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Herpetological Conservation and Biology 14(2):380–392.
Submitted: 29 January 2019; Accepted: 15 May 2019; Published 31 August 2019.

Origin of Invasive Populations of the Guttural Toad (*Sclerophrys gutturalis*) on Reunion and Mauritius Islands and in Constantia, South Africa

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Abstract.—**The Guttural Toad, *Sclerophrys gutturalis*, has three established invaded regions: on Mauritius and Reunion islands, and in Constantia, a peri-urban area of Cape Town, South Africa. The native range of this toad covers much of central and southern Africa. Here we use mitochondrial DNA (mtDNA) to sample across the range of the natural distribution (from Ethiopia to South Africa) and compare ND2 and 16S sequences to those from animals sampled from each of the three invaded regions. We show that all individuals in invaded regions refer to the same mtDNA clade, which is naturally distributed in north-eastern South Africa, but not from adjoining Mozambique or southernmost Eastern Cape areas. Our findings corroborate previous reports of deliberate introductions from South Africa to Mauritius, and from Mauritius to Reunion. Similarly, our results suggest a single accidental translocation within South Africa from the northeast to Constantia. Our findings highlight the combination of anthropophilic behavior, and extreme long-distance dispersal occurring with accidental translocation for this species. We caution that accidental pathways are likely to continue into the future, with increasing numbers of invasive populations of this species**.

Key Words.—Amphibia; Anura; biogeography; phylogeography; invasive species; mitochondrial DNA; pathways

Introduction

Biotic invasions have become one of the major drivers of biological extinctions (Clavero and García-Berthou 2005; Simberloff et al. 2013), and threats to biodiversity (United Nations Environment Programme [UNEP], 2011) in recipient habitats. The major environmental and economic impacts caused by invasive species (e.g., Pimental et al. 2000) has led to this risk being recognized as one of the four major threats to biodiversity, as outlined in the Convention on Biological Diversity (see UNEP 2011). Although the spread of invasive species is not a new phenomenon, understanding the impacts and the pathways of invasive species has only recently become a strategic priority (McGeoch et al. 2016). Understanding pathways is of particular interest as it affects the success of invasions (Wilson et al. 2009), and without this we have little hope of stemming the ever-increasing tide of invasive propagules. van Wilgen et al. (2018) provided a systematic review of published papers on herpetofaunal alien species, which calls for more investigations of pathways as these are currently under-represented in the literature.

Numerous amphibian introductions have occurred across the globe, and frogs (Anura) have the highest rate of successful establishment (56%: Kraus 2009). Although the literature on impacts comprises the greatest proportion (50%: van Wilgen et al. 2018), it still only represents a minority of species (41%: Measey et al. 2016). Pathways of introduction change over time; introduction of alien species as a means of biological control was once a common pathway of introduction, which has now dwindled to an insignificant level. In contrast the influence of the pet trade has risen to prominence (Wilson et al. 2009), particularly for herpetofauna (Schlaepfer et al. 2005). Some cases of invasions are so well documented that the number of individuals and the date of introduction was recorded, as was the case for the Cane Toad, *Rhinella marina* (see Easteal 1981). However, introductions of other, more cryptic, species require retrospective techniques that can uncover the origin and introduction pathway (e.g., Tolley et al. 2007; De Busschere et al. 2016; Vences et al. 2017; Mohanty and Measey 2018), to help build information on impact severity (Kulhanek et al. 2011).

The Guttural Toad, *Sclerophrys gutturalis* (Fig. 1), is a case in point. Measey et al. (2017) reviewed the invasion pathways for this and other alien amphibians in southern Africa. The first introductions of this species were an attempt of biocontrol for the cane beetle, *Phyllophaga smithi*, to the Republic of Mauritius in 1922 by Gabriel Regnard, a Director of the dock management company

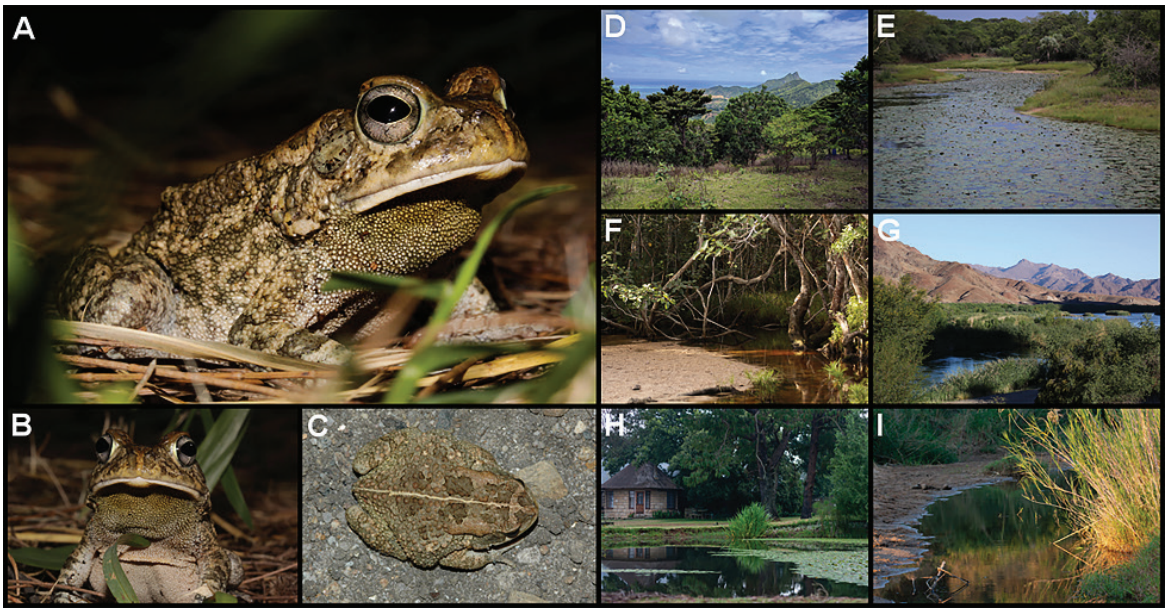

Figure 1. (A-C) The Guttural Toad, *Sclerophrys gutturalis*. Examples of suitable habitat for this species: (D) Mauritius Island forest, (E) perennial farm dam in KwaZulu-Natal, South Africa, (F) natural seep near Sodwana Bay in northern KwaZulu-Natal, South Africa, (G) the Orange River on the border between South Africa and Namibia, (H) urban pond in Chrissiesmeer, Mpumulanga Province, South Africa and (I) slow flowing tributary in the Hluhluwe-Umfolozi National Park, KwaZulu-Natal, South Africa (A, B, E-I: Photographed by Nicolas S. Telford; C and D Photographed by John Measey).

in Port Louis, Mauritius (Cheke and Hume 2008). Despite the detailed timing of this introduction, it is not known from where Regnard obtained his Guttural Toads. Toads were then moved from Mauritius to neighboring Reunion Island around 1927 as a biocontrol for malarial carrying mosquitoes (Cheke and Hume 2008), from where they quickly colonized the lower areas of the island. A more recent introduction of this species was to peri-urban Constantia, Cape Town, South Africa, where males were first heard calling from a private property in 2000 (De Villiers 2006). It is speculated that eggs or tadpoles were accidently introduced through a consignment of aquatic plants from Durban, South Africa (De Villiers 2006; Measey et al. 2017); however, an anecdotal record of deliberate movement of many adult toads from Eastern Cape Province, South Africa, is also present in the literature (De Villiers 2004).

