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Original Research

Frogs of the Makuleke Contractual Park, northern Kruger National Park



Authors:

Chad Keates^{1,2} Ryan J. Wasserman^{1,2} Werner Conradie^{3,4} Farai Dondofema⁵ Linton Munyai⁶ Eddie Riddell^{7,8} Tatenda Dalu^{2,6}

Affiliations:

¹Department of Zoology and Entomology, Faculty of Science, Rhodes University, Makhanda, South Africa

²South African Institute for Aquatic Biodiversity, Makhanda, South Africa

³Port Elizabeth Museum, Gqeberha, South Africa

⁴Department of Nature Conservation Management, Faculty of Science, Nelson Mandela University, George, South Africa

⁵Department of Geography and Environmental Sciences, Faculty of Science, University of Venda, Thohoyandou, South Africa

⁶School of Biology and Environmental Sciences, Faculty of Science, University of Mpumalanga, Nelspruit, South Africa

⁷Kruger National Park, SAN Parks, Skukuza, South Africa

⁸Centre for Water Resources Research, Faculty of Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa

Corresponding author: Chad Keates, chadkeates97@gmail.com



Scan this QR code with your smart phone or mobile device to read online. The Kruger National Park is the largest protected area in South Africa and one of the most extensively surveyed in sub-Saharan Africa. Scientific studies, passive sampling from rangers and citizen science records have resulted in comprehensive faunal species lists spanning the entire park. Albeit, numerous frog records from different sources exist, they reveal contrasting species assemblages for the northern reaches of the park. This inconsistency leads to problems in conducting ecological work and implementing conservation legislation, as the baseline data are not congruent across sources. This is problematic because the northern Kruger National Park is known as the Ramsar-declared Makuleke Wetland System. Although this system receives rigorous conservation efforts, there is a lack of a comprehensive and up-to-date list of frog species. In this study, we aimed to develop an updated regional baseline using a combination of published literature, citizen science and museum records, supplemented with active field surveys. Field surveys of the study region resulted in the identification of 18 species from 10 families of frogs. When combined with existing records, the Makuleke Contractual Park is expected to play host to at least 30 frog species. In addition to collating existing data into a single source, the field component of this study also revealed the first record of Tomopterna natalensis for the area during active surveys, reconfirmed the presence of several frogs, several of which have not been recorded in the region in over 50 years and provided the first confirmed national record of Afrixalus crotalus using phylogenetic reconstruction.

Conservation implications: Comprehensive species lists are fundamental for robust management protocols and ecological research. By collating data from multiple sources, this article presents an improved and updated frog list for the region, which will aid conservation, management and any long-term wetland ecosystem monitoring efforts in the region.

Keywords: amphibians; protected area; biodiversity; species list; African herpetofauna.

Introduction

Frogs are an integral component of most food webs and ecosystems because their complex life cycle enables them to influence energy exchanges within aquatic and terrestrial environments (Cuthbert et al. 2022). They are generally semi-aquatic, requiring water for reproduction purposes while using adjacent riparian and terrestrial habitats as adults (Du Preez & Carruthers 2017). As such, waterbodies (i.e. floodplain pans, vleis, reservoirs and rivers) and their riparian zones typically support an elevated diversity and communities of frogs at the landscape level.

The Kruger National Park (KNP) is one of the largest protected areas in Africa (Eckhardt, Van Wilgen & Biggs 2000), and is characterised by a sprawling network of rivers, streams and floodplain pans (Riddell et al. 2019). For these reasons, the area has been the focus of many scientific studies to elucidate the biodiversity associated with these aquatic ecosystems (Broomker 1994; Majdi et al. 2022; Pienaar 1968; Wolmarans & De Kock 2006). When coupled with eyewitness accounts from rangers, scientists and visitors, KNP has amassed an impressive species list spanning most taxa. The incorporation of technological advances and the popularisation of citizen science platforms has also improved our understanding of species assemblages within the region. This is particularly true of the frogs, with historical publications (e.g. Pienaar, Passmore & Carruthers 1976) and present citizen science platforms (e.g. FrogMap 2023; iNaturalist 2023) contributing to a robust dataset of records spanning the entire park.

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In the north of the KNP, the Makuleke Contractual National Park (MCNP) has received much interest from aquatic ecologists in recent years because of its expansive network of inter-connected permanent and ephemeral pans (Dyamond 2017; Kock 2017; Malherbe et al. 2019). The MCNP is a conservation area in the northern reaches of the KNP that has shared conservation management between South African National Parks (SANParks) and the community of Makuleke who own the land (Figure 1). The park is a product of a newly evolving conservation strategy termed 'community-based natural resource management' (CBNRM) (Reid & Turner 2013). The floodplain pan system, collectively referred to as the Makuleke Wetlands, is a Ramsar-declared wetland system, which can be found mainly within the geographical limits of the MCNP, with several pans located just south of the Luvuvhu River. Although relatively well sampled by aquatic ecologists, the area has received less attention from an amphibian point of view, with much of the frog-centric work being localised south of the Luvuvhu River.

