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Elizabeth C. Miller [a], Anna B. Sellas [b] & Robert C. Drewes [c]

[a] Department of Ecology, Behavior and Evolution, University of California, San Diego, CA, USA

[b] Center for Comparative Genomics, California Academy of Sciences, San Francisco, CA, USA

[c] Department of Herpetology, California Academy of Sciences, San Francisco, CA, USA

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A new species of *Hemidactylus* (Squamata: Gekkonidae) from Príncipe Island, Gulf of Guinea, West Africa with comments on the African-Atlantic clade of *Hemidactylus* geckos

Elizabeth C. Miller[1], Anna B. Sellas[2] & Robert C. Drewes[3]*

[1]*Department of Ecology, Behavior and Evolution, University of California, San Diego, CA, USA;* [2]*Center for Comparative Genomics, California Academy of Sciences, San Francisco, CA, USA;* [3]*Department of Herpetology, California Academy of Sciences, San Francisco, CA, USA*

Abstract.—The remote oceanic Gulf of Guinea islands of São Tomé and Príncipe are home to a highly endemic herpetofauna, which has become the focus of modern, on-going scientific analysis only during the past decade. Until now, the endemic *Hemidactylus greeffi* Bocage 1886 (Greeff's giant gecko) has been considered to occur on both islands. Herein, we employ both new and previously published molecular data, along with differences in morphological characters, to demonstrate that the Príncipe populations are a full species distinct from *H. greeffi.* We describe the new species and restrict the range of *H. greeffi* to the island of São Tomé. The original unnumbered holotype of *H. greeffii* held in the Museu Bocage was destroyed in Lisbon and we have been unable to determine the collection provenance of unnumbered material treated by subsequent authors. We therefore designate a neotype for *H. greeffi* from California Academy of Science collections and provide morphological information on that species additional to that of earlier workers, based upon 14 specimens collected in various localities on São Tomé during the past decade.

Key words.—Squamata, Gekkonidae, *Hemidactylus*, São Tomé, Príncipe, Gulf of Guinea Islands, new species, neotype

Resumo.–As ilhas de São Tomé e Príncipe, no Golfo da Guiné, possuem uma herpetofauna única e com elevada proporção de endemismos que tem sido alvo de estudos científicos, principalmente na última década. Até agora, a espécie endémica *Hemidactylus greeffi* Bocage 1886 (osga gigante de Greeff) tem sido considerada como ocorrendo em ambas as ilhas. No presente trabalho, foram utilizados novos dados moleculares e outros previamente publicados, conjuntamente com dados morfológicos, para demonstrar que as populações do Príncipe constituem uma espécie válida e distinta de *H. greeffii*. Com este estudo, *H. greeffi* passa a ter uma área de ocorrência restrita à Ilha de São Tomé. O holótipo original não-numerado de *H. greeffi*, depositado no Museu Bocage, foi destruído em Lisboa e foi impossível determinar a origem geográfica dos espécimes tratados por autores subsequentes. Consequentemente é designado um neótipo para *Hemidactylus greeffi*, sendo depositado na colecção da CAS (Academia de Ciências da Califórnia) e é fornecida informação morfológica adicional sobre aespécie, baseada em 14 espécimenes colhidos em várias localidades de São Tomé durante a última década.'

*Corresponding author. Email: rdrewes@calacademy.org

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INTRODUCTION

The Gulf of Guinea archipelago is unique in that it comprises both a continental island (Bioko) and three oceanic islands, all the result of the same orogenic processes of the Guinea Line (Marzoli *et al*. 2000). The oceanic islands of Príncipe, São Tomé and Annobón have been aerial for 31, 14 and 4.8 My respectively (Lee *et al*. 1994) and are geologically ancient compared to other oceanic archipelagos, such as the Galapagos or Hawaiian Islands. Since the islands have been isolated for millions of years, it is not surprising that the biota of the Republic of São Tomé and Príncipe is highly endemic. The herpetofauna includes 19 species of reptiles, 14 of which are currently considered to be endemic (73%, including this paper) and seven species of endemic amphibians of five families (Measey *et al.* 2007). The non-pelagic bird fauna includes 28 unique species, perhaps the highest concentration of endemic birds per unit area in the world (M. Melo, pers. comm.). Although there has been a renewal of interest in the natural history of the islands during the past decade (see for instance, Drewes & Stoelting 2004; Csuzdi 2005; Wirtz *et al.* 2007; Desjardin & Perry 2009; Figueiredo *et al.* 2011), the biodiversity of the Republic of São Tomé and Príncipe remains poorly documented.

Many of the island endemics, especially the amphibians, are notably poor dispersers across oceanic barriers; their presence on the Gulf of Guinea islands is intriguing and has led to a hypothesis of dispersal by rafting (Measey *et al.* 2007). However, several species of geckos of the widespread genus *Hemidactylus* also inhabit the islands and members of this group are notoriously good dispersers both by random, natural means and by anthropogenic transport. The *Hemidactylus* gecko fauna of São Tomé and Príncipe includes the African species, *H. longicephalus* (Carranza & Arnold 2006), the widespread *Hemidactylus mabouia* (Jesus *et al.* 2005a) and the endemic Greeff's giant gecko, *H. greeffi.* The endemic species is unique within the genus in lacking the terminal phalanx of the thumb, thus also the claw (Russell 1972) and hitherto has been thought to occur on both São Tomé and Príncipe islands. Bocage described *H. greeffi* in 1886 after long confusing his original São Tomé specimen with *H. mabouia.* After comparison with additional material at Coimbra, Portugal, he eventually realised that the absence of the terminal phalanx of the thumb was not a developmental anomaly.

Since 2001, the California Academy of Sciences (CAS) has been conducting a series of research expeditions to the two islands of the Republic of São Tomé and Príncipe in an attempt to document their unique biodiversity. Where endemic species have been assumed to exist on both islands, we have initiated morphological and molecular inter-island comparisons of samples, including purported closely related species from the African mainland (Uyeda *et al.* 2007, and ongoing studies of *Lamprophis* and *Panaspis* [*Afroablepharus*]). Similar inter-island comparisons have already been made by colleagues at the University of Madeira (Jesus *et al.* 2005a,b, 2006, 2007) but without morphological analysis.

