoral pores and dorsal scales (smaller in size) are associated with shade-tolerance in forest species, whereas small numbers of femoral pores and dorsals (larger) are related to heat-tolerance in open vegetation species (Gallagher *et al*. 1986). However, without phylogenetic analyses, the division of *Kentropyx* into groups and the interpretation of the evolution of morphological and ecological traits are merely speculative.

Gallagher & Dixon (1992) interpreted the current distributional patterns of *Kentropyx* as consistent with the 'Pleistocene

Refuge Hypothesis': successive climatic and vegetational cycles during the Pleistocene promoted the expansion and retraction of species ranges, with speciation occurring in forest refuges during dry/cold periods, and in savanna refuges, during wet/hot periods (Haffer 1969, 1982; Gallagher 1979; Gallagher & Dixon 1992). The presence of *K. striata* in open ecosystem enclaves within Amazon and Atlantic forests and the widely geographically separated populations of *K. calcarata* in Amazon and Atlantic forests apparently support this hypothesis. However, other events able to explain current distributional patterns, such as secondary dispersal, were not considered. Moreover, the importance of the 'Pleistocene Refuge Hypothesis' on the distributional patterns of the South American herpetofauna has been clearly overestimated (Colli 2005). Ancient historical events of the Tertiary, like marine transgressions, the arrival of immigrants from Central and North America, and the uplift of the Central Brazil Plateau, may have had more profound influences (Colli 2005).

Herein, we reconstruct phylogenetic relationships of *Kentropyx* based on morphology and mitochondrial DNA data (12S and 16S), using maximum parsimony and Bayesian methods, and evaluate biogeographic scenarios based on ancestral areas analyses and molecular dating by Bayesian methods. We also test the life-history hypothesis that open ecosystem species of *Kentropyx* produce larger clutches with smaller eggs and that forest ecosystems species produce clutches with fewer and larger eggs, using Stearns' phylogenetic-subtraction method and canonical phylogenetic ordination.

Materials and methods

Phylogeny and biogeography

Morphological data. We obtained reproductive and morphological data of *Ameiva ameiva* and *Cnemidophorus gramivagus* (used as outgroups in phylogenetic analyses), and *Kentropyx* from museum specimens (Appendix I; total of 1143 specimens of *Kentropyx*; Table 1). Morphological data included pholidosis and coloration patterns (for a detailed description of morphological characters and states see Appendix II).

We coded quantitative characters as continuous variables using step matrix gap-weighting for parsimony analysis (Wiens 2001). This method attributes different weights to intervals with different ranges, through a step matrix that shows costs of transitions between each character state. For each species sampled, we coded qualitative characters with intraspecific variation (polymorphism) using the frequency of derived states (Wiens 1995). We weighed qualitative characters with no polymorphism by 999 and polymorphic qualitative characters by 999 divided by the largest number of steps between two character states, and thus, the cost of a transformation in quantitative characters is equivalent to the weight of a polymorphic or no-polymorphic character (Wiens 2001). Consequently, all analyses using this weighting scheme produced cladograms with lengths (and Bremer branch support) multiplied by 999. Thus, we divided the length and Bremer branch support of those cladograms by 999, allowing comparisons with other studies. For Bayesian analyses, we gap-coded quantitative characters (Thiele 1993), using 0.5 standard deviation as cut-point and regarded them as ordered. We conducted Bayesian analyses using MrBayes-ordered standard model (Huelsenbeck & Ronquist 2001).

Molecular data. We used 12S and 16S mitochondrial DNA sequences previously published (GenBank–NCBI; www.ncbi.nlm.nih.gov/) or obtained by us (Table 2). We extracted whole genomic DNA from liver using DNeasy tissue kits (QIAGEN) and amplified fragments of nearly 350 bp of the 12S ribosomal gene and of nearly 500 bp of the 16S gene with 12Sa, 12Sb, 16SaR, and 16Sd primers, using the same polymerase chain reaction (PCR) conditions described in Reeder (1995). We sequenced PCR products on an ABI PRISM 377 automated DNA sequencer (Applied Biosystems) using DYEnamic ET terminator cycle sequencing kit (Amersham Pharmacia Biotech), according to manufacturer's instructions, and analysed and edited sequences using BioEdit 5.09 (Hall 1999). We obtained a multiple alignment based on parsimony with MALIGN 2.7 (Wheeler & Gladstein 1994). We assigned gap costs for internal gaps (2) and leading and trailing gaps (1), but equal weight for transitions and transversions. All alignments were submitted to TreeBase (study Accession no. SN3720). For both 12S and 16S mitochondrial DNA sequences, we chose the model of sequence evolution by hierarchical likelihood ratio tests (HLRTs) using ModelTest 3.7 (Posada & Crandall 1998). For the Bayesian combined molecular data (12S + 16S), each sequence had its own independent model of evolution and model parameters.

Phylogenetic analysis. We conducted phylogenetic analyses with maximum parsimony (MP) and Bayesian methods, using the species *A. ameiva* and *C. gramivagus* as outgroups. We excluded *Kentropyx borckiana* from analyses because of its hybrid origin (Cole *et al*. 1995; Reeder *et al*. 2002), which precludes a dichotomous tree to correctly represent its relations with other species of *Kentropyx* (Frost & Wright 1988). We analysed each character partition (morphology, 12S, 16S) separately and in combination, using PAUP* version 4.0b10 (Swofford 1999) and MrBayes version 3.0b4 (Huelsenbeck & Ronquist 2001). For MP analysis, we used branch-and-bound searches, coding gaps as a fifth state (Giribet & Wheeler 1999) and assessed the reliability of results with 1000 bootstrap samples (Felsenstein 1985a) and Bremer support (Bremer 1994), with MacClade 4.0

Table 1 Meristic characters of nine species of *Kentropyx*. Values indicate $\bar{x}$± SD, with range in parentheses

