Genetic and morphological variation of the leaf-tailed gecko *Uroplatus fimbriatus* from Madagascar, with description of a new giant species

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Abstract. Data on mitochondrial DNA sequences, external morphology and hemipenis morphology suggest that the Madagascan gecko *Uroplatus fimbriatus* is a complex of at least two species. We therefore describe *Uroplatus giganteus* sp. n. from the mid-altitude rainforest of the Montagne d'Ambre National Park in northern Madagascar. It differs from *U. fimbriatus* by its larger size (snout-vent length up to 200 mm vs. 186 mm, total length up to 322 mm vs. 295 mm), hemipenis morphology, colouration of iris, head and back, and strong genetic differentiation (4.8 % pairwise sequence divergence in a fragment of the 16S rRNA gene between *U. fimbriatus* from Nosy Mangabe and *U. giganteus* from Montagne d'Ambre). Although the level of molecular, morphological and chromatic differentiation might warrant the specific distinctness of both taxa, the inclusion of a specimen from Marojejy National Park and a further one without locality data in the analysis indicates significant intraspecific differentiation of both species and suggests that even more taxa might be involved. Strong differences in the hemipenial structure of the two studied, clearly conspecific males of *U. giganteus* suggest seasonal hemipenial variation which, to our knowledge, has not been demonstrated for geckos so far. *Uroplatus giganteus* appears to be the second largest living gecko in the world and might be seriously threatened.

Key words. Reptilia, Gekkonidae, *Uroplatus*, new species, size variation, genetic differentiation, seasonal hemipenis variation.

Introduction

The genus *Uroplatus* is an unusual group of nocturnal geckos endemic to the forests of Madagascar and currently includes 11 species and one subspecies (Böhle & Schönecker 2003). Its largest representative, the bizarre leaf-tailed gecko *Uroplatus fimbriatus* was first mentioned in the 17th century (Flacourt 1658). Since then, the species has received remarkable attention both from scientists and hobbyists who mainly studied its morphology and anatomy (e. g. Boulenger 1885, Siebenrock 1893, Schmidt 1913, Haedge 1917, Wellborn [1933] (1997), Bopp 1953, Rittenhouse et al. 1997, Russell et al. 2000) or focused on its behaviour, keeping and breeding (Rothschild 1903, Braun 1913, Krapp 1963, Kästle 1973, Meier 1984, Rickert 1986, Henkel 1992, Anony-

mus 1993, Schmidt & Müller 1994, Baldwin 1997, Aresté & Farriols 1998, Svatek & van Duin 2002). *U. fimbriatus* is certainly among the most famous reptile species from Madagascar but despite its prominence, relatively little is still known about its biology, geographic distribution and variation (e. g. Bauer & Russell 1989, Böhme & Ibisch 1990, Glaw & Vences 1994).

For several years a giant *Uroplatus* form has been imported from Madagascar by the pet trade and has been shown or mentioned in a few publications (Love 2001a, 2001b, Svatek & van Duin 2002). Although its exact origin was unknown at first, it soon became clear that it occurs in the region of Montagne d'Ambre in northern Madagascar (Svatek & van Duin 2002). In the year 2004 we were able to collect several specimens with exact locality data in this National Park. This

so-called "white-eye-*fimbriatus*" strongly resembled *U. fimbriatus*, but differed in its larger size and the whitish colour of its iris. Although the difference in iris colouration is relatively easy to recognize, other morphological differences remained unknown, and the large size of this form (> 300 mm total length) was claimed to be reached also by *U. fimbriatus* from Nosy Boraha (e. g. Henkel 1992). To clarify the situation we studied the size variation of *U. fimbriatus* from different localities. In 2005, we furthermore visited the (neo)type locality of *U. fimbriatus*, the small off-shore island Nosy Mangabe in northeastern Madagascar, in order to obtain tissue samples from this topotypical population for comparative DNA studies with the "white-eye-*fimbriatus*" and other populations. The results of this study are described in the following.

Materials and methods

The geckos were collected at night by opportunistic searching. They were killed using chlorobutanol, fixed in 10 % formalin and preserved in 70 % ethanol. Museum acronyms used are UADBA (Université d'Antananarivo, Département de Biologie Animale), ZFMK (Zoologisches Forschungsmuseum Alexander Koenig, Bonn), and ZSM (Zoologische Staatssammlung, München). FGZC and ZCMV are field numbers of F. Glaw and M. Vences, respectively. Morphological measurements were taken to the nearest mm or nearest 0.1 mm: SVL (snout-vent length, defined as distance between snout tip and cloaca opening), TaL (tail length, from cloaca to tail tip, ToL (total length), HL (head length, from snout tip to posteriormost margin of ear opening), HW (maximum head width), HH (maximum head height posterior to the eyes), TaW (maximum tail width), NSD (nostril-snout tip distance, from anterior margin of nostril to snout tip), END (eye-nostril distance, from anterior margin of eye to posterior margin of nostril), ESD (eye-snout tip distance, from anterior margin

of eye to snout tip), NND (nostril-nostril distance), ED (horizontal eye diameter), EOD (horizontal ear opening diameter), EED (eye-ear distance, from posterior margin of eye to anterior margin of ear opening), SL (number of supralabials), IL (number of infralabials), FoL (forelimb length, from the axil to the tip of the longest finger), HiL (hindlimb length, from the cloaca to the tip of the longest toe), AGD (axilla-groin distance), WFH (maximum width of lateral fringes at head sides), WFF (maximum width of lateral fringes at flanks). Sex was determined by inspection of the tail base.

DNA was extracted with QIAmp tissue extraction kits (Qiagen) from muscle or toe tissue previously preserved in 98 % ethanol. For amplification we used the following primer pairs and sequencing conditions: 16SA (light chain; 5' - CGC CTG TTT ATC AAA AAC AT - 3') and 16SB (heavy chain; 5' - CCG GTC TGA ACT CAG ATC ACG T - 3') of Palumbi et al. (1991) amplified a ca. 540 bp section of the mitochondrial 16S ribosomal RNA gene; PCR cycling procedure was as follows: initial denaturation step: 90 s at 94 °C, 33 cycles: denaturation 45 s at 94 °C, primer annealing for 45 s at 55 °C, extension for 90 s at 72 °C. The sequenced fragment is homologous to positions 3976-4554 of the *Xenopus laevis* mitochondrial genome (Roe et al. 1985). PCR products were purified with QIAquick purification kits (Qiagen). We sequenced single-stranded fragments using ABI 377 and ABI 3100 automatic sequencers. Sequences were validated and aligned with the software Sequence Navigator (Applied Biosystems), and deposited in GenBank.

