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Phylogeny and taxonomy of the African frog genus *Strongylopus* (Anura: Pyxicephalidae)

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Abstract: We present a molecular phylogeny of African stream frogs (genus *Strongylopus*), based on 12S rRNA, 16S rRNA, the nuclear recombination activating gene 1 (*RAG-1*) and tyrosinase exon 1 (*tyr*). Molecular data were supported by advertisement call analysis and morphology. We recognise six valid species: *Strongylopus bonaespei* (Dubois, 1981) from the southern and southwestern parts of the Western Cape Province, South Africa; *Strongylopus fasciatus* (Smith, 1849) from eastern South Africa to Zimbabwe; *Strongylopus grayii* (Smith, 1849) found throughout South Africa with older records in Naukluft, in central Namibia; *Strongylopus rhodesianus* (Hewitt, 1933) known from the eastern highlands of Zimbabwe and western Mozambique; *Strongylopus wageri* (Wager, 1961) from KwaZulu-Natal Province, South Africa and *Strongylopus merumontanus* (Lönnberg, 1907) from eastern Zambia, Malawi, northern Mozambique and Tanzania. *Strongylopus fuelleborni* (Nieden, 1911), *S. kitumbeine* Channing & Davenport, 2002 and *S. kilimanjaro* Clarke & Poynton, 2005 were shown to be junior synonyms of *Strongylopus merumontanus*. *Strongylopus springbokensis* Channing, 1986 is recovered as a junior synonym of *Strongylopus grayii*. Divergence ages were estimated, and discussed in terms of paleoclimatic events.

Keywords: Amphibia - Africa - phylogeny - integrative taxonomy - synonyms - advertisement calls - 12S rRNA - 16S rRNA - *tyr* - *RAG-1* - morphology - paleoclimate.

INTRODUCTION

Stream frogs in the genus *Strongylopus* are widespread in the south of Africa and at high elevations of tropical East Africa (Poynton, 2013), reaching low elevations only in the high latitudes. They are assigned to the endemic African family Pyxicephalidae (van der Meijden *et al.*, 2005), and presently 10 species are recognised (Frost, 2022).

Strongylopus bonaespei (Dubois, 1981), endemic to South Africa, is associated with the coastal lowlands and the higher mountainous regions running parallel with the Western Cape Province coast from west to east; *Strongylopus fasciatus* (Smith, 1849) is known from eastern South Africa to Mozambique, Zimbabwe, southern Zambia and the highlands of eastern Africa, including the Eastern Arc Mountains of Tanzania and the Nyika Plateau of Malawi; *Strongylopus fuelleborni* (Nieden, 1911) occurs on the isolated mountains in the south of Malawi and adjacent Mozambique; *Strongylopus grayii* (Smith, 1849) is widespread in South Africa at both lower and higher elevations, extending into Lesotho, and reaching the borders of Botswana in the north and an isolated population from Naukluft in central Namibia; *Strongylopus kilimanjaro* Clarke & Poynton, 2005 is only known from the high slopes (above 1700 m) of Mt. Kilimanjaro (Zancolli *et al*., 2013); *Strongylopus kitumbeine* Channing & Davenport, 2002 is restricted to an extinct volcano, Mt. Kitumbeine in northern Tanzania; *Strongylopus merumontanus* (Lönnberg, 1907) similarly occurs on the highlands of an extinct volcano, Mt. Meru

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in northern Tanzania; *Strongylopus rhodesianus* (Hewitt, 1933) is known from the Eastern Highlands of Zimbabwe and the Gorongosa massif in western Mozambique; *Strongylopus springbokensis* Channing, 1986 is known from the arid area of Namaqualand in South Africa; *Strongylopus wageri* (Wager, 1961) is found on the uplands of KwaZulu-Natal Province, including the slopes of the Drakensberg.

The taxon has an unsettled taxonomic history and was regarded as a 'problematic' genus by Poynton (2013) due to the similarity of many of the species. *Strongylopus* was described by Tschudi in 1838, although it was still placed in the genus *Rana* by most workers for the next century. The status of the genus *Strongylopus* was later confirmed based on tadpole morphology (Van Dijk, 1966), but subsequently placed as a subgenus of *Rana* (Dubois, 1988). The misidentification of an *Amietia* gene sequence as *S. grayii* resulted in the apparent polyphyly of *Strongylopus* (Bittencourt-Silva *et al*., 2016; Channing *et al*., 2016). Presently the genus *Strongylopus* is recognised as part of the endemic African family Pyxicephalidae, which is not closely related to *Rana* (Channing, 2001; Frost *et al*., 2006; Channing *et al*., 2016; Du Preez & Carruthers, 2017). Frost (2022) presents a history of the taxonomic changes applied to *Strongylopus*, and there are still questions concerning the taxonomy of many of the species.

One problem is the occurrence of colour variations. For instance, a common colour pattern is longitudinal lines of black, brown and yellow, which may have caused confusion in the identification of *S. bonaespei, S. fasciatus* and the three northern species (Poynton, 1964b; Greig *et al*., 1979; Clarke & Poynton, 2005). Colour patterns may distinguish some species, although these can be very variable. Further pattern elements that vary include ventral spotting, vertebral stripes, and cross-banding on legs. Some morphological criteria are useful in species diagnosis, for example, *Strongylopus grayii* can be distinguished from *S. rhodesianus* by the ratio of head width/length of fourth toe (Poynton, 1964a). Morphological characters previously used to differentiate between taxa (Poynton, 1964a; Poynton & Broadley, 1985) include relative head width, relative snout length, relative diameter of tympanum, relative foot length, relative tibia length, and amount of webbing on the fourth toe. Advertisement calls are useful taxonomic characters in most instances (Passmore, 1977), as morphologically similar species of *Strongylopus* can be easily distinguished acoustically (Channing, 1979).

While most species have small distributions, *Strongylopus fasciatus* (Smith, 1849) and *S. grayii* (Smith, 1849) are widespread and expected to show some genetic sub-structure. *Strongylopus fuelleborni* is known from Tanzania, Malawi and northeastern Zambia. Loveridge placed *S. fuelleborni* as a synonym of *Rana fasciata merumontana* (1933), and later as a subspecies of *S. fasciata* (1953). Poynton (1964b) compared six specimens from each of six localities, and showed that the ratio of head width/foot length revealed three groups, one in South Africa and Zimbabwe (as *S. f. fasciatus*), one on Mt. Mulanje and Nyika Plateau (as *S. fasciatus fuelleborni*) and the third in the north on the Usambara and Poroto Mountains (as *S. fasciatus merumontana*). The proportions of the central *S. fuelleborni* are between those of the southern *S. fasciatus* and the northern *S. merumontanus*. *Strongylopus fuelleborni* which occurs on many isolated highlands (Conradie *et al*., 2018) was elevated to a full species by Channing (2001) based on advertisement call differences. Channing & Davenport (2002) based on similarity in advertisement calls and morphology, subsequently placed *S. fuelleborni* as a junior synonym of *S. merumontanus* (Lönnberg, 1907), which was described from Mt. Meru in northern Tanzania. Poynton (2004) regarded *S. merumontanus* as restricted to high elevations on Mt. Meru. Pickersgill (2007) used the name *S. fuelleborni* for the northern species as it was geographically separate from *S. fasciatus*. However, the integrity of this species has not been tested phylogenetically.

The other common species, the Clicking Stream Frog *Strongylopus grayii* (Smith, 1849) is widespread in South Africa, known from many vegetation types and a range of elevations. Tolley *et al*. (2010) showed differences between southwestern (winter rainfall) and northern (summer rainfall) populations, which they suggested might represent cryptic species. *Strongylopus kitumbeine* Channing & Davenport, 2002 was described from Mt. Kitumbeine, an extinct volcano in northern Tanzania. It was diagnosed based on some unique characters in morphology and advertisement calls. *Strongylopus kilimanjaro* Clarke & Poynton, 2005 is the most recently described species, distinguished on small morphological differences from *S. kitumbeine*. It is only known from Mt Kilimanjaro in Tanzania. *Strongylopus rhodesianus* (Hewitt, 1933) was originally described as a subspecies of *Rana grayi* (Hewitt, 1933). However, small samples of typical *S. grayii* and *S. rhodesianus* showed significant differences in the ratio of head length/length of fourth toe (Poynton, 1964a). *Strongylopus rhodesianus* is not well known, occurring on the Eastern Highlands of Zimbabwe and the adjacent highlands of Mozambique, including Mt Gorongosa. Poynton & Broadley (1985) thought it very likely that there was genetic mixing with *S. fasciatus*. *Strongylopus bonaespei* (Dubois, 1981) was originally described as *Rana fasciata montana* FitzSimons, 1946, and later named *Rana montana* by Greig *et al*. (1979). However, these names were preoccupied by *Rana temporaria* var. *montanus* Koch, 1872. The species was renamed *Rana* (*Strongylopus*) *bonaespei* by Dubois (1981). It occurs along the coast and on the mountains in the southern and southwestern Cape Province, South Africa. The little-known *Strongylopus springbokensis* Channing, 1986 occurs in the montane, arid region of Namaqualand in South Africa. *Strongylopus wageri*

(Wager, 1961) is known from the midlands of KwaZulu-Natal Province and the slopes of the Drakensberg Mountains in South Africa. Although the species was inadvertently described by Wager, there seem to be no taxonomic problems in this taxon (Poynton, 1963). In contrast the status of the taxa isolated on relatively small areas in northern Tanzania (*Strongylopus kitumbeine, S. kilimanjaro* and *S. merumontanus)* is in need of further study, i.e. to test their genetic isolation. Poynton (2004) showed that the northern *S. fuelleborni* is not related to the southern *S. fasciatus*. The early changes in nomenclature of these species are reviewed by Poynton (2013).

The purpose of this study is to re-evaluate the taxonomy of stream frogs, using nuclear and mitochondrial gene sequences, advertisement calls, and morphology.

MATERIALS AND METHODS

Sampling: The specimens used for the molecular study are listed in Appendix 1. This includes material from South Africa, Lesotho, Zimbabwe, Malawi, Mozambique, and Tanzania. Localities where advertisement calls were obtained are listed in Appendix 2. Finally, all material used for the morphological study is listed in Appendix 3.

Molecular: DNA extraction and amplification of fragments of mitochondrial 12S rRNA, 16S rRNA, the nuclear-coding tyrosinase exon 1 (*tyr*), and a fragment of the nuclear recombination activating gene 1 (*RAG-1*) followed standard protocols (Channing *et al*., 2016). We developed new nuclear recombination activating gene 1 (*RAG-1*) primers for *Strongylopus*, Rag1-Str-F1 (TGC TGC AAA CCC CTT TGC CTA) and Rag1-Str-R1 (TTC CCT TCG CTG GCC CA) and modified 12S primers: 12Sam F (TCCTRGCCTTRTCARCT) and 12Sam R (ATAGTGGGGTATCTAATCCCAGTTT). The annealing temperature for the *RAG-1* primers was 55°C, and 51°C for the 12Sam primers. Some samples were sequenced in both directions, and some were sequenced on two separate occasions. Sequences were aligned using Sequencher 5.4. GenBank sequences and sources are listed in Appendix 1. Uncorrected *p*-distances were calculated using PAUP* (Swofford, 2002). The uncorrected *p* distances for the mitochondrial 16S rRNA, nuclear *tyr* and *RAG-1* genes were compared between all available *Strongylopus* sequences. The genus *Amietia* is the appropriate outgroup for *Strongylopus* (Frost *et al.,* 2006; Jetz & Pyron, 2018, 2019). The outgroup sequence is a chimaera of *Amietia nutti* from Kenya (12S KU69367, 16S KU693777, *tyr* KU694111), and *Amietia delalandii* from Lesotho (*RAG-1* HQ014422).

The two most likely haplotypes for each individual for the nuclear genes were determined by first submitting the aligned sequences, including IUPAC polymorphism symbols, to SeqPhase step 1 (Flot, 2010), which produces an input file for PHASE (Stephens & Donnely, 2003). PHASE calculates the likelihood of possible haplotypes. The output is submitted to SeqPhase step 2, which produces full sequences for each haplotype (accessed at https://eeg-ebe.github.io/SeqPHASE). Haplotype networks for both nuclear gene fragments and the mitochondrial genes were determined using TCS 1.21 (Clement *et al.,* 2000) and illustrated using PopArt (Population Analysis with Reticulate Trees; http://popart.otago.ac.nz).

Phylogenies based on each gene separately (phased nuclear genes), and then on all four genes combined, for samples where this was possible, were generated using the Maximum Likelihood (ML) software IQ-TREE (Nguyen *et al.,* 2015; Trifinopoulos *et al.,* 2016). Aligned sequences were submitted to the website W-IQ-TREE (http://iqtree.cibiv.univie.ac.at). The ultrafast bootstrap UFBoot2 (Minh *et al.,* 2013; Hoang *et al.,* 2017) was determined using 1000 replicates. The software determined the appropriate model for the ML analysis: TIM2e+I+G4. Support values for the phylogeny were calculated as SH-aLRT (%) and the ultrafast bootstrap (%). The tree was rooted and drawn using FigTree 1.4.4 (Rambaut, 2018). Divergence dates for representatives of each species were estimated in BEAST 2.6.3 (Hasegawa *et al*., 1985; Heled & Drummond, 2012; Bouckaert *et al*., 2019), calibrating the tree based on a mean rate of change of 0.16% per million years for Pyxicephalidae 16S rRNA, after Bittencourt-Silva *et al*. (2016).

Museum acronyms: The museum acronyms used in the literature and their current equivalents follows Sabaj (2019). TMP – Transvaal Museum, Pretoria (now DNMNH – Ditsong National Museum of Natural History, Pretoria); BMNH – Natural History Museum, London; PEM – Port Elizabeth Museum, Bayworld complex, Gqeberha; NRM – Swedish Museum of Natural History, Stockholm; ZMB – Museum für Naturkunde, Leibniz Institute for Evolution- and Biodiversity Science, Berlin; CAS – California Academy of Sciences.

Advertisement calls: Most calls were obtained in the field using a Marantz PMD660 digital recorder with a directional microphone. Older recordings using a Sony cassette recorder were also included. Air temperature was noted. Call analysis was carried out using Raven Pro 1.6.1 (Center for Conservation Bioacoustics, 2019). Calls consist of brief clicks or whistles. Call parameters were determined following Köhler *et al*. (2017). The following call parameters were noted: dominant harmonic mid-point, other emphasised harmonics, pulses per note, note duration and note rate. Calls were classified into guilds following Emmrich *et al*. (2020).

Morphology: Measurements were taken with a digital calliper accurate to 0.01 mm, rounded to 0.1 mm, with the aid of a dissecting microscope. The following measurements were recorded: SUL – snout-urostyle length, HW – maximum head width, HL – head length measured from the back of the lower jaw to the snout tip,

SL – snout length measured form the anterior corner of the eye to the snout tip, EW – horizontal eye diameter, EN – distance between eye and nostril, ET – distance between eye and tympanum, TYM – horizontal tympanum diameter, FOT – foot length measured to include the inner metatarsal tubercle to the tip of the fourth toe, TIB – length of tibiafibula, HND – hand length, measured to include the palmar tubercle to the tip of the third finger. In addition, notes were made of ventral, snout, back and limb patterns. The dorsal vertebral skin ridges may be absent, interrupted or continuous, and this was noted. Colour patterns were scored from photos of live animals. The following ratios were calculated: HW/FOT – relative foot length as used by Poynton (1964a), SUL/HW – relative head width, SUL/SL – relative snout length, HW/EW – relative eye size, ET/EW relative distance between eye and tympanum, TYM/ET – relative tympanum size, SUL/TIB – relative tibiafibula length, TIB/FOT – relative foot to tibia length, HW/HND – relative hand length, SL/EW – snout length relative to eye width, FOT/SUL – length of foot relative to body length, EN/TYM – relative size of tympanum.

RESULTS

Molecular data

Phylogeny: The software proposed TIM2e + I + G4 as the appropriate model for the ML analysis. The phylogeny based on four genes showed six well-supported clades. The same topology was recovered from the four genes when analysed separately. We show the 16S and *tyr* trees as they included most samples (Figs 1, 2, 3). The 16S rRNA sequences (Figs 1, 2) show slight variation between the highland populations of *S. fuelleborni* on Mt. Mulanje (Malawi), *S. fuelleborni* on the Mt. Namuli and Mt. Mabu complex (Mozambique), *S. fuelleborni* at Nyanga and the highlands of eastern Zimbabwe, *S. fuelleborni* on the Mbizi Hill highlands south of Lake Rukwa in Tanzania, the Livingstone Mountains in Tanzania, and the Udzungwa Mountains in Tanzania, *S. kilimanjaro* on Mt. Kilimanjaro (Tanzania), *S. kitumbeine* on Mt. Kitumbeine (Tanzania) and *S. merumontanus* on Mt. Meru (Tanzania)(Fig. 4). The nuclear *tyr* phylogeny (Fig. 3) shows that many sequences of *S. kilimanjaro, S. fuelleborni*, *S. merumontanus* and *S. kitumbeine* are identical.

The 16S rRNA phylogeny (Fig. 2) shows that *S. springbokensis* sequences from Rooiberg and Studer Pass on the Namaqualand highlands are embedded within sequences of *S. grayii* from the southern and southwestern Cape in South Africa. Sequences of the nuclear *tyr* gene from the same localities (Fig. 3) places them basal to *S. grayii* but in a well-supported clade. *Strongylopus springbokensis* is thus regarded as a junior synonym of *S. grayii*.

Uncorrected p distances: The uncorrected *p* distances for 16S and the two nuclear genes, *tyr* and *RAG-1* are presented in Table 1. The uncorrected interspecific *p* distances for 16S varied from 2.8-7.4%, for *tyr* 0.2-3.6%, and for *RAG-1* 0.2-6.7%. The intraspecific uncorrected *p* distances for 16S varied from 0-2.1%, with the largest

Table 1. Range of uncorrected p-distances as percentages for 16S rRNA, *tyr* and *RAG-1* between *Strongylopus* taxa. GRA – *S. grayii*, FAS – *S. fasciatus*, MER – *S. merumontanus*, WAG – *S. wageri*, BON – *S. bonaespei*. RHO – *S. rhodesianus*. Top line –16S, middle line – tyrosinase exon 1, lowest line – *RAG-1*.

	GRA	FAS	MER	WAG	BON	RHO
GRA	0-1.7 0.2-1.0 0-0.8					
FAS	4.2-6.0 2.4-3.4 2.8-5.5	0-0.8 0-0.6 0-1.1				
MER	4.8-6.6 2.6-3.6 2.9-4.7	2.8-4.8 0.2-1.4 3.3-5.4	0-2.4 0-1.0 0-1.4			
WAG	4.8-5.6 2.4-3.0 2.7-4.2	5.6-6.3 2.8 3-6.7	6.0-7.4 3.0-3.3 3.3-5.0	0-0.2 0 0.7-1.5		
BON	4.4-5.8 1.8-2.8 2.7-3.9	5.0-5.4 2.2-3 3.3-6.4	2.8-6.4 2.4-3.4 3.3-4.3	5.8-5.9 2.4-3.0 2.4-3.8	0 0-0.6 0.4	
RHO	5.7-7.0 1.0-3.3 2.6-4.3	3.9-5.1 0.6-1.0 3.5-4.3	3.3-4.6 0.6-2.5 0.2-1.5	7.7 3.1 3.7-4.3	5.5-5.7 2.3-3.3 3.9-4.5	0 0 0-0.2

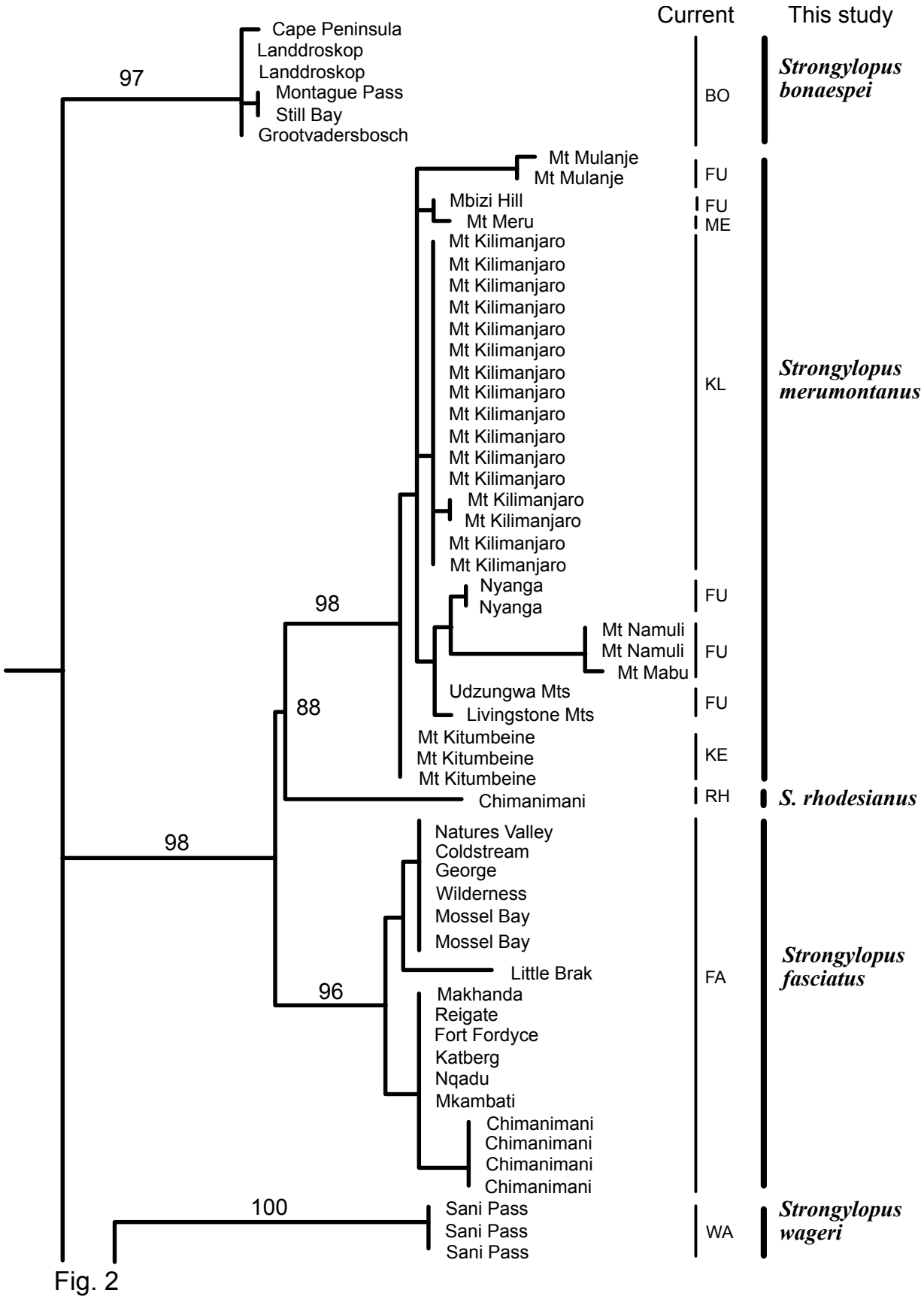


Fig. 1. Part 1 of Phylogeny of the genus *Strongylopus*, based on 16S rRNA. Current name codes: BO–*S. bonaespei*, FU–*S. fuelleborni*, ME – *S. merumontanus*, KL – *S. kilimanjaro*, KE – *S. kitumbeine*, RH – *S. rhodesianus*, FA – *S. fasciatus*, WA – *S. wageri*.

