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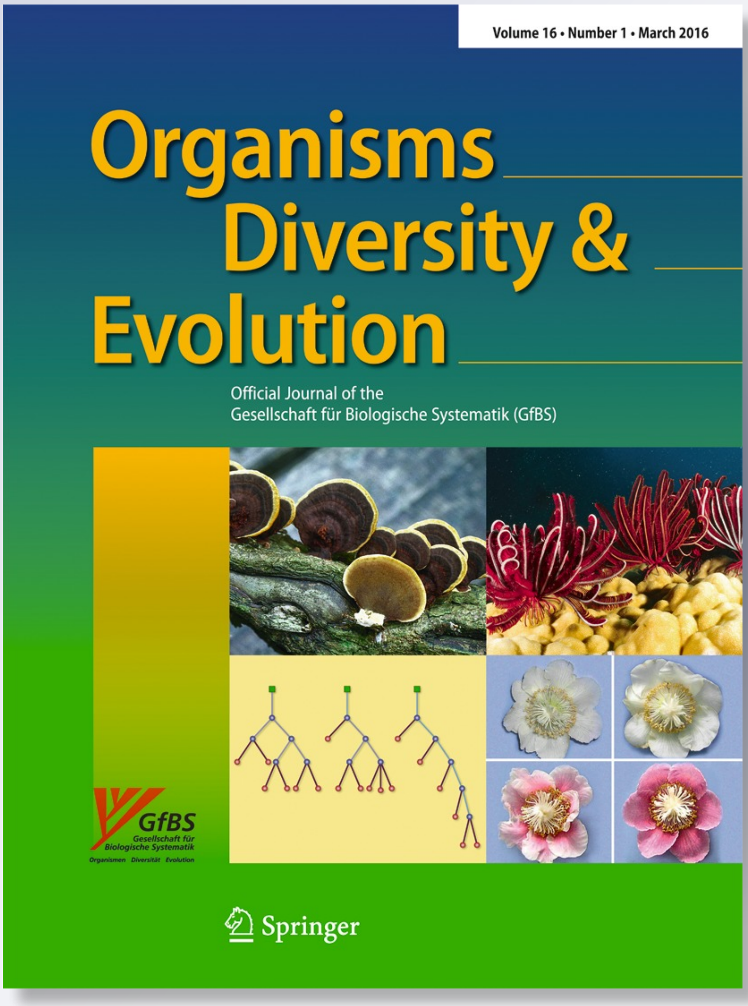

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ORGANISMS DIVERSITY & EVOLUTION

ORIGINAL ARTICLE

Resurrection of the Comoran fish scale gecko *Geckolepis humbloti* Vaillant, 1887 reveals a disjunct distribution caused by natural overseas dispersal

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Abstract Fish scale geckos (*Geckolepis*) are taxonomically poorly resolved, mainly because of the difficulty of applying standard morphological characters to diagnose taxa. Three species, *Geckolepis maculata*, *G. polylepis*, and *G. typica*, are currently recognized from Madagascar and the Comoro Islands. Molecular studies suggested a number of operational taxonomical units within the *G. maculata* complex, but none of these has been formally described. The Comoran population was described as *Geckolepis humbloti* Vaillant 1887 but later synonymized. Prior to our study, no genetic data and little other information were available for this taxon. We revised the status of *G. humbloti* using molecular genetics, external morphology, and osteological characters retrieved from 3D skeletal models created using micro-computed tomography (micro-CT). Our results demonstrate that *G. humbloti* represents a genetic lineage strongly distinct from all other *Geckolepis* species. It is furthermore distinguished by a combination of external morphological characters and probably by osteology. We therefore resurrect *G. humbloti* Vaillant, 1887 from synonymy with *G. maculata*. Remarkably, this lineage is not restricted to the Comoros: A specimen from Tsingy de Bemaraha in western Madagascar falls as a closely related sister lineage to all Comoran *Geckolepis* in our molecular phylogenetic analysis and is osteologically almost identical with a specimen from the type locality Grand Comoro. We therefore include it in *G. humbloti*. The phylogenetic topology and the intraspecific genetic divergences suggest that the Comoros were colonized naturally from western Madagascar by overseas dispersal. *G. humbloti* is not considered as threatened, but its presence is indicative of natural or near-natural habitats.

Keywords Biogeography · Madagascar · Mayotte · Reptiles · Taxonomy · Tsingy de Bemaraha

Introduction

The Malagasy faunal region has long been in the focus of herpetological interest because of its diversity and the high degree of endemism of its species and genera (Goodman and Benstead 2003). As a result, even many comparatively species-rich groups are now well studied in terms of their taxonomy, phylogeny, and biogeography. This is especially true for many gecko genera such as *Phelsuma* (Rocha et al. 2007, 2009), *Uroplatus* (Greenbaum et al. 2007; Raxworthy et al. 2008; Ratsoavina et al. 2012, 2015), and *Paroedura* (Jackman et al. 2008; Hawlitschek and Glaw 2013). In contrast, the genus *Geckolepis*, despite its apparent low diversity, is widely considered a taxonomically difficult group. The genus is widespread on Madagascar and the Comoro Islands, but the morphology of most populations is highly variable and biogeographically puzzling. As in most other geckos, scale characters play a major taxonomic role, but studying them is difficult in *Geckolepis* because of the remarkable tendency of these geckos to shed their scales when captured (Schubert et al. 1990). Consequently, there are only two modern studies on the genus. The work by Köhler et al. (2009) takes a purely

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morphological approach to taxonomy, whereas Lemme et al. (2013) integrate a molecular phylogeny with morphological analyses but call their results "preliminary," with the "aim to highlight inconsistencies and agreement between morphological and molecular data." Therefore, the taxonomy and phylogeny of *Geckolepis* are far from resolved, and there is urgent need of further studies.

