Herpetofaunal survey of the Khaudum–Ngamiland dispersal area in Namibia

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Abstract

An extensive Herpetofaunal survey of three under researched areas in North–eastern Namibia (all forming part of the Khaudum Ngamiland dispersal area) was conducted. The areas in question were the Nyae Nyae conservancy, Khaudum national park, and a small area of Mahango–Divundu, which borders the Okavango river approximately 75 km into the Caprivi strip. A preliminary checklist and desktop study of the areas was conducted based on known ranges and occurrences of Southern–African amphibian and reptile species, supplementing the survey. During the expedition 17 amphibian species representing 13 genera and 10 families, and 22 reptile species representing 19 genera and 12 families were encountered. Genetic sequencing of the 16S ribosomal gene was done for 20 specimens to confirm their identity. Phylogenetic trees of two species and ecological niche models of four species were created, supplementing scientific knowledge regarding the herpetofauna of this part of Namibia. This study provides the first record of adult specimens of an undescribed *Pyxicephalus* specie, as well as the first genetic data of *Ichnotropis grandiceps*.

Keywords

Namibia, Khaudum, Nyae Nyae Conservancy, Mahango, reptile, amphibian, herpetofauna, 16S

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Chapter 1

Introduction, aim and objectives

1.1 Introduction

Southern Africa is blessed with an incredible terrestrial and marine biodiversity, with countries such as South Africa, Namibia, Botswana, Zimbabwe and Mozambique possessing incredible biodiversity and endemism, with rich diversities of mammals, birds, plants, aquatic life, and herpetofauna (amphibians and reptiles). Frogs and reptiles are vital components of the ecosystem, acting as producers (in systems where they act as prey), and regulators and consumers (where they act as predators). In almost all cases, frogs and reptiles are somewhere in the middle of the food chain, providing a food source for birds of prey and other small predators, and also acting as predators and regulators for a variety of insects and other small frogs and reptiles. The environment and environmental conditions of a particular area is a major factor in determining the presence of not only particular species, but whole groups of animals. Frogs, due to their aquatic to semi aquatic nature and reliance on water for breeding, lifecycles, effective locomotion, and osmoregulation among other factors, rely heavily on the presence of water as a key factor in their habitat and may be expected to prefer areas with higher or more frequent precipitation, more suitable vegetation, and less drainage. Amphibians are much less abundant in desert and arid areas as they are much more water dependant in comparison to reptiles. These factors are a few among many substantiating frogs' prevalence in and affinity to wetter areas.

On the other hand, one may expect reptiles to prefer warmer and drier areas in comparison to amphibians. Reptiles are not able to self–regulate their temperature and rely on the sun to increase their body temperature to a level able to sustain their metabolism and movements. Drier areas are usually so due to higher temperatures, more drainage, and less rainfall. For many reptile species these conditions are preferable, though there are some semi aquatic reptile species reliant on water primarily for finding prey (E.g. Brown Water Snake, *Lycodonomorphus rufulus* (Lichtenstein, 1823)) as well as frogs found far from waterbodies, with adaptations of their metabolism and reproductive biology making them suitable to live in arid environments (E.g. the Desert Rain Frog *Breviceps macrops* Boulenger 1907) (du Preez & Carruthers, 2017). According to Herman & Branch (2013) reptiles play an important role in tropical and subtropical arid areas as these environments usually are not able to support large diversities and numbers of mammals, due to their dependency on water and nutrients, this high–energy requirement not being sustainable by such desert environments. Based upon a comprehensive checklist and summary of work on the herpetofaunal diversity of Namibia, Herman & Branch (2013) showed that Namibia is home to a diverse assemblage of reptiles and to a lesser extent amphibian species. Namibia is home to about 270 species of reptiles and 61 species of frogs (Herman & Branch, 2013). There is an especially high diversity of amphibians and reptiles in the Namib desert (Griffin, 2003) which stretches from the southern

point of the Namibian coastline in a narrow belt northward into Angola as far as the Namibe province. This desert boasts a particularly high reptile biodiversity with a richness of lizard, skink, burrowing lizard, gecko, and snake species.

Whilst highlighting the biodiversity and depth of herpetofaunal research in the Namib desert and western part of Namibia Herman & Branch (2013) also highlights the lack thereof in Eastern Namibia and the Caprivi. These authors note that as of 2013, no herpetofaunal survey or specific herpetological research has been done with North–eastern Namibia or any of its parks, reserves, conservancies, or areas as a focus area, nor had there been any published data describing the herpetofauna of these areas in a specific manner (Fig. 1). Factors contributing to the lack of more formal data are the extreme remoteness and inhospitality of North–eastern Namibia, as well as the lack of roads and effort needed to travel and camp in the large areas between settlements. A herpetofaunal survey of three under–researched areas in North–eastern Namibia was conducted for the goal of benefitting the knowledge of the governing bodies to help manage these areas and lay the basis for future scientific study. The areas in question are the Nyae Nyae Conservancy (henceforth referred to as the Nyae Nyae) (Fig. 1), Khaudum National Park (henceforth referred to as the Khaudum) (Fig. 2), and Mahango area next to the Okavango river in the Kavango region of Namibia. (Fig. 2). All three aforementioned areas are located in the far North–eastern corner of Namibia with the Nyae Nyae being the furthest South, the Khaudum directly to its North, and Mahango approximately 75 km East from the mouth of the Caprivi strip. The Khaudum and associated areas around it make up the Khaudum–Ngamiland dispersal area (Fig. 2) and due to the fact that all study areas are located within this dispersal area, this study marks the first formal investigation into the herpetofauna of this dispersal area and, accordingly, is titled as such.

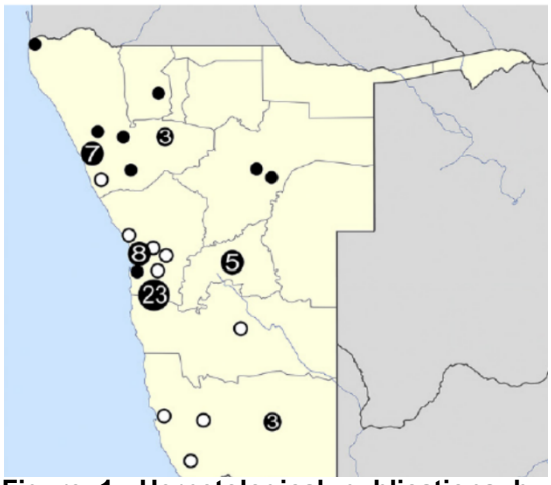

Figure 1: Herpetological publications by study location in Namibia. Enclosed numbers indicate number of studies, dark circles equal two studies, white circles equal one study (Herman & Branch, 2013)

Herpetofaunal surveys provide a valuable tool to determine the composition of an area's herpetofauna, describing which species of amphibians and reptiles are present in a particular area or reserve. The use of such an activity is to generate scientific knowledge, identify undescribed species, identify endangered species, and identify particular areas of vulnerability within the areas' herpetofaunal population (HCV Africa, 2022). Understanding the

population dynamics of an areas' herpetofauna can help develop conservation efforts and decrease biodiversity loss, increase scientific knowledge regarding a specific ecosystem and its activities (such as transmission pathways and pathogens' hosts), increase public knowledge and general respect for an area, describe new and possibly endangered species, and present authorities with crucial information beneficial to the management and legislation regarding a specific area.

There are some data such as sightings by locals, herpetologists, officials, etc. as well as recorded databases such as that of the IUCN red list (IUCN, 2022) and the predicted ranges of some species that give an indication of the herpetofauna expected to reside in the study areas. Lee (2013) noted that there are 25 species of amphibians and reptiles known to the Ju/'hoansi bushpeople of the Greater Dobe area, an area that transects large parts of the Khaudum and Nyae Nyae, though he does not list the specific species. He further mentions that six species of venomous snakes loom large in these Bushpeople's lives, also without specifically mentioning the species. These are more than likely the Black Mamba, Puffadder, Mozambique Spitting Cobra, Boomslang, Anchieta's Cobra, and Vine Snake, although there are some other possible culprits such as the Night Adder. Furthermore, the Ju/'hoansi also confirm the presence of lizards, tortoises, and chameleons, the latter of which would probably be the Flap–necked chameleon *Chameleo dilepis* Leach, 1819, in the Dobe area.

Presently, no formal species checklists of the former two reserves are available. A desktop study, describing the species expected to occur in the study area in question, may prove a valuable tool to determine the herpetofauna of the areas, which in turn may benefit the herpetofaunal survey to determine the presence of endangered or undescribed species, identify vulnerable areas in the herpetofaunal population, and help with the development of conservation efforts by the management of the Nyae Nyae and Khaudum. These efforts in turn may combat loss of biodiversity and a lack of public knowledge and interest, as well as help secure funding and grants for conservation. In this way this study hopes to provide more information for the improvement of managerial decisions, implementation of conservation efforts, as well as increase general scientific and public to benefit further conservation in Namibia. The design of this study is that of an analytical, observational study as it did not entail any active intervention (only documentation and identification of herpetofauna).

Accompanying the herpetofaunal survey it may be necessary to genetically sequence species that may need to be identified molecularly, as well as other species of note such as undescribed, genetically distinct, or rarely seen species, just to confirm their identities. Key species identified to be present in the study area are also the subjects of various ecological

niche models (henceforth referred to as ENMs) to visualize possible suitable habitat for the species in question. These niche models cross–referenced known occurrences of particular species (along with any supplementary occurrences we provide) with environmental data, resulting in maps visualizing similar suitable environmental conditions and providing a tool for future researchers to streamline their efforts and focus on areas with known environmental suitability for whatever species that may be the focus of their studies.

1.2 Research aim and objectives

1.2.1 Research aim

The aim of this study is to provide data regarding the herpetofaunal species composition of the Khaudum National Park, Nyae Nyae Conservancy, and Mahango–Divundu area in North–eastern Namibia. This goal was achieved by way of a desktop study, an extensive herpetofaunal survey, as well as phylogenetic analysis of cryptic, unknown, and unexpected species to determine the identity of all specific specimens, as well as determine the presence of unknown species. To supplement this study, ENMs (Ecological niche models) will be created to visualize possible suitable habitat for key species in the North–eastern part of Namibia.

1.2.2 Research objectives

- Create preliminary herpetofaunal checklist of the Khaudum–Ngamiland dispersal area (study area) by way of literature review.
- Sample (actively and passively) and identify reptile species of the study areas.
- Sample (actively and passively) and identify amphibian species of the study areas
  - Identify amphibian species of the study areas by use of a SongMeter.
  - Identify amphibian species of the study areas via systematic identification of tadpoles.
- Identify possible undescribed and/or endangered species in the study areas morphological identification and phylogenetic analysis.
- Calculate and determine range extensions for species where applicable.
- Identify key species of the study areas for the creation of ENMs and Create ENMs for aforementioned key species for the visualization of possible suitable habitat
- Provide recommendations for future study.

Chapter 2

Study area, Materials and Methods

2.1 Study areas

The Nyae Nyae conservancy (central coordinates: 19.76˚S; 20.62˚E), is the oldest registered conservancy in Namibia and comprises approximately 9000 km^2 of broadleaf and acacia woodlands and savanna, forming a sandy Kalahari bushveld (Giess, 1971; du Plessis, 1992). It is interspersed with multiple large pans that fill up during the rainy months of December to March (Matson, 2006; NACSO, 2022). These pans are one of its most prominent features, along with its presence of approximately 3000 Ju/'hoansi Bushpeople. These people are completely dependent on the conservation of the Nyae Nyae and greater Dobe area to continue their existence by way of hunting and foraging, as well as the selling of crafts and a tiny amount of agriculture (cattle) (Matson, 2006). The Nyae Nyae falls in a semi–arid zone that receives about 300 mm–500 mm of rain yearly (Fig. 3) and has very little deviation in biome and climate (mean temperature 19.7˚C – 20.7˚C (Fig. 4) (Kaseke *et al.*, 2016). Its inhospitality and dry climate makes agriculture extremely difficult, further emphasizing the importance of conservation of the traditional bushpeople way of life, and by extension the Nyae Nyae.

The Khaudum National Park (central coordinates: 18,79˚S; 20,78˚E) is a 3864 km^2 Namibian park comprised mostly of forest and shrub savanna woodland (Giess, 1971), and borders the Nyae Nyae to the South and Botswana to the East. It is an extremely remote and sandy area of woodland and Kalahari sand (Laurenson *et al.*, 1997). It is also home to a single small seasonal spring and two permanent springs. During the latter half of the wet season (Jan–Mar) the rainwater and runoff accumulate in salt pans interspersed throughout the park (Stander, 2004). Along with 10 artificial watering holes created to support the wildlife, these are the only pieces of suitable habitat for many frog species, providing a limiting factor in regards to what species may occur. The Khaudum National Park is also the sole reserve in Namibia that conserves the animals and plants of the Namibian Northern Kalahari Sandveld (Wanke, 2007), lending weight to the importance of a herpetofaunal survey and more associated conservation efforts for this area. One of the Khaudums' most prominent features is its elephant population of approximately three to four thousand elephants (Stander, 2004) almost a third of the total amount of elephants in Namibia (Matson, 2006). The Khaudum has a similar annual precipitation and temperature to that of the Nyae Nyae (Fig. 3 & 4) (Kaseke *et al.*, 2016).

The last study area in question is a small portion of the Mahango–Divundu area (central coordinates: 18,79˚S; 20,78˚E) in the Kavango region of Namibia, bordering the Cubango (a.k.a. Okavango) river. Even though the Mahango area is situated in the Dry Woodlands and Forest Savanna vegetation type of Namibia, it is represented by riverine

vegetation as the Cubango River flows through and adjacent to it, eventually emptying out into the Okavango Delta. A unique part of this area is its floodplain vegetation, acting as a highly productive resource for the people of the area to graze their cattle (du Plessis, 1992). These floodplains also house valuable plant species which have high seed yields and are adaptable to periodic flooding. Amphibians act as important biotic indicators due to their permeable skin and sensitivity to changes in environmental conditions. Accordingly, it is important to be aware of, monitor, and conserve the herpetofauna of this area as they form an important link and part of the food chain and ecosystem in which the aforementioned plant species occur, as well as act as key indicators of ecosystem stressors. This area has a similar mean annual precipitation and temperature to that of the Khaudum and Nyae Nyae (Fig. 3 & 4) (Kaseke *et al.*, 2016). All three areas, as well as the boundaries of the Khaudum-Ngamiland dispersal area is visualized in Fig 2.

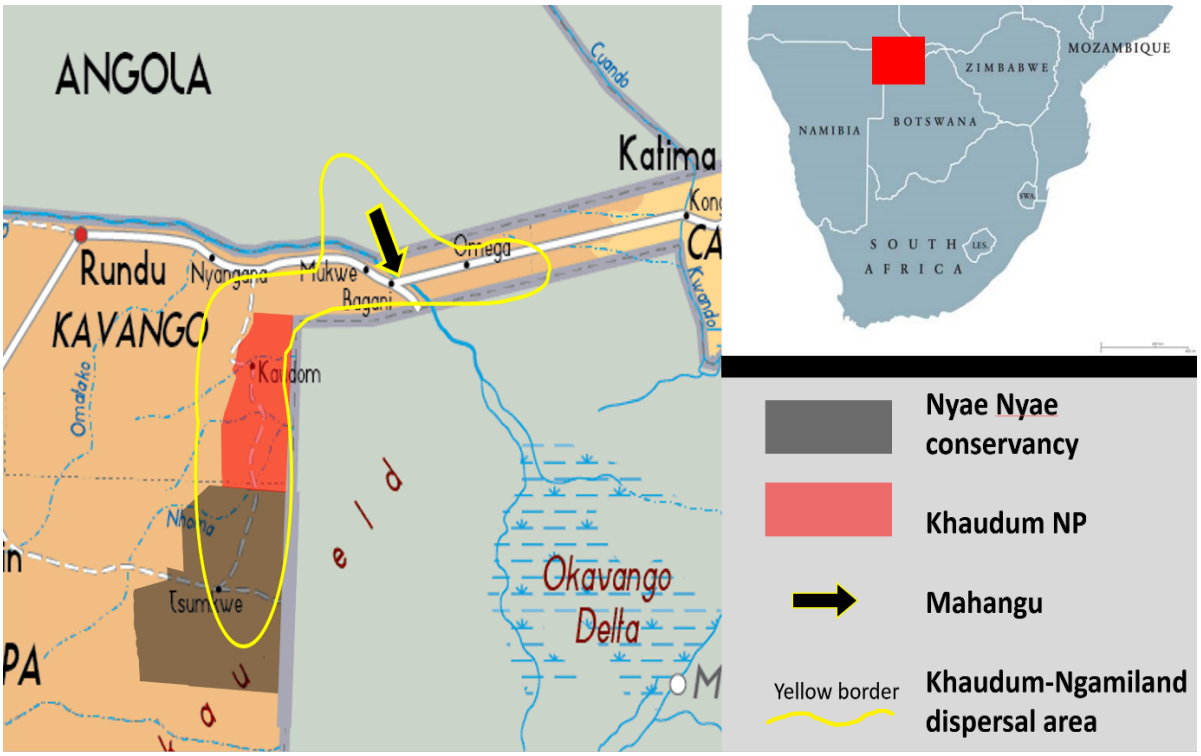

Figure 2: Nyae Nyae conservancy (dark brown area), Khaudum National Park (red area), Mahango (Tip of black and yellow arrow), and Khaudum–Ngamiland dispersal area (Yellow outline), the latter of which was the focal area of this study.

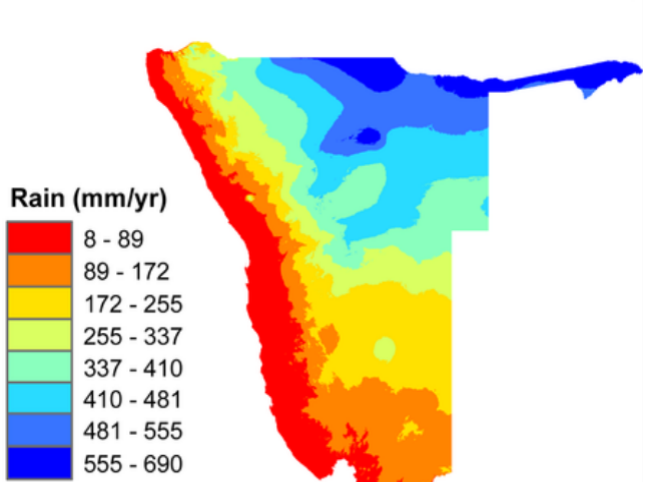

Figure 3: Mean annual precipitation of Namibia (Kaseke *et al.*, 2016).

The preceding figure (Fig. 3) shows the annual average rainfall of Namibia (mm/year). The Nyae Nyae falls mostly in an area of 410 – 481 mm per year, whilst the Khaudum falls partially within this same area, but mostly receives 481 – 555 mm per year. Mahango is the wettest of the study areas, receiving approximately 55 – 690 mm per year. All three of the study areas have a mean annual temperature (Fig. 3) of 20.8 – 21.8 ˚C.

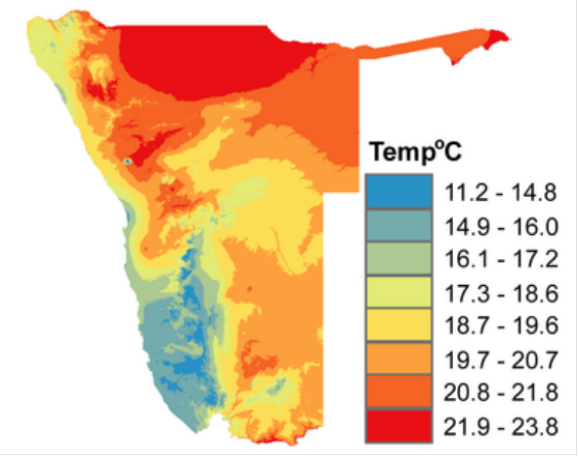

Figure 4: Mean annual temperature of Namibia (Kaseke *et al.*, 2016).

2.2 Desktop study

Based on the work of Branch (1998), Griffin (2003) Alexander & Marais (2007), du Preez & Carruthers (2017) as well as data from the IUCN Red List (2022), African Snakebite Institute (2022) and Reptile Database (Uetz *et al.*, 2022), the expected species checklist of the Khaudum–Ngamiland dispersal area was compiled. Literature was examined and species were included in the checklist if their range visualised on a map in the literature overlapped the Khaudum Ngamiland dispersal area, either fully, partially, or came close to doing so. The desktop study is not by any means a final word on the herpetofauna of this area of North–

eastern Namibia, it was simply created for the purpose of background for the researchers. The goal was to create some expectation of what may be encountered, helping both with preparation (i.e. preparing to encounter venomous snakes), as well as field identification (saving time in the field).

2.3 Sampling sites

Detail on the various localities used in the present study is provided in Table 1. Localities were numbered in the general order that they were visited in, and the term 'locality' and their associated numbering is used to provide a name for a specific geographical point a specimen was collected at. In total, 40 distinct localities provided specimens or sightings contributing to the survey. Unfortunately, limited time was allowed as we were part of a bigger research team with a set schedule. Sites had to be located around or at accessible areas along the route travelled from Tsumkwe up through the Nyae Nyae, Khaudum, and into the Caprivi to Mahango. From the base camp in the Nyae Nyae (locality 7), a series of short excursions were made to pans and fountains all within a reasonable driving distance. At these waterbodies the convoy was stopped whilst all participants employed sampling techniques to locate and capture, or at least identify any amphibian or reptile species. Rocky areas and manmade structures were also identified and examined as reptiles prefer such structures. Some localities that produced herpetofauna were not selected for any particular qualities. At rest and comfort stops during the expeditions active search also took place and some specimens were located by chance. Sampling sites included in and around full and dry pans, ponds, springs, pools on the side or on the road, mud pools, swampland, rocky outcrops, manmade buildings and watch posts, and areas with wooden debris such as logs. Localities are visualized in Figure 5.

Table 1: Coordinates of study sites where herpetofauna were sampled during the expedition

NR.	BASIC FEATURES	LATITUDE	LONGITUDE		NR.	BASIC FEATURES	LATITUDE	LONGITUDE
1	Tsumkwe Lodge Camp Site	–19.600510	20.495422		**21**	Campsite–1. Khaudum	-19.080724	20.700769
2	Dirt Road	–19.739227	20.484267		**22**	Bush	–19.075588	20.703025
3	Flooded vlei	–19.726200	20.485453		**23**	Vlei	–19.080884	20.696554
4	Pan	–19.665303	20.500515		**24**	Roadside pool	–18.988	20.707290
5	Dirt road pool	–19.643866	20.502059		**25**	Roadside pool	–18.986497	20.707927
6	Dirt road pool	–19.617064	20.510375		**26**	Elephant drinking site	–18.956980	20.713089
7	Nyae–nyae pan	–19.749949	20.482376		**27**	Elephant drinking site	–18.91125	20.79716
8	Fountain pond	–19.823779	20.416457		**28**	Bush	–18.851962	20.894202

9	Muddy pool	–19.472550	20.387852		**29**	Khaudum lodge camp	–18.500976	20.818285
10	Grassland pan	–19.741010	20.427360		**30**	Road	–18.500233	20.818500
11	Grassland pan	–19.738819	20.477739		**31**	pan	–18.480775	20.834619
12	Grassland pan	–19.742957	20.479020		**32**	Kiaat forest	–18.287593	20.989673
13	Bush	–19.703928	20.496166		**33**	Kifi pan	–18.162079	21.683225
14	Pool on road	–19.72091	20.58874		**34**	Mahango lodge	–18.140118	21.681536
15	Grassland muddy pan	–19.81204	20.58063		**35**	Kifi fish ponds	–18.159380	21.685468
16	Dirt road	–19.372831	20.499893		**36**	Muddy pool	–18.148751	21.685182
17	Bush	–19.135334	20.485842		**37**	Gautcha pan	–19.442900	20.423107
18	Bush	–19.232616	20.485842		**38**	Gautcha pan far side	–19.49091	20.341873
19	Fountain pool	–19.095563	20.589990		**39**	Muddy roadside pool	–19.431164	20.354679
20	Small muddy pool	–19.092987	20.620146		**40**	Sandy clearing	–19.392984	20.47553

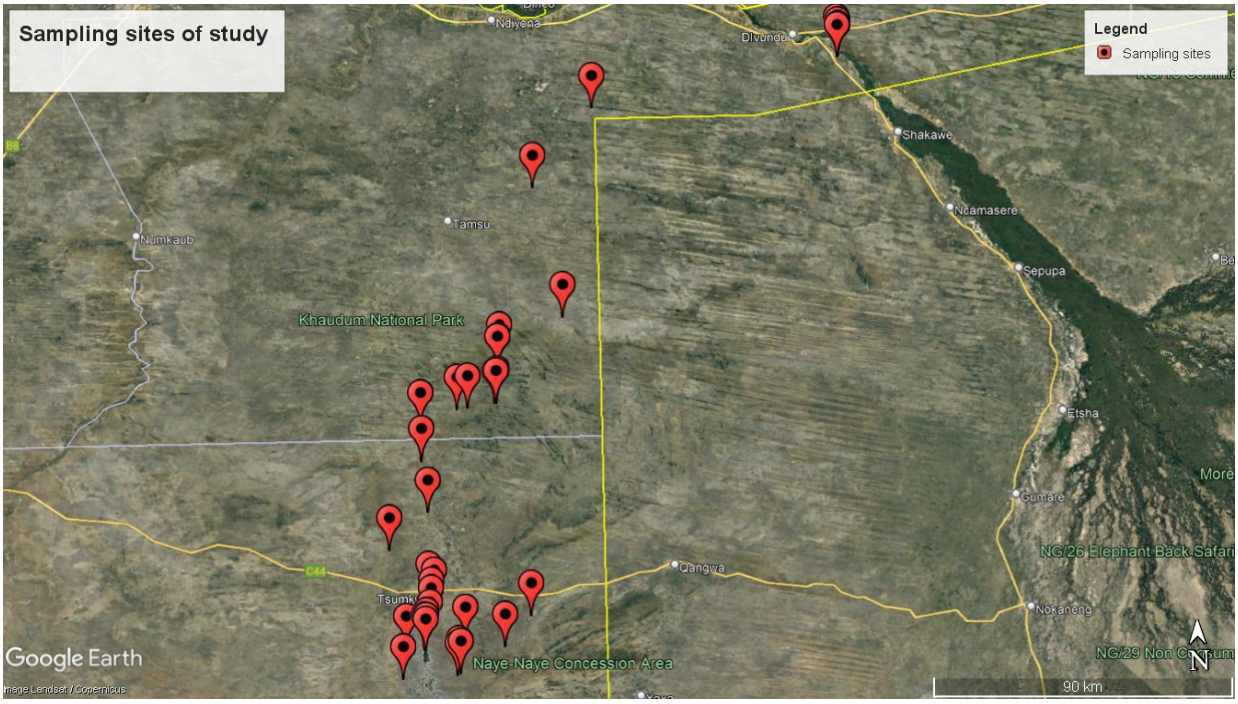

Figure 5: Sampling sites, birds eye view of North–eastern Namibia and Botswana, with Namibia on the left hand side and Botswana on the right hand side, with the border line running through the middle (Google Earth, 2022).

