

## Reverse taxonomy of fish-scale geckos (*Geckolepis*) using shotgun sequencing for museomics-based assignment of historical names to genetic lineages

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### Abstract

Fish-scale geckos (*Geckolepis*), endemic to Madagascar and the Comoro Islands, are nocturnal, arboreal lizards with large, imbricate scales that can be shed and regenerated in their entirety, most likely as an antipredator mechanism. Their species-level taxonomy has remained partly unresolved because morphology alone has proven insufficient to reliably assign genetic lineages to scientific names. Here, we use a museomics approach to extract and shotgun-sequence DNA from twelve liquid-preserved historical and mostly name-bearing type specimens of six historical *nomina* in the genus, and assemble sequences of the mitochondrial genes for 12S rRNA, 16S rRNA, and *nd4* for ten of these specimens. Despite relatively low coverage, the assembled sequences robustly placed all these type specimens in a phylogenetic tree with 113 ingroup samples collected across Madagascar. Our results demonstrate the efficiency of this approach to recover mitochondrial evidence for taxonomic purposes, but also show its failure to recover reads matching single-copy nuclear genes. We discuss the promise of a renewed focus on nuclear ribosomal genes as complementary nuclear-encoded DNA barcodes for vertebrates, as we found 18S rRNA

sequences to be well-represented among the reads of the historical specimens studied here. Based on evidence from the mitochondrial tree, two nuclear-encoded genes (*C-MOS*, *RAG-1*), and morphology, we resurrect *G. typica* var. *modesta* Methuen & Hewitt, 1913 as a full species, *G. modesta*, from north-western Madagascar, confirm the current use of the name *G. maculata* Peters, 1880 for specimens from northern and eastern Madagascar, of *G. polylepis* Boettger, 1893 for specimens from western and north-western Madagascar, and of *G. typica* Grandidier, 1867 for specimens from southern and south-western Madagascar. We also recognize *G. typica* var. *anomala* Mocquard, 1902 as a subspecies, *G. typica anomala*, and confirm *G. petiti* Angel, 1942 to be a synonym of this subspecies. We also describe two new species, *G. bekira* sp. nov. and *G. spania* sp. nov. from northern Madagascar. The new classification provides a robust baseline to identify the remaining taxonomic issues in the genus and tackle them, preferably using genomic approaches which are also promising to better understand the molecular mechanisms of scarless wound healing and skin regeneration in these geckos.

**Key words:** Squamata, Gekkonidae, *Geckolepis spania* sp. nov., *Geckolepis bekira* sp. nov., Madagascar, museomics, archival DNA.

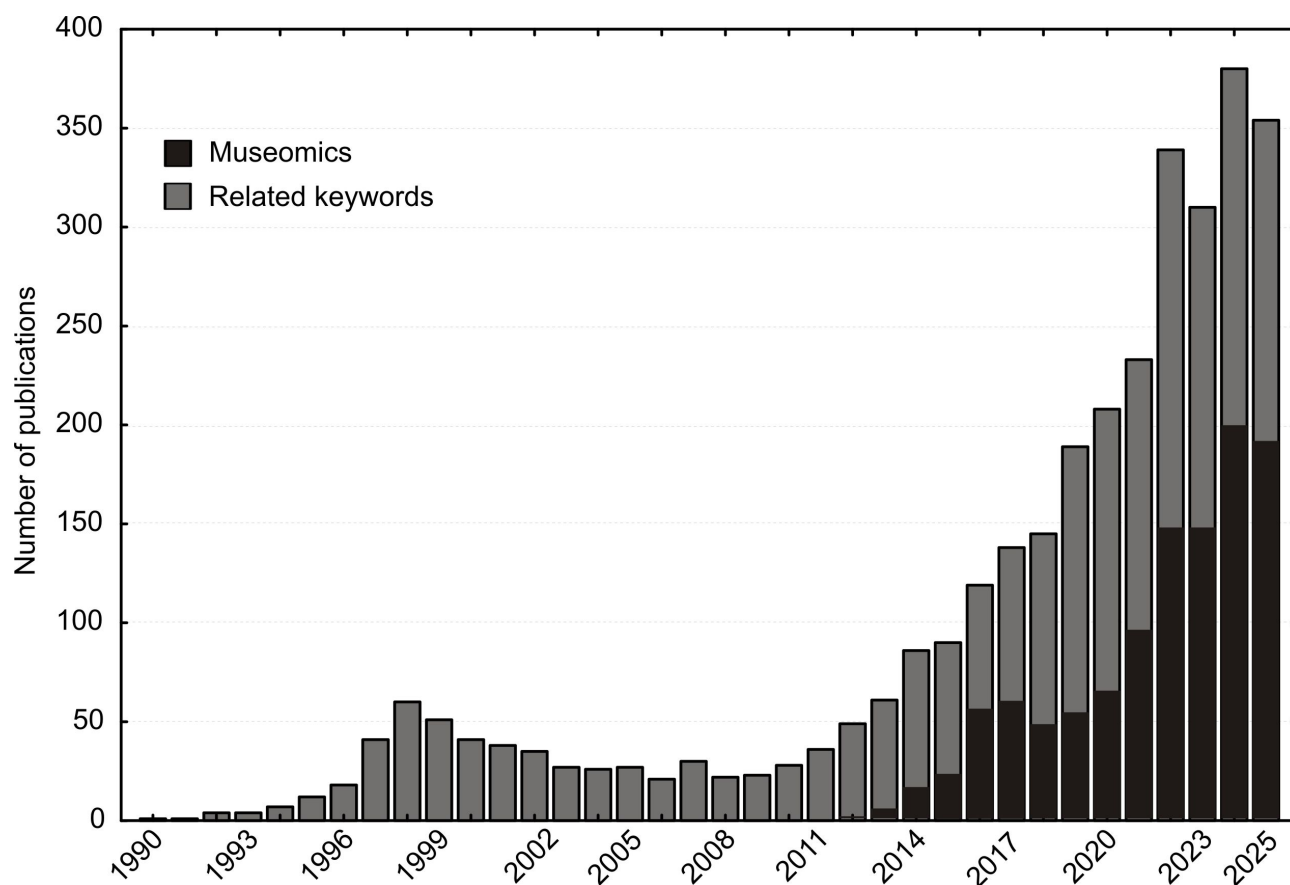
## Introduction

Over the past two decades, the increasing availability of molecular data has profoundly advanced biological taxonomy, providing powerful tools for resolving cryptic species diversity, reconstructing evolutionary relationships, and testing hypotheses on species delimitation and biogeography. However, in many cases, progress in our understanding of genetically delimited species and species-level classification is impeded by nomenclatural obstacles (Scherz *et al.* 2017, 2020, 2021)—specifically, the inability to match historical name-bearing type specimens, and thus scientific nomina, with genetic lineages. This difficulty often arises from the absence of reliable external diagnostic characters and, in some cases, from ambiguous type locality data (Scherz *et al.* 2017), compounded by the inaccessibility of these types to PCR-based Sanger sequencing due to the high degree of DNA fragmentation in liquid-preserved specimens (Straube *et al.* 2021).

To address such impediments, museomics—the

application of high-throughput sequencing techniques to natural history collections—has rapidly emerged as a transformative tool for taxonomy (Lalueza-Fox 2022; Kapun *et al.* 2025). As of 20 October 2025, a total of 3258 papers with museomics-related keywords were included in the Scopus database, with a rapid increase in the number of publications per year after 2010 and a maximum of 380 papers in 2024 (Fig. 1). Since the term was coined by S. C. Schuster and W. Miller in 2008–2009 (see Fong *et al.* 2023) in the context of their work on mammoth museum-specimens, and first used as a keyword in a Scopus-indexed paper in 2009 (Schaefer *et al.* 2009; registered in Scopus as 2012), the use of this term and approach has become widespread. Our search returned 1112 papers referring to museomics, with 200 mentions in 2024 alone (Fig. 1).

Advances in non-PCR based ancient DNA methods (Orlando *et al.* 2021; Hofreiter *et al.* 2021), along with new DNA extraction protocols (Straube *et al.* 2021; Hahn *et al.* 2024), now enable the recovery of genomic data from century-old museum specimens, offering unprecedented opportunities to resolve long-standing taxonomic and nomenclatural questions (Bi *et al.* 2013; Holmes *et al.* 2016). Especially in amphibians and reptiles, where specimens are usually liquid-preserved, often after fixation in formalin, museomics has proven highly effective in



**FIGURE 1.** Number of papers found in the Scopus database by searches for museomics-related keywords (“museomics”, “museum genomics”, “collection-based genomics”, “herbarium genomics”, “archival DNA”, “historic(al) DNA”, “herbarium DNA”, “barcode fishing”), plotted by years of publication. Black partial columns show the proportion of papers containing the term “museomics”. Note that data for 2025 are incomplete as searches were carried out on 20 October 2025

clarifying the identity of name-bearing types and other specimens previously inaccessible to molecular study (Staats *et al.* 2013; Ruane & Austin 2017). Numerous recent studies have demonstrated that even highly degraded DNA can be used to place historical specimens into robust phylogenetic frameworks, thereby stabilizing nomenclature and guiding conservation priorities (e.g., McGuire *et al.* 2018; Rowe *et al.* 2020; Rancilhac *et al.* 2020; Scherz *et al.* 2020, 2022). Museomics thus holds great promise for resolving long-standing ambiguities in herpetological taxonomy.

Although museomics allows one to obtain genome-scale datasets from historical specimens for instance via targeted enrichment followed by high-throughput sequencing (Bi *et al.* 2013; Staats *et al.* 2013; Ruane & Austin 2017; Rancilhac *et al.* 2020; Scherz *et al.* 2020), such approaches are not always necessary. When specimens only need to be matched to genetic lineages already identified from recent field collections, more cost-effective strategies focusing on short sequences of single DNA barcode markers can be sufficient (typically mitochondrial genes such as *cox1*, *cytb* or 16S rRNA in vertebrates; e.g., Stuart & Fritz 2008; Mutanen *et al.* 2015; Prosser *et al.* 2016). Targeted enrichment of these loci, called “DNA barcode fishing” (Scherz *et al.* 2020) has been proven to be very efficient (e.g., Rancilhac *et al.* 2020; Vences *et al.* 2021; Scherz *et al.* 2020, 2022), but so too has extraction of mitochondrial markers from shallow shotgun sequencing approaches (e.g. Hoban *et al.* 2022, Rakotoarison *et al.* 2023).

Fish-scale geckos of the genus *Geckolepis* are a remarkable component of Madagascar’s diverse gecko fauna. These nocturnal lizards are characterized by their large, imbricate scales, which can be shed in their entirety (along with the underlying epidermis) when grasped by a predator, leaving exposed the underlying dermis that subsequently regenerates rapidly and without scarring (Schubert & Christophers 1985; Schubert *et al.* 1990). This dermolytic defense mechanism, first described by Schmidt (1911), in this extreme form is unique among geckos and has long fascinated herpetologists, both for its adaptive value in predator escape and its implications for understanding integumentary regeneration in reptiles (Paluh *et al.* 2017; Scherz *et al.* 2017).

While of high biological interest due to its regeneration capacities, the unique structure of *Geckolepis* scales and skin (see also Paluh *et al.* 2017), as well as the dermolytic autotomy of these geckos constitute substantial impediments to resolving their taxonomy (Scherz *et al.* 2017). Many characters of pholidosis commonly used for morphological diagnosis of gecko species cannot easily be scored in fish-scale geckos, due to the irregular scale arrangements with injured or regenerated areas in many specimens (Köhler *et al.* 2009). Even micro-CT-based osteological analysis has failed to yield particularly useful diagnostic features (Hawlitshchek *et al.* 2016; Scherz *et al.* 2017). A total of eight species-level taxa have been named in *Geckolepis*, of which five are currently considered valid (Uetz *et al.* 2026). A morphological revision recognized three species only (Köhler *et al.* 2009), but

a subsequent molecular survey (Lemme *et al.* 2013) identified up to 12 operational taxonomic units (OTUs) in *Geckolepis*, herein referred to as mitochondrial lineages, which could only partly be reconciled with the available names. Subsequent studies resurrected one species from synonymy (Hawlitshchek *et al.* 2016) and named another, previously morphologically identified and genetically divergent lineage (Scherz *et al.* 2017), adding two distinct fish-scale gecko species. However, the overall taxonomy of the genus and the precise definition and distribution range of species such as *G. maculata* and *G. typica* remained largely unclarified—mainly due to the inability to match historical name-bearing type specimens with genetic lineages, making this genus an ideal model to evaluate the efficiency of comprehensive museomics approaches for taxonomic revision.

