Zootaxa 4300 (2): 195–216
http://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4300.2.3
http://zoobank.org/urn:lsid:zoobank.org:pub:9FEDD063-A972-49A2-8305-BDABD53C11F8

Uncovering a hidden diversity: two new species of *Breviceps* (Anura: Brevicipitidae) from northern KwaZulu-Natal, South Africa

LESLIE R. MINTER[1], EDWARD C. NETHERLANDS[1,2] & LOUIS H. DU PREEZ[1,3]

[1]*Unit for Environmental Sciences and Management, North-West University, Potchefstroom 2520, South Africa.*
[2]*Laboratory of Aquatic Ecology, Evolution and Conservation, University of Leuven, Charles Debériotstraat 32, B-3000 Leuven, Belgium.*
[3]*South African Institute for Aquatic Biodiversity, Somerset Street, Grahamstown 6140, South Africa.*

Abstract

Breviceps carruthersi **sp. nov.** and *Breviceps passmorei* **sp. nov.** are described from northern KwaZulu-Natal. Both new species are distinguished from *B. adspersus*, *B. mossambicus*, *B. poweri* and *B.* cf. *sopranus* by substantial interspecific genetic divergence (> 6.8 uncorrected p-distance) in the mitochondrial 16S rRNA gene, differences in advertisement call structure and limited morphological variation. *Breviceps carruthersi* **sp. nov.** has a short, pulsatile call with minimal amplitude modulation and groups its calls. *Breviceps passmorei* **sp. nov.** differs significantly from *B. adspersus* and *B. mossambicus* in call duration and pulse number, but not in pulse rate or dominant frequency. For both new species morphological characters are of limited diagnostic value, necessitating a re-evaluation of the taxonomic status of populations currently assigned to *B. adspersus* and *B. mossambicus* in KwaZulu-Natal.

Key words: Amphibia, *Breviceps carruthersi* **sp. nov.**, *Breviceps passmorei* **sp. nov.**, advertisement calls, bioacoustics, cryptic species, molecular genetics, morphology, Zululand

Introduction

The existence of cryptic species, i.e. two or more species that have been classified as a single nominal species because they are, at least superficially, morphologically indistinguishable (Bickford *et al.* 2007), has always presented a problem for morphological taxonomists. The advent of molecular methods for determining genetic distance between populations has provided a valuable tool for detecting the presence of such species and has resulted in very significant increases in estimated species richness in many regions (Bickford *et al.* 2007, Fouquet *et al.* 2007, Funk *et al.* 2012, Dawood *et al.* 2002). Identification of cryptic species has important implications in other fields such as conservation, biogeography, agriculture and medicine (Angula & Icochea 2010, Bickford *et al.* 2007, Paterson 1991).

Once cryptic species are identified, subsequent examination often reveals subtle but consistent morphological differences (eg. Elmer & Connatella 2008), but it is usually necessary to adopt an integrated approach by including characteristic behavioural and/or ecological differences in the species descriptions. Bioacoustics has proved valuable in this regard due to the function of the advertisement call as a species specific mate recognition signal (Passmore 1985, Paterson 1985), and is now widely used in frog taxonomy (eg. Köhler *et al.* 2015).

Breviceps Merrem, 1820 currently comprises 16 species, of which 12 are endemic to South Africa, Lesotho and Swaziland (Frost *et al*. 2017). As Poynton (1964:70) noted, "the diagnosis of the different forms of *Breviceps* is particularly difficult on account of the variation and slight morphological differentiation". However, the inclusion of advertisement calls as a diagnostic character resulted in the discovery of two cryptic species (Minter 2003) and facilitated reliable identification in the field. Genetic characterisation of several *Breviceps* populations in Mozambique and north-eastern South Africa has identified additional divergent lineages which may represent undescribed cryptic species (Nielsen *et al.* unpub. data).

In 1988, LM collected a sample of 22 *Breviceps* specimens (19 with associated calls) from three locations west

Accepted by M. Vences: 26 Jun. 2017; published: 2 Aug. 2017

of Tembe Elephant Reserve in the vicinity of the Phongolo River, which he provisionally assigned to *B. adspersus* Peters, 1882 on the basis of morphological and advertisement call characters (Minter 1998). At one locality, some individuals produced single, strongly pulsed calls, longer than that of a typical *B. adspersus* advertisement call, while others gave relatively short calls, which were usually grouped. When the combined call sample was analysed, the range of variation in several call parameters overlapped broadly with those of the Polokwane population assigned to typical *B. adspersus*. He noted, however, that "the unusually large coefficients of variation in call duration and pulse number and the unusually long calls emitted by some individuals invite further investigation" (Minter 1998: 80).

In 2015 a population of a *Breviceps* with an unusual advertisement call was discovered at Hluhluwe, and an additional specimen was photographed and its call recorded by Darel Dell at Phinda Private Game Reserve, about 35 km north of Hluhluwe. These individuals are also morphologically similar to *B. adspersus*, but whereas the latter produces pulsed calls, in this species the calls are pulsatile, i.e. neither tonal nor pulsed, but exhibiting barely quantifiable amplitude modulation (sensu Köhler *et. al.* 2017). Additional specimens, tissues and calls of both the Tembe and the Hluhluwe populations were collected in 2017. This study uses molecular, bioacoustic and morphological data to describe two new cryptic *Breviceps* species.

Materials and methods

Sampling. Specimens were collected in northern KwaZulu-Natal, South Africa (Table 1; Fig. 1). After calls were recorded, air temperature was measured at ground level using a Sensortek Bat-12 digital thermometer (accurate to 0.1°C). Captured specimens were weighed on a Sartorius PT120 portable balance (accurate to 0.01g), photographed, euthanased using tricaine methanesulfonate (MS222) solution, fixed in 10% buffered formalin and preserved in 70% ethanol. Liver samples were taken following euthanasia and were preserved in 100% ethanol for genetic analysis. Holotype, holotype advertisement calls and paratypes were deposited at the South African Institute for Aquatic Biodiversity (SAIAB); additional paratypes were deposited in the herpetological collection of the Port Elizabeth Museum (PEM), while those paratypes collected in 1988 by LM remain in the Ditsong Museum of Natural History (TM).

Bioacoustics. Advertisement calls were recorded in the field using various recorders (Marantz PMD671 digital recorder set to PCM 24 bit audio format and a sampling frequency of 44.1 kHz, Sony TC-D5PROII tape recorder, Nagra-AresM digital recorder) and an external microphone (Sennheiser ME80). Analogue to digital conversion was carried out on the Marantz recorder and the digital signals were analysed using Raven Pro Version 1.5.0 (www.birds.cornell.edu/raven). Spectrograms were generated using a Hanning window, set at Window size 45 and 300; DFT size 512. One to three call bouts were recorded before collecting each specimen and three advertisement calls per male were analysed. Because of the small sample sizes, it was not possible to calculate and apply correction factors for temperature and mass.

The bioacoustic terminology used here follows Köhler *et al*. (2017), and is call-centred, i.e., the call is defined as "the main coherent sound unit", separated from other calls by an interval of silence that is usually the same length, or longer, than the call. In the genus *Breviceps* males advertise their presence by producing a series of identical, repeated calls. Each call is emitted during a single expiration, and may be tonal, pulsatile or pulsed. The period of vocal activity during which the series of calls is produced is referred to as a call bout: these are usually separated by relatively long intervals of silence (1.5 to 10 minutes in *B. adspersus*, n = 100, Minter 1995) which become shorter as the chorus intensity increases. A call bout may contain a series of single calls or, in some species, the calls may be condensed into call groups consisting of two, three or more, evenly spaced calls. The size of the call group is related to chorus strength: in low intensity choruses, the call bout may contain predominantly single calls. The interval between calls (call period) within a group is shorter and less variable than the call period between single calls or between two call groups (Minter, 1995, 1997, 1998). Parameters within the call group and call, that show the least variation, such as call period, call rate, call duration, pulse number, pulse rate and dominant frequency, are likely to function in mate recognition.

The following call parameters were measured: call period (call interval *sensu* Minter 1995, 1997, 1998) within call groups (s), defined as the interval between two consecutive calls within a group, measured from the beginning of one call to the beginning of the consecutive call; call period between single calls/call groups (s), defined as the

call period between consecutive single calls, or between the beginning of the last call in a group and the beginning of the first call of the consecutive group or single call; call (repetition) rate within call groups (min^{-1}), calculated as

$$\frac{x-1}{y} X\ 60$$

where x = the number of calls in a call group, y = time (s) from the beginning of the first to the beginning of the last call in the group; call duration (s) measured, in an oscillogram, from the beginning of the first to the end of the last recognisable pulse (in pulsatile and tonal calls, the entire call), and pulse (repetition) rate (s^{-1}) defined as the time between the beginning of the first and the beginning of the last recognisable pulse, divided by the number of pulses between these points. Dominant frequency (Hz) was measured as the peak frequency of the call in a spectrogram slice view taken through the midpoint of the call. The same call parameters were used by Minter (1995, 1997, 1998) in the analysis of calls of *B. adspersus*, *B. mossambicus* Peters, 1854 and *B. poweri* Parker, 1934, referred to in this publication.

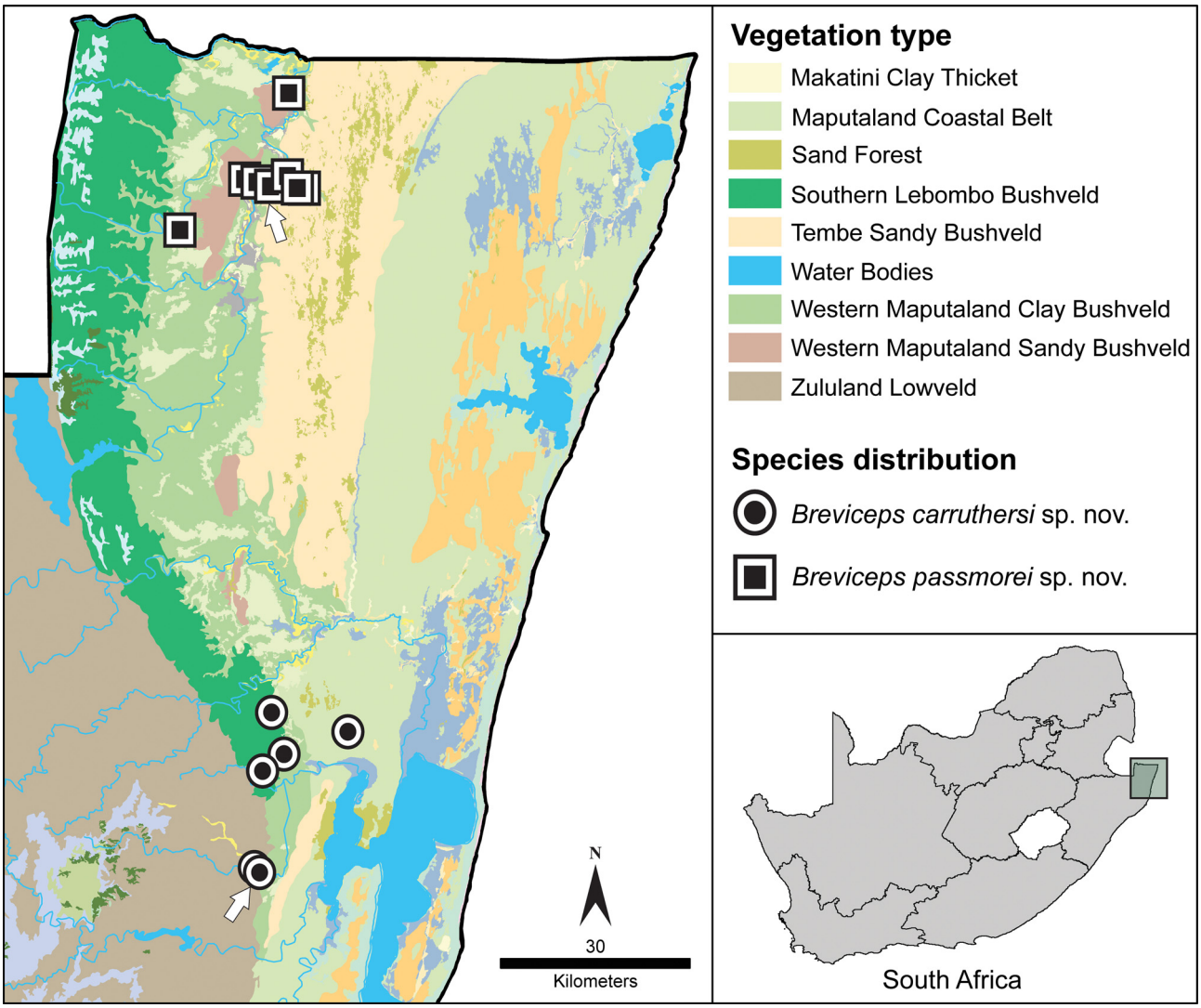

FIGURE 1. Map of northern KwaZulu-Natal province showing the vegetation types according to Mucina & Rutherford (2006) and the distribution records for *Breviceps carruthersi* **sp. nov.** and *B. passmorei* **sp. nov.** The type localities are indicated by white arrows.