2.4 Field sampling techniques

For the herpetofaunal survey, active sampling was mostly opportunistic and took place near water sources, when animals were seen during driving, near campsites etc. Techniques used were time constrained search (spending varying amounts of time looking for animals in promising areas, usually until no progress could be made and majority of area was searched)

as well as occasional encounters, whilst passive sampling was conducted via the utilization of pitfall traps dug and left over the 4 day stay at the Nyae Nyae, turtle traps baited with chicken liver and left over night at water sources near camping sites as well as in water sources near rest stops that were visited for extended times, and the use of a SongMeter ($SM3^+$, Wildlife Acoustics, Inc.) set up over a period of days during the stay at the Nyae Nyae and Mahangu. These methods entailed animals (reptiles and frogs) safely being captured and identified by the use of Frogs of Southern Africa (du Preez & Carruthers, 2017), Field guide to Snakes and Reptiles of Southern Africa (Branch, 1998), the African Snakebite Institute Application (ASI, 2022) (available on Google and Apple playstores), and the Complete Guide to Frogs of Southern Africa Application (available on Google and Apple playstores). Further insight to the species composition was achieved by analysing recordings by a SongMeter ($SM3^+$, Wildlife Acoustics, Inc.), which were set at specific high interest sites (flooded and wetland areas with high amphibian acttivity) and recorded for 10 minutes at the start of every hour between 16:00 and 06:00 the next day. Sites had to be close to campsites that were stayed at for at least 3 nights (only the Nyae Nyae camp and Mahangu), so that SongMeters could collect data for a number of consecutive nights. SongMeter data was intended both to identify (by identification of calls) species not encountered by active sampling, as well as observe any trends in calling activity, with only the latter goal being achieved.

For genetic analysis of specimens, a small volume of blood was taken from each animal (from the ventral caudal vein in snakes and lizards, from the subcarapacial sinuses in tortoises, and from the femoral artery in frogs). Stringent biosecurity measures were applied, including single use needles, gloves, alcohol solution for disinfecting hands and other equipment, monitoring animals to ensure recovery etc. For large reptiles and frogs (>10 cm body length) 25 gauge needles were used whilst 27 gauge needles were used for smaller animals (<10 cm body length) for reptiles. In the field muscle tissue samples (and tissue separated by autonomy when available) were also taken using sterile dissection tools, including tweezers of varying sizes and scalpels. Two Voucher specimens (a male and female of most species) were taken to the NWU and analysed further, where after they were sent to be deposited in the Namibian National Museum in Windhoek. Both reptile and amphibian vouchers were humanly and ethically euthanized with appropriate volumes of Tricaine Methanesulfonate (MS222) followed by pithing. Samples were fixed in 10% neutral buffered formalin. Back in the laboratory at the NWU specimens were rinsed in water and transferred to 70% ethanol and stored in glass jars. Blood and tissue samples were fixed in 70% ethanol and refrigerated and later stored in a –4˚C freezer until further molecular analysis could be performed. Animals for which a blood sample only was collected were processed on site and released where collected.

2.5 Laboratory and analysis techniques

Under Laboratory conditions, DNA extraction took place following the standard protocol method for nucleated blood and/or animal tissue as detailed in the DNeasy Tissue Kit (QIAGEN, Germany). For nucleated blood 20 μl of blood and ethanol mixture was centrifuged to be able to separate and discard the ethanol, which was also measured to determine the volume of remaining blood. To the blood was added Proteinase K (20 μl), BioFluid & Cell Buffer Red (200 μl), and DNA Elution Buffer (200 μl). This mixture was then vortexed for homogeneity and incubated at 55˚C for a minimum of 30 min, until such a time that all blood cells had sufficiently lysed and no solid biological material remained in the sample. One volume (the same volume of fluid already in the tube, in these cases about 430 μl) of Genomic Binding Buffer was added to the sample, which was then transferred to a spin column and centrifuged for one minute at 12000 rpm. The supernatant was discarded, DNA Pre–Wash (400 μl) added to the spin column and again centrifuged for one minute at 12000 rpm. The process of emptying the collection tube, adding the chemical indicated by the kit, and centrifuging at 12000 rpm for one minute is then repeated twice more with 700 μl and then 200 μl respectively of g–DNA Wash Buffer. Lastly 50μl of Elution Buffer that had been incubating at 70˚C was added to the spin column, incubated at room temperature for 3 minutes, and then centrifuged into a new clean Eppendorf tube at 15000 rpm for one minute. This last step was replicated to ensure a higher yield of usable DNA in the sample. The process for solid tissue samples differed only from that of nucleated blood in the beginning steps where a tiny split sample was made from the main tissue sample, added to a mixture of Proteinase K (10 μl) Solid Tissue Buffer Blue (95 μl), and water (95 μl), and incubated at 55˚C overnight until all solid biological matter had completely dissolved. To this mixture was added two volumes of Genomic Binding Buffer and from here the steps were the same as with nucleated blood, including the chemicals used and centrifuge cycles, speeds, and durations. The quality of the DNA samples was tested using a Nanodrop. All samples were stored at –4˚C when not in use. PCR amplification was then performed under strict and sterile laboratory conditions using a primer targeting the 16S rRNA gene in both the reptile and amphibian samples. For PCR products 1.25 μl of both the forward and reverse primers were added to 12.5 μl of Master Mix (as well as 6 μl of denucleated water), creating a mixture with a volume of 21 μl. To each particular sample 4 μl of an individual DNA sample was added to increase the volume to 25 μl. These samples were then placed in a ProFlex PCR thermal cycler and amplified with the parameters expressed in Table 2.

Table 2: PCR conditions used for all samples and their sequences.

Cycles	**x1**	**x34**			**x1**
Process	Denaturation	Denaturation	Annealing	Extension	Extension
Temperature	95 ˚C	95 ˚C	51 ˚C	72 ˚C	72 ˚C
Time	1 min 30 sec	45 sec	45 sec	1 min 30 sec	5 min

To check whether or not DNA amplification was successful gel electrophoresis was done using a 1% Agarose gel loaded with a 1 KBp ladder and 2 μl samples of PCR product. The aforementioned electrophoresis took place at 100 volts for 30 min. The gels produced were placed in a BioRad GelDoc spectrophotometer and viewed under illumination. Singular, unbroken illuminated bands indicated a high concentration of DNA present in the PCR sample, a necessary requirement for sequencing. Samples were then sent to INQABA Biotec for purification and sequencing of the 16S gene amplified in the samples. The resulting forward and reverse sequences were used to create consensus sequences in Geneious (Version: 2022.2.2) which were then processed in BLAST (NCBI, 2022), an online database comparing our sequences to known sequences already uploaded to the NCBI database (Genbank), allowing us to determine the identity of a species as well as see how much it may differ from other individuals of the same species, or from the nearest related species. Phylogenetic alignment was done in Mega 11 (Tamura *et al.*, 2021), aligning sequences by Clustal W and trimming sequences to be of similar length (roughly 490 bp). For *Ichnotropis*, maximum likelihood trees were created using 11 sequences from available *I. capensis* (Smith*,* 1838) and *I. bivittata* Bocage*,* 1866 samples from GenBank, as well as our sampled sequence (sample 28), and unpublished sequence from an *Ichnotropis* specimen from Angola supplied by Werner Conradie. A *Pyxicephalus* tree was constructed using our three *P. adspersus* Tschudi, 1838 and four unknown *Pyxicephalus* sp. sequences (all collected during the fieldwork of this study), as well as 18 other sequences of *P. adspersus* and *P. edulis* Peters, 1854 from GenBank. For both trees the model was used with the lowest BIC (Bayesian Information Criterion) scores, indicated by MEGA 11. The model in question was Hasegawa–Kishino–Yano (HKY) with a discrete Gamma distribution and five rate categories (Nei & Kumar, 2000) (Tamura *et al.*, 2021).

Maximum entropy distribution models were created in Maxent Ver.3.4.1 (Philips *et al*., 2022) using occurrence data from GBIF (2022) which was supplemented by our own occurrence data points (and in some cases occurrences from other publications) and 19 variables of environmental and elevation data (Table 3) (WorldClim, 2022). Occurrence data was filtered to only include observations from the iNaturalist database that are of research–

grade quality (includes photo, coordinates, and an ID that is verified by one or more experts). Localities were visualised in Google Earth Pro (Ver.7.3.4.8642) and further trimmed to ensure no outliers or mistakes (impossible coordinates) were included, no occurrences (except in the case of *Pyxicephalus* sp. where there are only 3 occurrences) were located extremely close to each other (to avoid bias in the model), and a broad representation of occurrences were included across a species' entire known range, not purposefully excluding any that may prove valuable to the model. The amount of occurrences varied per species, as some species are not as frequently sighted or sampled nor their data uploaded to any applicable databases. Climate variables were clipped to include only Southern–and Central African countries (South Africa, Namibia, Zambia, Zimbabwe, Mozambique, Tanzania, Angola, DRC, Burundi, Rwanda, Swaziland, Lesotho). In Maxent, a first model was created using all 19 BioClim variables, with a jack–knife output and response curves also being generated. Based on these results, certain variables were excluded, whereupon a second model was made with 10 replications cross validating to create an average of the results. This result was processed in QGIS (V: 3.26.3) to create an eligible map to be used for discussion.

Table 3: Description of BioClim variables used for maximum entropy distribution models.

Variable Number	Variable
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp – min temp))
BIO3	Isothermality (BIO2/BIO7) (×100)
BIO4	Temperature Seasonality (standard deviation ×100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5–BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter

BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

2.6 Ecological niche modelling

Not all environmental variables are of equal importance for the Maxent model, and it is beneficial to exclude those with non–existing or insignificant gains to the model so it may be created quicker, and more emphasis can be placed on the variables with a more marketed effect. There are a few factors that can be used when deciding which characteristics and variables to exclude from the model. The curves of Fig. 6 show how each environmental variable affects the Maxent prediction. The curves show how the predicted probability of presence changes as each environmental variable is varied, keeping all other environmental variables at their average sample value. These (Fig. 6) are examples of response curves for the model created for *Pyxicephalus adspersus* Tschudi, 1838. In both the first and second curves there is no movement whatsoever, indicating that this variable does not affect the Maxent model in any meaningful way.

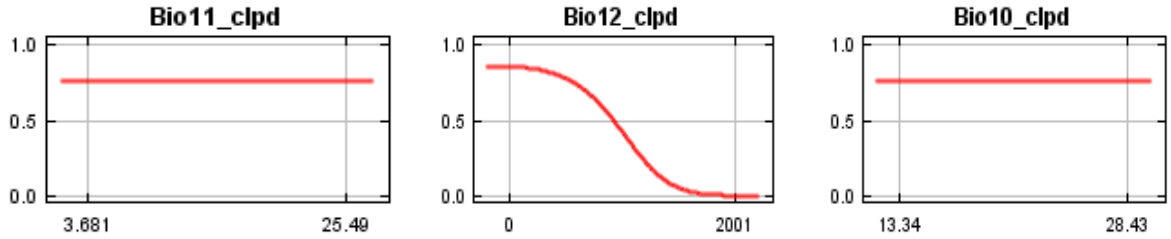

Figure 6: Maxent response curves.

In contrast to the above marginal response curves, each of the following curves represents a different model, namely, a Maxent model created using only the corresponding variable. These plots reflect the dependence of predicted suitability both on the selected variable and on dependencies induced by correlations between the selected variable and other variables, and are also examples of the model created for *P. adspersus*.

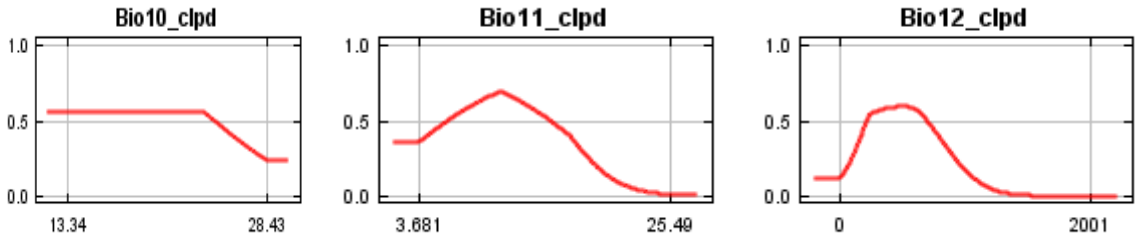

Figure 7: Maxent response curves for three variables, exemplifying the effect of particular variables on the model.

The method used to evaluate variable importance was examining the jackknife graph for each species' first run. Figure 8 shows the results of the jackknife test of variable importance for the first replicate run of another species for which an ENM was created. *Ichnotropis grandiceps*. In this case, the environmental variable with the highest gain when used in isolation was BIO5 (Max Temperature of Warmest Month) which therefore appears to have the most useful information by itself. The environmental variable that decreases the gain the most when it is omitted is BIO15 (Precipitation Seasonality (Coefficient of Variation)), which therefore appears to have the most information that isn't present in the other variables. To determine which variables to include a threshold value of the regularized training gain with that variable as the only variable is chosen, and although no particular rule of thumb is applied, the threshold value generally is a value that only includes variables on the high end of the spectrum of regularized training gain, contributing the most to the model. In the case of *I. grandiceps* all values with a regularized training gain lower than 0.6 were excluded from the model.

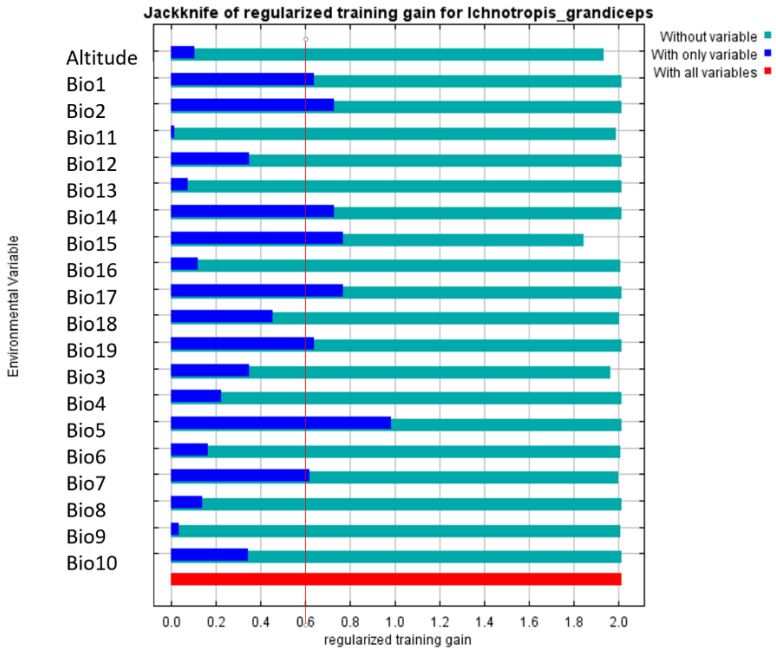

Figure 8: Jackknife graph for *I. grandiceps*, including they red exclusion linear regularized training gain value of 0.6 (Maxent).

Chapter 3

Results

3.1 Desk top study on expected amphibian and reptile species

3.1.1 Amphibians

Based on the desktop study conducted, 32 amphibian species representing 14 genera and 10 families may occur in the study area. This comprises roughly half the number of species thought to occur in Namibia as a whole (Herman & Branch, 2013). The expected amphibian species are listed in Table 4. These species represent one member of the family Arthroleptidae, one of the Brevicipitidae, six of the Bufonidae, two of the Hemisotidae, four of the Hyperoliidae, two of the Microhylidae, two of the Phrynobatrachidae, six of the Ptychadenidae, three of the Pipidae, and five of the Pyxicephalidae.

Table 4: Expected amphibians of the study areas.

Family	Scientific name	Common name
Arthroleptidae	*Leptopelis bocagii* (Günther, 1864)	Bocage's Tree Frog
Brevicipitidae	*Breviceps adspersus* Peters, 1882	Bushveld Rain Frog
Bufonidae	*Poyntonophrynus fenoulheti* (Hewitt & Methuen, 1913)	Northern Pygmy Toad
	Poyntonophrynus kavangensis (Poynton & Broadley, 1988)	Kavango Pygmy Toad
	Sclerophrys lemairii (Boulenger, 1901)	Lemaire's Toad
	Sclerophrys gutturalis (Power, 1927)	Guttural Toad
	Sclerophrys poweri (Hewitt, 1935)	Western Olive Toad
	Sclerophrys pusilla (Mertens, 1937)	Flat–backed Toad
Hemisotidae	*Hemisus guineensis* Cope, 1865	Guinea Shovel–nosed Frog
	Hemisus marmoratus (Peters, 1854)	Mottled Shovel–nosed Frog
Hyperoliidae	*Kassina senegalensis* (Duméril & Bibron, 1841)	Bubbling Kassina
	Hyperolius nasutus Günther, 1864	Long Reed Frog
	Hyperolius parallelus Günther, 1858	Angolan Reed Frog
	Hyperolius benguellensis Bocage, 1893	Bocage's Sharp–nosed Reed Frog
Microhylidae	*Phrynomantis affinis* Boulenger, 1901	Spotted Rubber Frog

	Phrynomantis bifasciatus (Smith, 1847)	Banded Rubber Frog
Phrynobatrachidae	*Phrynobatrachus mababiensis* FitzSimons, 1932	Dwarf Puddle Frog
	Phrynobatrachus parvulus (Boulenger, 1905)	Small Puddle Frog
Ptychadenidae	*Hildebrandtia ornata* (Peters, 1878)	Ornate Frog
	Ptychadena nilotica (Seetzen, 1855)	Nile Grass Frog
	Ptychadena subpunctata (Bocage, 1866)	Speckled–bellied Grass Frog
	Ptychadena mossambica (Peters, 1854)	Broad–banded Grass Frog
	Ptychadena oxyrynchus (Smith, 1849)	Sharp–nosed Grass Frog
	Ptychadena taenioscelis Laurent, 1954	Dwarf Grass Frog
Pipidae	*Xenopus laevis* (Daudin, 1802)	Common Platanna
	Xenopus muelleri (Peters, 1844)	Müller's Platanna
	Xenopus poweri Hewitt, 1927	Power's Platanna
Pyxicephalidae	*Pyxicephalus adspersus* Tschudi, 1838	Giant Bullfrog
	Cacosternum boettgeri (Boulenger, 1882)	Boettger's Caco
	Tomopterna tandyi (Channing & Bogart, 1996)	Tandy's Sand Frog
	Tomopterna cryptotis (Boulenger, 1907)	Tremolo Sand Frog
	Tomopterna krugerensis Passmore & Carruthers, 1975	Knocking Sand Frog

3.1.2 Reptiles

The expected reptiles of the study areas are listed in Table 5 and comprise 69 species representing 48 genera and 23 families, roughly one quarter of the species thought to occur in Namibia as a whole. These species include one Crocodile, two shelled–reptiles, seven Geckos, three of the Agamidae, one Chameleon, eight of the Scincidae, seven of the Lacertidae, three Burrowing Lizards, two of the Varanidae and thirty–five snakes.

Table 5: Expected reptiles of the study areas

Family	Scientific name	Common name

Crocodylidae	*Crocodylus niloticus* Laurenti, 1768	Nile crocodile
Pelomedusidae	*Pelomedusa subrufa* (Bonnaterre, 1789)	Helmeted Turtle
Testudinidae	*Stigmochelys pardalis* (Bell, 1828)	Leopard Tortoise
Gekkonidae	*Chondrodactylus turneri* (Gray, 1864)	Turner's Thick–toed Gecko
	Chondrodactylus laevigatus (Fischer, 1888)	Fischer's Thick–toed Gecko
	Lygodactylus bradfieldi Hewitt, 1932	Bradfield's Dwarf Gecko
	Lygodactylus capensis (Smith, 1849)	Common Dwarf Gecko
	Pachydactylus punctatus Peters, 1854	Pointed Thick–toed Gecko
	Pachydactylus wahlbergii (Peters, 1869)	Kalahari Ground Gecko
	Pachydactylus capensis (Smith, 1846)	Cape Gecko
Agamidae	*Agama aculeata* Merrem, 1820	Western Ground Agama
	Acanthocercus cyanocephalus (Falk, 1925)	Falk's Blue–headed Tree Agama
	Acanthocercus atricollis (Smith, 1849)	Southern Tree Agama
Chameleonidae	*Chameleo dilepis* (Smith, 1831)	Flap–neck Chameleon
Scincidae	*Acontias kgalagadi* Lamb, Biswas & Bauer, 2010	Kalahari Burrowing Skink
	Mochlus sundevallii (Smith, 1849)	Sundevall's Writhing Skink
	Trachylepis spilogaster (Peters, 1882)	Kalahari Tree Skink
	Trachylepis binotata (Bocage, 1867)	Ovambo Tree Skink
	Trachylepis varia (Peters, 1867)	Variable Skink
	Trachylepis damarana (Peters, 1870)	Damara Skink
	Trachylepis punctulata (Bocage, 1872)	Speckled Skink
	Typhlacontias rohani (Angel, 1923)	Rohan's Blind Dart Skink
Lacertidae	*Heliobolus lugubris* (Smith, 1838)	Bushveld Lizard

	Ichnotropis capensis Smith, 1838	Ornate Rough–scaled Lizard
	Ichnotropis grandiceps Broadley, 1967	Caprivi Rough–scaled Lizard
	Meroles squamulosus (Peters, 1854)	Savanna Lizard
	Pedioplanis lineoocellata (Duméril and Bibron, 1839)	Spotted Sand Lizard
Gerrhosauridae	*Gerrhosaurus nigrolineatus* (Hallowell, 1857)	Black–lined Plated Lizard
	Gerrhosaurus auritus (Bettger, 1887)	Kalahari Plated Lizard
Amphisbaenidae	*Monopeltis sphenorhynchus* (Peters, 1879)	Slender Spade–snouted Worm Lizard
	Dalophia longicauda (Werner, 1915)	N/A
	Zygaspis quadrifons (Peters, 1862)	Kalahari Dwarf Worm Lizard
Varanidae	*Varanus albigularis* Daudin, 1802	White–throated Monitor
	Varanus niloticus (Linnaeus, 1766)	Nile Monitor
Typhlopidae	*Afrotyphlops schlegelii* (Bianconi, 1849)	Schlegel's Beaked Blind Snake
Leptotyphlopidae	*Leptotyphlops scutifrons* (Peters, 1854)	Peter's Thread Snake
Boidae	*Python natalensis* Smith, 1840	Southern African Python
Colubridae	*Dasypeltis scabra* (Linnaeus, 1758)	Rhombic Egg Easter
	Dispholidus typus (Smith, 1828)	Boomslang
	Philothamnus angolensis Bocage, 1882	Angola Green Snake
	Philothamnus semivariegatus (Smith, 1840)	Spotted Bush Snake
	Telescopus semiannulatus Smith, 1849	Eastern Tiger Snake
	Thelotornis capensis (Smith, 1849	Southern Twig Snake
Natricidae	*Natriciteres olivacea* Peters, 1854)	Olive Marsh Snake
Lamprophiidae	*Boaedon capensis* Duméril & Bibron, 1854	Brown House Snake
	Lycophidion capense (Smith, 1831)	Cape Wolf Snake

	Limaformosa capensis (Smith, 1847)	Cape File Snake
	Gracililima nyassae (Günther, 1888)	Black File Snake
Prosymnidae	*Prosymna angolensis* Boulenger, 1915	Angola Shovel–snout
Pseudaspididae	*Pseudaspis cana* (Linnaeus, 1758)	Mole Snake
Psammophiidae	*Hemirhagerrhis nototaenia* (Günther, 1864)	Eastern Bark Snake
	Psammophis angolensis (Bocage, 1872)	Dwarf Sand Snake
	Psammophis mossambicus Peters, 1882	Olive Grass Snake
	Psammophis subtaeniatus Peters, 1882	Western Yellow–bellied Sand Snake
	Psammophis trinasalis (Werner, 1902)	Fork–marked Sand Snake
	Psammophis jallae Peracca, 1896	Jalla's Sand Snake
	Psammophylax tritaeniatus (Günther, 1868)	Three–lined Grass Snake
Atractaspididae	*Amblyodipsas ventrimaculata* (Roux, 1907)	Kalahari Purple–glossed Snake
	Aparallactus capensis Smith, 1849	Black–headed Centipede Eater
	Xenocalamus mechowii Peters, 1881	Elongate Quill–snouted Snake
	Xenocalamus bicolor Günther, 1868	Slender Quill–snouted Snake
Elapidae	*Aspidelaps scutatus* Smith, 1849	Common Shield Cobra
	Aspidelaps lubricus (Laurenti, 1768)	Angolan Coral Snake
	Dendroaspis polylepis Günther, 1864	Black Mamba
	Elapsoidea semiannulata Bocage, 1882	Angolan Garter Snake
	Naja anchietae Bocage, 1879	Anchieta's Cobra
	Naja mossambica Peters, 1854	Mozambique Spitting Cobra
Viperidae	*Bitis arietans* Merrem, 1820	Puff Adder
	Causus rhombeatus (Lichtenstein, 1823)	Rhombic Night Adder

3.2 Results of genetic sequencing

Not all species were immediately identifiable and thus in some cases the 16S genetic sequences were amplified to be compared to known sequences on Genbank. Table 6 exhibits the results of the genetic sequencing.

Table 6: Results of blast, including initial identifications, field codes, and details regarding their closest matches from Genbank.

