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Molecules and morphology suggest cryptic species diversity and an overall complex taxonomy of fish scale geckos, genus *Geckolepis*

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Abstract The current classification of the Malagasy-Comoroan geckos of the genus *Geckolepis* recognizes three valid species and is based on morphological differences only. Species allocation of individuals is difficult, mainly because of the insufficiently known degree of variation and the frequent partial loss of the integument in preserved specimens. Here we study *Geckolepis* specimens from almost their entire known range. We combine molecular and morphological data to estimate species richness in the genus, and to assess the taxonomic validity of the morphological differences proposed. Analyses of mitochondrial (12S, ND4) and nuclear (RAG1, CMOS) gene sequences support three major clades in addition to the morphologically distinct *G. polylepis* (included for 12S only), and the presence of various divergent lineages within these clades, some occurring in sympatry. Among lineages, the external morphology seems to be comparatively conservative, and differences are faint in many cases. For each major lineage, we summarize the most diagnostic morphological characters that distinguish it from other lineages. The combined molecular and morphological data strongly indicate the presence of highly divergent lineages, three of which are tentatively referred to the names *Geckolepis maculata, G. polylepis* and *G. typica*, whereas three other major lineages are considered Confirmed Candidate Species. Among the remaining lineages, differentiation is shallower, and these may qualify for Deep Conspecific Lineages. In conclusion, our analysis provides evidence for an underestimation of species richness in the genus and a complex evolutionary history, not reflected by current *Geckolepis* species-level taxonomy.

Keywords Squamata · Gekkonidae · *Geckolepis* · Madagascar · Molecular genetics · Morphology · Systematics · Species diversity

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Introduction

Fish-scale geckos of the genus *Geckolepis* are endemic to Madagascar and the Comoros. These small to medium-sized nocturnal geckos are characterized by their large overlapping body scales and the ability to rapidly shed off these scales when touched or grasped, which can be interpreted as an efficient mechanism to escape from potential predators (e.g., Schubert and Christophers 1985; Schubert et al. 1990). As a consequence, many voucher specimens deposited in museum collections exhibit a partly damaged integument, a fact that may hamper proper morphological analyses and species identification. Furthermore, it seems that scale characters among individuals of *Geckolepis* are rather variable,

and it is so far unknown if the numbers and patterns of the original scales equal those of scale regenerates.

The taxonomy of *Geckolepis* has been considered to be problematic (Glaw and Vences 2007), and over time some flux in numbers of recognized species is evident. In total, seven names are available in the genus: *G. anomala* Mocquard, 1902, *G. humbloti* Vaillant, 1887, *G. maculata* Peters, 1880, *G. modesta* Methuen and Hewitt, 1913, *G. petiti* Angel, 1942, *G. polylepis* Boettger, 1893, and *G. typica* Grandidier, 1867. The distinction among these taxa was mainly based on only a few faint scale characters, and the reliability of these characters had not been assessed until the revision of Köhler et al. (2009). Whereas former authors tentatively followed the classification of Angel (1942) in recognizing five species (e.g., Wermuth 1965; Brygoo 1987; Glaw and Vences 1994, 2007), with *G. humbloti* placed as a synonym of *G. maculata*, and *G. modesta* as a subspecies of *G. typica*, Köhler et al. (2009) in their revision recognized three valid species only, namely *G. maculata*, *G. polylepis* and *G. typica*, by placing the taxa *anomala, modesta* and *petiti* in the synonymy of *G. typica*. This revision was based on the examination of a great number of museum specimens including types, but relied on morphology only.

As is known from molecular and integrative studies, the herpetofauna of Madagascar holds a considerable portion of cryptic taxonomic diversity (e.g., Greenbaum et al. 2007a; Jackman et al. 2008; Vieites et al. 2009; Crottini et al. 2011; Nagy et al. 2012), and in many cases morphology alone is known to be insufficient to decipher the actual diversity at the species level (Padial et al. 2009; 2010). Given this fact and considering the difficulties involved in the morphological identification of *Geckolepis* species, we felt the necessity to start a molecular screening of the diversity in these geckos and to combine the results with an assessment of morphological variation. Our goal was to obtain a first realistic estimate of actual species richness in the genus and to assess the taxonomic validity of the morphological differences proposed by previous studies. In the following, we present preliminary results from this analysis including *Geckolepis* samples from various localities within its known range in Madagascar. The goal of this first study is not to fully resolve *Geckolepis* systematics. We rather aim to highlight inconsistencies and agreement between morphological and molecular data, and thereby provide a roadmap for a more comprehensive future analysis involving more molecular markers and specimens.

Materials and methods

Tissue samples of specimens collected in the field were stored in 90–99 % ethanol and DNA extracted using standard salt extraction protocols.