TABLE 1. Collection localities.

Locality	GPS coordinates	Description	Elevation (m)
Bambanana Gas Station	S -27.10528° E 32.15944°	Intersection of roads P443 and P522 (Mbodla/ Siweni)	98
Hluhluwe 1	S -28.02278° E 32.27306°	Open field adjacent to the playing fields of the Hluhluwe Sports Club	88
Hluhluwe 2	S -28.02111° E 32.27167°	Roadside through Hluhluwe town	100
Phinda Game Reserve 1	S -27.79062° E 32.29045°	Calling from a stand of *Dichrostachys cinerea* in open woodland	123
Phinda Game Reserve 2	S -27.85097° E 32.30819°	Near the reserve airstrip, calling from open woodland dominated by *Acacia* spp.	52
Phinda Game Reserve 3	S -27.87453° E 32.27734°	Calling from closed woodland in hilly terrain	89
Ngwenya	S -27.81778° E 32.39889°	30 km NNE of Hluhluwe on R22 road to Sodwana	39
Lulwane 1	S -27.04319° E 32.28836°	2.33 km E of bridge over Phongolo River on road P522	64
Lulwane 2	S -27.04333° E 32.29056°	2.53 km E of bridge over Phongolo	63
Lulwane 3	S -27.02838° E 32.31263°	Near bridge over Phongolo River on road D1861	36
Mpophomeni 1	S -27.04639° E 32.33389°	6.85 km E of bridge over Phongolo River on road P522	70
Mpophomeni 2	S -27.04597° E 32.32722°	6.2 km E of bridge over Phongolo River on road P522	77
Ndumo	S -26.90963° E 32.31396°	Ndumo Rest Camp	94
Shemula Gata 1	S -27.03278° E 32.25222°	1.5 km W of bridge over Phongolo River on road P522	98
Shemula Gata 2	S -27.03719° E 32.26944°	0.35 km E of bridge over Phongolo River on road P522	40

Morphology. Linear morphometric measurements of body length were taken using a manual vernier caliper while the head, feet and toes were measured using a Nikon AZ100 (Nikon, Amsterdam) microscope fitted with NIS Elements software. Measurements included the following variables, measured on both sides of the specimen: distance between anterior corners of eyes (EAD); distance between posterior corners of eyes (EPD); eye-snout distance measured as distance from the tip of head to anterior corner of eye (ES); fifth toe length (T5L); fifth toe width (T5W); first toe length (T1L); first toe width (T1W); foot length measured from proximal edge of inner metatarsal tubercle to tip of longest toe (FL); fourth toe length (T4L); inner metatarsal tubercle length (IMTL); internarial distance measured as distance between medial margins of nostrils (IND); maximum head-width (HW); nostril-ocular distance (NOD); nostril-upper lip distance measured as distance from medial margin of nostril to margin of upper lip (NL); palpebral fissure length (PFL); snout–vent length (SVL). Standard statistics were calculated using Microsoft Excel.

Molecular analysis. Total genomic DNA was extracted from liver tissue following the standard protocol for human or animal tissue and cultured cells as detailed in the NucleoSpin®Tissue Genomic DNA Tissue Kit (Macherey-Nagel, Düren, Germany). The primer sets, 16SaR-F (5'-CGCCTGTTTAYCAAAAACAT-3') and 16SbR-R (5'-CCGGTYTGAACTCAGATCAYGT-3') sourced from Kocher *et al*. (1989), as modified by Bossuyt & Milinkovitch (2000) were used to amplify a fragment of the mitochondrial 16S rRNA gene. In amphibians, these are widely accepted markers in DNA barcoding (Vences *et al*. 2005). We performed Polymerase Chain Reaction (PCR), using 12.5 µl Thermo Scientific DreamTaq PCR master mix (2×) (2× DreamTaq buffer, 0.4 mM of each dNTP, and 4 mM MgCl2), 1.25 µl of each primer (10 µM), and 1 µl DNA. The final reaction volume was made up with PCR-grade nuclease free water (Thermo Scientific, Vilnius, Lithuania) to adjust the volume to 25 µl. PCR reactions were carried out using a ProFlex™ PCR thermal cycler (Applied Biosystems by Life Technologies). Conditions were: initial denaturation at 95°C for 90 seconds, followed by 34 cycles entailing a 95°C denaturation

for 45 seconds, annealing at 51°C for 45 seconds, with an end extension of 72°C for 90 seconds, and a final extension at 72°C for 5min. PCR products were sent to a commercial sequencing company (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa) for purification and sequencing in both directions. Resultant sequences were assembled, and chromatogram-based contigs were generated and trimmed using Geneious R9.1 (http://www.geneious.com, Kearse *et al.* 2012). Sequence and species identity was verified against previously published sequences using the Basic Local Alignment Search Tool (BLAST) (Altshul *et al.* 1990).

Also, 16S rDNA sequences from four comparative *Breviceps* species (Table 2), namely *B. adspersus, B. mossambicus, B. poweri* and *B.* cf. *sopranus* Minter, 2003 were obtained for comparison, from Nielsen *et al*. (unpub. data) and added to the analysis, due to their close morphological similarity, advertisement call structure or their geographical distribution. We further selected *Breviceps gibbosus* (Linnaeus, 1758) (see Peloso *et al*. 2015: KM509100) and *Breviceps macrops* Boulenger, 1907 (see Channing & Wahlberg 2011: HQ111340) as outgroup species, due to their phylogenetic placement within the *B. gibbosus* complex, a sister group to the *B. mossambicus* complex (Nielsen *et al*. unpub. data). In all analyses, only nucleotide sequences with intraspecies variation were selected, to avoid the use of redundant sequences from the same haplotype. Sequences were aligned using the MUSCLE alignment tool (Edgar 2004) implemented in Geneious R9.1. The dataset consisted of 20 sequences (Table 2) with a total length of 415 nt. Bayesian inference (BI) analysis was used to infer the phylogenetic relationships. Prior to the analysis a model test was performed to determine the most suitable nucleotide substitution model, according to the Akaike Information Criterion (AIC) using jModelTest 2.1.7 (Guindon & Gascuel 2003; Darriba *et al*. 2012). The best model identified by the best AIC score was the General Time Reversible model with estimates of invariable sites and a discrete Gamma distribution (GTR+I+Γ). The BI analysis was implemented from within Geneious R9.1 using MrBayes 3.2.2 (Ronquist & Huelsenbeck 2003). For the BI analysis, posterior probability values were calculated using the Markov Chain Monte Carlo (MCMC) algorithm which was run for 10^7 generations, sampling with a frequency of 10^2 generations and using the default parameters. The first 25% of the trees were discarded as 'burn-in' with no 'burn-in' samples being retained. Results were visualised in Trace (implemented from within Geneious R9.1), to assess convergence and the burn-in period. Uncorrected pairwise distances (p-distance) were determined with PAUP version 4.0a152 (Swofford 2002). The analysis comprised the 20 samples used in the phylogenetic analysis. We deposited all sequences obtained in the current study in the NCBI GenBank database under the accession numbers: MF288975 – MF288989.

Results

The results show substantial genetic divergence (> 6.8% uncorrected p-distance) between and among the candidate new species and the potentially sympatric and/or morphologically similar *B. adspersus*, *B. mossambicus*, *B. poweri* and *B.* cf. *sopranus*. Bioacoustic analysis revealed significant differences from these species in several relatively stable advertisement call parameters, such as call duration, pulse number and amplitude modulation, supporting the hypothesis that two undescribed cryptic species are represented in the samples. Although a few morphological characters showed minimal intraspecific variation in the candidate species, they were of limited diagnostic value in distinguishing them from *B. adspersus*, *B. mossambicus*, *B. poweri* and *B.* cf. *sopranus*.

Phylogenetic analysis. Amplicons of between 553–560 nt of the mitochondrial 16S rRNA gene were derived from liver tissue of *B. carruthersi* **sp. nov.** (n=10) and *B. passmorei* **sp. nov.** (n=5). Based on the uncorrected p-distance the closest relative to *B. carruthersi* **sp. nov.** is *B.* cf. *sopranus* with an interspecific divergence of 6.8–7.3%, while the closest relative to *B. passmorei* **sp. nov.** is *B. carruthersi* **sp. nov.**, with an interspecific divergence of between 7.3–7.5% (Table 3). The average intraspecific divergence for *B. carruthersi* **sp. nov.** and *B. passmorei* **sp. nov.** was 0.2–1.5% and 0.2%, respectively (Table 3). In the phylogenetic analysis, the BI tree showed polytomy separating *B. adspersus, B. mossambicus* and *B. poweri* in distinct clades, with *Breviceps carruthersi* **sp. nov.**, *B. passmorei* **sp. nov.**, and *B.* cf. *sopranus* shown to form a well-supported monophyletic clade (Fig. 2).

TABLE 2. List of partial 16S rDNA sequences from the *Breviceps* species used for molecular analysis, with accession or field numbers, species assignment, locality and references.

Accession number or field number	Species	Locality	Reference
AB777216	*Breviceps adspersus*	Pet trade, probably Mozambique	Kurabayashi & Sumida 2013.
BAd_08	*Breviceps adspersus*	Zambezi Region [=Caprivi], Namibia	Nielsen *et al*. unpublished data
BAd_09	*Breviceps adspersus*	Zambezi Region [=Caprivi], Namibia	Nielsen *et al*. unpublished data
MF288975	*Breviceps carruthersi* **sp. nov.**	Hluhluwe 1, see Table 1*	Current study
MF288976	*Breviceps carruthersi* **sp. nov.**	Hluhluwe 1, see Table 1*	Current study
MF288980	*Breviceps carruthersi* **sp. nov.**	Hluhluwe 1, see Table 1*	Current study
MF288983	*Breviceps carruthersi* **sp. nov.**	Hluhluwe 2, see Table 1	Current study
KM509100	*Breviceps gibbosus*	Stellenbosch, South Africa	Peloso *et al*. 2015
HQ111340	*Breviceps macrops*	Kleinzee, South Africa	Channing & Wahlberg 2011
BMs_02	*Breviceps mossambicus*	Mount Mulanje, Malawi	Nielsen *et al*. unpublished data
BMs_03	*Breviceps mossambicus*	Ilha de Mozambique, Mozambique*	Nielsen *et al*. unpublished data
BMs_05	*Breviceps mossambicus*	Pemba, Mozambique	Nielsen *et al*. unpublished data
BMs_06	*Breviceps mossambicus*	Pemba, Mozambique	Nielsen *et al*. unpublished data
BMs_09	*Breviceps mossambicus*	Lichinga, Mozambique	Nielsen *et al*. unpublished data
BMs_10	*Breviceps mossambicus*	Balama, Mozambique	Nielsen *et al*. unpublished data
MF288985	*Breviceps passmorei* **sp. nov.**	Lulwane 2, see Table 1	Current study
MF288986	*Breviceps passmorei* **sp. nov.**	Lulwane 1, see Table 1*	Current study
BPo_01	*Breviceps poweri*	Katanga, Democratic Republic of the Congo	Nielsen *et al*. unpublished data
BPo_03	*Breviceps poweri*	Lusaka, Zambia	Nielsen *et al*. unpublished data
BSo_01	*Breviceps* cf. *sopranus*	Hluhluwe, Mfolozi, South Africa	Nielsen *et al*. unpublished data

