Cryptic diversity in South American Teiinae (Squamata, Teiidae) lizards

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Based on phylogenetic and molecular dating analyses of several species of *Cnemidophorus* and *Ameiva*, representing major groups of species of these two genera, we uncover a previously unrecognized *Ameiva* lineage, which includes described *Cnemidophorus parecis* from south-western Amazonia. We discuss the diagnosis of *Ameiva* and *Cnemidophorus* and the implications of the new taxonomic rearrangement of genera from Teiidae for the monophyly of *Ameiva*. Based on the conclusion of our analyses, we provide description of a new species named *Ameiva jacuba* from the central Brazilian Cerrado and a detailed diagnosis for the relocation of *C. parecis* to *Ameiva*. We do not adopt here recent taxonomic changes proposed for Teiidae and provide a discussion about them. Finally, based on molecular dating and the distribution of living species, we propose an evolutionary scenario for the origins of South American cis-Andean *Ameiva* lineages, associated with the topographic subdivision of the Cerrado region during Miocene marine introgressions.

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Introduction

Knowledge on patterns of diversity and distribution of species is crucial for efficient conservation planning; therefore, overcoming the Linnean and Wallacean shortfalls (Brown & Lomolino 1998; Lomolino 2004; Brito 2010) has become a priority, in face of the global biodiversity crisis (Walpole *et al.* 2009; Rands *et al.* 2010; Barnosky *et al.* 2011). These shortfalls stem primarily from insufficient sampling, including the reduced number of sampled localities and sampled individuals in each locality (Costa *et al.* 2010; Funk *et al.* 2012) and the existence of a large number of cryptic species (Bickford *et al.* 2007; Gamble *et al.* 2012; Werneck *et al.* 2012). Further, our restricted understanding of phylogenetic relationships, the Darwinian shortfall, limits the knowledge on spatial variation of phylogenetic

diversity (Pace 2009; Davies & Buckley 2011; Morlon *et al.* 2011). This situation is especially critical in the so-called hotspots of discovery, regions where our knowledge of biodiversity is most limited but where biodiversity is highly threatened by habitat destruction (Guénard *et al.* 2012).

The Cerrado of South America (Oliveira & Marquis 2002), the largest tropical savanna, has lost approximately 80% of its original cover (ca. two million km^2) to habitat destruction (Cavalcanti & Joly 2002; Klink & Machado 2005; Marris 2005), resulting in its inclusion as one of the global biodiversity 'hotspots' (Mittermeier *et al.* 1998, 2004; Myers *et al.* 2000). Many new squamate species have been described in Cerrado (Colli *et al.* 2009; Pinna *et al.* 2010; De Freitas *et al.* 2011), and many more await description. As a result of a series of herpetological surveys

in Cerrado, we discovered an undescribed species, with a puzzling combination of characters intermediate between *Ameiva ameiva* (Linnaeus, 1758) and *Cnemidophorus ocellifer* (Spix, 1825). This demanded phylogenetic analyses including the new species and representatives of the major species groups of *Cnemidophorus* and *Ameiva*.

Living Teiidae comprise 10 genera (*sensu* Reeder *et al.* 2002) that are restricted to the New World, from Argentina to north-eastern United States (Vitt & Caldwell 2009). *Callopistes*, *Crocodilurus*, *Dicrodon*, *Dracaena*, *Kentropyx*, *Teius* and *Tupinambis* are limited to South America, whereas *Cnemidophorus* is also found in West Indies and *Ameiva* reaches Central and North America. *Aspidoscelis* ranges from north-western Costa Rica to the United States (Presch 1974; Krause 1985; Reeder *et al.* 2002). *Ameiva*, *Aspidoscelis*, *Cnemidophorus* and *Kentropyx* form a well-supported monophyletic group, the 'cnemidophorines' (Presch 1974; Reeder *et al.* 2002; Giugliano *et al.* 2007). A recent taxonomic review, based only on 137 morphological characters for almost one hundred species, increased to 14, the number of teiid genera. Herein, we do not follow this new nomenclature, but discuss some of the proposed changes.

With about 37 described species (Ugueto & Harvey 2011), *Ameiva* ranges in the West Indies, Central and South America. A phylogenetic analyses based on mtDNA sequences, allozymes and morphologic data indicated the paraphyly of *Ameiva* (Reeder *et al.* 2002); however, only six species of the genus were included. Later, a molecular phylogeny based on 12S and 16S regions of the mtDNA, including most of the Antillean species, indicated the monophyly of this insular assemblage and divided it into four groups (Hower & Hedges 2003). Little is known about the phylogenetic relationships of the trans-Andean species. *Ameiva festiva*, the only trans-Andean species included in a molecular phylogenetic analysis, is apparently related to *A. undulata*, a Central American species (Hower & Hedges 2003). Based on a phylogenetic analysis of morphological characters, Harvey *et al.* (2012) split the trans-Andean species in two genera: *Holcosus*, which includes other Central American species, and *Medopheos*, a monospecific genus for *Ameiva edracantha*. Reeder *et al.* (2002) suggested that the Antillean species of *Ameiva* are closely related to *Aspidoscelis*, whereas continental species are more related to *Cnemidophorus* and *Kentropyx*. Nevertheless, Harvey *et al.* (2012) grouped the Antillean and cis-Andean species under *Ameiva*. The alpha diversity of cis-Andean teiids is rapidly increasing: more than 40% of the 'cnemidophorines' in this region were described during the last 20 years. From the seven known species of cis-Andean *Ameiva*, two (28%) were described since 1993 (Uetz *et al.* 2012).

Ameiva ameiva, the type species of the genus, is widely distributed in cis-Andean South America, from Venezuela and Colombia to Argentina. Several subspecies were recognized (Barbour & Noble 1915; Donoso-Barros 1968; Peters & Donoso-Barros 1970), but most adopt a conservative view considering a monotypic, widespread *A. ameiva* (e.g. Vanzolini 1986; Vitt & Colli 1994; Ávila-Pires 1995). *Ameiva ameiva* is the single species in most of South America, and is easily found in a wide range of environments, from open savannas to forest edges and clearings, and also anthropogenic areas (Vitt 1982; Colli 1991; Sartorius *et al.* 1999). Vitt & Colli (1994) found large morphological similarities among eight populations of *A. ameiva* in four different habitats, concluding that body size and shape are very conservative throughout its range. However, no study has yet examined the genetic structure of this widespread species. Based on external morphology, Ugueto & Harvey (2011) concluded that *A. ameiva* in Venezuela comprised at least four evolutionary species, elevated *A. a. ameiva*, *A. a. atrigularis* and *A. a. praesignis* to the species rank, and also described a new species, *A. pantherina*, from southern Monagas savannas.