The Guttural Toad is a common and large (up to 140 mm snout-vent length) bufonid, widely distributed in a variety of habitats from sea level to about 1,900 m in southern Africa (Channing 2001; du Preez et al. 2004). It is a habitat generalist that occurs in grasslands, thickets, various savannahs, and agricultural lands, as well as being abundant in peri-urban areas, where it often breeds in garden ponds (Channing 2001; du Preez et al. 2004; Fig. 1). Since their introduction, Guttural Toads have become widespread across both Reunion and Mauritius (Cheke and Hume 2008; Sanchez and Probst 2016), and it was suggested that that they have impacted endemic snail populations (Griffiths and Florens 2006). In Constantia, South Africa, their population has consistently expanded by up to 8 km^2 through leading-edge dispersal (Vimercati et al. 2017a). More recently a jump dispersal of 10 km was facilitated to the nearby suburb of Noordhoek (Measey et al. 2017), opening up the possibility that this species may invade a far larger area of the Western Cape. In this study, we aim to clarify the invasion history of the Guttural Toad, specifically to identify the source population(s) and determine whether the introduction to Constantia was deliberate from the Eastern Cape Province or accidental from the Durban region of the KwaZulu-Natal province. We do this by using a dataset of homologous mtDNA sequences from across its range, determine the area likely used for introductions to Mauritius and Cape Town, and by contrasting the genetic diversity from areas of deliberate introduction in Mauritius and Reunion with that of the presumed accidental introduction into Constantia.

Materials and Methods

Data collection.—We obtained tissue samples in the form of liver, thigh muscle, or toe clips from across the Guttural Toad natural range from the herpetological tissue bank at the South African National Biodiversity Institute (SANBI), or our own collections. In addition, we collected toe clips from the Guttural Toad range within South Africa between January and March 2014 and Mauritius in December 2015. The Invasive Alien Animal Working Group of the Cape Action for People

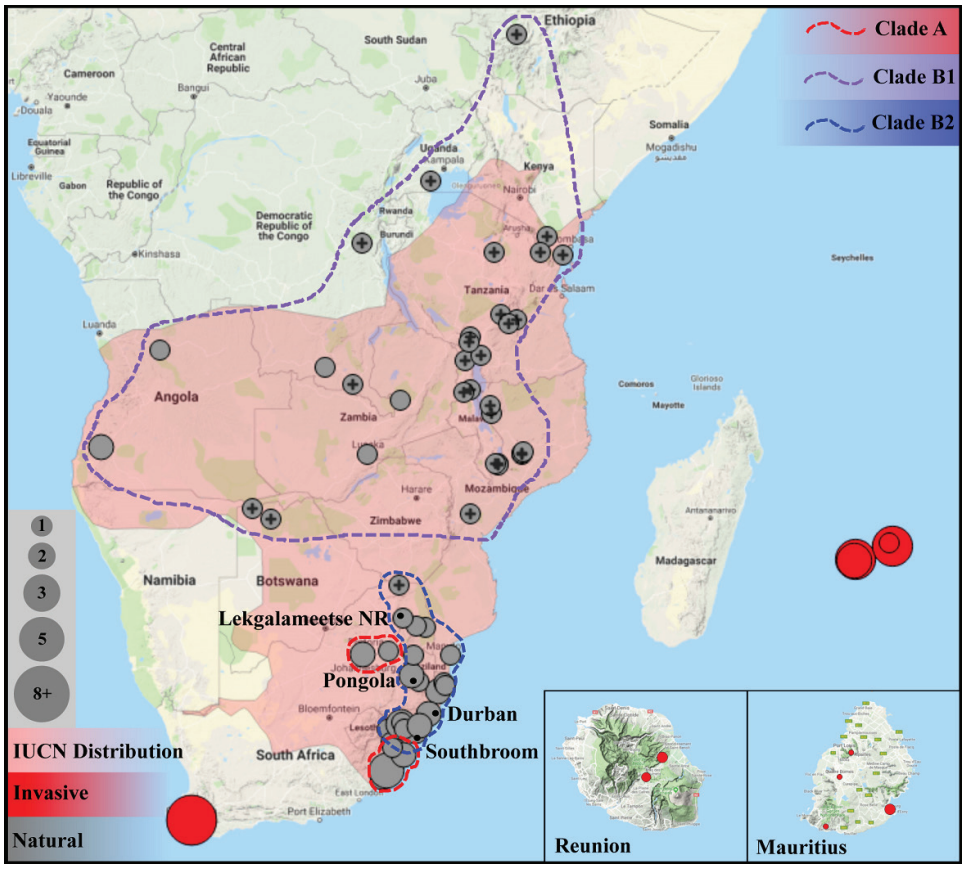

Figure 2. Sample localities used in this study. Grey filled circles are Guttural Toad (*Sclerophrys gutturalis*) samples from within their native range sequenced for ND2 and 16S. Circles with black crosses represent sample sites where only 16S data was available. Red circles are Guttural Toads from their invasive range. The sizes of circles indicate sample size at each locality. The area shaded in red represents the current International Union for the Conservation of Nature (IUCN) distribution for S. gutturalis (IUCN SSC-ASG. 2016. Sclerophrys gutturalis IUCN Red List of Threatened Species. Available from http://www.iucnredlist.org [Accessed 19 January 2018]). Dotted lines represent the approximate distribution of the identified clades.

and the Environment (CAPE) supplied samples from the Cape Town invasive population collected during their extirpation campaign. We obtained further tissue samples from Mauritius and Reunion from Claudia Baider and Giovanni Vimercati, respectively. We consider all three introduced Guttural Toad populations as invasive, as they meet the criteria provided in the definition by Richardson et al. (2011).

Because of the reported origins for the invasions, we focused our sampling efforts along the eastern seaboard of South Africa and achieved good sampling coverage from central Eastern Cape northwards through KwaZulu-Natal and into the Mpumulanga and Limpopo Provinces (Fig. 2). For the remainder of the South African range of the species and outside of South Africa, we managed to get sparse coverage with large distances between samples where sequence data from both our chosen genetic markers were available (Fig. 2). Our sampling efforts yielded 100 Guttural Toad samples and two outgroup samples that we used in our analyses. Of these, we also included 44 samples from GenBank (including 42 published by Liedtke et al. 2016; see Appendix Table) and the remaining 98 were tissue samples. A further 42 samples, whose sequences were available for only one of our chosen markers, improved our sample coverage across the species range outside of South Africa (Fig. 2).

