



## Research Article

## Three decades later in the northern Kruger National Park: multiple acoustic and capture surveys may underestimate the true local richness of bats based on historical collections

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### Abstract

The Kruger National Park (KNP) is considered an important biodiversity hotspot, with insectivorous bats representing about twenty percent of the total mammalian diversity of South Africa. Historically, 40 bat species have been documented in the northern region of the Park between 1960 and 1990. However, it has been three decades since the last comprehensive assessment. To aid the long-term monitoring of bats within KNP, our study re-surveyed the bat community of northern KNP over two years, incorporated the latest acoustic technologies, compared changes in bat species richness with historical data, and tested the use of an automated classifier for the acoustic data. We captured bats and recorded echolocation calls at 26 sites, between March and October in 2017 and 2018. Kaleidoscope Pro software was used to identify each bat call series recorded. To enhance the accuracy of this tool, a northern KNP-specific classifier was developed. We recorded 27 distinct species during this study, of which 13 were live-captured. The historical data therefore show a much higher richness of bat species within the study area (40 species) than recorded during our study (27 species), although the former were collected over a much longer period of time during numerous collecting trips by staff of the former Transvaal Museum (Ditsong National Museum of Natural History). Total sample effort, environmental effects, biological aspects and overall study limitations likely contributed to the observed differences. The classifier tool had a relatively high percentage accuracy (80%) but manual identification was required to avoid the misidentification of rare species and to detect new species not previously recorded. Future studies should focus more effort on live-capturing, given the high species richness of the region and the limitation of bat detectors to record high frequency and low intensity echolocation calls, which are common in many southern African species.

## Introduction

The world-famous Kruger National Park (KNP) in South Africa protects some of Africa's most endangered plant and animal species and is considered an important biodiversity hotspot (Gelderblom et al., 1995). Insectivorous bat species represent about twenty percent of the mammalian diversity in the KNP, with 40 bat species historically documented in the northern regions of the Park (Monadjem et al., 2020; Adams et al., 2015; Mickleburgh et al., 2002; Aldridge and Rautenbach, 1987; Rautenbach et al., 1984, 1985). Due to its sub-tropical climate, the northern KNP is predicted to have the highest bat species richness in South Africa (Venter and Gertenbach, 1986; Rautenbach et al., 1985). Bat surveys in this area were last conducted in the 1980's to determine the number of species occurring in the region (Aldridge and Rautenbach, 1987; Rautenbach et al., 1984, 1985), but museum specimens were collected between 1960 and 1990 (Monadjem et al., 2020). In 1985, one QMC S-200 bat detector (Ultrasound Advice, UK) was used to record release calls from 16 bat species of five different families, but no comprehensive acoustic methods were used in the assessment of KNP bat communities historically (Aldridge and Rautenbach, 1987; Rautenbach et al., 1984, 1985).

Bats are ideal indicator species and constant monitoring of bat communities provides important insights into environmental health (Russo and Voigt, 2016; Mickleburgh et al., 2002; Fukui et al., 2001). Bats are also important keystone species providing many ecosystem services such as pest control, pollination and seed dispersal (Russo and Voigt, 2016; Jones et al., 2013). Unfortunately, about one quarter of all bat species are threatened with extinction and due to their nocturnal behaviour, use of multiple, often inaccessible roosts, and their variation in flight patterns, bats can be difficult to survey and are often overlooked in diversity studies (Adams et al., 2012; Monadjem and Reside, 2008; Berry et al., 2004; Mickleburgh et al., 2002; Gelderblom et al., 1995). Most African bat species are poorly studied with about 15% of all bat species in southern African listed under Data Deficient by the IUCN (Monadjem et al., 2020). Likewise, bat diversity in the KNP region has been understudied and since the last comprehensive assessment, it has been more than three decades (Monadjem and Reside, 2008).

Bats are sensitive to changes in climate, temperature, resource abundance, and habitat change, which makes them valuable indicators for monitoring environmental health (Foord et al., 2015; Russo and Ancillotto, 2015). Increases or decreases in bat populations can indicate the loss of vegetation communities, pollution, diseases, changes in the availability of water sources, and climate change (Stahlschmidt and Brühl, 2012). The abundance of bats around riparian habitats can also indicate the quality of water sources (McCain, 2007). Bats rely

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on water sources to drink and to hunt for prey above the water surface (Sherwin et al., 2013; Downs and Racey, 2006). Declines in water quality can result in lower abundances of prey and can cause decreases in bat activity while certain urban adapters might benefit, in the short-term, from increases in insect abundances of species such as chironomid (Jones et al., 2009; Naidoo et al., 2013; Vaughan et al., 1996). Streams contaminated from pollution can be monitored, however, by looking at bat activity in combination with the community composition at species level (Jones et al., 2009). Continuous and long-term monitoring of bat communities therefore provides important insight into environmental health (Fukui et al., 2001; Mickleburgh et al., 2002). This is especially important in the southern African region, which is more severely affected by accelerating climate and land use change (IPCC, 2019; Conway et al., 2015; Midgley and Bond, 2015).

There have been major advances in the technology of bat detectors in the last two decades, which facilitate the study of echolocating insectivorous bat species (Russo and Voigt, 2016). However, there are still a number of limitations in echolocation call analyses, as bat calls can vary intra- and inter-specifically depending on many factors such as habitat and overall species composition. Calls between species also vary greatly in their detectability depending on their amplitude (Monadjem et al., 2017; Jones et al., 2009). These limitations are important and must be considered in the analyses of the recorded calls. The earliest bat detectors used a heterodyne system, which relied on the observer to recognize the produced sound, and only worked within a pre-selected frequency range. This led to very subjective analyses and species that fell outside the set frequency range were missed (Jones et al., 2013). Nowadays, the most common detectors use time-expansion and record the sound, which can be played back, as well as all the information necessary for species identification such as the duration and frequency of calls (Jones et al., 2013). This information allows the active and passive recording of huge datasets that then have to be analysed, which has given rise to a vast range of automated classifiers (Russo and Voigt, 2016). However, very few of these classifiers have been validated and none of them provide 100% accuracy (Russo and Voigt, 2016; Clement et al., 2014). We therefore developed and tested a KNP-specific classifier using subsequent manual verification of all automated classifications.

