THE DISTRIBUTION OF THE DESERT RAIN FROG (*Breviceps macrops*) IN SOUTH AFRICA

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ABSTRACT

The Distribution of the Desert Rain Frog (*Breviceps macrops*) in South Africa

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The desert rain frog (*Breviceps macrops*) is an arid adapted anuran found on the west coast of southern Africa occurring within the Sandveld of the Succulent Karoo Biome. It is associated with white aeolian sand deposits, sparse desert vegetation and coastal fog. Little is known of its behaviour and life history strategy. Its distribution is recognised in the Atlas and Red Data Book of the Frogs of South Africa, Lesotho, and Swaziland as stretching from Koiingnaas in the South to Lüderitz in the North and 10 km inland. This distribution has been called into question due to misidentification and ambiguous historical records. This study examines the distribution of *B. macrops* in order to clarify these discrepancies, and found that its distribution does not stretch beyond 2 km south of the town of Kleinzee nor further than 6 km inland throughout its range in South Africa. The reasons for this are not clear, as there appears to be adequate habitat south of this point. Habitat suitability, food availability and competition, anthropogenic disturbance, and historical distribution patterns are discussed in terms of their impact on *B. macrops* distribution however no significant correlations are found. In addition, examination of the available habitat within South Africa reveals that the anthropogenic impact of strip mining for alluvial diamonds has greatly transformed much of the west coast of southern Africa including vast tracts of *B. macrops* habitat. Previous estimates of distribution as pertains to available habitat are found to be overly generous and this study estimates that only 21.84% of the original area remains. Thus the conservation status of this species is dire and should be reviewed by the IUCN in light of current findings. It is the assertion of the author that the current status of Vulnerable (VU) be elevated to Endangered (EN).

January 2009

DECLARATION

I declare that The Distribution of the Desert Rain Frog (*Breviceps macrops*) in South Africa is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Kirsty Jane Bell January 2009

Signed.....

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CONTENTS

CHAPTER 1: INTRODUCTION

1.1 The Study Animal

1.1.1 Amphibians

The class Amphibia is distinguished by a biphasic lifecycle, where species undergo metamorphosis from a water-dependant larval stage to that of a terrestrial adult (Duellman & Trueb, 1986). Amphibians occur in almost every habitat across the globe, increasing in abundance and diversity towards the tropics (Duellman, 1999). There are three orders that make up the amphibians of the world; the Gymnophiona, worm-like animals, the Caudata which comprises newts and salamanders, and the Anura, frogs and toads (Duellman & Trueb, 1986; Carruthers, 2001). Of the three orders, anurans are currently the most successful, being the most abundant, with over 5602 species worldwide (Frost, 2008). They are also the only order found in southern Africa (Poynton, 1999; Frost, 2008).

Features, which distinguish frogs and toads from the rest of the amphibians, include having no tail in the adult phase of their lifecycle, producing vocalisations during the breeding season, and most have well developed limbs that are used in many different ways (Duellman & Trueb, 1986; Carruthers, 2001). It must be noted here that other amphibians also have well developed limbs, such as members of the Caudata, however, their uses are less varied. All anurans are generalist predators, with feeding behaviour that is usually opportunistic, predominantly consuming invertebrates, although there are many exceptions to this rule (Toft, 1980; Santos *et al.*, 2004). Their methods of foraging vary from ambush to active hunting (Duellman & Trueb, 1986). Camouflage is one of their main methods of avoiding detection, although to confuse predators they also employ other methods such as flash and aposematic colouration and polymorphism (Duellman & Trueb, 1986; Inger *et al.*, 1995; Summers & Clough, 2001; Vences *et al.*, 2003; Hoffman & Blouin, 2008).

1.1.2 Genus: *Breviceps*

This genus belongs to the family Brevicipitidae, within which the genus *Breviceps* is made up of 16 described species, distributed from South Africa to Tanzania, and across to Angola (Channing & Minter, 2004; Minter *et al.*, 2004; Frost, 2008). All of the species described are burrowing species, with entirely terrestrial life cycles (Poynton, 1964; De Villiers, 1988; Minter *et al.*, 2004). As such they share several morphological adaptations. In general, *Breviceps* spp have rotund bodies and short stumpy legs with well-developed metatarsal tubercles. They have characteristically flattened faces with drooping mouths, and relatively large eyes with horizontal pupils. They cannot jump or swim, but walk across the ground (Carruthers, 2001; Minter, 2003; Minter *et al.*, 2004). During the day they retreat underground, some into branching tunnels beneath rocks or fallen logs and some into loose sand in open dunes (Minter *et al.*, 2004). They bury themselves into the ground by moving soil from beneath their posterior using their hind legs, causing them to disappear backwards into the sand as they dig. At night they return to the surface to forage and to mate, when the conditions are suitable (Channing *et al.*, 2004; Minter *et al.*, 2004).

1.1.3 *Breviceps macrops*

The desert rain frog (*Breviceps macrops*) resides in the arid parts of southern Africa, specifically the Succulent Karoo Biome on the west coast of the continent (Carruthers & Passmore, 1978; Channing, 1987; De Villiers, 1988; Poynton, 1999; Carruthers, 2001; Minter *et al.*, 2004; Frost, 2008). The distinguishing features that define *B. macrops* include webbing on its hind feet; a lack of tubercles on the palms of the forefeet; a hidden tympanum; and an interorbital distance approximately half the horizontal diameter of the eye (De Villiers, 1988). Its skin is smooth and generally pale cream all over, however, the dorsal surface is often mottled in a darker brown. This colouring blends in particularly well with the colour of the sand and can be used to identify individuals (Channing, 1987; De Villiers, 1988). In addition, sand adheres to the skin of the frog, leaving only the eyes, mouth and nostrils visible, making it very difficult to discern from the rest of the substrate found in its habitat (Figure 1.1).

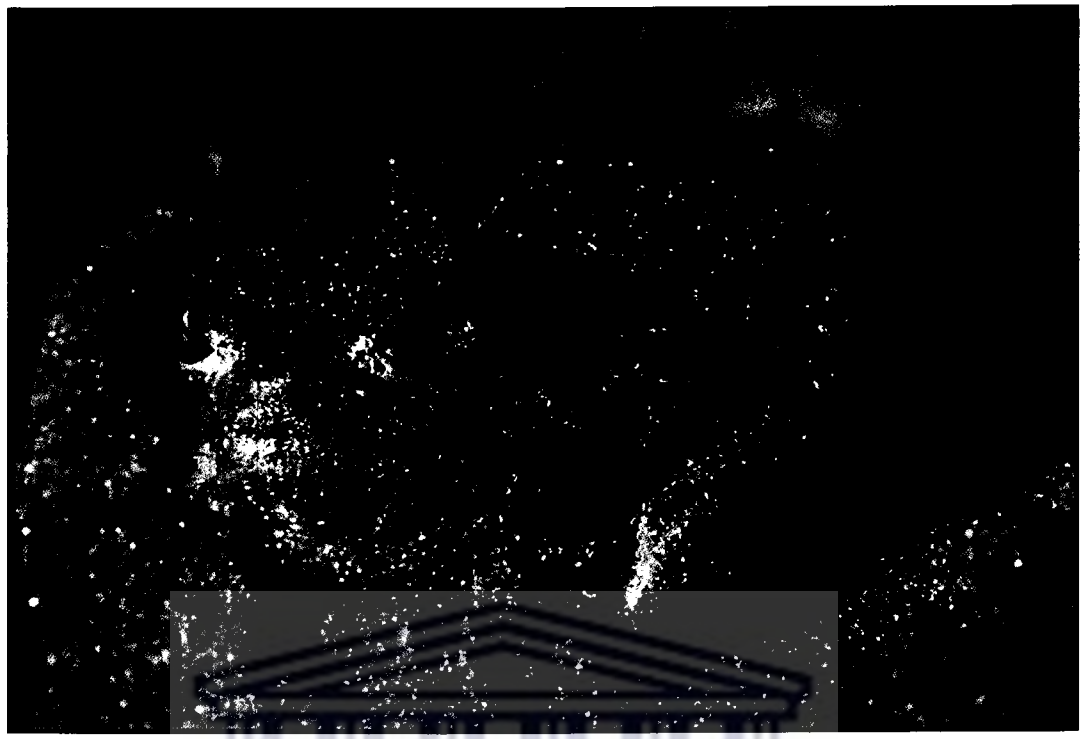

Figure 1.1. *B. macrops*, camouflaged in its natural habitat.

1.2 Adaptations for Living in Arid Areas

Deserts and semi-deserts generally receive little rainfall, and are characteristically dry with extreme temperature fluctuations (Rutherford *et al.*, 2006). Species occurring in this environment have evolved both physiological and behavioural adaptations that allow them to survive such harsh surroundings. Of all the species that one would expect to occur here, perhaps anurans are the least likely, being mostly soft-skinned water-dependant animals. Those species that have managed to colonise these areas have of necessity developed similar methods of coping with the inherent stresses. They are able to absorb water and a small amount of oxygen through the skin. However, this permeability can make them more prone to dehydration.

Anurans living in dry areas have had to adapt their life-history strategies in order to both reduce water loss and maximise water uptake. Examples of these adaptations are found worldwide and include increased permeability of the skin and avoidance of the harshest conditions through hibernation, aestivation, and sheltering. In Arizona certain frogs such as *Spea hammondii* hibernate in

underground burrows for up to nine months of the year (Ruibal *et al.*, 1969). In Australia, *Notaden nichollsi* and *Uperoleia micromeles* also hibernate (Thompson *et al.*, 2005), whilst other Australian anurans take it a step further by forming cocoons whilst in aestivation (Lee & Mercer, 1976; Thompson *et al.*, 2005). Similarly in Africa, *Pyxicephalus adspersus* and *Leptopelis bocagii* have been found to form a cocoon during aestivation (Loveridge & Craye, 1979). However, some anurans cannot burrow as they lack well developed metatarsal tubercles on the feet, and hence they utilise the shelter of cracks and crevices in rocks and trees, for example *Poyntonophrynus hoeschi* (Channing, 1976; 1988).

As concerns adaptations to its arid environment *B. macrops* has specific morphological characteristics that allow it to survive. It is the only species of the *Breviceps* genus that has webbing between the toes, which possibly provides increased purchase on the loose, soft sand in which it is found (Carruthers & Passmore, 1978; De Villiers, 1988; Minter *et al.*, 2004). Another very distinctive adaptation is the 'belly patch', it is located towards the posterior end of the ventral side, being a large pink area of translucent un-pigmented skin, surrounded by white, pigmented skin (Carruthers & Passmore, 1978; De Villiers, 1988). There are two theories concerning the function of the 'belly patch'. Firstly, Carruthers and Passmore suggested that the 'belly patch' could be part of a heat exchange mechanism between the body and the ground although there is little evidence supporting this theory (Carruthers & Passmore, 1978). Secondly, it is possible that, being made up of well vascularised skin, it maximises the amount of water absorbed from the surrounding sand, aided by the fact that it is this part of the belly that remains in contact with the ground when the frog is stationary (Carruthers, 2001).

Certain anurans exhibit a behavioural adaptation similar to the latter of these theories. Brekke *et al.* (1991) demonstrated that the toad *Anaxyrus punctatus* displays a certain water absorption response when on moist ground. This response involves the adduction of their hind limbs, causing the skin on their ventral side to be pressed into the moist ground (Brekke *et al.*, 1991). In 1993 Parsons *et al.* proved that *Bufo marinus* have an increase in water uptake across the skin of the pelvis in proportion to a reduction in bladder size (Parsons *et al.*, 1993). There are

many more findings involving various aspects of the pelvic patch and the water absorption response in several other anuran species (Hillyard *et al.*, 1998; Parsons & Schwartz, 1991; Sullivan *et al.*, 2000; Word & Hillman, 2005). It is thus most likely that *B. macrops* also uses the 'belly patch' for increased cutaneous water uptake.

Figure 1.2. The 'belly patch' of *B. macrops*.

Many animals and plants living in arid environments are dependant on ephemeral sources of water such as dew, mist and fog. Not only is it necessary for the limited plant growth, which feeds the insects that are the staple diet of most herpetofauna, but some herpetofauna, consume this water source directly. Cooper and Robinson (1990) postulated that the Namib Desert Sand Dune Lizard, *Aporosaura anchietae*, maintains its water balance by drinking fog water that has condensed on surrounding vegetation. Similarly, *B. macrops* obtains most of its water from both its food and by direct absorption through its skin of condensed fog water on and below the ground.

B. macrops conserves water and reduces water loss by burying under the sand, and appears to emerge at night only when the climatic conditions are suitable for foraging (De Villiers, 1988). To date the burrowing depths of *B. macrops* are unknown. They have been found between 12.5 and 20 cm below the surface (Minter *et al.*, 2004) which most likely corresponds to the minimum depth of the boundary layer. A number of studies have shown that environmental factors other then air temperature or relative humidity, such as surrounding moisture content, are more influential in stimulating certain anurans to leave their diurnal burrows to forage (Cree, 1989; Seebacher & Alford, 1999). Ruibal *et al.* (1969) demonstrated that *Spea hammondii* is prompted to emerge by coming to the surface first, assessing whether conditions are suitable, and then deciding to leave its retreat or not. It is possible that *B. macrops* is stimulated in a similar way, however, the causal factors that stimulate this response have not been studied. One factor that could be partly responsible might be the characteristic coastal fog of the area that occurs for more than 100 days within a year. This fog is the main factor influencing air and soil moisture in the coastal strip (De Villiers, 1988; Olivier, 2002).

1.3 Conservation

1.3.1 Global Significance of Anuran Conservation and Threats

Amphibians are an integral part of the planet's ecosystem. They act both as predator and prey for a variety of animals. They have been part of human culture for millennia, in the form of folklore, as aids in hunting and more recently in scientific research linked to medical progress (Branch & Harrison, 2004; Minter *et al.*, 2004). With our burgeoning human population, and increased resource demands we are destroying vast tracts of the natural world. This destruction takes many different forms, including deforestation, pollution of rivers, global warming and habitat destruction (Flannery, 2005). The worldwide decline in amphibian populations has been attributed to many of these same factors, with the recent addition of disease transportation (Kiesecker *et al.*, 2001; Collins & Storfer, 2003;

Branch & Harrison, 2004; Pounds *et al.*, 2005). Due to their moist soft skin through which water and oxygen can be absorbed, amphibians are susceptible to toxic elements that are bi-products and waste materials of human consumerism (Rohr *et al.*, 2008). Many of them are so dependant on their specific niche that when their habitat is threatened they are unable to migrate to other locations and are forced towards extinction. These factors make them highly susceptible to environmental impacts and as such they have been used as indicator species in scientific assessments of the health of many different ecosystems (Hartwell & Olivier, 1998; Collins & Storfer, 2003; Branch & Harrison, 2004). It is imperative to ensure that global anuran populations are monitored and afforded adequate protection.

1.3.2 IUCN Conservation Status

In order to assess the IUCN conservation status of an animal a number of parameters must be taken into account, such as population size, reduction in habitat, distribution, endemism, habitat requirements and continued threats to the species (Branch & Harrison, 2004; Minter *et al.*, 2004). From this assessment species can be placed into categories that indicate the level of threat they face. The IUCN Red List Categories and Criteria is the system used to classify species in terms of their risk of global extinction (IUCN, 2001, Version 3.1). The structure of the categories is shown in Figure 1.3. *B. macrops* is listed as Vulnerable (VU) according to the IUCN Red List (Minter *et al.* 2004 (b)). However, the exact range and distribution of the frog is uncertain. Before correct and adequate protection can be provided for this frog, it is essential that its distribution is accurately known.

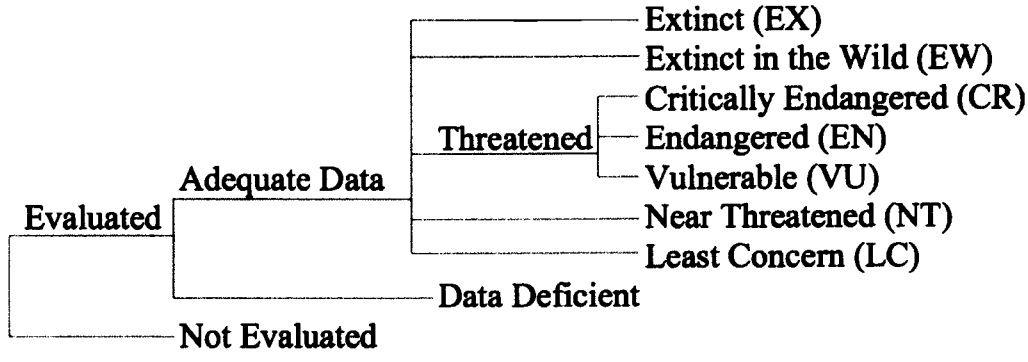

Figure 1.3. IUCN Red List Categories (IUCN, 2001, Version 3.1).