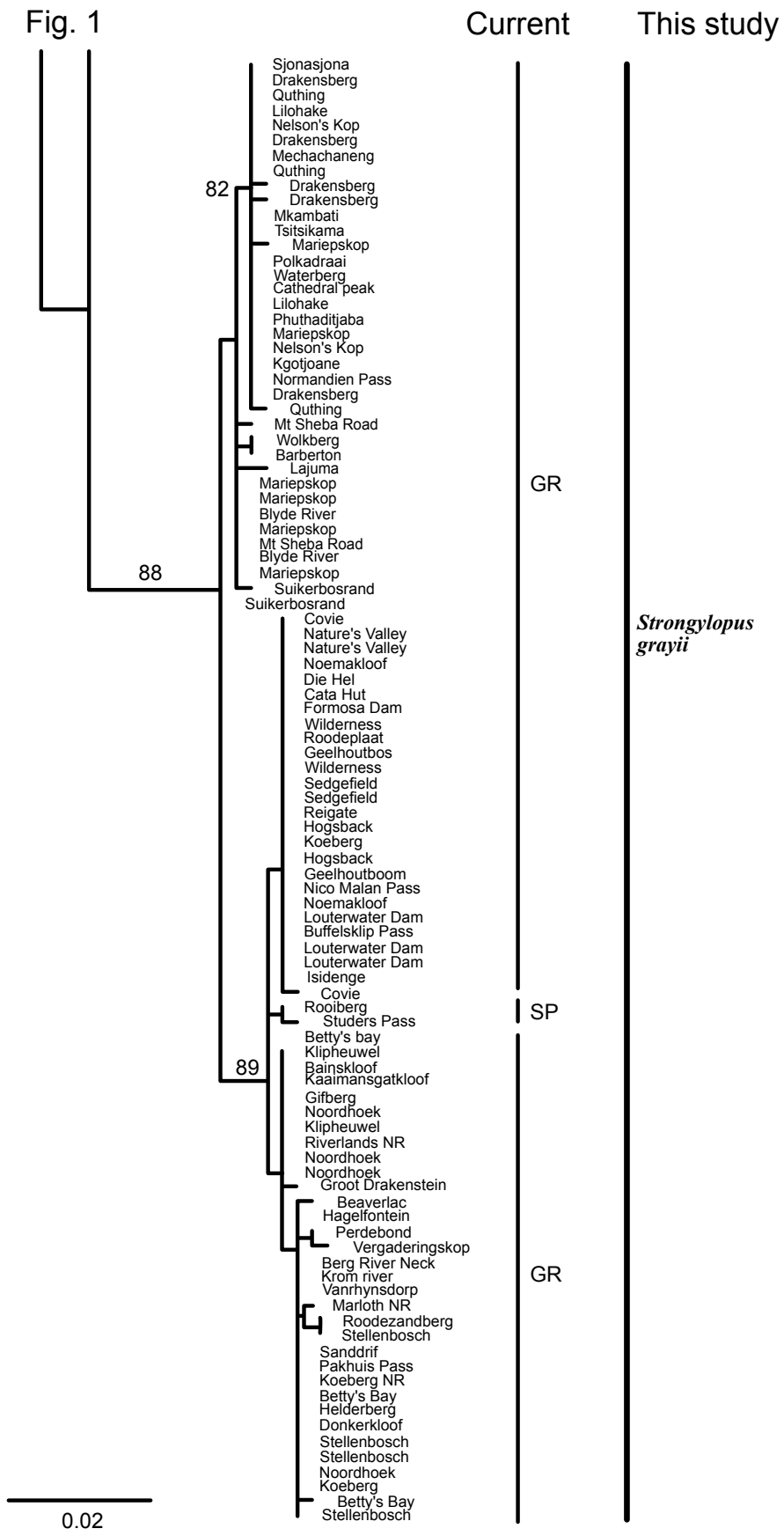


Fig. 2. Part 2 of Phylogeny of the genus *Strongylopus*, based on 16S rRNA. Current name codes: GR – *S. grayii*, SP – *S. springbokensis*.

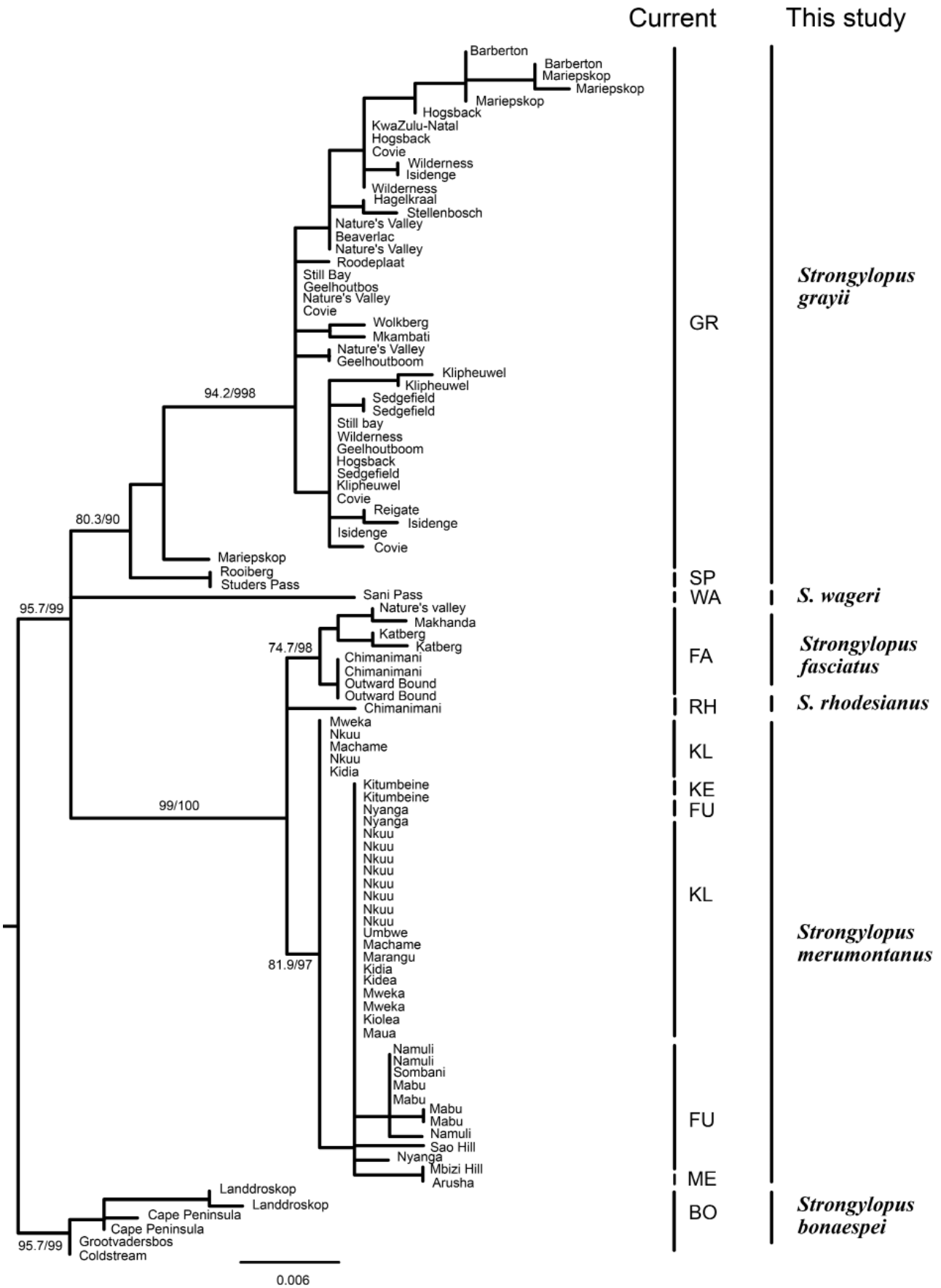


Fig. 3. Phylogeny of the genus *Strongylopus*, based on tyrosinase exon 1. Current name codes: See Figs 1 and 2.

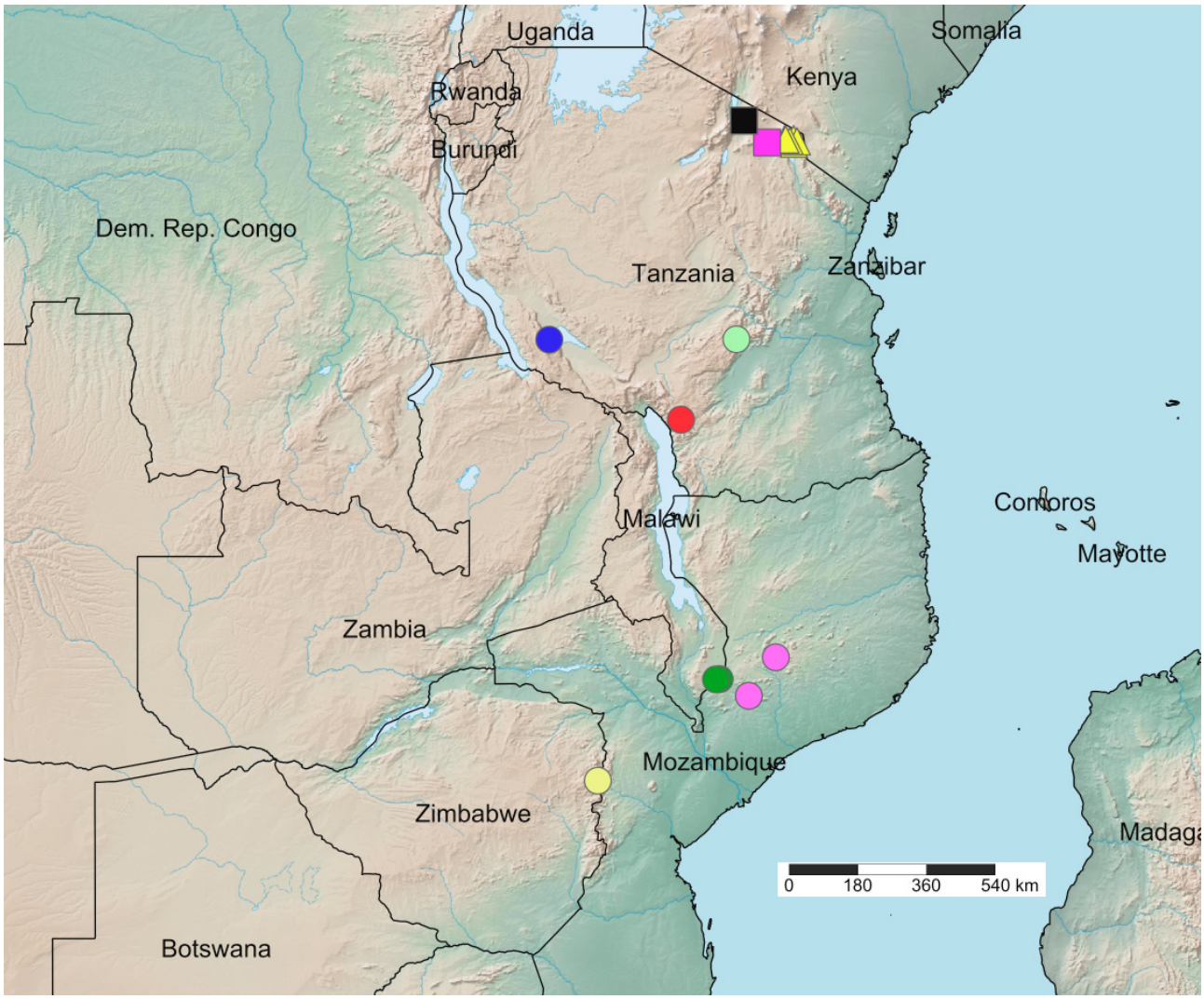


Fig. 4. Location of the sampled populations now recognised as *S. merumontanus*. *Strongylopus merumontanus* – purple square, *S. kitumbeine* – black square, *S. kilimanjaro* – yellow triangles, *S. fuelleborni* Mbizi Hill – blue circle, *S. fuelleborni* Udzungwa Mts. – light green circle, *S. fuelleborni* Livingstone Mts. – red circle, *S. fuelleborni* Mt. Mulanje – green circles, *S. fuelleborni* Mts. Mabu and Namuli–purple circles, *S. fuelleborni* Nyanga – yellow circle.

differences revealed between isolated populations of *S. merumontanus*. Table 4 lists the uncorrected *p* distances between *S. kitumbeine, S. kilimanjaro, S. merumontanus,* and populations of *S. fuelleborni* on Mt. Mulanje, Mts. Namuli and Mabu, Nyanga, Mbizi Hill, and the Udzungwa Mountains. The distances between *S. merumontanus* and the populations of *S. fuelleborni* on Mt. Mulanje and Mts. Namuli and Mabu vary from 1.5 to 2.1%. The distances between *S. merumontanus*, *S. kitumbeine*, *S. kilimanjaro*, and populations of *S. fuelleborni* from Nyanga, Mbizi Hill, the Livingstone Mts. and the Udzungwa Mts. are very similar, varying 0-0.8%. The intraspecific distances for *tyr* and *RAG-1* varied from 0-1.0%, and 0-1.4%, respectively.

Haplotype networks: Haplotype networks using the TCS algorithm are shown in Fig. 5. The mitochondrial 12S rRNA and 16S rRNA, as well as the two nuclear genes showed similar patterns. There was a single shared *RAG-1* haplotype between *S. rhodesianus* and *S. merumontanus* from Nyanga in Zimbabwe. Due to low genetic differences, *S. merumontanus*, *S. fuelleborni*, *S. kitumbeine*, and *S. kilimanjaro* were combined and treated as a single species. The minimum base changes between closely related species are listed in Table 2.

Discordance: Some recent studies have shown discordance between the phylogenies derived from mitochondrial and nuclear genes (Toews & Brelsford, 2012), which is often related to incomplete lineage sorting (Streicher & Day, 2020). The faster rate of divergence of mitochondrial genes compared to nuclear genes in amphibians (Crawford, 2003), should not change the relationships recovered where there is no discordance. Hybridization has been proposed to account for discordance in *Polygonia* butterflies (Wahlberg *et*

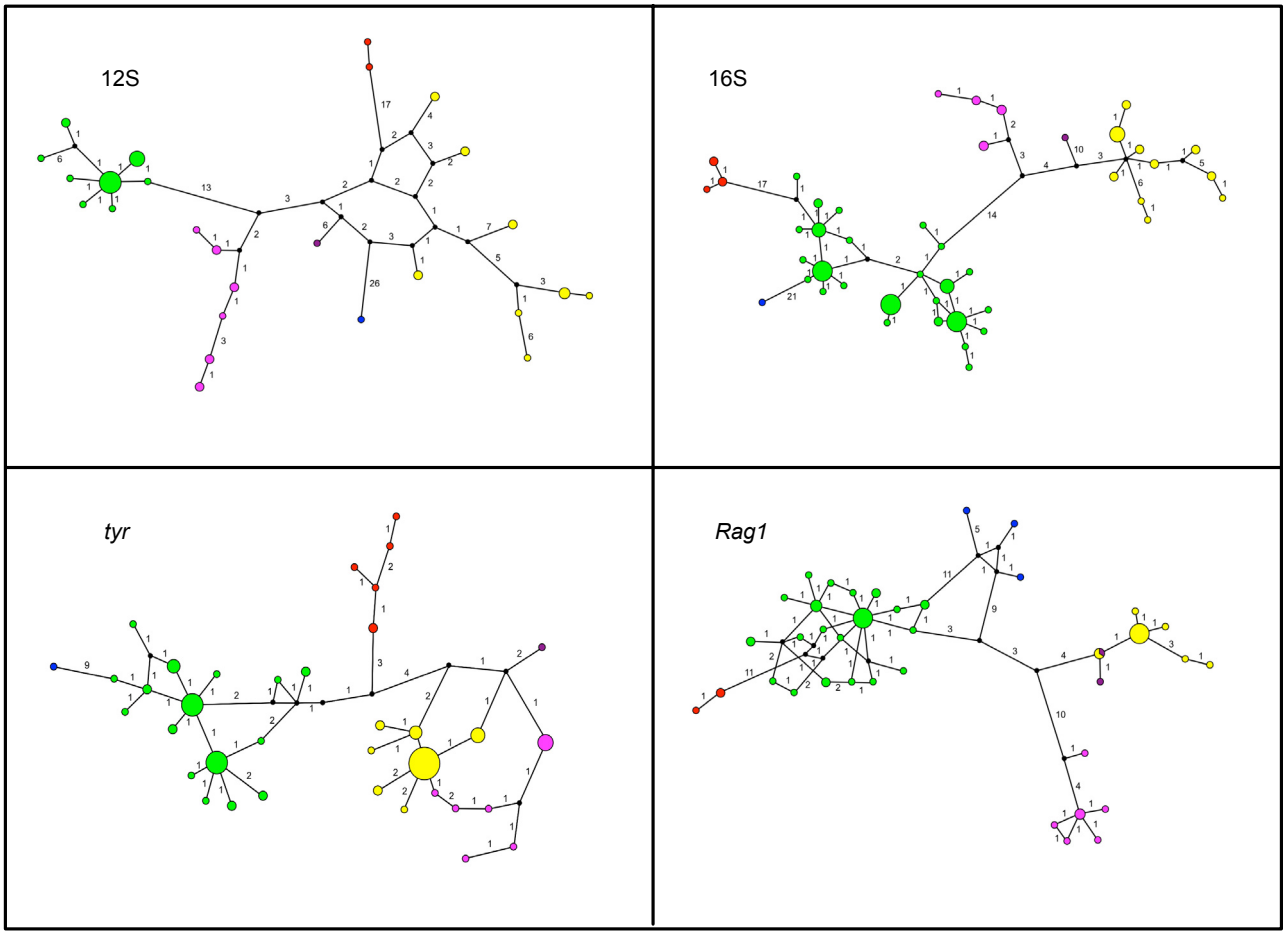


Fig. 5. Haplotype networks for 12S, 16S, tyrosinase exon 1, and *RAG-1*. Size of symbol is relative to number of individuals. Numbers indicate base changes along branch. Black symbols are hypothesised intermediate haplotypes. Green – *S. grayii*, red – *S. bonaespei*, blue – *S. wageri*, violet – *S. fasciatus*, yellow – *S. merumontanus*, purple – *S. rhodesianus*.

Table 2. Minimum base differences between species of *Strongylopus*. See Table 1 for species codes. Data presented as 12S rRNA/16S rRNA/*tyr*/*RAG-1*.

	GRA	FAS	RHO	WAG	MER
GRA					
FAS	19/20/-/-				
RHO		18/22/5/18			
WAG	-/22/10/16		36/-/-/-		
MER		-/-/1/18	-/17/5/0	31/-/-/-	
BON	-/20/10/16				26/-/12/-

al., 2009) and for many cases in amphibians (Toews & Brelsford, 2012). Gonçalves *et al*. (2007) suggested that a deep coalescence was responsible for the discordance in midwife toads *Alytes*, but could not discount the possibility of putative hybridization events causing a reticulated pattern of evolution. Although there may be a difference in the relationships recovered from mitochondrial and nuclear genes, there was no difference in the composition of the clades (species) recovered here. No discordance was found in *Strongylopus*, with the clades (species) strongly supported, similar to the findings of Stöck *et al*. (2008) in the species of *Hyla* found around the Mediterranean, and *Pleuroderma* in South America (Faivovich *et al*., 2012).

Dating (Fig. 6): The split of *Strongylopus* from *Amietia* happened during the early Eocene, 53 Mya (95% HPD [highest posterior density] 83.4-26.8 Mya). The next split during the middle Oligocene was between the ancestor of southern *S. bonaespei* + *S. grayii* + *S. wageri* from the ancestor of northern *S. merumontanus* + *S. fasciatus* + *S. rhodesianus* at 27 Mya (95% HPD 41.3-15.6 Mya). Further splits during the Miocene included the *Strongylopus bonaespei* split from *S. grayii* + *S. wageri* around 22 Mya (95% HPD 34.3-11.7 Mya), followed by the split of *S. grayii* from *S. wageri* around 19 Mya (95% HPD 29.6-8.7 Mya). The *S. rhodesianus* split from the ancestor of *S. fasciatus* + *S. merumontanus* took place at around 16 MYA (95% HPD 22.6-10.8 Mya), followed by the split of *S. fasciatus* from *S. merumontanus* at around 11 Mya (95% HPD 11.7-10.2 Mya). The paleoclimate correlates are discussed below.