Our study re-surveyed the insectivorous bat community of northern KNP by visiting the majority of the same localities sampled historically in the northern Kruger between 1960 and 1990 (Monadjem et al., 2020; Aldridge and Rautenbach, 1987; Rautenbach et al., 1984, 1985), incorporating the latest acoustic technologies and aiming to (1) compare changes in bat species richness with historical data, (2) develop a site-specific echolocation release call library to be used for current and future acoustic surveys in the area, (3) generate baseline data to be used for long-term monitoring of bats within KNP, and (4) test the use of an automated classifier for this important bat biodiversity hotspot.

## Materials and Methods

### Study area

Our study took place in northern KNP in the north-eastern corner of South Africa, bordering Zimbabwe and Mozambique. Northern KNP is located in a sub-tropical climate (SANParks 2018) with the main rains occurring between October and April. The region experiences hot, humid summers and mild, frost-free winters (Tshiala et al., 2011; Wessels et al., 2011). The daily average temperature in the summer has a mean of 27°C and the winter season is mild with a mean of 20°C (Tshiala et al., 2011; Venter and Gertenbach, 1986).

KNP is located within the Savanna Biome (Du Toit et al., 2003) which occurs at altitudes mostly below 1,500 m (Mucina and Rutherford, 2006). There is a distinct difference in the vegetation of the northern region (north of the Olifants River) of the park, where the current study took place, compared to the southern region (Codron et al., 2007). The northern region is dominated by broad-leaved trees while the southern region is dominated by fine-leaved trees (Codron et al., 2007).

### Data collection

Data were collected in the form of morphometric measurements from live-captured bats and echolocation call recordings using bat detectors (Taylor et al., 2013a), during four field trips per year over two years (2017–2018), sampling a total of 26 sites. Site selection was based on covering the broad range of historical sampling sites in the northern KNP (Aldridge and Rautenbach, 1987; Rautenbach et al., 1984, 1985). In 2017, twelve sites (eight acoustic-only, one live-capture only, and three using both techniques) were sampled in the Pafuri region, while 14 sites (eleven acoustic-only, one live-capture only and two using both techniques) were sampled in the Punda Maria/ Shingwedzi region in 2018 (Fig. 1). Sampling took place in March, April, May, August and October 2017 and in March, May, September and October 2018 (Tab. 2 and 3). Due to logistical constraints our sampling dates did not all match the months in which the historical surveys were conducted.

### Live captures

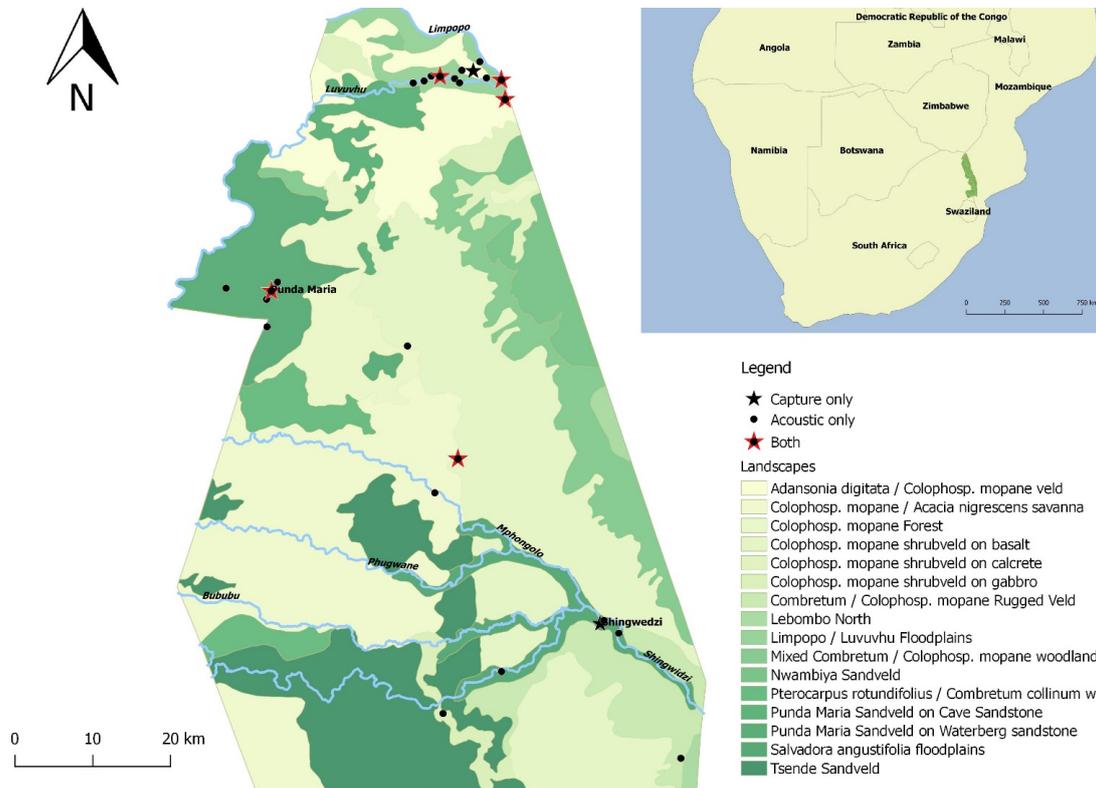
Mist-nets (either 6, 9, or 12 m in length; Ecotone, Gdynia, Poland) were utilized within fenced rest camps and a two-bank harp trap (Faunatech, Victoria, Australia) was used within rest camps and at designated picnic sites in the northern KNP. The mist nets and the harp trap were set up no later than 30 minutes before sunset near either a water source (swimming pool, pan or stream) or a man-made structure that displayed evidence of bat activity. Mist-nets were operated for two to four hours and were monitored throughout this time. The harp trap was left on site for 12 to 13 hours and was checked at sunrise (Taylor et al., 2013a,b). Eight separate sites were sampled for a total of 33 nights during the two year study. Captured bats were placed individually in cloth bags for processing and released the following day (Taylor et al., 2013a,b).

All captured bats were identified to species level using the key of Monadjem et al. (2020). Morphometric measurements that were recorded for each individual included body mass (g), forearm length (mm), sex and age (adult, sub-adult and juvenile). After processing, bats were released about half an hour before sunset. Upon release, echolocation calls from each bat were recorded using either an Anabat SD2 (Titley Electronics, Australia) and/ or Echo Meter Touch 2Pro (Wildlife Acoustics, USA). The recordings of the calls were used to confirm the identification of the bat species and to generate a call reference library. Certain individuals that could not be identified in the field (2017, n=9; 2018, n=3) were taken as museum specimens to confirm identification using cranial and dental characteristics (Taylor et al., 2013b). All specimens were sent to the Durban Natural Science Museum to be cleaned, analysed and accessioned. Permission to conduct the research within KNP was granted by SANParks (Permit #: PARD1401). All live-captures were approved by the SANParks Animal Use and Care Committee (Approval #: 025/16) and fell under the provincial capture and handling permit issued to PJT (Permit code: 0089-MKT001-00004). Ethical clearance for the study was also granted by the University of Venda (Permit No. SMNS/17/SARC H1/01/2006).

