African Journal of Herpetology,
Vol. 61, No. 1, April 2012, 81–90

UNISA university of south africa PRESS
Taylor & Francis
Taylor & Francis Group

Short communication

Karyotypes of two species of Malagasy ground gecko (*Paroedura*: Gekkonidae)

Hana Main[1], Daniel P. Scantlebury[2], David Zarkower[3] & Tony Gamble[3,4]*

[1]*College of Biological Sciences, University of Minnesota, St. Paul, MN, USA;* [2]*Department of Biology, University of Rochester, New York, USA;* [3]*Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN, USA;* [4]*Bell Museum of Natural History, University of Minnesota, Minneapolis, MN, USA*

Abstract.—The karyotypes of Malagasy geckos are poorly known. Herein, we describe the karyotypes of two Malagasy gecko species, *Paroedura picta* and an undescribed or currently unrecognised *Paroedura* species. These are the first karyotypes described for the genus *Paroedura*. Each species had a distinct karyotype; *P. picta* had 2n = 36 and *Paroedura* sp. had 2n = 34. We used a fragment of the mitochondrial *ND2* gene to conduct a phylogenetic analysis of 17 described and putative *Paroedura* species and show that the *Paroedura* sp. examined here is unique and related to *P. bastardi*, *P. ibityensis, P. tanjaka* and another undescribed *Paroedura* species from Tsingy de Bemaraha. Our results highlight the need for continued research into the basic biology and taxonomy of Malagasy lizards.

Key words.—Chromosome, cytogenetics, Gekkota, lizard, Madagascar, phylogeny

The gecko genus *Paroedura* consists of 16 described species and occurs on Madagascar and the Comoros (Nussbaum & Raxworthy 2000; Jackman *et al.* 2008). The biology of this genus is poorly known even though many species are kept as pets and one species, *Paroedura picta*, is commonly used as a model for studying development, physiology and behaviour (Brillet 1993; Blumberg *et al.* 2002; Kubicka & Kratochvil 2009; Noro *et al.* 2009; Starostova *et al.* 2010). New species continue to be discovered and several forms await description (Nussbaum & Raxworthy 2000; Glaw & Vences 2007; Jackman *et al.* 2008). This general lack of knowledge extends to cytogenetics as no *Paroedura* species have published karyotypes. Indeed, only seven species of Malagasy geckos have been karyotyped to date, all in the genus *Phelsuma* (Aprea *et al.* 1996). Herein, we describe the karyotypes of two *Paroedura* species and present evidence for another undescribed species in the genus that has not been identified in recent investigations of the group (Jackman *et al.* 2008).

We acquired a captive-born male and female *Paroedura picta* and two males and two females of an undescribed species of *Paroedura* through the pet trade. Metaphase chromosome spreads were obtained from fibroblast tissue cultures derived from tail tissue. We cultured cells at 28–30° C in DMEM 1X (Invitrogen, Carlsbad, CA, USA) with 4.5 g/l glucose and L-glutamine without sodium pyruvate, 20% foetal bovine serum and anti-anti (Invitrogen), which contains penicillin, streptomycin, and

*Corresponding author. Email: gambl007@umn.edu

ISSN 2156-4574 print/ISSN 2153-3660 online

http://dx.doi.org/10.1080/21564574.2012.667837
http://www.tandfonline.com

amphotericin. Cells were arrested in metaphase using vinblastine sulphate (1 μg/ml) and incubated in hypotonic solution (0.07 M KCl) for 20 min at 37°C. After hypotonic treatment, cells were centrifuged and fixed in methanol:acetic acid (3:1). We washed the cell suspension in fresh fixative a total of five times. We also prepared meiotic chromosomes from the male *P. picta*. The male gecko was killed using an intraperitoneal injection of MS-222 dissolved in water (Conroy *et al.* 2009) and the testes were immediately removed into phosphate-buffered saline. Tissue was cut into small pieces and the cell suspension incubated in hypotonic solution (0.07 M KCl) for 30 min at 37°C. The cell suspension was centrifuged and fixed in methanol:acetic acid solution as above. Cell suspensions were dropped onto cleaned glass slides and air dried. Slides were stained with 4,6-diamidino-2-phenylindole (DAPI) and mounted with Perma?uor (Lab Vision) and a cover slip. We used reverse fluorescence counterstaining with chromomycin A3 (CA3) and DAPI to visualise GC-rich regions of the chromosomes (Schweizer 1976; Ezaz *et al.* 2005). We incubated slides for 3 h in a humid chamber at room temperature with 200 μl of 0.5 mg/ml of CA3 dissolved in McIlvaine's buffer (pH 7.0). Slides were rinsed in McIlvaine's buffer, stained with DAPI and mounted with Permafluor (Lab Vision) and a cover slip.