1.4 Aims and Research Questions

1.4.1 Aims

The aim of this study is to more accurately define the distribution of *B. macrops* in order to aid its correct placement in the IUCN Red Data List categories and ensure the species and its habitat gains the level of protection it requires. This will be achieved by assessing its southerly distribution, and the maximum distance it occurs from the coast. In addition a number of environmental factors are assessed that may influence this distribution, and the genetics of three disparate *B. macrops* populations are investigated.

1.4.2 Research Questions

To direct this study the folowing questions were asked:

1. What are the limits of *B. macrops* distribution, both south of Kleinzee and inland?
2. What are the possible causes limiting *B. macrops* range?
3. Is there a correlation between environmental/physiographic factors and the distribution of *B. macrops*?
4. How much undisturbed habitat is left to the species?
5. Is there a significant genetic difference between the known populations of *B. macrops* in South Africa?
6. How do the results inform the conservation of the species and its IUCN Red Data status?

CHAPTER 2: MATERIALS AND METHODS

2.1 Study Area

2.1.1 Study Sites

The current distribution of *B. macrops* within South Africa is listed as stretching from Alexander Bay in the north, to Skulpfontein, in the south, and extends from the high water mark to approximately 10 km inland (Minter *et al.*, 2004). This area falls within the Sandveld of the Succulent Karoo along the north-west coast of South Africa (Figure 2.1). Three study sites were chosen to encompass both the distribution and genetic components:

1) The primary study site was the area between Kleinzee and Koiingnaas from the coast up to 8 km inland (29° 41′ 01.6″ S; 17° 03′ 16.6″ E to 30° 12′ 22.0″ S; 17° 14′ 00.0″ E and 29° 41′ 00.0″ S; 17° 09′ 30.0″ E to 30° 12′ 21.7″ S; 17° 19′ 00.0″ E) ranging between 9 m and 151 m in altitude.

2) The secondary study sites were:
   - Next to the coast at McDougall's Bay near Port Nolloth (29° 16′ 09.2″ S; 16° 52′ 22.4″ E) at approximately 16 m in altitude,
   - The sand dunes on the northern side of the Holgat River, from the mouth up to 1.5. km inland (28° 58′ 30.8″ S; 16° 43′ 00.2″ E to 28° 58′ 00.8″ S; 16° 43′ 42.6″ E) ranging between 12 m and 46 m in altitude.

The primary study site covered a distance of approximately 60 km north to south, and 10 km east to west. It was delineated as such to determine the distance the species occurs from the coast and the extent of its southerly range. The two secondary study sites were chosen as they have known populations of *B. macrops* which could be used firstly to ground-truth the methodology used in the distribution study and secondly to provide genetic samples for a comparison of extant *B. macrops* populations. Photographs of the study sites are shown in Figures 2.2, 2.3, 2.4 and 2.5.

The three study sites are separated by two rivers, the Holgat River and the Buffels River, both of which could have been substantial natural barriers against the migration of *B. macrops*. Thus it was envisioned that samples taken from these three disparate populations might be genetically distinct.

2.1.2 Geology

Within Namaqualand, from the Olifants River in the south to the Orange River in the north there is a thin coastal strip of loose white sand which can be up to 30 km in width (Le Roux, 2005). This strip is often referred to as the Coastal Plain of Namaqualand or the Sandveld (Le Roux, 2005). The geology of Namaqualand in general is fairly complex, with the mountainous desert of the Richtersveld in the north-west comprised of pre-Gondwanan rocks that are extensively intruded. The escarpment zone in the south is dominated by nubbins and castle-koppies, and derived from the erosion of granite and gneiss of the Namaqua Metamorphic Province (Cowling *et al.*, 1999). This area is commonly referred to as the Hardeveld with high peaks in this granitic landscape measuring up to 1700 m in the Kamiesberg (Cowling *et al.*, 1999). The geology includes portions of sedimentary rocks of the Gariep, Numees and Nama Formations which are found to the west of the level coastal plain, the Sandveld. The Sandveld is composed of a complex sequence of marine and aeolian sands derived from weathered, fine-grained deposits of the later Tertiary age, and more recent white calcareous sands in the coastal margin (Desmet, 1996). These white wind-blown sands are underlain by hardpans of siliceous (dorbank) or calcareous (calcrete) materials, with exposed outcrops of Tertiary origin silcretes (Partridge, 1997).

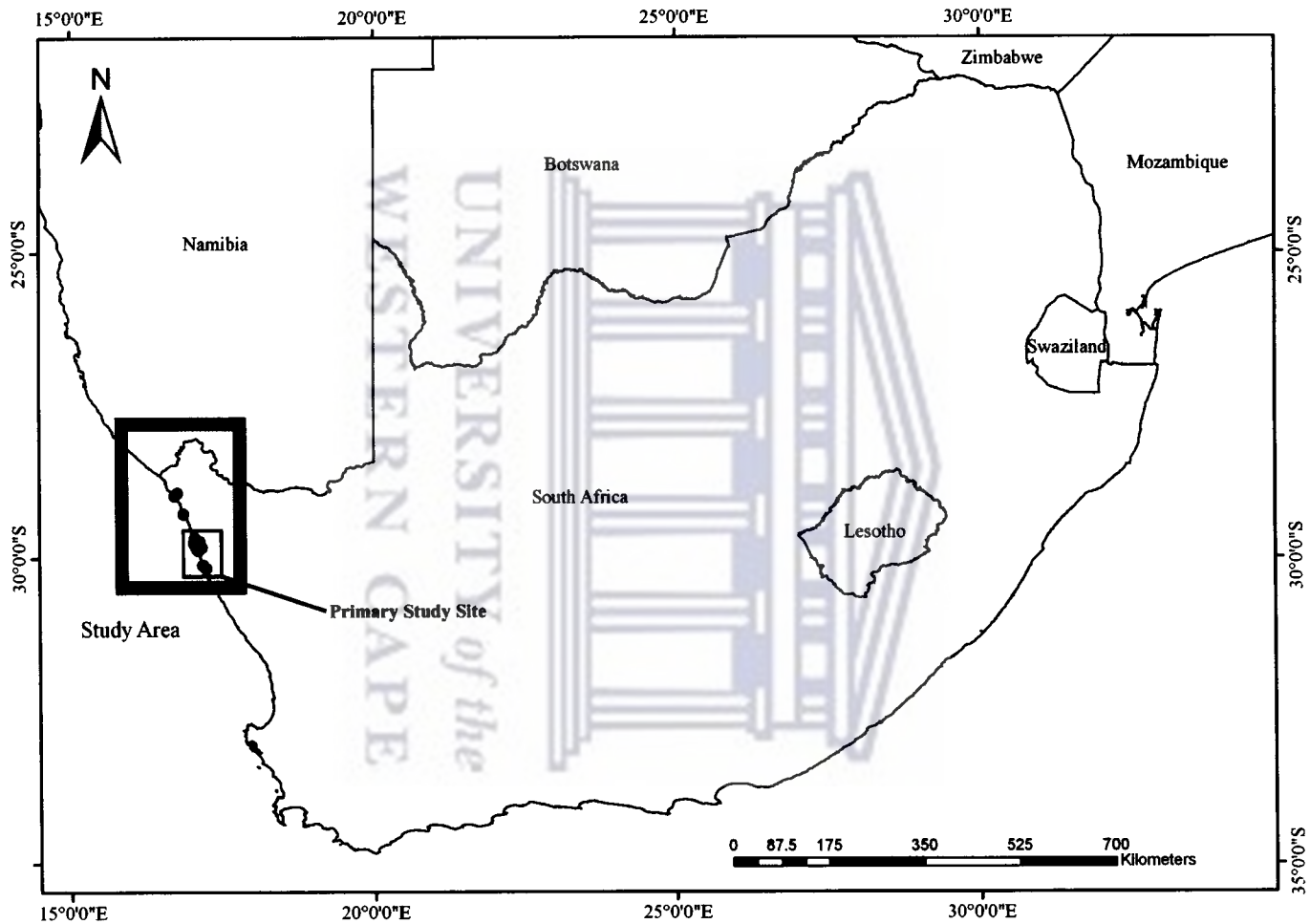

Figure 2.1. Location of the study area on the west coast of South Africa.

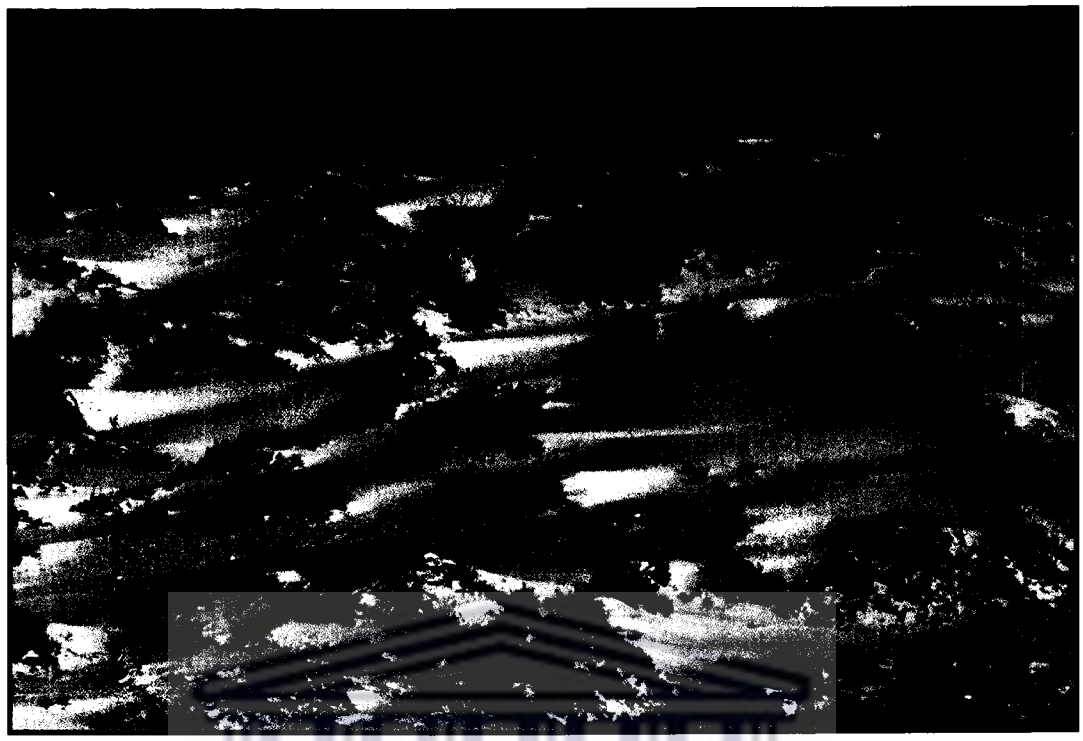

Figure 2.2. Habitat investigated at the Holgat River mouth.

Figure 2.3. Habitat investigated south of Kleinzee.

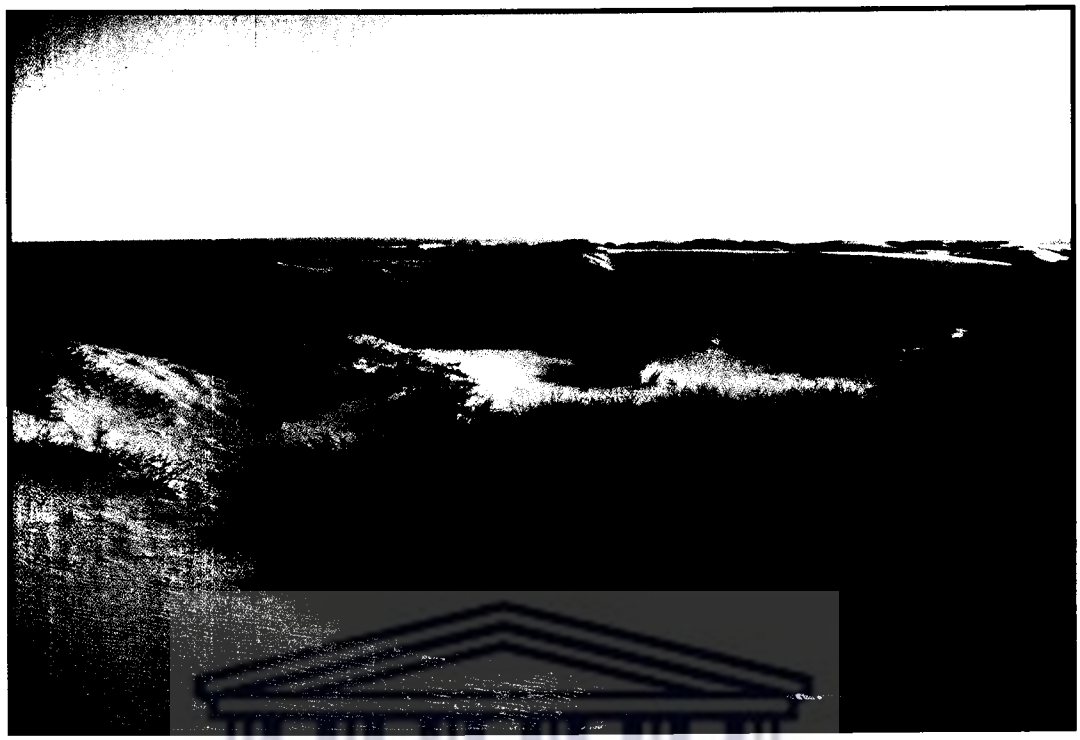

Figure 2.4. Habitat investigated north of Koiingnaas (Noup).

Figure 2.5. Habitat investigated at McDougall's Bay, Port Nolloth.

2.1.3 Climate

The Succulent Karoo is a semi-desert region strongly influenced by its proximity to the Atlantic Ocean, characterised by an even, mild climate. As with the Fynbos biome it receives winter rainfall, however, its Mean Annual Precipitation (MAP) is much less, between 100 and 200 mm (Mucina *et al.*, 2006). The coastal strip of the Sandveld region between Alexander Bay and half way between Port Nolloth and Kleinzee receives a MAP of 50-80 mm (Mucina *et al.*, 2006). South of Port Nolloth to just beyond the Groen River mouth the MAP rises to 114 mm (Mucina *et al.*, 2006). Of interest is the predictability of this rainfall, which tends to preclude any prolonged droughts. This reliability is unique and allows for several of the biologically unusual patterns and processes of this region (Hoffman & Cowling, 1987).

In addition the Sandveld is characterised by a highly predictable fog that is often a nightly occurrence. The high humidity coupled with cool temperatures at night result in large volumes of dewfall. This dew is the main source of moisture within the Sandveld (Le Roux, 2005).

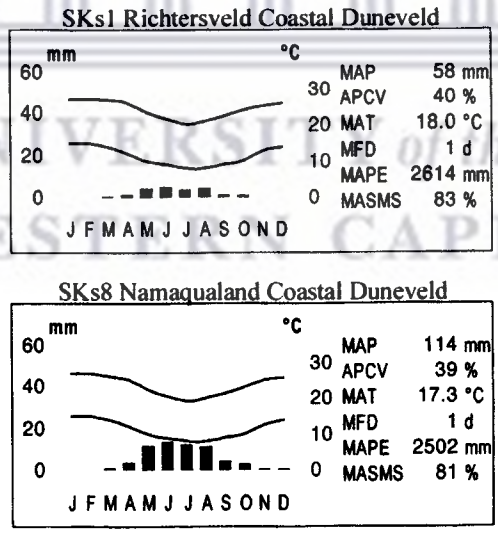

Figure 2.6. Climatic diagrams of two Namaqualand Sandveld Bioregion units that fall within the study area. The blue bars illustrate the monthly median rainfall. The upper red line illustrates the mean daily maximum temperature, and the lower red line illustrates the mean daily minimum temperature. MAP: Mean Annual Precipitation; APCV: Annual Precipitation Coefficient of Variation; MAT: Mean

Annual Temperature; MFD: Mean Frost Days (days when temperature dropped below 0°C); MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress (% days when evaporative demand was more than double the soil moisture supply). These climatic diagrams have been taken from Mucina *et al.* (2006).

