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First evidence of chromosomal variation within *Chelonoidis chilensis* (Testudines: Testudinidae)

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Chelonoidis chilensis is an endangered tortoise that inhabits arid regions in Argentina, Bolivia and Paraguay. Blood samples were obtained from wild specimens from the Argentinan distribution range together with samples from specimens of known morphotype but unknown provenance. Cytogenetic analysis using Giemsa staining showed that the diploid chromosome complement was 2*n*=52 for all twenty-five tortoises analysed. Two different karyomorphs, termed A and B, were identified, with a karyotypic formulae of 7:5:14 and 6:5:15, respectively. G-band analysis suggests that karyomorph B may originate from a chromosomal fission event involving chromosome pair 7 of karyomorph A. In addition, all specimens analysed using Fluorescence In Situ Hybridisation (FISH) with a telomeric probe showed telomeric signals only at the terminal regions of chromosomes. This evidence suggests that the karyotype of *C. chilensis* does not have telocentric chromosomes, and that interstitial telomeric sequences have not played a major role during the recent chromosomal evolution of this species. Our data agree with recent molecular evidence supporting the existence of one instead several species for the *C. chilensis* complex. Our data further suggest a possible correlation between chromosomal variation and geographical distribution: karyomorph A is present in tortoises from the Dry Chaco Eco-region, whereas karyomorph B characterises tortoises living in the Monte of Steps and Plains Eco-region. Morphology appears to vary independently of cytomorph variation.

Key words: Chelonoidis chilensis, cryptodira, Fluorescence In Situ hybridisation (FISH), karyotypic evolution, testudines

INTRODUCTION

Living South American tortoises of the family Testudinidae are represented by four species belonging to a single genus, *Chelonoidis* (Fritz & Havaš, 2007): *Chelonoidis carbonaria*, *C. chilensis* (both present in the Argentine Dry Chaco Eco-region, the latter also in the Monte of Steps and Plains Eco-region, Cabrera, 1998), *C. denticulata* and *C. nigra*. *Chelonoidis chilensis* is the most abundant tortoise from Argentina, with 95% of its range occurring in this country. Nevertheless *C. chilensis* is currently included in the Appendix II of the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES), and is considered Vulnerable by the IUCN (2012), and by national assessments (Prado et al., 2012). Two main factors put *C. chilensis* populations at risk: multipurpose extraction (mostly tortoises commercialised as pets) and habitat loss due to agricultural expansion. Until recently, up to three species were considered within the *C. chilensis* complex: *C. chilensis*, *C. donosobarrosi* and *C. petersi*, distinguished from each other by carapacial design (Cei, 1986; Fig. 1). The three species of the *C. chilensis* complex were however subsequently reduced to two species (Fernández, 1988; Cabrera, 1998; Richard, 1999; Chébez, 2008). More recently, Fritz et al. (2012) found no significant genetic differences between the three morphs of the *C. chilensis* complex, and proposed *C. chilensis* as valid species with *C. donosobarrosi* and *C. petersi* as junior synonyms.

In reptiles, chromosomal analysis is a very useful tool for species recognition (Chevalier et al., 1979; Reed et al., 1991; Machado Pellegrino et al., 1994; Kupriyanova et al., 2006). However, contrary to the widespread used in other groups (Chevalier et al., 1979; Machado Pellegrino et al., 1994; Rico Medeiros et al., 2013), chromosomal characters are rarely employed in both phylogenetic and phylogeographic studies within chelonians (Caccone et al., 1999, 2002; Le et al., 2006; Vargas-Ramírez et al., 2008). Regarding *C. chilensis*, Bickham & Carr (1983) describe the chromosome number of the species using conventional staining techniques on metaphase samples from specimens from unknown localities, and Martínez et al. (2009) describe the karyotype of two specimens from the southern part of the species range (*donosobarrosi* morph). In the present study, we describe chromosomal variation across the range of *C. chilensis* in Argentina. We support the conclusion that the taxon comprises a single species (Fritz et al., 2012), and discuss the role of chromosomes during evolution.

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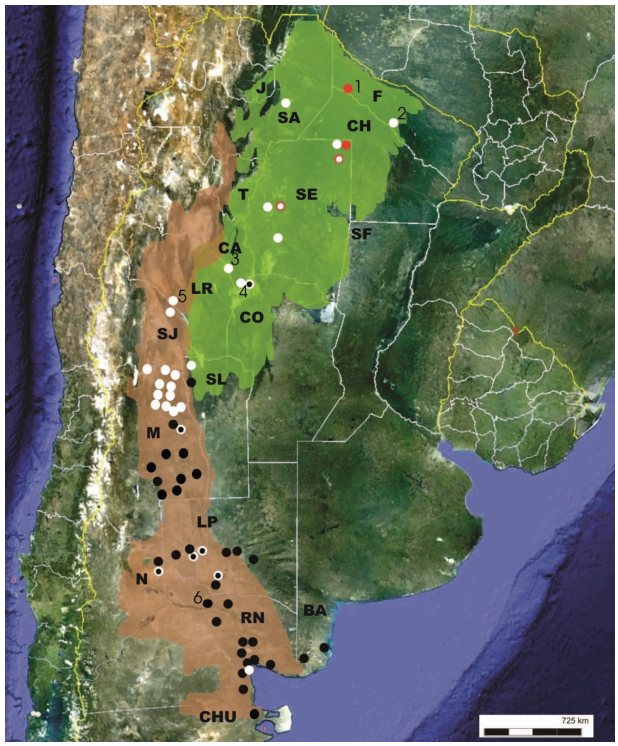