To address the knowledge gap, we aim to investigate the frog diversity within the Makuleke Wetlands region using published literature, citizen science platforms, museum records and active field surveys. In doing so, we will develop an updated baseline to inform conservation practices.

Research methods and design Study site

The Makuleke Contractual National Park is found in the northernmost reaches of the KNP. The area is characterised by low rainfall and high temperatures, which have given rise to a vegetation type that is the dominated by Mopane bushveld vegetation unit (Mucina & Rutherford 2006; Tinley 1981). The area's topography is strongly undulated with rugged low mountains and slopes with sandstone sediments in the central and western areas (Nortje 2014). The southern, northern and eastern regions are slightly undulating and concave with alluvial deposits flanking the Luvuvhu River and Limpopo River (Nortje 2014). The area plays host to an enormous diversity of faunal and floral diversity, largely because of the high densities of ephemeral pans that characterise the region. This resulted in the area being designated as a Ramsar wetland of international importance in 2007 (Matthews 2018).

Field surveys

Field surveys were conducted within the Makuleke Wetlands as part of a larger ecological study of temporary pans within the region. Ten pans were identified; eight north of Luvuvhu River (in the MCNP) and two south of the Luvuvhu River - all of which were considered part of the Makuleke Wetlands system. The pans were surveyed opportunistically over four separate field trips; 07-11 April 2021, 10-16 December 2021, 20-23 March 2022 and 05-07 September 2022, with each pan receiving at least two diurnal and one nocturnal survey over the course of the study. Because of the logistical constraints of working in the park's northern sections, each pan was not surveyed equally, and thus several pans received more than one nocturnal survey over the course of the study. To confirm the presence of several generalist taxa within the study area (not found at pans), one nocturnal search of the Luvuvhu River was conducted during the September field trip.

Desktop assessment

Species records were gleaned from multiple sources within the northern reaches of KNP. Quarter-degree squares (QDS) of approximately 27 km × 27 km (729 km²) were used to determine the presence of species within a particular area, as is standard practice for most South African atlas projects. The extent of QDS 2231AC and QDS 2231AD enveloped the entire study area. Any species recorded within these quarterdegree grid cells, from the different data sources, were considered present in the study area. A combination of published literature, published reports, citizen science records and museum records was used to ensure a thorough representation of the frogs within the region. These included all specimen records from the Skukuza Museum, South African Institute for Aquatic Biodiversity (SAIAB) and the Port Elizabeth Museum (PEM) collection, as well as all records from the 'Atlas and red data book of the frogs of South Africa, Lesotho and Swaziland' (Minter et al. 2004), the latest edition of 'The frogs of the Kruger National Park' (Pienaar et al. 1976) and the most recent park-wide frog assessment commissioned by the Water Research Commission (Vlok et al. 2013). Lastly, this study also included all citizen science records from iNaturalist (iNaturalist 2023) and FrogMap (FrogMap 2023). Citizen science records were treated with caution as they are based on photos, which may not show the diagnostic features necessary for teasing apart cryptic taxa. Additionally, all citizen science records were verified by W.C. and C.K., with questionable records being omitted, and outdated taxonomy being updated. All records were grouped into three categories, namely pre-2004 (all records in Minter et al. 2004), post-2004 (all records post Minter et al. 2004) and this study (all physical records conducted during field surveys conducted by authors).

Phylogenetic identification of an unidentified *Afrixalus*

The phylogenetic placement of the unidentified *Afrixalus* specimen (PEM A12181) collected at Mapimbana pan in the MCNP was estimated by extracting DNA from the liver sample collected and the amplification of the partial mitochondrial ribosomal gene *16S* rRNA. In addition to the newly generated sequence produced for this study, the dataset was supplemented with published *Afrixalus* sequences from GenBank (ncbi.nlm.nih.gov/genbank).

A standard salt extraction (Bruford et al. 1992) was used to isolate DNA from tissues using lysis (Buffer ATL; Qiagen) and elution (Buffer AE; Qiagen) buffers. Standard PCR procedures were utilised to amplify one partial mitochondrial ribosomal gene *16S* rRNA (Forward primer, L2510: 5'-CGCCTGTTTATCAAAAACAT-3' and reverse primer, H3080: 5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi 1996). Amplification was carried out using 20 ng/ μ L–50 ng/ μ L extracted genomic DNA with a total PCR mixture volume of 25 μ L. Each PCR contained 12.5 μ L TopTaq Mastermix (Qiagen; containing 10x PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs and 0.75 U Taq polymerase), 2 μ L