* Type locality

TABLE 3. Average estimates of evolutionary divergence, given as a percentage, between partial 16S rDNA sequences from the *Breviceps* species used in the current study. Matrix showing ranges for uncorrected p-distances per site between sequences (below diagonal). Numbers presented as average percentage.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.
1. *B. adspersus* AB777216																				
2. *B. adspersus* BAd 08	1.0																			
3. *B. adspersus* BAd 09	0.5	1.2																		
4. *B. carruthersi* **sp. nov.** MF288976	9.3	9.3	9.3																	
5. *B. carruthersi* **sp. nov.** MF288975	9.5	9.5	9.5	0.5																
6. *B. carruthersi* **sp. nov.** MF288980	10.0	10.0	9.8	1.5	1.0															
7. *B. carruthersi* **sp. nov.** MF288983	9.3	9.3	9.3	0.2	0.2	1.2														
8. *B. mossambicus* BMs 02	9.5	9.7	9.7	9.0	9.5	9.5	9.2													
9. *B. mossambicus* BMs 03	9.0	9.3	9.2	9.5	9.7	10.0	9.5	2.2												
10. *B. mossambicus* BMs 05	9.0	9.3	9.0	10.0	10.2	9.7	10.0	1.9	1.2											
11. *B. mossambicus* BMs 06	8.8	9.0	9.0	9.5	9.7	9.7	9.5	1.9	1.2	0.5										
12. *B. mossambicus* BMs 09	9.0	9.3	9.2	9.7	10.0	10.5	9.7	2.4	1.7	1.5	1.5									
13. *B. mossambicus* BMs 10	9.2	9.5	9.5	10.0	10.2	10.2	10.0	1.9	1.2	0.5	0.5	1.5								
14. *B. passmorei* **sp. nov.** MF288986	10.0	10.2	9.7	7.3	7.5	7.5	7.3	10.7	10.0	9.7	10.0	10.0	10.2							
15. *B. passmorei* **sp. nov.** MF288985	9.7	10.0	9.5	7.1	7.3	7.3	7.1	10.4	9.7	9.5	9.7	9.7	10.0	0.2						
16. *B. poweri* BPo 01	11.7	12.4	11.9	11.4	11.4	11.4	11.4	10.2	10.4	10.7	10.2	10.4	10.7	13.1	12.9					
17. *B. poweri* BPo 03	12.0	12.7	12.2	10.5	10.5	11.0	10.5	10.5	11.2	11.0	10.5	10.2	11.0	13.7	13.4	2.9				
18. *B.* cf. *sopranus* BSo 01	9.0	8.8	9.0	6.8	7.1	7.3	6.8	9.2	10.2	9.7	9.5	9.7	10.0	9.5	9.2	12.1	11.7			
19. *B. macrops* 16S HQ111340	13.1	13.1	13.6	14.3	14.6	15.3	14.6	14.3	13.8	14.3	14.1	14.3	14.6	16.7	16.5	14.8	15.1	14.8		
20. *B. gibbosus* KM509100	13.9	13.9	13.9	13.9	14.1	14.6	14.1	14.9	13.9	14.1	14.4	14.4	14.6	15.8	15.6	16.3	16.6	16.1	9.5	

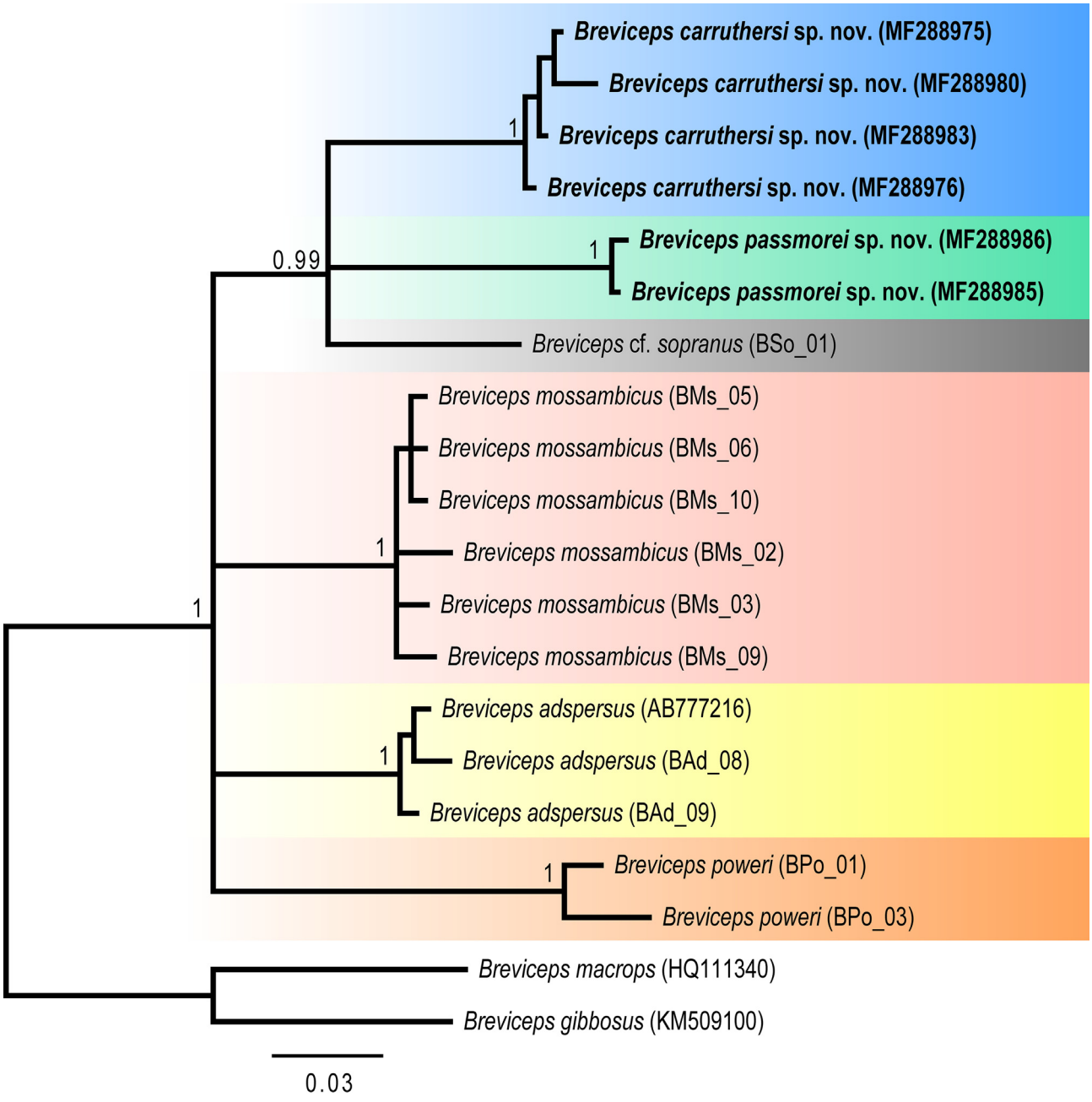

FIGURE 2. Phylogenetic analysis of eight *Breviceps* species based on mitochondrial 16S rDNA sequences. Bayesian inference analysis showing the phylogenetic relationships for *Breviceps carruthersi* **sp. nov.** and *B. passmorei* **sp. nov.** (represented in bold), four *Breviceps* species from the *Breviceps mossambicus* complex, and two *Breviceps* species, namely *Breviceps gibbosus* and *Breviceps macrops* used as the outgroup. Node support with posterior probabilities > 0.95 shown. The scale bar represents 0.03 nucleotide substitutions presite.

***Breviceps carruthersi* sp. nov. Du Preez, Netherlands & Minter**

http://zoobank.org/urn:lsid:zoobank.org:act:BC8E4F04-CC6F-4E44-8C39-AD2E7A340A25

Tables 4–5, Figs. 3–4

Holotype. Adult male (SAIAB 204591) collected by LM & LdP on 24 January 2017 at the type locality (Hluhluwe 1), an open field covered by short, grassy and herbaceous vegetation on red, sandy, clay loam soil, adjacent to the playing fields of Hluhluwe Sports Club, Town of Hluhluwe, KwaZulu-Natal, South Africa (S -28.02278°, E 32.27306°, elevation 88 m).

Paratypes (12 males, 2 females). Three adult males (PEM A11993–11995), collected 14 December 2015 by LM at Hluhluwe 1; (see Table 1 for locality details); Six adult males (PEM A11996–11998; SAIAB 204592–204594) and one adult female (SAIAB 204595), collected 24 January 2017 by LM & LdP at Hluhluwe 1; two adult males (PEM A11999; PEM A12000), collected 24 January 2017 by Francois Becker at Hluhluwe 2; one adult male (PEM A12001) and one adult female (PEM A12002), collected 24 January 2017 by L. Verburgt, U. Verburgt and A. Coetzer at Ngwenya.

Differential diagnosis. This species is placed in the genus *Breviceps* on the following grounds: snout extremely abbreviated; mouth narrow and downturned; short limbs which, at rest, are held close to the body, not projecting beyond the body outline; digits tapering to apex; inner and outer toes very short or rudimentary; inner and outer metatarsal tubercles well developed, confluent or separated by a narrow groove; vent terminal, not deflected downwards. Furthermore, the placement of these species is supported by monophyly of the mitochondrial 16S marker with other *Breviceps* taxa (Fig. 2).

Breviceps carruthersi is geographically isolated by substantial distances (180–3600 km) from all other known species except *B. adspersus*, *B. passmorei*, *B. mossambicus* and *B. sopranus* (Fig 1., Minter *et al.* 2004 a-c). *Breviceps bagginsi* Minter, 2003 and *B. verrucosus,* Rapp, 1842, which have been recorded as close as 180 km from Hluhluwe, have different habitat preferences: open, moist grassland, and forest or forest fringes, respectively.

Morphologically, *B. carruthersi* can be distinguished from *B. macrops* Boulenger, 1907 and *B. namaquensis* Power, 1926 and *B. branchi* Channing, 2012, by its by its relatively small eyes; the prominent facial mask separates it from *B. acutirostris* Poynton, 1963*, B. fuscus* Hewitt, 1925*, B. gibbosus* and *B. macrops*; the very short outer toe (as long as it is wide) separates this species from all other known species except *B. acutirostris, B. adspersus*, *B. bagginsi*, *B. mossambicus*, *B. passmorei* **sp. nov.***, B. poweri*, *B. sopranus* and *B. rosei* Power, 1926. The dorsal colouration and markings of *B. carruthersi* closely resemble that of *B. adspersus*, *B. poweri* and *B. bagginsi* and to a lesser extent those of *B. mossambicus*, which may lack paravertebral patches while retaining dorsolateral patches, and *B. sopranus*, in which both markings are present but indistinct.

The advertisement call of *B. carruthersi* is pulsatile, distinguishing it from all other *Breviceps* species (except *B. branchi*), which have either pulsed or tonal calls. The advertisement call of *B. branchi* is unknown: the holotype specimen was collected north of Port Nolloth, 1800km from Hluhluwe, and resembles *B. namaquensis* and *B. macrops* in morphology, having large eyes and fleshy webbing). The calls of *B. carruthersi* are grouped within the call bout, with up to 28 calls/group. In *B. poweri* , which has grouped, tonal calls (Minter 1997), the mean call duration is 0.14 s (0.07 s in *B. carruthersi*) and occupies a lower frequency range of 1557–1903 Hz (2182–2481 Hz in *B. carruthersi*). *Breviceps adspersus*, *B. bagginsi, B. mossambicus* (Mozambique Island) and *B. passmorei* differ from *B. carruthersi* in having pulsed calls with a mean duration of 0.193, 0.198, 0.05 and 0.305 s, respectively (Minter 1997, 2003, this publication), while *B. sopranus* has a very long tonal whistle with a mean duration of 1.5 s and a mean dominant frequency of 3332 Hz (Minter 2003).

This new species differs from other species within the *Breviceps mossambicus* complex with regard to the 16S marker, by a net uncorrected p-distance value of 6.8–11.3% (see Table 3). In the case of *B. bagginsi* reliable identified tissue was not available but this species differs from *B. carruthersi* in advertisement call structure (see above).