### Acoustic sampling

SM4 and SM2 Songmeters (Wildlife Acoustics, USA) were used to record the echolocation calls of insectivorous bats in northern KNP (Adams et al., 2012). Each detector was equipped with a waterproof case and an SMM-U1 or SMX-US ultrasonic full spectrum, omnidirectional microphone (Wildlife Acoustics, Concord, MA, USA), which was placed at a 45-degree angle, at least 1.5 meters above the ground, by mounting it onto a pole or a tree (Clement et al., 2014).

Each detector was programmed to record passively from sunset to sunrise, at a sample rate of 384 kHz and a gain of zero (Wildlife Acoustics, 2018). Site selection was determined before the first trip of each year and was based on covering the broad range of historical sampling sites in northern KNP (Aldridge and Rautenbach, 1987; Rautenbach et al., 1984, 1985). Each site was located near a water source such as a pan, stream or river to enhance sampling success of insectivorous bats (Monadjem et al., 2020). A total of 24 sites were acoustically sampled over the two years. Eleven of these sites were located in the Pafuri region (surveyed in 2017; Tab. 2) and 13 were located in the Punda



**Figure 1** – Study area in the northern Kruger National Park showing the location of sampling sites (either acoustic, live-captures or both) and the inset showing the location of the study area in northern South Africa.

Maria/ Shingwedzi region (surveyed in 2018; Tab. 3). In 2017, there was a total of 148 nights of sampling and in 2018 a total of 130 nights.

### Historical data review

Historical data were collated based on the three bat surveys conducted in northern KNP during the years 1979, 1982, 1983, 1984, 1985, and 1987 (Aldridge and Rautenbach, 1987; Rautenbach et al., 1984, 1985). Additional data were drawn from Monadjem et al. (2020), describing the majority of bat specimens collected within the study area between 1960 and 1990, and were predominantly from the Ditsong National Museum of Natural History (formerly Transvaal Museum). All references provided detailed accounts of each of the species captured including the number of individuals, location of capture, sex, and morphometric descriptions (Monadjem et al., 2020; Aldridge and Rautenbach, 1987; Rautenbach et al., 1984, 1985). Several species names have changed over the past thirty years due to additional taxonomic information available. Thus, the most current and accepted species names were used based on Monadjem et al. (2020) and other papers (Taylor et al., 2018; Goodman et al., 2017).

### Sampling efficiency

A rarefaction curve was constructed to provide a meaningful interpretation of the species richness found during the current survey for northern KNP (Colwell et al., 2012). A rarefaction curve is a linear model that calculates an estimated number of species within an area compared to the number of sample sites surveyed (Gotelli and Colwell, 2001). The model presumes that as the number of sample sites increases, the number of observed new species within the study area will decrease (Skalak et al., 2012). EstimateS (version 9.1; Colwell, 2013) was used to generate the individual-based accumulation curve (Chao et al., 2000). EstimateS is a programme that calculates species richness and various diversities such as the Shannon and Simpson index within a sample site (Colwell and Elsensohn, 2014). EstimateS computes a mean and variance for species richness by randomly selecting a sample size from the total data sample to generate an  $S(\text{est})$ , Incidence Coverage-based Estimator (ICE), and Chao2 line (Gotelli and Colwell, 2001).  $S(\text{est})$

calculates the expected number of species based on the sample. Both ICE and Chao2 calculate the sampling efficiency to generate an estimated species richness within the study area (Gotelli and Colwell, 2001; Chao et al., 2000).

### Data analyses

#### Echolocation call identification

The Kaleidoscope Pro software version 5 (Wildlife Acoustics, Concord, MA, USA) was used to analyse and identify each bat call series recorded. Kaleidoscope Pro is able to automatically identify recordings based on the characteristics of each call series through the use of a built-in classifier tool for different regions of the world (Rydell et al., 2017). To enhance the accuracy of this automated identification tool, a northern KNP-specific bat species classifier was developed and uploaded into the programme. A comprehensive call reference library was created using the echolocation calls of bats that had been recorded upon release, during this study. In addition, unpublished echolocation calls of identified bat species recorded by PJT and DMP were also included to supplement the library. The call reference library was used as an identification guide for the development of the KNP-specific classifier using call sequences from 516 individual bats of 29 species and seven families (Tab. 1, Tab.S1).

To develop the KNP-specific classifier, a subset of 8,118 individual call sequences was used, collected during the first night of sampling in 2017 from nine sites across the Pafuri area. The classifier is a function used as a filter to speed up sorting and subsequent final identification of each call. Using the built-in “cluster tool” of Kaleidoscope, the 8,118 recordings were clustered into 43 initial clusters based on their call characteristics. Call characteristics included total duration of the call (Dur), characteristic frequency (Fc), minimum call frequency (Fmin), maximum call frequency (Fmax) and the frequency at the change in slope of the call (Fk) (Monadjem et al., 2020). Each of the individual bat call sequences within each of the newly recognized clusters was then manually identified to species level using the call reference library. Recorded call sequences were only positively identified/labelled to species level if there was a sequence of three or more pulses recorded, to

avoid wrong identification of species due to possible noise, social calls or insufficient call statistics. If a call sequence could not be positively identified, it was left blank. Call sequences were also left blank when there was clearly more than one bat species that had been recorded in a call sequence. This conservative approach was adopted to avoid committing a Type I statistical error (Parker and Bernard, 2019; Clement et al., 2014) and to improve the overall accuracy of the classifier. All manual species assignments were then verified by two additional observers (DMP and PJT). Using these new species identifications, the ‘cluster tool’ in Kaleidoscope was then re-run on the same subset of call sequences to produce a final .kml file (i.e. the KNP-specific classifier) that could be used to cluster all echolocation call recordings from each site (over both years) into putative species categories based on their call characteristics. Even though Kaleidoscope automatically identifies each recording, the software is not completely accurate (Rydell et al., 2017). Therefore, each putative species identification made by Kaleidoscope was manually verified using the call reference library and the conservative criteria described above. Using this procedure, passing bats could be identified to species level and the accuracy of the KNP-specific classifier (i.e. % of correct identifications) assessed. To keep the automated identification consistent throughout the study in order to test the accuracy of the classifier, new species and unidentifiable echolocation calls were not added to the classifier tool.

