Facilitating golden mole conservation in South African highland grasslands: A predictive modelling approach

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A thesis submitted to the Department of Biological Sciences for the fulfilment of the requirements for the degree

*Magister Scientiae*

at the University of Cape Town, Cape Town, South Africa

September 2015

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Co-supervisors: Prof. N. C. Bennett, Prof. P. Bloomer, Dr I. T. Little, Prof. M. P. Robertson
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The scientist is a builder. Collecting scientific data can be compared to gathering stones for a house; a stack of data is no more 'science' than a heap of stones is a house. Unstudied scientific results are just a dead heap of stones.

Kristian Olaf Birkeland, 1903
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Preface

This study was initiated by the Threatened Grassland Species Programme of the Endangered Wildlife Trust (EWT), which secured seed funding from the Mohamed bin Zayed Species Conservation Fund. The fieldwork described was carried out across the Mpumalanga Province, South Africa from October 2013 - April 2014. All tissue and genetic work was carried out in the Departments of Zoology and Genetics, respectively, at the University of Pretoria, Gauteng. The study was supervised by Dr G. N. Bronner at the University of Cape Town, and co-supervised by Professors N. C. Bennett, P. Bloomer and M. P. Robertson (University of Pretoria) and Dr I. T. Little (Endangered Wildlife Trust).

The research described in this dissertation represents the original work of the author and has not otherwise been submitted in any form for any degree or diploma at any university. Where use has been made of the work of others, it is duly acknowledged in the text.

The author acknowledges the meaning of plagiarism and declares that all work in this thesis is her own.

Signed

Chanel Rampartab

Dr G. N. Bronner

University of Cape Town

2015
I, Dr G. N. Bronner, as the principal supervisor of Ms C. Rampartab’s M. Sc. study, have examined the full Originality report for her dissertation entitled "Facilitating golden mole conservation in South African highland grasslands: A predictive modelling approach" that was generated by the Turnitin.com today. The overall similarity index (excluding bibliographic and quoted materials) was 4%, reflecting only small phrasing matches inherent to the jargon of the discipline, and I am convinced that there is no indication of plagiarism as defined under the relevant UCT policy.

Dr G.N. Bronner
7 September 2015
Summary

Golden moles are subterranean mammals endemic to sub-Saharan Africa and threatened by anthropogenic habitat loss. At present, little is known about the biology, taxonomy, distribution and severity of threats faced by many of these taxa. In an attempt to raise awareness of these elusive grassland flagship taxa, the Endangered Wildlife Trust’s Threatened Grassland Species Programme (EWT-TGSP) identified the need for more information on the distributions and conservation status of four poorly-known golden mole taxa (*Amblysomus hottentotus longiceps*, *A. h. meesteri*, *A. robustus*, *A. septentrionalis*) that are endemic to the Grassland Biome, and which may be heavily impacted by anthropogenic habitat alteration in the Highveld regions of Mpumalanga Province. This study employed species distribution modelling to predict the distributional ranges of these taxa, and involved four main processes: (i) creating initial models trained on sparse museum data records; (ii) ground-truthing field surveys during austral spring/summer to gather additional specimens at additional localities; (iii) genetic analyses (using cytochrome-*b*) to determine the species identities of the newly-acquired specimens, as these taxa are morphologically indistinguishable; and (iv) refining the models and determining the conservation status of these Highveld golden moles.

Initial species distribution models were developed using occurrence records for 38 specimens, based on interpolated data for 19 bioclimatic variables, continuous altitude data, as well as categorical spatial data for landtypes, WWF ecoregions and vegetation types. These initial models helped to effectively focus survey efforts within a vast study area, with surveying during the austral spring-summer of 2013-4 resulting in the acquisition of 25 specimens from across Mpumalanga, nine individuals of which (*A. h. meesteri* *n* = 2; *A. septentrionalis* *n* = 5; unknown *n* = 2) were captured in five new quarter-degree-squares (QDSs) where no previous golden moles have been recorded. Additionally, observed activity was also recorded in nine new QDSs (see Appendix 3), showing that the model refinement methods used (variable selection, auto-correlation, non-repeated versus cross-validated models, jackknife of variable importance and localities, independent data testing) were effective in locating golden mole populations. By using genetically-identified historical golden mole records, predictive distribution models were calibrated in maximum entropy (MaxEnt) software to focus ground-truthing efforts.
Genetic analysis of the mitochondrial DNA (mtDNA) cytochrome-\(b\) gene sequences allowed unequivocal discrimination between the four cryptic taxa. While probabilistic support values for the delineation of \(A.\) \(h.\) \(longiceps\) and \(A.\) \(h.\) \(meesteri\) were strong, distinguishing between \(A.\) \(robustus\) and \(A.\) \(septentrionalis\) was less robust, with the identifications of two specimens (CR18 and CR25) being equivocal. Despite these problems arising from the use of only cyt-\(b\), which was a compromise given time and financial constraints, only two of the 13 specimens of the latter species (Table 3.2) could not be identified with 95% confidence. Given the close phylogenetic relatedness of \(A.\) \(septentrionalis\) and \(A.\) \(robustus\), and their predicted geographic niche overlap along the southern parts of the escarpment, the use of additional molecular markers is recommended for any possible future ground-truthing survey identifications.

Refined models based on 59 genetically-identified specimens were developed through a rigorous variable selection and model evaluation process involving 33 model iterations. Autocorrelation analyses ensured that only the most biologically relevant contributing climatic variables (precipitation of warmest quarter and coldest quarter) were used. The use of an appropriate background (landtypes) and spatial filtering (1 km\(^2\) grid size low vagility and limited dispersal abilities of golden moles) served to minimalize sampling biases inherent in the data and minimize omission rates. Stringent model comparisons based on area under curve (AUC) of the receiver operating characteristic (ROC) values for the final SDM models for all taxa were significant (0.997 – 0.999), and showed realistic predicted geographic distributions with simple response curves. Maxent internal jackknife statistics and a locality jackknife method showed that the final models have 95% significance (except for \(A.\) \(robustus\) where significance was marginal (\(p = 0.06\)). Omission error for activity points (where species identifications were not possible but golden mole presence was confirmed) was 13.21 %, indicating that the final models are fairly robust at predicting areas where golden moles probably occur. Based on these lines of evidence, I concluded that the refined models, while relatively robust, are at best a first approximation of golden mole distributional ranges in Mpumalanga grasslands given deficiencies of the data on which the models are based (e.g. small sample sizes with probable high geographic sampling biases; presence-only data; only interpolated climatic data; coarse-scale categorical data for soil organic carbon content, and primary production). Nonetheless, these models, which provide predictions for 19 184 cells at a 4 km\(^2\) grid size, are arguably better than a scant database of 59 distribution records at a 1 km\(^2\) locality grid size for predicting the distributional ranges of...
the four targeted golden mole taxa, and thus provide a valuable conservation assessment and planning tool.

Based on spatial analyses employing the refined models, the current protected areas network in Mpumalanga conserves sufficient area (> 28 %) of the distributional ranges of all four targeted chrysochlorid taxa if the "> 5 – 10% range conservation goal" is applied. Of the four ecoregions that coincide with the predicted ranges of these taxa, three (Drakensberg Montane grasslands, woodlands and forests ecoregion - 11.7 %; Highveld grasslands - 5.2 %; Southern Africa Bushveld - 14 %) are adequately conserved if this criterion is used. The predicted distributional ranges of three of the four taxa (excluding *A. septentrionalis*) are highly coincident not only with two grassland ecoregions (Drakensberg and Highveld grasslands), but also with mountain catchment areas having soils with high organic carbon contents (> 4 %) that support high primary production (> 6 t/ha/an). The prime habitats of the taxa studied (as here defined) coincide with areas of high soil organic carbon content (> 3 %) and primary productivity (> 6 t/ha/an) that experience warm summers (> 16 °C) and high precipitation (> 370 mm) associated within mountain catchment areas (MCA) and the Grassland Biome. The percentage overlap of the predicted taxon ranges with protected areas fulfilling these criteria is low (*A. h. longiceps*: 2.4 %; *A. h. meesteri*: 4.4 %; *A. robustus*: 3.9 %; *A. septentrionalis*: 7.8 %), suggesting that prime habitats are under-conserved within the existing protected area network. The value of ecological corridors and conservancy areas is assessed, and found to be of marginal conservation importance for these taxa and would not greatly increase the protection of golden moles should they be declared formally protected. Given deficiencies of the data on which the models were based, further ground-truthing is required not only to increase the number of verified occurrence records for each taxon, but also to collect presence-absence data and to develop higher-resolution spatial protected area layers (with continuous soil organic carbon content and primary productivity data) on a scale appropriate for analysing the geographic configuration and extent (including inter-connectedness) of prime habitats that these taxa apparently prefer.
Acknowledgments

This study was made possible with the support of the following people and organisations:

To the National Research Foundation and to the Mohamed bin Zayed Species Conservation Fund for investing in this project, which is proudly affiliated with the University of Cape Town (UCT), University of Pretoria (UP), Endangered Wildlife Trust (EWT) and Mpumalanga Tourism and Parks Agency (MTPA).

My supervisor, Gary N. Bronner (UCT) has provided exceptional guidance during this intensive and exciting study. To Nigel C. Bennett (UP), thank you for providing field support, experiences and wisdom. To Ian T. Little (EWT), I thank you for organising funding and publicity surrounding the project, and for rallying the volunteer troops. Thank you to Mark P. Robertson (UP) for providing his insight into predictive models. To Paulette Bloomer, Sarita Maree and Samantha Mynhardt (UP), I thank you for your guidance with genetic analyses. It has certainly been a pleasure working with such an esteemed and diverse group of scientists.

Many people contributed to my field work. First and foremost, my heartfelt thanks go out to Lientjie Cohen, Geelbooi Mashilo and Vaino Prinsloo (MTPA) for putting 110% into golden mole hunting. Many thanks to Jiba Magwaza (EWT) for firing up the donkey every night in Carolina. A big thank you to my colleagues who helped me in the field: Bradley Gibbons (EWT), Low de Vries (UP), Michael Staegemann (University of KwaZulu-Natal, UKZN), Lindile Cele (UKZN), Sibongakonke Lucky Myeni (EWT), German Andres Montoya Sanhueza (UCT), Glenn Ramke (EWT) and Ayanda Ndlela (UKZN). I am grateful to the landowners of Mpumalanga who not only allowed me to dig through their well-manicured gardens and freshly-ploughed farms, but also went out of their way to accommodate my team and me. A big thank you to Mervyn Lötter (MTPA) for providing spatial datasets for Mpumalanga, to Nicholas Lindenberg (UCT) for GIS advice, and Wynand Smit for assisting with formatting.

I would like to thank all the dogs, sunsets, waterfalls and wildlife of Mpumalanga for keeping me sane during seven gruelling months of field work.

Finally and most importantly, I would like to thank my big, loving family who have all taken me into their homes as I roamed to and from Durban, Cape Town and Johannesburg. Thank you especially to my parents, who have supported me through the past 19 years of studying.
Chapter 1: Introduction

Species distribution models are being increasingly used in conservation biology and planning owing to the excessive cost and effort needed to conduct biological surveys (Guisan et al. 2006, 2013, Rebelo and Jones 2010). This is especially true in cases where targeted taxa are cryptic and occur across a potentially broad geographic area, making specimens difficult to both detect and capture. The four golden mole taxa endemic to the grassland biome of South Africa satisfy all of these criteria. These taxa belong to the genus *Amblysomus* (Table 1.1) and each is known from only a few (< 10) scattered localities in the Highveld (flat highland grasslands) of Mpumalanga Province in South Africa. Little is known about the biology, habitat requirements, niche tolerances and distribution limits of these chrysochlorid taxa. The grasslands they inhabit are heavily impacted by anthropogenic activities, resulting in 44% of grasslands having been transformed (Ferrar and Lotter 2007), underpinning the inclusion of two species in threatened categories (IUCN 2015). This augured the development and application of species distribution models to better understand their niche requirements and to estimate their geographic ranges, with a view to better assessing their conservation status and facilitating improved conservation management strategies.

**Table 1.1:** Species frontal views and profile data for four cryptic *Amblysomus* – note that *A. h. meesteri* often has a recognisable dorsal stripe. Photos: Rampartab, C. Mpumalanga 2014.

<table>
<thead>
<tr>
<th>Species</th>
<th><em>A. h. longiceps</em></th>
<th><em>A. h. meesteri</em></th>
<th><em>A. robustus</em></th>
<th><em>A. septentrionalis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Known habitat</td>
<td>Grasslands, forests, cultivated land, gardens; widespread in Eastern Cape and KwaZulu-Natal</td>
<td>Restricted to north eastern mountain grassland and Afromontane forests of Mpumalanga</td>
<td>Few localities in grasslands; Mpumalanga</td>
<td>Montane grasslands; Free State and Mpumalanga</td>
</tr>
<tr>
<td>Body length (mm)</td>
<td>104-135</td>
<td>107-145</td>
<td>109-143</td>
<td>105-145</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>37-85</td>
<td>41-57</td>
<td>61-98</td>
<td>52-86</td>
</tr>
<tr>
<td>IUCN (2015)</td>
<td>Least Concern</td>
<td>Least concern</td>
<td>Vulnerable</td>
<td>Near Threatened</td>
</tr>
</tbody>
</table>
1.1. Highveld grasslands

Highveld grasslands occur in north-eastern South Africa between 1400 m and 1800 m above sea level (a.s.l.) and dominate four provinces of South Africa, viz. Mpumalanga, KwaZulu-Natal, Free State and Gauteng (Figure 1.1). Epeirogenic upliftment of the Great Escarpment by 600 m – 900 m between 5 – 3 million years ago (Partridge 1997, Partridge and Maud 2000) led to the development of drier and cooler highlands with increased rainfall seasonality during episodic wet-dry global climatic oscillations. This allowed C4 grasses to outcompete C3 grasses and woodland specialists during the Plio-Pleistocene global cooling/warming periods, with the result that Highveld grasslands are now dominated by C4 grasses (Partridge and Maud 2000, McCarthy and Rubidge 2005, Clark et al. 2011).

Figure 1.1: The Grassland Biome covers much of the eastern interior of South Africa. The study area of interest is in the Mpumalanga Province, dominated by mesic Highveld grasslands (Mucina and Rutherford 2006).
Grassland landscapes in South Africa are dominated by grasses punctuated by shrubs, and are widespread through Montane/Highveld regions with broad precipitation ranges (Rutherford et al. 2006, Bond and Parr 2010). South African grasslands cover 360 149 km$^2$ (Mucina and Rutherford 2006), 185 863 km$^2$ of which forms the official Highveld grassland ecoregion (Olson et al. 2001). Grasslands cover the largest area (17 %) of any biome in South Africa (Neke and du Plessis 2004). An estimated 3 800 plant and animal species exist in 112 000 km$^2$ of the Highveld (Cowling et al. 1989, Cowling and Hilton-Taylor 1994, Neke and du Plessis 2004, Bond and Parr 2010). These grasslands, although dominated by a single-layered herbaceous community of tussocked (bunch) grasses, support a high diversity of non-grassy herbs, most of which are perennial plants with large underground storage structures, and which can live for several decades (Stephens 2008, Bond and Parr 2010). Climates are cool and dry with annual summer rainfall exceeding 500 mm and infrequent extreme weather including frost, hail and lightning storms (Ferrar and Lotter 2007). Grazing by animals and, to a greater extent, regular austral winter fire regimes, facilitate the existence of rich grassland assemblages of taxa such as birds and arthropods (Little et al. 2013), but also releases carbon stores and soil organic carbon from burning plants and leaf litter, respectively (O'Connor and Kuyler 2009). There are very few annual species, the pioneer plants needed to start ecological succession and repair disturbed habitats. Consequently, these ecosystems are particularly vulnerable to destruction and are under threat, placing this biome and its suite of endemic flora and fauna in high ranks in the conservation arena (Ferrar and Lotter 2007, Matsika 2007, Ceballos et al. 2010).

Conservation projects such as the South African National Biodiversity Institute’s (SANBI) Biodiversity Assessment and Grassland Programme aim to draw attention to threatened ecosystems and prioritise conservation strategies by expanding the protected areas network (Global Environment Facility et al. 2007, Driver et al. 2011). Similarly, the Endangered Wildlife Trust’s Threatened Grassland Species Programme (EWT-TGSP) aims to mitigate environmental damage by promoting the conservation of flagship species that inhabit these grasslands (EWT
One of the most threatened grassland regions is located on the Highveld in Mpumalanga at altitudes > 1000 m. Mpumalanga spans over 76 735 km², of which 61 % was historically covered by grasslands, but transformation has reduced the extent of natural grasslands to just 44 %, spanning 23 of Mpumalanga’s 64 vegetation types (Ferrar and Lotter 2007). Some of this anthropogenic transformation is so severe that it is irreversible (O’Connor and Kuyler 2005, Ferrar and Lotter 2007). The Mpumalanga Biodiversity Conservation Plan (MBCP) aims to remediate some of the damage by focussing conservation efforts in the grasslands’ sandy soils and rocky outcrops and the species within them (Ferrar and Lotter 2007, Stephens and Tau 2013). The MBCP layers (http://bgis.sanbi.org/MBCP/project.asp) were imported into ArcGIS and area of conservation layers were calculated using the projected co-ordinate system PCS WGS 1984 UTM 35 S that is congruent with the study area location on earth (Section 2.2.1).

At present, the protected area network of Mpumalanga spans 20.53 % of the province’s grasslands and Savannah habitats (excluding polygon overlap) and is important in the conservation of golden moles as urban and industrial developments are managed and/or prohibited here. Other areas of conservation concern such as ecological corridors and conservancies (12.91 and 5.50 % area of Mpumalanga, respectively) are not formally protected, but could offer protection of golden moles should they be incorporated into the formal protected areas network in the future. Due to their elusive nature, it is currently unknown from the existing occurrence records whether the golden moles studied here are well-conserved by the MBCP’s priority conservation regions, or whether ecological corridors and/or conservancies are important areas for golden mole protection. This study provides insight into the potential distributions of four golden mole taxa largely endemic to the Mpumalanga grasslands, thereby focussing efforts aimed at conservation planning and implementation. The models will hopefully also facilitate decision-making to ensure that future proclamation of protected environments preserves the most feasible and crucial habitats of threatened golden moles.
South African grasslands support distinct floral and faunal assemblages, including 45% of the country’s endemic mammals (Matsika 2007). Of the 34 mammal species endemic to South Africa, 15 occur in the Grassland Biome, including five golden mole taxa (family Chrysochloridae). Four of these are morphologically indistinguishable (Table 1.1) and were traditionally assigned to the species Amblysomus hottentotus. Two (A. robustus and A. septentrionalis) were elevated to full species based on karyotypic and craniometric differences (Bronner 2000), and are listed as Vulnerable and Near-Threatened respectively (Rampartab 2015a, 2015b)(Table 1.1). Recent chromosomal and genetic analyses have also shown that the other two taxa (Amblysomus hottentotus longiceps and A. h. meesteri), currently classified as subspecies of the widespread A. hottentotus, should be elevated to full species (Gilbert et al. 2008, Maree et al., in prep. Mynhardt et al., submitted). However, as the genetic results have not yet been published, these taxa are here provisionally retained as subspecies following Bronner (2013). While A. h. longiceps and A. h. meesteri are currently not yet listed as distinct species owing to their classification as only subspecies within the widespread A. hottentotus (Bronner and Mynhardt 2015), both are likely to qualify for threatened status once assessed as full species (Mynhardt et al., submitted). Chrysochlorids are thus an important component of the Grassland Biome’s endemic mammal fauna, and an ideal flagship taxon for conservation assessments given their low vagility and high habitat specificity (Bronner 2013). This study therefore focused on these four golden mole taxa.

1.2 Chrysochlorid biology

Along with sengis (elephant shrews), tenrecs, hyraxes, aardvark, manatees and elephants, golden moles represent an ancient clade of endemic African mammals known as the Afrotheria (Kuntner et al. 2010). Golden moles (order Afrotheria, suborder Chrysochloridae) comprise 21 of the 89 species within Afrotheria (Kingdon et al. 2013), and thus represent a significant proportion (~ 30 %) of the extant diversity of this clade. All chrysochlorids are
endemic to sub-Saharan Africa, with 18 species occurring only in southern Africa (Bronner 2013).

Like many other fossorial mammals, chrysochlorids have very specific habitat requirements including friable soils to reduce the energetic costs of digging (Vleck 1981, Jackson et al. 2008b, 2008a), and lead K-selected lifestyles (Schoeman et al. 2004), investing energy and resources in the birth and upbringing of only a few offspring annually to increase their chances of survival (Pianka 1970) in nests ~30 cm deep. Their specialised diets of energy-rich invertebrate prey is influenced by prey availability (Kuyper 1985), which restricts the range of niches they can inhabit. These factors collectively reduce their ability to recover from disturbance events as shown with specialist butterflies (Kitahara and Fujii 1994, Kitahara et al. 2000) and plants (Bohn et al. 2014) in disturbed habitats.

This "life in the slow lane" has some negative implications for the persistence of golden moles in a changing environment. While a few species occur over wide geographic ranges (e.g. Chrysochloris asiatica and A. hottentotus), most are range-restricted and some species (e.g. Cryptochloris wintoni and A. robustus) are known from only a few localities (Bronner and Bennett 2005). Due to their underground lifestyle, creating a burrow system is energy-consuming: compared with surface foraging, sub-surface foraging expends at least 3 500 times more energy, more so in harder, drier soils (Vleck 1979, Busch et al. 2000). Consequently, small fossorial mammals generally exhibit spatially limited movements and dispersal abilities, with further constraints imposed by the availability of friable soils (Collis-George 1959, Gaines and McClenaghan Jr 1980, Busch et al. 1997), factors that exacerbate any potentially negative effects of habitat fragmentation and population vicariance (Spellerberg 1998, Johnston et al. 2012).