Sample nr.	Fieldcode	Field identification	Closest match	Percentage identity	Locality of match	Accession
1	AL211207A2	*Poyntonophrynus sp.*	*Sclerophrys pusilla*	100	Uganda	KF665136
2	AL211202H1	*Phrynomantis bifasciatus*	*Phrynomantis bifasciatus*	99.82	Mozambique	KM509174
3	AL211202J4	*Ptychadena mapatcha*	*Ptychadena mossambica*	100	Zambia	MK464337.1
4	AL211202J5	*Ptychadena c.f. mapatcha*	*Ptychadena mossambica*	100	Zambia	MK464306.1
5	AL211204G1	*Pyxicephalus c.f. adspersus*	*Pyxicephalus sp.*	100	Zambia	MK464306.1
6	AL211204A1	*Pyxicephalus c.f. adspersus*	*Pyxicephalus sp.*	100	Zambia	MK464306.1
7	AL211204H2	*Pyxicephalus c.f. adspersus*	*Pyxicephalus sp.*	100	Zambia	MK464306.1
8	AL211204H1	*Pyxicephalus c.f. adspersus*	*Pyxicephalus sp.*	100	Zambia	MK464306.1
9	AL211202C2	*Pyxicephalus adspersus*	*Pyxicephalus adspersus*	99.83	N/A	LC640564
10	AL211202G1	*Pyxicephalus adspersus*	*Pyxicephalus adspersus*	99.83	N/A	LC640564
11	AL211202L1	*Pyxicephalus adspersus*	*Pyxicephalus adspersus*	99.83	N/A	LC640564
12	AL211201K1	*Tomopterna cryptotis*	*Tomopterna cryptotis*	99.81	Zambia	MK464284
13	AL211202K2	*Tomopterna cryptotis*	*Tomopterna cryptotis*	99.81	Zambia	MK464284
14	AL211204D1	*Tomopterna cryptotis*	*Tomopterna cryptotis*	99.81	Zambia	MK464284
15	RE211206C1	*Ichnotropis sp.*	*Ichnotropis bivittata*	90.42%	Angola	HF547775
16	AL211206B5	*Ptychadena nilotica*	*Ptychadena nilotica*	100	Botswana	KX836495

17	AL211204C1	*Breviceps adspersus*	*Breviceps adspersus*	99.58	Namibia	MT944251
18	RE211204D1	Lacertidae sp.	*Heliobolus lugubris*	99.60	N/A	DQ871142.1
19	AL211204E1	*Leptopelis bocagii*	*Leptopelis bocagii*	99.46	Angola	MK036434
20	AL211204A1	*Leptopelis bocagii*	*Leptopelis bocagii*	99.46	Angola	MK036434

Sample 1 (Field code: AL211207A2) exhibits multiple 100% pairwise identity matches with *S. pusilla,* confirming its identity as such. Sample 2, a *P. bifasciatus* (FC: AL211202H1) with very unusual patterning exhibits multiple strong matches with other *P. bifasciatus* sequences, with a 99.82% pairwise identity match to a *P. bifasciatus* (Accession: KM509174) from Chumpanga in Mozambique (Peloso *et al.*, 2015). The small difference is the result of a single basepair mismatch. Sample 3 & 4, two *Ptychadena* c.f. *mapatcha* (FC: AL211202J4 & AL211202J5) are identical to one another and have multiple high percentage pairwise identity matches with specimens labelled as *P.* cf. *mossambica* from Zambia (Bittencourt– Silva, 2019). Two of these samples match 100% with ours (A: MK464337.1; MK464340.1), whilst the lowest match is 99.61% (A: MK464336). Other high percentage matches are to *P. mossambica* specimens from Selesele Pan in Northern KwaZulu–Natal, South Africa (A: MH115762; MH115764; MH115761; MH115763) (Reeder *et al.*, 2015). Samples 5, 6, 7, and 8 (FC: AL211204G1; AL211204A1; AL211204H2; AL211204H1) which are identical and identified as *Pyxicephalus* c.f. *adspersus*, exhibit three 100% pairwise identity matches (A: MK464306.1; MK464307; MK464308) with juvenile *Pyxicephalus* c.f. *adspersus* specimens from Western Zambia (Bittencourt–Silva, 2019). These specimens themselves are at most 94.75% identical to the applicable gene of the *P. adspersus* complete mitochondrial genome (A: NC04480) (Cai *et al.*, 2019), whilst ours is 94.62% identical to the same sequence. Samples 9, 10, and 11 (FC: AL211202C2; AL211202G1; AL211202L1) which are all identical and identified as *Pyxicephalus* c.f. *adspersus* exhibit a 100% match with a *P.* c.f. *adspersus* (A: DQ347304) sequence (Bossuyt *et al.*, 2006) (origins vague) as well as with *P. adspersus* (A: LC640564) from (Kambayashi *et al.*, 2022) (Origins also vague). The next best match (99.83%) is to the *P. adspersus* sequence from the complete mitochondrial genome (A: NC044480) (Cai *et al.*, 2019), differing by a single basepair in the applicable barcode. Samples 12, 13, and 14 (FC: AL211201K1; AL211202K2; AL211204D1), which are all identical to one another and identified as *Tomopterna* c.f. *cryptotis*, exhibit two 100% pairwise identity matches with specimens identified as *T. cryptotis* from Angola (A: MN057687; MN057689) (Channing & du Preez, 2020) as well as a 100% match with a *Tomopterna* specimen from Northern Namibia, that being *Tomopterna* sp. "*Shankara*" (identified as the "true *T. cryptotis*) (A:

MK335430) (Wilson & Channing, 2019). It exhibits very high percentage matches (99,81% in all cases) with *Tomopterna* specimens from Western Zambia (A: MK464284; MK464285; MK464282) (Bittencourt–Silva, 2019), which most closely matches the *Tomopterna* "*Shankara*" species from Northern Namibia (A: AY255095). Sample 15, (FC: RE211206C1) an *Ichnotropis* c.f. *grandiceps,* exhibits at best a 90.42% match with the only available *I. bivittata* (from Angola) sequence on Genbank (A: HF547775) (Edwards *et al.*, 2012), followed by a 90.37% match with an *I. capensis* from Namibia (A: DQ871149) (Garcia–Porta *et al.*, 2019). Sample 16 (FC: AL211206B5), a *Ptychadena* c.f. *nilotica*, exhibits the highest percentage pairwise identity matches (upwards of 98%) with various *P. nilotica* as well as *P. mascariensis* sequences from Genbank, with a particular 100% match to *P. nilotica* (A: KX836495) from Vumbura, Botswana (Zimkus *et al.*, 2017). Sample 17 (FC: AL211204C1), a *Breviceps adspersus,* exhibits many (>20) high quality (>98% pairwise identity similarity) matches to *B. adspersus* sequences from Genbank, the strongest of which is a 99.77% match (differing by a single basepair) to a *B. adspersus* from south of the Congo basin (A: MH340372) (Nielsen *et al.*, 2018), followed by a 99.58% match with a *B. adspersus* from Namibia (A: MT944251) (Nielsen *et al.*, 2020). Sample 18, a lizard of the family Lacertidae (FC: RE211204D1), exhibits a few high percentage matches (99.60% & 99.19%) with sequences from *Heliobolus lugubris* haplotype specimens (A: DQ871142.1; DQ871141.1) (Makokha *et al.*, 2007). Samples 19 and 20 (FC: AL211204E1 & AL211204E2), two *Leptopelis bocagii,* are identical to one another and exhibit multiple high (>90%) matches with *L. bocagii* sequences from Genbank, the strongest of which is a 99.46% match to a specimen from Malanje Province, Angola (A: MK036434) (Hayes *et al.*, 2018).

3.3. Field observations

Table 7 shows at which localities (described in Table 1) specific species were found during the fieldwork of this study. The most common species to occur were all amphibians, with *Poyntonophrynus kavangensis, Kassina senegalensis, Ptychadena mossambica, Pyxicephalus adspersus*, and *Cacosternum boettgeri* being extremely prevalent and present in multiple localities in the Khaudum, Naye Nyae, and Mahango. A total of 17 amphibian species from 13 genera and 10 families were confirmed during the expedition. The most prevalent reptile encountered was *Pelomedusa subrufa*, and was present in most permanent and seasonal water sources in the Nyae Nyae and Khaudum. A total of 22 reptile species from 19 genera and 12 families were encountered across the study areas.

Table 7: Reptile and amphibian species encountered during the expedition

Family	Scientific name	Localities (table 1) present
Arthroleptidae	*Leptopelis bocagii*	23
Brevicpitidae	*Breviceps adspersus adsperus*	13, 22
Bufonidae	*Poyntonophrynus kavangensis*	1, 3, 7, 8, 10, 13, 21
	Sclerophrys gutturalis	33
	Sclerophrys poweri	33
	Sclerophrys pusilla	34, 36
Hyperoliidae	*Kassina senegalensis*	1, 4, 7, 10, 12, 13, 18, 33, 34
Microhylidae	*Phrynomantis bifasciatus*	12, 13, 33 ,34
Phrynobatrachidae	*Phrynobatrachus mababiensis*	33
Ptychadenidae	*Ptychadena mossambica*	9, 13, 14, 20, 26, 27, 37
	Ptychadena nilotica	33
Pipidae	*Xenopus muelleri*	33
Pyxicephalidae	*Pyxicephalus adspersus*	3, 13, 20, 23, 33
	Pyxicephalus sp.	20, 21
	Cacosternum boettgeri	11, 12, 20, 21, 26, 34, 38
	Tomopterna cryptotis	12, 14, 22
Rhacophoridae	*Chiromantis xerampelina*	35
Crocodylidae	*Crocodylus niloticus*	34
Pelomedusidae	*Pelomedusa subrufa*	6, 23, 24, 25, 30, 39
Testudinidae	*Stigmochelys pardalis*	2, 30, 36
	Psammobates oculifer	16
Gekkonidae	*Chondrodactylus laevigatus*	1, 10, 11
	Lygodactylus capensis	7, 15, 34
Agamidae	*Agama aculeata*	5, 10, 12
Scincidae	*Mochlus sundevallii*	8, 27
	Trachylepis damarana	4, 7, 15, 32, 34, 36, 40
Lacertidae	*Heliobolus lugubris*	37
	Ichnotropis capensis	17, 32
	Ichnotropis grandiceps	32
	Meroles squamulosa	16
Amphisbaenidae	*Monopeltis anchietae*	28

Varanidae	*Varanus niloticus*	34
Boidae	*Python natalensis*	4
Colubridae	*Philothamnus angolensis*	34
	Philothamnus semivariegatus	34
Psammophiidae	*Psammophis subtaeniatus*	3,32
	Psammophylax tritaeniatus	7, 15, 37
Elapidae	*Dendroaspis polylepis*	16
	Naja anchietae	7

3.3.1 Amphibian occurrences.

Leptopelis bocagii. Bocage's tree frog (Fig. 9) was found in a single small field in the Khaudum (locality 23). The field in question consisted of sharp dry grass growing from an old pan or lakebed, pockmarked throughout with deep elephant tracks. These frogs were identified by their toad–like body, large eyes, blunt head with a rounded snout, and dark horseshoe mark on their backside. They can be distinguished from other *Leptopelis* species by having very small terminal discs on their fingertips, an interorbital bar without a triangle on the head, a large inner metatarsal tubercle, limited webbing, and lastly being the only *Leptopelis* with a range close to or transecting the study areas in question (du Preez & Carruthers, 2017). The locality where our specimens were encountered was still well south of their known range, representing a range extension.

Figure 9: *Leptopelis bocagii* (Photo: L. du Preez) and occurrence record from this study.

Breviceps adspersus: The Bushveld Rain Frog (Fig. 10) was confirmed in the Nyae Nyae at locality 13 only by their call, a series of short pulsed whistles uttered in groups of three or more. Two specimens were collected in the Khaudum at locality 22 and genetically

confirmed to be *B. adspersus*. Characteristics used to identify this species included their prominent facial masks, smooth and unmarked undersides, an outer toe that is as wide as it is long, single basal tubercles on the hand, and small eyes (du Preez & Carruthers, 2017).

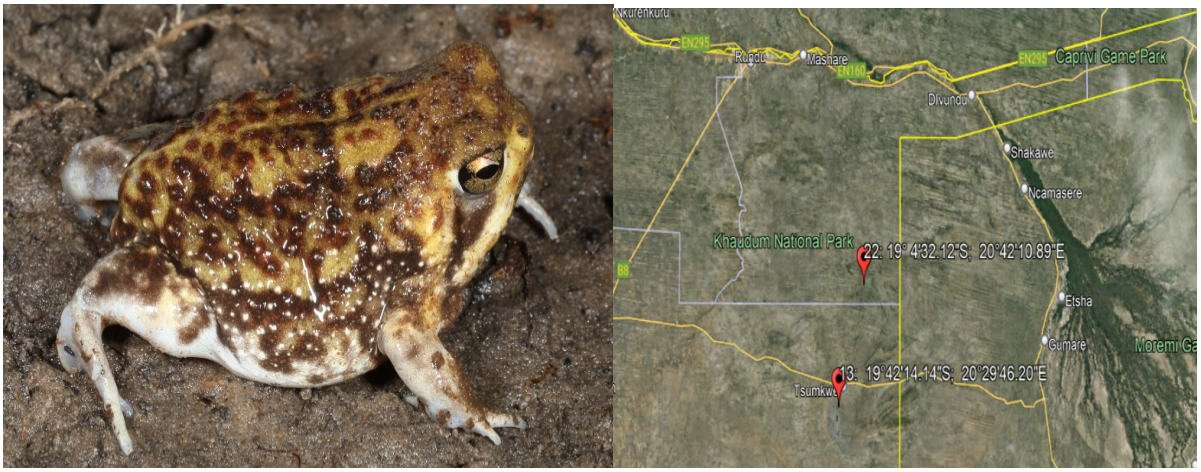

Figure 10: *Breviceps adspersus* (Photo: L. du Preez) and occurrence records from this study.

***Poyntonophrynus kavangensis*;** Another very prevalent amphibian species in the study areas was the Kavango Pygmy Toad (Fig. 11). These small, flattened, warty toads usually exhibit three pairs of dark patches as well as a pale vertebral line on their backside. This species was encountered at localities 1, 3, 7, 8, 10, 13, and 21. Characteristics that were used to discern them from other *Poyntonophrynus* species included their indistinct tympanum, underside lacking in dark blotches, flattened ventral warts, as well as their known range which is confined to the northern part of Botswana, Western Zambia, and North–eastern Namibia (du Preez & Carruthers, 2017).

Figure 11: *Poyntonophrynus kavangensis* (Photo: L. du Preez) and occurrence records from this study.

***Sclerophrys gutturalis*:** Quite a few Guttural Toads (Fig. 12) were both seen and heard in Mahango at locality 33, in the swampy areas bordering the river, and in sympatry with

many other frog species, including *S. capensis* and *S. poweri.* These toads are distinguishable from *S. pusilla* by a pale cross on the back of the head, red infusion on the backs of their legs, and an absence of a fused bar of dark patterning on the back of the head (du Preez & Carruthers, 2017).

Figure 12: *Sclerophrys gutturalis* from another locality (Photo: L. du Preez) and occurrence record from this study.

Sclerophrys poweri: A single Western Olive Toad (Fig. 13) was encountered in the same locality (Loc. 33) and in sympatry with the same species of the aforementioned *S. gutturalis*. An absence of dark patches on the snout, absence of fused bar behind the eyes, presence of dark infusions on the back of the legs, and dark–edged red–brown to brown patches in pairs on the dorsum of this toad make it easily identifiable as *S. poweri*.

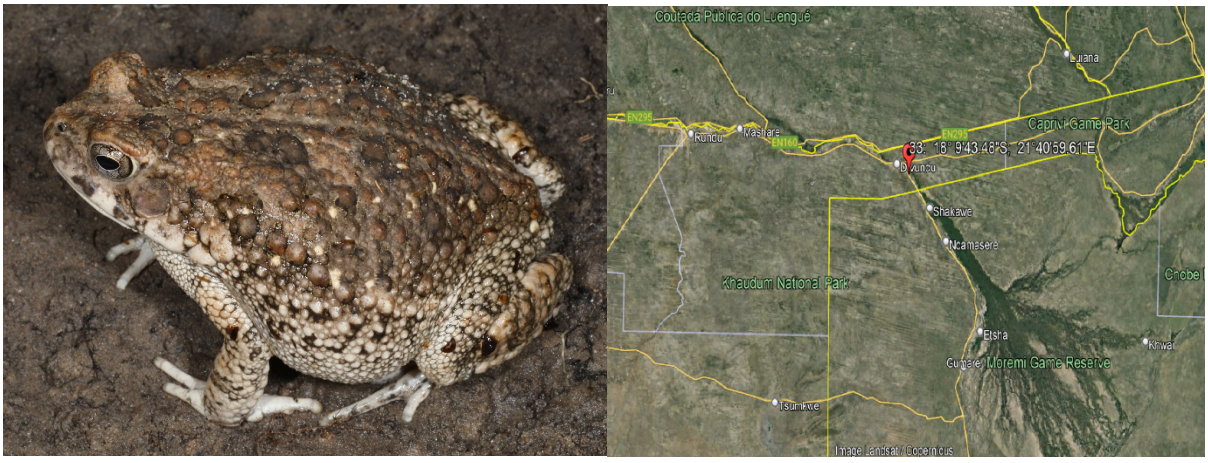

Figure 13: *Sclerophrys poweri* (Photo: L. du Preez) and occurrence record from this study.

Sclerophrys pusilla: Flat backed toads (Fig. 14) were quite prevalent in Mahango, with multiple specimens seen, heard, and sampled at both the river and inland water areas at localities 34 and 36.

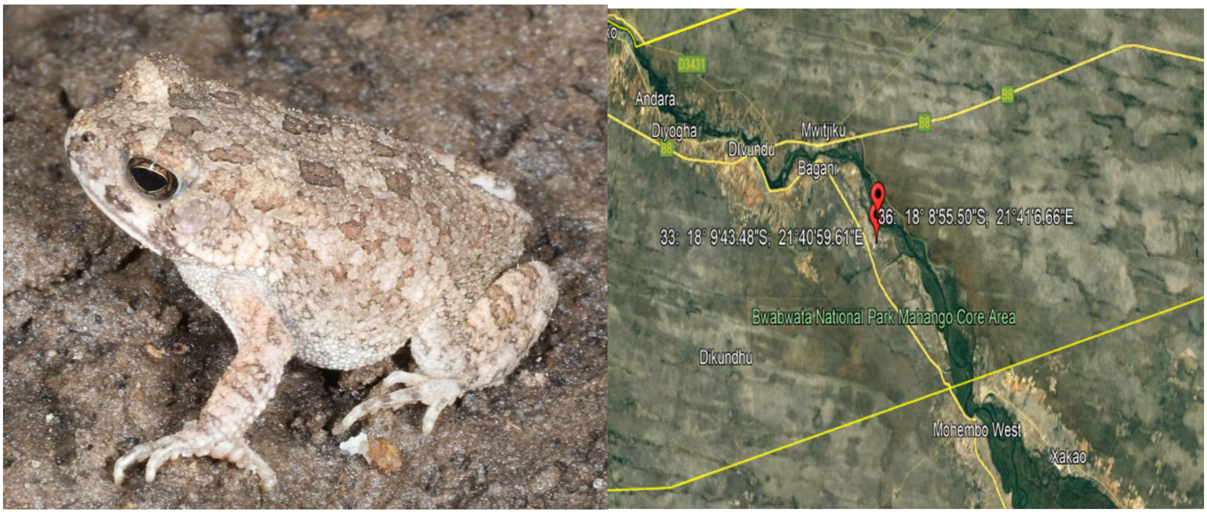

Figure 14: *Sclerophrys pusilla* (Photo: L. du Preez) and occurrence records from this study.

Kassina senegalensis: Throughout this survey Bubbling Kassinas (Fig. 15) was probably the most prevalent frog specie encountered, appearing in the Naye Naye, Khaudum, as well as Mahango area at localities 1, 4, 7, 10, 12, 13, 18, 33, and 34. They are easily identifiable by their smooth, bullet shaped bodies and dark paravertebral bands on a yellow or olive background. At night time their calls produce a beautiful cacophony of bubbling "bloip" sounds, hence the name Bubbling Kassina (du Preez & Carruthers, 2017), and these distinct calls also made it very easy to identify presence localities where physical frogs weren't encountered.

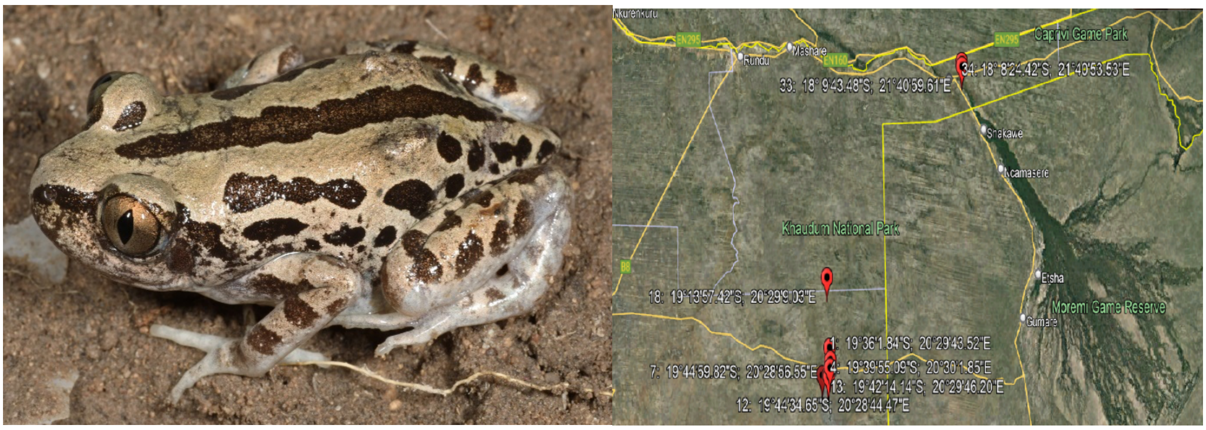

Figure 15: *Kassina senegalensis* (Photo: L. du Preez) and occurrence records from this study.

Phrynomantis bifasciatus. The Banded Rubber Frog (Fig. 16) was found in a few places during the study at localities 12, 13, 33, and 34 with a particularly interesting specimen (Fig. 17) being found in the Khaudum. *P. bifasciatus* is very easily recognizable by its smooth skin and bright orange bands on a black background, and is distinct from other *Phrynomantis* species by having numerous white spots on their grey underside, slightly webbed toes, and disc shaped ends of their fingers and toes (du Preez & Carruthers, 2017).

Figure 16: *Phrynomantis bifasciatus* (Photo: L. du Preez) and occurrence records from this study.

Figure 17: *Phrynomantis bifasciatus* with unusual patterning (Photo: L. du Preez) from this study.

Phrynobatrachus mababiensis: Dwarf Puddle Frogs (Fig. 18) were identified on the riverbank in camp in Mahango at locality 33 solely by their call, a distinct insect–like buzz that lasts for about a second and is followed by a few clicks (du Preez & Carruthers, 2017).

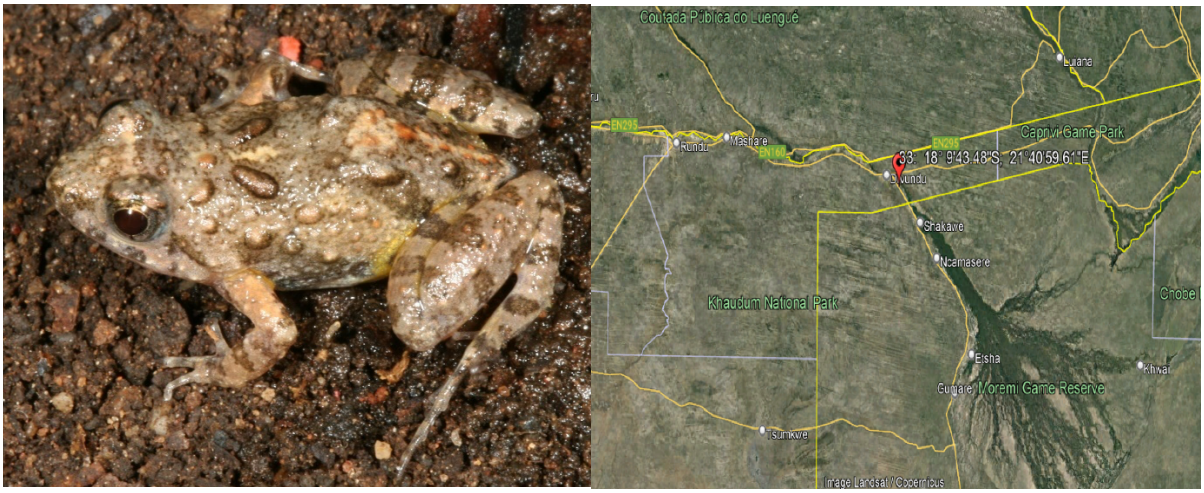

Figure 18: *Phrynobatrachus mababiensis* from another locality (Photo: L. du Preez) and occurrence record from this study.

Ptychadena mossambica*:* Broad–banded Grass Frogs (Fig. 19) were the most prevalent species of *Ptychadena* encountered during this study, occurring in the Nyae Nyae, Khaudum, and Mahango at localities 9, 13, 14, 20, 26, 27, and 37. These *Ptychadena* are distinguishable by the yellow mottling on the back of their thighs that may form irregular lines, an internarial distance greater than the snout–nostril distance, foot length greater than the tibia length, presence of an outer metatarsal tubercle, and a broad vertebral band stretching from the snout to vent (du Preez & Carruthers, 2017). Variants with vertebral bands varying in colour (cream, orange, and green) were encountered, which originally posed questions as to their identity and necessitated genetic sequencing.

Figure 19: *Ptychadena mossambica* (Photo: L. du Preez) and occurrence records from this study.

***Ptychadena nilotica*:** Nile Grass Frogs (Fig. 20) were encountered and sampled in the swampland areas bordering the river of Mahango at locality 33 in sympatry with various other frog and toad species, including *P. mossambica. P. nilotica* is identifiable by longitudinal black and yellow stripes on the back of their thighs, an internarial distance equal to the snout–nostril distance, foot length greater than the tibia length, absence of outer metatarsal tubercle, and a snout that is not paler than the rest of the body.