## Results

### Bats of the northern KNP

Three surveys were conducted in northern KNP in the 1980’s. Combined, the three published records describe bat captures representing 37 species in eight families (Tab. 1: Vespertilionidae, Molossidae, Miniopteridae, Emballonuridae, Nycteridae, Pteropodidae, Rhinolophidae and Hipposideridae).

Additional data were drawn from Monadjem et al. (2020) that describes all bat specimens collected within the study area between 1960 and 1990, including some of the specimens from the 1980’s surveys mentioned previously. A total of 142 individuals were added from this dataset, representing 35 species from nine families (Tab. 1: Vespertilionidae, Molossidae, Miniopteridae, Nycteridae, Rhinolophidae, Rhinonycteridae, Emballonuridae, Pteropodidae, and Hipposideridae). Thus, as a whole, the region had a total of 40 individual species recorded historically.

In 2017, a total of 106 individual bats were captured within the Pafuri area. Eight species, representing four families (Molossidae, Vespertilionidae, Rhinolophidae and Pteropodidae) were captured in total (Tab. 1). In 2018, a total of 49 individuals, representing nine species from five families, were captured (Tab. 1: Molossidae, Vespertilionidae, Hipposideridae, Rhinonycteridae, and Pteropodidae). One new and endangered species was added to the northern KNP species list, which was not recorded in KNP until 2012 (Balona et al., 2016; Jubber, 2012), namely *Clootis percivali* (Rhinonycteridae).

In 2017, a total of 71,512 echolocation call sequences were recorded within the Pafuri area. Twenty-two species, representing six families (Emballonuridae, Hipposideridae, Miniopteridae, Molossidae, Rhinolophidae, and Vespertilionidae), and one unknown call, labelled Unknown 75, were recorded (Tab. 1). In 2018, a total of 48,982 echolocation call sequences were recorded within the Punda Maria/Shingwedzi area. Twenty-two species, representing six families (Emballonuridae, Hipposideridae, Miniopteridae, Molossidae, Rhinolophidae and Vespertilionidae), and two unknown calls, labelled Unknown 75 and Unknown 34, were recorded (Tab. 1; Fig.S2). One new species (*Otomops martiensseni*, Molossidae) and two possibly new species (Unknown 34 and 75 kHz) were added to the species list of northern KNP by using the acoustic methods. This brings the total number of species recorded in northern KNP to at least 42 but possibly 44.

### Sampling efficiency

The rarefaction curves for the live-captures of this study showed a noticeably broad standard deviation at most sampling sites and curves do

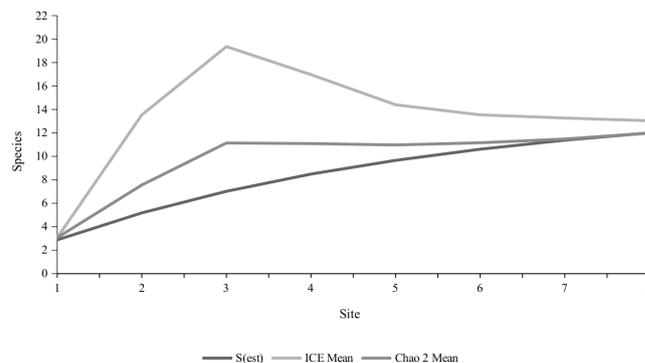


Figure 2 – Species rarefaction curve for all live-capture data recorded from current survey within northern Kruger National Park. S(est) is the observed data and ICE mean and Chao 2 mean are the estimated species richness. Error bars represent standard deviation.

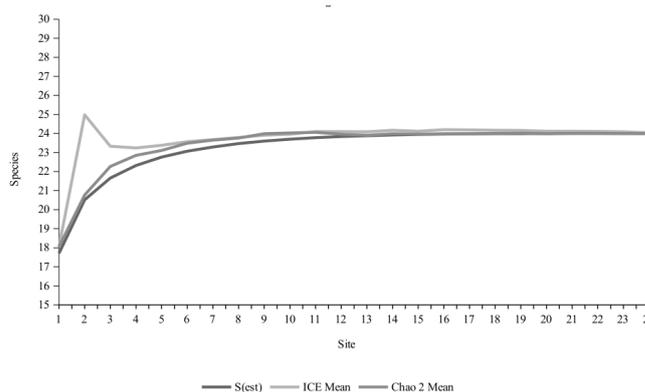


Figure 3 – Species rarefaction curve for all acoustic data recorded within the northern Kruger National Park. S(est) is the observed data and ICE mean and Chao 2 mean are the estimated species richness. Error bars represent standard deviation.

not seem to visibly level off, which indicates that sampling was not complete for this study method (Fig.2). However, the rarefaction curves for the acoustic sampling show rarefaction curves levelled off and the standard deviation was low at most sites (Fig.3), indicating sampling completeness.

### The use of an automated classifier

The classifier tool in Kaleidoscope software had a relatively high average accuracy in terms of identifying echolocation calls to species level. The classifier tool had an average percent accuracy of 79.3% in 2017 and 80.0% in 2018. Overall, the classifier tool demonstrated a 79.7% accuracy in correctly identifying the echolocation calls to species level. The cluster tool demonstrated to have the highest identification accuracy for the families Hipposideridae (99.5%) and Miniopteridae (98.8%). The families Molossidae (83.7%) and Vespertilionidae (83.4%) both had a moderate identification accuracy. The identification of Rhinolophidae (60.2%) and Emballonuridae (51.8%) had the lowest accuracy percentage.