Köhler et al. (2009) recognized only three valid species of *Geckolepis*: *Geckolepis typica* Grandidier 1867, the type species of the genus, *Geckolepis maculata* Peters 1880, and *Geckolepis polylepis* Boettger 1893. Based on their morphological data, Köhler et al. (2009) considered *Geckolepis petiti* Angel, 1942; *Geckolepis typica anomala* Mocquard 1909; and *Geckolepis typica modesta* Methuen and Hewitt 1913, to be junior synonyms of *G. typica*, and *Geckolepis humbloti* Vaillant, 1887 as a junior synonym of *G. maculata*. Because of the limited genetic sampling available, correlating these synonyms with samples included in DNA studies was not yet possible. Lemme et al. (2013) found that *G.* "*maculata*" comprises a number of distinct genetic lineages but did not make any taxonomic changes. The only taxon that can be clearly delimited biogeographically is *G. humbloti*, whose type locality is on the Comoro Islands (Grand Comoro) instead of Madagascar. Köhler et al. (2009) studied the type series (three specimens) of *G. humbloti* but did not find any significant differences to *G. maculata*, and therefore followed Angel (1942) in treating *G. humbloti* as conspecific with *G. maculata*. Lemme et al. (2013) did not include DNA of Comoran *Geckolepis* in their study.

A DNA barcoding study of Hawlitschek et al. (2013) showed a deep divergence in the barcodes of *Geckolepis* from the Comoros and *G. maculata* from Madagascar as well as some haplotype diversity within Comoran *Geckolepis*, suggesting that the Comoran populations of *Geckolepis* resulted from natural colonization and not from recent human-mediated introduction. The Comoros are an archipelago of four oceanic islands of volcanic origin, Anjouan, Grand Comoro, Mayotte, and Mohéli, situated between the north tip of Madagascar and the East African coast, at distances of ~300 km to both of these landmasses. Currently, 30 species of terrestrial squamate reptiles are recognized from the Comoros, 18 being considered native (16 endemic) to the archipelago (Hawlitschek et al. 2011, 2012; Hawlitschek and Glaw 2013). The majority of introduced and native species are most closely related to taxa from Madagascar. Within Madagascar, the phylogenetic sister taxa of some Comoran species are found in the north (*Amphiglossus*: Hawlitschek et al. 2013, *Paroedura*: Hawlitschek and Glaw 2013, *Phelsuma v-nigra*: Rocha et al. 2007, 2009). This part of Madagascar is geographically closest to the Comoros, and dispersal is easily explainable by marine currents (Louette et al. 2004). Other species have the closest affinities to west Madagascar (*Oplurus*: Münchenberg et al. 2008, *Phelsuma comorensis*, *Phelsuma nigristriata*: Rocha et al. 2009). *Geckolepis* is widespread at low altitudes and drier climates around Madagascar, and so far, no link could be established between the populations from the Comoros and any Malagasy population from a specific region.

Our study has the following objectives: (1) to revise the taxonomy of Comoran *Geckolepis* and test the validity of *G. humbloti*, (2) to detect the phylogenetic affinities of Comoran *Geckolepis*, and (3) to test the hypothesis that *Geckolepis* colonized the Comoros by natural means, and discuss possible biogeographical explanations for this scenario.

Material and methods

Tissue samples of specimens collected in the field were stored in 96 % ethanol. We extracted DNA using the standard protocol of the Macherey & Nagel NucleoSpin® 96 Tissue kit and edited the chromatogram data in Sequencher 4.9. We amplified the mitochondrial 12S and ND4 markers following Lemme et al. (2013) using protocols for PCR amplification and DNA sequencing described in Hawlitschek et al. (2012) with an annealing temperature of 50 °C for 12S. Sequence data were deposited in GenBank and are available under accession numbers KT823681 to 692 and KT878502 to 512. Locality data is published in Hawlitschek et al. (2011) with further data from Hawlitschek and Glaw (2014).

Alignments of the sequences generated for this project, together with GenBank sequences from Lemme et al. (2013) for *G. maculata*, *G. typica*, and *G. polylepis* (12S only) were created in Geneious® 7.1.7 using the built in Geneious alignment tool. *Blaesodactylus antongilensis* (the clade of *Blaesodactylus* + *Homopholis* is the sister group to *Geckolepis* according to Gamble et al. (2012)), *Paroedura stumpffi*, *Phelsuma lineata*, and *Uroplatus lineatus* were added as outgroups to root the tree. The alignments were quality checked in Mesquite (Maddison and Maddison 2015). Phylograms were constructed using the concatenated dataset of 1256 bp and the maximum likelihood (ML) method in RaxML GUI vers. 1.3 (Silvestro 2012; Stamatakis 2014) under the GTR+G model with 1000 bootstrap repeats. GTR+G was chosen because GTR is the most general and efficient substitution model, and GTR+G incorporates rate heterogeneity, which makes estimating the proportion of invariables sites unnecessary (Stamatakis 2015). To avoid overparametrization of the mitochondrial dataset, we did not partition by codon positions (following Lemme et al. 2013). We then used the Species Delimitation plugin v.1.03 (Rosenberg 2007; Ross et al. 2008) provided for Geneious® 7.1.7 to analyze phylogenetic support for a priori user-defined taxonomic units (i.e., *G. maculata* complex, *G. typica*, *G. humbloti*, and *G. polylepis*) based on the k2p model.

