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An assessment of alien terrestrial invertebrate species in the pet trade in South Africa

By

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General abstract

Many species have been introduced throughout the globe for the pet trade and some have escaped or been released from captivity and become invasive. In South Africa, different terrestrial invertebrate species are offered for sale in pet stores, on websites and by breeders. It is not known which alien terrestrial invertebrate species are traded, if the names used are correctly applied or whether these species could pose the risk of becoming invasive should they be released or escape. To determine which species are in the South African pet trade, species names were collected from pet stores, websites and via private breeders. Twenty-seven specimens from 11 species were purchased from different pet stores for DNA barcoding to determine if the species were correctly labelled. To determine if any parts of South Africa would be climatically suitable, climate match (13 species) and species distribution models (23 species) were developed for 36 terrestrial invertebrate species. The Socio-Economic Impact Classification of Alien Taxa (SEICAT) and Environmental Impact Classification for Alien Taxa (EICAT) were used to assess the recorded impacts of terrestrial invertebrate species. A total of 53 (36 recognized and 17 unrecognized) terrestrial invertebrate species were recorded as being for sale in South Africa. The most popular species were mealworms (*Tenebrio molitor*) (89 availability index score), superworms (*Zophobas morio*) (78 availability index score) and dubia roach (*Blaptica dubia*) (49 availability index score). Terrestrial invertebrate species are used for various purposes such as pets, food for pets, cleaners of cages and soil improvement. Out of the 11 species that were sequenced, nine species were correctly identified. The phylogenetic tree indicated that nine species clustered with reference sequences of the same species with high nodal support values while two species clustered with the same genus but not the same species names. Nine species assessed following the climate match method had suitable climate in South Africa. The model performance indicated that models were successful in predicting areas that are climatically suitable for 15 species. Impact records were available for only 18 species. The recorded socio-economic impacts were generally higher than the recorded environmental impacts. We did not find any species that had large areas that were climatically suitable and high availability and that also had high environmental or socioeconomic impacts. Overall, the risk of invasion from terrestrial invertebrate species in the pet trade appears to be low based on the species considered in this study. However, the trade is dynamic which could mean that species availability could change in future or new species could be introduced. Therefore, long term sampling is necessary to obtain a more complete list as the market appears to be dynamic.

Keywords: Availability index, climatic match, DNA barcoding, invasion, pet trade, risk assessment, species distribution modelling (SDM), terrestrial invertebrates.

Declaration

I, **Takalani Nelufule**, declare that the thesis/dissertation, which I hereby submit for the degree **Master of Science in Zoology** at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: ______________________

DATE: 01 October 2018

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List of abbreviations

AIC	Akaike Information Criterion
COI	Cytochrome c oxidase subunit I
BIC	Bayesian Information Criterion
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Data System
DNA	Deoxyribonucleic acid
GBIF	Global Biodiversity Information Facility
IT IS	Integrated Taxonomic Information System
MC	Minimal Concern
MN	Minor
MO	Moderate
MR	Major
MV	Massive
MaxEnt	Maximum Entropy
MP	Maximum Parsimony
NJ	Neighbor-joining
NCBI	National Center for Biotechnology Information
SDM	Species distribution modelling

List of definitions

Common names	A common name of a taxon or organism (also known as a vernacular name, English name) used by people to call the species in different countries, e.g. Mealworm.
Trade names	Refers to the names used in the pet trade that were not recognized by GBIF or ITIS, also regarded here as unrecognized species e.g. *Opistachanthus wahlbergi*.
Recognized	Scientific names that appear in GBIF and/or ITIS e.g. *Blatta lateralis.*
Availability index	This is the number of times that a species was available when visiting pet stores, on websites and from expositions.

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Disclaimer

This thesis is comprised of a series of chapters of which two chapters have been prepared for submission to scientific journals. Therefore, some of the tables and figures overlap between the chapters. The reference style follows that of the journal "Biological Invasions" to which chapter two will be submitted. The tables and figures are presented at the end of each chapter (chapter two and three) while the appendices are presented at the end of the thesis.

Chapter 1: General introduction

Rationale and background

Humans are responsible for deliberately or unintentionally moving species to areas outside their native ranges (Levine and D'Antonio 2003; Hulme 2009). These species are introduced for different purposes such as agriculture, forestry (Richardson 1998), ornamental purposes (Ng et al. 2016) and for the pet trade (Hou et al. 2006). If these species find suitable habitat in their introduced area, some are able to establish and spread (Blackburn et al. 2011; Pyšek and Richardson 2010). Species with the ability to produce self-sustaining populations and spread into a new environment are referred to as invasive species, with the whole process termed biological invasion (Blackburn et al. 2011). Only a small proportion of species that are introduced from elsewhere actually become invasive.

Invasive species can threaten ecosystems and cause economic and biodiversity loss (Oslon 2006; Vila et al. 2011). It is usually costly to eradicate or control alien species once they establish in new areas (Fagestone 2003; Navia et al. 2010). In the United States of America, Pimentel et al. (2005) estimated that annual costs associated with invasive species are about US$120 billion. In South Africa's Cape Floristic Region, an estimate of R564 million (approximately US$39 million using a 2018 exchange rate) was spent on controlling invasive species over the past 20 years (van Wilgen et al. 2016) and this is nowhere near the actual cost associated with alien species. It is important to manage introductions of alien species in such a way that the risk of invasion is minimized. Therefore, preventing harmful species from being introduced into a country is an important way to prevent invasions (Faulkner et al. 2014).

There are many different pathways by which alien species can be introduced, which can complicate management. Hulme (2009) identified six overarching categories of pathways by which alien species can be introduced namely; release (introduction of species intentionally via release), escape (introduction of species intentionally but escapes unintentionally), containment (unintentional introduction with specific object), stowaway (unintentional introduction attached to a transport vector), corridor (unintentional introduction via human infrastructure linking previously unconnected regions) and unaided (unintentional introduction through natural dispersal of alien species across political borders from areas where a species is not native). The pet trade is one of the leading pathways by which species can be introduced into a new area, whereupon the alien species may escape captivity or in a few cases be deliberately released (Ng et al. 2016).

The demand for a species is thought to increase its availability, which in turn could lead to a greater likelihood of release or escape (Chucholl and Wendler 2017). Availability refers to the number of times that a species is available through various sources, including pet stores and websites. The likelihood of a species to becoming established once it is outside of captivity is influenced by the number of individuals of a species that are introduced into the new area, known as propagule pressure (Lockwood et al. 2009). Species with a high availability within a country or region are thus likely have a high propagule pressure.

Many species of different taxa are traded in large numbers around the world as pets (Carpenter et al. 2014; Su et al. 2015). The pet trade has increased in size and is estimated at a value of 20 billion US dollars with 350 million live animals traded globally per year (Karesh et al. 2007). Different taxa such as birds, reptiles and invertebrates have been introduced into many countries through the pet trade (Peacock et al. 2007; van Wilgen et al. 2008; Kumschick et al. 2016). Some of these species have either escaped or been released and have subsequently become invasive and caused signficant negative impacts (Carrete and Tella 2008). For example, the invasion of burmese pythons (*Python bivittatus*) has disrupted the local natural ecosystem, causing declines in native mammal and bird species in Florida (USA) (Dove et al. 2011). The red-eared slider turtle (*Trachemys scripta elegans*) became invasive in many parts of the world and has caused negative impacts on native turtle species (Ernst and Lovich 2009). The introduced ring-necked parakeet (*Psittacula krameri*) has established in many parts of the world and competes for nesting sites with native birds in Belgium (Strubbe and Matthysen 2007). Furthermore, also in Europe, the introduction of the American grey squirrel (*Sciurus carolinensis*) has resulted in the decline of native squirrel species (Gurnell et al. 2004). In South Africa, the common myna (*Acridotheres tristis*) was introduced into the pet trade and later escaped, and has subsequently spread throughout southern Africa (Peacock et al. 2007). In South Africa and Swaziland, the Australian redclaw crayfish (*Cherax quadricarinatus*) has established several population in several river system and has been reported to be spreading into new areas (Nunes et al. 2017). Moreover, the Indian walking stick insect (*Carausius morosus*) was introduced to South Africa for laboratory use, the pet trade and for school demonstrations and it has subsequently established populations locally (Brock 1999). It has also established in other parts of the world where it was introduced like Florida, California, the Azores and Madeira (Brock 1999; Aguiar et al. 2014).

Some species in the pet trade have been reported to pose a threat to biosecurity and public health. For example, tarantulas have been reported to be a vector of nematodes such as *Halicephalobus* sp. and *Haycocknema* sp. that have negative impacts on human health (Pizzi 2009). In Belgium, the illegal introduction of parakeets has caused psittacosis in humans after exposure to birds (De Schrijver

1998). Psittacosis is a zoonotic infectious disease caused by bacteria such as *Chlamydia psittaci*. The symptoms of psittacosis include diarrhea, weakness, nausea, vomiting, fatigue, fever and chills (NASPHV 2010). The import of African rodents from Ghana for the pet trade has led to the introduction of Monkeypox to humans and native rodents in the United States of America (Guarner et al. 2004). In addition, the introduction of finches, parrots and lovebirds from Pakistan resulted in the introduction of Newcastle disease which affects humans and poultry into Italy (World Parrot Trust 2004). In the United States of America (USA), different zoonotic diseases have been detected from pet species, including salmonellosis which has been reported to infect young children (CDC 2003; Chomel et al. 2007).