Data analysis.—Using standard proteinase K/SDS procedures (Palumbi 1996), we digested all tissue samples (n = 98; about 10 mg) and extracted genomic DNA using either the standard phenol/chloroform method (Palumbi 1996), or a salt extraction (Aljanabi and Martinez 1997). We used the Polymerase Chain Reaction (PCR) to amplify segments of both the 16S rRNA (16S) and NADH dehydrogenase subunit 2 (ND2) markers. To amplify the 16S rRNA and ND2 mitochondrial gene fragments, we used the 16SaR (5'-CGC CTG TTT ATC AAA AAC AT-3') and 16SbR (5'-CCG GTC TGA ACT CAG ATC ACG T-3'; Palumbi et al. 2002) primer pair and the vMet2 (5'-GCT AAA CAA GCT TTC GGG CCC ATA CC-3') and vTrp (5'-CTC

CTG CTT AGG GCT TTG AAG GC-3'; Cunningham and Cherry 2004) primer pair respectively.

For most PCR reaction mixes, we prepared 25 µl reactions using 4 µl of about 20 ng/µl DNA template, 1 µl of each forward and reverse primer, 6.5 µl ddH20 and 12.5 µl FastTaq polymerase ready mix (Kapa Biosystems) with an MgCl2 concentration of 1.5 mM/µl. For all other PCR reactions, we prepared a 25 µl PCR mix containing 2 µl of about 20 ng/µl DNA template, 2.5 µl Buffer, 2.5 µl 1.5 mM/µl MgCl2, 0.4 µl dNTP, 0.3 µl of both the forward and reverse primers, 0.15 µl taq polymerase (SuperTherm) and 17 µl ddH20. We used standard PCR conditions with 35 cycles and annealing at 51° C for 16S and 57° C for ND2 to amplify products, which we viewed under ultra-violet light on 0.7–1% agarose gels stained with ethidium bromide. We sent successfully amplified PCR product to either the Central Analytical Facility (CAF) at Stellenbosch University or Macrogen Inc. (Amsterdam, Netherlands) for Sanger sequencing of the forward strands and deposited all new sequence data in GenBank (MK759949 - MK760065; MK806289 – MK806386; see Appendix Table for a detailed list).

We included all samples from GenBank where both 16S and ND2 sequences were available (AF220875, AF220878, AF463777 and AF463778) and used the Kisolo Toad, *Sclerophrys kisoloensis*, (AF220891 and AF463788) and the Cameroon Toad, *S. camerunensis*, (AF220893 and AF463789) as outgroups, following Cunningham and Cherry (2004). We aligned and edited sequences in Geneious Pro v4.8.5 (http://www.geneious.com; Kearse et al. 2012) and translated the alignment of the protein coding ND2 marker into amino acids to detect for stop codons. We did not detect any stop codons, which suggested that no pseudogenes were sequenced. The final alignment lengths were 547 bp and 861 bp for the 16S and ND2 markers, respectively.

We inferred phylogenetic relationships using Bayesian inference and maximum likelihood (ML) methods. To assess the evolutionary model that best fitted each partition using the Akaike Information Criterion (AIC) test, we used jModelTest2 (Guindon and Gascuel 2003; Darriba et al. 2012) on the Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway v3.3 (Miller et al. 2010). We identified the best substitution models for each marker as HKY + G and TIM1 + G for the 16S and ND2 fragments, respectively. We conducted the analysis using MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003) and RAxML v.7.3 (Stamatakis 2006) through the CIPRES Science Gateway (Miller et al. 2010). We assigned the best fit models identified for each marker to each partition when running MrBayes and ran the Markov Chain Monte Carlo (MCMC) for 10 million generations with a 10% burn-in. We used Tracer v1.7.1 (Rambaut et al. 2018) to verify that the effective sample size (ESS) was above 200 for all parameters. For both partitions, we used the GTR + I + G model with 1000 bootstrap replicates for the ML analysis.

To further investigate the possible source populations of the invasive populations, we created a TCS haplotype network (Clement et al. 2002) in PopART v1.7 (Leigh and Bryant 2015). We did not include samples from Botswana, Zimbabwe Malawi, Namibia, Kenya, or Tanzania as well as southern Ethiopia at the extreme edge of the species range as we had only 16S sequences for these individuals. To use all available data and to further corroborate our results, we created a second TCS network using all available 16S sequence data in PopART v1.7 (Leigh and Bryant 2015; Appendix Figure).

To investigate intra-clade diversity, we used standard measures of genetic variation. Using the software Arlequin 3.5 (Excoffier and Lischer 2010), we calculated nucleotide diversity (π), the probability that two randomly chosen homologous nucleotides are different (Tajima 1983; Nei 1987) and haplotype diversity (h), the probability that two randomly chosen haplotypes are different (Nei 1987). Using the concatenated data set of 1,408 bp, we ran jModelTest2 (Guindon and Gascuel 2003; Darriba et al. 2012) via the CIPRES Science Gateway v3.3 (Miller et al. 2010) to identify the best model and gamma shape and found the TVM + G model with $\alpha = 0.02$ to be the best model. Arlequin 3.5 (Excoffier and Lischer 2010) does not support this model and we therefore implemented the closest available model (TrN + G) with the same gamma shape for this analysis.

Results

The Bayesian and Maximum Likelihood phylogenetic analyses produced similar topologies, with all major nodes having good support for both bootstrap and posterior probability (Fig. 3). The resulting topology indicates that the Guttural Toad is monophyletic with respect to the outgroups but is comprised of two major mtDNA clades (A and B; Fig. 3). Clade A contained animals captured from Gauteng Province and in a portion of Eastern Cape Province (South Africa), indicating the possibility of two disjunct populations. Further sampling in the gap between these populations is necessary to further infer the distribution limits of this clade. For convenience, and to further isolate specific geographic regions, we divided clade B into two geographically separate sub-clades (B1 and B2; Figs. 2 and 3). Clade B1 contained all specimens from the northern part of the Guttural Toad range: Angola, Zambia, Democratic Republic of the Congo, and Mozambique; with clade B2 distributed through the KwaZulu-Natal, Mpumulanga, and Limpopo provinces of eastern South Africa (Fig.