Fig. 1. Map of Argentina (excluding the southern part of Patagonia) showing the two Eco-regions at which *Chelonoidis chilensis* is mainly found (brown: Monte of Steps and Plains; green: Dry Chaco). Black dots represent locations for the *donosobarrosi* morph; white dots indicate locations for the *chilensis* morph; red dots indicate locations for the *petersi* morph. Colour combinations represent the intermediate morphs (white - red dots: *chilensis* - *petersi* morph; black - white dots: *donosobarrosi* - *chilensis* morph). The distribution of the morphs is based on field observations and literature (Freiberg, 1973; Cabrera 1998 for the *donosobarrosi* morph; Richard 1999 for the *chilensis* and *donosobarrosi* morphs from Mendoza). Locations for which we have obtained blood cultures for kariology: 1) Reserva Natural Formosa National Park; 2) Comandante Fontana; 3) San Martin; 4) northeastern part of the Salinas Grandes; 5) Talampaya National Park; 6) Chelforó. Abbreviations of the provinces at which the species is present: BA, Buenos Aires; CA, Catamarca; CH, Chaco; CHU, Chubut; CO, Córdoba; F, Formosa; J, Jujuy; LP, La Pampa; LR, La Rioja; M, Mendoza; N, Neuquén; RN, Rio Negro; S, Salta; SE, Santiago del Estero; SF, Santa Fe; SJ, San Juan; SL, San Luis; T, Tucumán.

MATERIALS AND METHODS

Blood samples from 17 captive and 8 wild specimens of *C. chilensis* were extracted from the dorsal coccygeal vein using a heparinised sterile syringe. Samples were arranged according to geographic provenance (wild tortoises only), sex, and phenotype (all cases). Phenotypes were categorised considering the three species of Freiberg (1973) as morphs of a single species, *C. chilensis*: "*chilensis*", "*donosobarrosi*" and "*petersi*" (Fig. 2). In addition, we considered two intermediate morphs (Fig. 3), termed here "*donosobarrosi-chilensis*" and "*chilensis-petersi*". Sex was determined based on presence / absence of plastral concavity, as suggested by Freiberg (1973, Table 1).

Blood samples from captive tortoises were obtained from the Zoo of Mendoza province (*n*=9) and private pets (*n*=8) from La Plata (Buenos Aires province). Argentine Eco-regions were considered following Burkart et al. (1999). Samples from wild specimens were collected at Monte of Steps and Plains Eco-region (*n*=5); Chelforó, Río Negro province (*n*=1); Talampaya National Park, La Rioja province (*n*=2); northeastern part of the Salinas Grandes, Catamarca province (*n*=2); Dry Chaco Eco-region (*n*=3): San Martin, Catamarca province (*n*=1); Reserva Natural Formosa National Park, Formosa province (*n*=1); and