With the increased demand for new species worldwide, alien species are now available for sale in pet stores and on websites (Bush et al. 2014). Many species are also traded illegally on the internet, for example, salamanders have been recorded as for sale on a South African website (Measey et al. 2017). According to Harrison et al. (2016), illegal wildlife trade has been detected on the dark-web. In addition, several other species including invasive species have been reported to be traded illegally over the internet without any controls and regulation (Martin and Coetzee 2011; Humair et al. 2015). In addition, species prohibited for import under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and International Union for Conservation of Nature (IUCN) are known to be introduced via the pet trade (Gong et al. 2009). This will have implications for the management of alien and invasive species.

In South Africa, many species are offered for sale in pet stores, on websites and at public shows (expositions). Aquatic plants are also often sold in pet stores because they are used in fish tanks (Martin and Coetzee 2011). A few studies have assessed the connection between the pet trade and invasion in South Africa, e.g. amphibians (Measey et al. 2017), reptiles (van Wilgen et al. 2008) and tarantulas (Shivambu 2018). Some species introduced to South Africa have been reported to have established wild populations and spread (Petersen et al. 2017; Marr et al. 2017). To ensure that management actions are in place to manage alien species in the pet trade, every country should have up-to-date lists of introduced species. This can help identify the introduction of new species that could pose invasion risks and manage species that are already in the country (Latombe et al. 2016).

Although research has been undertaken for several vertebrate groups (e.g. reptiles (van Wilgen et al. 2008), amphibians (Measey et al. 2017) and tarantulas (Shivambu 2018), it is not known which terrestrial invertebrate species are offered for sale in the South African pet trade. One of the major challenges of understanding the pet trade is that species are often misidentified and common names are used in preference to scientific names (Keller and Lodge 2007; Patoka et al. 2014). Several terrestrial invertebrate species are traded using a variety of generic names, and common names (pers. obs.). Some species have more than one common name and it creates confusion for species identification (van der Walt 2012). Furthermore, identification of the species in the pet trade has been reported to be a challenge, for example, many fish species have been misidentified in the aquarium trade (van der Walt 2012). The identification of terrestrial invertebrates based on their morphological characteristics is often challenging and requires taxonomic expertise (Shivambu 2018). Molecular techniques can be used to determine the correct identity of these species.

Molecular techniques such as DNA barcoding are increasingly being used to identify species globally (Hebert et al. 2003) for different taxa (Cywinska et al. 2006; Seifert 2009; Joly et al. 2014). DNA barcoding is a taxonomic technique that uses short genetic markers in an organism's DNA to determine the species to which a specimen belongs (Herbert et al. 2003). Many risk assessments rely on information from the scientific literature in order to assess a number of traits and if the species cannot be identified or is incorrectly named then this information might either not be available or be incorrect. The correct species name (scientific name) is therefore necessary to unlock information about the species.

According to the International Plant Protection Convention and World organization for Animal Health (FAO 1996; OIE 1924), any species that is being considered for introduction is required to go through a risk assessment before being imported into most countries (Phelong et al. 1999). Risk assessment is the process that evaluates the likelihood that alien species will become invasive and the potential consequences in terms of impacts to native flora and fauna (Simons and De Poorter 2009). Risk assessments have been used to prevent high-risk species from being introduced and guide management decisions for alien and invasive species (Kumschick and Richardson 2013). Risk assessments follow different approaches to assess risks, including trait scoring and rapid screening approaches (Keller and Kumschick 2017). As part of risk assessments, species distribution modeling makes use of several climatic and environmental variables to predict the potential suitability of the climate in an area (Jacobs et al. 2015). Impact assessments form an integral part of risk assessment as they evaluate the consequences of species introductions in terms of their impact (Bacher et al. 2017).

Some of the species introduced to new areas have caused negative impacts on the ecosystems, human well-being and the economy of the country (Jeschke et al. 2014). From a management perspective, it is essential to understand the likely negative impacts of each introduced species (Vila et al. 2010). In order to prevent or minimize negative impacts, several steps have been proposed by the Convention on Biological Diversity in its Aichi target 9, which includes management and prioritization strategies (CDC 2013). Impact assessments attempt to evaluate the current and potential impacts of alien species and thereby assist decision-making processes and prioritization (Jeschke et al. 2014, Kumschick and Richardson 2013). These impact assessment schemes rely on published literature about the negative impacts caused by the species from their native or introduced ranges (Kumschick et al. 2015). Impact assessment protocols have been used to assess different species such as birds, mammals, plants and invertebrates (Nentwig et al. 2010; Kumschick et al. 2015; Vaes-Petignat and Nentwig 2014).

Many countries have incorporated risk assessment protocols into their legislation as a means of preventing the introduction of potentially harmful species (McGeoch et al. 2010), e.g. Hawaii (Daehler et al. 2004); Australia (Bomford and Hart 1999); New Zealand (Kolar 2004) and Europe (Weber and Gut 2004). Species-based screening and risk assessments have also been implemented for different taxa, e.g. fishes (Copp et al. 2005), plants (Pheloung et al. 1999) and both plants and animals (D'hondt et al. 2015).

The intentional introduction of alien species into South Africa is regulated under the National Environmental Management Biodiversity (NEMBA) Act 10 of 2004, and specifically the Alien and Invasive Species Regulations (hereafter the A&IS Regulations) that were promulgated in October 2014 (DEA 2014). The A&IS Regulations have categories by which different taxa are listed: category 1a, 1b, 2 and 3. Category 1a are species that cannot be owned and imported to South Africa, and must be combatted and eradicated; 1b are species that are not supposed to be owned, imported to South Africa and should be controlled and wherever possible, removed and destroyed. Category 2 species are species that need a permit for any activity and individuals of category 3 species are allowed to remain, but there must be no further propagation or sale (DEA 2014). Some species found in the pet trade are listed under these regulations, for example, redclaw crayfish (*Cherax quadricarinatus*) (listed as category1b) (Nunes et al. 2017) and the green-and-black poison arrow frog (*Dendrobates auratus*) (listed as categeory 2) (Measey et al. 2017). The species included in the A&IS lists were chosen based on a process of expert consultation followed by public consultation. However, the decisions underpinning the lists were not clearly documented, and there are several discrepencies. For example, the whole order Phasmatodea, including the Indian stick insect (*Carausis morosus*), is listed, and any exemption for native stick-insect species is not immediately clear from the lists. The regulations under which intentional introductions are managed may need to be revised so that native species are explicitly excluded and to ensure that all species are listed based on documented scientific evidence.

There has been an increase in the introduction of alien organisms through the pet trade in South Africa (Martin and Coetzee 2011; Jones et al. 2013). These increases are a cause for biosecurity concern because species with the potential of causing negative impacts to native species and the economy of South Africa may be introduced. Therefore, there is a need for research that will identify newly introduced species and identify risks associated with these introductions. This will inform alien species management programmes and assist in the development of effective legislation, risk assessments and policies that will regulate the introduction of alien species in the pet trade (van Wilgen et al. 2008).

Thesis outline

In this study, chapter 1 (this chapter) introduces the pet trade and discusses potential risks associated with the introduction of species for the pet trade. Chapter 2 investigates which alien terrestrial invertebrates are being offered for sale in the pet trade in South Africa. The aims of this chapter are to (1) to compile a list of alien terrestrial invertebrates and, (2) determine the extent to which these species have been correctly identified.Chapter 3 assesses aspects of risks associated with alien terrestrial invertebrates. The aims of this chapter are to (1) assess climatic suitability for these species should they escape or be released, and (2) assess the potential impacts of alien terrestrial invertebrates using a standard scoring system based on impacts throughout the world. Lastly, the thesis concludes with a general discussion, conclusion and recommendations (Chapter 4).