We sequenced a fragment of mitochondrial DNA as a DNA 'barcode' (Hebert *et al.* 2004) to compare with sequences from a recent analysis of *Paroedura* phylogeny (Jackman *et al.* 2008) and confirm identification of the species used in this study. We chose the *ND2* gene because it had the most *Paroedura* sequences available on Genbank for comparison. We extracted DNA from tissues from one *P. picta* and three *Paroedura* sp. (two of the karyotyped *Paroedura* sp. specimens and an additional, non-karyotyped, specimen – MCZ-R189500) using the Qiagen DNeasy Blood and Tissue kit (Qiagen Inc., Venlo, The Netherlands) following the manufacturer's instructions. We amplified a fragment of the mitochondrial *ND2* gene using primers L4437b (5'-AAGCAGTTGGGCCCATRCC-3') and H5934 (5'-AGRGTGCCAATGTCTTTGTGRTT-3') (Macey *et al.* 1997). Polymerase chain reaction products were purified using Exonuclease I and Shrimp Alkaline Phosphatase (Hanke & Wink 1994) and sequenced using Big Dye Terminator 3.1 chemistry on an ABI 3730xl at the Biomedical Genomics Center at the University of Minnesota. Sequences were assembled and checked for accuracy using Sequencher 4.8 (Gene Codes Corp., Ann Arbor, MI, USA). Protein coding sequences were aligned using ClustalW (Thompson *et al.* 1994) and DNA data translated to amino acids using MacClade 4.0.8 (Maddison & Maddison 1992) to confirm alignment and ensure there were no premature stop codons. Non-coding sequences were aligned using R-Coffee (Moretti *et al.* 2008), which uses predicted secondary structures when making the alignment. We conducted phylogenetic analysis of the *ND2* data using partitioned maximum likelihood, implemented in RAxML 7.2.6 (Stamatakis 2006). Data were partitioned by codon with a fourth partition for tRNAs. All partitions were assigned the GTR + G model of sequence evolution. We assessed nodal support using 100 non-parametric bootstrap replicates (Felsenstein 1985). We calculated net among group genetic distances in *ND2* data among sampled *Paroedura* species. We calculated both uncorrected (*p*) distances and ML corrected distances, using the GTR + G model, with standard errors calculated using 1 000 bootstrap replicates using MEGA 5.05 (Tamura *et al.* 2011).

We examined between four and 20 metaphase spreads for each individual. Diploid chromosome number of *P. picta* was 36 (Figs. 1–2). *Paroedura picta* had one

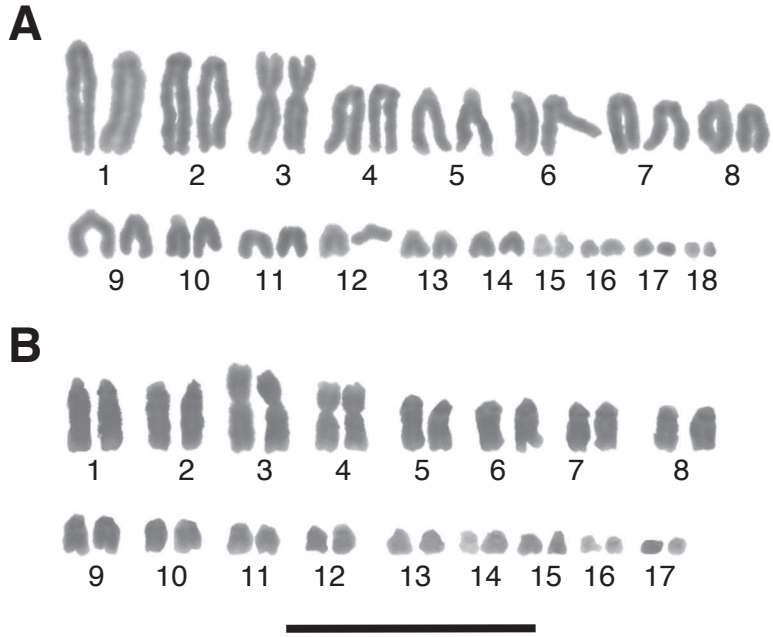

Figure 1. DAPI stained karyotypes of (A) female *Paroedura picta*, TG766, 2n = 36 and (B) female *Paroedura* sp., TG1064, 2n = 34.
Note: Bar = 20 μm.

pair of sub-metacentric chromosomes with the remaining chromosomes being acrocentric and gradually decreasing in size. Diploid chromosome number of *Paroedura* sp. was 34 (Fig 1). *Paroedura* sp. had two pairs of sub-metacentric chromosomes with remaining chromosomes acrocentric and gradually decreasing in size. Reverse fluorescence counterstaining showed no defined bands although some increased CA3 staining and decreased DAPI signal was found near centromeres and telomeres on larger chromosomes for both *Paroedura* species (Fig 3). This pattern is typical of staining with GC-specific fluorochromes in amphibians and reptiles (Schmid & Guttenbach 1988), including other gecko species such as *Gekko gecko* (Solleder & Schmid 1984) and *Coleonyx elegans* (Pokorná *et al.* 2011).