All morphological measurements of voucher specimens were taken by MDS using a digital caliper (0.01-mm precision) to the nearest 0.1 mm. Scale characters were studied using a stereomicroscope, excluding specimens whose integument was severely damaged. All characters studied in Köhler et al. (2009) and Lemme et al. (2013) were analyzed. A list and an explanation of all characters are given in Appendix A1 (supplementary file). Out of this total of 29 characters, the following 7 were selected for further analysis either because they are recognized as standard or because of their high diagnostic value: snout–vent length (SVL), number of scale rows around midbody (MBS), number of ventral scale rows from postmentals to vent (VS), number of infralabials (ILAB), number of supralabials (SLAB), the number and fraction of infralabials to level of the anterior margin of the eye (IFL), and rostral and mental scale conditions according to the classification used in Köhler et al. (2009). All specimens examined are deposited in the Zoologische Staatssammlung München, Germany (ZSM) (urn:lsid:biocol.org: col:34660) and the Zoologisches Museum, Berlin, Germany (ZMB) .

Two specimens, ZSM 81/2006 and ZSM 80/2010, were scanned using X-ray micro-computed tomography (micro-CT) for osteological comparisons and to produce 3D skeletal models. Scanning was performed on a nanotom m (GE Measurement & Control, Wunstorf, Germany) cone-beam micro-CT machine using a diamond target. Specimens were scanned in a plastic housing using a polystyrene base and braced in position using polystyrene and small wooden struts, with a few ml of alcohol in the bottom of the vessel to achieve air saturation. Full body scans were performed for 18 min, using 1440 projections, at 140 kV and 80 mA. Skull-only scans were performed for 20 min, using 2440 projections, at 140 kV and 80 mA. Scans were visualized and examined in VGStudio 2.2 (Volume Graphics GmbH, Heidelberg, Germany). Skeletal surface meshes and 3D volumes were produced in Amira 5.4.5 (FEI Visual Sciences Group, Burlington MA, USA), and models prepared in Adobe 3D Toolkit (Adobe Systems Inc., San Jose CA, USA), and analyzed in Adobe Acrobat Pro 10.1.12. Osteological terminology follows that of Romer (1956), Daza et al. (2008, 2012), Russell and Bauer (2008), and Jerez et al. (2010). The scans were compared to an existing scan of the holotype of *G. maculata* (ZMB 9655) prepared by MDS. This scan will be published in an upcoming manuscript.

In an integrative taxonomic approach (modified from Miralles et al. 2011) we use two lines of evidence, mtDNA and morphology, for species delimitation, i.e., to test the taxonomic status of *G. humbloti*. In our study, the mtDNA line of evidence is met when an operational taxonomical unit (OTU) is represented in the phylogram by a recognizable cluster with support values of >80 % bootstrap support. The morphology line of evidence is met when an OTU can be distinguished from other OTUs by a combination of diagnostic characters. The only OTU analyzed here is the *Geckolepis* form from the Comoros which is compared to the previously recognized species *G. polylepis* and *G. typica*. Since the taxonomy of the *G. maculata* complex remains unclear, Comoran *Geckolepis* are only compared to the holotype of this species, using the values given in Köhler et al. (2009). While this does not provide any information on intraspecific variation (due to the difficulty of defining species here), the data are useful for differential diagnoses. Other OTUs within *G. maculata*, as studied in Lemme et al. (2013) are not analyzed here. Lemme et al. (2013) provide data of some morphological characters that can be used to differentiate Comoran *Geckolepis* from the OTUs within the *G. maculata* complex.

Results

Molecular phylogeny

The topology of the phylogenetic tree recovered from the ML analysis (Fig. 1) shows all specimens of *Geckolepis* from the Comoros as members of a highly supported monophyletic group. Most closely related to this group is the specimen ZSM 81/2006 from Tsingy de Bemaraha, Madagascar (TB). Within the Comoran clade, specimens from Mayotte and Anjouan each form a clearly supported clade and the single sequences included from the islands of Mohéli and Grand Comoro cluster separately as well. *G. polylepis* and *G. typica* are retrieved as monophyletic groups with high bootstrap support. All other *Geckolepis* specimens are here assigned to the *G. maculata* complex and form a poorly supported clade with subdivisions mostly congruent to the topologies retrieved by Lemme et al. (2013). The relationships between *Geckolepis* from the Comoros + TB, *G. polylepis*, *G. typica*, and the *G. maculata* complex are also poorly resolved.

The genetic k2p distances between species are much larger than the intraspecific distances of any species (see Table 1 and Fig. 2). The largest intraspecific divergences are found in the taxonomically unresolved *G. maculata* complex. The intraspecific divergences between the island populations fall within the ranges found in *G. polylepis* or *G. typica* (approximately 0.01–0.03 in 12S and 0.04–0.07 in ND4). The TB specimen is clearly more divergent from the Comoran populations (0.03 in 12S and 0.11 in ND4) than any of these populations among one another.