Advertisement calls

The calls could be divided into four types. A single amplitude modulated (AM) click, a brief high-pitched frequency modulated (FM) whistle, a chuckle, and a trill (Fig. 7). *Strongylopus grayii* usually produces clicking calls. Brief whistle calls are known for *S. fasciatus* and *S. merumontanus*. A trill is a series of repeated whistles, common in *S. merumontanus*. The chuckle call is part of the *S. bonaespei* vocalisation, often interspersed with croaks. The call of *S. wageri* is best described as a very variable cackle, with an infrequent loud clack or trill. The call of *S. rhodesianus* is unknown. It was previously described as a trill (Channing, 2001) but this was not based on a male whose identification was confirmed by DNA, and may have been a misidentified *S. fasciatus*. Species-specific call characteristics are given with the species summaries below, including the call guilds after Emmrich *et al*. (2020). The call parameters of *S. fuelleborni*, *S. kitumbeine*, and *S. kilimanjaro* are

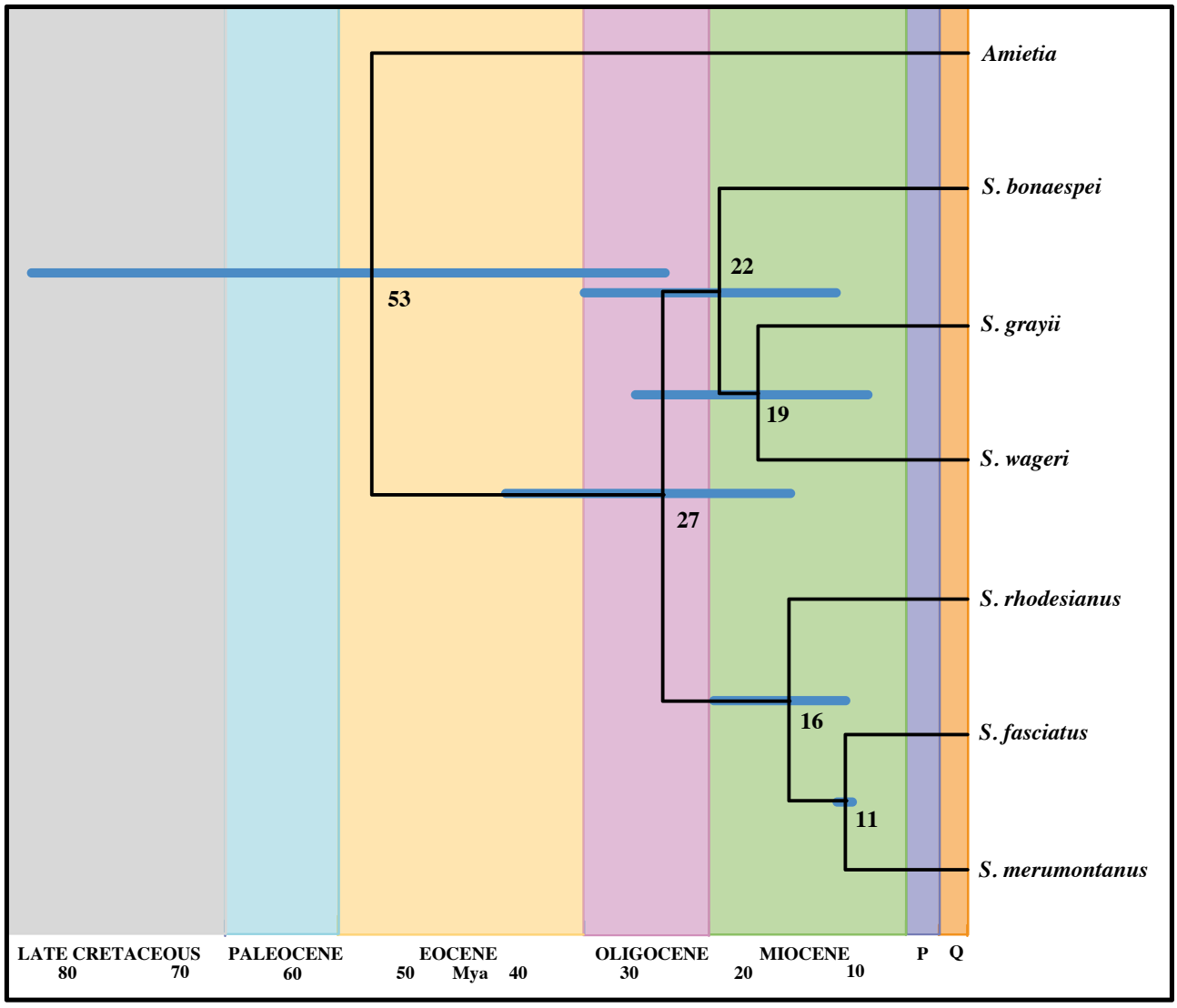


Fig. 6. Divergence estimates based on 16S rRNA. Blue bars indicate 95% HPD. Scale in Ma.

discussed under *H. merumontanus* below, as they are very similar and overlap in structure.

Greig *et al*. (1979) showed differences between the calls of *S. fasciatus* and *S. bonaespei*. Poynton (1964a) remarked that the southern and northern populations of *S. grayii* had slightly different advertisement calls with the northern population having slightly lower pitched calls. Our large sample (474 calls) shows that the range of dominant frequencies, 1464-3030 Hz was not related to latitude (Fig. 8) with a range of frequencies shown in both northern and southern populations.

Morphology

The available frogs of all taxa showed remarkable similarity in most body proportions (Table 3), although there was considerable variation. The largest species in our sample was *S. wageri* with a snout-urostyle length of 50.6 mm. It was closely followed by *S. fuelleborni* (49.3), *S. kilimanjaro* (46.5) and *S. bonaespei* (46.5). Poynton (1964a) gives a maximum size for *S. bonaespei* of 47.5 mm.

The mean snout-urostyle lengths were very similar. *Strongylopus merumontanus* is the shortest (25 mm) while *S. grayii*, *S. springbokensis*, *S. fasciatus*, *S. fuelleborni*, *S. kitumbeine*, *S. kilimanjaro*, *S. wageri*, *S. bonaespei* and *S. rhodesianus* all varied 31.4-40.5 mm.

Strongylopus springbokensis had the shortest legs, as shown by the average value of 0.7 for HW/FOT, although there was considerable overlap shown by all other species of 0.4-0.6.

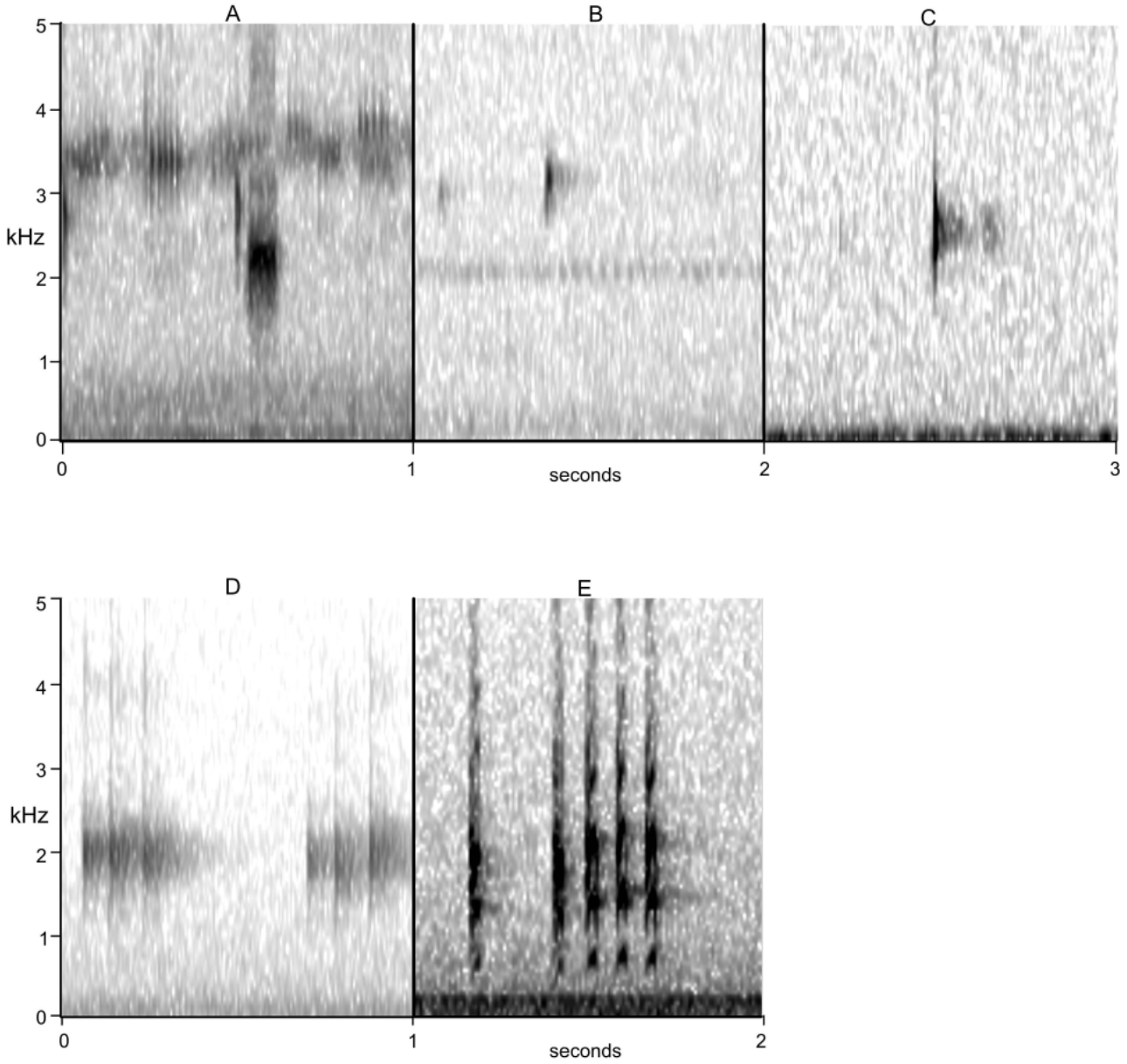


Fig. 7. Representative advertisement calls of the species of *Strongylopus*. A – *S. bonaespei,* B – *S. fasciatus*, C – *S. grayii*, D – *S. merumontanus* (Mt. Kitumbeine), E – *S. wageri*.

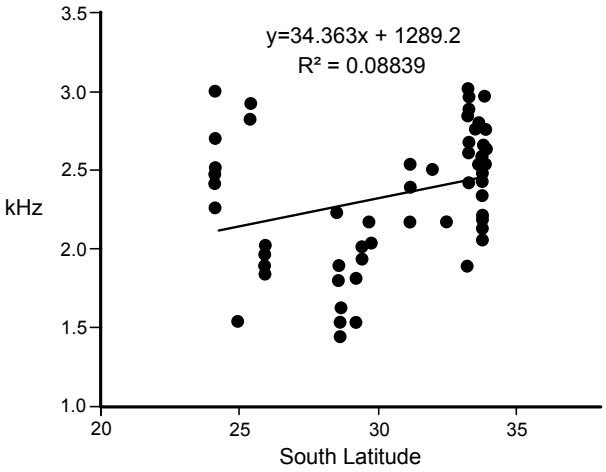


Fig. 8. Dominant frequency of the calls of *S. grayii* vs latitude.

Strongylopus springbokensis also had the shortest hands relative to head width (HW/HND=1.3-2.1) while *S. fasciatus* had the longest hands (HW/HND=0.8-1.2). The other eight species showed intermediate but overlapping values for relative hand length.

Head width as a proportion of body length varied evenly across the species, as shown by the overlap in the proportion SUL/HW (Table 3) for all species.

The largest eyes, as indicated by HW/EW were found in *S. merumontanus* (mean HW/EW=12.0), with the smallest eyes (mean HW/EW=18.3) were found in *S. springbokensis*. However, all species have overlapping ranges in this proportion.

The largest distance between tympanum and eye, as ET/EW, occurred in *S. bonaespei* (mean ET/EW=0.4). All the other species had mean values of 0.3. Body proportions in general were not useful for species identification as there was considerable variation and overlap between all species (Table 3).

The amount of webbing, as indicated by the number of phalanges free of webbing on the fourth (longest) toe, varied from 3-4 within most species, with the most webbing shown by *S. wageri*, where there are 2-3 phalanges free in our sample, often with a margin of webbing to the toe tip. Poynton (1964a) reported 1-3 phalanges free of web in *S. wageri*. Breeding males tend to have more webbing, but this needs to be investigated with a larger sample, or with a long-term field study.

The presence and completeness of dorsal ridges have been used as characters in most species descriptions. Our data revealed that *S. fasciatus* has unbroken vertebral dorsal ridges while *S. grayii*, *S. springbokensis, S. rhodesianus* and *S. bonaespei* have broken ridges, sometimes with a smooth back. *Strongylopus merumontanus, S. fuelleborni, S. kitumbeine* and *S. kilimajaro* have dorsal ridges which are usually continuous, but are often broken or absent. *Strongylopus wageri* has no dorsal ridges.

Colour patterns: Colour patterns have previously been used as diagnostic taxonomic characters by all authors (Figs 9-35). Variable pattern elements in most species include a thin vertebral stripe which may be present or absent, a wide vertebral band, the development of the 'mask' a dark stripe from the nostril through the eye to the arm insertion, a pale triangle on the snout, longitudinal dark stripes running parallel to the dorsal midline, the presence and arrangement of lateral stripes, sometimes broken into rows of spots, the presence and arrangement of dorsal spots or blotches, and the presence of a pale mark running from the anterior corner of the eye to the lower jaw. Overall colour varies from golden brown through dark brown, grey, yellow, orange to red. Stripes and spots are often contrasting in colour. Longitudinal dark stripes along the dorsal midline and laterally are common in *S. fasciatus* and *S. merumontanus*. The variation shown by the different species is detailed below.

Taxonomy

The northern species (*S. merumontanus, S. fuelleborni, S. kitumbeine,* and *S. kilimanjaro*) are similar in genetics, morphology, and advertisement call. They are recognised here as a single species, with *S. merumontanus* being the senior synonym. *Strongylopus springbokensis* is recognised as a junior synonym of *S. grayii*.

***Strongylopus bonaespei* (Dubois, 1981)**

Figs 9-10

Rana (*Strongylopus*) *bonaespei* Dubois, 1981: 929. *Nomen novum* for *Rana fasciata montana* FitzSimons, 1946.

Rana fasciata montana FitzSimons, 1946: 351 (type TMP 20223).

Rana montana. – Greig, Boycott & De Villiers, 1979: 2, figs 7, 10-12 .

Strongylopus montanus. – Channing, 1979: 797, fig. 25.

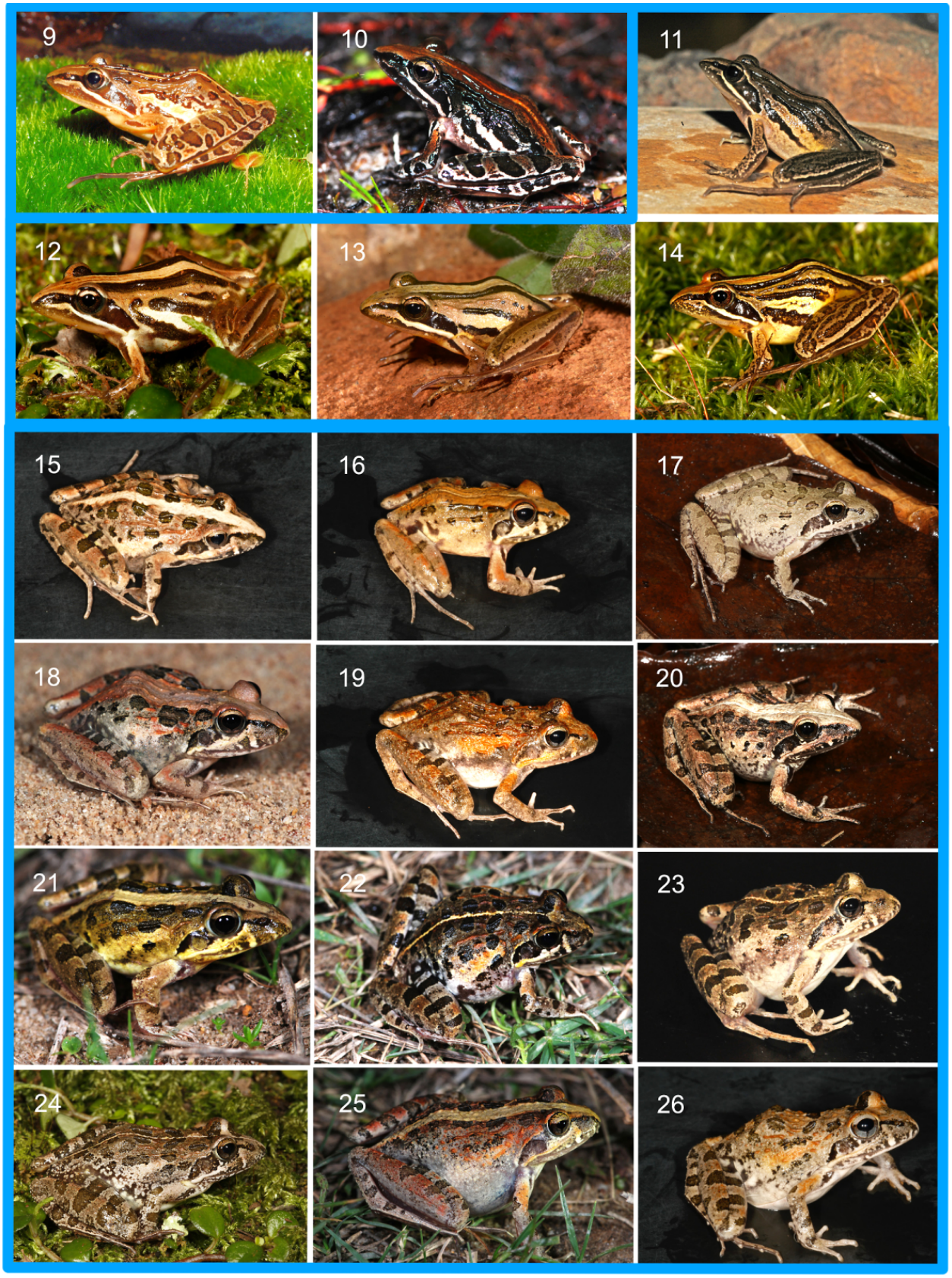
Strongylopus bonaespei. – Channing, 2001: p. 352, fig. 22.6.

Molecular: The mitochondrial 16S rRNA, nuclear *tyr* and *RAG-1* sequences are remarkably uniform across the range of the species, from the Cape Peninsula in the west to Grootvadersbosch in the east (Table 1, Figs 1, 3).

Morphology: Maximum SUL is 35 mm for males and 48 mm for females (Poynton, 1964a). Snout-urostyle length 3.2 times head width (3.0-3.2). Snout length 1.9 times eye width (1.8-2.1). Eye-nostril distance 1.5 times tympanum width (1.3-1.6). Length of foot 0.8 of SUL (0.7-0.8). Head width 0.41 of foot length (0.40-0.44). Four phalanges of fourth toe free of web. The overall colour is golden brown, although very dark morphs are known. Snout mottled or a pale triangle. A thin dark line runs from the snout through the eye, over the tympanum to the arm. The dark pair of dorsal stripes usually has paired breaks (Poynton, 1964a); cross-banding present on the tibia. There are broken rows of dark paravertebral stripes, or silver and dark stripes (Figs 9, 10). A

Table 3. *Strongylopus* body proportions. Mean and range shown. GRA – *Strongylopus grayii*, SPR – *S. springbokensis*, FAS – *S. fasciatus*, FUE – *S. fuelleborni*, KIT – *S. kitumbeine*, KIL – *S. kilimanjaro*, MER – *S. merumontanus*, WAG – *S. wageri*, BON – *S. bonaespei*, RHO – *S. rhodesianus*. See text for morphological abbreviations.

	GRA (n=29)	SPR (n=7)	FAS (n=21)	FUE (n=15)	KIT (n=2)	KIL (n=5)	MER (n=4)	WAG (n=11)	BON (n=4)	RHO (n=4)
SUL	34.2 (20.8-45.5)	38.0 (33.8-43.3)	36.2 (28.3-41.8)	38.2 (27.4-49.3)	31.4 (23.7-39.2	36.2 (24.4-46.5)	25.0 (20.5-35.2)	37.3 (24.6-50.6)	40.5 (31.7-46.5)	36.1 (30.2-42.3)
HW/FOT	0.5 (0.4-0.6)	0.7 (0.7-0.7)	0.4 (0.3-0.4)	0.4 (0.4-0.5)	0.6 (0.5-0.6)	0.4 (0.4-0.4)	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.4 (0.4-0.4)	0.43 (0.41-0.6)
SUL/HW	3.0 (2.6-3.3)	2.7 (2.5-2.8)	3.7 (3.1-4.4)	3.2 (2.7-3.7)	2.4 (2.0-2.7)	3.6 (3.4-4.0)	2.8 (2.6-3.0)	2.9 (2.4-3.5)	3.15 (3.0-3.3)	3.2 (3.0-3.5)
SUL/SL	40.4 (24.9-53.1)	45.6 (40.6-51.8)	42.6 (33.7-49.6)	45.4 (32.6-58.0)	38.4 (30.6-46.2)	42.4 (29.2-54.0)	30.1 (24.7-42.1)	44.6 (29.5-60.1)	48.4 (38.2-55.0)	42.8 (36.0-50.2)
HW/EW	14.8 (9.4-20.2)	18.3 (16.6-21.8)	13.5 (11.2-17.3)	15.7 (10.6-19.3)	17.2 (15.8-18.6)	13.7 (9.6-18.2)	12.0 (10.2-15.8)	17.7 (11.6-24.3)	17.0 (13.3-19.3)	15.4 (13.3-17.9)
ET/EW	0.3 (0.2-0.5)	0.3 (0.2-0.4)	0.34 (0.22-0.5)	0.3 (0.2-0.4)	0.3 (0.3-0.4)	0.3 (0.3-0.4)	0.3 (0.2-0.3)	0.3 (0.2-0.4)	0.4 (0.4-0.5)	0.3 (0.3-0.4)
TYM/ET	2.0 (1.3-2.8)	2.4 (1.8-3.0)	1.8 (1.1-2.8)	1.9 (1.5-2.8)	1.7 (1.3-2.2)	1.6 (1.4-2.0)	1.6 (1.1-2.0)	1.67 (1.07-2.4)	1.2 (1.1-1.2)	1.3 (1.2-1.6)
SUL/TIB	1.7 (1.5-2.2)	1.8 (1.7-1.9)	1.5 (1.4-1.8)	1.5 (1.4-1.8)	1.5 (1.2-1.8)	1.6 (1.5-1.7)	1.5 (1.5-1.6)	1.4 (1.3-1.5)	1.5 (1.5-1.5)	1.5 (1.4-1.6)
TIB/FOT	1.0 (0.8-1.0)	1.0 (1.0-1.1)	0.9 (0.7-1.0)	0.9 (0.7-1.0)	0.9 (0.9-0.9)	0.9 (0.9-1.0)	1.0 (0.9-1.0)	1.0 (1.0-1.0)	0.9 (0.9-0.9)	0.9 (0.9-0.9)
HW/HND	1.3 (1.1-1.6)	1.5 (1.3-2.1)	1.0 (0.8-1.2)	1.1 (0.9-1.3)	1.3 (1.3-1.3)	1.0 (0.9-1.1)	1.1 (1.0-1.2)	1.2 (0.9-1.4)	1.2 (1.1-1.3)	1.1 (1.0-1.1)
SL/EW	1.8 (1.5-2.3)	1.9 (1.7-2.9)	1.9 (1.4-2.3)	1.9 (1.5-2.2)	1.7 (1.6-1.8)	1.7 (1.6-2.1)	1.6 (1.5-1.7)	1.6 (1.4-1.9)	1.9 (1.8-2.1)	1.6 (1.5-1.7)
FOT/SUL	0.6 (0.6-0.7)	0.5 (0.5-0.6)	0.8 (0.7-0.8)	0.7 (0.7-0.8)	0.8 (0.6-1.0)	0.7 (0.7-0.7)	0.7 (0.6-0.8)	0.77 (0.6-0.8)	0.8 (0.8-0.8)	0.7 (0.7-0.8)
EN/TYM	1.2 (0.8-1.8)	1.2 (0.8-1.6)	1.3 (1.0-1.9)	1.2 (1.0-1.4)	1.2 (1.1-1.3)	1.4 (1.3-1.8)	1.6 (1.3-2.0)	1.5 (1.1-1.9)	1.5 (1.3-1.6)	1.5 (1.3-2.0)



Figs 9-26. Variation in colour patterns. (9-10) *S. bonaespei*, 9 – Landdroskop, 10 – Cape Peninsula. (11-14) *S. fasciatus,* 11 – George, 12 – Nquado, 13 – Chimanimani, 14 – Sandile's Rest. (15-26) *S. grayii*, 15 – Stellenbosch, 16 – Stellenbosch, 17 – Baviaanskloof, 18 – Little Karoo, 19 – Stellenbosch, 20 – Baviaanskloof, 21 – Cape Town, 22 – Cape Town, 23 –Hogsback, 24 – Langeni, 25 – Cape Point, 26 – Stellenbosch.

vertebral stripe is usually present. There are no brown stripes from behind eyes to urostyle. The ventral surface is immaculate.