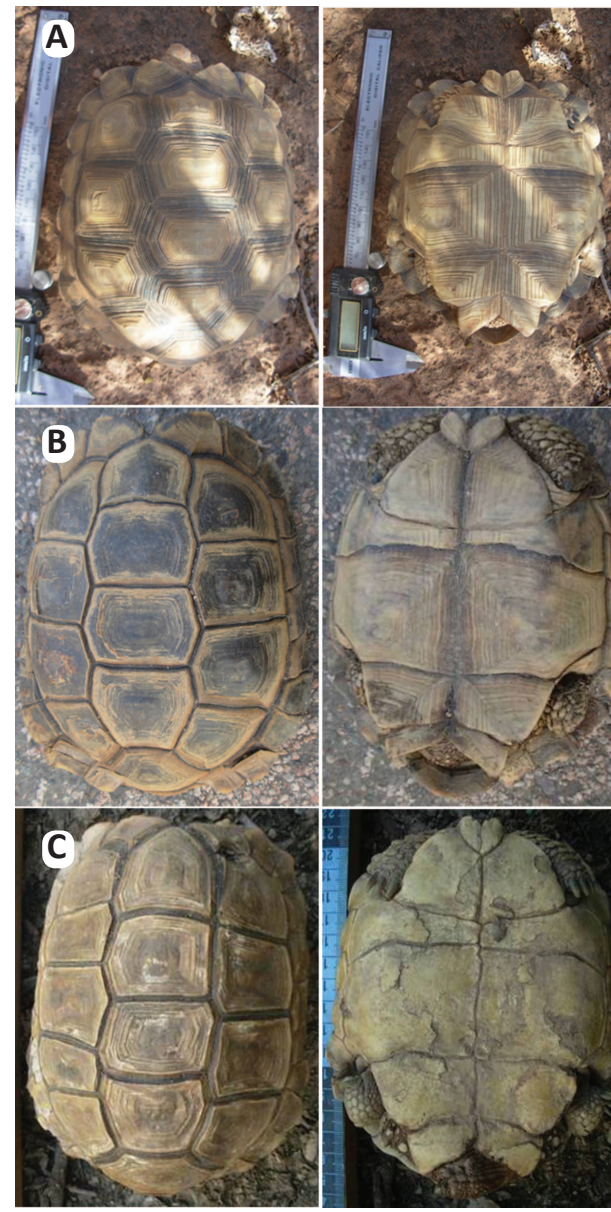

Fig. 2. Dorsal (left side) and ventral (right side) of the carapacial design characteristic of each of the three morphs as they are appreciated in adult specimens of *Chelonoidis chilensis*: (A) *chilensis*; (B) *donosobarrosi;* (C) *petersi.* It is important to remark that recently hatched and juvenile specimens lack the typical design of each morph, a feature that is acquired with age.

Table 1. Samples of *Chelonoidis chilensis* included in the present study indicating sex, locality, eco-region, karyomorph, and morph of each specimen. DC, dry chaco; F, female; M, male; MSP, monte of steps and plains.

Specimen	Sex	Locality / Eco-region	Karyomorph / Morph
CAPTIVE SPECIMENS			
1	F	Family houses - Buenos Aires	A / *chilensis - petersi*
2	F		A / *chilensis*
3	M		A / *donosobarrosi - chilensis*
4	F		A / *donosobarrosi - chilensis*
5	F		A / *chilensis*
6	F		A / *donosobarrosi – chilensis*
7	F		A / *donosobarrosi - chilensis*
8	M		A / *chilensis*
9	F	Mendoza Zoo	B / *donosobarrosi*
10	M		A / *donosobarrosi - chilensis*
11	F		A / *donosobarrosi - chilensis*
12	?		B / *donosobarrosi*
13	F		B / *donosobarrosi*
14	F		B / *donosobarrosi - chilensis*
15	M		A / *donosobarrosi*
16	?		A / *chilensis*
17	F		A / *chilensis*
WILD SPECIMENS			
18	M	Reserva Natural Formosa/DC	A / *petersi*
19	M	Comandante Fontana/DC	A / *chilensis - petersi*
20	M	San Martin/DC	A / *chilensis*
21	F	Salinas grandes/MSP	A / *donosobarrosi - chilensis*
22	F		B / *donosobarrosi - chilensis*
23	?	Talampaya/MSP	B / *chilensis*
24	M		B / *chilensis*
25	F	Chelforó/MSP	B / *donosobarrosi*

Comandante Fontana, Formosa province (*n*=1, Table 1, Fig. 1).

Whole blood (0.5–1.0 ml) was cultured following Ulsh et al. (2001). Six hours before harvesting, 10 µg/ml of colchicine was added to each culture flask. Cells were collected and processed using standard methods, including hypotonic solution (water/culture medium 3:1, 75 min at 28°C) and methanol/acetic acid (3:1) fixative. Routine karyotypic analysis was performed to preparations stained with 5% Giemsa solution. The diploid modal number of chromosomes (2*n*) was calculated from the observation of 5–10 metaphases per specimen in Giemsa-stained slides. Metaphase cells were photographed using a Nikon Eclipse 50i microscope mounted by a Nikon DN-100 digital camera, and karyotypes were constructed using Adobe Photoshop CS® software. The nomenclature used for karyotypic construction follows Bickham (1975), and the chromosome morphology was established according to Levan et al. (1964). Blood samples of ten captive tortoises (Mendoza Zoo) and two wild specimens (Salinas Grandes - Catamarca and Reserva Formosa - Formosa) were processed for G banding according the protocol of Ezaz et al. (2005).

The Fluorescence In Situ Hybridisation (FISH) technique using Cy3-conjugated peptide nucleic acid (PNA) pan-telomeric probe (i.e., which identifies all telomeres in the chromosomes of a metaphase cell) was employed to analyse the distribution of telomeric sequences (TTAGGG) *n* of the chromosomes. Six samples from captive tortoises were analysed. PNA-FISH was performed following the instructions provided by the supplier (DAKO Corporation, CA, USA). Signals were observed using a Nikon Eclipse 50i epifluorescence microscope equipped with a HBO 100 W mercury lamp and filters for DAPI and Cy3 (Chroma Technology, USA). A Nikon DN-100 digital camera was used for photography. Images were processed using Adobe Photoshop CS® software.