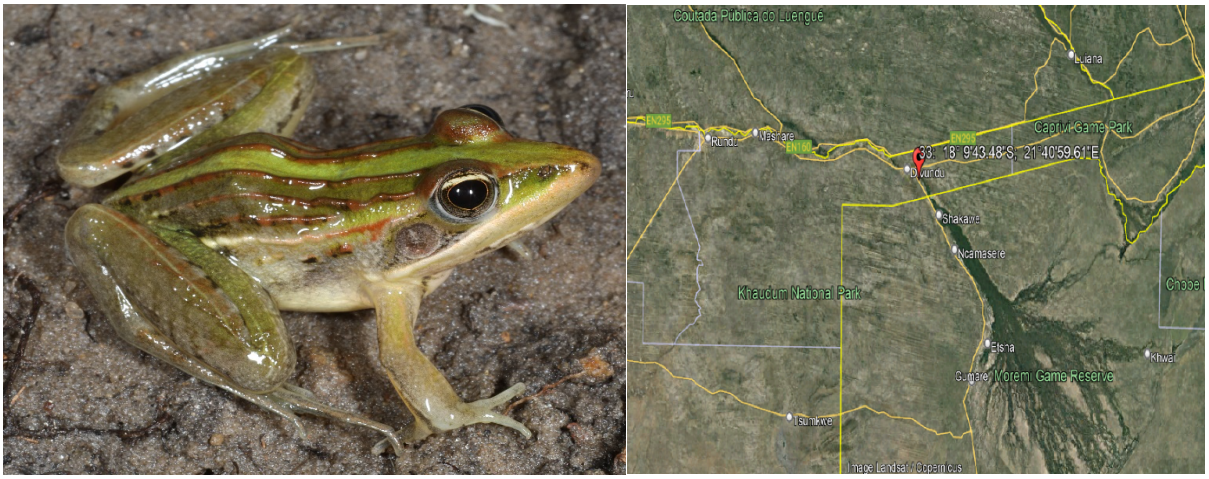

Figure 20: *Ptychadena nilotica* (Photo: L. du Preez) and occurrence record from this study.

***Xenopus muelleri*:** Müller's Platanna (Fig 21) was encountered only in the swampland bordering the river in Mahango at locality 33 and, surprisingly, was the only *Xenopus* species encountered during the entire study. *X. muelleri* is distinguished from other *Xenopus* species by their longish subocular tentacles that are at least half as long as the diameter of the eye. The greyish underside of their pectoral region blends to a deep orange–yellow towards the belly and the underside of the thighs. Its snout is rounded and eyes face dorsally, with a brown to grey colouration with irregular dark patches on its backside (du Preez & Carruthers, 2017).

Figure 21: *Xenopus muelleri* from another locality (Photo: L. du Preez) and occurrence record from this study.

Pyxicephalus adspersus: The Giant Bullfrog (Fig. 22) was encountered in the Nyae Nyae, Khaudum, and Mahango at localities 3, 13, 20, 23, and 33 occurring in sympatry to another unidentified *Pyxicephalus* species at sites in the Khaudum and Mahango. These enormous bullfrogs are distinct from other well–known *Pyxicephalus* species due to an upper jaw void of pale, irregular vertical bars, odontoids longer than they are wide, tympanum void of a white spot, absence of pale interorbital bar, distance from the eye to tympanum roughly twice the diameter of the eye (in adults), and a large inner metatarsal tubercle shaped and used as a spade.

Figure 22: *P. adspersus* (Photo: L. du Preez) and occurrence records from this study.

***Pyxicephalus* sp:** The second *Pyxicephalus* species encountered during the study were four *Pyxicephalus* Bullfrogs (Fig. 23) from the Khaudum at localities 20 and 21, where they were encountered in sympatry with *P. adspersus*. They are largely similar to and identified as *Pyxicephalus* cf. *adspersus*, with the key difference being their prominent leopard like dorsal and lateral spots, and in the case of the largest individual, bright orange colouration.

Figure 23: *Pyxicephalus sp.* (Photo: L. du Preez) and occurrence record from this study.

Cacosternum boettgeri: A very prevalent species of frog encountered in the Nyae Nyae, Khaudum, and Mahango was Boettger's Caco (Fig. 24). This species was encountered at localities 11, 12, 21, 20, 26, and 34. They are identifiable by a smooth to slightly granular dorsum, small grey to black spots on the underside (except on the throat), presence of small subarticular tubercles, absence of a metatarsal tubercle, and a tympanum that is not visible (du Preez & Carruthers, 2017). They also have a very distinct call (a series of rapid high–pitched clicks, sounding like a tin can rapidly being tapped) which was useful in identifying localities where they are present, as well as finding the frogs themselves.

Figure 24: *Cacosternum boettgeri* (Photo: L. du Preez) and occurrence records from this study.

***Tomopterna cryptotis*:** Tremolo Sand Frogs (Fig. 25) were encountered in the Nyae Nyae and Khaudum, yet it was not immediately easy to identify them beyond genus level. This species can be identified by asymmetrical blotches on the dorsum, warty dorsal skin, a pale scapular patch, single subarticular tubercle on the first finger, and an absence of a glandular ridge above the tympanum. The fact that there was some morphological variation between our *Tomopterna* specimens (tympanum ranging from moderately discernible to indiscernible; dorsal skin ranging from moderately smooth to warty; absence and presence of vertebral line) as well as the fact that *T. adiostola* and *T. tandyi* are morphologically identical necessitated genetic sequencing of our samples for species identification.

Figure 25: *Tomopterna cryptotis* (Photo: L. du Preez) and occurrence records from this study.

***Chiromantis xerampelina*:** A single individual of the last amphibian specimen encountered during this study, the Southern Foam Nest Frog (Fig. 26), was encountered on a building at the Kifi fisheries in Mahango (Loc. 36). They are very easy to identify by their colour, size, and posture when clinging to structures. A dark grey to whitish frog with scattered dark markings, with a protruding pelvic girdle that forms a characteristic hump, and soft but slightly warty skin. This species has a horizontal pupil, fingers arranged in pairs, terminal discs on their toes and fingers, and extensive toe webbing (du Preez & Carruthers, 2017).

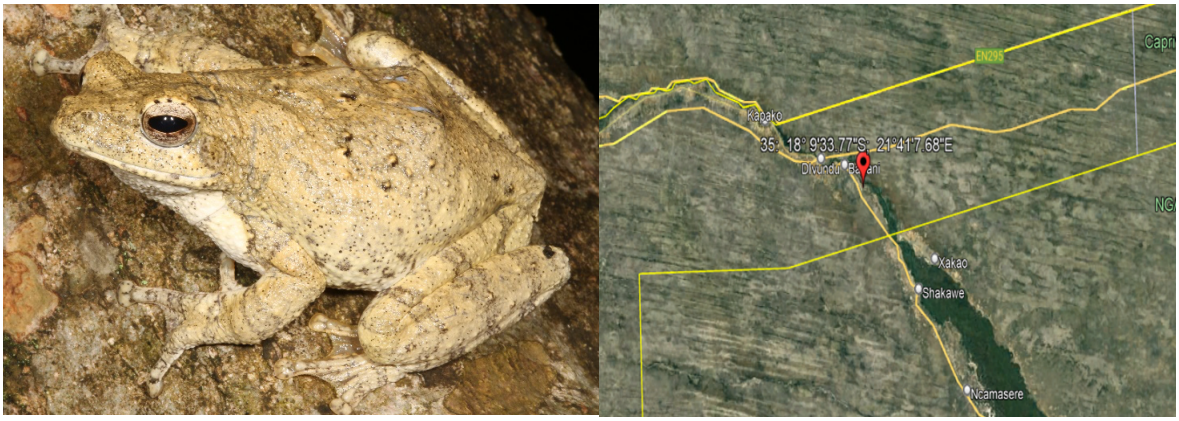

Figure 26: *Chiromantis xerampelina* from another locality (Photo: L. du Preez) and occurrence record from this study.

3.3.2 Reptile occurences

***Crocodylus niloticus*:** Many Nile Crocodiles (Fig. 27) were seen in the Okavango river in Mahango, yet none were present in any of the waterbodies in the Khaudum or Nyae Nyae, as none of these waterbodies are connected to any waterbodies where *C. nilotica* are already present, and many of these waterbodies are seasonal and unsuitable for many aquatic animals. The locality where specimens were observed in Figure 27 is visualized by a green pin. This species would hypothetically be present throughout the Okavango river (red pins).

Figure 27: *Crocodylus nilotica* (not from this study) and occurrence record from this study. Green pin is locality where C. nilotica was observed, whilst red ins indicate Okavango river, an area that would most likely also be populated by this species.

***Pelomedusa subrufa*:** Marsh terrapins (Fig. 28) were seen and sampled in many water bodies in the Nyae Nyae and particularly the Khaudum, including small ponds, roadside pools, and shallow pans. Baited terrapin traps and dragnets were used to determine their presence in a pond, whilst it was also possible to confirm their presence visually, as these turtles come up to the surface to breath.

Figure 28: *Pelomedusa subrufa* (Photo: R. van Breda) and occurrence records from this study.

Stigmochelys pardalis: Leopard tortoises (Fig. 29) were encountered in the Nyae Nyae, Khaudum, and Mahango at localities 2, 30, and 36, and were one of two tortoise species encountered during this study.

Figure 29: *Stigmochelys pardalis* (Photo: R. van Breda) and occurrence records from this study.

***Psammobates oculifer*:** The Kalahari Tent Tortoise (Fig. 30) is the second species of tortoise sampled during the trip and only a single specimen was found on the way to the Khaudum at locality 16. This genus is characterised by raised scutes and geometric patterning on the shell (Marais & Carruthers, 2007), and characteristics particular to *P. oculifer* include a nuchal scute divided in two, serrated anterior and posterior marginal scutes, and buttock tubercles.

Figure 30: *Psammobates oculifer* (Photo: L. du Preez) and occurrence record from this study.

***Chondrodactylus laevigatus*:** Tubercled Geckos (Fig. 31) were encountered in the Nyae Nyae at localities 1, 10, and 11 and identified as *C. laevigatus based* on their morphology and a revision of the genus by Heinz *et al.* (2021), which concluded that this species does not occur n sympatry with any other Chondrodactylus species in the study areas.

Figure 31: *Chondrodactylus laevigatus* (Photo: R. van Breda) and occurrence records from this study.

Lygodactylus capensis: The widespread Cape Dwarf Gecko (Fig. 32) was found in the Nyae Nyae (locality 7) and in camp at Mahango (locality 34). In accordance with this species relatively low specificity to particular habitat, they were found both in trees as well as in and around stone and wooden manmade structures.

Figure 32: *Lygodactylus capensis* (Photo: R. van Breda) and occurrence records from this study.

Agama aculeata: The Common Ground Agama (Fig. 33) was encountered at various sandy and rocky sites in the Naye Naye at localities 5, 10, and 12, exclusively moving on the ground and relying on their speed and camouflage to evade perceived danger.

Figure 33: *Agama aculeata* (Photo: R. van Breda) and occurrence records from this study. Colouration of deceased individual varies greatly from live specimen.

Mochlus sundevalli: Sundevall's Writhing Skink (Fig. 34) was encountered once in the Naye Nyae at locality 8 and once in the Khaudum at locality 27. These muscular, smooth–scaled writhing skinks were both found under rocks, and their tight fitting, smooth scales made them exceedingly difficult to handle and capture.

Figure 34: *Mochlus sundevalli* (Photo: R. van Breda) and occurrence records from this study.

***Trachylepis damarana*:** Many skinks were encountered throughout the expedition, with their identity most likely being that of *T. damarana (*Fig. 35), based on species distribution modelling by Weinell & Bauer (2015).

Figure 35: *Trachylepis damarana* (not from this study) (Photo: T. Ping) and occurrence records from this study.

***Heliobolus lugubris*:** A Bushveld Lizard (Fig. 36) was encountered in the Nyae Nyae at locality 37.

Figure 36: *Heliobolus lugubris* (Photo: R. van Breda) and occurrence record from this study.

***Ichnotropis capensis*:** Two members of the genus *Ichnotropis* was encountered in the study, the first of which being *I. capensis* (Fig. 37) which was encountered at localities 17 and 32 in the Khaudum. They can be identified by their relatively small size (body length generally not exceeding 60 mm (Graham & Alexander, 2007), 28–43 scale rows at midbody, usually 4 upper labial scales anterior to the subocular scale, 16–17 lamellae under the fourth toe (Broadley, 1977), as well as their colouration and patterning. According to Marais & Alexander (2007), *I. capensis* possesses striking colours and patterns such as a copper shaded dorsum, white–edged black stripes along the flank continuing to the tail with yellow and red on the flanks of the males during the breeding season. The study produced three occurrences of *I. capensis*, all of which were in the Khaudum.

Figure 37: *Ichnotropis capensis* (Photo: R. van Breda) and occurrence records from this study.

***Ichnotropis grandiceps*:** The second member of the *Ichnotropis* genus found during the study is highly likely to be an *I. grandiceps* specimen. Our specimen (Fig. 38) was found in sympatry to *I. capensis* in a Kiaatwood forest (Loc. 32) roughly 45 km west of the type locality of *I. grandiceps* (described as 25 miles west of Mohembo, Botswana, on the Caprivi border) (Broadley, 1967). This specimens' 16S gene was amplified and compared to known sequences from GenBank and was found to exhibit at best a 90.42% match with the only available *I. bivittata* sequence on GenBank, a specimen from Angola (A: HF547775) (Edwards *et al.*, 2012), followed by a 90.37% match with an *I. capensis* from Namibia (A: DQ871149) (Garcia–Porta *et al.*, 2019), as well as some other <91%, matches to *I. capensis.* These results exclude our specimen from being either *I. bivittata* or *I. capensis*, as the genetic dissimilarity is too large. These results, along with the fact that the type specimens from Broadley (1967) have never been sequenced or published on GenBank, does not give us a clear answer as to what it may be based on its genetics. Our specimen shares the following characteristics with the holotype and paratype specimens of *I. grandiceps* described by Broadley (1967) (Fig. 39): single frontonasal (1); subocular scale bordering the upper lip beneath the eye (2); a single anterior loreal scale (3); prefrontal scales (4) separated from the anterior supraocular scale (5); supracilliarie scales (6) separated from the supraocular by a series of small scales (7); five upper labial scales (8) anterior to the subocular scale (ours having 5 on one side and 4 on the other side, similar to two of four more *I. grandiceps* specimens examined by Haacke (1970)); a large trapeziform occipital scale (9) wedged between the parietals (10); upper head shields feebly striated; nostril pierced between three nasal scales (11); supranasal scales (12) in broad contact behind the rostral scale (13); the frontonasal scale one and a half times as broad as it is long; the prefrontal scales twice as long as they are broad and in broad contact mesially, as well as not reaching the anterior of the two large supraocular scales and in contact with (on the right side) and separated from (on the left side) the anterior loreal scale; the frontal scale (14) twice as long as its maximum width between the posterior tips of the prefrontals and strongly narrowed posteriorly; frontoparietals (15) longer than they are broad and widely separated by a large intraparietal (16) and occipital scale that is trapeziform in shape and its posterior margin level with the posterior borders of the parietals; an elongate keeled upper temporal shield (17) borders the parietals; three supraoculars (18), the third small, separated from the supracilliaries by 15–17 (n12–17 in other specimens) small keeled scales; five supracilliaries, the anterior two much the longest and forming a long oblique structure, three upper labials (19) posterior to the subocular; temporal scales (20) strongly keeled; a narrow tympanic shield on the upper anterior edge of the vertically elongated ear opening (21) ; lower eyelid with a median series of vertically elongated scales (22); seven (n7–8 in other specimens) lower labials (23); five pairs of large chin shields (24), the first three pairs in median contact; gular scales (25) imbricate; dorsal scales (26) rhombic, strongly keeled and imbricate;

laterals (27) smaller and feebly keeled, passing gradually into the smooth, rounded ventral scales (28), which are broader than they are long; 46 (n44–47 in other specimens) scales around the middle of the body; ventrals in about 10 longitudinal and 30 transverse tows between fore and hind limbs; preanal scales (29) irregular; scales on upper surfaces of limbs rhombic, strongly keeled and imbricate; 13 (n12–13 in other specimens) femoral pores on each side; subdigital lamellae pluricarnate and spinulose, 24 lamellae (n23–26 in other specimens) under the fourth toe; caudal scales strongly keeled except those just posterior to the vent, which are smooth; body moderately depressed; head not depressed, one and a half times as long as it is broad, its length equivalent to 26% (29–34% in other specimens) of the snout vent length, expanded in the temporal region and very distinct from the neck. The coloration in alcohol is described as pale grey brown above, with darker stippling and a few scattered dark spots (not covering more than one scale) on the body and tail; a poorly defined dark dorsolateral band extends from neck to groin, where it breaks up into a line of lateral spots on the tail. The side of its head and lower flanks are white with dark stippling and a white ventrum. In the paratypes the dark lateral band is absent. Haacke (1970) described a further 4 museum specimens collected between 1965 and 1970. One 15 km North of the Aha hills at the Botswana–Namibia border (approximately 167 km south from our specimen), two from a farm approximately 220 km West Southwest from our specimen, and the last from a poorly described area somewhere in the Caprivi. Of these four specimens, two possessed 4 upper labial scales on one side and 5 on the other (similar to our specimen) whilst the other two had 5+5. The rows of scales at midbody ranged from 44 to 46 in all specimens.

Figure 38: *Ichnotropis grandiceps* (Photo: L. du Preez) and occurrence records from this study.

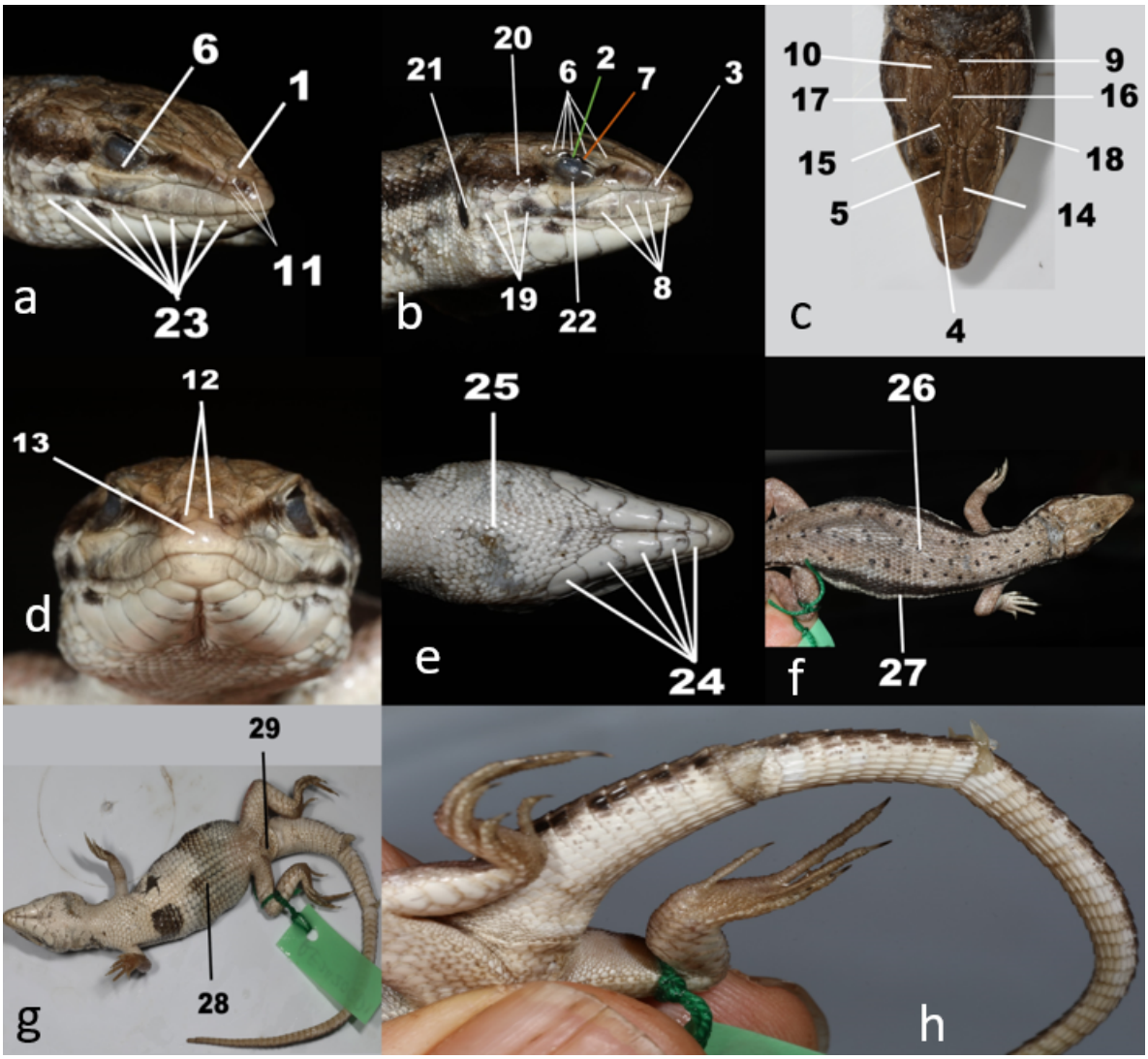

Figure 39: ***Ichnotropis grandiceps*** **(Photos: L. du Preez) scale patterning. (a) head front side view, (b) head lateral view, (c) head dorsal view, (d) Head frontal view. (e) Head ventral view, (f) body dorsal view, (g) body ventral view, (h) tail and feet ventral view.**

***Meroles squamulosa*:** A Common Rough–scaled Lizard (Fig. 40) was encountered on the way to the Khaudum at locality 16. This species of lizard is similar to and of the same family as *Ichnotropis.* Key characteristics used in identifying them include a frontonasal scale divided into two, subocular scale that does not reach the lip, occipital scale that is absent or very small, and 46–58 scales around the middle of the body.

Figure 40: *Meroles squamulosa* (Photo: R. van Breda) and occurrence record from this study.

***Monopeltis anchietae*:** An arboreal Anchieta's Spade Snouted Worm Lizard (Fig. 41) was found in the Nyae Nyae at locality 28.

Figure 41: *Monopeltis anchietae* (Photo: L. du Preez) and occurrence record from this study.

Varanus nilotica: Multiple Nile monitors (Fig. 42) were seen in camp in Mahango at locality 34.

Figure 42: *Varanus nilotica* (Photo: R. van Breda) and occurrence record from this study.

***Python natalensis*:** The largest Southern African snake, the Southern African Python (Fig. 43), was found in the Nyae Nyae. A medium sized, newly shed female was found curled up in a tree in the middle of a shallow pan (Loc. 4). These snakes are easily identifiable as they are large and bulky, possess many small and shiny scales, an off white belly, light stripe on the end of the tail, and two light lines spanning from the nose to the back of the head across the eye.

Figure 43: *Python natalensis* (Photo: B. Jordaan) and occurrence record from this study.

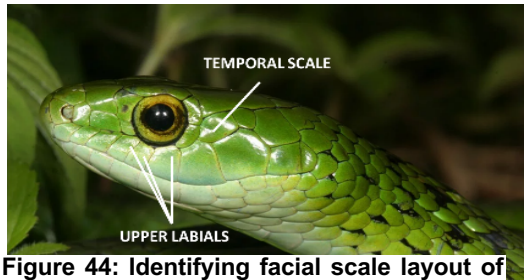

Figure 44: Identifying facial scale layout of *Philothamnus angolensis*

Philothamnus angolensis: An Angolan green snake (Fig. 44 & 45) was encountered in Mahango at camp (Loc. 34). At first it was identified as *Philothamnus* c.f. *angolensis*, but was later confirmed as this species through the pattern of head scalation (Fig. 44). *P. angolensis* possesses

three upper labial scales in contact with the eye, and only one temporal scale. The three upper labials in contact with the eye distinguishes our specimen from the similar looking *Philothamnus hoplogaster*, which only has two upper labials, whilst the colouration excludes it from *Philothamnus ornatus*. The range of this snake further distinguishes it from *P. hoplogaster*, the most likely candidate with which it may be confused (ASI, 2022).

Figure 45: *Philothamnus angolensis* (not from this study) (Photo: ASI) and occurrence record from this study.

***Philothamnus semivariegatus*:** A Spotted Bush Snake (Fig. 46) was encountered in Mahango camp (Loc. 34)**.** This snake is easily identifiable by its red to yellow iris, copper coloured tale in northern specimens, metallic blue flecks in the interstitial skin, blackish spots on the front half of the body, a double row of temporal scales, and three upper labial scales in contact with the eye (ASI, 2022).

Figure 46: *Philothamnus semivariegatus* (not from this study) (Photo: T. Ping) and occurrence record from this study.

***Psammophis subtaeniatus*:** The extremely fast moving Western Yellow–bellied Sand Snake (Fig. 47) was encountered at two locations, once in a very wet waterlogged vlei area in the Nyae Nyae (Loc. 3), and once in a Kiaat forest in the Khaudum (Loc. 32). These snakes can be identified by their brown, beige, and black longitudinal stripes, black spots on the upper lip, elongated head, patterning on the neck, and bright yellow underbelly (ASI, 2022).

Figure 47: *Psammophis subtaeniatus* (not from this study) (Photo: ASI) and occurrence records from this study.

Psammophylax tritaeniatus: Multiple Striped Skaapstekers (Fig. 48) were encountered only in the Naye Nyae at localities 7, 15, and 37. These snakes can be identified by dark and caramel stripes stretching the entire length of the body (including a dark band that spans the eye), a thin vertebral line that may be divided by small dots, and a cream coloured underside (ASI, 2022).

Figure 48: *Psammophylax tritaeniatus* (not from this study) (Photo: ASI) and occurrence records from this study.

***Dendroaspis polylepis*:** A particularly large Black Mamba (Fig. 49) was encountered on the road just inside the entrance to the Khaudum National Park (Loc. 17). The extremely potent neurotoxin of this species, coupled with the remoteness of the study area, discouraged any type of handling or sampling of this specimen. This species is easy to identify by its long and slender size, colouration ranging from dark brown to dark grey, and coffin shaped head.

Figure 49: *Dendroaspis polylepis* (not from this study) (Photo: ASI) and occurrence record from this study.