Jesus *et al.* (2005a) analysed the relationships among the *Hemidactylus* geckos of the Gulf of Guinea using mitochondrial and nuclear sequences. Their samples included tissues from two *H. greeffi* from São Tomé, 12 from Príncipe, three *H. bouvieri* from the Cape Verde Islands, one *H. newtoni* (endemic to Annobón) and 16 *H. mabouia* from all three oceanic Gulf of Guinea Islands. The authors found that

the two island populations of *H. greeffi* were monophyletic clades, genetically distinct from one another based on an average of 3.3% genetic divergence in mitochondrial sequences. However, the authors were unable to distinguish the populations based on nuclear gene c-mos sequences and declined to formally recognise the Príncipe population as distinct and undescribed. Nevertheless, the genetic divergence of *H. greeffi* from other taxa in their study supported the status of *H. greeffi* (*sensu lato*) as an endemic species, long isolated from ancestral island colonisers.

No morphological comparison of the two island populations has been attempted and the most recent description of multiple specimens (three of uncertain museum provenance, A. Rosado, pers. comm.) was by Loveridge in 1947. The sample of Jesus *et al.* (2005a) was heavily biased toward the island of Príncipe (12 vs. two from São Tomé) and, in recent years, CAS material from both São Tomé and Príncipe islands has been used in a number of broad studies (e.g. Carranza & Arnold 2006; Bauer *et al.* 2010) under the assumption that *H. greeffi* is present and monophyletic on both islands. We utilised the large series from both islands collected during the CAS Gulf of Guinea Expeditions; we believe this to be the largest collection extant. With this larger sample size, we combined morphological characters with molecular data to re-evaluate the evidence of Jesus *et al.* (2005a) that the island populations might represent two genetically distinct species.

MATERIALS AND METHODS

We collected a total of 25 individuals by hand during the CAS' first and second Gulf of Guinea expeditions (March–June 2001 and March–June 2006) and an additional Príncipe specimen during the third expedition in 2008. All specimens were euthanized with veterinary buthanol and preserved in 10% buffered formalin. The total collection includes 12 geckos from Príncipe collected from six localities and 14 geckos from São Tomé collected from nine localities. Liver samples taken from 10 geckos from Príncipe and 12 geckos from São Tomé were preserved in 95% ethanol. Hi-resolution laboratory images were made with the Visionary Digital BK-100 system. X-rays were made by Faxitron 43855c using Kodak MX 125 film.

Morphology

We measured 14 characters for each specimen in our collection using a Mitutoyo digital calliper (precise to 0.01 mm). We also counted 18 meristic characters under a dissection microscope. Morphological characters measured include: snout-vent length (SVL); head length (HL); head width (HW); head height, eye–ear distance; eye–snout distance (ES); eye diameter; ear diameter; interlimb distance; tail base width; internarial distance; body width (BW); palm–elbow length; heel–knee length. Meristic counts included: preanal-femoral pores; longitudinal rows of tubercle scales; supralabials on the right and left sides; infralabials on the right and left sides; divided lamellae on each finger and each toe on the right side; anterior chin shields. These data were compared with Bocage's (1886a) original description and subsequent contributions (1886b,c), Bedriaga's more detailed analysis (1892) and data in

Loveridge's (1947) treatment. We ran analysis of variance on the continuous morphological data using JMP software (version 7) to test for significant character differences between island populations. We used two-tailed Student's *t* test for significance of differences between meristic counts. When drawing conclusions as to whether the populations differed on the basis of continuous morphological or meristic data, probability values of each pairwise comparison were compared against Bonferroni's corrected alpha levels, that is, 0.05 divided by the number of tests. The single juvenile individual in our collection (CAS 233490) was excluded from these analyses.

Molecular

DNA was extracted from liver tissue of 22 specimens (12 from São Tomé and 10 from Príncipe) using the Qiagen DNeasy tissue extraction kit (Qiagen Inc.). A 230 bp fragment of the nuclear α-Enolase gene was amplified using primers EnolL731 (5'-TGGACTTCAAATCCCCCGATGATCCCAGC-3') and EnolH912 (5'-CCAG GCACCCCAGTCTACCTGGTCAAA-3') from Friesen *et al.* (1997). Polymerase chain reaction (PCR; 25 µl) consisted of 1x PCR Buffer (20 mM Tris-HCl, 50 mM KCl), 3.0 mM $MgCl_2$, 0.4 uM primer, 200 uM each dNTP, 1.25 units of Taq polymerase, and approximately 25 ng of extracted DNA. Thermal cycling was as follows: initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 45 s. PCR products were cleaned using the ExoSAP-IT reagent (Affymetrix). DNA sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were precipitated with ethanol and 125 mM EDTA and run on an ABI 3130 DNA Analyzer.

Forward and reverse sequences were edited using Sequencher 4.7 (Gene Codes, Ann Arbor, MI, USA). Consensus sequences were then aligned using the MAFFT alignment tool in Geneious 4.6 (Drummond *et al.* 1999). To obtain an F_{ST} estimate from the α-Enolase data, an analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) was run using Arlequin 2.0. Owing to the presence of multiple heterozygous sites, PHASE 2.1 was used to analytically phase the α-Enolase data before calculating sequence divergence estimates using DnaSP 5.0 (Librado & Rozas 2009). Finally, a haplotype network was constructed using the program Network v4.6.