Distribution: The distribution falls within the Cape Fold ecoregion of Abell *et al*. (2008). They are known from sea level to the high plateaux of the mountains, in the south-west, extending north along the Cederberg and east to the Tsitsikama Mountains in South Africa (Fig. 36).

Advertisement call: *Strongylopus bonaespei* produces a brief croaking call and a chuckle call (Fig. 7). The duration of the croaking note is 0.083-0.139 s, with 8 or 9 pulses, at a mean pulse rate of 60.8 s^{-1} (50.3-84.3 s^{-1}, n=13). The mean emphasised frequency is 2115 Hz (1987-2231 Hz; n=14). Some calls have the first harmonic suppressed, with the second visible on the sound spectrogram. One individual emphasised both the fundamental frequency and the first harmonic. The chuckle call consists of an initial two notes, followed by three rapidly repeated notes, within 0.8 s (n=1). The call falls into guild E of Emmrich *et al*. (2020).

Strongylopus fasciatus **(Smith, 1849)**

Figs 11-14

Rana fasciata Smith, 1849: 333, pl. 78, fig. 1; (neotype BMNH 58.11.25.127).
Rana fasciata fasciata. – Barbour & Loveridge, 1928: 197 (by implication).
Strongylopus fasciatus fasciatus. – Van Dijk, 1966: 259.
Rana (*Strongylopus*) *fasciata*. – Dubois, 1981: 250.
Strongylopus fasciatus. – Channing, 2001: 353, fig. 22.7.

Molecular: There are four 16S rRNA haplotypes, of which three overlap geographically, and differ by 1.8% or less. The samples from eastern Zimbabwe are 1.3-2.2% different from the southern population, but are all included in a well-supported clade (Fig. 1). The nuclear *RAG-1* and *tyr* haplotypes show differences of 0-1.1% and show a well-supported clade (Fig. 3).

Morphology: Maximum SUL is 37 mm for males and 50 mm for females (Poynton, 1964a). Snout-urostyle length 3.7 times head width (3.1-4.4). Snout length 1.9 eye width (1.4-2.3). Eye-nostril distance 1.3 times tympanum width (1.0-1.9). Length of foot 0.8 of SUL (0.7-0.8). Head width 0.4 of foot length (0.3-0.4). Fourth toe with 3.5-4 phalanges free of web. Poynton (2013) described this species as possessing characteristic dark-edged skin ridging and stripes. Our samples are overall brown and yellow, with some darker morphs known. There is a pale triangle on the snout. There are two dark continuous paravertebral stripes with a pale vertebral band. A thin dark line runs from the snout through the eye, over the tympanum to the arm. A dark stripe runs from behind the eye to the urostyle, and there are two or three dark lateral stripes. The tibia has mottling or longitudinal stripes. The ventral surface is immaculate.

Distribution: This widespread species is found in six of the ecoregions of Abell *et al.* (2008): Cape Fold, Amatolo-Winterberg Highlands, Southern Temperate Highveld, Zambezian Lowveld, Eastern Zimbabwe Highlands and Zambezian Highveld (Fig. 36). It is known from Zimbabwe, to the South African highveld and east coast.

Advertisement call: *Strongylopus fasciatus* produces a very brief whistle that shows a rise in frequency, or sometimes a high-pitched click (Fig. 7). The mean emphasised frequency is 2854 Hz (1650-3200 Hz, n=361), although a few calls had the fundamental frequency suppressed, with the lowest emphasised frequency being the first harmonic, 5130-6140 Hz (n=30). In three cases, the sound spectrogram showed no harmonics, but only the fundamental frequency. Air temperature does not affect the frequency. The calls fall into guild B of Emmrich *et al*. (2020).

Strongylopus grayii **(Smith, 1849)**

Figs 15-26

Rana grayii Smith, 1849: 335, plate 78, fig. 2 (lectotype BMNH 58.11.25.138).
Strongylopus grayi. – Steindachner, 1867: 21.
Rana grayi var. *dorsalis* Werner, 1910: 297 (type unknown).
Rana grayi grayi. – Hewitt, 1933.
Dicroglossus grayi. – Deckert, 1938: 138.
Strongylopus grayii. – Van Dijk, 1966: 259
Rana grayii grayii. – Passmore & Carruthers, 1979: 138.
Rana (Strongylopus) grayii. – Dubois, 1981: 250.
Strongylopus springbokensis Channing, 1986: 128, fig. 1 (type PEM A963). **New synonym**
Rana (*Strongylopus*) *springbokensis*. – Dubois, 1992: 259.

Molecular: The 16S rRNA haplotype variation shows a maximum difference of 1.7%. These sequences fall into three groups (Fig. 37): a southwestern clade extending from Namaqualand in the northwest, to the southern Cape (blue square symbols); a southern Cape clade (red triangles); and a northern clade (yellow circles) which extends from northwestern South Africa to the south in the Cape Town area. *Strongylopus springbokensis* is embedded within the southwestern clade. These clades overlap geographically; see the discussion below.
The nuclear *RAG-1* and *tyr* sequences are 0-1.0% different. The *tyr* phylogeny places *S. springbokensis* basal but within a well-supported clade of *S. grayii* sequences (Fig. 3).

Morphology: Values are given for *S. grayii* and *S. springbokensis* separately in Table 3. Body proportions are listed here as *S. grayii*/*S. springbokensis*. Maximum SUL is 42/42 mm for males and 64/44 mm for females (Channing & Rödel,

2019). Snout-urostyle length is 3.0/2.7 times head width (2.6-3.3/2.5-2.8). Snout length 1.8/1.9 eye width (1.5-2.3/1.7-2.9). Eye-nostril distance 1.2/1.2 times tympanum width (0.8-1.8/0.8-1.8). Length of foot 0.6/0.5 of SUL (0.6-0.7/0.5-0.6). Head width 0.5/0.7 of foot length (0.4-0.6/0.7-0.7). Fourth toe with four phalanges free of web, infrequently three (Poynton, 1964a). These two species are remarkably similar in body proportions, supporting the placement of *S. springbokensis* as a synonym of *S. grayii*.

Our samples (Figs 15-26) are overall brown and grey with yellow, orange or red tinges. There is sometimes a pale triangle on the snout. A thin pale vertebral stripe or wide vertebral band is common. There are no paravertebral stripes, but irregular dark blotches, usually in pairs. A thin dark line runs from the snout through the eye, over the tympanum to the arm. There is usually a thin pale line from the anterior corner of the eye to the upper jaw. A dark stripe runs from behind the eye, through the tympanum to the arm. The tibia has dark cross-banding. An unusual morph with a red dorsum was found in the Langvlei dunes near Wilderness (Jacobsen, 2013). The ventral surface is immaculate, rarely with grey speckles.

Distribution: This widespread species is found in eight of the ecoregions of Abell *et al.* (2008): Karoo, Cape Fold, Amatolo-Winterberg Highlands, Drakensberg-Maluti Highlands, Southern Temperate Highveld, Western Orange (extended to Naukluft). Karoo and Zambesian Lowveld (Figs 36, 37). It is widespread in South Africa, excluding the arid interior. This species was introduced to St Helena Island around 1883 by Miss Phoebe Mary Moss (Barbour, 1934; Mertens, 1971; Ashmole & Ashmole, 2000; Lever, 2003) and they are still present. There are records from Naukluft in central Namibia from 1979 (Channing, 2020), which might represent an introduction. It has not been recently recorded from Naukluft.

Advertisement call: The call of *S. grayii* consists of a sharp, energetic click (Fig. 7). Sometimes the clicks are run together into a trill. The call of *S. springbokensis* from Springbok in Namaqualand is a trill or single click. The dominant frequency of *S. springbokensis* varies from 975 Hz to 1862 Hz (n males=12), while that of *S. grayii* varies from 1464 Hz to 3030 Hz (n males=61). The overlap in call structure and dominant frequency supports placing *S. springbokensis* as a junior synonym of *S. grayii.*

In summary, the duration of the click varies from 0.011-0.028 s. The mean emphasised frequency visible on the sound spectrogram is 2182 Hz (975-3030 Hz, n calls=560), with high energy visible up to 8 kHz. Air temperature does not affect the emphasised frequency. The calls are often produced in chorus, and frequently as a trill. The trills consist of 2-12 notes, at a pulse rate of 12.5-14.9 s^{-1}. Females produce a twittering call during amplexus. The call is classified into guild A (Emmrich *et al*., 2020).

Strongylopus merumontanus **(Lönnberg, 1907)**

Figs 27-33

Rana merumontana Lönnberg, 1907: 21, fig. 4 (type NRM 1367).

Rana (*Ptychadena*) *merumontana*. – De Witte, 1921: 7.

Rana fasciata merumontana. – Barbour & Loveridge, 1928: 197.

Strongylopus merumontanus. – Channing & Davenport, 2002: 140.

Rana fülleborni Nieden, 1911: 436 (type ZMB 21773). **New synonym**

Rana (*Ptychadena*) *fuelleborni*. – De Witte, 1921: 7.

Rana fasciata fülleborni. – Loveridge, 1953: 373. – Poynton, 1964a: 203.

Strongylopus fuelleborni. – Channing, 2001: 354, fig. 22.8.

Strongylopus kitumbeine Channing & Davenport, 2002: 137, figs 2-5 (type CAS 225064). **New synonym**

Strongylopus kilimanjaro Clarke & Poynton, 2005: 54, figs 1-2 (type BMNH 1936.2.2.2). **New synonym**

Molecular: The 16S rRNA sequences form a well-supported clade, with recognisable sub-structure with differences varying from 0-2.1%. Isolated populations on the highlands of Mt. Mulanje, Mt. Kilimanjaro, the eastern highlands of Zimbabwe, Mt. Kilimanjaro, Mt. Namuli and Mt. Mabu in Mozambique, and Mt. Kitumbeine (Fig. 4) each show slight differences, with the greatest *p*-distances between Mt. Mulanje in Malawi and Mts Namuli and Mabu in Mozambique and the Udzungwa Mountains in Tanzania (Table 4).

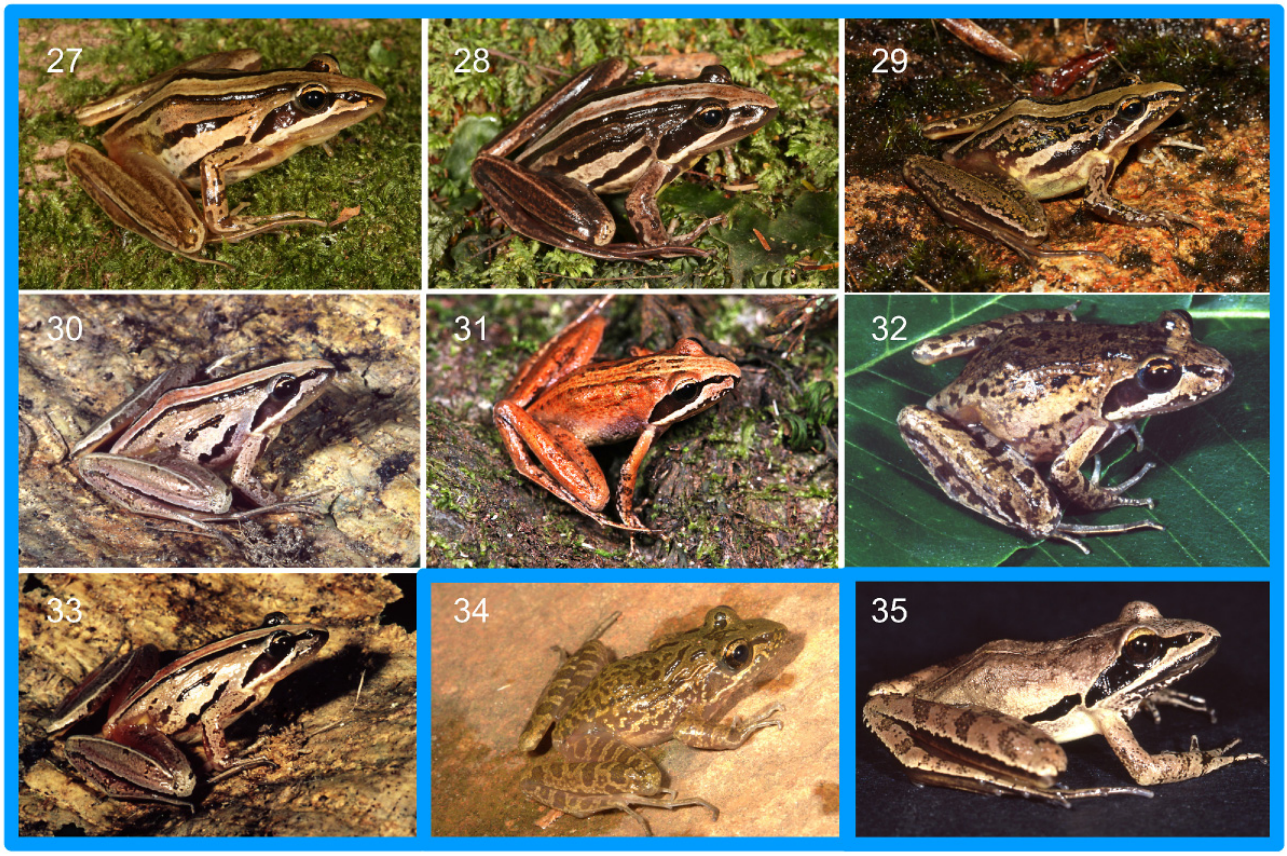
The nuclear *RAG-1* and *tyr* sequences differ by 0-1.4%.

Morphology: The body proportions of *S. merumontanus*, *S. fuelleborni*, *S. kitumbeine* and *S. kilimanjaro* are listed separately in Table 3. The combined values are presented as *S. merumontanus* here. Maximum SUL is 40 mm for males and 53 mm for females (Channing & Rödel, 2019). Snout-urostyle length 3.2 times head width (2.0-4.0). Snout length 1.8 eye width (1.5-2.2). Eye-nostril distance 1.3 times tympanum width (1.0-2.0). Length of foot 0.7 of SUL (0.6-1.0). Head width 0.4 of foot length (0.4-0.6). Fourth toe with four phalanges free of web, infrequently slightly less. Our samples (Figs 27-33) are overall brown to reddish orange. There is sometimes a pale triangle on the snout. A thin pale vertebral stripe or wide vertebral band is common. There is a pair of paravertebral stripes in many populations. A thin dark line runs from the snout through the eye, over the tympanum to the arm. The tibia has cross-banding or a thin longitudinal line. Material from the northern volcanic mountains lacks the ridged paravertebral stripes of the southern *S. fasciatus* (Poynton, 2013). The ventral surface is immaculate or with grey speckles.

Distribution: This widespread species is found in six of the ecoregions of Abell *et al*. (2008): Eastern Zimbabwe Highlands, Mulanje, Coastal East Africa, Lake Malawi, Lake Rukwa, Pangani. It is known from the eastern

Table 4. Uncorrected 16S rRNA *p* distances between populations included in *Strongylopus merumontanus*. Species codes: MER – *S. merumontanus*, FUE – *S. fuelleborni*, KIT – *S. kitumbeine*, KIL – *S. kilimanjaro*.

	MER Mt Meru	FUE Mt. Mulanje	FUE Mts. Namuli & Mabu	FUE Nyanga	FUE Mbizi Hill	FUE Livingstone Mts.	FUE Udzungwa Mts.	KIT Mt Kitumbeine	KIL Mt Kilimanjaro
S. merumontanus	-								
S. fuelleborni Mt. Mulanje	1.5-1.7	0.2							
S. fuelleborni Mts. Namuli & Mabu	1.7-1.9	1.7-2.1	0-0.2						
S. fuelleborni Nyanga	0.8	1.9-2.1	1.3-1.5	0					
S. fuelleborni Mbizi Hill	0	1.5-1.7	1.7-1.9	0.8	-				
S. fuelleborni Livingstone Mts.	0.4	1.5-1.7	1.3-1.5	0.4	0.4	-			
S. fuelleborni Udzungwa Mts.	0.6	1.7-1.9	1.5	0.6	0.6	0.2	-		
S. kitumbeine	0.4	1.5-1.7	1.7-1.9	0.8	0.4	0.4	0.6	0	
S. kilimanjaro	0.4-0.6	1.5-1.9	1.5-1.9	0.8-1.1	0.4-0.6	0.4-0.6	0.6-0.8	0.4-0.6	0-0.2



Figs 27-35. Variation in colour patterns. (27-33) *S. merumontanus*, 27 – *S. fuelleborni* Mt Namuli, 28 – *S. fuelleborni* Mt Mabu, 29 – *S. fuelleborni* Mt Mulanje, 30 – *S. fuelleborni* Kitetele, 31 – *S. kilimanjaro* Mt Kilimanjaro, 32 – *S. kitumbeine* Mt Kitumbeine, 33 – *S. fuelleborni* Mbeya. (34) *S. rhodesianus* – Chimanimani. (35) *S. wageri* – Sani Pass.

highlands of Zimbabwe, the isolated highlands and old volcanic mountains of Malawi, northern Mozambique inselbergs, southern Tanzania, and the Eastern Arc Mountains. It has not been found in the drier lowlands between the Eastern Arc highlands (Fig. 36).

Advertisement call: The advertisement call of *S. fuelleborni* consists of a brief whistle or series of whistles combined into a trill. Each note is frequency modulated, with a rise in pitch, and may contain 1-10 pulses. The dominant frequency varies from 2061 Hz to 3145 Hz (n males=31). The advertisement call of *S. kilimanjaro* consists of a brief whistle or series of whistles combined into a trill. Each note is frequency modulated, with a rise in pitch. The dominant frequency varies from 2667 Hz to 2940 Hz (n males=6). The advertisement call of *S. kitumbeine* consists of a trill with two to eight notes. The dominant frequency varies from 2135 Hz to 2325 Hz (n males=15). The only recorded call of *S. merumontanus* consists of a brief whistle with a dominant frequency of 2663 Hz. The advertisement calls of these northern species overlap in structure and dominant frequency, supporting placing them into a single species.

In summary, the call of *S. merumontanus* consists of a brief whistle or pulsed note, that may be repeated as a trill (Fig. 7). The mean emphasised frequency is 2493 Hz (2061-3145 Hz, n calls=298). The trill consists of two to eight notes at a mean pulse rate of 9.4 s^{-1} (7-11.6 s^{-1}). Whistles are frequency modulated, rising in frequency. Both trills and whistles are heard in the same chorus. The function of the different calls is not known. These calls fall into guilds B and F of Emmrich *et al*. (2020).

***Strongylopus rhodesianus* (Hewitt, 1933)**

Fig. 34

Rana grayi rhodesiana Hewitt, 1933: 12, plate 1 (Type not traced).

Strongylopus grayii rhodesianus. – Van Dijk, 1966: 259.

Strongylopus rhodesianus. – Channing, 2001: 359, fig. 23.3.

Molecular: Only a single specimen was available for sequencing. The two *RAG-1* haplotypes were 0.2% different.

Morphology: Maximum length 40 mm for males and 50 mm for females (Channing & Rödel, 2019). Snout-urostyle length 3.2 times head width (3.0-3.5). Snout length 1.6 eye width (1.5-1.7). Eye-nostril distance 1.5

times tympanum width (1.3-2.0). Length of foot 0.7 of SUL (0.7-0.8). Head width 0.4 of foot length (0.4-0.6). Fourth toe with 3.7-4 phalanges free of web. Our samples (Fig. 34) are overall brown and grey. There is usually a pale triangle on the snout, often continuous with a broad vertebral band. A thin vertebral stripe is absent. The back is usually plain, or with darker irregular blotches. There is a thin pale line from the anterior corner of the eye to the upper jaw. A thin dark line runs from the snout through the eye, over the tympanum to the arm. The tibia has dark cross-bands. The ventral surface is immaculate or with grey speckles.

Distribution: This species is only known from a single ecoregion of Abell *et al*. (2008), the Eastern Zimbabwe Highlands (Fig. 38).

Advertisement call: In light of the presence of three species in the area (*S. merumontanus, S. fasciatus* and *S. rhodesianus*) and the similarity in the calls, there is no confirmed recording of a call where the male has been positively identified by sequencing.

***Strongylopus wageri* (Wager, 1961)**
Fig. 35

Rana wageri Wager, 1961: 151, figs 151, 154 (type NMP 1145).
Strongylopus wageri. – Van Dijk, 1966: 259.
Rana (*Strongylopus*) *wageri*. – Dubois, 1981: 233.

Molecular: Three 16S rRNA sequences were available from the Sani Pass area, which were identical. The nuclear *RAG-1* phased sequences varied from 0.7-1.5%. Only a single *tyr* sequence was available.

Morphology: Maximum SUL is 42 mm for males and 48 mm for females (Channing & Rödel, 2019). Snout-urostyle length 2.9 times head width (2.4-3.5). Snout length 1.6 eye width (1.4-1.9). Eye-nostril distance 1.5 times tympanum width (1.1-1.9). Length of foot 0.7 of SUL (0.6-0.8). Head width 0.5 of foot length (0.4-0.6). Fourth toe with one to three phalanges free of web. There are no dorsal skin ridges. Our samples (Fig. 35) are overall brown. There is a pale triangle or spotting on the snout. A thin pale vertebral stripe is often present. There are irregular dark blotches or fine spots on a uniform dorsal background. A thin dark line runs from the snout through the eye, over the tympanum to the arm. A dark lateral line runs from the arm to the leg. The tibia has dark cross-banding. The ventral surface is immaculate.

Distribution: This species is found in two of the ecoregions of Abell *et al*. (2008): Drakensberg-Maluti Highlands and Southern Temperate Highveld (Fig. 38). It is known from forested areas and mountain slopes.

Advertisement call: *Strongylopus wageri* produces a series of notes that can best be described as a cackle, as the notes are irregularly spaced (Fig. 7). The individual calls (n calls=36) may sound like a cough in some cases. The note rate for cackles varies from 3.6-13.6 s^{-1}, while the coughs show individual pulses with pulse rates of 36.7-129.0 s^{-1}. The mean emphasised frequency is 1319 Hz (682-1563 Hz). The calls fall into guild E of Emmrich *et al*. (2020).