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Chapter 2: Alien terrestrial invertebrates in the pet trade in South Africa

Abstract

Many species have been introduced throughout the globe for the pet trade and some have escaped or been released from captivity and become invasive. In this study we use the terrestrial invertebrate pet trade in South Africa as a case-study to highlight trends in sale patterns and to try to update lists of alien species in the country. A total of 489 terrestrial invertebrate names including duplicates were obtained from three sources in the pet trade. A total of 53 (36 recognized and 17 unrecognized species) terrestrial invertebrate species names were found to be offered for sale, of which *Acheta domestica*, *Zophobas morio* and *Blaptica dubia* were used for food for pets, *Alphitobius diaperinus* for cleaners of cages, earthworms for soil improvement, and *Pandinus imperator* for pets. The most popular species were mealworms (*Tenebrio molitor*) (89 availabilty index score), superworms (*Zophobas morio*) (78) and dubia roach (*Blaptica dubia*) (49). Twenty-seven specimens from 11 species were selected for DNA barcoding to determine if the species were correctly labelled. A phylogenetic tree was used to determine the similarity of the species name on the label with the actual identity of the species. The phylogenetic tree indicated that 22 out of 27 specimens clustered with reference sequences of the same species with high nodal support values confirming the identity of nine of the 11 species sequenced. Long term sampling is necessary to obtain a more complete list as the market appears to be dynamic. The availability of the species appeared to be linked to their use, with the most available species being used as food for pets. Most of the specimens that were selected for DNA barcoding in this study matched with their reference sequence. Although none of the species that were found in the pet trade have been reported to be invasive elsewhere, several species could not be linked to recognized species names. The use of common names in the pet trade presents a challenge for compiling species lists.

Keywords: DNA barcoding, pet trade, terrestrial invertebrates

Introduction

Every year, massive number of live animals are transported around the world as part of the pet trade industry (Broad et al. 2003). Species are traded for ornamental (Ng et al. 2016) and recreational purposes (Cambray 2003), and for pet food. The increased popularity of alien pets has led to more introductions of alien species globally (Papavlasopoulou et al. 2013; van Wilgen et al. 2008; Mori et al. 2017). A large variety of species are offered for sale in pet stores. The pet trade is, therefore, a significant pathway for introducing alien species into new areas (Papavlasopoulou et al. 2013; Kumschick et al. 2016; Mori et. al. 2017), and is recognized as one of the CBD's introduction pathway sub-categories (Harrower et al. 2017). Once these species are in the pet trade, they are often released into the wild or they escape from captivity (Cadi and Joly 2003; Faulkes 2010). When released, some of these species can outcompete native species (Cadi and Joly 2004; Polo-Cavia et al. 2008), cause biodiversity loss (Engeman et al. 2007), and spread diseases and pathogens that are harmful to human health (Haenen et al. 2004; Weir et al. 2012).

While much of the trade has traditionally been through formal shops, more exotic organisms tend to be traded via expositions or directly from breeders to enthusiasts. In-line with other trades, there is an increasing amount of person-to-person trade over the internet (Derraik and Phillips 2010). For example, over 500 terrestrial invertebrate species are offered on one website alone (https://www.bugzuk.com). Internet companies are increasing access to a wider variety of taxa, leading to a demand for more novel pets (Bush et al. 2014). Many species of different taxa are offered for sale over the internet throughout the world (Kikillus et al. 2012), including mammals, insects, aquatic species and plants (Secretariat of the Convention on Biological Diversity 2010).

Some terrestrial invertebrates have managed to establish in areas where they have been introduced and are causing negative impacts. In Brazil, the introduced giant African land snail (*Achatina fulica*) has become a widespread pest by infesting urban areas, large areas surrounding cities and natural ecosystems (Thiengo et al. 2007). In Florida, a major infestation was caused by an introduction of these snails from Hawaii in the 1960s (Cowie 2011). In Florida, Mexican red rump tarantula (*Brachypelma vagans*) is reported to have established populations in the wild (Edward and Hibbard 1999). In South Africa, the Indian walking stick insect (*Carausius morosus*) was introduced for laboratory purposes and as a pet species, and subsequently established a population in the Cape Floristic Region (Picker et al. 2002; Picker and Griffiths 2017).

In South Africa, species that are popular in the pet trade are reptiles (van Wilgen et al. 2008), and amphibians (Measey et al. 2017). There appears to have been very little research on terrestrial invertebrates in the pet trade to date.

It is important for each country to compile an inventory of alien species as it serves as a first reference point in the management of biological invasions (Roy et al. 2014). These inventories are needed in order to know what to manage and determine what is present and potentially harmful (Latombe et al. 2016; McGeoch et al. 2012; Regan et al. 2002). Accurate and taxonomically correct lists are important because they can help prevent future invasions, thus increasing biosecurity for the country or region.

Even lists of supposedly well known invasive plant species contain many errors and inconsistencies (Magona et al. 2018). It is important to know which species are traded in order to assess their risks, but there is often inconsistency in the application of species names in the pet trade (Bartlett et al. 2001). Furthermore, many species have been misidentified (Keller and Lodge 2007). In South Africa, van der Walt et al. (2017) reported that fishes were misidentified in the pet trade. Tarantula species were also traded under inaccurate names in South Africa (Shivambu 2018). Misidentification of species can lead to the accidental introduction of problematic species.

The demand for species in the pet trade has been reported to increase species availabilty. Propagule pressure is known as the number of individuals of a species which are introduced to a new area coupled with the number of introduction events (Lockwood et al. 2009). Species with a high availability within a country or region are assumed to have a high propagule pressure, which could increase the likelihood that these species will escape from captivity or be released (Marr et al. 2017).

DNA barcoding has become a widely used molecular technique for identifying different species (Hebert et al. 2004a; Ward et al. 2005; Chase and Fay 2009). It has, for example, been used in identifying molluscs found in the pet trade in Indonesia (Ng et al. 2016). In other parts of the world, the DNA barcode region of mitochondrial CO1 has been used to identify and distinguish closely related and cryptic species in the pet trade (Hebert et al. 2004b; Tavares and Baker 2008; Mendoza and Francke 2017).

The aims of this study were to: 1) determine which alien terrestrial invertebrate species (excluding tarantulas, as as they were the subject of another study, by Shivambu (2018) are present in the pet trade in South Africa, 2) test the accuracy of identification of terrestrial invertebrates in the South

African pet trade using DNA barcoding, and 3) determine which species are most suitable as a proxy for propagule pressure.

Materials and methods

Data collection

Pet stores

We visited private breeders, pet stores and websites to obtain a list of alien terrestrial invertebrates offered for sale. Breeders were found at pet expositions, for example SOS^2 Annual Reptile's Expo (Emperor's Palace, 64 Jones RD, Kempton Park, in Gauteng province in South Africa on the 07th May of 2016 and 06th of May 2017) and Spring Reptile's Exotic Pet Expo (Jabulani Recreation Centre, Sandringham in Johannesburg Gauteng province, South Africa on the 10th September 2016 and 7th September 2017). Breeders are defined as people who breed these species and sell them to other people. They may also keep certain species as pets which they do not sell. Initially, pet store locations were gathered by searching the internet via Google search, with additional pet stores being found in the vicinity of pet stores that were visited. Key words like "pet stores around Pretoria" (search string) and "pet stores in Johannesburg", were used to search for pet store locations. Pet stores were investigated in four cities in South Africa, namely Pretoria, Johannesburg, Cape Town and Durban over two consecutive years (2016 - 2017) (Fig. S4). We visited these cities because they are the largest cities in South Africa, they are located in three provinces across the country and represent different climatic regions. Furthermore, internet search results showed more pet stores found around these cities than elsewhere in South Africa. We spent over four days in each city surveying pet stores [May 2016 for Gauteng (Pretoria and Johannesburg), August 2016 in the Western Cape (Cape Town) and December 2016 in KwaZulu-Natal (Durban)]. In October 2017, we also visited stores in Gauteng Province to purchase specimens for DNA barcoding. Species names were recorded as provided by the pet stores.

Websites

Google was used to search for South African online pet stores (hereafter referred to as "websites"). "Exotic pets", "insects" and "invertebrate pets for sale" were used as search terms to search for the species. Other online advertising sites such as Facebook (https://www.facebook.com), Gumtree (https://www.gumtree.co.za), Ananzi (https://www.ananzi.co.za), Junk mail (https://www.junkmail.co.za), and Olx (https://www.olx.co.za), were also searched for terrestrial invertebrates offered for sale using “exotic pets, insects and invertebrate pets for sale,”. Additionally, we used “cockroaches”, “scorpions”, “stick insects” and “terrestrial invertebrates for sale” as search terms. We only searched websites with a South African domain name, (i.e. addresses ending in co.za), except for Facebook. Websites were investigated over a period of two years, in 2016 and 2017. The searches were performed two months apart, starting in June 2016, on the last day of each month indicated above. Species names (common and scientific names) were then verified using the Integrated Taxonomic Information System (ITIS 2017) and Global Biodiversity Information Facility (GBIF 2017), using common names to search for species on GBIF. Some species were offered for sale using names that were not recognized by these databases in which case the species were recorded as “trade names” (e.g. Agrillus crickets).

Species accumulation curve

We ran species accumulation curves on the species data that were collected in order to assess sampling adequacy. These accumulation curves were generated used the vegan package in R (R core 2014).

Availability index

The availability index was calculated by counting the number of times that a species was available when visiting pet stores, on websites and from expositions. Only five pet stores in Pretoria were revisited and species re-counted. Species sold as food for pets were often sold in bulk quantities in a container (e.g. beetle larvae and cockroaches) which makes them hard to count, while species sold as pets were generally sold with fewer than 10 individuals per container (e.g. scorpions and centipedes). The number of individuals per species was not analysed, as many pet stores did not include the number of individuals of a species, for those that were sold in containers with more than one individual. This has made it difficult to collect prices for the individuals per species and therefore we do not have this information for many species.