forward primer (10 µM), 2 µL reverse primer (10 µM) and 8.5 µL of the genomic DNA and de-nucleated water combined. The standard cycling profile comprised an initial denaturing step at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 50°C for 45 s and 72°C for 45 s, with a final extension at 72°C for 8 min. The final PCR products were sequenced (after purification) by Macrogen Corp. in Amsterdam, the Netherlands with the forward primers only. The sequence trace files were checked using BioEdit Sequence Alignment Editor v.7.2.5 (Hall 1999) and aligned on MEGA v.7.0 (Kumar et al. 2016), using the ClustalW plugin function, along with the sequences acquired from GenBank. The optimal scheme and best-fitting models of molecular evolution were selected using ModelFinder, implemented in IQ-TREE v.2.1.2 (Minh et al. 2021), with the following settings: -p partition file (each partition has own evolution rate), a greedy strategy and the FreeRate heterogeneity model excluded (only invariable site and Gamma rate heterogeneity considered) (Chernomor, Von Haeseler & Minh 2016; Kalyaanamoorthy et al. 2017). The best-fitting model scheme for the 16S alignment was TIM2+I+G. Maximum likelihood (ML) analysis was conducted using IQ-TREE v.2.1.2 (Nguyen et al. 2015). The ML analysis was implemented using a random starting tree and assessed using the ultra-fast bootstrap approximation (UFBoot) method (Hoang et al. 2018) and 1000 bootstrap replicates. The final tree was rendered in Figtree v.1.4.2 (Rambaut 2014).

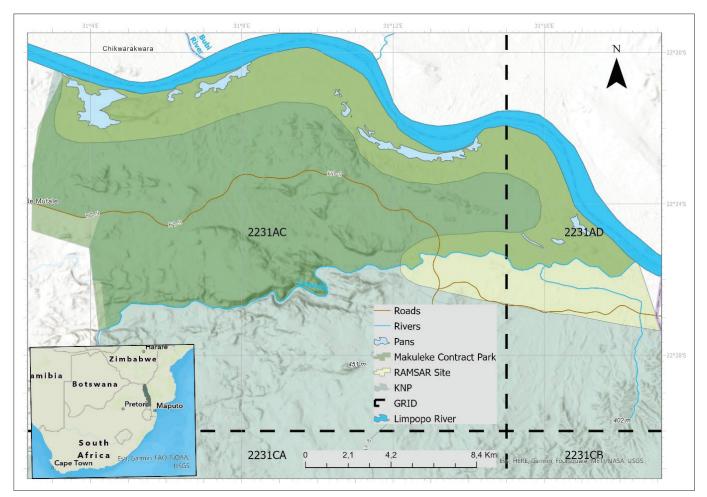


FIGURE 1: Map illustrating the Makuleke Contractual Park (Kruger National Park) with quarter-degree squares (QDS) overlaid.

Ethical considerations

All procedures performed in this study followed all international, national and/or institutional guidelines for the care and use of animals. The research permits were granted by the South African National Parks Agency and Kruger National Park (Reference numbers: SKZ132 and DALT1635). Research ethical clearance was granted by the University of Venda Animal, Environmental and Biosafety Research Ethics Committee (Reference numbers: SES/20/ERM/14/1611 and SES/18/ERM/10/1009).

Results

Field surveys of the study region resulted in the documentation of 18 species comprising 10 families of frogs (Table 1; Figure 2) Voucher specimens representing the amphibian diversity of the region were taken, resulting in the cataloguing of 83 specimens into the PEM herpetological collection (Online Appendix Table 1-OA1). The collection was also supplemented with an additional 58 DNA samples, resulting in a total of 141 novel DNA samples for the region (that can be used in future phylogenetic and conservation research). Using published literature and citizen science records, the most comprehensive species lists for the region came from 'pre-2004' with 28 species being recorded in the region. This was followed by 'post-2004' with 24 species

and 'this study' with 18 species. Using all available records from the literature, citizen science platforms and this study, the total species list for the MCNP was found to be 30 species (Table 1). One of the more notable records from our field surveys was the collection of a Natal sand frog – *Tomopterna natalensis* within the study area (confirmed using keys from Du Preez & Carruthers 2017), as it represented the first record of the species for QDS 2231AD, and the first confirmed record of the species for the region. Phylogenetic reconstruction of *Afrixalus* using the maximum likelihood algorithm (ML) revealed a well-supported sister relationship between GPN02 + PEM A12181 and *A. aureus* Genbank Accession Number - PP913975) (Figure 3).