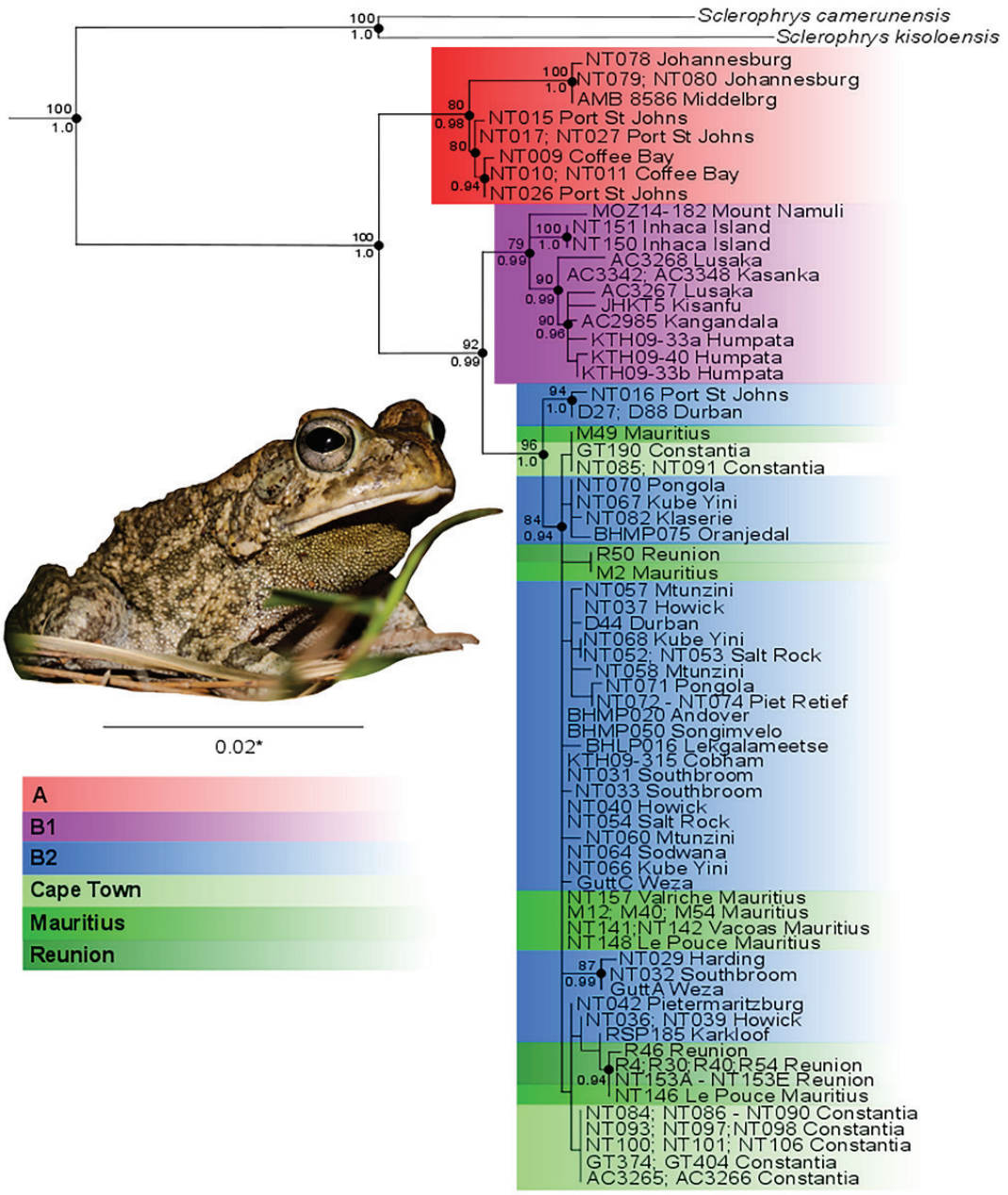

Figure 3. Maximum Clade Credibility (MCC) Bayesian inference phylogeny of the Guttural Toad (*Sclerophrys gutturalis*). Supported nodes are indicated by black circles. Maximum likelihood bootstrap (≥ 75) and Bayesian posterior probability (≥ 0.95) values are indicated above and below, respectively. The color key indicates the clades and where each invasive population is found in the phylogeny. (Photographed by Nicolas S. Telford).

2). The presence of a single sample (NT016) from Port St. Johns (in the Eastern Cape of South Africa) in haplogroup B2 is noteworthy as this animal appears to belong to the Durban region (Fig. 3).

All samples collected from the three invaded regions were found to cluster within clade B2 (Figs. 3 and 4). Samples from this clade were exclusive to a geographic area in north-eastern South Africa: Southbroom in southern KwaZulu-Natal north through Durban to Pongola in northern KwaZulu-Natal and into Mpumulanga with the most northern sample collected from Lekgalameetse Nature Reserve in Limpopo. The geographic region from Southbroom in the south to Lekgalameetse Nature Reserve in the north is the source for all three Guttural Toad invasive populations. We recovered 36 haplotypes from a concatenated data set of 102 samples. Each of the clades are separated by a minimum of five mutational steps. All clades (A, B1,

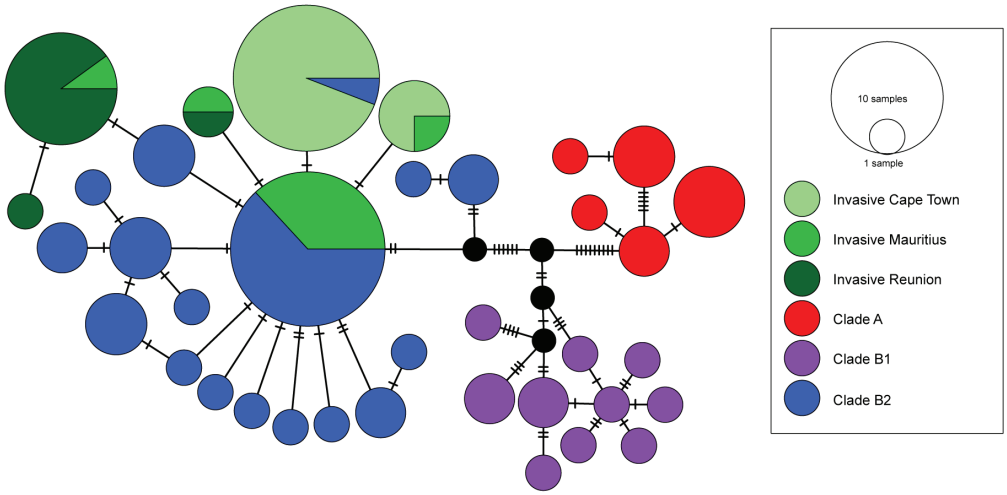

Figure 4. TCS haplotype network of the Guttural Toad (*Sclerophrys gutturalis*). Dashes on the network indicate single mutational steps and black circles represent inferred missing haplotypes. Haplotypes are colored according to the phylogeny and the number of samples in each haplotype is indicated by the size of the circle.

and B2) occupied distinct geographic regions.