Whilst relatively protected against environmental fluctuations (especially in ambient temperature) owing to buffering offered by the subterranean ecotype, subsurface foraging by golden moles exposes them narrowly to the surface climatic milieu (Adhikari et al. 2014,
As a result of physiological adaptations to reduce energy expenditure, such as daily and/or seasonal torpor (Scantlebury et al. 2005, 2008), most golden mole species are active only in the wetter seasons when soils are friable and invertebrate prey resources are not limiting. This further constrains the dispersal capabilities and distributions of golden moles and other subterranean mammals alike (Bennett 1990, Nevo 1995, Busch et al. 1997, Herbst 2002), and thus gene flow between demes (Aars and Ims 2000, Wang et al. 2013). These factors underpin why five of the twelve endangered and critically endangered mammals in South Africa are golden moles (IUCN 2015). Of the 21 species of golden moles, one is critically endangered, four are endangered, five are vulnerable and two are near threatened (IUCN 2015).

The four Amblysomus taxa that occur in the Mpumalanga grasslands form a cryptic species complex, i.e. taxa that are morphologically indistinguishable and can only be separated using chromosomal characters (Gilbert et al. 2006, 2008), fast-evolving genetic markers (Maree 2002, Hebert et al. 2004, Bickford et al. 2006, Engelbrecht et al. 2011, Bastos et al. 2011, Faulkes et al. 2011) and/or multivariate craniometrics (Bronner 1996, 2000, Taylor et al. 2009). At a finer resolution, genetic analyses of mitochondrial DNA (mtDNA) markers (such as ND2, cytochrome b and 12S rRNA) can detect changes between generations and populations (Hebert et al. 2004, Bickford et al. 2006, Jackson and Robertson 2010, Faulkes et al. 2011, Pauperio et al. 2012).

The distributions of species are strongly influenced by both biotic and abiotic factors, for example, prey density, competition, temperature and rainfall. A study by Slatyer et al. (2013) used meta-analysis of 64 studies to determine the relationship between niche breadth and geographical range size and showed that environmental tolerance breadth, habitat breadth and diet breadth were the strongest influences of range size. Therefore, specialist species are more vulnerable to extinction whereas generalist species are more likely to thrive across many niches over a wide geographic range (Slatyer et al. 2013). Over geological time, vicariance and disturbance events separate populations and each adapt to a separate niche. Eventually, new species with a suite of traits developed to suit their environment arise (Holt 2003, Gotelli 2008,
Sexton et al. 2009). Due to their poor dispersal and low vagility, golden moles are especially susceptible to this separation (Nevo 1979, 1995, Jackson et al. 2008b).

1.3 Species distribution models

Many floral and faunal distributional assessments involve physically surveying species to delimit their ranges and areas of occupancy. However, surveying the distributions of golden moles is difficult owing to their cryptic, subterranean lifestyles and trap shyness (Bronner 2013). Given also the large extent of Mpumalanga’s grasslands (46,808 km²) in which the four targeted golden mole taxa potentially occur, systematic surveying is unfeasible owing to high costs and effort required. Methods for selecting survey sites that will maximise the return on resources invested in surveying efforts are thus needed. Hence, with the use of statistical niche modelling software, predictive distributions of species are created.

Available predictive distribution modelling tools are tailored to address different questions under different circumstances. To date, several reviews have compared the accuracy, precision and application of these modelling techniques (Jesús and Felicísimo 2004, Guisan and Thuiller 2005, Peterson et al. 2007). MaxEnt is one such modelling tool that uses algorithms to link environmental variables (e.g. altitude, vegetation, soil types, temperature and rainfall) to occurrence records whilst limiting spatial autocorrelation, thereby creating gradients of probability of locating a species in an area (Elith et al. 2006, 2011, Phillips et al. 2006). For example, in comparison to BIOCLIM and GARP, MaxEnt has been shown to create more robust and accurate models of expected distribution ranges of taxa (Stockwell and Peterson 2002, Anderson et al. 2003, Elith et al. 2006, Peterson et al. 2007, Phillips and Dudik 2008, White and Newell 2009, Pio et al. 2011). This type of habitat modelling is becoming increasingly popular in ecology and conservation, especially due to its autocorrelation-resistant models and works
particularly well in cases with few occurrence records and presence-only data (Pearson et al. 2007, Williams et al. 2009, Wilting et al. 2010).

While MaxEnt is the most suitable software for this study, there are a few precautions to keep in mind when modelling. These centre on sampling bias, presence-only versus presence-absence data and species with few occurrence records. Whilst bias grids in MaxEnt can compensate for disparate sampling effort (Elith et al. 2011), MaxEnt models are vulnerable to other sampling biases. For the initial models, occurrence records from museum collections are likely to have spatial bias by being collected close to roads and throughways (Freitag et al. 1998). When refining models, sample effort is purposely concentrated on areas predetermined by the initial models to have a high probability of occurrence, so collecting biases associated with museum records may influence ground-truthing surveying. Additionally, all of the targeted golden mole species share similar habitat preferences for sandy and loamy grasslands (Bronner 2013), and the models will therefore likely show co-occurrence of species – which may not be the case in reality as chrysochlorids existing in broad sympatry often favour different habitats.

Using presence-only data, as opposed to presence-absence data (Fielding and Bell 1997, Hirzel et al. 2006, Phillips et al. 2009) is also problematic. Presence-absence data are more precise and allow the elimination of species from sites, but previous sampling efforts possibly bias the model as absences cannot be confirmed without exhaustive sampling at each site (Hirzel et al. 2002, Liu et al. 2005, Elith et al. 2006, Gibson et al. 2007), which is near-impossible given the extent of the study area and the elusive nature of golden moles. Hence, ground-truthing, or physically validating the model predictions, was necessary (Anderson et al. 2009, Rebelo and Jones 2010) to refine the golden mole database and predictive models.

MaxEnt modelling has been employed successfully by several studies to delimit ranges of flora and fauna, including those of species at risk of extinction, and other small mammals (Bombi et al. 2009, Williams et al. 2009, Wilting et al. 2010, Meynard et al. 2012). More
specifically, this method has been successfully employed on the limited occurrence dataset of
the threatened Juliana’s golden mole (*Neamblysomus julianae*) (Jackson and Robertson 2010).
Their initial model predictions allowed identification of suitable patches of habitats to survey
during ground-truthing, resulting in the discovery of two previously-unknown populations.
Calibration of the initial model with these two new records allowed the quantification of how
well this species is being protected and identified areas at high risk of habitat loss that deserve
priority conservation effort. Thus MaxEnt has excellent potential to model the distributional
range of the four golden mole taxa from the Mpumalanga Highveld grasslands using existing but
limited occurrence records.

### 1.4 Aims and hypotheses

My study aimed to: model the likely distributions of four of the *Amblysomus* taxa
(*Amblysomus hottentotus longiceps* and *A. h. meesteri, A. robustus* and *A. septentrionalis*) endemic
to grasslands in Mpumalanga and surrounding regions in South Africa; enhance current
understanding of the niche requirements of these taxa; identify habitat patches with areas of
high chrysochlorid diversity to focus conservation efforts; and provide an accurate predicted
distribution database for use by conservation planners, environmental managers and
environmental assessors. The approach involved running initial MaxEnt models based on
current museum records and ground-truthing these predictions to test initial model efficacy.
The specimens added from the field expeditions were genetically analysed to identify the
species affiliations of the four cryptic taxa and to refine the existing golden mole database. These
updated data were used to refine MaxEnt models to heuristically assess the distribution of these
golden moles for guiding environmental management and conservation efforts.
The following hypotheses were tested in this study:

- Distributional ranges of the four golden mole species in threatened Highveld grasslands are constrained by numerous bioclimatic factors, most importantly soil properties, and can thus be modelled effectively using geospatial data. Therefore it is expected that the models will be ground-truthed successfully, i.e. presence of golden moles will be confirmed at sites highlighted by the models as potential presences, and reveal unknown populations of each of the four taxa.

- Genotyping will reveal conclusive differences between the morphologically indistinguishable *Amblysomus* species, and also show variation within the two *A. hottentotus* subspecies in Mpumalanga, thereby contributing to the reclassification of these taxa as full species.

- Protected areas, ecological corridors and conservancies currently cover 20.53 %, 12.91 % and 5.50 % of Mpumalanga respectively. Given that the golden mole species are patchily distributed in grasslands and along the escarpment in Mpumalanga, I hypothesise that golden mole distributional areas will coincide with conservation areas in smaller proportions than the respective proportions of conservation areas within Mpumalanga.

Such information is needed to understand how effectively current conservation and land use practices are protecting endemic flagship species of the Highveld. Whereas current genetic reference records for the four *Amblysomus* taxa suggest that they do not occur sympatrically, the addition of craniometrically identified records (which have a confidence level of only 90%: Bronner 2000) suggest that certain areas may be potential contact zones where more than one taxon co-occurs. These reference collections are useful in providing insight on the difficult problem of spatial delimitation based on species identification alone. Hence, further genetic investigation is required to further clarify species identifications and therefore species ranges and conservation status. This dissertation augments the existing distribution and genetic data for the four *Amblysomus* taxa in the Highveld grasslands and makes inferences on their conservation status.
Chapter 2: Materials and Methods

2.1 Initial species distribution models development

2.1.1 Species data and profiles

Thirty-eight occurrence records from Ditsong Museum of Natural History specimens and the Molecular Ecology and Evolutionary Program (MEEP) lab, Department of Genetics, University of Pretoria (Table 2.1) of the four *Amblysomus* taxa (Chapter 1, Table 1.1) based on chromosomal characters (Bronner 1996) or cytochrome-\(b\) sequences obtained during a separate study (Maree et al., in prep.) were collated into a database for the development of species distribution models. Since only three genetic records at the same GPS position existed for *A. h. meesteri*, four additional morphometric records (Bronner 2000) were included in order to train the initial models.

2.1.2 Initial species distribution models (SDM)

Preliminary geographic distribution models were created using MaxEnt (version 3.3.3, Phillips et al. 2006), which is the preferred modelling technique for presence data of this nature (Elith et al. 2006, Pearson et al. 2007, Wisz et al. 2008). Nineteen continuous interpolated bioclimatic variables (Table 2.2) from WorldClim (Hijmans et al. 2005) were incorporated into the initial model to maximize both the scope of possible environmental and climatic variables that may influence the niches and distributions of the four targeted chrysochlorid taxa, and the number of possible habitat patches to survey during subsequent ground-truthing. The Mpumalanga winter is synonymous with dry, cool conditions and summer with wet, warm conditions. Hence, variables Bio5, 8, 10, 11, 13, 16, 18 are ‘summer’ variables (Table 2.2) and Bio 6, 9, 11, 14, 17, 19 are ‘winter’ variables.
Table 2.1: Species occurrence records and associated metadata used to train initial distribution models. Co-ordinates are given in decimal degree format. TM = Transvaal museum, now known as Ditsong Museum of Natural History (DMNH); GM = golden mole number; Prov = province; Lat = latitude; Lon = longitude; MPU = Mpumalanga; KZN = KwaZulu-Natal; FS = Free State. * Sourced from MEEP lab. **For *A. h. meesteri*, four morphometric records were included for initial model calibration.

<table>
<thead>
<tr>
<th>Species</th>
<th>Collector/Accession number</th>
<th>Prov</th>
<th>Locality</th>
<th>Lat</th>
<th>Lon</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. h. longiceps</em></td>
<td>S. Maree, G. Bronner, N. Bennett*</td>
<td>MPU</td>
<td>Tafelkop Farm, Wakkerstroom</td>
<td>-27.2833</td>
<td>30.2667</td>
</tr>
<tr>
<td></td>
<td>GM051</td>
<td>MPU</td>
<td>BirdLife Africa, Wakkerstroom</td>
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<tr>
<td></td>
<td>GM026</td>
<td>FS</td>
<td>Kiara Lodge, Clarens</td>
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<td>28.4167</td>
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<tr>
<td></td>
<td>GM027</td>
<td>FS</td>
<td>Francois Bester Farm, Tweeling</td>
<td>-27.5500</td>
<td>28.5000</td>
</tr>
<tr>
<td></td>
<td>GM028</td>
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<td>30.3335</td>
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<tr>
<td></td>
<td>GM029-30</td>
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<td></td>
<td>J. Wilson*</td>
<td>KZN</td>
<td>Sani Pass, Drakensberg</td>
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<td></td>
<td>J. Wilson*</td>
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<td></td>
<td>S. Maree, TM42132</td>
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<td>Graskop</td>
<td>-24.9333</td>
<td>30.8333</td>
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<tr>
<td></td>
<td>TM44177**</td>
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<tr>
<td><em>A. h. meesteri</em></td>
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<td>TM12748-50**</td>
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<td>Mariepskop</td>
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<tr>
<td></td>
<td>TM42392**</td>
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<td>Farm De Gama, White River</td>
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<td>L. Cohen</td>
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<tr>
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<td>J. Wilson*</td>
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<td>Mariepskop</td>
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<tr>
<td></td>
<td>TM39163, TM41661, TM41666</td>
<td>MPU</td>
<td>Verlorenvallei NR, Dullstroom</td>
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<td>30.1333</td>
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<tr>
<td></td>
<td>TM40904</td>
<td>MPU</td>
<td>Groenvlei Farm, Belfast</td>
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<td></td>
<td>TM39847</td>
<td>KZN</td>
<td>Ngome Forest, Vryheid</td>
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<tr>
<td><em>A. robustus</em></td>
<td>S. Maree, G. Bronner, N. Bennett*</td>
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<td>BirdLife, Wakkerstroom</td>
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<td>30.1500</td>
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<tr>
<td></td>
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<td>MPU</td>
<td>Ermelo Dam</td>
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<td>S. Maree*</td>
<td>MPU</td>
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<td>30.1500</td>
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<tr>
<td><em>A. septentrionalis</em></td>
<td>S. Maree, G. Bronner, N. Bennett*</td>
<td>MPU</td>
<td>BirdLife, Wakkerstroom</td>
<td>-27.3500</td>
<td>30.1500</td>
</tr>
</tbody>
</table>
Additionally, continuous altitude data from the shuttle radar topography mission (SRTM) (Farr et al. 2007) were used. Categorical spatial landtype data for soils (ARC-ISCW 2003), WWF ecoregions (Olson et al. 2001) and vegetation types (Mucina and Rutherford 2006) were used as predictor variables (Table 2.3) in the models. All the above environmental data were used at the same spatial grid resolution of 30 arc seconds. The logistic models converged after 1000 maximum iterations, with a regularization multiplier of 1, and models calibrated using auto features. Subsamples were created using 25% of the dataset. Area under the Receiver Operating Characteristic (ROC) curve (AUC) was used to test model performances in defining suitable habitats. Where AUC = 0.5, the model is not different from random distributions; where AUC is closer to 1, the model fits the data more closely. An AUC value > 0.7 indicated suitability of the curve to the data and thus that the models were useful (Liu et al. 2005, Freeman and Moisen 2008), and AUC > 0.9 is considered highly significant (Swets 1988). The contribution of the variables to the model was determined by jackknife analyses of variable importance in MaxEnt. This removes each variable in turn and tests the variable alone to determine its relative and absolute contribution to the model (Elith et al. 2011).

Table 2.2: Bioclimatic variables downloaded from WorldClim (http://www.worldclim.org/bioclim) for use in initial model development.

<table>
<thead>
<tr>
<th>Code</th>
<th>Data contained</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio1</td>
<td>Annual mean temperature</td>
<td>Mean monthly difference between max and min temp.</td>
</tr>
<tr>
<td>Bio2</td>
<td>Mean diurnal range</td>
<td>(Bio2/Bio7) x 100</td>
</tr>
<tr>
<td>Bio3</td>
<td>Isothermality</td>
<td>Standard deviation x 100</td>
</tr>
<tr>
<td>Bio4</td>
<td>Temperature seasonality</td>
<td>Bio5 – Bio6</td>
</tr>
<tr>
<td>Bio5</td>
<td>Maximum temperature of warmest month</td>
<td></td>
</tr>
<tr>
<td>Bio6</td>
<td>Minimum temperature of coldest month</td>
<td></td>
</tr>
<tr>
<td>Bio7</td>
<td>Annual temperature range</td>
<td></td>
</tr>
<tr>
<td>Bio8</td>
<td>Mean temperature of wettest quarter</td>
<td></td>
</tr>
<tr>
<td>Bio9</td>
<td>Mean temperature of driest quarter</td>
<td></td>
</tr>
<tr>
<td>Bio10</td>
<td>Mean temperature of warmest quarter</td>
<td></td>
</tr>
<tr>
<td>Bio11</td>
<td>Mean temperature of coldest quarter</td>
<td></td>
</tr>
<tr>
<td>Bio12</td>
<td>Annual precipitation</td>
<td></td>
</tr>
<tr>
<td>Bio13</td>
<td>Precipitation of wettest month</td>
<td></td>
</tr>
<tr>
<td>Bio14</td>
<td>Precipitation of driest month</td>
<td></td>
</tr>
<tr>
<td>Bio15</td>
<td>Precipitation seasonality</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>Bio16</td>
<td>Precipitation of wettest quarter</td>
<td></td>
</tr>
<tr>
<td>Bio17</td>
<td>Precipitation of driest quarter</td>
<td></td>
</tr>
<tr>
<td>Bio18</td>
<td>Precipitation of warmest quarter</td>
<td></td>
</tr>
<tr>
<td>Bio19</td>
<td>Precipitation of coldest quarter</td>
<td></td>
</tr>
<tr>
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<td>Environmental variable</td>
<td>Data contained</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Continuous</td>
<td>Bioclimatic variables</td>
<td>Temperature and precipitation ranges</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Altitudinal gradients</td>
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<tr>
<td>Categorical</td>
<td>Altitude</td>
<td>Distribution of soil classes</td>
</tr>
<tr>
<td></td>
<td>Landtype</td>
<td>Distribution of vegetation types and biomes</td>
</tr>
<tr>
<td></td>
<td>Vegetation type</td>
<td>Dominant species assemblages</td>
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<tr>
<td></td>
<td>Ecoregions</td>
<td></td>
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</tbody>
</table>

### 2.1.3 Spatial analyses

Using ArcMap™ version 10.1 (ESRI 2012), a map was drawn using projected coordinate system PCS WGS 1984 UTM 35S that corresponds to the study area. Initial model outputs were converted to raster format as a float output data type. The raw outputs presented (Chapter 3, Figure 3.2) were used to determine the lowest presence threshold (LPT) for each taxon, i.e. the lowest predicted probability of occurrence that intersects with an occurrence record used for model training. Taxon-specific LPTs were applied as a selection criterion for the threshold to reclassify (spatial analyst toolbox) the continuous dataset into binary presence and absence predictions. Raster calculator (spatial analyst toolbox) was used to produce a sum of all taxa distributions (Figure 3.3). This species richness map highlighted hotspot areas within the study region most likely to have golden moles, and was used to direct ground-truthing effort and intensity (Table 2.4). Areas of high richness were sampled most intensively, whereas areas of low richness were searched for signs of activity and less effort placed on trap-setting.

### 2.2 Ground-truthing the models

The resultant MaxEnt models highlighted areas with a high probability of occurrence of one or more golden mole taxa (see Figure 3.3). The validity of the models was tested during seven austral spring/summer months of ground-truthing (October 2013-April 2014); new records were being used to subsequently revise the existing database and refine the predictive models.
2.2.1 Study area

The Mpumalanga Province in South Africa borders Mozambique and Swaziland onto the east and Gauteng on the west. Based on the MapLibrary shapefile of Mpumalanga, projected onto a Universal Transverse Mercator (UTM) at 35 S latitude, Mpumalanga spans over 76 735 km$^2$, 6.3 % of South Africa's land area. The temperate vegetation is dominated by high plateau grassland spanning 49 878 km$^2$ (Lötter et al. 2014). The Drakensberg region of the eastern escarpment, where afromontane forests and grasslands transition into the grassland-savannah vegetation of the lowveld, forms the eastern border of the Mpumalanga grasslands. Substrates are dominated by sandy and loamy soils, with intermittent utisols (red clays) and vertisols (black clays) (Dijkshoorn et al. 2008, Jackson et al. 2008a). The region generally experiences warm, wet summers and mild, dry winters, and coupled with the buffering capacity of soil and leaf litter, there is little temperature fluctuation in the underground microhabitat (Jackson et al. 2008b).

Mpumalanga has a complex road and railway infrastructure; with the coal-mining industry centred in eMalahleni, and twelve coal-fired power stations (Eskom 2013) that form the hub of South Africa’s power generation network. The agricultural sector includes a large dairy industry in Standerton, as well as tropical fruit and sugar production in Piet Retief and Malelane. Another major agricultural product is wool, based in Ermelo, close to the forestry region of Sabie (GCIS 2014).

2.2.2 Field surveys

Quarter degree squares (QDS) were used as basic geographic units for regional site selection. These grid cells represent $1/16$ of a degree square and have a dimension of $\sim 27 \times 24$ km and an area of 648 km$^2$ (Figure 2.1). Targeted QDS were selected based on suitable habitat; maximal geographic coverage and ease of access, within 90 QDS of the Highveld, escarpment.