DISCUSSION

Genetic variation: The 16S gene is widely used as a yardstick when discussing species boundaries with 3% difference a general indication of potential species difference (Vences *et al*., 2005; Vietes *et al*., 2009). In the Pyxicephalidae, the 16S uncorrected p-distances between species within a genus varied as follows: *Amietia* 1.3-10.0% (Channing *et al*., 2016), *Anhydrophryne* 6.3-7.6% (Dawood & Stam, 2016), *Arthroleptella* 1.1-6.6% (Turner & Channing, 2008), *Cacosternum* 1.1-7.3% (Channing *et al.,* 2013), *Nothophryne* 3.0-6.5% (Bittencourt-Silva *et al*., 2016), and *Tomopterna* 1.0-9.2% (Wilson & Channing, 2019). The distances found between species of *Strongylopus* (2.8-7.4%) found in our study are thus in line with the other pyxicephalid genera.

Substructure in *Strongylopus grayii*: Tolley *et al*. (2010) suggests that gene flow may be restricted between the northern (summer breeding) and southern (winter breeding) populations of *S. grayii*. They recognise a southern clade, which was divided into Southwest (Group 1) and South (Group 2), and a northern clade divided into East (Group 3), Drakensberg (Group 4) and North (Group 5). They also had two samples from the far north, which were not classified. Our study recognised three clades, equivalent to groups 1 and 2 and the northern clade (groups 3+4+5) of Tolley *et al*. (2010) and supports the recognition of a southern and northern clade, with their far northern samples included in the northern clade. However, our study included more samples than were available to Tolley *et al*. (2010), and shows geographical overlap between the northern and southern clades (Fig. 37), with the 'northern clade' extending to Cape Town at the southern tip of Africa, and a greater *p*-distance between individuals of up to 1.7%.

The northern and southern populations of *S. grayii* showed a mean 0.6% difference between populations in 16S sequences (Tolley *et al*., 2010). Tolley *et al.* (2010) found this difference to be "comparable to the level of divergence found between other amphibian species (Turner *et al*., 2004; Vences *et al*., 2005; Measey *et al*., 2007; Channing & Schmitz 2008; Turner & Channing, 2008; Blackburn, 2009)." However, this is a misreading of the literature. Turner *et al*. (2004) found a mean difference between combined 12S and 16S sequences between *Arthroleptella* species of 3.9%; Vences *et al*. (2005) found a mean difference between sibling species of mantellid frogs of about 7% for 16S; Measey *et al*.

(2007) reported a 16S difference between two species of *Ptychadena* of 5.2-5.6%; Channing & Schmitz (2008) found *Cacosternum kinangopensis* to be 4% different for 16S from *C. plimptoni*; Turner & Channing (2008) found 16S differences between species of *Arthroleptella* of 1.7-6.6%; and Blackburn (2009) reported a mean divergence between *Arthroleptis* species of 12.8%, and between *Cardioglossa* species of 8.8%, using the 12S to 16S fragment. The 0.6% difference in 16S sequences found between some northern and southern populations of *Strongylopus* (Tolley *et al*., 2010) or the 1.7% difference in our larger sample is the kind of variation that is expected in a widespread species and reflects intraspecific variation.

Substructure in *Strongylopus merumontanus*: Many of the populations are found on isolated highlands, for example the extinct volcanoes Mt. Kitumbeine

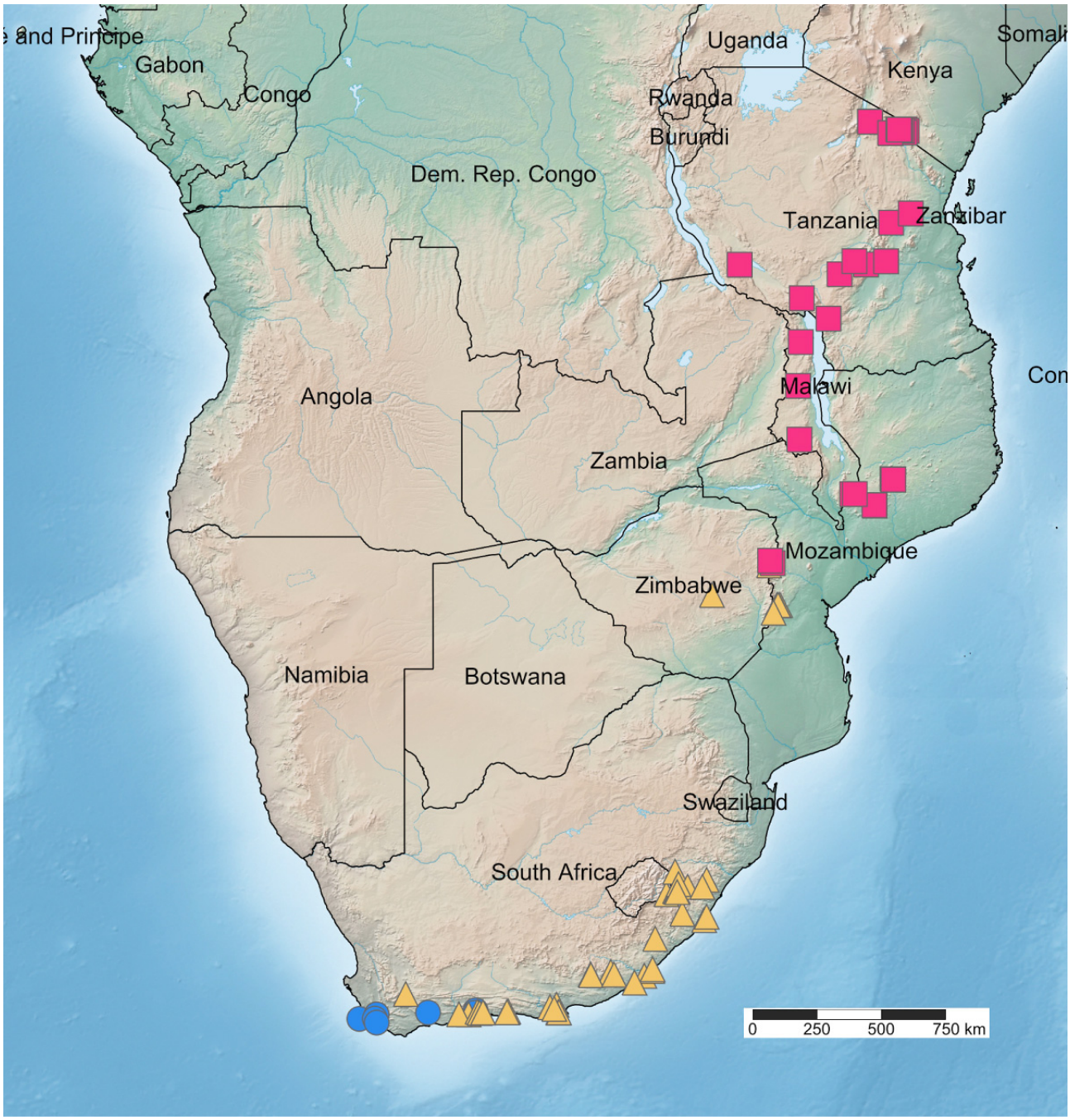


Fig. 36. Distribution of samples. Specimens examined for morphology, advertisement calls, and/or molecular sequences. *Strongylopus merumontanus* – red, *S. fasciatus* – orange, *S. bonaespei* – blue.

(*S. kitumbeine*) Mt. Kilimanjaro (*S. kilimanjaro*) and Mt. Meru (*S. merumontanus*), while *S. fuelleborni* is widely distributed in northeastern Zambia, Malawi and Tanzania (Fig. 4). Although these four populations have previously been recognised as different species, this study has demonstrated a similarity in genetics, advertisement call and morphology. The 16S rRNA uncorrected *p* distances between populations are not large enough to maintain specific status for the northern volcanic species. *Strongylopus merumontanus* has uncorrected *p* values of 0-1.9% compared to all other species in this group. *Strongylopus fuelleborni* from Mt. Mulanje has uncorrected *p* values of 1.5-1.9% between all other populations (Table 4), while the other populations of *S. fuelleborni* have uncorrected *p* values of 0.2-1.9%. The nuclear *tyr* sequences between the populations vary 0-1.0%.

The greater uncorrected 16SrRNA *p* distance of the population of *S. fuelleborni* on Mt. Mulanje compared to other populations may be related to the age of the inselberg. While the volcanoes of northern Tanzania are less than 2 million years old, Mt. Mulanje was formed by erosion of softer soil around the granites of the inselberg that were formed 140 Mya. The long period of isolation is reflected in the presence of at least 69 endemic plant species on Mt. Mulanje (Strugnell, 2002).

New relationships: The relationship between *Strongylopus fasciatus* and *S. merumontanus* has been documented since Barbour & Loveridge (1928) recognised *Rana fasciata merumontana.* Our molecular phylogeny confirmed the relationship, placing these taxa as sister species. *Strongylopus bonaespei* was also described as a subspecies of *Rana fasciata* by FitzSimons (1946), but the molecular phylogeny places *S. bonaespei* basal to all the other species. *Strongylopus rhodesianus* was initially believed to be related to *S. grayii* when it was described by Hewitt (1933) as *Rana grayi rhodesiana.* The molecular phylogeny, however, places *S. rhodesianus* and *S. grayii* in different clades.

Origin of the species: Poynton & Broadley (1985) suggested that the genus shows a progression from primitive taxa in the south, with more derived taxa in the north. Poynton later doubted that there was support for this suggestion, stating that the genus does not easily fit the commonly held pattern of radiation from the south (Poynton, 2013). The southern African rainfall gradient from >1000 mm in the east to <20 mm in the west is reflected in the presence of *Strongylopus* only in the east of the continent, with the exception of a possible introduction of *S. grayii* in central Namibia (Channing, 2020).

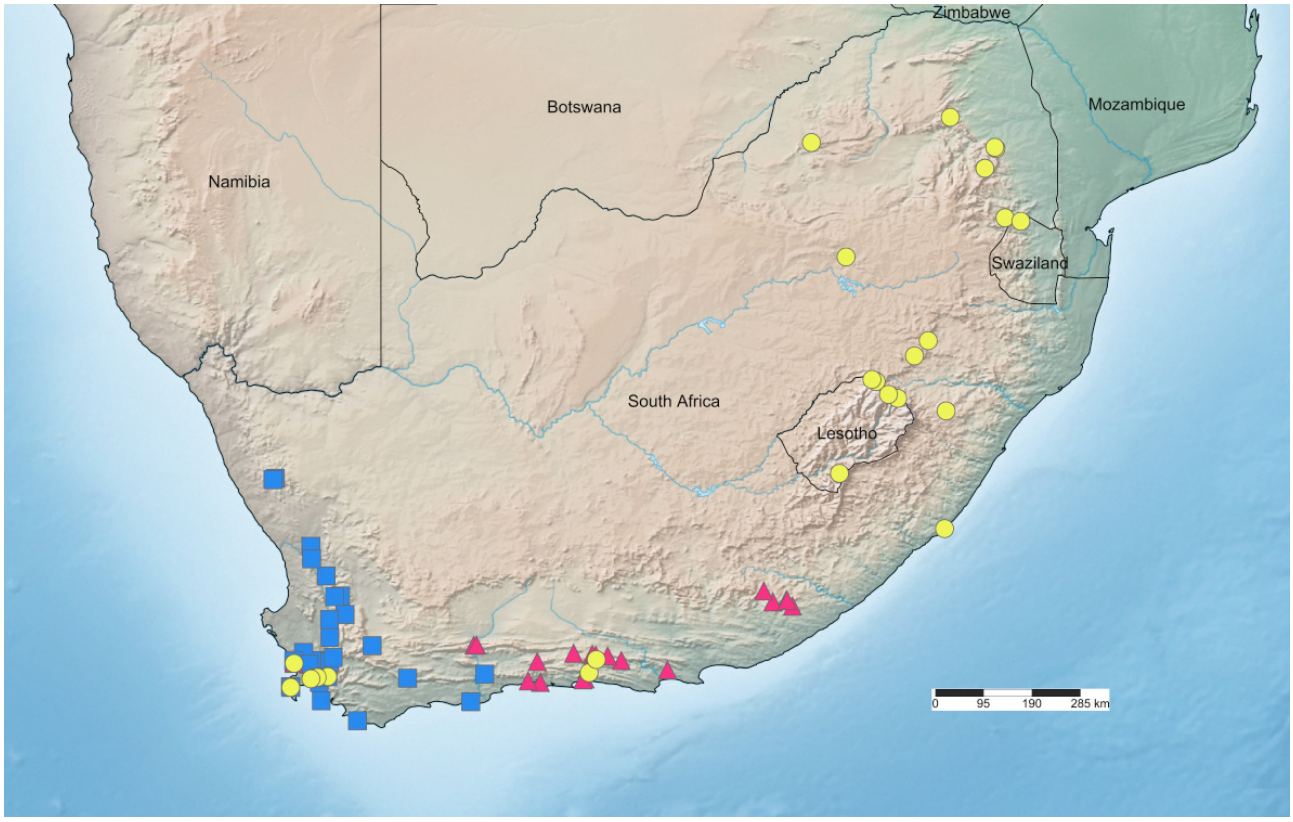


Fig. 37. Distribution of the clades within *S. grayii*. Blue symbols – southwest, red symbols – southern, yellow symbols –northern.

We estimated the split of the genus *Strongylopus* from *Amietia* to have happened 53 Mya (95% HPD 26.8-83.4 Mya), during the middle Eocene. At this time all major rifting, igneous intrusions and mountain building had ceased (McCarthy & Rubidge, 2005), when the vegetation in Africa was mostly woodland in areas with intermediate rainfall. The Orange River was formed by the Kalahari River that captured the Karoo River. In the late Eocene the climate began to cool, due to the opening of the Drake Passage, which permitted the circulation of cold oceanic water as the Antarctic ice cap reached a maximum 25-35 Mya (McCarthy & Rubidge, 2005). The cold Benguela current developed at this time (Feakins & de Menocal, 2010), leading to increased southern African aridity. *Strongylopus* is represented mostly by highland species, with the exception of the widespread *S. grayii,* which occurs in a range of habitats from coastal lowlands to the southern African inland plateau. This transition from the Eocene-Oligocene ice-free greenhouse world, to an icehouse climate with the first major glaciation of Antarctica occurring around 34 Mya, lasting only 0.5 Ma (Hutchinson *et al*., 2021).

The earliest split separated the ancestor of the southern species from the ancestral widespread species, which may have been limited to the north. The ancestor of *S. bonaespei* + *S. grayii* + *S. wageri* split from the ancestor of *S. merumontanus* + *S. fasciatus* + *S. rhodesianus* at 27 Mya (95% HPD 15.6-41.3 Mya), during the mid-Oligocene. At around 30 Mya grasslands developed in drier areas, possibly isolating the northern and southern clades, although frog fossils are not sufficient or varied enough to understand what effect, if any, the conditions leading to this climate change had on semi-terrestrial amphibians (McCarthy & Rubidge, 2005).

The early Miocene from about 20 Mya was characterised by warmer global climates (Jacobs, 2004), with grasslands expanding, along with deserts and tundra. During the early Miocene the southwest coast and the Cape Peninsula was an open riparian forest and swamp vegetation until the middle Miocene (Sciscio *et al*., 2016). Uplift of the eastern side of southern Africa started around 20 Mya (McCarthy & Rubidge, 2005). Rain forest trees showed a similar split around 17 Mya (95% HPD 10.5-23.4 Mya) (Couvreur *et al*., 2008). The beginning of the Miocene saw the evolution of mammals into niches left vacant by the Eocene extinction event (McCarthy & Rubidge, 2005). This period was also the start of the final evolution events leading to the six species recognised today.

The central high plateau was formed by uplifting around 20 Mya (Partridge, 1997). Rifting started in east Africa during the Miocene. The uplift of the eastern side of southern Africa reduced rainfall to the interior, leading to a rain shadow to the west of the escarpment (McCarthy & Rubidge, 2005), and a rain shadow in East Africa. An arid corridor started to develop between Namibia and northeast Africa (Bobe, 2006). The reduced rainfall led to the expansion of grasslands, which allowed *S. grayii* to occupy seasonal wetlands. These experienced a stronger rainfall over a shorter period (McCarthy & Rubidge, 2005).

The last two splitting events occurred close together. *Strongylopus bonaespei* split from *S. grayii* + *S. wageri* around 22 Mya, followed by the split between *S. grayii* and *S. wageri* around 19 Mya that may have been related to the expansion of grasslands and reduced rainfall that isolated the two species in highland forest, leading to allopatric speciation. The southwest coast and the Cape Peninsula were covered in riparian forest from the early to middle Miocene (Sciscio *et al.,* 2016). The present *S. bonaespei* distribution largely coincides with the reconstructed Miocene distribution of tropical/sub-tropical forest patches (Roberts *et al.,* 2013).

After 16 Mya there was a climax of warmth (Flower & Kennet, 1994), the mid Miocene Climatic Optimum (Feakins & de Menocal, 2010), followed by the Benguela current becoming intensified (Flower & Kennett, 1994), with the further expansion of grasslands, associated with the growth of the Antarctic ice cap and rapid cooling (McCarthy & Rubidge, 2005). This may have isolated the northern *S. merumontanus* from the southern *S. fasciatus*. *Strongylopus rhodesianus* split from the ancestor of *S. fasciatus* + *S. merumontanus* around 16 Mya. The populations presently on the recent volcanic mountains of northern Tanzania are presumably successful due to orographic rainfall (Poynton, 2013), surrounded by more arid lowlands.

There was a mid-Miocene climatic optimum around 15 Mya. Subsequent climate changes include a major cooling event at 14.1-14.8 Mya, leading to the increase of the East Antarctic ice sheet, with increased seasonality, and a mixture of environments (Hill, 1987). Forests were still common in East Africa at 12 Mya (Bobe, 2006). Mid-Miocene warming was followed by cooling, which led to the retreat of tropical ecosystems and increased seasonality. The split between *Strongylopus fasciatus* and *S. merumontanus* occurred around 11 Mya, with *S. fasciatus* distributed in the grasslands south of the moist mountains where *S. merumontanus* was found. They presently overlap on the Eastern Highlands of Zimbabwe, along with *S. rhodesianus*. This may be the result of recent northward expansion of *S. fasciatus* aided by a stable climate (Cohen *et al*., 2007).

Intense volcanic activity started north of Lake Tanganyika at 10 Mya, which continued until 4 Mya. In East Africa an increase in grassland around 5.4-8.4 Mya was implied by a study of Annonaceae rain forest trees (Couvreur *et al.,* 2008; Senut *et al*., 2009). Drier conditions were indicated by the first appearance of C4 grasses in East Africa around 10 Mya (Uno *et al*., 2006). *Strongylopus bonaespei* and *S. wageri* occur in forest (or where forest was recently present), and their ancestor may have been restricted to moist high elevations. An arid corridor running from Namibia to northeast Africa continued to develop during the Miocene, separating the Central

African forest block from the coastal and montane forests of East Africa (Bobe, 2006).

Between 7-8 Mya the Arctic ice cap expanded, shifting climatic belts to the south (Senut *et al*., 2009). The Sahara became arid although parts of southern Africa remained relatively tropical. In southern Africa a strong seasonal west coast rainfall regime developed (Hoffmann *et al*., 2015). The C4 grass expansion that occurred around 10 Mya in East Africa only occurred much later in southern Africa (Hopley *et al.,* 2019). This is shown by the presence of predominantly C3 vegetation in the Hoogland cave in South Africa, 5.3-7.3 Mya (Hopley *et al*., 2019). During the late Miocene there was increased aridity around 6 Mya (Bobe, 2006), with expansion of grasslands, and evidence of local varied habitats.

The southern African coast was uplifted about 600 m during the last 5 Ma (Partridge, 1997), resulting in the development of the Great Escarpment. East Africa 3-5 Mya was warmer and wetter (Danley *et al*., 2012), while in southern Africa the cold water Benguela upwelling was initiated 3-5 Mya, resulting in aridification of the adjacent west coast (Hoffmann *et al*., 2015).

A shift from woodland to grassland occurred just before 4 Mya (de Menocal & Bloemendal, 1995). The populations of *S. fasciatus* have subsequently been able to expand to the south and north, overlapping the northern *S. merumontanus* on the Eastern Highlands of Zimbabwe, with 3-5 Mya characterised by warmer and wetter conditions (Danley *et al*., 2012). In the northern parts of its range, *S. merumontanus* is restricted to moist highlands. Many of these highlands, such as Mt. Kitumbeine, Mt. Meru and Mt. Kilimanjaro, are relatively recent in origin, having formed in the last two million years (Nonnotte *et al*., 2008).