RESULTS

Giemsa preparations showed that the diploid chromosome complement of 25 tortoises we analysed was 2*n*=52. We were able to identify two different karyomorphs, termed A and B. Karyomorph A presented a karyotypic formula of 7:5:14 (metacentrics:acrocentrics or telocentrics:microchromosomes), whereas karyomorph B exhibited a karyotypic formula of 6:5:15 (Fig. 4A and 4B, respectively). Morphological analysis of the karyotype revealed three different groups of chromosomes for each karyomorph. Within karyomorph A, Group A consisted of seven pairs of large to medium size metacentric macrochromosomes; Group B presented five pairs of medium size macrochromosomes (pairs 1 and 2 acrocentrics; and pairs 3, 4 and 5 are strict telocentrics), and Group C was characterised by fourteen pairs of microchromosomes (Fig. 4A). In karyomorph B, only Group B was identical to karyomorph A. Group A consisted of six pairs of large to medium size metacentric macrochromosomes, whereas Group C presented fifteen

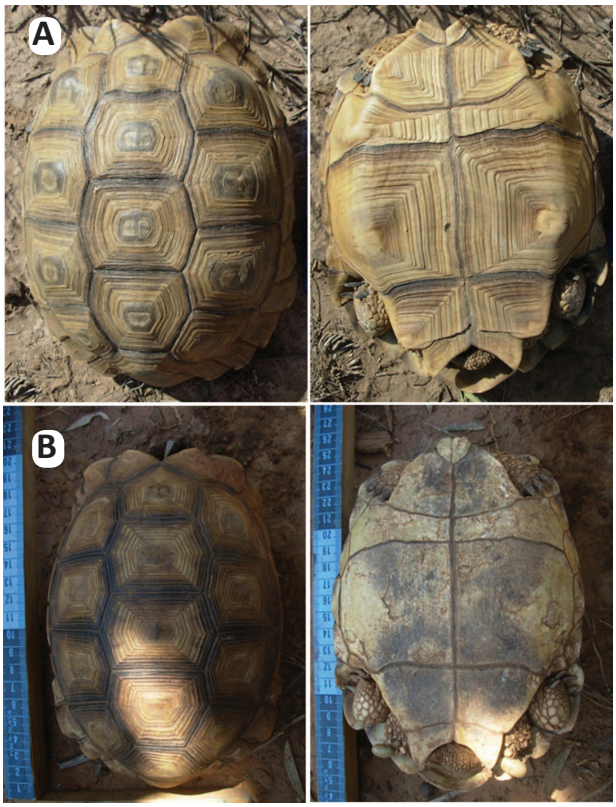

Fig. 3. Dorsal (left side) and ventral (right side) of the carapacial design of intermediate morphs of adults of *Chelonoidis chilensis*: (A) *donosobarrosi - chilensis*; (B) *chilensis - petersi.*

pairs of microchromosomes (Fig. 4B). Therefore, the karyotype of *C. chilensis* consisted of 12 (karyomorph A) or 11 (karyomorph B) pairs of macrochromosomes and 14 (karyomorph A) or 15 (karyomorph B) pairs of microchromosomes. Because of the size of the microchromosomes, their centromeres could not be accurately detected.

Interestingly, karyomorph A was present in three wild tortoises from the Dry Chaco Eco-region, with the three individuals represent one typical *chilensis* morph (San Martin, Catamarca) and two morphs which could be either assigned to *petersi* (Reserva Formosa NP, Formosa) or the intermediate *chilensis-petersi* (Comandante Fontana, Formosa). Karyomorph B also characterised the five wild tortoises from the Monte of Steps and Plains Eco-region (Río Negro, La Rioja and Catamarca provinces) independent of their morph (*chilensis*, *donosobarrosi*, and the intermediate *donosobarrosi - chilensis*; Table 1).

Due to the small size of microchromosomes, the G-banding pattern could only be determined for macrochromsomes and a few pairs of microchromosomes only. Both karyomorphs (A and B) have similar banding patterns that differ in at least a single chromosome rearrangement. The G-banding showed that the long arm of the seventh pair of macrochromosomes from karyomorph A exhibited a pattern similar to the long arm of the first pair of microchromosomes of karyomorph B, which lack the G negative band present in the short arm of the former (Fig. 5). Such difference were found in both males and females. Thus, the additional pair of microchromosomes found in karyomorph B could be derived from a fission event involving the (macro) chromosome pair 7 of karyomorph A.

All chromosomes (karyomorphs A and B) showed four telomeric signals, two at each end (Fig. 6A and B). No interstitial telomeric signals were revealed using PNA-FISH. Contrary to the information obtained using non-banded karyotypes, the FISH technique revealed that *C. chilensis* lacks telocentric chromosomes.