Discussion

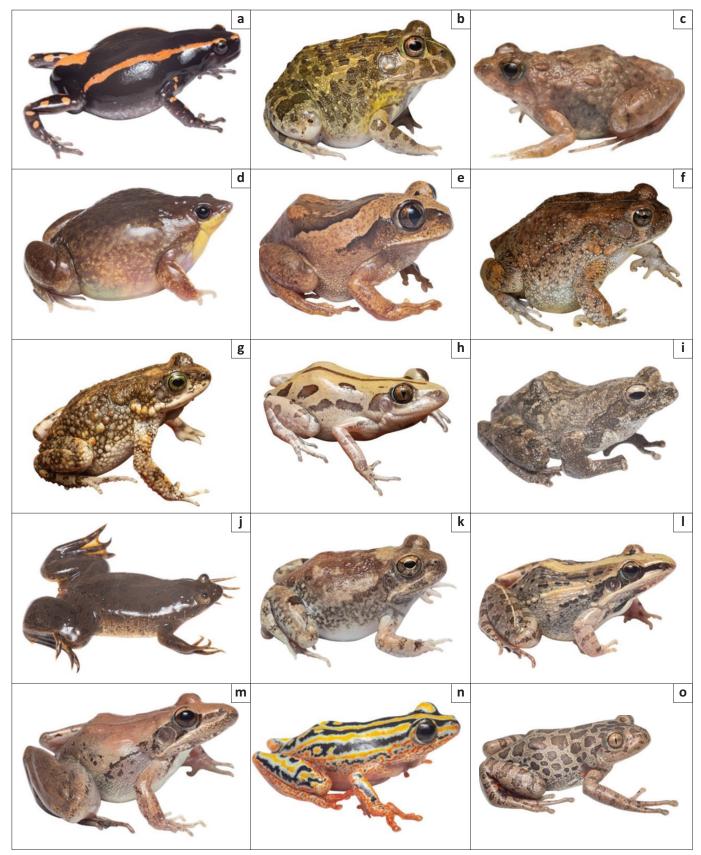
The field surveys combined with historical records produced the most comprehensive species list for the area to date, with a total of 30 frog species within the MCNP. In addition to increasing the known species diversity of the area, this study highlights discrepancies in our understanding of the biodiversity of a region when individual sources are utilised in isolation.

The most comprehensive source, Minter et al. (2004), which filtered into FrogMap (2023), collated data from citizen scientists and museum records to provide nationwide

TABLE 1: List of frog species encountered during surveys and all records from the desktop assessment for Makuleke Contractual Park.

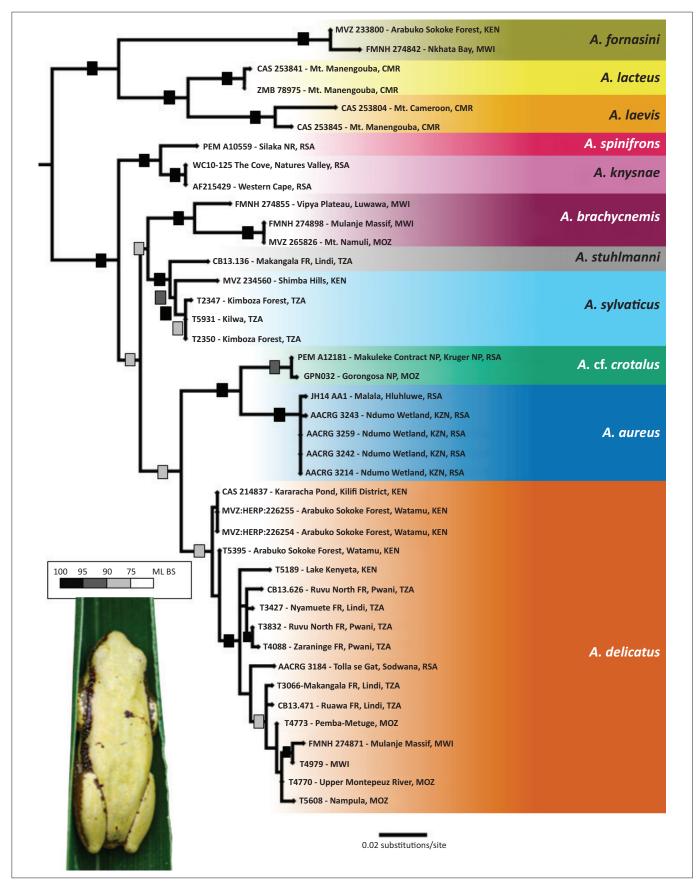
Scientific/current name	Old name (Pienaar et al. 1976)	Common name	Species records		
			This study	Post-2004	Pre-2004
Afrixalus cf. crotalus	Afrixalus brachycnemis brachycnemis	Snoring leaf-folding frog	х	х	x
Amietia delalandii	Rana angolensis	Delalande's river frog	-	х	х
Arthroleptis stenodactylus	-	Common squeaker	-	х	х
Breviceps adspersus†	Breviceps mossambicus adspersus	Bushveld rain frog	-	х	Х
Breviceps mossambicus†	-	Mozambique rain frog	-	-	х
Cacosternum boettgeri	-	Boettgers' caco	-	-	х
Chiromantis xerampelina	-	Southern foam-nest frog	Х	х	х
Hemisus marmoratus	Hemisus marmoratum	Mottled shovel-nosed frog	х	х	х
Hildebrandtia ornata	Hildebrandtia ornata ornata	Ornate frog	-	-	х
Hylambates maculatus	Kassina maculata	Red-legged kassina	х	-	х
Hyperolius marmoratus	Hyperolis viridiflavus	Painted reed frog	Х	х	х
Hyperolius pusillus	-	Water lily frog	-	-	х
Kassina senegalensis	-	Bubbling kassina	Х	х	х
Leptopelis mossambica	Leptopelis? cinnamomeus	Brown-backed tree frog	х	х	х
Phrynobatrachus mababiensis	Phrynobatrachus ukingensis mababiensis	Dwarf puddle frog	Х	х	х
Phrynobatrachus natalensis	-	Snoring puddle frog	-	х	х
Phrynomantis bifasciatus	Phrynomantis bifasciatus bifasciatus	Banded rubber frog	Х	х	х
Poyntonophrynus fenoulheti	Bufo vertebralis fenoulheti	Northern pygmy toad	х	х	х
Ptychadena anchietae	Ptychadena superciliaris	Plain grass frog	Х	х	х
Ptychadena mossambica	-	Broad-banded grass frog	х	х	х
Pyxicephalus edulis	-	African bullfrog	Х	х	х
Schismaderma carens	Bufo carens	Red toad	-	х	х
Sclerophrys garmani	Bufo garmani	Olive toad	Х	х	х
Sclerophrys gutturalis	Bufo regularis	Guttural toad	-	х	-
Sclerophrys pusilla	Bufo pusillus	Eastern flat-backed toad	Х	х	х
Tomopterna adiastola	Tomopterna delalandei cryptotis	Southern sand frog	-	х	х
Tomopterna krugerensis	-	Knocking sand frog	Х	х	х
Tomopterna marmorata	-	Marbled sand frog	-	х	х
Tomopterna natalensis	-	Natal sand frog	х	-	-
Xenopus muelleri	-	Mueller's clawed frog	Х	х	х