Results

Morphological

Morphometrically, adult specimens of *H. greeffi* from São Tomé Island are larger than Príncipe adults; mean SVL for adult São Tomé males is 76.7 mm and for females 62.0 mm; males from Príncipe are 65.9, females are 54.2 mm. Most parameters measured appeared to be size-dependent including all body proportions; when standardised by SVL, the two populations did not differ greatly with the following exceptions: São Tomé *H.greeffi* has longer forelimbs (a 1% difference, ($F = 20.85$, $p = 0.0001$) and longer hind limbs (a 1% difference, ($F = 15.07$ $p = 0.0007$;

alpha level of 0.003). These differences in proportion, while interesting, are insufficient to distinguish the species on brief inspection. However, our meristic observations yielded rather striking differences between the two island populations; these morphological characters are non-overlapping between the geckos of the two islands. São Tomé *H. greeffi* males have more preano-femoral pores (42 to 48) than those on Príncipe (26 to 39), more rows of divided sub-digital lamellae beneath the third finger and third toe (São Tomé: 7 to 8 and 8 respectively; Príncipe: 6 and 6 to 7) and the enlarged slightly keeled tubercles arranged in longitudinal rows on the dorsal and lateral surfaces of the trunk are more than four times the size of the surrounding granular scales in São Tomé *H. greeffi,*, but two to three times the size in Príncipe individuals. A consistent difference in the iris colour of living specimens was noted. Diagnostic and additional meristic and morphometric data are presented in Appendix 1.

Molecular

Based on results from mitochondrial sequence data from 12S rRNA, 16S rRNA and cytochrome b, Jesus *et al.* (2005a) noted that *H. greeffi* specimens had an average of 3.3% genetic divergence between islands, higher than what is reported for some other lizard species (Austin 2004). However, they were unable to differentiate the island populations based on nuclear sequence data from the intron of α-Enolase. We were able to sequence the α-Enolase fragment for 10 Príncipe and 12 São Tomé specimens. Sequences were trimmed and a final 212 bp fragment was used for analyses. Three unique haplotypes were found within the São Tomé specimens, while only one haplotype was found in the Príncipe specimens. There were no shared haplotypes between the two island populations. In addition, a higher number of heterozygous sites was found in the São Tomé sequences vs. those of Príncipe. While only one individual had one heterozygous site in the Príncipe samples, five individuals had a total of 10 heterozygous sites in the São Tomé specimens (Fig 1). Sequence divergence estimate between the islands was approximately 1% and AMOVA results indicated highly significant population structure ($F_{ST} = 0.843$, $p < 0.00001$). All sequences have been deposited to GenBank under accession numbers (JQ611732 – JQ611753).

Hemidactylus principensis Miller, Sellas & Drewes sp. nov.

Holotype.—CAS 219212, an adult male collected on a tree trunk along the beach below Sao Joaquim, Príncipe Island, Republic of São Tomé and Príncipe (1°37′19.87'N, 7°22′13.10'E). Collected by R.E. Stoelting (Fig 2).

Diagnosis.—Differs from all other members of the genus except *Hemidactylus greeffi* in the absence of the terminal phalanx and claw of the first digit (thumb) of the manus, (Fig 3) and from *H. greeffi* in a gold-coloured iris (Fig 4A), smaller adult body size (SVL: ♂♂$\bar{x} = 61.4$ mm;♀♀$= 54.2$ mm), smaller size of enlarged dorsal tubercles (Fig 5A), lower number of divided lamellae beneath the third digit of the manus (six; Fig 6A) and third digit of the pes (six; Fig 6B), lower number of preano-femoral pores (26 to 39, $\bar{x} = 34.7$) and some superciliary scales noticeably pointed (Fig 4A, insert).

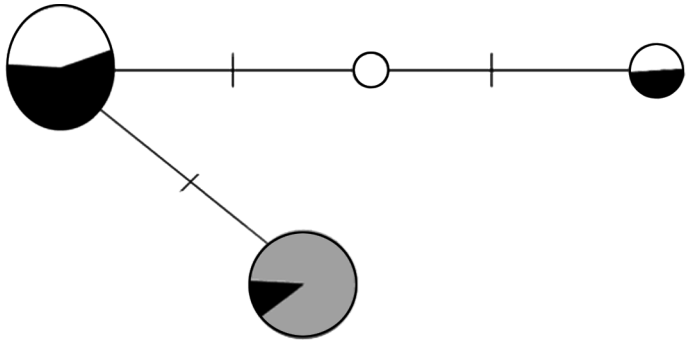

Figure 1. Haplotype reconstruction network of *Hemidactylus* α-Enolase sequences from São Tomé (open circles) and Principe (grey circle). Each circle represents a unique haplotype with black regions representing proportion of sequences containing one or more heterozygous sites. Mutational steps are indicated by hash marks. The size of the smallest circle represents a haplotype frequency of 1. There were no shared haplotypes between the islands.

Description of holotype.—Adult male, 64.55 mm SVL. The tail, 69.0 mm long, is regenerated. The head is short (HL/SVL = 0.27) and wide (HW/HL = 0.68), with a relatively short snout (ES/HL = 0.38). The ear opening is an oblique oval approximately one-third the diameter of the eye. Scales of occipital and inter-orbital

Figure 2. Holotype: *Hemidactylus principensis* ♂. CAS 219212. (A) Dorsal. (B) Ventral.

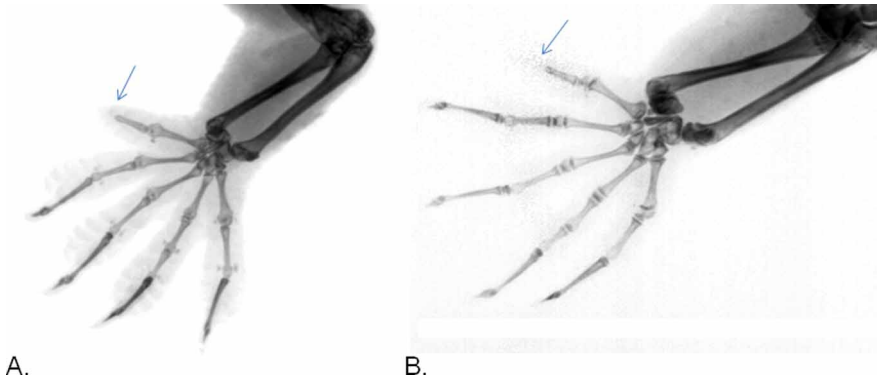

Figure 3. Radiographs: (A) Left manus of *Hemidactylus principensis* holotype CAS 219212. (B) Left manus of *Hemidactylus greeffi* neotype CAS 219062.