For the captive tortoises, karyomorph A corresponded to the *chilensis* or *donosobarrosi* morphs, and to the intermediate morphs *donosobarrosi-chilensis* and *chilensis-petersi*. The four captive tortoises displayed karyomorph B correspond to the *donosobarrosi* morph and to the intermediate morph *donosobarrosi-chilensis*.

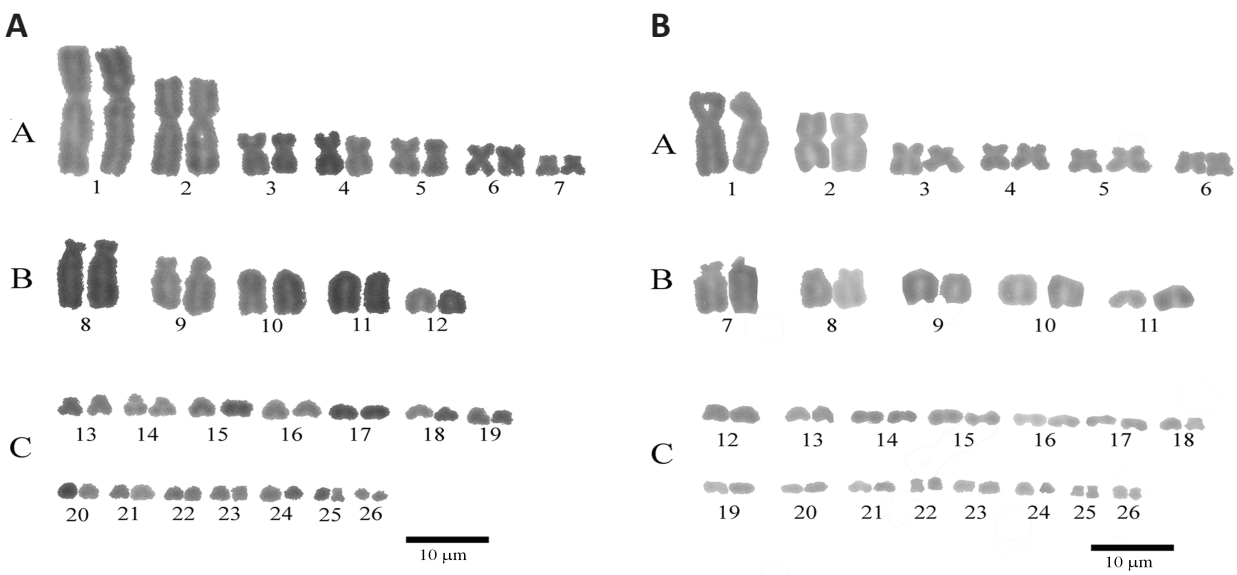

Fig. 4. Non-banded karyotypes of a male (A) and female (B) specimens of *Chelonoidis chilensis* (2*n*=52). (A) = karyomorph A; (B) = karyomorph B. A, B and C correspond to the different chromosome groups (metacentric, submetacentric or acrocentric, and microchromosomes, respectively).