Here, we undertake a comprehensive “reverse taxonomy” (Markmann & Tautz 2005) of the genus *Geckolepis*. We extend the dataset of Lemme *et al.* (2013) by combining DNA sequences of three mitochondrial gene fragments, adding sequences of numerous additional samples, and delimit species-level mitochondrial lineages and their geographic distribution from this molecular dataset. We then assign scientific names to these lineages, based on DNA sequences of type specimens of almost all *nomina* in the genus, obtained using shotgun sequencing, and formally name and describe two new species for which no names are available. We use this case study to discuss future directions of cost-effective museomics approaches, such as shotgun sequencing rather than targeted enrichment, for addressing nomenclatural challenges and accelerating taxonomic progress.

## Materials and Methods

### Field work and sampling

Specimens and samples of *Geckolepis* used in this study originated from field collections undertaken in the period from 2000–2025 in various regions of Madagascar. Specimens were collected during opportunistic searches in their habitat, either during the night with the aid of flashlights on the trunks of trees and bushes, or during the day under the bark of dead trees or in tree holes. Representative voucher specimens were anesthetized by intra-coelomic (IC) injection of 250–500 mg/kg pH-neutralized 1% MS-222 or ketamine solution, and subsequently euthanized by injection of 50% unbuffered MS-222 (Conroy *et al.* 2009) or ketamine overdose. Tissue samples (usually leg or tongue muscle, or sometimes liver) were excised and preserved separately in cryovials pre-filled with pure ethanol, ahead of specimen fixation in 90–98% ethanol followed by long-term preservation in 70% ethanol. For several specimens collected after 2020, only tissue samples in the form of a few shed scales were taken in situ and preserved in pure ethanol, and specimens then released at the site of capture.

Voucher specimens were deposited in the collections of the Zoologische Staatssammlung München, Germany (ZSM) and the Université d’Antananarivo, Mention



Zoologie et Biodiversité Animale, Antananarivo, Madagascar (UADBA). Additional specimens from the Ditsong National Museum of Natural History (DNMNH), formerly Transvaal Museum, Pretoria, South Africa; the Museum National d'Histoire Naturelle, Paris, France (MNHN); the Senckenberg Naturmuseum, Frankfurt, Germany (SMF); the Museum für Naturkunde, Berlin, Germany (ZMB); and the Natural History Museum, London, UK (BMNH, now NHMUK) were studied as well; for the latter, we here give numbers (of historical types only) under the BMNH acronym to ensure consistency with previous studies. Field numbers of preserved specimens and tissue samples examined and sequenced herein refer to the collections of A. Crottini (ACZC / ACP), M.D. Scherz (MSZC), F. Glaw (FGMV, FGZC) and M. Vences (FGMV, ZCMV, MVTIS), D.R. Vieites (DRV), and A. Miralles (MIRZC). For acronyms of sequences downloaded from GenBank, see the original GenBank records and associated publications.

For this study, we rely largely on previously published morphometric data (Köhler *et al.* 2009; Lemme *et al.* 2013; Hawlitschek *et al.* 2016; Scherz *et al.* 2017), and make reference to the rostral and mental scale conditions/types of Köhler *et al.* (2009) and Lemme *et al.* (2013).

#### Molecular datasets

For this study, we mainly rely on a molecular dataset consisting of DNA sequences of three concatenated mitochondrial gene fragments: NADH dehydrogenase subunit 4 (*nd4*), and 12S rRNA and 16S rRNA (*12S*, *16S*). Sequences of freshly collected samples were obtained by Sanger sequencing after PCR, and combined with sequences downloaded from GenBank, mainly from the papers by Greenbaum *et al.* (2007), Lemme *et al.* (2013), Hawlitschek *et al.* (2016); Scherz *et al.* (2017), Mezzasalma *et al.* (2018), and Fulgence *et al.* (2022). The dataset was complemented by sequences derived from several historical type specimens obtained by a museomics approach. In addition, we used a dataset of *nd4* (available for the largest number of samples), after excluding sequences with missing data and with all sequences trimmed to similar lengths, for analysis with ASAP (Puillandre *et al.* 2021) and calculation of diagnostic sites with MolD (Fedosov *et al.* 2022); a set of only 16S sequences trimmed to similar lengths, for the calculation of genetic distances; and a set of DNA sequences of a fragment of the nuclear-encoded gene for oocyte maturation factor *mos* (*C-MOS*) and the recombination-activating gene 1 (*RAG-1*) for assessment of concordant mitochondrial and nuclear differentiation. All alignments are available from Zenodo (<https://doi.org/10.5281/zenodo.21984699>). Detailed information on the respective laboratory protocols and analytical methods is given in the following sections.

#### Sanger sequencing

We extracted genomic DNA and amplified the gene fragments using standard protocols as described in Lemme *et al.* (2013) and Vences *et al.* (2025), using the primers ND4 (5'-CACCTATGACTACCAAAGCTCATGTAGA-3')

and LeutRNA (5'-AGCCATTACTTTTACTTGGATT TGCACC-3') (originally from Arevalo *et al.* 1994) for *nd4*; 16Sar-L (5'-AAACTGGGATTAGATACCCCACTAT -3') and 16Sbr-H (5'-CCGGTCTGAACTCAGATCACGT-3'), from Kocher *et al.* (1989) for *12S*; 16Sar-L (5'-CGCCTGTTTATCAAAAACAT-3') and 16SBH-new (5'-CCTGGATTACTCCGGTCTGA-3'), modified from Palumbi *et al.* (1991) for *16S*; primers UropRAG1-F1 (5'-GAAAACCTGGAGCGGTATGA-3') and UropRAG1-R1 (5'-GCAACTCTGCAAACGTTGA-3') (Bauer *et al.* 2011) for *RAG-1*; and primers CO8 (5'-GCTTGGTGTTCAATAGACTGG-3') and CO9 (5'-TTGGGAGCATCCAAAGTCTC-3') for *C-MOS* (Han *et al.* 2004). We purified PCR products with ExoSAPIT (Thermo Fisher Scientific, Waltham, MA, USA) and sent them for sequencing on an automated capillary DNA sequencer at Microsynth Seqlab GmbH (Göttingen, Germany). The software CodonCode Aligner version v3.7.1 (CodonCode Corporation) was used to check and correct errors in the DNA sequences based on the chromatograms, and to trim poor-quality stretches and primer sequences from the beginning and end of the sequences. We verified the absence of stop codons in the protein-coding gene fragments (*nd4*, *C-MOS*). New sequences obtained in the present study were submitted to GenBank (accession numbers PZ867833–PZ867911, PZ872761–PZ872781, PZ882254–PZ882259). A full table with all sequences used, accession numbers and metadata is available from Zenodo (<https://doi.org/10.5281/zenodo.21984699>).

#### Museomics

Name-bearing types of several *nomina* in the genus *Geckolepis* were sampled for DNA sequencing using a museomics (archival DNA high-throughput sequencing) approach. Specimens were sampled between 2023–2025 for a small piece of thigh muscle tissue after making a small incision and reflecting the ventral skin of the thigh. Sampling was done with sterile scalpels, scissors and tweezers, and the samples stored in a vial with pure ethanol. For the extraction of genomic DNA, each sample was weighed prior to incubation in a Guanidine Thiocyanate (GuSCN) based extraction buffer solution, adapted from Rohland *et al.* (2004), at 37°C overnight. The next day, the lysate was centrifuged, and processed following the consecutive steps as described in Straube *et al.* (2021), and DNA was eluted twice with TET buffer (10 mM Tris-HCl, 1 mM EDTA, 0.05% Tween-20) for a total of 25 µl DNA extract per sample. DNA yield was quantified using 1 µl DNA extract with the Qubit dsDNA HS Assay Kit 0.2–100 ng/µl (Life Technologies, Carlsbad, California, US) according to the instructions of the manufacturer. Subsequently, single-stranded library preparation was carried out following the protocol of Gansauge *et al.* (2017) according to the steps described in Straube *et al.* (2021). All lab work prior to qPCR was conducted in a dedicated DNA facility at the University of Potsdam, Germany, which meets all requirements to work with historical samples (see Fulton & Shapiro 2019) and extraction and library blanks were run alongside



all samples to check for contamination. Final library concentrations and fragment length distributions were assessed using a 2200 TapeStation (Agilent Technologies) assay. Sequence data was obtained through shotgun-sequencing of approximately five million 75-bp single-end reads (or one million reads for some samples) using an Illumina Nextseq 500/550 sequencing platform at the University of Potsdam, following the procedure described in Paijmans *et al.* (2017). The quality of the obtained reads was visualized twice using FastQC (<https://www.bioinformatics.babraham.ac.uk>), both before and after trimming of Illumina adapter sequences and discarding reads shorter than 30 bp with cutadapt v1.12 (Martin 2011).

We used local Blast (Blast+; Camacho *et al.* 2009) as implemented in BlasTax (part of iTaxoTools: Vences *et al.* 2021) on the raw reads against a library of *nd4*, *12S* and *16S* sequences of all *Geckolepis* mitochondrial lineages and species and collected the matching reads in FASTA files. For comparative purposes, we also ran the same BLAST analysis against reference databases containing fragments of the *cox1* and *18S* genes for which reference sequences of one and seven *Geckolepis* lineages, respectively, were available, and of the nuclear-encoded *C-MOS* and *RAG-1* gene fragments. We then used CodonCode Aligner v 3.7.1 (CodonCode Corporation) to map the matching reads separately to reference sequences of various *Geckolepis* lineages (option: “align to reference”), verified that the resulting assemblies were congruent (thus, no reference bias was introduced), and used the most complete consensus sequences obtained as representing the respective type specimens, with missing sections in-between partial contigs coded by the letter “N”.

#### Phylogenetics and species delimitation

Sequences of the three mitochondrial gene fragments (*nd4*, *12S*, *16S*), including sequences of these gene fragments obtained from the name-bearing types using the museomics approach, were concatenated after MAFFT alignment (Kato & Standley 2013) with the G-INS-i option as implemented in Concatenator (Vences *et al.* 2022b). Total alignment length was 1847 bp, of which 866 bp corresponded to *nd4*, 435 bp to *12S*, and 546 bp to *16S* (however, many sequences had shorter lengths for the respective fragments).

The concatenated alignment was used to infer a Maximum Likelihood tree using IQ-Tree v2.4.0 (Minh *et al.* 2020), determining substitution models and data partitions using ModelFinder (Kalyaanamoorthy *et al.* 2017) and assessing bootstrap support with 2000 full non-parametric bootstrap replicates. We used a partitioning scheme by gene (not by codon) to avoid overparameterization given the relatively short length of the fragments used.

Uncorrected pairwise genetic distances were calculated from the trimmed *16S* alignment (488 bp for 40 sequences) using MEGA7 (Kumar *et al.* 2016). Detailed distance tables and treefiles are available from <https://doi.org/10.5281/zenodo.21984699>. We also used the trimmed

*nd4* alignment (519 bp for 76 sequences) for analysis with ASAP (Puillandre *et al.* 2021) and diagnostic nucleotide sites using MolD (Fedosov *et al.* 2022).

The relationships among alleles (haplotypes) of the nuclear-encoded gene fragments were illustrated as haplotype genealogies (networks). For this, we trimmed the alignments by removing regions of poor quality and sequences with missing data, and inferred alleles using the PHASE algorithm (Stephens *et al.* 2001) implemented in the software Hapsolutely (Vences *et al.* 2024b). Haplotype genealogies were then reconstructed by the Fitch tree algorithm (Salzburger *et al.* 2011; Matschiner 2015) using the same program.

To delimit new species, we broadly follow the general lineage concept (de Queiroz 1998, 2007). In the absence of data derived from hybrid zones or whole genomes for *Geckolepis*, we here apply a pragmatic approach that starts with using ASAP (Puillandre *et al.* 2021) on the trimmed *nd4* dataset to computationally determine a primary species partition (i.e., a series of primary species hypotheses that can then be confronted with other evidence). We then examine the *RAG-1* and *C-MOS* haplotype networks and morphological data from Lemme *et al.* (2013) to evaluate concordance with these primary hypotheses. Species status is then assigned to lineages based on congruence between genetic and morphological evidence (Padial *et al.* 2010). We follow recent suggestions of Hillis (2020), de Queiroz (2020), and Dufresnes *et al.* (2023) to use the subspecies category (and nomenclatural rank) for lineages that can be defined by genetic or phenotypic means but have not yet reached complete evolutionary independence.