The deliberate introduction of Guttural Toads to Mauritius resulted in the highest nucleotide diversity among invasive populations (Table 1). Our results conform to the reports of colonization of toads to Reunion from Mauritius, with Reunion having a reduced nucleotide diversity than Mauritius (Table 1), which mostly fits with haplotypes also found within Mauritius (Fig. 3; Clade B2 - Mauritius M2, NT146 with Reunion R50, R30, respectively); however, Reunion holds an additional 16S haplotype (R46) not recorded elsewhere in our samples (see Appendix Figure). Toads introduced to Constantia had the lowest nucleotide diversity, consistent with an accidental introduction (Fig. 4; Table 1).

We identified 20 haplotypes in clade B2, of which only four were haplotypes found in the three invasive populations, and not recovered from the native range. Although clade B2 consisted of the majority of recovered haplotypes in the best sampled area, it represented the lowest nucleotide diversity (0.0028 ± 0.0015 [standard deviation]). This was in contrast to clades A and B1, which had twice the nucleotide diversity (Table 1). Whereas clade A was from a very small area (61 682 km^2), clade B1 represents the largest area of the distribution with sparse sampling (2,419,460 km^2). Clade A was separated from clade B1 by 14 mutations and from clade B2 by 17 mutations of the concatenated dataset.

The Cape Town invasive population was found to contain only two haplotypes, of which one was not recovered in the natural population and the other shared a haplotype with a sample from Southbroom in KwaZulu-Natal. Four haplotypes were found in the Mauritius invasive population, of which three were not recovered in the natural populations and one matched with the common haplotype that was found throughout the geographic range of clade B2. One haplotype was shared with the sample recovered from Cape Town. Three haplotypes were recovered from the Reunion invasive population, of which two were shared with haplotypes found in Mauritius and one was not recovered from the natural populations or from Mauritius. Nucleotide diversity for both the Cape Town and Reunion invasive populations was significantly lower than the source

Table 1. Relative sample sizes (n) and respective haplotypes (HAP) recorded for the 16S and ND2 markers and the concatenated data set (C). Genetic diversity indicated by nucleotide (π) and haplotype (h) diversity for the three invasive populations of the Guttural Toad (*Sclerophrys gutturalis*) as well as each clade.

		HAP					
Group	n	16S	ND2	C	π (95% CI)	h (95% CI)	Area (km^2)
Cape Town	19	1	2	2	0.000460 ± 0.000419	0.2807 ± 0.1163	9.4
Mauritius	10	1	4	4	0.001478 ± 0.001021	0.5333 ± 0.1801	2,040
Reunion	11	2	2	3	0.001141 ± 0.000827	0.3455 ± 0.1722	2,512
Clade A	11	3	4	5	0.007551 ± 0.004207	0.8182 ± 0.0826	61,682.67
Clade B1	12	7	8	10	0.007079 ± 0.003976	0.9697 ± 0.0443	2,419,460.97
Clade B2	37	7	13	17	0.002801 ± 0.001598	0.8829 ± 0.0438	148,945.19

population (clade B2; Table 1). In contrast, nucleotide diversity of the Mauritius population was found to be slightly lower but similar to the population from the source region (Table 1). A similar pattern was found for haplotype diversity between the invasive populations and the population from the source region (Table 1).

Discussion

Our sampling of Guttural Toads from across their natural range and all known invasive populations suggests that all invasive populations have the same or nearby sources in the northeast of South Africa. As common haplotypes are widespread in this area, and some of the haplotypes of invasive populations were not recovered, we are not able to precisely locate the source population to a specific area or town. Our data, however, do not reject the hypothesis (De Villiers 2006; Vimercati et al. 2017b; Measey et al. 2017) that the source population for the Constantia invasion was an accidental introduction from Durban. Nevertheless, it does seem unlikely that many adults were deliberately translocated from the Eastern Cape as our Eastern Cape samples came from a different clade, and the number of haplotypes found in Constantia was low, indicating a point source was more likely, also reflected by the low nucleotide diversity.

The introduction of Guttural Toads to Mauritius in 1922 was made by Gabriel Regnard, a man with good connections at international ports (Cheke and Hume 2008). Of the potential ports from which Regnard could have obtained Guttural Toads, our data discount Mombassa, Dar es Salaam, Beira, and Maputo. This leaves the ports of Richard's Bay and Durban, both in South Africa, as the two ports that could have acted as sources for Regnard, and have the requisite haplotypes found in our study. Of these, Durban appears most likely being the larger port most frequently connected with Mauritius in the 1920s (Rodrigue 2017). The nucleotide diversities for Guttural Toads from Mauritius and Reunion are both much larger than that of Constantia, corroborating the difference between a deliberate introduction and an accidental introduction, respectively (e.g., De Busschere et al. 2016). That Reunion nucleotide diversity is lower than that of Mauritius substantiates the report that the former was introduced from the latter (Cheke and Hume 2008). Reunion does have a unique allele, representing a single mutational step from a haplotype also found on Mauritius. This could represent incomplete sampling of haplotypes from Mauritius, a genuine mutation that has occurred since introduction to Mauritius and/or Reunion, now nearly 100 y, or it could be a sequencing error. All three invasive populations had haplotypes not recovered in the natural distribution, suggesting that the full genetic diversity of the natural population was not recovered in our sampling, even though we sampled intensely within the clade from which these populations were undoubtedly drawn.

Our geographic sampling also revealed a divergent clade (clade A) within the Guttural Toad phylogeny. Animals from this clade were recovered from two disjunct localities in Gauteng Province (in and around Johannesburg) and on the coast of Eastern Cape Province in the vicinity of Coffee Bay and Port St. Johns. Hewitt (1935) noted that collections from Port St. Johns comprised animals that were notably smaller than elsewhere such that they represented another taxonomic unit. Du Preez et al. (2004) recognized that these same animals corresponded with this same divergent clade (see also Cunningham and Cherry 2004). Although our sampling does not allow us to exclude the possibility that animals from this clade exist in the intervening area between the Gauteng Province and the coastal region of the Eastern Cape Province, it is noteworthy that animals in this area were only recorded between 1996 and 2003 during the South African Frog Atlas Project (du Preez et al. 2004), and that this reflects the suggested range for the species mapped by the International Union for Conservation of Nature (IUCN, 2004). Similarly, Poynton (1964) did not recognize records of Guttural Toads (then *Bufo regularis*) south of Port St. Johns in the Eastern Cape, such that this may represent a relatively recent incursion and even a movement from Gauteng Province to Port St. Johns and Coffee Bay, which are both popular holiday destinations. Further research is required to clarify if the toads in Port St Johns and Coffee Bay form part of the species natural distribution or if they were introduced to the area.