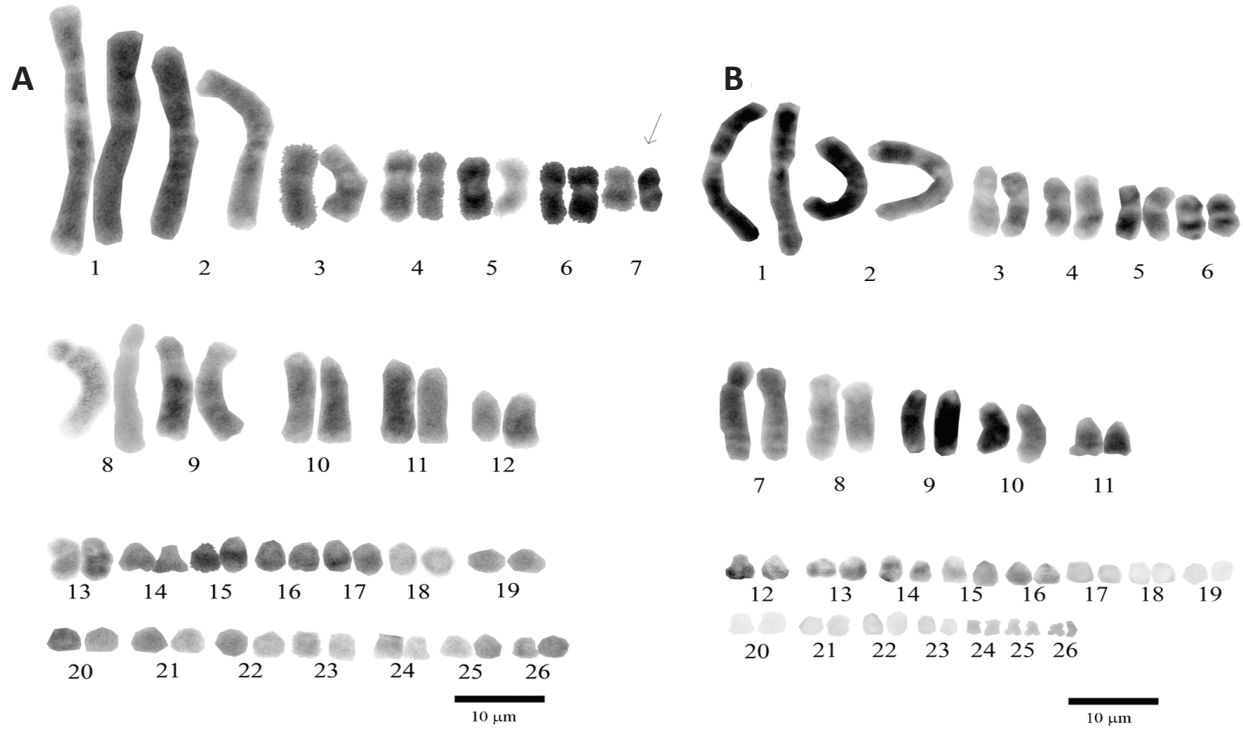

Fig. 5. G-banded karyotypes of a male (A) and female (B) specimens of *Chelonoidis chilensis*. (A) = karyomorph A; (B) = karyomorph B. Scale bar represents 5 um. Arrows indicate the chromosome pair 7 of karyomorph A and the first pair of microchromosomes in karyomorph B

DISCUSSION

We confirm the diploid number of 2*n*=52 previously reported for *Chelonoidis* (Forbes, 1966; Sampaio et al., 1971; Goldstein & Lin, 1972; Bickham, 1976; Benirschke et al., 1976; Bickham & Baker, 1976; Dowler & Bickham, 1982; Bickham and Carr, 1983; Martinez et al. 2009). However, we report the first evidence for chromosomal variation within *C. chilensis*, with the karyotype consisting of 12 (karyomorph A) or 11 (karyomorph B) pairs of macrochromosomes and 14 (karyomorph A) or 15 (karyomorph B) pairs of microchromosomes.

G-banding revealed that the karyomorphs of *C. chilensis* differ in at least one chromosome rearrangement. This is likely due to a fission event involving chromosome pair 7 of karyomorph A, with subsequent fusion of these fragments with other chromosomes. The fact that such rearrangement occurs in both sexes indicates that sexual dimorphism is not expressed at chromosome level. The cytogenetic analysis employed in the present work allowed us to identify differences in karyotype organisation, but did not allow us to determine the rearrangement that generated variants A and B. Chelonians usually have low variability in chromosome number, morphology and G-banding patterns (Olmo, 2005, 2008), and *Chelonoidis* is one of the few chelonian genera with demonstrated intrageneric chromosomal variation (Bickham & Baker, 1976). In *C. carbonaria* and *C. denticulata* karyotypic variation corresponds to clearly defined species (Farías et al., 2007; Vargas-Ramírez

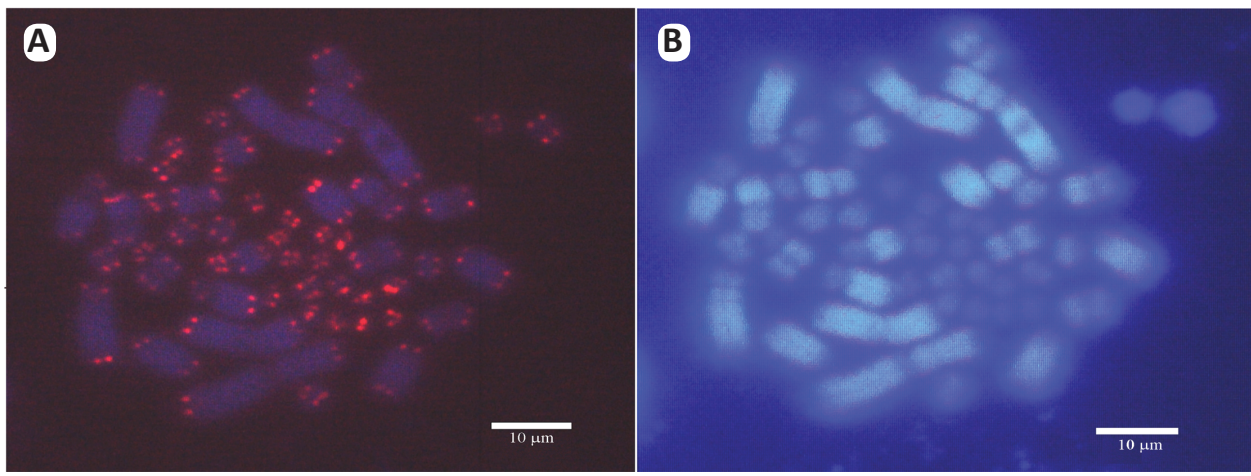

Fig. 6. Location of the telomeric sequence (TTAGGG)n in a metaphase spread of a female specimen of *Chelonoidis chilensis* after hybridisation with a Cy3-labeled PNA telomeric probe. The chromosomes were counterstained with DAPI. (A) PNA-FISH plus DAPI-stained metaphase; (B) DAPI-stained metaphase.