The warmest phase of the Pliocene occurred between 3-4 Mya, with sea levels about 30 m higher than today,

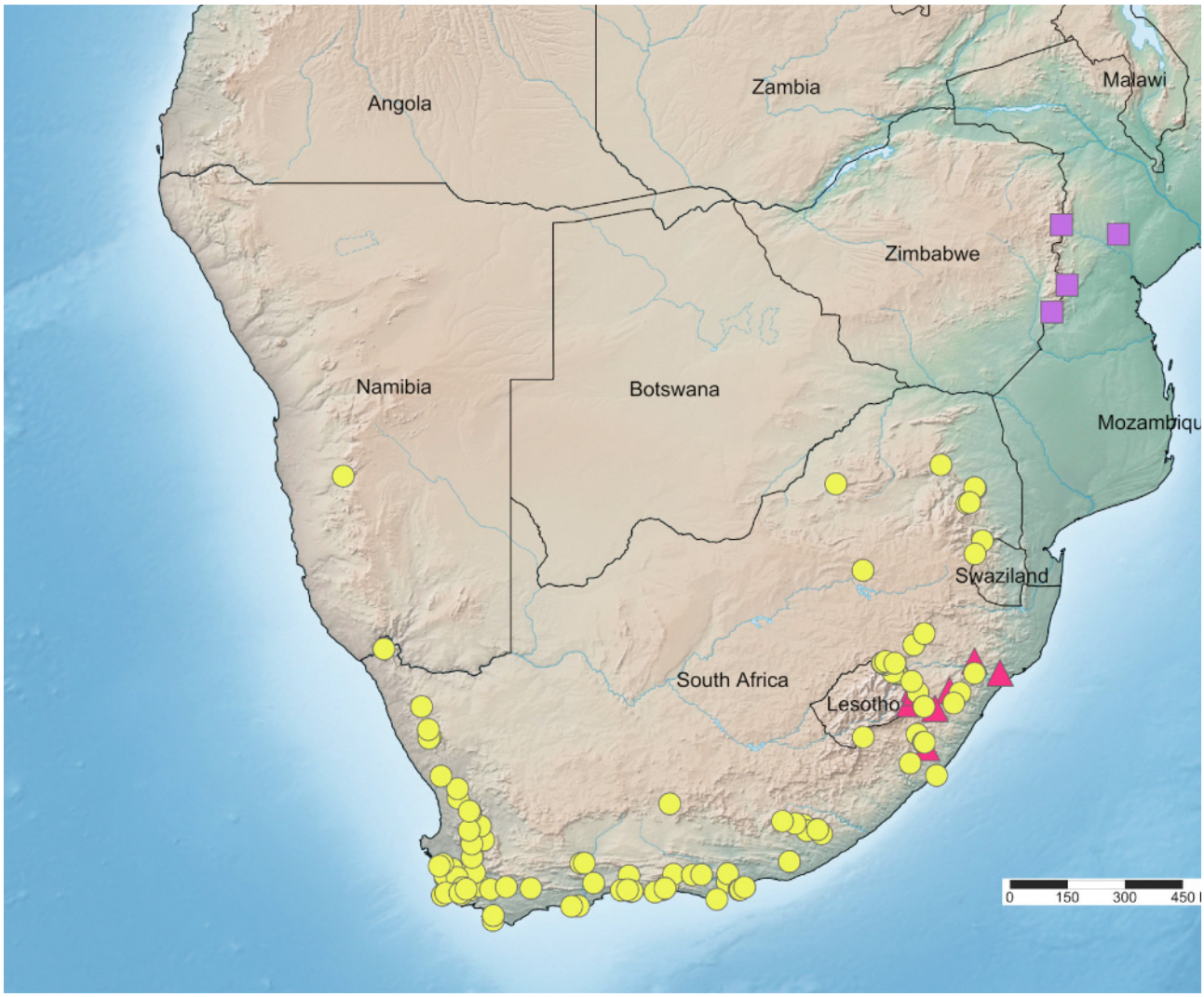


Fig. 38. Distribution of samples. Specimens examined for morphology, advertisement calls, and/or molecular sequences. *Strongylopus grayii* – yellow, *S. wageri* – red, *S. rhodesianus* – purple.

and with high latitudes experiencing mild climates (Partridge, 1997). A series of ice ages followed, resulting in desertification starting in East Africa (Senut *et al*., 2009). The present-day arid conditions along the west coast of southern Africa started around 2.8 Mya.

East Africa and the Lake Malawi basin experienced prolonged drought 135-127 ka when the surrounding watershed was a semidesert, followed by the expansion of grasslands between 120-75 ka around Lake Malawi (Cohen *et al*., 2007). These conditions would have continued the isolation of populations of *S. merumontanus* on the moist highlands of Mt. Mulanje and surrounding granite inselbergs, the Eastern Arc Mountains and the volcanic highlands of Mt. Kilimanjaro and Mt. Kitumbeine. In the last 70 ka conditions have become more humid and stable (Cohen *et al*., 2007).

Southern Africa has been called a 'cradle of faunal diversity' (Tolley *et al*., 2008). *Strongylopus* evolution took place during the Miocene, which is similar to the ages of splits found in *Bradypodion* chameleons (Tolley *et al*., 2008), *Psammobates tentorius* tortoises (Zhao *et al*., 2020), killifish in the genera *Nothobranchius* (van der Merwe *et al*., 2021) and *Poropanchax* (Bragança & Costa, 2019), lizards in the genus *Agama* (Matthee & Flemming, 2002), *Platypleura* cicadas (Price *et al*., 2007) and sengis in the genus *Elephantulus* (Smit *et al*., 2007).

Distribution: The distribution of our molecular, morphological and advertisement call samples is shown in Figs 36, 38. The presumed species ranges can be found based on museum records in Poynton (1964a, 2013), Poynton & Broadley (1985), Channing (1986), Channing & Davenport (2002), and Clarke & Poynton (2005). The overall distribution of the genus closely follows the ecosystem regions of Abell *et al*. (2008), which are based on freshwater fish ranges (Table 5). For example, *Strongylopus* species occupy many of the habitats where killifish (*Nothobrachius*) occur, such as headwater floodplains, and marshes, seepage areas and slow-flowing seasonal stream channels (van der Merwe *et al*., 2021). These ecoregions reflect drainage basins and may prove to be useful for examining distributions of amphibians. The present *S. bonaespei* distribution largely coincides with the reconstructed Miocene distribution of tropical/sub-tropical forest patches (Roberts *et al*., 2013).

A broad division of Africa into biogeographical regions based on plants, birds, amphibians, reptiles and mammals (Linder *et al*., 2012) did not recognise the highlands occupied by *Strongylopus*. Poynton (2013) pointed out that the one degree scale of the Linder *et al*. (2012) study was too coarse to separate the Afrotemperate region of the high mountains of Tanzania where *Strongylopus* occurs.

The distribution of genetically similar populations of *S. merumontanus* across the presently isolated mountain blocks of East Africa, including the West and East Usambaras, the Nguru and Nguu mountains, the Ulugurus, the Rubeho, Malundwe and Udzungwas is similar to populations of reed frogs *Hyperolius* that also occur on moist montane blocks of the Eastern Arc. Lawson (2010) showed that lineages within *Hyperolius puncticulatus*, the *H. spinigularis* complex, and *H. mitchelli* had separate histories with splits dating from 2.1-15.2 Mya, even though they showed similar distributions.

Field identification of *S. fasciatus* and *S. merumontanus* is not easy, and older museum records may be confused. Overlap in body proportions and colour patterns between species indicates that morphology alone is

Table 5. The distribution of *Strongylopus* species in the ecoregions of Abell *et al*. (2008).

Code	Ecoregion	Species present
559	Lake Malawi	*S. merumontanus* (as *S. fuelleborni*)
560	Zambezian Highveld	*S. fasciatus*
561	Lower Zambezi	*S. merumontanus* (as *S. fuelleborni*)
562	Mulanje	*S. merumontanus* (as *S. fuelleborni*)
563	Eastern Zimbabwe Highlands	*S. fasciatus, S. merumontanus* (as *S. fuelleborni*)*, S. rhodesianus*
564	Coastal East Africa	*S. merumontanus* (as *S. fuelleborni*)
565	Lake Rukwa	*S. merumontanus* (as *S. fuelleborni*)
568	Pangani	*S. merumontanus* (as *S. merumontanus, S. kitumbeine,* and *S. kilimanjaro*)
572	Western Orange	*S. grayii*
573	Karoo	*S. grayii* (including *S. springbokensis*)
574	Drakensberg-Maloti Highlands	*S. grayii, S. wageri*
575	Southern Temperate Highveld	*S. fasciatus, S. grayii, S. wageri*
576	Zambezian Lowveld	*S. fasciatus, S. grayii*
577	Amatolo-Winterberg Highlands	*S. fasciatus, S. grayii*
578	Cape Fold	*S. bonaespei, S. fasciatus, S. grayii*

not a useful means of identification. The discovery of *S. merumontanus* on the Eastern Highlands of Zimbabwe close to *S. fasciatus* suggests that more detailed genetic studies are required where these two species overlap. As little as a single base difference separates some *tyr* haplotypes of *S. fasciatus* and *S. merumontanus*, suggesting relatively recent gene flow between these species. Further sampling may discover nuclear haplotypes shared between these two species.

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REFERENCES

Abell R. Thieme M.L., Revenga C., Bryer M., Kottelat M., Bogutskaya N., Coad B., Mandrak N., Balderas S.C., Bussing W., Stiassny M.L.J., Skelton P., Allen G.R., Unmack P., Naseka A., Ng R., Sindorf N., Robertson J., Armijo E., Higgins J.V., Heibel T.J., Wikramanayake E., Olson D., López H.L. Reis R.E., Lundberg J.G., Pérez M.H.S., Petry P. 2008. Freshwater ecoregions of the world: A new map of biogeographic units for freshwater biodiversity conservation. *BioScience* 58: 403-414.

Ashmole P., Ashmole M.J. 2000. St Helena and Ascension Island: a natural history. *Anthony Nelson, Oswestry,* 500 pp.

Barbour T. 1934. The St. Helena frog. *Copeia* 1934: 183.

Barbour T., Loveridge A. 1928. A comparative study of the herpetological faunae of the Ulugure and Usambara Mountains, Tanganyika Territory with descriptions of new species. *Memoirs of the Museum of Comparative Zoology, Cambridge, Massachusetts* 50: 87-265.

Bittencourt-Silva G.B., Conradie W., Siu-Ting K., Tolley K.A., Channing A., Cunningham M., Farooq H.M., Menegon M., Loader S.P. 2016. The phylogenetic position and diversity of the enigmatic mongrel frog *Nothophryne* Poynton, 1963 (Amphibia, Anura). *Molecular Phylogenetics and Evolution* 99: 89-102.

Blackburn D.C. 2009. Description and phylogenetic relationships of two new species of miniature *Arthroleptis* (Anura: Arthroleptidae) from the Eastern Arc Mountains of Tanzania. *Breviora* 517: 1-17.

Bobe R. 2006. The evolution of arid ecosystems in eastern Africa. *Journal of Arid Environments* 66: 564-584.

Bouckaert R., Vaughan T.G., Barido-Sottani J., Duchêne S., Fourment M., Gavryushkina A., Heled J., Jones G., Kühnert D., De Maio N., Matschiner M., Mendes F.K., Müller N.F., Ogilvie H.A., du Plessis L., Popinga A., Rambaut A., Rasmussen D., Siveroni I., Suchard M.A., Wu C.-H., Xie D., Zhang C., Stadler T., Drummond A.J. 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 15: e1006650. DOI: 10.1371/journal.pcbi.1006650

Bragança P.H.N., Costa W.J.E.M. 2019. Multigene fossil-calibrated analysis of the African lampeyes (Cyprinodontoidei: Procatopodidae) reveals an early Oligocene origin and Neogene diversification driven by palaeogeographic and palaeoclimatic events. *Organisms, Diversity and Evolution* 19: 303-320.

Center for Conservation Bioacoustics 2019. Raven Pro: Interactive Sound Analysis Software (Version 1.6.1) *Ithaca, NY: The Cornell Lab of Ornithology*.

Channing A. 1979. Ecological and systematic relationships of *Rana* and *Strongylopus* in southern Natal (Amphibian: Anura). *Annals of the Natal Museum* 23: 797-831.

Channing A. 1986. A new species of the genus *Strongylopus* Tschudi from Namaqualand, Cape Province, South Africa (Anura: Ranidae). *Annals of the Cape Provincial Museums (Natural History)* 16: 127-135.

Channing A. 2001. Amphibians of Central and Southern Africa. *Ithaca. Cornell University Press*, 470 pp.

Channing A. 2020. Pyxicephalidae *Strongylopus grayii* (Smith, 1849) Clicking Stream Frog in Namibia. *African Herp News* 75: 61-66.

Channing A., Davenport T.R.B. 2002. A new stream frog from Tanzania (Anura: Ranidae: *Strongylopus*). *African Journal of Herpetology* 51: 135-142.

Channing A., Rödel M.-O. 2019. Field guide to the frogs and other amphibians of Africa. *Struik Nature, Cape Town*, 408 pp.

Channing A., Schmitz A. 2008. Hiding in plain sight: another cryptic dainty frog from the highlands of Kenya (Anura: Pyxicephalidae: *Cacosternum*). *African Journal of Herpetology* 57: 75-84.

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: 518-550.

Channing A., Dehling J.M., Lötters S., Ernst R. 2016. Species boundaries and taxonomy of the African river frogs (Amphibia: Pyxicephalidae: *Amietia*). *Zootaxa* 4155: 1-76.

Clarke B.T., Poynton J.C. 2005. A new species of stream frog, *Strongylopus* (Anura: Ranidae) from Mount Kilimanjaro, Tanzania, with comments on a 'northern volcanic mountains group' within the genus. *African Journal of Herpetology* 54: 53-60.

Clement M., Posada D., Crandall K.A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1660.

Cohen A.S., Stone J.R., Beuning K.R.M., Park L.E., Reinthal P.N., Dettmen D., Scholz C.A., Johnson T.C., King J.W., Talbot M.R., Brown E.T., Ivory S.J. 2007. Ecological consequences of early Late Pleistocene megadroughts in tropical Africa. *Proceedings of the National Academy of Sciences USA* 104: 16422-16427.

Conradie W., Bittencourt-Silva G.B., Farooq H.M., Loader S.P., Menegon M., Tolley K.A. 2018. New species of mongrel frogs (Pyxicephalidae: *Nothophryne*) for northern Mozambique inselbergs. *African Journal of Herpetology* 67: 61-85.

Couvreur T.L.P., Chatrou L.W., Sosef M.S.M., Richardson J.E. 2008. Molecular phylogenetics reveal multiple tertiary vicariance origins of the African rain forest trees. *BMV Biology* 6: 54. DOI: 10.1186/1741-7007-6-54

Crawford A.J. 2003. Relative rates of nucleotide substitution in frogs. *Journal of Molecular Evolution* 57: 636-641.

Danley P.D., Husemann M., Ding B., DiPietro L.M., Beverly E.J., Peppe D.J. 2012. The impact of the geologic history and paleoclimate on the diversification of East African cichlids. *International Journal of Evolutionary Biology* (ID 574851), 20 pp. DOI: 10.1155/2012/574851.

Dawood A., Stam E.M. 2006. The taxonomic status of the monotypic frog genus *Anhydrophryne* Hewitt from South Africa: a molecular perspective. *South African Journal of Science* 102: 249-253.

Deckert K. 1938. Beiträge zur Osteologie und Systematik ranider Froschlurche. *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin* 1938: 127-184.

deMenocal P.B., Bloemendal J. 1995. Plio-Pleistocene subtropical African climate variability and the paleoenvironment of hominid evolution: A combined data-model approach. *In:* Vrba E.S., Denton E.G. Burckle L., Partridge T. (Eds), Paleoclimate and Evolution With Emphasis on Human Origins. *Yale University Press, New Haven, CT,* pp. 262-288.

De Witte G.F. 1921. Description de batraciens nouveaux du Congo belge. *Revue Zoologique Africaine*, *Bruxelles* 9: 1-22.

Dubois A. 1981. Deux noms d'espèces préoccupés dans le genre *Rana* (Amphibiens, Anoures). *Bulletin du Muséum National d'Histoire Naturelle. Paris. Section A, Zoologie, Biologie et Ecologie Animales* 2: 927-931.

Dubois A. 1988 [1987]. Miscelanea Nomenclatorica Batrachologica (XVII). *Alytes. Paris* 7: 1-5.

Dubois A. 1992. Notes sur la classification des Ranidae (Amphibiens anoures). *Bulletin Mensuel de la Société Linéenne de Lyon* 61: 305-352.

Du Preez L., Carruthers V. 2017. Frogs of southern Africa. A complete guide. *Struik Nature, Cape Town*, 519 pp.

Emmrich M., Vences M., Ernst R., Köhler J., Barej M.F., Glaw F., Jansen M., Rödel M.-O. 2020. A guild classification system proposed for anuran advertisement calls. *Zoosystematics and Evolution* 96: 515-525.

Faivovich J., Ferraro D.P., Basso N.G., Haddad C.F.B., Rodrigues M.T., Wheeler W.C., Lavilla E.O. 2012. A phylogenetic analysis of *Pleuroderma* (Anura: Leptodactylidae: Leiuperinae) based on mitochondrial and nuclear gene sequences, with comments on the evolution of anuran foam nests. *Cladistics* 28: 460-482.

Feakins S.J., de Menocal P.B. 2010. Global and African regional climate during the Cenozoic. *In:* Werdelin L., Sanders WJ. (eds), Cenozoic mammals of Africa. *University of California Press, Berkeley*: 45-55.

FitzSimons V. 1946. An account of the reptiles and amphibans collected on an expedition to the Cape Province, October to December, 1940. *Annals of the Transvaal Museum* 20: 351-377.

Flot J.-F. 2010. SeqPHASE: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. *Molecular Ecology Resources* 10: 162-166.

Flower B.P., Kennett J.P. 1994. The middle Miocene climatic transition: East Antarctic ice sheet development, deep ocean circulation and global carbon cycling. *Palaeogeography, Palaeoclimatology, Palaeoecology* 108: 537-555.

Frost D.R. 2022. Amphibian species of the World: an online reference. Version 6.1 (27 January 2022). Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php. *American Museum of Natural History, New York, USA*.

Frost D.R., Grant T., Faivovich J., Bain R.H., Haas A., Haddad C.F.B., De Sa R.O., Channing A., Wilkinson M., Donnellan S.C., Raxworthy C.J., Campbell J.A., Blotto B.L., Moler P., Drewes R.C., Nussbaum R.A., Lynch J.D., Green D.M., Wheeler W.C. 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History* 297: 1-370.

Gonçalves H., Martínez-Solano I., Farrand N., García-Paris M. 2007. Conflicting phylogenetic signal of nuclear *vs* mitochondrial DNA markers in midwife toads (Anura, Discoglossidae, *Alytes*): Deep coalescence or ancestral hybridization? *Molecular Phylogenetics and Evolution* 44: 494-500.

Greig J.C., Boycott R.C., de Villiers A.L. 1979. Notes on the elevation of *Rana fasciata montana* FitzSimons, 1946 to specific rank and on the identity of *Rana fasciata* sensu Burchell, 1824 (Anura: Ranidae). *Annals of the Cape Provincial Museums, Natural History* 13: 1-30.

Hasegawa M., Kishino H., Yano T. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22: 160-174.

Heled J., Drummond A.J. 2012. Calibrated tree priors for relaxed phylogenetics and divergence time estimation. *Systematic Biology* 61: 138-149.

Hewitt J. 1933. On new and rare frogs from Chirinda Forest.

Occasional Papers of the National Museum of Southern Rhodesia 6: 12-13.

Hill A. 1987. Causes of perceived faunal change in the later Neogene of East Africa. *Journal of Human Evolution* 16: 583-596.

Hoang D.T., Chernomor O., Von Haeseler A., Minh B.Q., Vinh L.S. 2017. UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35: 518-522.

Hoffman V., Verboom G.A., Cotterill F.P.D. 2015. Dated plant phylogenies resolve Neogene climate and landscape evolution in the Cape Floristic Region. *PLoS ONE* 10(9): e)137847. DOI: 10.1371/journal.pone.0137847.

Hopley P.J., Reade H., Parrish R., De Kock M., Adams J.W. 2019. Spelthem evidence for C3 dominated vegetation during the Late Miocene (Messinian) of South Africa. *Review of Palaeobotany and Palynology* DOI: 10.1016/j.revpalbo.2019.02.006.

Hutchinson D.K., Coxall H.K., Luntm D.J., Steinthorsdottir M., de Boer A.M., Baatsen M., van der Heydt A., Huber M., Kennedy-Asser A.T., Kunzmann L., Ladant J.-B., Lear C.H., Moraweck K., Pearson P.N., Piga E., Pound M.J., Salzmann U., Scher H.D., Sijp W.P., Śliwińska K.K., Wilson P.A., Zhang Z. 2021. The Eocene-Oligocene transition: a review of marine and terrestrial proxy data, models and model-data comparisons. *Climate of the Past* 17: 269-315.

Jacobs B.F. 2004. Palaeobotanical studies from tropical Africa: relevance to the evolution of forest, woodland and savannah biomes. *Philosophical Transactions of the Royal Society, London B* 359: 1573-1583.

Jacobsen N. 2013. *Strongylopus grayii* Smith 1849, Clicking Stream Frog, Colour. *African Herp News* 59: 16-18.

Jetz W., Pyron R.A. 2018. The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nature Ecology & Evolution* 2: 850-858.

Jetz W., Pyron R.A. 2019. Data from: The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life, *Dryad*, Dataset. DOI: 10.5061/dryad.cc3n6j5.

Koch C. 1872. Formen und Wandlungen der ecaudaten Batrachier des Unter-Main und Lahn-Gebietes. *Bericht der Senckenbergischen Naturforschenden Gesellschaft in Frankfurt am Main 1871-72*: 122-183.

Köhler J., Jansen M., Rodríquez A., Kok P.J.Rr., 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: 1-124. DOI: 10.11646/zootaxa.4251.1.1

Lawson L.P. 2010. The discordance of diversification: evolution in the tropical-montane frogs of the Eastern Arc Mountains of Tanzania. *Molecular Ecology* 19: 4046-4060.

Lever C. 2003 Naturalized reptiles and amphibians of the world. *Oxford University Press*, *Oxford*, 318 pp.

Linder H.P., De Klerk H.M., Born J., Burgess N.D., Fjeldså J., Rahbek C. 2012. The partitioning of Africa: statistically defined biogeographical regions in sub-Saharan Africa. *Journal of Biogeography* 39: 1189-1205.

Lönnberg E. 1907. Reptilia and Batrachia. *In:* Sjöstedt Y. (ed.), Wissenschaftliche Ergebnisse der Schwedischen zoologischen Expedition nach dem Kilimandjaro, dem Meru und den umgebenden Massaisteppen Deutsch-Ostafrikas 1905-1906, unter Leitung von Prof. Dr. Yngve Sjöstedt. Hrsg. mit Unterstützung von der Königl. schwedischen Akademie der Wissenschaften Vol. 1. No. 4: 1-28. *Stockholm, P. Palmquists*.

Loveridge A. 1933. Reports on the scientific results of an expedition to the southwestern highlands of Tanganyika Territory. VII. Herpetology. *Bulletin of the Museum of Comparative Zoology* 74: 197-416.

Loveridge A. 1953. Zoological results of a fifth expedition to East Africa. IV Amphibians from Nyasaland and Tete. *Bulletin of the Museum of Comparative Zoology* 110: 325-406, with 4 plates.

Matthee C.A., Flemming A.F. 2002. Population fragmentation in the southern rock agama, *Agama atra*: more evidence for vicariance in Southern Africa. *Molecular Ecology* 11: 465-471.

McCarthy T., Rubidge B. 2005. The story of Earth and Life. *Struik, Cape Town*, 333 pp.

Measey G.J., Vences M., Drewes R.C., Chiari Y., Melo M., Bourles B. 2007. Freshwater paths across the ocean: molecular phylogeny of the frog *Ptychadena newtoni* gives insights into amphibian colonization of oceanic islands. *Journal of Biogeography* 34: 7-20.

Mertens R. 1971. Der Frosch von St. Helena. *Natur und Museum* 101: 472-473.

Minh B.Q., Nguyen M.A.T., von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* 30: 1188-1195.

Nguyen L.-T., Schmidt H.A., von Haeseler A., Minh B.Q. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution* 32: 268-274.

Nieden F. 1911 "1910". Neue ostafrikanische Frösche aus dem Kgl. Zool. Museum in Berlin. *Sitzungsberichte der Gesellschaft Naturforschende Freunde zu Berlin* 1910: 436-441.

Nonnotte P., Guillou H., Le Gall B., Benoit M., Cotten J., Scaillet S. 2008. New K-Ar age determinations of Kilimanjaro volcano in the North Tanzania diverging rift, East Africa. *Journal of Volcanology and Geothermal Research* 173: 99-112.