Voucher specimens and accession numbers are as follows: *Uroplatus giganteus* (paratypes ZSM 267/2004 [=FGZC 514], DQ857328; UADBA 27490 [=FGZC 516], DQ857330; and ZFMK 75753, DQ857329), *U*. cf. *giganteus* from Marojejy National Park (ZSM 55/2005 [=ZCMV 864], DQ857331), *U. sikorae* from Montagne d'Ambre (ZSM 264/2004 [=FGZC 508], DQ857326; ZSM 265/2004 [=FGZC 509], DQ857327), *U.*

Voucher number	Sex	locality	snout-vent length	total length
ZFMK 48169	M	Andambe, Fianarantsoa	168	–
ZFMK 48170	F	Andambe, Fianarantsoa	178	258
ZFMK 51101	F	Andambe, Fianarantsoa	183	(255) reg.?
ZFMK 51102	F	Andambe, Fianarantsoa	174	272
ZFMK 50656	M	Nosy Boraha	176	277
ZFMK 50657	F	Nosy Boraha	152	250
ZFMK 50658	F	Nosy Boraha	178	277
ZFMK 51110	M	Nosy Boraha	175	– (reg.)
ZFMK 55026	F	Nosy Boraha	186	295
ZFMK 55027	F	Nosy Boraha	176	285
ZFMK 58169	M	Nosy Boraha	170	– (reg.)
ZFMK 58170	F	Nosy Boraha	185	290
ZFMK 58171	F	Nosy Boraha	180	289
ZFMK 58172	F	Nosy Boraha	175	280
ZFMK 48146	M	Nosy Boraha	163	–
ZFMK 48147	F	Nosy Boraha	185	–
ZFMK 48148	F	Nosy Boraha	170	266
ZFMK 48149	F	Nosy Boraha	170	277
ZFMK 64824	M	Nosy Boraha	162	273
ZFMK 47256	M	Nosy Boraha	164	270
ZFMK 47257	F	Nosy Boraha	180	267
ZFMK 47546	M	Nosy Boraha	172	–
ZFMK 47547	F	Nosy Boraha	181	286
ZFMK 47548	F	Nosy Boraha	171	250
ZFMK 52461	M	Nosy Boraha	168	279
ZFMK 52462	F	Nosy Boraha	174	–
ZFMK 46008	M	Nosy Boraha	177	–
ZFMK 46009	F	Nosy Boraha	180	–
ZFMK 46104	F	Nosy Boraha	180	–
ZFMK 46105	F	Nosy Boraha	178	–
ZFMK 47011	F	Nosy Boraha	150	–
ZFMK 36503*	F	Nosy Mangabe	172	264
ZFMK 29072	F	Nosy Mangabe	173	241
ZFMK 53414	M	Nosy Mangabe	154	250
ZFMK 53415	M	Nosy Mangabe	153	247
ZFMK 53416	F	Nosy Mangabe	168	264
ZSM 56/2005	F	Nosy Mangabe	172	263
ZSM 57/2005	M	Nosy Mangabe	157	250
ZSM 55/2005**	F	Marojejy	173	256

Tab. 1. Snout-vent length and total length (in mm) of adult *Uroplatus fimbriatus* from different populations. *Data of the neotype fide Bauer & Russell (1989), **specific attribution uncertain. M = male; F = female.

Fig. 1. Colour variability of an individual of *Uroplatus giganteus* sp. n. (either holotype or UADBA paratype): (a) unstressed; (b) stressed (both photographs were taken within a few minutes during the day); (c) in sleeping position adpressed on a tree at dusk, before being captured. Photographs: F. Glaw.

fimbriatus from Nosy Mangabe (UADBA, uncatalogued [=ZCMV 2199], DQ857334 and ZFMK uncatalogued, DQ857333), *U. fimbriatus* without locality data (ZFMK, uncatalogued, DQ857332), *U. ebenaui* from Berara (taken from Genbank: DQ270586), *U. henkeli* from Nosy Be (no voucher collected, DQ857337), *U. lineatus* from Nosy Boraha (no voucher collected, DQ857336), *U. guentheri* without locality data (ZFMK, uncatalogued, DQ857335), *Phelsuma mada-gascariensis grandis* from Sambava (no voucher, DQ270569), *Gehyra mutilata* from Antananarivo (no voucher, DQ857338).

Phylogenetic analysis was carried out using PAUP* version 4b10 (Swofford 2002). Using MODELTEST version 3.06 (Posada & Crandall 1998), we determined the best-fitting substitution models for our different alignments from a set of 56 nested models (decisions were made based on the Akaike information criterion). We applied this model

Fig. 2. *Uroplatus giganteus* sp. n., paratype (ZFMK 75753). Photograph: F.-W. Henkel.

to construct a neighbour-joining (NJ) tree. Topology robustness was estimated by running 2000 bootstrap replicates each in NJ and maximum parsimony, and 100 in maximum likelyhood for comparison (Felsenstein 1985).

Results

Genetic differentiation of *Uroplatus*: The dataset comprised 436 characters, of which 217 were invariant and 137 were parsimony-informative. Based on the Akaike Information Criterion MODELTEST selected the GTR+G substitution model (Rodríguez et al. 1990) as being most likely (-lnL=2434.7134), with base frequencies $\pi A=0.3442$, $\pi C=0.2647$, $\pi G=0.1827$ and $\pi T=0.2083$, and substitution rates R[A-C]=5.9375, R[A-G]=11.3196, R[A-T]=4.02538, R[C-G]=0, R[C-T]=23.25206 and R[G-T]=1.0000. The gamma shape parameter for rate heterogeneity among sites was set to $\alpha=0.3161$.

The molecular analyses produced only partially resolved phylogenetic trees. The topology (Fig. 8) received a consistency index of 0.693 and a retention index of 0.578, and required 473 steps. The genetic differences between the described *Uroplatus* species that were included in our study are rather large (9.6 % uncorrected pairwise sequence divergence between *U. sikorae* and *U. fimbriatus* to 22.8 % between *U. henkeli* and

Fig. 3. Portrait of *Uroplatus fimbriatus* from Nosy Boraha (a) and *Uroplatus giganteus* sp. n. (b) demonstrating the differences in the iris and head colouration. Photographs: F. Glaw & M. Vences.