2.1.4 Soils

Along the coastline, the white aeolian sand deposits form dunes that stretch north often alongside old river mouths. Slightly further inland the sand becomes redder in colour and increasingly calcareous and undulating in terms of landscape (Le Roux, 2005).

Although the salt content of the Sandveld is unknown, it is very likely that these sands are high in salts, as the probable parent rock is composed of marine deposits that have been uplifted in earlier geological periods. In addition, through sea-spray and wind transportation, further salts are likely to be laid down along this coastal strip. Due to the lack of rain in arid areas such as these the process of leaching is much reduced and the rate of evaporation is much increased. Together these processes leave a more saline soil than in other more humid areas (Allison *et al.*, 1969). This results in a very harsh environment for plants and animals to survive in.

2.1.5 Vegetation

The Sandveld is known for its pristine, white sand dunes that meet the sea, which are dotted with patches of low succulents that, like the rest of the region, flower at the end of the winter rains. For a semi-desert it is surprisingly high in diversity in terms of both flora and fauna, many of which are associated with the Namib Desert. This high diversity is likely due to the regular and frequent fog that blankets the coast on an almost nightly basis (Carruthers & Passmore, 1978; Cowling *et al.*, 1999, Mucina *et al.*, 2006). The vegetation consists of low succulents and annual flowering plants approximately 30 cm in height (Le Roux, 2005). It is very high in diversity being home to over 2400 endemic plants, and is uniquely characterised by high numbers of Mesembryanthemaceae, and relatively

high numbers of Iridaceae and Geraniaceae (Cowling *et al.*, 1999; Le Roux, 2005). The vegetation classification depicted in Figure 3.5 is taken from Mucina *et al.* (2006). From Alexander Bay to half way between Port Nolloth and Kleinzee the vegetation consists of SKs 1, Ritchersveld Coastal Duneveld; a band running parallel to the coast between 1-12 km in diameter. Here the vegetation is relatively homogenous on the stable sheets of sand and correlates closely to the satellite imagery (Figure 3.4). Often *Stoeberia utilis* can be found at the crest of the dunes, whilst *S. beetzii* is generally found on more stabilised sand (Mucina *et al.*, 2006). Endemic vegetation species include *Crassula brevifolia psammophila* and *Bassia dinteri* (Mucina *et al.*, 2006). Further south stretching from just north of Kleinzee to just below the Groen River the vegetation type changes to SKs 8, Namaqualand Coastal Duneveld (Mucina *et al.*, 2006). Here *Cladoraphis spp*, spiny grasses, are common on semi-stable sands and endemic taxa include the succulent shrub *Wooleya farinose* and the herb *Gazania* sp (Mucina *et al.*, 2006).

2.2 Distribution Analysis

Observational surveys and pitfall traps were used within the primary study site to sample for presence or absence of *B. macrops*. These methods were calibrated at M^{c}Dougall's Bay with a known population of individuals. Observational surveys were conducted at the Holgat River mouth to sample the population for genetic analysis.

B. macrops is active throughout the year, but sampling was conducted during the winter months as communications with the locals of Kleinzee and with Alan Channing (2008) indicated that temperature might be a factor involved in their activity patterns. It is likely that *B. macrops* is most active when the temperature is below 15°C (Minter *et al.*, 2004).

2.2.1 Pitfall Traps

Pitfall traps with drift fences were used in the primary study site. This site was divided into transects that ran from the coast up to 8 km inland. The first transect

was established at Kleinzee Beach just south of existing mining areas, with each subsequent transect placed at 5 km intervals south thereof. The transects were divided into 6 sample points spaced 2 km apart, the first sample point located at the coast. Traps were placed in the afternoon of the first day and remained in place for a period of three nights, traps were checked each morning for the presence of *B. macrops* and other animals. Extraneous animals were removed from the traps each morning. After three nights the traps were moved to the next sampling point. The co-ordinates for the 'proposed' transect points are listed in Appendix A. It was decided to sample every point on each transect, provided that it fell outside restricted-access mining areas. However, initial transects indicated that this type of random sampling was utilising far too much time and effort in areas where it was clearly apparent that *B. macrops* did not occur, i.e. far from the coast and on substrates that were too hard for burrowing. Thus the sampling technique was altered to a more stratified sample as follows: once on site, the traps were only placed where the substrate being sampled was suitable for *B. macrops*. Hence the traps were placed within one kilometre of a proposed sample point according to three habitat assumptions:

1. The sand must be soft enough for a frog to burrow into,
2. There must be evidence of insect life,
3. The location must be outside of mining areas, historical or extant.

If neither the 'proposed' point nor the immediate area surrounding it within one kilometre fitted these three assumptions, then traps were not placed in that location. The coordinates of the sites actually sampled are listed in Appendix B, and the locations of the sites are shown in Figure 2.7.

In addition to the above criteria it was found that a large tract of land between Kleinzee and Koiingnaas held no suitable habitat whatsoever. Due to the fact that the study was governed by time constraints and the need to investigate areas where *B. macrops* was thought to occur it was decided to exempt this area from investigation, evidenced in Figure 2.7.

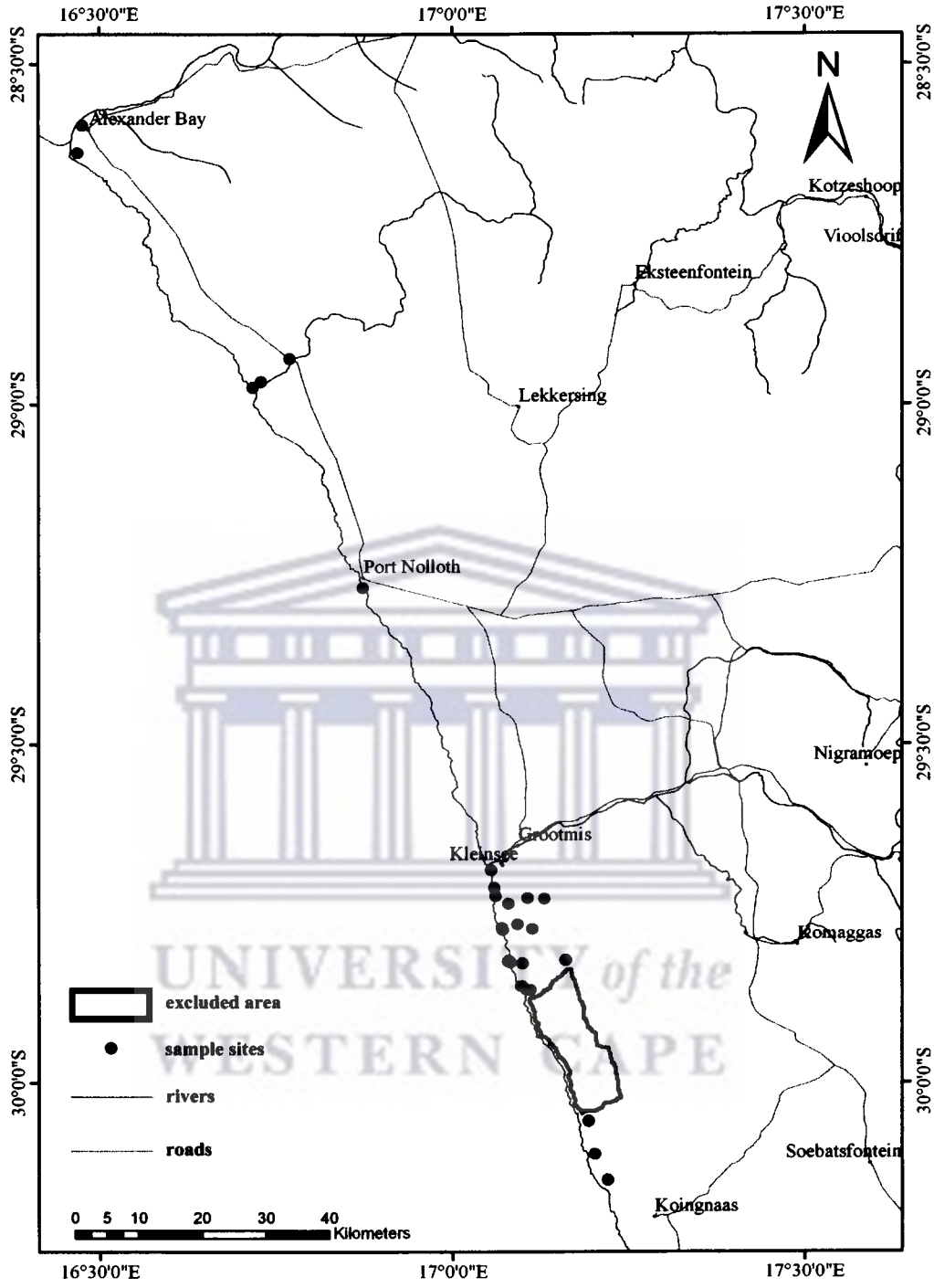

Figure 2.7. Location of the points sampled.

The traps were composed of 5 plastic household buckets, approximately 30 cm in diameter, and 40 cm in depth (10 litres, orange or blue in colour). The drift fences were made of thick, black building plastic, cut to 5 m in length and 25 cm in height. The plastic was stabilised with 8 wooden dowels, 10 mm in

diameter and 45 cm in height. A layer of sand, approximately 5 cm in depth was placed in the base of each bucket to provide shelter to any *Breviceps* spp should a predator, such as a dwarf adder, also fall into the trap. The arrangement of the traps is detailed in Figure 2.8, and a photograph of a trap in situ is depicted in Figure 2.9.

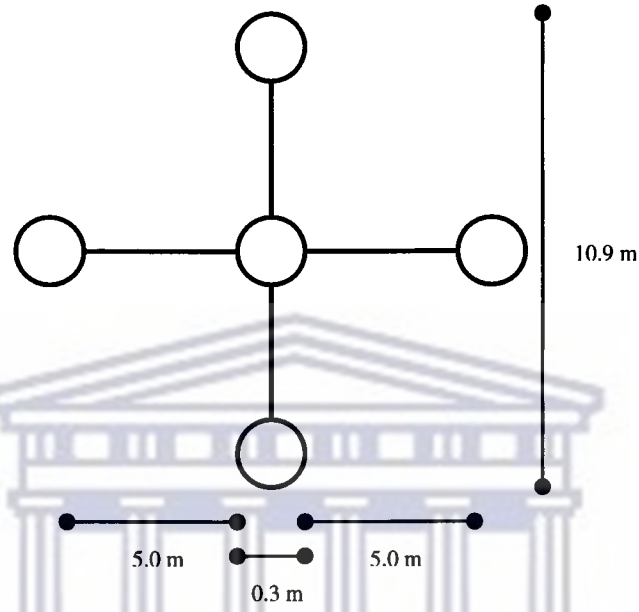

Figure 2.8. Illustration of the arrangement of the pitfall traps.

Figure 2.9. Example of a trap.

2.2.2 Observational Surveys

Observational surveys were defined in person-hours, with one person-hour being the equivalent of one person surveying for one hour, or two people surveying for half an hour. The observations were conducted at night when the temperature was at its lowest, using a 3 D-Cell Mag-LED flashlight, and observers searching the area in a random fashion for individuals as well as tracks. Both the immediate area surrounding each trap site and several areas between transects which adhered to the three habitat assumptions were searched. It must be noted that due to morphological similarities between *B. macrops* and *B. namaquensis*, and the possibility of habitat range overlap, tracks observed could not be reliably attributed to one or the other species. Therefore, a positive identification of *B. macrops* could only be made when a specimen was sighted.

2.2.3 Calibration of Sampling Techniques

Starting in 2002 and continuing through to 2007, Channing conducted a demographic study of the *B. macrops* population at McDougall's Bay (29° 16′ 39.1″ S; 16° 52′ 43.7″ E), 5 km south of Port Nolloth. The aim of the study was to elucidate the density, dispersal ability and reproductive rate of the species using a mark-recapture technique. Individuals were identified by their unique dorsal patterns, and their geographic positions were recorded. Channing found that the population of *B. macrops* in McDougall's Bay, consisted of 885 individuals per hectare, of which 521 were adults (Channing, unpublished). This was considered to be a healthy, viable population and thus chosen for calibration of the methods employed at the primary study site. Utilisation of the pitfall traps at the McDougall's Bay site resulted in a frequency of capture of no less than one *B. macrops* per night per trap. This was calculated by placing the traps out over a period of three nights with each night yielding a single capture. Likewise, observational surveys conducted showed that the observers involved in the study could find *B. macrops* in their natural habitat at a frequency of at least one frog per person every thirty minutes. Thus these results provided a yardstick for measuring the capture and observational success of the methods employed for sampling *B. macrops* south of Kleinzee in the primary study site.

2.2.4 Museum and Literature Records

Museum and literature records from South Africa and Namibia were used to investigate and where possible corroborate the presence of *B. macrops*. Co-ordinates and localities referenced in the Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland for 2004 (RDB 2004) (Minter *et al.*, 2004) and the South African Red Data Book – Reptiles and Amphibians for 1988 (De Villiers, 1988) were verified by direct communications with the original authors of the texts. The primary study site was chosen due to the fact that records indicated that *B. macrops* occurred as far south as Skulpfontein (30° 2′ 21.01″ S; 17° 15′ 7.84″ E), a farm just north of the town of Koiingnaas.

2.2.5 Revised Distribution

In determining the distribution of *B. macrops* the following methods were utilised. First an estimation of the currently recognised distribution, as described in the RDB 2004 (Minter *et al.*, 2004), was defined by drawing a polygon using ArcGIS 9.2 around the distribution space, ArcGIS 9.2 then calculated the exact area of the polygon. This area extended from the coast to the limit of the described range inland, between the South African-Namibian border to the most southerly point of the recognised range. The same method was utilised to calculate the revised distribution once the easterly and southerly limits of *B. macrops* range were shown by this study.

2.3 Environmental Factors

2.3.1 Aeolian Sand Deposits

Using satellite imagery obtained from Google Earth (version 4.3), and GLCF: Earth Science Data Interface, a polygon was drawn to encompass the white aeolian sand deposits along the coast (NASA Landsat Program, 1999, 2000). These polygons were over-laid with ArcGIS 9.2 to see if there was a correlation with the distribution of *B. macrops*.

2.3.2 Vegetation

Data sets were taken from shape files provided in Mucina *et al.* (2006). These shape files were over-laid using ArcGIS 9.2 in order to investigate the possibility of a relationship with the distribution of *B. macrops*.

2.3.3 Soil Analyses

2.3.3.1 Moisture Content

At each trap site and at every point where a *B. macrops* specimen was found 3-5 samples of soil were collected at a depth of approximately 15 cm from the surface. After removing the lids, the samples were weighed in the plastic jar they were collected in. The average weight of the jars was measured using 15 jars, and this average was subtracted from every weight recorded. They were then oven dried at 80°C for a minimum of 24 hrs, and the weights were recorded again. The percentage change in weight was recorded as the moisture content of each sample according to the equation below:

$$\frac{\text{(Initial weight – Dry weight)}}{\text{Initial weight}} \times 100 = \%\ \text{moisture content}$$

2.3.3.2 pH

Each sand sample was mixed with distilled water to its 'sticky point' as defined in the Agricultural Handbook No. 60 (Allison *et al,* 1969). From this mixture both the pH and the salinity of the samples were measured. Once mixed with distilled water, each sample was left for a minimum of fifteen minutes to allow the salts and minerals to dissolve into the water. The pH was measured first, using a PHM64 Research pH Meter (Radiometer, Copenhagen, NV).

2.3.3.3 Salinity

Salinity was calculated by measuring the resistance of each sample using a YSI Model 35 Conductance Meter. The results were inverted into conductance, which were then converted to a measurement of salinity using the graph shown in Figure 2.10.

2.3.3.4 Colour and Texture

The colour of the samples was assessed on the basis of whether they fell into three basic visual categories: white, red or pale. These categories were based upon a comparison between the samples themselves within the laboratory.

The size of the sand grains was categorised by the texture of the sand being soft, coarse or intermediate. These categories were based upon a subjective comparison between the sample sites themselves whilst in the field.