Description of holotype (Table 5; Figs. 3A–C, E). Morphometrics are given in Table 5. A male, SVL 34 mm. Snout extremely abbreviated; pupils horizontally elliptic; tympanum not distinguishable. Vent terminal. Skin of dorsum glandular: its surface consists of irregular folds and numerous small and large tubercles, giving it a densely granular appearance; the openings of the dermal glands are closely spaced and evenly distributed. Ventrum smooth. Limbs short. Fourth (outer) finger reaches the distal subarticular tubercle of the third finger; subarticular tubercles of third finger undivided. Webbing absent. No divided subarticular tubercles on hands or feet were observed. Well-developed inner metatarsal tubercle separated from outer metatarsal tubercle by a deep cleft. Outer toe very short, barely reaching basal subarticular tubercle of fourth toe.

Colour in life: dorsum of body light orange-brown to dark brown, with very dark tubercles forming an incomplete border to the anterior pair of light brown paravertebral patches. Interocular bar present but indistinct; light paravertebral patches distinct; three light dorsolateral spots present, not sharply demarcated. Light vertebral line absent; indistinct heel-to-heel line and small, poorly demarcated cream patch over urostyle present. A broad black stripe runs obliquely downwards, from margin of lower eyelid towards base of arm, not reaching it; anterior to this, a broad white stripe runs down to angle of mouth and onto upper and lower lips and separates dark stripe

from gular patch. Gular patch uniformly dark anteriorly, becoming mottled posteriorly. Sides of body between limbs light brown with scattered white speckles. Pectoral region and ventrum immaculate, white. In the preserved specimen orange-brown has faded to cream and shades of grey (Figs. 3B, C).

TABLE 4. Measurements of advertisement call variables of *Breviceps carruthersi* **sp. nov.** Abbreviations: s = seconds; min = minute; Hz = hertz.

Variable	n	Mean	SD	Range	CV%
No. of calls in call group	9	9.85	9.12	2–28	92.6
Call period within call group (s)	9	0.21	0.03	0.18–0.28	14.0
Call period between single calls/call groups (s)	9	4.77	3.26	0.47–15.47	68.3
Call rate within call group (min^{-1})	9	285	46.8	203–344	16.4
Call duration (s)	9	0.07	0.01	0.05–0.10	14.3
Dominant frequency (Hz)	9	2378	122	2182–2481	5.1

TABLE 5. Measurements of the type series of *Breviceps carruthersi* **sp. nov.** All linear measurements in millimetres (mm). See Material and methods for measurement abbreviations.

Measurement	Holotype	Paratypes							
		Females				Males			
		n	Mean	Range	SD	n	Mean	Range	SD
Mass (g)	5.3	2		15.2; 32.9		9	15.2	3.4–7.6	1.2
EAD	5.6	2		7.5; 8.0		12	5.9	4.7–6.6	0.5
EPD	10.0	2		12.8;14.2		12	10.4	9.0–11.3	0.6
ES	1.4	2		2.8; 2.5		12	1.8	1.4–2.2	0.3
FL	12.5; 11.7	4	17.8	16.7–18.5	0.8	24	11.7	9.2–13.5	0.9
HW	11.2	2		13.0; 14.6		12	11.4	9.6–12.9	0.9
IMTL	3.3; 2.8	4	4.2	3.8–5.1	0.6	23	2.9	2.0–3.5	0.4
IND	1.8	2		2.5; 3.1		12	2.0	1.6–2.5	0.3
NL	2.1; 1.9	4	2.7	2.2–3.2	0.5	24	1.8	1.3–2.1	0.2
NOD	1.8; 1.4	4	2.5	2.2–2.8	0.3	24	2.0	1.7–2.5	0.3
PFL	3.7; 3.8	4	4.8	3.5–5.7	1.0	24	3.7	3.0–4.2	0.4
SVL	34	2		59; 47		9	33.9	28–39	3.3
T1L	0.6; 0.6	4	1.0	1.0–1.1	0.1	24	0.6	0.4–0.8	0.1
T1W	0.7; 0.5	4	1.35	1.0–2.4	0.7	24	0.7	0.5–0.9	0.1
T4L	6.2; 6.3	4	9.2	8.4–10.1	0.7	22	6.0	4.9–7.5	0.6
T5L	0.7; 0.7	4	1.4	1.2–1.6	0.2	23	0.7	0.4–0.8	0.1
T5W	0.7; 0.7	4	0.9	0.7–1.0	0.1	24	0.6	0.4–0.8	0.1

Holotype advertisement call (Fig. 4). Several call bouts consisting of 16 call groups were recorded at the Sports Club in Hluhluwe, on 24th January 2017 at 23h38; air temperature 24 °C. It was raining heavily and a strong chorus had developed. The calls are pulsatile, with amplitude modulation barely distinguishable in most calls, while a few are more strongly modulated; amplitude increases gradually from the start, falling rapidly at the end of the call. Frequency modulation absent. Two to three harmonics are present. All calls in the call bout are condensed into groups consisting of 8–21 calls (mean 12, sd 4). Means and ranges for three calls/call groups are: call period within call group 0.211 s (0.204–0.227); call rate 294 min^{-1} (290–301); call duration 0.078 s (0.074–0.086); dominant frequency 2378 Hz (2350–2392).

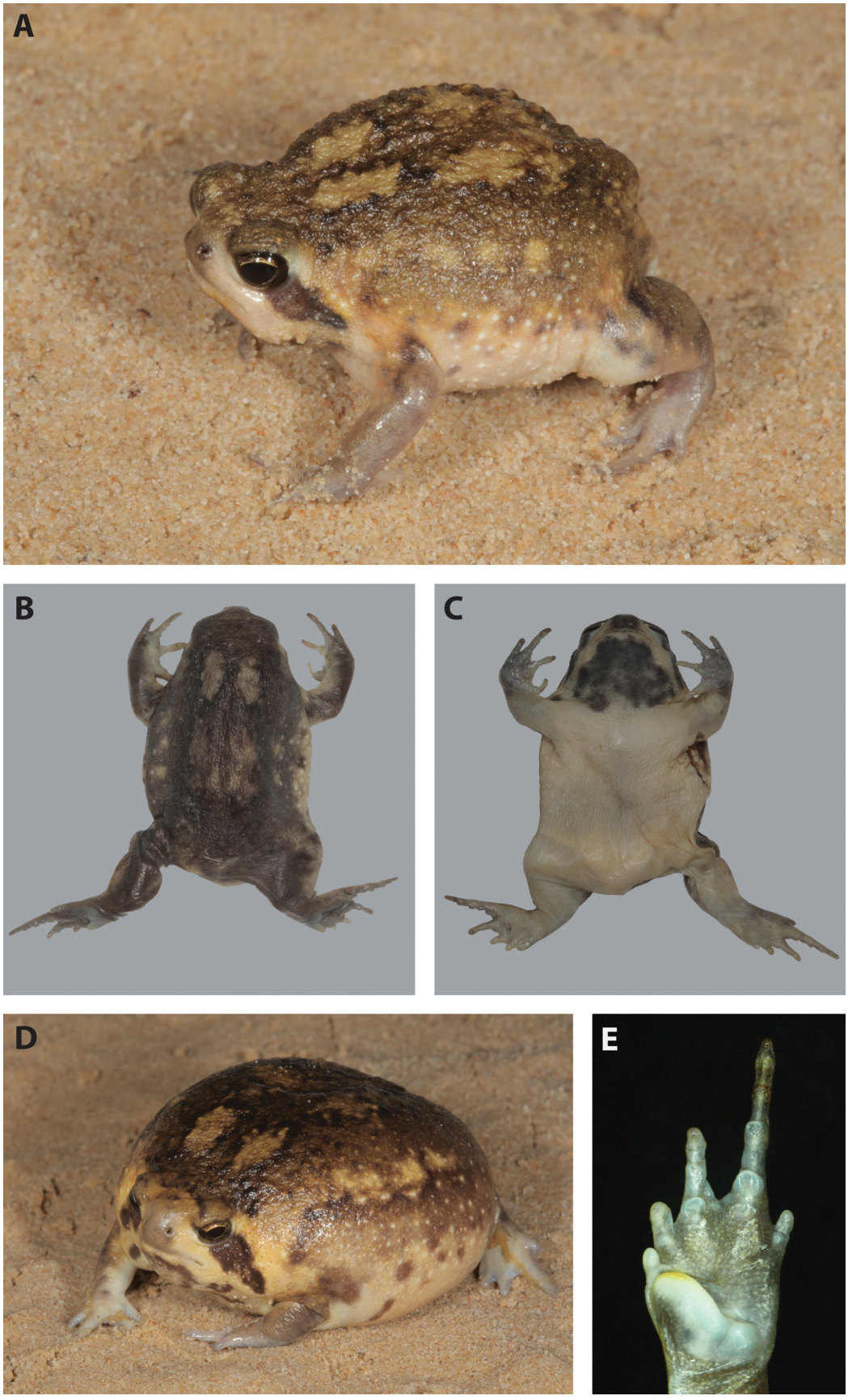

FIGURE 3. *Breviceps carruthersi* **sp. nov.** from Hluhluwe, KwaZulu-Natal**:** A, holotype male (SAIAB 204591); B, preserved holotype, dorsal view; C, preserved holotype, ventral view; D, paratype female (SAIAB 204595); E, left foot of holotype.

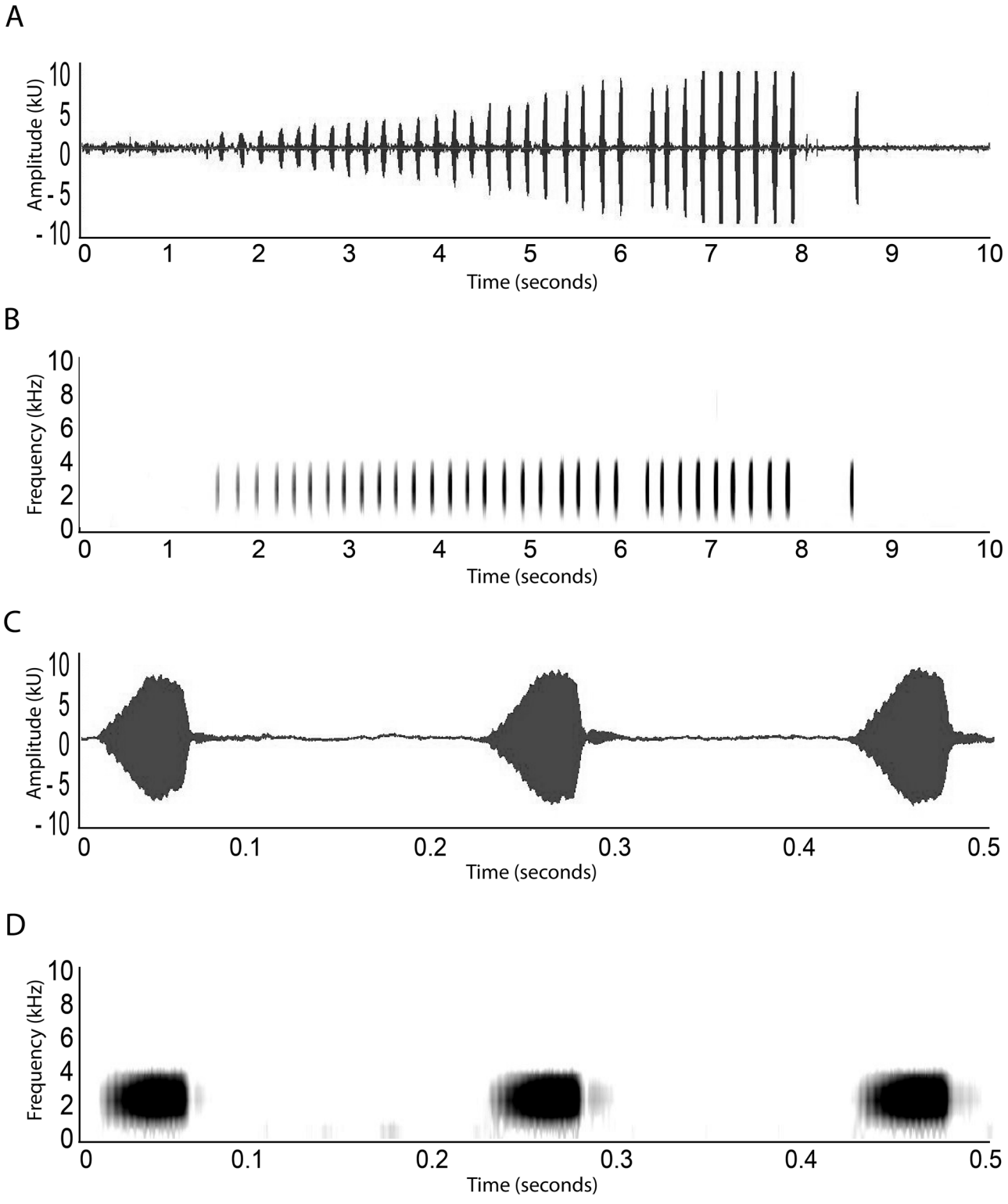

FIGURE 4. Advertisement call structure of *Breviceps carruthersi* **sp. nov.** paratype (PEM A11997)**:** A–Oscillogram of call bout; B–Spectrogram of call bout; C–Oscillogram of three calls; D–Spectrogram of three calls**.** Spectrogram parameters, using a Hanning window, were set at Window size 300 (Fig. 4B) and 45 (Fig. 4D); DFT size 512.