Morphological analyses

A morphological comparison of *Geckolepis* species is given in Table 2. A full list of all characters examined for every individual is given in Appendix A1. The morphological distinction of all *Geckolepis* species recognized by Köhler et al. (2009) and of Comoran *Geckolepis* is difficult, as most

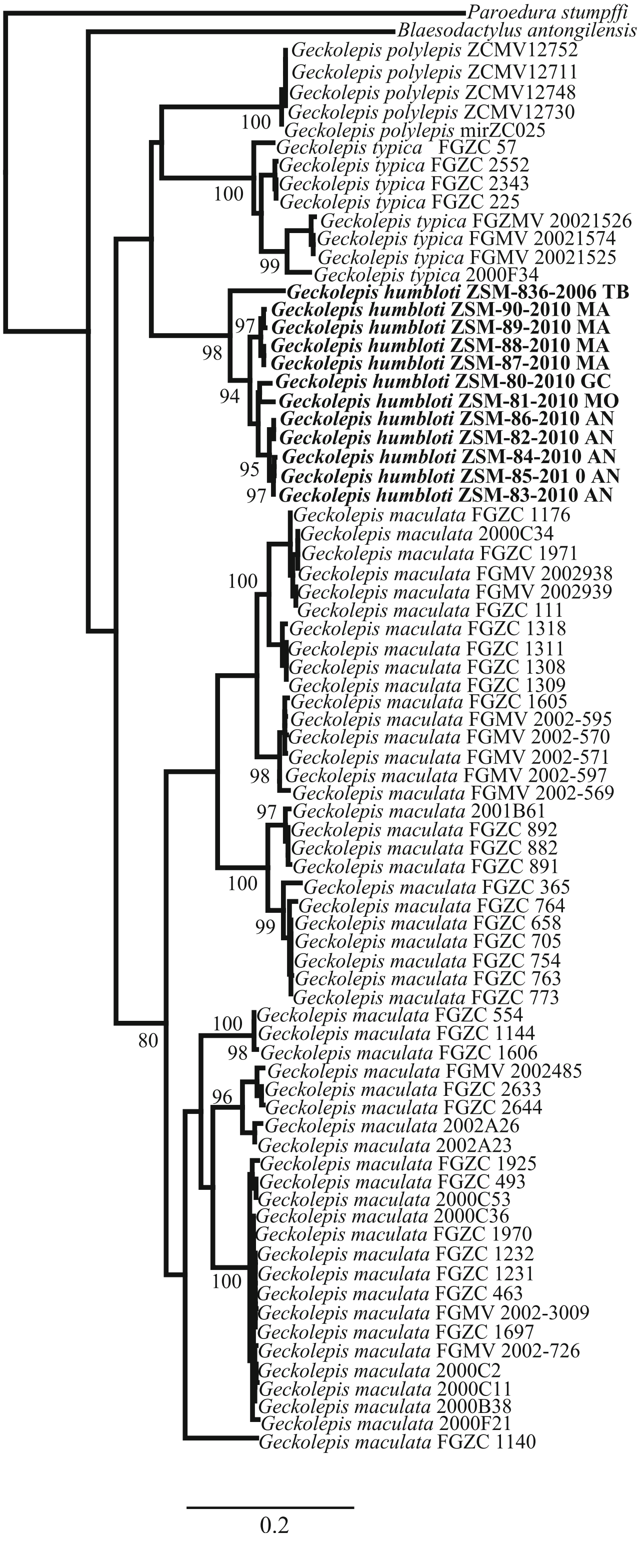

Fig. 1 Results of the molecular genetic analysis of *Geckolepis*. The maximum likelihood tree is based on 12S and ND4 sequences. Only closely related outgroups are shown. Support values of 1000 bootstrap repeats are given below nodes. *TB* Tsingy de Bemaraha, *AN* Anjouan, *GC* Grand Comoro, *MA* Mayotte, *Mo* Mohéli. All representatives of the taxonomically unresolved *Geckolepis maculata* complex are named *Geckolepis maculata* in the tree

measurements and scale counts are highly overlapping between the species. However, a distinction of species based on a combination of several characters is possible.

Comoran *Geckolepis* and the TB specimen resemble *G. maculata* most closely. Notably, the TB specimen shows the mental scale configuration F (sensu Köhler et al. 2009) with a small scale between the large mental scales, whereas all other studied individuals from the Comoros show configuration E with the mental scales in contact. Köhler et al. (2009) found configuration E in most and F in some specimens of the *G. maculata* complex, but no locality data were given. It is therefore possible that the specimens of Köhler et al. (2009) with configuration F belong to the same clade as the TB specimen studied here. In this case, the mental scale configuration could be considered a diagnostic character.

Taxonomy

The clade comprising all studied *Geckolepis* specimens from the Comoros Archipelago + the single TB specimen from Madagascar (Fig. 3) forms a clearly delimited and well-supported cluster and is distinguished from all previously recognized species of the genus by a combination of morphological characters (Table 2). Therefore, the two lines of evidence for species delimitation are met, and we recognize the clade as a species distinct from all *Geckolepis* species recognized in Köhler et al. (2009) and Lemme et al. (2013). The clade includes specimens from the type locality of *G. humbloti* (Grand Comoro), which is therefore resurrected.

Diagnosis of *Geckolepis humbloti* Vaillant, 1887

Geckolepis humbloti is a species of *Geckolepis* that differs from all other species of the genus by the following combination of characters (see also Table 2): maximum SVL (65.3 mm), number of scale rows around midbody (22–30), number of infralabials to level of anterior margin of eye (3.0–4.0), number of ventral scale rows from postmentals to vent (33–41), and mental scale condition—innermost pair of large postmental scales in contact with each other posterior to mental scale (type E) or separated by a median postmental scale (type F).