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1 http://www.mapmakerdata.co.uk.s3-website-eu-west-1.amazonaws.com/library/stacks/Africa/South%20Africa/Mpumalanga/index.htm
and partially into lowveld in Mpumalanga, hence covering an area of 540 km² (see Chapter 3, section 3.2). QDS were surveyed with effort intensity (Figure 3.3) according to their species richness (Table 2.4) and resultant on-site activity. Each QDS was driven through to search for potential golden mole habitat. Local golden mole sites were then surveyed on foot in at least six 1 km² sites within each QDS for observable activity in the form of subsurface foraging tunnels and fresh mole hills (Figure 2.2). Survey effort was concentrated in areas of high predicted species richness, where trapping was done for 60 to 90 trap nights (up to thirty traps were placed per night) per QDS, or until an individual was captured (Table 2.4). In medium richness areas, less survey effort (30-60 trap-nights) was placed on trap setting, and more on covering as much ground as possible to locate observable activity (Figure 2.2). In low richness areas, survey effort was focused predominantly on surveying for activity (Table 2.4).

Figure 2.1: The Quarter Degree Squares (QDS) geographic system represents 1/16th of a grid cell. The cell reference used for the above shaded QDS is 2830AA, and represents 27 x 24 km. Adapted from the Avian Demography Unit (2014) http://web.uct.ac.za/depts/stats/adu/qdgc.htm.

Table 2.4: The survey effort intensity method used was based primarily on the species richness map of the initial models (Figure 3.3).

<table>
<thead>
<tr>
<th></th>
<th>Number of QDSs</th>
<th>Survey area (6 x 1 km²)</th>
<th>Trap nights</th>
</tr>
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<tbody>
<tr>
<td>High</td>
<td>20</td>
<td>120</td>
<td>60-90</td>
</tr>
<tr>
<td>Medium</td>
<td>43</td>
<td>258</td>
<td>30-60</td>
</tr>
<tr>
<td>Low</td>
<td>27</td>
<td>162</td>
<td>0-30</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>90</strong></td>
<td><strong>540</strong></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.2: Signs of golden mole activity included fresh heaps 10-15 cm in diameter (left) and subsurface foraging tunnels (right).

Modified Hickman live traps (Figure 2.3; taken from Hickman 1979) consist of a PVC pipe sealed at one end and a metal trap door on the other. These traps are common in studies involving small fossorial mammal trapping as they cause little distress to the animals. Once fresh activity was located and landowner permission sought, the tunnel was excavated carefully using shovels and trowels. Each day, up to 30 traps were laid out carefully in fresh tunnels, often baited with earthworms, insects or pieces of lamb kidney, and checked twice daily each morning and afternoon (Figure 2.4). Once captured, each animal was immediately placed into a 20 l bucket with 20 cm of soil and allowed to rest in the shade. Earthworms, beetles, larvae and chopped lamb kidney were provided ad libitum and if necessary, the soil was dampened with cool water (see further guidelines on captive mammals: Sikes et al. 2011). All animal capture, holding and euthanasia procedures were approved by the Science Faculty Animal Ethics Committee of the University of Cape Town (clearance number: 2013/V14/GNB), and authorized by a permit (MPG5366) issued by the Mpumalanga Tourism and Parks Agency.
Given possible sympatry of *Amblysomus* cryptic taxa, a maximum of three live *Amblysomus* per QDS were collected and prepared as voucher specimens to confirm their species identity using variation in mtDNA sequences. Any pregnant or lactating females were released immediately at capture sites without further processing. Specimens were euthanized using Halothane and dissected to collect tissues (heart, liver, kidney, muscle, gut, reproductive tract).
that were stored in 1.5 ml cryostorage Nunc® vials containing tissue buffer or 96% EtOH. Whole specimens were then frozen at -4 °C (when possible) and prepared for deposition in the Ditsong National Museum of Natural History. These voucher specimens are thus available to future researchers who may wish to verify species identifications and distribution records.

Where fresh golden mole activity was found, but yielded unsuccessful trapping, GPS points were recorded into an independent activity database. These points served as a means of refined model evaluation test file (see Section 2.4., below.) These points were plotted in relation to the Great Escarpment rim and mountain catchment areas (MCA) along with points where specimens were obtained successfully, to determine whether the ranges of golden mole taxa are possibly constrained by these physiographic parameters (see Figure 3.4).

2.3 Genetic analyses

The tissue samples collected in the field were processed in the MEEP lab (Department of Genetics, University of Pretoria). Under the supervision of Prof. P. Bloomer, Ms S. Mynhardt and Dr S. Maree, sequences of mtDNA cytochrome- b were used as proxies for cryptic taxon delimitation within Amblysomus.

2.3.1 DNA extraction and PCR amplification

The QIAGEN DNeasy® Blood and Tissue Kit was used to extract DNA from heart samples (Table 2.5). Tissues were lysed and homogenised using 20 mg heart tissue and 180 µl Buffer ATL and 20 µl Proteinase K. Samples were then vortexed thoroughly before, during and after incubation at 55 °C for 5-10 hours until solutions were clear. To remove RNA, 16 µl RNase was added to each tube and incubated for 2 minutes at room temperature before vortexing.
Table 2.5: DNA was extracted according to the following protocol (adapted from QIAGEN DNeasy® Blood and Tissue Handbook [http://mvz.berkeley.edu/egl inserts/DNeasy_Blood_&_Tissue_Handbook.pdf]

<table>
<thead>
<tr>
<th>Action</th>
<th>Reagent/s</th>
<th>Volume (µl)</th>
<th>Centrifuge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyse cell membrane</td>
<td>Buffer AL</td>
<td>200</td>
<td>8000 rpm x 1 min</td>
</tr>
<tr>
<td></td>
<td>96% EtOH</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Remove impurities</td>
<td>Buffer AW1</td>
<td>500</td>
<td>8000 rpm x 1 min</td>
</tr>
<tr>
<td></td>
<td>Buffer AW2</td>
<td>500</td>
<td>13000 rpm x 3 min</td>
</tr>
<tr>
<td>Elute DNA</td>
<td>Buffer AE*</td>
<td>200</td>
<td>8000 rpm x 1 min</td>
</tr>
</tbody>
</table>

* This step was repeated to extract all remaining DNA from the filter membrane.

To visualise the genomic DNA present in the elutions (indicative of a successful extraction process), agarose gel electrophoresis was carried out using 2 % agarose gel run at 100 V for 30 minutes and viewed on a UV Gel Dock. Each well contained 2 µl of either DNA or 100 bp molecular weight ladder and 2.5 µl GelRed™ loading dye. To test DNA quantity and quality, the brightness of the band was matched against the known concentration of the size markers.

Successful elutions were then subjected to polymerase chain reactions (PCR) to amplify the diagnostic cytochrome-b genes. Each 20 µl reaction, containing a volume of DNA (x), reagents and a volume of distilled water (y), was pipetted into a 1.5 ml microcentrifuge tube whilst on ice (Table 2.6). Reaction tubes were incubated in a thermal cycler to denature DNA at 94 ºC (4:00), followed by 25 cycles of 94 ºC (0:30), annealing of primers at 50 ºC (0:30) and fragment extension by the free nucleotides at 72 ºC (0:20); with a final extension at 72 ºC (20:00). Agarose gel electrophoresis was conducted to ensure that: i) the PCR amplified the correct sequence as indicated by a bright band at the 1143 bp position; ii) there was no non-specific binding, as indicated by ‘shadow’ bands; and iii) the DNA was not over- or under-concentrated, as indicated by genomic DNA was stuck in the wells or no band present, respectively. If the amplification was not successful, PCRs were rerun and visualised using $T^{\circ}_{\text{anneal}} = 52$ ºC and $\text{MgCl}_2 = 1.5$ µl is more stringent to correct for i) and ii) above; or $T^{\circ}_{\text{anneal}} = 50$ ºC and $\text{MgCl}_2 = 2.5$ µl is less stringent to correct for iii) above.
Table 2.6: PCR reactions were made up using the following reagent volumes.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>x</td>
</tr>
<tr>
<td>Buffer</td>
<td>2.5</td>
</tr>
<tr>
<td>MgCl(_2) (stock 25 mM)</td>
<td>1.5</td>
</tr>
<tr>
<td>dNTP (stock 2 mM)</td>
<td>2.5</td>
</tr>
<tr>
<td>Forward Primer 14724 (25 pmol)</td>
<td>0.5</td>
</tr>
<tr>
<td>Reverse Primer 15915 (25 pmol)</td>
<td>0.5</td>
</tr>
<tr>
<td>SuperTherm™ Taq Polymerase (5 units)</td>
<td>0.15</td>
</tr>
<tr>
<td>dH(_2)O</td>
<td>y</td>
</tr>
</tbody>
</table>

DNA was purified by precipitating after PCR. To each PCR product tube, 10 µl dH\(_2\)O, 2 µl 3 mM NaAc and 70 µl 100 % EtOH was added and centrifuged at 13 000 rpm x 30 min and the supernatant carefully discarded via micropipetting. Following this, 90 µl 70 % EtOH was added and centrifuged at 13 000 rpm x 20 min. The supernatants were discarded and the tubes dried on a 55°C dry bath x 20 min. The dry pellets were re-suspended in 15 µl of d\(_2\)H\(_2\)O and visualised on agarose gel to ensure all non-specific amplification had been removed.

Samples underwent separate cycle sequencing with the forward and reverse primers using quarter reactions (2 µl Big Dye, 2 µl Buffer, 1 µl 3.2 pm primer, 2 µl DNA and 3 µl d2H\(_2\)O into each tube to make up a 10 µl reaction). Products were precipitated in 2 µl NaAc and 70 µl 100 % EtOH, centrifuged at 9 600 rpm x 30 min and the supernatant discarded. Following this, 90 µl of 70 % EtOH were added and centrifuged at 8 600 rpm x 15 min. The supernatant was discarded and the dried pellets stored at 4 °C.

2.3.2 Cytochrome-b sequencing and analysis

Reaction tubes containing the final forward and reverse sequence pellets were sent to the DNA Sequencing of the Faculty of Natural and Agricultural Sciences, at the University of Pretoria. The resulting DNA chromatograms were uploaded for the MEEP Lab group on http://seqserve.bi.up.ac.za/, CLC Bio Main Workbench v.7.0.3.64 (CLC Inc, Aarhus, Denmark, http://www.clcbio.com) was used to create contiguous sequences and imported to Clustal Omega software (Sievers et al. 2011) to create alignments to reference sequences (Appendix 1:
genetics references localities) within Chrysochloridae. Following the initial alignment, gaps and other errors were corrected and the sequences realigned and exported for use in Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura et al. 2013). MEGA was used to infer phylogenetic histories using bootstrapped neighbour joining and maximum likelihood trees. Nodes with support > 70 translate to ~ 95 % confidence limit (Hillis and Bull 1993). The sequences were then imported into TCS (Clement et al. 2000) to infer allele networks based on statistical parsimony, i.e. showing the minimum number of alleles connecting groups of individuals.

2.4 Model refinement

Genetic species identifications based on DNA sequences (Section 2.3 and Table 3.2) were assigned to each caught specimen and used to revise the existing occurrence record database (Section 3.4). MaxEnt models were re-calibrated to produce conservative and maximally accurate estimations of possible golden mole presences based on the extremely limited number of records available, for the purpose of informing future conservation management practices.

Based on the works of Merow et al. (2014), simple models based on small sample sizes are suitable for predicting SDM ranges but potentially including bias when applied over a large spatial extent, which increases the likelihood of spatial autocorrelation, especially if based on interpolated environmental data. Whilst spatial autocorrelation may pose a concern, MaxEnt is robust against this and manual calculations were run in Microsoft Excel to select environmental variables with no strong correlation (see 2.4.1 Variable Selection). Hence, the criteria posed by Merow et al. (2014) match with the occurrence database for the four Amblysomus taxa and associated predictor variables, and were hence used to calibrate simple models. Simple models are conservative and easier to control and adapt, and the effect of distribution drivers on the occurrence pattern is easier to interpret (Elith et al. 2006). Model analysis by way of response
curves sheds light on how the model was derived and how possible environmental predictors influence occurrence probability (i.e. a niche characterisation), whereas the projection onto a specified geographical area (in this case, Mpumalanga) limits the potential predicted distribution (i.e. shows the species range within this province).

To create reliable models, the following issues were addressed and are discussed below: (i) variable selection (spatial autocorrelation, biologically-relevant variables and resolution limits of categorical variables); (ii) selection of a background to which model predictions were limited; (iii) comparison of single-run non-repeated models versus five-fold cross-validation models; (iv) model evaluations using area under the curves (AUC) of the receiver operating characteristic (ROC), response curves, MaxEnt’s internal jackknife of variable importance to model training and the jackknife of localities approach (Pearson et al. 2007), and omission rates using an independent activity database.

2.4.1 Variable selection

The process involved thirty three trial iterations with differing environmental variable and background selections, and including some uncorrected occurrence record data, viz.: (i) initial cyt-b analyses which offered an unconfirmed first approximation of the identity of two caught specimens (A. h. longiceps: CR23; A. h. meesteri: CR1, see Table 3.2), and (ii) specimens of dubious species affiliation (A. robustus: Malelane); with subsequent model evaluations.

The first step was ensuring the most suitable predictor variables were selected for each taxon and to optimize predictive power of the model. Whilst the initial models were based on all nineteen bioclimatic variables (Table 2.2) and three categorical variables (Table 2.3), variable selection for the refined models was restricted to those environmental predictors that were deemed most reliable and biologically-relevant. Categorical variables, in particular, should be used with caution due to possible spatial autocorrelation, lack of resolution, or as is the case with global data, inaccurate/outrated databases (Elith and Leathwick 2009, Williams et al. 2009).
Equifinality is the phenomenon describing the many potential paths that lead to a common state (Beven and Freer 2001). In the case of species distribution models (SDMs), this is interpreted as the ability of an environmental predictor suite to correctly predict the species distribution in question; i.e. if the selected predictor variables do not contain sufficient information related to the species’ range limits, the models will not reflect the true distribution (Dormann et al. 2012). Conversely, introducing too many potential predictors (over-parameterization) can lead to biased model outputs as a result of over-fitting (Araújo and Guisan 2006, Jiménez-Valverde et al. 2008), whereby pseudo-relationships are inferred from the environmental variables, falsely limiting the prediction.

Only variables that contributed to each model were included to reduce potential noise and false positives (commissions errors) and negatives (omission errors) by over- or under-predicting, respectively (Elith et al. 2006, Phillips et al. 2006, Pearson 2007). This ensured that model predictions were determined by variables upon which the taxon is dependent. For example, if the variable *average day-length* measured at background points (any grid cell within the study area not occupied by an occurrence point) has the same value as at an occurrence point, then all background points sharing this value could be potential habitat and have a high probability of occurrence. If the background point does not share the same *average day-length* value range as an occurrence point, the models would disregard the distal and indirect influence of *average day-length* on occurrence. Biological knowledge of the species and its environment aids in the variable selection (Williams et al. 2012). In these cases, the prediction pattern of the MaxEnt output is examined for biogeographic imprecision; i.e. whether the high probability areas (fundamental niche areas) are plausible given the current species distribution (occupied niche areas) (Araújo & Guisan, 2006; Hutchinson, 1957, Pearson, 2007). Upon inspection of the initial models and subsequent refined model trials, altitude was excluded from the environmental suite as it is unlikely to have any direct influence on the distribution of any of the four *Amblysomus* taxa (Robertson pers. comm. 2015).
Furthermore, several factors driving community assembly are not included in the models because they require in-depth knowledge of the behaviour (e.g. inter- and intra-specific competition), historical dispersal (e.g. Founder effects) and present dispersal (e.g. ecological corridors and habitat fragmentation), data for which are often unavailable or preliminary (Perault and Lomolino 2000, Haddad et al. 2003, Kamffer 2004, Cavender-Bares et al. 2009, Peterson et al. 2013).

Seven of the original 11 variables that were associated with ambient temperatures (annual mean temperature (Bio 1); mean diurnal range (Bio 2); isothermality (Bio 3); temperature seasonality (Bio 4); annual temperature range (Bio 7); mean temperature of wettest quarter (Bio 8); mean temperature of driest quarter (Bio 9)) were omitted as the golden moles studied are mainly nocturnal (Bronner 2013) and occupy a thermally-buffered subterranean ecotype (Adhikari et al. 2014, Murphy 2014). Further, soil organic carbon content (SOCC) at the soil-surface interface is inversely proportional to bulk density (soil dry mass per unit volume), which increases thermal conductivity and diffusivity, thereby insulating the subsurface soil environment from aboveground temperature and precipitation variations (Adhikari et al. 2014, Murphy 2014). All *Amblysomus* taxa in the study area were obtained from substrates with medium-high SOCC > 2% (0.6 mm) (Figure 2.5 C), and so may benefit from thermal buffering even at the soil-surface interface when they forage in subsurface tunnels. In deeper permanent tunnels (20 - 30 cm), where nests are located, temperature variability is likely to be minimal (Poudel et al. 2012). Consequently, only four variables (maximum temperature of warmest month (Bio5); minimum temperature of coldest month (Bio6); mean temperature of warmest quarter (Bio10); mean temperature of coldest quarter (Bio11)) that summarize the extremes of surface ambient temperatures were selected as bioclimatic predictor variables likely to summarize their buffered thermal milieu.

Of the eight precipitation variables originally used, four (annual precipitation (Bio12); precipitation of wettest month (Bio13); precipitation of driest month (Bio14); precipitation
seasonality (Bio15)) were omitted from analyses as these variables summarize variations in surface precipitation that are likely buffered by the water-holding capacities of the loamy soils that the targeted chrysochlorid taxa prefer (Bronner 2013). Consequently, the remaining four precipitation variables (precipitation of wettest quarter (Bio16); precipitation of driest quarter (Bio17); precipitation of warmest quarter (Bio18); precipitation of coldest quarter (Bio19)) that likely summarized seasonal changes in soil moisture availability were selected as bioclimatic predictors for further analysis. A similar suite of temperature and precipitation predictor variables was used when developing a distribution model for Juliana’s golden mole *N. julianae* (Jackson and Robertson 2010).

The shortlisted continuous bioclimatic predictor variables (temperature: Bio5-6, 10-11; precipitation: Bio16-19) were then subjected to autocorrelation analyses (Table 2.7). All bioclimatic variables except Bio5 (maximum temperature of warmest month) were strongly and significantly autocorrelated for *A. h. meesteri*, which may be attributed to the small sample size comprising of only 10 localities from higher-altitude MCAs likely to have similar climates in the northern (24S30E) and southern (27S30E) escarpment, dominated by undulating kloofs and wetlands respectively. Comparison of bioclimatic variables for the northern escarpment (NE) and southern *A. h. meesteri* populations (Table 2.8) showed that while summer temperatures (maximum temperature (Bio5) and mean temperature of warmest quarter (Bio10)) do not differ markedly, the southern escarpment (SE) population experiences colder winter minima, with differences of up to 23 °C in the minimum temperature of coldest month (Bio6)) and means of up to 3 °C in mean temperature of coldest quarter (Bio11)).

For the other three taxa, the number of strongly correlated variables (> |0.8|) were counted and averaged for sets of temperature variables to select only one variable based on its biological relevance and interactions with remaining variables (Table 2.9). However, correlation coefficients are usually normally distributed only when sample sizes are large (n > 500) Consequently, correlation coefficients (r) were converted to Fisher’s z-transformations (Table
to achieve an approximate normal distribution, and to stabilize the variance (Schulze 2004), thereby reducing any biases compared to using untransformed r values (Silver and Dunlap 1987, Corey et al. 1998). The averages were then back-transformed from Z-to-r and averaged for sets of temperature and precipitation variables as above. This Fisher z-transformation was performed on all taxa; all three taxa except A. h. meesteri; and separately for each of the three taxa except A. h. meesteri to select four suitable continuous bioclimatic variables (Table 2.9). The spatial autocorrelation analysis of the selected environmental predictor variables (Table 2.7) underwent Fisher-z transformation on all taxa, all taxa together except A. h. meesteri and separately for all taxa except A. h. meesteri.

These five averaged matrices support the same conclusions: Bio10-11 and Bio18-19 had fewer correlations (one and three, respectively) than Bio5-6 and Bio16-17 (four and five, respectively). Bio10-11 (mean temperature of warmest quarter and coldest quarter) were thus selected as temperature predictor variables. Though Bio16-17 were more correlated than Bio18-19, average r autocorrelations of wettest and warmest quarter precipitation variables were similar (Bio16 = 0.514, Bio18 = 0.505), whereas those for driest and coldest quarter variables (Bio17 = 0.419, Bio19 = 0.419) were identical. For this reason, coupled with the fact that precipitation in Mpumalanga occurs during the austral summer months when temperatures were highest, Bio16-17 (precipitation of warmest quarter and coldest quarter) were selected.

Categorical variables cannot be tested for spatial autocorrelation and so must be included only if necessary. Soil organic carbon content (SOCC) was not available at the time of initial model refinement but was explored as a potential variable in the model refining process. These data served as a proxy for invertebrate prey density, available as broad categorises, viz.: 1 % (0 - 0.5 mm); 2 % (0.6 - 1 mm); 3 % (1.1 - 2 mm); > 4 % (> 2 mm) (Du Preez et al. 2011). The poor resolution of SOCC and vegetation type data and their indirect effects on golden mole distribution resulted in exclusion from subsequent models. Landtype is the most important distributional driver of golden moles (Jackson and Robertson 2010) and was thus included.
Table 2.7: Correlation coefficients (r) among winter and summer temperature and precipitation variables (Bio 5-6, 10-11, 16-19) across occurrence points for each taxon, calculated in MS Excel. Variables exhibiting correlation coefficients > |0.8| were excluded from the models. $T^\circ$ = temperature; max = maximum; min = minimum; $\bar{x}$ = mean; Ppt = precipitation; qtr = quarter.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>$T_{\text{max}}$</th>
<th>$T_{\text{min}}$</th>
<th>$\bar{x} \ T^\circ$</th>
<th>Ppt wettest</th>
<th>Ppt driest</th>
<th>Ppt warmest</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{min}}$ coldest month</td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{x} \ T^\circ$ warmest qtr</td>
<td>0.9</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{x} \ T^\circ$ coldest qtr</td>
<td>0.4</td>
<td>1.0</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ppt wettest qtr</td>
<td>-0.7</td>
<td>-0.2</td>
<td>-0.6</td>
<td>-0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ppt driest qtr</td>
<td>0.0</td>
<td>0.4</td>
<td>0.1</td>
<td>0.4</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Ppt warmest qtr</td>
<td>-0.7</td>
<td>-0.2</td>
<td>-0.5</td>
<td>-0.2</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Ppt coolest qtr</td>
<td>0.0</td>
<td>0.4</td>
<td>0.1</td>
<td>0.4</td>
<td>0.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 2.8: Differences in ranges of predictor variable values between the northern (NE) and southern (SE) escarpment for $A. h. meesteri$. $T^\circ$ = temperature; max = maximum; min = minimum; $\bar{x}$ = mean; Ppt = precipitation; qtr = quarter.
Table 2.9: Average correlation for each variable set of temperature (Bio 5-6 vs Bio 10-11) or precipitation (Bio 16-17 vs Bio 18-19), based on Fisher Z-back-transformations. Infinite values are an artefact of the asymptotic Z conversion where r=1.

