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Frog research at Ndumo Game Reserve: A late start with leapfrogs in species discovery and socially inclusive herpetology

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Abstract: In the context of most game reserves, the establishment of the Ndumo Game Reserve (NGR) was likely aimed at preserving larger, charismatic wildlife species. In its 100-year existence however, this reserve has gained popularity among wildlife researchers and enthusiasts in invertebrates and smaller vertebrates, such as spiders, dung beetles, birds, and frogs. Notably, research focussed on frogs at NGR has experienced significant growth during the last decade. Within this 10-year period, numerous research advancements have been made in frog research at NGR, extending its implications and applicability beyond the reserve's boundaries. These leapfrogs in NGR anuran research have established models for comprehensive herpetological conservation strategies, expanded the repertoire of ecological indicators, and contributed to a better understanding of the diversity of South African frogs and the organisms that parasitise them.

Keywords: Amphibians; Cryptic species; Ecotoxicology; Haemoparasites; Maputaland-Pondoland-Albany

Ndumo Game Reserve as a station for anuran research

The proclamation of Ndumo Game Reserve (NGR) seemed to follow the norm of giving conservation priority to megafauna. Still, the reserve later became one of the preferred protected areas for tourists and biologists interested in smaller vertebrates as well as invertebrates. The proclamation of NGR in 1924 is widely interpreted as being for the protection of Hippopotamus (*Hippopotamus amphibious*) however, to validate this interpretation put forth by several authors, a comprehensive historical analysis is necessary (Ezemvelo KZN Wildlife, 2009). Regardless of the main reason for proclaiming NGR, the reserve is an important protected area for birding ecotourism (Torquebiau, Dosso, Nakagwa & Philippon, 2012) and research focused on non-charismatic wildlife such as spiders (Haddad, Dippenaar-Schoeman & Wesolowska, 2006; Haddad, 2022), butterflies (Pringle & Kyle, 2002), reptiles (Cook, Netherlands & Smit, 2016; Cook, Netherlands, Van As & Smit, 2018; Kyle, Alexander, & Du Preez, 2021; Jordaan, van As & Netherlands, 2023) and frogs (Alexander, Harrison, Fairbanks & Navarro, 2004; Ezemvelo KZN Wildlife, 2009; Phaka, Netherlands, Kruger & Du Preez, 2017; Acosta et al., 2020; Kyle & Du Preez, 2020). Regarding research focused on frogs (anuran amphibians), NGR holds importance due to its strategic geographic location contributing to the reserve's high biodiversity. Furthermore, the surrounding regions are characterised by swift land use changes, making the reserve an ideal site for adopting a landscape approach to study amphibian biodiversity.

South Africa generally boasts a remarkable diversity of frog species attributed to its diverse topography, favourable climate, and diverse habitats. Notably, this frog diversity increases northwards along the coast to northern KwaZulu-Natal (Du Preez & Carruthers, 2017), where NGR is situated. The northern region of KwaZulu-Natal includes the Phongolo Floodplain, renowned for its aquatic biodiversity (Lankford *et al.*, 2010), with NGR forming the lower reaches of this floodplain (Ezemvelo KZN Wildlife, 2009). Another contributor to biodiversity at NGR is its location at the confluence of two rivers systems (Usuthu and Phongolo rivers) and additional streams in the southwest part of the reserve, thus making it three principal water sources that are part of NGR (Ezemvelo KZN Wildlife,

2009). The location of this 11 898 ha reserve on South Africa's border with Mozambique (Ezemvelo KZN Wildlife, 2009), and within a province with at least 43% of its natural habitat transformed to meet human needs (Jewitt, Goodman, Erasmus, O'Connor & Witkowski, 2015), provides researchers with an opportunity to incorporate landscape dynamics to anuran conservation research in a protected area. The rapid and human-induced alterations in the landscape surrounding NGR underscore the need for anuran research that considers external impacts on the reserve. Such research is significant, as the distribution of frog species and the factors affecting the survival of frog populations are often not confined by the boundaries of protected areas alone.