Fig. 4. *Uroplatus* cf. *giganteus* from Marojejy National Park. Photograph: F. Glaw.

U. ebenaui). On the other hand, conspecific specimens from the same locality have virtually identical sequences (Fig. 8). There is also remarkable genetic variability between populations hitherto attributed to *U. fimbriatus* (up to 4.2 % uncorrected pairwise sequence

Locality	SVL males	SVL females	ToL males	ToL females
Andambe/ Fianarantsoa	168 n=1	174-183 n=3	–	258-272 n=2
Nosy Boraha	162-177 n=9	150-186 n=18	270-279 n=4	250-295 n=12
Nosy Mangabe	153-157 n=3	168-173 n=4	247-250 n=3	241-264 n=4
Marojejy	–	173 n=1	–	256 n=1
Montagne d'Ambre and unknown	188-198 n=2	182-200 n=2	317 n=2	286-322 n=2

Tab. 2. Summary of size variation (in mm) in different populations of *Uroplatus fimbriatus* and *U. giganteus*.

divergence, 10 transitions, 6 transversions) indicating that this taxon does comprise more than one species. The differences between the typical *Uroplatus fimbriatus* from Nosy Mangabe and the "white-eye-*fimbriatus*" from Montagne d'Ambre (that will be described as *U. giganteus* below) are especially distinct (4.8 % uncorrected pairwise sequence divergence; 15 transitions, 5 transversions) and leave no doubts that the latter population represents a new species. The specimen from Marojejy has a similar distance to the population of Montagne d'Ambre (3.6 % uncorrected pairwise sequence divergence; 12 transitions, 3 transversions) and to that from Nosy Mangabe (4.1 % uncorrected pairwise sequence divergence; 12 transitions, 5 transversions).

Morphological variation: In the following, we used the Mann-Whitney U-Test to test for statistic significance. The available four specimens of the "white-eye-*fimbriatus*" are significantly larger in SVL (p < 0.05) and ToL (p < 0.05) than the studied specimens of *U. fimbriatus* from three localities (n=38) (Tab. 1). With one exception, their ToL is furthermore larger than those of all other specimens measured by Angel (1929), Bauer & Russell (1989) and Böhme & Ibisch (1990). *U. fimbriatus* specimens from Nosy Boraha are significantly larger than those

from the neotype locality Nosy Mangabe in SVL (p < 0.05) and ToL (p < 0.05). Throughout the studied populations females reach a significantly larger maximum SVL (p < 0.05) than males (Tab. 1 and 2), but ToL is not significantly different between sexes. There are also distinct differences in hemipenis morphology and colouration between the "white-eye-*fimbriatus*" and typical *U. fimbriatus* (see below). We therefore describe the giant population from Montagne d'Ambre as new species:

Uroplatus giganteus sp. n.
(Figs. 1, 2, 3b)

Holotype: ZSM 269/2004 (field number FGZC 517), adult female in good condition, collected at Montagne d'Ambre National Park, below Antamboka (12°29'S, 49°10'E, ca. 800 m alt.), northern Madagascar, on 22 February 2004 by F. Glaw, M. Puente, R. D. Randrianiaina & A. Razafimanantsoa.

Paratypes: ZFMK 75753, adult male, without locality data, provided by A. Grund in 2001. ZSM 267/2004 (field number FGZC 514), adult male with well preserved body and tail, but with partly mazerated head, collected at Montagne d'Ambre National Park (12°29'S, 49°10'E, ca. 850 m alt.), northern Madagas-

	ZSM 269/2004	ZSM 267/2004	ZFMK 75753
Status	holotype	paratype	paratype
Sex	female	male	male
SVL	200	198	188
TaL	122	119	129
ToL	322	317	317
HL	56.7	57.5	56.5
HW	41.0	39.3	39.8
HH	19.3	18.5	18.2
TaW	35.1	41.0	26.2
NSD	5.9	4.9	5.0
END	22.6	21.5	22.1
ESD	28.4	26.5	26.1
NND	6.3	5.8	6.1
ED	ca. 11	–	13.0
EOD	2.0	1.8	3.1
EED	ca. 17	–	15.5
SL (left/right)	36/34	–/–	35/32
IL (left/right)	30/29	–/30	–/–
FoL	76	81	72.5
HiL	102	99	97.5
AGD	92	95.5	88.2
WFH	5.4	–	3.3
WFF	6.1	4.1	3.5

Tab. 3. Morphological measurements (all in mm) and counts of three type specimens of *Uroplatus giganteus*. For abbreviations of measurements see Materials and Methods.

car, on 21 February 2004 by F. GLAW, M. PUENTE, R. D. RANDRIANIAINA & A. RAZAFI-MANANTSOA. UADBA 27490 (field number FGZC 516), adult female, same locality and collecting data as holotype, but collected on 21 February 2004.

Diagnosis: *Uroplatus giganteus* sp. n. differs from most other *Uroplatus* species (*U. ebenaui, U. phantasticus, U. malama, U. alluaudi, U. guentheri, U. malahelo, U. pietschmanni*) in its much larger size, lateral membranous fringes on head, body and limbs, and numerous other morphological and chromatic characters. The new species is similar to the other species of the *U. fimbria-tus* species group (*U. fimbriatus, U. henkeli, U. sikorae*) which share a relatively large size (adult SVL at least 85 mm) and a similar general morphology including lateral membranous fringes. *U. giganteus* differs from *U. sikorae* and *U. henkeli* by larger size (SVL 182-200 mm vs. 85-160 mm), hemipenis morphology (see Fig. 7) and brownish, concentrically arranged lines around the iris (see Fig. 3). It differs from the most similar species, *U. fimbriatus*, by larger size (SVL 182-200 mm vs. 150-189 mm, ToL up to 322 mm vs. 295 mm), white ground colour of the iris (vs. yellowish, Fig. 3), a special head colouration (see Fig. 3), terminal elements of hemipenes (shape almost parallel

Fig. 5. Holotype of *Uroplatus fimbriatus lichenius* (plate 3 from ROTHSCHILD 1903), showing the typical iris colouration of *Uroplatus fimbriatus*.

