Zoologica Scripta

KUNGL. VETENSKAPS-AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

A new perspective on the reduction of cephalic scales in fossorial legless skinks (Squamata, Scincidae)

Aurélien Miralles, Teppei Jono, Akira Mori, Robert Gandola, Jesse Erens, Jörn Köhler, Frank Glaw & Miguel Vences

Submitted: 31 May 2015
Accepted: 16 November 2015
doi:10.1111/zsc.12164

Miralles A., Jono T., Mori A., Gandola R., Erens J., Köhler J., Glaw F., Vences M. (2016). A new perspective on the reduction of cephalic scales in fossorial legless skinks (Squamata, Scincidae). —*Zoologica Scripta*, *45*, 380–393.

In this study, we provide an extended multilocus phylogenetic analysis combining mitochondrial and nuclear DNA of a group of fossorial and miniaturized legless lizards (genus *Paracontias*) from Madagascar, including the description of two species new to science, *P. ampijoroensis* sp. nov. and *P. mahamavo* sp. nov. Our analyses revealed the existence of two distinct, parapatric and diagnosable clades within the genus: (i) the 'kankana clade' (including *P. kankana* and the two newly described species), located in the north (but absent from the extreme northern tip) of the island and characterized by a pattern of cephalic scales very unusual for Malagasy Scincinae, with large loreal scales extending to and meeting each other at dorsal midline, and (ii) the 'brocchii clade' (including all other studied species), endemic to the north of Madagascar and characterized by small loreal scales separated from each other by the rostral and the frontonasal scale. By combining phylogenetic results with morphological traits observed among species, we develop novel hypotheses on the simplification of the cephalic scalation pattern within this genus, a trend frequently encountered among various lineages of legless squamates that convergently adapted to a burrowing lifestyle. Additionally, a user-friendly graphical identification key for species of *Paracontias* is provided and made available as supplementary information.

Corresponding author: *Aurélien Miralles, CNRS-UMR 5175 CEFE, Centre d'Ecologie Fonctionelle et Evolutive, 1919 route de Mende, 34293 Montpellier Cedex 5, France. E-mail: miralles.skink@gmail.com*

Aurélien Miralles, Division of Evolutionary Biology, Zoological Institute, Technical University of Braunschweig, Mendelssohnstr. 4, Braunschweig 38106, Germany. E-mail: miralles.skink@gmail.com

Teppei Jono, Department of Zoology, Graduate School of Science, Kyoto University, Kyoto, 606-8502 Japan and Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China. E-mail: mjusinondo@gmail.com

Akira Mori, Department of Zoology, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan. E-mail: gappa@ethol.zool.kyoto-u.ac.jp

Robert Gandola, Ocean and Earth Sciences, National Oceanography Centre, University of Southampton, European Way, Southampton SO13 3ZH, UK. E-mail: rg3g13@soton.ac.uk

Jesse Erens, Division of Evolutionary Biology, Zoological Institute, Technical University of Braunschweig, Mendelssohnstr. 4, Braunschweig 38106, Germany and Biosystematics Group, Wageningen University, Droevendaalsesteeg 1, Wageningen 6708PB, The Netherlands. E-mail: jesse.erens@wur.nl

Jörn Köhler, Hessisches Landesmuseum Darmstadt, Friedensplatz 1, Darmstadt 64283, Germany. E-mail: joern.koehler@hlmd.de

Frank Glaw, Zoologische Staatssammlung München (ZSM-SNSB), Münchhausenstr. 21, München 81247, Germany. E-mail: Frank.Glaw@zsm.mwn.de

Miguel Vences, Division of Evolutionary Biology, Zoological Institute, Technical University of Braunschweig, Mendelssohnstr. 4, Braunschweig 38106, Germany. E-mail: m.vences@tu-bs.de

Introduction

Convergent adaptations to a highly specialized burrowing lifestyle have repeatedly occurred in the evolutionary history of squamate reptiles (e.g. Wiens & Slingluff 2001; Whiting *et al.* 2003; Kohlsdorf & Wagner 2006; Wiens *et al.* 2006; Brandley *et al.* 2008; Carranza *et al.* 2008; Skinner *et al.* 2008; Crottini *et al.* 2009; Mott & Vieites 2009; Siler & Brown 2011), and a cortege of remarkable morphological transformations (most frequently loss or regressions of pre-existing structures) is usually characterizing these organisms evolving in an extreme subterranean environment: reduction or loss of the limbs, eyes, ear-openings or skin pigmentation, and body miniaturization, with an increase in the number of vertebrae and elongation of the body shape (Gans 1974, 1975; Lee 1998; Pianka & Vitt 2003; Sakata & Hikida 2003; Miralles *et al.* 2012). Important modifications affecting the cephalic scalation are also frequently observed in these head-first digging species, likely because of strong biomechanical constraints exerted on their cephalic integument, as it has been recently shown in the genus *Paracontias* (Miralles *et al.* 2011a), in which all the cephalic scales located in the snout have fused into a remarkable tegumentary structure, the rostral shield.

Paracontias Mocquard is an enigmatic genus of legless and mostly miniaturized wormlike skinks endemic to Madagascar. Until recently, most of the taxonomic knowledge published on this genus dated from the second half of the 19th century and the very first years of the 20th century (*cf.* Fig. S1). In the following 100 years, this genus received little attention from taxonomists, with only a single new species described between 1907 and 2001 (Angel 1949). In contrast, the beginning of the 21st century marks a remarkable second wave of taxonomic discoveries, with more than half of the currently recognized species diversity of *Paracontias* described during the last 13 years (Andreone & Greer 2002; Köhler *et al.* 2009, 2010; Miralles *et al.* 2011a). A similar pattern, characterized by a slowdown in the description rates during the 20th century followed by a recent increase is also observed for Malagasy scincine lizards in general (Miralles *et al.* 2011a) and suggests that their actual species-level diversity – especially in *Paracontias* – is far from being satisfactorily understood.

Recently, two populations of *Paracontias* superficially similar to *Paracontias kankana* have been discovered in the dry deciduous forests of north-western Madagascar: (i) one population was found during zoological surveys conducted nearby Ampijoroa village, Ankarafantsika National Park whereas (ii) the other population was discovered in the Matsedroy forest within the Mahamavo watershed, as part of an ongoing biodiversity monitoring programme in the area. Both these populations, recognized herein as species new to science, share with *Paracontias kankana* a very peculiar cephalic scalation, characterized by the presence of large loreal scales extending to and meeting each other at dorsal midline, whereas other *Paracontias* – and apparently all other species of Malagasy Scincinae – have small loreal scales separated from each other by the rostral and the frontonasal scale.

Herein, we first formally describe these two recently discovered populations as new species and assess their phylogenetic position based on a new and fully resolved molecular phylogenetic tree of the genus *Paracontias*. Subsequently, we take the opportunity offered by this enriched data set to propose complementary homology hypotheses for the derived cephalic scale states of some *Paracontias*. We develop a stepwise evolutionary hypothesis leading from the plesiomorphic cephalic scalation pattern observed in the majority of Madagascar's tetrapodal skinks towards the remarkably reduced cephalic scalation pattern of *Paracontias*. Additionally, a graphical identification key is provided allowing non-specialists (e.g. conservationists, naturalists) to identify *Paracontias* species without a particular expertise.

Materials and methods

Taxonomic framework

Species in the genus *Paracontias* have been confirmed as a monophyletic Malagasy radiation of limbless skinks (Crottini *et al.* 2009; Miralles *et al.* 2011a). Taxonomic concepts and morphological data of *Paracontias fasika*, *P. hafa*, *P. hildebrandti*, *P. kankana*, *P. manify*, *P. minimus*, *P. tsararano* and *P. vermisaurus* herein are based on our own morphological examination of their respective type specimens. The definition of *P. milloti* is only based on the original description; the definitions of *P. rothschildi* and *P. holomelas* are based on their original descriptions completed with the redescriptions made by Angel (1942); and the definition of *P. brocchii* is based on the original description and the data published by Brygoo (1980) and Andreone & Greer (2002). We here take the opportunity to correct a mistake previously published in Miralles *et al.* (2011a) concerning the collection numbers of the holotype and paratype specimens of *P. vermisaurus*, which actually are ZSM 597/2009 and ZSM 598/2009, respectively (instead of ZSM 597/2008 and 598/2008 which were in error).

Morphological study

The specimens examined (all preserved in 70% ethanol) are deposited in the Museum National d'Histoire Naturelle, Paris (MNHN), Museo Regionale di Scienze Naturali, Turin (MRSN), Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt am Main (SMF), Museum für Naturkunde, Berlin (ZMB), Zoologisches Forschungsmuseum

Alexander Koenig, Bonn (ZFMK), Zoologische Staatssammlung München (ZSM) and Kyoto University Museum (KUZ). ZCMV, DRV and AMP refer to M. Vences, D. R. Vieites and T. Jono field numbers, respectively. Measurements of specimens were recorded to the nearest 0.1 mm using a digital caliper. Meristic, mensural and qualitative characters examined here are routinely used in the taxonomy of Scincidae, such as scale counts, the presence or absence of homologous scale 'fusions' or the variability in colour patterns (*cf.* Data S1 for more details and Data S2 for the list of specimens examined).