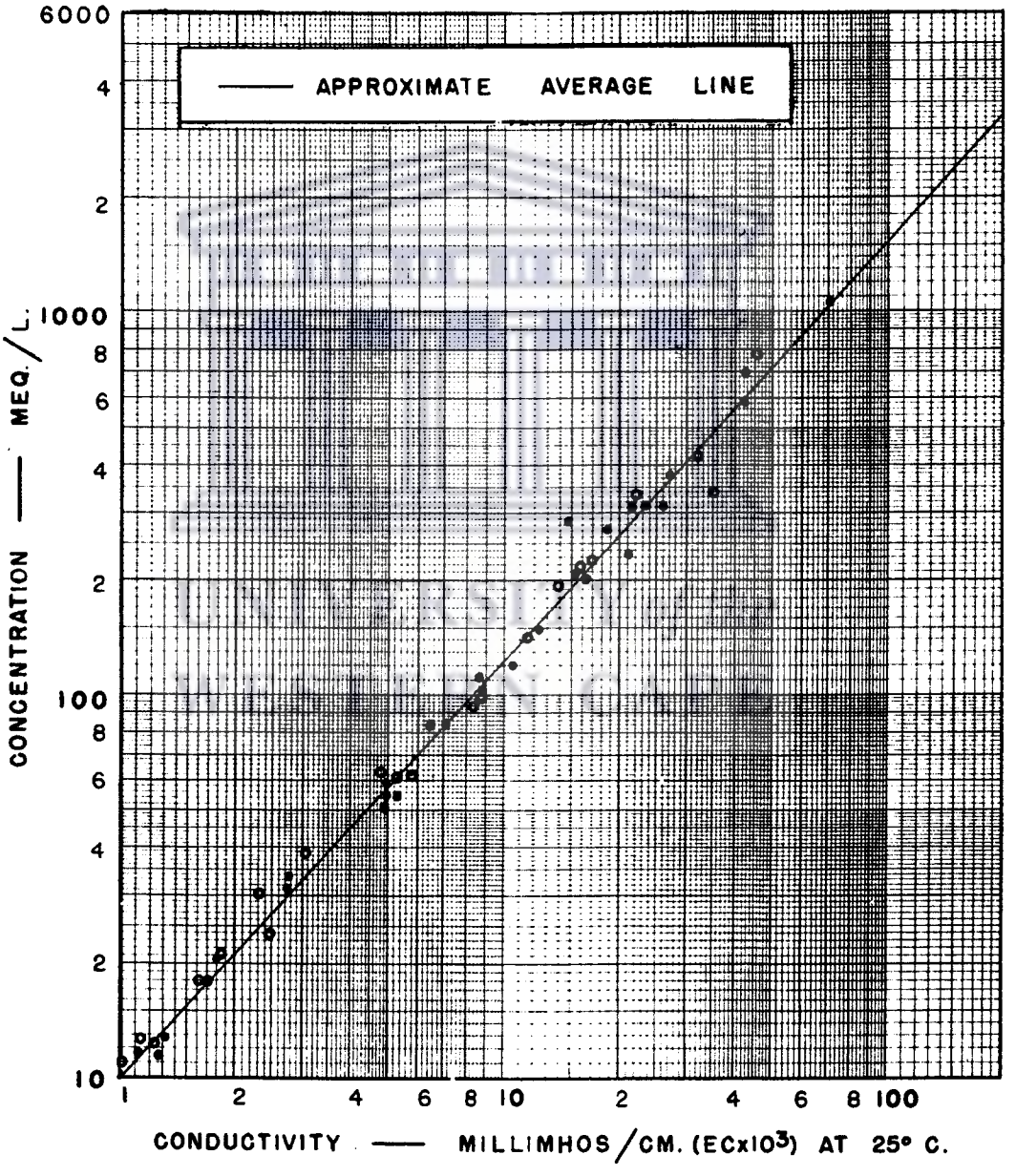

Figure 2.10. Concentration of saturation extracts of soils in milliequivalents per litre as related to electrical conductivity, taken from Allison *et al* (1969).

2.3.4 Remaining Undisturbed Habitat

The amount of undisturbed habitat remaining to the frogs was determined using Google Earth (version 4.3) by excluding areas that were totally transformed by strip mining for alluvial diamonds. These areas contain no suitable *B. macrops* habitat. Polygons were superimposed over the areas that have not been mined, and then transferred to ArcGIS 9.2 for digitizing and analysis.

2.4 Molecular Data

2.4.1 Sampling Strategy

A minimum of two individuals was sampled for genetic analysis from each of the study sites. Two specimens were collected south of Kleinzee and three at M^{c}Dougall's Bay. The specimens used to represent the Holgat study site were caught during observational surveys. In all cases Dr. Alan Channing handled and processed the specimens.

2.4.2 DNA Extraction

Each specimen was anaesthetised in MS222 (tricaine methane sulphonate) until a pinch-test of the toes indicated that it was dead. The left thigh muscle was used for genetic analysis, which was dissected and stored in analytical grade 100% ethanol. The specimen was then fixed in 10% formaldehyde for 12-24 hours, rinsed in 70% ethanol, and preserved in 70% ethanol.

DNA was extracted by incubating each tissue sample at 55°C in 0.5 ml extraction buffer (SDS 0.5%; 50 mM Tris; 0.4 M EDTA, pH 8.0) and a minimum of 20 µl proteinase K (0.1%) until no solid was visible in the solution. 0.5 ml PCI (phenol 25:chloroform 24:isoamyl alcohol 1) was added and the mixture shaken. It was then centrifuged at 5000 x g for 10 minutes. The supernatant was removed and shaken with 0.5 ml CI (chloroform 24:isoamyl alcohol 1). This mixture was centrifuged at 5000 x g for 10 minutes. The supernatant was removed and added to 45 µl 3M sodium acetate and 650 µl ice cold 100% ethanol, and incubated in a deep freeze overnight. The solution was then centrifuged at 13000 x g for 10 min.

The supernatant was removed and discarded, and the DNA pellet was air-dried for a minimum of 20 minutes. The pellet was finally resuspended in 50 µl TE buffer (0.12 g Tris (10 mM); 0.037 g EDTA (1 mM); 100 ml H_2O (pH 8.0). The concentrations of these final solutions of DNA were measured using a fluorometer (Invitrogen).

2.4.3 Polymerase Chain Reaction (PCR)

A fragment of the 16S gene was amplified using the polymerase chain reaction (PCR). The concentration of a working solution for PCR was made up to 2 $ng.\mu l^{-1}$ (DNA: template) using appropriate amounts of TE buffer (as detailed above). Each PCR solution had a final volume of 25 µl containing:

Primer 16Sa	1.0 µl
Primer 16Sb	1.0 µl
ReadyMix™	12.5 µl
Water	6.5 µl
Template	4.0 µl

A negative control, containing no tissue extract but distilled water in place of the template, was included for each PCR set that was run.

PCR reactions were performed using a Techne TC-512 thermocycler, with the following parameters: an initial denaturation at 95°C for 4 minutes; followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 90 seconds; and a final extension at 72°C for 5 minutes.

The PCR products underwent electrophoresis on a 0.8% agarose gel with ethidium bromide for 15 minutes at 90 V.

2.4.4 Sequencing and Analysis

All PCR products were sent to MacroGen, a Korean commercial company, for sequencing. Sequences were then edited and aligned using the software packages CLC Sequence Viewer (Version 5.1.1) and 4 Peaks (Version 1.7.2) respectively. Genetic distances were measured using the software package PAUP (Version 4.10b).

CHAPTER 3: RESULTS

3.1 Distribution

3.1.1 Pitfall Traps

In total the pitfall traps sampled 576 trap-hours over a six-month period (see Table 3.1), during which time one *B. macrops* and one *B. namaquensis* were caught. The *B. macrops* was caught during the sampling of the first transect, on the 13th May 2008, 130 m inland of Kleinzee Beach, 9 m in altitude (29° 41′ 01.6″ S; 17° 03′ 16.1″ E). This individual fell into the trap on the third night that the trap was out. Subsequently no further individuals were captured despite the placing of traps in what was deemed to be good *B. macrops* habitat. The *B. namaquensis* was caught in a trap 15 km south of Kleinzee town, along Transect 4 (29° 49′ 17.7″ S; 17° 06′ 11.7″ E), on the 9th July 2008, 2300 m from the coast, 49 m in altitude on the first night of trapping.

Table 3.1. Cumulative time spent trapping for frogs in transects south of Kleinzee, assuming that frogs in general are active for eight hours a night, and therefore one trap-night is equivalent to eight trap-hours.

Transect No.	Distance from Coast (m)	Time Out (nights)	Active Trap Hours	Cumulative Hours
1	130	6	48	48
2	133	3	24	72
2	130	3	24	96
2	2000	3	24	120
2	4500	3	24	144
2	6800	3	24	168
2	8000	3	24	192
3	235	3	24	216
3	116	3	24	240
3	2350	3	24	264
3	2340	3	24	288
3	4330	3	24	312
3	4340	3	24	336
4	93	3	24	360
4	153	3	24	384
4	270	3	24	408
4	2250	3	24	432

Transect No.	Distance from Coast (m)	Time Out (nights)	Active Trap Hours	Cumulative Hours
4	7880	3	24	456
4	7900	3	24	480
5	117	3	24	504
5	122	3	24	528
5	400	3	24	552
5	1000	3	24	576

3.1.2 Observational Surveys

During the six-month field work period, 47 person-hours were spent on observational surveys between Kleinzee and Koiingnaas, the details of which are listed in Table 3.2.

Table 3.2. Amount of person-hours spent on observational surveys.

Night of - Date	Transect No.	Distance from coast (m)	No. Persons	Walked (min)	Overall Hours
22.6.08	2	6800	2	30	1
22.6.08	2	8000	2	30	2
23.6.08	2	130	2	40	3.33
23.6.08	2	2000	2	30	4.33
23.6.08	2	4500	2	20	5
24.6.08	3	235	2	30	6
24.6.08	3	116	2	30	7
25.6.08	1	130	2	40	8.33
8.7.08	1-2	110	2	60	10.33
9.7.08	4	93	2	15	10.83
9.7.08	4	2250	2	30	11.83
10.7.08	2	130	2	45	13.33
28.7.08	5	117	2	30	14.33
28.7.08	5	122	2	30	15.33
14.8.08	11	50	2	60	17.33
15.8.08	12	70	2	150	22.33
16.8.08	11	50	2	60	24.33
16.8.08	12	70	2	90	27.33
17.8.08	12	70	2	120	31.33
6.9.08	1-2	30	3	60	34.33
6.9.08	1-2	110	3	90	38.83
7.9.08	2	130	3	120	44.83
8.9.08	3	235	3	30	46.33
8.9.08	2-3	200	3	15	47.08

Transects began on the southern side of Kleinzee at the beach. In total only two *B. macrops* were found within the primary study site. The first specimen was caught on the 8th July 2008, slightly inland of the Kleinzee Angling Club, 2 km south of Kleinzee town, between Transects 1 and 2 (29° 42′ 32.1″ S; 17° 03′ 31.8″ E), 259 m from the coast, 12 m in altitude. This individual was found in two hours with two people searching the dune, representing one frog in four person-hours. The second *B. macrops* was found in the same dune after 90 minutes of three people searching, representing one frog in four and a half person-hours. The sampling proceeded south from this point up to 60 km south of Kleinzee town with no further success at finding any more *B. macrops*. A number of *Breviceps* tracks were noted at various locations, however, a visual confirmation of the species was not made. The first track was spotted 2 km south of Kleinzee, between Transects 1 and 2 (29° 41′ 01.6″ S; 17° 03′ 16.1″E) on the 12th May 2008, but due to location and track width, they were assumed to have been made by the same, first individual that was caught in the trap the following day. The next tracks were spotted 5 km south of Kleinzee, on Transect 2 (29° 43′ 32.5″ S; 17° 06′ 22.4″ E), 4500 m from the coast, 96 m in altitude, on the 1st June 2008, however, no individual was seen, and so no positive identification could be made. Several tracks were seen and followed next to the coast 55 km south of Kleinzee, on Transect 11 (30° 09′ 27.5″ S; 17° 13′ 09.9″ E) on the 15th August 2008. These tracks were approximately 50 m from the coast and 20 m in altitude. However, again no individuals were found.

In addition to the study undertaken between Kleinzee and Koiingnaas, genetic samples were collected at previously known locations. Preliminary investigations revealed a healthy population of *B. macrops* at McDougall's Bay, 5 km south of Port Nolloth (29° 16′ 52.9″ S; 16° 52′ 49.7″ E), 50 m from the coast and 11 m in altitude. Tracks of *Breviceps* were also found slightly north of the town of Port Nolloth 1000 m from the coast and 35 m in altitude (29° 13′ 38.0″ S; 16° 51′ 30.1″ E). Although no specimens were found during this study, positive identification by a reliable source (pers. comm. Van Wyk, 2008) suggests that *B. macrops* occurs in the town of Alexander Bay (approximately 28° 36′ 13.5″ S; 16° 28′ 55.5″ E), 4-6 km inland and up to 30 m in altitude. Observational surveys

were also carried out along the Holgat River. Two *B. macrops* were found at the mouth of the river, in the dunes on the northern side (28° 58′ 30.8″ S; 16° 43′ 00.2″ E), 130 m from the coast and 46 m in altitude, within 15 minutes of three people searching, representing 0.75 person hours. A third *B. macrops* was found at Daberas, a soft, pale dune, on the northern side of the Holgat River (28° 58′ 00.8″ S; 16° 43′ 42.6″ E), 1530 m from the coast and 12 m in altitude, within 10 minutes of 3 people searching, representing 0.5 person hours. Further inland, on the northern side of the Holgat River, one *B. namaquensis* was found on relatively hard, red sand, 7000 m from the coast and 112 m in altitude (28° 55′ 58.4″ S; 16° 46′ 08.9″ E).

The map in Figure 3.1 illustrates the locations in which all individual frogs were caught throughout the project; Table 3.3 lists information concerning these locations.

3.1.3 Museum and Literature Records

The following museums were approached to investigate existing records of *B. macrops*:

- The State Museum, Windhoek, Namibia.....1 specimen
- National Flagship Institution, Pretoria, South Africa.....18 specimens
- Durban Natural History Museum, Durban, South Africa..... 1 specimen
- Natal Museum, Pietermaritzburg, South Africa..... 0 specimens
- M^{c}Gregor Museum, Kimberley, South Africa.....0 specimens
- Iziko (South African Museum), Cape Town, South Africa..... 8 specimens

Where records existed the specimens were examined and identification verified. The details of these records are shown in Table 3.4. Figure 3.2 compares the locations at which *B. macrops* have been found during the current study in conjunction with previous records obtained from museums and literature. The plotting of these points relies on quarter-degree-square geographic references, which is accurate to within approximately 30 km.