In their review, Measey et al. (2017) highlighted the potential for some southern African anurans to become invasive on other continents, as pathways for trade already exist. Large bodied toads, such asGuttural Toads, are not unusual stowaways in shipping containers (Tingley et al. 2017). Given that they are now present in several international shipping ports, including two of the three invasive populations, it is increasingly likely that they will be transported elsewhere. Modelling the potential distribution of Guttural Toads would likely change dramatically depending on whether the entire native range is used, or only the clade from which invasive populations originated (see Kulhanek et al. 2011). For example, finding a wider range of haplotypes of an invasive population of the Common Platanna (*Xenopus laevis*) in France (De Busschere et al. 2016) has been used as a possible explanation as to why their niche has expanded beyond the native range (Rödder et al. 2017).

Conclusion.—With a growing body of research on the Cape Town Guttural Toad invasion, it is clear that the population has been present since the late 1990s (De Villiers 2006), the population has expanded considerably and it has reached a dominant demographic phase (Vimercati et al. 2017a,b), and has undergone physiological (i.e., greater ability to take up water faster in response to an increase of evaporative water loss) and behavioral (i.e., postural adjustments to minimize water loss) adaptations (Vimercati et al. 2018). The species has been classified as having a moderate impact (Kumschick et al. 2017) using the Hawkins et al. (2015) Environmental Impact Classification of Alien Taxa (EICAT) scheme. The anthropophilic nature of toads means that future accidental movements are likely. The population is expanding through leading edge dispersal as well as two recent cases of jump dispersal (Measey et al. 2017). This study adds to this growing body of research by isolating the source of the invasive population. All three invasive populations are found to stem from the same genetic clade that is distributed in the northeast of South Africa, probably Durban. Although this geographic region is small relative to the entire natural distribution of the species it still covers an area of 148,945 km^2. Previous reports of a deliberate introduction to Mauritius are corroborated by relatively diverse nucleotide diversity, and that animals on Reunion were likely introduced from Mauritius. There is no genetic evidence of a deliberate introduction of Guttural Toad adults into Constantia, Cape Town.

Acknowledgments.—AC and NST would like to thank the National Research Foundation of South Africa for providing funding for this project. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Claudia Bader, Vincent Florens, and Giovanni Vimercati are thanked for collecting and providing tissue samples from Mauritius and Reunion. JM would like to thank the Centre of Excellence for Invasion Biology of the Department of Science and Technology - National Research Foundation (DST-NRF). We would like to thank Anthony Cheke for providing useful discussion and literature. Permits to collect tissue samples were acquired for the Eastern Cape (CRO 21/14CR and 22/14CR) and KwaZulu-Natal (OP 1510/2014) provinces of South Africa.

Literature Cited

Aljanabi, S.M., and I. Martinez. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Research 25:4692–4693.

Channing, A. 2001. Amphibians of Central and Southern Africa. Cornell University Press, Ithaca, New York, USA.

Cheke, A., and J.P. Hume. 2008. Lost Land of the Dodo: An Ecological History of Mauritius, Réunion and Rodrigues. T. and A.D. Poyser, London, UK.

Clavero, M., and E. García-Berthou. 2005. Invasive species are a leading cause of animal extinctions. Trends in Ecology and Evolution 20:110.

Clement, M., Q. Snell, P. Walke, D. Posada, and K. Crandall. 2002. TCS: estimating gene genealogies. Proceedings of the 16th International Parallel Distribution Process Symposium 2:184.

Cunningham, M., and M.I. Cherry. 2004. Molecular systematic of African 20-chromosome toads (Anura: Bufonidae). Molecular Phylogenetics and Evolution 32:671–685.

Darriba D., G.L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772.

De Busschere, C., J. Courant, A. Herrel, R. Rebelo, D. Rödder, G.J. Measey, and T. Backeljau. 2016. Unequal contribution of native South African phylogeographic lineages to the invasion of the African Clawed Frog, *Xenopus laevis*, in Europe. PeerJ 4:e1659. doi:10.7717/peerj.1659

De Villiers, A. 2006. *Bufo gutturalis* Power, 1927. Guttural Toad. Introduced population. African Herp News 40:28–29.

De Villiers, A. 2004. *Bufo pantherinus* A. Smith, 1828. Pp. 71–74 *In* Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland SI/MAB Series #9. Minter, L.R., M. Burger, J.A. Harrison, H.H. Braack, P.J. Bishop, and D. Kloepfer (Eds.). Smithsonian Institution, Washington, D.C., USA.

Du Preez, L.H., C. Weldon, M.J. Cunningham, and A.A. Turner. 2004. *Bufo gutturalis* Power, 1927. Pp. 67–69. *In* Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland SI/MAB Series #9. Minter, L.R., M. Burger, J.A. Harrison, H.H. Braack, P.J. Bishop, and D. Kloepfer (Eds.). Smithsonian Institution, Washington, D.C., USA.

Easteal, S. 1981. The history of introductions of *Bufo marinus* (Amphibia: Anura); a natural experiment in evolution. Biological Journal of the Linnean Society 16:93–113.

Excoffier, L., and H.E.L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources. 10:564–567.

Griffiths, O.L., and V.F. Florens. 2006. A Field Guide to the Non-marine Molluscs of the Mascarene Islands: (Mauritius, Rodrigues, and Réunion) and the Northern Dependencies of Mauritius. Bioculture Press, Rivière des Anguilles, Mauritius.

Guindon, S., and O. Gascuel. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology 52:696–704.

Hawkins, C.L., S. Bacher, F. Essl, P.E. Hulme, J.M. Jeschke, I. Kühn, S. Kumschick, W. Nentwig, J. Pergl, P. Pyšek, and W. Rabitsch. 2015. Framework and guidelines for implementing the proposed IUCN Environmental Impact Classification for Alien Taxa (EICAT). Diversity and Distributions 21:1360–1363.

Hewitt, J. 1935. Some new forms of batrachians and reptiles from South Africa. Records of the Albany Museum 4:283–357.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.

Kraus, F. 2009. Alien reptiles and amphibians: a scientific compendium and analysis. Springer Series in Invasion Ecology 4. Springer Science and Business Media B.V., Netherlands.

Kulhanek, S.A., A. Ricciardi, and B. Leung. 2011. Is invasion history a useful tool for predicting the impacts of the world's worst aquatic invasive species? Ecological Applications 21:189–202.

Kumschick, S., G. Vimercati, F.A. de Villiers, M.M. Mokhatla, S.J. Davies, C.J. Thorp, A.D. Rebelo, and G.J. Measey. 2017. Impact assessment with different scoring tools: how well do alien amphibian assessments match? Neobiota 33:53–66.

Leigh, J.W., and D. Bryant. 2015. PopART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution 6:1110–1116.