Variables	*K. altamazonica* (*n* = 233)	*K. borckiana* (*n* = 4)	*K. calcarata* (*n* = 231)	*K. paulensis* (*n* = 96)	*K. pelviceps* (*n* = 157)	*K. striata* (*n* = 150)	*K. vanzoi* (*n* = 160)	*K. viridistriga* (*n* = 21)	*Kentropyx sp.* (*n* = 21)
Supralabials	12.2 ± 0.6 (10–14)	12.0 ± 0.0 (12–12)	12.1 ± 0.5 (10–15)	12.2 ± 0.6 (11–15)	12.3 ± 0.6 (10–15)	12.0 ± 0.2 (12–13)	12.0 ± 0.3 (11–14)	12.3 ± 0.6 (12–14)	12.1 ± 0.7 (10–14)
Infralabials	10.2 ± 1.5 (8–15)	8.2 ± 0.5 (8–9)	9.9 ± 1.3 (8–12)	8.7 ± 1.4 (6–14)	10.3 ± 1.7 (8–14)	9.9 ± 1.4 (6–13)	8.7 ± 1.2 (7–13)	7.8 ± 0.7 (6–9)	8.0 ± 0.0 (8–8)
Collar scales	16.6 ± 1.5 (13–22)	17.5 ± 0.6 (17–18)	16.4 ± 1.6 (13–22)	16.2 ± 1.6 (12–21)	16.9 ± 1.6 (11–22)	13.9 ± 1.1 (11–17)	14.3 ± 1.2 (12–17)	16.1 ± 1.8 (12–19)	15.8 ± 1.5 (13–18)
Supraoculars	3.1 ± 0.3 (3–4)	3.2 ± 0.5 (3–4)	3.0 ± 0.1 (3–4)	3.1 ± 0.2 (3–4)	3.1 ± 0.3 (3–5)	3.1 ± 0.3 (3–4)	3.1 ± 0.3 (3–4)	3.2 ± 0.4 (3–4)	3.0 ± 0.0 (3–3)
Parietals	3.0 ± 0.0 (3–3)	3.0 ± 0.0 (3–3)	3.0 ± 0.1 (3–5)	3.0 ± 0.2 (3–4)	3.0 ± 0.1 (3–4)	3.0 ± 0.0 (3–3)	3.0 ± 0.0 (3–3)	3.0 ± 0.0 (3–3)	3.0 ± 0.0 (3–3)
Postparietals	2.5 ± 0.8 (2–6)	2.7 ± 0.5 (2–3)	2.2 ± 0.4 (2–5)	2.5 ± 0.7 (2–5)	2.3 ± 0.5 (2–4)	2.1 ± 0.4 (2–5)	2.3 ± 0.5 (2–4)	2.6 ± 0.7 (2–5)	2.5 ± 0.7 (2–4)
Scales around midbody	107.7 ± 7.8 (89–135)	74.7 ± 2.1 (72–77)	113.8 ± 9.7 (93–140)	78.4 ± 7.9 (61–100)	111.9 ± 7.5 (94–132)	47.8 ± 4.3 (38–64)	83.8 ± 6.6 (71–106)	75.0 ± 5.1 (66–83)	71.8 ± 7.2 (61–90)
Transverse rows of ventrals	33.3 ± 1.1 (30–36)	30.3 ± 0.5 (30–31)	32.5 ± 1.2 (29–35)	32.2 ± 1.1 (30–35)	31.2 ± 1.1 (29–34)	31.7 ± 0.9 (29–34)	31.6 ± 1.1 (29–35)	33.9 ± 1.2 (32–36)	32.7 ± 0.8 (31–34)
Ventrals in transverse row	15.6 ± 0.8 (13–17)	16.0 ± 0.0 (16–16)	14.3 ± 0.7 (13–16)	13.9 ± 0.7 (12–16)	14.7 ± 0.9 (14–16)	14.6 ± 0.9 (13–16)	12.7 ± 0.9 (12–14)	14.5 ± 0.8 (14–16)	14.0 ± 0.0 (14–14)
Femoral pores	33.1 ± 2.8 (20–40)	25.5 ± 2.4 (23–28)	37.8 ± 3.4 (28–46)	18.7 ± 2.5 (12–24)	40.3 ± 3.3 (32–49)	13.1 ± 1.2 (10–16)	10.3 ± 1.9 (6–16)	23.1 ± 2.5 (18–28)	21.1 ± 1.3 (19–24)
Prefemorals	12.7 ± 1.8 (9–19)	10.0 ± 0.0 (10–10)	12.4 ± 1.7 (7–17)	8.6 ± 1.1 (6–11)	11.9 ± 1.4 (8–16)	7.3 ± 0.6 (6–9)	7.6 ± 0.9 (6–10)	8.9 ± 0.9 (7–11)	8.8 ± 0.6 (8–10)
Prefemorals rows	15.4 ± 1.2 (12–20)	14.3 ± 0.5 (14–15)	16.2 ± 1.2 (12–19)	12.9 ± 1.0 (11–15)	16.1 ± 1.0 (14–18)	13.9 ± 0.8 (12–16)	12.2 ± 0.9 (10–14)	15.0 ± 1.5 (12–18)	13.0 ± 0.7 (11–14)
Infratibiais rows	11.6 ± 0.9 (9–14)	11.5 ± 1.0 (10–12)	11.0 ± 0.9 (9–15)	9.3 ± 0.9 (8–11)	11.5 ± 1.2 (9–15)	9.0 ± 0.8 (7–11)	8.4 ± 0.7 (7–11)	9.6 ± 0.8 (8–11)	7.9 ± 0.6 (7–9)
Preanals	4.7 ± 0.6 (4–6)	4.5 ± 0.6 (4–5)	4.6 ± 0.6 (4–6)	4.0 ± 0.5 (3–5)	4.6 ± 0.5 (3–6)	4.3 ± 0.5 (3–5)	3.8 ± 0.5 (3–5)	4.3 ± 0.5 (4–5)	4.5 ± 0.6 (4–6)
Fourth finger lamellae	18.8 ± 1.4 (15–22)	18.0 ± 0.8 (17–19)	17.1 ± 1.1 (15–23)	15.1 ± 1.2 (12–18)	17.4 ± 1.2 (14–20)	16.1 ± 1.0 (13–19)	15.8 ± 1.0 (13–18)	16.2 ± 1.3 (14–20)	15.4 ± 0.7 (14–17)
Fourth toe lamellae	27.3 ± 1.7 (20–33)	28.0 ± 0.8 (27–29)	26.5 ± 1.5 (22–32)	22.9 ± 1.9 (18–28)	25.8 ± 1.7 (21–31)	24.5 ± 1.3 (22–28)	23.4 ± 1.4 (20–28)	25.1 ± 1.5 (23–29)	21.7 ± 1.2 (20–24)
Dorsals	164.0 ± 17.4 (130–207)	118.0 ± 3.5 (115–121)	157.6 ± 10.0 (132–186)	129.5 ± 10.6 (106–155)	143.9 ± 9.4 (119–182)	84.1 ± 3.9 (75–93)	143.7 ± 9.2 (123–164)	134.0 ± 11.4 (116–156)	118.3 ± 5.7 (108–129)
Scales around tail (15)	19. ± 1.6 (16–22)	16.5 ± 0.6 (16–17)	17.2 ± 1.6 (14–22)	15.5 ± 1.6 (13–19)	19.6 ± 1.5 (16–23)	18.2 ± 1.0 (15–28)	14.7 ± 1.2 (12–19)	17.6 ± 1.4 (15–20)	16.8 ± 1.1 (14–18)

(Maddison & Maddison 1999) and PAUP*. Bayesian analyses started with randomly generated trees and ran for 5.0×10^6 generations, implementing the Metropolis-coupled Markov chain Monte Carlo method (MC3) (Altekar *et al*. 2004). We sampled trees at intervals of 100 generations, producing 50 000 trees. We plotted the log-likelihood scores of the 50 000 trees against generation time to detect stationarity using Tracer 1.4 (Rambaut & Drummond 2007). We regarded all sample points before stationarity as burn-in samples (until 6500th generation) that contained no useful information about parameters. For each analysis, we conducted four independent runs to avoid trapping in local optima. The frequency of any particular clade in the majority-rule consensus tree of the stationarity stage, from the four independent runs, represented the posterior probability of that node (Huelsenbeck & Ronquist 2001).

Molecular dating. We estimated divergence times based on a Bayesian relaxed molecular clock approach implemented in MULTIDISTRIBUTE (Thorne *et al*. 1998; Kishino *et al*. 2001; Thorne & Kishino 2002). This approach allows the incorporation of multiple time constraints, and takes into account both molecular and palaeontological uncertainties to estimate the variance of divergence times. For this analysis, we used the most parsimonious tree topology of the combined analysis (morphological + 12S and 16S mitochondrial DNA sequences). We calibrated the origin of the genus based on Giugliano *et al*. (2007) estimate [29.8

Table 2 Species, locality, collection, collection number and GenBank Accession number

Species	Locality	Collection	Tag	GenBank Accession no.
Ameiva ameiva 1	Peru: Cuzco Amazônico	SBH	267103	12S – AY359473, 16S – AY359493
Cnemidophorus gramivagus	Venezuela: Portuguesa	ALM	8199	12S – AY046432, 16S – AY046474
Kentropyx altamazonica	Peru: Loreto	KU	205015	12S – AY046456, 16S – AY046498
Kentropyx altamazonica	Venezuela: Tapirapeco	AMNH	R-134175	12S – AY046455, 16S – AY046497
Kentropyx calcarata 1	Guyana: Warniabo Creek	AMNH	R-140967	12S – AY046458, 16S – AY046500
Kentropyx calcarata 2	Brazil: Vila Rica-MT	MTR	978224	12S – AF420707, 16S – AF420760
Kentropyx pelviceps	Ecuador: Sucumbios	OMNH	36502	12S – AY046459, 16 s – AY046501
Kentropyx striata	Guyana: Southern Rupununi Savanna	AMNH	R-139881	12S – AY046460, 16S – AY046502
Kentropyx paulensis 1*	Brazil: Paracatu -MG	CHUNB	26031	12S – EU345185, 16S – EU345179
Kentropyx paulensis 2*	Brazil: Paracatu -MG	CHUNB	26032	12S – EU345187, 16S – EU345181
Kentropyx vanzoi 1*	Brazil: Vilhena – RO	CHUNB	11631	12S – EU345191, 16S – EU345177
Kentropyx vanzoi 2*	Brazil: Vilhena – RO	CHUNB	11644	12S – EU345188, 16S – EU345178
Kentropyx sp. 1*	Brazil: Mateiros-TO	CHUNB	41296	12S – EU345192, 16S – EU345184
Kentropyx sp. 2*	Brazil: Mateiros-TO	CHUNB	41299	12S – EU345190, 16S – EU345180
K. viridistriga 1*	Brazil: Mato Grosso	UFMT	1270	12S – EU345189, 16S – EU345182
K. viridistriga 2*	Brazil: Mato Grosso	UFMT	2375	12S – EU345186, 16S – EU345183

ALM, field series of Allan L. Markezich, Black Hawk College, Moline, IL; AMNH, American Museum of Natural History; CHUNB, Coleção Herpetológica da Universidade de Brasília; KU, Natural History Museum, University of Kansas; MTRs, from Miguel Trefaut Rodrigues (IBUSP and MZUSP, São Paulo, Brazil), OMNH, Oklahoma Museum of Natural History, University of Oklahoma; SBH, Tissue collection of S. Blair Hedges, Pennsylvania State University; UFMT, Universidade Federal do Mato Grosso, Mato Grosso, Brazil. Asterisks correspond to sequences provided by our study.

million years ago (Ma)] and confidence intervals (lower bound 15.7 Ma and upper bound 48.4 Ma).