## Discussion

### Completeness of the current survey and bat species richness

A total of eight sites were sampled using live-capture within the study area of northern KNP over a two-year period. From the eight sample sites, 13 species were detected. The expected number of species and Chao 2 (11.99) estimator lines demonstrate that species richness continued to increase throughout the sampling (Fig.2). Both the expected number of species and Chao2 curves suggest that sampling was incomplete. In addition, the ICE (19.36) estimator indicated that the survey missed approximately six species over the eight sampling sites (Fig.2). By expanding efforts through increasing the numbers of capture sites

**Table 1** – Bat species recorded within the northern parts of Kruger National Park, South Africa, historically and during this study in 2017/2018. Showing the total number of calls recorded for the acoustic surveys as well as the years of historic and current live-captures (Rautenbach et al. 1984, 1985, Aldridge and Rautenbach 1987, Monadjem et al. 2020). The X indicates an absence of records. Species are given with their current global IUCN status (LC= least concern/NT= near threatened) and population trend (iucnredlist.org). (\*) Due to current or previous taxonomic uncertainties it is unclear if the echolocation call of this species has been recorded .

| Species                          | Historical live-captures                 | Acoustics 2017 | Acoustics 2018 | Live-capture | IUCN status   |
|----------------------------------|--|----------------|----------------|--------------|---------------|
| <b>Emballonuridae</b>            |  |                |                |              |               |
| <i>Taphozous mauritanus</i>      | 1983, 1984, 1985                         | 895            | 624            | X            | LC-unknown    |
| <b>Hipposideridae</b>            |  |                |                |              |               |
| <i>Hipposideros caffer</i>       | 1979, 1982, 1984, 1985, 1988             | 1083           | 262            | 2018         | LC-decreasing |
| <i>Hipposideros vittatus</i>     | 1985, 1987, 1988, 1990                   | X              | X              | X            | NT-decreasing |
| <b>Miniopteridae</b>             |  |                |                |              |               |
| <i>Miniopterus natalensis</i>    | 1979, 1984                               | 611            | 1673           | X            | LC-unknown    |
| <b>Molossidae</b>                |  |                |                |              |               |
| <i>Chaerephon ansorgei</i>       | 1985, 1986, 1988                         | 9649           | 3660           | X            | LC-stable     |
| <i>Chaerephon pumilus</i>        | 1960, 1961, 1979, 1983, 1984, 1985       | 8036           | 6345           | 2017, 2018   | LC-unknown    |
| <i>Mops condylurus</i>           | 1984, 1985                               | 15106          | 8384           | 2017, 2018   | LC-unknown    |
| <i>Mops midas</i>                | 1979, 1983, 1984, 1985                   | 5681           | 1371           | X            | LC-decreasing |
| <i>Otomops martiensseni</i>      | X  | 39             | 33             | X            | NT-decreasing |
| <i>Tadarida aegyptiaca</i>       | 1982, 1984                               | 18596          | 12992          | X            | LC-unknown    |
| <i>Tadarida fulminans</i>        | 1984, 1985                               | X              | X              | X            | LC-stable     |
| <b>Nycteridae</b>                |  |                |                |              |               |
| <i>Nycteris thebaica</i>         | 1960, 1985                               | X              | X              | X            | LC-unknown    |
| <i>Nycteris woodi</i>            | 1979                                     | X              | X              | X            | LC-decreasing |
| <b>Pteropodidae</b>              |  |                |                |              |               |
| <i>Epomophorus wahlbergi</i>     | 1975, 1979                               | NA             | NA             | 2018         | LC-stable     |
| <i>Rousettus aegyptiacus</i>     | 1979, 1983, 1983                         | NA             | NA             | 2017         | LC-stable     |
| <b>Rhinocysteridae</b>           |  |                |                |              |               |
| <i>Cloeotis percivali</i>        | 2012                                     | X              | X              | 2018         | LC-unknown    |
| <b>Rhinolophidae</b>             |  |                |                |              |               |
| <i>Rhinolophus clivosus</i>      | 1990                                     | X              | X              | X            | LC-unknown    |
| <i>Rhinolophus darlingi</i>      | 1984, 1985, 1990                         | 1              | 33             | X            | LC-unknown    |
| <i>Rhinolophus fumigatus</i>     | 1975, 1983, 1985, 1989, 1990             | 307            | 49             | 2017         | LC-unknown    |
| <i>Rhinolophus landeri</i>       | 1985                                     | X              | X              | X            | LC-unknown    |
| <i>Rhinolophus simulator</i>     | 1983, 1985, 1986                         | 56             | 154            | X            | LC-decreasing |
| <i>Rhinolophus smithersi</i>     | 1979, 1982, 1983, 1984, 1985, 1989       | 1194           | 541            | X            | NT-stable     |
| <i>Rhinolophus swinnyi</i>       | 1985                                     | X              | X              | X            | LC-unknown    |
| <b>Vespertilionidae</b>          |  |                |                |              |               |
| <i>Eptesicus hottentotus</i>     | 1982, 1984, 1985                         | 1194           | 1270           | X            | LC-unknown    |
| <i>Glauconycteris variegata</i>  | 1979, 1982, 1982, 1983, 1984, 1985, 1986 | X              | X              | X            | LC-unknown    |
| <i>Kerivoula argentata</i>       | 1985                                     | X              | X              | X            | LC-unknown    |
| <i>Kerivoula lanosa</i>          | 1984                                     | X              | X              | X            | LC-unknown    |
| <i>Laephotis botswanae</i>       | 1985                                     | X              | X              | X            | LC-unknown    |
| <i>Myotis bocagii</i>            | 1979, 1982, 1982, 1983, 1984, 1985, 1986 | X              | X              | X            | LC-unknown    |
| <i>Myotis tricolor</i>           | 1979, 1984, 1985                         | 104            | 98             | X            | LC-unknown    |
| <i>Myotis welwitschii</i>        | Date Unknown                             | X              | X              | X            | LC-unknown    |
| <i>Neoromicia capensis</i>       | 1979, 1985, Date Unknown                 | 950            | 4387           | 2018         | LC-stable     |
| <i>Neoromicia nana</i>           | 1985, Date Unknown                       | 4563           | 1758           | 2017, 2018   | LC-unknown    |
| <i>Neoromicia stanleyi</i> *     | 1985, Date Unknown                       | NA             | NA             | X            | NA            |
| <i>Neoromicia zuluensis</i>      | 1985, Date Unknown                       | 369            | 2323           | 2018         | LC-unknown    |
| <i>Nycticeinops schlieffeni</i>  | 1979, 1982, 1985, 1988, 1990             | 257            | 1219           | 2017         | LC-unknown    |
| <i>Pipistrellus hesperidus</i>   | 1990                                     | 35             | 14             | X            | LC-unknown    |
| <i>Pipistrellus rueppelli</i>    | 1983, 1984, 1985                         | X              | X              | X            | LC-unknown    |
| <i>Pipistrellus rusticus</i>     | 1985, Date Unknown                       | 54             | 593            | 2018         | LC-unknown    |
| <i>Scotophilus dinganii</i>      | 1979, 1983, 1984, 1985                   | 1895           | 1659           | 2017, 2018   | LC-unknown    |
| <i>Scotophilus leucogaster</i> * | 1979, 1982, 1984, 1988                   | NA             | NA             | X            | LC-unknown    |
| <i>Scotophilus viridis</i>       | 1985                                     | X              | X              | X            | LC-unknown    |
| <b>Unidentified species</b>      |  |                |                |              |               |
| Unknown 34 kHz                   | NA                                       | 0              | 88             | X            | NA            |
| Unknown 75 kHz                   | NA                                       | 38             | 3              | X            | NA            |