DNA barcoding for confirmation of identity

To determine the correct identity of species in the pet trade we performed molecular analysis of a selected number of species. We selected 13 species for DNA barcoding, using the following criteria: (1) only a genus or common name was given by the pet stores, (2) species with available reference sequences in publicly accessible databases (BOLD 2018 and NCBI 2018), and (3) species prohibited for import under the South African National Environmental Management: Biodiversity Act, Alien and Invasive Species Regulations (DEA 2014). Three specimens of each species were purchased for barcoding, each specimen from a different pet store to ensure that sources were independent. The majority of the proposed species had reference sequences available on publicly accessible databases. For this study, 39 specimens belonging to 13 species with three specimens for each species were selected for DNA barcoding. For some species that were selected for DNA barcoding, the required three specimens could not be found for purchase. It was not possible to obtain three specimens of the same species for *Extatosoma tiaratum*, *Hermetia illucens*, *Achatina immaculata* and *Gryllus assimilis*. In addition, DNA did not amplify for three specimens of *Blaptica dubia*. As a result the final dataset consisted of 27 specimens belonging to 11 species.

DNA extraction, amplification and sequencing

The samples were euthanized and preserved in 99.9% ethanol at the University of Pretoria, South Africa. Total genomic DNA was extracted from a leg of each specimen using a Machery-Nagel NucleoSpin Tissue Kit (Duren, Germany), following the manufacturer's specifications. The mitochondrial COI gene was amplified by polymerase chain reaction (PCR) using the forward primer LC0-1490 (GGTCAACAAATCATAAAGATATTGG) and the reverse primer HCO-2198 (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994). The PCR reaction contained 20 pmol of each primer (forward and reverse), a single unit of TakaraTaq (Emerald Amp®MAX HS PCR Master mix, TAKARA BIO INC., Otsu, Shiga, Japan), 50 – 100 ng of DNA template made up to 25ul with distilled water.

The PCR denaturation step occurred at 94 °C for 9 minutes; 35 cycles of 94 °C for 45 seconds; annealing at 50 °C for 45 seconds and extension at 72 °C for 60 seconds with a final elongation step at 72 °C for 10 minutes. Following PCR, the samples were checked using agarose gel electrophoresis stained with goldview. The PCR products were purified using Machery-Nagel NucleoSpin Gel and PCR clean-up kit following the manufacturer's specifications. Purified PCR products were sequenced in both directions using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) and analyzed on the ABI PRISM 3130XL sequencer. The DNA Sequences were aligned with CLC Workbench Version 7.9.1 (QIAGEN Aarhus A/S: www.qiagenbioinformatics.com) and trimmed using MEGA7 (Kumar et al. 2016).

Molecular analysis

A BLAST search (Altschul et al. 1997) for highly similar sequences was performed on the Barcode of Life System (BOLD 2018) in order to confirm the accuracy of species identification. A high match was considered for species with a BLAST match of greater than 98%, medium match for a match between 90 - 98% and low match for species with a BLAST match of below 90% (Altschul et al. 1990).

Two COI reference sequences were downloaded from the publicly accessible databases for each specimen. In cases where there were no available reference sequences, congeners were incorporated. Reference sequences were used in order to confirm the accuracy of names used for the specimens obtained in the South Africa pet trade. The downloaded sequences were also incorporated in phylogenetic analyses for comparison with the sequences generated from the terrestrial invertebrate specimens. Two CO1 gene sequences from closely related genera to the terrestrial invertebrate sequences, the red wood ant (*Formica rufa*) (KR928491) and emperor moth (*Gonimbrasia belina*) (SATW060), were downloaded and included as outgroups to root the phylogeny.

The best model of sequence evolution was determined using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) by implementing jModelTest (Posada 2008). Phylogenetic analyses were performed using different tree building methods implemented in MEGA7 (Kumar et al. 2016). Initially the Neighbor-joining method (Saitou and Nei 1987) based on uncorrected p-distance (Nei and Kumar 2000) was used and thereafter, Parsimony (Tamura et al. 2011) and Maximum likelihood (ML) (Guindon et al. 2010) were implemented. A pairwise distance was used to calculate interspecific P-distance. Parsimony search used Subtree-Pruning-Regrafting (SPR) while the ML approach used the GTR+I+G model estimated in jModel test with a discrete gamma distribution (Darriba et al. 2012). Nodal support for all resultant topologies was assessed using 500 bootstrap replicates (Felsenstein 1981) and branches with less than 50 % bootstrap support were collapsed.

All specimens are being stored in the Department of Zoology and Entomology and will be lodged in the Agricultural Research Council's National Insect Collection. All sequences will be uploaded to the BOLD database when the manuscript has been accepted for publication.

Results

A total of 489 terrestrial invertebrate records including duplicates were obtained (Table S2). Of these 489 records, 340 were collected from websites, 98 from pet stores and 51 from breeders (Appendix, Table S2). When the duplicate names were removed, a total of 53 species belonging to 6 classes and 11 orders were obtained (Table 1) of which 36 species alien to South Africa (belonging to 11 orders and 5 classes) were recognized in GBIF and ITIS, while 17 species were not recognized (Table S2).

Most species (62%) belong to the class Insecta (18 recognized and seven unrecognized species) with 16.8% of those used as food for pets, while only two species (2.2%) were used as cleaners (Table 1).

All seven unrecognized species were sold as pet food. Under class Insecta, the most commonly traded groups were crickets (three species), cockroaches (six species), mealworms (one species) and superworms (one species). Unrecognized species were used for various purposes such as food for pets, as pets and for soil improvement.

Table 1. The names of alien terrestrial invertebrates per class and order obtained from the South African pet trade (online, pet stores and breeders) in 2016 and 2017. *Common name* is a common name of a taxon or organism (also known as a vernacular name, English name). *Source* is the source where invertebrates were obtained. *Recognized* are the names recognized by GBIF or ITIS while *unrecognized* represent the names that were not recognized by GBIF or ITIS. An asterisk indicates species for which DNA barcoding was performed.

Class	Order	Scientific name	Common name	Source	Recognized/ unrecognized	Availability
Arachnida	Scorpiones	*Pandinus imperator** Koch, 1843	Emperor scorpion	Websites, pet stores & breeders	Recognized	19
Arachnida	Scorpiones	*Pandinus cavimanus* Pocock, 1888	Tanzanian red clawed scorpion	Websites	Recognized	6
Arachnida	Scorpiones	*Hottentota saulcyi* Simon, 1880	None	Websites	Recognized	3
Arachnida	Scorpiones	*Heterometrus longimanus* Herbst, 1800	Black emperor scorp; Asian forest scorpion	Websites	Recognized	2
Arachnida	Scorpiones	*Hottentotta tamulus* Fabricius, 1798	Indian red scorpion; eastern Indian scorpion	Websites	Recognized	2
Arachnida	Scorpiones	*Leiurusquin questriatus* Hemprich & Ehrenberg, 1829	Deathstalker scorpion	Websites	Recognized	2
Arachnida	Scorpiones	*Mesobuthus eupeus* Koch, 1839	Lesser Asian scorpion; mottled scorpion	Websites	Recognized	2
Arachnida	Scorpiones	*Pandinus viatoris* Pocock, 1890	None	Websites	Recognized	1
Arachnida	Scorpiones	NA	Black forest scorpion	Websites	Unrecognized	3

Class	Order	Scientific name	Common name	Source	Recognized/ unrecognized	Availability
Arachnida	Scorpiones	NA	Hadogenes sp.	Websites	Unrecognized	4
Arachnida	Scorpiones	NA	Opistachanthus sp.	Websites	Unrecognized	2
Arachnida	Scorpiones	Opistachanthus wahlbergi	N/A	Websites	Unrecognized	3
Chilopoda	Scolopendromorpha	*Scolopendra subspinipes* Leach, 1815	Vietnamese centipede	Websites	Recognized	1
Chilopoda	Scolopendromorpha	NA	Blue leg centipede	Breeder	Unrecognized	1
Chilopoda	Scolopendromorpha	NA	Red leg centipede	Breeder	Unrecognized	2
Chilopoda	Scolopendromorpha	NA	Yellow leg centipede	Breeder	Unrecognized	2
Clitellata	Haplotaxida	*Eisenia fetida* Savigny, 1826	Brandling worm; common dung-worm	Websites	Recognized	2
Gastropoda	Eupulmonata	*Achatina immaculata** Lamarck, 1822	None	Websites & breeders	Recognized	4
Gastropoda	Eupulmonata	*Cornu aspersum* O.F. Muller, 1774	Brown garden snail; European brown snail	Websites	Recognized	2
Gastropoda	Eupulmonata	NA	Giant African snails	Breeder	Unrecognized	1
Insecta	Coleoptera	*Tenebrio molitor** Linnaeus, 1760	Yellow mealworm; mealworm	Websites, pet stores & breeders	Recognized	87
Insecta	Coleoptera	*Zophobas morio** Fabricius, 1776	Superworm; zophobas	Websites, pet stores & breeders	Recognized	76