Phylogenetic relationships among *Paroedura* species from *ND2* data (Fig 4) were largely congruent with results from Jackman *et al.* (2008). DNA sequence data confirmed the identification of the *P. picta* specimen sequenced, which formed a well-supported clade with other *P. picta* sequences. *Paroedura* sp. formed a clade with *P. bastardi*, *P. ibityensis, P. tanjaka* and another undescribed *Paroedura* species from Tsingy de Bemaraha (*Paroedura* sp. 2). *Paroedura* sp. does not belong to any of the other sampled species in the genus and uncorrected genetic distances between *Paroedura* sp. and other sampled *Paroedura* species ranged from 18–31% (Table 1).

No heteromorphic sex chromosomes were evident in either of the *Paroedura* species we examined. *Paroedura picta* has been shown to have genotypic sex determination based on incubation experiments (Blumberg *et al.* 2002). Although we did not observe sex chromosomes with DAPI or CA3 staining, additional

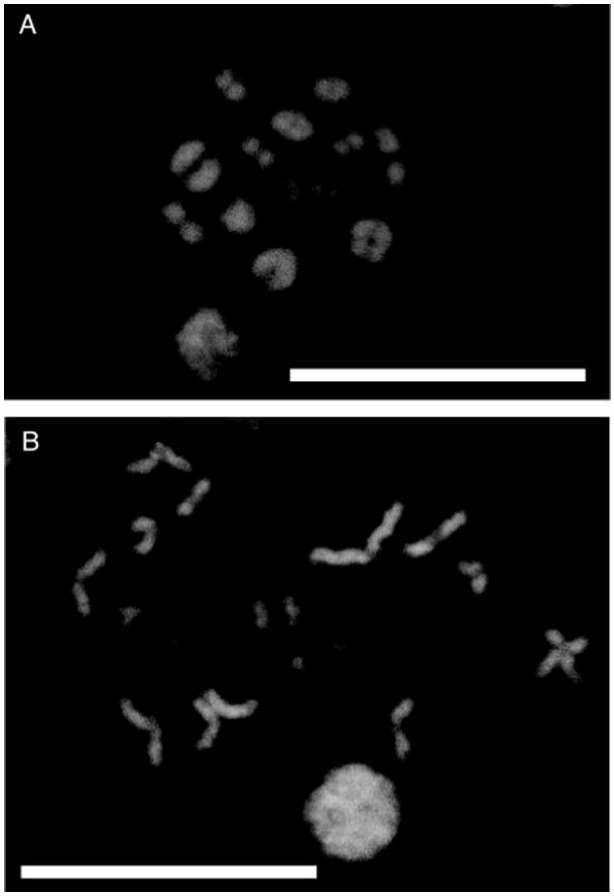

Figure 2. Meiotic chromosomes of male *Paroedura picta*, TG1433 (A) diplotene with 18 bivalents and (B) metaphase II with 18 chromosome pairs.
Note: Bar = 20 μm.

cytogenetic methods such as comparative genomic hybridisation, as described by Ezaz *et al.* (2005), may prove useful to confirm the presence of sex chromosomes.

Paroedura shares some superficial cytogenetic similarities with other related gecko species. *Paroedura* belongs to a clade of Afro-Madagascan geckos (Joger 1985; Gamble *et al.* 2011) and other karyotyped species in this group, including *Phelsuma*, *Homopholis* and *Chondrodactylus*, like *P. picta*, have 36 pairs of chromosomes (De Smet 1981; Aprea *et al.* 1996). The similarity ends with chromosome number, however, and all of these genera differ substantially in fundamental number and chromosomal morphology (De Smet 1981; Aprea *et al.* 1996). The nine species of karyotyped *Phelsuma* all have karyotypes consisting solely of acrocentric chromosomes (Aprea *et al.* 1996). *Homopholis wahlbergii* has a 2n = 36 karyotype with three pairs of sub-metacentric chromosomes and one pair of metacentric chromosomes (De Smet 1981). *Chondrodactylus bibronii* also has a 2n = 36 karyotype but has one pair of sub-metacentric chromosomes, three pairs of sub-telocentric chromosomes and four pairs of metacentric chromosomes (De Smet 1981). The relative stability of chromosome number in this group belies an underlying variability and numerous