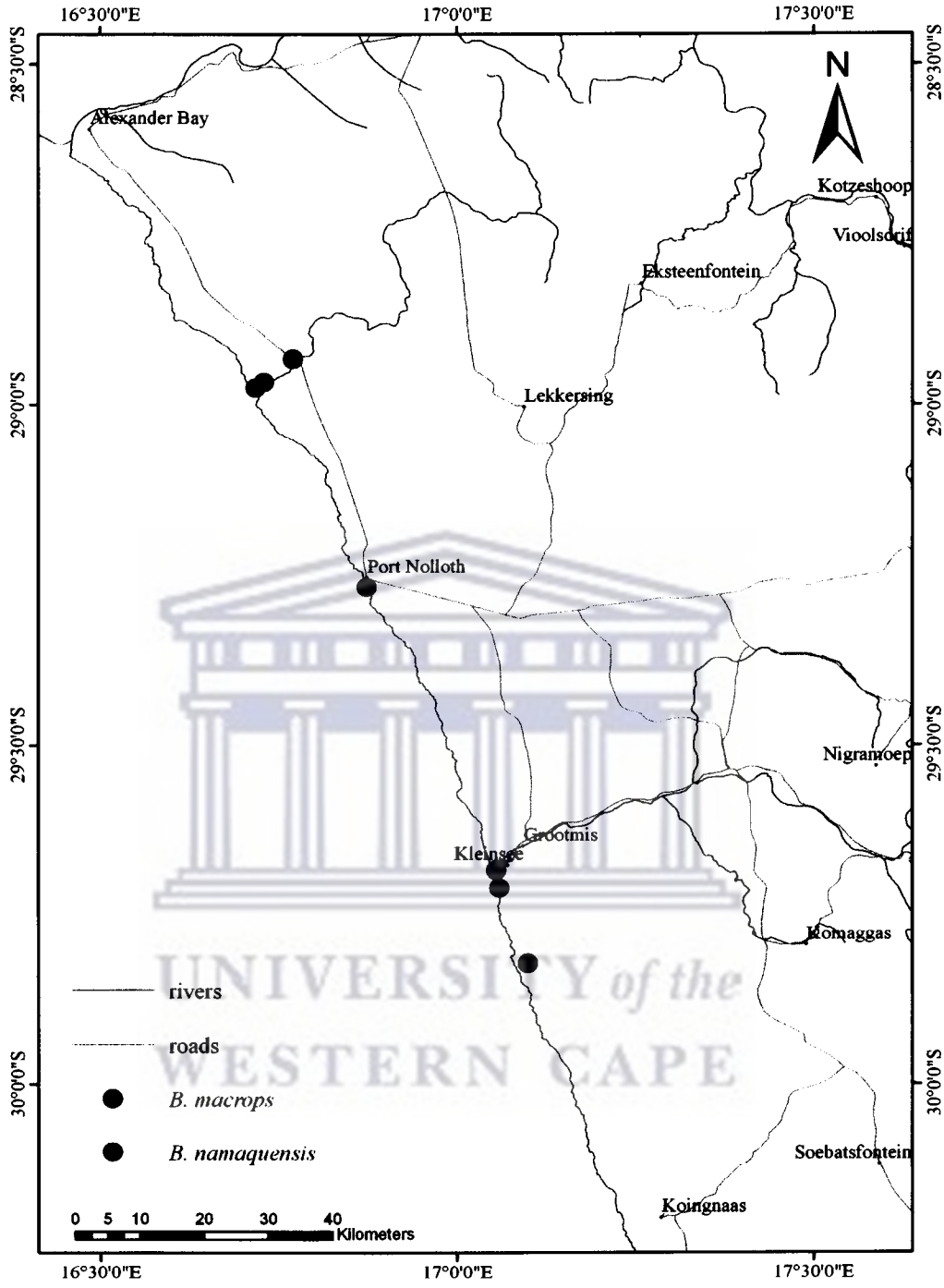

Figure 3.1. Locations at which frogs were found.

Table 3.3. List of the location and conditions of the frog capture sites.

Species	Date	Location	Coordinates		Sand:	Distance
			S	E	Colour, Texture	from coast (m)
B. macrops	27.3.08	M^{c}Dougall's Bay	29.28689	16.88496	White, soft	628
B. macrops	16.5.08	Kleinzee	29.68378	17.05447	White, soft	130
B. macrops	6.9.08	Kleinzee	29.70891	17.05884	White, soft	110
B. macrops	8.7.09	Kleinzee	29.70891	17.05884	White soft	110
B. namaquensis	9.7.08	Brazil Farm	29.82157	17.10326	Pale, soft	2250
B. macrops	10.09.08	McDougall's Bay	29.26922	16.87276	White, soft	15
B. macrops	10.09.08	McDougall's Bay	29.26922	16.87276	White, soft	15
B. macrops	16.10.08	Holgat River mouth	28.97523	16.71672	Pale, soft	130
B. macrops	16.10.08	Holgat River mouth	28.97523	16.71672	Pale, soft	130
B. macrops	16.10.08	Holgat, 1.5 km inland	28.96688	16.72851	Pale, firm	1920
B. namaquensis	16.10.08	Holgat gate	28.93290	16.76915	Red, hard	7330

Table 3.4. Distribution records of B. macrops obtained from museums and literature. NFI: National Flagship Institute, Pretoria; SM: State Museum, Windhoek; SAM: South African Museum (Iziko), Cape Town; DNH: Durban Natural History Museum, Durban; RDB 1988: The Red Data Book of 1988.

Specimen No.	Museum	Locality	District	Country	Grid Reference.	Lat. (S)	Long. (E)	Date	Collector
25717	SM	Boegoeberg	Luderitz	Namibia	2715 Dd	27 54′	15 56′	20/08/1983	J. Irish
9425	SAM		Namaqualand	South Africa				16/03/1906	FC Kolbe
9426	SAM		Namaqualand	South Africa				16/03/1906	FC Kolbe
9503	SAM		Namaqualand	South Africa				0/12/1906	FC Kolbe
9505	SAM		Namaqualand	South Africa				0/12/1906	FC Kolbe
12208	SAM		Port Nolloth	South Africa	2916 Bd			0/0/1912	CL Biden
12209	SAM		Port Nolloth	South Africa	2916 Bd			0/0/1912	CL Biden
13572	NFI	Alexander Bay	Namaqualand	South Africa				0/12/1928	IB Cilliers
17985	NFI		Port Nolloth	South Africa				20/08/1937	V FitzSimons
17986	NFI		Port Nolloth	South Africa				20/08/1938	V FitzSimons
17987	NFI		Port Nolloth	South Africa				20/08/1939	V FitzSimons
17988	NFI		Port Nolloth	South Africa				20/08/1940	V FitzSimons
17989	NFI		Port Nolloth	South Africa				20/08/1941	V FitzSimons
17990	NFI		Port Nolloth	South Africa				20/08/1942	V FitzSimons
17991	NFI		Port Nolloth	South Africa				20/08/1943	V FitzSimons
34102	NFI		Port Nolloth	South Africa				18/09/1967	WD Haacke
34103	NFI		Port Nolloth	South Africa				18/09/1967	WD Haacke
34104	NFI		Port Nolloth	South Africa				18/09/1967	HD Brown
34105	NFI		Port Nolloth	South Africa				18/09/1967	HD Brown
34106	NFI		Port Nolloth	South Africa				18/09/1967	HD Brown
34107	NFI		Port Nolloth	South Africa				18/09/1967	HD Brown
34200	NFI	Daberas, Holgat	Richtersveld	South Africa				25/09/1967	WD Haacke

Specimen No.	Museum	Locality	District	Country	Grid Reference.	Lat. (S)	Long. (E)	Date	Collector
34201	NFI	Daberas, Holgat	Richtersveld	South Africa				25/09/1967	WD Haacke
33977	NFI	Daberas, Holgat	Richtersveld	South Africa		28 52´	16 56´	26/09/1967	WD Haacke
33979	NFI	Daberas, Holgat	Richtersveld	South Africa		28 52´	16 56´	25/09/1967	WD Haacke
196	DNH	Port Nolloth		South Africa				0/06/1962	WJ Lawson
	RDB 1988	Port Nolloth		South Africa	2916 Bd				
	RDB 1988	Cliffs		South Africa	2916 Bb				
	RDB 1988	Daberas, Holgat		South Africa	2816 Dd				
	RDB 1988	Grootderm	Alexander Bay	South Africa	2816 Da			0/0/1964	Poynton
	RDB 1988	Kleinzee		South Africa	2917 Ca			0/0/1987	Channing & Van Wyk
	RDB 1988		Oranjemund	Namibia	2715 Dd			0/0/1987	Berger-Dell'mour

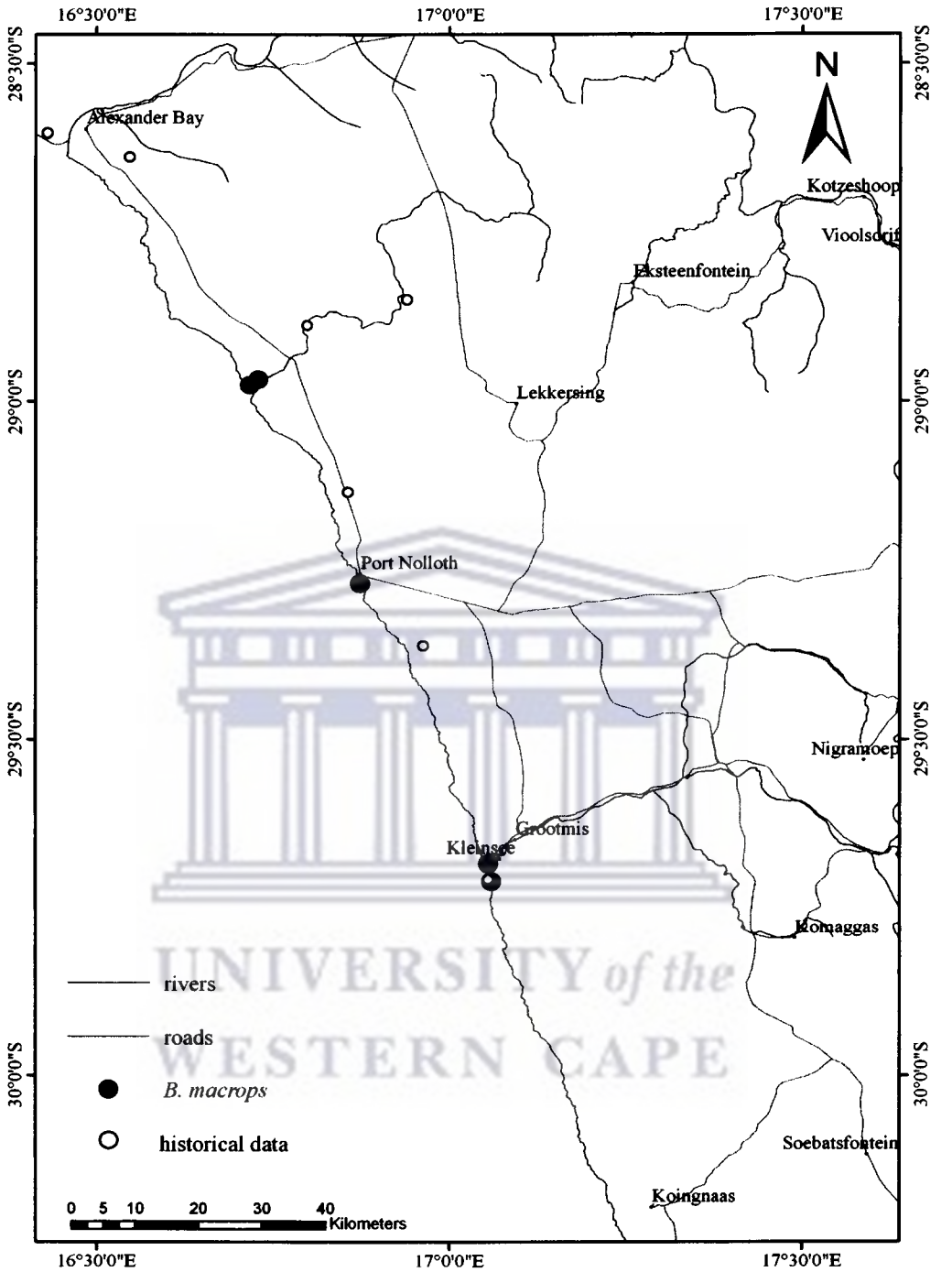

Figure 3.2. Location of all sites at which *B. macrops* has been found in the study with historical locations overlayed. All ambiguous or generalised location data have been omitted.

3.1.4 Revised Distribution

The most southerly point where *B. macrops* was found during this study was 2 km south of Kleinzee town, between Transects 1 and 2 (29° 42´ 32.1˝ S; 17° 03´ 31.8˝ E). The RDB 2004 (Minter, *et al.*, 2004) reports that *B. macrops* was recorded within the farm Skulpfontein (3017Aa/Ab). A specimen from this location is held at the National Flagship Institute, Pretoria, listed as No. 69964. However, it is currently labelled as *B. namaquensis* (pers. comm. Haacke, 2009). In addition, photographs, provided by the collector, Wulf Haacke, do not provide a clear identification of the specimen, as it is necessary to view the hind feet for a positive identification. Therefore, for the purpose of this study this record is assumed to be that of *B. namaquesis*. The most inland specimen collected in this study was 1.5 km inland, adjacent to the Holgat River. Mr Pieter van Wyk (pers. comm., 2008) has recorded an individual within the town of Alexander Bay, between 4.5 km to 6 km inland (28° 36´ 13.5˝ S; 16° 28´ 55.5˝ E). The reference in the RDB 2004 (Minter *et al.*, 2004) to the occurrence of *B. macrops* up to 10 km from the coast, has been refuted by the original observer (Prof S. Hanrahan pers. comm. via Channing, 2008).

There have been several references to the occurrence of *B. macrops* within Namibia. However, The State Museum in Windhoek holds only one specimen (SM-25717), which was collected at the Boegoeberg, in the Lüderitz District (27° 54´ S; 15° 56´ E). Its authenticity was verified in this study.

Figure 3.3 compares the difference between the *B. macrops* distribution currently recognised, estimated at 1239.48 km^2, with the revised distribution as shown by this study, estimated at 841.85 km^2.

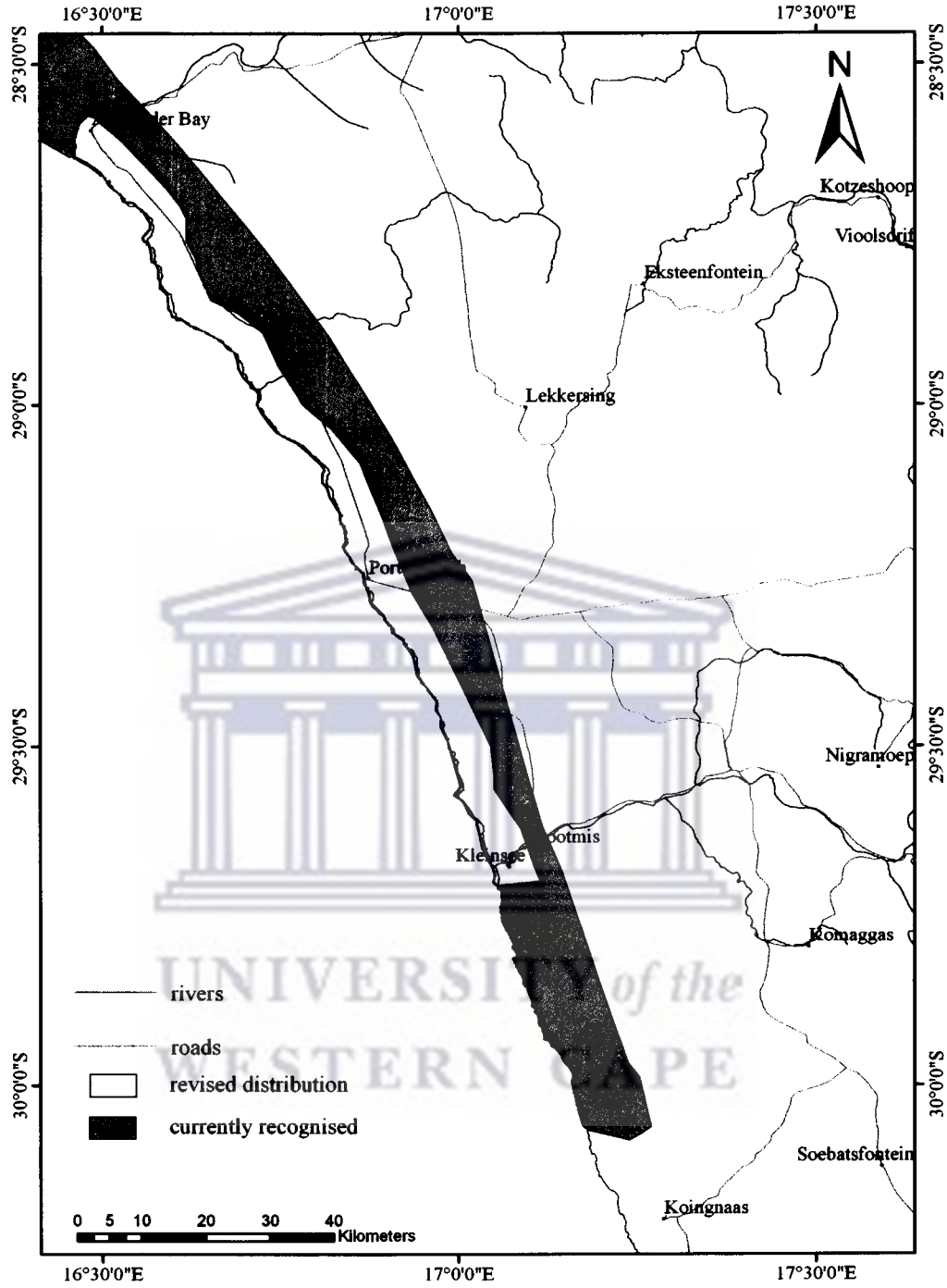

Figure 3.3. Comparison between the currently recognised distribution of *B. macrops* and the revised distribution as estimated by this study.