<table>
<thead>
<tr>
<th></th>
<th>Temperature</th>
<th>Precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All taxa</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Z</td>
<td>0.612</td>
<td>1.216</td>
</tr>
<tr>
<td>Back-transform r</td>
<td>0.546</td>
<td>0.838</td>
</tr>
<tr>
<td><strong>Average per set</strong></td>
<td>0.692</td>
<td>0.632</td>
</tr>
<tr>
<td><strong>All taxa except A. h. meesteri</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Z</td>
<td>0.715</td>
<td>0.790</td>
</tr>
<tr>
<td>Back-transform r</td>
<td>0.614</td>
<td>0.658</td>
</tr>
<tr>
<td><strong>Average per set</strong></td>
<td>0.636</td>
<td>0.400</td>
</tr>
<tr>
<td><strong>A. h. longiceps</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Z</td>
<td>0.598</td>
<td>0.615</td>
</tr>
<tr>
<td>Back-transform r</td>
<td>0.535</td>
<td>0.547</td>
</tr>
<tr>
<td><strong>Average per set</strong></td>
<td>0.541</td>
<td>0.357</td>
</tr>
<tr>
<td><strong>A. robustus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Z</td>
<td>0.988</td>
<td>0.714</td>
</tr>
<tr>
<td>Back-transform r</td>
<td>0.756</td>
<td>0.613</td>
</tr>
<tr>
<td><strong>Average per set</strong></td>
<td>0.685</td>
<td>0.379</td>
</tr>
<tr>
<td><strong>A. septentrionalis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Z</td>
<td>0.560</td>
<td>1.041</td>
</tr>
<tr>
<td>Back-transform r</td>
<td>0.508</td>
<td>0.778</td>
</tr>
<tr>
<td><strong>Average per set</strong></td>
<td>0.643</td>
<td>0.444</td>
</tr>
</tbody>
</table>

2.4.2. Background selection

Though MaxEnt is robust to some extent against sampling bias (Phillips et al. 2006, 2009), and occurrence data are often skewed to well-sampled areas (Freitag et al. 1998, Loiselle et al. 2007), spatial bias can and should be corrected for where possible (Reddy and Davalos 2003, Skarpaas and Stabbetorp 2011, Hijmans 2012). While the effects of spatial bias are masked as sample sizes increases, spatial filtering should be employed to reduce omission (false negative) and commission (false positive) errors (Loiselle et al. 2007, Kramer-Schadt et al. 2013). Sample sizes for the four golden mole taxa studied were very small, even with additions from the field surveys (Table 3.3). Roadsides and towns are heavily sampled as spotting activity and setting traps involve low sampling effort in such areas, which explains why many of the records were from near human habitations and infrastructure. Specimens caught in the field survey were found in areas with deciduous forests, woodlands and shrublands that have high primary productivity due to high radiant energy and rainfall levels (Bond and Parr 2010). This is perhaps
an indication of the preference of golden moles for microhabitats containing ground cover and leaf litter under which they can forage safely for invertebrates (Jackson 2007). Very few golden moles have been captured in open grasslands, as the height of golden mole activity period coincides with that of tallest grass length, making sampling vast expanses of grassland impractical. Sampling bias in this study included possible sampling intensity and dispersion biases in the on initial occurrence database (Freitag et al. 1998) and models, and imperfect detection of golden moles due to trapping and genetic identification constraints during the field survey.

To reduce false positives possibly resulting from sampling biases, a method of background selection (Figure 2.5) was used. Kramer-Schadt et al. (2013) have shown that this approach can increase model robustness when tested against an uncorrected sampling bias dataset, though when predictions are weak some high commission errors may be unavoidable (Kramer-Schadt et al. 2013). Background selection of a biogeographically-relevant area reduces the possibility of over-prediction (false-positives: predicts presence in geographic space matching the species’ preferred environmental suite, but which the species does not occupy); and the possibility of omission errors (false-negatives: where an occurrence record falls outside the predicted area). Background selection therefore influences model fit: overly-broad backgrounds tend to fit very tightly and vice versa. To create the background, polygons of four potential backgrounds were selected if they coincided with an occurrence point and exported as a layer with equal environmental parameters as all other variables. Of the possible backgrounds where any of the four golden mole taxa has been confirmed to occur (Figure 2.5), World Wide Fund for Nature (WWF) ecoregions polygons (Olson et al. 2001) was selected because it is wider than known golden mole distributions, but encapsulate large areas where characteristic taxa and environments occur. All environmental predictors were extracted by the ecoregions mask and converted to ASCII. The models trained on this large background were projected onto the study area, i.e. within the provincial boundaries of Mpumalanga.
Specimens deposited at museums often lack spatial resolution and are lumped together in the closest GPS record, e.g. seven *A. h. longiceps* from Glengarry, Rosetta (Table 2.1), making only one record useful for modelling purposes and the rest pseudo-replicates. Sampling bias is generally found in areas that are easily accessible and known to be typical habitat of the target species with little effort on range limit exploration (Reddy and Davalos 2003, Kramer-Schadt et al. 2013). Hence, spatial bias arises when several occurrence records are very close to each other. A locality grid area is then defined to eliminate duplicates at a resolution relevant to the size and mobility of the animal in question, thereby preventing bias where many specimens were obtained from geographically indistinct locations (Table 2.10). While a 16 km$^2$ grid is the standard used for most chrysochlorid taxa by the Afrotheria Specialist Group (IUCN), using this would have excluded more than 50% of records (for *A. h. longiceps*) and this grid size is probably too large given the specialized habitat requirements, low vagility and limited dispersal abilities of golden moles (Bronner 2013). Therefore a 1 km$^2$ grid size was used as the best trade-off between spatial resolution and sample sizes. Even at this small grid size (1 km$^2$), there was a 47% reduction in sample sizes.

**Table 2.10**: Sample size differed from number of records available depending on the spatial grid size used.

<table>
<thead>
<tr>
<th>Species</th>
<th>n(records)</th>
<th>n(1 km$^2$)</th>
<th>n(16 km$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. h. longiceps</em></td>
<td>25</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td><em>A. h. meesteri</em></td>
<td>10</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td><em>A. robustus</em></td>
<td>9</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><em>A. septentrionalis</em></td>
<td>15</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>68</td>
<td>39</td>
<td>35</td>
</tr>
</tbody>
</table>
Figure 2.5: Backgrounds selections investigated for refined MaxEnt models, based on landtypes (A), biomes (B), soil organic carbon content (C) and WWF ecoregion (D) polygons where golden moles have been located.
2.4.3 Model comparisons

Model trials using the selected variables were calibrated to refine liberal and exploratory models (excluding categorical variables) into more conservative and accurate models (including categorical variables). Liberal trials made use of continuous data only and were not constrained by the resolution limits of categorical variables. These models are often useful for uncovering hidden populations (Pearson et al. 2007), as was the case with the initial models pre-ground truthing. However, for cases where stringent conservation matters are the focus, over-prediction resulting from false-positives should not detract from key areas (Pearson et al. 2007). Using the approach outlined below, Conservative trial I (using landtype as the sole categorical variable and four continuous bioclimatic variables) was selected as the most suitable environmental suite for developing the refined MaxEnt models.

While categorical variables have been shown to improve commission rates of MaxEnt models and correctly identify species ranges, they should be used with caution as they create fragmented outputs (Elith and Graham 2009, Rebelo and Jones 2010), and cannot be tested for spatial autocorrelation using conventional methods as the values given to each nomination are arbitrary. Categorical databases with few classes lack the resolution needed to clarify its relationship with the species’ distribution (Slatyer et al. 2013), hence the extensive landtype survey was selected as the resolution was greater than SOCC. Landtype is used here as it is arguably a biologically-relevant distribution driver (Jackson and Robertson 2010).

Table 2.12 outlines the process used, which first tested the effect of single-run non-repeated (1x) models against five-fold cross-validated repeated (5x) models (Liberal I and II, respectively), and then the effects of the inclusion of categorical variables landtype (Conservative I), SOCC (Conservative II) or both (Highly Conservative). Cross-validation splits the occurrence data into training and testing points using k-fold sampling to test the model predictions independently by determining whether the removed test record falls within an area predicted as present by the model, thereby increasing model robustness (Efron and Gong 1983,
Phillips and Dudik 2008). Elith and Leathwick (2009) showed that cross-validation gives the highest AUC value and explains the most deviance compared to other approaches (AIC – Akaike Information Criterion; MARS - Multivariate Adaptive Regression Splines). However, splitting data decreases the sample size available for training considerably with small sample sizes (up to 66 %: A. h. meesteri, Table 2.11), and is more suitable for databases with larger sample sizes. A meta-study has shown that cross-validated models can lead to erroneous conclusions if spatial sorting bias is not quantified by the use of, for example, a null model (Hijmans 2012). They stress the need for data quality and biological relevance in combination with other testing methods to prevent over-extrapolation of any single model (Hijmans 2012). It is therefore preferable to use a cross-validation repetition method in conjunction with a single-run model and additional model evaluation techniques (see below). Table 2.12 shows model performances between single run versus cross-validated models with no categorical variables (liberal I and II, respectively) and trials including the landtype, soil organic carbon content (SOCC) categorical variables (Conservative I and II), or both (Highly Conservative).

**Table 2.11**: Sample size of training and testing repeats used in the liberal single run (liberal I) models versus the 5x cross-validations. *n based on 1 km² localities contained 13 and 9 occurrence records respectively. The n-1 reduction in the models is a limitation of the environmental variable resolution.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample size</th>
<th>Single-run</th>
<th>5x Cross-validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. h. longiceps*</td>
<td>Training</td>
<td>12</td>
<td>9/10</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>-</td>
<td>2/3</td>
</tr>
<tr>
<td>A. h. meesteri</td>
<td>Training</td>
<td>6</td>
<td>4/5</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>-</td>
<td>1/2</td>
</tr>
<tr>
<td>A. robustus*</td>
<td>Training</td>
<td>8</td>
<td>6/7</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>-</td>
<td>1/2</td>
</tr>
<tr>
<td>A. septentrionalis</td>
<td>Training</td>
<td>11</td>
<td>8/9</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>-</td>
<td>2/3</td>
</tr>
</tbody>
</table>

Single-run (1x) and replicated (5x) cross-validated trials (Liberal trials I and II, respectively) were not constrained by categorical variables and were dominated by the mean temperature of the warmest month (Bio 10), indicating that summer temperatures are an important distribution driver or limiter. This is possibly linked to the increased availability of
invertebrate prey in the upper soil layers during the rainy season when soils are moist and cool under more dense vegetation (Choi et al. 2002, Ivask et al. 2006, Jackson 2007). Though there is no direct evidence for the summer activity patterns of golden moles in the Mpumalanga grasslands, the warm (34 °C maximum temperature, WorldClim Bio 5) and wet (682 mm summer precipitation, WorldClim Bio 16) summer conditions differ greatly from the cold (-1.5 °C minimum temperature, WorldClim Bio 6) and dry (13 mm winter precipitation, WorldClim Bio 17) winters, and this differential could influence invertebrate prey densities in the upper soil layers, making golden moles more difficult to detect. For most trials, mean temperature of the coolest month and precipitation of the driest quarter (Bio 11 and 17, respectively) did not contribute substantially to the models, indicating that the distributions of the four taxa are not markedly influenced by winter temperatures and precipitation; this seems plausible given that invertebrate prey density decreases and remaining prey items burrow into deep soil horizons to overwinter and pupate where soil temperature is buffered against surface fluctuation and retains moisture (Choi et al. 2002).

With the introduction of a categorical landtype variable (Conservative I), models were constrained to a smaller geographical range more likely to represent golden mole habitat preferences, poor vagility and dispersal habits (Chapter 1, Section 1.2), despite average AUC values being reduced from 0.94 in the liberal trials to 0.90 (Table 2.12). These landtype categorical data were collected over 12 years and include 16 179 entries on pH, texture, degree of weathering, and for the purpose of this study, soil type (e.g. granites, clays and dolerites) available at 1:250 000 (ARC–ISCW 2003). With soil parameters potentially playing such a large role in golden mole feeding and possibly breeding and dispersal, it is likely that landtype is an important distribution driver, as found by Jackson and Robertson (2010) for N. julianae.
Table 2.12: Summary of model trials run with continuous variables (Bio 10-11; 16-17) in the absence of categorical variables (Liberal models) using both single-run (1x) versus cross-validated (5x) options, and single-run Conservative models employing either or both landtype and SOCC categorical variables. Conservative I model (shaded) was selected as the most appropriate method.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Species</th>
<th>AUC</th>
<th>Realistic prediction</th>
<th>Response curves</th>
<th>Top contributing variable, %</th>
<th>Low contributing variables &lt;1 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liberal I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 categorical variables; 1x</td>
<td>A. h. longiceps</td>
<td>0.921</td>
<td>No</td>
<td>Yes</td>
<td>Bio10, 63</td>
<td>Bio17</td>
</tr>
<tr>
<td></td>
<td>A. h. meesteri</td>
<td>0.983</td>
<td>Yes</td>
<td>Yes</td>
<td>Bio10, 72</td>
<td>Bio11, 17</td>
</tr>
<tr>
<td></td>
<td>A. robustus</td>
<td>0.873</td>
<td>No</td>
<td>Yes</td>
<td>Bio10, 77</td>
<td>Bio11, Bio17</td>
</tr>
<tr>
<td></td>
<td>A. septentrionalis</td>
<td>0.973</td>
<td>No</td>
<td>Yes</td>
<td>Bio10, 80</td>
<td>-</td>
</tr>
<tr>
<td>Liberal II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 categorical variables; 5x</td>
<td>A. h. longiceps</td>
<td>0.917</td>
<td>No</td>
<td>Yes</td>
<td>Bio10, 70</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>A. h. meesteri</td>
<td>0.979</td>
<td>Yes</td>
<td>Yes</td>
<td>Bio10, 74</td>
<td>Bio11, Bio17</td>
</tr>
<tr>
<td></td>
<td>A. robustus</td>
<td>0.884</td>
<td>Yes</td>
<td>Yes</td>
<td>Bio10, 76</td>
<td>Bio11</td>
</tr>
<tr>
<td></td>
<td>A. septentrionalis</td>
<td>0.967</td>
<td>No</td>
<td>Yes</td>
<td>Bio10, 89</td>
<td>Bio11</td>
</tr>
<tr>
<td>Conservative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Landtype; 1x</td>
<td>A. h. longiceps</td>
<td>0.999</td>
<td>Yes</td>
<td>Yes</td>
<td>Landtype, 95</td>
<td>Bio11, Bio17</td>
</tr>
<tr>
<td></td>
<td>A. h. meesteri</td>
<td>0.999</td>
<td>Yes</td>
<td>Yes</td>
<td>Landtype, 76</td>
<td>Bio11, Bio17</td>
</tr>
<tr>
<td></td>
<td>A. robustus</td>
<td>0.999</td>
<td>Yes</td>
<td>Yes</td>
<td>Landtype, 94</td>
<td>Bio11, Bio17</td>
</tr>
<tr>
<td></td>
<td>A. septentrionalis</td>
<td>0.997</td>
<td>Yes</td>
<td>Yes</td>
<td>Landtype, 80</td>
<td>Bio11</td>
</tr>
<tr>
<td>Conservative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOCC; 1x</td>
<td>A. h. longiceps</td>
<td>0.823</td>
<td>No</td>
<td>Yes</td>
<td>Bio11, 43</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>A. h. meesteri</td>
<td>0.972</td>
<td>Yes</td>
<td>Yes</td>
<td>SOCC, 75</td>
<td>Bio11</td>
</tr>
<tr>
<td></td>
<td>A. robustus</td>
<td>0.854</td>
<td>No</td>
<td>Yes</td>
<td>SOCC, 78</td>
<td>Bio11, Bio17</td>
</tr>
<tr>
<td></td>
<td>A. septentrionalis</td>
<td>0.936</td>
<td>Yes</td>
<td>Yes</td>
<td>SOCC, 60</td>
<td>Bio11</td>
</tr>
<tr>
<td>Highly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>conservative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Landtype &amp; SOCC; 1x</td>
<td>A. h. longiceps</td>
<td>0.780</td>
<td>Yes</td>
<td>Yes</td>
<td>Landtype, 90</td>
<td>Bio16, 17</td>
</tr>
<tr>
<td></td>
<td>A. h. meesteri</td>
<td>0.987</td>
<td>Yes</td>
<td>Yes</td>
<td>Landtype, 58</td>
<td>Bio11, Bio17</td>
</tr>
<tr>
<td></td>
<td>A. robustus</td>
<td>0.900</td>
<td>Yes</td>
<td>Yes</td>
<td>Landtype, 78</td>
<td>Bio 10, 11, 16, 17</td>
</tr>
<tr>
<td></td>
<td>A. septentrionalis</td>
<td>0.926</td>
<td>Yes</td>
<td>Yes</td>
<td>Landtype, 73</td>
<td>Bio11, 16, 17</td>
</tr>
</tbody>
</table>

Two other conservative trials were run using SOCC as a variable on its own (Conservative trial II) and in conjunction with landtype (Highly Conservative). While this variable can be seen as a proxy for invertebrate prey density due to the role invertebrates play in decomposition, nutrient cycling and soil organic matter accumulation (Rawlins et al. 2007, Corsi et al. 2012), the low-resolution interpolated database available in only four broad categories/states (Figure 2.5) made the outputs questionable (Merow et al. 2013); these data were hence used only in the post-modelling spatial analyses. Further investigation of this variable is recommended should the full raw continuous SOCC dataset (as used by Du Preez et al. (2011)) become available.

Results of model trials run using various combinations of environmental predictors and settings, highlights of which are summarized in Table 2.12. The two liberal trials (liberal I and II)
were not constrained by categorical variables and thus compared the effects of single-run (1x) models with no repeats against models using five-fold (5x) cross-validation, whereby models are reiterated five times, splitting occurrence records into training and independent testing data subsets. Though there was no change in model outcomes between the two approaches, the single-run method was opted for as sample sizes were too small to split into training and testing datasets (Pearson et al. 2007); sample size reduced by up to 66 % in very small databases (A. h. meesteri, Table3.8).

2.4.4 Model evaluations

In this study, the single-run models with the categorical landtype variable was opted for mainly due to small sample sizes; and the following methods of evaluation were employed: (i) AUC values analysis; (ii) response curve analysis; (iii) internal MaxEnt jackknife of variable importance; (iv) jackknife test of localities (Pearson et al. 2007) and (v) omission testing using an independent database containing observed activity.

In addition to comparing single-run and the average model of five cross-validated runs, predictive model performance was measured using the area under the curve (AUC) of the receiver operating characteristic (ROC) (Elith et al. 2011). AUC values > 0.7 are indicative of goodness-of-fit of the model to the data, though not necessarily indicative of good model performance (Lobo et al. 2008, Freeman and Moisen 2008). AUC is based on non-dichotomous scales of the raw MaxEnt output and is independent of the selected threshold type, e.g. minimum training presence. This method is useful in comparing different models (Allouche et al. 2006), but it compares presences with background data, not true absences (Merow et al. 2014). In cases concerning conservation, such as those presented here, the non-dichotomous scales were transformed into dichotomous (presence-absence) using the lowest presence threshold. To assess the accuracy of these transformed models, the True Skill Statistic (TSS) method (Allouche et al. 2006) calculates sensitivity and specificity, thereby quantifying the omission and commission rates respectively. This is similar to the Kappa method (Cohen 1960), but more
useful for models of rare species because it is not dependent on prevalence (Allouche et al. 2006). Unfortunately, however, independent validation data (presence and absence points) required for the TSS method was not available for the golden mole data used here, hence only AUC was used. AUC values for all taxa were significant (0.997 – 0.999), and showed realistic predicted geographic distributions with simple response curves (Table 2.12). AUC is the most commonly reported accuracy statistic of SDMs (Kharouba et al. 2013), with several studies reporting AUC with no Kappa or TSS (Thuiller et al. 2006, Vega et al. 2010, Calkins et al. 2012, Bland et al. 2014, Taylor et al. 2015).