Molecular procedures

Our sampling represents 10 of the 14 species of *Paracontias* recognized in the present study (tissue samples or sequences were not available for *P. hafa*, *P. holomelas*, *P. milloti* and *P. tsararano*). One sample has been used per species, except for the two new taxa for which two specimens each have been genotyped. A total of 94 new DNA sequences were generated and combined with DNA sequences previously published (Whiting *et al.* 2004; Schmitz *et al.* 2005; Crottini *et al.* 2009; Köhler *et al.* 2010; Miralles *et al.* 2011a) to include 13 specimens in the analysis (but see Discussion). The data set is 95% complete (160 sequences of a theoretical total of 169).

Total genomic DNA was extracted using proteinase K (10 mg/mL) digestion followed by a standard salt-extraction protocol (Bruford *et al.* 1992). From the mitochondrial DNA (mtDNA), we amplified fragments of the 12S rRNA, 16S rRNA and ND1 genes with adjacent tRNAs, including the full $tRNA^{Gln}$ and $tRNA^{Ile}$ genes and partial $tRNA^{Met}$. Additionally, fragments of nine nuclear DNA genes (nuDNA) were amplified: brain-derived neurotrophic factor (BDNF); oocyte maturation factor (CMOS); recombination activating gene 1 (RAG1); recombination activating gene 2 (RAG2); α-enolase (ENOL); phosducin (PDC); leucine-rich repeat and WD repeat-containing protein (KIAA1239); sacsin (SACS) and titin (TTN). Standard polymerase chain reactions were performed in a final volume of 11 μL containing 0.3 μL each of 10 pmol primer, 0.25 μL of total dNTP 10 mM, 0.1 μL of 5 U/mL GoTaq DNA polymerase and 2.5 μL of 5× GoTaq Reaction Buffer (Promega). Template DNA was added in various amount for optimizations and with MQ water added to meet the final volume. See Crottini *et al.* (2009) for primers and PCR conditions used for the amplification of mtDNA, as well as BDNF, CMOS, RAG2, ENOL and PDC. The genes KIAA1239, SACS and TTN were amplified with nested PCR using primers and conditions by Shen *et al.* (2012), amplifying both SACS fragments and the first fragment of KIAA1239 and TTN, respectively. We amplified RAG1 with a customized nested PCR assay, using the primers Mart FL1 (5′-AGCTGCAGYCARTA YCAYAARATGTA-3′) and Mart R6 (5′-GTGTAGAGCC ARTGRTGYTT-3′) for the first amplification round, and Amp F2 (5′-ACNGGNMGICARATCTTYCARCC-3′) and RAG1_DRV_UCR (5′-TTGGACTGCCTGGCAT TCAT-3′) for the second amplification round (see Chiari *et al.* 2004 and Crottini *et al.* 2012 for primer references). PCR conditions for both rounds were as follows: 94 °C for 120 s, followed by 40 cycles of 94 °C (20 s), 52–55 °C (50 s), 72 °C (180 s) and a final extension phase of 72 °C (600 s) – with the annealing temperature being 52 °C in the first and 55 °C in the second amplification round. The successfully amplified products were purified using ExoSAP-IT purification kit according to the manufacturer's instruction. Purified PCR templates were sequenced using dye-labelled dideoxy terminator cycle sequencing on an ABI 3130 automated DNA sequencer. Chromatograms were checked and sequences corrected if necessary using CodonCode aligner (v. 3.5.6, Codon Code Corporation). gblocks (Castresana 2000) was used under the stringent (default) settings to identify and exclude ambiguously aligned saturated regions in the mtDNA. Ninety-four newly determined sequences were deposited in GenBank under accession numbers KT828558 to KT828651. See Table S1, for an overview of all included sequences and taxa.

Phylogenetic analyses

Phylogenetic analyses were conducted on the concatenated data set using maximum parsimony (MP), partitioned maximum likelihood (ML) and Bayesian inference (BI) methods. In addition, unpartitioned BI analyses were performed to reconstruct single-gene trees, treating the concatenated mtDNA genes as a single locus. Models of sequence evolution and the best partitioning scheme were estimated under the BIC criterion with Partitionfinder 1.1.1 (Lanfear *et al.* 2012). All protein-coding genes were partitioned per codon position, per single gene or by treating the third codon position separately from the first and second codon position. In addition, the tested models of sequence evolution were limited to those that can be implemented in mrbayes 3.2 (Ronquist *et al.* 2012). The best scoring partitioning scheme (cf. Table S2) was subsequently used for Bayesian inference of the concatenated alignment. We used jmodeltest 2.1.4 (Darriba *et al.* 2012) to infer best-fitting models of sequence evolution for gene tree reconstructions: GTR+I+G for mtDNA, Jukes Cantor for BDNF, K80+G for CMOS, HKY+I for RAG1 and KIAA1239, K80 for RAG2 and ENOL, K80+I for PDC, HKY+G for SACS and HKY for TTN (*cf.* Table S2 for more details). BI was carried out in mrbayes 3.2 with two independent runs of 20 million generations

and four incrementally heated Markov chains each (at default heating values) for the concatenated analyses, and 10 million generations for the gene tree reconstructions. Starting from a random tree, the chains were sampled every 1000th generation. 50% of the sampled trees were conservatively discarded as burnin, with the remaining post-burn-in samples being used to reconstruct a majority rule consensus tree. ML analyses were performed in RAXML 7.0.4 (Stamatakis 2006). Partitions were defined per codon position following the partitioning scheme in Partitionfinder, but given the model of sequence evolution as implemented by RAXML, using the GTR+G nucleotide substitution model for all partitions. Bootstrap support was obtained through 1000 pseudoreplicates using the GTRCAT model. We used PAUP* 4.0b10 (Swofford 2002) to perform MP analyses with 1000 random addition sequence replicates, tree bisection and reconnection (TBR) branch-swapping, equal character weighting and gaps denoted as missing data. Bootstrap support was based on 1000 pseudoreplicates, 10 random addition sequence replicates and TBR branch-swapping.

Outgroups for the concatenated analyses comprised an elaborate data set of taxa, which is part of an ongoing more comprehensive phylogenetic study on Malagasy scincines currently in preparation. Gene tree reconstructions were performed using *Madascincus mouroundavae* as outgoup, following the placement of the genus *Madascincus* as sister to *Paracontias* (Crottini *et al.* 2009). Uncorrected p-distances were estimated with MEGA 6 (Tamura *et al.* 2013) for the 16S mtDNA fragment to provide an overview of the genetic divergence among taxa.

Results

Molecular results

The concatenated phylogenetic trees obtained are very consistent across the methods of reconstruction and strongly supported (Fig. 1A): all nodes are supported by posterior probability (PP) values of 1.0 for BI, nine of ten are supported by bootstrap values ≥99 for ML analyses and eight of ten are supported by bootstrap values ≥98 for MP analyses. The topology obtained is highly similar to the one previously published by Miralles *et al.* (2011a) based on a lower number of markers, only differing by the placement of *P. manify* (here placed sister to the (*P. minimus* + *P. brocchii*) clade, whereas it was placed sister to *P. hildebrandti* in the previous study).