***Naja anchietae*:** A young Anchieta's Cobra (Fig. 50) was encountered just outside camp in the Nyae Nyae (Loc. 7). Even though *Naja anchietae* is similar in appearance and behaviour to *Naja annulifera,* these species do not occur in sympatry with one another in the study areas.

Figure 50: *Naja anchietae* (Photo: B. Jordaan) and occurrence record from this study.

3.4 SongMeter results

The SongMeter recordings were attentively analysed to try and identify any frog species that weren't visually encountered during active sampling by their call, to make sure no species were accidentally left out, yet at no localities were any calls heard of frogs that

hadn't already been confirmed (by encountering individuals at that location) to be present there. Secondly, the recordings were analysed to identify any trends or quantitative tendencies of the frogs' calls. The only species that was able to provide quantifiable data (data with some sort of identifiable trend) was *K. senegalensis*. The following graph (Fig. 51) visualizes *K. senegalensis'* call at locality 3 in the Nyae Nyae conservancy. Recording took place for the first 10 minutes of each hour from 16:00 on 30/11/21 to 06:00 on 01/12/2. Calling began at 20:00 and ended at around 00:00, with only sporadic and isolated calls taking place between 00:00 and 02:00. At the beginning of the calling the amount of frogs concurrently calling was also the highest, gradually declining along with their frequency of calling to only a single frog from 00:00 to 02:00. Initially, roughly 20 Kassinas are actively calling, with this number declining at each interval on the recordings. At the start of the cacophony calls can be heard roughly every three seconds, with about 20 calls per frog per minute. The amount of calls per hour also decreases at each hour interval, with there eventually being only about 1 or 2 calls every few minutes. In Figure 51, a clear starting time is visualized at 20:00, with gradual decline in calling frequency and the amount of frogs thought to be calling, eventually ceasing meaningful calling at around midnight.

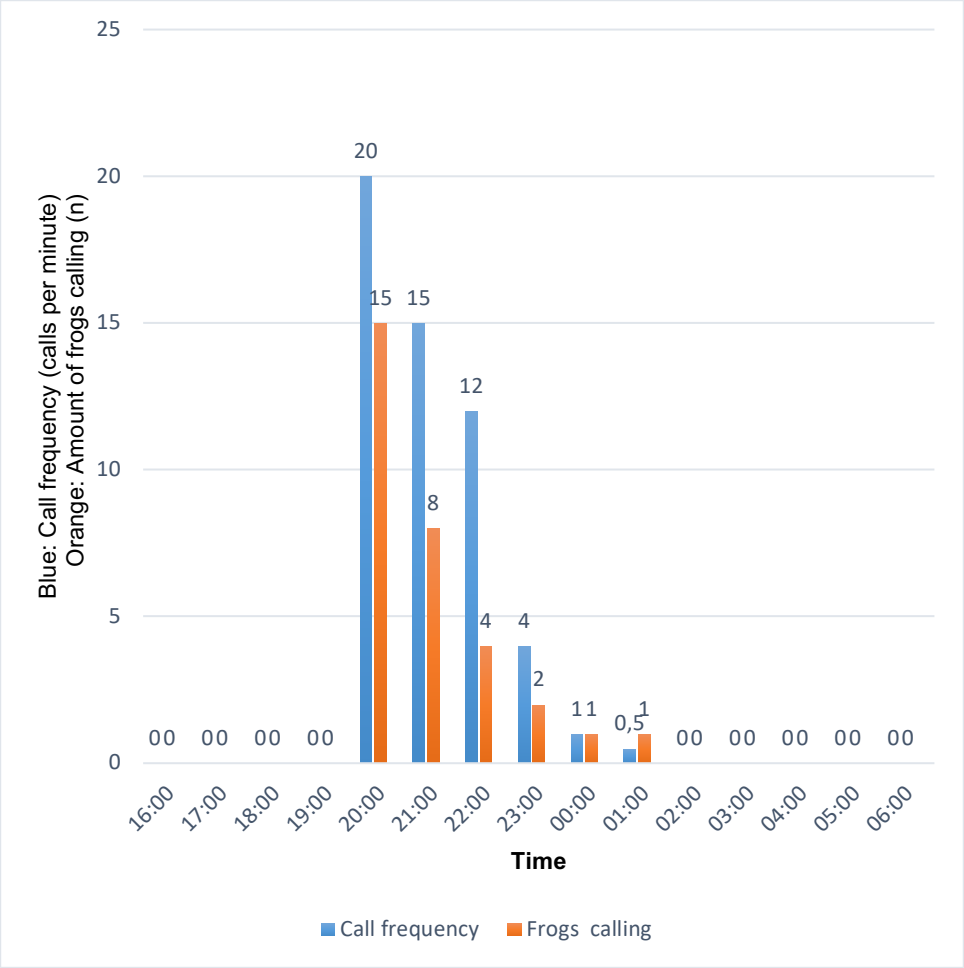

Figure 51: Visualization of *Kassina senegalensis* calling tendency in the Nyae Nyae conservancy, Namibia.

3.5 Phylogenetic analysis

Two species, a *Pyxicephalus* and an *Ichnotropis*, remained difficult to identify, both morphologically as well as genetically, as their 16S ribosomal sequences were not wholly identical to any on GenBank. Tables 7 & 8 and Figures 52 and 53 are applicable to a phylogenetic analysis done on both these genera.

3.5.1 Ichnotropis

Table 7 describes all *Ichnotropis* sequences compared with ours and formed part of the phylogenetic tree that was created for *Ichnotropis*. Genbank accession numbers, species, countries of origin, and a reference are included.

Table 8: *Ichnotropis* sequences used for phylogenetic analysis

Sequence name (GenBank accession)	Species	Country	Reference

MK464416	*I. capensis*	Zambia	Bittencourt–Silva (2019)
MK464417	*I. capensis*	Zambia	Bittencourt–Silva (2019)
MK464415	*I. capensis*	Zambia	Bittencourt–Silva (2019)
JX962898	*I. capensis*	Namibia	Engleder *et al.* (2013)
MK464418	*I. capensis*	Zambia	Bittencourt–Silva (2019)
DQ871148	*I. capensis*		Makhoka (2007)
MN015330	*I. capensis*	Namibia	Garcia–Portia (2007)
DQ871149	*I. capensis*		Makhoka (2007)
HF547775	*I. bivittata*	Angola	Edwards *et al.* (2012)
Sample 28	*I. grandiceps*	Namibia	This study
4056 16Sa L2510	*Ichnotropis sp.*	Angola	Unpublished

Looking at the phylogenetic tree (Fig. 52) constructed with our *Ichnotropis* sequence (Sample 28), an unidentified *Ichnotropis* sequence from Conradie (unpublished), and the remaining *I. capensis* and *I. bivittata* sequences from Genbank, our *Ichnotropis* sequence immediately diverges from all other clades (Clades 1 and 2) along with a sequence of the undescribed *Ichnotropis* from Angola (Conradie, unpublished). These two samples form a clade (Clade 3) separate to all other available *Ichnotropis* sequences. A second clade, rather pathway of divergence, that occurs in this tree occurs at the only available sample for *I. bivittata*, forming what is indicated as clade 2 in Figure 52. Lastly, the *I. capensis* sequences used in this tree diverge and form clade 1. Our *Ichnotropis* sequence and the unknown sequence from Conradie (unpublished) are also quite divergent from one another, indicated by the long arm length of the branches they rest on and high percentage (95%) surety of their divergence at that point in the evolution. This is indicative that our specimen is not the same species as that collected by Conradie, nor is it the same as any species with published genetic data, which strengthens the claim of our specimens proposed identity as *I. grandiceps*, provides the first genetic data of this species, and accordingly carries significant scientific weight.

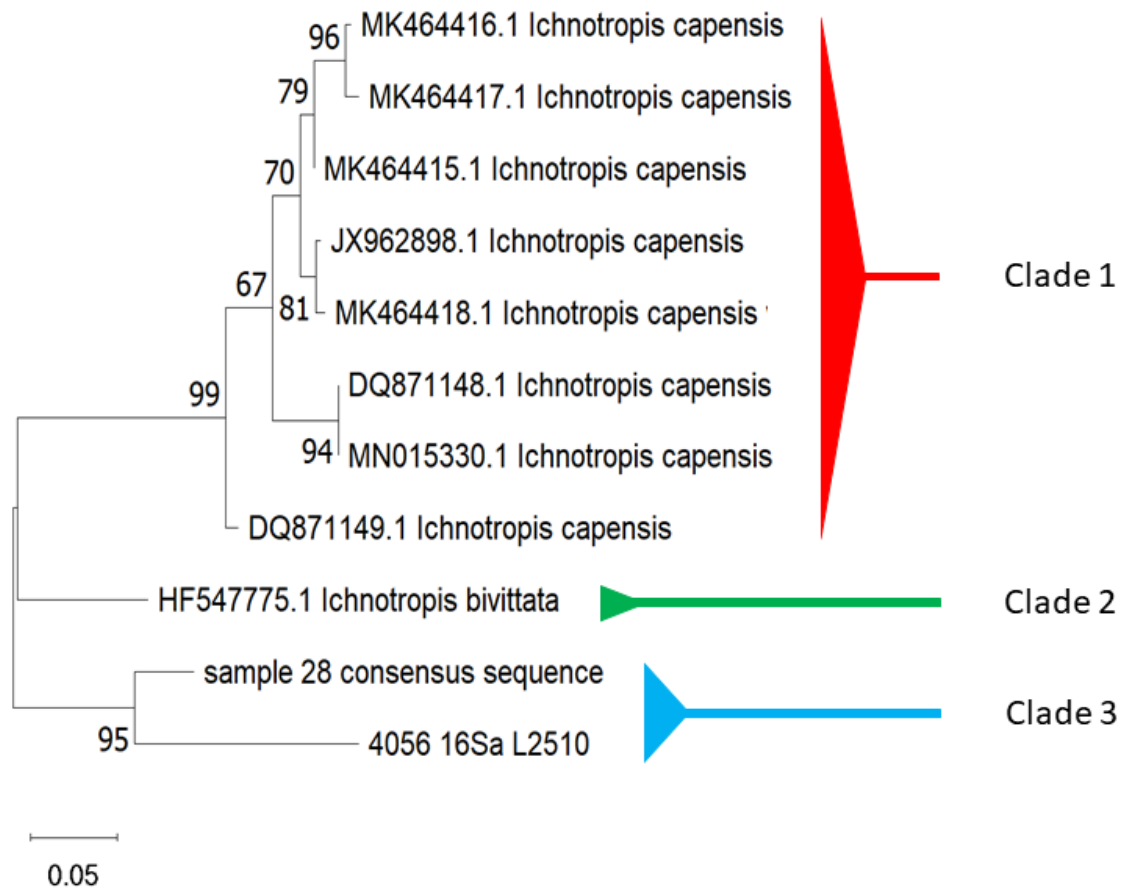

Figure 52: Phylogenetic tree for *Ichnotropis*.

3.5.2 *Pyxicephalus*

Table 9 displays all of the *Pyxicephalus* sequences included in the phylogenetic tree for the genus. GenBank accession numbers, PCR products, countries of origin, and a reference are included.

Table 9: *Pyxicephalus* sequences used for phylogenetic analysis.

Sequence name (GenBank accession)	Species	Origin	Reference
Sample 18	*Pyxicephalus sp.*	Namibia	This study
Sample 2	*Pyxicephalus sp.*	Namibia	This study
Sample 13	*Pyxicephalus sp.*	Namibia	This study
Sample 20	*Pyxicephalus sp.*	Namibia	This study
MK464309	*Pyxicephalus c.f. adspersus*	Zambia	Bittencourt–Silva (2019)

MK464305	*Pyxicephalus c.f. adspersus*	Zambia	Bittencourt–Silva (2019)
MK464306	*Pyxicephalus c.f. adspersus*	Zambia	Bittencourt–Silva (2019)
MK464307	*Pyxicephalus c.f. adspersus*	Zambia	Bittencourt–Silva (2019)
MK464308	*Pyxicephalus c.f. adspersus*	Zambia	Bittencourt–Silva (2019)
Sample 1	*P. adspersus*	Namibia	This study
Sample 3	*P. adspersus*	Namibia	This study
Sample 19	*P. adspersus*	Namibia	This study
DQ022366	*P. edulis*	Africa	Scott (2005)
LC440402	*P. adspersus*	Africa	Unpublished
LC640564	*P. adspersus*	Africa	Kambayashi *et al.* (2022)
DQ283157	*P. edulis*	Africa	Frost *et al.* (2006)
KY177062	*P. edulis*	Africa	Unpublished
AF206472	*P. adspersus*	Africa	Chen *et al.* (2005)
EF107211	*P. edulis*	Africa	Roelants *et al.* (2007)
KF991277	*P. edulis*	Africa	Barej *et al*. (2014)
KY177061	*P. edulis*	Africa	Barratt *et al*. (2017)
MH115769	*P. edulis*	Africa	Reeder *et al*. (2019)
MH115770	*P. edulis*	Africa	Reeder *et al*. (2019)
MH115761	*P. adspersus*	Africa	Reeder *et al*. (2019)
AF215505	*P. edulis*	Africa	Vences (1999)

In the phylogenetic tree of *Pyxicephalus* (Fig. 53), four clear clades can be distinguished. Clade 1 and 2 are the only ones of importance to our study. A very clear and divergent Clade is formed by our *Pyxicephalus* sp. samples 18, 2, 13, and 20, and *Pyxicephalus* specimens from Western Zambia (Bittencourt–Silva, 2019), forming Clade 1. This clade is more closely related to the *P. adspersus* clade (Clade 2) when compared to the

P. edulis clades (Clades 3 and 4) yet is still extremely divergent and represents a distinct species. Our *P. adspersus* sequences (sample 1, 3, 19) form a distinct clade (Clade 2) with other *P. adspersus* sequences from Genbank. Clade 1 represents a unique and presently undescribed *Pyxicephalus* species with some small amounts of genetic variation. Our *P. adspersus* sequences are part of another distinct clade with other *Pyxicephalus* sequences and one (probably misidentified) *P. edulis* sequence (A: DQ022366.1), forming Clade 2. The other two clades formed in this tree (Clades 3 and 4) are composed of mostly *P. edulis* sequences and some (probably misidentified) *P. adspersus* sequences. These two clades are indicative of the genetic variation in the *Pyxicephalus* genus and certainly merits further study as this was not within the scope of this study. The importance of this phylogenetic tree to this study is that it, in conjunction with the BLAST results of our specimens and their variance in phenotype to one another, makes is clear that more than two *Pyxicephalus* species are represented by the occurrences from this study, all of which were initially identified as *P. adspersus* or *P*. c.f. *adspersus*. This of course means that an undescribed (and in terms of published data) new *Pyxicephalus* species was encountered during this study, a discovery of significant scientific weight.

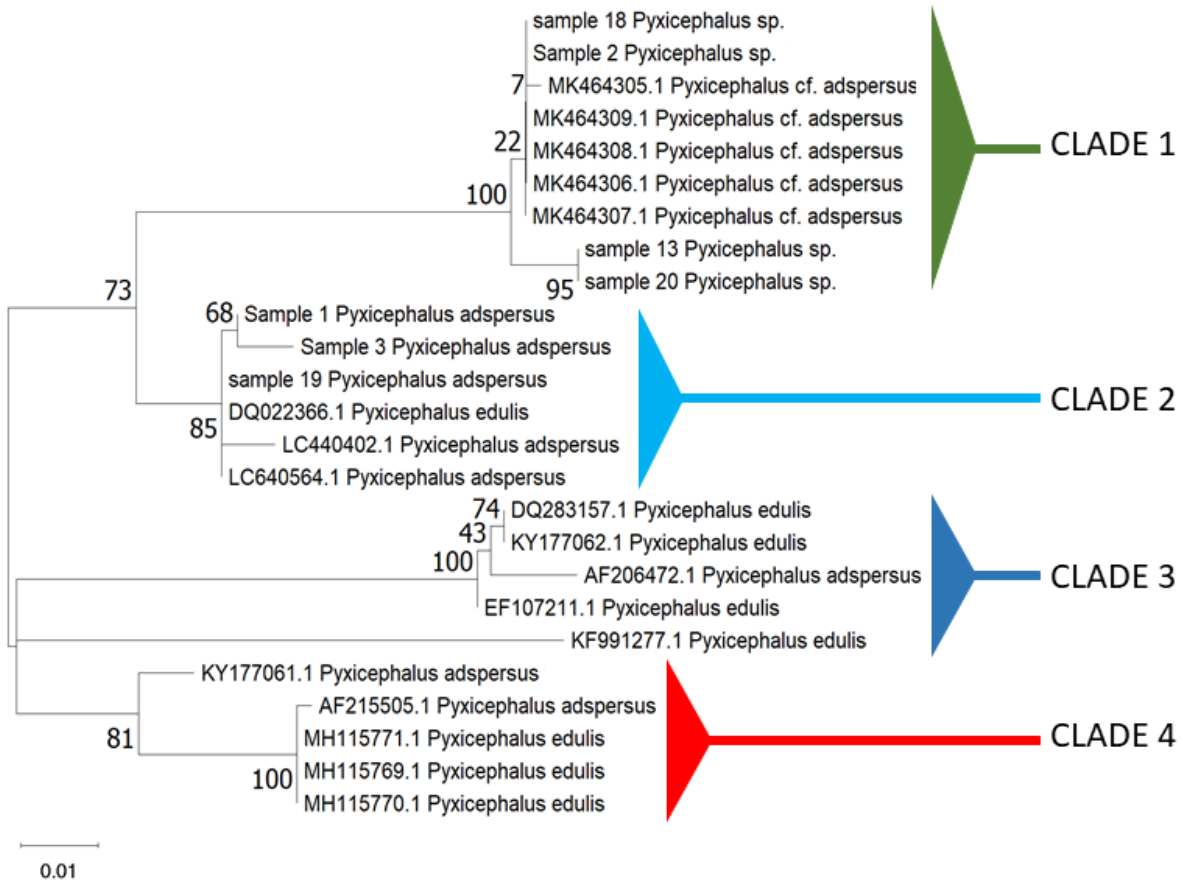

Figure 53: Phylogenetic tree for *Pyxicephalus.*

3.6 Ecological niche modelling

Species that ecologial niche modelling was applied for included *L*. *bocagii*, where a possible range extention may be calculated in the future, as well as the two significant discoveries (*Pyxicephalus* sp. and *Ichnotropis grandiceps*) and the species they could most likely be confused with (*Pyxicephalus adspersus* & *Icnotropis capensis*, respectively) to supplement data for these species.

3.6.1 *Ichnotropis capensis*

For *I. capensis*, 19 occurrence records were used including our study sites where *I. capensis* was present. For the first replicate all variables were used and both jack–knife and response curves were created. The variables that were selected to be used for the replicate runs were Altitude, Isothermality (BIO3), Temperature (BIO4), precipitation of wettest quarter (BIO16), precipitation of wettest month (BIO13), and annual precipitation (BIO12) (Table 3), as these had the highest influence on the model. Replicates using the aforementioned variables and occurrence data resulted in the following map (Fig. 54). Warmer colours show areas with better predicted conditions. Occurrences are represented by green dots, and cities are also included to facilitate georeferencing.

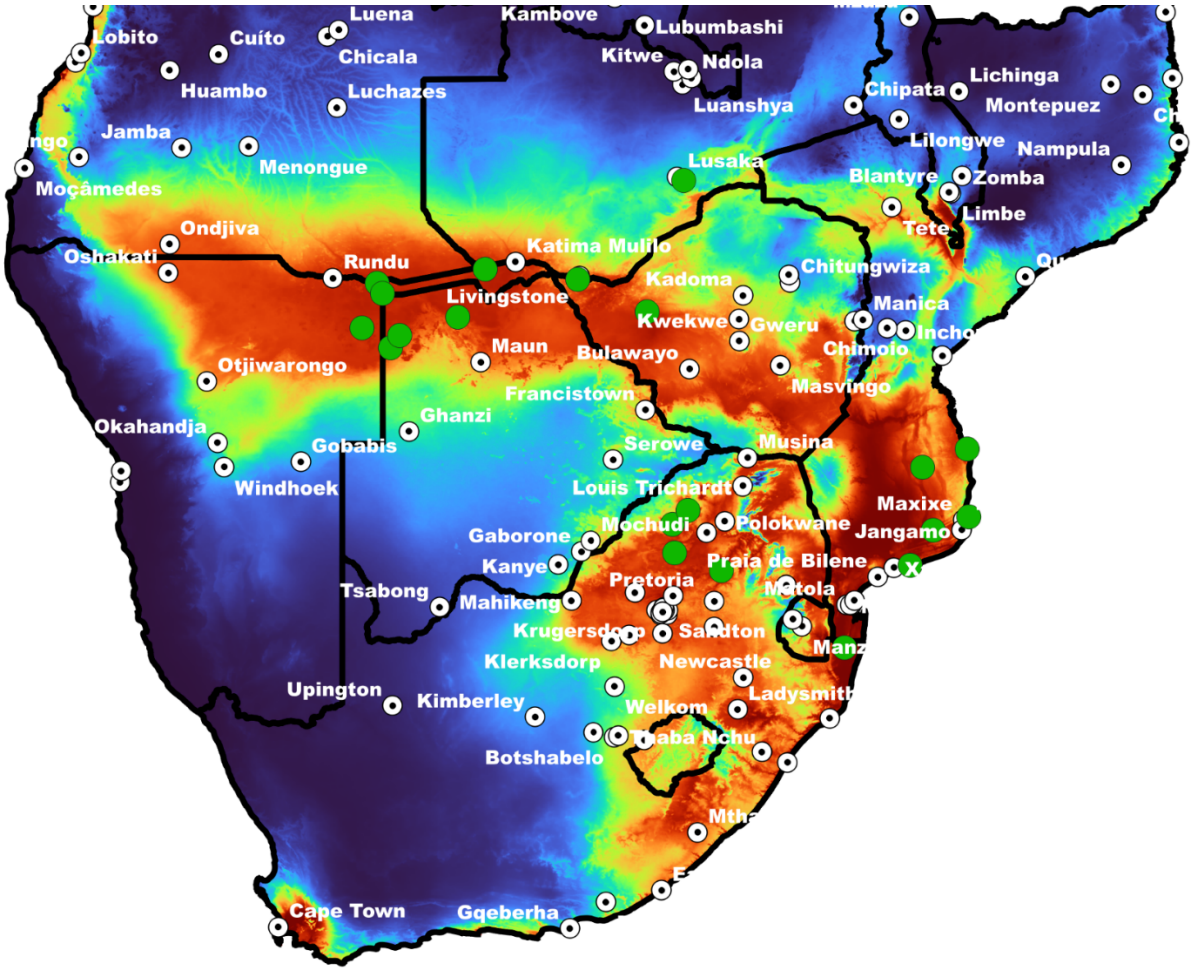

Figure 54: Areas of Southern Africa with Environmental conditions suitable for *I. capensis.*

The Maxent model predicts suitable climate for *I. capensis* throughout the Eastern half of South Africa from Mafikeng, Krugersdorp, and Botshabelo eastward. Suitable habitat for this species stretches over most of Kwazulu–Natal, Gauteng, Northwest, Mpumalanga, Lesotho, and Swaziland, as well as an isolated area around Cape Town. Suitable environmental conditions also occur in Southern Mozambique as far north as Chimolo. A broad belt of suitable environmental conditions occurs in Northern Botswana (north of Maun), Northern Namibia (north of Windhoek into Southern Angola (south of Menongue), and Southern Zambia.

3.6.2. *Ichnotropis grandiceps*

For *Ichnotropis grandiceps*, the first occurrence that was used was our locality where we found our specimen (locality 32). The second is a point derived from the type locality described by Broadley (1967), that being "25 miles west of Mohembo" in Botswana on the Caprivi border (rough coordinates –18.31678889; 21.43025833). The third locality was described as 15 km North of the Aha Mountains on the Botswana border, (rough coordinates –19.65967; 20.99876), a fourth on Deo–volente farm in Grootfontein district in Namibia (rough

coordinates –18.90312; 18.91493), and a last 19 km east of the Caprivi Botswana corner beacon, on the border of Namibia and Botswana (rough coordinates –18.31679; 21.43026). The environmental variables with the highest influence on the model and selected for the replicates were Altitude, annual mean temperature (BIO1), mean diurnal range (BIO2), max temperature of warmest month (BIO5), temperature annual range (BIO7), precipitation of driest month (BIO14), precipitation seasonality (BIO15), precipitation of driest quarter (BIO17), and precipitation of coldest quarter (BIO19) (Table 3). Replicates using the abovementioned variables and occurrence data resulted in the following map (Fig. 55). Warmer colours show areas with better predicted conditions. Occurrences are represented by green dots, and cities are also included to facilitate georeferencing.