Paratype variation (Fig. 3D; Tables 4–5). Morphometrics are given in Table 5. All specimens resemble the holotype in the absence of a visible tympanum; skin densely granular dorsally and laterally, smooth ventrally; one specimen granular laterally, not dorsally. Outer finger reaches distal subarticular tubercle of third finger in 13 specimens, falling just short in one; subarticular tubercles on third finger not divided. Cleft separating inner and outer metatarsal tubercles deep in seven specimens, shallow in seven. Outer toe very short, wider than long, falling short of basal subarticular tubercle of fourth toe in 10 specimens; reaching it in four.

Colour in life: dorsum orange-brown in eight specimens, dark brown in five and pale cream in one unpigmented individual; dark tubercles on dorsum scattered in four, forming a dark border to the lighter paravertebral patches in 10; white lateral speckles present in 13, absent in one. Interocular bar present in seven specimens, indistinct in six, not visible in the unpigmented specimen; light paravertebral patches present in eight, indistinct in six; three light, dorsolateral spots present in 11, indistinct in three. Light mid-vertebral line absent in 12 specimens, present posteriorly in two. A distinct, light-yellowish patch present over the urostyle in four specimens, indistinct in eight, absent in two; light heel-to-heel line indistinct in three, absent in eleven. A broad, black stripe runs obliquely downwards from margin of lower eyelid towards base of arm, reaching it in seven specimens, falling short in seven; anterior to this, a broad white stripe runs down to angle of mouth and onto upper and lower lips in all individuals, separating the dark stripe from the gular patch. Gular patch with scattered, dark-grey mottles on a white background in the two female specimens. In males the gular region is unpigmented in one, uniformly grey or darkened along the lower jaw, becoming lighter medially, in six; in five specimens the gular patch is mottled posteriorly, on a grey to yellowish-brown background. Pectoral region white, with scattered spots in three males. Ventrum immaculate white in 13 specimens; a few scattered spots in one.

Colour in preservative: dorsum of body medium to dark grey. Paravertebral patches beige to dark brown or dark grey in some. Interocular bar indistinct and beige to dark brown. Dorsolateral patches distinct and pale. Heel-to-heel line indistinct to absent. Cream patch over urostyle distinct, present in all but two specimens. Facial stripe prominent, dark grey to black. Gular patch uniformly dark anteriorly, becoming mottled posteriorly in most males, mottled in females. Ventrum immaculate, creamy-white.

Advertisement call data for the paratype males is presented in Table 3. No significant differences between the calls of the holotype male and the paratypes were found.

Etymology. This species is named for Vincent Carruthers who, through his numerous books and articles on the natural history of southern Africa in general, and frogs in particular, has done much to stimulate interest in these much-maligned creatures.

Distribution and habitat. Currently known only from the area around Hluhluwe and Phinda Game Reserve (Fig. 1; Table 1). (Vegetation types as defined by Mucina & Rutherford, 2006). Hluhluwe is situated in vegetation type SVI 23 (Zululand Lowveld), which grades into SVI 20 (Western Maputaland Clay Bushveld) on the eastern perimeter of the town. At Phinda Private Game Reserve *B. carruthersi* occurs mainly in the south, on SVI 20 and SVI 23, as well as SVI 6 (Southern Lebombo Bushveld), less commonly in the sandy soils of CB 1 (Maputaland Coastal Belt) to the north (Daryl Dell 2017, pers. comm.). The first 18 km of the R22 between Hluhluwe and Sodwana consists of SVI 21 (Makatini Clay Thicket), continuing northwards as CB 1.

Field observations. Calls were recorded between 18h45 and 01h15, during and after moderate to heavy rain. Two males were calling from shallow depressions at the base of grasstufts, while the remainder called from exposed positions on the surface. When the vocal sac is inflated, the white stripe separating the gular patch from the dark eyestripe is conspicuous and may function as a visual signal.

Available earlier names. *Breviceps adspersus adspersus* Pienaar, 1963, *B. adspersus pentheri* Poynton, 1964, *B. mossambicus* var. *occidentalis* Werner, 1903, *B. parvus caffer* Hewitt, 1932, *B. parvus* Hewitt, 1925, *B. pentheri caffer* Parker, 1934, *B. pentheri pentheri* Parker, 1934, *B. pentheri* Werner, 1899, *B. pretoriensis* FitzSimons, 1930 and *B. mossambicus adspersus* Broadley, 1971, considered to be junior synonyms of *B. adspersus* (see Poynton 1964); *B. mitchelli* Hoffman, 1944, *Engystoma granosum* Cuvier, 1829 and *Systoma granosum* Parker, 1868 considered to be junior synonyms of *B. mossambicus*.

***Breviceps passmorei* sp. nov. Minter, Netherlands & Du Preez**

http://zoobank.org/urn:lsid:zoobank.org:act:F836CD13-8351-476F-8C90-EE1DFD691A2F

Tables 6–7, Figs. 5–6.

Holotype. Adult male (SAIAB 204596) collected by LM & EN on 26 January 2017 at the type locality (Lulwane 1, KwaZulu-Natal, South Africa) (S -27.04319°, E 32.28836°, elevation 64 m), a narrow strip of natural vegetation alongside the tarred road (P522) in a fairly heavily populated, rural area. The vegetation type is Tembe Sandy Bushveld: open leguminous woodland with a species-rich shrub layer and grassy undergrowth.

Paratypes (18 males, 2 females). Five adult males (TM 70333, TM 70335–6, TM 70341, TM 70343), collected 13 December 1988 by LM at Bambanana (see Table 1 for locality details); one adult female (SAIAB 204597), collected 26 January 2017 by EN at Bambanana; one adult female (PEM A12003), collected 26 January 2017 by LM at Lulwane 2; two adult males (PEM A12004; SAIAB 204617), collected 18 November 2016 by LdP & EN at Lulwane 3; two adult males (SAIAB 204598; SAIAB 204599), collected 13 December 2015 by LM at Mpophomeni 1; three adult males (SAIAB 204618; PEM A12005; PEM A12006), collected 11 December 2015 by LM at Mpophomeni 2; one adult male (PEM A12007), collected 17 February 2017 by EN at Ndumo; four adult males (TM 70362–3, TM 70365, TM 70367), collected 23 October 1988 by LM at Shemula Gata 1; one adult male (TM 70361), collected 22 October 1988 by LM at Shemula Gata 2.

Differential diagnosis. This species is placed in the genus *Breviceps* on the following grounds: snout extremely abbreviated; mouth narrow and downturned; short limbs which, at rest, are held close to the body, not projecting beyond the body outline; digits tapering to apex; inner and outer toes very short or rudimentary; inner and outer metatarsal tubercles well developed, confluent or separated by a narrow groove; vent terminal, not deflected downwards. Furthermore, the placement of these species is supported by monophyly of the mitochondrial 16S marker with other *Breviceps* taxa (Fig. 2).

Breviceps passmorei **sp. nov.** is geographically isolated by substantial distances (320–3600 km) from all other known species except *B. adspersus, B. carruthersi, B. mossambicus* and *B. sopranus* (Fig. 1., Minter *et al.* 2004a-c). *B. bagginsi* and *B. verrucosus*, which have been recorded as close as 320 km from Ndumo, have different habitat preferences: open, moist grassland, and forest or forest fringes, respectively.

Morphologically, *B. passmorei* can be distinguished from *B. macrops*, *B. namaquensis* and *B. branchi* by its relatively small eyes; the prominent facial mask separates it from *B. acutirostris*, *B. fuscus, B. gibbosus* and *B. macrops*; the very short outer toe (as long as it is wide) separates this species from all other known species except *B. acutirostris, B. adspersus*, *B. bagginsi, B. carruthersi* **sp. nov.**, *B. mossambicus, B. poweri*, *B. rosei* and *B. sopranus*. The dorsal colouration and markings of *B. passmorei* closely resemble that of *B. adspersus*, *B. bagginsi*, *B. carruthersi* and *B. poweri* and, to a lesser, extent those of *B. mossambicus*, which may lack paravertebral patches while retaining dorsolateral patches, and *B. sopranus*, in which both markings are present but indistinct.

The advertisement call of *B. passmorei* is strongly pulsed, distinguishing it from *B. macrops*, *B. namaquensis*, *B. poweri* and *B. sopranus*, which have tonal calls, and *B. carruthersi*, in which the call is pulsatile. Under optimum conditions, *B. passmorei* may condense the calls in the call bout into groups of two to three calls, as do *B. adspersus* and *B. mossambicus*, but its call has a longer mean duration: 0.305 s compared with 0.193 s in *B. adspersus*, 0.198 s in *B. bagginsi* and 0.05 s in *B. mossambicus* (Minter 1997), and a shorter mean duration than that of *B. verrucosis* (0.612 s, Minter 2003). The advertisement calls of *B. bagginsi* are not arranged in groups, but consist of a single, rapidly repeated series of pulsed calls (numbering 7 – 19, Minter 2003).

This new species differs from other species within the *Breviceps mossambicus* complex with regard to the 16S marker, by a net uncorrected p-distance value of 7.1–13.7% (see Table 3). In the case of *B. bagginsi* reliable identified tissue was not available but this species differs from *B. passmorei* in advertisement call structure (see above).

Description of holotype (Tables 6–7, Figs. 5A–C, E). Morphometrics are given in Table 7. A male, SVL 36.4 mm. Snout extremely abbreviated; pupils horizontally elliptic; tympanum not distinguishable. Vent terminal. Skin of dorsum very glandular, with closely spaced openings of dermal glands that produce the adhesive secretion during amplexus; its surface consists of irregular small folds and tubercles with scattered larger tubercles, giving it a granular appearance. Skin of ventrum glandular, relatively smooth.

Limbs short. Fourth (outer) finger reaches distal subarticular tubercle of third finger; subarticular tubercles of third finger undivided. Webbing absent. No divided subarticular tubercles on hands or feet were observed. Well-developed inner metatarsal tubercle separated from outer metatarsal tubercle by a shallow cleft. Outer toe falls short of basal subarticular tubercle of fourth toe.

TABLE 6. Measurements of paratype advertisement call variables of *Breviceps passmorei* **sp. nov.** Abbreviations: s = seconds; min = minute; Hz = hertz.

Variable	n	Mean	SD	Range	CV%
Call period within call group (s)	8	0.528	0.072	0.411–0.638	13.63
Call period between single calls/call groups (s)	16	1.638	0.626	0.824–3.058	38.20
Call rate within call group (min^{-1})	14	51.49	24.01	19.73–98.6	46.60
Call duration (s)	18	0.305	0.05	0.192–0.432	15.40
No. of pulses	18	34.59	8.36	21–63	24.12
Pulse rate (s^{-1})	18	113.29	23.02	86.47–163.27	20.32
Dominant frequency (Hz)	18	1848	80.45	1690–2110	4.35

TABLE 7. Measurements of the type series of *Breviceps passmorei* **sp. nov.** All linear measurements in mm.