The differences between *Ameiva* and *Cnemidophorus* have been extensively debated, as well as the validity of these two closely related genera (Presch 1974). With few exceptions, the lingual sheath (present in *Ameiva* and absent in *Cnemidophorus*) and the number of longitudinal ventral plates (eight in *Cnemidophorus* and 10 or more in *Ameiva*) seem to be important diagnostic characters (Boulenger 1885; Burt 1931; Presch 1971; Cei & Scrocchi 1991). However, no single character correctly classifies all species from both genera (Burt 1931; Presch 1974). As a consequence, several synonyms involving *Ameiva* and *Cnemidophorus* are known. *Ameiva bifrontata* and *A. praesignis* were classified, respectively, as *C. divisa* and *C. maculatus* by Fischer (1879). *Ameiva vitatta* was described as *C. vittatus* (Boulenger 1902) and relocated to *Ameiva* (Burt 1931; Vance 1978). *Cnemidophorus lacertoides* was named *A. lacertoides* (Burt 1931) and relocated to *Cnemidophorus* (Gallardo 1966). *Cnemidophorus longicauda* and *C. vanzoi* were originally described as *A. longicauda* (Bell 1843) and *A. vanzoi* (Baskin & Williams 1966), respectively. Further, a new species from a Cerrado enclave in south-western Amazonia was described as *C. parecis* (Colli *et al.* 2003b), but allozyme data indicated it is more related to *A. ameiva* than to other species of *Cnemidophorus* (Giugliano *et al.* 2006). Based only on our previous study (Giugliano *et al.* 2006) and without examining any specimen, Harvey *et al.* (2012) allocated *C. parecis* to *Ameiva incertae sedis*. In this context, herein we (1) recover the phylogenetic relationships of *C. parecis* and the new species of *Ameiva* among teiid genera, (2) discuss the diagnosis of *Ameiva* and *Cnemidophorus* and the new taxonomic arrangement in the light of a well-supported molecular phylogeny, (3) describe the new species of *Amei-*

va and re-evaluate the taxonomic position of *C. parecis*, (4) recover a biogeographical scenario for the evolution of South American cis-Andean *Ameiva* and (5) present new data on the pattern and timing of diversification of the herpetofauna of Cerrado.

Materials and methods

Taxon sampling and molecular data

We assembled a molecular data set from 12S and 16S mtDNA genes and c-mos nuclear gene, previously published on GenBank or obtained by us. The data set includes 17 species, representing all "cnemidophorine" genera (*Ameiva*, *Aspidoscelis*, *Cnemidophorus* and *Kentropyx*) and all *Cnemidophorus* species complexes: *lemniscatus*, *longicauda*, *ocellifer* and *lacertoides* (Cei & Scrocchi 1991; Cole & Dessauer 1993; Feltrim & Lema 2000; Dias *et al.* 2002). We used *Tupinambis teguixin* (Linnaeus 1758) as outgroup (Supporting Information, Table S1). We deposited sequences in GenBank (Supporting Information, Table S1) and the trees in TreeBase (accession numbers). Samples were obtained through fieldwork led by GRC, CN and PHV, and vouchers were deposited in Coleção Herpetológica da Universidade de Brasília (CHUNB).

We extracted genomic DNA from liver samples using the DNeasyTM Tissue kit (QIAGEN; Valencia, CA, USA) and used PCR to amplify a fragment of nearly 350 bp of the 12S gene and nearly 500 bp of the 16S gene, using the 12Sa, 12Sb, 16SaR and 16Sd primers and the same PCR conditions described in Reeder (1995). The nuclear gene c-mos was amplified with G73 and G74 primers as described by Saint *et al.* (1998). PCR products were sequenced on an ABI Prism 377 automated DNA sequencer (Applied Biosystems; Foster City, CA, USA) using DYEnamicTM ET terminator cycle sequencing kit (GE HealthCare Corp.; Stockholm, Sweden), according to the manufacturer's instructions. We analysed and edited sequences with SEQSCAPE V2.1 (Applied Biosystems) and obtained a multiple alignment for the mtDNA sequences based on the RNA secondary structure with R-Coffee, using the fast approximation mode (Moretti *et al.* 2008; Wilm *et al.* 2008). We aligned nuclear sequences with T-Coffee (Notredame *et al.* 2000) and found no internal gaps among ingroup taxa.

Phylogenetic analysis and divergence dating

We conducted concatenated phylogenetic analyses based on maximum-likelihood (ML) and Bayesian inference (BI) methods. We assessed the model of sequence evolution of 12S and 16S sequences based on the Akaike's Information Criterion (AIC) with jModelTest (Posada 2008). For the concatenated data, each sequence data set had its own independent model of evolution and model parameters. We excluded gaps from all analyses. We conducted ML analy-

ses with MEGA5 (Tamura *et al.* 2011) and assessed the reliability of results with 1000 bootstrap replications (Felsenstein 1985).

For BI, we used MRBAYES v.3.0b4 (Huelsenbeck & Ronquist 2001) with a randomly generated starting tree. We conducted two independent runs, each consisting of twenty million generations, sampled every 1000 generations. We assessed the burn-in phase of 2 million generations with TRACER v. 1.4 (Rambaut & Drummond 2007). We regarded the frequency of any particular clade in the majority-rule consensus tree of the stationary stage, from the two independent runs, as the posterior probability of that node (Huelsenbeck *et al.* 2001).

We estimated divergence times with BEAST v. 1.6.1 (Drummond & Rambaut 2007) using an uncorrelated relaxed clock, to allow for rate heterogeneity among lineages, and a Yule prior (Aldous 2001). We conducted all analyses using the best model of sequence evolution selected by jModelTest. For calibration, we used normal priors based on estimates from Giugliano *et al.* (2007) for the most recent common ancestor of 'cnemidophorines' (mean = 33.7 Ma [millions of years ago], SD = 3.37 Ma). We implemented three hundred million generations and discarded the first thirty million burn-in generations as assessed by TRACER v. 1.4 (Rambaut & Drummond 2007).

Morphological data

We examined patterns of scalation and external coloration for interspecific comparisons. We used data on *Cnemidophorus parecis*, *C. ocellifer*, *C. lemniscatus* (Linnaeus, 1758), *C. gramivagus* McCrystal and Dixon, 1987, *C. lacertoides* Duméril and Bibron, 1839, *C. longicauda* (Bell 1843) and *A. ameiva* housed at CHUNB and Centro Nacional Patagónico-CENPAT, Puerto Madryn, Argentina (Supporting Information, Appendix S1). From each specimen, we recorded the following meristic counts: 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), chinshields (most anterior pair of chinshields separated from infralabials by row of small scales), gular folds (number of folds in gular region), supraoculars (number of supraoculars on right side), parietals (number of parietals plus interparietal scale), scales around mid-body (counted midway between fore- and hind limbs, excluding ventrals), transverse rows of ventrals (counted along the midline, from gular fold to anterior margin of hind limbs), ventrals in transverse row (counted midway between fore- and hind limbs), 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), rows of prefemorals

(counted from hip to knee), rows of infratibials (number of enlarged scales on longitudinal row, from knee to base of first metatarsal), precloacals (number of enlarged scales on precloacal plate, from level of medial femoral pores to vent), fourth finger lamellae (counted under the finger), fourth toe lamellae (counted under the toe), scales around tail (counted on fifth transverse row) and dorsals (counted along the midline, from occiput to first transverse row of scales around tail).