For molecular diagnosis, we used the trimmed *nd4* alignment in MolD (Fedosov *et al.* 2022). Diagnostic sites are given relative to the full *nd4* sequence from a mitochondrial genome of *G. typica* determined by S. Miura and Y. Kumazawa (unpublished; GenBank accession AB661666).

#### Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: [lsid:zoobank.org:pub:FFE286CE-30AA-40F3-9A7A-ABBF041C23C7](https://zoobank.org/pub:FFE286CE-30AA-40F3-9A7A-ABBF041C23C7). The electronic edition of this work was published in a journal with an ISSN, has been archived and is available from the Biotaxa digital repository.

## Results

#### Recovery of mitochondrial fragments of historical types

Except for two specimens (the lectotype and one paralectotype of *G. polylepis*), we recovered reads matching the target mitochondrial fragments (*12S*, *16S* and *nd4*) for all historical types included in the

**TABLE 1.** Number of Illumina reads matching reference sequences of *Geckolepis* for the three mitochondrial target genes (*12S*, *16S*, *nd4*) and two nuclear-encoded genes (*C-MOS*, *RAG-1*). Numbers are numbers of unique reads compared by BLAST to a database containing sequences of equal length of all major mitochondrial lineages in *Geckolepis*. Abbreviations used: HT = holotype, ST = syntype, LT = lectotype, PLT = paralectotype.

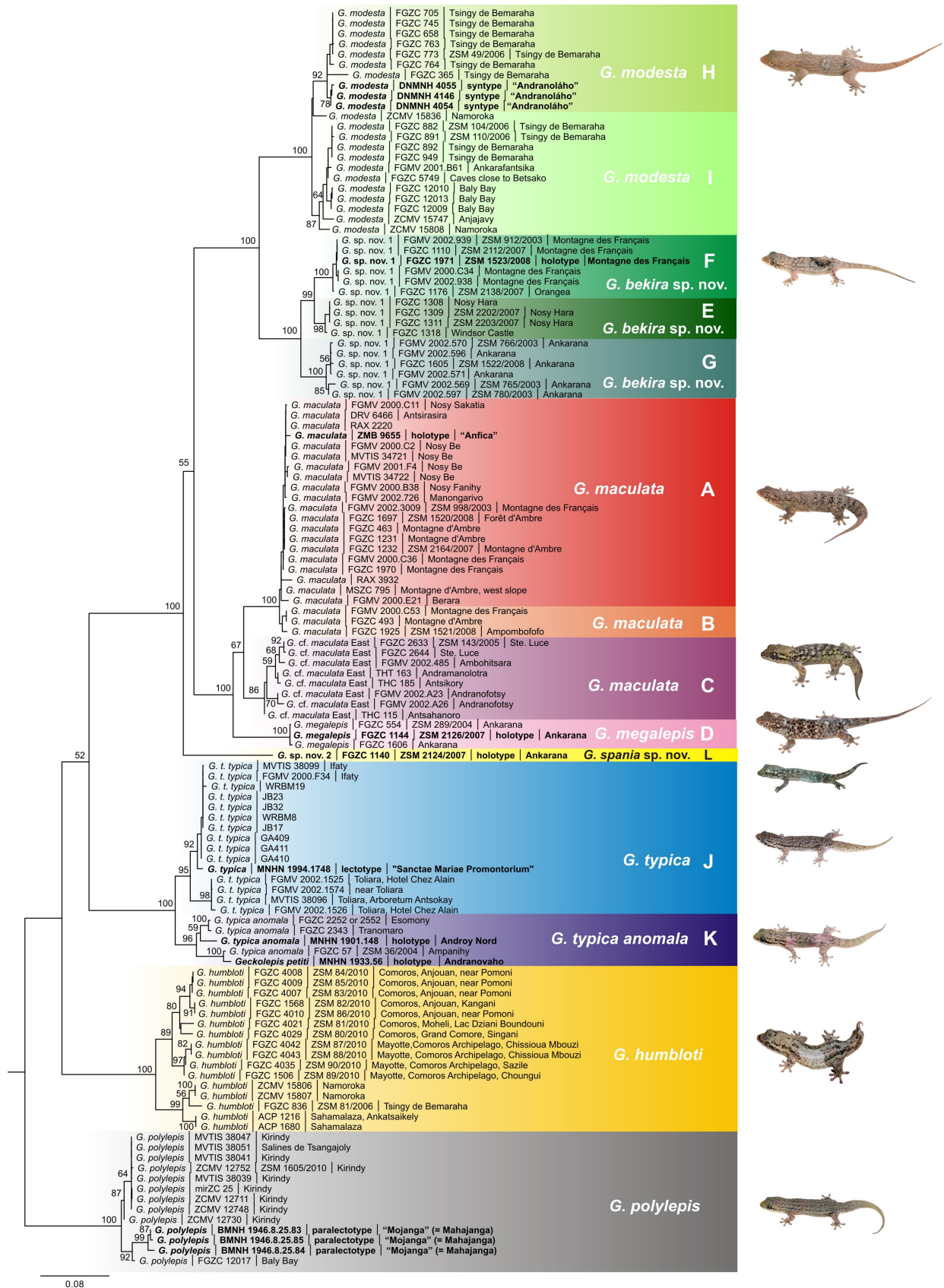
| Nomen                                                       | Catalogue number  | Status | Total reads | Reads <i>12S</i> | Reads <i>16S</i> | Reads <i>nd4</i> | Reads <i>C-MOS</i> | Reads <i>RAG-1</i> |
|-------------------------------------------------------------|-------------------|--------|-------------|------------------|------------------|------------------|--------------------|--------------------|
| <i>G. maculata</i> Peters, 1880                             | ZMB 9655          | HT     | 3802955     | 9                | 13               | 24               | 0                  | 0                  |
| <i>G. typica</i> var. <i>modesta</i> Methuen & Hewitt, 1913 | DNMNH 4054        | ST     | 3701258     | 10               | 20               | 21               | 0                  | 0                  |
| <i>G. typica</i> var. <i>modesta</i> Methuen & Hewitt, 1913 | DNMNH 4055        | ST     | 4599899     | 5                | 3                | 13               | 0                  | 0                  |
| <i>G. typica</i> var. <i>modesta</i> Methuen & Hewitt, 1913 | DNMNH 4146        | ST     | 3538918     | 34               | 47               | 32               | 0                  | 0                  |
| <i>G. petiti</i> Angel, 1942                                | MNHN 1933.56      | HT     | 4373555     | 5                | 1                | 4                | 0                  | 0                  |
| <i>G. polylepis</i> Boettger, 1893                          | BMNH 1946.8.25.82 | PLT    | 1042980     | 0                | 0                | 0                | 0                  | 0                  |
| <i>G. polylepis</i> Boettger, 1893                          | BMNH 1946.8.25.83 | PLT    | 1216609     | 68               | 99               | 139              | 0                  | 0                  |
| <i>G. polylepis</i> Boettger, 1893                          | BMNH 1946.8.25.84 | PLT    | 1506528     | 23               | 58               | 100              | 0                  | 1                  |
| <i>G. polylepis</i> Boettger, 1893                          | BMNH 1946.8.25.85 | PLT    | 1075663     | 44               | 80               | 80               | 0                  | 1                  |
| <i>G. polylepis</i> Boettger, 1893                          | SMF 9217          | LT     | 3817921     | 0                | 0                | 0                | 0                  | 0                  |
| <i>G. typica</i> Grandidier, 1867                           | MNHN 1994.1748    | LT     | 3871911     | 13               | 18               | 7                | 0                  | 0                  |
| <i>G. typica</i> var. <i>anomala</i> Mocquard, 1902         | MNHN 1901.148     | HT     | 4428089     | 16               | 12               | 20               | 0                  | 0                  |

museomics analysis (Table 1). Success rate ranged from a single read matching *16S* in the *G. petiti* holotype to 139 unique reads matching *nd4* in one *G. polylepis* paralectotype. There were no consistent differences in the number of reads found for the two rRNA genes (*12S* and *16S*) and the protein-coding gene (*nd4*): in some samples, BLAST searches for the rRNA genes were slightly more successful (e.g., 13 and 18 vs. 7 reads in the *G. typica* lectotype) while in many others, those for *nd4* were more successful (e.g., 100 vs. 23 and 58 in one *G. polylepis* paralectotype), and this remained true when standardizing the number of matching reads by reference fragment length (Table S1 available from <https://doi.org/10.5281/zenodo.21984699>). Practically no matches were found for the nuclear-encoded single-copy *C-MOS* and *RAG-1* fragments (single matches in two *G. polylepis* paralectotypes), while the nuclear-encoded 18S fragment consistently yielded matches in all samples, and often more than the mitochondrial genes (Table S1). Matches for the mitochondrial *cox1* gene were largely restricted to those samples for which conspecific reference sequences were available (i.e., only 0–3 matches for the holotype of *G. maculata* and syntypes of *G. typica* var. *modesta* for which no reference *cox1* sequence of the same genetic lineages were available; Table S1).

#### Molecular systematics of *Geckolepis*

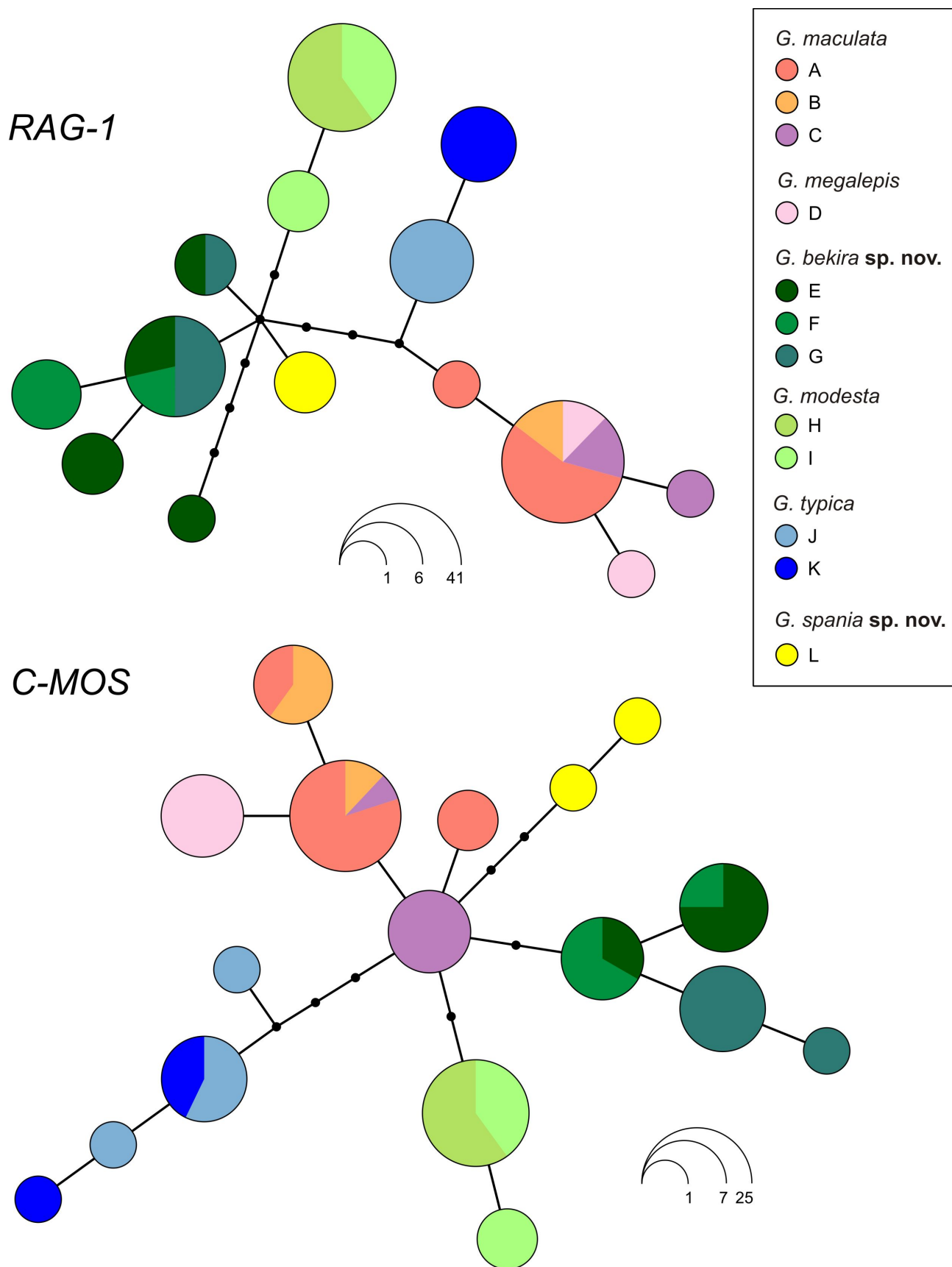
The maximum likelihood tree inferred from 1847 bp of three concatenated mitochondrial gene fragments (Fig. 2) largely agrees with previous molecular phylogenies (Lemme *et al.* 2013; Hawlitschek *et al.* 2016; Scherz *et al.* 2017) but due to its more complete geographic sampling improves the understanding of mitochondrial diversity and lineage distribution in the genus. The tree distinguishes four deep clades in the genus whose interrelationships are not or are very poorly supported by the bootstrap analysis (bootstrap values BS ≤ 52% between clades). These clades, each maximally supported (BS = 100%), correspond to (i) *G. polylepis* (PO), (ii) *G. humbloti* (HO), (iii) a set of samples from the South West of Madagascar corresponding to mitochondrial lineages J and K of Lemme *et al.* (2013); and (iv) a clade containing *G. maculata* (sensu Lemme *et al.* 2013), *G. megalepis*, and several other lineages whose identity has so far remained unclarified (lineages A through I of Lemme *et al.* 2013, plus a new lineage here named L). New phylogeographic results include the finding of *G. humbloti* in Namoroka. In addition, one sample from Ankarana (FGZC 1140) was confirmed as a strongly distinct lineage based on additional gene fragments and new sequences of the nuclear-encoded DNA fragments, where it differed from all other included species by at least two (*RAG-1*) and three (*C-MOS*) mutational steps (Fig. 3).