Dispersal-vicariance analysis. We inferred ancestral areas based on parsimony, using DIVA 1.1 (Ronquist 1997), which searches for optimal distribution of ancestral nodes that minimize dispersal and extinction events (higher costs events) (Ronquist 1997). We used five areas in the analysis, corresponding to four large geological areas of the South American Platform mostly formed during the Tertiary (Almeida *et al*. 2000) and important for the diversification of the South American herpetofauna (Colli 2005). We also included the Atlantic Forest, corresponding to peripheral records of *Kentropyx calcarata*. Thus, the areas were: (A) Guianan Shield, (B) Amazon Basin, (C) Atlantic Forest, (D) Brazilian Shield, and (E) Chaco-Paraná Basin (Fig. 1).

Life-history parameters

We considered females containing oviductal eggs, vitellogenic follicles or *corpora lutea* as reproductive, and estimated clutch size based on the number of eggs or vitellogenic follicles. For reproductive analyses, we removed, counted, and measured length and width (with digital calipers to 0.01 mm) of oviductal eggs. We calculated egg volume with the formula for a spheroid:

$$V = \frac{4}{3}\pi\left(\frac{w}{2}\right)^2\left(\frac{l}{2}\right),$$

where w is egg width and l is egg length. For each individual lizard, we also measured the snout-vent length (SVL) to 1 mm, with digital calipers.

We assessed interspecific differences in clutch size and mean egg volume of *Kentropyx*, using the analysis of covariance, with SVL as the covariate, and the Tukey HSD test, for a posteriori multiple comparisons of species means. To assess differences in clutch size and mean egg volume of *Kentropyx* between forest and open vegetation ecosystems (*calcarata* group in forests; *paulensis* and *striata* groups in open vegetations) and among all species of *Kentropyx*, we built linear mixed-effects models, with species as a nested random effect and SVL as a covariate. We chose this approach (i) because of significant correlations between SVL vs. clutch size ($r = 0.62$, $t_{207} = 11.32$, $P < 0.001$) and SVL vs. mean egg volume ($r = 0.45$, $t_{50} = 3.54$, $P < 0.001$), (ii) because the design was unbalanced, and (iii) to avoid inflation of type I Error by pseudoreplication (degrees of freedom should be based on species, not on individual lizards). We performed these statistical analyses using R version 2.7.0 (R DCT 2008).

Stearns phylogenetic-subtraction method and canonical phylogenetic ordination

We used Stearns' phylogenetic subtraction method (SPSM, Stearns 1983; Harvey & Pagel 1991) and canonical phylogenetic ordination (CPO; Giannini 2003) to examine the influence of habitat (major vegetation type of occurrence) on

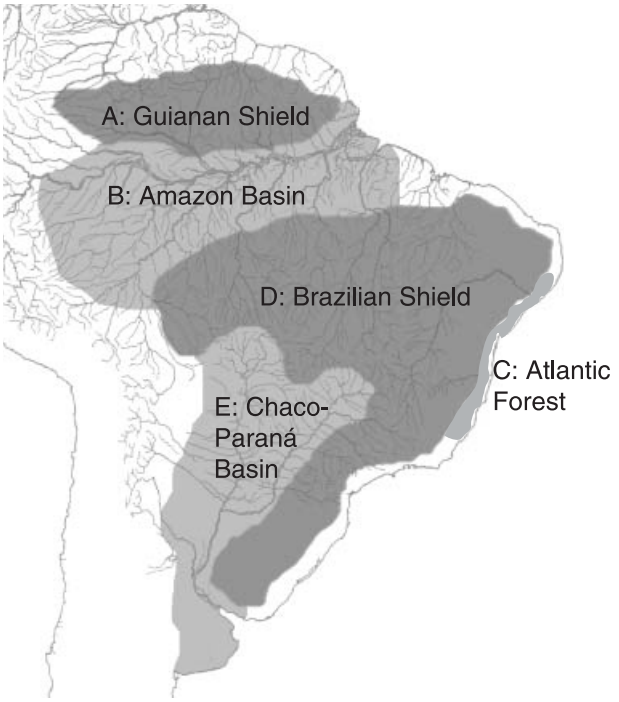

Fig. 1 Geographic areas used in the DIVA analysis. A: Guianan Shield, B: Amazon Basin, C: Atlantic Forest, D: Brazilian Shield, E: Chaco-Paraná Basin.

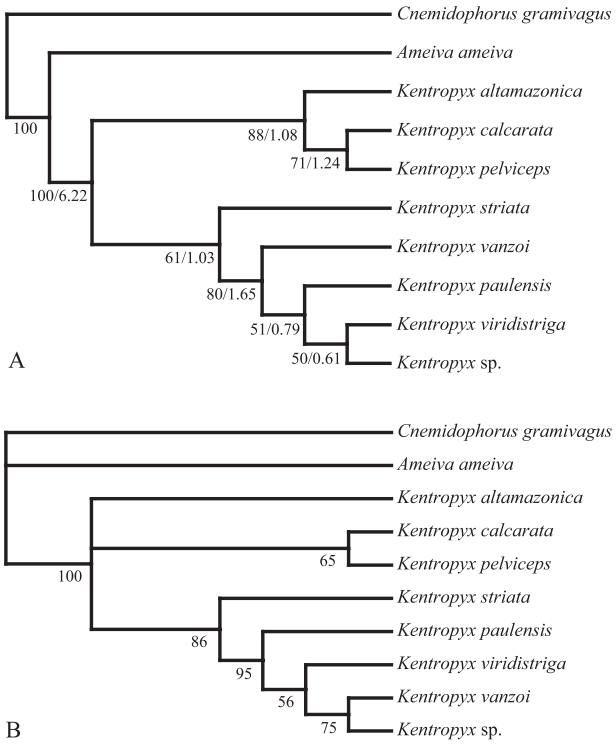

Fig. 2 *Kentropyx* phylogeny inferred from morphological data. (A) Most parsimonious tree, with bootstrap and Bremer support values, respectively. Bremer support values are not absolute numbers because they were divided by 999 in order to compensate the character weighting. (B) Tree inferred by Bayesian analysis, with posterior probability values.

clutch size and egg volume, independently of phylogenetic relationships. We performed SPSM through multiple linear regressions between clutch size and egg volume (dependent variables) and the phylogenetic information (independent variables), which consisted of binary variables representing all monophyletic groups of *Kentropyx*, based on a given topology (defined in Fig. 4A). Next, we used regression residuals, representing the variation not attributed to phylogenetic effects, to evaluate the influence of vegetation type upon clutch size and egg volume, using the analysis of covariance (ANCOVA) with SVL as covariate. We conducted these analyses using R version 2.7.0 (R DCT 2008).

CPO is a modification of canonical correspondence analysis (CCA, Ter Braak 1986), a constrained multivariate ordination technique that relates the variation in a matrix of dependent variables with another matrix of independent variables, maximizing their correlations (Ter Braak 1986; Giannini 2003). The significance of the association between each monophyletic group and variables of interest is tested by randomization of one or both of the data sets. In our CPO, one of the matrices (Y) contained reproductive data (clutch size and egg volume) measured over the species of *Kentropyx*, whereas the other matrix (X) consisted of a tree matrix that contained all monophyletic groups of a given topology, each coded separately as a binary variable (Fig. 4A) and major vegetation type of occurrence of each species of *Kentropyx*. We used SVL as a covariate in CPO. The analysis thus consisted of finding the subset of groups (columns of X) that best explained the variation in Y, independently of SVL, using CCA coupled with Monte Carlo permutations. We performed CPO in Canoco 4.5 for Windows (Ter Braak & Smilauer 2002), using the following parameters: symmetric scaling, biplot scaling, downweighting of rare species, manual selection of environmental variables (monophyletic groups), 9999 permutations, and unrestricted permutations.