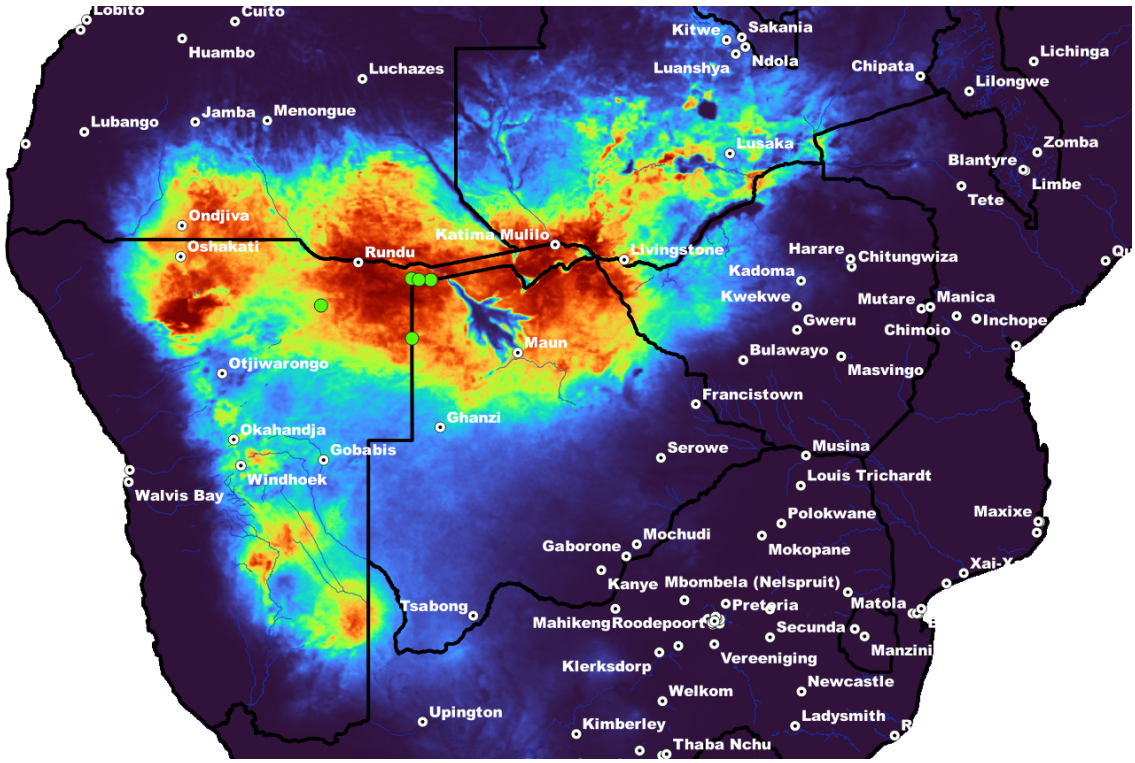

Figure 55: Areas of Southern Africa with Environmental conditions suitable for *I. grandiceps.*

Based on this Maxent model a relatively large area of Southern Africa seems to possess the necessary suitable environmental conditions for *I. grandiceps* to occur. The North–eastern corner of Namibia seems to exhibit the most suitable habitat, with four out of the five known occurrences for this species occurring on the border between Namibia and Botswana. Suitable environmental conditions span the entire Caprivi and south into Botswana all the way to just north of Ghanzi. Interestingly, the Okavango delta is clearly outlined as an area with environmental conditions wholly unsuitable for *I. grandiceps*. There are some sporadic areas of suitability in the south of Zambia (slightly further north than Lusaka), as well as Southern Angola (up to just south of Menongue and Jamba. Other small areas of suitability are in the very left hand corner of Zimbabwe near Livingstone and in Namibia, south of Windhoek.

3.6.3 *Leptopelis bocagii*

For *Leptopelis bocagii*, 14 observations were used. Of these observations, 13 were data points downloaded from GBIF and included only research grade observations, with coordinates, no geospatial issues, and originating from occurrence records from Southern African aquatic biodiversity, iNaturalist research–grade observations, and Rwanda's wetlands biodiversity data from different inventories that were conducted between years 2008 and 2017. One occurrence was added to represent the *Leptopelis* occurrence in this study. The environmental variables with the highest influence on the model and selected for the replicates were Altitude, annual mean temperature (BIO1), mean diurnal range (BIO2), min temperature of coldest month (BIO6), temperature annual range (BIO7), mean temperature of driest quarter (BIO8), mean temperature of coldest quarter (BIO11), precipitation of driest month (BIO14), precipitation seasonality (BIO15), precipitation of driest quarter (BIO17), and precipitation of coldest quarter (BIO19) (Table 3). Replicates using the aforementioned variables and occurrence data resulted in the following map (Fig 56). Warmer colours show areas with better predicted conditions. Occurrences are represented by green dots, and cities are also included to facilitate georeferencing.

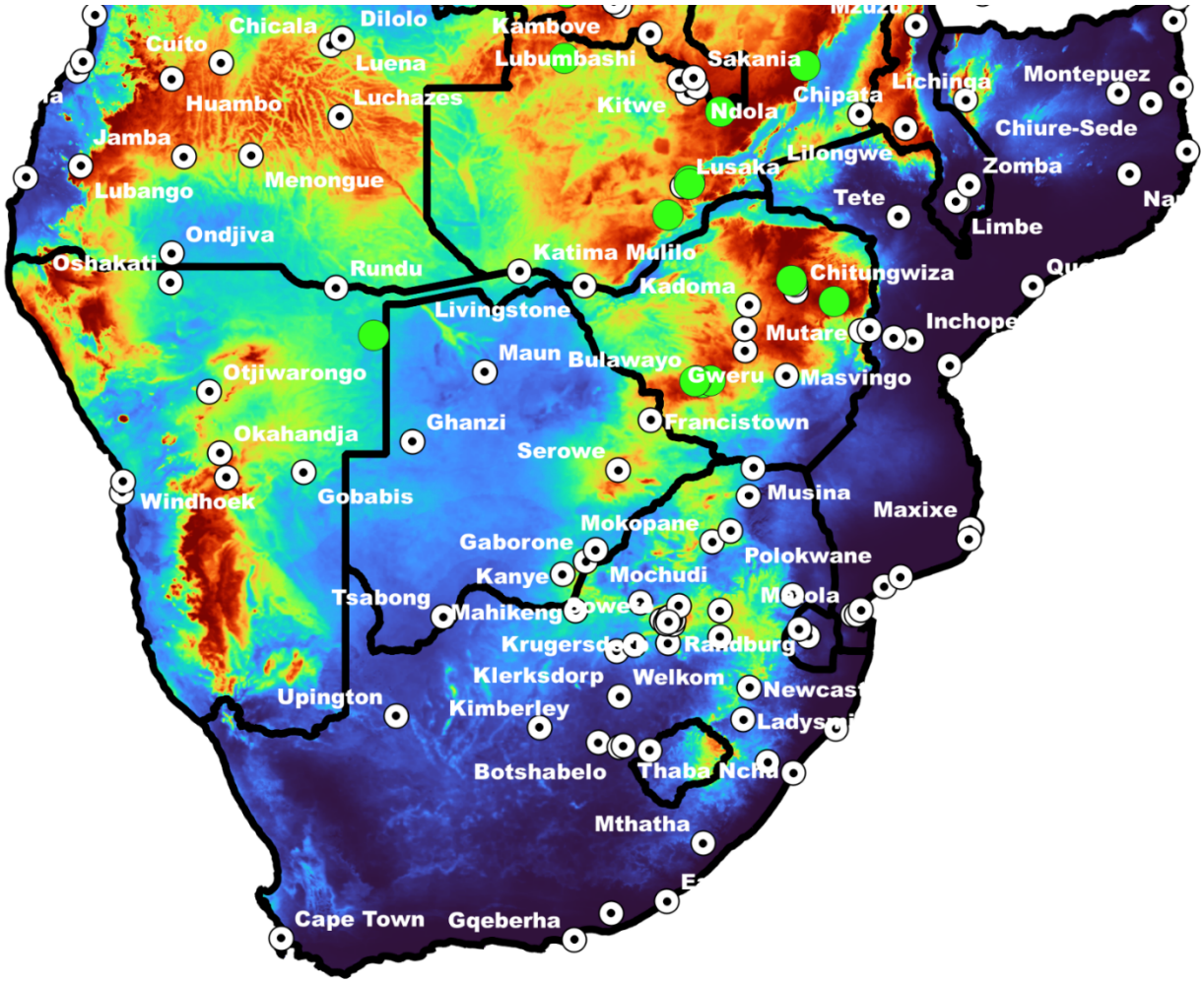

Figure 56: Areas of Southern Africa with Environmental conditions suitable for *L. bocagii.*

The Maxent model predicts areas with environmental conditions suitable for *Leptopelis bocagii* throughout large areas of central Namibia (near and south of Windhoek) and North–western Namibia (West of Oshakati). Botswana seems to have very little areas of suitable environmental conditions, with only a small area surrounding Serowe. Conversely, most of Zimbabwe, Zambia, Angola, and areas northward beyond the scope of the map is very suitable for *L. bocagii*. Suitable environmental conditions for this species are also predicted to occur in some of Eastern South Africa and Swaziland, yet no specimens have ever been recorded in South Africa. Looking at this Maxent model it is surprising that *L. bocagii* was encountered in the Khaudum national park, as the model predicts only moderately suitable conditions for this species in this area.

3.6.4 *Pyxicephalus adsperus*

For *P. adspersus*, 24 observations were used of which 20 were downloaded from GBIF and four were occurrences from our study. The environmental variables with the highest

influence on the model and selected for the replicates were Altitude, mean diurnal range (BIO2), temperature seasonality (BIO4), min temperature of coldest month (BIO6), temperature annual range (BIO7), mean temperature of driest quarter (BIO9), mean temperature of coldest quarter (BIO11), annual precipitation (BIO12), precipitation of wettest month (BIO13), and precipitation of wettest quarter (BIO16) (Table 3). Replicates using the aforementioned variables and occurrence data resulted in the following map (Fig. 57). Warmer colours show areas with better predicted conditions. Occurrences are represented by green dots, and cities are also included to facilitate georeferencing.

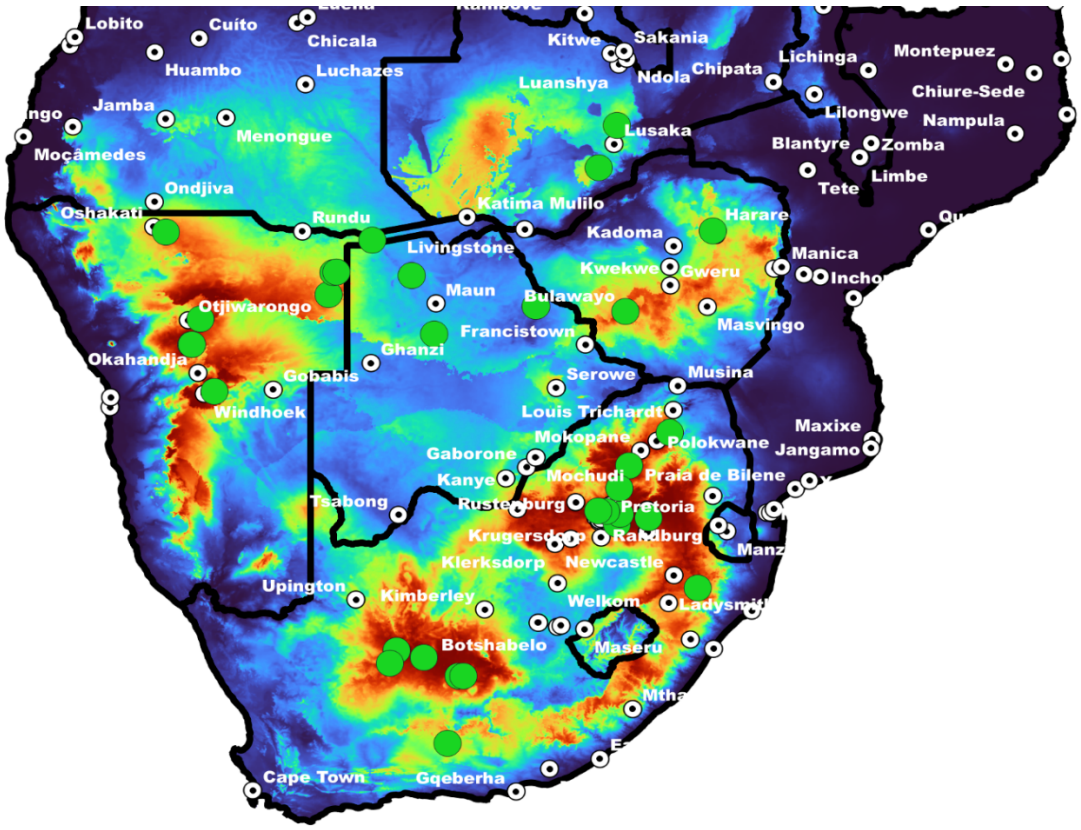

Figure 57: Areas of Southern Africa with Environmental conditions suitable for *P. adspersus.*

This map indicates suitable climate for *Pyxicephalus adspersus* throughout most of Southern Africa, with most of Namibia (bar the Namib Desert), Zimbabwe, and some parts of Zambia also possessing highly suitable environmental conditions for *P. adspersus*. The southern half of Angola (south of Luchazes) as well as most of Botswana also has moderately suitable environmental conditions for this species. Particular hotspots include much of Limpopo and Mpumalanga, as well as the centre of the Northern Cape, Central Namibia and central Zimbabwe. Moderately suitable climate constitutes most of South Africa, Zimbabwe, Namibia, Botswana, and the southern parts of Zambia and Angola

3.6.5 *Pyxicephalus* sp.

For the last ENM for the unknown *Pyxicephalus* sp. there are only three occurrences, two of which are from this study and the remaining one from Bittencourt–Silva (2019), who collected five juveniles of a *Pyxicephalus* sp. that is genetically identical to our specimens, all from the same locality in Western Zambia. The environmental variables with the highest influence on the model and selected for the replicates were Altitude, annual mean temperature (BIO1), mean diurnal range (BIO2), max temperature of warmest month (BIO5), temperature annual range (BIO7), precipitation of driest month (BIO14), precipitation seasonality (BIO15), precipitation of driest quarter (BIO17), and precipitation of coldest quarter (BIO19) (Table 3). Replicates using the aforementioned variables and occurrence data resulted in the following map (Fig. 58). Warmer colours show areas with better predicted conditions. Occurrences are represented by green dots, and cities are also included to facilitate georeferencing.

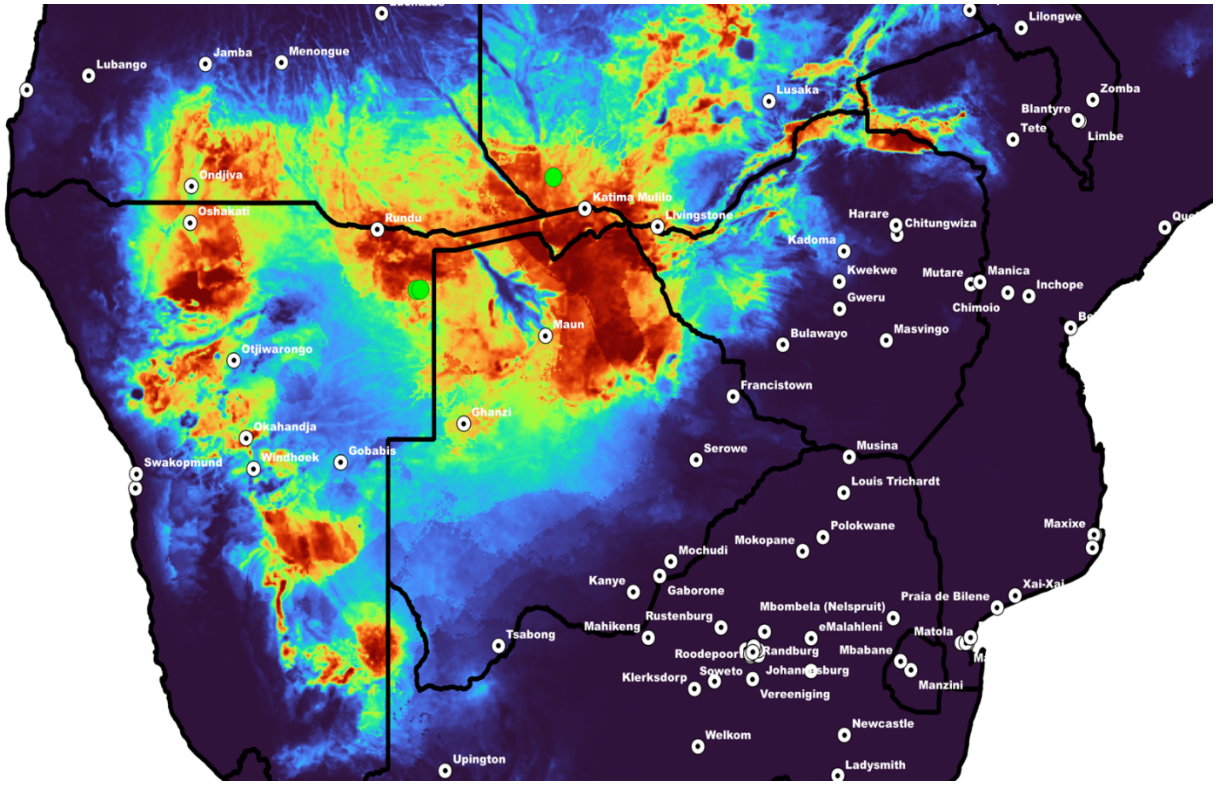

Figure 58: Areas of Southern Africa with Environmental conditions suitable for *Pyxicephalus* sp.

The model predicts suitable environmental conditions for this species in a limited area in Southern Africa, with similar yet more broken up environmentally suitable areas as *I. grandiceps* (Fig. 55). Highly suitable areas occur in Northern Botswana, Southern Zambia and Angola, as well as in a broad stretch down central Namibia. Interestingly, the Okavango delta is singled out as possessing unsuitable environmental conditions for this species.

Chapter 4

Discussion

Even though this is a non–comprehensive study and was conducted slightly before the optimal time to encounter herpetofaunal diversity, this study does provide a valuable insight in to the herpetofaunal biodiversity of the Khaudum–Ngamiland dispersal area, in particular the Nyae Nyae conservancy, Khaudum National park, and Mahango area bordering the Okavango River near Divundu. During this study, representatives from at least 39 species were encountered across 40 study sites. Whilst, for a checklist, this species count is relatively good, it still falls short of the estimated 69 species of reptiles and 32 species of amphibians hypothesized to be present in the study areas. A massive limiting factor to any study in these areas is the extreme remoteness and nature of the study area. These areas are incredibly remote with the nearest developed towns being hundreds of km's away into Namibia, or across a national border into Botswana. 4X4 vehicles are an absolute necessity as the deep sand of the areas subject vehicles to getting stuck quite easily, which is amplified when laden when supplies and equipment. Large amounts of food, water, and fuel need to be taken with along with the camping gear, utensils, traps and sampling equipment, microscopes, generators, cooler boxes etc. This makes the vehicles very heavy, and the slow movement of a convoy and careful driving, as well as the time it takes to set up camp and cook meals, severely limit the amount of time that can be spent actively sampling and processing data in the field. The remoteness and ecology of the area also resulted in extreme care and safety having to be a priority for all participants. The Khaudum and Naye Naye are home to the largest concentration of elephants in the world, as well as hyenas, lions, leopard, buffalo, and many other species of large game. The Okavango river in Mahango is populated by Nile crocodile and many hippopotami. The remoteness of the areas also made working with venomous snakes out of the question, as a bite from a mamba or cobra so far from a hospital would certainly prove fatal. Another major factor severely limiting the herpetofaunal diversity of this study was rainfall. Whilst the Naye Nyae still had some water left in select areas from the previous rainy season, it had not yet rained recently at the time of the sampling for this study. This drought (effectively) severely limited the activity of amphibians and to a lesser extent all reptile species, especially the snakes. High amounts of rainfall would have had the effect of much more active amphibians (both in movement and calling), and quite a few more species may have been encountered. The amphibian activity may in turn have benefitted the snake activity, as many snake species rely heavily on amphibians as a prey source. The fieldwork of this study was done in conjunction with other researchers from a variety of projects, and was perhaps a month too early for proper rainfall in the area. Most of the samples were released, whist voucher samples of at least a male and female of as many species as possible were collected

for the collection of the Namibian National Museum in Windhoek. These specimens proved valuable for identification, as we were able to systematically examine them under laboratory conditions at a later time. This study allows us to majorly contribute to the governing bodies of Namibia in regards to the herpetofauna of these areas, and in some cases we are able to contribute our genetic data to global databases such as GenBank. For the specimens that required genetic sequencing to determine or confirm their identification, barcoding of their 16S genes proved an invaluable exercise, yet should not be the only tool relied upon in such a study. Bittencourt–Silva (2019) highlights that identification using barcoding may be somewhat simplistic, with proposed sequence divergence thresholds not always taking into account the broad range of inter–and intraspecific divergence values present in some groups of animals. When making use of these techniques the limit of available sequences, as well as accuracy of tentative identifications, also come into play and may have an effect on your identification, in turn affecting other studies along the line. Bittencourt–Silva (2019) quotes Bridge *et al.* (2003) and Vilgalys (2004) to highlight the inaccuracy of public genetic databases such as Genbank and BOLD (the latter of which was not used in this study due to its lack of data in comparison to GenBank). Morphlogical review for Southern African reptile genera sampled in this study may be necessary, especially *Monopeltis, Mochlus, Agama*, and *Lygodactylus,* (for ease of identification between species, especially in the field) whilst recent taxonomic revisions highly benefitted this study in terms of the ease and speed of identification (in the case of *Trachylepis* and *Chondrodactylus*).

4.1 Amphibians

In total, 17 species of amphibians from 13 genera and 10 families were identified in this study, representing roughly 53 % of the species expected to occur in the study area. Whilst this would not be regarded as particularly successful if the expected species were already known to be present in the areas, it is highly likely that not all species expected to occur in the areas (based on their ranges identified by literature) actually occur in the area. Climate variables, habitat suitability, and the fact that some areas may just not be accessible, may have the effect that ranges are smaller and more refined than expected. The only way to prove a species' inhabitation of its expected range is through ground proofing (visiting the area and trying to find a particular species). This is the essence of what happened during this study. All of the above also applies to the reptile species encountered during this study. Observations regarding the habitat and true nature of the study areas (beyond the scope of this study) may affect future expected species' checklists. While most of the species were easily identifiable, some did pose some difficulty due to morphological variations from their known characteristics. In such cases, genetic sequencing of the 16S ribosomal gene and comparison to known sequences on GenBank provided answers as to their identity.

Leptopelis bocagii: Our two *L. bocagii* specimens that were genetically sequenced exhibited multiple very high (>99%) matches with *L. bocagii* sequences from Genbank, the strongest of which is a 99.46% match to a specimen from Malanje Province, Angola (A: MK036434) (Hayes *et al.*, 2018). These results, as well as the morphological characteristics described in the results section, confirm the identity of our specimens *as L. bocagii.*

Breviceps adspersus: Based on the ranges provided by du Preez & Carruthers (2017), *B. adspersus* is the only member of the genus *Breviceps* with a range that transects the study areas in question, and the only that inhabits the savanna biome, which simplified the process of identifying them in the field. One of the two *Breviceps* specimens sequenced exhibited many (>20) high quality (>98%) pairwise identity similarity matches to *B. adspersus* sequences from Genbank, the strongest of which is a 99.77% match (differing by a single basepair to A: MH340372) to a *B. adspersus* from south of the Congo basin in Sub–Saharan Africa (Nielsen *et al.*, 2018), followed by a 99.58% match with a *B. adspersus* from Namibia (A: MT944251) (Nielsen *et al.*, 2020). These results, as well as the physical characteristics described under the results section, confirm our *Breviceps* specimens as *B. adspersus.*

Sclerophrys pusilla: One of two small toads (FC: AL211207A2) found in camp in Mahango were sequenced due to their identity being questionable. These toads were originally thought to be *Poyntonophrynus* specimens, with a similar size, colouration, and patterning to *P. kavangensis*, a species that was quite prevalent during the study. Careful examination of the morphological characteristics of these frogs disproved them from being *P. kavangensis*, as they had a smoother backside with sparser warts, unflattened belly warts, and large palmar tubercles. Genetically it exhibited multiple 100% pairwise identity matches with *Sclerophrys pusilla*, allowing us to identify these toads as juveniles of this species.

Phrynomantis bifasciatus. The interesting individual in question (FC: AL211202H1) was phenotypically identical to other *P. bifasciatus* specimens, except for the patterning and brokenness of the usual orange dorsal markings. In this individual they were broken and divided, resembling patches or spots of the same orange hue. Molecular sequencing of the 16S ribosomal gene revealed a 99.82% pairwise identity match to a *P. bifasciatus* (A: KM509174) from Chumpanga in Mozambique (Peloso, *et al.*, 2015), suggesting our specimen as *P. bifasciatus,* and providing an example of a genetic variation or mutation of the phenotype that this species may incur.

Ptychadena mossambica*:* Two *Ptychadena* specimens (one unquestionably *P. mossambica and* the other more cryptic) were genetically sampled and compared to known sequences from Genbank (NCBI, 2022). They were proven to be identical to one another and have multiple high percentage pairwise identity matches with specimens labelled as *Ptychadena* c.f. *mossambica* from Zambia (Bittencourt– Silva, 2019). These particular

specimens were identified as *P. mossambica* using a key from Poynton and Broadley (1985), yet are labelled as c.f. due to their skin folds not being continuous, as Poynton and Broadley would suggest. Two of these samples match 100% with ours (A: MK464337.1; MK464340.1), whilst the lowest match is 99.61% (A: MK464336), suggesting our *Ptychadenas* from North–eastern Namibia are the same species as the *Ptychadena* identified by Bittencourt–Silva (2019). Other high percentage matches are to *Ptychadena mossambica* specimens from Selesele Pan in Northern KwaZulu–Natal, South Africa (A: MH115762; MH115764; MH115761; MH115763) (Reeder *et al.*, 2015). These results suggest the identity of our specimens as *Ptychadena mossambica,* and provide some insight as to the morphological variation that this species may incur.

***Ptychadena nilotica*:** To be sure of the identity of some of the *Ptychadena* specimens encountered in Mahango, a representative of the *P. nilotica* specimens sampled was genetically sequenced and exhibited high (>98%) percentage pairwise identity matches with various *P. nilotica* and *P. mascariensis (*now *P. nilotica*) sequences on GenBank, with a particular 100% match to a *P. nilotica* (A: KX836495) from Vumbura, Botswana (Zimkus *et al.*, 2017). These matches suggest this sample to be *Ptychadena nilotica* as well as the second *Ptychadena* species encountered during this study.