Two lineages defined by Lemme *et al.* (2013) based



**FIGURE 2.** Maximum likelihood tree showing evolutionary relationships in *Geckolepis*, inferred from 1847 bp DNA sequences of three concatenated mitochondrial gene fragments (*12S*, *16S*, *nd4*). Colors indicate mitochondrial lineages (OTUs) from Lemme *et al.* (2013) plus *G. polylepis*, *G. humbloti*, and *G. spania sp. nov.* (lineage L). Name-bearing type specimens (and paralectotypes of *G. polylepis*) are set in boldface. Numbers at nodes are bootstrap support values in percent (not shown for values <50% and at shallowest nodes). A sequence of *Blaesodactylus sakalava* was used as the outgroup (graphically removed from the tree to better show branch lengths within the ingroup).





**FIGURE 3.** Haplotype genealogies (networks) for *Geckolepis* inferred from two nuclear-encoded DNA fragments of the genes for *C-MOS* (409 bp, 50 individuals) and *RAG-1* (399 bp, 50 individuals), recalculated from the dataset of Lemme *et al.* (2013) with addition of some newly determined sequences. Note that sequences of *G. humbloti* and *G. polylepis* are missing from the network. Networks were calculated from phased sequences, i.e., each individual is represented twice in each network.

**TABLE 2.** Uncorrected pairwise distances (in percent) calculated from a 488 bp DNA fragment of the mitochondrial 16S rRNA gene for 40 sequences of *Geckolepis*. Values are means, with min–max in parentheses. A–K, PO and HU are abbreviations for mitochondrial lineages, expanded from Lemme *et al.* (2013). Intra-lineage divergences are marked with gray shade. Reddish shade marks distances between lineages that we here consider as conspecific.

|                             | A                   | B                   | C                   | D                   | E                   | F                   | G                   | H                   | I                   | L                   | HU                  | PO                  | K                | J                |
|-----------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|------------------|------------------|
| <i>G. maculata</i> A        | 1.0<br>(0.0–1.9)    |                     |                     |                     |                     |                     |                     |                     |                     |                     |                     |                     |                  |                  |
| <i>G. maculata</i> B        | 1.5<br>(1.3–1.9)    | NA                  |                     |                     |                     |                     |                     |                     |                     |                     |                     |                     |                  |                  |
| <i>G. maculata</i> C        | 5.9<br>(5.7–6.2)    | 6.2<br>(6.2–6.2)    | 0.8<br>(0.8–0.8)    |                     |                     |                     |                     |                     |                     |                     |                     |                     |                  |                  |
| <i>G. megalepis</i> D       | 8.6<br>(8.3–9.2)    | 8.7<br>(8.5–8.8)    | 7.2<br>(6.8–7.5)    | 0.2<br>(0.2–0.2)    |                     |                     |                     |                     |                     |                     |                     |                     |                  |                  |
| <i>G. bekira</i> sp. nov. E | 12.8<br>(12.6–13.2) | 13.0<br>(12.8–13.2) | 12.7<br>(12.5–13.0) | 13.6<br>(13.5–13.7) | 0.4<br>(0.4–0.4)    |                     |                     |                     |                     |                     |                     |                     |                  |                  |
| <i>G. bekira</i> sp. nov. F | 13.0<br>(12.8–13.6) | 12.7<br>(12.6–12.8) | 13.3<br>(13.2–13.6) | 13.6<br>(13.5–13.9) | 1.8<br>(1.5–2.1)    | 0.1<br>(0.0–0.2)    |                     |                     |                     |                     |                     |                     |                  |                  |
| <i>G. bekira</i> sp. nov. G | 12.7<br>(12.6–13.0) | 13.3<br>(13.2–13.4) | 12.5<br>(12.3–12.7) | 13.5<br>(13.2–13.7) | 5.2<br>(5.1–5.3)    | 5.5<br>(5.3–5.7)    | 0.2<br>(0.2–0.2)    |                     |                     |                     |                     |                     |                  |                  |
| <i>G. modesta</i> H         | 12.8<br>(12.6–13.0) | 13.0<br>(12.9–13.0) | 12.2<br>(12.0–12.3) | 13.9<br>(13.8–14.1) | 8.0<br>(7.6–8.3)    | 8.3<br>(8.1–8.5)    | 8.1<br>(7.9–8.3)    | 0.4<br>(0.4–0.4)    |                     |                     |                     |                     |                  |                  |
| <i>G. modesta</i> I         | 12.6<br>(11.8–13.3) | 12.8<br>(12.0–13.3) | 12.2<br>(11.1–12.6) | 14.4<br>(13.5–14.8) | 9.4<br>(8.3–10.2)   | 9.7<br>(8.7–10.4)   | 9.0<br>(7.7–9.8)    | 2.3<br>(1.5–2.8)    | 1.6<br>(0.0–3.0)    |                     |                     |                     |                  |                  |
| <i>G. spania</i> sp. nov. L | 8.4<br>(8.4–8.6)    | 8.6<br>(8.6–8.6)    | 9.2<br>(9.0–9.4)    | 11.7<br>(11.6–11.8) | 10.7<br>(10.5–10.9) | 11.2<br>(11.1–11.3) | 11.6<br>(11.5–11.8) | 11.1<br>(11.0–11.1) | 11.0<br>(10.5–11.4) | NA                  |                     |                     |                  |                  |
| <i>G. humbloti</i> HU       | 17.9<br>(17.7–18.1) | 17.9<br>(17.9–17.9) | 19.0<br>(18.9–19.1) | 19.2<br>(19.1–19.3) | 19.3<br>(19.3–19.3) | 18.8<br>(18.7–18.9) | 19.0<br>(18.9–19.1) | 18.3<br>(18.1–18.5) | 18.5<br>(18.3–18.8) | 18.0<br>(18.0–18.0) | 0.0<br>(0.0–0.0)    |                     |                  |                  |
| <i>G. polylepis</i> PO      | 15.7<br>(15.4–16.2) | 16.1<br>(15.8–16.2) | 15.6<br>(15.1–16.6) | 16.7<br>(16.3–17.3) | 18.0<br>(17.9–18.3) | 18.2<br>(18.1–18.5) | 16.7<br>(16.6–17.0) | 14.5<br>(14.3–14.8) | 14.4<br>(13.9–14.8) | 15.1<br>(14.1–15.4) | 16.8<br>(16.4–17.1) | 0.8<br>(0.0–2.8)    |                  |                  |
| <i>G. typica anomala</i> K  | 15.1<br>(14.9–16.0) | 15.0<br>(14.9–15.1) | 14.2<br>(14.0–14.4) | 15.1<br>(15.0–15.2) | 18.1<br>(18.0–18.2) | 17.9<br>(17.8–18.0) | 18.1<br>(18.0–18.2) | 14.5<br>(14.4–14.6) | 14.3<br>(14.1–14.7) | 15.1<br>(15.0–15.4) | 15.6<br>(15.3–15.7) | 13.5<br>(13.2–14.0) | 2.7<br>(0.4–3.8) |                  |
| <i>G. t. typica</i> J       | 12.2<br>(11.9–12.6) | 12.1<br>(12.0–12.4) | 12.3<br>(12.1–12.5) | 14.0<br>(13.7–14.3) | 16.1<br>(15.3–16.8) | 16.3<br>(15.5–17.0) | 16.0<br>(15.5–16.3) | 13.0<br>(12.3–13.7) | 12.9<br>(12.1–13.5) | 13.2<br>(13.1–13.5) | 14.3<br>(14.3–14.4) | 13.2<br>(13.0–13.6) | 6.0<br>(5.3–6.7) | 1.0<br>(0.0–1.5) |

on a tree constructed with *nd4* sequences were much less obvious in our tree that also incorporated *12S* and *16S*: lineage B did not receive relevant bootstrap support and was separated by only minimal branch lengths from lineage A. Similarly, due to the inclusion of new samples from Namoroka and other geographically intermediate sites, lineage I now appears merely as a weakly divergent phylogroup within lineage H.

Inter-lineage genetic distances in the *16S* fragment, previously not available for most *Geckolepis* lineages, ranged from 1.3–19.3% (Table 2). Differences between lineages A and B, H and I, and E and F, respectively, were very low (1.3–2.8%) and similar to distances within some clades (e.g., up to 1.9% within lineage A). Slightly higher distances were observed for lineage C vs. A and B (5.7–6.2%), lineage G vs. E and F (5.1–5.7%), and lineage K vs. J (5.3–6.7%). Distances between lineages clearly representing distinct species based on morphology ranged between 6.8% (*G. megalepis*, D, vs. *G. maculata*, C) and 19.3% (*G. megalepis* vs. *G. humbloti*).

### Species delimitation

The three best partitions inferred by ASAP based on the *nd4* alignment suggested high numbers of species-level subsets (primary species hypotheses), i.e., 23 (ASAP-score 2.5), 19 (ASAP-score 6.5) and 21 (ASAP score 7.0). These partitions recovered all lineages (OTUs) of Lemme *et al.* (2013) as distinct subsets but in addition also split *G. humbloti* into six subsets which we here consider to represent oversplitting. A more realistic scenario was only ranked 8th (ASAP score 10.0), suggesting nine subsets as follows: A+B, C, D, H+I, E+F+G, L, HU, PO, J+K. We conservatively chose this as our preferred partition for comparison with evidence from nuclear-encoded DNA, morphology, and geography. Nevertheless, as will be discussed below, future studies should test whether a finer taxonomic resolution as suggested by the higher-ranking ASAP partitions may be warranted, especially for *G. humbloti*.

Examination of the haplotype genealogies of the two nuclear-encoded gene fragments (Fig. 3) supports this more conservative species partition. Both networks show wide haplotype sharing within the subset E+F+G (with G not sharing haplotypes with E or F in *C-MOS*), and within A+B+C and H+I, respectively. Lineages J+K share haplotypes in *RAG-1* but not in *C-MOS*. There is almost no haplotype sharing between species-level subsets in this conservative partition, only *G. maculata* (A+B and C) and *G. megalepis* (D) share a centrally placed allele in *RAG-1*, and lineage C shares haplotypes with A+B.

Morphologically, taking into account data from Köhler *et al.* (2009), Lemme *et al.* (2013), Hawlitschek *et al.* (2016), and Scherz *et al.* (2017), there are consistent morphological differences between the subsets in our preferred species partition, such as particularly low (*G. megalepis*) or high (*G. polylepis*) counts of midbody and ventral scales, or unique states of mental and postmental scales (J+K). Also other subsets such as E+F+G or L are differentiated from all others by subtle morphological differences (see Diagnoses of new species below).