region of the head are rounded, subequal in size, gradually increasing in size anteriorly to the nasals and laterally in the canthal region. A few slightly enlarged granular scales in the postorbital cheek region. Two enlarged postmentals, followed

Figure 4. (A) *Hemidactylus principensis* paratype in life, CAS 233430♀; arrows in insert indicate enlarged pointed superciliary scales. (B) *Hemidactylus greeffi*, in life, CAS 233488♂

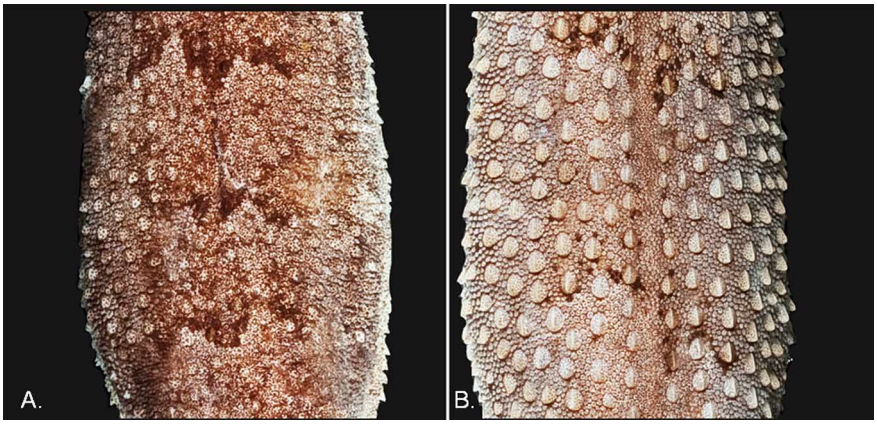

Figure 5. Mid-dorsal tubercles of (A) *Hemidactylus principensis*: holotype CAS 219212 and (B) *Hemidactylus greeffi* neotype CAS 219062.

by a single row of moderately enlarged scales bordering the medial edge of the infralabials. Rostral scale partially divided by a vertical fissure. Nostril bordered by the rostral, first supralabial, and three small nasals. Superciliaries numerous and fine edged. Supralabials 11/10, infralabials 10/8.

Dorsal and lateral surfaces of the trunk covered with small subequal granular scales, within which are 21 longitudinal rows of slightly keeled enlarged tubercles, each two to three times the size of surrounding granular scales. A ventrolateral fold of skin continuous between axillae of the limbs, along which are aligned slightly enlarged, pointed but unkeeled scales; the latter are slightly smaller than the enlarged tubercles of the dorsum but larger than adjacent lateral scales (Fig 5A).

Body relatively gracile (BW/SVL = 0.21). Dorsal surface of forelimbs with moderately enlarged scales, some weekly keeled, becoming increasingly more imbricate distally; anterior, medio-dorsal and posterior surfaces of the hindlimbs beset with enlarged, conical, unkeeled tubercles extending distally to the ankle. Limbs are relatively short, with the forelimb distance 14% and hindlimb distance 15% of SVL. Individual scansors of divided lamellae rounded distally. Six divided lamellae under the third, fourth and fifth digits of the pes, with three basal undivided lamellae under the fourth and fifth digit. Digits of pes slightly webbed. Very small, narrow, oblique preano-femoral pores in 36 continuous rows. Hemipenial bulges present but not well developed.

Colouration (in preservative; Fig 2): medium-brownish grey body with nine roughly 'M-shaped' chevrons across the dorsum between the snout and the base of the regenerated tail. Individual chevron bands more intensely pigmented at posterior margins. Regenerated portion of the tail without pattern; unregenerated tails are broadly banded throughout their length, with the distal-most bands being the widest. Ventrum immaculate, whitish.

Etymology.—This species is named for the Island of Príncipe of the Republic of São Tomé e Príncipe, to which it is endemic.