3.2 Environmental Factors

3.2.1 Aeolian Sand Deposits

All of the *B. macrops* found in this study were on soft sand, white or pale in colour, which could suggest that they occur only on aeolian sand deposits (Figure 3.4). The majority of the historical records are also located on the aeolian sand deposits, however, not exclusively. This could be due to the wide margin of error in the grid references of these historical records.

3.2.2 Vegetation

The known locations of *B. macrops* are shown in relation to the different vegetation types across the study area in Figure 3.5. All *B. macrops* found during this study were within SKs 1 and SKs 8, Richtersveld Coastal Duneveld and Namaqualand Coastal Duneveld respectively. SKs 1 is a 1-12 km wide strip, along the coast, from between Boegoe Twins and Alexander Bay to between Port Nolloth and Kleinzee. SKs 8 is composed of coastal plains on or near the sea, from just south of Port Nolloth to south of the Groen River mouth. Historical recordings obtained during the study are located in several other vegetation types. However, again, some may be due to the ambiguity of the grid references of the historical records. Notably SKs 1 closely matches the white sands north of Kleinzee with a more patchy distribution south of this town. This could indicate that the obvious correlation between vegetation and soil type might also influence the distribution pattern of *B.macrops*.

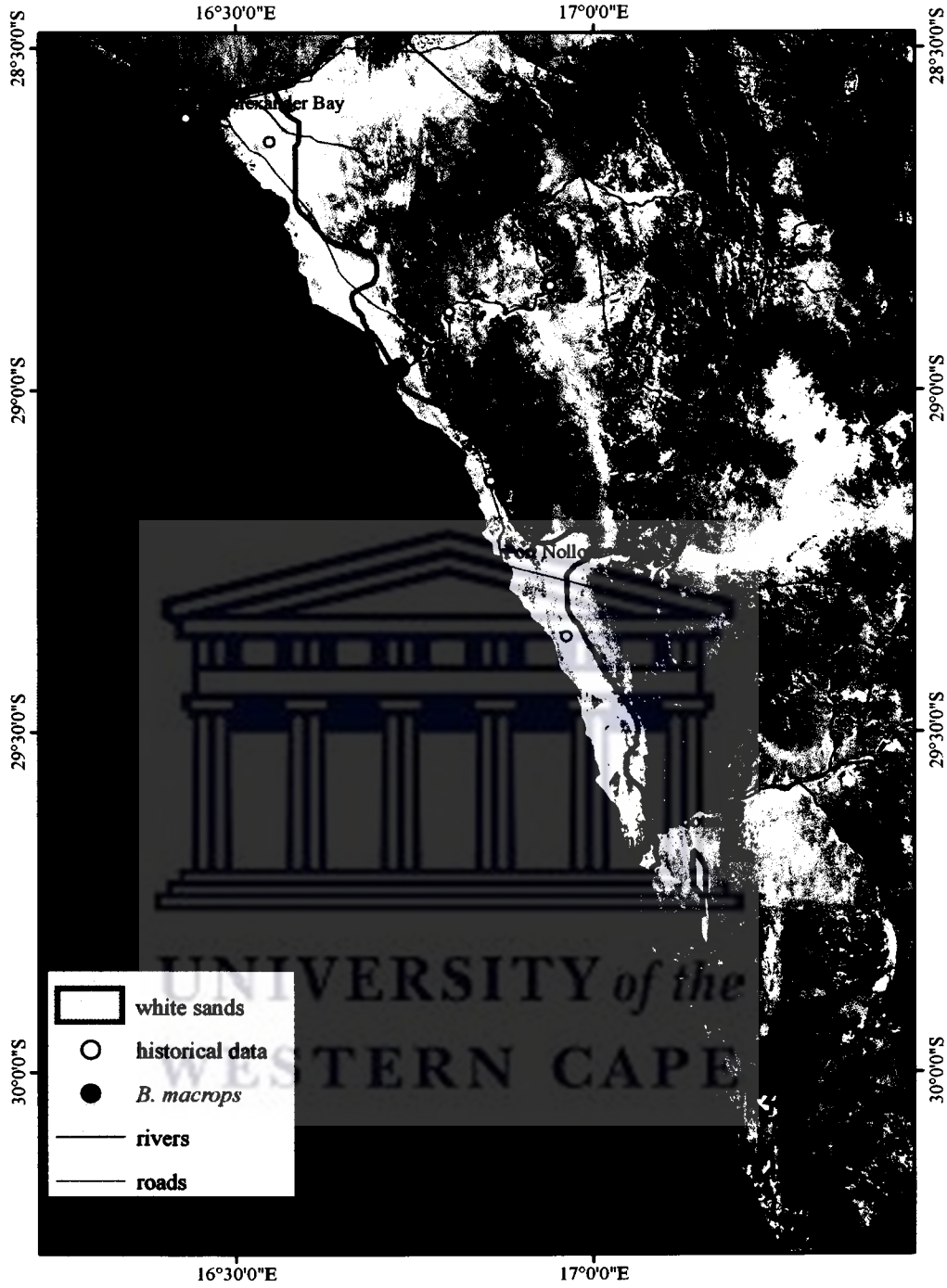

Figure 3.4. Locations in which *B. macrops* has been found in relation to aeolian sand deposits.

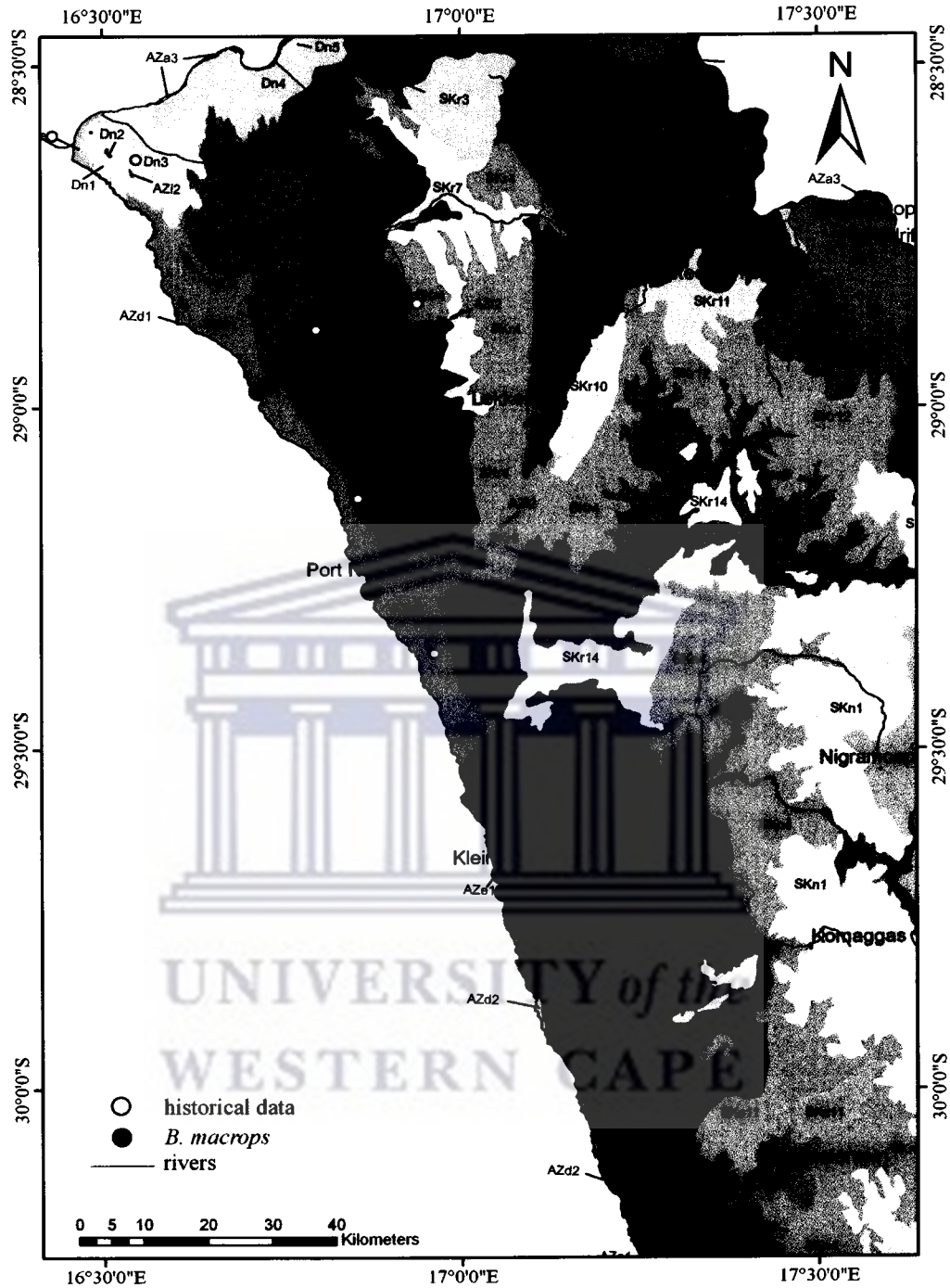

Figure 3.5. Main vegetation types of the Sandveld, showing locations at which *B. macrops* has been found. This vegetation data set is taken from Mucina *et al.* (2006).

3.2.3 Soil Analyses

3.2.3.1 Moisture Content

All *B. macrops* found were located on dry sand, as Table 3.5 indicates. In no cases were the frogs found on particularly moist sand, however not all pale, dry sand investigated revealed a frog population. It was observed that during and after rain the sand approximately 15 cm from the surface maintained a relatively constant level of moisture, i.e. the rain did not appear to percolate down further than 15 cm. Nearer the coast and on the white sands the percolation appeared to be even less.

Table 3.5. Table showing soil moisture (%) as defined by the following location categories: 1) where *B. macrops* were found, 2) where *B. namaquensis* were found, 3) where no frogs were found, but the soil was similar in either colour or texture to 1) (white or pale sand), and 4) where no frogs were found and the soil was not similar in either colour or texture to 1) (red sand).

Moisture Content (%) / Location category	0-1	1-2	2-3	3-4	4-5
1	5	0	0	0	0
2	0	1	0	0	0
3	8	4	1	0	0
4	4	0	1	0	2

3.2.3.2 pH

As can be seen in Table 3.6 there were too few samples analysed where frogs were found to isolate any significant correlations. In addition, where there were sufficient data (no frogs, white and pale sand; no frogs red sand) the distribution of data points is very similar to a neutral pH range.

Table 3.6. Table showing soil acidity (pH) as defined by the following location categories: 1) where *B. macrops* was found, 2) where *B. namaquensis* was found, 3) where no frogs were found and the sand was similar in colour to 1) (white or pale sand), and 4) no frogs were found and the sand was different in colour to 1) (red sand).

Acidity (pH) / Location category	0-4	4-5	5-6	6-7	7-8	8-9
1	0	0	0	0	1	4
2	0	0	0	1	0	0
3	0	1	1	2	8	1
4	0	2	0	2	3	0

3.2.3.3 Salinity

Similarly to the analysis of the soil acidity, and as can be seen in Table 3.7 very few samples were analysed, therefore no conclusions can be draw from this data set. Where there were sufficient data (no frogs, white and pale sand; no frogs red sand), the distribution of data points illustrates a wide range of salinity concentrations.

Table 3.7. Soil salinity (milli-Equivalents per litre) as defined by the following location categories: 1) *B. macrops* was found, 2) *B. namaquensis* was found, 3) no frogs were found and the sand was similar in colour to the locations to 1) (white or pale sand), and 4) no frogs were found and the sand was different in colour from 1) (red sand).

Salinity ($mEq.l^{-1}$) / Location category	0-20	20-40	40-60	60-80	80-100	100-120	120-140
1	3	1	0	1	0	0	0
2	0	0	0	0	0	0	1
3	1	2	2	5	2	1	0
4	1	0	1	1	2	1	1

3.2.3.4 Colour and Texture

All of the *B. macrops* were found in white or pale sand, none were found on red sand. In comparison, *B. namaquensis* was not found on white sand, but in pale and red sand.

3.2.4 Remaining Undisturbed Habitat

Figure 3.6 gives a basic idea of the areas in which *B. macrops* has been reported to occur (distribution according to RDB, 2004), the areas in which *B. macrops* is likely to occur according to the current study (revised distribution), and the area of remaining undisturbed habitat once mining has been taken into account (undisturbed habitat) (Table 3.8). These areas were estimated using Google Earth, version 4.3. A total of 270.72 km^2 of undisturbed habitat remains to the frogs. Of the currently recognised distribution (1239.48 km^2) and the revised distribution (841.85 km^2) this represents 21.84% and 32.16% respectively.

Table 3.8. The size of the previously recorded distribution of *B. macrops*, the revised distribution and the sizes of the remaining fragments of undisturbed habitat.

Area	Polygon	Km^2
Study Area	1	531.04
Revised distribution	2	841.85
Undisturbed habitat	3	20.04
Undisturbed habitat	4	28.44
Undisturbed habitat	5	12.85
Undisturbed habitat	6	196.84
Undisturbed habitat	7	12.55
Distribution according to RDB, 2004	8	1239.48
Total Undisturbed habitat	-	270.72

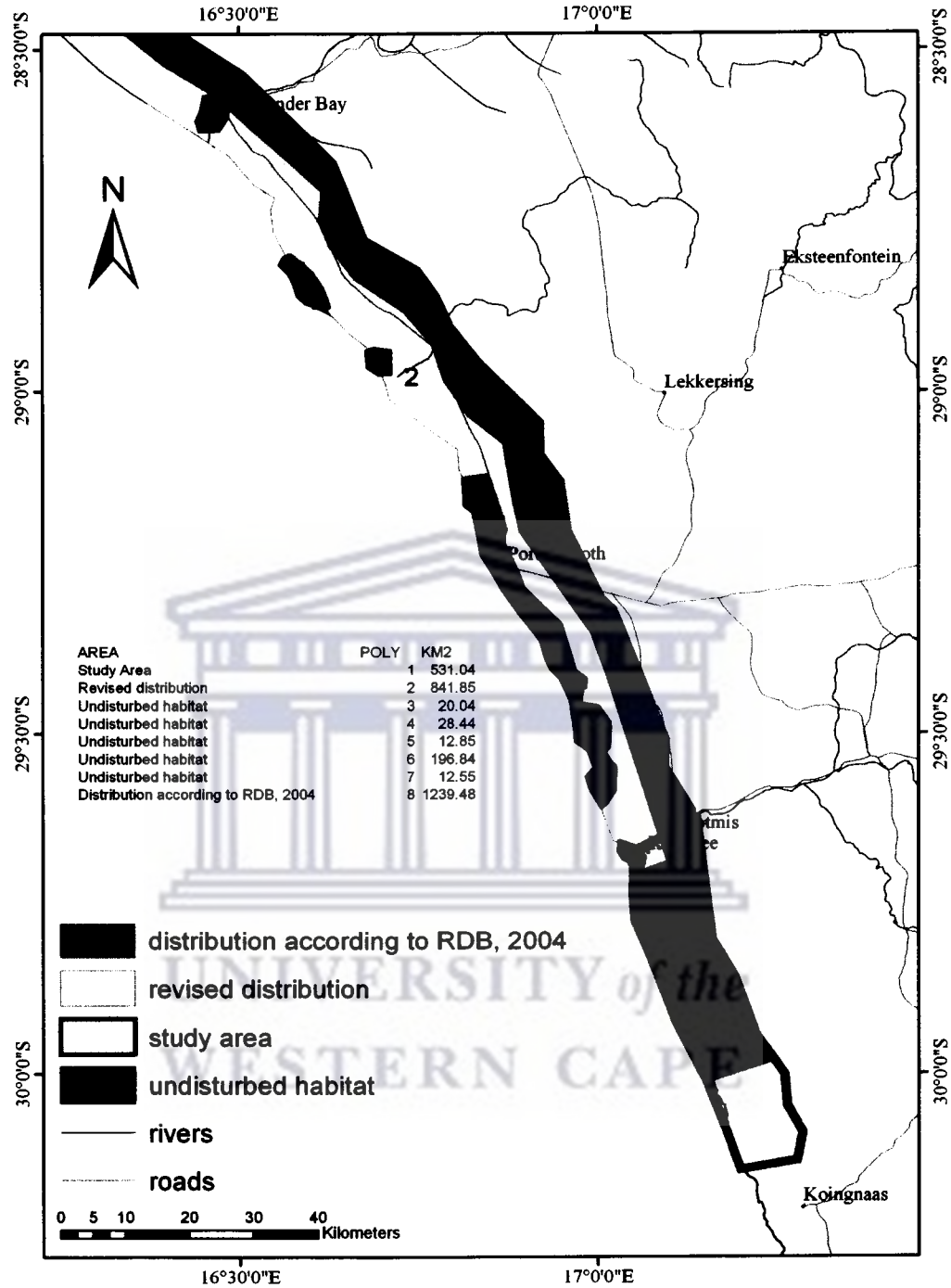

Figure 3.6. The distribution of *B. macrops* according to previous records (Brown), the results of the present study (Beige), and remaining undisturbed habitat within this area (Red).