Largely in line with current classification, it is

therefore appropriate to consider these subsets (= genetic lineages) as differentiated at the species level, except for lineage C which we consider as a deep genetic lineage conspecific with A+B. The species status of several of these lineages is further corroborated by their sympatric occurrence (without apparent admixture) (Fig. 4). For instance, in the North West, there is a wide overlap of the ranges of HU, PO and lineage H. Additionally, HU and lineage H occur in syntopy in both Bemaraha and Namoroka. In the Sambirano and North regions, there is overlap of the ranges of A+B, D, E+F+G, and L, with three of these occurring in close sympatry at Ankarana.

### Reverse taxonomy based on DNA barcodes from type specimens

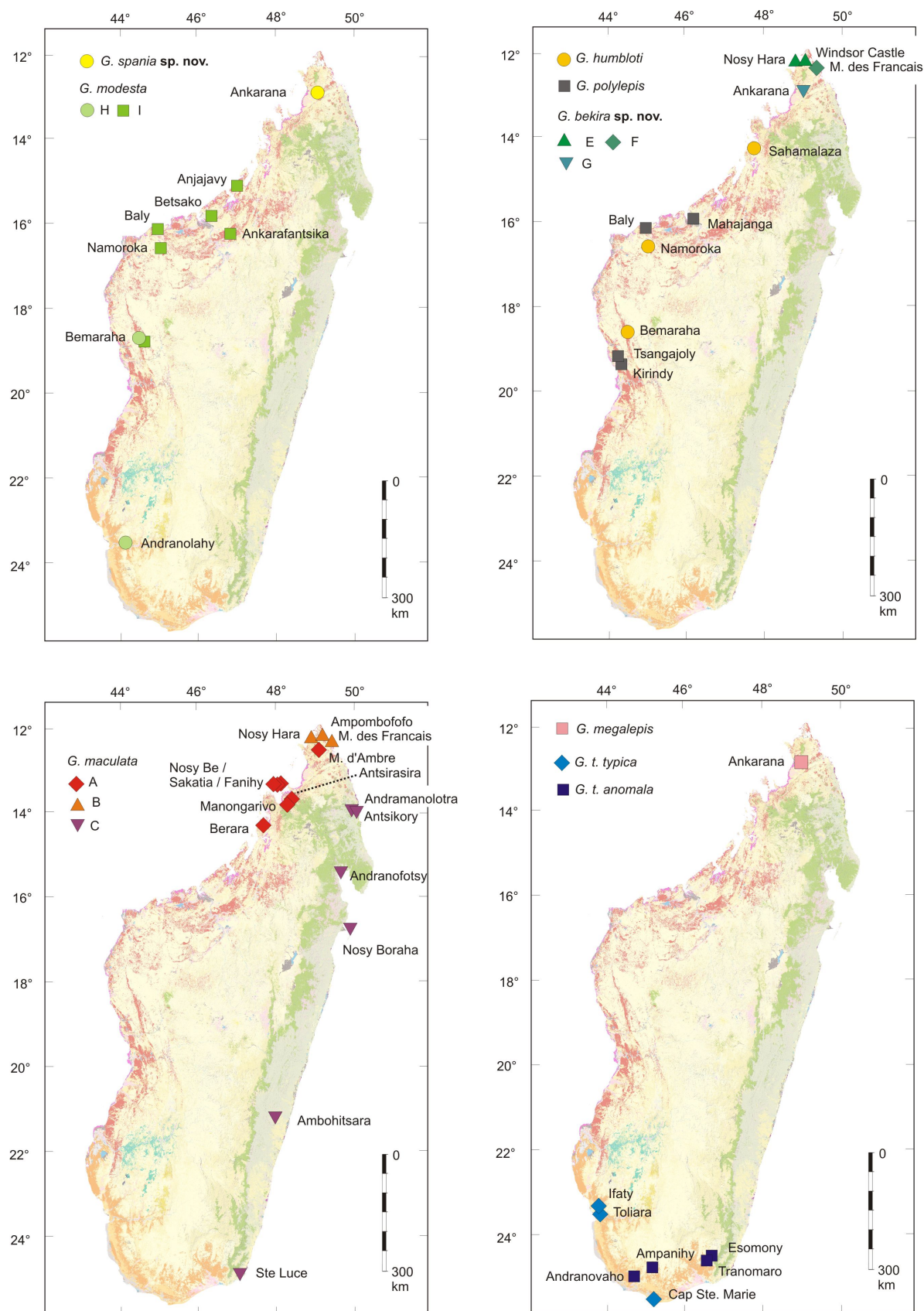
All sequenced historical type specimens clustered closely and unambiguously within one of the main mitochondrial lineages.

(i) The three historical specimens of *G. polylepis* Boettger, 1893 from “Mojanga” (= Mahajanga) in the North West (BMNH 1946.8.25.83–85; same locality as that of lectotype SMF 9217) sequenced herein, plus a further specimen BMNH 1946.8.25.82, are catalogued in the Natural History Museum London as “types”, with a reference to the Senckenberg Museum (P. Campbell pers. comm. in 2025). They were catalogued in 1893 (the year of the *G. polylepis* description) under original numbers 93.11.1.1–4. We therefore consider these specimens as paralectotypes, although some inconsistencies remain about the original type series, not discussed by Köhler *et al.* (2009) who only listed the lectotype. The original description (Boettger 1893) mentions six adult and one juvenile specimens, all obtained by A. Voeltzkow in 1893. In addition to the SMF lectotype and the four BMNH paralectotypes, three specimens have been catalogued as types in the ZSM (ZSM 309–310/0, 24/1921 of which 309/0 is still present), suggesting that one of these (perhaps ZSM 24/1921) was not actually a paralectotype.

The three BMNH specimens analysed herein clustered with samples from Kirindy and nearby sites in the West that previously had been assigned to this species. One newly sequenced specimen from the geographically intermediate Baly Bay confirms that the species does occur in the North West, thus supporting correctness of the historical type locality.

(ii) The lectotype of *G. typica* Grandidier, 1867 (MNHN-RA-1994.1748, formerly MNHN 1454A) from “Sanctae Mariae promontorium” (referring to Cap Ste. Marie, not to be confounded with Sainte-Marie island, cf. Brygoo 1990; Fig. 4) was placed in lineage J from the South West, and specifically in a phylogroup from Ifaty north of the town of Toliara. The types of both *G. typica* var. *anomala* Mocquard, 1902 (MNHN-RA-1901.148 from the “Androy Nord” region) and *G. petiti* Angel, 1942 (MNHN-RA-1933.56 from “Andranovaho, Falaise calcaire près d’Ampotaka, pays Mahafaly, Madagascar”) cluster within lineage K from the southernmost arid areas of Madagascar. Given the rather low genetic divergences between J and K and the apparent absence of morphological differences between them (Lemme *et al.*





**FIGURE 4.** Distribution maps of all species of *Geckolepis* recognized herein, showing only localities verified by DNA sequence analysis. The base map shows vegetation across Madagascar from the Madagascar Vegetation Mapping Project (Moat & Smith 2007; available at <https://web.archive.org/web/20180419112513/http://www.vegmad.org/>). Vegetation is colored as follows: green, humid forest (rainforest); red, western dry deciduous forest; pink, mangroves; orange, spiny forest-thicket; greenish blue, western subhumid forest. The locality Nosy Boraha for *G. maculata* lineage C is based on a *12S* sequence of Lemme *et al.* (2013) not included herein.

2013), we here consider all these samples as conspecific and apply the oldest available name *G. typica* to them; furthermore, we suggest considering K as a subspecies, *G. typica anomala*, and regard *G. petiti* as a junior synonym of *G. typica* (more specifically, of *G. typica anomala*).

(iii) The holotype of *G. maculata* Peters, 1880 (ZMB 9655) from the locality “Anfica” in northwestern Madagascar, is placed within lineage A, which agrees with the morphological assessment of Lemme *et al.* (2013). It specifically clusters close to samples from Nosy Be which was one of the main historical collecting regions in northern Madagascar. We therefore apply the name *G. maculata* in a strict sense to lineage A+B; the species according to the molecular data is thus known in the North and Sambirano regions, reaching into the North West at Sahamalaza. The status of lineage C, which comprises all samples collected along Madagascar’s eastern coast, is uncertain. Lemme *et al.* (2013) did not find morphological differences between A+B and C, and haplotype sharing between them occurs in both nuclear gene fragments. We here provisionally include lineage C in *G. maculata*, but it may represent a distinct taxon at subspecies or species level.

(iv) The three sequenced syntypes of *G. typica* var. *modesta* Methuen & Hewitt, 1913 (DNMNH 4054, 4055 and 4146) from “Andranoláho, on the River Oniláhy” according to the original description (probably corresponding to Andranolahy), are placed within lineage H and cluster with some of the samples from the Tsingy de Bemaraha. As we found lineages H+I to represent a separate unit at the species level, we here propose to resurrect this name as *G. modesta* for the fish-scale gecko populations corresponding to the mitochondrial lineages H+I.

(v) The identity of *G. megalepis* Scherz, Daza, Köhler, Vences & Glaw, 2017 unambiguously corresponds to the lineage D, as the holotype (ZSM 2126/2007) was already sequenced for the original description (Scherz *et al.* 2017).

(vi) We did not obtain sequences of three syntypes of *G. humbloti* Vaillant, 1887 (MNHN-RA-1885.0480 to 0482 from Grande Comore), but the identity of this species was clarified by Hawlitschek *et al.* (2016) based on geographical and morphological data.

(vii) Two deep lineages remain to which no available scientific name can be assigned: one of these being E+F+G from northern Madagascar, and the second one being lineage L represented by a single sample (FGZC 1140) from the Ankarana Massif. Based on the concordance of molecular markers and morphological differentiation (see Diagnoses below), we name and describe these lineages formally as new species in the following.

### ***Geckolepis bekira* sp. nov.**

urn:lsid:zoobank.org:act:3E840BCB-F11B-473C-819E-FB2884E24A54

Figs 5–7

**Remark.** This species subsumes specimens of the genetic lineages defined as OTUs E, F and G by Lemme *et al.* (2013) and referred to under these names herein.

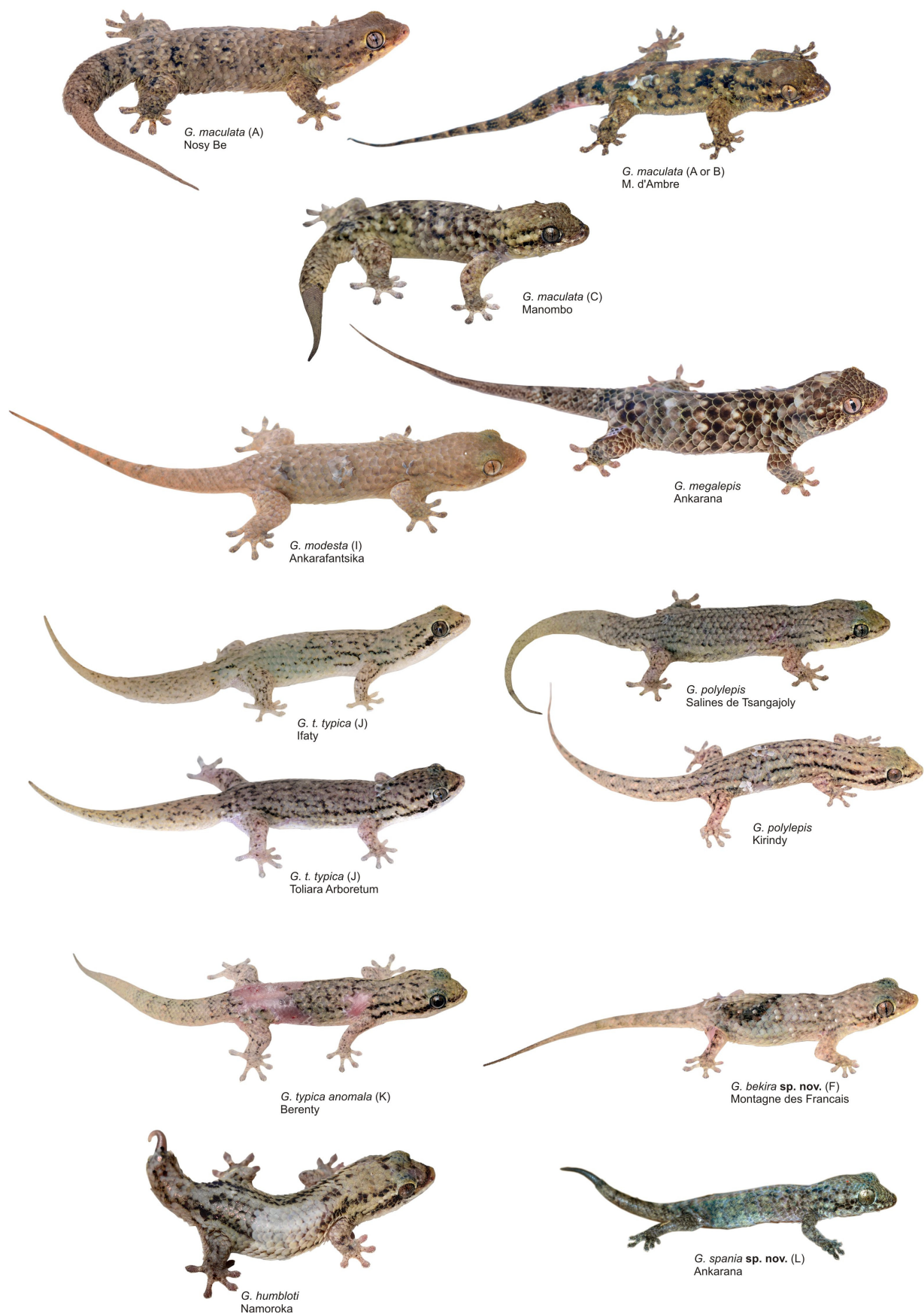
**Holotype.** ZSM 1523/2008 (field number FGZC 1971), adult specimen, collected by F. Glaw & J. Köhler on 3 March 2008 on Montagne des Français (near French Fort; 12.3258°S, 49.3381°E, 260 m above sea level), northern Madagascar.