We recorded the following categorical variables: vertebral line (light stripe from interparietal scale to base of tail: absent [A], interrupted [B, not reaching the interparietal scale or the base of tail], continuous [C, complete from interparietal scale to base of tail] or double [D]), paravertebral stripes (one on each side, light stripe from parietal scale to first third of tail, between vertebral and dorsolateral fields: absent [A], interrupted [B], continuous [C] or double [D]), dorsolateral stripes (one on each side, light stripe from superciliaries to first third of tail, between dorsolateral and upper lateral fields: absent [A], interrupted [B] or continuous [C]), upper lateral stripes (one on each side, light stripe from suborbital region to hind limb, between upper and lower lateral fields: absent [A], interrupted [B] or continuous [C]), lower lateral stripes (one on each side, light stripe from axilla to hip, between lower lateral field and ventrals: absent [A], interrupted [B] or continuous [C]), lateral spots (rounded light areas on flanks: absent [A], present [B]), hind limb spots (rounded light areas on hind limbs: absent [A], present [B]), chinshield contact (degree of contact between most anterior pair of chinshields: no contact [A], contact smaller than half of their lengths [B] or contact greater than half of their lengths [C]), semicircles (degree of contact between supraoculars and medial head scales: no contact [A], no contact with semicircles isolating first supraocular [B], supraoculars contacting frontal and parietals [C], supraoculars contacting frontal [D], supraoculars contacting parietals [E]), dorsal caudals (keels on dorsal, caudal scales, from most anterior third of tail: absent [A], present [B]) and ventral caudals (keels on ventral, caudal scales, from most anterior third of tail: absent [A], present [B]).

From the 29 morphological variables recorded on 525 individuals, 533 of the 15225 data points were missing data (3.5%), which we replaced using multivariate imputations by chained equations (Van Buuren et al. 1999, 2006; Van Buuren 2007) implemented with the *mice* package of R v. 2.12.2 (R Development Core Team 2011). To investigate morphological differences between *Ameiva* and *Cnemidophorus* and between the new species and its closer relatives, we used a logistic regression analysis (Tabachnick & Fidell 2001) based on all morphological variables with R v. 2.12.2 (R Development Core Team 2011). To assess the statistical significance of the full model, we compared it against a con-

stant-only (null) model using a chi-square test of the scaled deviance (Chambers & Hastie 1992; Faraway 2006). We also assessed the importance of each variable in the model-by-model selection via single term addition (Chambers & Hastie 1992) as follows: (1) the full model was tested against a constant-only model; (2) the significant term with the lowest AIC value was added to the null model; (3) step two was repeated; (4) any non-significant terms were dropped from the model; (5) steps three and four were repeated until no significant terms could be added or no non-significant terms could be dropped from the model.

Tongue structure

Historically, two tongue characters (tongue sheath and posterior edge of scaly portion of tongue) have been used to differentiate *Ameiva* and *Cnemidophorus*. To assess the diagnostic usefulness of these characters, we dissected the tongue of one preserved individual of each of the following species: *C. parecis*, *Ameiva* sp. n., *A. ameiva*, *C. lemniscatus* (representing the *lemniscatus* complex) and *C. ocellifer* (representing the *ocellifer* complex). We photographed tongues on a stereomicroscope and coded the two characters as following: tongue sheath, absent [A] or present [B]; posterior edge of scaly portion of the tongue, not forked or slightly forked [A], or clearly forked, with free or nearly free lateral posterior extensions [B].

Results

Taxon sampling and molecular data

The concatenated molecular data set consisted of 1201 aligned base pairs from three loci for 18 teiid taxa (Supporting Information, Table S1). The aligned 12S sequences consisted of 334 base pairs, with 123 variable sites, whereas the aligned 16S sequences consisted of 492 base pairs, with 149 variable sites. The aligned c-mos nuclear sequences consisted of 375 base pairs, with 72 variable sites.

Phylogenetic analysis and divergence dating

The estimated model of evolution for 12S was GTR + G and for 16S was GTR + G + I. For the nuclear c-mos, the simpler HKY model was selected. ML and BI analyses of the concatenated data set yielded largely congruent topologies (Fig. 1). *Cnemidophorus parecis* and *Ameiva* sp. n. were placed in a clade more closely related to *A. ameiva* than to *Cnemidophorus*, with high branch support in both analyses (Fig. 1). Both analyses indicated the paraphyly of *Ameiva* and *Cnemidophorus*, but diverged on the phylogenetic position of some taxa, with low support values. We also analysed the nuclear gene separated from the mitochondrial fragments and the high supported clade formed by *Ameiva* sp. n., *A. ameiva* and *C. parecis* were corroborated in both data sets (Fig. S1–S2).
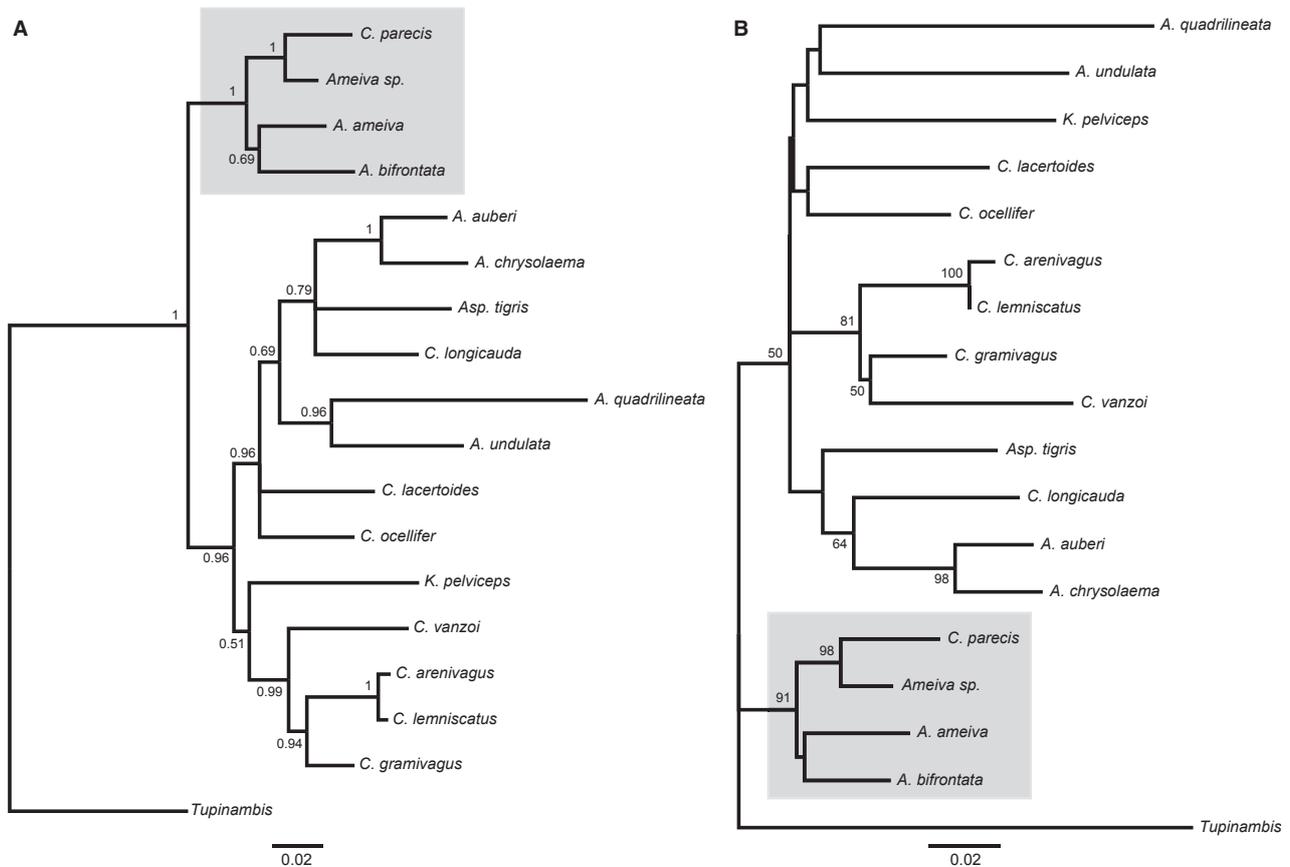
Fig. 1 'Cnemidophorine' phylogeny based on A. Bayesian inference analysis with posterior probabilities and B. maximum likelihood with 1000 replicates bootstrap values.