We PCR-amplified fragments of four different genes to assess the molecular systematics of *Geckolepis*: (1) a fragment of the mitochondrial NADH dehydrogenase subunit 4 and tRNAHis genes (in the following abbreviated ND4 for simplicity) was used as the main marker and sequenced from 48 available samples. This DNA fragment is here used to calculate a gene tree of *Geckolepis*. (2) A fragment of the mitochondrial 12S gene was sequenced from 56 available samples. Because the 12S data set contained a relatively large number of missing data and the ND4 sequences were sufficient to obtain a good node support in the resulting mitochondrial tree, we did not combine it with ND4 for phylogenetic reconstruction. However, a few individuals, including all specimens of *G. polylepis*, were available only for 12S, but not for ND4 or for the nuclear genes, and we discuss this 12S evidence in the text and use it to assign these samples and localities to the respective mitochondrial lineages. (3) Sequences of the nuclear oocyte maturation factor (CMOS) and (4) of the nuclear recombination activating gene 1 (RAG1) were used from 54 and 51 samples, respectively, to test for genealogical concordance (Avise and Ball 1990), i.e., to understand which of the major mitochondrial lineages does not share with other lineages haplotypes in these two unlinked loci, which would support a lack of gene flow among these lineages. Our main goal here is not to study phylogenetic relationships within *Geckolepis,* but to assess differentiation among populations using three unlinked and thus partly independently evolving markers (mitochondrial-CMOS-RAG1), and to use arguments of congruence among taxonomic characters to delimit independent evolutionary lineages (see Padial et al. 2010). We therefore analyzed the various DNA fragments independently. We chose a network analysis for the only weakly variable nuclear sequences and a Bayesian phylogenetic analysis for the ND4 fragment which had too many differences among taxa for a meaningful network reconstruction.

The following primers and PCR conditions were used for the different gene fragments: ND4 was amplified using the primers ND4 (CAC CTA TGA CTA CCA AAA GCT CAT GTA GA) and leutRNA (AGC CAT TAC TTT TAC TTG GAT TTG CAC C) (originally from Arévalo et al. 1994; modified primer sequences developed by Ed Louis, Omaha's Henry Doorly Zoo) and the following thermal profile: initial denaturation at 94 °C for 90 s, 33 cycles of denaturation at 94 °C for 45 s, annealing at 47 °C for 45 s and elongation at 72 °C for 90 s, followed by 10 min of final elongation. 12S was amplified using the primers 12SAL (AAA CTG GGA TTA GAT ACC CCA CTA T) and 12SBH (GAG GGT GAC GGG CGG TGT GT) of Palumbi et al. (1991) as follows: 35 cycles (94 °C for 45 s, 50 °C for 60 s, 74 °C for 120 s), without initial denaturation and final elongation. RAG1 was amplified with UropRAG1-F1 (GAA AAC CTG GAG CGG TAT GA) and UropRAG1-

R1 (GCA ACT CTG CAA AAC GTT GA) (Bauer et al. 2011) as follows: initial denaturation at 94 °C for 120 s, 39 cycles (94 °C for 20 s, 51 °C for 50 s, 72 °C for 180 s) and 10 min of final elongation. CMOS was amplified with CO8 (GCT TGG TGT TCA ATA GAC TGG) and CO9 (TTT GGG AGC ATC CAA AGT CTC) (Han et al. 2004) as follows: initial denaturation at 94 °C for 120 s, 35 cycles (94 °C for 45 s, 49 °C for 45 s, 72 °C for 60 s) and 6 min of final elongation.

Sequences were resolved on an ABI 3130XL automated sequencer (Applied Biosystems). For the ND4 and 12S fragments only the light strand was sequenced, while the RAG1 and CMOS fragments were sequenced in both directions in order to verify possible heterozygote sites. All sequences were checked for their quality by eye in the program CodonCode Aligner and aligned manually. After alignment and exclusion of incomplete positions at the beginning and end of sequences, the fragments consisted of 626 (ND4), ca. 380 (12S), 465 (RAG1) and 481 (CMOS) nucleotides, respectively.

All sequences were deposited in GenBank. Accession numbers are as follows: JQ974264–JQ974311 (ND4); JQ974108–JQ974158 (12S); JQ974213–JQ974263 (RAG1); JQ974159–JQ974212 (CMOS). For phylogenetic analysis, we used as hierarchical outgroup a sequence of *Phelsuma lineata* (to be submitted to GenBank in the framework of a forthcoming study) and a sequence of *Blaesodactylus antongilensis* (EU054182). Greenbaum et al. (2007b) confirmed that *Blaesodactylus*+*Homopholis* is the immediate sister group to *Geckolepis*.

MrModeltest version 2.3 (Nylander 2004) was used to select the best fitting nucleotide model of evolution under the Akaike information criterion (a GTR+I+G model for both ND4 and 12S). To avoid overparametrization in the analysis of the altogether rather short ND4 sequences, a single model was assigned to the entire (unpartitioned) fragment, including coding positions and a very short piece of tRNAHis. Phylogenetic trees based on Bayesian inference were computed with MrBayes v3.0b4 (Ronquist and Huelsenbeck 2003) using Markov chain Monte Carlo (MCMC) sets for 20×10^6 generations and sampled every 1,000 generations. The trees corresponding to the first 10×10^6 generations were conservatively discarded as burn-in after empirically assessing the log-likelihood values of the sampled trees.