Measurement	Holotype	Paratypes							
		Females				Males			
		n	Mean	Range	SD	n	Mean	Range	SD
Mass (g)	8.7	2		13.4; 17.6		13	6.6	4.1–10.5	0.2
EAD	8.0	2		7.0; 7.3		11	6.0	5.3–7.7	0.7
EPD	12	2		12.2; 12.8		11	10.9	10.0–11.7	0.7
ES	1.9	2		1.8; 2.0		11	1.8	1.4–2.4	0.3
FL	13.3; 12.9	4	14.7	14.3–15.2	0.5	20	11.6	9.9–12.9	0.7
HW	16.1	2		13.9; 13.2		11	12.0	11.0–12.5	0.5
IMTL	3.3; 3.4	4	4.1	3.6–4.7	0.5	18	2.9	2.0–3.4	0.4
IND	2.1	2		2.9; 2.7		11	2.0	1.8–2.3	0.2
NL	2.7	2		2.6; 2.7		16	1.8	1.6–2.1	0.14
NOD	1.7; 1.7	4	2.2	1.9–2.5	0.3	21	1.9	1.4–2.3	0.3
PFL	3.8; 3.7	4	5.0	4.6–5.6	0.4	22	4.3	3.7–4.8	0.3
SVL	36.4	2		43.2; 46.9		19	32.1	25.5–35.9	4.0
T1L	0.7; 0.7	4	0.9	0.8–1.0	0.1	22	0.7	0.4–0.9	0.1
T1W	0.8; 0.7	4	1.0	0.9–1.0	0.1	19	0.7	0.5–1.1	0.2
T4L	6.4; 6.4	4	7.6	7.2–8.1	0.4	22	5.7	4.6–6.5	0.5
T5L	0.5; 0.5	4	0.7	0.6–0.7	0.1	22	0.6	0.4–0.8	0.1
T5W	0.3; 0.4	4	0.7	0.6–0.8	0.1	19	0.5	0.4–0.8	0.1

Colour in life, dorsum of body light brown to orange-brown; scattered dark blotches join to form a reticulated pattern; tubercles light to dark brown, becoming almost black around the light paravertebral and dorsolateral patches; laterally, some tubercles are unpigmented, forming conspicuous white speckles; six light brown paravertebral, and four dorsolateral patches present; interocular bar present, from which a light vertebral line runs posteriorly to urostyle, joining a distinct heel-to-heel line; there is an indistinct, creamy-yellow spot on urostyle at its junction. A broad, black stripe runs obliquely downwards, from margin of lower eyelid towards base of arm, not reaching it; anterior to this, a broad white stripe runs down to angle of mouth and onto upper and lower lips, separating the dark stripe from gular patch. Gular region not heavily pigmented, with confluent, dark spots anteriorly and laterally.

Sides of body between limbs, light brown with large dark blotches bordering the light dorsolateral patches; unpigmented tubercles form scattered white speckles. Pectoral region and ventrum immaculate, white. In the preserved specimen, orange-brown and brown has faded to cream and shades of grey (Figs. 5 B, C).

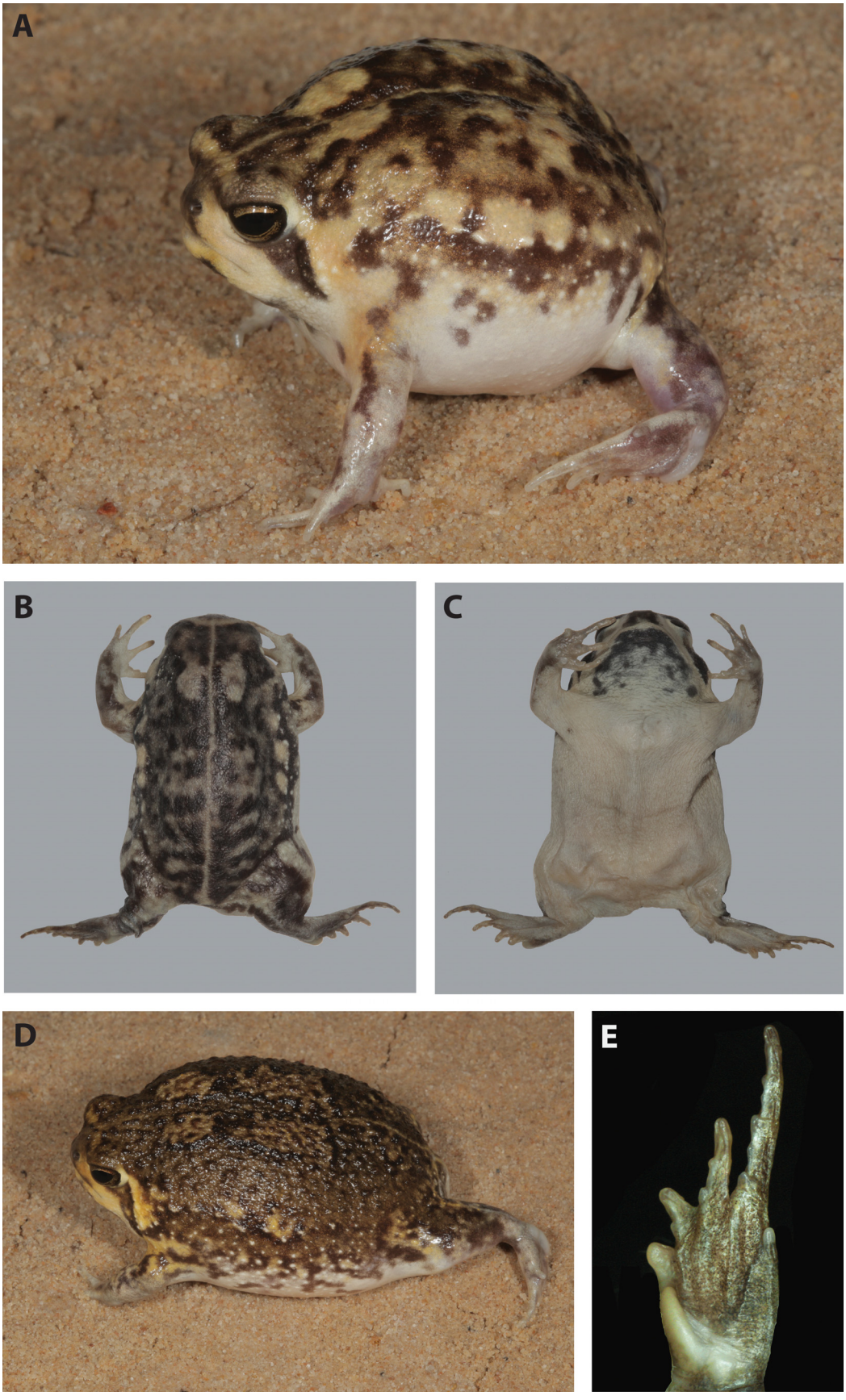

FIGURE 5. *Breviceps passmorei* **sp. nov.** from northern KwaZulu-Natal**:** A, holotype male (SAIAB204596); B, preserved holotype, dorsal view; C, preserved holotype, ventral view; D, paratype female (SAIAB204597); E, left foot of holotype.

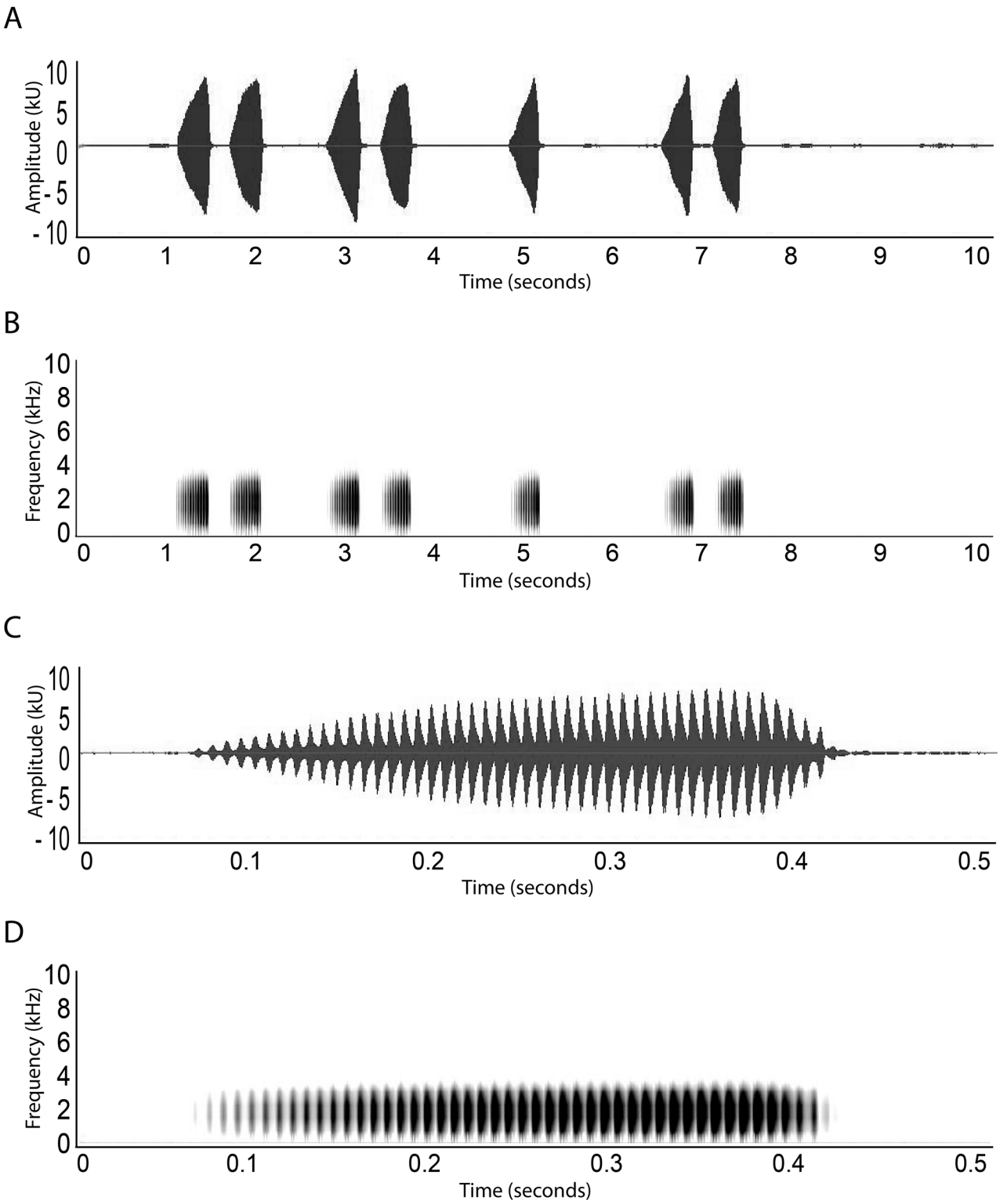

FIGURE 6. Advertisement call structure of *Breviceps passmorei* **sp. nov.** holotype (SAIAB 204596)**:** A—Oscillogram of call bout; B—Spectrogram of call bout; C—Oscillogram of single call; D—Spectrogram of single call. Spectrogram parameters, using a Hanning window, were set at Window size 300 (Fig. 6B) and 45 (Fig. 6D); DFT size 512.

Holotype advertisement call (Fig. 6). A bout of seven calls, comprising three groups of two calls and a single call, were recorded at Lulwane 1 on 26 January 2017 at 01h30, following light rain. Air temperature was not recorded. Three of these calls were analysed. Several other males were calling sporadically in the vicinity, but the chorus was waning. The calls are strongly pulsed and are not frequency modulated; 2–3 harmonics are present; the

amplitude increases gradually throughout the call, falling rapidly at the end. Call rate for the entire bout is 62 min^{-1}; call period within a group 0.571 (0.559–0.584) s; call period between two call groups or between a call group and a single call 1.36 (1.046–1.645) s; call duration 0.352 (0.328–0,373) s; number of pulses 47 (44–50); pulse rate 133.5 (131.7–134.7) s^{-1}; dominant frequency 1833 (1820–1850) Hz.