G. humbloti is distinguished from the holotype of *G. maculata* (ZMB 9655) by a combination of maximum SVL (65.3 vs. 58.5 mm) and number of ventral scale rows from postmentals to vent (33–41 vs. 32). The following osteological characters are also noted to differ between the skulls of two scanned *G. humbloti* specimens and that of ZMB 9655: anterolateral corner of nasal bulging laterally (versus straight), frontoparietal suture straight (vs. curved posteriorly), subolfactory process ventromedially closed (vs. not closed), frontoparietal suture straight (vs. curved posteriorly), and posterior edge of fused paired parietals curved (vs. more or less straight). These are to be considered "potentially diagnostic"

Table 1 Genetic k2p distances between *Geckolepis* species and populations of *G. humbloti*, calculated with the species delimitation plugin for Geneious 7.1.7

Species	12S			ND4		
	Closest	Intra	Inter	Closest	Intra	Inter
G. typica	*G. humbloti*	0.028	0.166	*G. humbloti*	0.065	0.192
G. polylepis	*G. humbloti*	0.008	0.103	n/a	n/a	n/a
G. humbloti	*G. polylepis*	0.013	0.103	*G. typica*	0.063	0.192
G. maculata complex	*G. humbloti*	0.087	0.166	*G. typica*	0.113	0.209
G. humbloti TB	*G. humbloti* Anjouan/Mayotte	0.00	0.03	*G. humbloti* Anjouan/Mayotte	0.00	0.11
G. humbloti Grand Comoro	*G. humbloti* Mohéli/Anjouan/Mayotte	0.00	0.01	*G. humbloti* Anjouan	0.00	0.04
G. humbloti Mohéli	*G. humbloti* Grand Comoro/Anjouan	0.00	0.01	*G. humbloti* Mayotte	0.00	0.04
G. humbloti Anjouan	*G. humbloti* Grand Comoro/Mohéli	0.00	0.01	*G. humbloti* Grand Comoro	0.01	0.04
G. humbloti Mayotte	*G. humbloti* Grand Comoro	0.01	0.01	*G. humbloti* Mohéli/Anjouan	0.01	0.04

For each cluster (species or population), all data is given for the 12S and the ND4 marker

Closest most closely related cluster, *Intra* largest intra-cluster divergence, *Inter* largest inter-cluster distance to closest relative

until further data on the variation of osteology in these taxa are available.

G. humbloti is distinguished from *G. typica* by a combination of the following characters: maximum SVL (65.3 vs. 57 mm), number of scale rows around midbody (22–30 vs. 26–36), number of ventral scale rows from postmentals to vent (33–41 vs. 37–48), number of supralabial scales (5–7 vs. 6–7), number of infralabial scales (4–6 vs. 5–7), and arrangement of the postmental scales posterior to the mental scale (types E, F vs. types A, B, C, D).

G. humbloti is distinguished from *G. polylepis* by a combination of the following characters: maximum SVL (65.3 vs. 51 mm), number of scale rows around midbody (22–30 vs. 30–37), number of ventral scale rows from postmentals to vent (33–41 vs. 40–50), and number of infralabials to level of the anterior margin of the eye (3.0–4.0 vs. 4.0–5.5).

Osteological description

We compare the osteology of *Geckolepis humbloti* with CT models of the holotype of *G. maculata* (ZMB 9655). Most diagnostically valuable characters are situated in the skull. We therefore present a description only of the skull, along with PDF-embedded interactive 3D models in Appendix A2. Rather than a full osteological description, which is planned for a future publication (Scherz et al. in prep.), we give a summary of features that are noted to differ between *G. maculata* and *G. humbloti*. PDF-embedded 3D models of the full skeletons of the scanned specimens are presented in Appendix A3. We also include notes on differences between *G. humbloti* from Madagascar (ZSM 81/2006) and the type locality, Grand Comoro (ZSM 80/2010). The condition for *G. maculata* is noted in parentheses when differing.

Skull (Fig. 4 and Appendix A3)

Calcium sacs of both specimens are large and extremely dense (by far, the most X-ray absorptive structures in the body), overlapping the squamosal in ZSM 80/2010, leaving only limited space for the stapes. This specimen also has large,

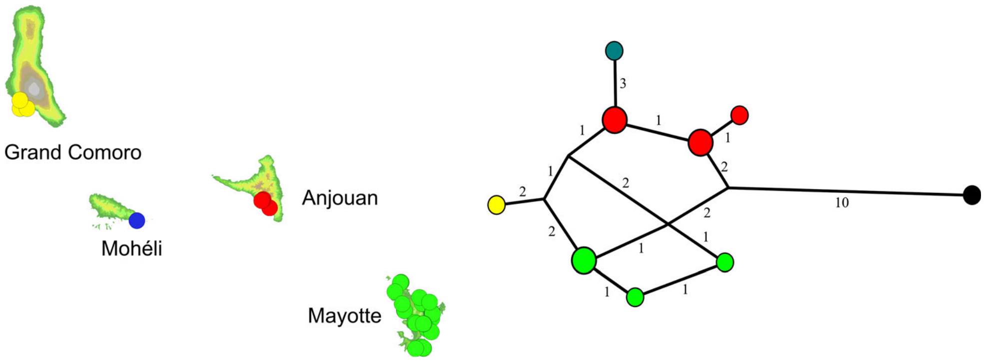

Fig. 2 Parsimony haplotype network of *G. humbloti* based on 12S sequences. *Colors* code for sampling localities: *red* = Anjouan, *blue* = Mohéli, *green* = Mayotte, *yellow* = Grand Comoro, *black* = Madagascar (Tsingy de Bemaraha). *Sizes of circles* represent numbers of identical haplotypes (1 or 2); *numbers* along branches indicate mutation steps (color figure online)