The distribution of frogs is also not restricted by national boundaries thus presenting the unique opportunity to pilot transboundary bilateral amphibian conservation research initiatives. Adopting a bilateral approach to biodiversity research would contribute to the mandate of the transfrontier conservation area (Lubombo Transfrontier Conservation Area) which NGR is a part of (Sandwith, Shine, Hamilton & Sheppard, 2001). This would not be the first time NGR is subject to unique amphibian-focused studies. Research in NGR by North-West University's (NWU) African Amphibian Conservation Research Group (AACRG) that began in 2012 culminated in advances towards a template for socially inclusive approaches to herpetological conservation with outcomes that demonstrate ecosystem services of amphibians and has the potential to inform integrative conservation planning that is encouraged by South African environmental policy (Phaka, 2018).

A late start

Prior to the leapfrogs in species discovery and socially inclusive herpetology, the earliest publications focused on NGR frog species included the work of Pooley, Pooley, Hadley & Gans (1973), and Measey, Armstrong & Hanekom (2009). Since 2012, the AACRG has conducted 12 research expeditions within NGR, and in the process trained Masters and PhD students in anuran studies. These postgraduate anuran studies encompassed research areas

such as species diversity, habitat utilisation, acoustic monitoring, disease and parasite monitoring, and general biology and ecology of anurans.

A search for publications about frogs at NGR was conducted on three scholarly databases; Web of Science (<https://www.webofscience.com>), Google Scholar (<https://scholar.google.com>), and Scopus (<https://www.scopus.com>) using a combination of the keywords ‘frog’ (including variations amphibia, anura, herpetofauna, herptiles and toad) and ‘Ndumo’ (including variations Nduma and Ndumu). To take into consideration that historical NGR research might not be indexed on popular scholarly databases, an additional search for literature was conducted on the Biodiversity Heritage Library (<https://www.biodiversitylibrary.org>) using the same keyword combinations used on the three scholarly databases. The combined results from the Biodiversity Heritage Library and the scholarly databases had 35 publications with two among them using the Ndumu variation of the NGR spelling and no suitable publications used the Nduma spelling. Grouping the sample of 35 publications according to similarities in their focus revealed two categories (Figure 1).

In the first category with 26 publications, the focus revolves around NGR frog species as the central topic or research subject (e.g., anuran frog diversity studies, frog parasite survey or frogs as ecological indicators). The second category with nine publications only mentions NGR frog species, but they are not the research focus (e.g., a publication focused on non-amphibian taxa mentioning that NGR is important for anuran diversity). From 1924 until 2024 (May 20th), 26 publications (15 scientific articles, eight postgraduate theses, one conference paper, one book chapter and one magazine article) focused on NGR frog species, while nine publications (three scientific articles, five postgraduate theses and one magazine article) with a different taxonomic focus mentioned NGR frog diversity in their text (Supplementary Materials 1; 2). These publications exclude wildlife guides that are an introduction to the biology of South African frog species including those occurring within boundaries of NGR (e.g., Lambiris, 1988; Du Preez & Carruthers, 2017; Phaka *et al.*, 2017).

Student research highlights based on projects

Species Diversity: The tropical environment of NGR is a prime location for anuran species diversity studies. Several student projects of the AACRG have focused on anuran species diversity in the NGR. Based on multiple student projects, spanning several years, the most current and expected number of anuran species is 37, with 14 being especially notable during surveys (Netherlands, 2014; Phaka, 2018; Pretorius, 2019; Reeder, 2019).

Reeder (2019) conducted a phylogenetic study using DNA barcoding to understand amphibian diversity in northern KwaZulu-Natal, including the NGR. The research aimed to uncover cryptic species and confirm species identity. For example, this study helped resolve the occurrence of species such as *Afrixalus delicatus*, (Table 1) which was shown to be restricted to the coast as opposed to inland as previously thought (Reeder, 2019; Pretorius, 2019) and confirmed historic presence/absence records of several anuran species inside NGR.

Habitat Utilization and Ecology: Several projects focused on frogs' habitat preferences, revealed the crucial role of distinct environments in supporting specific species. Botha (2014) investigated ecomorphological guilds and diet of anuran tadpoles, illuminating their ecological role in determining ecosystem function. Wolmarans (2015) explored the impact of organochlorine pesticide exposure on amphibians, uncovering seasonal variation in pesticide levels and associations with biomarker responses.

Acoustic Monitoring: The deployment of "song meters" for Passive Acoustic Monitoring (PAM) augmented research capabilities, facilitating the identification of calling patterns, breeding seasons, and species presence throughout the year. Netherlands (2014) pioneered the use of permanent song meters, providing insights into breeding behaviour and temporal calling patterns. Pretorius (2019) harnessed automated recorders to understand anuran diversity and its connection to meteorological variables, contributing to our understanding of South African anuran behaviour.