Paratype variation (Fig. 5D; Tables 6-7). Morphometrics are given in Table 7. All specimens resemble the holotype in having an extremely abbreviated snout, horizontally elliptic pupil, absence of a visible tympanum, terminal vent and glandular skin. Skin of dorsum granular in 10 individuals, densely granular in five, granular laterally in five; tubercles light to dark brown, becoming almost black around the light paravertebral and dorsolateral patches; dark tubercles on dorsum randomly scattered in 11, confluent in seven, forming a dark border to the lighter paravertebral patches, and absent in two; laterally, some tubercles are unpigmented, forming conspicuous white speckles. Skin of ventrum glandular, relatively smooth. Outer finger reaches distal subarticular tubercle of third finger in 17 individuals, nearly reaching it in two and falling short in one; subarticular tubercles of third finger undivided in all individuals. Well-developed inner metatarsal tubercle separated from outer metatarsal tubercle by deep cleft in 12 specimens and by a shallow cleft in eight. Outer toe falls just short of basal subarticular tubercle of fourth toe in 19 specimens, extending beyond it in one.

Colour in life: dorsum of body light orange-brown to dark brown with scattered dark blotches joining to form a reticulated pattern in seven specimens; three to six paravertebral, and two to four dorsolateral patches present, indistinct in two; interocular bar present in 17 specimens, indistinct in three; light vertebral line distinct in 14 specimens, indistinct in three, present posteriorly in one and absent in two; heel-to-heel line distinct in five, indistinct in eight and absent in seven individuals. A broad, black stripe runs obliquely downwards from margin of lower eyelid, reaching the base of arm in 10 individuals, nearly reaching it in 10; anterior to this, a broad white stripe runs down to angle of mouth and onto upper and lower lips, separating the dark stripe from gular patch in 17 specimens. Gular region: in the two females, freckled and streaked on a white background; in the 18 males, heavy mottling is present in four, mottling and streaking in two, while dark pigmentation partially, to completely, obscures the mottling anteriorly and laterally in 12. Pectoral region and ventrum immaculate, white, with a few scattered spots in two males.

Colour in preservative: dorsum of body medium brown to dark grey, scattered dark blotches join to form a reticulated pattern. Paravertebral patches beige to dark grey. Interocular bar indistinct and beige to dark brown. Dorsolateral patches distinct and pale. Vertebral line prominent in most specimens, only visible posteriorly in a few and absent in two. Heel-to-heel line indistinct to prominent. Indistinct cream patch over urostyle where vertebral line and heel-to-heel line meet. Facial stripe prominent: dark grey to black. Gular patch of male uniformly dark in six, becoming mottled posteriorly in 12; mottled in females. Ventrum immaculate, creamy-white.

Advertisement call data for the paratype males is presented in Table 6. No significant differences between the calls of the holotype male and the paratypes were found.

Etymology. This species is named for Neville Passmore in recognition of his contributions to South African herpetology in the field of bioacoustics, and for instilling a lifelong interest in frogs among his students, many of whom have also made significant contributions in this and other fields.

Distribution and habitat. Currently known only from the area West of Tembe Elephant Reserve, in the vicinity of the Phongolo River (Fig. 1, Table 1). (Vegetation types as defined in Mucina & Rutherford, 2006. *Breviceps passmorei* **sp. nov.** occurs in SVI 18 (Tembe Sandy Bushveld), SVI 19 (Western Maputaland Sandy Bushveld) and SVI 20 (Western Maputaland Clay Bushveld). All sites were situated in natural but disturbed roadside vegetation on sandy loam to clay loam soils.

Field observations. Calls were recorded after rain in summer, between 17h40 and 00h15. Calling continued for several days following heavy rain. Four males were calling from shallow depressions, concealed under vegetation, while the majority called from exposed sites on the surface.

Available earlier names. *Breviceps adspersus adspersus* Pienaar, 1963, *B. adspersus pentheri* Poynton, 1964, *B. mossambicus* var. *occidentalis* Werner, 1903, *B. parvus caffer* Hewitt, 1932, *B. parvus* Hewitt, 1925, *B. pentheri caffer* Parker, 1934, *B. pentheri pentheri* Parker, 1934, *B. pentheri* Werner, 1899, *B. pretoriensis* FitzSimons, 1930 and *B. mossambicus adspersus* Broadley, 1971, all considered to be junior synonyms of *B. adspersus* (see Poynton 1964); *B. mitchelli* Hoffman, 1944, *Engystoma granosum* Cuvier, 1829 and *Systoma granosum* Parker, 1868 considered to be junior synonyms of *B. mossambicus*.

Discussion

The lack of distinct morphological variation between *B. adspersus*, *B. mossambicus* and *B. poweri* and the apparently high morphological variation within these species have created difficulties in finding reliable diagnostic morphological characters. Taxonomists were obliged to use combinations of somewhat variable characters in their attempts to characterise these species. The presence of individuals that did not conform to these combinations were seen as an indication of hybridisation. For example, Poynton (1964, 1982) and Poynton & Broadley (1985) postulated that *B. adspersus* and *B. mossambicus* hybridise extensively in areas of sympatry, producing hybrid swarms. Relatively "pure" *adspersus* was said to occur in the southwest (Okavango area), while "pure" *mossambicus* was restricted to the northeast (northern Mozambique). In the intervening "hybrid zone", individuals possessed various combinations of *adspersus* and *mossambicus* characters. They used the light paravertebral and dorsolateral patches as an index of hybridisation in the hybrid zone: both were said to be present in "pure" *adspersus"*, neither, in "pure" *mossambicus*, and only dorsolateral patches in hybrids (Poynton & Broadley, 1985). The discovery of a hidden cryptic diversity within the genus *Breviceps* in Zululand, may account for some of the taxonomic difficulties encountered in the past. The fact that this study did not succeed in identifying reliable diagnostic morphological characters for distinguishing between *B. adspersus*, *B. carruthersi*, *B.mossambicus*, *B. passmorei*, *B. poweri* and *B. sopranus*, may be accounted for by the relatively small sample sizes, or attributed to morphologically static cladogenesis, i.e. diversification of new species without morphological change (Bickford *et al.* 2007). Nevertheless, it has emphasised the need for a more careful morphological investigation of larger samples identified by call or molecular methods.

Frog advertisement calls provide reliable diagnostic characters, as they are species specific and subject to stabilising selection due to their role in mate recognition. In those species of *Breviceps* for which sufficient data for statistical analysis exist, call duration and pulse number show a high degree of interspecific differentiation, whereas pulse rate and, in most cases, dominant frequency, are relatively undifferentiated: this is also the case in the new species described here.

In this study, the advertisement call data was not adjusted to a common temperature or mass because the sample sizes were too small for the calculation of reliable correction factors. The ranges of call parameters measured at different average temperatures in different populations may show a greater degree of overlap than would be the case if the data was adjusted to a common temperature. For example, in the case of *B. adspersus*, call duration is negatively correlated with air temperature (Minter 1995). The mean air temperature at which the *B. adspersus* sample from Polokwane was recorded was 15.7ºC (range 9.5–20.3, n=100, Minter 1995), while that of the *B. passmorei* sample was 18.9ºC (range 15.2–28, n=18, paratype sample). When the *B. adspersus* sample is adjusted to 18.9ºC (the average temperature of the *B. passmorei* sample), using the linear regression of call duration on air temperature (Minter 1995, Fig. 4), the mean call duration drops from 0.207 s to 0.175 s (ct. 0.305 s in *B. passmorei*).

Molecular analyses using 16S rDNA sequences have proven useful in several studies identifying new anuran taxa in South Africa. Recently, taxa such as *Amietia* (see Channing & Baptista 2013; Channing *et al.* 2016), *Cacosternum* (see Channing *et al.* 2013; Conradie 2014) and *Capensibufo* (see Channing *et al.* 2017) have used the 16S marker to resolve various taxonomic issues, including the identification of cryptic species. Likewise this marker has played an important role in the identification of two new cryptic species of *Breviceps* in the current study. Both new species are distinguished from *B. adspersus*, *B. mossambicus*, *B. poweri* and *B.* cf. *sopranus* by a substantial interspecific genetic divergence of > 6.8% in the targeted fragment of the 16S rRNA gene.

Although our phylogenetic analysis is not well resolved (multiple polytomies), all the *Breviceps* species clusters within our analysis were shown to form well-supported monophyletic clades, with relatively high interspecies genetic variation (6.8–13.7%), and low intraspecies genetic variation (0.2–2.9%) within the *B. mossambicus* complex, as compared to the genera *Cacosternum* and *Capensibufo* with interspecies genetic variation of between 1.7–5.6% and 2.5–4.6%, respectively (see Channing *et al.* 2013, 2017). This indicates that although the phylogeny shows low resolution for the evolutionary relationships within the *Breviceps* species complex, the 16S mitochondrial marker is able to distinguish between species within this group. However, for future phylogenetic studies on this group we suggest that a the combination of several markers (both mitochondrial and nuclear) may provide better insight into the evolutionary relationships within the *Breviceps* species complex.

Four cryptic *Breviceps* species have now been described from KwaZulu-Natal and there is strong evidence that

several additional undescribed species exist in this region and elsewhere (Nielsen *et al*. unpub. data.). As noted in the Introduction, samples of *B. passmorei* were provisionally referred to *B. adspersus* by Minter (1998, 2003: Fig 7), together with syntopic individuals with a shorter advertisement call. The taxonomic status of the latter is currently under investigation as it also appears to differ genetically from *B. adspersus* populations closer to the type locality for *B. adspersus* (see Nielsen *et al*., unpub. data).

Breviceps populations from the Tembe Elephant Reserve and other localities in coastal Zululand and southern Mozambique, that were referred to *B. mossambicus* by Minter (1998, 2003, 2004b), differ genetically from samples collected at the type locality, Mozambique Island (Nielsen *et al*, unpub. data) and require further investigation. This candidate species has not yet been found to occur in sympatry with *B. passmorei* but occurs in its close proximity; advertisement calls from Maputo, Kosi Bay and Tembe Elephant Reserve (Minter 1998) have a short duration (mean 0.079s, range 0.046–0.104) and relatively few pulses (mean 11, range 10–14) and therefore cannot be confused with *B. passmorei.*

Pending additional distribution and other data, *B. carruthersi* and *B. passmorei* should be assigned to the IUCN category Data Deficient.

Acknowledgements

Werner Conradie and Stu Nilson kindly gave us access to unpublished sequences of several *Breviceps* species for the purposes of comparison with the new species described in this study. We are grateful to Daryl Dell of Phinda Private Game Reserve for his photograph, call recordings, and locality and habitat information pertaining to *B. carruthersi* at Phinda. Willie Landman, Fortunate Phaka, Wentzel Pretorius and Jani Reeder assisted in the collection of specimens and calls, while, Francois Becker, Andre Coetzer and Luke and Ursula Verburgt provided additional paratype material used in the descriptions. Permits for the collection of material in KwaZulu-Natal were issued by Ezemvelo KZN Wildlife. The study was financially supported by the South African National Biodiversity Institute (SANBI) and the National Research Foundation (NRF), Grant UID 98114. Financial assistance to EN who was supported by the DAAD-NRF Doctoral scholarship (Grant UID: 108803), and the VLIR-OUS university scholarship (ID 0620854 / Contract 000000076310). Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the funding bodies.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410.
https://doi.org/10.1016/S0022-2836(05)80360-2

Angulo, A. & Icochea, J. (2010) Cryptic species complexes, widespread species and conservation: lessons from Amazonian frogs of the *Leptodactylus marmoratus* group (Anura: Leptodactylidae). *Systematics and Biodiversity*, 8, 357–370.
https://doi.org/10.1080/14772000.2010.507264

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winkler, K., Ingram, K.K. & Das, I. (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, 22, 148–155.
https://doi.org/10.1016/j.tree.2006.11.004

Bossuyt, F. & Milinkovitch, M.C. (2000) Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. *Proceedings of the National Academy of Sciences*, 97, 6585–6590.
https://doi.org/10.1073/pnas.97.12.6585

Boulenger, G.A. (1907) Description of a new Engystomatid frog of the genus *Breviceps* from Namaqualand. *The Annals & Magazine of Natural History*, Series 7, 20, 46–47.