Passmore N.I. 1977. Mating calls and other vocalisations of five species of *Ptychadena* (Anura: Ranidae). *South African Journal of Science* 73: 212-214.

Passmore N.I., Carruthers V.C. 1979. South African Frogs. *Witwatersrand University Press, Johannesburg*, 270 pp.

Partridge T.C. 1997. Late neogene uplift in Eastern and Southern Africa and its paleoclimatic implications. *In:* Ruddiman W.F. (ed.), Tectonic Uplift and Climate Change, *Plenum Press, New York*. pp. 63-86.

Pickersgill M. 2007. Frog search. Results of expeditions to southern and eastern Africa from 1993-1999. Frankfurt Contributions to Natural History Volume 28. *Edition Chimaira, Frankfurt am Main*, 574 pp.

Poynton J.C. 1963. Descriptions of southern African amphibians. *Annals of the Natal Museum* 15: 319-332.

Poynton J.C. 1964a. The Amphibia of Southern Africa. *Annals of the Natal Museum* 17: 1-334.

Poynton J.C. 1964b. Amphibia of the Nyasa-Luangwa region of Africa. *Senckenbergiana Biologica* 45: 193-225.

Poynton J.C. 2004. Stream frogs in Tanzania (Ranidae: *Strongylopus*): The case of *S. merumontanus* and *S. fuelleborni*. *African Journal of Herpetology* 53: 29-34.

Poynton J.C. 2013. Afrotemperate amphibians in southern and eastern Africa: a critical review. *African Journal of Herpetology* 62: 5-20.

Poynton J.C., Broadley D.G. 1985. Amphibia Zambesiaca 2. Ranidae. *Annals of the Natal Museum* 27: 115-181.

Price B.W., Barker N.P., Villet M.H. 2007. Patterns and processes underlying evolutionary significant units in the *Platypleura stridula* L. species complex (Hemiptera: Cicadidae) in the Cape Floristic Region, South Africa. *Molecular Ecology* 16: 2574-2588.

Rambaut A. 2018. FigTree. Tree Figure Drawing Tool Version 1.4.4 available from http://tree.bio.ed.ac.uk/.

Roberts D.L., Sciscio L., Herries A.I.R., Scott L., Bamford M.K., Musekiwa C., Tsikos H. 2013. Miocene fluvial systems and palynofloras at the southestern tip of Africa: Implications for regional and global fluctuations in climate and ecosystems. *Earth-Science Reviews* 124: 184-201.

Sabaj M.H. 2019. Standard symbolic codes for institutional resource collections in herpetology and ichthyology: An Online Reference. Version 7.1 (21 March 2019). Electronically accessible at https://asih.org, American Society of Ichthyologists and Herpetologists, Washington, DC.

Sciscio L., Tsikos H., Roberts D.L., Scott L., van Breugel Y., Damste J.S.S., Scouten S., Grocke D.R. 2016. Miocene climate and vegetation changes in the Cape Peninsula, South Africa: Evidence from biogeochemistry and palynology. *Palaeogeography Palaeoclimatology, Palaeoecology* 445: 124-137.

Senut B., Pickford M., Ségalen L. 2009. Neogene desertification of Africa. *Comptes Rendus Geoscience* 341: 591-602.

Smit H.A, Robinson T.J., Van Vuuren B.J. 2007. Coalescence methods reveal the impact of vicariance on the spatial genetic structure of *Elephantulus edwardii* (Afrotheria, Macroscelidae). *Molecular Ecology* 16: 2680-2692.

Smith A. 1849. Illustrations of the Zoology of South Africa; Consisting Chiefly of Figures and Descriptions of the Objects of Natural History Collected during and Expedition into the Interior of South Africa, in the Years 1834, 1835, and 1836. Vol. III, Reptilia, Part 28. *London: Smith, Elder & Co*.

Steindachner F. 1867. Reise der österreichischen Fregatte Novara um die Erde in den Jahren 1857, 1858, 1859 unter den Befehlen des Commodore B. von Wüllerstorf-Urbair. Pt 9, Bd. 1, Abt. 4, Zoologischer Theil. Amphibien. *Wien: K.K. Hof- und Staatsdruckerei.*

Stephens M., Donnelly P. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *American Journal of Human Genetics* 73: 1162-1169.

Stöck M., Dubey S., Klütsch C., Litvinchuk S.N., Scheidt U., Perrin N. 2008. Mitochondrial and nuclear phylogeny of circum-Mediterranean tree frogs from the *Hyla arborea* group. *Molecular Phylogenetics and Evolution* 49: 1019-1024.

Streicher J.W., Day J.J. 2020. The toad's warts: discordance creates bumpy expectations of mitochondrial-nuclear evolution between species. *Molecular Ecology* 29: 3400-3402.

Strugnell A.M. 2002. Endemics of Mt. Mulanje. The endemic spermatophytes of Mt. Mulanje, Malawi. *Systematics and Geography of Plants* 72: 11-26.

Swofford D.L. 2002. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. *Sinauer Associates, Sunderland, Massachusetts*.

Toews D.P.L., Brelsford A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology* 21: 3907-3930.

Tolley K.A., Chase B.M., Forest F. 2008. Speciation and radiations track climate transitions since the Miocene Climatic Optimum: a case study of southern African chameleons. *Journal of Biogeography* 35: 1402-1414.

Tolley K.A., Braae A., Cunningham M. 2010. Phylogeography of the Clicking Stream Frog *Strongylopus grayii*, (Anura, Pyxicephalidae) reveals cryptic divergence across climatic zones in an abundant and widespread taxon. *African Journal of Herpetology* 59: 17-32.

Trifinopoulos J., Nguyen L.-T., Von Haeseler A., Minh B.Q. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44: W1, W232-W235.

Tschudi J.J. von. 1838. Classification der Batrachier mit Berücksichtigung der fossilen Thiere dieser Abtheilung der Reptilien. *Neuchâtel: Petitpierre*.

Turner A.A., Channing A. 2008. A new species of *Arthroleptella* Hewitt, 1926 (Anura: Pyxicephalidae) from the Klein Swartberg Mountain, Caledon, South Africa. *African Journal of Herpetology* 57: 1-12.

Turner A.A., De Villiers A.L., Dawood A., Channing A. 2004. A new species of *Arthroleptella* Hewitt, 1926 (Anura: Ranidae) from the Groot Winterhoek Mountains of the Western Cape Province, South Africa. *African Journal of Herpetology* 53: 1-12.

Uno K.T., Polissar P.J., Jackson K.E., de Menocal P.B. 2016. Neogene biomarker record of vegetation change in eastern Africa. *Proceedings of the National Academy of Sciences USA* 113: 6355-6363.

van der Meijden A., Vences M., Hoegg S., Meyer A. 2005. A previously unrecognized radiation of ranid frogs in Southern Africa revealed by nuclear and mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 37: 674-685.

van der Merwe P.d.W., Cotterill F.P.D., Kandziora M., Watters B.R., Nagy B., Genade T., Flügel T.J., Svendsen D.S., Bellstedt D.U. 2021. Genomic fingerprints of palaeogeographic history: The tempo and mode of rift tectonics across tropical Africa has shaped the diversification of the killifish genus *Nothobranchius* (Teleostei: Cyprinodontiformes). *Molecular Phylogenetics and Evolution* 158: 106988.

van Dijk D.E. 1966. Systematic and field keys to the families, genera and described species of southern African anuran tadpoles. *Annals of the Natal Museum* 18: 231-286.

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.

Vieites D.R., Wollenberg K.C., Andreone F., Köhler J., Glaw F., Vences M. 2009. Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences USA* 106: 8267-8272.

Wager V.A., 1961. The Plain *Rana*. *African Wildlife* 15: 151-155.

Wahlberg N., Weingartner E., Warren A.D., Nylin S. 2009. Timing major conflict between mitochondrial and nuclear

genes in species relationships of *Polygonia* butterflies (Nymphalidae: Nymphalini). *BMC Evolutionary Biology* 9: 92.

Werner F. 1910. Reptilia et Amphibia. *Denkschriften. Medicinisch-naturwissenschaftliche Gesellschaft zu Jena* 16: 279-370.

Wilson L., Channing A. 2019. A new sand frog from Namaqualand, South Africa (Pyxicephalidae: *Tomopterna*). *Zootaxa* 4609: 225-246.

Zancolli G., Steffan-Dewenter I., Rödel M.-O. 2013. Amphibian diversity on the roof of Africa: unveiling the effects of habitat degradation, altitude and biogeography. *Diversity and Distributions* 20: 297-308.

Zhao Z., Heideman N., Bester P., Jordaan A., Hofmeyr M.D. 2020. Climatic and topographic changes since the Miocene influenced the diversification and biogeography of the tent tortoise (*Psammobates tentorius*) species complex in Southern Africa. *BMC Evolutionary Biology* 20: 153.

Appendix 1. Gene sequences of *Strongylopus* taxa used in this study, listing species, voucher, locality, co-ordinates, and GenBank accession numbers.

Species	Voucher	Locality	Latitude	Longitude	12S	16S	*RAG-1*	*Tyr*
S. bonaespei	VUB1221	Cape Peninsula, South Africa	-34.2054	18.4061		DQ347345	DQ347288	DQ347196
S. bonaespei	PEM A14996	Landdroskop, South Africa	-34.0491	19.0096	OK649508	OK649433		OK648617
S. bonaespei	AC2965	Grootvadersbosch NR, South Africa	-33.9848	20.8077	OK649513	OK649437	OK648576	OK648622
S. bonaespei	AC3380	Montague Pass, South Africa	-33.9039	22.4628		OK649479		
S. fasciatus	ZFMK 66444	Little Brak, South Africa	-34.0485	22.2198	DQ019594	AF215412	DQ019513	
S. fasciatus	PEM A15007	Saarsveld, South Africa	-33.9577	22.5325		OK649480		
S. fasciatus	PEM A15009	Wilderness railway line, South Africa	-33.9948	22.5921	OK649544	OK649482		
S. fasciatus	PEM A11167	Reigate Farm; Travisscock wetland, South Africa	-32.5701	27.1832	OK649546	OK649485		
S. fasciatus	PEM A12087	Nqadu Forest, South Africa	-31.4125	28.7327	OK649553	OK649494		
S. fasciatus	GNP_2009_T2	Chimanimani, Mozambique	-19.7644	33.0892		OK605285		
S. fasciatus	ZMB 90085	Chimanimani, Mozambique	-19.7644	33.0892		OK605286		
S. fasciatus	PEM A09073	Nature's Valley, South Africa	-33.9688	23.5598	OK649526	OK649449	OK648588	OK648636
S. fasciatus	AC3217	Hogsback, South Africa	-19.7693	32.9816	OK649541	OK649475	OK648606	OK648663
S. fasciatus	PEM A11424	Fort Fordyce Nature Reserve; Katberg Gate, South Africa	-32.6631	26.4788	OK649545	OK649484	OK648610	OK648666
S. fasciatus	PEM A11799	Fort Fordyce Nature Reserve	-32.6631	26.4788	OK649547	OK649486		
S. fasciatus	PEM A10173	Cape Morgan Nature Reserve, South Africa	-32.7022	28.3508	OK649549	OK649488		OK648668
S. fasciatus	SAIAB 193036	Kweza Trail, Chimanimani, Zimbabwe	-19.7826	33.0408	OK649562	OK649503	OK648612	OK648682
S. fasciatus	AC3220	Above Outward Bound, Zimbabwe	-19.7681	33.0236	OK649534	OK649457	OK648605	OK648644, OK648662

Species	Voucher	Locality	Latitude	Longitude	12S	16S	*RAG-1*	*Tyr*
S. fasciatus	KTH06-01	Gondwana Game Reserve, South Africa	-34.0466	21.8827		GU952052		
S. fasciatus	KTH06-02	Mossel Bay, South Africa	-34.0466	21.8827		GU952053		
S. grayii	ATSLBSGO1	Vergaderingskop Private NR, South Africa	-34.3635	21.5746		GU952048		
S. grayii	STGRHLDB	Driekoppe Farm, Helderberg, South Africa	-34.022	18.8851		GU952077		
S. grayii	MH0846	Berg River Neck, South Africa	-33.9983	19.0114		EF136559		
S. grayii	No voucher	Polka Draai, South Africa	-33.9646	18.7519		HQ014420	HQ014428	
S. grayii	KTH571	Marloth Nature Reserve, South Africa	-33.9469	20.4539		EF136544		
S. grayii	AB009	Stellenbosch, South Africa	-33.9346	18.8667		GU952038		
S. grayii	AB010	Stellenbosch, South Africa	-33.9346	18.8667		GU952039		
S. grayii	AB013	Stellenbosch, South Africa	-33.9346	18.8667		GU952040		
S. grayii	GDKSG1	Groot Drakenstein Mountains, South Africa	-33.9196	19.0391		GU952051		
S. grayii	CF050	Kaaimansgatkloof, South Africa	-33.8992	19.2611		EF136558		
S. grayii	MH1196	Formosa Dam, South Africa	-33.8508	23.6733		EF136557		
S. grayii	MH0325	Louterwater Dam, South Africa	-33.85	23.6731		GU952063		
S. grayii	MH1065	Louterwater Dam, South Africa	-33.85	23.6731		GU952071		
S. grayii	MH1147	Louterwater Dam, South Africa	-33.85	23.6731		GU952072		
S. grayii	MH1108	Perdebond, South Africa	-33.825	21.9264		EF136556		
S. grayii	QRFA1063	Koeberg Nature Reserve, South Africa	-33.6845	18.4365		GU952075		
S. grayii	V222	Buffelsklip Pass, Kamanassieberg, South Africa	-33.6525	22.7542		GU952078		
S. grayii	AB001	Betty's Bay, South Africa	-33.6358	18.8661		GU952035		
S. grayii	AB003	Betty's Bay, South Africa	-33.6358	18.8661		GU952036		
S. grayii	AB006	Betty's Bay, South Africa	-33.6358	18.8661		GU952037		
S. grayii	SGBK2B	Noemakloof, headwaters, South Africa	-33.6175	23.8014		EF136561, GU952076		
S. grayii	AMNH A144979	Bainskloof, South Africa	-33.5798	19.1351		DQ283068		

Species	Voucher	Locality	Latitude	Longitude	12S	16S	*RAG-1*	*Tyr*
S. grayii	ATRVLSG1	Riverlands Nature Reserve, South Africa	-33.4917	18.6047		GU952046		
S. grayii	V174	Die Hel, South Africa	-33.3635	21.6283		EF136560		
S. grayii	ATLAJSG1	Lajuma, South Africa	-33.2225	19.0732		GU952045		
S. grayii	ATRZBSG1	Roodezandberg, Tulbagh, South Africa	-33.2225	19.0732		GU952047		
S. grayii	CF072	Donkerkloof, South Africa	-32.8169	19.3453		GU952049		
S. grayii	MH1336	Cata Hut, South Africa	-32.5647	27.1033		EF136539		
S. grayii	CF519	Upper Krom River, South Africa	-32.5025	19.1625		EF136546		
S. grayii	CF108	Sanddrif, South Africa	-32.4906	19.2656		GU952050		
S. grayii	MEEP XS077	Nico Malan Pass top, Winterberg, South Africa	-32.4583	26.8472		GU952079		
S. grayii	KTH293	Pakhuis Pass, South Africa	-32.1642	19.0367		GU952061		
S. grayii	V194	Gifberg, South Africa	-31.8316	18.7462		EF136536		
S. grayii	V146	Van Rhynsdorp, South Africa	-31.6106	18.7334		EF136537		
S. grayii	MH0644	Upper Quthing Drainage, Lesotho	-30.3994	28.2573		EF136542		
S. grayii	MH0648	Upper Quthing Drainage, Lesotho	-30.3994	28.2573		GU952069		
S. grayii	MH0668	Upper Quthing Drainage, Lesotho	-30.3994	28.2573		EF136543		
S. grayii	QQ0119	Drakensberg, ridge top tarn, South Africa	-29.3513	29.5388		EF136550		
S. grayii	MH0320	Kokoatsane Valley, back of the saddle, Lesotho	-28.9204	29.0658		EF136540		
S. grayii	MH0487	Above Cathedral Peak, South Africa	-28.8973	29.0258		GU952066		
S. grayii	MH0642	Drakensberg, South Africa	-28.877	28.9543		EF136549		
S. grayii	MH0638	Liholohake, Lesotho	-28.6897	28.7621		EF136538		
S. grayii	MH0643	Liholohake, Lesotho	-28.6897	28.7621		GU952068		
S. grayii	MH0450	Upper Kgotjoane, Lesotho border, South Africa	-28.6594	28.70391		GU952065		
S. grayii	QQ0126	Mechachaneng Stream, Lesotho	-28.6515	28.7008		EF136547		
S. grayii	MH0608	Drakensberg, South Africa	-28.6486	28.714		EF136552		
S. grayii	MH0449	Phuthaditjaba, South Africa	-28.6223	28.7795		GU952064		
S. grayii	MH0633	Nelson's Kop, South Africa	-28.2336	29.4422		EF136541		

Species	Voucher	Locality	Latitude	Longitude	12S	16S	*RAG-1*	*Tyr*
S. grayii	MH0636	Nelson's Kop, South Africa	-28.2336	29.4422		GU952067		
S. grayii	QQ0359	Normandien Pass, South Africa	-27.9661	29.6855		GU952074		
S. grayii	MH0315	Suikerbosrand, Heidelberg, South Africa	-26.4833	28.25		GU952062		
S. grayii	MH0733	Suikerbosrand, Heidelberg, South Africa	-26.4833	28.25		GU952070		
S. grayii	MPU027	Mt Sheba Road, South Africa	-24.9094	30.6939		EF136554		
S. grayii	MPU030	Mt Sheba Road, South Africa	-24.9094	30.6939		GU952073		
S. grayii	SGMK1	Quarry near Waterberg summit, South Africa	-24.4547	27.6147		EF136553		
S. grayii	MA10	Stellenbosch, South Africa	-33.9346	18.8667	DQ022342			
S. grayii	MHNG 2690.028	Klipheuwel, South Africa	-33.7009	18.6988	OK649509	KC756281	OK648572	OK648618
S. grayii	TM 84854	Barberton, South Africa	-25.7884	31.0532	OK649512	DQ022367		OK648621
S. grayii	AC2966	Stillbay, South Africa	-34.3693	21.4074	OK649514	OK649438	OK648577	OK648623
S. grayii	AC3083	Groot Hagelkraal, South Africa	-34.7	19.5666	OK649516	OK649439	OK648578	OK648625
S. grayii	AC3137	Klipheuwel, South Africa	-33.7009	18.6988	OK649518	OK649441	OK648581	OK648628
S. grayii	PEM A14999	Stellenbosch, South Africa	-33.9346	18.8667		OK649481	OK648580	OK648627
S. grayii	PEM A15003	Hogsback, South Africa	-32.5951	26.9322	OK649524	OK649447	OK648586	OK648634
S. grayii	PEM A15004	Klipheuwel, South Africa	-32.5951	26.9322	OK649525	OK649448	OK648587	OK648635
S. grayii	PEM A09074	Nature's Valley, South Africa	-33.9761	23.5611	OK649527	OK649450	OK648589	OK648637
S. grayii	PEM A09071	Covie, South Africa	-33.95	23.6	OK649528	OK649451	OK648590	OK648638
S. grayii	PEM A09072	Covie, South Africa	-33.95	23.6	OK649529	OK649452	OK648591	OK648639
S. grayii	PEM A13446	Nature's Valley, South Africa	-33.9688	23.5598	OK649530	OK649453	OK648592	OK648640
S. grayii	PEM A09387	Geelhoutboom River, South Africa	-33.7983	25.0619	OK649531	OK649454		OK648641
S. grayii	PEM A15001	Wilderness, South Africa	-33.994	22.5748	OK649535	OK649458	OK648595	OK648645
S. grayii	PEM A15002	Wilderness, South Africa	-33.994	22.5748	OK649536	OK649459		OK648646
S. grayii	PEM A15000	Sedgefield, South Africa	-34.0226	22.8075	OK649519	OK649442	OK648609, OK648582	OK648665, OK648629
S. grayii	PEM A11948	Mkambati Nature Reserve, South Africa	-31.2967	29.9758	OK649548	OK649487		OK648667
S. grayii	PEM A10977	Geelhoutbos Baviaanskloof, South Africa	-33.6338	24.2439	OK649550	OK649489		OK648669
S. grayii	PEM A11805	Isidenge State Forest, South Africa	-32.67	27.2783	OK649551	OK649490		OK648670

Species	Voucher	Locality	Latitude	Longitude	12S	16S	*RAG-1*	*Tyr*
S. grayii	PEM A11163	Roodeplaat, Baviaanskloof, South Africa	-33.6036	25.0753	OK649552	OK649491		OK648671
S. grayii	PEM A11164	Reigate Farm, South Africa	-32.5701	27.1832		OK649492		OK648672
S. grayii	PEM A13468	Coldstream, South Africa	-31.9646	23.7152		OK649493		OK648673
S. grayii	SAIAB 96953	Grey Dam, Makhanda, South Africa	33.3205	26.5208	OK649559	OK649500		OK648679
S. grayii	SAIAB 200301	Beaverlac, South Africa	-32.9079	19.0672	OK649560	OK649501	OK648611	OK648680
S. grayii	NMB A8071	Wolkberg, South Africa	-24.0072	30.0764	OK649515, OK649506	OK649478	OK648608	OK648624
S. grayii	KTH06-48	Noordhoek, South Africa	-34.1094	18.3765		GU952054		
S. grayii	KTH06-50	Noordhoek, South Africa	-34.1094	18.3765		GU952055		
S. grayii	KTH06-52	Noordhoek, South Africa	-34.1094	18.3765		GU952056		
S. grayii	KTH06-54	Noordhoek, South Africa	-34.1094	18.3765		EF136551		
S. grayii	KTH07-83	Koeberg Nature Reserve, South Africa	-33.6846	18.4366		GU952057		
S. grayii	KTH07-86	Koeberg Nature Reserve, South Africa	-33.6401	18.4316		GU952058		
S. grayii	AB08-01	Blyde River Nature Reserve, South Africa	-27.9663	29.6855		GU952041		
S. grayii	AB08-01	Mariepskop, South Africa	-27.9663	29.6855	OK649520	OK649443	OK648583	OK648630
S. grayii	AB08-02	Blyde River Nature Reserve, South Africa	-24.5619	30.8931		GU952042		
S. grayii	KTH08-02	Eastern Escarpment, KwaZulu-Natal, South Africa	-27.9663	29.6856	OK649521	GU952059		OK648631
S. grayii	KTH08-03	Mariepskop, South Africa	-27.9663	29.6856		GU952060		
S. grayii	AB08-03	Mariepskop, South Africa	-27.9663	29.6855	OK649522	GU952043	OK648584	OK648632
S. grayii	AB08-04	Mariepskop, South Africa	-24.5396	30.8725		GU952044		
S. grayii	NMB A8073	Rooiberg, South Africa	-30.4044	18.1016	OK649523	OK649446	OK648585	OK648633
S. grayii	PEM A15008	Studers Pass, Namaqualand, South Africa	-30.4281	18.0616	OK649561	OK649502		OK648681
S. merumontanus	RdS 994	Sao Hill, Tanzania	-8.2233	35.1671				KC180300
S. merumontanus	G56	Nkuu, Mt Kilimanjaro, Tanzania	-3.1797	37.2492			OK648603	OK648659
S. merumontanus	G57	Nkuu, Mt Kilimanjaro, Tanzania	-3.1797	37.2492			OK648604	OK648660