Results

Phylogenetic analysis and biogeographic scenarios

Morphological phylogeny. The maximum-parsimony analysis recovered a single most-parsimonious tree (Fig. 2A) with 77 steps (CI = 0.634, RI = 0.570). Despite low branch support values, the topology indicated the monophyly of two groups: a forest clade consisting of *K. altamazonica*, *K. calcarata*, and *K. pelviceps* and an open vegetation clade consisting of *K. striata*, *K. vanzoi*, *K. paulensis*, *K. viridistriga*, and *Kentropyx* sp. (Fig. 2A). Within the open vegetation clade, *K. striata* is sister to a clade comprising *K. vanzoi*, *K.*

Table 3 Parameters of molecular substitution model selected by ModelTest for 12S and 16S regions

DNA region	Base frequencies	Substitution frequency	Gamma distribution (G)
12S	A = 0.3323	A-C = 1.0000	0.2503
	C = 0.2428	A-G = 3.8806	
	G = 0. 1721	A-T = 1.0000	
	T = 0.2288	C-G = 1.0000	
		C-T = 12.9889	
		G-T = 1.0000	
16S	A = 0.3499	A-C = 1.0000	0.1266
	C = 0.2571	A-G = 6.1200	
	G = 0.1642	A-T = 1.0000	
	T = 0.2288	C-G = 1.0000	
		C-T = 8.3267	
		G-T = 1.0000	

paulensis, *K. viridistriga*, and *Kentropyx* sp., occupying a basal position among these species. The Bayesian analysis produced a similar topology with a polytomy uniting species of the forest clade (Fig. 2B).

Molecular data. We obtained five equally parsimonious alignments for 12S sequences, with slight differences among them, but only one most parsimonious alignment was found for 16S sequences. We carried out phylogenetic analyses on each of the five 12S alignments and obtained a single topology, with small differences in bootstrap indices and Bremer support (results not shown). Thus, we arbitrarily chose one of the alignments to be used in the following analyses (TreeBase Accession no. SN3720). The likelihood-ratio test implemented in ModelTest favoured the TrN + G model of sequence evolution [Tamura–Nei model with a gamma distribution parameter; (Tamura & Nei 1993)] for both 12S and 16S. Table 3 depicts the inferred base frequencies, the ratio of invariable sites, and the gamma distribution parameter.

The multiple alignments of 12S sequences generated a fragment of 333 base-pair characters, with 67 informative characters. An unweighted branch-and-bound search produced a single most parsimonious tree with 185 steps (CI = 0.762, RI = 0.777), placing *K. striata* at the base of the tree, followed by a clade containing *K. viridistriga, K. paulensis*, and *Kentropyx* sp., and another formed by *K. vanzoi*, *K. altamazonica, K. pelviceps*, *and K. calcarata*. Except for the placement of *K. vanzoi*, the other groupings had high branch support values. The consensus tree obtained by Bayesian analysis under the TrN + G model of evolution had some incongruences with the MP tree, with a single well-supported group formed by *K. viridistriga*, *K. vanzoi*, *K. paulensis*, and *Kentropyx* sp. (*paulensis* group).

For the 16S gene, we obtained a fragment with 446 positions and 84 informative characters. We found two

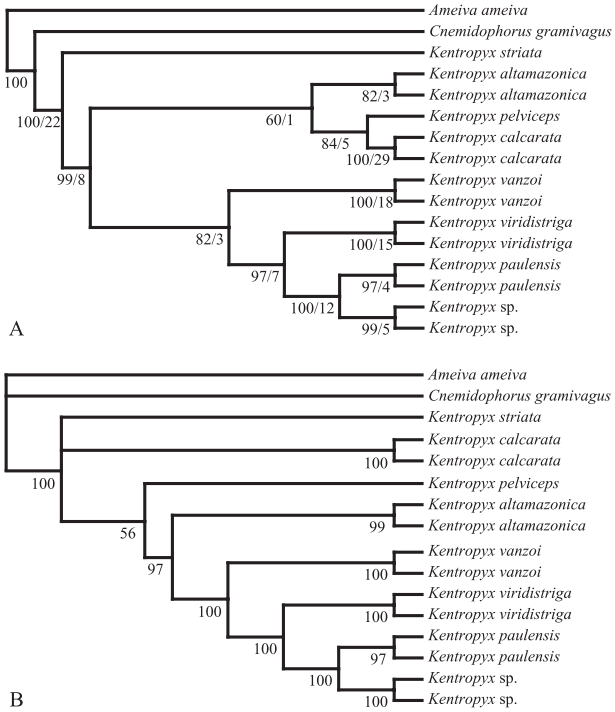

Fig. 3 *Kentropyx* combined mtDNA phylogeny inferred from combined 12S and 16S sequences. (A) Most parsimonious tree, with bootstrap and Bremer support values, respectively. (B) Tree inferred by Bayesian analysis using the TrN + G model, with posterior probability values.

equally most parsimonious trees with 224 steps (CI = 0.665, RI = 0.706), with three well-supported groups: a clade formed by *K. viridistriga*, *K. paulensis*, and *Kentropyx* sp., another formed by *K. paulensis* and *Kentropyx* sp. and a third consisting of *K. striata* in basal position (*striata* group). These monophyletic groups were also recovered by MP and Bayesian analyses based on the 12S sequences. The consensus tree obtained by Bayesian analysis under the TrN + G model of evolution was consistent with the MP tree, containing the same three groups.

The MP analysis of the combined molecular data (12S + 16S) resulted in one most parsimonious tree (Fig. 3A) with 413 steps (CI = 0.702, RI = 0.728). The MP tree strongly supported *K. striata* as the basal species (*striata* group), followed by a clade consisting of *K. altamazonica*, *K. calcarata* and *K. pelviceps* (the forest-dwelling *calcarata* group) and another formed by *K. vanzoi*, *K. viridistriga*, *K. paulensis*, and *Kentropyx* sp. (*paulensis* group). The Bayesian analysis resulted in a different topology (Fig. 3B) but also strongly supported the monophyly of the *paulensis* group.

Combined data: DNA and morphology. The combined data included 779 molecular and 49 morphological characters, with 156 informative characters. The MP analysis produced a single most parsimonious tree with 454 steps (CI = 0.714,

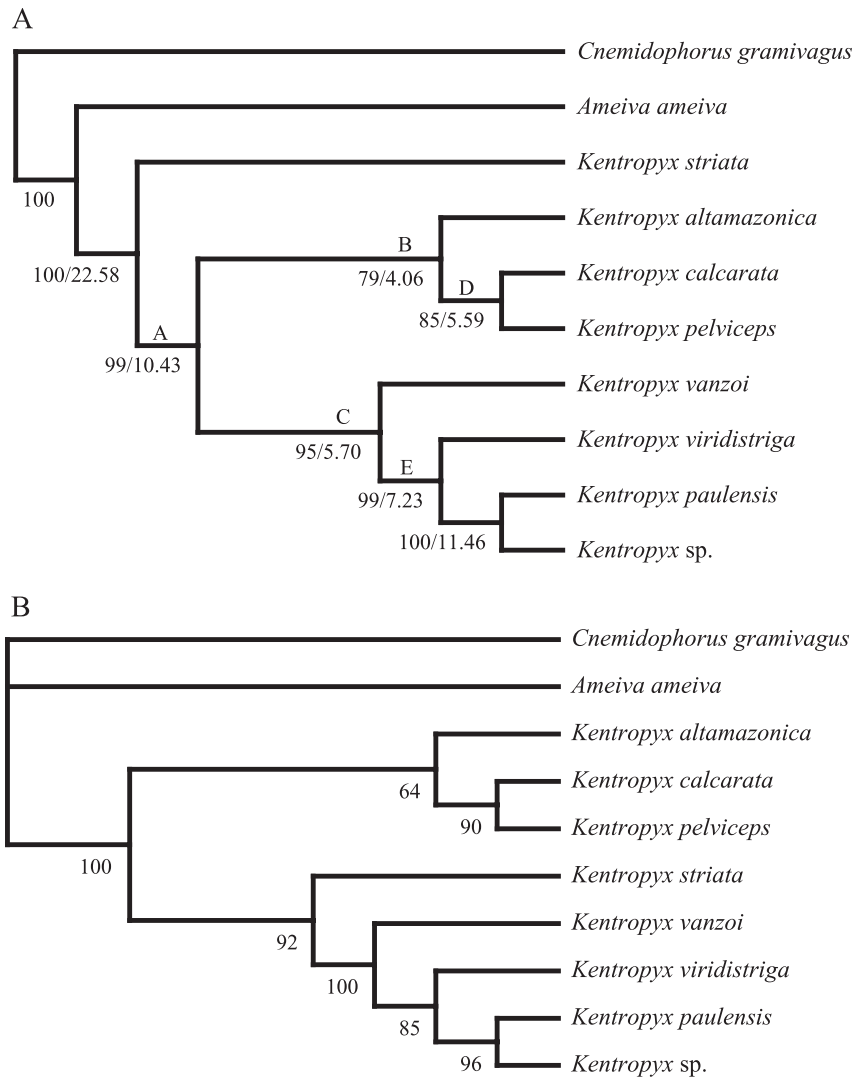

Fig. 4 *Kentropyx* phylogeny inferred from combined molecular (12S + 16S) and morphological data. (A) Most parsimonious tree, with bootstrap and Bremer support values, respectively. Bremer support values are not absolute numbers because they were divided by 999 in order to compensate the character weighting. (B) Tree inferred by Bayesian analysis using the TrN + G model, with posterior probability values. Letters above clades correspond to monophyletic groups of *Kentropyx* used as individual groups in canonical phylogenetic ordination.