†, Recently the taxonomy of Breviceps has undergone several changes (see Discussion). The status of northern KNP Breviceps thus still requires verification.



Source: Photographs taken by C. Keates

FIGURE 2: Select frogs sampled during surveys of the Makuleke Contractual Park: (a) *Phrynomantis bifasciatus* (Banded rubber frog), (b) *Pyxicephalus edulis* (African bull frog), (c) *Phrynobatrachus mababiensis* (Dwarf puddle frog), (d) *Hemisus marmoratus* (Mottled shovel-nosed frog), (e) *Leptopelis mossambicus* (Brown-backed tree frog), (f) *Sclerophrys pusilla* (Flat-backed toad), (g) *Poyntonophrynus fenoulheti* (Northern pygmy toad), (h) *Kassina senegalensis* (Bubbling kassina), (i) *Chiromantis xerampelina* (Southern foam-nest frog), (j) *Xenopus muelleri* (Mueller's platanna), (k) *Tomopterna natalensis* (Natal sand frog), (l) *Ptychadena mossambica* (Broad-banded grass frog), (m) *Ptychadena anchietae* (Plain grass frog), (n) *Hyperolius marmoratus taeniatus* (Painted reed frog) *and* (o) *Hylambates maculatus* (Red-legged kassina).



Source: Photograph taken by Ian Rijsdijk

Note: The insert picture to the phylogeny is the A. cf. crotalus specimen from iNaturalist.

MOZ, Mozambique; RSA, South Africa; MWI, Malawi; TZA, Tanzania; KEN, Kenya; CMR, Cameroon; ML, maximum likelihood; BS, bootstrap.

FIGURE 3: Phylogenetic reconstruction of *Afrixalus* using the *16S* marker and the maximum likelihood algorithm.

biodiversity baselines. While Minter et al. (2004) acknowledged the presence of an Afrixalus sp. north of the Letaba River, these records were not attributed to species level and did not make their way into any recent publications. In Pienaar et al. (1976), however, there are several records of Afrixalus brachycnemis brachycnemis flanking the Luvuvhu River, with one historical record within QDS 2231AD. Since the publication of Pienaar et al. (1976), Afrixalus b. brachycnemis has been split into three species, namely, Afrixalus delicatus, Afrixalus crotalus and Afrixalus aureus (Pickersgill 1984). The only record since Pienaar et al. (1976) is an iNaturalist record from 2022 (www.inaturalist.org/observations/59764353), initially identified as Afrixalus aureus based on its proximity to specimens of the same species from southern KNP. Additionally, our sample (PEM A12181) was also assumed to belong to this species based on its close geographic proximity to the iNaturalist record and all records from Pienaar et al. (1976), which had been recognised as A. aureus in Minter et al. (2004) and Vlok et al. (2013). A superficial look at the morphology and the results of gene blasting (high similarity of PEM A12181 to KZN A. aureus) also suggested A. aureus. Closer inspection of the specimen, however, revealed it to be a different species of Afrixalus entirely, based on the absence of a dorsal tibia band.

Before this study, there were no publicly available records of A. crotalus on GenBank, but based on our phylogenetic reconstruction, the record from Gorongosa National Park (GPN02), which was identified as A. delicatus in Portik et al. (2019), is a misidentified A. crotalus. Delicate leaf-folding frog - Afrixalus delicatus is a widely distributed species, occurring from eastern South Africa, into northern Tanzania. Snoring leaf-folding frog - Afrixalus crotalus is a small, cryptic leaf-folding frog, which according to its original description (Pickersgill 1984) is distributed from eastern Zimbabwe northwards to southern Malawi and eastwards through central Mozambique to the coast. The availability of a more robust dataset has revealed a more expansive distribution that stretches from southern Zambia to the northernmost tip of South Africa (Channing & Rödel 2019). In addition to being morphologically similar, both species are sympatric in central Mozambique, which can confound field identification for these animals (Channing & Rödel 2019).