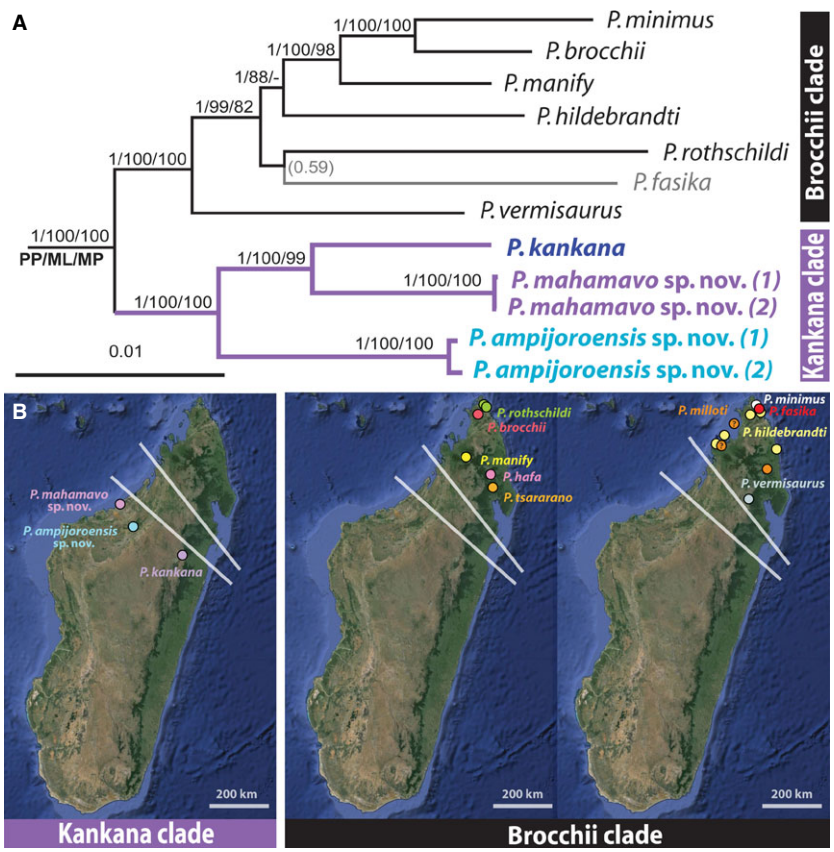

Fig. 1 (A) Phylogeny of the genus *Paracontias* resulting from Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses combining ten independent loci concatenated. The greycoloured branch indicates the poorly supported position of *P. fasika* (for which DNA sequences of only a reduced number of markers were available), as suggested by a separate BI analysis. Values are posterior probabilities (PP) from BI, and ML and MP bootstrap proportions in percent. (B) Distribution ranges of *Paracontias* species (exclusive of *P. holomelas*), illustrating the distinct distribution of the 'brocchii clade' (restricted to the north of Madagascar) and the 'kankana clade' ("subnorthern" distribution). The putative distribution gap separating both clades is delimited by two grey lines (after Glaw & Vences 2007; Köhler *et al.* 2009, 2010; Miralles *et al.* 2011a and the present study). Question marks refer to two uncertain localities of *P. milloti*.

The basalmost node separates *Paracontias* into two main clades: the first one, here called the 'kankana clade', includes *P. kankana* and two long branches corresponding, respectively, to the two new populations from Ampijoroa and Mahamavo, with *P. kankana* being sister to the Mahamavo lineage. Branches connecting the three main lineages within the 'kankana clade' are longer than those connecting well-differentiated species such as *P. minimus*, *P. manify* and *P. brocchii*. Genetic divergences – uncorrected p-distances of the 16S mtDNA fragment (mean ± SD) – observed between these three lineages (*P. kankana*/Mahamavo lineage: 6.3 ± 0.0%; *P. kankana*/Ampijoroa lineage: 4.7 ± 0.0%; Mahamavo lineage/Ampijoroa lineage: 3.9 ± 0.0%) are consistent with interspecific divergences observed between the other recognized species of *Paracontias*. In contrast, the divergences within each of the new populations from Mahamavo and Ampijoroa (two sympatric samples each) are null, unambiguously indicating that each pair of sympatric samples is conspecific. The second main clade is grouping together all the remaining species of *Paracontias*, including the type species of *Paracontias*, *P. brocchii*, and is therefore herein coined 'brocchii clade' for convenience.

The trees suggested by the ten separated analyses (nine nuclear markers and the concatenated mitochondrial data set) show less phylogenetic resolution but in their majority are concordant with the combined tree (*cf.* Fig. S2): nine markers (all but PDC) congruently support the monophyly of the 'kankana clade' and five markers (mtDNA, SACS, TTN, Enol and BDNF) support the monophyly of the 'brocchii clade'. Markers not supporting the monophyly of these two clades do not agree in any consensual alternative topology. This might be interpreted as a lack of phylogenetic signal accumulated during DNA sequence evolution, ancestral polymorphism and/or past introgressions, or an insufficient number of informative positions. In three cases (RAG1, KIAA and PDC), the absence of support for the monophyly of the two main clades is caused by the inclusion of *P. vermisaurus* within (or sister to) the 'kankana clade', but always with a rather low support (PP inferior to 0.95), a result not retrieved by the concatenated analysis.

Morphological results

The two newly discovered populations, from Ampijoroa and Mahamavo, present several morphological characteristics allowing to differentiate them from all other species of *Paracontias* and from each other (cf. Table 1, Fig. 2 and species diagnosis below). Together with *P. kankana*, they present enlarged loreals extending and meeting each other at dorsal midline (vs. loreals absent in *P. milloti*, or small loreals separated from each other by the rostral and the frontonasal in all the other species), representing an unambiguous diagnostic trait characterizing the 'kankana clade' and separating it from the 'brocchii clade'.

Description of two new species

Given the deep genetic differentiation of these lineages found in mitochondrial and nuclear genes, and the morphological characters separating them, we conclude that they represent distinct species-level evolutionary units, scientifically named in the following.

***Paracontias ampijoroensis* sp. nov. (Fig. 2A, see also Data S3 and Fig. S3)**

Holotype. KUZ R069565 (field number AMP2012-parabr), adult, from Ampijoroa, Ankarafantsika National Park, Mahajanga Province, MADAGASCAR, 16°19′00.9″S, 46°48′22.4″E, 153 m above sea level, collected on 24 January 2012, by H. R. Maheritafika.

Table 1 Intraspecific and interspecific variation within and between the three species of the 'kankana clade'

	P. kankana	*P. ampijoroensis* sp. nov.			*P. mahamavo* sp. nov.		
	ZSM 1810/2008 (holotype)	KUZ R069566 (paratype)	KUZ R69565 (holotype)	KUZ R069567 (paratype)	ZSM 2904/2011 (paratype)	ZSM 2905/2011 (holotype)	ZSM 166/2013 (paratype)
Scale rows around midbody	21	16	16	16	18	18	18
V scales	105	104	109	98	112	109	110
SVL (mm)	59	61.1	62.9	59.7	60.5	61.5	52.5
TL (mm) (*regenerated)	22.6*	29.7*	38.4*	58.8	Missing	26*	45.5
Enlarged nuchals (right/left side).	0/0	2/2	2/2	2/2	1/1	1/1	1/1
Supraciliaries (right/left side).	4/4	4/4	4/4	4/4	4/4	4/5	4/4
FN shape[a]	Pentagonal	Pentagonal	Pentagonal	Pentagonal	Triangular	Triangular	Triangular
Contact between F and 3rd SO (right/left side)	No/no	Short/no	Short/short	Short/no	Point contact/no	Short/no	Short/short

F, frontal scale; FN, frontonasal scale; SO, supraocular scale; SVL, snout-vent length; TL, tail length; V, ventral scales (mental not included).

[a]The FN shape is considered as *pentagonal* when its most lateral corners are projecting between the loreal and the first supraciliary, and as *triangular*, when its most lateral corners are projecting between the first supraciliary and the first supraocular.

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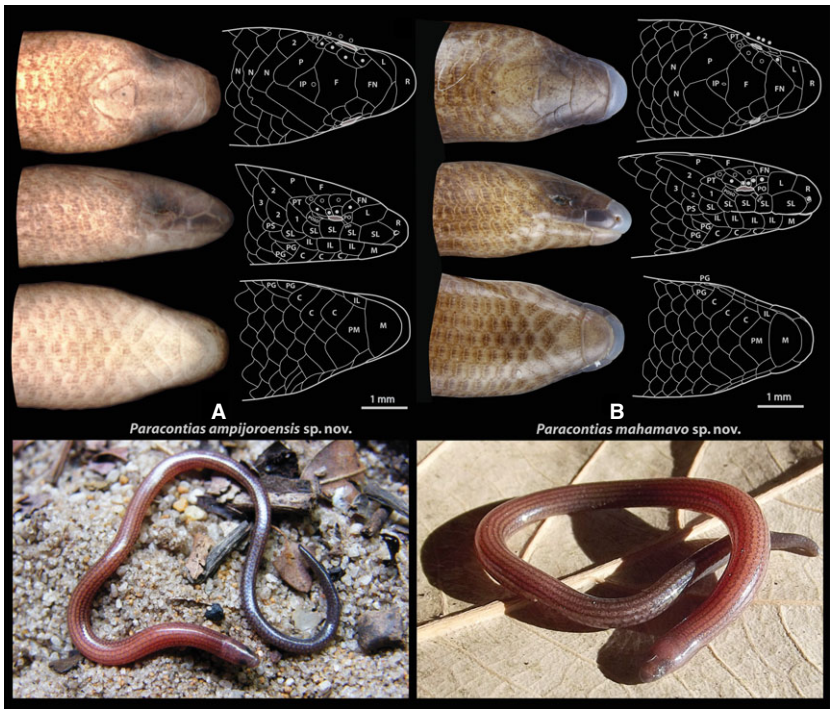

Fig. 2 Holotypes of (A) *P. ampijoroensis* sp. nov. (KUZ R069565) and (B) *P. mahamavo* sp. nov. (ZSM 2905/2011). Above: Photographs and the corresponding schematic drawings (dorsal, lateral and ventral view) of the heads of the preserved holotype specimens (for *P. mahamavo* sp. nov., the lateral view of the head having been symmetrically reversed and therefore representing the left side). C: chin scale, F: frontal, FN: frontonasal, IL: infralabial, IP: interparietal, L: loreal, M: mental, N: nuchal, P: parietal, PG: postgenial, PM: postmental, POSO: postsubocular, PS: postsupralabial, PSO: presubocular, PT: pretemporal, R: rostral, SL: supralabial, 1 to 3: primary to tertiary temporals, ○: supraocular, ●: supraciliary. Below: Living holotype specimens (photo credits Y. Kojima and C. Hendry, respectively).