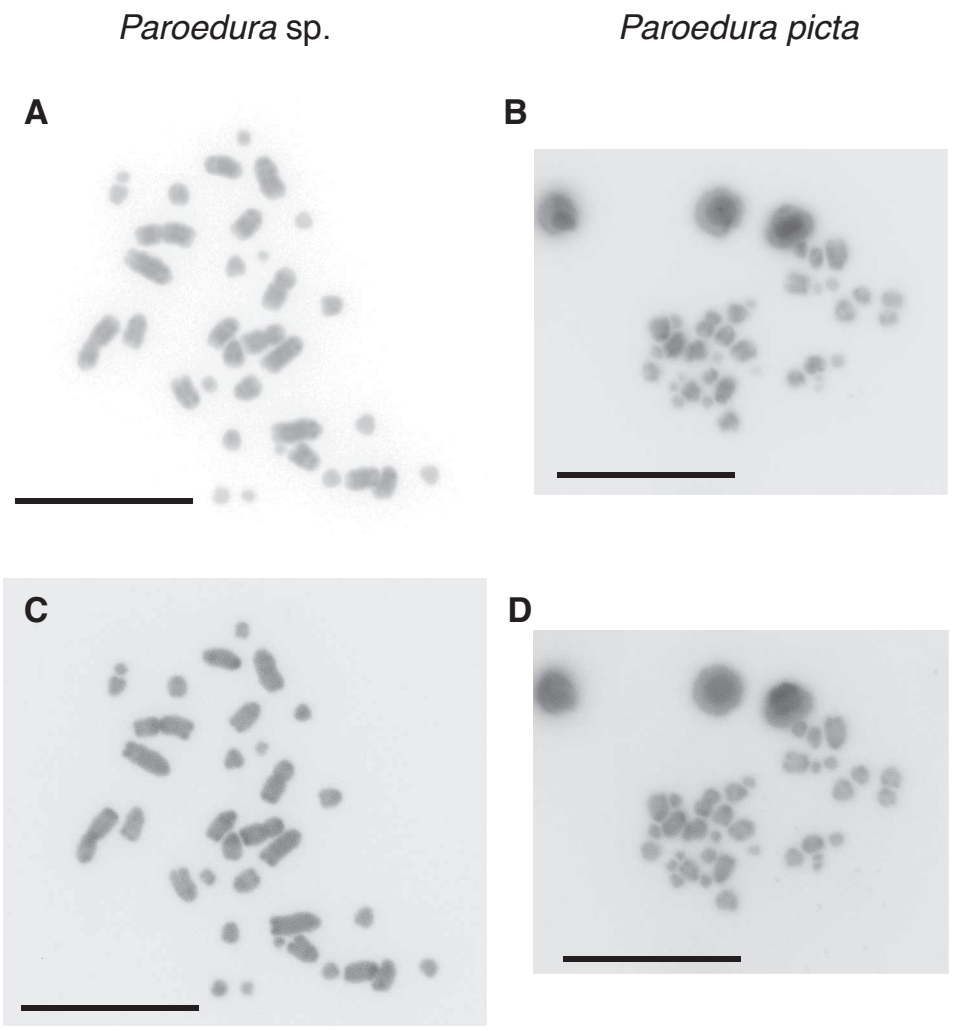

Figure 3. Metaphase chromosome spreads with reverse fluorescence counterstaining using DAPI and CA3 for two *Paroedura* species. DAPI stained (A) and CA3 (C) metaphase chromosomes from female *Paroedura* sp., TG1064. DAPI stained (B) and CA3 (D) metaphase chromosomes from male *Paroedura picta,* TG1433.
Note: Bar = 20 μm.

rearrangements. This variation extends to our observations within *Paroedura*. The 2n = 34 karyotype of *Paroedura* sp. can be derived from the *P. picta* 2n = 36 by a fusion of two chromosome pairs. Conversely, the 2n = 36 karyotype of *P. picta* can be derived by a fission of the sub-metacentric pair of chromosomes in *Paroedura* sp., 2n = 34. Differentiating between these two scenarios will require cytogenetic data from additional *Paroedura* species and other related Afro-Madagascan geckos.

These results add further evidence for the underestimation of species diversity in Madagascar (Glaw & Vences 2007; Vieites *et al.* 2009) and highlight the need for additional herpetological surveys in the region. Phylogenetic analyses of mtDNA