Fig. 6. *Rhacoessa hypoxantha* (plate 35 from WAGLER 1833), showing an iris colouration that clearly does not fit with *Uroplatus fimbriatus* or *U. giganteus*.

and not pointed vs. converging and pointed; median notch shallow vs. deep; 7-8 vs. 5-6 serrated longitudinal ridges on sulcal side; basal hardened papilla less strong vs. strong, see also Fig. 7) and strong genetic differentiation (4.8 % pairwise sequence divergence in a fragment of the 16S rRNA gene).

Description of the holotype: Adult female, in good condition. Measurements and counts are given in Tab. 3. Head large, triangular and distinctly depressed. Head, body, limbs, and tail covered with small, irregular-sized, juxtaposed, flat scales (the largest scales with a diameter of less than 2 mm). Distinct membranous, serrated fringes of 1-6 mm width along flanks, inner and outer sides of lower arms, outer sides of upper and lower legs, and

along head sides, but almost entirely absent along inner sides of upper and lower legs. Distinct, partly spine-like dermal fringes at skin surrounding the eyes, but absent from other parts of body. Lateral parts of the tail with little serrations, tail terminating in a small tubercle of ca. 1 mm length. Axillar indentations at the forelimb insertion ("Achseltaschen") poorly recognizable. Venter, and ventral parts of the legs and tail with fine, rather homogeneous, granular scalation. One pointed tubercle of ca. 1 mm length lateral of the cloaca. Throat with many small granular scales, mental scale not clearly recognizable, no enlarged postmental and chin shields. Paired, lateral postcranial endolymphatic sacs oval, hard and well developed (11 x 6 mm left; 10 x 8 mm right), whitish. Limbs slender, very fine scaled webbing between the toes for less than half toe length and between the fingers for at least half finger length. 13 adhesive subdigital lamellae on each side below fourth toe. Rostral scale entire, much wider (5.2 mm) than tall (1.8 mm). Eyes large, pupil vertical with crenate borders, nares directed dorsolaterally, separated from each other by ca. 9 small granular scales, from first supralabial scale by 1-2 scales, from rostral scale by 3 scales.

Colouration: After fixation with formalin and preservation in 70 % ethanol for more than two years, dorsal ground colour beige, with brown and blackish marbling. A series of indistinct brown spots on the neck above the insertion of arms. A large, triangular-shaped, light middorsal spot on the anterior back and a further large spot at middorsum. Both these spots only moderately distinct with partly poorly defined borders. Dorsal tail base and posterior parts of upper legs dark brown. A large light spot on head between eyes and nostrils. Nostrils and region of rostral scale blackish. Ventral surface whitish.

After preservation the colouration of the two ZSM specimens has become remarkably different from that in life. For this reason, and due to the ability of enormous colour change of living individuals (see Fig. 1a-c),

it was not possible to identify the holotype unambiguously from the photographs of the living animals. Therefore, the colouration in life will be described below for all photographed type specimens.

Variation: Measurements and counts of two paratypes (ZFMK 75753 and ZSM 267/2004) are given in Tab. 3. The third paratype, UADBA 27490, has a SVL of 182 mm and a total length of 286 mm (P. BORA, pers. comm.).

There is remarkable dorsal colour variation in preservative. The back colour of ZSM 267/2004 is even less contrasting than in the holotype and only the light middorsal spot and the light spot on the head are recognizable, but the posterior parts of the shanks are dark as in the holotype. The colour of ZFMK 75753 which died in captivity is, by far, more contrasting: Three large and well delimited, symmetrical beige spots are present on the back, a further one on the head between eyes and nostrils. The upper arms and parts of the flanks are beige as well, whereas the remaining dorsal parts of body and limbs are largely brown. The venter is white with a dark, indistinct, broken longitudinal stripe. The throat is white marbled with light brown.

Colouration in life: Although photographs are available for only relatively few individuals, both significant colour variation within and between individuals on the one hand and constant, species-specific pattern on the other hand are evident. The intra-individual colour variation is documented in Fig 1a-c. The dorsal surface of unstressed specimens is mainly covered with brown, grey, and black, often reminiscent the bark of trees. Similar to *U. fimbriatus*, *U. henkeli* and also to chameleons the colouration of stressed individuals is much more contrasting: the brownish and greyish spots mostly have changed to yellow and a black reticulation or marbling covers most dorsal parts of back, tail, head and limbs. Most of the photographed individuals agree in having one large beige symmetrical patch in the neck, one at middorsum, and one in the sacral region. These patches can
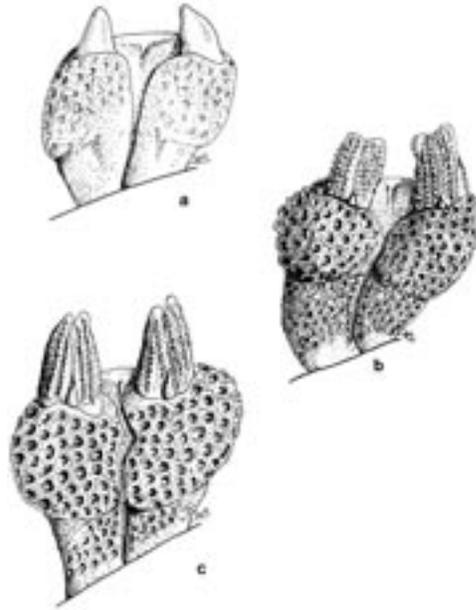

Fig. 7. Sulcal view of the hemipenes of (a) *Uroplatus giganteus* sp. n. (paratype ZSM 264/2004); (b) *U. giganteus* sp. n. (paratype ZFMK 75753) and (c) *U. fimbriatus* from Nosy Boraha (ZFMK 48169). Drawings: W. BÖHME.