Paratypes. KUZ R069566 (AMP2012-093), adult, from a locality very close to the type locality, 16°19′03.1″S, 46°48′40.7″E, 148 m above sea level, collected on 16 January 2012, by H. R. Maheritafika. KUZ R069567 (AMP2012-parawh), adult, from a locality very close to the type locality, 16°18′52.4″S, 46°49′05.5″E, 89 m above sea level, collected on 2 February 2012, by R. Ito.

Diagnosis. Small brownish apodous scincine species of the genus *Paracontias*, as revealed by DNA sequence analyses of mitochondrial and nuclear genes, and by the absence of legs and of distinct supranasals and postnasals, the main morphological synapomorphies that in combination differentiate the genus from other Malagasy scincines.

As a member of the 'kankana clade' (together with *P. kankana* and the second new species from Mahamavo), *P. ampijoroensis* sp. nov. differs from the other clade of *Paracontias* by the presence of large loreals (likely resulting from the fusion of loreals with supranasals) extending and meeting each other at dorsal midline (vs. small loreal separated from each other by the rostral and the frontonasal in *P. fasika*, *P. hafa*, *P. hildebrandti*, *P. holomelas*, *P. manify*, *P. minimus*, *P. rothschildi*, *P. tsararano*, *P. vermisaurus*, or loreals absent [likely fused with the frontonasal] in *P. milloti*).

Within the 'kankana clade', *Paracontias ampijoroensis* can also be differentiated from the two other species by the number of enlarged nuchals (two pairs vs. absence in *P. kankana* and one pair in the second new species from Mahamavo), the shape of the frontonasal (pentagonal vs. triangular in the new species from Mahamavo), and the number of scale rows around midbody (16 vs. 21 in *kankana* and 18 in the new species from Mahamavo), see Table 1 and Fig. S3.

Description of holotype. KUZ R069565 (Fig. 2A). Snout-vent length 62.9 mm, tail length 38.4 mm (including 9.7 mm that have probably been regenerated), width at midbody 2.3 mm, head width at level of parietal eye 2.6 mm. Supranasals absent, apparently fused with loreal. Frontonasal roughly pentagonal (diamond-shaped), wider than long, contacting loreals, first supraciliaries and first supraoculars (the most lateral corner being inserted between the loreal and the first supraciliaries). Prefrontals absent. Supraoculars three. Frontoparietals absent. Two pairs of enlarged nuchals. Nasal contacting rostral and first supralabial. Postnasals absent. A single pair of loreals meeting dorsomedially. Four supraciliaries on both sides. Supralabials five, the third being the enlarged subocular contacting the lower eyelid. 16 scale rows around midbody, 109 ventrals. In preservative, ground colouration reddish/light brown.

Variation. All the specimens found, including KUZ R069565 and 069566, were brownish whereas KUZ R069567 was uniformly whitish, this colouration probably

corresponding to stage shortly preceding moulting. For variation in measurements of type specimens, refer to Table 1.

Distribution, habitat and habits. The specimens of *P. ampijoroensis* sp. nov. were collected in a dry deciduous forest in Ampijoroa, Ankarafantsika National Park, north-western Madagascar (Fig. 1B, see also Fig. S3). Ampijoroa forest covers an area of ca. 20 000 ha, approximately 100–200 m above sea level, and the vegetation consists of a deciduous canopy and fairly sparce understory surrounded by savanna and agricultural land (Mori *et al.* 2006). The forest floor was covered by whitish sand with some leaf litter. Including the three specimens examined here, 13 individuals were collected from November to February, the first half of the rainy season in this area (Ikeuchi *et al.* 2012). Twelve of them were captured in pitfall traps with drift fences and one by hand from inside a populated ant nest. Although *P. ampijoroensis* sp. nov. is seemingly not rare in Ampijoroa, due to a paucity of current knowledge on this new species, we propose an IUCN Red List status of 'Data Deficient' for the species.

Etymology. The specific epithet *ampijoroensis* is an adjective, derived from Ampijoroa, where this species was collected (the last vowel of the locality name having been removed for euphonic reasons).

***Paracontias mahamavo* sp. nov. (Fig 2B, see also Data S3 and Fig. S5)**

Holotype. ZSM 2905/2011, adult, from Matsedroy, Mahajanga Province, MADAGASCAR, 15°29′13.7″S, 46°38′48.9″E, 27 m above sea level, collected on 30 June 2011, by J. Coates.

Paratypes. ZSM 2904/2011, adult, same data as holotype. ZSM 166/2013 (fieldnumber 1RMG14), adult, from Matsedroy, Mahajanga Province, Madagascar, 15°29′13.9″ S, 46°38′48.8″E, 27 m above sea level, collected on 20 July 2013 by M. Rabenoro.

Diagnosis. Small brownish apodous scincine species of *Paracontias*, as revealed by DNA sequence analyses of mitochondrial and nuclear genes, and by the absence of legs, supranasals and postnasals, the main morphological synapomorphies that in combination differentiate the genus from other Malagasy scincines.

As a member of the 'kankana clade' (together with *P. kankana* and *P. ampijoroensis* sp. nov.), *Paracontias mahamavo* sp. nov. differs from the other clade of *Paracontias* by the presence of large loreals (likely resulting from the fusion of loreals with supranasals) extending and meeting each other at dorsal midline (vs. small loreal separated from each other by the rostral and the frontonasal in *P. fasika*, *P. hafa*, *P. hildebrandti*, *P. holomelas*, *P. manify*, *P. minimus*, *P. rothschildi*, *P. tsararano*, *P. vermisaurus*, or loreals absent (likely fused with the frontonasal in *P. milloti*).

More specifically, within the 'kankana clade', *P. mahamavo* sp. nov. can also be differentiated from the other species by the number of enlarged nuchals (one pair vs. two pairs in *P. ampijoroensis* sp. nov., and the absence in *P. kankana*), the shape of the frontonasal (triangular vs. pentagonal in *P. ampijoroensis* sp. nov. and *P. kankana*) and the number of scale rows around midbody (18 vs. 21 in *kankana* and 16 in *ampijoroensis*), see Table 1 and Fig. S4.

Description of holotype. ZSM 2905/2011 (Fig. 2B). Snout-vent length 61.5 mm, tail lengh 26 mm including 10.5 mm that has probably been regenerated, width at midbody 3 mm, head width at level of parietal eye 3 mm. Supranasals absent, apparently fused with loreal. Frontonasal roughly triangular, wider than long, contacting loreals, first supraciliaries and first supraoculars (the most lateral corner being inserted between the first SC and the first SO). Prefrontals absent. Supraoculars three. Frontoparietals absent. One pair of enlarged nuchals. Nasal contacting rostral and first supralabial. Postnasals absent. A single pair of loreals meeting dorsomedially. Four supraciliaries on the right side, five on the left side. Supralabials five, the third being the enlarged subocular contacting the lower eyelid. 18 scale rows around midbody, 109 ventrals. In preservative, ground colouration beige/light brown.

Variation. See Table 1, for measurements and scale characters.

Distribution, habitat and habits. This species is only known from the type locality. Matsedroy forest is an unprotected fragment of dry deciduous forest near Mariarano, north-western Madagascar (Fig. 1B, see also Fig. S5). It is ~1758 ha in size, surrounded by a mosaic of wooded grass and scrubland, savanna and agricultural land (Moat & Smith 2007; Washington *et al.* 2009). The Mariarano and Mahamavo rivers may act as barriers to dispersal for *P. mahamavo* sp. nov., effectively causing the Matsedroy forest fragment to behave like a savannah island for this species. Despite extensive surveys in the area, no individuals have been encountered north of the Mariarano River to date; however, their distribution southwards towards the Mahamavo River remains unknown. Based on the data available, we recommend listing this species under the IUCN Red List criteria as 'Vulnerable' due to the apparently restricted range of this species (which is confined to a single location)

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and the continued degradation of its habitat at the type locality from slash and burn practices (tavy) for charcoal production and selective logging.

Etymology. The new species is named after the Mahamavo watershed in which the species was found and, most likely is restricted to. Mahamavo also means 'to make yellow' in the Malagasy language, in reference to the dusty nature of the area. The epithet is used as a noun in apposition.