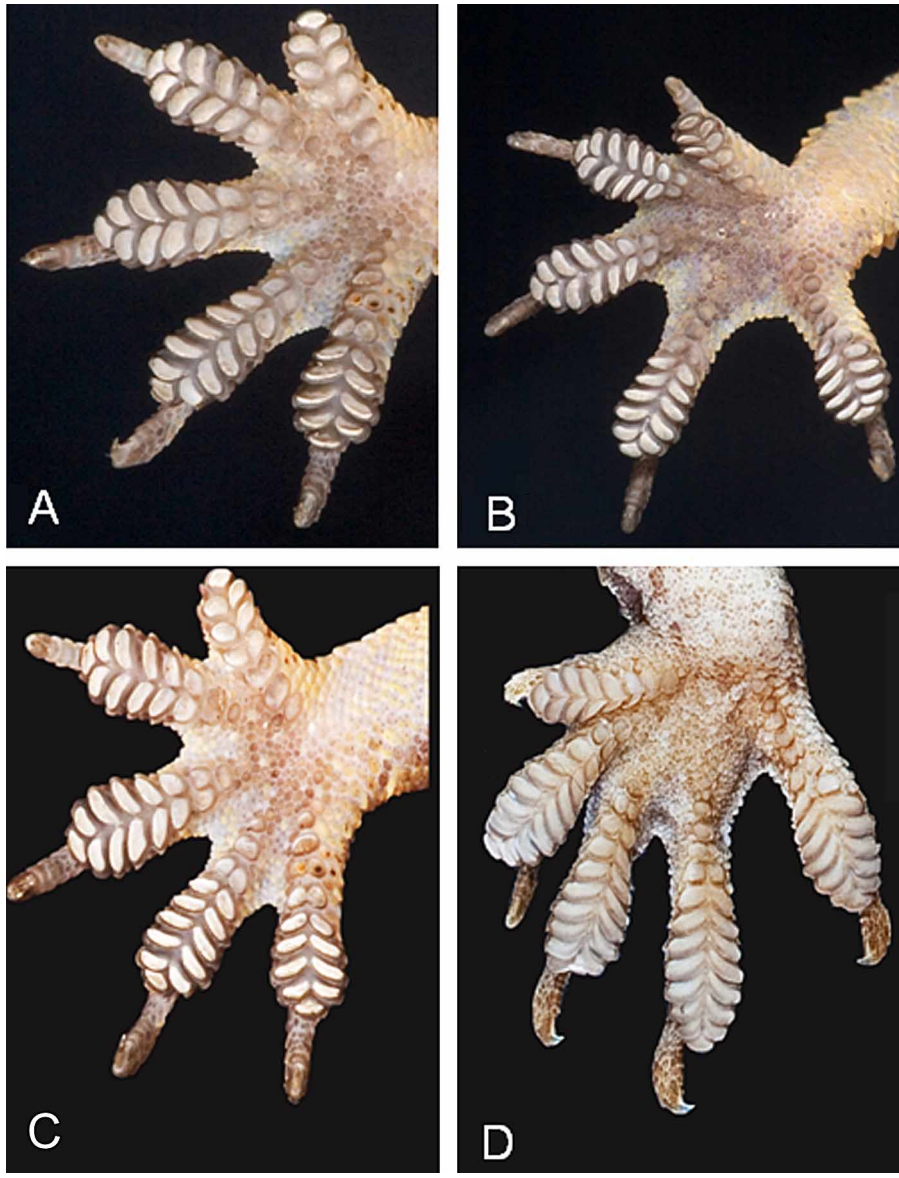

Figure 6. *Hemidactylus principensis* paratype CAS 233430: ventral views of right manus (A) and left pes (B); *Hemidactylus greeffi* neotype CAS 219062: ventral views of left manus (C) and right pes (D).

Paratypes.—CAS 219213 ♂ collected from type locality (paratopotype); CAS 233420 ♀, 233421 ♂, collected from 1°39′ 39.84’ N, 7° 23′ 38.7’ E, elevation 176 m. CAS 219198 ♀ collected 3.55 km NW of Santo Antonio (1°39′ 38.8’ N, 7°23′ 39.8’ E). CAS 233429–30, ♀♀, collected along a road between Santo Antonio and Sao Joaquim (1° 37′ 26.2’ N, 7° 23′ 29.3’ E), elevation 180 m. CAS 233524–26, all ♀♀, collected on road along the Papagaio River (1° 37′ 44.4’ N, 7° 25′ 1.1’ E), elevation 12 m. CAS 238894 ♂ collected on road to Bom Bom (1° 41′ 18.61’ N, 7° 24′ 13.88’ E).

Variation in paratypes.—SVL in males ranges from 53.8 to 65.9 mm; in females it is 50.2 to 62.8 mm. Among those specimens with complete tails (CAS 233524, CAS 233526 and CAS 233430♀♀), the tail ranges from 57 to 72 mm in length (slightly longer than SVL), is strongly depressed, cyclotetragonal in shape and is arranged in a series of 31 to 43 whorls with a markedly elongate spine at dorsolateral and ventrolateral corner of each whorl. The tails are thin compared to the body, half the width of the trunk at the base and gradually tapering.

Supralabials and infralabials vary from 8 to 11 and 8 to 10, respectively. Number of divided lamellae on the third, fourth and fifth toes ranges from 6 to 7. All males in the collection have enlarged jaw muscles that give the appearance of a broader head than in females and may be a secondary sexual character. Male preano-femoral pores are small flattened ovals and in some specimens can be quite inconspicuous. CAS 233421 has an additional row of three pores to the left of the vent underneath the near-continuous row of preano-femoral pores. CAS 233420, CAS 233429–30, CAS 233524–26, all ♀♀, have conspicuous bulges on either side of the neck, possibly calcium deposits. Enlarged dorsal tubercles range from two to three times larger than surrounding scales.

A ventrolateral fold is present in all material examined, but the slightly enlarged, pointed scales along it are most noticeable on the posterior half of the fold.

Colouration in preservative of dorsum ranges from light ashy beige (CAS 233429) to a deep brown (CAS 233420). Ventral colouration ranges from creamy white to dark brown. The pattern on the dorsum in the majority of the specimens is much more pronounced than that of *H. greeffi,* with seven to eight blotchy, wavy bands. The pattern is faded on five of the specimens. All specimens have a beige band across the eye and snout, a trait that is not common in *H. greeffi.* In specimens with unregenerated tails, the tails are broadly banded throughout their length, with the distal-most bands being the widest.

Colouration in life (Fig 4A,). Iris of the eye coppery-gold; overall colour is a combination of dark brown and light pink-toned beige. A continuous, broad, undulating mid-lateral stripe runs the length of the trunk, beginning at the nostril and passing through the eye, broadening at the insertion of the forelimbs and terminating at the insertion of the hind limb. Several thick brown chevron bands on the dorsal surface beginning immediately behind the head and continuing along the tail, with some bands ending in dark brown blotches or lines. Thinner brown bands along the limbs and digits. Ventral surface is patternless.

Distribution.—Restricted to Príncipe Island, Republic of São Tomé and Príncipe, West Africa.

Habitat.—Unlike *H. mabouia* and perhaps *H. longicephalus, Hemidactylus principensis* does not appear to occur in inhabited human dwellings. Our specimens were generally collected ca. 1 to 1.5 m above ground on tree and banana trunks, stone bridges and small boulder outcroppings along a dirt road.

History of *Hemidactylus greeffi* and designation of neotype

Bocage described *H. greeffi* in a brief article in Portuguese (1886a), then wrote a more detailed description in the same volume in French (*op. cit*. b). The original type

was a single male specimen (total length of 126 mm) collected by Dr Greeff on the island of São Tomé. The type was evidently not registered; no identifying number or descriptor was furnished. In the latter French description, Bocage mentioned two additional specimens (in his possession?) as sharing the condition of the reduced thumb, which he felt served to diagnose *H. greeffi* from all its congeners. In addition to this character, he also stated that the type possessed 12 preanal pores and lacked femoral pores, an observation at variance with our own data and subsequently corrected by Bedriaga (1892). The number of preano-femoral pores is a non-overlapping diagnostic character between *H. greeffi* and *H. principensis*. Other meristic characters provided by Bocage (*op.cit.*b) are equivocal with regard to the two species. Later, in the same volume (*op.cit.*c), Bocage compared his *H. greeffi* characters with 12 specimens of *H. mabouia* collected on São Tomé by M. Newton and housed at the museum in Coimbra, Portugal. None of this material was identified by number and no specific locality data nor meristic data were provided that would preclude the possibility that any of the material mentioned above might actually have been collected on Príncipe rather than the larger São Tomé Island.