RI = 0.573, Fig. 4A). The MP tree presented three major well-supported clades, corresponding to: (i) *striata* group (at the base of the tree); (ii) *calcarata* group, and (iii) *paulensis* group. The Bayesian analysis resulted in a similar topology, except for the position of *K. striata*, which is a sister species of the *paulensis* group forming a clade that includes all open vegetations species (Fig. 4B). To investigate if different coding strategies adopted for MP and Bayesian analysis could be influencing the incongruent results, we repeated MP using gap-coding for quantitative characters (Thiele 1993), but we found exactly the same topology, with small differences in branch support (results not shown).

In summary, relationships within and between *calcarata* and *paulensis* groups are well established in both MP and Bayesian analysis (Fig. 4). Conversely, the two approaches disagree only in the placement of *K. striata*, either placed in a basal position related to all other species (MP) or in a more derived position as sister taxon of the *paulensis* group (Bayesian). Based on the larger number of informative characters supporting the relationships of *K. striata* (6 morphological and 13 molecular in the MP topology; 5/0 in the Bayesian topology), on higher nodal support values for the placement of *K. striata* (even if nodal support and posterior probabilities are not directly comparable), and on the smaller number of assumptions, we favoured the topology recovered by MP for performing the analyses that follow.

Molecular dating. The molecular dating analysis indicated an early diversification of *Kentropyx* species mostly during the Miocene (Fig. 5). According to our analysis, *K. striata* was the first species to diverge during the Late Oligocene–Early Miocene, and the last divergence was between *K. paulensis* and *Kentropyx* sp. during the Late Miocene–Early Pliocene. The *calcarata* and *paulensis* groups probably diverged in the Early–Middle Miocene and the only diversification that took place during the Quaternary was among populations within species (Fig. 5).

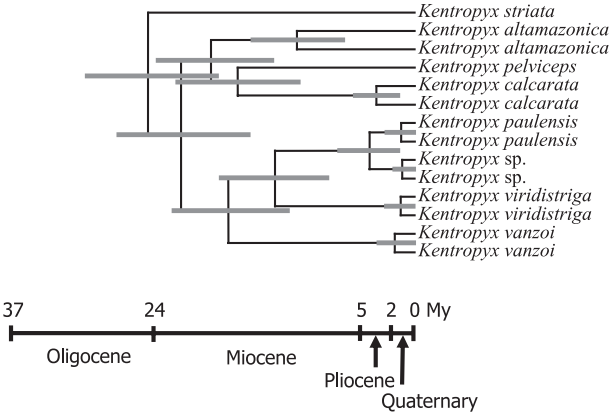

Fig. 5 Chronogram of *Kentropyx* evolution based on the combined morphological and molecular data, with divergence times estimated from a Bayesian relaxed molecular clock approach. Boxes indicate mean divergence time ± one standard deviation.

Dispersal–vicariance analysis. The DIVA analysis found two equally most parsimonious reconstructions, with four dispersal events each during the evolution of *Kentropyx* (Fig. 6). In both reconstructions, the divergence of *K. striata* was due to a vicariance event that isolated this group in the Guianan Shield. In addition, both reconstructions indicate that the divergence of the *calcarata* (in the Amazon Basin) and *paulensis* (in the Brazilian Shield) groups was due to vicariance. The two reconstructions differ in whether the common ancestor of all living species of *Kentropyx* was restricted to the Guianan and Brazilian Shields (Fig. 6A) or if it also inhabited the Amazon Basin (Fig. 6B). The first reconstruction implies that, after the divergence of *K. striata* by vicariance and isolation in the Guianan Shield, the common ancestor of the *calcarata* and *paulensis* groups occupied the Amazon Basin via dispersal (Fig. 6A). According to the second reconstruction, the common ancestor of all living species of *Kentropyx* was widespread, occupying the Amazon Basin and the Brazilian and Guianan Shields due to an earlier dispersal event (Fig. 6B). Both reconstructions require one dispersal event of *K. calcarata* into the Atlantic Forest and another involving the common ancestor of *K. viridistriga*, *K. paulensis*, and *Kentropyx* sp. into the Chaco-Paraná Basin.

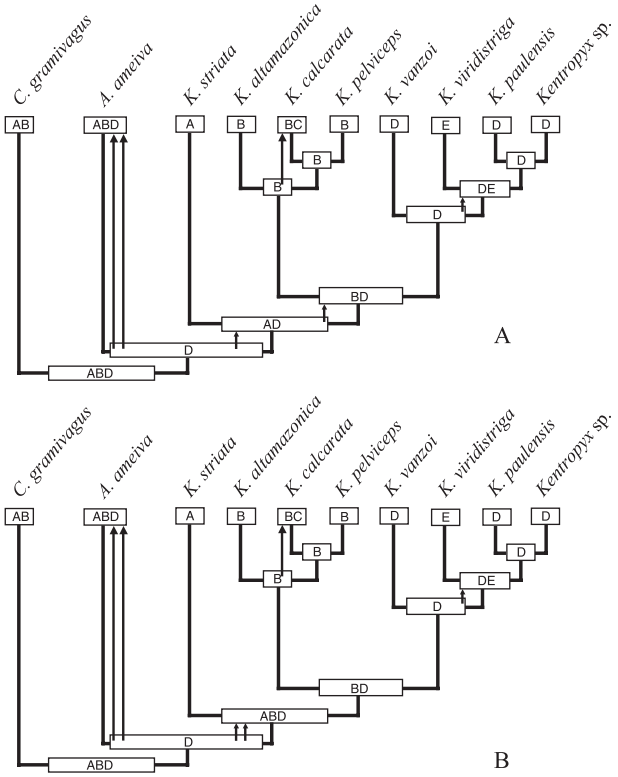

Fig. 6 Reconstructed ancestral distributions for each node on the most parsimonious solutions obtained that consider (A) Guianan and Brazilian Shield as ancestral areas or (B) Amazon Basin as an ancestral area as well.

Table 4 Distribution of females of nine species of *Kentropyx*, according to the reproductive condition

Species	Non reproductive females	Reproductive females	Total of females
K. altamazonica†	70	38	108
K. borckiana§	1	1	1
K. calcarata†	34	56	90
K. paulensis‡	8	19	27
K. pelviceps†	33	31	64
K. striata§	68	45	113
K. vanzoi‡	26	13	39
K. viridistriga‡	1	7	8
Kentropyx sp.‡	9	0	9

†*calcarata* group; ‡*paulensis* group; §*striata* group.