Disease and Parasite Monitoring: Several projects investigated presence and impact of diseases and parasites on frog populations, contributing to a comprehensive understanding of their health and conservation. Netherlands (2014) screened 436 frogs from diverse species for blood parasites, revealing insights into the diversity and host, parasites and vector interactions. These studies have led to the discovery of several new or undescribed blood parasites species (Netherlands *et al.*, 2014; 2020).

General Biology and Behaviour: Distinctive behaviours, like the unique breeding biology and parental care of the mottled shovel-nosed frog (*Hemisus marmoratus*), were also undertaken in NGR. Novel observations, such as the mother's active role in carrying tadpoles to open water, have provided fresh insights into these behaviours (Kyle & Du Preez, 2020).

Taxonomy: Members of the AACRG also expanded the known diversity of rain frogs within NGR by describing a new cryptic species the Ndumo Rain Frog, *Breviceps passmorei*. Once cryptic species are identified, subsequent examination often reveals subtle but consistent morphological differences. This description followed an integrated approach using morphology, acoustics and molecular data to distinguish it from other closely related taxa, contributing to the broader understanding of taxonomy within the genus (Minter, Netherlands & Du Preez, 2017).

Community engagement and ecotourism: Phaka (2018) explored the amphibian diversity from the perspective of communities surrounding NGR and considered how this diversity can be used for community-based ecotourism in the reserve. The major outcomes from this study were the publishing of an English-isiZulu handbook on Zululand frogs aimed to bridge the gap between people and amphibians while advocating for ecotourism as a tool for conservation.

Through these student projects, the significance of NGR as a research hotspot transcends individual species, encompassing broader landscape dynamics, transboundary conservation efforts, and holistic planning. As research persists, NGR continues to play a vital role in safeguarding and unravelling the intricate tapestry of life within its confines.

Bibliometric analysis of frog research at Ndumo Game Reserve

As literature on a certain topic increases, researchers might struggle to grasp the bigger picture, and to summarise the state of knowledge requires a map that connects research information and relationships to inform future research (Arruda, Silva, Lessa, Proença Jr & Bartholo, 2022). A bibliometric analysis is useful for exploration of a research topic and analysis of scientific data (Donthu, Kumar, Mukherjee, Pandey & Lim, 2021). To explore NGR frog diversity as a research topic the VOSviewer software tool was used for constructing and visualizing bibliometric networks (Van Eck & Waltman, 2010) of the 26 publications focused on NGR frogs that were found through the abovementioned search on Web of Science, Google Scholar, Scopus and Biodiversity Heritage Library. The following data from the sample of 26 publications were saved on a tab delimited file; author name(s), publication title, publication year, author affiliation(s), keywords, and article type. This file with NGR frog research data was subsequently imported into VOSviewer for analysis of co-authorship relationships, co-authorship frequency, frequency of affiliations appearing in publications, publication keyword co-occurrence and keyword usage frequency. The obtained results were visualised and exported as a collaboration map for authors (Figure 2), affiliation density map for author affiliations (Figure 3) and a keyword map (Figure 4).

The bibliometric analysis shows that the 26 publications exclusively focused on NGR frog species since the reserve's establishment have emerged from the collective efforts of 54 researchers (as depicted in Figure 2). These researchers are affiliated with 24 institutions worldwide (Figure 3). From these 54 researchers focused on NGR frog species and 15 published at least two publications. Twelve researchers collaborated at least twice with

other researchers, and Du Preez, Netherlands, Smit and Wepener had at least five collaborations among themselves or with other researchers (Figure 2).

The author affiliations of publications focused on NGR frog species are illustrated in Figure 3; North-West University (appeared 19 times) was the most common affiliation, followed by the South African Institute for Aquatic Biodiversity (appeared seven times). Authors mostly used keywords relating to Anura, conservation, Anuran parasites and pesticides in their publications' abstracts (Figure 4). Some of these commonly used keywords are also the topics in which anuran research in NGR leapfrogged expectations (as outlined in the subsequent section).