3.3 Molecular Data

Ten *B. macrops* specimens were collected in the study area for genetic analysis. These included one specimen from Kleinzee Beach, immediately south of the town, and one specimen from a dune 2 km south of the town. Three specimens were collected from M^{c}Dougall's Bay. Two specimens were collected from the mouth of the Holgat River, and one specimen from the Daberas dune 1.5 km inland along the Holgat River. In addition, two *B. namaquensis* specimens were also retained for genetic analysis: one specimen from the farm, Brazil, and one specimen 150 km north from the Holgat River area.

3.3.1 Genetic Differences

There was no genetic variation in the 16s gene fragment in the *B. macrops* samples in this study. The complete sequences are shown in Appendix C. However, there was an average of 4.7% genetic variation in the 16s gene between the *B. namaquensis* and *B. macrops* populations (Table 3.8). The GenBank accession number for the 16s gene fragment sequenced will be included in publications resulting from this work.

Table 3.8. Genetic differences (as percentages) for a 16S gene fragment between all *Breviceps* specimens in this study. All individuals are *B. macrops* specimens except those labelled 'nama', which denotes the species *B. namaquensis*. Specimen 1: Individuals found along the Holgat river, 1.5 km from the coast; Specimens 2, 6 and 7: Individuals caught at M[c] Dougall's Bay in a dune next to the coast; Specimens 3 and 4: Individuals found at the mouth of the Holgat river; Specimen 5: *B. namaquensis* found along the Holgat river, at the gate, 7 km from the coast; Specimens 8 and 9: Individuals found near Kleinzee, one immediately south of the town and the other 2 km south of the town; Specimen 10: *B. namaquensis* found on the farm, Brazil, 2.3 km from the coast.

	1	2	3	4	5	6	7	8	9
1 Holgat, 1.5 inland	-								
2 McDougall's Bay	0	-							
3 Holgat River mouth	0	0	-						
4 Holgat River mouth	0	0	0	-					
5 Holgat Gate 'nama'	4.7	4.7	4.7	4.7	-				
6 McDougall's Bay	0	0	0	0	4.7	-			
7 McDougall's Bay	0	0	0	0	4.7		-		
8 Kleinzee	0	0	0	0	4.7	0	0	-	
9 Kleinzee	0	0	0	0	4.7	0	0	0	-
10 Brazil 'nama'	5.1	5.1	5.1	5.1	4.2	5.1	5.1	5.1	5.1

CHAPTER 4: DISCUSSION

4.1 What is the distribution of *B. macrops*?

B. macrops is reported as occurring in the Sandveld of the Succulent Karoo Biome in the arid north-west of Namaqualand. Specifically along the coastal strip from Lüderitz (Namibia) in the north to the farm Skulpfontein, near Koiingnaas (South Africa) in the south, and from the high water mark to approximately 10 km inland (Channing, 1987; De Villiers, 1988; Minter, *et al.*, 2004). The findings of this study indicate that this north-south distribution is much reduced. The distribution of *B. macrops* should be revised to just south of Kleinzee and the inland range limited to a maximum of 6 km from the coast. This revised distribution is 67.84% of the currently recognised area of occupancy as described in the RDB 2004 (Minter *et al.*, 2004).

Thus the range available to *B. macrops* is almost half that previously stated. Reasons include misidentification and inaccurate reporting in the past (see below), a distribution based on piece-meal sampling, as well as the massive degree of fragmentation of the available habitat (De Villiers, 1988). *Breviceps namaquensis* (The Namaqua Rain Frog) is relatively similar in appearance yet more widespread than *B. macrops* (De Villiers, 1988). This has led to some uncertainty with regards to the reliability of the recorded range of *B. macrops* (De Villiers, 1988). Museum records, such as those held at the National Flagship Institute indicate that in the past *B. namaquensis* has been mistaken for *B. macrops*. For example, specimens No. 34202 and No. 33978, caught by Wulf Haacke in 1967, near Clanwilliam, have since been reassessed by R. C. Boycott in 1987, and found to be *B. namaquensis* (Museum card from The National Flagship Institute, Pretoria: *Breviceps macrops*, Boulenger). The current distribution is based on museum specimens gathered by collectors or scientists who obtained them in the course of fieldwork not related to the study of the distribution of the species. Many of them are not geographically accurate due to historical reporting techniques, which suggests that the basis for the current reported range is mostly circumstantial. In the past data collection in the area has been patchy because

geographically it is relatively isolated. It is also highly fragmented by strip mining for alluvial diamonds and access is greatly restricted in these areas (De Villiers, 1988).

Therefore we are left with a situation in which the current range is based on data that is notable more for its paucity and lack of reliability than its accuracy. However before we accept that the southerly distribution of *B. macrops* ceases at Kleinzee we need to examine whether the results presented in this study are valid, and deliberate on the factors which may contribute to this restricted range.

4.2 Why does *B. macrops* not occur south of Kleinzee?

This question is relevant to the study in that the negative result obtained during the fieldwork is considered unlikely when viewed in the light of the potential habitat available. As can be seen in Figure 2.3 (Dunes south of Kleinzee) and Figure 2.4 (Dunes of Noup) and Figure 2.5 (Port Nolloth Dunes) the available habitat south of Kleinzee at Noup appears to be very similar if not identical. Also in all of these areas there is evidence of large numbers of insects (potential food), and other herpetofaunal species that have similar life history strategies (similar niche requirements), such as Austen's Thick-toed Gecko (*Pachydactylus austeni*). The explanation for this apparent discrepancy could be the result of sampling error, habitat suitability, food availability and competition, anthropogenic disturbance, or historical distribution patterns. These factors are reviewed below to assess their validity.

4.2.1 Study Limitations

The sampling methods utilised could potentially result in an incorrect representation of the distribution of *B macrops* south of Kleinzee. Of the two techniques utilised the observational surveys were the least likely to incur methodological error. This is due to the amount of time spent searching for *B. macrops*, its sedentary nature and the ease with which it is encountered using this technique. In addition the observers involved spent time at a location with a

known population of *B. macrops* in order to validate both the method and their ability at applying it.

The pitfall traps are more susceptible to method error as they require the introduction of a foreign body into the frog's habitat. Crawford and Kurta (2000) investigated whether anurans differentiate the colour of pitfall traps from the surrounding substrate. Specifically they investigated whether lighter coloured traps were actively avoided or whether darker coloured traps were sought out as sites of refuge. They found that dark coloured traps in a light substrate repelled their study animal. This could introduce a degree of method error as concerns the trapping environment. However, their investigation was conducted in a forest environment and is thus not directly applicable to the present study. It could be argued that the present study was concerned with a nocturnal anuran where colour was less of an influencing factor. In light of the validation experiment conducted at Port Nolloth it is the opinion of the author that the colour of the buckets did not significantly influence the results. If the colour of the buckets could influence the effectiveness of the method, one would still have expected to find evidence of frogs in the form of tracks near the pitfall traps. This however was not the case.

The area sampled for the study was fairly extensive, and as such needed to be broken down into manageable units in the form of transects. Potentially not enough pitfall traps were placed or observational surveys conducted for the results to be conclusive. The sample size, however, was substantial enough to not preclude some frogs from being encountered with either of the two methods. Owing to the fact that no frogs were found south of Kleinzee, I believe that the southern population distribution of *B. macrops* halts just south of the town of Kleinzee. Further work in the area utilising both these methods could concentrate more heavily in the area just south of Kleinzee in order to define the geographically accurate southern-most point of distribution.

4.2.2 Habitat Suitability

Habitat condition and the way it influences the life history strategy of *B. macrops* might be implicated in the distribution anomalies. In certain areas where initial sampling took place the habitat was neither pristine nor indeed suitable for *B.*

macrops. At the start, the study was structured to be completely random, so as not to specifically sample areas where *B. macrops* was thought to occur. However, after the initial transects produced no evidence of *B. macrops*, and given the size of the study area and time constraints, the technique was altered to a more stratified random sample. This allowed the study to target the most likely *B. macrops* habitat for investigation. Hence habitat suitability in terms of the sampling procedure cannot be blamed for the result.

Studies have shown that various physical attributes can and do affect the distribution of anurans (Ling *et al.*, 1986; Grant & Licht, 1993). During the course of this investigation soil moisture content, pH and salinity were assessed at all trap sites and where frogs were found, but no obvious correlations were evident. This result is however biased by the limited number of frogs found during the study and consequently the number of soil samples taken at finds, therefore no relationships can be inferred from this data.

4.2.3 Ecological Considerations

Linked to the suitability of the habitat is also the fact that potentially there is not the correct or right amount of food available to *B. macrops*, south of Kleinzee. This could be the reason for the sudden cessation of *B. macrops* populations in the region as there is not a huge amount known about the diet of the frog. However, it appears to be an unlikely causal factor as the habitat appears, to all intents and purposes, to be very similar to that found at McDougall's Bay, indicated by both the vegetation type and the abundance of invertebrates that fall into the category of prey items likely to be suitable to *B. macrops*. For example Carruthers and Passmore recorded *B. macrops* tracks around dung heaps, which could indicate that *B. macrops* actively seeks out areas where insects congregate, in this case flies, fly larvae and dung beetles (Carruthers & Passmore, 1978). Channing reported on scat analysis for *B. macrops* from a number of captive specimens. The majority of the scat was composed of beetles, and one ant (Channing, 1987). Other species of *Breviceps* are known to eat small invertebrates such as termites, beetles, fly larvae and ants (Minter *et al,* 2004 (a)). Thus although the exact content of the diet of *B. macrops* is unknown, we can assume that it is composed

of both soft and hard bodied invertebrates of which numerous were noted both north and south of Kleinzee indicating that food availability is not affecting their distribution.

More likely, and related to information available on the distribution of *B. namaquensis* is the fact that to date no record has been made of these two species overlapping in distribution range. It is possible that there has been some confusion in the past due to the reported type locality of *B. namaquensis* as 'Port Nolloth' by Power in 1926, being misconstrued as the actual location of the type specimen as opposed to it being a reference to the nearest large town from where the specimen was located (Frost, 2008).

It is possible that *B. namaquensis* out-competes *B. macrops* in similar habitat where environmental tolerances of the two species meet. However one would then have expected to encounter more individuals of *B. namaquensis* during the sampling. In addition, the degree of overlap between these two species has only been speculated on in the past. This study found no evidence that such an overlap between their habitats occurs.

As part of the life history strategy of *B. macrops* it is apparent that they do not move very far. A study by Channing was conducted on the population dynamics of *B. macrops* at McDougall's Bay over a number of years (Channing, unpublished). In this time the maximum one individual moved was 379 m in one year, and the minimum one individual moved in one year was close to zero metres. The mean distance moved by a single individual was 15.4 m per annum (Channing, unpublished). This small and localised movement of the frog could theoretically have influenced the results of the pitfall traps, however the calibration of the method conducted at Port Nolloth indicates that the method is sound in areas where frogs occur in a healthy population. In addition, it indicates that as a species they may not be able to sufficiently cope with large-scale anthropogenic disturbance by simply moving away. In addition they may not actively migrate to colonise new areas of suitable habitat, or if they do so this movement is likely to be very slow.

4.2.4 Time of Sampling

It is possible that the time of sampling was not ideal for reasons associated with *B. macrops* behaviour and hence delivered a negative result. However, this is unlikely, since in previous work Channing found them to be active throughout the year excluding the month of February (Channing, unpublished).

4.2.5 Mining Influences

It is clear that historical and present mining impacts have had an effect on the distribution of *B. macrops*, however, just south of Kleinzee where frogs were encountered, the mining operation is immediately adjacent to the frog habitat. Potentially disparate mining operations might have destroyed localised populations of *B. macrops* south of Kleinzee, or split them from other populations thus creating a situation where immigration and emigration was halted, or where the remaining populations were not viable in the long term. This seems to be one of the most likely explanations for the seemingly inexplicable absence of *B. macrops* south of Kleinzee, however, it is difficult to define and isolate as an impact without more in-depth information concerning exactly where mining has taken place.

4.3 Are there correlations between environmental variables and the distribution of *B. macrops*?

If it is accepted that *B. macrops* does not occur south of Kleinzee then there are certain environmental variables which may explain this distribution.

4.3.1 Aeolian Sand Deposits

All *B. macrops* found in this study were located on pale sand dunes that run parallel to the coast and are the result of aeolian sand deposits (De Villiers, 1988). These dunes are greatly fragmented. As can be seen in Figure 3.4 most of the known sites for *B. macrops* fall within the aeolian deposits which would tend to suggest that this substrate and its associated fauna and flora are a base

requirement for the species. However, these deposits stretch south of Kleinzee and are therefore not the only requirement for colonisation by *B. macrops*.

4.3.2 Fog

The characteristic fog of the coastal strip along the west coast of South Africa most likely influences various aspects of this ecosystem, however there is no empirical data freely available for analysis. The limitation is partly due to the nature of fog, being variable over short distances, and partly due to the lack of weather stations in the less-populated areas of the west coast. Therefore although there may exist a significant correlation between the occurrence of fog and the distribution of *B. macrops* it is impossible to establish at this point. Work in the field is ongoing and may yield some positive outcomes in the near future.

4.3.3 Vegetation

All known locations in which *B. macrops* has been found occur within either SKs 1 or SKs 8. However due to the limited number of samples within either of these two vegetation types one cannot correlate distribution of *B. macrops* to either. Potentially the change from SKs1 in the vicinity of Kleinzee to SKs 8 may influence the densities of *B. macrops* close to and south of Kleinzee. Knowing that *B. macrops* occurs in high density in Port Nolloth, an area surrounded by SKs 1, it is possible that although the frogs can survive in SKs 8, they are unable to maintain the high densities seen in SKs 1. This will need further investigation.

4.3.4 Soils

The results of the soil moisture content comparison suggest that *B. macrops* may occur in areas with drier sand than surrounding areas. This could be linked to other climatic conditions that change south of Kleinzee. Due to the limited amount of data obtained from locations where *B. macrops* was found, it is not possible to suggest any significant trends. There were also no clear trends in terms of the pH and salinity.

4.4 Molecular Data

All individuals of *B. macrops* sampled displayed identical genetic structure based on the sequences of a 550 bp fragment of the 16S gene. The 16S gene is used as a standard for genetic studies in anurans as it provides an adequate amount of variation across the order (Goebel *et al.*, 1999). The most northern sample was separated by a distance of 150 km from the southern sample which suggests that at some point in the past the species was fairly widely and contiguously distributed over this area, and that natural barriers such as rivers did not restrict their movement therein. However, from a species conservation point of view the extant populations of *B. macrops* can be managed similarly due to this genetic uniformity.

4.5 Conservation of *B. macrops* and current threats

4.5.1 Diamond Mining

During the Cretaceous period Kimberlite erosion resulted in the deposition of diamonds amongst the gravel of ancient riverbeds. The rivers washed these diamonds down towards the coast, where they were re-distributed by tidal currents up and down the coast (McCarthy & Rubidge, 2005). The diamonds were then overlayed with aeolian deposits of sand and thus became the diamond fields of South Africa and Namibia.

The diamond mining industry in South Africa has now overtaken all other forms of land use in terms of local habitat destruction (Carrick & Krüger, 2007). As reported by Carrick and Krüger (2007) half the western coastline of South Africa, 400 km of 800 km, from Cape Point to the Gariep River in fragmented sections are either in the process of being mined or prospected, and this destruction continues into Namibia as far north as Lüderitz. In the areas where diamond mining has taken place the habitat destruction is extensive. By the end of the process the sand layers have been totally churned up destroying all vegetation and animal life that previously existed in that locality.

Virtually all of the available habitat for *B. macrops* is located within the diamond mining fields of South Africa and Namibia, where it has been under threat for the past 90 years (Branch, 1988; De Villiers, 1988). Figure 4.1 shows a satellite image of a section of the land transformation caused by diamond mining north of Port Nolloth, Figure 4.2 (a) and (b) show the impact at ground level. This level of destruction continues unbroken up the coast into Namibia. It is clear that vast tracts of otherwise good *B. macrops* habitat have been drastically altered and made unsuitable for survival.