Broadley, D.G. (1971) *The reptiles and amphibians of Zambia.* Puku, Chilange, 143 pp.

Channing, A. & Wahlberg, K. (2011) Distribution and conservation status of the desert rain frog *Breviceps macrops*. *African Journal of Herpetology*, 60, 101–112.
https://doi.org/10.1080/21564574.2011.608383

Channing, A. (2012) A new species of Rain Frog from Namaqualand, South Africa (Anura: Brevicipitidae: Breviceps). *Zootaxa*, 3381, 62–68.

Channing, A. & Baptista, N. (2013) *Amietia angolensis* and *A. fuscigula* (Anura: Pyxicephalidae) in southern Africa: A cold case reheated. *Zootaxa*, 3640 (4), 501–520.
https://doi.org/10.11646/zootaxa.3640.4.1

Channing, A., Schmitz, A., Burger, M. & Kielgast, J. (2013) A molecular phylogeny of African Dainty Frogs, with the description

of four new species (Anura: Pyxicephalidae: *Cacosternum*). *Zootaxa*, 3701 (5), 518–550.
https://doi.org/10.11646/zootaxa.3701.5.2

Channing, A., Measey, G.J., De Villiers, A.L., Turner, A.A. & Tolley, K.A. (2017) Taxonomy of the *Capensibufo rosei* group (Anura: Bufonidae) from South Africa. *Zootaxa*, 4232 (2), 282–292.
https://doi.org/10.11646/zootaxa.4232.2.11

Conradie, W. (2014) The King of the Dwarves: a new cryptic species of Dainty Frog (Anura: Pyxicephalidae: *Cacosternum*) from the eastern Great Escarpment of South Africa. *Zootaxa*, 3785 (3), 438–452.
https://doi.org/10.11646/zootaxa.3785.3.6

Cuvier, P.M.L.B. (1829) *Le Règne Animal Distribué d'Après son Organisation, pour Servir de Base à l'Histoire Naturelle des Animaux et d'Introduction à l'Anatomie Comparée. Vol. 2. Nouvelle Edition, Revue et Augmentée par P.A. Latreille*. Deterville, Paris, 406 pp.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature methods*, 9, 772–772.

Dawood, A., Channing, A. & Bogart, J.P. (2002) A molecular phylogeny of the frog genus *Tomopterna* in southern Africa: examining species boundaries with mitochondrial 12S rRNA sequence data. *Molecular Phylogenetics & Evolution*, 22, 407–413.
https://doi.org/10.1006/mpev.2001.1060

Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and 546 high throughputs. *Nucleic Acids Research*, 32, 4673–4680.
https://doi.org/10.1093/nar/gkh340

Elmer, K.R. & Cannatella, D.C. (2008) Three new species of leaflitter frogs from the upper Amazon forests: cryptic diversity within *Pristimantis "ockendeni"* (Anura: Strabomantidae) in Ecuador. *Zootaxa*, 1784, 11–38.

FitzSimons, V. (1930) Descriptions of new South African Reptilia and Batrachia, with distribution records of allied species in the Transvaal Museum collection. *Annals of the Transvaal Museum*, 14, 20–48.

Fouquet, A., Gilles, A., Vences, M., Marty, C., Blanc, M. & Gemmell, N.J. (2007) Underestimation of species richness in Neotropical frogs revealed by mtDNA analysis. *PLoS ONE*, 2, e1109.
https://doi.org/10.1371/journal.pone.0001109

Frost, D.R. (2017) *Amphibian Species of the World: an Online Reference.* Version 6.0. American Museum of Natural History, New York, USA. Electronic Database. Available from: http://research.amnh.org/herpetology/amphibia/index.html (accessed 12 June 2017)

Funk, W.C., Caminer, M. & Ron, S.R. (2012) High levels of cryptic species diversity uncovered in Amazonian frogs. *Proceedings of the Royal Society B: Biological Sciences*, 279, 1806–1814.
https://doi.org/10.1098/rspb.2011.1653

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, 52, 696–704.
https://doi.org/10.1080/10635150390235520

Hewitt, J. (1925) Descriptions of three new toads belonging to the genus *Breviceps* Merrem. *Annals of the Natal Museum*, 5, 189–194.

Hewitt, J. (1932) Some new species and subspecies of South African batrachians and lizards. *Annals of the Natal Museum*, 7, 105–128.

Hoffman, A.C. (1944) Frogs from Chitiala, Nyasaland, together with description of new species. *Indago*, 1, 173–182.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649.
https://doi.org/10.1093/bioinformatics/bts199

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X. & Wilson, A.C. (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences*, 86, 6196–6200.
https://doi.org/10.1073/pnas.86.16.6196

Köhler, J., Glaw, F., Pabijan, M. & Vences, M. (2015) Integrative taxonomic revision of mantellid frogs of the genus Aglyptodactylus (Anura: Mantellidae*). Zootaxa*, 4006 (3), 401–438.
https://doi.org/10.11646/zootaxa.4006.3.1

Köhler, J., Jansen, M., Rodriguez, A., Kok, P.J., Toledo, L.F., Emmrich, M., Glaw, F., Haddad, C.F.B., Rödel, M.-O. & Vences, M. (2017) The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa*, 4251 (1), 1–124.
https://doi.org/10.11646/zootaxa.4251.1.1

Kurabayashi, A. & Sumida, M. (2013) Afrobatrachian mitochondrial genomes: genome reorganization, gene rearrangement mechanisms, and evolutionary trends of duplicated and rearranged genes. *BioMed Central Genomics*, 14, 633.
https://doi.org/10.1186/1471-2164-14-633

Lambiris, A.J.L. (1989) A review of the amphibians of Natal. *Lammergeyer*, 1989, 391–212.

Linnaeus, C. (1758) *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Vol. 1. 10th Edition*. L. Salvii. Stockholm, 824 pp.

Merrem, B. (1820) *Tentamen Systematis Amphibiorum.* J.C. Krieger, Marburg, Hesse, 191 pp.
Minter, L.R. (1995) The advertisement call and other aspects of the reproductive biology of *Breviceps adspersus* Peters (Anura, Microhylidae). *Madoqua*, 19, 37–44.
Minter, L.R. (1997) Advertisement call structure and morphology of *Breviceps mossambicus* Peters and *B. poweri* Parker (Anura: Microhylidae) from northern Mozambique. *Annals of the Natal Museum*, 38, 5–19.
Minter, LR. (1998) *Aspects of the reproductive biology of* Breviceps. Unpublished PhD thesis. University of the Witwatersrand, Johannesburg, 480 pp.
Minter, L.R. (2003) Two new cryptic species of *Breviceps* (Anura: Microhylidae) from southern Africa. *African Journal of Herpetology*, 49, 3–8.
https://doi.org/10.1080/21564574.2000.9650011
Minter, L.R. (2004a) *Breviceps adspersus* Peters, 1882. *In*: Minter, L.R., Burger, M., Harrison, J.A., Braack, H.H., Bishop, P.J. & Kloepfer, D. (Eds.), *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland. SI/MAB Series #9*. Smithsonian Institution, Washington, D.C., pp 172–173.
Minter, L.R. (2004b) *Breviceps mossambicus* Peters, 1854. *In*: Minter, L.R., Burger, M., Harrison, J.A., Braack, H.H., Bishop, P.J. & Kloepfer, D. (Eds.), *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland. SI/MAB Series #9*. Smithsonian Institution, Washington, D.C., pp 184–186.
Minter, L.R. (2004c) *Breviceps sopranus* Minter, 2003. *In*: Minter, L.R., Burger, M., Harrison, J.A., Braack, H.H., Bishop, P.J. & Kloepfer, D. (Eds.), *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland. SI/MAB Series #9*. Smithsonian Institution, Washington, D.C., pp 189–191.
Mucina, L. & Rutherford, M.C. (2006) *The vegetation of South Africa, Lesotho and Swaziland. Strelitzia. Vol. 19*. South African National Biodiversity Institute, Pretoria, 805 pp.
Parker, H.W. (1934) *A monograph of the frogs of the family Microhylidae*. Trustees of the British Museum, London, 208 pp.
Parker, W.K. (1868) *A monograph on the structure and development of the shoulder girdle and sternum in the Vertebrata. No. 42. Ray Society Series*. Robert Hardwicke, London, 237 pp.
Passmore, N.I. (1985) Sibling species, the acoustic environment and the anuran specific-mate recognition system. *In:* Vrba, E.S. (Eds.), Species and speciation. *Transvaal Museum Monograph No. 4.* Transvaal Museum, Pretoria, pp. 125–127.
Paterson, H.E.H. (1985) The recognition concept of species. *In:* Vrba, E.S., (Eds.), Species and speciation. *Transvaal Museum Monograph No. 4.* Transvaal Museum, Pretoria, pp. 21–29.
Paterson, H.E.H. (1991) The recognition of cryptic species among economically important insects. *In:* Zalucki, M.P., (Eds.), *Heliothis: Research Methods and Prospects*, 9, pp. 1–10.
https://doi.org/10.1007/978-1-4612-3016-8_1
Peloso, P.L., Frost, D.R., Richards, S.J., Rodrigues, M.T., Donnellan, S., Matsui, M., Raxworthy, C.J., Biju, S.D., Lemmon, E.M., Lemmon, A.R. & Wheeler, W.C. (2015) The impact of anchored phylogenomics and taxon sampling on phylogenetic inference in narrow-mouthed frogs (Anura, Microhylidae). *Cladistics*, 32, 113–140.
https://doi.org/10.1111/cla.12118
Peters, W.C.H. (1854) *Diagnosen neuer Batrachier, welche zusammen mit der früher (24. Juli und 18. August) gegebenen Übersicht der Schlangen und Eidechsen mitgetheilt werden.* Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlich Preußischen Akademie der Wissenschaften zu Berlin, Berlin, 15 pp. [pp. 614–628]
Peters, W.C.H. (1882) *Naturwissenschaftlich Reise nach Mossambique auf befehl Seiner Mäjestat des Königs Friedrich Wilhelm IV in den Jahren 1842 bis 1848 ausgeführt*. *Zoologie* 3 (Amphibien). Berlin: G. Reimer.
Pienaar, U.d.V. (1963) The zoogeography and systematic list of Amphibia in the Kruger National Park. *Koedoe*, 6, 76–82.
Power, J.H. (1926) A monographic revision of the genus *Breviceps*, with distribution records and descriptions of new species. *Annals of the South African Museum*, 20, 451–471.
Poynton, J.C. (1963) Descriptions of southern African amphibians. *Annals of the Natal Museum*, 15, 319–332.
Poynton, J.C. (1964) The amphibians of southern Africa: a faunal study. *Annals of the Natal Museum*, 17, 1–334.
Poynton, J.C. (1982) On species pairs among southern African amphibians. *South African Journal of Zoology*, 17, 67–74.
https://doi.org/10.1080/02541858.1982.11447782
Poynton, J.C. & Broadley, D.G. (1985) Amphibia Zambeziaca I. Scolecomorphidae, Pipidae, Microhylidae, Hemisidae, Arthroleptidae. *Annals of the Natal Museum*, 26, 503–553.
Ronquist, F. & Huelsenbeck, J.P. (2003) Mr Bayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
https://doi.org/10.1093/bioinformatics/btg180
Swofford, D. L. (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Ver. 4. Sinauer Associates, Sunderland, Massachusetts.
Vences, M., Thomas, M., Van der Meijden, A., Chiari, Y. & Vieites, D.R. (2005) Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology*, 2, 5.
https://doi.org/10.1186/1742-9994-2-5
Werner, F. (1899) Beschreibung einiger neuer Schlangen und Batrachier. *Zoologischer Anzeiger*, 22, 114–117.
Werner, F. (1903) *Über Reptilien und Batrachier aus Guatemala und China in der zoologischen Staats-Sammlung in München nebst einem Abhang über seltene Formen aus Anderer gebieten.* Abhandlungen der Mathematisch-Physikalischen Classe der Königlich Bayerischen Akademie der Wissenschaften, München, 43 pp. [pp. 342–384]