Bedriaga (1892) published a much more comprehensive description of a male and female collected by M.A.F. Moller on São Tomé. His specimens conform to the range of variation of *H. greeffi* not *H. principensis* in at least two of the characters we regard as diagnostic between the two species: number of preano-femoral pores (46) in the male and number of subdigital lamellae (Appendix 1). As already stated, preano-femoral pores in *H. principensis* are small flattened ovals in shape and can be easily overlooked. Again, the specimens were not numbered, no specific locality data were provided and their museum provenance is not known. No examples of *H. greeffi* exist at the Natural History Museum in Coimbra (Dr P.G. Mota, pers. comm.), and all of the scientific collections in the Museum of Natural History in Lisbon (Museu Bocage) were totally destroyed by fire in 1978.

Loveridge's (1947) account of *H. greeffi* was based on three specimens with specific localities on São Tomé (Lagoa Amelia and Roca Sandade [*sic*: = Saudade] and Príncipe (Sao Matheus). His count of preano-femoral pores (34 to 36) conforms to *H. principensis* of Príncipe. The provenance of the material he examined was not specified, and the collections of the Museum of Comparative Zoology (Harvard) contain no specimens of *H. greeffi* (J. Rosado, *pers. comm.*) The Natural History Museum in London (BMNH) collections contain three specimens of *H. greeffi;* coincidentally, there are two from São Tomé and one from Príncipe as in Loveridge (1947) but these are associated with island names only and not with specific locality data. Thus, it is unlikely they provided the basis of Loveridge's treatment (D. Gower, pers. com.).

The material analysed by Jesus *et al.* (2005a) was collected in 2002 and vouchers are housed in the University of Madeira – some tissue came from samples of gecko tails only (Jesus, pers. com.); their analysis was heavily biased toward Príncipe, with sequences from 12 *H. principensis* vs. two *H. greeffii*. In recent years, the tissue collections resulting from the CAS expeditions to São Tomé and Príncipe (2001–2011) have been utilised by other researchers at institutions worldwide. Among these have been a number of studies, including purported '*Hemidactylus greeffi*,' under the assumption that populations of this taxon on both islands were the same species. Several of these studies present broad phylogeographic hypotheses, including

Carranza & Arnold (2006 – three *H. principensis*), Bauer *et al.* (2010) – one *H. greeffi*) and Gamble *et al.* (2010 – one *H. greeffi*).

Given the loss of the *H. greeffi* type material and the uncertain provenance of material subsequently treated, together with our new understanding that there are two presumably old lineages of *Hemidactylus* rather than one (both diagnosable by the loss of the terminal phalanx of the thumb), we designate a neotype for *H. greeffi* below and provide additional information on both taxa in Appendix 1.

Neotype

Hemidactylus greeffi Bocage 1886. CAS 219062♂. Republic of São Tomé and Príncipe: São Tomé. Id: Contador Valley, slope on W side of Rio Contador viaduct, within south entrance of tunnel. 00 18 24.0 N, 006 33 04.6 E. Col: R.C. Drewes, R.E. Stoelting and J.V. Vindum (Fig 7).

An adult male, SVL = 83.5 mm; TL = 180.5 mm (distal 17.8 mm of tail regenerated). Iris of eye light moss-green in life (Fig 4B insert). Enlarged keeled tubercles in 20 dorsolateral rows, each tubercle ca. four times the size of the surrounding granules (Fig 5B); eight divided lamellae beneath the third finger (Fig 6C), nine beneath the third toe (Fig 6D); 48 preano-femoral pores. Bocage (1886b,c) described a longitudinal ventrolateral fold of skin between the axillae, along which are arranged enlarged tubercles. Bedriaga (1892) suggested that this fold and the

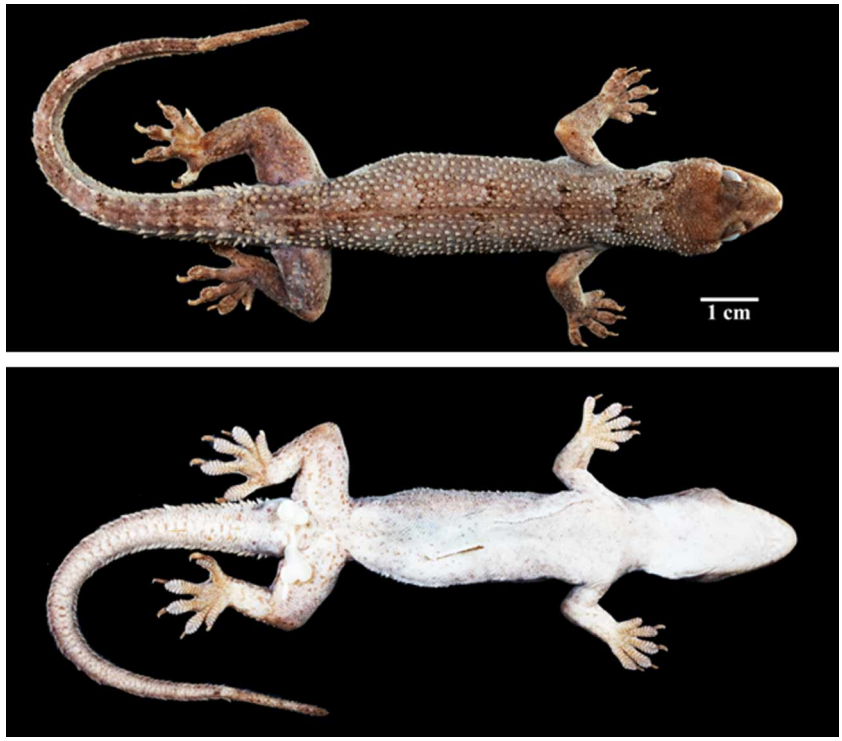

Figure 7. *Hemidactylus greeffi* neotype CAS 219062♂ (A) dorsal; (B) ventral.

enlarged scales are more developed in males than in females. While the fold itself is present and obvious in most specimens examined, the size and number of enlarged pointed scales along it is variable. In most specimens, these scales are noticeably enlarged along the posterior half of the fold; in one (CAS 219281♀) they are present on the posterior two-thirds of the fold on the left side of the specimen and but one-third on the right. Superciliary scales not pointed, not overlapping eye in dorsal view. All other parameters fall within the ranges for *H. greeffi* in Appendix 1.