vary from poorly defined (e. g. Fig. 1) to very distinct (ZFMK 75753, Fig. 2) and their borders can be well delimited by black or poorly recognizable. The light patches are often filled with brownish pattern and their shape is variable. Similar patches can also occur in *U. fimbriatus* and *U. sikorae* but appear to be rare or absent in *U. henkeli*. The head colouration of *U. giganteus* is rather characteristic and appears to be unknown from other *Uroplatus* species: There are two distinct, chevron-shaped, blackish markings, both pointing posteriorly, one between the eyes and the other in front of the eyes. One, often poorly delimited, blackish spot is generally present between the nostrils and a further one posterior to the nostrils. The area between these spots and the two chevrons is usually beige, brown or yellow. Two black spots are present behind the posterior chevron, and the combined appearance often
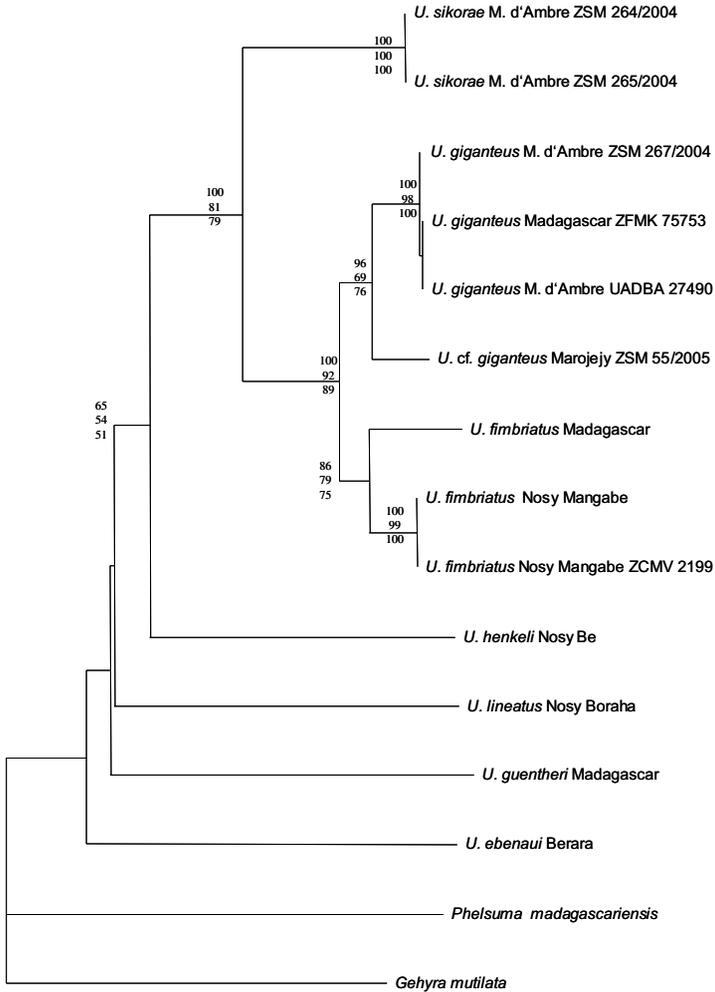
Fig. 8. Phylogenetic tree (NJ), based on 436 bp of the mitochondrial 16S rRNA gene. Bootstrap support values (NJ, MP, ML) are given for each node if higher than 50.

resembles a "sad smiley" symbol. The iris is whitish with brownish lines around the vertical pupil and is less distinct and colourful than in *U. fimbriatus*. No dark colour was noted in the open mouth. The ventral surface is whitish. In addition to the colour photographs presented here others have been published by LOVE (2001a, 2001b) and SVATEK & VAN DUIN (2002).

Description of hemipenes: Two paratypes

with everted hemipenes were available for study, ZSM 264/2004 and ZFMK 75753. Both organs, although from two fully adult males, differ markedly from each other. The stout hemipenis of ZSM 264/2004 (Fig. 7a) has two differentiated apical lobes between which the sperm groove is ending. Each lobe bears a pointed, coniform terminal element with a smooth surface. Its length is approximately the half of apical lobe's height. The apical lobes are covered with indistinct shal-

low calyces; the truncus below them has a smooth surface (for terminology see Böhme 1988).

ZFMK 75753 has a similarly stout organ with much thicker apical lobes (Fig. 7b). Here, these lobes are covered by very distinct, deep calyces, and the ridges between them are a bit elongate and have slightly serrated margins. Also the proximal trunk of the organ is adorned with distinct calyces. The two terminal elements on the tip of the apical lobes, which resemble the pedunculi of Madagascan chameleons of the genus *Furcifer* (Böhme 1988, Böhme & Ibisch 1990), are roughly rectangular in shape, with subparallel lateral margins. The upper margin has a slight, medial notch, from which a shallow groove separates each of these elements into two halves. Viewed from the sulcal side, each element bears 7-8 longitudinal serrated ridges. Basal to these elements is a hardened (keratinized or calcified?) papilla which is not visible in ZSM 264/2004.

Etymology: The specific name is derived from the Latin adjective "giganteus" and refers to the giant size of the species.

Habitat and habits: The type specimens and several other individuals were observed active at night on stems of small to large trees in pristine rainforest at the type locality. This habits on tree stems might be due to the large size and weight of *U. giganteus* and is in contrast with the three distinctly smaller syntopic *Uroplatus* species which were mainly seen on smaller branches. One *U. giganteus* which was discovered shortly after dusk was still in sleeping position with the head downwards, less than one meter above the ground, whereas all others were encountered at heights of ca. 2-4 m. Captured specimens tried to bite intensively. *U. giganteus* was found to be moderately common during our stay in its habitat from 21-24 February 2004 (we observed about 4-5 specimens during a single night excursion). Whether the relatively high abundance of *Uroplatus giganteus* and the high density of the syntopic species *U. siko-*

rae and *U.* cf. *ebenaui* in the isolated rainforest Montagne d'Ambre is due to reduced predation pressure or other factors related to island effects remains to be studied.

Distribution: The new species is only known from the rainforest of the Montagne d'Ambre National Park in northern Madagascar which is completely isolated from the major rainforest blocks of the north and east and covers a surface area of 182 km^2 (Nicoll & Langrand 1989, Raxworthy & Nussbaum 1994). It is unknown whether *U. giganteus* also occurs in the adjacent Foret d'Ambre Special Reserve with a surface area of further 48 km^2. Raxworthy & Nussbaum (1994) give an altitudinal range of 650-800 m for *U. fimbriatus* from Montagne d'Ambre and their data almost certainly refer to *U. giganteus*. Our records of this species are restricted to an elevational range of about 800-850 m altitude. During herpetological surveys at higher altitudes (1000 m or higher) in the National Park in the years 1994, 2000, 2003 and 2004 we did not find a single specimen of *U. giganteus*, indicating that the species might occur in a rather small forest belt. The lower limit of the altitudinal range at 650 m is probably due to the absence of rain forest at low elevation whereas the upper limit at about 850 m might be due to the decreasing temperature at higher altitude (only few nocturnal forest geckos in Madagascar are known from altitudes of more than 1000 m). During intensive surveys in the vicinity of Montagne d'Ambre (Ankarana Special Reserve, Manongarivo Special Reserve, Montagne des Francais) we did not detect further localities of this species. The population from Marojejy National Park shows distinct genetic differences to *U. giganteus* and furthermore chromatic differences to both *U. giganteus* and *U. fimbriatus* appear to exist (Gehring & Kubik 2005, pers. obs., see Fig. 4). Although the status of the Marojejy population needs further study, it is probably not conspecific with *U. giganteus*. It is therefore likely that *U. giganteus* is actually endemic to the isolated forest of Montagne d'Ambre.