Haplotypes of the nuclear gene fragments were inferred using the PHASE algorithm (Stephens et al. 2001) implemented in DnaSP software (Version 5.10.3; Librado and Rozas 2009). Haplotype network reconstruction of phased sequences of the CMOS and RAG1 fragments was performed using the software TCS version 1.21 (Clement et al. 2000) based on statistical parsimony (Templeton et al. 1992).

Specimens studied are listed in Appendix 1 and deposited in the Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main (SMF), Museum für Naturkunde, Berlin (ZMB), Université d'Antananarivo, Département de Biologie Animale, Antananarivo (UADBA), Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK) and Zoologische Staatssammlung München (ZSM). Field numbers used are FGMV, FGZC, MV and ZCMV (field numbers of F. Glaw and M. Vences, respectively).

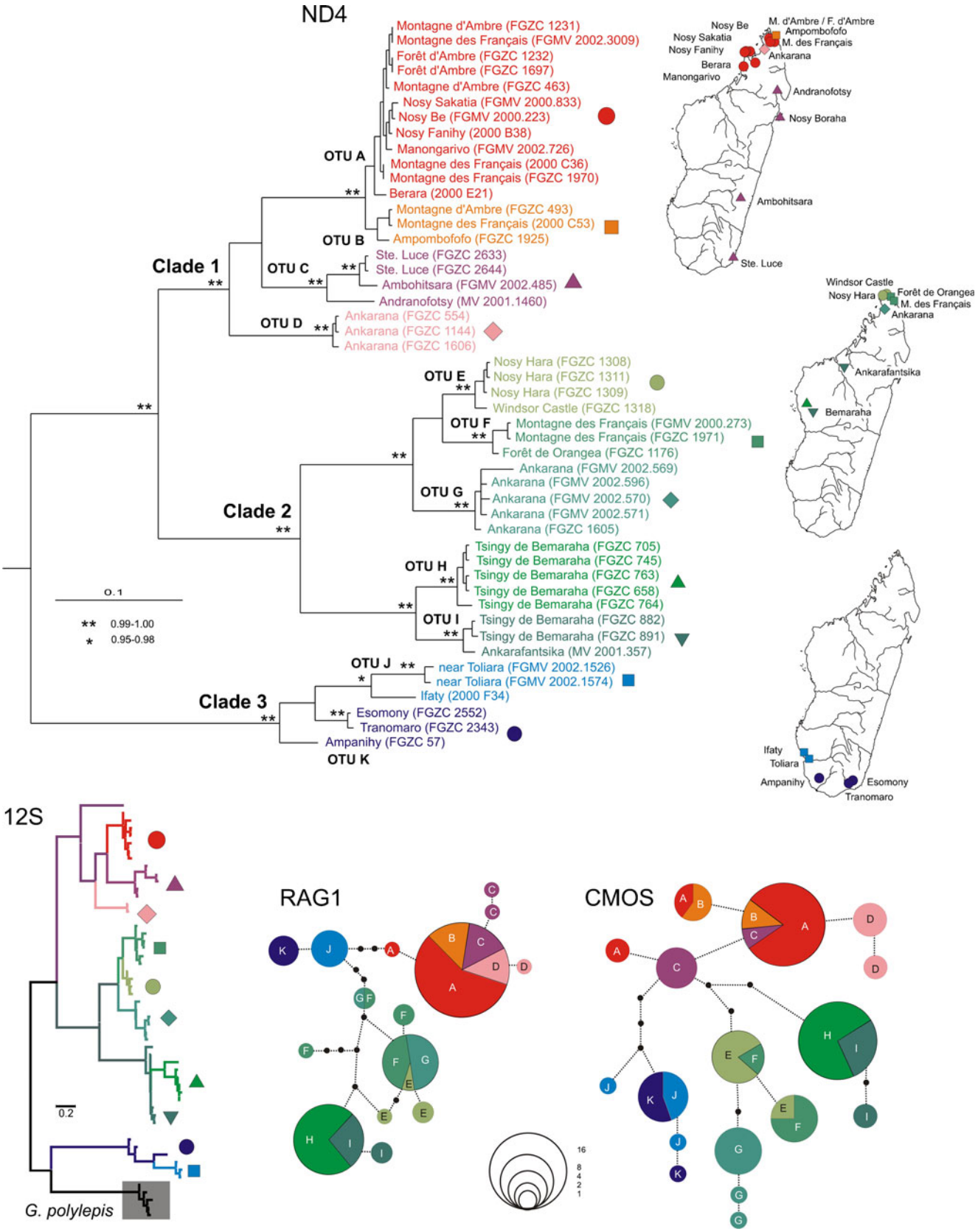
Morphological measurements of voucher specimens were all done by the same person (ME) using a digital caliper (0.01-mm precision) to the nearest 0.1 mm. Measurements taken were snout-vent length (SVL), greatest head width, measured at the widest point of the cranium (HW), and tail length, measured from cloaca to the tip of the tail (TaL). Scale characters were studied only in specimens with intact integument using a Leica MZ6 stereomicroscope. Scale counts included scale rows around the midbody, transversal ventral scale rows from the throat to cloaca, number of infralabials, number of supralabials, and number of lamellae under the fingers and toes. Furthermore, rostral and mental scale conditions were checked and classified, generally following the scheme of Köhler et al. (2009).