Leapfrogs in anuran research

Anuran research in NGR made several notable advances in the fields of conservation, parasitology, and toxicology between 2014 and 2022. Minter *et al.*, (2017) described a new *Breviceps* species with one of the original specimens (a paratype) used for this description sampled in NGR. The newly described Ndumo Rain Frog (*B. passmorei*) could not be morphologically differentiated from five other *Breviceps* species thus relying on molecular techniques to confirm its identity within a complex of cryptic species (Minter *et al.*, 2017). The identification of cryptic species has implications for conservation (Angulo & Icochea, 2010), and particularly demonstrates the importance of NGR for the conservation of known and undescribed anuran species.

Methods for inclusive approaches to anuran conservation (and herpetological conservation in general) were piloted through a postgraduate NGR anuran research project which showed that local cultural norms and practices can serve as a tool for understanding and incorporating local wildlife perspectives into conservation planning (Phaka, 2018). Among its outputs, this pilot provided future conservation initiatives with a previously unavailable comprehensive list of IsiZulu names for the frogs of NGR and surrounds while also demonstrating that some local folklore about frogs was likely premised on explanations of

observed anuran behaviour without the knowledge of anuran biology needed for such explanations (Phaka, Netherlands, Kruger & Du Preez, 2019). An example of this folklore is the consideration of frogs as harbingers of rain when the biologically accurate explanation is that humid and moderate conditions before the onset of rain are favourable for frogs to start being active before any rain starts falling (Phaka *et al.*, 2019).

The ecosystem services provided by anuran diversity in NGR and the Zululand region in general, besides frogs being part of cultural practices, was demonstrated with a first of its kind bilingual (IsiZulu and English) field guide for frogs that was developed in consultation with communities surrounding NGR (Phaka *et al.*, 2017). In addition to the educational benefits of anuran diversity for local communities demonstrated by the bilingual frog book, its development contributed to promoting African languages as languages of teaching and learning (Phaka & Ovid, 2022). Further conservation value of anuran research was surprisingly demonstrated in anuran parasite diversity studies at NGR; habitats inside the protected area had higher anuran parasite diversity when compared to habitats with greater human impact exposure outside NGR (Netherlands, Cook, Kruger, Du Preez & Smit, 2015), thus suggesting anuran parasites could be useful as ecological indicators.

Studies on blood parasite diversity have led to the discovery of several new parasites and advances in African parasitology. A new species of *Hepatozoon* (*Hepatozoon ixoxo*) that parasitises Bufonid frogs was found in Flat-backed Toad (*Sclerophrys maculata*) and Eastern Olive Toad (*Sclerophrys garmani*) sampled in NGR, and this study also had the first morphological and molecular characterisation of a *Hepatozoon* species found in South African Bufonids (Netherlands, Cook & Smit, 2014). *Hepatozoon ixoxo* was only the second haemogregarine with a frog host to be described from South Africa (Netherlands *et al.*, 2014). Subsequently, another haemogregarine was described from a Plain Grass Frog (*Ptychadena anchietae*) from NGR. This was the first description of a species of *Dactylosoma* (*Dactylosoma kermi*) parasitising an African anuran (Netherlands *et al.*, 2020). Additionally, the description of *D. kermi* by Netherlands *et al.* (2020) was the first molecular characterisation of an African *Dactylosoma* species and subsequent phylogenetic

analysis showed *Dactylosoma* species with anuran hosts to be a monophyletic group that is separate from other haemogregarines.

The value of frogs in toxicology was demonstrated with Müller's Platanna (*Xenopus muelleri*) sampled in NGR being used as a case study for the assessment of organochlorine pesticide accumulation in aquatic ecosystems. Bioaccumulation of organochlorine pesticides, including dichlorodiphenyltrichloroethane (DDT), had no relationship to Malaria vector control measures in the area surrounding NGR but showed a negative correlation to rainfall (Wolmarans *et al.*, 2018) thus highlighting a need to monitor the impact of droughts on the accumulation of harmful pesticides in aquatic ecosystems.

Anuran conservation research prospects in Ndumo Game Reserve

The conservation research project piloted at NGR demonstrated its future value when it was developed into a long-term national research project aimed at understanding whether cultural practices can provide science-based tools for collaborative conservation planning and social inclusion in herpetological matters (Phaka, 2022). The conservation impact (both negative and positive) of these cultural practices on herpetofaunal diversity at NGR and elsewhere has been catalogued by Phaka (2022) but there is a research gap for the quantification of those impacts.