**Paratypes.** We here designate six genotyped specimens belonging to lineages F and E as paratypes: ZSM 2112/2007 (FGZC 1110), collected by F. Glaw, P. Bora, H. Enting, J. Köhler & A. Knoll on 27 February 2007 at the type locality. ZSM 912/2003 (FGMV 2002.939), adult male, collected by F. Glaw & R.D. Randrianaina on 20 February 2003 on Montagne des Français. ZSM 522/2000 (FGMV 2000.273), collected by F. Glaw, K. Schmidt & M. Vences on 13 March 2000 on Montagne des Français. ZSM 2138/2007 (FGZC 1176), collected by F. Glaw, P. Bora, H. Enting, J. Köhler & A. Knoll on 5 March 2007 at Forêt de Orangea (east of Ramena), northern Madagascar. ZSM 2202/2007 (FGZC 1309) and ZSM 2203/2007 (FGZC 1311), collected by F. Glaw, J. Köhler & H. Enting on 7 March 2007 on Nosy Hara islet (12.2426°S, 49.0129°E, near sea level).

**Additional material.** The following four specimens belonging to lineage G are not included in the type series due to their somewhat larger genetic differentiation as they may be found to be taxonomically distinct in the future: ZSM 780/2003 (FG/MV 2002.597), ZSM 765/2003 (FGMV 2002.569), and ZSM 766/2003 (FGMV 2002.570), collected by F. Glaw, R.D. Randrianaina & A. Razafimanantsoa on 12 February 2003 in Ankarana National Park. ZSM 1522/2008 (FGZC 1605), collected by F. Glaw, M. Franzen, J. Köhler & N. D’Cruze on 12 February 2008 in Ankarana National Park (Petit Tsingy).

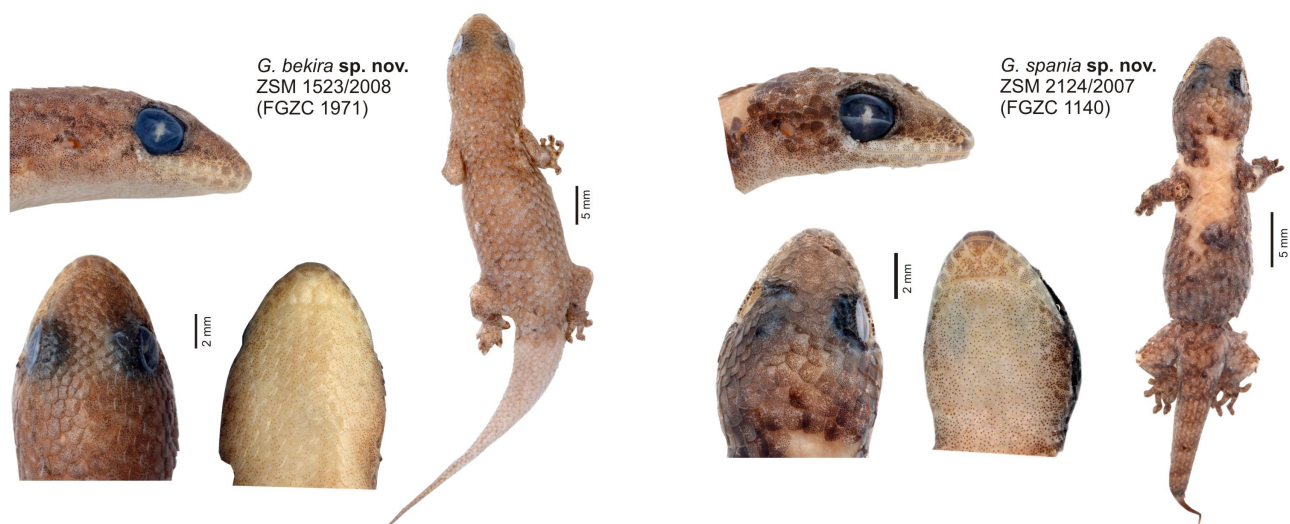
**Diagnosis.** Assigned to the genus *Geckolepis* based on molecular phylogenetic relationships and body covered with large, imbricated scales. Based on morphological data of Lemme *et al.* (2013), characterized by snout–vent length <54 mm, 25–28 scales around midbody, 33–43 ventral scale rows, condition A or B of mental scale, eight supralabials, and usually a dark stripe from eye to ear opening. The new species differs from *G. humbloti*, *G. maculata*, *G. megalepis* and *G. modesta* by a smaller body size (SVL in these species reaching up to 65–75 mm), from *G. megalepis* and *G. modesta* furthermore by more scales around midbody (25–28 vs. 17–25), and from *G. megalepis* also by more ventral scale rows (33–43 vs. 27–32); from *G. typica* by a different condition of mental scale (A or B vs. D); from *G. polylepis* by having fewer ventral scale rows (33–43 vs. 48–55) and more scales around midbody (25–28 vs. 35–38). The new species can be differentiated from all other species and lineages in the genus complex by numerous diagnostic nucleotide positions in the mitochondrial genome. MolD identified for the *nd4* fragment a robust diagnostic nucleotide combination of an “A” at site 904, a “C” at site 975, and a “T” at site 1002 (positions relative to the full *nd4* gene of *Geckolepis*).

**Description of the holotype.** A medium-sized specimen in excellent state of preservation (Fig. 6). Few scales missing dorsally above insertion of right forearm; left forearm missing (removed as tissue sample); tail



**FIGURE 5.** Representative photos in life of all species and subspecies of *Geckolepis* according to the classification proposed herein. All specimens except *G. modesta* and *G. maculata* from Manombo correspond to genotyped individuals. Not to scale.





**FIGURE 6.** Photos of the preserved holotypes of the two new species described herein, *Geckolepis spania* **sp. nov.** and *Geckolepis bekira* **sp. nov.** For each species, lateral, dorsal and ventral views of head, and dorsal view of entire specimen are shown.



**FIGURE 7.** Photos in life of the holotypes of the two new species described herein. (A, B) *Geckolepis spania* **sp. nov.**, holotype, probably a subadult, ZSM 2124/2007 (FGZC 1140) from Ankarana. (C, D) *Geckolepis bekira* **sp. nov.**, the adult male holotype ZSM 1523/2008 (FGZC 1971) from Montagne des Français.

complete and attached. SVL 42.0 mm; tail length 37.6 mm; axilla–groin distance 14.4 mm; head length 11.9 mm; head width 9.6 mm; head height 6.1 mm; snout length 5.7 mm; eye diameter 3.4 mm; interorbital distance 2.0 mm; eye–ear distance 3.5 mm; rostral large, convex, visible from above; medium-sized postrostrals, separated by a thin oblong scale anteriorly and two small scales posteriorly (type D of Köhler *et al.* 2009); nostril bordered by rostral, first supralabial, two postnasals, and one supranasal (=postrostral); postnasals slightly larger in

size than anterior loreals; postnasals and loreals separated by one row of small scales; scales on snout and on loreal region almost all with rounded posterior margins, slightly convex, imbricate; eight canthal scales in a line on the canthal ridge including the postnasals, between nostril and anterior margin of eye; scales at supraorbital region similar to and continuous with those on top of head; scales increase two times in size from level of mid-eye to occipital region; ten scales along a straight line dorsally between orbits; pupil vertical; eight supralabials on each



side, all of roughly equal width, the posterior-most two smaller than the rest; ear opening much smaller than eye, horizontally oval; four infralabials to below level of anterior margin of eye, decreasing in size posteriorly; mental scale medium-sized, triangular, with convex anterior border; postmentals symmetrical, one large pair immediately posterior to mental scale, with medial contact (condition B of Lemme *et al.* 2013); one row of small scales separating postmentals from anterior chin scales; chin and ventral body scales rhomboid with rounded posterior margins, imbricate, only slightly increasing in size posteriorly, arranged in roughly regular rows; 36 scales along the midventral line between postmentals and vent, not including smaller scales adjacent to postmentals and anterior to vent; dorsal scales cycloid, larger than lateral or ventral scales; 26 scales around midbody; anterior half of tail flattened and rather wide (ca. 60% of body width), decreasing in width gradually, down to a very thin tip; tail covered with scales similar to body scales, but slightly decreasing in size posteriorly, a series of transversely expanded median subcaudals absent; 9 lamellae under first toe, extending from one fourth of the toe's length to the claw, and 12 lamellae under fourth toe; claws exposed, non-retractile.

After 17 years in preservative (Fig. 6), head and body dorsally homogeneously pale brown; a faint darker postocular lateral stripe, extending to level of insertion of forearm; dorsal scales brown, with regularly scattered blackish spots; legs as dorsum; dorsal surfaces of tail dirty cream; ventrally pale cream, with scales bearing only fine scattered brown spots; exposed dorsal skin cream. In life (Fig. 7), the specimen was light brown to beige with weakly expressed dark brown to blackish pattern which included two stripes starting roughly behind the eye and running backwards, one of them to the ear and from there to forelimb insertion. Tail laterally with a reddish brown tint which also extended onto the posterior hindlimbs, and dorsally bordered by a distinct dark line. Iris copper.

**Etymology.** The species name is derived from the Malagasy words *be* (= big) and *kira* (= scale), referring to the large imbricated scales that distinguish this and other species of *Geckolepis* from other Malagasy geckos. The species epithet is an invariable noun in apposition to the generic name.

**Natural history.** At the type locality Montagne des Français, the species is not uncommon and is regularly seen at night on relatively thin trunks of thorny bushes where it is very difficult to catch. Nothing else is known.

**Distribution.** Based on genotyped individuals (Fig. 2) the species is known from five localities in the North region of Madagascar (Fig. 4): (1) the type locality, Montagne des Français (lineage F); (2) Forêt de Orangea (F), (3) Nosy Hara (E), (4) Windsor Castle, a rock formation close to Antsiranana (E), and (5) Ankarana (G).

### *Geckolepis spania* sp. nov.

urn:lsid:zoobank.org:act:13EB3678-09CF-4D23-A108-E7E2C9A3E288

Figs 5–7

**Remark.** This species corresponds to the genetic lineage L in the present study, formed by the holotype only (ZSM 2124/2007), and mentioned by Lemme *et al.* (2013) as a divergent lineage based on its 12S sequence only, under the field number FGZC 1140.

**Holotype.** ZSM 2124/2007 (field number FGZC 1140), collected by F. Glaw, P. Bora, H. Enting, J. Köhler & A. Knoll on 1 March 2007 in Ankarana National Park (on the way to Petit Tsingy and Grotte des Chauves Souries, 12.9569°S, 49.1183°E, 90 m above sea level), northern Madagascar.