Results

Molecular analyses

The 50 % majority consensus tree inferred by Bayesian analysis of the ND4 sequences (Fig. 1) provided a highly resolved phylogenetic hypothesis for the included *Geckolepis* samples, with high posterior probabilities of 0.99–1.0 for most nodes. The samples were subdivided into three major clades, named clades 1–3 in Fig. 1. Of these, clade 3 contains samples from the subarid areas of southern and southwestern Madagascar, clade 2 contains specimens from the north and west, and clade 1 contains samples from the north and from lowland localities along the eastern coast. At two sites of northern Madagascar (Ankarana, Montagne des Français), specimens belonging to clades 1 and 2, respectively, occur syntopically.

Each of the three major clades is subdivided into further mitochondrial lineages of substantial differentiation. We partitioned this variation manually into 11 terminal lineages here considered as provisional operational taxonomic units, OTUs A–K, based on branch lengths and geographical occurrence. These subjective units were established as a means for further analysis but not following strict delimitation criteria. In some cases OTUs of relatively low differentiation were defined to test their relevance with respect to taxonomy, particularly in cases where some genetic differentiation occurs at a single locality and could potentially indicate sympatric occurrence of recently diverged species. Distances between OTUs clustering as each other's closest


ND4
Montagne d'Ambre (FGZC 1231)
Montagne des Français (FGMV 2002.3009)
Forêt d'Ambre (FGZC 1232)
Forêt d'Ambre (FGZC 1697)
Montagne d'Ambre (FGZC 463)
Nosy Sakatia (FGMV 2000.833)
Nosy Be (FGMV 2000.223)
Nosy Fanihy (2000 B38)
Manongarivo (FGMV 2002.726)
Montagne des Français (2000 C36)
Montagne des Français (FGZC 1970)
Berara (2000 E21)
Montagne d'Ambre (FGZC 493)
Montagne des Français (2000 C53)
Ampombofofo (FGZC 1925)
Ste. Luce (FGZC 2633)
Ste. Luce (FGZC 2644)
Ambohitsara (FGMV 2002.485)
Andranofotsy (MV 2001.1460)
Ankarana (FGZC 554)
Ankarana (FGZC 1144)
Ankarana (FGZC 1606)
Nosy Hara (FGZC 1308)
Nosy Hara (FGZC 1311)
Nosy Hara (FGZC 1309)
Windsor Castle (FGZC 1318)
Montagne des Français (FGMV 2000.273)
Montagne des Français (FGZC 1971)
Forêt de Orangea (FGZC 1176)
Ankarana (FGMV 2002.569)
Ankarana (FGMV 2002.596)
Ankarana (FGMV 2002.570)
Ankarana (FGMV 2002.571)
Ankarana (FGZC 1605)
Tsingy de Bemaraha (FGZC 705)
Tsingy de Bemaraha (FGZC 745)
Tsingy de Bemaraha (FGZC 763)
Tsingy de Bemaraha (FGZC 658)
Tsingy de Bemaraha (FGZC 764)
Tsingy de Bemaraha (FGZC 882)
Tsingy de Bemaraha (FGZC 891)
Ankarafantsika (MV 2001.357)
near Toliara (FGMV 2002.1526)
near Toliara (FGMV 2002.1574)
Ifaty (2000 F34)
Esomony (FGZC 2552)
Tranomaro (FGZC 2343)
Ampanihy (FGZC 57)
OTU A
OTU B
OTU C
OTU D
OTU E
OTU F
OTU G
OTU H
OTU I
OTU J
OTU K
Clade 1
Clade 2
Clade 3
0.1
** 0.99-1.00
* 0.95-0.98
Nosy Be
Nosy Sakatia
Nosy Fanihy
Berara
Manongarivo
M. d'Ambre / F. d'Ambre
Ampombofofo
M. des Français
Ankarana
Andranofotsy
Nosy Boraha
Ambohitsara
Ste. Luce
Windsor Castle
Nosy Hara
Forêt de Orangea
M. des Français
Ankarana
Ankarafantsika
Bemaraha
Ifaty
Toliara
Ampanihy
Esomony
Tranomaro
12S
0.2
G. polylepis
RAG1
CMOS
16
8
4
2
1