Table 2 Diagnostic morphological characters of *Geckolepis*

	G. humbloti (Comoros)	*G. humbloti* (TB)	*G. maculata* (holotype)	*G. typica*	*G. polylepis*
Max. SVL (mm)	65.3	47.9	58.5	57	51
MBS	22–30	28	25	26–36	30–37
VS	33–41	36	32	37–48	40–50
ILAB	4–6	5	7	5–7	7
SLAB	5–7	6	8	6–7	8
IFL	3.0–4.0	3.5	4.1	2.8–4.6	4.0–5.5
PM	E	F	E	A, B, C, D	E

SVL snout–vent length, *MBS* number of scale rows around the midbody, *VS* number of ventral scale rows from postmentals to vent, *ILAB* number of infralabials, *SLAB* number of supralabials, *IFL* the number and fraction of infralabials to level of the anterior margin of the eye, *PM* rostral and mental scale conditions according to the classification used in Köhler et al. (2009)

dense structures (presumably additional calcium reservoirs) dorsally to the sphenoid and medially to the epipterygoids, which contact the ventrolateral surface of the *crista alaris*. Premaxilla possessing approximately 8 teeth; maxilla bearing approximately 31–35 teeth, with some gaps where teeth have been lost or are not resolved by CT scan; anterolateral corner of nasal bulging laterally (straight in *G. maculata*); ectopterygoid not participating in lacrimal foramen; vomer possessing a medial anterior fenestra; maxilla-prefrontal suture straight in ZSM 81/2006 (more so than any other *Geckolepis* thus-far examined), but strongly curved in ZSM 80/2010 (as in *G. maculata*); subolfactory process ventromedially closed (not closed in the type specimen of *G. maculata*, but possibly damaged); frontoparietal suture straight (more so in ZSM 80/2010 than ZSM 81/2006; posteriorly curved in *G. maculata*); posterior edge of paired parietals curved (more or less straight in *G. maculata*); postparietal process long and thin, dorsolaterally contacted by squamosal (as in *G. maculata*); squamosal in contact with postparietal process anteriorly and paroccipital process posteriorly, almost in contact with dorsal-most end of quadrate ventrally. Coronoid process of mandible dorsally boxy in ZSM 80/2010 and rounded in ZSM 81/2006; retroarticular process posteriorly broad in ZSM 80/2010 and thin in ZSM 81/2006.

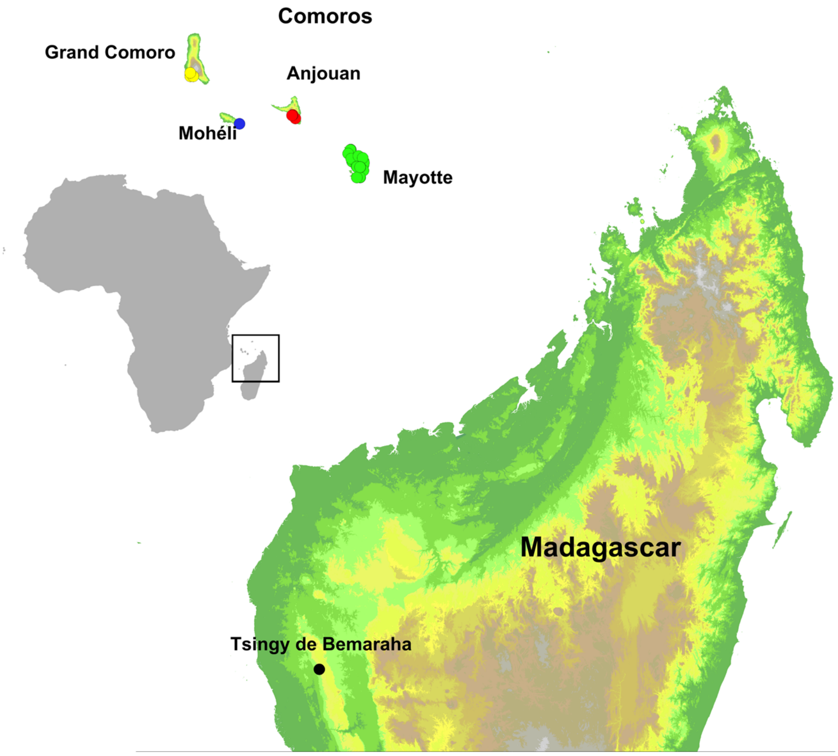

Fig. 3 Map created in QGIS 2.8.1 showing the known distribution of *Geckolepis humbloti*. *Colored dots* represent known localities: *Red* = Anjouan, *blue* = Mohéli, *green* = Mayotte, *yellow* = Grand Comoro, *black* = Madagascar (Tsingy de Bemaraha). In addition to the samples used in this study, locality data was taken from Hawlitschek et al. (2011), Hawlitschek and Glaw (2014), and Wang et al. (2015). Inlay shows the position in the context of the African continent