Although the presence of *A. crotalus*, in South Africa, was alluded to in Channing and Rödel (2019), this is yet to be confirmed with any verifiable records. The well-supported relationship between GPN02 + PEM A12181 and *A. aureus* coupled with the morphological characteristics in our sample, and the iNaturalist record lends strong support to the presence of *A. crotalus* in northern South Africa. While we were unable to confirm the IDs of the northern *Afrixalus* specimens from Pienaar et al. (1976), it is highly likely that these specimens will also represent specimens of *A. crotalus*. This would suggest that *A. crotalus* is restricted to the northern fringes of Limpopo within South Africa while *A. aureus* does not extend north of the Letaba River.

Sclerophrys gutturalis is a widespread and generalist toad species that is broadly expected to occur in the region based on the proximity of records on FrogMap (2023), north and west of the target QDS's and the distribution maps supplied by both Du Preez & Carruthers (2017) and Channing and Rödel (2019). In Vlok et al. (2013), S. guturalis was recorded within the region. Interestingly, neither the combined efforts from our study nor previous research (excluding Vlok et al. 2013) managed to document the species within the MCNP. Sclerophrys gutturalis is one of only two species absent from the pre-2004 list, even though the species is considered locally abundant throughout much of its range. Unlike S. pusilla, which is common in low-lying areas, S gutturalis is less common in these areas and tends to be abundant in the higher-lying, western parts of KNP (Pienaar et al. 1976). Given that much of our sampling was focussed on lowerlying areas, near ephemeral pans, it is unsurprising that we did not encounter S. gutturalis here. Increased sampling in the western parts of the MCNP would likely produce S. gutturalis records.

When comparing the specimen records following the completion of the amphibian red atlas (Minter et al. 2004), several species are yet to be documented again. These include Breviceps mossambicus, Cacosternum boettgeri, Hildebrandtia ornata and Hyperolius pusillus. While the absence of the latter three species is surprising given their presence in adjacent QDS's, the absence of B. mossambicus is likely a product of their cryptic nature. Their terrestrial lifestyle coupled with their specialised reproductive strategy (water-independent tadpole stage) (Du Preez & Carruthers 2017; Passmore & Carruthers 1995) precludes these animals from water edges, which decreases encounter probability. Additionally, rain frogs often call from concealed positions in highly heterogeneous terrestrial environments near burrows. Combined with their preference to call during or after bouts of rain, this makes the discovery of these animals very challenging, with trained herpetologists often resorting to triangulation to locate these animals (Passmore & Carruthers 1995). Although infrequently encountered by past researchers, the environment and microhabitats offered by MCNP are ideal for *Breviceps* spp. In addition to their cryptic nature, the taxonomy of Breviceps spp. is currently in flux and the specimens regarded as B. mossambicus in Pienaar et al. (1976) are likely B. passmorei as B. mossambicus is not expected to occur in South Africa (Nielsen et al. 2018).

The presence of *Tomopterna natalensis* from just north of Pafuri Research Camp, on the boundary of the MCNP was not only the first confirmed record of the species for QDS 2231AD, but the first record for the MCNP. The species has been recorded south of the study area on iNaturalist (www. inaturalist.org/observations/71876167; www.inaturalist. org/observations/70820437), further corroborating our morphological identification of the specimen. *Tomopterna spp.* encountered during the field trips included *Tomopterna krugerensis, Tomopterna adiastola* and *Tomopterna marmorata*. The latter two were omitted from our study survey list as they were recovered close to the Punda Maria Camp Site,

Competing interests

them in writing this article.

Authors' contributions

outside of the designated area for this study. The same applies to *Phrynobatrachus natalensis*, which was also only recovered in the Punda Maria Camp Site during our survey of the region. These samples have, however, been included in Online Appendix Table 1-OA1 for completeness. Although not all documented within the MCNP, all four *Tomopterna* spp. and *P. natalensis* are expected to be found within the Ramsar-declared wetland system based on previous surveys of the region (Table 1).

Conclusion

While the field surveys conducted during this study could not locate and confirm the presence of all the frogs previously recorded from the area, we are confident that a more robust sampling strategy (more time and more sites) would reveal all 30 species. While frogs are often associated with water, their ecologies and behaviours are complex as they also use terrestrial landscapes during their adult stages. Seasonality and climatic conditions coupled with topography and a varying suite of abiotic and biotic conditions work synergistically to create the ideal conditions and microhabitats for different frog species. As we could not cover all sections of the park and focussed on the ephemeral pan systems, it is unsurprising that certain species were not encountered.