et al., 2010), but the variable phenotypes described for *C. chilensis* are not concordant with cytochrome b (Fritz et al., 2012) and chromosomal variation. A more comprehensive study is required to determine whether the two karyomorphs for example represent ancestral populations which inhabited the Monte of Steps and Plains and the Dry Chaco, and whether they may have led to the standing morphological variation. It is noteworthy that the two karyomorphs were differentially distributed across the study area (karyomorph A in Dry Chaco, and karyomorph B in Monte of Steps and Plains), but were unlinked with morphs. We consider our data to be consistent with recent DNA analysis supporting the existence of only one species for the *C. chilensis* complex (Fritz et al. 2012).

Martinez et al. (2009) suggested that interstitial telomeric sequences have played no role in the chromosomal evolution of *C. chilensis*. While we found no evidence for interstitial telomeric sequences, additional evidence is required for general conclusions for chelonians. We do not exclude the possibility that interstitial telomeric sequences and chromosome fusions not involving telomeric sequences (Nanda et al., 1995; Slijepcevic, 1998) might have played a role in the karyotypic evolution of other Testudines. Alternatively, the absence of interstitial telomeric sequences in *C. chilensis* may be due to a loss of telomeric repeats during chromosomal evolution, or low copy numbers which remained undetected by PNA-FISH.

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REFERENCES

Benirschke, R.J., Quinn, A.D. & Sekulovich, R.E. (1976). Chromosomal studies in Geochelone (Testudinidae–Reptilia). *Chromosome Information Service* 12, 14–16.

Bickham, J.W. (1975). A cytosystematic study of turtles in the genera *Clemmys, Mauremys* and *Sacalia*. *Herpetologica* 31, 198–204

Bickham, J.W. (1976). A meiotic analysis of four species of turtles. *Genetica* 46, 193–198.

Bickham, J.W. & Baker, R.J. (1976). Karyotypes of some neotropical turtles. *Copeia* 1976, 703–708.

Bickham, J.W. & Carr, J.L. (1983). Taxonomy and phylogeny of the higher categories of cryptodiran turtles based on a cladistic analysis of chromosomal data. *Copeia* 4, 918–932.

Burkart, R., Bárbaro, N.O., Sánchez, R.O. & Gómez, D.A. (1999). *Ecorregiones de la Argentina.* Administración de Parques Nacionales y Secretaria de Recursos Naturales y Desarrollo Sustentable. Argentina: Buenos Aires.

Cabrera, M.R. (1998). *Las tortugas continentales de Sudamérica austral*. Argentina: Córdoba.

Caccone, A., Amato, G., Gratry, O.C., Behler, J. & Powell, J.R. (1999). A molecular phylogeny of four endangered Madagascar tortoises based on mtDNA sequences. *Molecular Phylogenetics and Evolution* 12, 1-9.

Caccone, A., Gentile, G., Gibbs, J.P., Fritts, T.H., et al. (2002). Phylogeography and history of Giant Galápagos Tortoises. *Evolution* 56, 2052–2066.

Cei, J.M. (1986). *Reptiles del centro, centro-oeste y sur de la Argentina. Herpetofauna de las zonas áridas y semiáridas.* Monografie di Museo Regionale di Scienze Naturali, Torino 4, 1–527.

Chébez, J.C. (2008). *Los que se van. Fauna argentina amenazada. I.* Buenos Aires, Argentina.

Chevalier, M., Dufaure, J.P. & Lecher, P. (1979). Cytogenetic study of several species of *Lacerta* (Lacertidae, Reptilia) with particular reference to sex chromosomes. *Genetica* 50, 11–18.

Dowler, R.C. & Bickham, J.W. (1982). Chromosomal relationships of the tortoises (family Testudinidae). *Genetica* 58, 189–197.

Ezaz, T., Quinn, A.E., Miura, I., Sarre, S.D., et al. (2005). The dragon Lizard *Pogona vitticeps* has ZZ/ZW micro-sex chromosomes. *Chromosome Research* 13, 763–776.

Farías, I.P., Jerozolimski, A., Melo, A., das Neves Viana, M., et al. (2007). Population genetics of the Amazonian tortoises, *Chelonoidis denticulata* and *C. carbonaria,* (Cryptodira: Testudinidae) in an area of sympatry. *Amphibia-Reptilia* 28, 357–365.

Fernández, M.S. (1988). *Las Testudinidae (Reptilia: Chelonii) argentinas: Osteología, sistemática y distribución geográfica.* PhD thesis. Universidad Nacional de La Plata.