Range.—Restricted to the island of São Tomé, Republic of São Tomé and Príncipe, West Africa.

Habitat.—So far as is known, *H. greefi* does not occur in inhabited human dwellings but, like *H. principensis*, is frequently found on man-made structures, such as stone walls, road culverts, the Contador Valley aqueduct tunnel, but tree trunks as well. It does not appear to co-occur with *H. longicephalus* on the open basalt cliffs at Praia Mutamba, just north of the road culverts, from which nine of our specimens were taken (CAS 219044–45; 219280–83; 233488–90. The undocumented specimen recorded by Loveridge (1947) from Lagoa Amelia suggests that the known altitudinal range is from near sea level to ca. 1400 m.

One of our larger specimens, CAS 233674 (SVL = 85.2 mm), was collected at mid-day as it was being devoured by a São Tomé green-snake, *Philothamnus thomensis* (CAS 233675).

Snout-ventlengths for CAS males range from 66.9 to 86.2 mm; females are from 56.0 to 73.1. Known as Greeff's giant gecko, *H. greeffi* has been cited in error as the largest African member of the genus (Drewes & Wilkinson 2004); our largest male specimen, CAS 219062, is 86.2 mm SVL. This specimen is surpassed in size, however, by two other specimens cited in Loveridge (1947): the male type of *Hemidactylus formosus* Hallowell from Liberia (SVL = 95 mm) and by the female paratype of *Hemidactylus fasciatus ituriensis* Schmidt (SVL = 89 mm).

Our genetic data, in combination with differences in morphological characters, support our hypothesis that the two island populations are species distinct from one another and that the Príncipe species has previously been unrecognised.

DISCUSSION

Previous authors (Bocage 1886a,b,c; Bedriaga 1892; Loveridge 1947; Schätti & Loumont 1992) have had access to preserved specimens only or, in the case of Jesus *et al.* (2005a), have conducted molecular analysis only without morphological examination. As a result, earlier workers have overlooked the obvious fact that, in *H. greeffi,* the colour of the iris is invariably a distinctive light moss-green (Fig 4B, insert), whereas that of *H. principensis* is coppery-gold (Fig 4A, insert). In addition to the other unequivocal morphological differences cited above, this particular character serves to distinguish *H. greeffi* from all other squamates on both islands. *H. greeffi* and *H. principensis* share the unique synapomorphy of the loss of the penultimate digit of the thumb (Fig 3), which would suggest that the two species are each other's closest relatives. However, our current genetic data are not yet robust enough to confirm this assertion (see below).

The relatively higher degree of genetic variability in α-Enolase sequences of *H. greeffi* of São Tomé with regard to number of haplotypes and heterozygous sites may be simply a reflection of differences in overall population size and habitat diversity or may reflect time of colonisation. Although both islands are geomorphologically complex, the island of São Tomé is currently more than seven times the area of Príncipe (836 km^2 vs. 128 km^2). It is also possible that samples collected from São Tomé Island are from more than one genetically distinct population and this is contributing to the overall diversity. Significant population structure has been found on this island for another lizard species, *Mabuya maculilabris* (Jesus *et al*. 2005b).

As stated above, tissues from both species, *H. greeffi* and *H. principensis*, have been included in studies as '*H. greeffi*.' Three of these are phylogeographic studies that hypothesise ancient trans-Atlantic dispersal by members of the genus *Hemidactylus*. Carranza & Arnold (2006) defined an African–Atlantic clade that included *H. longicephalus* (mainland Africa and Gulf of Guinea – samples from São Tomé), *H. greeffi*, (Príncipe samples [= *H. principensis*]), *H.* (*Briba*) *brasileanus* (Brazil) and *H. bouvieri* (three different Cape Verde Islands localities). They placed *H. greeffi* and *H. longicephalus* in a clade that is sister to *H. brasileanus* plus three distinct populations of *H. bouvieri*. Bauer *et al.* (2010) found *H. brasileanus* to form a clade with *H. greeffi* (São Tomé sample), which is sister to *H. longicephalus* (São Tomé sample). Analysing the same samples, Gamble *et al.* (2010) arrived at the same relationship: (*H. longicephalus* (*H. greeffi* + *H. brasileanus*)). They stated that the Brazilian members of the African–Atlantic clade diverged from their Old World sister taxa (*H. greeffi* and *H. longicephalus*) between 7.4 ma and 21 ma. This range of divergence time of the Neotropical members of the clade from the African members includes a period of seven my, during which the island of São Tomé did not exist and the island of Príncipe was the only oceanic member of the archipelago as it exists today. The data presented above indicate that there is an additional member of the African–Atlantic clade, *H. principensis,* which has hitherto been overlooked in the studies cited, and the question arises as to which oceanic Gulf of Guinea island was colonised first by the common ancestor of members of the African–Atlantic clade.