Conservation status: If *U. giganteus* is indeed endemic to the low and mid-altitude forest of Montagne d'Ambre its area of occupancy (which is defined by IUCN as the area within the extent of occurrence which is occupied by a taxon) would be very small for this large species (less than 150 km²). Furthermore, its island-like distribution might be a further important factor to consider the species as endangered. Future studies should clarify the actual distribution of the species and its population density to estimate its extinction risk more reliably. A further factor of unknown importance is the pet trade. Until a few years ago, *U. giganteus* was regularly offered by the international pet trade. Since commercial collecting in nature reserves is prohibited in Madagascar we can only speculate that these specimens either came from unknown and unprotected localities or that they were collected illegally in the National Park. In any case, the trade of this species was apparently stopped after the inclusion of *Uroplatus* in CITES.

Available older names: Although *U. fimbriatus* was among the first Madagascan reptiles that became known to science, only very few synonyms are available (Russell & Bauer 1988, Bauer & Russell 1989). *Rhacoessa hypoxantha* Wagler, 1833 (Fig. 6), apparently was published after Wagler's deadly accident in 1832 and it is not clear if he intended to erect this taxon as new species or as nomen substitutum. Wermuth (1965: 186) considered this name as (unjustified) nomen substitutum for *Stellio fimbriatus* probably because Wagler (1833) provided a list of synonyms in his description. We follow the opinion of Wermuth and consider *hypoxantha* as an unavailable nomen substitutum rather than a junior synonym of *Stellio fimbriatus*. The ventral side of *Rhacoessa hypoxantha* was described and figured as being yellow which is unknown for any species of the *U. fimbriatus* group but was apparently considered typical by Wagler as both the scientific species name "*hypoxantha*" and the German common name "Gelbbauchiger

Franzengecko" refer to this character. The colour plate (Fig. 6) shows an olive-greenish iris with a single darker band around the pupil. Reddish lines, as typical for the iris of *U. fimbriatus* and *U. giganteus* are not visible. It therefore appears clear that *Rhacoessa hypoxantha* refers to neither *U. fimbriatus* nor *U. giganteus*. *U. henkeli* differs from *Rhacoessa hypoxantha* by the non-yellow ventral colouration.

Uroplatus fimbriatus lichenius Rothschild, 1903 (Fig. 5) was described rather tersely with the imprecise type locality "Madagascar" and without providing data on size or any other morphological characters of the single known specimen (the holotype by monotypy). However, the two colour plates (Rothschild 1903: plates 3 and 4) clearly show the characteristic iris colouration of the typical, yellow-eyed *Uroplatus fimbriatus* from eastern Madagascar (Fig. 5). Furthermore, it appears that the holotype on plate 3 is shown in its original size which agrees well with the size of *U. fimbriatus*, but is smaller than *U. giganteus*. We therefore continue considering *Uroplatus fimbriatus lichenius* a synonym of *U. fimbriatus*. The holotype of *U. fimbriatus lichenius* is unknown from any collection and might be considered as lost. We conclude that neither *Rhacoessa hypoxantha* nor *Uroplatus fimbriatus lichenius* are available as earlier name of *Uroplatus giganteus*.

Discussion

Variation in the *Uroplatus fimbriatus* complex: Our molecular analysis has demonstrated that *Uroplatus fimbriatus* as hitherto defined includes at least two species, *U. fimbriatus* and *U. giganteus*.

Although the distinct molecular, morphological and chromatic differentiation might warrant the specific distinctness of both taxa, the inclusion of a specimen from Marojejy National Park and a further one without locality data in the analysis indicates enormous intraspecific differentiation of both species

and suggests that even more species might be involved. This is especially true for the Marojejy population which lies geographically between Montagne d'Ambre (*U. giganteus*) and Nosy Mangabe (*U. fimbriatus*). The molecular relationships of the studied Marojejy specimen with either species are not well resolved and the genetic distance to both species is similar (see Results). The size (SVL and ToL) of the only available specimen resembles that of *U. fimbriatus*, whereas the colouration of the iris and a part of the head colouration (the "smiley" pattern) are more similar to *U. giganteus*. However, the remaining colour pattern features do not agree with either species and indicate that it might represent its own taxon. Further studies are necessary to clarify this question.

Hemipenis morphology: One remarkable result of our hemipenis comparisons is the strong difference in the hemipenial structure of the two clearly conspecific males of *Uroplatus giganteus* which are almost identical in the 16S rRNA gene (see Fig. 8). We regard these differences as seasonal, implying that the two males were in a different reproductive state when preserved. Seasonal hemipenial variation is certainly widely distributed among lizards, but has not yet been demonstrated for geckos; it is, however, known from many lacertids, few iguanids and some chameleons (BÖHME 1988). Among the latter, there are at least two Madagascan species (*Furcifer tuzetae* and *Brookesia brygooi*, formerly considered as *B. ebenaui*) for which seasonal variation of hemipenial structures has been suggested (BÖHME 1988) which corroborates our conclusion with regards to *U. giganteus* sp. n.