Forbes, W.C. Jr (1966). *A cytological study of the Chelonia.* Unpublished PhD dissertation. University of Connecticut, Storrs.

Freiberg, M.A. (1973). Dos nuevas tortugas terrestres de Argentina. *Boletín de la Sociedad de Biología de Concepción* 46, 81-93.

Fritz, U., Alcalde, L., Vargas-Ramírez, M., Goode, E., et al. (2012). Northern genetic richness and southern purity, but just one species in the *Chelonoidis chilensis* complex. *Zoologica Scripta* 41, 220–232.

Fritz, U. & Havaš, P. (2007). Checklist of chelonians of the world. *Vertebrate Zoology* 57, 149–368.

Goldstein, S. & Lin, C.C. (1972). Somatic cell hybrids between cultured fibroblast from the Galapagos tortoise and the golden hamster. *Experimental Cell Research* 73, 266–269.

IUCN (2012). *IUCN Red List of Threatened Species*. Version 2012. Available from: <http://www.iucnredlist.org>.

Kupriyanova, L.A., Mayer, W. & Böhme, W. (2006). Karyotype diversity of the Eurasian lizard *Zootoca vivipara* (Jacquin,

1787) from Central Europe and the evolution of viviparity. In *Proceedings of the 13th Congress of the Societas Europaea Herpetologica*. Edited by Köhler, T. & Böhme, W.

Le, M., Raxworthy, C.J., McCord, W.P. & Mertz, L. (2006). A molecular phylogeny of tortoises (Testudines: Testudinidae) based on mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* 40, 517–531.

Levan, A., Fredga, K. & Sandberg, A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* 52, 201–220.

Machado Pellegrino, K.C., Yonenaga-Yassuda, Y. & Trefaut Rodrigues, M. (1994). Cytogenetic studies in six species of Tropiduridae (Sauria). *Revista Brasileira de Genética* 17, 401–408.

Martínez, P.A., Boeris, J.M., Sánchez, J., Bolzán, A.D. & Ledesma, M.A. (2009). Karyotypic characterization of *Trachemys dorbigni* (Testudines: Emydidae) and *Chelonoidis donosobarrosi* (Testudines: Testudinidae), two species of Cryptodiran turtles from Argentina. *Genetica* 137, 277–283.

Nanda, I., Schneider-Rasp, S., Winking, H. & Schmid, M. (1995). Loss of telomeric sites in the chromosomes of *Mus musculus domesticus* (Rodentia: Muridae) during Robertsonian rearrangements. *Chromosome Research* 3, 399–409.

Olmo, E. (2005). Rate of chromosome changes and speciation in reptiles. *Genetica* 125, 185–203.

Olmo, E. (2008). Trends in the evolution of reptilian chromosomes. *Integrative and Comparative Biology* 48, 486-496.

Prado, W.S., Waller, T., Albareda, D.A., Cabrera, M.R., et al. (2012). Categorización del estado de conservación de las Tortugas de la República Argentina. *Cuadernos de Herpetología* 26, 375–387.

Reed, K.M., Hanks, B.G., Bickham, J.W., Rhodin, A.G.J., et al. (1991). Cytogenetic analysis of the pleurodine turtle *Phrynops hogei* and its taxonomic implications. *Amphibia-Reptilia* 12, 203–212.

Richard, E. (1999). *Tortugas de las regiones áridas de Argentina*. Buenos Aires: LOLA Press.

Rico Medeiros, L., Bolsoni Lourenço, L., Cerqueira Rossa-Feres, D., Lima, A.P, et al. (2013) Comparative cytogenetic analysis of some species of the *Dendropsophus microcephalus* group (Anura, Hylidae) in the light of phylogenetic inferences. *BMC Genetics* 14, 59.

Sampaio, M.M., Barros, R.M., Ayres, M. & Cunha, O.R. (1971). A karyological study of two species of tortoises from the Amazon region of Brazil. *Cytologia* 36,199–204.

Slijepcevic, P. (1998). Telomeres and mechanisms of Robertsonian fusion. *Chromosoma* 107, 136–140.

Ulsh, B.A., Congdon, J.D., Hinton, T.G., Whicker, F.W. & Bedford, J.S. (2001). Culture methods for turtle lymphocytes. *Methods in Cell Science* 22, 285-297.

Vargas-Ramírez, M., Castaño-Mora, O.V. & Fritz, U. (2008). Molecular phylogeny and divergence times of ancient South American and Malagasy river turtles (Testudines: Pleurodira: Podocnemidae). *Organisms, Diversity & Evolution* 8, 388–398.

Vargas-Ramírez, M., Maran, J. & Fritz, U. (2010). Red- and yellow-footed tortoises, *Chelonoidis carbonaria* and *C. denticulata* (Reptilia: Testudines: Testudinidae), in South American savannahs and forests: do their phylogeographies reflect distinct habitats? *Organisms, Diversity & Evolution* 10, 161–172.

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