Response curves outline the response of the occurrence probability predicted by the model against each environmental predictor variable when all other variables are at their mean or median (Merow et al. 2014). Both simple and complex models can show broad-scale patterns as well as underlying mechanisms that drive the patterns (Evans et al. 2013). Unimodal and smooth bell-shaped response curves are indicative of simple models, where occurrence is limited by both extremes of a particular variable, e.g. golden moles caught in this study are found in areas with summer temperatures between 15 °C to 27 °C, and are less likely to occur outside this summer temperature range. While this generality allows for broad-scale pattern interpretation, it also runs the risk of oversimplifying models if all potential distribution drivers are not considered. Complex multivariate curves with jagged edges are indicative of model complexity, and imply that fitness increases in an unlikely pattern in relation to a predictor (Merow et al. 2014). These signals of complex models are often weak or confounded by sampling bias, noise or autocorrelation, making them more difficult to interpret, extrapolate and adapt to new data (Merow et al. 2014). In the case of *A. h. meesteri*, which occupies a small geographic range that can be sampled exhaustively in the future, a complex model could potentially shed light on the exact factors underlying this taxon’s distribution. As it currently stands, the sample size for this taxon is insufficient for complex modelling, and the use of interpolated data would render model accuracy questionable.
MaxEnt's internal jackknife of variable importance (Figure 2.6) and percentage contribution (Table 2.12) quantifies the influence of each variable on model robustness. The jackknife removes one variable per iteration and calculates the training gain; higher gain is representative of greater fit of the model to the data. All models have high training gain (A. h. *longiceps*: 2.782; A. h. *meesteri*: 3.148; A. *robustus*: 2.774; A. *septentrionalis*: 2.834), and this is attributed mainly to the addition of the landtype categorical variable (75.7 – 94.5 % contribution). Landtype contains the most useful information that is not present in other environmental predictors. Mean temperature of warmest quarter (Bio 10) and precipitation of wettest quarter (Bio 16) contribute more to all models than mean temperature of coolest quarter (Bio 11) and precipitation of driest quarter (Bio 17), indicating that summer variables affect the training gain more than winter variables. Bio 11 and Bio 17 did not contribute (< 1 %) to A. h. *longiceps*, A. h. *meesteri* and A. *robustus*, and Bio 11 did not contribute to A. *septentrionalis*. The above results are evidence that the simple models calibrated with these four continuous and one categorical variable are well-fitted and robust.

Since the selected single-run method is not as robust as cross-validation, models were evaluated using the jackknife of localities approach (Pearson et al. 2007) To validate the models, the occurrence data for each species was split into training (n-1) and test (removed n) data to create (n) datasets for each species. This method tests the models’ capability of correctly predicting the presence of any test record. To reduce spatial bias, only one record per species in a locality was selected for modelling. The Afrotheria Specialist Group (IUCN) uses a locality grid of 16km² for Red Data Assessments for most species; however, this reduced the sample size greatly (Table 2.10). A more realistic yet somewhat arbitrary 1 km² grid was opted for, reflective of the poor vagility and dispersal of golden moles; grid cells this size were used for some chrysochlorid species with few distributional records during the latest IUCN Red List assessments (Bronner pers. comm. 2015).
Figure 2.6: Maxent internal jackknife values to illustrate variable importance in the refined models for each taxon. The increase of training gain is shown without the variable (light blue), as the only variable (dark blue) and with all variables (red). Bio 10 = mean temperature of warmest quarter; Bio 11 = mean temperature of coolest quarter; Bio 16 = precipitation of wettest quarter; Bio 17 = precipitation of driest quarter; LT = landtype.

An R-script (Robertson pers. comm. 2015 - Appendix 4) and methods from the package ‘sp’ (Pebesma and Bivand 2005) were used in R programming software (R Core Team 2013) to create auto-generated databases of (n) localities per species and run in MaxEnt matching the settings of trial Conservative I (Table 2.12) to produce (n) models. The resulting ASCII files were used by the R-script to assign each locality with a presence or absence using the lowest training presence threshold (equivalent to MaxEnt’s minimum presence threshold) for all model runs for each species. A P-value was then calculated for a matrix of (n) runs per species using pValueCompute.exe (Pearson et al. 2007, available online at:
http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2699.2006.01594.x/suppinfo). See schematic diagram of the above process outlined in Figure 2.7.
Figure 2.7: Schematic representation of the jackknife n-1 model evaluation process. Locality records (A) are used to auto-generate model training and test data (B) which are converted to presence/absence based on the lowest presence threshold (C). A P-value is generated based on the frequency of test points correctly predicted as present (D).

Table 2.13: Results of a jackknife model evaluation method (developed by Pearson et al. 2007) run on auto-generated data by an R-script (Robertson pers. comm. 2015).

<table>
<thead>
<tr>
<th>Species</th>
<th>Success rate</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. h. longiceps</em></td>
<td>0.923077</td>
<td>0.000264</td>
</tr>
<tr>
<td><em>A. h. meesteri</em></td>
<td>0.857143</td>
<td>0</td>
</tr>
<tr>
<td><em>A. robustus</em></td>
<td>0.875</td>
<td>0.059146</td>
</tr>
<tr>
<td><em>A. septentrionalis</em></td>
<td>0.818181</td>
<td>0.001412</td>
</tr>
</tbody>
</table>

The results of the jackknife of localities model evaluation (Table 2.13) show significant P-values for all taxa, except *A. robustus* where significance was marginal (p = 0.06). This confirms that the models correctly predicted the n<sup>th</sup> removed testing locality as present at least 81% of the time using the lowest presence threshold (LPT). This threshold converts continuous probability values into binary presence (0) and absence (1) data using the lowest predicted probability at any occurrence point, i.e. if a cell with probability 0.34 coinciding with an
occurrence point is lower than all other cells containing occurrence points, it is the LPT. Upon
inspection of the map (Figure 3.7), models showed an omission rate of zero. Thus, despite low
sample size and bias concerns, appropriate variable and background selections ensured that the
refined models are robust and accurate.

Finally, to further test the accuracy of the model outputs, the activity database containing
GPS points of observed golden mole activity was used to verify the model predictions. The
activity points were overlaid with the four species’ raster MaxEnt predictions. Points were
extracted if they intersected with raster cells containing a probability of occurrence below
species-specific lowest presence thresholds (LPT) per species and for all four species, i.e. if an
activity point is present in an area below the LPT of each or all four species, it is considered an
omission. The omission rate was calculated as the ratio of omitted activity points to the total
number of activity points. *A. h. longiceps* is mainly distributed outside Mpumalanga, and so its
LPT is outside Mpumalanga. *A. h. meesteri, A. robustus* and *A. septentrionalis* are all distributed
predominantly within the study area, along with their respective LPTs. The omission rates per
species are high especially with range-restricted *A. h. meesteri* and *A. robustus* because activity is
not species-specific (Table 2.14). Omission for those activity points which have been omitted
from all four species models was 13.21%, indicating that the models are fairly robust at
predicting areas golden moles are observed to occur, without placing taxon-specific constraints
on habitat selection.

**Table 2.14:** Activity omission rates of refined models used for model evaluations.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>LPT</th>
<th>Omission rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. h. longiceps</em></td>
<td>0.05</td>
<td>26.41</td>
</tr>
<tr>
<td><em>A. h. meesteri</em></td>
<td>0.11</td>
<td>52.83</td>
</tr>
<tr>
<td><em>A. robustus</em></td>
<td>0.10</td>
<td>62.26</td>
</tr>
<tr>
<td><em>A. septentrionalis</em></td>
<td>0.09</td>
<td>32.08</td>
</tr>
<tr>
<td>All taxa</td>
<td>0.05</td>
<td>13.21</td>
</tr>
</tbody>
</table>
2.5 Spatial analyses

Model outputs were imported into ArcGIS Desktop 10.1 (ESRI 2012) for map construction and rendering. The minimum training presence threshold ASCII's created by MaxEnt were imported and converted to an integer raster to represent presence distributions. To assess the predicted species distribution within areas of conservation concern, the lowest presence threshold distributions were cross-tabulated (zonal area tabulation, spatial analyst toolbox) against the categories of environmental (geography and soil), vegetation and conservation layers as (Table 2.15). This identified the percentage area overlap between the thresholded distributional ranges of each taxon and the various layers. Independent layers containing discontinuous polygons are measured as presence-absence states only, and are buffered by one cell size (~ 4.7 km²) to account for raster cell edge cases where the zonal tabulation only selects cells if the cell-centre is present in the polygon. This buffering ensures co-operation with the cell-centre rule of the extraction and is applied consistently in the escarpment, MCA, PAs, conservancies and EC polygon layers. Soil organic carbon content (SOCC), predicted soil loss (PSL), primary productivity (PP), ecoregions, biomes and terrestrial biodiversity assessment (TBA) (Ferrar and Lotter 2007) have polygon classes across the entire study area and were not buffered because this would result in overlap of one or more classes.

Presence thresholds for each species were then extracted according to the mask of these layers and cross-tabulated (zonal area tabulation, spatial analyst toolbox) against the total layer area. Percent area of presence was calculated based on the total area within the layer and the total presence layer. These findings were used to determine the most important bioclimatic factors and current conservation/land-use types associated with the distributions of the different golden mole species, and to assess the areas of occupancy of these species. The resulting distribution maps were used to assess the conservation status of each species, and to
identify areas of high conservation concern, thus facilitating improved environmental strategies in a region and biome disturbed by anthropogenic degradation.

**Table 2.15:** Categorical environmental and conservation/land-use types subjected to spatial analyses to calculate the percent overlap with thresholded species distributions.

<table>
<thead>
<tr>
<th>Group</th>
<th>Layer name</th>
<th>States</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geography and soil</td>
<td>Escarpment (Escarp)</td>
<td>Presence</td>
</tr>
<tr>
<td></td>
<td>Mountain catchment area (MCA)</td>
<td>Presence</td>
</tr>
<tr>
<td></td>
<td>Soil organic carbon content (SOCC)</td>
<td>2 – 4+ %</td>
</tr>
<tr>
<td></td>
<td>Predicted soil loss (PSL)</td>
<td>Very low; low; medium; high; very high</td>
</tr>
<tr>
<td>Vegetation</td>
<td>Ecoregions</td>
<td>Woodlands; Bushveld; Montane grasslands; Highveld grasslands</td>
</tr>
<tr>
<td></td>
<td>Biomes</td>
<td>Grasslands; Forests; Savannah</td>
</tr>
<tr>
<td></td>
<td>Primary production</td>
<td>3 – 10+ t/ha/an</td>
</tr>
<tr>
<td>Conservation</td>
<td>Protected areas (PAs)</td>
<td>Presence</td>
</tr>
<tr>
<td></td>
<td>Conservancies (Cons)</td>
<td>Presence</td>
</tr>
<tr>
<td></td>
<td>Ecological corridors (EC)</td>
<td>Presence</td>
</tr>
<tr>
<td></td>
<td>Terrestrial biodiversity assessment (TBA)</td>
<td>Least concern (LC); protected; highly significant (HS); important and necessary (I&amp;N); irreplaceable; no natural habitat remaining (NNHR)</td>
</tr>
</tbody>
</table>
3.1. Initial model outputs

Thirty-eight genetic and four morphometric reference points for the targeted golden mole taxa (Table 2.1; Figure 3.1) were obtained from museum and MEEP lab records; however, only 25 were usable as the remaining 13 constituted duplicate GPS records using the 1 km² locality grid. The training of initial MaxEnt models used the following number of records per species: A. h. longiceps n = 10; A. h. meesteri n = 5; A. robustus n = 6; A. septentrionalis n = 4.

These records are sparsely distributed throughout Mpumalanga with occurrences of A. h. longiceps also southwards in the Free State and KwaZulu-Natal. Some locality records had the same GPS co-ordinates (n = 14 – A. h. longiceps: 7; A. h. meesteri: 2; A. robustus: 1; A. septentrionalis: 4), which reduced the already small sample size even further. Most records for A. h. longiceps were from interior grasslands of KwaZulu-Natal, with the northernmost records at the southern Mpumalanga border near Wakkerstroom which coincides with the southern escarpment (SE). A. h. meesteri records coincided with the northern escarpment (NE) region in mountain catchment areas (MCA). The few A. robustus records were from the type locality (near Dullstroom) and surroundings areas to the west of those for A. h. meesteri, with the exception of two localities in KwaZulu-Natal (Ngome Forest, not shown) and eastern Mpumalanga (Malelane) which were based on preliminary genetic identifications lacking morphological verification.

Records for A. septentrionalis were mainly from the western Gert Sibande district of Mpumalanga, which is dominated by Highveld grasslands. Records for this species from the Ermelo region are the only confirmed reference specimens occurring outside the escarpment and MCA regions.

Based on multivariate craniometry, Bronner (1995) tentatively identified five specimens of Amblysomus from Swaziland as A. septentrionalis. His craniometrics discrimination technique
was, however, only 90% accurate so it will remain unclear which taxa occur there until chromosome and/or genetic identifications have been done, thereby precluding the use of those records in this study. Swaziland was also excluded from the models because geospatial environmental data layers of comparable quality to those available for South Africa are not available. To gain a trans-frontier perspective on potential golden mole distributions and conservation priority areas, variable data could be merged with local data across countries, but this compromises data quality. Alternatively, models must be limited to global interpolated data which often lacks accuracy. Since the geographical focus area for this study was the Mpumalanga grasslands, emphasis was placed on modelling golden mole distributions with maximum rigor given data available in this region only.

**Table 3.1:** The top three environmental predictor contributors of the training of initial models. Ppt = precipitation; T° = temperature; qtr = quarter

<table>
<thead>
<tr>
<th>Species</th>
<th>Top contributing variables (contribution %)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A. h. longiceps</td>
<td>Landtype (45%) Ecoregions (28%) Annual ppt (13%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. h. meesteri</td>
<td>Vegetation (52%) Ppt of warmest qtr (22%) Annual T° range (8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. robustus</td>
<td>Landtype (48%) Ecoregions (27%) Ppt of wettest month (15%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. septentrionalis</td>
<td>Vegetation (54%) Ecoregions (19%) Landtype (13%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.1: Genetic references for all four taxa and morphometric records for *A. h. meesteri* were obtained from museum collections (Ditsong Museum of Natural History) and MEEP lab (University of Pretoria) in relation to district boundaries within Mpumalanga (http://conservation3.arcgisonline.com/Apps/MBSP), mountain catchment areas (MCA) (Rabie and Burgers 1997) and the Great Escarpment (MTPA pers. comm. 2015). Additional records in KwaZulu-Natal and Free State for *A. h. longiceps* and *A. robustus* are not shown.
The initial model outputs all had AUC values > 0.8, indicating reasonably good model fits to the data. For all four taxa, the top three variables contributed to > 80% of the model (Table 3.1). Categorical variables landtype, ecoregions and vegetation contributed most to the models for all four taxa, with lesser contributions from annual temperature and precipitation and summer precipitation. Specific polygons of categorical variables landtype and vegetation type that intersected with the models’ thresholded outputs are given in Appendix 2.

MaxEnt models trained on confirmed genetic records for all species and additional morphometric records for A. h. meesteri were converted to raster layers to visualise the habitat patches suitable for each targeted taxon (Figure 3.2). Smoother maps (A. h. longiceps; A. septentrionalis) were obtained for taxa with more occurrence records, whereas more pixelated images were obtained when occurrence records were few (A. h. meesteri; A. robustus). This is likely the effect of sample size limitation on the ability to represent fully the suite of bioclimatic preferences of the species.

The initial probability distribution maps suggested that A. h. longiceps and A. septentrionalis are likely to be more widespread throughout most of the Mpumalanga grasslands than the sparse locality records available (Bronner 2013) indicate, but with the highest probabilities of occurrence in the eastern areas adjoining the rim of the Great Escarpment (which also forms the boundary between the Grassland and Moist Savannah biomes). The maps for A. h. meesteri and A. robustus, although trained on few records, similarly suggested that these taxa have restricted ranges in the north-eastern grasslands and along the rim of the Great Escarpment, especially in mountain catchment areas (Figure 3.1), but again that these taxa are probably much more widespread than the few available records (see Table 2.1) indicate.
Figure 3.2: Initial species distribution probability maps for A. h. longiceps n = 10 (a), A. h. meesteri n = 5 (b), A. robustus n = 6 (c) and A. septentrionalis n = 4 (d) in Mpumalanga and bordering areas of adjoining provinces. Deep blue areas indicate the highest probability of species occurrences.
3.2 Species richness hotspots and field surveys

A map showing probable areas of high golden mole species richness based on combined initial MaxEnt model spatial predictions for all four target taxa is shown in Figure 3.3. Predicted maximal diversity (three species) was concentrated in two hotspots that coincide with the escarpment and MCA. The first hotspot, encircling the area bordering the rim of the northeastern escarpment (NE) in Mpumalanga (24S30E), is likely to contain two species (*A. h. longiceps* and *A. h. meesteri* or *A. robustus*) in the eco-touristic regions of Lydenburg, Pilgrim’s Rest, Graskop, Sabie and Mariepskop; and discontinuously towards Kaapsehoop (*A. h. longiceps* and *A. septentrionalis*). The second major hotspot was in the southern escarpment (SE) area, encircling the wetland-dominated Wakkerstroom, Ermelo and Chrissiesmeer regions (26S30E and 27S30E), and is likely inhabited by up to two golden mole taxa (*A. h. longiceps* and *A. septentrionalis*).

In the NE hotspot, diverse grassland and forest biomes with fertile soils and high carbon biomass are intersected geographically by undulating kloofs (White et al. 2000, Mentis 2006, Mucina and Rutherford 2006). These areas therefore presented great potential for uncovering new populations and were the focus of survey efforts. Many of the habitats along the northeastern escarpment (NE) area (25S30E) have been transformed into *Pinus* sp. and *Eucalyptus* sp. plantations which render soils dry and compact (Mills and Fey 2003, GCIS 2014), and thus probably unfavourable for golden moles. However, there are designated natural areas within these plantations that serve as island refugia for existing wildlife, e.g. Komatiland Forests has implemented a conservation management plan to conserve 27% of the property for non-commercial use and such areas undergo routine grassland and forest monitoring (Komatiland Forests, 2008). Up to 30% of the land owned by York Timbers is similarly dedicated to conservation of streams and land (York, [http://www.york.co.za/home.asp](http://www.york.co.za/home.asp)). These and 38 other enterprises and governance bodies in Mpumalanga are members of the Forest Stewardship Council.
Council (FSC, https://ic.fsc.org/preview.list-of-south-africa-stakeholders.a-2366.pdf), a global independent body dedicated to monitoring and evaluating companies for forest conservation. Such conserved and untransformed areas within the agroforestry landscape are likely to be important refugia for the golden mole taxa present in the northern hotspot.

Field survey efforts were concentrated in these two hotspots, which cover ~10% of the area of Mpumalanga, with trapping being undertaken for up to three consecutive nights (Table 2.4) based on initial model species richness (Figure 3.3) using the maximum number of traps (n ≤ 30) in at least six 1 km² sites within the targeted QDSs. Survey effort was concentrated on areas where two species coexist and therefore afforded high sampling effort; where one species was predicted, sampling effort was intermediate with areas where no model probability exceeded that of the taxon’s LPT.

Figure 3.3: Map showing probable areas of high species richness based on the initial MaxEnt model spatial predictions of known locality records for the four chrysochlorid species targeted shown in Figure 3.2. Ground-truthing efforts were focused primarily on areas of high predicted richness.
Habitats coinciding with the southern escarpment (SE) hotspot are less degraded and have been subjected to only mild transformation owing largely to ranching and agricultural activities, which have relatively low biodiversity impacts compared to other land uses in South Africa (O’Connor and Kuyler 2009), and which are therefore unlikely to be so severe as to exclude any of the targeted fossorial golden mole taxa. Agricultural lands often have moist, high carbon content soils as a result of soil nutrient enrichment, tilling and artificial irrigation, but failure to regulate livestock grazing, nutrient leaching, and controlled fires potentially causes long-term organic carbon loss and erosion of the top soil (Mills and Fey 2003, Bai and Dent 2007, Dijkshoorn et al. 2008, Du Preez et al. 2011, O’Mara 2012). Since invertebrate prey such as earthworms prefer high carbon content soils (Ivask et al. 2006), golden moles often thrive on farms, but face the risk of local extirpation by domestic animals and combine harvesters (see ‘threats’: Bronner and Mynhardt (2015), Rampartab (2015a, 2015b)). The Chrissiesmeer and Wakkerstroom regions are dominated by wetlands, providing ample invertebrate prey in moist and nutrient rich soils.

Areas of medium potential richness were offered second priority in ground-truthing efforts. These areas fall along the easternmost Great Escarpment at the Swaziland-Mpumalanga border, and areas surrounding the NE hotspot. This medium richness area bridges the gap between two high richness areas (Dullstroom and Lydenburg) in the NE hotspot, a possible indication of undersampling in this vast agricultural expanse. From Kaapsehoop (26S30E) south to Piet Retief (27S30E), the initial models predicted that occurrence of *A. robustus* is likely, but that occurrence of *A. h. longiceps* and *A. h. meesteri* might occur intermittently. Kaapsehoop and surrounding regions are dominated by eco-touristic villages, punctuated by large-scale mills and power stations; the latter increases in frequency southward toward Piet Retief, which is dominated by plantations and industrial towns.

Finally, in sampling areas with low diversity in the far east and west of Mpumalanga, covering the widest area (more than four degree squares in light blue, Figure 3.3), more
emphasis was placed on surveying for activity and setting traps for no more than one night. Low-probability areas such as the Secunda-Bethal region (26S29E) are dominated by large-scale mining operations (Mentis 2006, Pandor 2008), and black vertisol clays (ARC–ISCW 2003, Dijkshoorn et al. 2008, C. Rampartab, pers. obs.) known as ‘turf’ unsuitable for golden moles. These areas were surveyed for activity and traps were set for only one night as most soils were highly compacted.