Reproduction life-history evolution

Female reproduction. We obtained reproductive data from all nine species of *Kentropyx*, but had no reproductive female of *Kentropyx* sp. (Table 4). For data analysis, we considered only reproductive females containing oviductal eggs or vitellogenic follicles. Mean clutch size ranged from 3.31 (*K. vanzoi*) to 7.33 (*K. viridistriga*) (Table 5). For some species, our results indicated clutch sizes largely different from previous literature reports. For instance, previous studies indicate clutches of *K. viridistriga* of 6–7 eggs, whereas we recorded a maximum clutch size of 12 eggs (Table 5). Clutch size differed significantly among species (irrespective of habitat), independently of SVL (ANCOVA $F_{7,200} = 12.97$, $P < 0.001$). Based on *post hoc* Tukey HSD tests, we found that clutch size of *Kentropyx pelviceps* (adjusted mean ± SE: 3.84 ± 0.23) was significantly smaller than

Table 5 Clutch size and egg volume (in mm³) of eight species of *Kentropyx* observed in this study and obtained from the literature. Values indicate $\bar{x}$ ± SD, sample size (in parentheses), and range (only for clutch size)

Species	Clutch size (this study)	Egg volume (this study)	Clutch size (literature)	Source†
K. altamazonica	5.45 ± 1.11 (38) 3–9	713.45 ± 127.94 (14)	2,4	1
K. borckiana	6 (1)	—	5,9	2
K. calcarata	5.63 ± 1.23 (56) 3–9	921.16 ± 149.13 (16)	3,7	1,2,3,4
K. paulensis	3.90 ± 0.78 (19) 3–6	528.94 ± 189.10 (4)	3–5	5
K. pelviceps	5.52 ± 0.85 (31) 4–7	1089.39 ± 200.25 (6)	5–8	6
K. striata	5.84 ± 1.72 (45) 3–12	670.65 ± 135.69 (8)	3–10	1,7,8
K. vanzoi	3.31 ± 1.18 (13) (1–6)	510.11 (1)	—	—
K. viridistriga	7.33 ± 2.34 (6) (6–12)	804.06 ± 87.42 (3)	6–7	2

†1- Ávila-Pires (1995); 2- Gallagher & Dixon (1992); 3- Vitt (1991); 4- Magnusson & Lima (1984); 5- Anjos *et al*. (2002); 6- Vitt *et al*. (1995); 7- Dixon *et al*. (1975); 8- Vitt & Carvalho (1992).

K. altamazonica (5.70 ± 0.16), *K. calcarata* (5.02 ± 0.14), and *K. striata* (5.89 ± 0.15), whereas *K. striata* had significantly larger clutches than *K. calcarata* (Tukey HSD, $P < 0.05$). In addition, clutches of *K. viridistriga* (7.67 ± 0.41) were significantly larger than all other species of *Kentropyx*. Species differ significantly in mean egg volume, independently of SVL (ANCOVA $F_{6,44} = 6.60$, $P < 0.001$). Based on *post hoc* Tukey HSD tests, mean egg volume of *K. striata* (adjusted mean ± SE: 671.40 ± 53.03 mm³) was significantly smaller than *K. calcarata* (928.45 ± 39.20 mm³) and *K. pelviceps* (1112.59 ± 71.29 mm³), whereas mean egg volume of *K. pelviceps* was larger than *K. altamazonica* (709.28 ± 40.61 mm³). However, there was no difference between forest and open-vegetation species in clutch size (forest: 5.5 ± 1.1; open-vegetation: 4.9 ± 1.6; $F_{1,6} = 5.22$; $P = 0.06$), or egg volume (forest: $\bar{X} = 868.42 \pm 201.46$ mm³, $n = 36$; open-vegetation: $\bar{X} = 650.20 \pm 164.17$ mm³, $n = 16$; $F_{1,5} = 4.12$; $P = 0.10$), independently of SVL.

CPO and stearns phylogenetic-subtraction method. Multiple linear regressions from the Stearns' phylogenetic subtraction method revealed no significant phylogenetic effects on clutch size ($F_{4,2} = 0.645$, $P = 0.683$) or egg volume ($F_{4,2} = 2.003$, $P = 0.359$) of *Kentropyx*. An ANCOVA on the regression residuals revealed no significant influence of major habitat type on clutch size ($F_{1,4} = 0.313$, $P = 0.605$) or egg volume ($F_{1,4} = 0.603$, $P = 0.481$), independently of phylogenetic structure. Moreover, SVL was significantly correlated with both clutch size ($r = 0.768$, $t = 2.683$, $P = 0.044$) and egg volume ($r = 0.936$, $t = 5.927$, $P < 0.001$). Monte Carlo permutations from CPO revealed no significant effects of phylogenetic structure or habitat type on reproductive parameters of *Kentropyx* (Table 6).

Table 6 Effect of monophyletic groups and ecosystems on the reproductive features of *Kentropyx*. Clade labels according to Fig. 4

Groups	Variation	*F*	*P*
A	< 0.01	0.190	0.7692
B	< 0.01	0.122	0.7143
D	< 0.01	0.411	0.5225
E	< 0.01	0.050	0.8132
Ecosystems	< 0.01	0.122	0.7063

Discussion

Phylogenetic relationships and historical biogeography of Kentropyx

The total evidence reconstructions, based on morphological and molecular data, supported the monophyly of the three phenetic groups of *Kentropyx* previously recognized (Gallagher 1979), both using MP and Bayesian methods. However, our results differ fundamentally from previous proposals in the placement of *K. striata* (which represents the *striata* group). According to our MP combined analysis, *K. striata* is the most basal, and not the most derived species of *Kentropyx*. Gallagher & Dixon (1992) advocated the

lower number of dorsals (because of their larger sizes) and femoral pores of *K. striata* as an adaptation for dry, open ecosystems and as a derived condition relative to the *paulensis* and *calcarata* groups, since other teiid genera do not share these character states (Gallagher & Dixon 1992). However, even if the phenetic grouping proposed previously (Gallagher & Dixon 1992) matches the phylogenetic relationships (this study), the relations among groups should not necessarily follow the evolution of a single character. The same sort of gene tree vs. species tree incongruence problems deriving from single gene phylogenies (Doyle 1997; Maddison 1997) can also occur for a single morphological character phylogeny. Furthermore, correlations between scale counts and surface area available for thermoregulation or environmental properties are not clear and straight. Controversial results indicate both positive (Soulé & Kerfoot 1972; Malhotra & Thorpe 1997; Sanders *et al*. 2004) and negative (Horton 1972; Lister 1976) correlations between number of scales (inversely proportional to their sizes) and drier environments. In addition, Gallagher & Dixon (1992) used this character evolution scenario and the current species distribution to conclude that the ancestral *Kentropyx* 'proceeded from a forest proto-*Kentropyx* stock, derived from an *Ameiva-Cnemidophorus*-like ancestor' and that Quaternary refuge events promoted the diversification of the genus, with secondary colonization of drier, open environments. In summary, previous studies addressing *Kentropyx* evolution proposed phylogenetic relationships and biogeographic scenarios for the genus without implementing rigorous phylogenetic analyses, using alternative data sets, or including any biogeographic reconstruction.

Our evolutionary scenario implies that *Kentropyx striata* was the first species to diverge in the genus, at Late Oligocene–Early Miocene, and that enlargement of dorsal scales occurred early in the evolution the genus, with a possible reversal occurring later in the *calcarata* group. The basal divergence between *K. striata* (a Guianan Shield species) and other species of *Kentropyx* is paralleled by other vertebrate groups and concordant with a basal Brazilian/Guianan Shield split, frequently attributed to Miocene marine introgressions (Rasanen *et al*. 1995; Webb 1995; Ribas *et al*. 2005; Noonan & Wray 2006; Garda & Cannatella 2007). Most of *Kentropyx* diversification occurred at the Oligocene/Miocene, a period fundamentally relevant for the diversification of South America's fauna (Gamble *et al*. 2008).

The period of origin of *Kentropyx* (Eocene/Oligocene) was marked by savanna expansion in South America (Giugliano *et al*. 2007) and is much more ancient than the previously suggested origin and diversification during the Quaternary (Gallagher & Dixon 1992). Thus, the 'Pleistocene Refuge Hypothesis' has only limited importance for the diversification of *Kentropyx* species, being able to explain only the recent diversification of populations. This and the DIVA results suggest that the ancestor of *Kentropyx* was not a forest-dweller as previously proposed (might be both present in the Amazon Basin or totally non-forest). Given that the close relatives of *Kentropyx* are primarily open vegetation taxa even when occurring in the Amazon Basin, an open vegetation ancestor is more plausible (Fig. 6A). Therefore, savannas were likely the centre of origin of the genus, instead of Amazonian forest, and successive Tertiary events played a significant role in the differentiation of living species. Accordingly, the distribution of species of the *calcarata* group in the Amazon Basin is better explained as a more recent dispersal, after the beginning of the marine retraction.