Discussion

Remarks on Paracontias holomelas

When discussing relationships and biogeography in the genus *Paracontias*, the largest known and morphologically most distinct species in the genus, *Paracontias holomelas*, requires some comments. It was described by Günther (1877) from Anzahamaru, a locality according to the original description most probably corresponding to a village near Mahanoro, south of Tamatave (= Toamasina). Brygoo (1980) apparently examined all specimens present in collections at that time, including the types. He stated that the original description is accurate and that the series of nine specimens examined by him is very homogenous with respect to morphology. In the map provided by Brygoo (1980), most records of *P. holomelas* originate from eastern localities not far from Toamasina, whereas one more northern locality probably refers to Fandrarazana (at the east coast at the level of Nosy Boraha), a locality already mentioned by Kaudern (1922) and Angel (1942), and one north-eastern locality possibly refers to Marojejy. More recently collected specimens considered to represent *P. holomelas* originate from the Masoala peninsula (Andreone & Greer 2002) and Marojejy (Andreone *et al.* 2000; Whiting *et al.* 2004; Schmitz *et al.* 2005).

DNA sequences for *P. holomelas* were published by Whiting *et al.* (2004) and Schmitz *et al.* (2005) and all originate from the same specimen (UMMZ 201644) from Marojejy Reserve, Manantenina River. Moreover, Austin & Arnold (2006) included *P. holomelas* sequences originating from a tail tip from Antsiranana (province?), but without providing more exact voucher information. When describing *P. kankana*, Köhler *et al.* (2009) discovered that published mtDNA sequences of UMMZ 201644 were identical with those from the holotype of *P. kankana* (a species collected later and considerably differing from *P. holomelas* in morphology) and concluded that most probably the supposed *P. holomelas* sequences from Marojejy correspond to a misidentified specimen of *P. kankana*. Therefore, Crottini *et al.* (2009) in their analyses partly used the *P. holomelas* sequences provided by Whiting *et al.* (2004) for the *P. kankana* data set (tentatively named *P.* sp. aff. *tsararano* in their article). However, our new data revealed that the ND1 sequence of *P. holomelas* by Whiting *et al.* (2004) is considerably different from the sequence of the same gene in *P. kankana*, strongly arguing for a mixture of samples or other problems with the molecular *P. holomelas* data set of Whiting *et al.* (2004). We therefore regard the species identity of these sequences as in need of confirmation and conclude, given the incomplete voucher information of Austin & Arnold (2006), that there are no sequences available reliably referring to *P. holomelas*. The species was therefore completely excluded from our molecular analyses, as were the Whiting *et al.* (2004) sequences used by Crottini *et al.* (2009) for *P. kankana* (GenBank accession numbers AY315509, AY391227).

According to records vouchered by museum specimens, the distribution of *P. holomelas* ranges along Madagascar's east coast from central Madagascar northwards to the Marojejy massif at 850 m above sea level (Brygoo 1980; Andreone *et al.* 2000; Glaw & Vences 2007). Given that all other known species of *Paracontias* are distributed exclusively in northern Madagascar and most of them appear to be microendemic, inhabiting a small area only, the supposed range of *P. holomelas* is rather remarkable, representing by far the largest of all known ranges and including the southernmost records for the genus. However, given the lack of molecular data and partly difficult access of collected specimens for detailed morphological examination, we are unable at this stage to confirm that all the large *Paracontias* specimens from the mentioned range really correspond to *P. holomelas*. Summarizing, there is no verified information on the distribution, nor the phylogenetic relationships of *P. holomelas*. For practical reasons, and given the possibility that the name *P. holomelas* as currently recognized may constitute a complex of species, we here exclude it from the discussion of biogeography and phylogeny, being aware that future research on *P. holomelas* may partly require modification of the hypotheses provided below.

Two deep lineages in Paracontias

Based on the molecular phylogeny shown in Fig. 1A, the 'kankana clade' represents an old lineage within the genus *Paracontias*, being sister lineage of the 'brocchii' clade comprising all the other species. In addition, one unambiguous morphological character and a contrasting geographical pattern provide evidence that these two major lineages have diverged a long time ago and might represent two different evolutionary trajectories within the genus. In a complementary paper (Miralles *et al.* 2015), the divergence beween the two main *Paracontias* clades is estimated as mid-Oligocene, around 27 Mya.

New insights into the evolution of the cephalic scalation in Paracontias

Legless fossorial tetrapods (squamates and amphibians) essentially use their snout for digging into the soil. In response to the strong mechanical constraints exerting on the tip of the animal's head, highly derived skull characters (compact bones, solidly enclosed braincases and reduced arcades) have evolved in many of these lineages specialized in a head-first burrowing lifestyle (e.g. Uropeltoidea and scolecophidian snakes, amphisbaenians, dibamids and caecilians; Gans 1974, 1975; Rieppel 1984; Measey & Herrel 2006; Rieppel & Maisano 2007; Rieppel *et al.* 2009). The cephalic integument is also expected to present signs of anatomical adaptation to a burrowing lifestyle via a process of simplification of the cephalic scalation (Miralles 2001; Miralles *et al.* 2011a). Indeed, many distinct lineages have convergently followed the general trend of reduction in the number of scales (fusion) on the anteriormost part of the head. This phenomenon has led to a remarkable diversity of cephalic scalation patterns characterized by the presence of bigger scales, conferring a smooth aspect to the cephalic tegument and likely a higher penetration coefficient to the head (e.g., scolecophidians and several colubrid snakes, amphisbaenians, dibamids, scincids or the gymnophthalmid genus *Bachia*; Marx & Rabb 1970; Gans 1974; Savitzky 1983; Avila-Pires 1995; Miralles 2001; Broadley & Wallach 2009; Miralles *et al.* 2011a, 2012; present study). Nevertheless, and despite the fact that cephalic scalation has always constituted one of the most important sources of morphological characters for comparative taxonomy, relatively few studies have paid attention to the evolutionary processes at the origin of these tegumentary structures, or to the reliability of the homologies hypothesized in a phylogenetic context for each of these scales covering the head.

In a previous study (Miralles *et al.* 2011a), *Paracontias* has been used as a model to hypothesize the evolution of the cephalic scales having led to the formation of a very peculiar tegumentary structure that convergently evolved in several highly fossorial legless squamates, the rostral shield (a large, smooth and conical plate covering the snout, totally encompassing the nostrils, and with a horizontal groove running posteriorly from either nostril). Here we take the opportunity offered by the two newly described species of *Paracontias* to propose complementary hypotheses about the morphological transformations affecting the cephalic scalation within this highly derived genus. In a first step, homology hypotheses are proposed for the scales located in the loreal region (lateral sides of the upper jaw located between the eyes and the nostrils) of the 'kankana' and the 'brocchii' clades of *Paracontias*, then in a second step, a scenario is proposed for the evolutionary sequence from the plesiomorphic cephalic scalation pattern of the majority of quadrupedal Malagasy skinks towards the reduced pattern of *Paracontias*.

Traditionally, herpetologists have used the notion of 'fusion' of scales to refer to the transition from a state with two or more little scales to a state with a single larger scale occupying more or less the same place, shape and expanse, and thus supposed to be homologous with the smaller scales (the notion of 'fragmentation' corresponding to the same phenomenon with an inverse transformation). In the present study, we chose to continue using these terms for obvious practical reasons. It is nevertheless essential to insist on the fact that it does not insinuate any morphogenetic processes, but only refers to an evolutionary transition from one pattern to another, whatever the ontogenetic nature of the mechanism involved.

Evolution of the loreals in Paracontias. Species of the 'kankana clade' are characterized by a peculiar scalation pattern apparently unique among Malagasy scincines (Fig. 3A). They have large loreals extending and meeting each other at dorsal midline, whereas almost all the species of the 'brocchii clade' have small loreals separated from each other by the rostral and the frontonasal (the only exception being *P. milloti* whose loreals are absent). As these two clades are separated at the basalmost node of the genus *Paracontias*, the tree topology does not allow unambiguously polarizing the evolution of the loreal scales within this taxon. The topology allows two alternative hypotheses (Fig. 3A), apparently equally parsimonious, to explain the origin of the 'extended loreal' pattern observed in the 'kankana clade': According to hypothesis I, this pattern would represent a plesiomorphic trait within the genus *Paracontias* (bI), intermediate between the ancestral pattern observed in most of the Malagasy scincines such as *Madascincus* and *Amphiglossus* (a) and a more derived pattern observed in the 'brocchii clade' of *Paracontias* (cI), characterized by the absence of supranasals, postnasals and with small loreals separated from each others. It would therefore result from the fusion of the loreals with the supranasals (L+SN) in the early history of *Paracontias* (based on homology of size and position), followed by a lateral regression of these resulting 'loreosupranasal' scales in the 'brocchii clade', allowing the rostral and the frontonasal to meet each other dorsally. According to hypothesis II, this pattern would, on the contrary, represent an autapomorphic trait of the 'kankana clade' (cII), derived from the pattern observed in the 'brocchii clade' (bII). It would result from the dorsal extension of the loreals, allowing them to meet each other and separating the so-called 'rostral' from the frontonasal [given this hypothesis, and based on size and position homologies, the loss of the supranasals characterizing all the species of