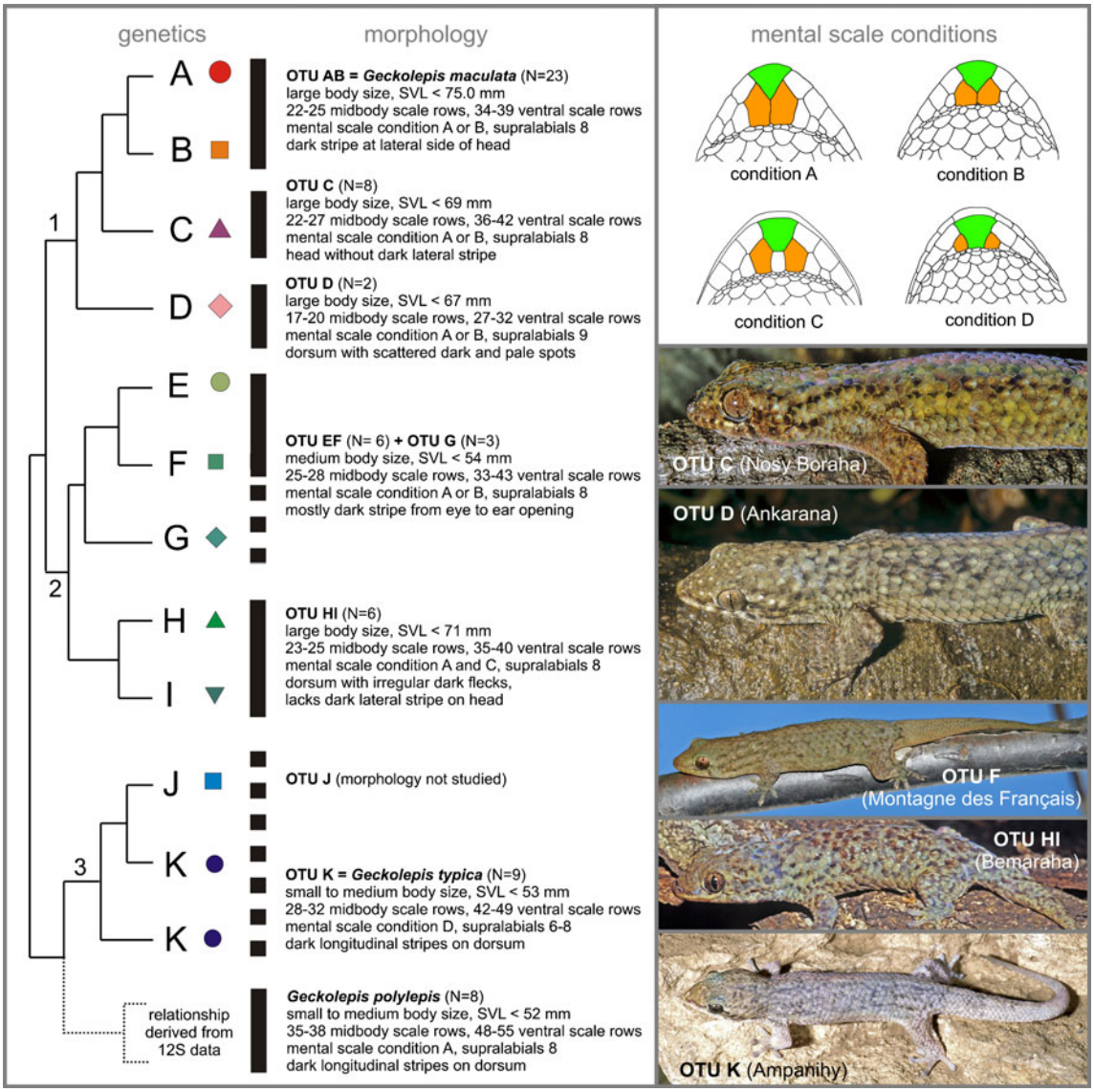


Fig. 2 *Left*: Simplified ND4 tree of *Geckolepis* showing major clades and lineages referred to as OTUs as shown in Fig. 1 (relationships of *G. polylepis* are derived from analyses of the 12S gene sequences). Major diagnostic morphological differences of each (or combined) OTU are summarized at the right of each respective clade (considers only adult specimens with intact integument). *Top right*: Schematic drawings of throat scalation with indication of different mental scale conditions (*green*=mental scale; *orange*=postmental scales). *Bottom right*: *Geckolepis* individuals in life, exemplifying certain OTUs

relatives was 1.8–14.7 % uncorrected pairwise distance (p-distance), while average within-OTU distances ranged from 0.2–4.0 %.

◀ **Fig. 1** Molecular variation of fish-scale geckos, *Geckolepis*, in DNA sequences of four gene fragments ND4, 12S (mitochondrial) and RAG1 and CMOS (nuclear). Based on the ND4 tree, 11 operational taxonomic units are defined (A–K) and haplotypes of CMOS and RAG1 are colored accordingly. Maps show the distribution of the various OTUs. Note that one locality (Nosy Boraha) for OTU C is based on additional evidence from the 12S rRNA gene shown at the lower left. Uppermost branch in 12S tree refers to divergent specimen FGZC 1140 (not included in ND4 tree). The ND4 tree is a 50 % majority-rule consensus tree from a Bayesian inference analysis. Posterior probabilities are coded by asterisks: ** 0.99–1.0, * 0.95–0.98

The complementary analyses of the 12S gene sequences generally produced the same tree topologies as those of the ND4 gene, but included several samples not included in the ND4, CMOS and RAG1 analyses (Appendix 2). These additional samples mainly originate from specimens morphologically referable to *G. polylepis*, forming a fourth major clade being sister to clade 3 comprising OTUs J and K.