**Table 2** – Sampling locations within the Pafuri region of northern Kruger National Park with the number of nights, months, sampling method and habitat type (sites which were sampled both acoustically and by live-capturing in bold).

| Site   | Nights | Habitat   | Method              | Month                       |
|--|--------|---|---------------------|-----------------------------|
| Rietbok Vlei                                 | 2      | <i>Adansonia digitata/Colophospermum</i> Mopane Rugged Veld | Mist Net            | April, August               |
| Pafuri Border Camp                           | 1      | <i>A. digitata/Colophospermum</i> Mopane Rugged Veld        | Mist Net, Harp Trap | May, October                |
| Pafuri camp                                  | 8      | Limpopo/Luvuvhu Floodplains                                 | Mist Net, Harp Trap | March, May, August          |
| Crooks Corner                                | 2      | Limpopo/Luvuvhu Floodplains                                 | Harp Trap           | October                     |
| Mapimbi Pan                                  | 14     | Limpopo/Luvuvhu Floodplains                                 | Acoustic            | March, May, August, October |
| <b>Crooks Corner</b>                         | 18     | Limpopo/Luvuvhu Floodplains                                 | Acoustic            | March, May, August, October |
| Pafuri Picnic Site                           | 13     | Limpopo/Luvuvhu Floodplains                                 | Acoustic            | May, August, October        |
| Nwambi Pan                                   | 16     | Limpopo/Luvuvhu Floodplains                                 | Acoustic            | August, October             |
| Luvuvhu River Bridge                         | 19     | Limpopo/Luvuvhu Floodplains                                 | Acoustic            | March, May, August, October |
| Opposite old Bohameng Campsite               | 14     | Limpopo/Luvuvhu Floodplains                                 | Acoustic            | March, May, August, October |
| Luvuvhu River near Thulamela                 | 3      | Limpopo/Luvuvhu Floodplains                                 | Acoustic            | March                       |
| Between Pafuri picnic site and Crooks Corner | 5      | Limpopo/Luvuvhu Floodplains                                 | Acoustic            | October                     |
| <b>Pafuri Border Camp</b>                    | 17     | <i>A. digitata/Colophospermum</i> Mopane Rugged Veld        | Acoustic            | March, May, August, October |
| Nyala Pan                                    | 16     | Limpopo/Luvuvhu Floodplains                                 | Acoustic            | March, October              |
| <b>Pafuri Camp</b>                           | 13     | Limpopo/Luvuvhu Floodplains                                 | Acoustic            | March, May, August          |

and sampling hours, the possibility of recording additional, less common bat species could increase (Larsen et al., 2007).

A total of 24 sites were sampled using acoustic detectors within northern KNP over a two-year period. From the 24 sample sites, 24 species were detected. The expected number of species line demonstrates that species richness no longer increased after the 20th sampling (Fig.3). The Chao2 (24.06) estimator indicated completeness of sampling after the inclusion of the 11th sampling site (Fig.3). However, the ICE (24.98) estimator indicated that the survey missed approximately one species over the 24 sampling sites. High frequency calls cannot travel further than a few meters thus decreasing the possibility of recording a species with a higher frequency call (Monadjem et al., 2017). Very high frequencies calls ranging between 80 to >200 kHz are often undetected by acoustic surveys unless the bat species flies close to the microphone (Monadjem et al., 2017).

Northern KNP has a high richness of bats, with 40 species documented historically using capture techniques (Monadjem et al., 2020; Aldridge and Rautenbach, 1987; Rautenbach et al., 1984, 1985). In our study during 2017 and 2018, we recorded a lower total number of 27 distinct species, of which only 13 species were live-captured. Sampling limitations, limitations of the bat detectors, behavioural aspects and environmental effects could have contributed to these observed differ-

ences in species richness (McCain, 2007). The time span of the historical data was 30 years, compared to the current assessment of only two years. Expanding the number of capture locations and sampling hours could increase the possibility of capturing more rare species (Larsen et al., 2007). Furthermore, for logistical reasons, we could not sample all the months in which historical surveys took place and might have missed species, which only seasonally occur in the area. However, as June and July are the dry and cold winter months in northern South Africa, with generally lowest bat activity, we choose to match the historical sampling sites for our acoustic survey rather than the historical sampling month, aiming to sample at least one winter and one summer month at each site.

As mentioned earlier, bat species that call at very high frequencies (80 to >200 kHz), such as *Kerivoula lanosa*, are able to go undetected by acoustic surveys (Monadjem et al., 2017). In addition, the family Nycteridae are also known as ‘whispering bats’ due to their soft, low-intensity echolocation calls (Monadjem et al., 2020). It is very difficult to record this family through acoustic technology even from a distance of <1 m (Monadjem et al., 2017). As such, this group is regularly under-represented in acoustic bat surveys (Parker and Bernard, 2019; Monadjem et al., 2017).

**Table 3** – Sampling locations within the Punda Maria/ Shingwedzi region of northern Kruger National Park with the number of nights, months, sampling method and habitat type (sites which were sampled both acoustically and by live-capturing in bold).

| Site                        | Nights | Habitat  | Method              | Month                 |
|-----------------------------|--------|--|---------------------|-----------------------|
| Shingwedzi Research camp    | 8      | <i>Salvadora angustifolia</i> Floodplains                      | Harp Trap, Mist Net | May, October          |
| Punda Maria restcamp        | 6      | Punda Maria Sandveld   | Mist Net, Harp Trap | March, September      |
| Babalala Picnic site        | 4      | <i>Colophospermum</i> Mopane Shrubveld                         | Harp Trap           | March, May            |
| Shingwedzi restcamp         | 2      | <i>S. angustifolia</i> Floodplains                             | Harp Trap           | October               |
| <b>Babalala Picnic site</b> | 16     | <i>Colophospermum</i> Mopane Shrubveld                         | Acoustic            | March, May, September |
| Kaniedood bird hide         | 13     | <i>S. angustifolia</i> Floodplains                             | Acoustic            | March, May, September |
| Tshanga lookout             | 8      | <i>Colophospermum</i> Mopane Shrubveld                         | Acoustic            | March, May            |
| <b>Shingwedzi restcamp</b>  | 17     | <i>S. angustifolia</i> Floodplains                             | Acoustic            | March, May, September |
| Nyawatsi bird hide          | 8      | <i>Combretum/Colophospermum</i> Mopane Rugged Veld             | Acoustic            | September             |
| Red Rocks lookout           | 8      | <i>S. angustifolia</i> Floodplains                             | Acoustic            | September             |
| Sirheni restcamp            | 8      | <i>Colophospermum</i> Mopane/ <i>Acacia nigrescens</i> savanna | Acoustic            | September             |
| Punda Maria restcamp        | 18     | Punda Maria Sandveld   | Acoustic            | March, May, September |
| Maritube Pan                | 10     | Punda Maria Sandveld   | Acoustic            | March, May, September |
| Matukwala Pan               | 7      | Punda Maria Sandveld   | Acoustic            | March, May            |
| Coetzer Pan                 | 7      | Punda Maria Sandveld   | Acoustic            | March                 |
| Dzundzwini Road             | 6      | <i>Colophospermum</i> Mopane Shrubveld                         | Acoustic            | May                   |
| Punda Maria Gate            | 4      | Punda Maria Sandveld   | Acoustic            | October               |