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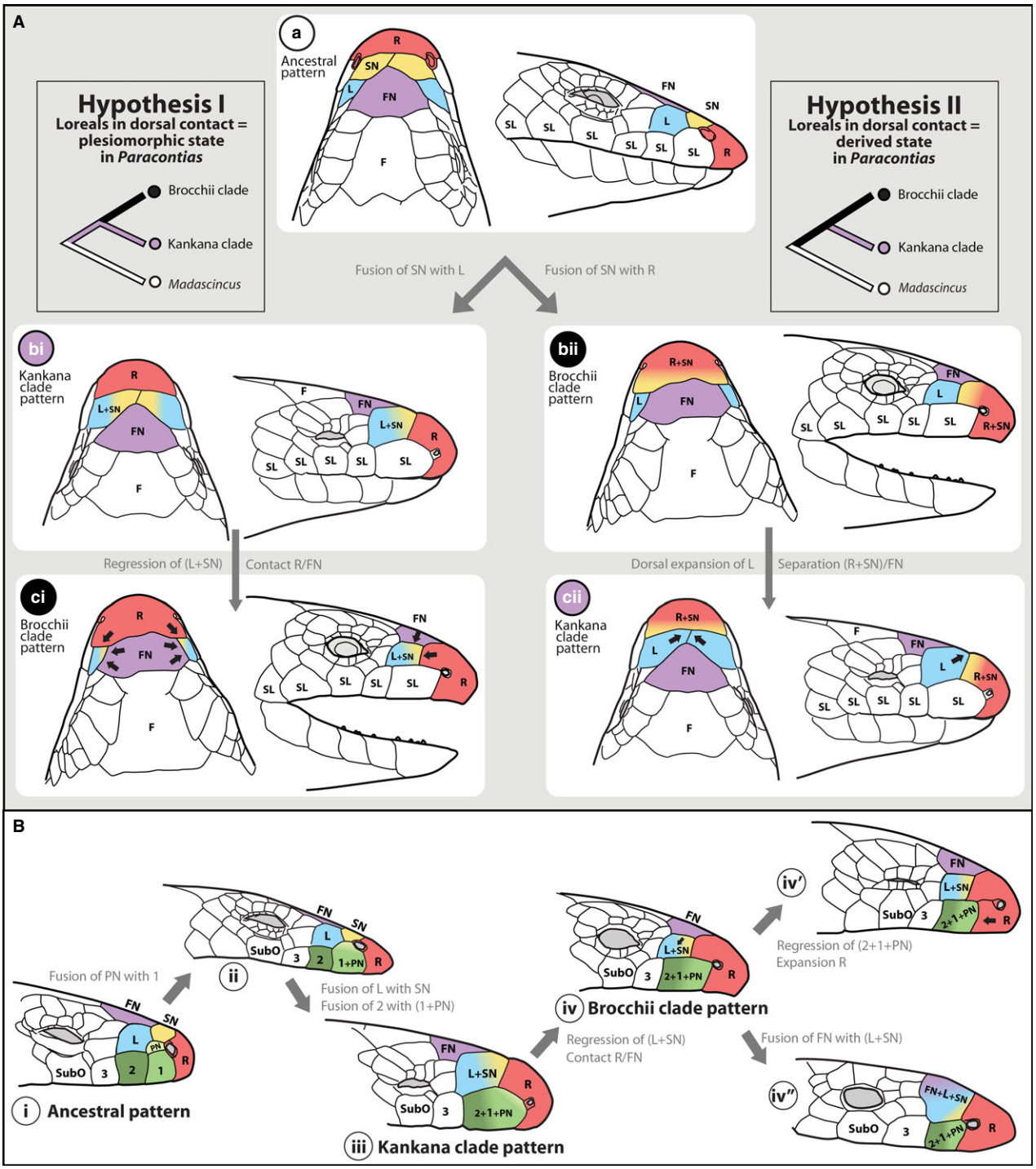

Fig. 3 Evolution of the cephalic scalation within the genus *Paracontias*. (A) Alternative hypotheses explaining the origin of the "extended loreal" pattern observed in the 'kankana clade': according to hypothesis I, this pattern would represent a plesiomorphic trait within the genus *Paracontias*, whereas hypothesis II considers it as an autapomorphic trait. Black arrows represents major changes (extension/regression) in scales configuration. (B) Hypothetical evolutionary sequence retracing the simplification of the cephalic scalation in *Paracontias*. Scale terminology (based on the ancestral pattern as referential): FN: frontonasal, L: loreal, PN: postnasal, R: rostral, SN: supranasal, SubO: subocular supralabial, 1 to 3: first to third supralabials.

Paracontias would likely result from their fusion with the rostral scale *sensu stricto* (R+SN)].

Both hypotheses involving one step of fusion followed by one step of regression/extension, might be regarded as equally parsimonious. Nevertheless, hypothesis I appears more likely to us than hypothesis II as it does not involve reversals and follows a general trend of cephalic scale simplification observed in many other legless fossorial Squamata (e.g. skinks of the genera *Acontias*, *Nessia*, *Voeltzkowia*, *Grandidierina* or *Typhlosaurus*, dibamids or fossorial snakes such as *Calamaria* or *Prosymna*, Chippaux 2006; Miralles *et al.* 2011a, 2012; Ziegler & Quyet 2005). On the contrary, hypothesis II would involve a simplification of the dorsal side of the snout (only two large median scales on the dorsal side of the snout, separated by wide and rather straight transversal sutures) followed by a backward step consisting in a return to a more complex pattern for the dorsal side of the snout, with relatively small scales (loreals) intercalating between the so-called rostral (actually R+SN) and the frontonasal, and conferring a complex pattern by mixing transversal, longitudinal and oblique sutures on the dorsal side of the snout. If cephalic scale simplification has adaptive value for fossorial lizards, then such a reversal to increased complexity of cephalic scalation would not be expected in a clade of continued fossorial habits.

Genesis of the cephalic scalation in Paracontias. Our preferred hypothesis (I) presented above for the evolution of the scales of the loreal region can be combined with the 'two-step hypothesis' formulated in Miralles *et al.* (2011a) for the evolution of the scales of the rostral region (Fig. 3B). This yields a hypothetical evolutionary sequence towards the highly simplified cephalic scalation of most *Paracontias*.

We consider the cephalic pattern observed in the genus *Madascincus* (a genus of quadrupedal skinks sister to *Paracontias*) as a good model to represent the ancestral cephalic scalation of the *Paracontias* ancestor (Fig 3B (i)): the rostral region has a rostral scale (R), a pair of supranasals in contact (SN), a pair of postnasals (PN), and three pairs of supralabials (1, 2, 3) between the rostral and the subocular supralabial (SubO). Interestingly, one partly fossorial *Madascincus* (*M. arenicola* (ii)), has a slightly simplified cephalic scalation (Miralles *et al.* 2011b) with absent postnasals (apparently fused with the first loreal (1+PN), based on size and position homology). We here propose that a similar event, likely convergently, might have led to the absence of postnasals in *Paracontias*. This modifies the hypothesis of Miralles *et al.* (2011a) who suggested that these scales might have fused with the supranasal and the rostral in an early step of cephalic scale reduction.

The next step of the scenario proposed here is the fusion of supranasals with loreals into a large 'loreosupranasal' (L+SN), and of the first (1+PN) and second (2) supralabial scales, leading to the pattern currently observed in the 'kankana clade' (iii). Then, a regression of the 'loreosupranasals' would have followed, allowing the frontonasal to meet the rostral, a pattern characterizing species of the 'brocchii clade' (iv). In some species of this clade, the simplification of the rostral scales continued either via the regression of the anteriormost supralabial (1+2+PN), leading to the formation of a true rostral shield sensu Miralles *et al.* (2011a) (iv), or via the fusion of loreosupranasals (L+SN) with the frontonasal as seen in *Paracontias milloti* (iv).

In this scenario, the transition from (ii) to (iii) involves two parallel phenomena of scale reduction (supranasal + loreal, and first + second SL). In both cases, the involved scales are located on the lateral sides of the snout (i.e. in the loreal region) and the fusion occurred along an anteroposterior axis (loss of a vertical suture). These characteristics suggest that similar morphogenetic processes might have been involved in the transformations of these scales, potentially simultaneously and concomitantly to a shortening of the snout.

This scenario requires further testing, and we suggest that detailed studies on the embryonic development of scales and the underlying gene expression would be a suitable approach for better understanding patterns of scale reduction and fusion in squamates.