Between October 2013 and April 2014, field surveying over 110 days/night in 90 QDSs lead to intensive trapping and active surveying of six 1 km$^2$ grid squares per QDS (6 x 90 = 540 km$^2$; Table 2.4) using up to 30 traps per night (maximum traps set: 3300). This ground truthing resulted in the acquisition of 25 new individuals, of which nine were captured in five QDSs where golden moles have not previously been recorded (Figure 3.3). Ground-truthing surveying thus increased the size of the initial distribution database of confirmed genetic specimens (Table 2.1) from 38 to 59 records, an increase of 61%, with four specimens of unknown species affinity. Additionally, golden mole activity (distinctive subsurface tunnels, Figure 2.2) was recorded in nine new QDSs (see Appendix 3). Species affiliations of the newly-captured specimens are outlined in Section 3.3 below.

**3.3 Cytochrome-**$b$ sequencing and analysis

Neighbour-joining (NJ) and maximum-likelihood (ML) phylogenetic trees derived from mitochondrial cytochrome-**$b$** sequences between 700 and 1100 bp are shown in Figure 3.4a. Although bootstrap support for many nodes was low (<70%), the trees show similar topologies with respect to the major clades. *A. marleyi* and *A. h. meesteri* (including specimens CR2, 8, 10-12, 19-20, Table 3.2) were retrieved as sister-taxa and formed a monophyletic lineage distinct from the other *Amblysomus*, confirming a close phylogenetic relationship between these species (Bronner 1995a).
In both trees, specimen CR9 was retrieved as distinct (with high bootstrap support) from other *Amblysomus* lineages; consequently, the species affinity of this specimen could not be determined based on the limited genetic data available. Similarly, the phylogenetic affinity of specimen CR15 relative to *A. h. longiceps*, *A. h. pondoliae*, *A. robustus* and *A. septentrionalis* was unclear and lacked probabilistic support. Specimen CR4 could not be identified due to very poor DNA quality collected from a decomposed individual, and CR22 did not yield a sufficiently long reverse sequence. These four specimens were treated as unidentifiable and were omitted from the model refinement analyses (Section 3.5).

While the *A. h. longiceps* reference specimens (together with CR23) were retrieved within a monophyletic lineage including *A. h. pondoliae* (from southern KwaZulu-Natal) in the ML tree, in the NJ phylogram the *A. h. longiceps* lineage was retrieved as sister to *A. septentrionalis* and *A. robustus*. However, considering the lack of bootstrap support, the trees do not differ much in topography and species placements. The single *A. robustus* reference specimen (together with CR6-7) formed a distinct lineage with high bootstrap support but within a broader clade including the *A. septentrionalis* reference specimens and CR1, 3, 5, 13-14, 16-17, 18, 24. This concurs with results of an ongoing phylogeographic study (Mynhardt et al., submitted) based on diversity of the mitochondrial NADH Dehydrogenase gene sequences within *Amblysomus*. As *A. robustus* and *A. septentrionalis* are cytogenetically distinct (Bronner 2000) and are retrieved as monophyletic but recently diverged (0.8 – 0.2 m.y.a.) lineages in a multigene study using both mitochondrial and nuclear markers (Maree et al., in preparation), the apparent lack of phylogenetic distinctness between these taxa in this study must be an artefact of including only a single gene in the current analysis. The asterisks in Figure 3.4a denotes a reference specimen from Malelane that was previously identified as *A. robustus* but is shown here to group with *A. septentrionalis*. The discrepancy of this occurrence record can be attributed to the use of only short sequences for different mitochondrial genes and lack of morphometric testing, and was therefore omitted from the model refinement.
Given low bootstrap support for many nodes in the NJ/ML trees, an allele network (Figure 3.4b) with confidence limits of 95% (Bloomer pers. comm. 2014) based on trimmed sequences to exclude any missing data (838 base pairs) was used to further clarify the species affinities of newly-collected specimens relative to reference specimens of *A. h. longiceps*, *A. septentrionalis* and *A. robustus*. This confirmed that specimens CR6-7 are separated from nominotypical *A. robustus* (from Dullstroom) by only 2-3 mutations; and that there are only 8 mutations between CR23 and nominotypical *A. h. longiceps* from the type locality (Queens Park, Pietermaritzburg), which is commensurate with the mutational distances among other reference demes of this relatively widespread taxon. The allele network further corroborated the NJ/ML trees in showing that nine of the newly-acquired specimens (CR1, 3, 5, 13-14, 16-17, 18, and 24) represent *A. septentrionalis* with a maximum of 7 mutations separating any locality samples. The species affinities of CR18 and CR25, however, were more equivocal. While both of these specimens fall within a broader *A. longiceps* lineage in the NJ/ML trees, in the allele network CR25 is most closely affiliated with CR24 (= *A. septentrionalis*). CR18 is separated by 5 mutations from the *A. h. longiceps* reference specimen (Wakkerstroom, Tafelkop) and 7 mutations from CR13 (= *A. septentrionalis*), being placed as *A. h. longiceps* in the NJ, and *A. septentrionalis* in the ML tree.

Based on a 16km² locality grid, 11 of 12 reference specimens used in Figure 3.4 and 15 of 25 caught specimens occur at a unique locality where golden moles have not been caught and/or genetically identified. Combined, 22 of 37 (70%) total localities are taxon-specific, with the exceptions being Wakkerstroom (with three species: *A. h. longiceps*, *A. septentrionalis* and *A. h. meesteri*) and the escarpment region from Dullstroom to Graskop (*A. h. meesteri*, *A. robustus* and/or *A. septentrionalis*). Conclusive genetic identifications were thus obtained for 21 of the 25 newly-acquired *Amblysomus* specimens, and the associated spatial records were used to refine the initial MaxEnt models.
Figure 3.4a: Neighbour-joining (A) and maximum-likelihood (B) trees with associated bootstrap nodal support values, using Neamblysomus juliana as an outgroup. Nodes with bootstrap values > 70 % (~ 95 % probabilities) are indicated by blue dots. Each branch is colour-coded according to the species it represents. Asterisks denote specimens whose species affinity was unresolved by the limited gene sequences available.
3.4 Model refinement

3.4.1 Revised distribution database

Tables 3.2-3 gives the specific identities of the 25 specimens acquired during ground-truthing surveys relative to the 38 species occurrence records used to develop the initial models. Distribution records for the four *Amblysomus* taxa, based on locality records for morphometric or genetic reference specimens and golden moles captured (or sites where activity was observed) are shown in Figure 3.5. As predicted by the initial models (Figures 3.2-3), there are two predicted chrysochlorid diversity hotspots in Mpumalanga: a broader zone (24S30E; 25S30E) with three taxa (*A. h. meesteri, A. robustus* and *A. septentrionalis*) ranging from the Dullstroom area along the northern escarpment to Sabie and Lydenburg; and a smaller southern hotspot, also containing three taxa (*A. h. longiceps, A. h. meesteri* and *A. septentrionalis*) in the vicinity of Wakkerstroom (27S30E; Gert Sibande district) of southern Mpumalanga (Figure...
3.5). In the southern hotspot the three *Amblysomus* taxa occur in broad sympatry together with two other chrysochlorid species (Rough-haired golden mole *Chrysospalax villosus*; Sclater’s golden mole *Chlorotalpa sclateri montana*) not studied here. Nowhere else in South Africa are five golden mole taxa known to occur in broad sympatry (Bronner pers. comm. 2014) making Wakkerstroom both a provincial and national hotspot of golden mole diversity.

Only two specimens of *A. robustus* were captured during ground-truthing surveys, both from MCAs between known locality records, suggesting that this species has a restricted range confined to higher-altitude areas receiving high precipitation. This species appears to be restricted to the northern escarpment hotspot in moist, undulating landscape (Emery et al. 2002) within MCAs associated with the Steenkampsberg Mountains, as predicted by Bronner (2000). The species identity (*A. robustus*) of the single genetically-identified reference specimen from Malelane (circled record in Figure 3.5), below the Great Escarpment, is contradicted by my results (Figure 3.4) showing a much closer affinity to reference specimens of *A. septentrionalis* in both the phylogenetic trees and allele network. Given uncertainty about the specific affiliation of this specimen, the Malelane record was excluded from further model analyses.

Eight new *A. h. meesteri* specimens were captured during ground-truthing, thereby increasing the number of distribution records threefold. The newly-acquired specimens included three (CR19-21) from two new localities in Wakkerstroom in southern Mpumalanga (Figure 3.5), based on their well-supported clustering with *A. h. meesteri* reference sequences in the phylogenetic trees with a 99% bootstrap support (Bloomer pers. comm. 2014) and the allele network (Figure 3.4b). The presence of this taxon so far south was unexpected as *A. h. meesteri* has hitherto been recorded only from the Graskop-Sabie districts (25S30E) in north-eastern Mpumalanga (Bronner 2000), and extends the known range of this taxon considerably from just 67 km in the northern escarpment (including morphometric records) to 310 km between the records in the northernmost and southern grasslands.
Table 3.2: Species affiliations and localities for the 25 specimens acquired during ground-truthing surveys. The co-ordinates are given in decimal degree format. AN = Accession number.

<table>
<thead>
<tr>
<th>Collection Date</th>
<th>Species ID</th>
<th>AN</th>
<th>Closest town</th>
<th>Locality</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/16/13</td>
<td><em>A. septentrionalis</em></td>
<td>CR1</td>
<td>Sabie</td>
<td>Misty Mountain Lodge</td>
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<tr>
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<td><em>A. septentrionalis</em></td>
<td>CR3</td>
<td>Dullstroom</td>
<td>Willow Creek Trout and Nature Reserve</td>
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<td>Lydenburg</td>
<td>Falcon Crest</td>
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<tr>
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<td>CR5</td>
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</tr>
<tr>
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<td><em>A. robustus</em></td>
<td>CR6</td>
<td>Belfast</td>
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<td>Marrietjie Property</td>
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<td>Wakkerstroom</td>
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<td>Belfast</td>
<td>HeavinForge Studio</td>
<td>-25.6901</td>
<td>30.0298</td>
</tr>
</tbody>
</table>

Table 3.3: Specimens caught during the field survey increased the number of existing golden mole occurrence records confirmed by genetic tests by 33%.

<table>
<thead>
<tr>
<th>Species</th>
<th>Genetic references</th>
<th>Caught specimens</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
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<td>23</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td><em>A. h. meesteri</em></td>
<td>2</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
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<td>7</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td><em>A. septentrionalis</em></td>
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</tr>
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<td>-</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>25</td>
<td>63</td>
</tr>
</tbody>
</table>
Figure 3.5: Geographic distribution of newly-caught specimens and existing reference specimens of Amblysomus taxa in Mpumalanga. The circled record (Malelane) represents a reference specimen whose species affiliation was questioned by my results, and was excluded from refined models. A. h. longiceps n = 12; A. h. meesteri n = 6; A. robustus n = 7; A. septentrionalis n = 11.
All of the confirmed records for this taxon are from mountain catchment areas along the rim of the Great Escarpment. This is consistent with results of a previous modelling study (Emery et al. 2002) that predicted a potential range extending from the Graskop region southwards along the escarpment, towards the north-western border of Swaziland, as well as Bronner’s (1995) suggestion that this taxon might be more widespread in areas bordering the Great Escarpment in Mpumalanga and north-eastern KwaZulu-Natal.

The majority of A. h. longiceps genetic reference records are from the interior grasslands of KwaZulu-Natal and Free State, suggesting only a marginal intrusion into southern Mpumalanga, so it is not surprising that only two new specimens were obtained, from the Wakkerstroom region in the southern escarpment. Based on craniometrically identified reference specimens (Bronner 1995b), however, A. h. longiceps, may range throughout south-western Mpumalanga, and possibly also along the central Highveld regions towards the escarpment, and southwards to wetlands and grasslands near Volksrust (where one confirmed specimen of this subspecies was caught).

Nine new A. septentrionalis were captured along the Highveld and southern Mpumalanga, increasing the number of confirmed distribution records for this species by 50%, suggesting that this is the most widespread chrysochlorid taxon in the study area, occurring at 11 widely scattered localities throughout central and southern Mpumalanga from the western to northern and north-eastern escarpment. Ground-truthing resulted in the first genetically-confirmed records of this taxon in the Kaapsehoop and Chrissiesmeer grassland and wetland regions (25S30E and 26S30E, respectively), as well as three new genetically confirmed records from the north-eastern escarpment and MCAs. This contrasts previous sparse evidence suggesting that A. septentrionalis is restricted to only three localities in south-central Mpumalanga (Bronner 2000, Rampartab 2015), and corroborates findings of a previous modelling study (Emery et al. 2002) that predicted a relatively widespread distribution throughout the Highveld areas of southern Mpumalanga. It is apparently the only chrysochlorid taxon not restricted to mountain catchment
areas associated with the Great Escarpment. If the Malelane specimen indeed represents *A. septentrionalis*, as my results suggest, it is possible that this species ranges marginally also into Savannah habitat localities below the Great Escarpment; however, this requires confirmation by further field sampling and DNA sequencing.

Eighty-eight percent of the individuals acquired, and 67% of observed activity sites recorded, during austral summer ground-truthing surveys occurred within mountain catchment areas associated with the rim of the Great Escarpment (Figure 3.6). Similarly, 64% of the genetic reference specimens were from E/MCA, a region characterised by high altitudes (982 m to 2176 m above sea level; (Farr et al. 2007), warm climates (mean temperature range of warmest quarter (Bio10) of 15 °C to 27 °C; WorldClim (Hijmans et al. 2005)) and high precipitation (Bio16 - precipitation range of wettest quarter 301 mm to 677 mm; WorldClim - (Hijmans et al. 2005)). This suggests that the most favourable habitats for *Amblysomus* in Mpumalanga are high altitude and temperate montane grasslands receiving high precipitation. The dominant landtype geologies of the MCA were andesite, biotite, dolerite, dolomite, mafic, sandstone and quartzite shale soils (ARC–ISCW 2003). Such soils occur with high organic carbon contents (SOCC) in excess of 2% (Du Preez et al. 2011) found widely throughout the Grassland biome (Mucina and Rutherford 2006), and the Drakensberg montane grasslands, woodlands and forest ecoregions (Olson et al., 2001).

SOCC, a proxy for soil invertebrate prey availability (Rawlins et al. 2007, Corsi et al. 2012), may be an important predictor of chrysochlorid distributions, but the resolution of the categorical data layer available was too coarse (only 4 states) for use in refining the MaxEnt models (Section 3.5). Individuals recorded from outside the MCA were collected from sites with altitudes ranging between 1475 - 1741 m a.s.l, and experiencing summer temperature ranges of 16 -27 ° C (WorldClim (Hijmans et al. 2005) Bio10) and 259 - 682 mm summer precipitation (WorldClim (Hijmans et al. 2005) Bio16). Dolerite, sandstone and quartzite shale were the dominant landtypes at these localities in addition to alluvium, basalt, granite, lava, migmitite and
rhyolite soils (ARC–ISCW 2003). As with those found in the MCA, individuals outside the MCA were also found within grasslands having SOCC > 2 % (Du Preez et al. 2011).

Figure 3.6 shows that existing museum references confirmed by genetic and morphometric analyses fall within 64 % and 50 % of the E/MCA region respectively, indicating that sampling biases inherent in museum records (Freitag et al. 1998), and possibly amplified during ground-truthing along the escarpment, may have exaggerated the dependence of golden moles to the E/MCA. While these bioclimatic and edaphic factors may be broadly indicative of golden mole distributions in Mpumalanga, it is worth noting that sampling effort was concentrated in areas of known occurrence based on the initial MaxEnt models, i.e. deliberately skewed toward areas where there was a high probability of capturing golden moles. This, along with ease of access, landowner permissions and weather conditions, contributed to sampling bias. Since the study area covered a vast 76 735 km$^2$, this was a necessary trade-off to uncover new populations as quickly and efficiently as possible. Measures employed to minimize such sampling biases is addressed in Section 2.4. Given that another more-restricted and independent modelling analysis(Emery et al. 2002) derived similar distributional predictions for the presence of golden moles in E/MCA to those presented here, it seems likely that sampling biases were effectively corrected for.

**Figure 3.6:** Proportion of records that fell within versus outside of the Great Escarpment rim (E) and associated mountain catchment areas (MCA).
3.4.2 Refined model outputs

The refined models in Figure 3.7 were trained on the revised distribution database and converted to raster to visualise the refined probability distributions of taxa in ArcGIS. Predictions were projected onto the Mpumalanga study area to prevent over-prediction and focus on conservation status within the province; however, a large part of the *A. h. longiceps* predicted distribution is not shown here as the range is primarily in sub-montane grasslands of KwaZulu-Natal and Free State provinces south and south-west of Mpumalanga, respectively. In the initial models (Figure 3.2) for all taxa except *A. septentrionalis* there was a high-probability area predicted to extend northwards into Limpopo province – where other golden mole species such as *N. julianae* and *N. gunningi* occur, a phenomenon probably reflective of the shared habitat requirements of these *Amblysomus* and *Neamblysomus*, hence the projection on to the study area removed these false positives. In all four taxa, predictions were limited by the landtype polygons which most summarized their distributions, giving the maps a pixelated appearance (blue pixels). The size and shape of these polygons is indicative of a resolution limitation, and is one of the reasons categorical data do not produce smooth maps. In areas with low-medium prediction (yellow-green pixels), maps are smoother than that of the initial maps (Figure 3.2), indicating qualitatively that models are better fitted to the data.

In addition to its known existing presence in the southern wetlands, *A. h. longiceps* is predicted to also potentially occur in the northern escarpment region (Figure 3.7a). However, this predicted probability is below the LPT, indicating the environmental suitability at these cells is below that of any cell where the species is known to occur. This threshold assigns a presence state to any grid cell in the background with a probability equal to or greater than the lowest probability of the grid cells containing training occurrence records.
Figure 3.7: Refined models trained according to Conservative trial I for four golden mole taxa: *A. h. longiceps* (A), *A. h. meesteri* (B), *A. robustus* (C) and *A. septentrionalis* (D).

The minimum training presence, also known as the lowest presence threshold, is also used in the jackknife n-1 localities model evaluation test and proves to be a good indicator for conservative models with low sample size (Anderson and Raza 2010, Thompson et al. 2011,
Merow et al. 2014), though other thresholds such as maximum training specificity versus sensitivity may also be useful in cases of over-prediction (Calkins et al. 2012), or nth percentile thresholds useful for exploring the degree to which species ranges can expand (Graham et al. 2010). In contrast to the initial models (Figure 3.2a), the refined models in Figure 3.7a predict only an intermediate probability of occurrence of *A. h. longiceps* in the eastern parts of the province (25S30 and 26S30E), and the southern range (27S30E) is far more concentrated to Wakkerstroom of the southern escarpment than the wide expanse extending into the central Highveld (26S30E).

The change from *A. h. longiceps* being widespread in the NE hotspot and along the Swaziland border in the initial models to the concentrated probability of occurrence in SE hotspot and major detraction from the NE (Figure 3.8a) stands testament to the rigorous nature of the model refinement process. It shows that with the addition of occurrence records and stringent model evaluation, the probability of occurrence becomes conservative and more useful from a strictly conservation perspective. At present without occurrence records from the NE region, this area of high probability falls below the minimum threshold and is thus excluded as a presence in the binary conversion (Figure 3.9). Nonetheless, this area cannot be excluded as a potential habitat for *A. h. longiceps*, especially given the case with *A. h. meesteri* which was only predicted in the north, but also found in the south, sympatrically with *A. h. longiceps* (Figure 3.2b and 3.7b).

In contrast to the initial models which predicted that *A. h. meesteri* occurs throughout the northern escarpment region, the refined model predicts that *A. h. meesteri* occurs only in two discrete areas: the eastern parts of the northern escarpment, southern wetlands near Wakkerstroom, with only a low probability of occurrence along the eastern rim of the Great Escarpment between these latitudinal extremes (Figure 3.8a). Given a lack of intermediate reference specimens, this prediction may be an artefact of inaccurate genetic identifications
Figure 3.8a: Initial models (left) compared with refined models (right) of *A. h. longiceps* and *A. h. meesteri*.
Figure 3.8b: Initial models (left) compared with refined models (right) of *A. robustus* and *A. septentrionalis*. 
based on a single mtDNA marker, though this is unlikely given the relatively robust genetic results. However, it is possible that the range of this taxon occurs southwards from the Graskop district to the Swaziland border mountains, as suggested by Emery et al. (2002), and extends along the Drakensberg mountains southwards to KwaZulu-Natal, as predicted by (Bronner 1995b). The lack of any high probability occurrence between the northern and southern areas of high probability occurrence could be an artefact of the very small sample sizes (with all specimens originating from a small area in the NE hotspot) for this taxon. An alternative (and not exclusive) explanation is that the similarity of bioclimatic conditions in the NE and SE areas of high probability may underlie the observed model predictions of high occurrence in these two areas, and that this taxon has relatively narrow habitat tolerances.

Both *A. h. meesteri* and *A. robustus* could potentially occur in the Barberton area, but field efforts in this area revealed few sites with golden mole activity/presence. This is perhaps because soil compactness and erosion are rife in these plantation regions. *A. robustus* is found westward of the northern escarpment (Figure 3.4). One reference specimen obtained in the Malelane region with the unconfirmed species identification as *A. robustus* was excluded from the refined models, thereby excluding the high probability areas associated in the Malelane region (Figure 3.8b). This suggests that *A. robustus* habitat characteristics of the Malelane site are not suitable for *A. robustus*, which is corroborated by the lack of evidence for a predicted dispersal route along the north-western escarpment down into the savannah and Lowveld. More genetic samples need to be processed from this excluded specimen and others in the area.

Both the initial and refined model outputs of *A. septentrionalis* suggest that this taxon is the most widespread of the four taxa, with three regions of high probability occurrence in central Mpumalanga that differ mainly latitudinally (Figure 3.8b). This finding agrees with the predictions of Emery et al. (2002), based on an independent modelling analysis with a smaller data suite. In addition to the high probability of occurrence in the southern wetlands and
northern escarpment, the addition of the first records of genetically identified golden mole records from the wetlands around Ermelo and Chrissiesmeer led to the refined models predicting *A. septentrionalis* to be strongly present in these areas, thereby identifying new population demes to the north and south.