The hemipenial characters of the fully differentiated hemipenis (ZFMK 75753) of *Uroplatus giganteus* (Fig. 7b) differ markedly from those of *U. fimbriatus* from Nosy Boraha (Fig. 1b in BÖHME & IBISCH 1990). The additional drawing of a *U. fimbriatus* male from Nosy Boraha (ZFMK 48146: Fig. 7c) emphasizes the main differences of both species. They concern the shape of the terminal elements which are pointed in *fimbriatus*

with converging (rather than almost parallel) lateral margins and a deep (rather than a shallow) median notch. The serrated ridges on the sulcal side of these elements, only 5-6 in number, are also converging towards the tip. The basal hardened papilla is stronger than that in our new species. Thus, the hemipenial characters provide good evidence to further diagnose *U. giganteus* sp. n. from its closest relative *U. fimbriatus*. The former suspicion (BÖHME & IBISCH 1990) that perhaps even more species may be masked in this complex is supported by observed apical hemipenial differences in a *U. fimbriatus* male (ZFMK 48169) from Andambé. Despite these differences, the hemipenes of the members of the *U. fimbriatus* species complex can easily be distinguished from those of *U. henkeli* and *U. sikorae* which both were earlier regarded as conspecifics of *U. fimbriatus*. RÖSLER & BÖHME (2006) found hemipenial bones, i.e. internal calcified structures, in the hemipenis of *U. lineatus* which within geckos were otherwise only known from the Caribbean genus *Aristelliger*. Here, the hardened papillae might answer the question whether this character is unique for *U. lineatus* or whether it may be shared also by the members of the *U. fimbriatus* species complex. A histological approach is necessary to address this question.

Maximum size of geckos: Although several Madagascan gecko species (*Phelsuma madagascariensis grandis*, *P. m. kochi*, *Blaesodactylus boivini* and *Uroplatus fimbriatus*) are known to exceptionally approach or surpass 300 mm total length, *Uroplatus giganteus* is the largest documented gecko species from Madagascar, reaching at least up to 200 mm snout-vent length and more than 320 mm total length. SVATEK & VAN DUIN (2002: 145) reported that the largest measured specimen of the "white-eye-*fimbriatus*" even reached 345 mm total length. According to HENKEL (1992) and HENKEL & SCHMIDT (1995) the size of *Uroplatus fimbriatus* is 300-330 mm total length, with the largest specimens being from Nosy Boraha. Our data (Tab. 1 and 2) confirm that the

largest *U. fimbriatus* are from this island and show that throughout the studied populations females generally reach a larger maximum SVL than males. However, voucher specimens documenting a ToL of 300 mm or more are unknown to us. Angel (1929: 21) provided measurements of 18 specimens he considered as *U. fimbriatus*, the three largest being females, reaching 285 mm ToL (173 mm SVL), 280 mm ToL (176 mm SVL) and 254 mm ToL (179 mm SVL). However, most of the specimens were distinctly smaller and might include specimens of *U. henkeli* and *U. sikorae*. Bauer & Russell (1989) provided measurements of 58 specimens they considered as *U. fimbriatus* (but also included *U. henkeli*), the three largest specimens measuring 189.5 mm SVL (female, tail absent), 296.4 mm ToL (female, 181.7 mm SVL), and 288.7 mm ToL (male, 180.1 mm SVL). The data of Angel (1929), Bauer & Russell (1989), Böhme & Ibisch (1990) and those presented herein (table 1) show that *Uroplatus fimbriatus* individuals of 300 mm ToL or more might be exceptional.

Bauer & Russell (1991) provided the following ranking of the largest geckos in the world surpassing 170 mm SVL: by far the largest is the extinct *Hoplodactylus delcourti* (370 mm SVL), followed by *Rhacodactylus leachianus* (240 mm SVL), the extinct *Phelsuma gigas* (190 mm SVL), *Uroplatus fimbriatus* (186 mm), *Gekko smithi* (180 mm SVL), *Gekko gecko* (176 mm SVL), *Cyrtodactylus novaeguineae* (172 mm SVL), and *Rhacodactylus trachyrhynchus* (170 mm). According to this account *Uroplatus giganteus* appears to be the second largest living gecko in the world with regard to SVL. It is remarkable that most of these largest geckos occur on islands and that two of the three largest geckos have already gone extinct during the past few centuries. This fact strongly confirms our assumption that *Uroplatus giganteus* might be a seriously threatened species.

References

Angel, M.F. (1929): Contribution à l'étude systématique des lézards appartenant aux genres *Uroplatus* et *Brookesia*. – Mémoires de l'Academie Malgache IX: 1-65 + planches.

Anonymus (1993): An introduction to the leaf-tailed gecko (*Uroplatus fimbriatus*). – Reptilian **2**(1): 24.

Aresté M. & J.L. Farriols (1998): *Uroplatus fimbriatus*. «Plattschwanzgecko». – Reptilia **3**(6): 47-50.

Baldwin, R. (1997): The demons of Madagascar: leaf-tailed geckos of the genus *Uroplatus*. – Reptiles **5**(5): 48-68.

Bauer, A.M. & A.P. Russell (1989): A systematic review of the genus *Uroplatus* (Reptilia: Gekkonidae) with comments on its biology. – J. Nat. Hist. **23**: 169-203.

Bauer, A.M. & A.P. Russell (1991): The maximum size of giant geckos: a cautionary tale. – Bull. Chicago Herp. Soc. **26**(2): 25-26.

Böhle, A. & P. Schönecker (2003): Eine neue Art der Gattung *Uroplatus* Duméril, 1805 aus Ost-Madagaskar (Reptilia: Squamata: Gekkonidae). – Salamandra, **39**(3/4): 129-138.

Böhme, W. (1988): Zur Genitalmorphologie der Sauria: funktionelle und stammesgeschichtliche Aspekte. – Bonn. zool. Monogr. **27**: 1-176.

Böhme, W. & P. Ibisch (1990): Studien an *Uroplatus*. I. Der *Uroplatus-fimbriatus*-Komplex.

– Salamandra **26**(4): 246-259.

BOPP, P. (1953): Röntgenaufnahmen zur Frage der Schwanzautotomie bei Reptilien. – Naturwissenschaften **40**(22): 586.

BOULENGER, G. A. (1885): Catalogue of the lizards in the British Museum (Natural History). – Vol. 1, 2nd ed., London.

BRAUN, M. (1913): *Uroplatus fimbriatus* (SCHNEIDER 1797) in Gefangenschaft. – pp. 259-268 in VOELTZKOW, A.: Reise in Ostafrika in den Jahren 1903-1905, Wissenschaftliche Ergebnisse. – Band III, Systematische Arbeiten, Stuttgart, Schweizerbart'sche Verlagsbuchhandlung

FELSENSTEIN, J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. – Evolution **39**: 783-791.