Pyxicephalus adspersus: Due to the fact that our *P. adspersus* specimens were very difficult to distinguish from the other *Pyxicephalus* species encountered in the study (particularly the juveniles), genetic identification of almost all *Pyxicephalus* specimens encountered in the study was necessary. Three specimens (FC: AL211202C2, AL211202G1, AL211202L1), all identified as *Pyxicephalus* c.f. *adspersus,* and all identical to one another, exhibit 100% matches with two *P.* c.f. *adspersus sequences* from GenBank (A: DQ347304, Bossuyt *et al.* (2006) and A: nh0564, Kambayashi *et al.* (2022). The next best match (99.83%) is to the *P. adspersus* sequence from the complete mitochondrial genome (A: NC044480) (Cai *et al.*, 2019), differing by a single basepair in the applicable barcode, suggesting these specimens as *Pyxicephalus adspersus.*

***Pyxicephalus* sp:** Our unknown Pyxicephalus specimens exhibited three 100% pairwise identity matches with juvenile *Pyxicephalus* c.f. *adspersus* specimens from Western Zambia (A: MK464306.1; MK464307; MK464308), which were tentatively identified as *P.* c.f. *adspersus* based on the specie's geographic range (Bittencourt–Silva, 2019). These specimens are at most 94.75% identical to the applicable gene of the *Pyxicephalus adspersus* complete mitochondrial genome (A: NC04480) (Cai *et al.*, 2019), whilst ours is 94.62% identical to the same sequence. This high genetic dissimilarity excludes our specimens from being *P. adspersus*, consequently suggesting them as members of a taxonomically undescribed species of *Pyxicephalus.* The phylogeny of this species (Fig. 53) provides further proof for this hypothesis. The phylogenetic tree forms four very clear and relatively divergent

groupings or clades, suggesting four different species or at least subspecies within the available data. The tree also suggests where specimens may have been misidentified as either *P. adspersus* or *P. edulis,* as within three of the aforementioned clades samples of both specimens are present. However, this study is only focused on our specimens and cannot provide further analysis of the clades where *P. adspersus* and *P. edulis* are present. Our specimens are part of the fourth and most divergent clade and extremely closely related to the five samples of *Pyxicephalus c*.f. *adspersus* identified by Bittencourt–Silva (2019) in Western Zambia. As mentioned, whilst superficially resembling *P. adspersus,* these frogs were all juveniles and identified as *P*. c.f. *adspersus* based on their geographic range, and Bittencourt–Silva (2019) does not provide much further commentary on their physical characteristics. Our phylogenetic, molecular, and morphological results suggest these specimens as an undescribed species of *Pyxicephalus*, with a known range at least from the Khaudum in North–eastern Namibia to the study sites of Bittencourt–Silva (2019) in Western Zambia.

***Tomopterna cryptotis*:** Three *Tomopterna* specimens (FC: AL211201K1, AL211202K2, AL211204D1), were proven to be identical to one another and exhibited two 100% pairwise identity matches with specimens identified as *Tomopterna cryptotis* from Angola (A: MN057687; MN057689) (Channing & du Preez, 2020) as well as a 100% match with a species of *Tomopterna* from Northern Namibia, *Tomopterna* sp. "*Shankara*" (A: MK335430), identified as the "true *Tomopterna cryptotis*" (Wilson & Channing, 2019). They exhibit very high percentage (99,81%) matches with *Tomopterna* specimens from Western Zambia (A: MK464284; MK464285; MK464282) (Bittencourt–Silva, 2019), who themselves most closely matches the *Tomopterna* sp. "*Shankara*" species from Northern Namibia (AY255095). The results of our morphological analysis allowed us to confirm our specimens as either *T. cryptotis* or *T. tandyi*, whilst the molecular results confirmed them as *Tomopterna cryptotis*.

4.2 Reptiles

The reptiles encountered during this study comprised specimens of 22 species from 20 genera and 12 families. Although some species, especially the snakes, were easily identifiable, there exists no literature with a key for the identification of lizards across a variety of families and species comparative to the Complete Guide to Frogs of Southern Africa (du Preez & Carruthers, 2017) for amphibians. Where literature detailing the morphological characteristics unique to species level of some genera existed these characteristics were used, with the known ranges of some species (e.g. *Monopeltis anchietae* and *Agama aculeata*) also playing an important factor in their identification. The reptile species whose weight carried the most importance during this study; *I. grandiceps*, was genetically sequenced and closely compared to known literature for a positive ID. The comparative

importance of this identification is due to the fact that prior to this study there were no genetic sequences of any kind available for *I. grandiceps*, as well as only very few and very old specimens of this species in museum collections. Another lizard, *Heliobolus lugubris*, was genetically sequenced both to help with identification as well as test the effectivity of the primers and PCR parameters for the amplification of lizard DNA.

***Chondrodactylus laevigatus*:** The multiple large Tubercled Geckos encountered in the study were identified to be *C. laevigatus*, based on a revision of the *Chondrodactylus* genus by Heinz *et al.* (2021). These authors compared genetic material from 234 *Chondrodactylus* specimens across Southern Africa to identify particular clades and distinction in the genus, provide range maps visualising an updated range of each specie, revise incorrect taxonomy, and attempt to provide some standardization for the identification of these geckos based on morphological characteristics. Heinz *et al.* (2021) identified three subclades within phylogeny based on *C. laevigatus*, all with 100% bootstrap support. The one applicable to our specimens is a clade which's' range stretches from the Erongo Region northward into Angola, encompassing the Caprivi strip and Khaudum–Ngamilamd dispersal area. Although *Chondrodactylus laevigatus, C. pulitzerae, C. fitzsimonsi, C. angulifer*, and *C. bibronii* occur in Namibia and they all (with the exclusion of *C. bibronii*) occur in sympatry in north–western Namibia (Heinz *et al.*, 2021). no examples of sympatry with *C. laevigatus* are known in the northeast of Namibia. Concurring with the diagnosis of *C. laevigatus* by Heinz *et al.* (2021) our *Chondrodactylus* species exhibits a large and relatively depressed head that is longer than it is broad and tubercles on the posterior crown that are very large and keeled and gradually become smaller towards the anterior crown and interorbital area. The anterior dorsal head tubercles are in contact with one another. The dorsal tubercles are large, rounded, and keeled and are smaller and more conical on the flanks, as well as well–separated by small granules. The dorsal tubercles form 14 rows on the trunk. The tubercles on the postaxial surface of the thigh are large and slightly flattened, whilst the scales on the upper part of the arms are non–tuberculate and imbricate and become tubercular on the forearms. Lastly, the specimens have a verticilliate tail with 6 large and keeled tubercles (similar to spikes), at each tail whorl. The aforementioned morphological similarities between our specimens and those highlighted by Heinz *et al.* (2021), and

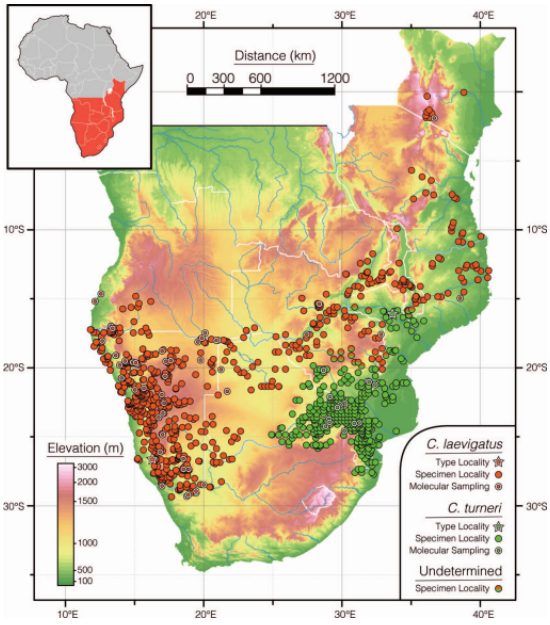

Figure 59: Occurrences of *C. laevigatus* and *C. turneri* (Heinz *et al*., 2021)

especially the species distribution based off of the phylogenetic analysis by the same author, strongly suggest the identity of our Tubercled Geckos as *C. laevigatus*.

***Trachylepis damarana*:** As for the identity of the skink species present in the study, it is likely that what is identified as T*rachylepis* c.f. *varia* may in fact be *T. damarana.* Weinell & Bauer (2015), conducted a systematic molecular study on the highly variable *Trachylepis varia* complex, identifying at least eight phenotypically distinct species by phylogenetic analysis and supporting these findings by indicating that these clades are also phenotypically distinct. Species distribution modelling was also conducted, with the resulting ranges indicating that members of the *T. varia* complex inhabiting North–eastern Namibia and the study area are all from a single clade with no examples of sympatry from other members of this clade. These results are indicative of our *T. varia* specimens in fact being *T. damarana*.

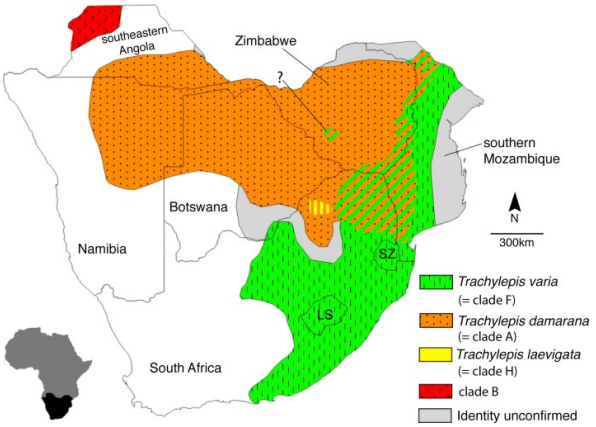

Figure 60: Species distribution model for multiple Trachylepis species (Weinell & Bauer, 2015)

***Heliobolus lugubris*:** A lizard identified to be part of the family Lacertidae was encountered and sampled in the Nyae Nyae. Subsequent sequencing and analysis of the 16S gene revealed its identity to be *H. lugubris*, the only member of the *Heliobolus* genus According to Marais & Alexander (2007), all members of the group containing the Bushveld Lizard (*Heliobolus*), Desert lizards (*Meroles*), Sandveld lizard (*Nucras*), and Sand lizards (*Pedioplanis*) have similar lifestyles and builds. All characteristically have strong, well developed legs, long toes, and rely on speed to evade predators. *Heliobolus* lizards have dark colouration with light spots during the juvenile phase, which gradually transitions to a lighter body with orange tail and hind legs, a light underside, longitudinal stripes, and pairs of dark paravertebral spots.

***Ichnotropis grandiceps*:** Due to the specimens' genetic dissimilarity to well–documented *Ichnotropis* specimens, as well as the high level of morphological similarity to *I. grandiceps* specimens sampled in the past, we submit our specimen as the most recently collected *I. grandiceps* specimen and provide its 16S genetic sequence.

4.3 Snakes of the Khaudum and Nyae Nyae

Piet Beytell, Chief Conservation Scientist for the Ministry of Environment & Tourism in Namibia made use of 15 years of life and work experience in the Nyae Nyae conservancy and Khaudum national park to confirm snake species, not encountered during this study, yet encountered by him on previous occasions in these study areas. These species are

Leptotyphlops scutifrons, *Dispholidus typus*, *Philothamnus semivariegatus*, *Telescopus semiannulatus*, *Thelotornis capensis*, *Boaedon capensis*, *Pseudaspis cana*, *Psammophis trinasalis*, *Psammophis jallae*, *Amblyodipsas ventrimaculata, Dendroaspis polylepis*, *Naja mossambica*, *Bitis arietans*, and *Causus rhombeatus*. Francois Jacobs, Senior Fisheries Biologist for the Ministry of Fisheries and Marine Resources in Namibia and resident of Mahango confirmed *Naja mossambica* to be present in this area. Whilst, unfortunately, no published records exist for these species' in this area, the personal expertise and experience of those involved in their identification serves as a good indication and start of herpetofaunal research regarding these species, especially in the study areas (and to a lesser degree Namibia). More concrete proof of their existence in the study areas may be obtained from online databases such as iNaturalist, especially where photographs and verification from other individuals play an important role. Whilst no such data was available at the time of this study, their theoretical value to a study such as this may prove very beneficial.

4.4 SongMeter analysis

Analysis of the calls of *Kassina senegalensis* in swampland areas in the Nyae Nyae reveal a gradual decline in calling frequency and estimated amount of frogs calling over a period of five hours throughout the night. Due to the fact that the SongMeter only records the first ten minutes of each hour it is unclear as to the particular minute that *Kassina senegalensis* begin to call. When calling, these frogs call continuously, with (in this locality) 15 frogs calling at an average frequency of twenty calls a minute during 20:00–20:10. Between 21:00 and 21:10 the frequency of calls have declined to around 15 a minute, whilst the estimated amount of frogs calling have decreased to about 8. The frequency of calling decreased to 12 calls per minute whilst the amount of frogs calling decreased to about 4 during 22:00–22:10, with a last decrease in calling frequency to 4 calls per minute and drop in frogs calling to 2 during 23:00–23:10. From midnight onwards only a single *Kassina* can be heard calling at one or less calls a minute, without any response, and in the late hours of the night only a single call could be heard with long periods of time in–between. These results indicate the best time to collect auditory data of this species, or to make use of their calling to supplement sampling, would from 20:00 to 22:00. These times may vary at different times of the year, accordingly we can only suggest this timeframe as optimal during November and December. The use of this technique may result in more of the expected species being confirmed, especially when data can be gathered from multiple areas over longer periods of time, analysing trends over time and in different seasons and weather conditions.

4.5. Ecological Niche modelling

The ecological niche models created in this study for *I. capensis, I. grandiceps, P. adspersus*, *Pyxicephalus* sp.*,* and *L. bocagii*, provide a valuable visualization of habitat with suitable environmental conditions for these species. Key among these were the models for *I. grandiceps* and the unknown *Pyxicephalus* sp. Previous to this study, only six recorded specimens of *I. grandiceps* have been sampled from 4 localities, all within a very limited area in North–eastern Namibia. This species model shows it may have a range stretching eastward into Botswana all the way into Zimbabwe as far as Harare at its furthest eastern limit, and Northward into Angola all the way to Menongue and northward into Zambia all the way to Lumbashi. The model makes it clear that the Okavango delta does not possess suitable environmental conditions for this specie, and the waterlogged nature of this area is probably the reason for this. According to Maxent this species distribution is most influenced by their habitats' annual mean temperature, mean diurnal range, max temperature of the warmest month, annual temperature range, precipitation of the driest month, precipitation seasonality, precipitation of the driest quarter, and the precipitation of the coldest quarter. This model can certainly be improved by including more occurrence data points. More points will give Maxent a larger pool of points to cross–reference with environmental data points, minimizing the influence of outlier specimens and providing a better representation for this species. This model may prove valuable for future studies, indicating areas where ground–truthing efforts should be focused as well as areas with little chance of being occupied by this specimen. The same can be said for the unknown *Pyxicephalus* sp. This frog is known from four adult specimens collected in this study and five juveniles collected previously, all of which from only three localities, of which two are extremely close together (this study). According to Maxent, the variables with the highest effect on this species' distribution are annual mean temperature, mean diurnal range, max temperature of the warmest month, annual temperature range, precipitation of the driest month, precipitation seasonality, precipitation of the driest quarter, and precipitation of the coldest quarter. These variables can be expected to have a big influence on an amphibian, as all the above may have an influence on water levels and water retention, water being a major deciding factor in amphibian presence and composition in an area. This species has a much more broken up and scattered map of suitable environmental conditions, suggesting a high specificity to particular environmental conditions. Even though the range of this species' suitable environmental conditions includes much of Northern Botswana, Namibia, Zambia, and Southern Angola, only eight public specimens are recorded. This species is likely to have a very limited and site specific range. There is a high chance that more specimens have been recorded, yet incorrectly identified as *Pyxicephalus adspersus*, due to their appearance being largely identical except for the dorsal patterning and colouration (our adult specimen was cream–orange with leopard–esque dark spots on the dorsum). As

with *I. grandiceps*, this model will certainly be subject to improvement by including more occurrence data points, as three does not give Maxent very much to work with. Ecological niche models for *P. adspersus* and *I. capensis* were created to both test the model and provide a basepoint with which to compare the niche distributions of *Pyxicephalus* sp. and *I. grandiceps*. For both *I. capensis* and *P. adspersus*, the niche models accurately reflect the species' known ranges. Both of these species have much larger niche ranges than their more cryptic and lesser known counterparts. This may indicate that these particular species are more specialized to the particular environmental conditions of Northern Botswana, the Caprivi, and thereabouts. The smaller niche generated by Maxent may also be due to the low amount of presence points, and will be improved in the future as more presences for these species are recorded. The last species for which an ENM was created was *Leptopelis bocagii*. This was because our occurrence in the Khaudum is much further south than the species had previously been recorded. The model shows our occurrence was in an only moderately suitable area, and also shows suitable conditions for this species in much of Namibia and some of Eastern South Africa, both of which are areas that this species has never been recorded

4.7 Recommendations for future studies

Due to the fact that this was the first herpetological study conducted in the particular study areas, and the first herpetological study focused on North–eastern Namibia in general, there is a plethora of research still to be done and information to be discovered in this remote part of the world. Building on this particular study, foremost would be further study of *I. grandiceps* and the *Pyxicephalus* sp., encountered in this study. *Ichnotropis grandiceps* has only produced a handful of published occurrences prior to this study, with no occurrences by tourists or other researchers on databases such as INaturalist. Of the published occurrences, the most recent was 52 years ago (at the time of this study). This seems to be a rare species, with what appears to be a relatively limited range, in an extremely remote are that is difficult to access and especially transport and use scientific and sampling equipment within, and is easily confused with *I. capensis* as well as occurring in sympatry with the aforementioned species. For futures studies it may be beneficial to search for this species within the predicted ecological niche visualized in Fig. 55, especially at the northern end of the Khaudum National Park, at the corner beacon of the border between Namibia and Botswana. More specimens need to be collected and their genome sequenced to supplement the meagre database of genetic information for the *Ichnotropis* genus, especially for other members of the genus than *I. capensis*. On GenBank there is presently only a single sequence of any kind for an

Ichnotropis other than *I. capensis* (that being one for *I. bivittata*), and this study provides the first for *I. grandiceps*. Furthermore, it may be beneficial to compare our specimen to the type specimens of the species, as this study only compared its characteristics to the characteristics published by Broadley (1967). Even though it was outside the scope of this study to compare the specimen to the type specimens (as they are housed in the British Museum of Natural History in London, England), the comparison between our physical specimen and the written word of Broadley (1967) was deemed sufficient to confirm our species as the same.

For the undescribed *Pyxicephalus* sp., it is extremely important that closer analysis of its morphology is studied (for example modelling of its skull and head structure), to uncover more disparities between it and *Pyxicephalus adspersus*, other than its colouration and patterning described in this study. This species is also only known from 9 specimens (four of which from this study) from three localities, of which two (the two from this study) are located within a very insignificant distance from each other. Similar to *I. grandiceps*, this species seems to be rare, occurs in the same difficultly accessible range, and is extremely similar and occurs in sympatry to its counterpart, *P. adspersus*. A formal description of this species is a clear opportunity for further publication. On the topic of *Pyxicephalus*, the phylogenetic analysis of the *Pyxicephalus* genus (Fig. 53) suggested the current database of *P. edulis* sequences on Genbank actually represents two different species, as two clear and separate clades are formed from the *P. edulis* sequences included in the analysis. Being outside the scope of this study, there definitely seems to be much more study necessary on the *Pyxicephalus* genus as a whole.

Longer expeditions into the Khaudum–Ngamiland dispersal area, during more appropriate times of the year (after high rainfall in December and January) may also result in some of the 15 amphibian species and 47 reptile species that were not encountered yet expected to occur within the study areas being confirmed. Genetic verification of the *Lygodactylus*, *Chondrodactylus, Agama* and *Trachylepis* specimens encountered in further studies may also be beneficial in confirming their identity, as the identifications from this study was based on a combination of morphological similarities and species distributions from other authors, which may not be as accurate as genetic sequencing.

Further studies in these areas should take special care in the planning and execution of any expeditions and fieldwork, as the nature of the areas pose exceptional challenges to sampling and traversing in these areas. 4x4 vehicles are essential, fuel, food, camping equipment, generators or sufficient battery packs, and cumbersome and expensive sampling equipment need to be transported everywhere, and any oversight of what may be useful or essential may prove detrimental to an expedition. Large amounts of time have to be spent in setting up camps and remote processing stations, ablutions, cooking food, and processing

samples, thus necessitating that any time actively sampling has to be used optimally. There is also a great degree of danger when working in the Khaudum and Nyae Nyae. Lion, leopard, hyena, large herbivores, and especially elephant are extremely prevalent and have to be taken into consideration, not to mention the estimated 8 species of deadly and potentially deadly snakes that occur in the areas. Deadly scorpions such as *Parabuthus* sp. also occur in the area. The “wild” nature and lack of anthropogenic disturbance in the areas have the result of dangerous animals being extremely commonplace and very likely to encounter on a daily basis. There is absolutely no medical assistance or hospitals located within 350 km of the Khaudum national park, and an encounter with a deadly animal, for example a bite from a Black Mamba (which was encountered in this study), would almost certainly prove fatal. Extreme care needs to be taken to ensure the safety and emergency preparation of all participants of another expedition into the Khaudum–Ngamiland dispersal area.

Chapter 5

References

5. References

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Appendix: Pairwise alignments of our sequences to sequences from GenBank

Pictured below is the pairwise alignment between our *Sclerophrys pusilla* (FC: AL211207A2) and a *S. pusilla* from Kampala, Uganda (A: KF665136) (Liedkte *et al.*, 2016).

```
Sample:     AGAGGTCCAGCCTGCCCAGTGACTCTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Reference:  AGAGGTCCAGCCTGCCCAGTGACTCTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGG  78
TAGCGTAATCACTTGTTCTTTAATTGGGGACTAGTATGAACGGCACCACGAGGGCTACAC  120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TAGCGTAATCACTTGTTCTTTAATTGGGGACTAGTATGAACGGCACCACGAGGGCTACAC  138
TGTCTCCTTTCTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGGATGAAAATAT  180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TGTCTCCTTTCTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGGATGAAAATAT  198
AAGACGAGAAGACCCTATGGAGCTTTAAACATTATGGCATCACCACACAACATATATTTT  240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAGACGAGAAGACCCTATGGAGCTTTAAACATTATGGCATCACCACACAACATATATTTT  258
TCCAGAACCACTTGCTCTTTAAGGTAGTGTGACCATGAGTTTTTGGTTGGGGTGACCGCG  300
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TCCAGAACCACTTGCTCTTTAAGGTAGTGTGACCATGAGTTTTTGGTTGGGGTGACCGCG  318
GAGTATAGTATAACCTCCACGCTGAAAGACACAGCTCTAAGCCAAGACCTACACTTCTAA  360
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GAGTATAGTATAACCTCCACGCTGAAAGACACAGCTCTAAGCCAAGACCTACACTTCTAA  378
GCATCAGCACACTGACATAAATTGACCCAATATATTTGATCAACGAACTAAGTTACCCTA  420
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GCATCAGCACACTGACATAAATTGACCCAATATATTTGATCAACGAACTAAGTTACCCTA  438
GGGATAACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGACCTCGAT  480
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GGGATAACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGACCTCGAT  498
GTTGGATCAGGGTATCCCAATGGTGCAGCCGCTATTAAAGGTTCGTTTGTTCAACGATTA  540
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GTTGGATCAGGGTATCCCAATGGTGCAGCCGCTATTAAAGGTTCGTTTGTTCAACGATTA  558
AAACCCTACG  550
||||||||||
AAACCCTACG  568
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Pictured below is the pairwise alignment between our *Phrynomantis* c.f. *bifasciatus (*FC: AL211202H1) sequence and a *P. bifasciatus* from Chumpanga in Mozambique (A: KM509174) (Peloso *et al*., 2015).

```
Sample:     AAAACATCGCCTCCTGATTTATTATAGGAGGTCCAGCCTGCCCAGTGACCCAGTTAAACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Reference:  AAAACATCGCCTCCTGATTTATTATAGGAGGTCCAGCCTGCCCAGTGACCCAGTTAAACG 545
GCCGCGGTACCCTAACCGTGCGAAGGTAGCGCAATCACTTGTTCTTTAAATGAGGACTAG  120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GCCGCGGTACCCTAACCGTGCGAAGGTAGCGCAATCACTTGTTCTTTAAATGAGGACTAG  605
TATGAATGGCATCACGAAGGCTACACTGTCTCCCCCCTCCAATCAGTGAAACTGATCTCC  180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TATGAATGGCATCACGAAGGCTACACTGTCTCCCCCCTCCAATCAGTGAAACTGATCTCC  665
CCGTGAAGAAGCGGGGATAAAACCATAAGACGAGAAGACCCCATGGAGCTTAAAACTCAG  240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CCGTGAAGAAGCGGGGATAAAACCATAAGACGAGAAGACCCCATGGAGCTTAAAACTCAG  725
TTTCACCTGCACACCAATATATCACATCAACCCTGCAGACCTGCTTACTAGTTTTCGGTT  300
||||||||||||||||||||||||||||||||||||||||x|||||||||||||||||||
TTTCACCTGCACACCAATATATCACATCAACCCTGCAGACCTGATTACTAGTTTTCGGTT  785
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GGGGTGACCACGGAGCAAAACAAAACCTCCACGACGAAAGGACATCTCCCTAATCCAAGA  360
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GGGGTGACCACGGAGCAAAACAAAACCTCCACGACGAAAGGACATCTCCCTAATCCAAGA  845
GCAACAACTCTAAGAATCAAAAAATTGACGTTACTTGATCCAAGTTTACTTGATCAACGA  420
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GCAACAACTCTAAGAATCAAAAAATTGACGTTACTTGATCCAAGTTTACTTGATCAACGA  905
ACCAAGTTACCCTGGGGATAACAGCGCAATCCATTTCAAGAGCTCCTATCGACAAATGGG  480
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACCAAGTTACCCTGGGGATAACAGCGCAATCCATTTCAAGAGCTCCTATCGACAAATGGG  965
TTTACGACCTCGATGTTGGATCAGGGTATCCTAGTGGTGCAGCCGCTACTAAAGGTTCGT  540
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTTACGACCTCGATGTTGGATCAGGGTATCCTAGTGGTGCAGCCGCTACTAAAGGTTCGT  1025
TTGTTCAACGATTAAAACCCT  561
||||||||||||||||||||
TTGTTCAACGATTAAAACCCT  1046
```

Pictured below is the pairwise alignment between our *Ptychadena* c.f. *mossambica* (FC: AL211202J4 & AL211202J5) sequences (which are identical to one another) and a *Ptychadena mossambica* from Zambia (A: MK464337.1) (Bittencourt–Silva, 2019).