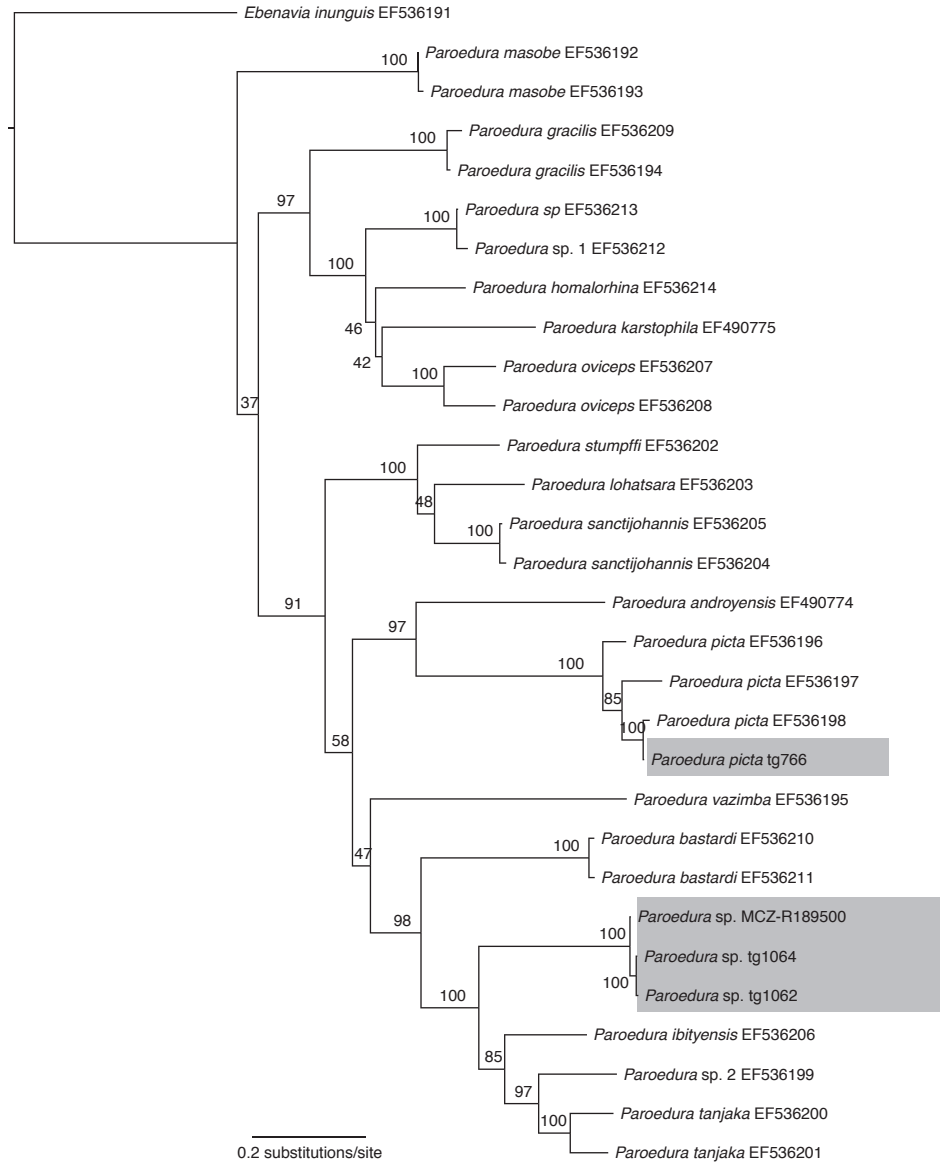

Figure 4. Maximum likelihood tree of sampled *Paroedura* species from mitochondrial *ND2* sequences. Numbers at nodes indicate bootstrap support. Sequences new to this study are enclosed in grey boxes.
Notes: tg, Tony Gamble; MCZ, Museum of Comparative Zoology, Harvard University.

confirmed that *Paroedura* sp. forms a well-supported clade with *P. bastardi*, *P. ibityensis*, *P. tanjaka* and an additional undescribed species (*Paroedura* sp. 2). *Paroedura* sp. (Fig 5) most closely resembles *P. ibityensis* although it shares some similarity to juvenile *P. bastardi*. Ultimately, further research will be necessary to confirm the identity of the undescribed or unrecognised *Paroedura* species examined here.

Table 1. Net among group distances of mitochondrial *ND2* gene among sampled *Paroedura* species. ML corrected distances using the GTR + G model are below the diagonal, uncorrected *p*-distances are above the diagonal. Standard error estimates, calculated with 1 000 bootstrap replicates, are shown in parentheses.