Overall sensitivity of microphones and the variability of echolocation calls between species and individual bats can cause limitations for the bat detectors thus causing under-representation of certain bat species (Adams et al., 2012). Lower call frequencies can travel greater distances and can be recorded further away from a microphone compared to higher call frequencies which can only be recorded from a short distance (up to a few meters) from the microphone (Monadjem et al., 2017). Highly sensitive microphones are able to detect bats from a greater distance but bats with lower frequency calls (e.g. those in the Molossidae) can appear to dominate a particular area of study (Adams et al., 2012). Less sensitive microphones have a shorter detection range and can only record bats that fly close to the microphone (Adams et al., 2012). Whether using highly sensitive or less sensitive microphones, maximum detection is dependent on placement of microphone and should be placed as close as possible to known bat activity locations such as a water sources (Agranat, 2014).

Due to these differences in sampling effort and other limitations in the current study, such as access to sites outside of designated rest camps and picnic sites, it is difficult to determine if the documented decline in bat richness has occurred due to environmental differences (e.g. climatic or vegetational) compared with the historical assessments. The majority of the historical individual bat captures were documented during the 1980's (Tab. 1). During this time, KNP experienced two years (1980 and 1984) of slightly higher than average precipitation while slightly lower than average precipitation was recorded in the years 1982, 1986 and 1988 (MacFadyen et al., 2018). The two years with high precipitation could have caused an increase in insect prey, resulting in an increase in bat activity (Monadjem et al., 2020; MacFadyen et al., 2018; Pinheiro et al., 2002). By contrast, in 2015 and 2016, KNP experienced a severe drought caused by the third strongest El Niño event recorded since the beginning of climate recordings (Urban et al., 2018). The decrease in precipitation leading up to the beginning of the current survey might have led to a decrease in insect abundance and a decline in bat activity (Pinheiro et al., 2002). We also have to acknowledge that the historical species richness is based on an accumulative total based on museums records, which does not provide error estimates for the sampling or a means to directly compare the approaches of the historical and current richness surveys.

Many of the species recorded historically are reliant on habitats with dense, woody vegetation (Monadjem et al., 2020; Aldridge and Rautenbach, 1987; Rautenbach et al., 1984, 1985). These so-called 'clutter-feeders', are dependent on large trees and shrubs as roosts and use the surrounding habitat as a food source while hunting (Monadjem et al., 2020; Taylor et al., 2020). In addition, foliage-roosting bat species, such as *Taphozous mauritanus* and *Neoromicia nana*, use the leaves, roots, trunks, and branches of trees and shrubs to roost in (Willis and Brigham, 2004; Fenton, 1992). KNP monitors tree and shrub density through aerial photography and data has shown a decline in the prevalence of large trees since the 1960's (Wigley et al., 2014; Trollope et al., 1998). Both, over-browsing by herbivores and fire, which restricts the growth processes of trees smaller than three meters in height, may have caused the recruitment of young trees to decline (Wigley et al., 2014; Trollope et al., 1998). Elephants (*Loxodonta africana*) are known to damage large trees by over-browsing, bark-stripping, snapping off branches and even killing trees by knocking them over (Midgley et al., 2005). Areas that have experienced increases in elephant populations, and fires, show significant declines in large trees (Trollope et al., 1998). In addition, many large riparian trees were severely destroyed by flood events around the Shingwedzi River in the year 2000 (Bonaccorso et al., 2014). The loss of several large trees has caused a decline in recruitment of young trees around affected areas (Bonaccorso et al., 2014). The historical trap locations were all within these riparian habitats, where bats are likely to forage (Monadjem and Reside, 2008; Aldridge and Rautenbach, 1987; Rautenbach et al., 1984, 1985). Even though the current survey sites were located within and around these historical sites, there were less clutter dependent bat species captured. Likewise, there was an increased number of the so-called 'open-air' and 'clutter-edge' foraging bat species captured in the current study. However, these

hypotheses are speculative and future studies should try to include variables of environmental change in order to test the effect of changes in vegetation due to over-browsing, fires and climate change.

### Community structure of the northern KNP bats

A total of 24 bat species were recorded acoustically from 24 sampling sites. Molossidae was the dominant family with 89,892 recorded calls in both years combined. The Molossidae are open-air flyers (i.e. they fly above tree canopy) and use low frequency calls <30 kHz to search for food (Monadjem et al., 2020; Norberg and Rayner, 1987). Due to their ability to call at relatively low frequencies, species from the family Molossidae can easily be detected through ultrasonic microphones (Monadjem et al., 2017). The family Molossidae was therefore most likely the dominant family recorded due to their low frequencies calls, potentially inflating their prevalence amongst the bat calls recorded (Monadjem et al., 2017, 2020).

Vespertilionidae was the second most dominant family with 22,744 calls recorded. Species of Vespertilionidae are clutter-edge foragers that fly between open air and dense vegetation with echolocation calls at intermediate frequencies (Russo et al., 2018; Lee and McCracken, 2004). Compared to the family Molossidae, Vespertilionidae have higher frequency calls with a lower maximum detection distance recorded by ultrasonic microphones (Monadjem et al., 2017). The family Hipposideridae was the family with the fewest calls (1,345) recorded. These bats hunt for prey within dense vegetation and use high frequency echolocation calls (Monadjem et al., 2020).