It is not inconceivable that the much older Príncipe Island was colonised first, prior to the emergence of São Tomé, and that the genetic uniformity of *H. principensis* vs. *H. greeffi* might be the result of a subsequent 'bottleneck' effect. The mid-Oligocene (31 mya) area of Príncipe can be estimated by measuring the dimensions of the island at the ca.100 m depth contour line (Krakstad *et al.* 2010). Approximately 15 my before the emergence of São Tomé, Príncipe was about 4 200 km^2 in area, roughly four times the combined area of the two islands today. Theoretically (geometrically), it is not impossible that Príncipe supported a volcanic peak of >4 000 m. Oligocene Príncipe was roughly twice the size of the Neogene continental island of Bioko today (Marzoli *et al.* 2000) and the latter is dominated by a 3 000 m peak. Thus, early Prìncipe provided an enormous, probably niche-rich target for colonisation from the mid-Oligocene to mid-Miocene epochs: in fact, the only equatorial Atlantic target.

Ongoing work is focusing on a deeper genetic analysis of all current members of the African–Atlantic clade, *H. longicephalus, H. greeffi, H. principensis, H. brasileanus, H. mabouia and H. palaichthus*, to determine first the phylogenetic

position of *H. principensis* within this clade and, second, to shed further light on the patterns of the trans-Atlantic dispersal of members of this clade, first hypothesised by Carranza & Arnold (2006). Future work will also investigate whether significant population structure exists among São Tomé samples. In addition, cloning of heterozygous individuals will allow us to determine whether there are multiple copies of the α-Enolase sequence in *H. greeffi* or whether many individuals do, in fact, possess multiple alleles.

Additional Material Examined

Hemidactylus greeffi: Republic of São Tomé e Príncipe: São Tomé Island: CAS 219006♀: Java 00° 15′ 39.9'N, 006° 39′ 03.2'E; CAS 219280♂, 219281–283♀♀, CAS 219044♂-045♀, CAS 233488–489♂♂, 233490♀: west coast road between Praia Mutamba and Ponta Barro Bobo 00° 23′ 29.8'N, 006° 36′ 02.7' E to 00° 23′ 30.5'N, 006° 36′ 02.5'E; CAS 219063♂; Contador Valley, west side Rio Contador in aquaduct segment entrance 00° 18′ 43.4' N, 006° 32′ 59.0'E; CAS 233674♂: bridge at Agua Panada 00° 16′ 04.9'N, 006° 38′ 56.0'E; CAS 233481; Pousada 00° 17′ 11.9″N, 006° 38′ 07.0″E

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APPENDIX 1: MALE AND FEMALE MORPHOMETRIC AND MERISTIC DATA FOR ALL SEXUALLY MATURE SPECIMENS EXAMINED

	São Tomé males ($n=7$)	São Tomé females ($n=7$)	Príncipe males ($n=4$)	Príncipe females ($n=7$)
SVL	66.9–86.2; 76.7±8.1	56.0–73.1; 62.0±6.3	53.8–65.9; 61.4±5.4	50.2–62.8; 54.2±4.3
Head length	15.8–22.9; 19.6±1.8	15.9–19.0; 17.2±1.1	14.5–17.7; 16.7±4.5	13.2–16.2; 14.5±1.1
Head width	12.1–16.3; 13.8±1.1	10.3–13.4; 11.5±1.0	10.2–12.7; 11.2±0.8	8.5–11.0; 9.3±1.5
Eye–ear distance	5.4–7.1; 6.4±0.7	4.1–5.2; 4.7±0.5	4.7–5.8; 4.9±0.6	4.4–5.6; 4.3±0.8
Eye–snout distance	7.2–9.5; 8.4±0.9	6.6–7.6; 6.9±0.3	5.6–7.0; 6.4±0.6	5.1–6.9; 5.4±0.7
Interlimb distance	28.1–39.3; 33.6±3.6	24.4–33.5; 27.9±2.5	21.4–29.5; 26.1±3.4	20.9–28.8; 21.6±5.5
Tail base width	5.9–8.7; 7.4±1.0	4.2–6.6; 5.1±0.8	4.8–6.8; 5.9±0.9	4.0–5.2; 4.8±0.4
Internarial distance	2.4–3.1; 2.8±0.3	2.0–2.9; 2.3±0.3	1.9–2.5; 2.2±0.2	1.8–2.2; 2.0±1.1
Body width	13.0–16.2; 14.5±1.4	8.8–12.6; 11.0±1.5	9.7–13.5; 11.5±1.6	9.5–12.3; 10.5±1.0
Head height	6.9–9.9; 8.5±0.9	5.8–7.9; 6.5±0.7	5.4–7.5; 6.4±0.9	4.8–6.6; 5.3±0.6
Eye diameter	4.1–4.6; 4.3±0.2	2.9–3.9; 3.5±0.4	2.6–3.3; 3.1±0.3	2.6–3.3; 3.0±0.2
Ear diameter	0.8–1.5; 1.1±0.3	0.8–1.5; 1.3±0.3	0.6–1.3; 1.0±0.3	0.6–1.2; 0.8±0.2
Palm-elbow length	9.9–13.3; 11.5±1.3	8.4–10.7; 9.3±0.8	7.2–10.0; 8.7±1.2	7.0–8.7; 7.5±0.5
Heel-knee length	11.8–14.2; 13.3±0.9	9.2–12.8; 10.5±1.2	7.7–10.8; 9.7±1.3	8.1–10.1; 8.6±0.7
# preanal/femoral pores (males)	42–48; 44.4±2.1	N/A	26–39; 34.7±6.0	N/A
# longitudinal rows of enlarged tubercles on dorsum	17–20; 18.4±1.5	17–21; 18.8±1.5	21; 21±0	18–21; 19.1±1.1
# supralabials (right side)	9–12; 10.7±1.1	9–11; 10.0±1.0	9–10; 9.7±0.5	9–11; 10.3±0.7
# infralabials (right side)	8–9; 8.6±0.5	8–9; 8.3±0.5	8–9; 8.2±0.5	8–10; 9±0.6
# divided lamellae on 3rd finger (right hand)	7–8; 7.1±0.4	7–8; 7.3±0.5	6; 6±0	6; 6±0
# divided lamellae on 3rd toe (right foot)	8–9; 8.3±0.5	8–9; 8.3±0.5	6–7; 6.2±0.5	6–7; 6.4±0.5

Notes: SVL, snout–vent length.
Measurements: range; mean±SD.