	P. masobe	*P. karstophila*	*P. gracilis*	*P. vazimba*	*P. androyensis*	*P. homalorhina*	*Paroedura* sp. 1	*P. oviceps*
P. masobe	–	0.277 (0.012)	0.234 (0.012)	0.303 (0.013)	0.305 (0.013)	0.258 (0.012)	0.256 (0.012)	0.209 (0.011)
P. karstophila	0.63 (0.058)	–	0.229 (0.012)	0.309 (0.013)	0.291 (0.012)	0.202 (0.011)	0.184 (0.01)	0.143 (0.01)
P. gracilis	0.513 (0.051)	0.452 (0.042)	–	0.283 (0.013)	0.271 (0.012)	0.21 (0.011)	0.202 (0.011)	0.157 (0.01)
P. vazimba	0.8 (0.078)	0.786 (0.081)	0.724 (0.076)	–	0.303 (0.013)	0.313 (0.013)	0.302 (0.012)	0.242 (0.011)
P. androyensis	0.809 (0.083)	0.692 (0.065)	0.637 (0.061)	0.737 (0.071)	–	0.291 (0.012)	0.28 (0.012)	0.233 (0.01)
P. homalorhina	0.57 (0.056)	0.342 (0.031)	0.384 (0.036)	0.826 (0.082)	0.68 (0.06)	–	0.166 (0.01)	0.121 (0.009)
Paroedura sp. 1	0.577 (0.055)	0.317 (0.028)	0.385 (0.036)	0.797 (0.077)	0.672 (0.06)	0.271 (0.025)	–	0.13 (0.009)
P. oviceps	0.516 (0.052)	0.268 (0.027)	0.323 (0.032)	0.656 (0.065)	0.604 (0.054)	0.206 (0.022)	0.239 (0.025)	–
P. sanctijohannis	0.577 (0.056)	0.608 (0.057)	0.516 (0.05)	0.615 (0.06)	0.553 (0.058)	0.553 (0.054)	0.558 (0.054)	0.475 (0.047)
P. lohatsara	0.646 (0.062)	0.649 (0.063)	0.558 (0.053)	0.668 (0.066)	0.619 (0.061)	0.58 (0.052)	0.664 (0.06)	0.48 (0.047)
P. stumpffi	0.642 (0.061)	0.577 (0.054)	0.575 (0.054)	0.609 (0.063)	0.569 (0.054)	0.618 (0.054)	0.59 (0.049)	0.474 (0.043)
P. picta	0.779 (0.079)	0.766 (0.077)	0.667 (0.069)	0.802 (0.08)	0.551 (0.049)	0.708 (0.071)	0.662 (0.065)	0.604 (0.062)
P. bastardi	0.724 (0.073)	0.75 (0.071)	0.652 (0.063)	0.713 (0.068)	0.687 (0.069)	0.687 (0.062)	0.672 (0.063)	0.616 (0.057)
P. ibityensis	0.761 (0.074)	0.752 (0.073)	0.665 (0.059)	0.679 (0.062)	0.665 (0.063)	0.687 (0.064)	0.721 (0.065)	0.621 (0.062)
P. tanjaka	0.652 (0.069)	0.714 (0.074)	0.587 (0.059)	0.586 (0.056)	0.617 (0.061)	0.617 (0.063)	0.66 (0.065)	0.553 (0.057)
Paroedura sp. 2	0.811 (0.078)	0.806 (0.077)	0.717 (0.064)	0.674 (0.06)	0.701 (0.063)	0.745 (0.068)	0.749 (0.065)	0.676 (0.065)
Paroedura sp. (this paper)	0.873 (0.087)	0.847 (0.083)	0.727 (0.064)	0.74 (0.073)	0.682 (0.064)	0.773 (0.067)	0.83 (0.076)	0.691 (0.066)

Table 1 (*Continued*)