These findings, coupled with the smaller size and lower perceived value of anurans, lend support to the possibility of new distributional records and even new species for the area given more sampling effort. This means that although well sampled, knowledge of the area is outdated and thus not subject to the same scrutiny and insights as modern findings. Following this line of reasoning, it is safe to assume that there is still much to uncover in the MCNP. Prior to this study, Hylambates maculatus, Xenopus muelleri and Sclerophrys pusilla (FrogMap 2023) had not been recorded in their quarter degree cells since 1960, 1973 and 1996, respectively. This highlights the importance of these studies in confirming species presence in areas through time, which empowers conservation practitioners with the tools necessary to make informed decisions regarding species assemblages. Lastly, in addition to providing a robust species and updated list for the MCNP, this study highlights the taxonomical changes (Table 1), since Pienaar et al. (1976), and provides the most up-to-date nomenclature for biologists, conservations and managers working in KNP.

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The authors declare that they have no financial or personal

relationship(s) that may have inappropriately influenced

C.K., T.D., F.D., R.J.W. and W.C. conceived the study. C.K.,

L.M., F.D. and T.D. carried out fieldwork and data capture.

C.K. interpreted the results with the help of all the other coauthors. T.D., F.D. and E.R. arranged permitting and all other

necessary permissions. T.D., R.J.W., F.D. and W.C. provided

funding for the project. C.K. wrote the first draft of the article.

C.K. collected and preserved the voucher samples and

W.C. confirmed identifications and catalogued material into

feedback and helped shape subsequent drafts of the article.

Data availability

The data that supports the findings from this study are openly available on citizen science platforms and voucher specimens can be viewed at the Port Elizabeth Museum Herpetological Collection. All genetic material can be found on Genbank (nih.org).

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

References

- Boomker, J., 1994, 'Parasites of South African freshwater fish, VI. Nematode parasites of some fish species in the Kruger National Park', Onderstepoort Journal of Veterinary Research 61, 35–43.
- Bruford, M.W., Hanotte, M., Brookfield, J.F.Y. & Burke, T., 1992, 'Single locus and multilocus DNA fingerprint', in A.R. Hoelzel (ed.), *Molecular genetic analysis of populations: A practical approach*, pp. 225–270, IRL Press, Oxford.
- Channing, A. & Rödel, M.O., 2019, Field guide to the frogs & other amphibians of Africa, Penguin Random House, South Africa.
- Chernomor, O., Von Haeseler, A. & Minh, B.Q., 2016, 'Terrace aware data structure for phylogenomic inference from supermatrices', *Systematic Biology* 65(6), 997–1008. https://doi.org/10.1093/sysbio/syw037
- Cuthbert, R.N., Wasserman, R.J., Keates, C. & Dalu, T., 2022, 'Food webs', in T. Dalu & R.J. Wasserman (eds.), *Fundamentals of tropical freshwater wetlands*, pp. 517–547, Elsevier, Cambridge.
- Du Preez, L. & Carruthers, V., 2017, A complete guide to the frogs of southern Africa, Struik Nature, Cape Town.
- Dyamond, K.S., 2017, Macro-invertebrate diversity within the Makuleke Wetlands in the Pafuri Region of Kruger National Park, University of Johannesburg, South Africa.
- Dzurume, T., Dube, T. & Shoko, C., 2022, 'Remotely sensed [sic] data for estimating chlorophyll-a concentration in wetlands located in the Limpopo Transboundary River Basin, South Africa', *Physics and Chemistry of the Earth, Parts A/B/C* 127, 103193. https://doi.org/10.1016/j.pce.2022.103193

- Eckhardt, H.C., Van Wilgen, B.W. & Biggs, H.C., 2000, 'Trends in woody vegetation cover in the Kruger National Park, South Africa, between 1940 and 1998', African Journal of Ecology 38(2), 108–115. https://doi.org/10.1046/j.1365-2028.2000.00217.x
- FrogMap, 2023, *FitzPatrick Institute of African Ornithology*, viewed 01 May 2023, from https://vmus.adu.org.za/?vm=FrogMAP.
- Hall, T.A., 1999, 'BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT', Nucleic Acids Symposium Series 41, 95–98.
- Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q. & Vinh, L.S., 2018, 'UFBoot2: Improving the ultrafast bootstrap approximation', *Molecular Biology and Evolution* 35(2), 518–522. https://doi.org/10.1093/molbev/msx281
- iNaturalist, 2023, iNaturalist, viewed 01 May 2023, from https://iNaturalist.co.za.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Von Haeseler, A. & Jermiin, L.S., 2017, 'ModelFinder: Fast model selection for accurate phylogenetic estimates', *Nature Methods* 14(6), 587–589. https://doi.org/10.1038/nmeth.4285
- Keates, C., Conradie, W., Dalu, T., Dondofema, F., Riddell, E.S. & Wasserman, R.J., 2022, 'Phylogenetic placement of the enigmatic Floodplain water snake, Lycodonomorphus obscuriventris FitzSimons, 1964', Koedoe 64(1), 1–9. https:// doi.org/10.4102/koedoe.v64i1.1698
- Kock, A., 2017, 'Diatom diversity and response to water quality within the Makuleke Wetlands and Lake Sibaya', Doctoral dissertation, North-West University, South Africa.
- Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K., 2018, 'MEGA X: Molecular evolutionary genetics analysis across computing platforms', *Molecular Biology* and Evolution 35(6), 1547–1549. https://doi.org/10.1093/molbev/msy096
- Majdi, N., De Necker, L., Fourie, H., Loggenberg, A., Netherlands, E.C., Bunte-Tschikin, J. et al., 2022, 'Diversity and distribution of benthic invertebrates dwelling rivers of the Kruger National Park, South Africa', Koedoe 64(1), 1–18. https://doi.org/ 10.4102/koedoe.v64i1.1702
- Malherbe, W., Christison, K.W., Wepener, V. & Smit, N.J., 2019, 'Epizootic ulcerative syndrome–First report of evidence from South Africa's largest and premier conservation area, the Kruger National Park', *International Journal for Parasitology: Parasites and Wildlife* 10, 207–210. https://doi.org/10.1016/ j.ijppaw.2019.08.007
- Matthews, S., 2018, 'Precious heritage-project quantifies the value of SA's Ramsar wetlands: Wetlands', Water Wheel 17(1), 16–20.
- Minh, B.Q., Lanfear, R., Trifinopoulos, J., Schrempf, D. & Schmidt, H.A., 2021, IQ-TREE version 2.1.2: Tutorials and manual phylogenomic software by Maximum Likelihood, viewed n.d., from http://www.iqtree.org/doc/iqtree-doc.pdf.
- Minter, L.R., Burger, M., Harrison, J.A., Braack, H.H., Bishop, P.J. & Kloepfer, D., 2004, Atlas and red data book of the frogs of South Africa, Lesotho and Swaziland, Smithsonian Institute and Avian Demography Unit.
- Mucina, L. & Rutherford, M.C., 2006, The vegetation of South Africa, Lesotho and Swaziland, South African National Biodiversity Institute.