The molecular dating analyses recovered a similar topology and indicated that the divergence between *A. ameiva* and the lineage leading to *C. parecis* and *Ameiva* sp. n. took place approximately 15 Ma, during the middle Miocene. This analysis also indicated a divergence between *C. parecis* and *Ameiva* sp. n. at approximately 8 Ma, on the late Miocene (Fig. 2).

Morphological data

A logistic regression analysis indicated that the 29 morphological variables taken together reliably ($P < 0.001$) differentiated *Ameiva* from *Cnemidophorus* (Tables S2–S4). The logistic regression scores of the two genera presented no overlap; thus, no misclassification is expected using the full model (Supporting Information, Fig. S3A). A model selection analysis indicated that scales around tail and ventrals in transverse row fully discriminate the two genera, with *Ameiva* having larger values of the two variables (Supporting Information Fig. S3B).

The logistic regression analysis performed on *Ameiva* sp. versus its closest relatives (*C. parecis* and *A. ameiva*, taken as a group) as outcome indicated that the 28 morphological variables taken together reliably ($P < 0.001$, Table S4) distinguish *Ameiva* sp. n. from *C. parecis* and *A. ameiva*. We excluded the vertebral line from this analysis because it is absent in the three species. The logistic regression scores of the two groups presented no overlap, indicating no misclassification using the full model (Supporting Information Fig. S4). A model selection analysis indicated that upper lateral stripes (interrupted in *Ameiva* sp. n., mostly absent in *A. ameiva* and mostly continuous in *C. parecis*), followed by dorsolateral stripes (mostly interrupted in *Ameiva* sp. n., mostly absent in *A. ameiva* and mostly continuous in *C. parecis*), and scales around tail (41–49 in *Ameiva* sp. n., 29–45 in *A. ameiva* and 34–47 in *C. parecis*) are the variables that best discriminate *Ameiva* sp. n. from its closest relatives.

In *Ameiva* sp. n., *A. ameiva* and *C. parecis*, the tongue sheath is developed and lacks a forked posterior edge of the scaly portion of tongue (Fig. S5 A–C). Conversely, in *C. lemniscatus* and *C. ocellifer*, the tongue sheath is absent and the scaly portion of tongue is clearly forked (Fig. S5 D–E), with free or nearly free lateral posterior extensions.
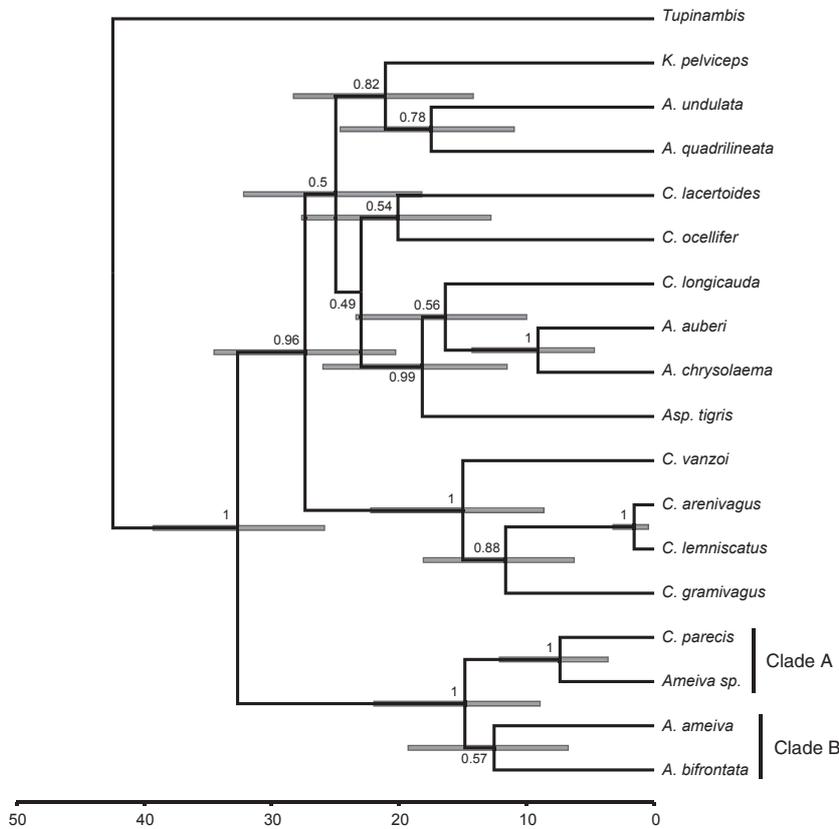
Fig. 2 Chronogram of 'cnemidophorine' evolution based on the combined molecular data (12S + 16S + c-mos), with divergence times estimated from a Bayesian relaxed molecular clock approach. Boxes indicate mean divergence time ± 95% confidence intervals.

Taxonomy

Three sources of evidence indicate that *C. parecis* and the new species actually belong to *Ameiva*. First, and most important, the molecular phylogenetic analyses showed that both species are more related to *Ameiva ameiva* than to the other 'cnemidophorines'. Second, the traditional characters used to distinguish *Ameiva* and *Cnemidophorus* (Boulenger 1885; Burt 1931; Presch 1971; Cei & Scrocchi 1991) are also consistent with the phylogenetic analyses: both *C. parecis* and the new species have a tongue sheath (absent in *Cnemidophorus*), lack a forked posterior edge of the scaly portion of tongue (forked in *Cnemidophorus*) and have 10 ventrals in a transverse row (generally eight in *Cnemidophorus*) (Table S2). Third, the number of scales around the tail supports this arrangement, which clearly discriminates *Ameiva* from *Cnemidophorus*. Below, we describe the new species and relocate *C. parecis* to *Ameiva*.

REPTILIA: SQUAMATA: TEIIDAE.

Genus *Ameiva* Meyer, 1795.

Ameiva jacuba sp. n. (Figs. 4 and S6).

Holotype. CHUNB 26586 (Fig. 3), adult male, Brazil, Goiás state, municipality of Chapadão do Céu, Parque Nacional das Emas, 821 m, pitfall trapping site in open, interfluvial Cerrado grassland ('campo limpo'), CL site (Valdujo *et al.* 2009), 18°13′ 08″ S, 52°46′17″ W (Fig. 4), collected between September 2001 and February 2002 by P. H. Valdujo.