**Diagnosis.** Assigned to the genus *Geckolepis* based on molecular phylogenetic relationships and body covered with large, imbricated scales. Characterized by small body size (but holotype is probably not adult), 26 scales around midbody, 34 ventral scale rows, condition A of mental scale, 7–8 supralabials, and presence of a dark stripe from eye to ear opening. The new species differs from *G. modesta* and *G. megalepis* by more scales around midbody (26 vs. 17–25), and from *G. megalepis* also by more ventral scale rows (34 vs. 27–32); from *G. maculata* (A+B) by more scales around midbody (26 vs. 22–25); from *G. maculata* (C) by fewer ventral scale rows (34 vs. 36–42); from *G. humbloti* by a larger number of supralabials compared to most specimens of that species (7–8 vs. 5–7); from *G. typica* by a different condition of mental scale (A vs. D); from *G. polylepis* by having fewer ventral scale rows (34 vs. 48–55) and fewer scales around midbody (26 vs. 35–38); reliable morphological differentiation from *G. bekira* is not possible at present. The new species can be differentiated from all other species and lineages in the genus complex by numerous diagnostic nucleotide positions in the mitochondrial genome. MolD identified for the *nd4* fragment a robust diagnostic nucleotide combination of an “A” at site 876, and a “C” at site 943 (positions relative to the full *nd4* gene of *Geckolepis*).

**Description of the holotype.** (Fig. 6) A small-sized specimen in reasonable state of preservation. Scales missing dorsally from scapular region to midbody, and on lower right forearm; tail complete and attached, its posteriormost tip regenerated. SVL 31.3 mm; tail length 17.5 mm; axilla–groin distance 12.3 mm; head length 9.6 mm; head width 6.9 mm; head height 4.3 mm; snout length 4.2 mm; eye diameter 2.4 mm; interorbital distance 1.7 mm; eye–ear distance 2.1 mm; rostral large, convex, visible from above; large postrostrals, anteriorly in contact, separated posteriorly by a rounded scale (type B of Köhler *et al.* 2009); nostril bordered by rostral, postrostral, first supralabial, two postnasals, and one supranasal (=postrostral); postnasals same size as anterior loreals; postnasals and loreals separated by one row of small scales; scales on snout and on loreal region almost all with triangular posterior margins, slightly convex, imbricate; eight canthal scales in a line on the canthal ridge including the postnasals, between nostril and anterior margin of eye; scales at supraorbital region similar to and continuous with those on top of head; scales increase two times in size from level of mid-eye to occipital region;

eight scales along a straight line dorsally between orbits; pupil vertical; eight supralabials on right side, seven on left side, all of roughly equal width, the posterior-most two smaller than the rest; ear opening much smaller than eye, almost circular in shape; 3.5 infralabials to below level of anterior margin of eye, decreasing in size posteriorly; mental scale medium-sized, perfectly triangular in shape, with convex anterior border; postmentals symmetric, one large pair immediately posterior to mental scale, with broad medial contact (condition A of Lemme *et al.* 2013); one row of small scales separating postmentals from anterior chin scales; chin and ventral body scales rhomboid with rounded posterior margins, imbricate, only slightly and gradually increasing in size posteriorly, arranged in roughly regular rows; 34 scales along the midventral line between postmentals and vent, not including smaller scales adjacent to postmentals and anterior to vent; dorsal scales cycloid, slightly larger than lateral or ventral scales; 26 scales around midbody; tail only slightly flattened and narrow (ca. 30% of body width), decreasing in width gradually, down to regenerated part, which is slightly desiccated and narrower than the original tail; tail covered with scales similar to body scales, but slightly decreasing in size posteriorly, a series of transversely expanded median subcaudals absent; 10 lamellae under first toe, and 13 lamellae under fourth toe; claws exposed, non-retractile.

After 18 years in preservative (Fig. 6), head and body dorsally brown, with some irregular and barely outlined dark brown markings; dorsal scales brown, densely scattered with dark brown spots; legs as dorsum; dorsal surfaces of tail dirty creamy brown with six transverse dark brown crossbands; labials and infralabials cream with sparsely scattered brown spots; ventrally cream, with scales bearing diffusely outlined dark brown spots; exposed dorsal skin cream.

Color in life (Fig. 7) similar to that in preservative, with bluish and reddish highlights on parts of the body caused by reflections of the flash used for photography, as typical for many *Geckolepis*. Iris silvery grayish.

**Etymology.** The species name is a latinized adjective derived from Greek σπάνιος (*spánios* = *rare*), used in the feminine gender in agreement with the genus name. It refers to the rare occurrence of this species, which is known from a single collected specimen, despite numerous herpetological explorations of its type locality, Ankarana National Park.

**Distribution.** Only known from Ankarana National Park (Fig. 4).

**Natural history.** Unknown.

## Discussion

**Molecular systematics and species diversity of *Geckolepis***  
Despite intense research activities focused on elucidating the taxonomy of Madagascar's squamate fauna (Glaw *et al.* 2022), *Geckolepis* has so far remained a taxonomic conundrum—especially because of uncertainties in assigning scientific names coined historically based

on faint morphological differences to genetic lineages identified from recent collections (Lemme *et al.* 2013; Hawlitschek *et al.* 2016; Scherz *et al.* 2017; Bauer *et al.* 2022). Here, we have contributed to overcoming these hindrances by providing sequences of name-bearing types of five scientific names, and of topotypical paralectotypes of one further *nomen*, using a shallow shotgun sequencing approach followed by bioinformatic extraction of target markers, which is less labor-intensive, potentially cheaper, and yields more potentially usable data than hybrid-enrichment “DNA barcode fishing” of mitochondrial gene sequences (Scherz *et al.* 2020). By this museomic approach, we were able to reliably assign these types to previously identified mitochondrial lineages, leading to the resurrection of *G. modesta* for lineages H+I, confirmation of the name *G. maculata* for lineages A+B+C, *G. typica* for J+K, confirmation of the name *G. polylepis* for those samples previously assigned to this species (Lemme *et al.* 2013), recognition of *G. typica* var. *anomala* as subspecies *G. typica anomala*, and confirmation of *G. petiti* as a junior synonym of *G. typica anomala*.

This solidified classification provides a robust baseline to identify the remaining taxonomic issues in the genus. We here described the two new species *G. bekira* and *G. spania*, for which no earlier scientific names were available, and proposed to distinguish two genetic lineages within *G. typica* in a preliminary way at the subspecies level. However, *Geckolepis* taxonomy will require refinement from analysis of more extensive, preferably genomic datasets. This applies especially to three apparently allopatric pairs of deep genetic lineages, i.e., (i) *G. t. typica* vs. *G. t. anomala*, (ii) *G. maculata* lineages A+B vs. C, and (iii) *G. bekira* lineages E+F vs. *G.* For all three lineage pairs, it should be tested whether they are differentiated at the species level, or, as here already hypothesized for the first pair, as subspecies. In these cases, identifying the contact or hybrid zone between the pairs of lineages, and formally testing for hybrid zone cline width based on genome-scale datasets, holds promise to conclusively demonstrate either a subspecies or species status of these lineages, depending on the amount of reproductive isolation (Dufresnes *et al.* 2021). Also, additional field data on the species rarely represented in collections, such as *G. spania*, which so far is known from a single specimen only, and the Malagasy populations of *G. humbloti*, are important for a full picture of *Geckolepis* natural history and biogeography. The description of *G. spania* based on a single specimen, which in addition is subadult, reflects an overall high number of species known only from singletons in biodiversity surveys (Lim *et al.* 2012) and taxonomic descriptions (Deng *et al.* 2019; Wells *et al.* 2019). Given that such singleton species may often be rare or difficult to observe, and often are not-recollected for considerable time spans (e.g., Wells *et al.* 2019), it seems reasonable to describe them as new species irrespective of sample size, as long as they can be thoroughly delimited (e.g., Jörger & Schrödl 2013; Cazabonne *et al.* 2024). For *Geckolepis*, the delimitation and the names of the species recognized herein are unlikely

to change with future phylogenomic studies, given that the primary mitochondrial DNA evidence herein is corroborated by differentiation in two nuclear genes plus faint but consistent morphological differences.

#### *Future directions of Geckolepis research*

While future genomic data will likely only refine, not revolutionize *Geckolepis* taxonomy, they could be of particular interest for better understanding the mechanisms of regeneration of tissue, especially skin, in vertebrates, in combination with detailed morphological analysis. By studying another gecko with the ability of integumentary loss, *Ailuronyx seychellensis*, Bauer *et al.* (1989) found a stratum compactum divided into inner and outer layers by intervening loose connective tissue, with the outer layer being much thicker and much weaker than the inner layer; they highlighted the probable importance of mechanical interaction of skin layers with one another and with the underlying body wall for an effective mechanism of integumentary loss. Spiny mice (*Acomys*), which also are able to shed and regenerate skin (Seifert *et al.* 2012), are as mammals more obvious model organisms for the biomedical community to investigate and clinically translate molecular mechanisms of scarless wound healing and skin regeneration (Gaire *et al.* 2021; Okamura *et al.* 2023). However, gene expression studies in *Geckolepis* could help identify common genes and pathways specifically involved in skin regeneration, beyond those involved in general tissue regeneration processes active for instance in gecko tails (Bateman & Fleming 2009; Nagumantri *et al.* 2021; Nurhidayat *et al.* 2025). *Geckolepis* would be tractable lab animals, as they have successfully been kept and bred in the pet trade.

From an ecological perspective, both the function of skin shedding as antipredator mechanism, and the consequences of skin regeneration for the affected *Geckolepis* specimen (i.e., longevity, reproduction) merit further study. Bauer & Russell (1992) assessed that integumentary loss is observed in at least 10 genera of geckos which mostly live on islands, and hypothesized that this antipredator strategy may be related to a predominance of predators on islands that grasp or pin their prey rather than kill it by initial bites or strikes. However, field observations on predation of *Geckolepis* are surprisingly scarce; Köhler *et al.* (2009) report that they never directly observed predatory attacks on *Geckolepis*, and did not find these geckos from snake stomach contents. Gardner & Jasper (2014) reported predation on *Geckolepis* by a bird, Gardner & Jasper (2015) reported on a failed predation attempt by a larger *Blaesodactylus* gecko on *Geckolepis*, and Glaw *et al.* (2002) observed predation by a scorpion. Here, we document what appears to be the first two records of snake predation on *Geckolepis*: (i) A *Mimophis occultus* preyed on a *Geckolepis* (probably *G. modesta*), in Ankarafantsika National Park in November 2022 (Fig. 8A–C). At the start of our observation, the snake had already started swallowing the gecko head first, and we therefore could not determine at which part of the body the initial bite took place. However, besides a small injury at the tail, we could not see any bitemarks on

the gecko, and the snake had no difficulties swallowing the specimen. (ii) A further observation of *Dromicodryas bernieri* preying upon an unidentified *Geckolepis* on 11 July 2025 at Mariarano was provided by Freddie Walker (pers. comm.). The snake seized the gecko by the head (Fig. 8D–E), coiled around it, and subsequently swallowed it head-first. Detached *Geckolepis* scales on the snake as well as large areas of shed skin in the belly area are visible. At least this second example, in which the snake secures its prey by constriction, appears to support Bauer & Russell's (1992) hypothesis, while also demonstrating that integumentary loss provides only imperfect protection against snakes employing this method of prey capture. These observations exemplify the importance of descriptive natural history observations to understand the adaptations of Madagascar's reptiles.

#### *Geckolepis biogeography and conservation*

In this study, we have also provided updated distribution maps of all *Geckolepis* species, based only on genotyped specimens. A comparison with previous compilations of locality records for *Geckolepis* (e.g., Glaw & Vences 2007; Köhler *et al.* 2009) highlights multiple biogeographic questions. For instance, Köhler *et al.* (2009) mapped multiple records of *G. typica* from the North West of Madagascar while we only recorded the species from the South West. The northernmost confirmed record is Ifaty near Toliara, but it is likely that the species' range extends farther north, especially since we lack any molecular data from the area between Ifaty and Morondava. A further interesting aspect is the occurrence of all of our *G. modesta* specimens in the West and North West of Madagascar, while its type locality is in the South West, close to Toliara. If correct, we expect the species to have a more or less continuous distribution area in western Madagascar, and its syntopic occurrence with *G. typica* at some sites in the South West is likely. According to our data it is probable that *G. maculata* (lineage C) is present all along Madagascar's eastern coast, despite the patchy distribution suggested by our scarce molecular sampling: own observations and records on iNaturalist (inaturalist.org; accessed 24 September 2025) exist from at least Nosy Boraha and Ankanin'ny Nofy, and Lemme *et al.* (2013) report a short *12S* sequence (not included here) from the former site. Several other enigmatic distributional patterns also require more scrutiny: For instance, no recent records of *G. polylepis* exist from its type locality, Mahajanga, and the distribution of *G. humbloti* in the North West of Madagascar appears to be very patchy despite an overall rather intensive sampling in this region (Fig. 4). These examples illustrate the need for continued exploration of Madagascar's biota, and in the case of taxa that cannot easily be identified by morphology, the need for large-scale DNA barcoding or metabarcoding approaches to obtain a reliable map of life for this island.