In 1964, Poynton classified the South African fauna into two distribution centres: tropical and Cape, with a transitional group in between (Poynton, 1964). Given the imminent climate changes affecting temperature and rainfall patterns, it is anticipated that frog communities will be impacted. Therefore, conducting long-term monitoring of frog populations becomes crucial. While frog populations in the Western Cape have been monitored for over a decade, this practice is largely lacking in the north-eastern regions of the country, where tropical species are found. Notably, NGR harbours anuran diversity characteristic of tropical fauna, making it an ideal location for long-term frog monitoring.

Across the African continent, vector-borne diseases continue to bear significant medical and economic implications. Although the initial discovery of blood parasites in the European Green frog dates back to 1842, it was the pioneering work of Alphonse Laveran in 1881 that firmly established the connection between bloodborne pathogens and diseases such as malaria (Netherlands, 2019). Research efforts have rightfully focused on blood parasites affecting humans and livestock due to their significance. However, blood parasites of ectotherms have received far less attention. Notably, much of the existing research on ectotherm blood parasites globally has centred on species descriptions. While taxonomy is integral to biological understanding, a critical gap in knowledge remains concerning the vectors and mechanisms that underlie transmission for these ectothermic blood parasites.

In addressing these gaps, the implementation of research in the actual environment of NGR holds the promise of making substantial contributions to life cycle experiments. This strategic approach has the potential to provide valuable insights into the intricate interplay of host-vector-parasite relationships, shedding light on the mechanisms that drive transmission. By leveraging and enriching our existing comprehension of the life history, diversity, evolutionary biology, and vector ecology of these parasites within the framework of southern Africa, this initiative possesses the capacity to usher in significant breakthroughs in knowledge.

Concluding remarks

Long-term research is required to investigate whether inclusive conservation approaches positively impact anuran conservation outlook at NGR and outcomes of such research would inform future collaborative conservation efforts for frogs and other wildlife in general. There is further need for research assessing the anuran conservation value of habitat connectivity provided by the transfrontier conservation area of which NGR is part. In addition to the noted anuran research gaps in NGR there is also unpublished research by the North-West University's African Amphibian Conservation Research Group. Some of this unpublished research carried out in the NGR indicates that the *adspersus* and

mozambicus groups within the genus *Breviceps* still encompass undescribed cryptic species. The necessity to investigate and resolve this species cluster has been long overdue, and NGR is an important locality for this research aimed at accurately delineating species of *Breviceps*. Which would, in turn, inform conservation prioritisation.

The progress made in resolving some *Breviceps*' taxonomic clusters, clarification of *A. delicatus*' distribution, and discovery of new amphibian parasites among other advancements have the protection of frog populations in NGR as a contribution factor. The value of NGR as a conservation area is underscored by the ongoing conversion of habitats for human settlement needs around the reserve. Furthermore, NGR is one of the oldest reserves in the KwaZulu-Natal province, which has experienced a rapid loss of natural habitats (Jewitt, Goodman, Erasmus, O'Connor & Witkowski, 2015). Without natural and protected strongholds such as the NGR, amphibian populations and associated parasites would not have a pristine refuge. Some of the herpetological advances highlighted here might not have been possible without the reserve, and the training of young herpetologists might not have progressed as well as it did when they had NGR as their study area.

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Tables

Table 1. Notable outcomes from Ndumo Game Reserve frog diversity surveys.

TAXON NAME	FAMILY	Lambiris 1988	Minter <i>et al.</i> (2004)	Frog App (2021)	AACRG surveys (2012- 2023)	Comment
<i>Afrixalus delicatus</i>	Hyperoliidae	✓	✓	✓	X*	*DNA barcoding shows exclusively coastal distribution
<i>Amietia delalandii</i>	Pyxicephalidae	✓*	✓*	✓	✓	*Taxonomic change: <i>Afrana angolensis</i> synonym of <i>A. delalandii</i>
<i>Breviceps adspersus</i>	Breviceptidae	*	✓	*	*	*Taxonomic change: <i>B. adspersus</i> synonym of <i>B. passmorei</i>
<i>Breviceps passmorei</i>	Breviceptidae	X*	X*	✓	✓	*See above
<i>Cacosternum nanum</i>	Pyxicephalidae	X	✓	✓	X	Species not detected
<i>Hyperolius poweri</i>	Hyperoliidae	*	*	✓	X	*Taxonomic change: <i>H. nasutus/H. acuticeps</i> synonym of <i>H. poweri</i>
<i>Ptychadena taenioscelis</i>	Ptychadenidae	✓	✓	✓	X	Species not detected
<i>Sclerophrys capensis</i>	Bufonidae	X	X	✓	X	*Taxonomic change: <i>Bufo rangeri</i> synonym of <i>S. capensis</i>
<i>Tomopterna adiastrata</i>	Pyxicephalidae	*	✓*	✓	✓	*Taxonomic change: <i>T. cryptotis</i> synonym of <i>T. adiastrata</i>
<i>Tomopterna marmorata</i>	Pyxicephalidae	✓	X	X	X	Species not detected
<i>Tomopterna natalensis</i>	Pyxicephalidae	X	✓	✓	✓	Presence confirmed
<i>Xenopus laevis</i>	Pipidae	X	✓	X	X	Species not detected