Paratypes. Twenty nine specimens; CHUNB 25734–35, 25737–39, 25741–49, 26505–06, 26584, 26586–87, 25736, 25740, 26583, 26585, 26588–89, 47996–99, 48052; all collected at the following sites within Parque Nacional das Emas (see Fig. 4), Goiás state: Mineiros municipality: CA: 18°15′ 05″ S, 52°53′06″ W, CS: 18°13′ 33″ S, 52°50′14″ W; P2: 18°06′ 07″ S, 52°55′21″ W; S3: 18°17′ 25″ S, 52°53′23″ W; W: 18°11′ 30″ S, 52°52′12″ W; C1: 18°14′ 42″ S, 52°52′57″ W; Chapadão do Céu municipality: CL: 18°13′ 08″ S, 52°46′17″ W C3: 18°18′ 23″ S, 52°48′24″ W. Site descriptions and coordinates in Valdujo *et al.* (2009). All sites between 800–850 m above sea level (see Fig. 4).

Etymology. Jacuba originates from the Tupi *y-acub*, which means warm water, and is also the name of a river with headwaters in Parque Nacional das Emas. In Portuguese, it further refers to a pap prepared from cassava flour, mixed with water, milk or aguardiente ('cachaça'), and sugar, honey or raw brown sugar ('rapadura'), which was popular among mule troopers in the eighteenth and nineteenth centuries.

*Diagnosis.* A species distinguished from other South American cis-Andean species of *Ameiva* by the following
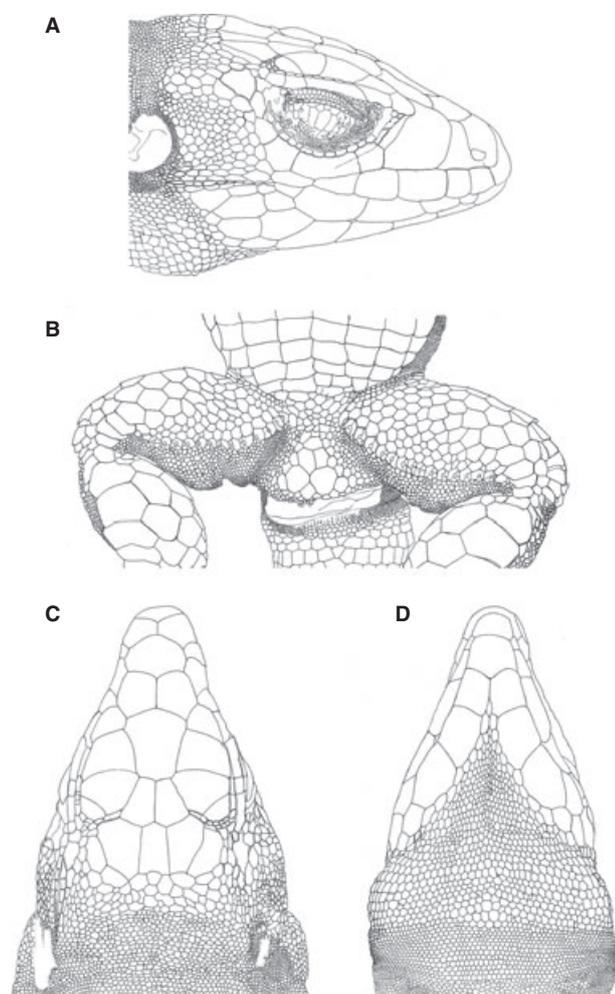
Fig. 3 *Ameiva jacuba*, holotype, CHUNB 26586, adult male, SVL 105 mm. A. lateral view of head; B. precloacal plate and femoral pores at each side; C. dorsal view of the head; D. ventral view of the head.

combination of characters: (1) 6–11 infralabials; (2) 0–2 chinshields; (3) 1–2 gular folds; (4) 3–6 parietals; (5) 31–35 transverse rows of ventrals; (6) 10–11 ventrals in transverse row; (7) 24–32 femoral pores; (8) 9–13 rows of prefemorals; (9) 2–60 precloacals; (10) 22–30 fourth toe lamellae; (11) 41–49 scales around the tail; (12) 201–284 dorsals; (13) dorsolateral stripes mostly interrupted; (14) upper lateral stripes interrupted; (15) lower lateral stripes mostly interrupted; (15) lateral spots absent; (16) hind limbs spotted; (17) chinshield contact greater than half of their lengths; 106 mm maximum SVL. *Ameiva jacuba* differs from *A. ameiva* in having 10–11 ventrals in transverse row (12), 24–32 femoral pores (34–43), lateral spots absent (present), smaller body size, maximum SVL 106 mm (190 mm, Vitt & Colli 1994). *Ameiva jacuba* differs from *A. parecis* in

having 6–11 infralabials (10–15 in *A. parecis*), upper lateral stripes interrupted (mostly continuous), spotted hind limbs (spots absent), mostly interrupted dorsolateral stripes (mostly continuous), larger body size, maximum SVL 106 mm (90 mm, Colli *et al.* 2003a,b), head brown with white spots (lime green laterally); ventral aspect of head predominantly white (yellowish), lateralmost longitudinal rows of ventrals white (vivid blue). *Ameiva jacuba* differs from *A. bifrontata* Cope 1862 in having a frontal undivided (divided transversally in the mid-region in *A. bifrontata*), granules between supraoculars and frontals absent (present), smaller size, maximum SVL 106 mm (101–116 mm), 6–11 infralabials (12) (Cope 1862; Boulenger 1885; Barbour & Noble 1915). *Ameiva jacuba* differs from *A. provitaae* Garcia-Pérez 1995; in having a frontal undivided (divided transversally in the mid-region in *A. provitaae*), smaller size, maximum SLV 106 mm (110–130 mm), 6–11 infralabials (12), 96–133 scales around mid-body (142 in *A. provitaae* holotype), vertebral line absent (present) (Garcia-Pérez 1995). *Ameiva jacuba* differs from *A. vittata* (Boulenger 1902) by having 96–133 scales around mid-body (74) and 24–32 femoral pores (18) (Boulenger 1902; Vance 1978). *Ameiva jacuba* differs from *A. atrigularis* (Garman 1887) by having 96–133 scales around mid-body (134–179 in *A. atrigularis*), 24–32 femoral pores (31–42), 22–30 fourth toe lamellae (32–41), 201–284 dorsals (263–361) and smaller size, maximum SVL 106 mm (186 mm) (Barbour & Noble 1915). *Ameiva jacuba* differs from *A. pantherina* Ugueto & Harvey 2011 by having 96–133 scales around mid-body (137–163 in *A. pantherina*), 24–32 femoral pores (32–40), 22–30 fourth toe lamellae (30–37), 201–284 dorsals (291–343) and smaller size, maximum SVL 106 mm (152 mm) (Ugueto & Harvey 2011). *Ameiva jacuba* differs from *A. praesignis* (Baird & Girard, 1852) by presenting 96–133 scales around mid-body (111–157 in *A. praesignis*), 24–32 femoral pores (28–42), 22–30 fourth toe lamellae (29–40), 201–284 dorsals (237–348) and smaller size and maximum SVL 106 mm (243 mm) (Ugueto & Harvey 2011).