- 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(1), 268–274. https://doi.org/10.1093/molbev/ msu300
- Nielsen, S.V., Daniels, S.R., Conradie, W., Heinicke, M.P. & Noonan, B.P., 2018, 'Multilocus phylogenetics in a widespread African anuran lineage (Brevicipitidae: *Breviceps*) reveals patterns of diversity reflecting geoclimatic change', *Journal of Biogeography* 45(9), 2067–2079. https://doi.org/10.1111/jbi.13394
- Nortje, G.P., 2014, 'Studies on the impacts of off-road driving and the influence of tourists' consciousness and attitudes on soil compaction and associated vegetation in the Makuleke Contractual Park, Kruger National Park', doctoral dissertation, University of Pretoria (South Africa).
- Passmore, N.I. & Carruthers, V.C., 1995, South African frogs: A complete guide, Witwatersrand University Press, Cape Town.
- Palumbi, S.R., 1996, 'The polymerase chain reaction', in D. Hillis, C. Moritz & B. Mable (eds.), *Molecular systematics*, 2nd edn., pp. 205–247, Sinauer Associates, Sunderland.
- Pickergill, M., 1984, 'Three new Afrixalus (Anura: Hyperoliidae) from South-Eastern Africa', Durban Museum Novitates 13, 203–220.
- Pienaar, U.V., 1968, 'The freshwater fishes of the Kruger National Park', Koedoe 11(1), 1–82. https://doi.org/10.4102/koedoe.v11i1.761
- Pienaar, U.V., Passmore, N.I. & Carruthers, V.C., 1976, The frogs of the Kruger National Park, National Parks Board of South Africa, Pretoria.
- Portik, D.M., Bell, R.C., Blackburn, D.C., Bauer, A.M., Barratt, C.D., Branch, W.R. et al., 2019, 'Sexual dichromatism drives diversification within a major radiation of African amphibians', *Systematic Biology* 68(6), 859–875. https://doi.org/10.1093/ sysbio/syz023
- Rambaut, A., 2014, Figtree, viewed 25 June 2023, from http://tree.bio.ed. ac.uk/ software/figtree/.
- Reid, H. & Turner, S., 2013, 'The Richtersveld and Makuleke contractual parks in South Africa: Win-win for communities and conservation?', in C. Fabricus, E. Koch, S. Turner & H. Magome (eds.), *Hector Magome Rights resources and rural* development, pp. 238–249, Routledge, London.
- Riddell, E.S., Govender, D., Botha, J., Sithole, H., Petersen, R.M. & Shikwambana, P., 2019, 'Pollution impacts on the aquatic ecosystems of the Kruger National Park, South Africa', *Scientific African* 6, e00195. https://doi.org/10.1016/j.sciaf.2019.e00195
- Tinley, K.L., 1981, 'The Northern Kruger National Park-an Ecological Inventory', African Wildlife-Special 35, 6–23.
- Vlok, W., Fouché, P.S.O., Cook, C.L., Wepener, V. & Wagenaar, G.M., 2013, An assessment of the current distribution, biodiversity and health of the frogs of the Kruger National Park in relation to physical and chemical factors, Report to the Water Research Commission, pp. 120–123.
- Wolmarans, C.T. & De Kock, K.N., 2006, 'The current status of freshwater molluscs in the Kruger National Park', Koedoe 49(2), 39–44. https://doi.org/10.4102/koedoe. v49i2.122