	P. sanctijohannis	*P. lohatsara*	*P. stumpffi*	*P. picta*	*P. bastardi*	*P. ibityensis*	*P. tanjaka*	*Paroedura* sp. 2	*Paroedura* sp. (this paper)
P. masobe	0.256 (0.013)	0.275 (0.012)	0.273 (0.012)	0.264 (0.011)	0.278 (0.012)	0.299 (0.012)	0.236 (0.012)	0.305 (0.012)	0.314 (0.013)
P. karstophila	0.268 (0.012)	0.282 (0.012)	0.267 (0.012)	0.27 (0.012)	0.291 (0.012)	0.3 (0.012)	0.254 (0.012)	0.31 (0.012)	0.313 (0.013)
P. gracilis	0.235 (0.012)	0.251 (0.011)	0.251 (0.011)	0.241 (0.011)	0.263 (0.012)	0.277 (0.011)	0.223 (0.011)	0.288 (0.011)	0.283 (0.011)
P. vazimba	0.271 (0.013)	0.283 (0.013)	0.269 (0.013)	0.277 (0.012)	0.291 (0.012)	0.291 (0.013)	0.232 (0.012)	0.289 (0.012)	0.299 (0.013)
P. androyensis	0.257 (0.014)	0.272 (0.013)	0.262 (0.013)	0.231 (0.011)	0.283 (0.013)	0.287 (0.013)	0.239 (0.012)	0.294 (0.012)	0.286 (0.013)
P. homalorhina	0.25 (0.012)	0.268 (0.011)	0.275 (0.011)	0.258 (0.011)	0.281 (0.012)	0.291 (0.012)	0.239 (0.012)	0.304 (0.012)	0.301 (0.011)
Paroedura sp. 1	0.247 (0.012)	0.277 (0.012)	0.263 (0.011)	0.244 (0.011)	0.27 (0.011)	0.288 (0.012)	0.238 (0.011)	0.295 (0.011)	0.303 (0.012)
P. oviceps	0.195 (0.011)	0.205 (0.011)	0.2 (0.01)	0.198 (0.01)	0.225 (0.011)	0.233 (0.012)	0.182 (0.01)	0.245 (0.011)	0.242 (0.011)
P. sanctijohannis	–	0.147 (0.011)	0.153 (0.011)	0.238 (0.013)	0.241 (0.012)	0.24 (0.012)	0.199 (0.011)	0.254 (0.012)	0.254 (0.013)
P. lohatsara	0.217 (0.023)	–	0.161 (0.01)	0.245 (0.011)	0.268 (0.011)	0.263 (0.012)	0.208 (0.011)	0.269 (0.011)	0.283 (0.012)
P. stumpffi	0.227 (0.023)	0.242 (0.024)	–	0.244 (0.01)	0.257 (0.012)	0.269 (0.013)	0.215 (0.011)	0.26 (0.012)	0.266 (0.012)
P. picta	0.631 (0.068)	0.642 (0.064)	0.631 (0.057)	–	0.242 (0.011)	0.261 (0.011)	0.199 (0.01)	0.259 (0.01)	0.271 (0.012)
P. bastardi	0.52 (0.049)	0.624 (0.057)	0.578 (0.052)	0.659 (0.065)	–	0.252 (0.011)	0.181 (0.01)	0.244 (0.011)	0.246 (0.011)
P. ibityensis	0.493 (0.047)	0.574 (0.053)	0.586 (0.055)	0.697 (0.062)	0.515 (0.044)	–	0.126 (0.009)	0.188 (0.011)	0.218 (0.011)
P. tanjaka	0.466 (0.046)	0.493 (0.049)	0.52 (0.051)	0.586 (0.058)	0.39 (0.035)	0.208 (0.021)	–	0.111 (0.008)	0.184 (0.011)
Paroedura sp. 2	0.54 (0.05)	0.591 (0.052)	0.555 (0.051)	0.689 (0.062)	0.486 (0.041)	0.292 (0.026)	0.172 (0.017)	–	0.23 (0.012)
Paroedura sp. (this paper)	0.563 (0.054)	0.683 (0.063)	0.604 (0.053)	0.77 (0.076)	0.528 (0.044)	0.39 (0.035)	0.374 (0.035)	0.43 (0.039)	–

Figure 5. Photograph of a live individual of the undescribed *Paroedura* species examined here, *Paroedura* sp.

Acknowledgements

Many thanks to T. Ezaz (University of Canberra) for the CA3 staining protocol and J. Rosado (Harvard University) for cataloguing specimens. DNA sequences for this study have been submitted to Genbank (JQ654965-JQ654968). Additional specimens of *Paroedura* sp. not used in this study have been catalogued at the Museum of Comparative Zoology, Harvard University (MCZ R-189496–9). D.P.S. was supported by NSF grant DEB-1110605. All experiments were carried out in accordance with University of Minnesota animal use protocol 0810A50001.

References

Aprea, G., G. Odierna, T. Capriglione, V. Caputo, A. Morescalchi & E. Olmo. 1996. Heterochromatin and NOR distribution in the chromosomes of six gekkonid species of the genus *Phelsuma* (Squamata:Gekkonidae). J. Afr. Zool. 110: 343–349.

Blumberg, M.S., S.J. Lewis & G. Sokoloff. 2002. Incubation temperature modulates post–hatching thermoregulatory behavior in the Madagascar ground gecko, *Paroedura pictus*. J. Exp. Biol. 205: 2777–2784.

Brillet, C. 1993. Behavioural cues in sex recognition by two species of nocturnal lizards: *Eublepharis macularius* and *Paroedura pictus*. Amphib.-Reptilia 14: 71–82.

Conroy, C.J., T. Papenfuss, J. Parker & N.E. Hahn. 2009. Use of tricaine methanesulfonate (MS222) for euthanasia of reptiles. J. Am. Assoc. Lab. Anim. Sci. 48: 28–32.

De Smet, W.H.O. 1981. Description of the orcien stained karyotypes of 27 lizard species (Lacertilia: Reptilia) belonging to the families Iguanidae, Agamidae Chameleontidae and Gekkonidae (Ascalabota). Acta. Zool. Pathol. Antverp. 76: 35–72.

Ezaz, T., A.E. Quinn, I. Miura, S.D. Sarre, A. Georges & J.A. Marshall Graves. 2005. The dragon lizard *Pogona vitticeps* has ZZ/ZW micro-sex chromosomes. Chromosome. Res. 13: 763.