Description of holotype

Adult male, 105 mm SVL, 421 mm total length, 36.0 g total mass (preserved specimen). Rostral pentagonal, as high as tall, visible from above, bordered posteriorly by nasals and first supralabials. Nasals in contact along midline; each nasal divided by an oblique suture. Nostril in lower part of suture, directed postero-superiorly, higher than wide. Frontonasal subelliptical with two concave insertions for the prefrontals, suture between nasals and prefrontals forming a semicircle. Prefrontals pentagonal, with medial suture approximately twice as long as that between nasals; in contact laterally with loreal, first supraciliary
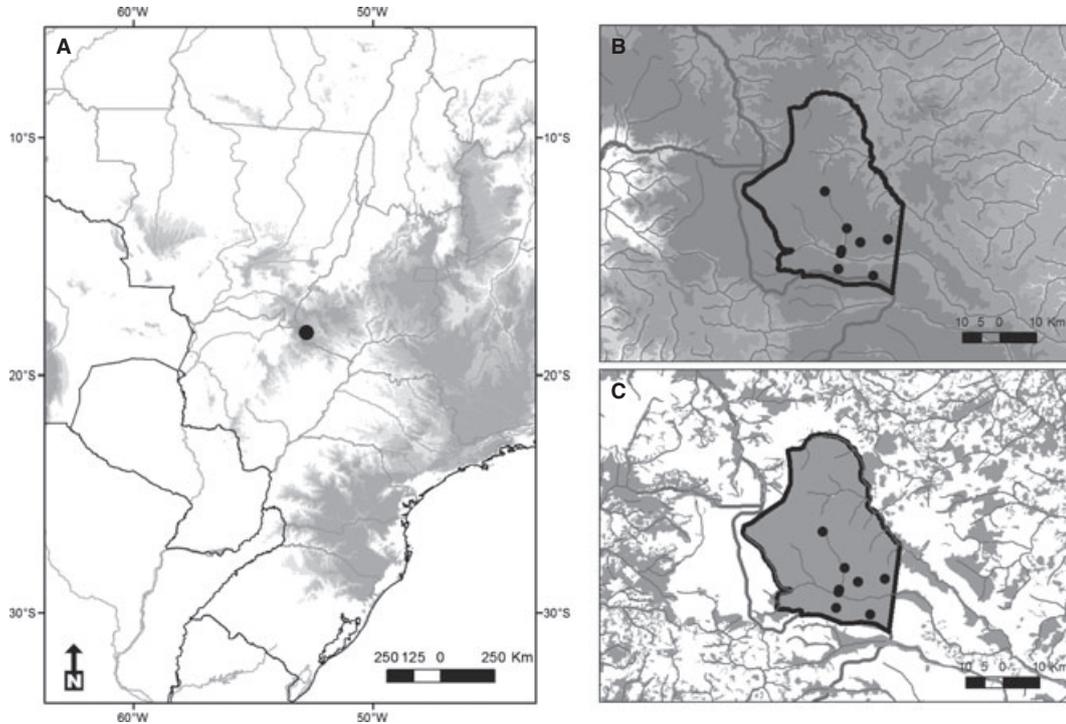
Fig. 4 Distribution of *A. jacuba* according to altitudinal variation in central Brazil. In (A) and (B), white areas correspond to depressions below 500 m, light grey 500–600 m, grey 600–800 m, dark grey 800–900 m above sea level. In (C), grey areas correspond to remaining Cerrado vegetation within and around Parque Nacional das Emas boundaries (dark line).

and first supraocular. Frontal approximately pentagonal, longer than wide, wider anteriorly; suture with prefrontals and frontoparietals approximately straight; in contact with first, second and third supraoculars. Frontoparietals pentagonal, longer than wide, with long straight medial suture and straight sutures with interparietal and parietals. Interparietal subpentagonal, bordered at each side by subpentagonal parietals. Occipitals irregular and variable in size. Four supraoculars on each side; fourth supraocular smallest; first supraocular in contact with prefrontal, frontal, first and second supraciliaries. Five supraciliaries on the left and six on the right side; first, second and third supraciliaries larger, others subequal. Loreal single, large, in contact with nasal, frontonasal, frontoparietal, first supraciliary, first and second suboculars (sensu Ugueto & Harvey 2010) and third and fourth supralabials. Five suboculars on both sides; first narrow, higher than wide, in contact with second subocular, loreal and scales in ocular region; third longest; all in contact with supralabials; a continuous keel runs from first to fifth subocular on both sides. Postoculars small, irregular, arranged in 3–4 rows, with four moderately larger scales. Lower eyelid with semi-opaque disc, formed by transversally enlarged, convex scales. Six enlarged supralabials on both sides, followed by

series of small scales extending to commissure of mouth; suture between fifth and sixth below centre of eye. Temporal region with irregular scales, granular centrally, moderately enlarged peripherally. Ear opening large, subcircular, with smooth margins, anterior margin forming a semicircle, posterior margin straight. Tympanum recessed in a short auditory meatus. All dorsal and lateral head scales juxtaposed, smooth (except for keeled suboculars).

Symphyseal anteriorly ellipsoid, posteriorly in contact with first infralabials and postsymphyseal, forming two wide angles. Postsymphyseal single, pentagonal, in contact with first and second infralabials on both sides, followed by three pairs of enlarged chinshields. First pair of chinshields in ample contact along midline; all pairs in contact with infralabials and followed posteriorly by enlarged scales. Medial chin scales small, smooth, elongate, arranged in longitudinal, posteriorly divergent rows; increasing in size and becoming roundish posteriorly. Four infralabials on right side, fourth below centre of eye; five infralabials on left side, fifth below centre of eye, followed posteriorly by series of small scales extending to commissure of mouth; first infralabials smallest. Gular region divided into two areas: anterior region with enlarged, round scales, in

transverse rows, delimited posteriorly by line uniting lower margin of ear openings; posterior region covered with granules, in transverse rows, bordered posteriorly by antegular fold. Gular and antegular folds marked by granules; scales between the two folds larger, increasing in size posteriorly, irregular.

Scales on nape and sides of neck similar to dorsals. Dorsals and scales on flanks granular, round, smooth, subimbricate; 246 scales from interparietal to base of tail. Scales around mid-body 119, excluding ventrals. Ventrals large, smooth, rectangular (wider than long), imbricate, in 10 longitudinal (at mid-body) and 32 transverse rows. Ventrals separated from scales on flanks by row of moderately large scales. Precloacal plate with three rows of enlarged scales, surrounded by small scales. Precloacal spurs absent. Femoral pores in a continuous row along each thigh, separated medially by short gap; 13 pores on right side, 14 pores on left side.

Scales on base of tail rectangular, smaller than ventrals, in transverse rows; keeled dorsally, smooth ventrally. All transverse rows continuous around tail, except first three, incomplete ventrally. Tail scales longer and narrower posteriorly; subcaudals keeled posteriorly. Limbs with large, smooth, imbricate scales on dorsal aspect of upper arms, anterodorsal aspect of forearms, anteroventral aspect of thighs and ventral aspect of lower legs; elsewhere scales small, granular. Larger scales on upper arms in longitudinal rows. Forearms with one row of enlarged scales, divided posteriorly, wider than long. Anterior scales on thigh decreasing in size proximally. Lower legs with two rows of enlarged, hexagonal scales. Ventral aspect of hands and feet granular; one enlarged tubercle at base of pollex. Subdigital lamellae single; lamellae under left fourth finger 13; under right fourth finger 16; under left fourth toe 23; under right fourth toe 26.