* Refer to comment for explanation

✓ Confirmed

X Not confirmed

Figures

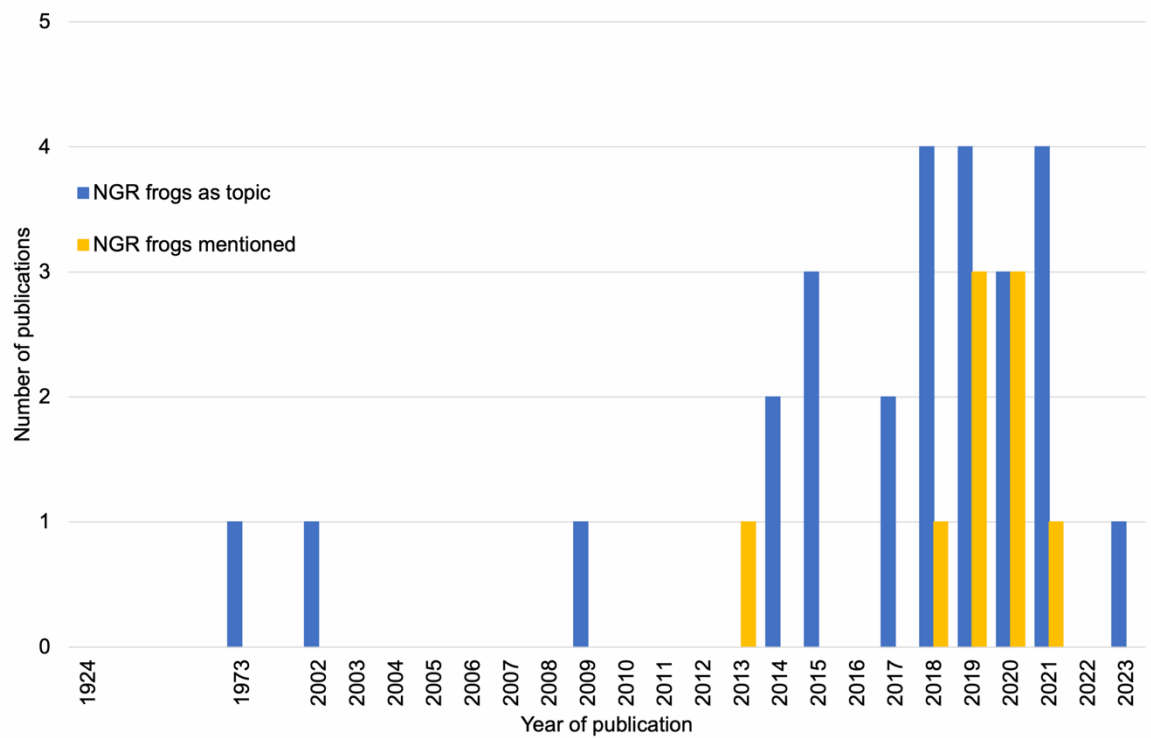


Figure 1: Publications about frogs of Ndumo Game Reserve since 1924.