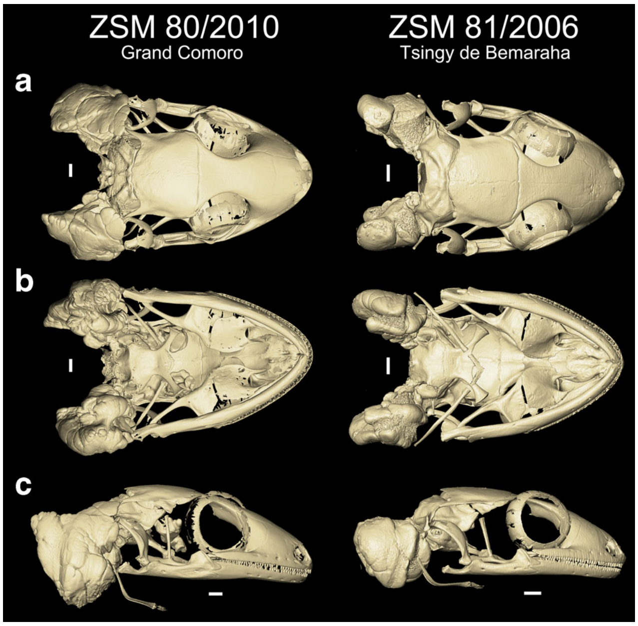

Fig. 4 The skulls of *Geckolepis humbloti* (ZSM 81/2006 and ZSM 80/2010) in **a** dorsal, **b** ventral, and **c** lateral view. Length of *bar* = 1 mm. See Supplementary A3 for PDF-embedded interactive 3D models of these skulls

Observations and conservation

G. humbloti was recorded on all four major islands of the Comoros Archipelago and at a single Malagasy locality at Tsingy de Bemaraha (Fig. 3). The majority of specimens were observed at night on trees or during the day in cavities or under bark (Fig. 5). Most localities were in areas of dry forest, shrub, or degraded dry forest at low elevation levels, as is typical for all species of the genus. On Mayotte, it has been found at high densities in mangrove forests (Wang et al. 2015). However, some specimens were observed at higher elevations of up to 632 m (ZSM 83–84/2010 on Anjouan), the highest known record of any *Geckolepis* species. Locally, *G. humbloti* can be abundant, and as many as eight individuals have been found beneath the bark of a single tree during the day. On Mayotte, one individual was observed on 11 Nov. 2014 after dusk, licking flowing sap off of an unidentified tree species, by MDS, C. Wang, and L. Montfort, as has recently been reported for another *Geckolepis* species from Ankarafantsika (Jono 2015).

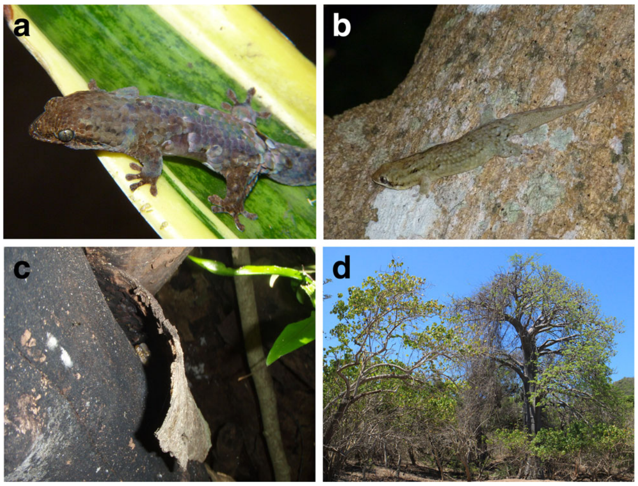

Fig. 5 Photos of *Geckolepis humbloti*. **a** ZSM 80/2010 from the type locality Grand Comoro. Lateral scales were shed in a defensive reaction during capturing. **b** ZSM 84/2010 at 632 m near Pomoni, Anjouan, the highest recorded locality of any *Geckolepis*. **c** ZSM 1699/2008 at Choungui, Mayotte, in a natural hiding place under the bark of a tree. **d** Habitat of *G. humbloti* at the dry forest of Saziley, Mayotte

The geographic distribution of *G. humbloti* is apparently rather restricted. The terrestrial area of the Comoros Archipelago is ~2000 km^2, and the restricted Malagasy distribution around Tsingy de Bemaraha is most likely less than 1000 km^2. Therefore, the extent of occurrence (EOO) of *G. humbloti* is below the threshold of 5000 km^2 for the status of Endangered according to the criteria of IUCN (2001). A number of potential threats, such as deforestation, collecting of dead wood for fire, and possibly invasive species, might affect the species in its natural habitats. However, *G. humbloti* has been observed also in degraded forest areas and in syntopy with invasive *Hemidactylus* species (Hawlitschek et al. 2011; Hawlitschek and Glaw 2014) and there is no evidence for any immediate threat of the habitats by severe degradation or fragmentation. Based on this rationale, *G. humbloti* (under the name of *G. maculata*) was evaluated as Least Concern in the regional Red List of Mayotte (IUCN France 2014) and we propose the same status for the global IUCN Red List.

Despite its relative abundance at least in parts of its range, *G. humbloti* was proposed as a protected species and as a determinant species for monitoring zones in Mayotte (Hawlitschek and Glaw 2014). This was done because the species is most common in mangroves, dry forests and shrubs, and natural mesic forests and is therefore highly indicative of natural habitats. Furthermore, *G. humbloti* has been observed much less frequently in other parts of its habitat (Grand Comoro, Mohéli; Hawlitschek et al. 2011) and may be affected by threats more strongly there.