Biogeography, microendemism and taxonomic research strategy

The 'kankana clade' and the 'brocchii clade' have distinct and as far as we know non-overlapping geographical patterns of distribution. The 'brocchii clade' of *Paracontias* is kown from the extreme north of Madagascar whereas the 'kankana clade' is apparently restricted to a more southern region. A very similar biogeography has been observed in the miniaturized species of the *Brookesia minima* group (Glaw *et al.* 2012). Both the genus *Paracontias* and the *B. minima* group are monophyletic groups restricted to the northern half of Madagascar, with the more southern clades including species from both the western dry forest and the eastern rainforest habitats. This suggests a primary north-south vicariance and a secondary east–west vicariance in both groups.

Although the distribution of *Paracontias* remains poorly known, microendemism appears to be frequent in the genus in accordance with a trend apparently generalizable to many other miniaturized and limb-regressed lizards (Lee *et al.* 2013). Most of the described species of *Paracontias* are known from restricted areas or even only from the original

type material collected in a single locality (Fig. 1B). The recent discovery of new species in unexplored localities supports the idea that many more new species are likely to be discovered in the future, and suggests that newly discovered isolated populations of *Paracontias* have a significant probability of representing new taxa. However, because these animals are small, morphologically cryptic and secretive, focused field campaigns are difficult and their discovery will continue to occur by chance in the course of general faunal inventories. We elaborated a 'user-friendly' graphical field identification key for *Paracontias* (available to all in the Supplementary information, Fig. S6.) which we anticipate will simplify the identification of described species and help revealing new candidate species that morphologically do not fit to any described species. Such unidentifiable specimens would therefore be worthwhile to be collected and studied for taxonomic purposes as part of ongoing biodiversity studies in Madagascar, and freshly collected material would also be paramount to assess the phylogenetic position of *P. holomelas*.

Acknowledgements

We are grateful to Annemarie Ohler and Ivan Ineich (MNHN) and Gunther Köhler (SMF) for providing us access to specimens under their care, Hiroki Sato, Ryo Ito and Hasina Rabe Maheritafika for their help in collecting specimens of *P. ampijoroensis* sp. nov., James Coates and Mamy Rabenoro for their help in collecting the holotype and paratypes of *P. mahamavo* sp. nov., respectively, Felix Rakotondraparany for the cooperation in conducting this research, and Yosuke Kojima and Taxi (MNHN) for having realized some of the original photographs of the Fig. 2. Parfait Bora, Neil D'Cruze, Charlie Ellis, Hildegard Enting, Michael Franzen, Stuart Graham, Cat Hendry, Angelika Knoll, Steven Megson, Randall Morrison, Zoltan Nagy, Jamie Neaves, R. Maheritafika, Angelin and Angeluc Razafimanantsoa were of invaluable help in the field. Andolalao Rakotoarison and Fanomezana Ratsoavina provided logistic support. We thank Frank Tillack and Mark-Oliver Rödel for information on a ZMB specimen of *P. holomelas*. The work was carried out in collaboration with the Département de Biologie Animale, Université d'Antananarivo, and Madagascar National Parks (MNP). We are grateful to the Malagasy authorities, in particular the Ministère de l'Environnement et des Eaux et Forêts, for research and export permits, and VOI Tanteraka (Mariarano) and VOI Tanandava for facilitating the research at Mahamavo. Aurélien Miralles was supported by a postdoctoral Research Fellowship from the Alexander von Humboldt Foundation and by a SYNTHESYS grant (FR–TAF–842). Akira Mori was supported by a Grant-in-Aid for International Scientific Research (B) (Nos. 24405008) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan; Teppei Jono was supported by JSPS Research Fellowships for Young Scientists (No. 231388) and a Fellowship for Young International Scientists of the Chinese Academy of Sciences (No. Y4J3011100). Robert Gandola was supported by funding from Operation Wallacea and logistics from DBCAM (Development and Biodiversity Conservation Action for Madagascar).

References

Andreone, F. & Greer, A. E. (2002). Malagasy scincid lizards: descriptions of nine new species, with notes on the morphology, reproduction and taxonomy of some previously described species (Reptilia, Squamata: Scincidae). *Journal of Zoology*, *258*, 139–181.

Andreone, F., Randrianirina, J., Jenkins, P. D. & Aprea, G. (2000). Species diversity of Amphibia, Reptilia and Lipotyphla (Mammalia) at Ambolokopatrika, a rainforest between the Anjanaharibe-Sud and Marojejy massifs, NE Madagascar. *Biodiversity and Conservation*, *9*, 1587–1622.

Angel, F. (1942). Les lézards de Madagascar. *Mémoires de l'Académie Malgache*, *36*, 1–139.

Angel, F. (1949). Description d'une espèce nouvelle du genre *Paracontias*. *Mémoire de l'Institut scientifique de Madagascar*, *Série A*, *3*, 81–87.

Austin, J. J. & Arnold, E. N. (2006). Using ancient and recent DNA to explore relationships of extinct and endangered *Leiolopisma* skinks (Reptilia: Scincidae) in the Mascarene islands. *Molecular Phylogenetics and Evolution*, *39*, 503–511.

Avila-Pires, T. C. S. (1995). Lizards of Brazilian Amazonia (Reptilia: Squamata). *Zoologische Verhandelingen*, *299*, 1–706.

Brandley, M. C., Huelsenbeck, J. P. & Wiens, J. J. (2008). Rates and patterns in the evolution of snake-like body form in squamate reptiles: evidence for repeated re-evolution of lost digits and long-term persistence of intermediate body forms. *Evolution*, *62*, 2042–2064.

Broadley, D. G. & Wallach, V. (2009). A review of the eastern and southern African blind-snakes (Serpentes: Typhlopidae), excluding *Letheobia* Cope, with the description of two new genera and a new species. *Zootaxa*, *2255*, 1–100.

Bruford, M. W., Hanotte, O., Brookfield, J. F. Y. & Burke, T. (1992). Single-locus and multilocus DNA fingerprinting. In A. R. Hoelzel (Ed.) *Molecular Genetic Analysis of Populations: A Practical Approach* (pp. 225–269). Oxford: IRL Press.

Brygoo, E. R. (1980). Systématique des lézards scincidés de la région malgache. III. Les «Acontias» de Madagascar: *Pseudoacontias* Barboza du Bocage, 1889, *Paracontias* Mocquard, 1894, *Pseudoacontias* Hewitt, 1929, et *Malacontias* Greer, 1970. IV. *Amphiglossus reticulatus* (Kaudern, 1922) nov. comb., troisième espèce du genre; ses rapports avec *Amphiglossus waterloti* (Angel, 1920). *Bulletin du Muséum national d'Histoire Naturelle*, *Section A Zoologie*, *Biologie et Ecologie Animale*, *2*, 905–918.

Carranza, S., Arnold, E. N., Geniez, P., Roca, J. & Mateo, J. A. (2008). Radiation, multiple dispersal and parallelism in Moroccan skinks, *Chalcides* and *Sphenops* (Squamata: Scincidae), with comments on *Scincus* and *Scincopus* and the age of the Sahara Desert. *Molecular Phylogenetics and Evolution*, *46*, 1071–1094.

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, *17*, 540–552.

Chiari, Y., Vences, M., Vieites, D. R., Rabemananjara, F., Bora, P., Ramilijaona Ravoahangimalala, O. & Meyer, A. (2004). New evidence for parallel evolution of colour patterns in Malagasy poison frogs (*Mantella*). *Molecular Ecology*, *13*, 3763–3774.

Chippaux, J. P. (2006). *Les Serpents d'Afrique Occidentale et Centrale*, 3rd edn. Paris: IRD.

Crottini, A., Dordel, J., Köhler, J., Glaw, F., Schmitz, A. & Vences, M. (2009). A multilocus phylogeny of Malagasy scincid lizards elucidates the relationships of the fossorial genera *Androngo* and *Cryptoscincus*. *Molecular Phylogenetics and Evolution*, *53*, 345–350.

Crottini, A., Madsen, O., Poux, C., Strauß, A., Vieites, D. R. & Vences, M. (2012). Vertebrate time-tree elucidates the biogeographic pattern of a major biotic change around the K–T boundary in Madagascar. *Proceedings of the National Academy of Sciences of the USA*, *109*, 5358–5363.

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, *9*, 772.

Gans, C. (1974). *Biomechanics: An Approach to Vertebrate Biology*. Philadelphia: Lippincott Co., 261 pp.

Gans, C. (1975). Tetrapod limblessness: evolution and functional corollaries. *American Zoologist*, *15*, 455–467.

Glaw, F. & Vences, M. (2007). *A Field Guide to the Amphibians and Reptiles of Madagascar*, 3rd edn. Cologne: Vences and Glaw Verlag.

Glaw, F., Köhler, J., Townsend, T. M. & Vences, M. (2012). Rivaling the world's smallest reptiles: discovery of miniaturized and microendemic new species of leaf chameleons (*Brookesia*) from northern Madagascar. *PLoS ONE*, 7, e31314.