Class	Order	Scientific name	Common name	Source	Recognized/ unrecognized	Availability
Insecta	Blattodea	*Blaptica dubia* Serville, 1838	Dubia roach; orange-spotted cockroach	Websites, pet stores & breeders	Recognized	49
Insecta	Blattodea	*Gromphadorhina portentosa** van Herrewege, 1973	Madagascar hissing cockroach	Websites, pet stores & breeders	Recognized	40
Insecta	Orthoptera	*Acheta domestica** Linnaeus, 1758	House crickets	Websites, pet stores & breeders	Recognized	32
Insecta	Blattodea	*Naupheota cinerea** Olivier, 1790	Lobster; speckled roach	Websites, pet stores & breeders	Recognized	30
Insecta	Blattodea	*Blatta lateralis** Walker, 1868	Turkestan cockroach; rusty red cockroach	Websites, pet stores & breeders	Recognized	29
Insecta	Orthoptera	*Gryllus bimaculatus* De Geer, 1773	Field crickets; two-spotted cricket	Websites	Recognized	14
Insecta	Blattodea	*Periplaneta americana* Linnaeus, 1758	American cockroach; waterbug	Websites	Recognized	14
Insecta	Orthoptera	*Gryllus assimilis** Fabricius, 1775	Jamaican field cricket	Websites	Recognized	12

Class	Order	Scientific name	Common name	Source	Recognized/ unrecognized	Availability
Insecta	Lepidoptera	*Bombyx mori* Linnaeus, 1761	Silkworms	Websites, pet stores & breeders	Recognized	11
Insecta	Blattodea	*Phoetalia pallida* Brunner von Wattenwyl, 1865	Palid roach	Websites, pet stores & breeders	Recognized	9
Insecta	Diptera	*Hermetia illucens** Linnaeus	Black soldier fly	Websites, pet stores & breeders	Recognized	9
Insecta	Diptera	*Drosophila hydei* Sturtevant, 1921	Flightless fruit fly	Websites	Recognized	6
Insecta	Coleoptera	*Alphitobius diaperinus* Panzer, 1797	Lesser mealworm; litter beetle	Websites	Recognized	6
Insecta	Blattodea	*Rhyparobia maderae*Fabricius, 1781	Madeiran cockroach	Websites	Recognized	4
Insecta	Coleoptera	*Dermestes maculatus* De Geer, 1774	Hide beetle	Websites	Recognized	3
Insecta	Phasmatodea	*Extatosoma tiaratum** Macleay, 1826	Macleay's spectre; spiny Leaf Insect	Websites & breeder	Recognized	3
Insecta	Lepidoptera	*Achroia grisella* Latreille, 1802	Lesser wax moth	Websites	Recognized	2
Insecta	Lepidoptera	*Manduca sexta* Linnaeus, 1763	Tobacco hornworm; goliath worm	Websites	Recognized	2
Insecta	Diptera	*Drosophila melanogaster* Meigen, 1830	Common fruit fly; vinegar fly	Websites & breeder	Recognized	2

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Class	Order	Scientific name	Common name	Source	Recognized/ unrecognized	Availability
Insecta	Blattodea	*Lucihormetica verrucosa* Brunner von Wattenwyl, 1865	Warty glowspot cockroach	Websites	Recognized	1
Insecta	Lepidoptera	*Samia cynthia* Drury, 1773	Ailanthus silkmoth	Breeder	Recognized	1
Insecta	Blattodea	*Blaberus discoidalis* Audinet-Serville, 1839	False death's head cockroach	Websites	Recognized	1
Insecta	Blattodea	*Blaberus fusca*	N/A	Breeder	Unrecognized	1
Insecta	Isoptera	NA	Termites	Websites	Unrecognized	4
Insecta	Coleoptera	*Dischista cinta*	N/A	Breeder	Unrecognized	1
Insecta	Diptera	NA	Maggots	Breeder	Unrecognized	1
Insecta	Orthoptera	NA	Crickets	Websites, pet stores & breeders	Unrecognized	34
Oligochaeta	Lubricina	NA	Composting worms	Breeder	Unrecognized	1
Oligochaeta	Lubricina	NA	Earthworms	Breeder	Unrecognized	2

The species accumulation curves for websites, pet stores and breeders did not attain an asymptote (Fig. 1). Websites and breeders had steep accumulation curves compared to pet stores. The lack of significant flattening suggests that either there might be many more species not included in our suveys, or there is significant turnover across sellers.

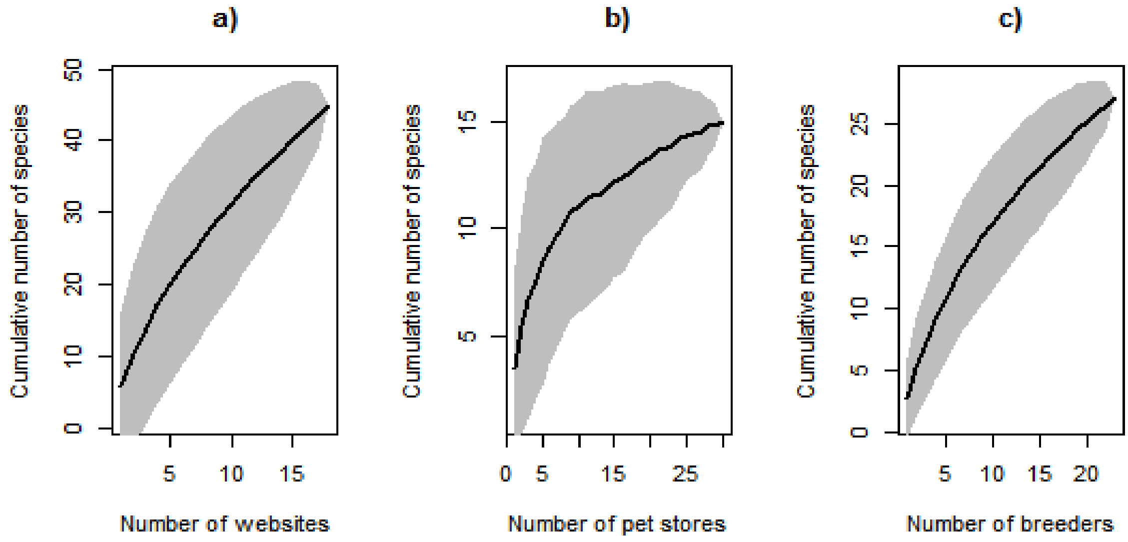

Figure 1. Species accumulation curves showing the number of terrestrial invertebrate species with the data obtained from a) websites, b) pet stores and c) breeders in the South African pet trade. The black line represents the mean accumulation curve while the grey shading represents standard deviation of the data from 100 random permutations.

Out of three sources, websites yielded the largest number of recognized terrestrial invertebrates with 36 species, followed by breeders with 17 and pet stores with 11 species (Fig. S2, Table 1). For the unrecognized species, websites had the highest number of terrestrial invertebrate species (n=9), followed by breeders (n=6) and pet stores (n=5) (Fig. S1).

We obtained a total of 95 trade names including duplicates and 35 names excluding duplicates from the three sources (Table S2). For both websites and pet stores, most of the species were sold using common names (e.g. “mealworms”), while some stores additionally provided scientific names.

The top seven species with the highest availability from all sources were mealworms (*Tenebrio molitor*) (n=89), superworms (*Zophobas morio*) (n=78), dubia roach (*Blaptica dubia*) (n=49), Madagascar hissing cockroach (*Gromphadorhina portentosa*) (n=40), house cricket (*Acheta domestica*) (n=32), lobster roach (*Nauphoeta cineria*) (n=31) and turkestan roach (*Blatta lateralis*) (n=30), which are all used for feeding pets (Fig. 2).