The two nuclear genes CMOS and RAG1 showed much less variation than the mitochondrial gene ND4, but in general were congruent with ND4 regarding the major groupings suggested. The haplotype networks (Fig. 1) indicate that no nuclear haplotype sharing occurs between

individuals assigned to the three major mitochondrial clades 1-3. The most shallowly differentiated OTUs, in contrast, had no or little differentiation in the nuclear genes: extensive haplotype sharing was found between the OTU pairs A and B, E and F, and H and I, respectively. These pairs had ND4 p-distances of only 1.8 %, 4.8 %, and 5.1 %, respectively. On the contrary, several of the OTUs with a stronger mtDNA differentiation also had exclusive haplotypes in one of the two nuclear genes, i.e., OTUs K and J in RAG1 (ND4 p-distance 7.6 %), and D and G in CMOS (minimum ND4 distance to other OTUs 10.1 % and 6.3 %, respectively). Specimens of the mtDNA lineage comprising the sister OTUs H and I did not share haplotypes with any other OTU in either nuclear gene.

Morphological analyses

Morphological examinations indicate that many characters studied seem to be highly variable, even within genetically well-supported clades. In conclusion, the majority of morphological characters are obviously not suited to either support or reject a certain clade or OTU. For this reason, we here refrain from presenting data of highly variable characters, but instead summarize only those morphological characters that seem to be suited to diagnose an OTU as revealed by the molecular analyses. However, to do so, we here fuse some of the sister clades to combined OTUs, which in combination very probably represent distinct taxonomic units and not only intraspecific genetic differentiation. In the following, we summarize the diagnostic morphological differences for each provisional OTU or, respectively, for clades of two or several OTUs where appropriate, as shown in Fig. 2.

OTU AB. — According to morphological comparisons with the type specimen of *Geckolepis maculata* (ZMB 9655) and its surmised type locality in northwestern Madagascar, we here tentatively consider this OTU to represent *G. maculata*. According to the material examined by us, this species (when defined as corresponding to OTU AB) is characterized by a large body size (SVL <75 mm), 22–25 scale rows around the midbody, 34–39 ventral scale rows, and mental scale condition A or B. The dorsal color usually consists of grey to beige with some darker markings, flecks or irregular transversal striping, extending to the tail. Most specimens show a dark streak at the lateral side of the head. *Geckolepis maculata* is morphologically very similar to and possibly conspecific with OTU C, its sister clade that contains members distributed along the east coast. It mainly differs from OTU C by faint differences in coloration, namely a dark streak at the lateral side of the head, missing in OTU C. *G. maculata* differs from OTU D from Ankarana by a higher number of ventral scale rows (34–39 vs. 27–32), a higher number of scale rows around the midbody (22–25 vs. 17–20) and a lower number of supralabials (8 vs. 9). *G. maculata* differs from OTU EFG by a larger body size (SVL <75 vs. <54 mm) and a lower number of midbody scale rows (22–25 vs. 25–28). It differs furthermore from OTU HI by a more slender body (robust and chunky in OTU HI) and a more clearly marked head-body transition. Some individuals of OTU HI show the mental scale condition C, not occurring in *G. maculata*. OTU K, here tentatively referred to as *Geckolepis typica*, differs from *G. maculata* by a smaller size (SVL <53 vs. <75 mm), a higher number of scales around the midbody (28–32 vs. 22–25) and mental scale condition D (vs. A or B). From *G. polylepis*, *G. maculata* mainly differs by a lower number of scales around the midbody (22–25 vs. 35–38), a lower number of ventral scale rows (34–39 vs. 48–55) and the lack of a dorsal pattern consisting of longitudinal parallel dark lines.

OTU C. — This OTU differs from OTU D from Ankarana by a higher number of ventral scale rows (36–42 vs. 27–32), a higher number of scale rows around the midbody (22–27 vs. 17–20) and a lower number of supralabials (8 vs. 9). OTU C differs from OTU EFG by a larger body size (SVL <69 vs. <54 mm). It differs from OTU HI by a more slender body (robust and chunky in OTU HI) and a more clearly marked head-body transition. Some individuals of OTU HI show the mental scale condition C, not occurring in OTU C. *Geckolepis typica* (OTU K) differs from OTU C by a smaller size (SVL <53 vs. <69 mm), a higher number of scales around the midbody (28–32 vs. 22–27) and by mental scale condition D (vs. A or B). From *G. polylepis*, OTU C mainly differs by a lower number of scales around the midbody (22–27 vs. 35–38), a lower number of ventral scale rows (36–42 vs. 48–55) and the lack of a dorsal pattern consisting of longitudinal parallel dark lines.