Discussion

Our results do not allow the clear determination of any phylogenetic position of *Geckolepis humbloti* within the genus. Like Lemme et al. (2013) we failed to find any clearly supported topology reflecting the relative phylogenetic positions of all *Geckolepis* species. The topology of the tree suggests a group including *G. typica*, *G. polylepis*, and *G. humbloti*, with the latter two species most closely related, and the *G. maculata* complex forming a distinct group. However, this is not statistically supported. Further studies with a larger molecular dataset will be required to solve this problem. The results still serve to demonstrate that *G. humbloti* represents an entity distinct from other clusters of *Geckolepis* specimens recognized as species. Unlike several of the OTUs detected by Lemme et al. (2013) within the *G. maculata* complex, *G. humbloti* forms a clearly distinct cluster with relatively large genetic divergences from congeners. This is supported by morphological characters and basically fits with the insular distribution of the species.

Most reptile species native to the Comoros show a genetic pattern that easily allows the distinction of island populations (Rocha et al. 2006, 2007; Hawlitschek et al. 2013). In *G. humbloti*, at least the populations from the islands of Mayotte and Anjouan form clusters, albeit with only very shallow genetic divergence in the genes so far investigated. There is clear divergence between all Comoran samples and the conspecific Malagasy TB specimen. This, together with a certain genetic diversity (up to 5 % ND4 divergence between Comoran haplotypes), suggests that *G. humbloti* was not introduced to the Comoros by human activity but colonized the archipelago by natural overseas dispersal originating from western Madagascar. Alternatively, the re-colonization of Madagascar from the Comoros might be speculated (see discussion in Hawlitschek and Glaw 2013) but in this case, we would expect the TB specimen to be nested within the Comoran cluster. Instead, it forms the sister clade to all Comoran specimens, making this scenario unlikely. The low haplotype diversity within the Comoran population compared to other native species (Hawlitschek et al. 2013) suggests a relatively recent natural colonization of the Comoros Archipelago, similar to the skinks of the genus *Cryptoblepharus* (Rocha et al. 2006).

The distinctiveness of the TB specimen is furthermore underlined by the fact that it is not the only lineage of *Geckolepis* living in its area: Lemme et al. (2013) also registered *Geckolepis* from two OTUs at Tsingy de Bemaraha. Furthermore, they showed that more than 1 OTU may also be present in other areas (e.g., Ankarana and Montagne des Français). As already discussed by Lemme et al. (2013) the sympatric occurrence of several lineages of *Geckolepis* in various localities suggests that the population genetic structure of this group does not only follow a geographical pattern but that other factors, e.g., ecological ones, may also play a role. However, deeper studies of these patterns will only be possible with a robust taxonomy of the group and much more data on the distributions of the species.

Despite relatively short genetic distances, we noted numerous differences in the osteology of the skull of the TB specimen, relative to that of a topotypical specimen. These may be associated with age, ZSM 81/2006 from TB being considerably smaller than ZSM 80/2010 from Grand Comoro, but may also reflect either strong intraspecific variation or differences between the Comoran and TB populations of *G. humbloti*. Some of these differences were greater than those observed between any known species of *Geckolepis*, indicating comparatively high intraspecific variation in this clade, which on the whole has strongly conserved cranial osteology. A more detailed assessment of intraspecific variation and age-associated changes is clearly needed.

The current data indicate a localized present-day distribution of *G. humbloti* on Madagascar. It appears remarkable that the Comoros were colonized by this and not by one of the more widespread species. However, it might be reasonable to assume that additional sampling may show that *G. humbloti* occupies a larger area in Madagascar than currently known. The fact that the species so far was found only in the highly specific Tsingy karst habitat also makes a disjunct distribution in suitable areas across Madagascar conceivable. Alternatively, *G. humbloti* may have occupied a larger area in Madagascar in the past.

The TB specimen serves as evidence for the affinity of Comoran *Geckolepis* to the group of native Comoran reptile species with Western Malagasy affinities. Dispersal from northern Madagascar seems more easily explainable: Marine currents favor drifting toward the Comoros, and a line of now sunken islands that may have served as stepping stones existed between Madagascar and the Comoros until the late Pleistocene (Battistini and Cremers 1972; Louette et al. 2004). There is no evidence for such beneficial circumstances for the dispersal from western Madagascar to the Comoros in recent geological times, but nevertheless, there are a number of Comoran reptile species with western Malagasy origins, perhaps transported by the large rivers discharging in western Madagascar (Goodman and Benstead 2003). Notably, in two of these species, *Oplurus cuvieri* and *Phelsuma comorensis*, the Comoran haplotypes were found to be nested within Malagasy clusters (Rocha et al. 2007, 2009; Münchenberg et al. 2008) suggesting a relatively recent colonization of the Comoros. Genetic evidence for older dispersal to the Comoros from northern and western Madagascar is found in other species (Rocha et al. 2009; Hawlitschek and Glaw 2013).

The detection of another native Comoran reptile species is a further step toward completing the biogeographical picture of the Comoros and highlights the value of this archipelago as a study system for historical biogeography and evolution, in line with other groups of oceanic islands. The recognition of *G. humbloti* as a species native to the Comoros also has direct conservation implications. First, it enables a status of legal protection, which is not possible for introduced species, and second, it allows for the use of *G. humbloti* as a determinant species for protected areas. This is important because among native Comoran reptiles, *G. humbloti* is the species that is most characteristic for the remaining patches of pristine or near-natural dry vegetation (Hawlitschek et al. 2011; Hawlitschek and Glaw 2014). All these findings were possible only with an integrative taxonomic approach. Our study therefore serves as another example for the wide importance of taxonomic work and expertise not only for other fields of evolutionary biology, such as biogeography, but also for conservation.

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Compliance with ethical standards

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Conflict of interest The authors declare that no conflict of interest exists.

Ethical approval All animal handling was conducted according to guidelines approved by an ethics committee.

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