Liedtke, H.C., H. Müller, M.O. Rödel, M. Menegon, L.N. Gonwouo, M.F. Barej, V. Gvoždík, A. Schmitz, A. Channing, P. Nagel, and S.P. Loader. 2016. No ecological opportunity signal on a continental scale? Diversification and life-history evolution of African true toads (Anura: Bufonidae). Evolution 70:1717–1733.

McGeoch, M.A., P. Genovesi, P.J. Bellingham, M.J. Costello, C. McGrannachan, and A. Sheppard. 2016. Prioritizing species, pathways, and sites to achieve conservation targets for biological invasion. Biological Invasions 18:299–314.

Measey, G.J., G. Vimercati, F.A. de Villiers, M. Mokhatla, S.J. Davies, C.J. Thorp, A.D. Rebelo and S. Kumschick. 2016. A global assessment of alien amphibian impacts in a formal framework. Diversity and Distributions 22:970–981.

Measey, G.J., S.J. Davies, G. Vimercati, A. Rebelo, W. Schmidt, and A. Turner. 2017. Invasive amphibians in southern Africa: a review of invasion pathways. Bothalia 47(2). a2117. https://doi.org/10.4102/abc.v47i2.2117.

Miller, M.A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 *In* Proceedings of the Gateway Computing Environments Workshop (GCE). New Orleans, Louisiana, USA. https://www.phylo.org/sub_sections/portal/sc2010_paper.pdf

Mohanty, N.P., and J. Measey. 2018. Reconstructing biological invasions using public surveys: a new approach to retrospectively assess spatio-temporal changes in invasive spread. Biological Invasions. https://doi.org/10.1007/s10530-018-1839-4.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, New York, USA.

Palumbi, S.R. 1996. Nucleic acids II: The polymerase chain reaction. Pp. 205–247 *In* Molecular Systematics. Hillis, D.M., C. Moritz, and B.K. Mable (Eds.). Sinauer Associates Inc., Sunderland, UK.

Palumbi, S., A. Martin, S. Romano, W.O. McMillan, L. Stice, and G. Grabowski. 2002. Simple Fool's Guide to PCR, Version 2.0. University of Hawaii, Honolulu, Hawaii, USA.

Pimental, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of non-indigenous species in the United States. BioScience 50:53–65.

Poynton, J.C. 1964. The amphibian of southern Africa: a faunal study. Annals of the Natal Museum 15:1–334.

Richardson, D.M., P. Pyšek, and J.T. Carlton. 2011. A compendium of essential concepts and terminology in invasion ecology. Pp. 409–420 *In* Fifty Years of Invasion Ecology: The Legacy of Charles Elton. Richardson, D.M. (Ed.). Blackwell Publishing, West Sussex, UK.

Rödder, D., F. Ihlow, J. Courant, J. Secondi, A. Herrel, R. Rebelo, G.J. Measey, F. Lillo, F.A. De Villiers, C. De Busschere, and T. Backeljau. 2017. Global realized niche divergence in the African Clawed Frog *Xenopus laevis*. Ecology and Evolution 7:4044–4058.

Rodrigue, J-P. 2017. The Geography of Transport Systems. 4th Edition. Routledge, New York, New York, USA.

Rambaut, A., A.J. Drummond, D. Xie, G. Baele, and M.A. Suchard. 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67:901–904.

Ronquist, F., and J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Sanchez, M., and J.M. Probst. 2016. L'herpétofaune allochtone de l'île de La Réunion (Océan Indien): état des connaissances en 2015. Bulletin de la Société Herpétologique de France 160:49–78.

Schlaepfer, M.A., C. Hoover, and C.K. Dodd, Jr. 2005. Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations. BioScience 55:256–264.

Simberloff, D., J.L. Martin, P. Genovesi, V. Maris, D.A. Wardle, J. Aronson, F. Courchamp, B. Galil, E. García-Berthou, M. Pascal, and P. Pyšek. 2013. Impacts of biological invasions: what's what and the way forward. Trends in Ecology and Evolution 28:58–66.

Stamatakis, A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105:437–460.

Tingley, R., P. García-Díaz, C.R.R. Arantes, and P. Cassey. 2017. Integrating transport pressure data and species distribution models to estimate invasion risk for alien stowaways. Ecography 41:635–646.

Tolley, K.A., S.J. Davies, and S.L. Chown. 2007. Deconstructing a controversial local range expansion: conservation biogeography of the Painted Reed Frog (*Hyperolius marmoratus*) in South Africa. Diversity and Distributions 14:400–411.

United Nations Environment Programme (UNEP). 2011. The strategic plan for biodiversity 2011–2020 and the Aichi biodiversity targets. UNEP/CBD/COP/DEC/X/2, Conference of the Parties to the Convention on Biological Diversity, Tenth Meeting, 18–29 October 2010, Nagoya, Japan. www.cbd.int/decisions/cop/?m=cop-10.

Van Wilgen, N., M. Gillespie, D.M. Richardson, and J. Measey. 2018. A taxonomically and geographically constrained information base limits non-native species risk assessment: the case of reptiles and amphibians. PeerJ 6:e5850. doi 10.7717/peerj.5850.

Vences, M., J.L. Brown, A. Lathrop, G.M. Rosa, A. Cameron, A. Crottini, R. Dolch, D. Edmonds, K.L.M. Freeman, F. Glaw, et al. 2017. Tracing a toad invasion: lack of mitochondrial DNA variation, haplotype origins, and potential distribution of introduced *Duttaphrynus melanostictus* in Madagascar. Amphibia-Reptilia 38:197–207.

Vimercati, G., S.J. Davies, C. Hui, and J. Measey. 2017b. Does restricted access limit management of invasive urban frogs? Biological Invasions 19:3659–3674.

Vimercati, G., S.J. Davies, and J. Measey. 2018. Rapid adaptive response to a Mediterranean environment reduces phenotypic mismatch in a recent amphibian invader. Journal of Experimental Biology (2018) 221, jeb174797. doi:10.1242/jeb.174797.

Vimercati, G., C. Hui, S.J. Davies, and G.J. Measey. 2017a. Integrating age structured and landscape resistance models to disentangle invasion dynamics of a pond-breeding anuran. Ecological Modelling 356:104–116.

Wilson, J.R., E.E. Dormontt, P.J. Prentis, A.J. Lowe, and D.M. Richardson. 2009. Something in the way you move: dispersal pathways affect invasion success. Trends in Ecology and Evolution 24:136–144.

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Appendix Table. Localities of all samples of Guttural Toads (*Sclerophrys gutturalis*) that were used for this study. The abbreviation n represents the number of samples from each locality and HAP is the number of haplotypes at each locality.