OTU D. — This OTU occurring at Ankarana differs from all other OTUs by the lower number of scale rows around the midbody (17–20 vs. 22–38 in all other OTUs) and a lower number of ventral scale rows (27–32 vs. 33–55 in all other OTUs). It furthermore differs from OTU EFG, *G. typica* and *G. polylepis* by a larger body size (SVL <67 vs. <54 mm).

OTU EFG. — This OTU differs from OTU HI by a smaller body size (SVL <54 vs. <71 mm) and a slightly lower number of scale rows around the midbody (23–25 vs. 25–28). It differs from *G. typica* by mental scale condition A or B (vs. D) and a dorsal color pattern. OTU EFG differs from *G. polylepis* by a lower number of scales around the midbody (25–28 vs. 35–38), a lower number of ventral scale rows (33–43 vs. 48–55) and in dorsal color pattern.

OTU HI. — This OTU from Bemaraha and Ankarafantsika differs from *G. typica* (OTU K) and *G. polylepis* by a larger body size (SVL <71 vs. <53 and <52 mm), a lower number of scale rows around the midbody (23–25 vs. 28–32 and 35–38) and a lower number of ventral scale rows (35–

40 vs. 42–49 and 48–55). Furthermore, it differs from *G. typica* by exhibiting mental scale condition A or C (vs. D). The unusual mental scale condition C is present in ZSM 50/2006 and 141/2006.

Geckolepis typica (OTU K) differs from *G. polylepis* by a lower number of scale rows around the midbody (28–32 vs. 35–38), a lower number of ventral scale rows (42–49 vs. 48–55) and mental scale condition D (vs. A). *Geckolepis polylepis* differs from all studied OTUs by the greatest number of midbody and ventral scale rows.

Discussion

The data presented herein suggest a highly complex pattern of *Geckolepis* taxonomy. Besides *G. polylepis*, a species that is well differentiated by morphology and that was included only in the 12S analyses, our data suggest the presence of at least three species of *Geckolepis*, corresponding to the mitochondrial clades 1–3 (Fig. 1). The reality of non-conspecificity of specimens belonging to these three clades is put in evidence by syntopic occurrence without admixture of populations of clades 1 and 2, and by a complete and concordant lack of haplotype sharing in three independent molecular markers.

Fish-scale geckos assigned to the three major clades also appear to be specialized to different bioclimatic regions of Madagascar. We only found clade 3 in subarid habitats of southwestern and southern Madagascar where its sister lineage (*G. polylepis*) also occurs, as revealed by our analysis. Despite co-occurrence of representatives of clade 1 and 2 at some sites, the data suggest that also the populations assigned to these two clades may have different ecological requirements. In fact, most collection localities of clade 1 are characterized by a tropical humid climate, whereas specimens of clade 2 occur at more arid sites. The two sites of syntopic occurrence, Ankarana and Montagne des Français, are also relatively arid but close to other, humid sites, suggesting that they are peripheral for the clade 1 specimens.

However, our data contain multiple though not fully conclusive indications for the existence of additional *Geckolepis* species. First, within our main clades, there are several subclades of substantial morphological and molecular differentiation that might deserve species status (Fig. 1). This concerns, for instance, OTU D from Ankarana in clade 1, which has in ND4 10.1 % minimum p-distance to all other OTUs, does not share haplotypes with other lineages in CMOS and has lower scale counts than other clade 1 OTUs, despite occurring in very close spatial proximity to OTUs A–B. Within clade 2, a similar situation concerns the subclade made up by OTUs H and I. Taken together, all specimens of this subclade are restricted to western Madagascar, have a minimum ND4 divergence of 11.5 % to all other OTUs, do not share haplotypes with any other OTU in CMOS and RAG1, and reach larger body sizes than other clade 2 OTUs.

When combining the molecular and morphological results (Fig. 2), we have good reason to tentatively allocate OTUs A and B to the name *Geckolepis maculata* and OTU K to *G. typica*. Furthermore, *G. polylepis* is unique in its morphological characters and is supported as a highly divergent clade by 12S sequence data. Considering the remaining divergent lineages that show some diagnostic morphological characters, we here may further classify three lineages as Confirmed Candidate Species (OTU D, OTU EFG and OTU HI) probably deserving formal species status. Some other divergent lineages (e.g., represented by FGZC 1140, which forms the uppermost branch in the 12S tree of Fig. 1) may likely turn out to represent independent evolutionary lineages too, whereas others here are tentatively considered to represent Deep Conspecific Lineages.

In summary, the present contribution can only be a first step towards a more complete taxonomic resolution in the genus *Geckolepis*. It demonstrates, however, that the evolutionary history in the genus is far more complex than previously thought and that the species diversity in the genus has been underestimated by studies considering morphology only. As a consequence, current *Geckolepis* taxonomy, proposing three valid species only (Köhler et al. 2009), does not adequately reflect their actual diversity.