Minimum presence thresholds from MaxEnt were converted to rasters and overlayed to show where high-probability areas for each species overlapped, i.e. occur in potential broad sympatry. Figure 3.9 shows that there are four regions where more than one species is likely to occur with high probability. The first three potentially sympatric regions occur in the northern escarpment and represent *A. septentrionalis* occurring with either *A. robustus* (banners 1 and 3) and *A. h. meesteri* (banner 2). The fourth region of broad sympatry, both from actual records and the model predictions, is in southern wetlands where three taxa occur: *A. h. longiceps*; *A. h. meesteri* and *A. septentrionalis* (banner 4). These four regions follow along the escarpment and MCA regions where high precipitation, soil organic carbon content and productivity (Figures 9 and 11, respectively); (Hijmans et al. 2005, Du Preez et al. 2011, MTPA pers. comm. 2015) facilitate high prey abundance and possibly low resource competition (Hubbell 2005, Ivask et al. 2006, Massol et al. 2011). These four areas with potentially high golden mole diversity and associated conservation concerns allow the focus of possible conservation and environmental management objectives.

### 3.5 Spatial analyses and conservation implications

The above thresholded taxon distributions were mapped and cross-tabulated with categorical layers (Table 2.15) to identify areas of high geographic coincidence. The thresholded distributions for *A. h longiceps* and *A. h. meesteri* were coincident with the escarpment and MCA (100 %) regions in northern and southern Mpumalanga (Figure 3.10-11), with *A. robustus*
Figure 3.9: Broad sympatric regions (red banners) show where two to three taxa potentially co-occur in geographic space based on the sum of five minimum presence thresholded outputs per species.
following closely (escarpment: 92 %, MCA: 78 %) and A. septentrionalis occurring mainly outside this area (escarpment: 44 %, MCA: 36 %). Notably, however, the thresholded distributions for all four taxa largely overlap (90 – 100 %) areas with high SOCC (> 4 %), with no predicted occurrences in SOCC < 3 %, confirming that SOCC (a proxy for invertebrate prey availability) is probably a major distribution driver (or limiter). Likewise, the thresholded distributions of all four taxa overlap broadly (63 – 89 %) with areas having very low predicted soil loss (PSL) values. While the distributional area of A. h. meesteri coincides mostly (81 %) with low or very low soil loss areas, of the four taxa studied it is most vulnerable to possible moderate predicted soil losses (19% overlap).

Vegetation community assemblages and structure likely do not influence golden moles directly (for example, by providing cover or food) as they are subsurface insectivores; however, vegetation properties such as high cover results in decreased water runoff and soil erosion, increased soil moisture and accumulation of nutrients through decomposition (Egoh et al. 2008), thereby increasing densities of the prey (Ivask et al. 2006, Rawlins et al. 2007) upon which golden moles are dependent (Kuyper 1985, Jackson et al. 2008a). Furthermore, vegetation provides shade and leaf litter (Rawlins et al. 2007, Murphy 2014), which influence the subsurface thermal milieu and moisture properties of soils, providing more suitable environmental conditions for golden moles to burrow and forage (Jackson et al. 2008a, Bronner 2013).

Primary productivity (plant production per unit area per year) is based on the Rosenzweig’s equation for estimating primary production (Schulze 2007). As the only primary productivity (PP) data layer available is based on an outdated data source of South African Veld Types (Acocks 1988), it was not used in the development of the MaxEnt models. Spatial analysis
Figure 3.10: Taxon distributions based on the sum of five cross-validated minimum presence thresholds in relation to (A) mountain catchment (MCA) and escarpment areas, and geographical gradients in (B) soil organic carbon content (SOCC) and (C) predicted soil loss (PSL).
Figure 3.11: Percent of taxon thresholded distributions overlapping with topographical and soil features (Table 2.16). MCA – mountain catchment areas; SOCC – soil organic carbon content; PSL – predicted soil loss.

(Figure 3.12b; 3.13) showed that the Mpumalanga region generally has medium-high primary productivity. The thresholded distributional ranges of three of the four Amblysomus taxa (A. h. longiceps, A. robustus and A. septentrionalis) coincide mostly (88 – 100 %) with areas of moderately high PP (6 – 8 t/ha/an), whereas 33 % of the range of A. h. meesteri coincides with areas of high PP (> 10 t/ha/an) that are especially concentrated along the escarpment.

Moreover, all of the thresholded distributional ranges for all four taxa coincide with areas having relatively mild and wet climates (a mean temperature of warmest quarter of 16.2 – 16.9 °C (Bio 10); precipitation of wettest quarter minima of 374 – 382 mm (Bio 16)) which may explain the relatively high primary productivity shown in the Mpumalanga region.

Three of the four taxa occur only in the Grassland biome (A. h. longiceps; A. h. meesteri; A. septentrionalis), where A. robustus also occurs predominantly (97 %), but this species is also found in Forests (3 %), which can be attributed to its predicted distribution along the Pilgrim’s Rest and Mariepskop regions along the rim of the escarpment where Afromontane forests adjoin...
Figure 3.12: Thresholded golden mole distributions in relation to (A) biomes (Mucina and Rutherford 2006), (B) primary productivity (Schulze 2007), and (C) ecoregions (Olson et al. 2001).
Figure 3.13: Percentage congruence between the thresholded distributions of the combined targeted golden mole taxa and biomes, ecoregions and primary production (PP). GL = grassland; F = forests; WL = woodland; BV = bushveld; DM = Drakensberg Montane; HV = Highveld.

grasslands (Figures 3.12-13). The thresholded distribution of *A. h. longiceps* is restricted exclusively to the Highveld grassland ecoregion (Figure 3.12c; 3.13), which also includes most of the thresholded distribution of *A. septentrionalis* (64 %), the remainder being in Montane grasslands. Similarly most of the thresholded distribution of *A. robustus* coincides with Montane grasslands (94 %) with 3 % each around Pilgrim’s Rest and Mariepskop where grasslands transitions into woodland, forest and Savannah habitats (Figure 3.12a; c; 3.13). In terms of ecoregion occurrences, *A. h. meesteri* is the least specialized of the four targeted chrysochlorid taxa, with 43 % of the thresholded distribution falling in Highveld grasslands and 29 % in both the woodlands and Montane grasslands.

Using the ecoregion framework, biologists can examine species richness and endemism patterns for taxa used in conservation planning, but some drawbacks of have been presented (Olson et al. 2001). First, accuracy of biogeographic frameworks varies for different taxa and designated ecoregions are a compromise to suit as many taxa as possible. Second, ecotones and mosaic habitats are more likely in reality than the strictly-defined boundaries of ecoregions. Thirdly, some microhabitats harbouring endemic species that are undetectable on a large scale
may exist within ecoregions (Olson et al. 2001). Thus, the scale and resolution of biogeographic units must be fine enough for the aim of the investigations (Olson et al. 2001). Despite these potential drawbacks, the high specificity (in 1 – 3 ecoregions) shown by all taxa suggests that the WWF ecoregions are a suitable conservation planning tool also for small subterranean mammals, such as chrysochlorids.

The current conservation plan for the province incorporates private, national and provincial protected areas, which cover 9 451 km² of grasslands (Global Environment Facility et al. 2007), as well as unprotected but demarcated ecological corridors, conservancies and proposed conservancies (Ferrar and Lotter 2007) to aid in the cohesion of habitats fragmented by anthropogenic activities. The distributional areas of all four *Amblysomus* taxa overlap extensively (28 – 91 %) with existing protected areas, this probably being because many protected areas coincide with mountain catchments that provide valuable water resources and also serve as hotspots of golden mole diversity (Ferrar and Lotter 2007). Given that globally a standard conservation goal in reserve design is to conserve 5 – 10 % of a species range within the reserve network (van Dyke 2008), and in Mpumalanga 10% is considered the threshold for adequate spatial conservation planning (Emery et al. 2002), it could be concluded that these taxa are adequately conserved by the Mpumalanga protected area network. However, ensuring that a minimum of 5-10 % of the geographic range of a taxon falls within reserves is only one of many conservation strategies, and does not necessarily translate into adequate protection of taxon-specific habitats, especially if these are patchily-dispersed and the taxa are ecological specialists with low vagility, as is the case with golden moles. Current emphasis is instead to ensure that the most ecologically suitable habitats are adequately protected and managed instead of simply preserving a quantity of land (van Dyke 2008). To confirm that the Mpumalanga protected area network is indeed adequately conserving the four *Amblysomus* taxa will entail ensuring that at least 5 – 10 % of their prime habitats are satisfactorily conserved and
managed to ensure that their ecological requirements will continue to be met, especially in the context of global climate change.

A recent study showed that climate change projections will likely result in the reduction and increase of vlei rat (*Otomys*) species due to grassland contraction and savannah expansion, respectively (Taylor et al. 2015). *Otomys* live, feed and disperse above ground in grasslands and vleis (Davis 1973) and are subjected to surface climates daily. The models in this study were calibrated to infer the distribution of golden moles in current climate, so climate change prediction datasets were not included. However, would climate change distribution models show similar effects on golden mole ranges? To understand the potential impacts of climate change on these grassland endemic taxa, I address here the main drivers of golden mole distributions that are predicted to change with climate change scenarios, viz. changes in precipitation and temperature, grassland range shifts and effects of vegetation on soil.

Whilst current trends show a decrease in summer precipitation over southern African grasslands (Warburton and Schulze 2005), climate projections instead predict higher, but delayed, precipitation in the grasslands as well as higher temperature (Christensen et al. 2007, Huntley and Barnard 2012) which may facilitate woody shrubs to outcompete grasses (Booth et al. 2003), thereby contracting grasslands and expanding savannah. However, no change in historic precipitation has been recorded in grasslands, and grasslands have not contracted along the shrubland ecotone (Masubelele et al. 2014). Contrary to studies localised to southern Africa, a study of global functional vegetation type dynamics predicted that southern African evergreen forests are likely to recede, and deciduous forests and grasses will take its place (Alo and Wang 2008).

In accordance with past research (Kuyper 1985, Jackson et al. 2008a), I have shown that the most important distribution drivers for the golden moles studied here are soil variables. Due to the buffering effect of soils (Adhikari et al. 2014, Murphy 2014), climate change projections of
temperature and precipitation variables alone would not provide a clear picture of golden mole distributions. Soil organic carbon content (SOCC) of topsoil is greater in grasslands than shrublands (Jobbágy and Jackson 2000), the latter of which is a functional vegetation type typical of savannah (Rutherford et al. 2006). Additionally, the projected higher temperatures and precipitation of the grasslands are associated with increased and decreased SOCC, respectively (Jobbágy and Jackson 2000). While it seems unclear what effect climate would have on SOCC (Davidson and Janssens 2006), vegetation type has a greater influence on SOCC than climate alone (Jobbágy and Jackson 2000). Hence, climate change events affecting soil-related variables such as SOCC and Predicted Soil Loss (PSL) could reduce the availability of prey items and habitat for golden moles. The inclusion of SOCC and PSL as cases of such events (Figure 3.10) suggests that golden moles are not currently affected by climate change scenarios.

With current climate change projections, species assemblages are likely to change due to changes in dispersal and extinction (Huntley et al. 2010, 2012), and those species with poor vagility or are of conservation concern face greater risk, especially in areas with high habitat loss (Erasmus et al. 2002, Huntley and Barnard 2012), such as the mining industries in Mpumalanga. Multiple climate scenarios showed that species richness hotspots of birds decrease in size particularly in the north and north-eastern regions (Huntley and Barnard 2012) congruent with Mpumalanga. The further shift of these hotspots into transformed grasslands (Huntley and Barnard 2012) could add additional pressure on grassland endemics. Ultimately, the effects of climate change on golden moles are likely attenuated; however, these taxa remain unassessed empirically.

In Mpumalanga, the percentage of ecoregions falling in protected areas that overlap with the by thresholded golden mole predicted presences are only 3 – 12 %. Of these four ecoregions that coincide with the predicted distributions of the four golden mole taxa targeted here, only the Drakensberg Montane grasslands, woodlands and forests ecoregion (11.7 %) and Highveld grasslands (5.2 %) are adequately conserved using the 5% criterion. While Southern Africa
Bushveld (14 %) appears to be adequately conserved, Zambezian and Mopane woodlands (3 %) is under-conserved; and these two ecoregions, which adjoin the Highveld grasslands to the east of the Great Escarpment, coincide only marginally with the thresholded distribution of only one of the four targeted taxa (*A. robustus*). Given the SOCC ≥ 3 % appears to be an important distribution limiter for golden moles in the Grassland Biome, and that the taxa occur mostly in areas with PP > 6 t/ha/an, then ideally at least 5 % of thresholded distributional areas of the golden moles should coincide with protected areas with ecoregions that also have SOCC ≥ 3% and PP > 6 t/ha/an (Table 3.4). The calculated overlap per taxon is as follows: *A. h. longiceps*: 2.44 %; *A. h. meesteri*: 4.39 %; *A. robustus*: 3.90 %; *A. septentrionalis*: 7.80 %. This emphasizes that while the sufficiently large areas of ecoregions (and landscapes – Emery et al. 2002) likely to harbour the targeted golden mole taxa may be adequately protected, it is unlikely that the prime habitats of these taxa except *A. septentrionalis* are adequately conserved.

**Table 3.4:** The percentage overlap of thresholded distribution for each taxon in relation to selected habitat characteristics (%) selected using the methods explained in the text. Method 1: PAs; SOCC > 4 %; PP > 6 t/ha/an; MCA. Method 2: PAs; SOCC > 3 %; PP > 6 t/ha/an; Grassland; Method 3: Conservancies; SOCC > 4 %; PP > 6 t/ha/an; Grassland; Method 4: PAs; SOCC > 3 %; PP > 6 t/ha/an; Grassland.

<table>
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</tr>
<tr>
<td><em>A. h. meesteri</em></td>
<td>10.45</td>
<td>4.39</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>A. robustus</em></td>
<td>0</td>
<td>3.90</td>
<td>1.67</td>
<td>1.19</td>
</tr>
<tr>
<td><em>A. septentrionalis</em></td>
<td>2.99</td>
<td>7.80</td>
<td>2.33</td>
<td>2.99</td>
</tr>
</tbody>
</table>

Despite its restricted range in two main fragments, 90 % of the thresholded distribution of *A. h. meesteri* coincides with protected areas, due largely to its location in the Panorama ecotourism route in the NE, and the wetlands conservation region in the SE (Figure 3.14; 3.15). However, 40 % of this taxon’s thresholded distribution coincides with areas in which there is no natural habitat remaining and 35 % in irreplaceable habitats. Furthermore, 61 % of the predicted distribution of *A. h. meesteri* and 50 % of *A. septentrionalis* falls within ecological
corridors, so formal protection of some corridors might be considered to ensure continued dispersal opportunities and gene flow between the NE to SE centres of this taxon’s distribution.

The importance of conserving ecological corridors between habitat patches has been well-documented (Bennett 1990, Perault and Lomolino 2000, Haddad et al. 2003, Carroll et al. 2012). However, conserving ecological corridors can have several disadvantages, including facilitating the spread of pathogens and invasive species between habitat fragments, and high disperser mortality owing to the presence of predators from adjoining areas and often lower-quality habitat, to the extent that overall population numbers may decline (van Dyke 2008). The persistence of a meta-population is sometimes also dependent on subunit populations having asynchronous demographics; dispersal through ecological corridors may compromise this independence and render subpopulations more vulnerable to regional environmental variation. The conservation effectiveness of corridors is thus taxon-specific, and depends on the characteristics of the corridors, especially the length from fragment to fragment relative to the dispersal abilities of the species (van Dyke 2008).

Given the low vagility and dispersal abilities of golden moles, the possible extent of corridor use as a means of dispersal is unknown but likely low. Consequently, on the basis of spatial analyses alone, the incorporation of some ecological corridors into the protected areas network to improve protection for *A. h. meesteri* and *A. septentrionalis* is not recommended. More surveying of the quality of suitable habitats, levels of predation and data on the dispersal abilities and the population dynamics of these taxa in habitat fragments would be needed before the purchase and management of corridors could be justified, especially since this can be counter-productive to conservation goals as it often means that resources are not optimally allocated to other measures (such as purchasing and managing additional land to enlarge existing reserves and habitat restoration) that may be more effective (van Dyke 2008).
The current network of conservancies, which are simply landowner coalitions that are not formally protected, and which can be transient, coincides only marginally with the distributional areas of two taxa (*A. robustus* – 17%; *A. septentrionalis* – 8%); based on geographic extent alone, conservancies appear to be of little conservation importance for these species. Supplementation of the existing protected areas network by incorporating some adjoining conservancies would only marginally enhance the conservation status of these taxa, given that their threshold distributional areas fall within 47% and 28% of current protected areas respectively. However, 66% and 56% (respectively) of the predicted distributions of *A. robustus* and *A. septentrionalis* overlap with areas of conservation concern (HS; I&N and I). This vindicates the IUCN Vulnerable status for *A. robustus*, which is largely confined to Montane grasslands subjected to overgrazing, too-frequent fires and mining activities (Rampartab 2015b). Furthermore, only 1.19% and 2.99% (*A. robustus* and *A. septentrionalis*, respectively) of the conservancies in which they are predicted to occur coincide with Grassland protected areas having SOCC ≥ 3% and PP > 6 t/ha/an. Thus, while supplementation of the existing protected area network through incorporation of adjacent conservancy lands be planned might be a desirable conservation goal for these taxa, areas with high soil organic carbon content and primary productivity should ideally be targeted.

In summary: *A. h. longiceps* has a marginal occurrence in Mpumalanga and appears to be adequately conserved in KwaZulu-Natal, so from a national and global perspective this taxon would probably not qualify for threatened status. However, from a provincial perspective, its limited range in Mpumalanga and limited occurrence in the conservation area network could be criteria warranting additional conservation focus. *A. h. meesteri* is adequately conserved by the existing protected area and land management network. Ecological corridors may be important
Figure 3.14: Taxon distribution thresholds in relation to (A) current ecological corridors, conservancies and protected areas network and (B) Mpumalanga Tourism and Parks Agency (MTPA) Terrestrial Biodiversity Assessment (TBA) areas of conservation concern (Ferrar and Lotter 2007).
Figure 3.15: Percentage congruence between the thresholded distributions of golden moles and various conservation parameters. EC – ecological corridors; Cons – conservancies; PAs – protected areas; TBA – terrestrial biodiversity assessment; LC – least concern; HS – highly significant; I&N – important and necessary; I – irreplaceable; NNHR – no natural habitat remaining.

for maintaining dispersal routes and possible gene flow between the NE and SE demes, thus supporting incorporation of these corridors into the formally protected network (van Dyke 2008), though more surveying of the quality of suitable habitats, levels of predation and data on the dispersal abilities and the population dynamics of this taxon in habitat fragments would be needed before the purchase and management of corridors could be justified. Conservancies are important for protecting suitable habitats for *A. robustus* more than other taxa, which may be important as this species is currently not adequately protected in selected Grassland habitat where SOCC > 3 % and PP > 6 t/ha/an (3.90 %) and may be a priority target for future conservation actions to supplement prime habitats (with high SOCC and PP in MCAs) of this species, given its endemic and vulnerable status (Rampartab 2015b). *A. septentrionalis*, likewise endemic and near-threatened (Rampartab 2015a), is marginally conserved in selected habitat by the existing PAs network (7.80 %) and efforts should be made to secure more land under conservation especially in the southern and central hotspots from Wakkerstroom towards Ermelo and Chrissiesmeer.
Chapter 4: Summary and Conclusions

The aim of this study was to develop and use species distribution models to assess bioclimatic parameters that circumscribe the niche requirements of four golden mole taxa (*Amblysomus hottentotus longiceps, A. h. meesteri, A. robustus* and *A. septentrionalis*) and to predict their distributions in the Highveld grasslands of Mpumalanga. Such information is needed to assess their conservation status and facilitate improved conservation and environmental management in the highly-impacted grasslands these taxa prefer. This study involved three main processes: (i) creating initial models trained on museum data; (ii) ground-truthing field survey during austral spring/summer to gather locality and genetic data; (iii) refining the models and determining the conservation status of these Highveld golden moles.

My first hypothesis (Section 1.4) was that the distributional ranges of these golden moles are constrained by numerous bioclimatic factors (especially soil properties), and thus can be modelled effectively using geospatial data so that the initial models (Figure 3.2 and 3.3) would accurately and precisely predict the distribution of golden moles based on museum data and a suite of environmental variables. By comparing these initial models to the field survey (Figure 3.5) and revised models (Figure 3.7 and 3.9) together in Figure 3.8, it is evident that the initial models helped to effectively focus survey efforts within a vast study area. A total of 25 new individuals were captured (Table 3.2), nine individuals of which (*A. meesteri* n = 2; *A. septentrionalis* n = 5; unknown n = 2) were captured in five new QDSs where no previous golden moles have been recorded. Additionally, observed activity was also recorded in 9 new QDSs (see Appendix 3). So this hypothesis is thus upheld by the results.