Colour in life. Head greenish brown dorsally, brown with white spots laterally and ventral aspect of head predominantly white (Fig. S6). Dorsum olive green, with black spots; dorsal aspect of tail, hind limbs and forearms brownish. Anterior aspect of thighs, proximal aspect of lower legs, lower flanks and lateralmost longitudinal rows of ventrals white, peppered with brown. Remainder of belly and ventral aspect of limbs and tail predominantly white. Lateral stripe whitish, continuous from suborbital region to hip; lower lateral stripe whitish, interrupted, extending from axilla to hip.

Colour in fixative (70% ethanol, after preservation in 10% formalin)— Head brownish dorsally, bluish white laterally; labial regions and ventral aspect of head bluish white. Dorsum bluish brown, dorsal aspect of tail bluish brown. Belly laterally bluish, with white centre; ventral aspect of tail, forelimbs and hind limbs immaculate.

Distribution and ecology.— Known only from several sites within Parque Nacional das Emas and one neighbouring private area, Fazenda Saramandaia, Alto Araguaia, Mato Grosso, Brazil, ca. 40 km NW of the park border (Fig. 4). *Ameiva jacuba* is found in fire-prone *campo limpo* vegetation (open grassland), usually dominated by the grass *Tristachya leiostachya* ('capim flecha'). *Campo limpo* grasslands are typical of flatlands on tabletop plateaus (locally 'chapadas' or 'chapadões') and are a major feature and typical habitat within Parque Nacional das Emas. Other syntopic lizard species at the type locality are the polychrotid *Anolis meridionalis*, the gymnophthalmid *Micrablepharus atticolus* and the teiids *Kentropyx paulensis* and *Tupinambis duseni*. Three other teiids are known from Parque Nacional das Emas: *A. ameiva*, *Cnemidophorus ocellifer* and *T. merianae*, but these are rarely found in syntopy with *A. jacuba*, being more frequently found in sandy soil cerrado, sandy soil scrubland and gallery forests, respectively. Although lower areas surrounding Parque Nacional das Emas were also sampled (Valdujo et al. 2009), *A. jacuba* was only found in interfluvial plateau grassland areas, a habitat type largely converted by agriculture outside the park limits (Fig. 4C). These tabletop grasslands are main targets for the expansion of mechanized agriculture and have suffered extensive losses conversion to cash crops in the last three decades (Redford 1985; Valdujo et al. 2009).

Family TEIIDAE.

Genus *Ameiva* Meyer, 1795.

Cnemidophorus parecis Colli, Costa, Garda, Kopp, Mesquita, Péres Jr., Valdujo, Vieira and Wiederhecker, 2003b: Figs 3–4 and S5–S6.

Ameiva parecis Harvey, Ugueto,Gutberlet, Jr., 2012.

Ameiva parecis
Abbreviated description: The species is characterized by 11–14 supralabials, 10–15 infralabials, 1–3 chinshields, 1–2 gular folds, 4–5 supraoculars, 2–5 parietals, 96–127 scales around mid-body, 29–35 transverse rows of ventrals, 8–10 ventrals in transverse row, 25–33 femoral pores, 5–12 prefemorals, 10–14 rows of prefemorals, 8–12 rows of infratibials, 4–5 precloacals, 13–19 fourth finger lamellae, 23–31 fourth toe lamellae, 34–47 scales around tail and 190–252 dorsals. The species is also characterized by vertebral line mostly absent, paravertebral stripes absent, dorsolateral stripes mostly continuous, upper lateral stripes mostly continuous, lower lateral stripes mostly interrupted, lateral spots absent, hind limb spots absent.

Coloration in life and preservative: see Colli et al. (2003a,b)
Comparison with other species of Ameiva: *Ameiva parecis* differs from *A. jacuba* in having 10–15 infralabials (6–11 in *Ameiva jacuba*), upper lateral stripes mostly continuous

(interrupted), hind limb spots absent (mostly present), mostly continuous dorsolateral stripes (mostly interrupted) and smaller size, maximum SVL 90 mm (106 mm), head lime green laterally (brown with white spots); ventral aspect of head yellowish (predominantly white), lateralmost longitudinal rows of ventrals vivid blue (white). *Ameiva parecis* differs from *A. ameiva* in having 96–127 scales around midbody (124–149 in *A. ameiva*), 8–10 ventrals in transverse row (12), 2533 femoral pores (34–43), lower lateral stripes mostly interrupted (absent), lateral spots absent (present), hind limb spots absent (present), smaller size, maximum SVL 90 mm (190 mm, Vitt & Colli 1994). *Ameiva parecis* differs from *A. bifrontata* by having a frontal undivided (divided transversally in the mid-region in *A. bifrontata*), granules between supraoculars and frontals absent (present), smaller size, maximum SVL 90 mm (102–116 mm), 34–47 scales around tail (49), hind limb spots absent (present) (Cope 1862; Boulenger 1885; Barbour & Noble 1915). *Ameiva parecis* differs from *A. provitaae* by having a frontal undivided (divided transversally in the mid-region in *A. provitaae*), smaller size, maximum SLV 90 mm (130 mm), 96–127 scales around the mid-body (142), 190–252 dorsals (286), absent of vertebral line (present in *A. provitaae*) (Garcia-Pérez 1995). *Ameiva parecis* differs from *A. vittata* by having 96–127 scales around mid-body (74 in *A. vittata*) and 25–33 femoral pores (18 in *A. vittata*) (Boulenger 1902; Vance 1978). *Ameiva parecis* differs from *A. atrigularis* by having 96–127 scales around mid-body (134–179 in *A. atrigularis*), 25–33 femoral pores (31–42), 23–31 fourth toe lamellae (32–41), 190–252 dorsals (263–361) and smaller size, maximum SVL 90 mm (186 mm) (Barbour & Noble 1915). *Ameiva parecis* differs from *A. pantherina* by presenting 96–127 scales around mid-body (137–163 in *A. pantherina*), 25–33 femoral pores (32–40), 23–31 fourth toe lamellae (30–37), 190–252 dorsals (291–343) and smaller size, maximum SVL 90 mm (152 mm) (Ugueto & Harvey 2011). *Ameiva parecis* differs from *A. praesignis* by having 96–127 scales around mid-body (111–157 in *A. praesignis*), 25–33 femoral pores (28–42), 23–31 fourth toe lamellae (29–40), 190–252 dorsals (237–348) and smaller size, maximum SVL 90 mm (243 mm) (Ugueto & Harvey 2011).