Figure 4.1. Satellite image of a section of land north of Port Nolloth, illustrating the extensive transformation caused by diamond mining.

Figure 4.2 (a) – Mining impact between Kleinzee and Port Nolloth

Figure 4.2 (b) – Mining impact at Alexander Bay

4.5.2 Residential Development

Since the start of mining in the area there has been the concomitant need for the erection of housing. With the decommissioning of certain mines and the establishment of tourism in the area further residential/holiday developments have followed and will continue to do so. In the areas surrounding Kleinzee and Port Nolloth this development could pose a risk to the coastal strip of dunes where *B. macrops* occurs as can be seen in Figure 4.3 where residential development is taking place directly within *B. macrops* habitat in Port Nolloth. As with mining, residential development involves the complete transformation of the immediate natural habitat where buildings are erected. After construction the inhabitants of the residences will add a continued impact through their access to the area. These developments are therefore likely to become the next major threat to *B. macrops* habitat when the mines finally close down. If residential development is to continue in these areas it must be properly managed so as not to destroy remaining habitat. Developments must be buffered from the habitat and controlled in terms of access to sensitive areas and their associated impacts.

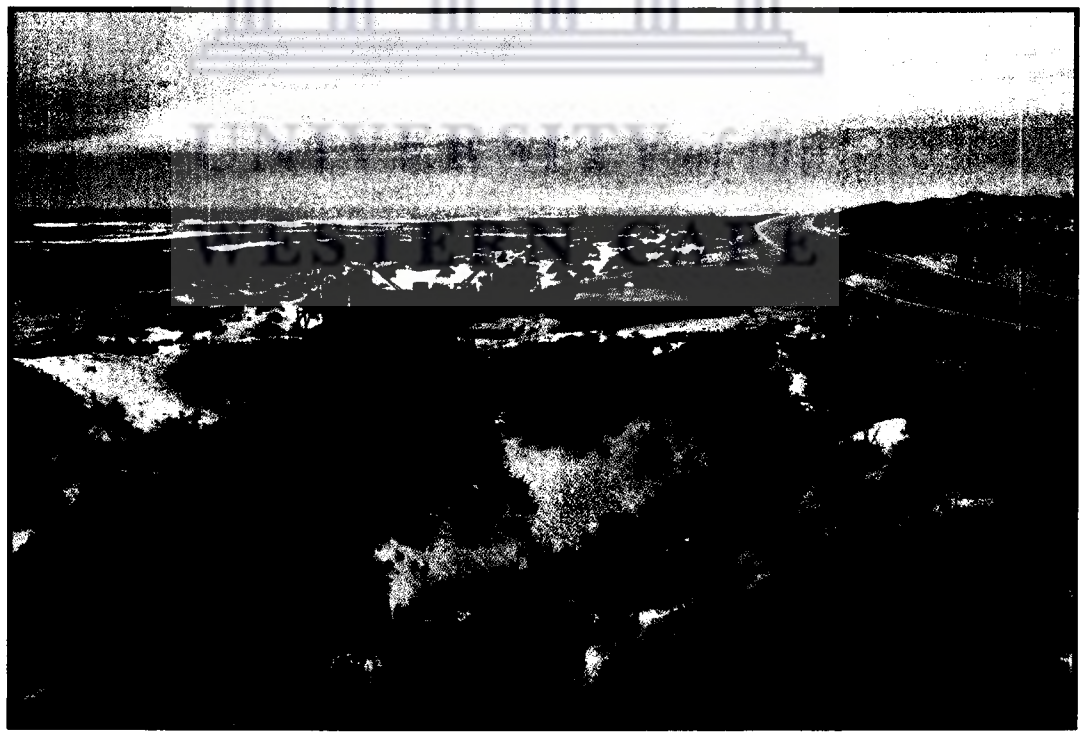

Figure 4.3. Human impact at the Port Nolloth study site, where construction is taking place in pristine *B. macrops* habitat.

4.5.3 Distribution in Namibia

B. macrops is known to occur in both South Africa and Namibia, with very little known of its distribution in the latter. Currently the northern distribution of *B. macrops* is listed as reaching up to Lüderitz, in Namibia. However, to date there have been very few confirmed sightings. Of concern is the extensive habitat destruction due to mining activities, evidenced in available satellite imagery. It is essential that the status of *B. macrops* be investigated in this country if we are to achieve a realistic conservation target for the species.

4.5.4 Revised IUCN Red Data Classification

B. macrops is currently listed as Vulnerable (VU) (Minter, *et al.*, 2004 (b)). This is based on a restricted distribution, with an area of occupancy of 501-2000 km^2, extensive habitat loss, and a predicted population decline of more than 50% in the next 30 years (from 2004). The area of occupancy currently accepted in the RDB 2004 (Mucina *et al.*, 2006) has been estimated at 1239.48 km^2 based on the original distribution of Koiingnaas in the South, and 10km inland. If we accept that the actual distribution is from Kleinzee in the south up to 6km inland, then the results now suggest that the area of occupancy should be revised to 841.85 km^2.

This represents 67.84% of the currently recognised area. However, it is highly unlikely that a viable population of frogs could survive within a mining site, unless part of the site encompasses some pristine ground. Analysis of the amount of habitat completely transformed by mining (no suitable habitat remaining) suggests that the actual area of occupancy remaining to *B. macrops* is in fact only 270.72 km^2. In addition very little is known of the actual distribution in Namibia and the similar threats it faces. Thus, if the percentage of pristine habitat remaining in Namibia is less than or similar to South Africa, the total area of habitat remaining to *B. macrops* may be as little as 541 km^2. It is therefore critical that the status of *B. macrops* be revised.

In light of this it is proposed that the conservation status of *B. macrops* should be elevated to that of Endangered (EN) based on; a restricted geographic

range (area of occupancy < 5000 km^2), severely fragmented habitat, and a continuing decline in area, extent and quality of habitat.

4.6 Conclusion

The results of this study were fairly conclusive in their rejection of the occurrence of *B. macrops* as far south as Skulpfontein and up to 10 km inland. The actual distribution was a maximum of 6 km inland and 2 km south of the town of Kleinzee.

The reasons for this restricted distribution seem to lie primarily at the door of historical misidentification and a lack of accurate reporting as well as anthropogenic disturbance. Factors were assessed which may shed some light on the reasons for *B. macrops* distribution ceasing south of the town of Kleinzee, when the habitat south of this point appears to be suitable, however no significant correlations could be identified.

There appears to be a slight correlation between the presence of *B. macrops* and aeolian sand deposits although this is no doubt combined with other habitat requirements. No trends were isolated from the soil analysis, due to the small sample size obtained for locations at which *B. macrops* was sampled.

B. macrops was found in two vegetation types, Richtersveld Coastal Duneveld (SKs 1) and Namaqualand Coastal Duneveld (SKs 8) (Mucina *et al.*, 2006). However, there were no obvious trends in the distribution of *B. macrops* within either of these two vegetation types, other than a superficial indication that SKs 1 may be a preferable vegetation type.

Habitat suitability south of Kleinzee appears to be adequate for colonisation by *B. macrops* but this has not occurred, which may indicate that there is some barrier to migration which is not readily observable.

Fog deposition along the coast may be implicated in the cessation of the distribution of *B. macrops* south of Kleinzee however there is insufficient data to derive any conclusions presently. Further research in these areas aimed at investigating these relationships would be useful.

The genetics of three disparate populations of *B. macrops* were analysed for differences in a fragment of the 16S gene, in order to examine their relatedness. It was clear from this analysis that there are no genetic differences between these populations. This could be problematic for the survival of the species as the lack of genetic plasticity in the genome may limit its ability to survive if placed under stress by habitat loss and fragmentation.

It is abundantly clear that large-scale transformation of the landscape has taken place throughout much of what was once suitable *B. macrops* habitat. This is almost solely due to strip mining for alluvial diamonds. The area of occupancy of *B. macrops* within South Africa is now predicted to be as little as 270.72 km^2. This represents a 78.16% reduction in what was believed to be available to the species. In addition the overall distribution of *B. macrops* in southern Africa may be limited to approximately 541 km^2. Thus the conservation status of this species should be revised and in the opinion of the author elevated to Endangered (EN).

The extant populations must be provided with the protection necessary to reduce the threat of extinction, specifically in areas where there still remain viable populations, such as McDougall's Bay. Additional studies need to be conducted to determine the viability of the Kleinzee population, and whether establishing populations of *B. macrops* south of this point, through facilitating the colonisation of apparently suitable areas, is necessary.

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APPENDIX A: Proposed survey coordinates for the primary study site.

Proposed Point	North/South	East/West
A1	S 29 41 00.0	E 17 03 15.0
A2	S 29 41 00.0	E 17 04 30.0
A3	S 29 41 00.0	E 17 05 45.0
A4	S 29 41 00.0	E 17 07 00.0
A5	S 29 41 00.0	E 17 08 15.0
A6	S 29 41 00.0	E 17 09 30.0

Proposed Point	North/South	East/West
B1	S 29 43 38.0	E 17 03 45.0
B2	S 29 43 38.0	E 17 05 00.0
B3	S 29 43 38.0	E 17 06 15.0
B4	S 29 43 38.0	E 17 07 30.0
B5	S 29 43 38.0	E 17 08 45.0
B6	S 29 43 38.0	E 17 10 00.0

C1	S 29 46 16.0	E 17 04 15.0
C2	S 29 46 16.0	E 17 05 30.0
C3	S 29 46 16.0	E 17 06 45.0
C4	S 29 46 16.0	E 17 08 00.0
C5	S 29 46 16.0	E 17 09 15.0
C6	S 29 46 16.0	E 17 10 30.0

D1	S 29 48 54.0	E 17 04 45.0
D2	S 29 48 54.0	E 17 06 00.0
D3	S 29 48 54.0	E 17 07 15.0
D4	S 29 48 54.0	E 17 08 30.0
D5	S 29 48 54.0	E 17 09 45.0
D6	S 29 48 54.0	E 17 11 00.0

E1	S 29 51 32.0	E 17 06 00.0
E2	S 29 51 32.0	E 17 07 15.0
E3	S 29 51 32.0	E 17 08 30.0
E4	S 29 51 32.0	E 17 09 45.0
E5	S 29 51 32.0	E 17 11 00.0
E6	S 29 51 32.0	E 17 12 15.0

F1	S 29 54 10.0	E 17 06 45.0
F2	S 29 54 10.0	E 17 08 00.0
F3	S 29 54 10.0	E 17 09 15.0
F4	S 29 54 10.0	E 17 10 30.0
F5	S 29 54 10.0	E 17 11 45.0
F6	S 29 54 10.0	E 17 13 00.0

G1	S 29 56 48.0	E 17 08 15.0
G2	S 29 56 48.0	E 17 09 30.0
G3	S 29 56 48.0	E 17 10 45.0
G4	S 29 56 48.0	E 17 12 00.0
G5	S 29 56 48.0	E 17 13 15.0
G6	S 29 56 48.0	E 17 14 30.0

H1	S 29 59 26.0	E 17 09 30.0
H2	S 29 59 26.0	E 17 10 45.0
H3	S 29 59 26.0	E 17 12 00.0
H4	S 29 59 26.0	E 17 13 15.0
H5	S 29 59 26.0	E 17 14 30.0
H6	S 29 59 26.0	E 17 15 45.0

I1	S 30 02 04.0	E 17 10 00.0
I2	S 30 02 04.0	E 17 11 15.0
I3	S 30 02 04.0	E 17 12 30.0
I4	S 30 02 04.0	E 17 13 45.0
I5	S 30 02 04.0	E 17 15 00.0
I6	S 30 02 04.0	E 17 16 15.0

J1	S30 04 42.0	E17 11 00.0
J2	S30 04 42.0	E17 12 15.0
J3	S30 04 42.0	E17 13 30.0
J4	S30 04 42.0	E17 14 45.0
J5	S30 04 42.0	E17 16 00.0
J6	S30 04 42.0	E17 17 15.0

K1	S30 07 20.0	E17 11 30.0
K2	S30 07 20.0	E17 12 45.0
K3	S30 07 20.0	E17 14 00.0
K4	S30 07 20.0	E17 15 15.0
K5	S30 07 20.0	E17 16 30.0
K6	S30 07 20.0	E17 17 45.0

L1	S30 09 58.0	E17 13 15.0
L2	S30 09 58.0	E17 14 30.0
L3	S30 09 58.0	E17 15 45.0
L4	S30 09 58.0	E17 17 00.0
L5	S30 09 58.0	E17 18 15.0
L6	S30 09 58.0	E17 19 30.0

APPENDIX B: Coordinates sampled within the primary study site once assumptions were adhered to. The initial letter relates to a transect ('A' directly south of Kleinzee, and 'B' 5 km south of 'A' etc), two letter before a number indicates a location between two transects. The number relates to the point along the transect ('1' being at the coast, '2' approximately 2 km from the coast etc). The second letter indicated multiple sample sites within close proximity to another.

Point	North/South	East/West	Elevation (m)	Distance from Coast (m)	Trap Hours	Person Minutes
A1	29 41 01.6	17 03 16.1	9	131	48	80
AB1	29 42 24.3	17 03 14.3	15	107	0	180
AB2	29 42 32.1	17 03 31.8	12	259	0	390
B1/A	29 43 21.7	17 03 38.2	8	133	24	260
B1/B	29 43 22.1	17 03 38.4	26	140	24	270
B2	29 44 02.4	17 04 45.2	64	2000	24	60
B3	29 43 32.5	17 06 22.4	96	4500	24	40
B4	29 43 36.8	17 07 49.3	97	6900	24	60
B5	29 43 38.2	17 08 54.3	116	8600	24	60
C1/A	29 46 26.3	17 04 15.3	9	236	24	150
C1/B	29 46 15.2	17 04 10.0	12	116	24	105
C2/A	29 45 55.2	17 05 29.9	150	2800	24	0
C2/B	29 45 56.6	17 05 30.0	150	2800	24	0
C3/A	29 46 20.7	17 06 47.1	132	4250	24	0
C3/B	29 46 21.6	17 06 47.3	98	4250	24	0
D1/A	29 49 11.2	17 04 41.8	2	93	24	30
D1/B	29 49 10.9	17 04 47.5	6	152	24	0
D1/C	29 49 18.4	17 04 54.8	16	270	24	0
D2	29 49 17.7	17 06 11.7	49	2300	24	60
D5/A	29 49 05.0	17 09 36.5	148	8000	24	0
D5/B	29 49 05.9	17 09 37.6	151	8000	24	0
E1/A	29 51 25.0	17 05 48.2	7	118	24	60
E1/B	29 51 26.6	17 05 54.4	11	123	24	60
E1/C	29 51 43.2	17 06 14.5	18	403	24	0
E2/A	29 51 45.1	17 06 37.4	27	1000	24	0
K1	30 07 59.4	17 11 54.2	20	30	0	240
L1	30 09 27.5	17 13 09.9	20	50	0	720

Appendix C: The genetic sequence for a fragment of the 16S gene of *B. macrops*, followed by the alignment of all eight specimens.

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   1 CCCAGATCCA ACATCGAGGT CGTAAACCCA TTTGTCGATA GGGGCTCTTG AAATGGATTG
  61 CGCTGTTATC CCCAGGGTAA CTAGGTTCGT TGATCAAGAG GTTTGGGTCA AGAGATGTCA
 121 TAATTATGAT CCTTTGGGGT GTGGCTCTTT GGTAAGAATT TAATGTCCTT TCAACGTGGA
 181 GGTTTTGTTA TACTCCGTGG TCGCCCCAGC CGAAAACTTT GAGGTCATAG CTAGTATATT
 241 TTGGGGTATT TTATTAAATA GTTGACTCTT AGTTTAAAGC TCCATGGGGT CTTCTCGTCT
 301 TATAGTTATA TCCCCGCTTC TTCACGGGGA GATCAGTTTA ACTGATTGAA GTTGGGAGAC
 361 AGTATAACCC TCGTGGGGCC ATTCATACTG GTCTATATTT AGTAGACAAG TGATTACGCT
 421 ACCTTCGCAC GGTTAGGGTA CCGCGGCCGT TGAATTAATC ACTGGGCAGG CTGGACCTCT
 481 TATAAGGGTG CAAGAGGCGA TGTTTTTGA
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