Both most parsimonious DIVA reconstructions required a dispersal event of *K. calcarata* into the Atlantic Forest. Faunal and floral affinities between Amazon and Atlantic forests are extensively documented (Andrade-Lima 1982; Oliveira-Filho & Ratter 1995; Silva 1995; Bates *et al*. 1998; Costa 2003). Older vicariance connections might be responsible for some of these affinities, but most might be attributed to one of the several more recent (Quaternary) forest corridors proposed, acting as dispersal routes linking these forests (Andrade-Lima 1982; Rizzini 1963, 1979; Bigarella *et al*. 1975; Oliveira-Filho & Ratter 1995). As a result, considering the recent divergence between the two forest populations of *K. calcarata* included here (3.4 Ma), the main distribution of this species in eastern Amazonia and the occurrence of Quaternary forest corridors previously connecting Amazon and Atlantic Forests, the dispersal scenario proposed by DIVA is supported.

Independent of the character partition analyzed and optimality criteria adopted, some relationships were typically recovered with high bootstrap and Bremer nodal support and posterior probabilities values, such as the sister relationship between *K. paulensis* and *Kentropyx* sp. and between these two species and *K. viridistriga*. Further, the monophyly of the *paulensis* group was well-supported, in contrast to the *calcarata* group. Genetic population studies might be useful to reveal higher levels of genetic similarity and possible gene flow among species of the *calcarata* group. The monophyly of the *paulensis* group corroborates the hypotheses that the three emergent large land blocks (Guianan Shield, Brazilian Shield, and Eastern base of the Andes) during marine introgressions in the Tertiary (Miocene) of South America would bear monophyletic taxa when compared to lowlands (Aleixo 2004; Rasanen *et al*. 1995; Webb 1995). This scenario was already corroborated from the point of view of different groups of vertebrates (Aleixo 2004; Ribas *et al*. 2005; Noonan & Wray 2006; Garda & Cannatella 2007).

In contrast to previous suggestions that *K. vanzoi* and *K. paulensis* are sister species, primarily distributed in Cerrado of Brazilian Shield (Colli 2005), our results indicate that *K. paulensis* is the sister species of *Kentropyx* sp. and is more

closely related to *K. viridistriga*, which inhabits the Chaco-Paraná depressions, than to *K. vanzoi*. The early divergence between *K. vanzoi* and the other species of the *paulensis* group might be attributed to isolation in the Parecis Plateau, an extensive sedimentary basin (Hasui & Almeida 1985; Bahia *et al*. 2006) which experienced a regional uplift during the Miocene (Costa *et al*. 1996; Westaway 2006). Further, our DIVA results indicate that the common ancestor of *K. viridistriga*, *K. paulensis*, and *Kentropyx* sp. was widely distributed in the Brazilian Shield and the Chaco-Paraná Basin, and that a later vicariance event, probably the final epeirogenic uplift of the Brazilian Shield during Middle–Late Tertiary (Colli 2005), promoted the divergence between *K. viridistriga* and the sister group, in the Pantanal and Guaporé depressions. More recently, a parapatric speciation event associated with sandy soils of the Tocantins depression might have promoted the divergence between *K. paulensis* and *Kentropyx* sp.

Reproduction life history evolution

Considering the direct comparisons between species, we found that clutch size and eggs volume can significantly differ between species of the same group (for instance for clutch size: *K. pelviceps* vs. other *calcarata* group species), as well as species of different groups (as *Kentropyx striata* vs. *K. calcarata*; *K. viridistriga* vs. all other species). Within the *paulensis* group the significantly lower clutch size of *Kentropyx paulensis* and *K. vanzoi*, relative to *K. viridistriga*, suggests a derived condition. This implies that low clutch size should have evolved twice within the *paulensis* group or this characteristic was secondarily lost in *K. viridistriga*, which has the greatest clutch size among all species of *Kentropyx* (Table 5).

Our results did not corroborate the hypothesis of Fitch (1982) that postulates larger clutch sizes and smaller eggs in open vegetation species and smaller clutch sizes with larger eggs in forest species, irrespective of phylogenetic structure. Thus, although forest and open vegetation species of *Kentropyx* form monophyletic groups, easily distinguished by meristic characters, such as femoral pores (Table 1), they show conservatism in life history traits. A possible explanation is that variation in reproductive parameters we studied is not affected by major habitat type where species occur. Consequently, species of *Kentropyx* did not diverge in a significant way with respect to their ancestral life history characters. Therefore, nonadaptive phylogenetic constraints and inertia seem to determine clutch size and egg volume in *Kentropyx*, instead of limitations on resource availability associated with different habitat types. Phylogenetic constraints might be recognized when a given trait was in the environment where it has originally evolved, but is under limits on the production of new phenotypic variants (Harvey & Pagel 1991; Blomberg & Garland Jr 2002). Phylogenetic constraints (instead of environmental and climatic variables) that might limit variation in reproductive parameters of *Kentropyx* include: female body size, availability of nest sites, foraging mode, thermoregulation requirements, pelvic constraints (characterized by the inability of large eggs to pass through a small pelvic aperture), life habits (some species have semi-arboreal and semi-aquatic habits), and locomotion performance, among others (Aubret *et al*. 2005; Vitt & Congdon 1978; Vitt 1981; Vitt & Price 1982; Shine & Schwarzkopf 1992; Oufiero *et al*. 2007; Pizzato *et al*. 2007). On the other hand, phylogenetic inertia is often invoked as an alternative hypothesis to adaptation by means of natural selection, to explain lack of interspecific variation in phenotypic traits (Blomberg & Garland Jr 2002). Hence, even after the ending of selective forces that have produced/maintained them, some traits might persist within a lineage (Blomberg & Garland 2002).

Accordingly, even accounting for phylogenetic influences, the major clades of *Kentropyx* present negligible variation in their reproductive strategies. It is essential to emphasize the importance of including species historical relationships in comparative analyses of life history traits. The current features of species and populations may reflect only past adaptations of their ancestors, phylogenetic inertia, and constraints, instead of current adaptations to environmental variation. Thus, ignoring the phylogenetic context may imply ignoring the determinant aspect, as shown for *Kentropyx*.

Conclusions

In summary, our results show that living species of *Kentropyx* form three monophyletic groups, which correspond to the phenetic grouping proposed earlier: *calcarata*, *paulensis* and *striata*. However, relationships among the groups differ from previous suggestions, with *K. striata* being the most basal species. The origin of the genus date back to the Tertiary (Eocene/Oligocene) and the 'Pleistocene Refuge Hypothesis' cannot account for the diversification of *Kentropyx*, and can only be associated with more recent divergence among populations. Ancestors of the genus were not restricted to forests as previously suggested and could be either present or absent from the Amazon Basin. We argue that a savanna ancestor is more likely and that the historical events which promoted the diversification of the genus include: (i) isolation of Brazilian/Guianan Shields attributed to Miocene marine introgressions, corresponding to the basal divergent between *K. striata* (a Guiana Shield species) and other *Kentropyx* species, specially the monophyletic *paulensis* group in the Brazilian Shield; (ii) distribution of *calcarata* species group in Amazon Basin possibly due to dispersion after the marine retraction; (iii) distribution of *K. calcarata* in Atlantic forest due to more recent (Quaternary) forest corridors acting as dispersion routes linking this

forests with the Amazon; (iv) differentiation of *K. vanzoi* from other species of the *paulensis* group occurring during the Miocene, coinciding with the isolation of the Parecis Plateau; (v) final epeirogenic uplift of the Brazilian Shield during the Late Tertiary, driving the differentiation of *K. viridistriga* in the Pantanal and Guaporé depressions and (vi) divergence between *K. paulensis* and *Kentropyx* sp. due to parapatric speciation in the Tocantins depression. SPSM and CPO showed that variation in reproductive parameters was not determined by the major habitat type where species occur, but may reflect past adaptations and phylogenetic inertia, essential aspects of life history evolution for *Kentropyx*.

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Fernanda P. Werneck is a Brazilian PhD student at Brigham Young University currently working on the phylogeography, niche modelling, and conservation genetics of lizards from Seasonally Dry Tropical Forests of South America. Her main research interests are biodiversity, phylogeography and biogeography of Neotropical herpetofauna. Lilian G. Giugliano is a PhD student at Universidade de Brasília focusing cnemidophorines phylogenetic relationships and evolution based on molecular and morphological data. Dr Rosane Garcia Collevatti is a geneticist who is interested in understanding population genetics and phylogeny of tropical species. Dr Guarino R. Colli is a professor in the Department of Zoology at the University of Brasília, with major research interests on the ecology, biogeography, and systematics of the Cerrado herpetofauna.

Appendix I

Specimens examined

The specimens are referred by their individual catalogue numbers, and initials for their respective collections are as follows: CHUNB (Coleção Herpetológica da Universidade de Brasília); FMNH (Field Museum of Natural History), INPA (Instituto Nacional de Pesquisas da Amazônia), KU (Natural History Museum, University of Kansas); MVZ (Museum of Vertebrate Zoology); MZUSP (Museu de Zoologia da Universidade de São Paulo).