Discussion

We are aware of the limitation of the concatenated approach that we applied to our molecular data set as it does not allow for genealogical independence of different genes (for a review, see Edwards 2009). However, all fragments in separated (Figs S1 and S2) and concatenated analysis indicated that the clade composed by *Ameiva jacuba* and *A. parecis* is the sister group of *A. ameiva* + *A. bifrontata*, which corroborates previous studies with allozymes and other morphological characters (Giugliano *et al.* 2006; Harvey *et al.* 2012). Both *A. parecis* and *A. jacuba* were first recognized as *Cnemidophorus* (Colli *et al.* 2003a,b; Valdujo *et al.* 2009), indicating that the distinction between the two genera is a difficult issue. Our results underscore the usefulness of molecular data to infer phylogenetic relationships and detect unknown lineages, enabling a more informative taxonomy. Historical characters also corroborate our taxonomic arrangement, and we propose the number of scales around the tail as a new, reliable and easily accessible character to distinguish *Ameiva* and *Cnemidophorus*. In spite of a trend for new Cerrado species to have small size, restricted range and inhabit poorly sampled areas (Diniz-Filho *et al.* 2005; Costa *et al.* 2007), we found a new species within a lineage of relatively large lizards that occurs in a well-sampled area (Costa *et al.* 2010). This indicates that knowledge of Cerrado biodiversity may be lower than previously thought.

A recent taxonomical review (Harvey *et al.* 2012) based only on morphological data corroborated the paraphyly of *Ameiva* and *Cnemidophorus* previously revealed by biochemical, morphological and genetic data sets (Reeder *et al.* 2002; Giugliano *et al.* 2006; Giugliano 2009). The proposed taxonomic arrangement split *Ameiva* species into four genera: *Ameiva*, *Medopheos* (only *A. edracantha*), *Holcosus* and *Contomastix* (*A. vittata* and *Cnemidophorus* of the *lacertoides* complex). In spite of evidence to the contrary (Reeder *et al.* 2002; Giugliano 2009) and to a no support clade (bootstrap lower than 50%), Harvey *et al.* (2012) grouped the Antillean and cis-Andeans species. Our results (Figs 1–2) further indicate this grouping is unwarranted, because it renders *Ameiva* paraphyletic. Further studies, with denser taxonomic sampling and using multiple loci, are necessary to clarify the evolutionary relationships among teiids. Until then, we consider broad taxonomic changes, as those proposed by Harvey *et al.* (2012), premature to say the least.

Colli (2005) argued that too much emphasis has been placed on Quaternary events (Haffer 1969; Vanzolini & Williams 1970) to explain the diversification of the Neotropical herpetofauna, suggesting that events in the Tertiary were more prominent. Recent studies support this position, indicating that Quaternary climate and vegetational changes had a limited influence in species diversification, having affected mostly postspeciation dispersal routes and differentiation at the population level (e.g. Garda & Cannatella 2007; Gamble *et al.* 2008, 2012; Werneck *et al.* 2009, 2012; Maciel *et al.* 2010; Prado *et al.* 2012). Our results indicate that *Ameiva* diversified mainly during the Miocene and Pliocene. The first divergence of the group occurred during Middle Miocene, originating clades B (*A. ameiva* and *A. bifrontata*) and *A. jacuba* and *A. parecis*) (Fig. 2). Presumably, this ensued from vicariance as a major

marine transgression separated the Guiana Shield from the Brazilian Shield (Räsänen *et al.* 1995; Webb 1995; Hernandez *et al.* 2005). Clade B likely differentiated and expanded its range in southern South America as widespread plains succeeded the surface flooded by the marine transgression during Late Miocene–Early Pliocene (Ortiz-Jaureguizar & Cladera 2006). Conversely, clade A likely differentiated in the highlands, due to the uplift of the Brazilian Central Shield associated with the subsidence of peripheral depressions from Late Tertiary to Early Quaternary (Ab'Saber 1983; Cole 1986; Silva 1997).

Squamate distribution patterns in Cerrado are strongly affected by topography and isolation between plateaus and depressions, which produce horizontal habitat stratification (Colli *et al.* 2002; Nogueira *et al.* 2011). Squamate endemism prevails in plateaus, whereas faunal interchange is stronger along peripheral depressions (Nogueira *et al.* 2011). Based on these observations, Werneck (2011) suggested that reciprocal monophyly is expected between groups of species associated with plateaus and depressions. Considering that *A. ameiva* is widespread in lowlands, including most of Amazonia (Ávila-Pires 1995), and *A. parecis* (Colli *et al.* 2003a,b) and *A. jacuba* only occur in higher elevations, never below 700 m, the prediction of reciprocal monophyly (Werneck 2011) was corroborated. Future studies are needed to confirm the monophyly of clade A and its endemism to interfluvial Cerrado plateaus, highly targeted areas for the expansion of mechanized agriculture in central Brazil. Other populations of these lizards, from southernmost localities on the Cerrado plateau, remain undescribed (referred to as *Cnemidophorus* aff. *parecis*), but have already been included in regional lists of threatened species (see Marques *et al.* 2009).

The recent discoveries in the Cerrado are not only relevant for highlighting rich and endemic faunas, but also for the discovery of previously neglected areas of high phylogenetic diversity (Faith 1992). In other words, this new scenario with three species of *Cnemidophorus* (Colli *et al.* 2003a,b, 2009) and three species of *Ameiva* in Cerrado, all belonging to different, deeply rooted teiid lineages (which differed at least 10 Ma), versus the old scenario with one species of *Ameiva* and four species of *Cnemidophorus*, is a clear indication that phylogenetic patterns were poorly described and acknowledged in previous studies of the Cerrado herpetofauna. These discoveries underline the usefulness of phylogenetic studies to calculate diversity indexes that incorporate phylogenetic relationships among species (Helmus *et al.* 2007).

In conclusion, our results reveal that: (1) there is a new *Ameiva* species at PNE, (2) *C. parecis* should be reallocated to *Ameiva*, (3) considering that *A. ameiva* are more abundant in lowlands, we found reciprocal monophyly between plateaus versus depressions for the Cerrado species of *Ameiva*, (4) the diversification of *Ameiva* probably occurred during the Late Miocene/Early Pliocene, (5) we propose an evolutionary scenario for South American cis-Andeans *Ameiva* associated with the Miocene marine introgression and the uplift of Cerrado plateaus, resulting in geomorphological and ecological segregation that probably promoted diversification in central Brazil.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Details of samples used in molecular analyses.

Table S2. Meristic characters of the eight species analyzed. Values indicate ± 1 SD, with range in parentheses.

Table S3. Categorical variables of the eight species analyzed. The character states are described in the Material and Methods section.

Table S4. Model evaluation using a Chi-square test of the scaled deviance.

Fig. S1. "Cnemidophorine" phylogeny based on mtDNA fragments obtained by Bayesian inference analysis with posterior probabilities.

Fig. S2. "Cnemidophorine" phylogeny based on the c-mos nuclear fragment obtained by Bayesian inference analysis with posterior probabilities.

Fig. S3. (A) Boxplot of logistic regression scores performed on genera as outcome. (B) Counts of ventrals in transverse row and scales around tail in *Ameiva* (A) and *Cnemidophorus* (C).

Fig. S4. Boxplot of logistic regression scores performed on *Ameiva jacuba* versus its congenerics (*A. parecis* e *A. ameiva* as a group) as outcome.

Fig. S5. Scaly portion of tongue of specimens from collection (A) *Ameiva jacuba* (25735), (B) *Cnemidophorus parecis* (9789), (C) *A. ameiva* (958), (D) *C. lemniscatus* (3518), (E) *C. ocellifer* (3312). The bar corresponds to approximately 1 mm. TS - tongue sheath, PE - posterior edge.

Fig. S6. Adult of *Ameiva jacuba*.

Appendix S1. Specimens Examined