Country	Locality	Latitude	Longitude	n	HAP	Genbank Accession Number
Angola	Kangandala	-9.82	16.65	1	1	MK759958; MK806294
	Humpata	-15.12	13.39	3	3	MK759959 – MK759961; MK806295 – MK806297
Botswana	Okavango Delta	-18.98	22.92	1	1	KF665453
	Shakawe	-18.38	21.85	1	1	AF220876
Democratic Republic of the Congo	Kisanfu	-10.76	25.95	1	1	MK759957; MK806293
	Itombwe Mts	-3.86	28.04	1	1	KF665124
	Kasongomukuli	NA	NA	1	1	KF665101
	Lubumbashi	NA	NA	1	1	KF665199
Ethiopia	Jimma	7.82	36.69	1	1	KF665283
Kenya	Taita Hills	-3.51	38.38	1	1	KF665221
	Kayemune Forest	-4.49	39.26	1	1	KF665474
Malawi	Mulanje 1	-16.01	35.65	2	2	KF665044; KF665183
	Mulanje 2	-16.02	35.52	1	1	KF665275
	Mulanje 3	-16.1	35.62	1	1	KF665317
	Nyika Valley	-10.35	33.82	2	2	KF665330; KF665369
	Nkhata Bay	-11.98	34.05	1	1	KF665297
	Luwawa	-12.11	33.72	2	2	KF665204; KF665402
Mauritius	Le Pouce	-20.19	57.53	2	2	MK760016; MK760017; MK806347; MK806348
	Vacoas	-20.29	57.48	2	1	MK760018; MK760020; MK806349; MK806350
	Villa Valriche	-20.49	57.42	1	1	MK760021; MK806351
	Mahebourg	-20.42	57.70	5	3	MK760022 – MK760026; MK806352 – MK806356
Mozambique	Inhaca Island	-26.02	32.96	2	1	MK759963; MK759964; MK806299; MK806300
	Mount Namuli	-15.38	37.04	1	1	MK759962; MK806298
	Ponta do Ouro	-22.42	30.1	2	2	KF665360; KF665487
	Gurue Town 1	-15.47	36.99	1	1	KF665487
	Gurue Town 2	-15.47	36.98	2	2	KF665493; KF665198
	Lichinga Town	-13.3	35.25	2	2	KF665315; KF665051
	Serra Jeci	-12.87	35.19	2	2	KF665042; KF665366
	Gorongosa Town	-18.68	34.07	1	1	KF665054
Reunion	Foret de Grand Etang	-21.09	55.65	6	3	MK760027 – MK760032; MK806357 – MK806362
	La Plaine des Cafres	-21.17	55.58	5	1	MK760033 – MK760037; MK806363 – MK806367
South Africa	Albert Falls	-29.44	30.43	1	1	AF220877

Appendix Table (continued). Localities of all samples of Guttural Toads (*Sclerophrys gutturalis*) that were used for this study. The abbreviation n represents the number of samples from each locality and HAP is the number of haplotypes at each locality.

Country	Locality	Latitude	Longitude	n	HAP	Genbank Accession Number
	Andover	-24.59	31.56	1	1	MK759978; MK806314
	Ashburton	-29.68	30.47	1	1	AF220875
	Cintsa	-30.32	29.61	1	1	KF665463
	Cobham	-29.68	29.39	1	1	MK760002; MK806342
	Coffee Bay	-31.98	29.14	3	1	MK759968; MK759969; MK759972; MK806303 – MK806305
	Constantia	-33.99	18.44	19	2	MK760038; MK760040; MK760044 – MK760046; MK760048 – MK760055; MK760057; MK760058; MK760060; MK760063 – MK760065; MK806368 – MK806386
	Durban North	-29.77	31.03	3	2	MK759999 – MK760001; MK806339 – MK806341
	Franklin	-30.32	29.61	1	1	KF665219
	Harding	-30.57	29.87	1	1	MK759998; MK806338
	Howick	-29.46	30.19	4	3	MK759993; MK759994; MK760011; MK760012; MK806330 – MK806333
	Johannesburg	-25.99	28.01	3	2	MK759967; MK759970; MK759971; MK806306 – MK806308
	Karkloof	-29.32	30.26	1	1	MK760009; MK806346
	Klaserie	-24.54	31.03	1	1	MK759966; MK806302
	Kube Yini	-27.81	32.23	3	2	MK760004; MK760005; MK760008; MK806343 – MK806345
	Lekgalameetse	-24.16	30.34	1	1	MK759983; MK806318
	Middelburg	-25.83	29.49	1	1	MK759965; MK806301
	Mtunzini	-28.93	31.73	3	3	MK759990 – MK759992; MK806327 – MK806329
	Oranjedal	-27.33	31.18	1	1	MK759985; MK806320
	Pietermaritzburg	-29.70	30.39	1	1	MK759995; MK806334
	Piet Retief	-27.01	30.80	3	3	MK759979 – MK759981; MK806315 – MK806317
	Pongola	-27.38	32.63	2	2	MK759988; MK760007; MK806325; MK806326
	Port St Johns	-31.67	29.38	5	4	MK759973 – MK759977; MK806309 – MK806313
	Salt Rock	-29.50	31.23	3	2	MK759987; MK760003; MK760010; MK806322 – MK806324
	Sodwana Bay	-27.51	32.65	1	1	MK759986; MK806321
	Songimvelo	-26.01	30.90	1	1	MK759984; MK806319
	Southbroom	-30.92	30.31	3	3	MK759996; MK759997; MK760015; MK806335 – MK806337
	Weza	-30.57	29.70	2	2	AF220875; AF220878; AF463777; AF463778

Appendix Table (continued). Localities of all samples of Guttural Toads (*Sclerophrys gutturalis*) that were used for this study. The abbreviation n represents the number of samples from each locality and HAP is the number of haplotypes at each locality.

Country	Locality	Latitude	Longitude	n	HAP	Genbank Accession Number
Swaziland	Malalotja	-26.14	31.12	1	1	AF220875
Tanzania	Kiswenbimbi	-7.81	35.8	1	1	KF665126
	Ifakara	-8.09	36.69	1	1	KF665280
	Kilombero	-8.35	36.26	1	1	KF665295
	Kipengere	-9.1	34.08	1	1	KF665159
	Same District	-4.33	38	1	1	KF665467
	Madehani	-9.34	33.99	1	1	KF665437
	Ludewa	-10.1	34.67	1	1	KF665389
	Mt. Hanang	-4.4	35.42	2	2	KF665494; KF665112
Uganda	Lake Nabugabo	-0.34	31.88	1	1	KF665364
Zambia	Kasanka	-12.55	30.16	2	1	MK759954 – MK759956; MK806289 – MK806292
	Lusaka	-15.50	28.27	2	2	MK759952; MK759953

Appendix Figure. 16S TCS haplotype network of the Guttural Toad (*Sclerophrys gutturalis*) using all available sequence data. Dashes on the network indicate single mutational steps and black circles represent inferred missing haplotypes. The number of samples in each haplotype is indicated by the size of the circle.