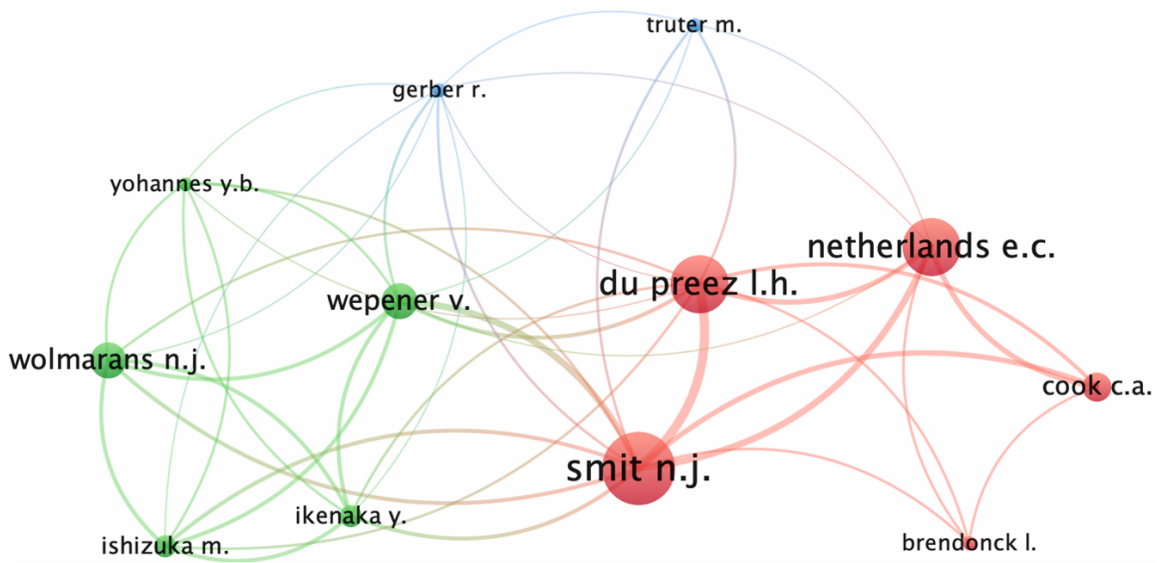


Figure 2: Collaboration map for researchers with minimum two collaborations and two co-authorships on publications focused on frogs in Ndumo Game Reserve since 1924. Bubble and text size represent the frequency of co-authorship, and line thickness represents collaboration relationships between researchers.

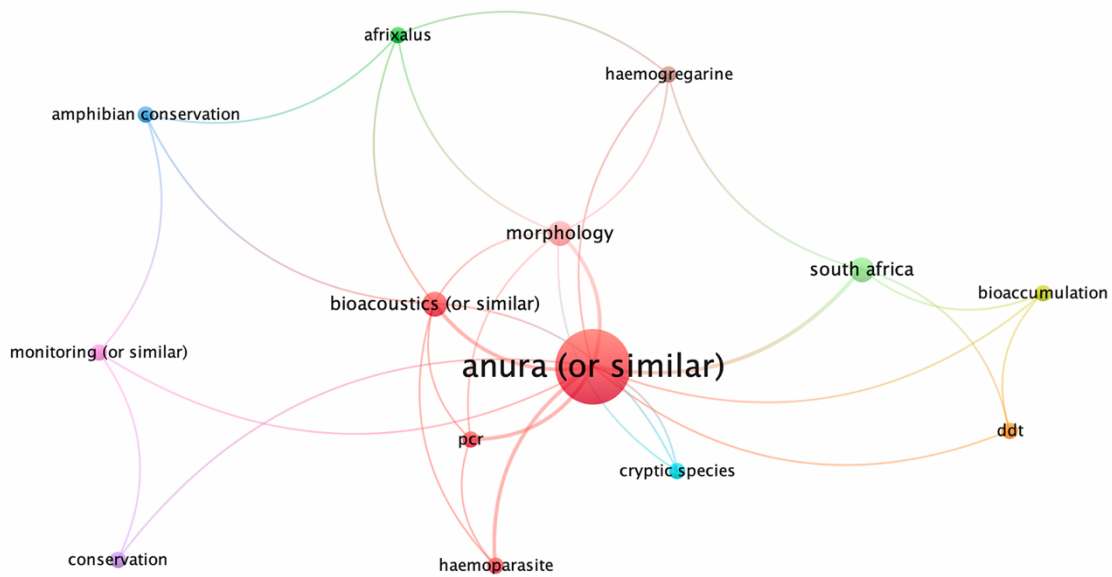


Figure 4: Author keywords used in at least two publications focused on frogs in Ndumo Game Reserve since 1924. Bubble and text size represent the frequency of keyword use in publication abstracts and lines represent the co-occurrence relationship between keywords.