FELSENSTEIN, J. 1985. Confidence-limits on phylogenies – an approach using the bootstrap. Evolution 39: 783–791.

GAMBLE, T., A.M. BAUER, G.R. COLLI, E. GREENBAUM, T.R. JACKMAN, L.J. VITT & A.M. SIMONS. 2011. Coming to America: multiple origins of New World geckos. J. Evol. Biol. 24: 231–244.

GLAW, F. & M. VENCES. 2007. A Field Guide to the Amphibians and Reptiles of Madagascar. Vences & Glaw Verlags GbR, Köln, Germany.

HANKE, M. & M. WINK. 1994. Direct DNA-sequencing of PCR-amplified vector inserts following enzymatic degradation of primer and dNTPs. BioTechniques 17: 858–860.

HEBERT, P.D.N., M.Y. STOECKLE, T.S. ZEMLAK & C.M. FRANCIS. 2004. Identification of birds through DNA barcodes. PLoS Biol. 2: e312.

JACKMAN, T.R., A.M. BAUER, E. GREENBAUM, F. GLAW & M. VENCES. 2008. Molecular phylogenetic relationships among species of the Malagasy-Comoran gecko genus *Paroedura* (Squamata: Gekkonidae). Mol. Phylogenet. Evol. 46: 74–81.

JOGER, U. 1985. The African gekkonine radiation: preliminary phylogenetic results, based on quantitative immunological comparisons of serum albumins. Pp. 479–494. In: K.L. SCHUCHMANN (Ed.) African Vertebrates: Systematics, Phylogeny and Evolutionary Ecology Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn, Germany.

KUBICKA, L. & L. KRATOCHVIL. 2009. First grow, then breed and finally get fat: hierarchical allocation to life-history traits in a lizard with invariant clutch size. Funct. Ecol. 23: 595–601.

MACEY, J.R., A. LARSON, N.B. ANANJEVA & T.J. PAPENFUSS. 1997. Evolutionary shifts in three major structural features of the mitochondrial genome among iguanian lizards. J. Mol. Evol. 44: 660–674.

MADDISON, W.P. & D.R. MADDISON. 1992. MacClade, Analysis of Phylogeny and Character Evolution. 3.0 edn. Sinauer, Sunderland, MA.

MORETTI, S., A. WILM, D.G. HIGGINS, I. XENARIOS & C. NOTREDAME. 2008. R-Coffee: a web server for accurately aligning noncoding RNA sequences. Nucleic. Acids. Res. 36: W10–W13.

NORO, M., A. UEJIMA, G. ABE, M. MANABE & K. TAMURA. 2009. Normal developmental stages of the Madagascar ground gecko *Paroedura pictus* with special reference to limb morphogenesis. Dev. Dyn. 238: 100–109.

NUSSBAUM, R.A. & C.J. RAXWORTHY. 2000. Systematic revision of the genus *Paroedura* Günther (Reptilia: Squamata: Gekkonidae), with the description of five new species. Misc. Publ. Mus. Zool. Univ. Mich.: 1–26.

POKORNÁ, M., M. RÁBOVÁ, P. RÁB, M. FERGUSON-SMITH, W. RENS & L. KRATOCHVÍL. 2011. Differentiation of sex chromosomes and karyotypic evolution in the eye-lid geckos (Squamata: Gekkota: Eublepharidae), a group with different modes of sex determination. Chromosome. Res. 18: 809–820.

SCHMID, M. & M. GUTTENBACH. 1988. Evolutionary diversity of reverse (R) fluorescent chromosome bands in vertebrates. Chromosoma 97: 101–114.

SCHWEIZER, D. 1976. Reverse fluorescent chromosome banding with chromomycin and DAPI. Chromosoma 58: 307–324.

SOLLEDER, E. & M. SCHMID. 1984. XX/XY sex chromosomes in *Gekko gecko* (Sauria, Reptilia). Amphib. Reptilia 5: 339–345.

STAMATAKIS, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.

STAROSTOVA, Z., L. KUBICKA & L. KRATOCHVIL. 2010. Macroevolutionary pattern of sexual size dimorphism in geckos corresponds to intraspecific temperature-induced variation. J. Evol. Biol. 23: 670–677.

TAMURA, K., D. PETERSON, N. PETERSON, G. STECHER, M. NEI & S. KUMAR. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28: 2731–2739.

THOMPSON, J.D., D.G. HIGGINS & T.J. GIBSON. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic. Acids. Res. 22: 4673–4680.

VIEITES, D.R., K.C. WOLLENBERG, F. ANDREONE, J. KÖHLER, F. GLAW & M. VENCES. 2009. Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. Proc. Natl. Acad. Sci. USA 106: 8267–8272.

Received: 20 November 2011; Final acceptance: 14 February 2012