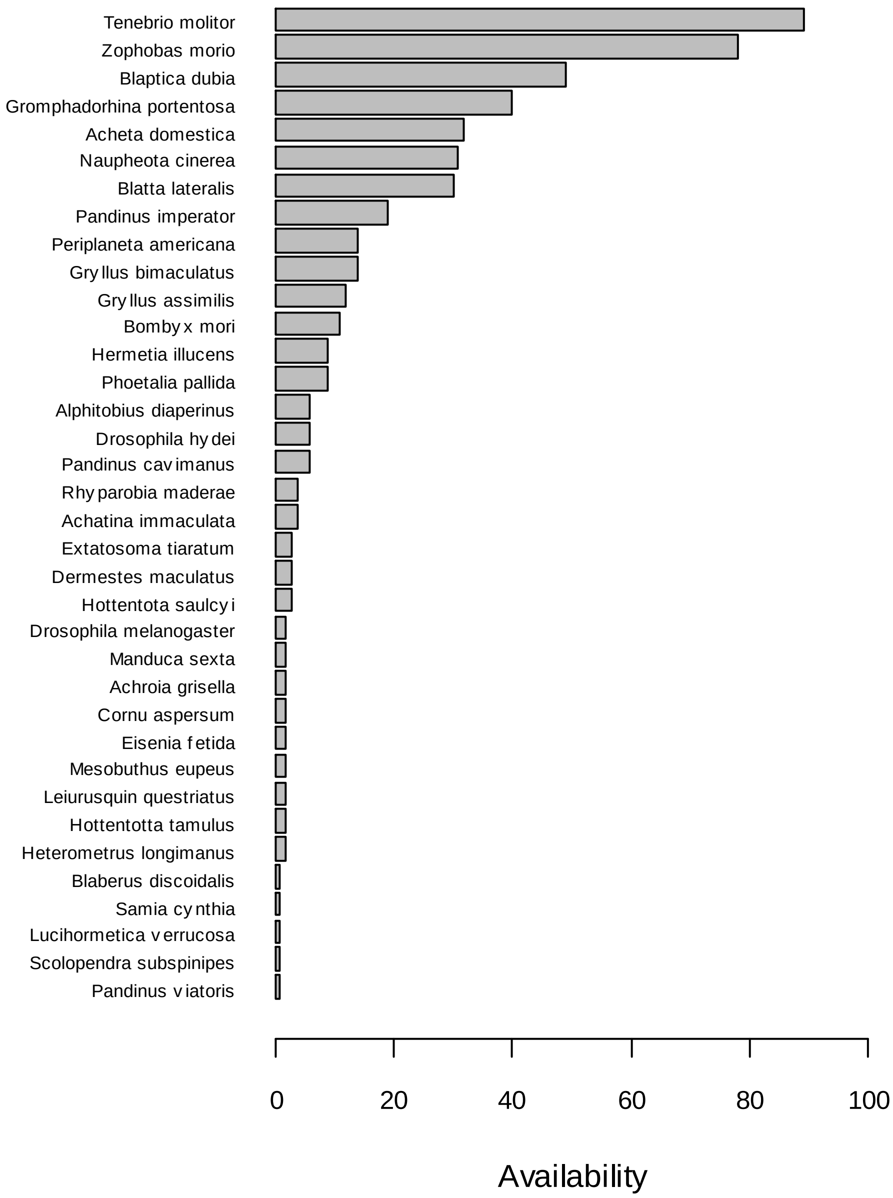

Figure 2. Availability index of terrestrial invertebrate species obtained from the South African pet trade. Availability index was calculated by counting the number of times that a species was available when visiting pet stores, on websites and from expositions.

We found three uses for terrestrial invertebrates in the pet trade, namely pets (e.g. emperor scorpion - *Pandinus imperator*), pet food (mealworms –*Tenebrio molitor*), and cleaners (species that are used for cleaning pet cages, e.g. hide beetle (*Dermestes maculatus*) and litter beetle (*Alphitobius diaperinus*) (Table S4). Out of the three uses, pet food had the highest number of species (n=24), followed by pets (n=12) and cleaners (n=2) (Table S4). Only three species, silkworms (*Bombyx mori*), Madagascar hissing cockroach (*Gromphadorhina portentosa*) and warty glowspot cockroach (*Lucihormetica verrucosa*) were used for two purposes—as pets and as pet food (Table S4).

Molecular results

BLAST search

Twenty-two out of 27 terrestrial invertebrate specimens matched with species of the same name from the BLAST search with identity matches of >93% (Table S1). Five specimens belonging to two species, (*Achatina immaculata* and *Pandinus imperator*) matched with species of the same genus but different species names. This is despite the presence of reference squences for these two species being available on the Genbank database. Out of these five specimens, three specimens of *Pandinus imperator* (specimen B7, B8 & B9) had a match of >94.95% to the reference sequence, while *Achatina immaculata* (Specimen D1 & D2) had a match of >80.74% (Table S1).

Model testing

To select the best-fit model with the best topology, 80 models were tested in jModelTest. The best-fit model of evolution for all terrestrial invertebrate specimens for COI was the Tamura 2-Parameter (S2P) model (Tamura 1992; Kumar et al. 2001), yielding the lowest AIC and BIC values.

Phylogenetic analysis

Sequences aligned with CLC Main Workbench and MEGA7 yielded sequence fragments of 545 base pairs (bp) for COI. A single well-supported tree was obtained through all data analyses methods (Fig.3). For all sites, the most parsimonious tree length was 1531, while the consistency index was 0.5361, the retention index 0.8627 and the composition index 0.4936. The mtDNA phylogenetic analysis formed clades whereby the majority had higher support from the nodal support values based on the ML bootstrap analyses. The sequences generated from the specimens acquired from pet stores produced a well-resolved phylogenetic tree with strong nodal support values (bootstrap values >70%) for species clades within Clade I and II. The results from the phylogenetic tree indicated that 27 terrestrial invertebrate barcodes obtained from the pet stores were grouped into two major clades (Clade I = 25 specimens; Clade II = 5 specimens) supported by good nodal support values (Fig. 3). The phylogenetic analysis indicated that 22 specimens out of 27 formed sister groups with the added reference sequences of the same species with strong nodal support values (NJ >100%, MP >99 %, ML >75%) (Fig. 3). In cases where reference sequences were not available, a reference sequence of closely related species was used. Only five specimens belonging to two species (*Achatina immaculata*: specimens D1 & D2) and (*Pandinus imperator:* B7, B8 & B9) formed a sister group with reference species of a different species (Fig. 3). For all the terrestrial invertebrate species, the estimated interspecific P-distance ranges between 0 - 0.785 for COI gene region.

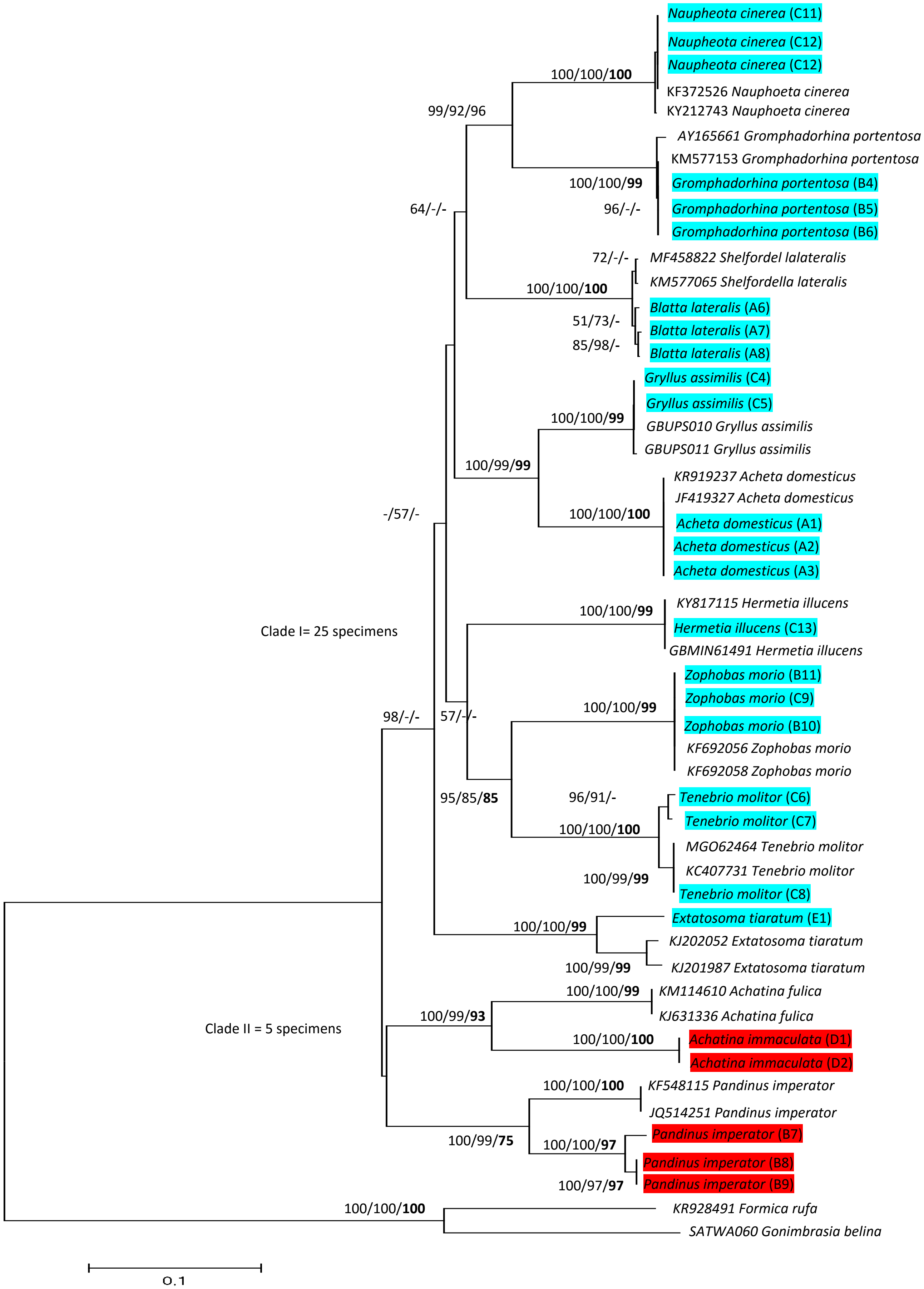
Naupheota cinerea (C11)
Naupheota cinerea (C12)
Naupheota cinerea (C12)
KF372526 Nauphoeta cinerea
KY212743 Nauphoeta cinerea
100/100/100
99/92/96
AY165661 Gromphadorhina portentosa
KM577153 Gromphadorhina portentosa
Gromphadorhina portentosa (B4)
Gromphadorhina portentosa (B5)
Gromphadorhina portentosa (B6)
100/100/99
96/-/-
64/-/-
72/-/-
MF458822 Shelfordel lalateralis
KM577065 Shelfordella lateralis
100/100/100
Blatta lateralis (A6)
Blatta lateralis (A7)
Blatta lateralis (A8)
51/73/-
85/98/-
Gryllus assimilis (C4)
Gryllus assimilis (C5)
GBUPS010 Gryllus assimilis
GBUPS011 Gryllus assimilis
100/100/99
100/99/99
KR919237 Acheta domesticus
JF419327 Acheta domesticus
Acheta domesticus (A1)
Acheta domesticus (A2)
Acheta domesticus (A3)
100/100/100
-/57/-
KY817115 Hermetia illucens
Hermetia illucens (C13)
GBMIN61491 Hermetia illucens
100/100/99
Clade I= 25 specimens
Zophobas morio (B11)
Zophobas morio (C9)
Zophobas morio (B10)
KF692056 Zophobas morio
KF692058 Zophobas morio
100/100/99
98/-/-
57/-/-
95/85/85
96/91/-
Tenebrio molitor (C6)
Tenebrio molitor (C7)
100/100/100
MGO62464 Tenebrio molitor
KC407731 Tenebrio molitor
Tenebrio molitor (C8)
100/99/99
Extatosoma tiaratum (E1)
KJ202052 Extatosoma tiaratum
KJ201987 Extatosoma tiaratum
100/100/99
100/99/99
KM114610 Achatina fulica
KJ631336 Achatina fulica
100/100/99
Clade II = 5 specimens
100/99/93
Achatina immaculata (D1)
Achatina immaculata (D2)
100/100/100
KF548115 Pandinus imperator
JQ514251 Pandinus imperator
100/100/100
Pandinus imperator (B7)
Pandinus imperator (B8)
Pandinus imperator (B9)
100/99/75
100/100/97
100/97/97
KR928491 Formica rufa
SATWA060 Gonimbrasia belina
100/100/100
0.1