Species	Voucher	Locality	Latitude	Longitude	12S	16S	*RAG-1*	*Tyr*
S. merumontanus	PEM A11187	Mount Mabu summit, Mozambique	-16.2968	36.3924				OK648675
S. merumontanus	CAS 225064	Mt Kitumbeine, Tanzania	-2.9044	36.235	OK649543	OK649431		OK648615
S. merumontanus	PEM A14994	Mbizi Hill Forest Reserve, Tanzania	-7.8932	31.6808	OK649507	OK649432	OK648571	OK648616
S. merumontanus	UDSM 2323	Maua Rt, Mount Kilimanjaro, Tanzania	-3.1699	37.4359	OK649510	OK649434	OK648573	OK648619
S. merumontanus	PEM A14997	Mount Kitumbeine, Tanzania	-2.9044	36.235		OK649435	OK648574	OK648685
S. merumontanus	CAS 225065	Mount Kitumbeine, Tanzania	-2.9044	36.235	OK649543	OK649511	OK648575	OK648620
S. merumontanus	AM5755	Nkweseko, Mt Kilimanjaro, Tanzania	-3.1824	37.2426		OK649461		
S. merumontanus	AM4758	Mweka Dam, Mt Kilimanjaro, Tanzania	-3.2167	37.3425		OK649464		
S. merumontanus	MTSN 9392	Mdandu FR, Livingstone Mts, Tanzania	-9.776	34.7818		OK649477		
S. merumontanus	PEM A11650	Mount Namuli, Mozambique	-15.3749	37.0168		OK649483		
S. merumontanus	SAIAB 96468	Thuchila hut, Mt Mulanje, Malawi	-15.8957	35.6096	OK649557	OK649498		
S. merumontanus	UDSM 1609	Mt Meru, Tanzania	3.2479	36.8103		OK649430		OK648614
S. merumontanus	RH001	Nyanga, Zimbabwe	-18.3061	32.8335	OK649532	OK649455	OK648593	OK648642
S. merumontanus	RH002	Nyanga, Zimbabwe	-18.22	32.7463	OK649533	OK649456	OK648594	OK648643
S. merumontanus	AM4654	Mweka Dam, Mt Kilimanjaro, Tanzania	-3.2167	37.3425	OK649537	OK649460	OK648596	OK648647
S. merumontanus	AM4756	Marangu Route, Mt Kilimanjaro, Tanzania	-3.2228	37.5146		OK649462	OK648597	OK648648
S. merumontanus	AM4757	Kidia Forest, Mt Kilimanjaro, Tanzania	-3.2803	37.4406		OK649463	OK648598	OK648649
S. merumontanus	G111	Kidia Forest, Mt Kilimanjaro, Tanzania	-3.2803	37.4406	OK649538	OK649465	OK648684	OK648650
S. merumontanus	GTASF	Machame, Mt Kilimanjaro, Tanzania	-3.1753	37.228	OK649539	OK649466	OK648599	OK648651
S. merumontanus	GL	Mweka Dam, Mt Kilimanjaro, Tanzania	-3.2166	37.3425		OK649467		OK648652
S. merumontanus	GL	Nkuu, Mt Kilimanjaro, Tanzania	-3.1797	37.2492	OK649540	OK649468	OK648600	OK648653
S. merumontanus	G51	Nkuu, Mt Kilimanjaro, Tanzania	-3.1797	37.2492		OK649469		OK648654

Species	Voucher	Locality	Latitude	Longitude	12S	16S	*RAG-1*	*Tyr*
S. merumontanus	G52	Nkuu, Mt Kilimanjaro, Tanzania	-3.1797	37.2492		OK649470	OK648601	OK648655
S. merumontanus	G53	Nkuu, Mt Kilimanjaro, Tanzania	-3.1797	37.2492		OK649471		OK648656
S. merumontanus	G54	Nkuu, Mt Kilimanjaro, Tanzania	-3.1797	37.2492		OK649472	OK648602	OK648657
S. merumontanus	G55	Nkuu, Mt Kilimanjaro, Tanzania	-3.1797	37.2492		OK649473		OK648658
S. merumontanus	ZMB 79033	Nkuu, Mt Kilimanjaro, Tanzania	-3.1797	37.2492	OK649505	OK649474		OK648661
S. merumontanus	MTSN 5298	Kiolela, Udzungwa Mts, Kilolo, Tanzania	-7.8835	36.0893	OK649542	OK649476	OK648607	OK648664
S. merumontanus	PEM A11359	Mount Namuli, Mozambique	-15.3877	37.0434	OK649554	OK649495		OK648674
S. merumontanus	PEM A11184	Mount Mabu, Mozambique	-16.2825	36.3816	OK649555	OK649496		OK648676
S. merumontanus	PEM A11352	Mount Namuli, Mozambique	-15.3877	37.0434	OK649556	OK649497		OK648677
S. merumontanus	SAIAB 96468	Between Sombani and Madzeka, Mt Mulanje, Malawi	-15.893	35.698	OK649558	OK649499		OK648678
S. rhodesianus	SAIAB 193037	Kweza Trail, Chimanimani, Zimbabwe	-19.7826	33.0408	OK649563	OK649504	OK648613	OK648683
S. wageri	J2J_Sw	Mkhomazana River, Sani Pass, South Africa	-29.6	29.35		FJ411441	FJ411457	
S. wageri	J3J_Sw	Mkhomazana River, Sani Pass, South Africa	-29.6	29.35		FJ411442	FJ411458	
S. wageri	AC3059	Sani Pass lower border, South Africa	-29.6128	29.3333	OK649517	OK649440	OK648579	OK648626

Appendix 2. Localities of *Strongylopus* advertisement call recordings, listing species, locality, co-ordinates and number of calls.

Species	Locality	Latitude	Longitude	n
S. bonaespei	George, South Africa	-33.9881	22.4529	1
S. bonaespei	Grootvadersbosch NR, South Africa	-33.9848	20.8077	5
S. bonaespei	Kleinmond, South Africa	-34.3353	19.0126	8
S. fasciatus	Fairview Farm, South Africa	-29.3552	30.5280	34
S. fasciatus	Geelhoutboom River, South Africa	-33.7983	25.0619	17
S. fasciatus	Gonubie, South Africa	-32.9422	28.0098	18
S. fasciatus	Himeville, South Africa	-29.7467	29.5136	54
S. fasciatus	Impendle, South Africa	-29.6005	29.8656	13
S. fasciatus	Lark Rise, South Africa	-29.7699	29.4641	122
S. fasciatus	Longmore Forest Station, South Africa	-33.8583	25.2080	14
S. fasciatus	Loteni Nature Reserve, South Africa	-29.5041	29.5462	20
S. fasciatus	Mazeppa Bay, South Africa	-32.4742	28.6416	11

Species	Locality	Latitude	Longitude	n
S. fasciatus	Oslo Beach, South Africa	-30.7585	30.4386	2
S. fasciatus	Pevensey, South Africa	-29.7830	29.5000	15
S. fasciatus	Pietermaritzburg, South Africa	-29.6006	30.3794	6
S. fasciatus	Pumula, South Africa	-30.6434	30.5264	16
S. fasciatus	The Lakes, South Africa	-33.9833	35.4666	13
S. fasciatus	Touws River, South Africa	-33.3398	20.0271	6
S. fasciatus	Rusito Mission, Zimbabwe	-20.0363	32.8621	40
S. grayii	Elandsbay, South Africa	-31.3141	18.3441	48
S. grayii	Fairview Farm, South Africa	-29.3552	30.5280	14
S. grayii	Firgrove, South Africa	-34.0561	18.7883	45
S. grayii	Gamkaskloof, South Africa	-33.3558	21.7081	2
S. grayii	Geelhoutboom River, South Africa	-33.7983	25.0619	28
S. grayii	Hogsback, South Africa	-32.5951	26.9322	10
S. grayii	Jonaskop, South Africa	-33.9716	19.5063	10
S. grayii	Jonkershoek, South Africa	-33.9686	18.9346	22
S. grayii	Klipheuwel, South Africa	-33.7009	18.6988	4
S. grayii	Lemoenputs, Namibia	-24.2666	16.0500	29
S. grayii	Mooiplaas, South Africa	-26.0961	30.8786	24
S. grayii	Pakhuis Pass, South Africa	-32.1363	19.0081	3
S. grayii	Pietermaritzburg, South Africa	-29.6006	30.3794	24
S. grayii	Rondeberg, South Africa	-33.4244	18.3047	74
S. grayii	Solitude, South Africa	-28.9000	30.8666	21
S. grayii	Stellenbosch, South Africa	-33.9346	18.8667	60
S. grayii	The Lakes, South Africa	-33.9833	35.4666	14
S. grayii	Vrolijkheid Nature Reserve, South Africa	-33.9195	19.8782	19
S. grayii	Bovlei Farm, South Africa	-30.2204	18.0475	31
S. grayii	Paradise Kloof, South Africa	-28.3275	17.0066	52
S. grayii	Springbok, South Africa	-29.6823	17.8872	5
S. grayii	Richtersveld National Park, South Africa	-28.3276	17.0063	32
S. merumontanus	Chelinda, Malawi	-10.5907	33.8136	37
S. merumontanus	Iringa, Tanzania	-7.7681	35.6861	19
S. merumontanus	Kitulo National Park, Tanzania	-9.0630	33.8479	15
S. merumontanus	Lilongwe, Malawi	-13.9943	33.7691	1
S. merumontanus	Luwawa, Malawi	-12.1199	33.7213	4
S. merumontanus	Mbizi Hill Forest Reserve, Tanzania	-7.8931	31.6808	3
S. merumontanus	Mount Kilimanjaro, Tanzania	-3.1753	37.2208	36
S. merumontanus	Mount Kitumbeine, Tanzania	-2.9044	36.2350	16
S. merumontanus	Mount Mabu, Mozambique	-16.2825	36.3816	54
S. merumontanus	Mount Meru, Tanzania	3.2479	36.8103	10
S. merumontanus	Nguu Forest Reserve, Tanzania	-3.1798	37.2492	26
S. merumontanus	Udzungwa Mountains, Tanzania	-7.7731	36.7941	23
S. merumontanus	Ukaguru Mountains, Tanzania	-6.4254	36.9670	15
S. merumontanus	Uluguru Mountains, Tanzania	-6.1050	37.6541	30
S. wageri	Entumeni, South Africa	-28.8889	31.4643	10
S. wageri	Lundy's Hill, South Africa	-29.7256	29.9372	26

Appendix 3. *Strongylopus* specimens examined for morphology, listing species, voucher, locality and co-ordinates.

Species	Voucher	Locality	Latitude	Longitude
S. bonaespei	PEM A14996	Landdroskop, South Africa	-34.0491	19.0119
S. bonaespei	PEM A14997	Landdroskop, South Africa	-34.0491	19.0119
S. bonaespei	PEM A15005	Rockview Dam, South Africa	-34.1956	18.9495
S. bonaespei	PEM A15006	Rockview Dam, South Africa	-34.1956	18.9495
S. fasciatus	PEM A15007	Saarsveld, South Africa	-33.9600	22.5300
S. fasciatus	PEM A15009	Wilderness, South Africa.	-33.9967	22.6001
S. fasciatus	PEM A11161	Groendal Nature Reserve, Rooikrans, South Africa	-33.7131	25.2900
S. fasciatus	PEM A11426	Next to dam at Katberg gate, Fort Fordyce Nature Reserve, South Africa	-32.6631	26.4789
S. fasciatus	PEM A11800	Fort Fordyce NR, vlei north of office, South Africa	-32.6786	26.4883
S. fasciatus	PEM A11803	Sandiles Rest, bottom trout dam, South Africa	-32.6639	27.2992
S. fasciatus	PEM A11804	Sandiles Rest, bottom trout dam, South Africa	-32.6639	27.2992
S. fasciatus	PEM A12357	Mazeppa Bay, pond midway to Manubi Forest, South Africa	-32.5297	28.6304
S. fasciatus	PEM A12359	Mazeppa Bay, pond midway to Manubi Forest, South Africa	-32.5297	28.6304
S. fasciatus	PEM A12416	The Island Nature Reserve, Trap 1, South Africa	-33.9875	25.3426
S. fasciatus	PEM A12422	The Island Nature Reserve, Trap 5, South Africa	-33.9792	25.3721
S. fasciatus	PEM A13872	Ingeli Lodge Outdoor Wedding Venue, South Africa	-30.5426	29.6801
S. fasciatus	PEM A8651	Driefontein, Mvuma, Zimbabwe	-19.4169	30.7144
S. fasciatus	PEM A8739	Natural Pool above (to east) Prime Minister's Lodge, Sehla-ba-Thebe, Lesotho	-29.8578	29.1236
S. fasciatus	PEM A9070	Covie, near Natures Valley, South Africa	-33.9500	23.6017
S. fasciatus	PEM A9073	Nature's valley, South Africa	-33.9761	23.5611
S. fasciatus	MHNG 1295.73	Selbourne, Nyanga, Zimbabwe	-18.3834	32.7044
S. fasciatus	MHNG 2162.80	Giant's Castle National Park, Drakensberg, 2000m, South Africa	-29.0720	29.4287
S. fasciatus	MHNG 2688.66	Drakensberg Gardens, road near Underberg, South Africa	-29.7533	29.4244
S. grayii	MHNG 2690.28	Klipheuwel flooded wheat field, South Africa	-33.6968	18.7243
S. grayii	PEM A14998	Stellenbosch, South Africa	-33.9321	18.8600
S. grayii	PEM A14999	Stellenbosch, South Africa	-33.9321	18.8601
S. grayii	PEM A15000	Sedgefield, South Africa	-26.9322	22.8328
S. grayii	PEM A15003	Hogsback, South Africa	-32.5951	26.9322
S. grayii	PEM A15004	Hogsback, South Africa	-32.5951	26.9322
S. grayii	PEM A11428	Top of Katberg pass, Devil's Bellow, Katberg, South Africa	-32.4272	26.6575
S. grayii	PEM A12229	Darling, pond at entrance to town, South Africa	-33.3775	18.3831
S. grayii	PEM A11678	Bergvliet, South Africa	-34.0572	18.4550
S. grayii	PEM A12418	The Island Nature Reserve, Trap 3, South Africa	-33.9835	25.3407
S. grayii	PEM A12420	The Island Nature Reserve, Trap 5, South Africa	-33.9792	25.3721
S. grayii	PEM A13559	Finella Falls, gate to falls, South Africa	-32.3729	26.3569
S. grayii	PEM A13863	Mpur Forestry, Franklin, South Africa	-30.3305	29.5184
S. grayii	PEM A13864	Mpur Forestry, Franklin, South Africa	-30.3305	29.5184
S. grayii	PEM A13870	Gomo Forest Stop 2, South Africa	-31.0102	29.3564
S. grayii	PEM A13871	Elandskuil Guest Farm, South Africa	-30.5206	29.6646
S. grayii	PEM A13881	Tower above Ingeli Forest, South Africa	-30.5216	29.6919
S. grayii	MHNG 2162.81	Giant's Castle National Park, Drakensberg, 2000 m, South Africa	-29.0887	29.3947

Species	Voucher	Locality	Latitude	Longitude
S. grayii	MHNG 2162.82	Giant's Castle National Park, Drakensberg, 2000 m, South Africa	-29.0887	29.3947
S. grayii	MHNG 2701.1	Sewefontein, South Africa	-32.5854	19.0039
S. grayii	MHNG 2701.2	Sewefontein, South Africa	-32.5854	19.0039
S. grayii	PEM A12228	Baviaanskloof, Bergplaas to Witte River Hike, South Africa	-33.6283	24.4623
S. grayii	PEM A5805	Mont aux Sources, South Africa	-28.6619	28.9993
S. grayii	PEM A5863	Strand, South Africa	-34.0946	18.8209
S. grayii	PEM A5885	Sabie, South Africa	-24.8911	30.7543
S. grayii	PEM A5886	Sabie, South Africa	-24.8911	30.7543
S. grayii	PEM A6421	Baardskeerdersbos, South Africa	-34.5875	19.5703
S. grayii	PEM A6895	Plettenberg Bay, Zenon street at Nature Conservation office, South Africa	-34.0347	23.3669
S. grayii	PEM A9072	Covie, near Nature's valley, South Africa	-33.9500	23.6017
S. grayii	PEM A9074	Nature's Valley, South Africa	-33.9761	23.5611
S. grayii	PEM A9542	Paapenkuils, Bridgemead, South Africa	-33.9241	25.4703
S. grayii	PEM A9664	Seal Point Nature Reserve, Cape St. Francis, South Africa	-34.2069	24.8186
S. grayii	PEM A963	Springbok, South Africa	-29.6800	29.6800
S. grayii	PEM A964	Springbok, South Africa	-29.6831	29.6831
S. grayii	PEM A965	Springbok, South Africa	-29.6831	29.6831
S. grayii	PEM A966	Springbok, South Africa	-29.6831	29.6831
S. grayii	PEM A967	Springbok, South Africa	-29.6831	29.6831
S. grayii	PEM A968	Springbok, South Africa	-29.6831	29.6831
S. grayii	PEM A15008	Studers Pass, South Africa	-30.4281	18.0616
S. merumontanus	MHNG 2619.86	Iringa, Udzungwa, Masisiwe, Tanzania	-8.2833	35.9000
S. merumontanus	MHNG 2699.29	Idete, Lukosi River, Tanzania	-8.1000	36.4833
S. merumontanus	PEM A14987	Kitetele, Mbeya Mountain, Tanzania	-8.8401	33.5331
S. merumontanus	PEM A14988	Kitetele, Mbeya Mountain, Tanzania	-8.8401	33.5331
S. merumontanus	PEM A14989	Kitetele stream. 1 km North, Tanzania	-8.8401	33.5331
S. merumontanus	PEM A14990	Ndumbe stream, Poroto mts, Tanzania	-9.0225	33.2122
S. merumontanus	PEM A14994	Mbizi Hill Forest Reserve, Tanzania	-7.8932	31.68
S. merumontanus	PEM A10547	Kitulo Plateau, Tanzania	-9.0772	33.8558
S. merumontanus	PEM A11185	Mabu Forest Summit Camp, Mozambique	-16.2968	36.3924
S. merumontanus	PEM A11186	Mabu Forest Summit Camp, Mozambique	-16.2968	36.3924
S. merumontanus	PEM A11187	Mabu Forest Summit Camp, Mozambique	-16.2968	36.3924
S. merumontanus	PEM A11352	Mt Namuli, Muretha Plateau, Mozambique	-15.3877	37.0434
S. merumontanus	PEM A11358	Mt Namuli, Muretha Plateau, near trap 1, Mozambique	-15.3877	37.0434
S. merumontanus	PEM A11359	Mt Namuli, Muretha Plateau, near trap 1, Mozambique	-15.3877	37.0434
S. merumontanus	PEM A11357	Mt Namuli, Muretha Plateau, Mozambique	-15.3881	37.0452
S. merumontanus	PEM A11650	Muretha Plateau camp, Mt Namuli, Mozambique	-15.3877	37.0434
S. merumontanus	ZMB 79033	Umbwe route, Mt Kilimanjaro, Tanzania	-3.1800	37.2800
S. merumontanus	ZMB 79034	Nkweseko, Mt Kilimanjaro, Tanzania	-3.1824	37.2426
S. merumontanus	ZMB 79035	Marango route, Mt Kilimanjaro, Tanzania	-3.2228	37.5146
S. merumontanus	ZMB 79036	Kidia Forest, Mt Kilimanjaro, Tanzania	-3.2803	37.4406
S. merumontanus	ZMB 79037	Mweka Dam, Mt Kilimanjaro, Tanzania	-3.2166	37.3425
S. merumontanus	CAS 225066	Kitumbeine Forest, Tanzania	-2.9000	36.23501
S. merumontanus	PEM A7499	Kitumbeine forest, Tanzania	-2.9044	36.2350
S. merumontanus	CAS 225065	Kitumbeine forest, Tanzania	-2.9044	36.2350
S. merumontanus	CAS 225066	Kitumbeine forest, Tanzania	-2.9044	36.2350
S. merumontanus	CAS 225067	Kitumbeine forest, Tanzania	-2.9044	36.2350

Species	Voucher	Locality	Latitude	Longitude
S. merumontanus	PEM A14991	Mt Meru, Tanzania	-3.2998	36.9366
S. merumontanus	PEM A14992	Mt Meru, Tanzania	-3.2998	36.9366
S. merumontanus	PEM A14993	Mt Meru, Tanzania	-3.2998	36.9366
S. rhodesianus	MHNG 1295.72	Gleneagles, Inyanga, Zimbabwe	-17.8700	30.9100
S. rhodesianus	PEM A2792	Mt Gorongosa, Mozambique	-18.5928	34.2421
S. rhodesianus	PEM A2793	Mt Gorongosa, Mozambique	-18.5928	34.2421
S. rhodesianus	PEM A6425	Chirinda Forest, Zimbabwe	-20.4193	32.6832
S. wageri	NMSA-HER-005343-D2	Weza Forest, Harding, South Africa	-30.6407	29.7450
S. wageri	NMSA-HER-005343-D5	Weza Forest, Harding, South Africa	-30.6407	29.7450
S. wageri	NMSA-HER-005980	Qudeni, South Africa	-28.6077	30.8650
S. wageri	NMSA-HER-006409	Lundy's Hill, South Africa	-29.7256	29.9370
S. wageri	NMSA-HER-006410	Lundy's Hill, South Africa	-29.7256	29.9370
S. wageri	NMSA-HER-006563	Bannerman Hut area, South Africa	-29.2514	29.4420
S. wageri	NMSA-HER-007330	Karkloof Nature Reserve, South Africa	-29.2999	30.2800
S. wageri	NMSA-HER-007352	Entumeni Nature Reserve, South Africa	-28.8889	31.4640
S. wageri	NMSA-HER-007353	Entumeni Nature Reserve, South Africa	-28.8889	31.4640
S. wageri	NMSA-HER-007356	Giant's Castle Game Reserve, South Africa	-29.3451	29.4820
S. wageri	NMSA-HER-007357	Giant's Castle Game Reserve, South Africa	-29.3451	29.4820