Additional sampling is required to fill geographical sampling gaps, e.g., in northeastern and western Madagascar, especially in potential contact zones of mitochondrial lineages that so far are known to occur in allopatry only, and to include type localities of available names currently considered to be synonyms (e.g., Andranovaho, the type locality of *G. petiti*). With more comprehensive data sets at hand, further differentiation within *Geckolepis* is likely to become evident, and we speculate that eventually the total number of recognized *Geckolepis* species may rise to around ten. But the forthcoming taxonomic revisions should be carried out carefully and integrate multiple data sets to make sure they correctly reflect independent evolutionary lineages within these fascinating geckos.

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Appendix 1

List of *Geckolepis* voucher specimens examined morphologically for this study. Order of listing follows the allocation to OTUs as mentioned in the text above. Field numbers in parentheses, as used in Fig. 1.

OTU AB (= *G. maculata*): ZSM 1521/2008 (FGZC 1925), Ampombofofo; ZSM 1520/2008 (FGZC 1697), ZSM 2164/2007 (FGZC 1232), Forêt d'Ambre; ZSM 523/2000 (FGMV 2000.275), ZSM 998/2003 (FGMV 2002.3009), Montagne des Français; ZMB 9655 (holotype of *G. maculata*), "Anfica"; SMF 9202-9204, SMF 9206–9207, SMF 57180, ZFMK 51814-815, ZSM 19/1921, ZSM 434/2000 (FGMV 2000.223), Nosy Be; ZFMK 61566–571, Nosy Mitsio; ZSM 435/2000 (FGMV 2000.833), Nosy Sakatia.

OTU C: ZSM 210/2002 (MV2001.1460), Andranofotsy; ZSM 143/2005 (FGZC 2633), Ste. Luce; SMF 9213, Ile aux Prunes; SMF 73582, ZFMK 47259–260, ZFMK 48157, ZFMK 51816, Nosy Boraha.

OTU D: ZSM 289/2004 (FGZC 554), ZSM 2126/2007 (FGZC 1144), Ankarana.

OTU EFG: ZSM 765–766/2003 (FGMV 2002.569-570), ZSM 780/2003 (FGMV 2002.597), ZSM 1522/2008 (FGZC 1605), Ankarana; ZSM 2138/2007 (FGZC 1176), Forêt de Orangea (east of Ramena); ZSM 2202–2203/2007 (FGZC 1309, 1311), Nosy Hara; ZSM 522/2000 (FGMV 2000.273), ZSM 998/2003 (FGMV 2002.3009), ZSM 2112/2007 (FGZC 1110), ZSM 1523/2008 (FGZC 1971), Montagne des Français.

OTU HI: ZSM 489/2001 (MV 2001.357), Ankarafantsika; ZSM 49–50/2006 (FGZC 773, 777), ZSM 104/2006 (FGZC 882), ZSM 110/2006 (FGZC 891), ZSM 141/2006 (FGZC 949), Tsingy de Bemaraha.

OTU K (= *G. typica*): SMF 22635, Amboasary, SMF 57182–183, Ambovombé; SMF 80772, Fort Dauphin; SMF 9222, Andranohinaly, ZSM 36/2004 (FGZC 57), Ampanihy; ZSM 582/2000 (FGMV 2000.565), Andatabo; ZSM 581/2000 (FGMV 2000.591), La Mangrove; ZSM 142/2005 (FGZC 2552), Esomony.

Geckolepis polylepis: SMF 9217 (lectotype), SMF 9218–9121 (paralectotypes), ZSM 309/0 (paralectotype), Mahajanga; ZSM 840/2012 (ZCMV 12753), ZSM 1605/2010 (ZCMV 12752), Kirindy.

Appendix 2

Additional specimens of *Geckolepis* sequenced for the mitochondrial 12S gene only and assigned by 12S data to the OTUs represented in Fig. 1 (field numbers in parentheses).

OTU A: UADBA (FGZC 3122), Forêt d'Ambre; UADBA (FGZC 1939) Montagne des Français. **OTU C:** UADBA (FGMV 2002.A23), Andranofotsy; ZSM 318/2006 (ZCMV 3385), Nosy Boraha. **OTU F:** ZSM 2112/2007 (FGZC 1110), UADBA (FGZC 1111, 1970), ZSM 912/2003 (FGMV 2002.939), UADBA (FGMV 2002.938), Montagne des Français. **OTU G:** ZSM 780/2003 (FGMV 2002.597), Ankarana. **OTU H:** ZSM 49–50/2006 (FGZC 773, 777), Tsingy de Bemaraha. **OTU I:** ZSM 141/2006 (FGZC 949) UADBA (FGZC 892), Tsingy de Bemaraha. **OTU J:** UADBA (FGMV 2002.1525), near Toliara. ***Geckolepis polylepis***: ZSM 1605/2010 (ZCMV 12752), UADBA (ZCMV 12711, 12730, 12748), Kirindy.

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