Secondly, I hypothesised that the cytochrome-*b* genotyping of the collected individuals would allow unequivocal discrimination between the four cryptic taxa. While probabilistic support values for the delineation of *A. h. longiceps* and *A. h. meesteri* were strong, distinguishing between *A. robustus* and *A. septentrionalis* was less robust, with the
identifications of two specimens (CR18 and CR25) being equivocal (Figure 3.4a; b). In hindsight, it would have been better to use multiple genetic markers as a previous genetic study employing a single marker (GHR; Asher et al. (2010)) was unable to discriminate between these species; likewise, an ongoing phylogeographic study has found it necessary to use two mtDNA markers (cytochrome-b and ND2) to successfully discriminate between these two taxa (Mynhardt et al. in review). *A. septentrionalis* and *A. robustus* are the most derived of all golden mole species, and based on rigorous molecular dating analyses based on 5 genes (S. Maree pers. comm., 2015) these taxa only diverged 0.4 – 0.8 m.y.a., whereas other *Amblysomus* species diverged 1.2 – 3.9 m.y.a. Given the close phylogenetic relatedness of *A. septentrionalis* and *A. robustus*, and their predicted geographic niche overlap along the southern parts of the escarpment, the use of additional molecular markers is recommended for any possible ground-truthing survey identifications. Despite these problems arising from the use of only cyt-b, which was a compromise given time and financial constraints, only two of the 13 specimens of these species (Table 3.3) could not be identified with 95% confidence using allele networks, so my hypothesis is largely supported by the results obtained.

I also hypothesized (Section 1.4) that since formal protected areas, ecological corridors and conservancies cover 20.53 %, 12.91 % and 5.50 % of Mpumalanga respectively, golden mole distributions would fall within these networks less than or equal to the given proportions owing to their patchy distribution and low vagility. This hypothesis is rejected by the relatively high (> 28 %; Table 4.1) overlap of the predicted distributional ranges of all four taxa that fall within protected areas, which can be attributed to their apparent preferences for habitats with high primary production and rich soils in mountain catchment areas – which have enjoyed preferential conservation owing to the ecosystem services they provide (Strydom and King 2009). Likewise, only the distributional range of *A. robustus* (8 %) coincides with ecological corridors to a lesser extent than I hypothesized, whereas the percentage overlap of the distributional areas of *A. h. meesteri* and *A. septentrionalis* that coincide with conservancies are
well above the 5.5% implied by my initial hypothesis, though these discrepancies are of little conservation importance as neither ecological corridors nor conservancies are currently formally protected.

**Table 4.1:** The percentage overlap between thresholded golden mole distributional areas and conservation areas in Mpumalanga based on data from Figure 3.14-15.

<table>
<thead>
<tr>
<th>Conservation area (%)</th>
<th><em>A. h. longiceps</em></th>
<th><em>A. h. meesteri</em></th>
<th><em>A. robustus</em></th>
<th><em>A. septentrionalis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protected areas (20.53)</td>
<td>65 %</td>
<td>90 %</td>
<td>47 %</td>
<td>28 %</td>
</tr>
<tr>
<td>Ecological corridors (12.91)</td>
<td>59 %</td>
<td>62 %</td>
<td>8 %</td>
<td>47 %</td>
</tr>
<tr>
<td>Conservancies (5.50)</td>
<td>0 %</td>
<td>17 %</td>
<td>0 %</td>
<td>8 %</td>
</tr>
</tbody>
</table>

### 4.1 How robust are the refined predictive distribution models?

Several factors may hamper the accuracy and precision of species distribution models (Elith and Graham 2009). The cryptic nature of chrysochlorids is such that much of their biology is unknown; and hence it is difficult to ascertain the most important environmental factors driving (or limiting) their distributions. Compounding this was the availability of only interpolated climatic data, which can be erroneous if not updated regularly at relevant scales, but use thereof was a necessary tradeoff as local climate data was neither available nor reliable for such a large study area. Categorical environmental data cannot be tested for spatial autocorrelation and potentially restricts the prediction area in ways seldom in nature (e.g., strictly-defined polygons representing sandy soils that golden moles prefer are in reality more likely to occur in gradients or mosaics rather than as homogenous units), so the use thereof in SDM is problematical if categorical classes are too coarse to reflect finer-scale geographic phenomena. Landtype categorical data was nonetheless included in deriving the refined models in this study, as landtypes are an arguably important environmental parameter affecting golden mole distributions (Jackson et al. 2008a), and the scale of the database available (16 179 landtype classes) was deemed appropriate. SOCC is arguably also a potentially important driver considering its influence on invertebrate prey density, but continuous or fine-scale categorical
data were not available (the available SOCC data layer used only 4 classes) for the formative models, although this is recommended for further refinement.

The use of presence-only data, as opposed to presence-absence data, was necessary in this study as confirming absences was practically impossible due to the large scale of the study area and difficulty of detecting, catching and correctly identifying specimens (Chapter 1 and Section 1.3.) While MaxEnt modelling is works particularly well in cases with few occurrence records and presence-only data (Pearson et al. 2007, Wisz et al. 2008, Williams et al. 2009, Wilting et al. 2010), the quality of any models derived is dependent largely on the veracity of the input data. Consequently, after ground-truthing, some of the available occurrence records were not included due to equivocal genetic identifications and/or duplicate records within the 1 km² locality grids used to reduce spatial bias. This was deemed necessary for data quality control, but reduced an already-small occurrence record database even further (Table 3.3). Although small occurrence record sample sizes may compromise model accuracy, using between 5 and 10 presence records often still permits the accurate modelling of rare species facing extinction risk (Pearson et al. 2007, Keith et al. 2008) as shown by Jackson et al (2010).

Given the limited number of distribution records available for calibrating the refined models, even after ground-truthing, I used a rigorous variable selection process (Section 2.4.1) to analyse variable contributions and spatial autocorrelations within the context of biological relevance. I also limited the prediction area by means of selecting a suitable projection background to improve spatial filtering and to reduce omission (false negative) and commission (false positive) errors (Section 2.4.2). I then also employed rigorous model comparison and evaluation techniques (Sections 2.4.3-4), including: comparing single-run models with five-fold cross-validated models; assessment of MaxEnt’s area under curve (AUC) of the receiver operating characteristic (ROC) to discriminate against poorly-fit models; examination of percentage contributions and MaxEnt internal jackknife values for variable importance, to ensure that only contributing factors were included in model training; as well as jackknife of
localities data to test if the refined model predictions could accurately identify presence of a removed occurrence record (Pearson et al. 2007), with subsequent omission testing.

The rigorous model development and evaluation procedure employed delivered encouraging results. Autocorrelation analyses ensured that only the most biologically relevant climatic variables contributed (Bio16-17: precipitation of warmest quarter and coldest quarter); given that precipitation is a major factor determining grasslands productivity; the use of these variables was justified (Rutherford 1980). The use of an appropriate background (landtypes) and spatial filtering (1 km² grid size due to low vagility and limited dispersal abilities of golden moles; Bronner 2013), although resulting in a 47% reduction in occurrence record sample sizes, served to minimalise sampling biases and omission rates. Stringent model comparisons employing jackknife analyses of variable importance (MaxEnt) and of localities (Pearson et al. 2007) showed that the final models have 95 % significance (except for *A. robustus* where significance was marginal (*p* = 0.06). Finally, omission error for activity points (where species identifications were not possible but golden mole presence was confirmed) was 13.21 %, indicating that the final models are fairly robust at predicting areas where golden moles probably occur. However an omission error as low as 5 % has been previously determined to be the ideal, but this is not necessarily prescriptive (Anderson et al. 2003, Phillips et al. 2006).

Lastly, AUC values for the final SDM models for all taxa were significant (0.997 – 0.999), and showed realistic predicted geographic distributions with simple response curves (Table 2.12). These are evidences of well-supported models, and follow a similar approach to that used by (Jackson and Robertson 2010).

### 4.2 Conservation implications and recommendations

So, what is the conservation value of the final models and findings from this study?

Notwithstanding the favourable model evaluation results, it is vital that deficiencies of the data on which the models are based (e.g. small sample sizes with probable high geographic sampling
biases; presence-only data; only interpolated climatic data, etc.) are borne in mind when assessing conservation implications. The maximally-conservative and fairly robust models I have developed, which provide predictions for 19 184 cells at a 4 km$^2$ grid size, are arguably better than a scant database of 59 distribution records at a 1km$^2$ locality grid size for the four targeted golden mole taxa (Table 3.3) when viewed from a conservation planning and environmental management perspective. However, given that no modelling algorithms predict consistently well with small taxon sample sizes (n < 30), conservation uses of model predictions should be highly conservative and largely restricted to exploratory applications (Wisz et al. 2008). Therefore, the refined models I have developed are at best a first approximation of golden mole distributional ranges in Mpumalanga grasslands. This proviso notwithstanding, my spatial analyses (Section 3.6) raise some noteworthy issues concerning the conservation of these taxa and management strategies for the environment that they occupy.

Based on my spatial analyses (Section 3.6), the current protected areas network in Mpumalanga conserves sufficient area (> 28 %) of the distributional ranges of all four targeted chrysochlorid taxa if the ">5-10% range conservation goal" is applied (Emery et al. 2002, van Dyke 2008). Likewise, of the four ecoregions that coincide with the predicted ranges of these taxa, three (Drakensberg Montane grasslands, woodlands and forests ecoregion - 11.7 %; Highveld grasslands - 5.2 %; Southern Africa Bushveld -14 %) are adequately conserved if this criterion is used, and while Zambeziand and Mopane woodlands (3 %) may seem under-conserved, this ecoregions probably includes only marginal habitat for only one of the four targeted taxa (*A. robustus*). However, given reservations about the scale, relevance and resolution of such macro-geographic units (Olson et al. 2001) when dealing with the conservation of range-restricted habitat specialists with low-vagility taxa that are habitat specialists, the "5-10% of range" criterion seems too lax.

Spatial analyses, however, also show that the predicted distributional ranges of most of the four taxa (excluding *A. septentrionalis*) are highly coincident not only with two grassland
ecoregions (Drakensberg and Highveld grasslands), but also with mountain catchment areas having soils with high organic carbon contents (> 4%) that support high primary production (> 6 t/ha/an.) and minima of 16 °C and 370 mm climatic minima for the warmest quarter and the wettest quarter, respectively. Assuming that these niche parameters circumscribe the prime habitats preferred by these taxa, the generally low coincidence of thresholded distributional ranges with protected areas having these properties (A. h. longiceps: 0%; A. h. meesteri: 10.45%; A. robustus: 0%; A. septentrionalis: 2.99%) suggests that their prime habitats are under-conserved by the existing protected areas network except for A. h. meesteri. Ecoregions were only used as a background to limit model predictions, and MCAs were only used to assess conservation status. These variables were not used during the development of refined models, because it would introduce an element of pseudo-replication and circularity into the assessment.

If one defines the prime habitat qualities of the taxa as being Grassland Biome areas having SOCC > 4% and PP > 6 t/ha/an, the percentage overlap with protected areas is only slightly higher (A. h. longiceps: 2.4%; A. h. meesteri 4.4%; A. robustus: 3.9%; A. septentrionalis: 7.8%, Figure 3.10), with only the predicted range of A. septentrionalis exceeding the 5% criterion. This suggests that prime habitats, as defined above, are under-conserved within the existing protected area network, and possibly occur only as discontinuous patches within formally conserved areas. Consequently, the “5-10% of range” conservation criterion may not be adequate to satisfactorily conserve and manage these areas to ensure that their ecological requirements of the chrysochlorid taxa, especially in the context of global change.
Figure 4.1: The prime habitat within Grassland Biome PAs where high SOCC and PP occur (> 3 % and > 6 t/ha/an, respectively.)

Applying these maximally conservative criteria from a precautionary perspective, it could therefore be concluded that the existing protected area network in Mpumalanga may not adequately conserve the four targeted chrysochlorid taxa, following IUCN council guidelines (IUCN Council 2007). However, the predicted golden mole distributional ranges, although based on fairly robust models, are a first approximation given the use of a small occurrence database, presence-only data and poor-resolution categorical SOCC data; caution should thus be exercised
when extrapolating therefrom. Furthermore, the SOCC categorical data used were coarse-grained (only 4 classes), and the PP data are, which could compound any inaccuracies inherent in model predictions and consequent conservation assessments.

Given also that all four of the golden mole taxa occur in areas of least concern or with no natural habitat remaining (LC: *A. h. longiceps*: 31%; *A. h. meesteri*: 15%; *A. robustus*: 42%; *A. septentrionalis*: 20%; NNHR: *A. h. longiceps*: 0%; *A. h. meesteri*: 40%; *A. robustus*: 14%; *A. septentrionalis*: 24%; Figure 3.15), and can survive in sub-optimal habitats subjected to mild transformation, the abovementioned definitions of “prime habitat” might be considered as too stringent and restrictive. However, a core principle in biological conservation is that it is best to protect the prime habitats of species, rather than sub-optimal and marginal habitats (van Dyke 2008). Moreover, if one applies the IUCN precautionary principle (IUCN 2015), which advocates against delaying conservation actions in the face of uncertainties and urges actions based on possible inaccurate predictions as these seldom result in significant environmental harm, then application of stringently conservative conservation criteria is warranted.

I therefore conclude that, based on my extensive analyses, the prime habitats of the four golden mole taxa largely endemic to the Highveld grasslands of Mpumalanga are probably not adequately conserved by the existing protected areas network. However, I stress that these conclusions are based on predictive models built with data that are, in many respects, lacking in quality and resolution; and thus that any application of the models and my findings by conservation or environmental managers should take cognizance thereof. Further ground-truthing is required not only to increase the number of verified occurrence records for each taxon, but also to collect absence-presence data, and also to develop higher-resolution spatial protected area layers (with continuous SOCC and PP data) on a scale appropriate for analysing the geographic configuration and extent (including inter-connectedness) of prime habitats these taxa apparently prefer.
All taxa except range-restricted *A. robustus* are well-distributed within ecological corridors, but the degree of corridor use by golden moles given their limited subterranean dispersal abilities raises doubt about the importance of ecological corridors for their conservation. Conservancies, although not formally protected, are declared areas in partnership with landowners to limit land-use activities and would link to existing neighbouring PAs and pristine habitat, aiding in the conservation of *A. robustus*, and marginally also *A. septentrionalis*.

The refined models presented here provide a best first-approximation of the spatial distribution of the four taxa studied. It has shown that models making use of past knowledge (Section 3.1) can be refined to create accurate (Section 3.2-3; 3.4.1) and conservative approximations of taxa distributions (Section 3.5). This approximation is the most applicable evidence for conservation planning as they are based on the most biogeographically-relevant distribution drivers available. Therefore, used in conjunction with current conservation management practices (Section 3.6), the protection of these endemic taxa can be optimised given environmental and anthropogenic development policy.
References


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Mammalia: 1–17.


Appendix 1: Genetic reference localities

Table A1: Accession codes for reference sequences used in NJ and ML trees and allele network. Sequences are available from MEEP lab (Department of Genetics, University of Pretoria.) *A. robustus* specimen is an unconfirmed specimen identity and was excluded from refined models.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. h. longiceps</em></td>
<td>Pietermaritzburg</td>
<td>AhlonQnsPrkPMBGM029</td>
</tr>
<tr>
<td></td>
<td>Wakkerstroom</td>
<td>AhlonTlkWksttm39876GM049</td>
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<tr>
<td></td>
<td>Ermelo</td>
<td>AhlonErmeloTM42136GM027</td>
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<td></td>
<td>Clarens</td>
<td>AhlongClarensGM026</td>
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<tr>
<td></td>
<td>Wakkerstroom</td>
<td>AhlonBirdLWksttmGM052</td>
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<tr>
<td></td>
<td>Van Reenen</td>
<td>AhlonVReenenGM034</td>
</tr>
<tr>
<td><em>A. robustus</em></td>
<td>Malelane</td>
<td>AmbMalelaneGM034</td>
</tr>
<tr>
<td><em>A. robustus</em></td>
<td>Dullstroom</td>
<td>ArobDullstTM41661GM047</td>
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<tr>
<td><em>A. septentrionalis</em></td>
<td>Ermelo</td>
<td>AsepErmeloTM42135GM203</td>
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<td><em>A. h. pondoliae</em></td>
<td>Margate</td>
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<td>Mariepskop</td>
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<tr>
<td></td>
<td>Graskop</td>
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<tr>
<td><em>N. juliana</em></td>
<td>Shere</td>
<td>NjulShere1TM40126GM125</td>
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</table>
### Appendix 2: Categorical variables overlapping model thresholds

**Table A2:** Overlap of landtype, ecoregion and biome with initial model thresholds, and landtype only with refined models.

<table>
<thead>
<tr>
<th>A. <em>h. longiceps</em></th>
<th>A. <em>h. meesteri</em></th>
<th>A. <em>robustus</em></th>
<th>A. <em>septentrionalis</em></th>
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<tbody>
<tr>
<td><strong>Landtypes</strong></td>
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<tr>
<td>Ab3; 27; 29; 30-40; 42-49; 56-59; 64-5; 67-8</td>
<td>Ab10; 30; 33; 35; 37-8; 40-1; 56-9</td>
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<td>Ab64</td>
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<td>Ba66</td>
<td>Ac39; 100</td>
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<td>Ad1; 9-11</td>
<td>Ae54</td>
<td>Ea75</td>
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<td>Ae54; 119; 121; 124</td>
<td>Fa329-30; 344; 353; 357-8</td>
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<tr>
<td>Ba14; 18-22; 34; 45; 51-7; 64-66</td>
<td>Ib161; 194</td>
<td>Fb162</td>
<td>Ca2; 17</td>
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<tr>
<td>Bb14-5; 21; 30; 35-39</td>
<td>Ic158</td>
<td>Fb65</td>
<td>Ea20; 23; 25</td>
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<td>Ca2; 3; 14; 17</td>
<td>Ib155</td>
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<td>Fa2; 50-1; 162-171; 24-5; 220; 310-330; 332; 336-8; 341; 343-4; 346; 352-3; 355; 357-8; 360-6; 393</td>
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<td>Fa162; 326-7; 343; 361</td>
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<tr>
<td>Bushveld</td>
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<td>Bushveld</td>
<td>Highveld</td>
</tr>
<tr>
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<td>Montane</td>
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<tr>
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<td>Grassland</td>
<td>Grassland</td>
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<td>Forests</td>
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<td><strong>Biome</strong></td>
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</tr>
<tr>
<td><strong>Azonal Vegetation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Refined Models** |                  |               |                     |
|-------------------|                  |               |                     |
| **Landtypes**     |                  |               |                     |
| Ab64 | Ab33; 35 | Ab32 | Ab47; 64 |
| Ac99 | Ac86; 89 | Ac1; 2; 75 | Ac1-2; 39; 75; 86 |
| Ca2; 17 | Ca17 | Ad1 | Ad1 |
| Fa24; 360-1 | Fa361 | Ba66 | Ba51 |
| Fa326-7; 343 | Bb21; 38-9 | Ib31 | Ca17 |
| Ib31 | Fa162; 326-7; 343; 361 |

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Appendix 3: Activity locality database

Table A3: Observed tunnelling activity characteristic of golden moles were logged at sites yielding unsuccessful trapping and used as an independent test file to verify model validity.

<table>
<thead>
<tr>
<th>Date</th>
<th>Closest Town</th>
<th>Locality</th>
<th>Latitude</th>
<th>Longitude</th>
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<tr>
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</table>
Appendix 4: R-script with comments (Robertson, pers. comm., 2015)

```r
setwd("F:/projects/MoleDistrib/Jacknife/")  #set the folder
aal <- read.table("trainjack6v2.csv", header=TRUE, sep=";")
ns <- nrow(aal)  #how many rows of data in the file?
no <- 1:ns  #numbers from 1 to ns
trdat <- {}  #empty variables
for (k in 1:ns) {  #loop to generate train & test set for all the species
  f <- setdiff(no, k)  #leave one out and take the rest for training
  tr <- aal[f,]  #for training
  spn <- "Amblysomus_hottentotus longiceps"  #species name to search for
  spp <- paste(spn, as.character(k), sep="")  #put a number onto each species name
  species <- sub(spn, spp, tr$species)  #  make the replacements
  trn <- cbind(species, tr[,2:3])  #combine the species names with the coordinates
  trdat <- rbind(trdat, trn)  #the training data
  tst <- aal[k,]  #select the testing point
  tst1 <- cbind(spp, tst[,2:3])  #combine the species name and coordinates
  tstdat <- rbind(tstdat, tst1)  #the testing data
}
fn2 <- "jack6v2.csv"  #file name
write.table(trdat, file=fn2, append=FALSE, sep=";", row.names=FALSE)  #write data to a csv file
# now run Maxent
# then evaluate
#fold1 <- choose.dir(default = "", caption = "Select folder")  #select the folder that contains the ascii files
fold1 <- "F:\projects\MoleDistrib\Jacknife\j6v2"  #folder to search for ascii files
fn <- list.files(fold1, pattern = "*.asc", full.names = TRUE)  #list of all the output ascii files in that folder (5x for each species)
tv1 <- {}  #empty variables used in loop
nf <- length(fn)  #how many files?
for (i in 1:nf) {  #for each file...
  p2 <- read.asciigrid(fn[i], as.image = FALSE, colname = basename("z"))  #read the output asciigrids, extract from predicted output
  p1 <- p2$z  #unique list of species
  f <- grep(us[i], trdat$species)  #row number for that rep
  d <- trdat[f,]  #training records for that rep
  coordinates(d) <- ~longitude+latitude  #sets spatial coordinates
  pp <- overlay(p1, d)  #extract values
  lpt <- min(pp$z)  #lowest presence threshold
  tv1 <- rbind(tv1, lpt)  #combine
  pl3 <- as.numeric(pl3[1:lpt])  #transform to presence-absence using lpt
  pl2 <- as.numeric(pl2[1:lpt])  #transform to presence-absence using 0.1
  ts <- tvstdat[i,]  #testing records for that rep
  coordinates(ts) <- ~longitude+latitude
  jk <- overlay(pl2, ts)  #extract test values
  w1 <- length(which(pl2$z==1))  #number of presence cells in map region
  w0 <- length(which(pl2$z==0))  #number of absence cells in map region
  pi <- pl2 / (pl2+w0)  #proportion of study area predicted present
  jack <- rbind(jk$z, pi)  #data for all reps
}
```

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