The uncertain taxonomy of *Geckolepis* has also long hindered a reliable assessment of their conservation status. The current Red List of the International Union for Conservation of Nature (IUCN) lists *G. maculata* and *G. typica* as Least Concern, and *G. polylepis* as Data





**FIGURE 8.** (A–C) Photos of a sequence of predation of a *Geckolepis* specimen (probably *G. modesta*) in Ankarafantsika National Park by the snake *Mimophis occultus*. Photographed 13 November 2022. (D–E) Predation of a *Geckolepis* specimen (species identity uncertain) by the snake *Dromicodryas bernieri*. Photographed by Freddie Walker at Mariarano on 11 July 2025.

Deficient, while Scherz *et al.* (2017) propose a status of Near Threatened for *G. megalepis*. The confirmation of widespread occurrence of *G. maculata*, *G. polylepis*, *G. typica* and probably also *G. modesta* and *G. humbloti*, would be in agreement with categorizing them as Least Concern. This is supported by their regular occurrence in secondary habitats, even human habitation, which we can confirm for genetically identified *G. polylepis* (Salines de Tsangajoly near Morondava), *G. maculata* (on Nosy Be and Andranofotsy), and *G. typica* (Antsokay Arboretum near Toliara). The situation may, however, be different for species restricted to karstic massifs such as Ankarana where *G. megalepis*, but also *G. spania* and lineage G of *G. bekira* appear to be microendemic to very small ranges, similar to other karst-specialized Malagasy geckos of the genera *Blaesodactylus*, *Lygodactylus*, and *Paroedura* (e.g., Jono *et al.* 2015; Köhler *et al.* 2019; Vences *et al.*

2022a). A status of Near Threatened (as suggested by Scherz *et al.* 2017) therefore appears to be justified for the *Geckolepis* species restricted to Ankarana.

#### *Cost-effective museomics for taxonomy*

In previous studies based on targeted enrichment barcode fishing, we found mitochondrial rRNA sequences (i.e., *16S*) more commonly represented in the datasets than mitochondrial protein coding genes (i.e., *cox1*, *cob*) (Rancilhac *et al.* 2020; Scherz *et al.* 2022). Here, we did not observe such a bias, and in fact sometimes found a higher coverage for the protein-coding *nd4* (Table 1). We suspect that the more variable mitochondrial protein-coding genes are less effectively recovered in targeted enrichment when the baits used do not fully correspond to the respective DNA sequence of the target. In contrast, in our *Geckolepis* study, we performed BLAST searches

with all lineages represented in the reference database, and therefore could recover the reads matching all three mitochondrial genes with similar success.

The numbers of reads matching *Geckolepis* references were quite low in our study (Table 1), yet the resulting assemblies placed the respective type specimens unambiguously and with high support into the respective lineages in the concatenated analysis. Our confidence in the accuracy of these results is supported by the following points: (i) exploratory single-gene trees (not shown) did not reveal any discrepancies regarding the placement of these specimens, (ii) in *G. polylepis* and *G. modesta* multiple sequenced type specimens clustered directly next to each other in the tree, and (iii) the placement of these specimens in no case is contradicted by morphological or biogeographic evidence. This suggests that even low-coverage shotgun sequencing, as performed here, has the potential to provide a reliable placement of historical types into mitochondrial lineages if interpreted cautiously.

Unsurprisingly, our simple shotgun sequencing approach, without previous targeted enrichment steps, was unsuccessful in recovering reads matching the two single-copy nuclear encoded gene fragments *RAG-1* and *C-MOS*. This is a dilemma, given the shortcomings of mtDNA as sole evidence in taxonomy and phylogenetics (Wüster 2025). Obtaining reliable evidence from nuclear DNA sequences from poorly preserved collection material is possible (Bi *et al.* 2013; Staats *et al.* 2013; Ruane & Austin 2017) but requires either much deeper sequencing than applied here, or laborious and sometimes expensive targeted enrichment steps, both of which are obstacles to their large-scale use in species-rich taxa.

Interestingly, we found a relatively high number of reads matching a fragment of the nuclear 18S rRNA gene. The nuclear 18S and 28S rRNA genes are commonly used as DNA barcode markers across eukaryotes (Pawlowski *et al.* 2012) and can be particularly useful for DNA metabarcoding of environmental samples (Machida *et al.* 2017). Probably due to the high copy number and tandem repeat nature of nuclear rRNA genes, these can be reliably recovered in low-coverage genome skimming (Hoban *et al.* 2022), as we showed herein also for *Geckolepis*. Although the 18S assemblies obtained for *Geckolepis* (<https://doi.org/10.5281/zenodo.21984699>) were largely uninformative due to a very small number of substitutions among species, we encourage a renewed focus on the somewhat more variable (e.g., Lv *et al.* 2020) 28S gene, which was suggested as DNA taxonomy marker by Tautz *et al.* (2003) and which could complement mitochondrial barcodes in assigning animal type specimens, including vertebrates, to genetic lineages. In fact, recent suggestions for a more universally applicable mixed bait set (Agne *et al.* 2022) also included nuclear rRNA baits usable for diverse animal groups, including both invertebrates and vertebrates.

Museum and herbarium collections have historically been invaluable for documenting phenotypic diversity and distinguishing species based on morphological traits, representing the foundation for taxonomy, comparative anatomy, and evolutionary studies. The rise of museomics

(Fig. 1) is adding new significance to these collections as molecular archives, underscoring their immense value for research within a comprehensive natural collections framework that embraces all data and knowledge derived from specimens (Sigwart *et al.* 2025). As an emerging field of research, museomics has opened new avenues for addressing scientific questions and has revolutionized biodiversity research (Fong *et al.* 2023) by adding a temporal perspective to the study of species' responses to environmental change while allowing us to include genetic information of rare or even extinct taxa (e.g. Hempel *et al.* 2022; Ernst *et al.* 2022; Stelbrink *et al.* 2024).

For decades, DNA analyses of museum specimens relied largely on dry-preserved specimens because the genetic information of wet-preserved specimens, particularly formalin-exposed material, has been considered inaccessible due the chemical alteration of their DNA (Raxworthy & Smith 2021; Holleley & Hahn 2025). Recent developments, however, have allowed us to deal with many of the hurdles imposed by this type of material. For instance, the application of ancient DNA methodologies, including optimized extraction chemistries, single-stranded library preparation, enzymatic removal of miscoding lesions, and damage-aware bioinformatic pipelines, has dramatically improved the recovery of genetic information from degraded samples (Gansauge *et al.* 2017; Lyra *et al.* 2020; Rancilhac *et al.* 2020; Straube *et al.* 2021). Several studies even reported the successful reconstruction of mitochondrial and nuclear genomes from formalin-fixed material, which represents a major leap forward in the genetic accessibility of museum specimens (Hykin *et al.* 2015; O'Connell *et al.* 2022; Hahn *et al.* 2022; Hahn *et al.* 2024).

Despite substantial technical progress over the past decade and a sharp increase in studies applying these approaches (Fig. 1), *museomics* remains a niche within taxonomy. Only a small—though steadily growing—fraction of the roughly 15,000–20,000 species newly described each year are delimited and diagnosed using molecular data (Miralles *et al.* 2020), and only a few of these studies employ museomic methods. The bottlenecks to access DNA of historical collection specimens are no longer primarily methodological but of logistical and budgetary nature: only a small number of institutions possess laboratory facilities meeting the standards for working with degraded samples (Fulton & Shapiro 2019) or at least have access to cryogenic storage for tissues of highly valuable specimens, e.g. name-bearing types, to prevent their DNA from further degradation until appropriate infrastructure becomes available. This huge gap in infrastructure and expertise is made even worse by the absence of service providers, as none among the multitude of commercial lab work or sequencing companies offers a museomics workflow. Particularly in the Global South, museums often lack both the infrastructure for optimal preservation of collection material and financial means to visit such academic laboratories or even to send samples abroad for processing.

Alongside logistical hurdles, museomics faces an imbalance between the ambition to generate large-scale



datasets and the practical needs and affordability for a broad scientific community. While whole-genome sequencing is now technically feasible, it remains costly and often exceeds what is necessary, particularly for taxonomic revisions. In taxonomic research, museum collections play a pivotal role, as they house name-bearing type specimens that serve as the physical reference for described species. By linking molecular data of these type specimens to genetically distinct lineages, museomics enables the identification of morphologically cryptic species and to the resolution of uncertainties in the allocation of historical species names (e.g. Scherz *et al.* 2022, Vences *et al.* 2024a, Petzold *et al.* 2025).

When designing a museomics-based research strategy for a taxonomic project, two goals must thus be balanced: (i) obtaining DNA barcodes to assign types and other collection material to genetic lineages identified from fresh collections, and (ii) incorporating the collection material in species delimitation or phylogenetic analyses. As long as the targeted type specimens are not thought to originate from geographical areas where hybridization and introgression is suspected, a focus on organellar (in animals: mitochondrial) DNA is usually sufficient. We here propose that genome skimming, i.e., a protocol combining (i) specialized DNA extraction optimized for historical specimens, (ii) high-throughput shotgun sequencing of single-stranded libraries, (iii) targeted BLAST searches for DNA fragments matching mitochondrial (and nuclear ribosomal) barcodes of the lineages identified from fresh samples, and (iv) reference-guided assembly of the (often incomplete) barcode sequences from the historical material, is often the most cost-effective and straightforward approach to assign historical specimens to lineages. It represents a more modest flavor of traditional “genome skimming” which usually aims at obtaining full mitochondrial or plastid genomes (Dodsworth 2015; Forrest *et al.* 2019).

In contrast, for convoluted hybridizing species complexes, at least some nuclear-encoded markers may be crucial. If obtaining nuclear markers from collection material is desired, reduced-representation strategies, such as the target enrichment approach (see Ferrari *et al.* 2023 for more examples), provide a scalable and cost-effective approach to generating nuclear datasets for reconstructing the evolutionary history of species. These methods have been shown to be effective in “barcode fishing” of specific mitochondrial markers from degraded DNA (Scherz *et al.* 2020; Vences *et al.* 2021), and bait sets for animal collection material exist that also integrate nuclear ribosomal genes (Agne *et al.* 2022). For modern material, several universal bait sets for targeted enrichment of large numbers of nuclear-encoded genes exist, e.g. SqCL for squamates (Singhal *et al.* 2017) and FrogCap for anurans (Hutter *et al.* 2022). Comparable resources for degraded material from museum specimens are still lacking, and could be developed by using shorter baits and denser tiling to account for DNA fragmentation, and by focusing on fewer loci that provide sufficient taxonomic resolution at the species level while ensuring compatibility across taxa and with earlier data sets, as e.g.

in the 117 “legacy markers” included in FrogCap (Hutter *et al.* 2022), the Rapidly Evolving Long Exon Capture (RELEC) loci suggested by Karin *et al.* (2020) or the universal single-copy ortholog (USCO) genes applicable across metazoans (Dietz *et al.* 2023, 2024). Even a small number of well-chosen nuclear loci may be sufficient to resolve species-level distinctions and estimate divergence times, particularly when complemented by more extensive datasets from modern specimens (e.g., Karin *et al.* 2020). Building on this, modular systems offering sets of 10, 20, or 50 markers would allow researchers to select loci according to their specific questions and budget, making museomic approaches more accessible to a broader scientific community. Given how underexplored museum collections remain at the molecular level, it would indeed be more effective in terms of time, computation, and costs to expand taxonomic and geographic coverage, rather than generate hundreds or thousands of markers for a few focal taxa.

In conclusion, despite the major methodological advances over the past decade, museomics continues to face logistical and structural challenges. Progress now is highly dependent on improving transparency, training, and infrastructure, for instance by establishing dedicated service facilities, offering workshops and training, and integration of museomics components into museum digitization initiatives. Large-scale digitization efforts should not only encompass high-quality imaging and scanning of specimens associated with metadata gathered from labels and catalogues, but further be extended by molecular data, linking physical specimens to sequence data in public databases. Only by expanding expertise, infrastructure, and accessibility, can the scientific community fully harness museum collections as comprehensive spatiotemporal genomic archives, capable of shedding light on evolutionary processes and documenting biodiversity change across centuries.

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