Figure 3. The Maximum Parsimony phylogenetic tree of 27 sequences of terrestrial invertebrate specimens purchased from South African pet stores in 2017. The values in the tree branch represent the bootstrap percentage values of Neighbor-joining, Maximum Parsimony and Maximum Likelihood. Reference sequences are the species with accession numbers. Red wood ant (*Formica rufa*) and emperor moth (*Gonimbrasia belina*) were used as an out-group. The numbers in brackets are the specimen identifiers (Table 3) used to distinguish species obtained from the pet stores. Blue colour indicates species that have formed sister groups. Red colour represent species that did not form sister groups.

Discussion

The number of species recorded during our surveys of the pet trade are likely to be a substaintial underestimate of what is available in trade as the species accumulation curves showed no sign of reaching an asymptote for all three sources. This could be because, 1) we sampled the pet stores over a limited period of time, 2) pet store sampling was only focused on four big cities and 3) each time when visiting the expositions (breeders in Fig. 1c) there were new species on display. This could mean that the market is dynamic and changes over time. Although some species of different taxa are also offered for sale on the dark web (Harrison et al. 2016), this was not explored here because we considered it to be beyond the scope of MSc study.

The number of species obtained in this study (n=53) is smaller than other groups studied in the South African pet trade e.g. almost 200 species of tarantulas were found (Shivambu 2018) compared with 266 species of alien reptile species from 30 families (van Wilgen et al. 2010). In contrast, only three amphibians have been reported in the pet trade (Measey et al. 2017). Picker and Griffiths (2017) reported a total of 138 terrestrial invertebrate species already intentionally introduced to South Africa mostly as biological control agents with only one species (Indian stick insect - *Carausius morosus*) reported to be introduced for the pet trade. There appears to be much less literature on terrestrial invertebrates in the pet trade in other parts of the world, but Thomas (1995) reports that cockroaches, scorpions, solpugids, centipedes, millipedes, mantids, walking sticks, spider wasps, velvet ants, dung and beetles that originate from Africa, Asia, Central America and South America were imported to Florida (USA) as part of the pet trade.

Kumschick et al. (2016) indicate that there are many uses for introduced terrestrial invertebrates including animal feed (Kenis et al. 2014), pet trade (Edward and Hibbard 1999), food for pets (Haggett 2013), habitat and soil improvement (Baker et al. 2006), live exhibits (Boppre and Vane-

Wright 2012), silk production (Liebhold 1989) and ornamental trade (New 2008). Our study revealed that most terrestrial invertebrates in the South African pet trade are being used as food for pets. Pet store owners and breeders reported that these species are commonly used as food for pets such as reptiles and tarantulas. Due to the increase of the reptile and tarantula trade, it is possible that this is increasing the demand for terrestrial invertebrates as food for these species (van Wilgen et al. 2009; Shivambu 2018).

Most species that were sold as pets were arachnids (Table 1). For the terrestrial invertebrate taxa used as pets in the South African pet trade, scorpions had the greatest number of species. *Pandinus imperator* was the most popular pet species in the current study, which is also a popular species in the pet trade globally (Dombrowski et al. 2007; Magalhaes and Sao-Pedro 2012). All species were available from websites while only emperor scorpion (*Pandinus imperato*r) was available in pet stores and from breeders.

Class Gastropoda had two recognized and one unrecognized species which were all sold as pet food, while class Clitellata only had one species sold as pet food followed by class Chilopoda that had only one recognized and four unrecognized species, which were sold as pets (Table S5). Breeders who were selling these centipedes were using only common names without any knowledge about the scientific names and the origin of the species.

Class Oligochaeta had only two unrecognized species which were sold as fishing bait and for soil improvement (Table S5) (Baker et al. 2006). Species sold for fishing bait were traded under the name, "earthworms", while species sold for soil improvement traded under the name, "composting worms". It is not clear what these unrecognized species are and where they come from which is a great concern for invasion. It has been reported that different species of earthworms are been traded in large numbers for soil restoration and improvement. Some of these earthworm species have been reported to be invasive in forests and agricultural fields where by they caused negative impacts to native species (Baker et al. 2006). It is not known if these earthworms that are currently traded in South Africa could initiate invasive populations. Nevertheless, some of these invasive species are currently traded for different purposes such as fishing bait on a global scale, e.g. *Eudrilus eugeniae* (Blakemore 1999).

Species availability

Most pet store owners and breeders recommended mealworms, grey crickets, superworms and the dubia roach as pet food for reptiles and tarantulas (pers. obs.). These species are preferred as they are highly nutritious and easy targets for pets such as lizards and tarantulas as they are slow moving and do not fly (Rumpold and Schluter 2013).

The availability of a species is associated with the demand for that species in the pet trade (Patoka et al. 2014; Chucholl and Wendler 2017). It has been reported that the higher the availability of a species, the greater the likelihood that the species may be released or escape from captivity due to propagule pressure, which in turn could lead to a species establishing a wild population (Reaser and Meyers 2007; Nunes et al. 2017). Pet species can be released from captivity due to inconveniences such as stress that the species causes to the owner and/or loss of interest towards the species (Cassey et al. 2004; Kikillus et al. 2012). Should these introduced species escape or be released and find a suitable environment, they can potentially establish and become invasive (Liang et al. 2006). Most of the species studied here are present in fairly large numbers and are therefore likely to have opportunities to escape.

What influences species availability?

We found a considerable variation in availability between species in our study. In Taiwan, Su et al. (2015) found that price plays an important role in influencing the number of birds introduced through the pet trade. However, Shivambu (2018) found that price was not a good predictor of species availability for the tarantula species in the pet trade in South Africa, i.e. cheaper species were not always available in higher numbers. Other factors that were suggested to influence availability are for example the preference of lentic habitats, bright colour and the ability to reproduce under warm artificial conditions, which were reported to explain species availability for freshwater crayfish in the pet trade (Chucholl and Wendler 2017). In a study on reptiles, van Wilgen et al. (2009) suggested that colour pattern, and species that are larger and easier to breed influence species availability in the pet trade in South Africa. Other variables which can explain species availability include the rarity of the species, size, longevity, productivity and higher profit (Courchamp et al. 2006; Chucholl 2013; Su et al. 2015; CEC 2017; Vall-llosera and Cassey 2017). These variables are more likely to increase the demand for a species hence influencing the massive breeding of the species for the pet trade (Courchamp et al. 2006; CEC 2017). The demand for terrestrial invertebrates could be linked to the availability of reptile species (van Wilgen et al. 2009), tarantula species (Shivambu 2018) and amphibians (Measey et al. 2017) in the pet trade in South Africa, as most of these species are sold as food for pets.