*Hipposideros caffer* was the only species from Family Hipposideridae recorded using bat detectors. With the highest call frequencies recorded during the survey, *H. caffer* has the shortest detection range and was most likely only recorded when an individual flew past close to the microphones (Parker and Bernard, 2019; Monadjem et al., 2017). The echolocation calls of *C. percivali* and *Hipposideros vittatus* were not recorded during the acoustic survey. *H. vittatus* mostly occurs in countries north of South Africa with Pafuri being the southernmost border of their distribution (Monadjem et al., 2020).

Throughout the survey, two sets of calls (Unknown 34 and Unknown 75) were recorded that could not be matched with certainty to any known species from the call reference library and current literature (Fig.S2; Monadjem et al., 2020). 'Unknown 34' appears to be a higher frequency Molossidae call that ranges between 33-35 kHz. The call was recorded during each trip in the 2018 survey throughout the Punda Maria/ Shingwedzi region. The call has also been recorded previous acoustic surveys within the Soutpansberg and Blouberg Mountains (Taylor et al., 2013a,b). Looking at distribution records the species could possibly be *Sauromys petrophilus*, that has a peak frequency of around 30 kHz (Monadjem et al., 2020).

Unknown 75 appears to be a higher frequency Vespertilionidae call that ranges between 73-76 kHz. The call was recorded during both, the 2017 and 2018, surveys. The call has also been recorded in previous acoustic surveys at Mapungubwe National Park, South Africa (Parker and Bernard, 2019) and in the Okavango Basin, Angola (Weier et al., 2020). Both unknown sets of calls cannot be identified through acoustic methods with certainty and a concerted effort should be made to capture and describe these species (Parker and Bernard, 2019; Taylor et al., 2018).

### Accuracy and use of the Kaleidoscope classifier tool

Even though the Kaleidoscope classifier tool had a relatively high percentage accuracy (79.7%), manual identification was still required to avoid misidentifications (Clement et al., 2014). Kaleidoscope was effective in identifying calls from common bat species such as *Mops condylurus* and *Neoromicia nana*, and in the identification of calls that are distinctive in terms of their characteristics (e.g. frequency and/or duration), which makes them unlikely to be incorrectly identified (e.g. *H. caffer*). However, Kaleidoscope was not as successful in distinguishing between common and non-common species that shared similar call characteristics of frequency and duration (Rydell et al., 2017). *Myotis tricolor*, for example, is a rare species and although it was placed into

the classifier tool as its own group, many calls were misidentified as *Neoromicia capensis*, a common species (Monadjem et al., 2020). It is possible to minimize misidentification of rare species when using the automatic identifier by placing more calls of those species into the cluster tool (Rydell et al., 2017). This will enable the classifier tool to better distinguish between the differences in each species' call parameters, thus minimizing misidentification (Rydell et al., 2017). In addition, the classifier tool misidentified species, such as *Taphozous mauritanus* (Emballonuridae) and *Rhinolophus darlingi* (Rhinolophidae), which overlapped in frequencies with other species but have a unique shape. The classifier tool seems to focus on frequency and duration of calls and is less sensitive to call shape parameters. When using the cluster tool, Kaleidoscope identifies all calls based only on the species used within the classifier tool (Rydell et al., 2017). This can cause false-negatives of species not included in the tool (Rydell et al., 2017). For example, *Otomops martiensseni* was not in the records of the 8,118 calls used to construct the cluster and was therefore always identified as *Mops midas*. *O. martiensseni* has only been recorded in northern KNP once before this study (Adams et al., 2015) and the current calls were detected through manual identification. Thus, after files are automatically identified, manual identification is important to minimize misidentification of rare species and to detect new species, which have not been previously recorded (Rydell et al., 2017; Russo and Voigt, 2016). Calls manually identified as new or non-common species can be collected throughout a study and added into the classifier tool to keep it updated, while aiding in improving the accuracy. Despite these limitations, the Kaleidoscope cluster tool was extremely valuable in streamlining the identification and processing of large volumes of bat calls from multiple sites (Rydell et al., 2017).

## Conclusion

Through both methods combined (acoustic and live-capture), our survey recorded a total of 27 species within the northern KNP. Of the total species richness count, 48% of the species were detected through the use of live-capture but most (89%) were recorded by bat detectors. Both acoustic detectors and live-capture methods can have biased results towards certain species and when using only one method, rare species are most likely to be overlooked (Monadjem et al., 2017; Flaquer et al., 2007; Francis, 1989). Combining techniques can result in a greater species richness estimate and a better understanding of bat diversity (Taylor et al., 2013a; Flaquer et al., 2007). Bat detectors are able to detect a greater species richness over a shorter period of time compared to live-capture methods (O'Farrell and Gannon, 1999). However, when identifying recorded echolocation calls, it is important to know and understand the call parameters of each species within the study area (Taylor et al., 2013a; Adams et al., 2012). Through releasing live-captured species, reference echolocation calls for call libraries can be collected (Taylor et al., 2013a). Having a strong reference library decreases the chances of mis-identifying calls recorded from acoustic detectors (Parker and Bernard, 2019). Our study was therefore successful in showing that through the combination of live-capture and bat detector methods, most (~68%) of the historically documented bat species were recorded within the study area.

In addition to a strong call reference library, an accurate classification tool is beneficial for the identification process of recorded echolocation calls. A reliable classifier tool is able to automatically sort through large datasets to select and identify quality bat calls, creating a quicker screening process (Rydell et al., 2017). With an accuracy of about 79%, the site-specific classifier tool developed for KNP was successful in the identification of common species but it was generally prone to mis-identifying rare species. Our research supports recent work that has demonstrated that manual identification of bat calls is essential to detect any false-negatives for rare species as well as species not included in the classifier tool (Rydell et al., 2017). As indicated by the rarefaction curves, sampling by means of live-captures is not complete and future studies should focus more effort on this method. Especially, given the limitation of bat detectors to record high frequency

echolocation calls, which are common in many southern African species.

Continuous monitoring of bat species can provide insight into the effects of climate change and overall health of the environment for bats species that face many global threats (Russo and Ancillotto, 2015; Mickleburgh et al., 2002). By re-surveying the insectivorous bats of northern KNP, we have been able to generate an important baseline dataset for the future monitoring of bat diversity. Through an increased research focus on bats, conservation efforts can continue to expand within KNP as well as throughout South Africa. ☞

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## Supplemental information

Additional Supplemental Information may be found in the online version of this article:

**Table S1** Summary of the echolocation call characteristics of released bats.

**Figure S2** Sonograms of each bat species (Figures S1–25).

**File S3** Classifier created with Kaleidoscope Pro 5.