Kentropyx altamazonica (235): CHUNB: 7505, 7507, 7508, 9816, 9821–9823, 9829, 9836, 11410–11431, 12775, 12776, 12778, 13327–13331, 13620, 18163–18210, 18212–18217, 22258, 22287, 22327. FMNH: 168016–168021, 168023, 168025, 168064–168066, 168069, 168071, 168075, 168131, 168175, 168177, 168225, 168230, 168232, 168235–168238, 168244, 168247, 168248, 168259, 168275, 168286, 168287, 168290, 168331, 168333–168336, 168338, 168343, 168345–168347, 168356, 168358, 168385–168388, 168390, 168393, 168395, 168397–168399, 168401, 168402, 168414, 168421, 168447, 168451, 168453, 168455, 168458, 208464, 218566, 229382, 229384. INPA: 491, 1466–1470, 1476–1479, 1490, 1494–1497, 1506–1509, 9480, 9481, 9483, 9492, 9493, 9496, 9498, 9499, 9676. KU: 205009, 205015, 209211–209214. MVZ: 163086–163088, 163090–163101, 163103–163113, 174856–174863. MZUSP: 52414, 60800, 70280.

Kentropyx borckiana (4): MZUSP: 51627–51630.

Kentropyx calcarata (231): CHUNB: 1653, 1654, 1656, 5215, 5225–5236, 7360–7362, 7500–7504, 7506, 7509, 9819, 9838, 11295, 11296, 12360, 12504, 12505, 13623, 13624, 13876–13878, 14095, 14096, 15131, 15137, 16145, 16959, 16960, 22239–22250, 22252–22257, 22259, 22260, 22281–22317, 22319–22326, 23822, 24653, 28972, 28994, 29046–29048, 29275. FMNH: 128956, 128958, 128961, 128965–128970, 134728. INPA: 62, 65, 68, 71, 74, 77, 78, 82, 131, 179, 194, 195, 225, 226, 814, 858, 859, 912, 919–923, 1083, 1128, 1274, 1275, 1309, 1310, 1480, 9023, 9591–9593, 9742, 10513, 11500, 11534, 11541, 11551. KU: 69806–69808, 97864, 124630, 127241–127244, 167544–167548. MZUSP: 885, 56785, 60795–60799, 60801, 67728, 68980–68982, 72655, 72658, 72840–72843, 72937–72949, 73280–73298.

Kentropyx paulensis (96): CHUNB: 1657, 5216, 8216, 9431, 9534, 11562–11566, 11568, 13628, 21755, 21756, 21758, 24529, 24541, 24549, 25672–25689, 26030–26033, 26512, 28010–28026, 30887. MZUSP: 10, 402, 629, 970, 986, 999, 1027, 2550, 2622, 4789–4792, 4794, 4795–4797, 4800–4804, 4850, 9944, 21464, 28427, 30716, 78162, 78163, 79655, 83204–83207, 87666, 93411.

Kentropyx pelviceps (156): INPA: 2183, 2184, 9413, 9482, 9484–9490, 9494, 9497, 9594, 9674, 9675, 9677, 9678, 10388, 10440, 11542. KU: 98948, 98949, 105376, 105377, 105379, 109713–109746, 122181–122188, 126793–126800, 144379, 147186, 148194–148204, 175341, 205007, 205008, 205010, 205012, 205013. MVZ: 163114–163138, 173758, 174869–174876, 174878, 174879, 174881, 174883, 174886, 174887, 174889, 174890, 175782, 199526. MZUSP: 12995, 32343, 32346, 32347, 32484, 41524, 41525, 41777, 42114, 42115, 42394–42396, 42399, 72652, 72653, 72656.

Kentropyx striata (219): CHUNB: 1197–1199, 1280–1292, 1300–1317, 1607–1652, 5217–5222, 5237–5243, 14093, 14094, 30825–30833. INPA: 1283, 1284, 10448–10464. MVZ: 84048–84050. MZUSP: 2158, 2977, 3000, 7145, 7214, 7215, 7217–7243, 7246–7248, 7730, 7735, 13525, 15074, 15368–15372, 16593, 16594, 18586, 18587, 23610, 35403, 66702–66704, 66849–66858, 66860–66878, 66985, 66997, 69085–69091, 72659.

Kentropyx vanzoi (160): CHUNB: 9824, 11591–11650, 12274–12280, 14057, 25289, 25290. MZUSP: 783, 801, 806–811, 834–838, 881, 898, 921–923, 941, 942, 64556–64570, 64572–64578, 64581–64605, 74988, 74989, 81614–81828, 88197, 88408–88410, 93410.

Kentropyx viridistriga (21): CHUNB: 29198, 29279. MVZ: 127394–127407. MZUSP: 45906, 45927, 57855, 57856, 74987.

Kentropx sp. (21): CHUNB 9996 10008 10009 10042 10043 10053 10070 10109 10160 10221 10225 10232–10235 10299 10407 10408 10448 10462 10497.

Ameiva ameiva (42): CHUNB: 00868–00877, 00920–00930, 00941–00950, 01553–01559, 01603–01606.

Cnemidophorus gramivagus (64): CHUNB: 3501–3508, 3511, 3513–3515, 3517, 3519, 3520–3522, 3525–3527, 3529–3533, 3535–3545, 3547–3553, 3555–3564, 3509, 3510, 3512, 3516, 3518, 3523, 3524, 3528, 3534, 3554, 7944.

Appendix II

Morphologial data description

From each specimen, we recorded the following quantitative meristic characters: supralabials (number of enlarged scales along the upper jaw, total on both sides), infralabials (number of enlarged scales along the lower jaw, total on both sides), gular folds (number of folds in the gular region), collar scales (number of enlarged scales present in the gular fold), supraoculars (number of supraocular scales on left side), parietals (number of parietal scales, including the interparietal scale), postparietals (number of postparietals scales contacting the interparietal scale, granular scales

were included when present), dorsals (counted along the midline, from occiput to first transverse row of scales around tail), scales around mid-body (counted midway between fore- and hindlimbs, excluding ventrals), transverse rows of ventrals (counted along the midline, from gular fold to anterior margin of hindlimbs), ventrals in one transverse row (counted midway between fore- and hindlimbs), femoral pores (total number on both sides), prefemorals (number of enlarged scales on anterior aspect of thigh, counted midway between the hip and the knee, on a row from femoral pores to granules on dorsal aspect of thigh), prefemoral rows (counted from hip to knee), infratibial rows (number of enlarged scales on longitudinal row from knee to base of first metatarsal), preanals (number of enlarged scales on preanal plate, from level of medialmost femoral pores to vent), fourth finger lamellae (counted under the finger), fourth toe lamellae (counted under the toe), scales around tail (counted on 15th transverse row).

We recorded the following qualitative characters, with no intraspecific variation (polymorphism): granular scales between chinshields and infralabials (absent or present), contact between supraciliaries and supraoculars (absent or present), precloacal spur in males (absent or present), keeled ventrals (absent or present), and dorsal scales of tail (smooth or keeled). Finally, we also scored the following qualitative characters, with intraspecific variation (polymorphism): shape of frontonasal (hexagonal or pentagonal); degree of contact between first pair of chinshields (no contact; contact smaller than half of their lengths or contact greater than half of their lengths); degree of contact between supraoculars and medial head scales (supraoculars contacting prefrontal, frontal, frontoparietals and parietals; supraoculars contacting prefrontal, frontal and frontoparietals; supraoculars contacting prefrontal and frontoparietal; no contact between supraoculars and medial head scales); shape of posterior margin of interparietal (flat, angular or rounded); condition of dorsals (granular dorsal and lateral scales, with a clear distinction between dorsals and keeled plate-like supracaudals; granular dorsal and lateral scales, gradually enlarging to the tail, where dorsal and supracaudals are almost indistinct; rows of enlarged plate-like dorsal and granular lateral scales); hindlimb spots (absent or present), lateral spots (absent or present) and pattern of stripes and fields. Fields are delimitated by stripes, and we considered the following states: absent (when stripes that delimit field are absent), dark, spotted, or light. The fields we scored were: vertebral fields (middorsal between paravertebral and vertebral stripes); dorsolateral fields (between paravertebral and dorsolateral stripes); upper lateral fields (between dorsolateral and upper lateral stripes); and lower lateral fields (between lateral stripes and ventral scales).