```
Sample:     ATTCAACGGCCGCGGTACTCTGACCGTGCAAAGGTAGCACAATCACTTGTTCTTTAAATT 126
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Reference:  ATTCAACGGCCGCGGTACTCTGACCGTGCAAAGGTAGCACAATCACTTGTTCTTTAAATT  60
AGGACTAGAATCAATGGCATCACGAGGGCCTCACTGTCTCCTTTTTCCAATCAGTGAAAC  186
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AGGACTAGAATCAATGGCATCACGAGGGCCTCACTGTCTCCTTTTTCCAATCAGTGAAAC  120
TGATCTCCCCGTGAAGAAGCGGGGATAACCTTATAAGACGAGAAGACCCCATGGAGCTTC  246
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TGATCTCCCCGTGAAGAAGCGGGGATAACCTTATAAGACGAGAAGACCCCATGGAGCTTC  180
AAACTCAACAGCTACCCCATTCAAACTACACGATAATTTAAGGGATTTAGCTATTAGTTT  306
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAACTCAACAGCTACCCCATTCAAACTACACGATAATTTAAGGGATTTAGCTATTAGTTT  240
TGGGTTGGGGTGACCACGGAGAATAGCAAAACCTCCGCAATGAAAGAATTAAAATTCTTA  366
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TGGGTTGGGGTGACCACGGAGAATAGCAAAACCTCCGCAATGAAAGAATTAAAATTCTTA  300
TCCAAGAGCAACACCTCTAAGAATTAACAAATTAACACACAGTGATCCGATATCTTTCGA  426
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TCCAAGAGCAACACCTCTAAGAATTAACAAATTAACACACAGTGATCCGATATCTTTCGA  360
TCAATGAACCAAGTTACCCTGGGGATAACAGCGCCATCCACTTTGAGAGTTCATATCGAC  486
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TCAATGAACCAAGTTACCCTGGGGATAACAGCGCCATCCACTTTGAGAGTTCATATCGAC  420
AAGTGGGTTTACGACCTCGATGTTGGATCAGGGTATCCCAGTGGTGCAGCCGCTACTAAA  546
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAGTGGGTTTACGACCTCGATGTTGGATCAGGGTATCCCAGTGGTGCAGCCGCTACTAAA  480
GGTTCGTTTGTTCAACGATTAAAACCCTACGTGAT  581
||||||||||||||||||||||||||||||||||
GGTTCGTTTGTTCAACGATTAAAACCCTACGTGAT  515
```

Pictured below is the pairwise alignment between our *Pyxicephalus* sp. (FC: AL211204G1; AL211204A1; AL211204H2; AL211204H1) sequences (which are identical to one another) and a *Pyxicephalus adspersus* (A: NC04480) (Cai *et al*., 2019).

```
Sample:    CCTGTTTTATCAAAAACATCGCCTCTTGCTAAACTATAAGAGGTCCAGCCTGCCCAGTGA  60
           ||||x||||x|||||||||||||||||||||||x|||||||||||||||||||||||||||
Reference:CCTG-TTTACCAAAAACATCGCCTCTTGCTAAATTATAAGAGGTCCAGCCTGCCCAGTGA 2178
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CAAAGTTCAACGGCCGCGGTACCCTAACCGTGCGAAGGTAGCATAATCACTTGTTCTTTA  120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CAAAGTTCAACGGCCGCGGTACCCTAACCGTGCGAAGGTAGCATAATCACTTGTTCTTTA 2238
AATAAGGACTAGTATCAACGGCATCACGAGGGCTATACTGTCTCCTTTCTCTAATCAGTG  180
|||||||||||||||||||||||||||||||||||||||||||||||||||x||||||||
AATAAGGACTAGTATCAACGGCATCACGAGGGCTATACTGTCTCCTTTCTCCAATCAGTG 2298
AAACTGATCTCCCCGTGAAGAAGCGGGGATCTAATTATTAGACGAGAAGACCCCATGGAG  240
|||||||||||||||||||||||||||||xx||x||||||||||||||||||||||||||
AAACTGATCTCCCCGTGAAGAAGCGGGGATTCAACTATTAGACGAGAAGACCCCATGGAG 2358
CTTCAAGCTCAATAGCAACTTA-TTATTCTACAACTTTTTAACCATCAAGTTATGCTAAT  299
||||||||x|||x|||||||||x||x|||x|x|||x|x|x|||||x||||||||||x||
CTTCAAGCCCAAAAGCAACTTACTTCTTC-ATAACCTCTAAACCACCAAGTTATGCTTAT 2417
TAGCTTTAGGTTGGGGTGACCGCGGAGCACAACACAGCCTCCACGATGTAAAGGATTTCC  359
|x|||||x|||||||||||||||||||x||||x|x|x|||||||||||||||||||||||
TGGCTTTGGGTTGGGGTGACCGCGGAGTACAATATAACCTCCACGATGTAAAGGATTTCC 2477
T--TTATCTAAGAACGACAGTTCAAAGAACCCTAAAAATGTCATAAAATGATCCGAACTT  417
||||||||||||x|||||||x|||||||x|||||||||||||||||||||||||||||||
CCCTTATCTAAGAACAACAGTTCGAAGAACCTTAAAAATGTCATAAAATGATCCGAACTT 2537
CGATCAACGGACCAAGTTACCCTGGGGATAACAGCGCAATCCATTTCAAGAGCCCCTATC  477
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CGATCAACGGACCAAGTTACCCTGGGGATAACAGCGCAATCCATTTCAAGAGCCCCTATC 2597
GACAAATGGGTTTACGACCTCGATGTTGGATCAGGGTGTCCTAGTGGTGCAGCCGCTACT  537
|||||||||||||||||||||||||||||||||||x||||||||||||||||||||||||
GACAAATGGGTTTACGACCTCGATGTTGGATCAGGGTATCCTAGTGGTGCAGCCGCTACT 2657
AAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAAACCGGAG  592
|||||||||||||||||||||||||||||||||||||||||||||||x|||||||
AAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAG 2712
```

Pictured below is the pairwise alignment between our *Pyxicephalus adsperus* (FC: AL211202C2; AL211202G1; AL211202L1) sequences (which are identical to one another) and a *Pyxicephalus adspersus* (A: NC044480) (Cai *et al*., 2019).

```
Sample:    AAAACATCGCCTCTTGCTAAATTATAAGAGGTCCAGCCTGCCCAGTGACAAAGTTCAACG  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Reference:AAAACATCGCCTCTTGCTAAATTATAAGAGGTCCAGCCTGCCCAGTGACAAAGTTCAACG 2190
GCCGCGGTACCCTAACCGTGCGAAGGTAGCATAATCACTTGTTCTTTAAATAAGGACTAG  120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GCCGCGGTACCCTAACCGTGCGAAGGTAGCATAATCACTTGTTCTTTAAATAAGGACTAG 2250
TATCAACGGCATCACGAGGGCTATACTGTCTCCTTTCTCCAATCAGTGAAACTGATCTCC  180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TATCAACGGCATCACGAGGGCTATACTGTCTCCTTTCTCCAATCAGTGAAACTGATCTCC 2310
CCGTGAAGAAGCGGGGATTCAACTATTAGACGAGAAGACCCCATGGAGCTTCAAGCCCAA  240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CCGTGAAGAAGCGGGGATTCAACTATTAGACGAGAAGACCCCATGGAGCTTCAAGCCCAA 2370
AAGCAACTTACTTCTTCATAACCTCTAAACCACCAAGTTATGCTTATTGGCTTTGGGTTG  300
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAGCAACTTACTTCTTCATAACCTCTAAACCACCAAGTTATGCTTATTGGCTTTGGGTTG 2430
GGGTGACCGCGGAGTACAATATAACCTCCACGATGTAAAGGATTTCCCCCTTATCTAAGA  360
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GGGTGACCGCGGAGTACAATATAACCTCCACGATGTAAAGGATTTCCCCCTTATCTAAGA 2490
ACAACAGTTCGAAGAACCTTAAAAATGTCATAAAATGATCCGAACTTCGATCAACGGACC  420
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACAACAGTTCGAAGAACCTTAAAAATGTCATAAAATGATCCGAACTTCGATCAACGGACC 2550
AAGTTACCCTGGGGATAACAGCGCAATCCATTTCAAGAGCCCCTATCGACAAATGGGTTT  480
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAGTTACCCTGGGGATAACAGCGCAATCCATTTCAAGAGCCCCTATCGACAAATGGGTTT 2610
ACGACCTCGATGTTGGATCAGGGTATCCTAGTGGTGCAGCCGCTACTAAAGGTTCGTTTG  540
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACGACCTCGATGTTGGATCAGGGTATCCTAGTGGTGCAGCCGCTACTAAAGGTTCGTTTG 2670
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```
TTCAACGATTAAAACCCTACGTGATCTGAGTTCAAACCGGAG  582
||||||||||||||||||||||||||||||||x|||||||
TTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAG  2712
```

Pictured below is the pairwise alignment between our *Tomopterna* c.f. *cryptotis* (FC: AL211201K1; AL211202K2; AL211204D1) sequences (which are identical to one another) and a *T. cryptotis* from Angola (A: MN057689) (Channing & du Preez, 2019).

```
Sample:    GTCCAGCCTGCCCGGTGACAAAGTTTAACGGCCGCGGTATCCTGACCGTGCGAAGGTAGC 101
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Reference: GTCCAGCCTGCCCGGTGACAAAGTTTAACGGCCGCGGTATCCTGACCGTGCGAAGGTAGC  60
ATAATCACTTGTTCTTTAAATAGGGACTAGTATCAACGGCATCACGAGGGTTACACTGTC  161
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ATAATCACTTGTTCTTTAAATAGGGACTAGTATCAACGGCATCACGAGGGTTACACTGTC  120
TCCTTTCCACAATCAGTGAAACTGATCTCCCCGTGAAGAAGCGGGGATACAACTATAAGA  221
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TCCTTTCCACAATCAGTGAAACTGATCTCCCCGTGAAGAAGCGGGGATACAACTATAAGA  180
CGAGAAGACCCCATGGAGCTTTAAGCTCAACAACACCTCCACGCATACACACCCTTATAG  281
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CGAGAAGACCCCATGGAGCTTTAAGCTCAACAACACCTCCACGCATACACACCCTTATAG  240
CCCACGAGCCCTGTATGTTAGCTTTAGGTTGGGGTGACCGCGGAGTATAACATAACCTCC  341
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CCCACGAGCCCTGTATGTTAGCTTTAGGTTGGGGTGACCGCGGAGTATAACATAACCTCC  300
ACGACGAATAGGCCTAAAACCTTTATCCAAGAGCAACTGCTCTAAGAATCATAAAATTGA  401
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACGACGAATAGGCCTAAAACCTTTATCCAAGAGCAACTGCTCTAAGAATCATAAAATTGA  360
CACTGAATGATCCGATCTTCGATCAACGGACCAAGTTACCCTGGGGATAACAGCGCAATC  461
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CACTGAATGATCCGATCTTCGATCAACGGACCAAGTTACCCTGGGGATAACAGCGCAATC  420
CATTTCAAGAGCTCCTATCGACAAATGGGTTTACGACCTCGATGTTGGATCAGGGTATCC  521
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CATTTCAAGAGCTCCTATCGACAAATGGGTTTACGACCTCGATGTTGGATCAGGGTATCC  480
CAGTGGTGCAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTA  572
||||||||||||||||||||||||||||||||||||||||||||||||||
CAGTGGTGCAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTA  531
```

Pictured below is the pairwise alignment between our *Ichnotropis* c.f. *grandiceps* (FC: RE211206C1) sequence and its closest known relative, an *I. bivittata* from Angola (A: HF547775) (Garcia–Porta *et al.*, 2019).

```
Sample:    TTCAACGGCCGCGGTATCCTAACCGTGCAAAGGTAGCATAATCACTTGTCCTATAAATAA 127
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Reference: TTCAACGGCCGCGGTATCCTAACCGTGCAAAGGTAGCATAATCACTTGTCCTATAAATAA  60
GGACTAGAATGAATGGTCAAATGAGGATCAAACTGTCTCTTACATCTGACCAATAAACCT  187
|||||x|||||||x|||||||||||||||x||||||||||||x|||xx||||||||||||
GGACTGGAATGAACGGTCAAATGAGGATCGAACTGTCTCTTATATCCAACCAATAAACCT  120
GATCTTTTAGTCCAAAAGCTAAAATAAACTCATAAGACGAGAAGACCCTGTGGAGCTTAA  247
|||||||||||||||||||||||||||xx||||||||||||||||||||||||||||||
GATCTTTTAGTCCAAAAGCTAAAATAAATCCATAAGACGAGAAGACCCTGTGGAGCTTAA  180
AACCAGACCCAATCATTTGGCCTCCTGGTTTTTAGTTGGGGCAACTCCGGAGCA-CAGAA  306
|||||x||||||x|xxx|||||x|||||||||||||||||||||||||||||x|xx|x||
AACCAAACCCAA-CCCCTGGCCCCCTGGTTTTTAGTTGGGGCAACTCCGGAGTATAATAA  239
ACCCTCCAGCATGGAACACACTCTTAGACCTACATATCAAAGAGCACTAAACCTTGACCC  366
|||||||xx||||||||xxx|x||x|||xx||||x||||||||||xx|x|x||xx|||||
ACCCTCCGACATGGAACCTGCCCTAAGATATACACATCAAAGAGCCATTACCCCCGACCC  299
```

```
AGTGGCCCTTA-AACCATCTGATCAATGAACCAAGTTACCCCAGGGATAACAGCGCCATC  425
||||xxxxx||x||x||||||||||x||||||||||||||||||||||||||||||||||
AGTG-ATTATACAATCATCTGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCCATC  358
CCCTTCTAGAGTCCATATCAACAAGGGGGTTTACGACCTCGATGTTGGATCAGGACACCC  485
||||xx||||||||||||||||||||||||||||||||||||||||||||||||||||||
CCCTCTTAGAGTCCATATCAACAAGGGGGTTTACGACCTCGATGTTGGATCAGGACACCC  418
CAATAGTGCAACCGCTATTAAAAGTTCGTTTGTTCAACGATTAA-AGTCCTACGTGATCT  544
|||||||||||||||||||||x||||||||||||||||||||x|||||||||||||||||
CAATAGTGCAACCGCTATTAAAGGTTCGTTTGTTCAACGATTAATAGTCCTACGTGATCT  478
```

Pictured below is its pairwise alignment between our *Ptychadena* c.f. *nilotica* (FC: AL211206B5) sequence to a *P. nilotica* from Vumbura, Botswana (A: KX836495) (Zimkus *et al.*, 2017).

```
Sample:    TTTAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCATAATCACTTGTTCTTTAAATGG 126
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Reference: TTTAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCATAATCACTTGTTCTTTAAATGG  60
GGACTAGAATCAACGGCATCACGAGGGTCTTACTGTCTCCTTTCCCCAATCAGTGAAACT  186
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GGACTAGAATCAACGGCATCACGAGGGTCTTACTGTCTCCTTTCCCCAATCAGTGAAACT  120
GATCTCTCCGTGAAGAAGCGGGGATAAAAATATAAGACGAGAAGACCCCATGGAGCTTCA  246
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GATCTCTCCGTGAAGAAGCGGGGATAAAAATATAAGACGAGAAGACCCCATGGAGCTTCA  180
AGCTCAACAAGTACTCTTCTTAACTAACCTAACAGACTCTAGAATCTATTTGTTAGCTTT  306
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AGCTCAACAAGTACTCTTCTTAACTAACCTAACAGACTCTAGAATCTATTTGTTAGCTTT  240
GGGTTGGGGTGACCGCGGAGAAAAACTTAACCTCCACAATGAAAAGAATAAAATCCTAAT  366
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GGGTTGGGGTGACCGCGGAGAAAAACTTAACCTCCACAATGAAAAGAATAAAATCCTAAT  300
CTATGAGCCTACACCTCTAAGAATCAATAAATTGGCATAAAATGACCCGATATTTCGATC  426
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CTATGAGCCTACACCTCTAAGAATCAATAAATTGGCATAAAATGACCCGATATTTCGATC  360
AATGAACCAAGTTACCCTGGGGATAACAGCGCCATCCACTTCAAGAGCCCATATCGACAA  486
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AATGAACCAAGTTACCCTGGGGATAACAGCGCCATCCACTTCAAGAGCCCATATCGACAA  420
GTGGGTTTACGACCTCGATGTTGGATCAGGGTGTCCCAGTGGTGCAGCCGCTACTAAAGG  546
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GTGGGTTTACGACCTCGATGTTGGATCAGGGTGTCCCAGTGGTGCAGCCGCTACTAAAGG  480
TTCGTTTGTTCAACGATTAAAACCCT  572
|||||||||||||||||||||||||
TTCGTTTGTTCAACGATTAAAACCCT  506
```

Pictured below is the pairwise alignment between our *Breviceps adspersus* (FC: MH340372) sequence and a *B. adspersus* from south of the Congo basin (A: MH340372) (Nielsen *et al.*, 2018).

```
Sample:    TTAAATGGCCGCGGTACCCTAACCGTGCAAAGGTAGCGTAATCACTTGTCTACTAAATAT 127
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Reference: TTAAATGGCCGCGGTACCCTAACCGTGCAAAGGTAGCGTAATCACTTGTCTACTAAATAT  60
AGACCTGTATGAACGGCACCACGAGGGCCACACTGTCTCCCCCCTTTAATCAGTAAAACT  187
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AGACCTGTATGAACGGCACCACGAGGGCCACACTGTCTCCCCCCTTTAATCAGTAAAACT  120
GATCCCCCCGTGAAGAAGCGGGGATTCAAATACAAGACGAGAAGACCCCATGGAGCTTTA  247
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GATCCCCCCGTGAAGAAGCGGGGATTCAAATACAAGACGAGAAGACCCCATGGAGCTTTA  180
```

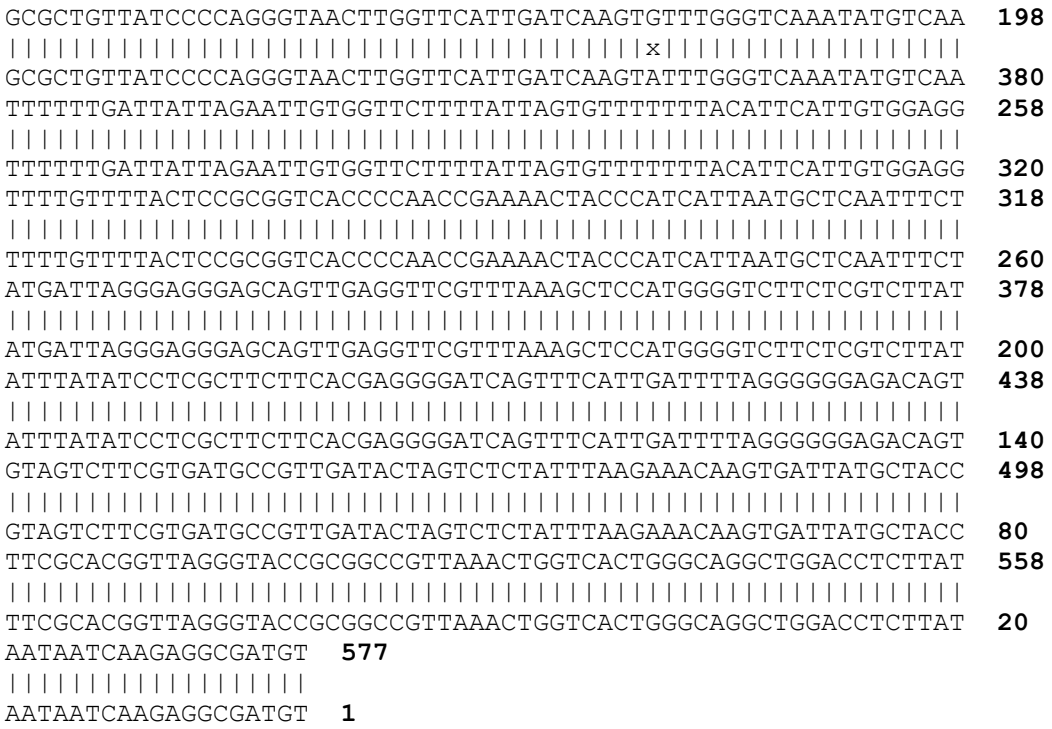
```
AACCAAAAGTCAACTGCTAAATTTTATATCATTAATACCGCAATCATGACTCAAAGTTTT  307
||||||  ||||||||||||||||||||||||||||||||||||||||||||||||||||
AACCAAGAGTCAACTGCTAAATTTTATATCATTAATACCGCAATCATGACTCAAAGTTTT  240
CGATTGGGGCGACCGCGGAGCATAACAAAACCTCCACGATGAAAGAACATAAAATTCTTA  367
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CGATTGGGGCGACCGCGGAGCATAACAAAACCTCCACGATGAAAGAACATAAAATTCTTA  300
CCCAAGAACCACACCACAAAGGACCACAAATGTGACATCCATTGACCCAAAAGCTTGATC  427
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CCCAAGAACCACACCACAAAGGACCACAAATGTGACATCCATTGACCCAAAAGCTTGATC  360
AACGAACCTAGTTACCCTGGGGATAACAGCGCAATCCATTTCAAGAGCCCATATCGACAA  487
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AACGAACCTAGTTACCCTGGGGATAACAGCGCAATCCATTTCAAGAGCCCATATCGACAA  420
ATGGGTTTACGACCTCGA  505
||||||||||||||||||
ATGGGTTTACGACCTCGA  438
```

Pictured below is the pairwise alignment between our lizard of the family Lacertidae (FC: RE211204D1) and the *Heliobolus lugubris* haplotype (A: DQ871142.1) (Makokha *et al*., 2007).

```
Sample:    GCCCAGTGAATTTTTTAACGGCCGCGGTATCCTAACCGTGCAAAGGTAGCATAATCACTT 116
           ||||||||||x||||||||||||||||||||||||||||||||||||||||||||||||||
Reference: GCCCAGTGAA-TTTTTAACGGCCGCGGTATCCTAACCGTGCAAAGGTAGCATAATCACTT  59
GTCTCCCAAATAGAGACTAGAATGAATGGCTTAATGAGGACAAAACTGTCTCTTACACTC  176
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GTCTCCCAAATAGAGACTAGAATGAATGGCTTAATGAGGACAAAACTGTCTCTTACACTC  119
AACCAATGAAACTGATCTTTCAGTACAAAAGCTGAAATATACACATAAGACGAGAAGACC  236
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AACCAATGAAACTGATCTTTCAGTACAAAAGCTGAAATATACACATAAGACGAGAAGACC  179
CTGTGGAGCTTCTAGATCAATATCACCATTATGATTTATCTGATCTTCAGTTGGGGCAAC  296
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CTGTGGAGCTTCTAGATCAATATCACCATTATGATTTATCTGATCTTCAGTTGGGGCAAC  239
TTCGGAGTATAAAAAACCCTCCGACAAATCAACTACTAATAAGATAAACAAATCAAACTT  356
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTCGGAGTATAAAAAACCCTCCGACAAATCAACTACTAATAAGATAAACAAATCAAACTT  299
AAAACCACCTGACCCAGTAATATTATAATTATCTGATCAACGGACCAAGTTACCCCAGGG  416
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAAACCACCTGACCCAGTAATATTATAATTATCTGATCAACGGACCAAGTTACCCCAGGG  359
ATAACAGCGCTATCCCCCTCTAGAGTCCTTATCGACAGGGGGGGTTTACGACCTCGATGTT  476
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ATAACAGCGCTATCCCCCTCTAGAGTCCTTATCGACAGGGGGGGTTTACGACCTCGATGTT  419
GGATCAGGACACCCCGATAGTGCAGCAGCTATCAAAGG-TCGTTTGTTCAACGATTAATA  535
||||||||||||||||||||||||||||||||||||x|||||||||||||||||||||||
GGATCAGGACACCCCGATAGTGCAGCAGCTATCAAAGGTTCGTTTGTTCAACGATTAATA  479
GTCCTACGTGATCTGA  551
||||||||||||||||
GTCCTACGTGATCTGA  495
```

Pictured below is the pairwise alignment between our *Leptopelis bocagii* (FC: AL211204E1 & AL211204E2) sequences (which are identical to one another) and a *L. bocagii* from Malanje Province, Angola (A: MK036434) (Hayes *et al*., 2018).

```
Sample:    TCACGTAGGGTTTTAATCGTTGAACAAACGAACCATTAGTAGCGGCTGCACCACTAGGAC  78
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Reference: TCACGTAGGGTTTTAATCGTTGAACAAACGAACCATTAGTAGCGGCTGCACCACTAGGAT 500
ACCCTGATCCAACATCGAGGTCGTAAACCCATTTGTCGATAGGGGCTCTTAAAATGGATT  138
||||||||||||||||||||||||||||||x|||||||||||||||||||||||||||||
ACCCTGATCCAACATCGAGGTCGTAAACCCACTTGTCGATAGGGGCTCTTAAAATGGATT  440
```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GCGCTGTTATCCCCAGGGTAACTTGGTTCATTGATCAAGTATTTGGGTCAAATATGTCAA 380
TTTTTTGATTATTAGAATTGTGGTTCTTTTATTAGTGTTTTTTTACATTCATTGTGGAGG 258
TTTTTTGATTATTAGAATTGTGGTTCTTTTATTAGTGTTTTTTTACATTCATTGTGGAGG 320
TTTTGTTTTACTCCGCGGTCACCCCAACCGAAAACTACCCATCATTAATGCTCAATTTCT 318
TTTTGTTTTACTCCGCGGTCACCCCAACCGAAAACTACCCATCATTAATGCTCAATTTCT 260
ATGATTAGGGAGGGAGCAGTTGAGGTTCGTTTAAAGCTCCATGGGGTCTTCTCGTCTTAT 378
ATGATTAGGGAGGGAGCAGTTGAGGTTCGTTTAAAGCTCCATGGGGTCTTCTCGTCTTAT 200
ATTTATATCCTCGCTTCTTCACGAGGGGATCAGTTTCATTGATTTTAGGGGGGAGACAGT 438
ATTTATATCCTCGCTTCTTCACGAGGGGATCAGTTTCATTGATTTTAGGGGGGAGACAGT 140
GTAGTCTTCGTGATGCCGTTGATACTAGTCTCTATTTAAGAAACAAGTGATTATGCTACC 498
GTAGTCTTCGTGATGCCGTTGATACTAGTCTCTATTTAAGAAACAAGTGATTATGCTACC 80
TTCGCACGGTTAGGGTACCGCGGCCGTTAAACTGGTCACTGGGCAGGCTGGACCTCTTAT 558
TTCGCACGGTTAGGGTACCGCGGCCGTTAAACTGGTCACTGGGCAGGCTGGACCTCTTAT 20
AATAATCAAGAGGCGATGT 577
AATAATCAAGAGGCGATGT 1