Ikeuchi, I., Hasegawa, M. & Mori, A. (2012). Characteristics of sleeping sites and timing of departure from them in a Madagascan diurnal gecko, *Lygodactylus tolampyae*. *Current Herpetology*, *31*, 107–116.

Kaudern, W. (1922). Sauropsiden aus Madagascar. *Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Tiere*, *45*, 395–458.

Köhler, J., Vieites, D. R., Glaw, F., Kaffenberger, N. & Vences, M. (2009). A further new species of limbless skink, genus *Paracontias*, from eastern Madagascar. *African Journal of Herpetology*, *58*, 98–105.

Köhler, J., Vences, M., Erbacher, M. & Glaw, F. (2010). Systematics of limbless scincid lizards from northern Madagascar: morphology, phylogenetic relationships and implications for classification (Squamata: Scincidae). *Organisms Diversity and Evolution*, *10*, 147–159.

Kohlsdorf, T. & Wagner, G. P. (2006). Evidence for the reversibility of digit loss: a phylogenetic study of limb evolution in *Bachia* (Gymnophthalmidae: Squamata). *Evolution*, *60*, 1896–1912.

Lanfear, R., Calcott, B., Ho, S. Y. & Guindon, S. (2012). Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, *29*, 1695–1701.

Lee, M. S. Y. (1998). Convergent evolution and character correlation in burrowing reptiles: towards a resolution of squamate relationships. *Biological Journal of the Linnean Society*, *65*, 369–453.

Lee, M. S. Y., Skinner, A. & Camacho, A. (2013). The relationship between reduction, body elongation and geographical range in lizards (*Lerista*, Scincidae). *Journal of Biogeography*, *40*, 1290–1297.

Marx, H. & Rabb, G. B. (1970). Character analysis: an empirical approach applied to advanced snakes. *Journal of Zoology*, *161*, 525–548.

Measey, G. J. & Herrel, A. (2006). Rotational feeding in caecilians: putting a spin on the evolution of cranial design. *Biology Letters*, *2*, 485–487.

Miralles, A. (2001). *L'évolution des plaques supra-céphaliques chez les serpents colubroïdes* [dissertation]. Paris : DEA de Systématique Animale et Végétale, Muséum national d'Histoire Naturelle.

Miralles, A., Köhler, J., Vieites, D. R., Glaw, F. & Vences, M. (2011a). Developing hypotheses on rostral shield evolution in head-first digging squamates from a molecular phylogeny and new species of the genus *Paracontias* (Scincidae). *Organisms Diversity and Evolution*, *11*, 135–150.

Miralles, A., Köhler, J., Glaw, F. & Vences, M. (2011b). A molecular phylogeny of the "*Madascincus polleni* species complex", with description of a new species of scincid lizard from the coastal dune area of northern Madagascar. *Zootaxa*, *2876*, 1–16.

Miralles, A., Anjeriniana, M., Hipsley, C. A., Müller, J., Glaw, F. & Vences, M. (2012). Variations on a bodyplan: description of a new Malagasy "mermaid skink" with flipper-like forelimbs only (Scincidae: *Sirenoscincus*). *Zoosystema*, *34*, 701–710.

Miralles, A., Hipsley, C. A., Erens, J., Gehara, M., Rakotoarison, A., Glaw, F., Müller, J. & Vences, M. (2015). Distinct patterns of desynchronized limb regression in Malagasy scincine lizards (Squamata, Scincidae). *PLoS ONE*, *10*, e0126074.

Moat, J. & Smith, P. P. (2007). *Atlas of the Vegetation of Madagascar/Atlas de la Vegetation de Madagascar*. London: Royal Botanic Gardens, Kew. 1–124 pp.

Mori, A., Hasegawa, M. & Ikeuchi, I. (2006). Herpetofauna of Ampijoroa, Ankarafantsika Strict Nature Reserve, a dry forest in northwestern Madagascar. *Herpetological Natural History*, *10*, 31–60.

Mott, T. & Vieites, D. R. (2009). Molecular phylogenetics reveals extreme morphological homoplasy in Brazilian worm lizards challenging current taxonomy. *Molecular Phylogenetics and Evolution*, *51*, 190–200.

Pianka, E. R. & Vitt, L. J. (2003). *Lizards: Windows to the Evolution of Diversity*. Berkeley, Los Angeles: University of California Press.

Rieppel, O. (1984). The cranial morphology of the fossorial lizard genus *Dibamus* with a consideration of its phylogenetic relationships. *Journal of Zoology*, *204*, 289–327.

Rieppel, O. & Maisano, J. A. (2007). The skull of the rare Malaysian snake *Anomochilus leonardi* Smith, based on high-resolution X-ray computed tomography. *Zoological Journal of the Linnean Society*, *149*, 671–685.

Rieppel, O., Kley, N. J. & Maisano, J. A. (2009). Morphology of the skull of the white-nosed blindsnake, *Liotyphlops albirostris* (Scolecophidia: Anomalepididae). *Journal of Morphology*, *270*, 536–557.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. &

Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542.

Sakata, S. & Hikida, T. (2003). A fossorial lizard with forelimbs only: description of a new genus and species of Malagasy skink (Reptilia: Squamata: Scincidae). *Current Herpetology*, *22*, 9–15.

Savitzky, A. H. (1983). Coadapted characters complexes among snakes: fossariality, piscivory, and durophagy. *American Zoologist*, *23*, 397–409.

Schmitz, A., Brandley, M. C., Mausfeld, P., Vences, M., Glaw, F., Nussbaum, R. A. & Reeder, T. W. (2005). Opening the black box: phylogenetics and morphological evolution of the Malagasy fossorial lizards of the subfamily 'Scincinae'. *Molecular Phylogenetics and Evolution*, *34*, 118–133.

Shen, X.-X., Liang, D. & Zhang, P. (2012). The development of three long universal nuclear protein-coding locus markers and their application to osteichthyan phylogenetics with nested PCR. *PLoS ONE*, 7, e39256.

Siler, C. D. & Brown, R. M. (2011). Evidence for repeated acquisition and loss of complex body-form characters in an insular clade of Southeast Asian semi-fossorial skinks. *Evolution*, *65*, 2641–2663.

Skinner, A., Lee, S. Y. M. & Hutchinson, M. N. (2008). Rapid and repeated limb loss in a clade of scincid lizards. *BMC Evolutionary Biology*, *8*, 310.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*, 2688–2690.

Swofford, D. L. (2002). *PAUP*. Phylogenetic Analysis Using Parsimony (*and other Methods) Version 4.0.b10*. Sunderland: Sinauer Associates.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, *30*, 2725–2729.

Washington, H., Rabenoro, M., Andriambololona, N., Griffin, O., Higgins, F., Osmond, T., Shah, P. & Clementine, V. M. (2009). *University of Bath Mahamavo Expedition 2009*. University of Bath, Bath, UK.

Whiting, A. S., Bauer, A. M. & Sites, J. W., Jr (2003). Phylogenetic relationships and limb loss in sub-Saharan African scincine lizards (Squamata: Scincidae). *Molecular Phylogenetics and Evolution*, *29*, 582–598.

Whiting, A. S., Sites, J. W., Jr & Bauer, A. M. (2004). Molecular phylogenetics of Malagasy skinks (Squamata: Scincidae). *African Journal of Herpetology*, *53*, 135–146.

Wiens, J. J. & Slingluff, J. L. (2001). How lizards turn into snakes: a phylogenetic analysis of body-form evolution in anguid lizards. *Evolution*, *55*, 2303–2318.

Wiens, J. J., Brandley, M. C. & Reeder, T. W. (2006). Why does a trait evolve multiple times within a clade? Repeated evolution of snakelike body form in squamate reptiles *Evolution*, *60*, 123–141.

Ziegler, T. & Quyet, L. K. (2005). A new species of reed snake, *Calamaria* (Squamata: Colubridae), from the Central Truong Son (Annamite mountain range), Vietnam. *Zootaxa*, *1042*, 27–38.

Supporting Information

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

Fig. S1. Number of *Paracontias* species described per decade in Madagascar.

Data S1. Detailed procedure for morphological study.

Data S2. List of additional specimens examined.

Table S1. List of taxa included in the molecular analyses

Table S2. Partitioning scheme used for the phylogenetic analyses

Fig. S2. Separated phylogenetic analyses of the genus *Paracontias*.

Data S3. Taxonomic descriptions (full versions).

Fig. S3. Living specimen and habitats of *Paracontias ampijoroensis*

Fig. S4. Schematic drawings of the holotypes of *P. kankana*, *P. ampijoroensis* and *P. mahamavo*.

Fig. S5. Living specimen and habitats of *Paracontias mahamavo*.

Fig. S6. Graphical identification key of the species of *Paracontias*.