FLACOURT, E. DE (1658): Histoire de la Grande Isle de Madagascar, Composée par le Sieur de Flacourt, Directeur Général de la Compagnie Française de l'Orient et Commandant pour su Majesté dans la Dite Isle et Isles Adjacentes. – Paris (Alexandre Lesselin).

GEHRING, P.S. & A.-L. KUBIK (2005): Herpetologische Beobachtungen aus Madagaskar. – Elaphe **13**(3): 60-66.

GLAW, F. & M. VENCES (1994): A fieldguide to the amphibians and reptiles of Madagascar. 2nd edition. – Vences und Glaw Verlag, Köln, Germany, 480 pp.

HAEDGE, H. (1917): Das Urogenitalsystem von *Uroplatus fimbriatus*. – pp. 487-518 in VOELTZKOW, A.: Reise in Ostafrika in den Jahren 1903-1905, Wissenschaftliche Ergebnisse,. – Band III, Heft 5, Systematische Arbeiten, Stuttgart, Schweizerbart'sche Verlagsbuchhandlung.

HENKEL, F.-W. (1992): *Uroplatus fimbriatus* (SCHNEIDER). – Sauria, Suppl., **14**(1-4): 229-232.

HENKEL, F.-W. & W. SCHMIDT (1995): Amphibien und Reptilien Madagaskars, der Maskarenen, Seychellen und Komoren. – Ulmer Verlag, Stuttgart, 311 pp.

KÄSTLE, W. (1973): Meister der Tarnung: Madagassische Plattschwanzgeckonen. – Aquarien-Magazin **7**: 192-195.

KRAPP, F. (1963): Beobachtungen am Plattschwanzgecko. – Natur und Museum **93**(11): 435-442.

LOVE, B. (2001a): Stalking the action (part one). – Reptiles **9**(12): Cover, 48-61.

LOVE, B. (2001b): Madagascar's houdinis. – Fauna (July/August 2001): 44-59.

MEIER, H. (1984): Sind Nachzuchterfolge bei *Uroplatus fimbriatus* (SCHNEIDER, 1797) möglich? – Herpetofauna **6**(33): 20-27.

NICOLL, M.E. & O. LANGRAND (1989): Madagascar: Revue de la conservation et des aires protégées. – World Wide Fund for Nature, Gland, Switzerland.

PALUMBI, S.R., A. MARTIN, S. ROMANO, W.O. MCMILLAN, L. STICE & G. GRABOWSKI (1991): The Simple Fool's Guide to PCR, Version 2.0. – Privately published document compiled by S. Palumbi, Dept. Zoology, Univ. Hawaii, Honolulu.

POSADA, D. & K.A. CRANDALL (1998): Modeltest: testing the model of DNA substitution. – Bioinformatics **14**: 817-818.

RAXWORTHY, C.J. & R.A. NUSSBAUM (1994): A rainforest survey of amphibians, reptiles and small mammals at Montagne d'Ambre, Madagascar. – Biol. Conservation **69**: 65-73.

RICKERT, M. (1996): Der Blattschwanzgecko *Uroplatus fimbriatus* aus Madagaskar. – TI-Magazin **130**: 45-47.

RITTENHOUSE, D.R., A.P. RUSSELL & A.M. BAUER (1997): Laryngotracheal morphology of the *Uroplatus fimbriatus* complex (Reptilia: Gekkonidae): yet another autapomorphic organ system in a group of highly aberrant geckos. – Afr. J. Herpetol. **46**(1): 36-48.

RODRÍGUEZ, F., J.F. OLIVER, A. MARIN & J.R. MEDINA (1990): The general stochastic model of nucleotide substitution. – J. Theoret. Biol. **142**: 485-501.

ROE, B.A., M. DIN-POW, R.K. WILSON & J.F. WONG (1985): The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. – J. Biol. Chemistry **260**: 9759-9774.

RÖSLER, H. & W. BÖHME (2006): Peculiarities of the hemipenes of the gekkonid lizard genera *Aristelliger* COPE, 1891 and *Uroplatus* DUMÉRIL, 1806. – Herpetologia Bonnensis II (Proc. 13th Ord. Gen. Meet. SEH), Bonn, in press.

ROTHSCHILD, W. (1903): Note on *Uroplates fimbriatus lichenius* subspec. nov. – Novitates Zoologicae **10**: 490, plates III, IV.

RUSSELL, A.P. & A.M. BAUER (1988): An early description of a member of the genus *Phelsuma* (Reptilia: Gekkonidae), with comments on names erreonously applied to *Uroplatus fim-*

briatus. – Amphibia-Reptilia **9**(2): 107-116.

Russell, A.P., D.R. Rittenhouse & A.M. Bauer (2000): Laryngotracheal morphology of Afro-Madagascan geckos: a comparative survey. – J. Morphol. **245**: 241-268.

Schmidt, W.J. (1913): Studien am Integument der Reptilien. IV. *Uroplatus fimbriatus* (Schneid.) und die Geckoniden. – Zool. Jahrb. Anat. Ontog. Tiere **36**(3): 377-464, Tafeln 33-36.

Schmidt, W. & V. Müller (1994): Freilandbeobachtungen und Bemerkungen zur Fortpflanzungsbiologie von *Uroplatus henkeli* und *Uroplatus fimbriatus* im Terrarium. – Elaphe (N. F.) **2**(4): 26-30.

Siebenrock, F. (1893): Das Skelet von *Uroplatus fimbriatus* Schneid. – Annalen des k. k. naturhistorischen Hofmuseums Wien **8**(3/4): 517-536.

Svatek, S. & S. van Duin (2002): Plattschwanz-geckos. Die Gattung *Uroplatus*. – Brähmer Verlag, 161 pp.

Swofford, D.L. (2002): PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4 beta 10. – Sunderland MA: Sinauer.

Wagler, J. (1833): Descriptiones et icones amphibiorum. Part III. – Monachii, Stuttgartiae et Tübingae: pls. XXV-XXXVI, Text

Wellborn, V. [1933] (1997): Comparative osteological examinations of geckonids, eublepharids and uroplatids (translated by A.R. Russell, A.M. Bauer & A. Deufel). – Bibliomania (Breck Bartholomew), Herpetological Translations No. 1.

Wermuth, H. (1965): Liste der rezenten Amphibien und Reptilien, Gekkonidae, Pygopodidae, Xantusiidae. – Das Tierreich, Berlin, 80: I-XXII, 1-246.

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