Many terrestrial invertebrate species sold as food for pets were cheap. For example, mealworms were sold for 0.50 South African rands individually while Madagascar hissing roaches were sold for ZAR 4.00 (0.28 USD) (pers. obs.). Unlike reptile and tarantula species which are relatively expensive to purchase (van Wilgen et al. 2009; Shivambu 2018), terrestrial invertebrate pet species are more likely to be released because they are cheap (van Wilgen et al. 2009). Furthermore, once pet owners are tired of keeping the species, they are more likely to release them into the wild than to kill them because of the love they had for their pets (Hulme et al. 2008). According to van Wilgen et al. (2009), large animals were dumped outside a Zoo by their owners when it became inconvenient to keep them. Many species are released by their owners because they tend to lose their beautiful colours when they are adults or they become unmanageable, for example, Burmese pythons (Snow et al. 2007). However, the species studied here are more likely to escape because they are bred in large numbers, sold in large quantities and owners spend little on their housing (pers. obs.).

There appears to be a relationship between the purpose for which terrestrial invertebrates are used and their availability in the South African pet trade. Species with high availability were used as food for pets, which could indicate that the demand for food for pets such as tarantulas and reptiles influences species availabilty.

In this study, seven species (*Parabuthus capensis*, *Parabuthus transvaalicus*, *Hadogenes troglodytes* "Scorpiones", *Oxyhaloa deusta* "Blattodea", *Anisorrhina flavomaculata, Rhabdotis aulica* and *Pachnoda sinuate "*Coleoptera") native to South Africa were found in the pet trade, while 36 species were alien to the country. We found one species under ther order Phasmatodea, the spiny Leaf Insect (*Extatosoma tiaratum*) which is prohibited for import under The National Environmental Management: Biodiversity Act (10/2004) Alien and Invasive Species regulations (DEA 2014) in South Africa. We did not find any species which were reported to be invasive elsewhere, however, this does not mean that these species will definitely not become invasive in South Africa. Also, insects and other terrestrial invertebrates are generally not well studied as aliens (except when introduced for biocontrol), and they are inconspicuous, which might mean they are not easily detected if in the wild.

Furthermore, a total of 17 unrecognized species were found, some of which could potentially be invasive. Of these species, two snails were sold under the common names "Giant African land snail" and "African land snail" without any knowledge of the scientific names or where they come from. Giant African land snails (*Achatina fulica*) are known to cause large problems elsewhere. For example in the 1960s, importation of giant African land snails from Hawaii led to an infestation in Florida which was later eradicated (Cowie 2011). In Brazil, they were introduced as pet food and became a widespread problem by infesting urban areas, large areas surrounding cities and natural ecosystems (Thiengo et al. 2007).

Names used

The names of species in the pet trade were not consistent throughout the pet stores. We found several terrestrial invertebrate species sold only under trade names, different common names for single species and inaccurate names. This can create problems when compiling a list of species that are available through the pet trade (Patoka et al. 2014; pers. obs.). In the Czech Republic, crayfish were reported to be sold under inaccurate names, names of other species, outdated names and only by a trade name in the pet trade (Patoka et al. 2014). Similarly, Chucholl (2013) reported the use of inappropriate trade names in the crayfish trade in Germany. The use of different names and spelling errors could lead to misidentification of species in the pet trade (Rabemananjara et al. 2008). Van der Walt (2012) reported that many fish species were misidentified in the aquarium pet trade. This can lead to the introduction of species with invasion potential, create confusion when compiling alien species inventories and can therefore cause identification uncertainties in the pet trade.

In order to monitor species in the pet trade effectively, the identity of the species needs to be known (Schlaepfer et al. 2005; Sanders et al. 2008), as this plays an important role in risk assessment as scientific names are a link to the literature on species traits and behaviour (Schlaepfer et al. 2005; van Riemsdijk et al. 2017). In the pet trade, pet store owners and breeders can misidentify species due to morphological similarities and lack of appropriate expertise for species identification (Lydeard et al. 2004; Natusch and Lyons 2012). Many species have been misidentified in the pet trade across a variety of taxa, including tarantulas (Shivambu 2018), crayfish (Chucholl 2013), turtles (Stuart and Parham 2007), aquatic plants (Keller and Lodge 2007) and fish (van der Walt et al. 2017). An additional problem occurs if species are incorrectly identified and hybridization occurs as a result of species mixing (Natusch and Lyons 2012). With regard to the snail *Achatina immaculata*, all species sold as *Achatina immaculata* were identified as *Achatina fulica*, albeit with a low percentage match which could indicate that these species could be a hybrid of *Atchatina fulica* or another species under genus *Achatina* (Fig. 3).

Confirming that the species are in South Africa

This study successfully reported a high number of specimens that matched with the barcodes of species from the same proposed species using the COI gene region. Shortage of reference sequences in these databases is a problem for many taxa and in an attempt to deal with the shortage, researchers around the world created and deposited reference sequences of various taxa into publicly accessible databases (Meier et al. 2016). This study was made possible because of the many barcodes deposited in these publicly accessible databases (Steinke et al. 2009). Identification of species using DNA barcodes can be easily performed when reference sequences are available and the taxonomy of the species is well resolved; both of which were true for the current study (Meier 2008).

The inclusion of other gene regions (such as 12s rDNA (12S), 16s rDNA (16S), cytochrome-b (CYTB), and ND1-16S) could make molecular identification possible for other species which were excluded from this study (Hamilton et al. 2011; Wenner et al. 2012; Mwale et al. 2016). The process of extracting and amplifying DNA from beetle larvae such as *Tenebrio molitor* and *Zophobas morio* was difficult as the legs were hard to cut because they are less than 1 mm long (Winnepenninckx et al. 1993).

It seems as if we do not have *Pandinus imperator* (emperor scorpion) in the South African pet trade as none of the three specimens matched with reference sequences of this species on BOLD. This could indicate that all *Pandinus imperator* specimens in the South African pet trade are in fact a different species, most likely *Pandinus viatoris.*

Conclusion

The study found 36 recognized species and 17 unrecognized species. This list of species is likely to be an underestimate of what is actually being traded. Long term sampling is necessary to obtain a more complete list as the market appears to be dynamic. Species in the pet trade are used as food for pets, as pets, as cage cleaners and for soil improvement. The availability of the species appeared to be linked to their use, with the most available species being used as food for pets. Availability of terrestrial invertebrates could be related to their demand as food for pets such as tarantulas and reptiles. Most of the specimens that were selected for DNA barcoding in this study matched with their reference sequence. Although none of the species that were found in the pet trade have been reported to be invasive elsewhere, several species could not be linked to recognized species names. In addition, specimens of *Achatina immaculata* matched with the reference sequence of the highly invasive *Achatina fulica* with the percentage match of 80.74%. The use of common names in the pet trade presents a challenge for compiling species inventories as it is often difficult to link the common name to the correct scientific name.

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Appendix 1

Table S1. Results of species identification from BLAST search for 27 terrestrial invertebrate specimen sequences acquired from pet stores. *Specimen ID* is the unique specimen identifier. *Specimen names* are the scientific names of the specimens. *COI* (*Blast Match %)* is the reference sequences with match percentage, which were found when the sequences from this study were blasted on the NCBI. *Accession number* is the accession numbers of the reference sequences found in NCBI.

Specimen ID	Specimen names	COI (Blast Match %)	Accession number
D1	*Achatina immaculata*	*Achatina fulica* (80.74)	GBMIN98725-17
D2	*Achatina immaculata*	*Achatina fulica* (80.74)	GBMIN98725-17
A1	*Acheta domesticus*	*Acheta domesticus* (100)	KR919237
A2	*Acheta domesticus*	*Acheta domesticus* (100)	JF419327
A3	*Acheta domesticus*	*Acheta domesticus* (100)	KR919237
A6	*Blatta lateralis*	*Shelfordella lateralis* (99)	KM577065
A7	*Blatta lateralis*	*Shelfordella lateralis* (99)	MF458822
A8	*Blatta lateralis*	*Shelfordella lateralis* (99)	KM577065
B3	*Extatosoma tiaratum*	*Extatosoma tiaratum* (93)	KJ202052
C4	*Gryllus assimilis*	*Gryllus assimilis* (100)	GBUPS010
C5	*Gryllus assimilis*	*Gryllus assimilis* (99.82)	GBUPS011
B4	*Gromphadorhina portentosa*	*Gromphadorhina portentosa* (100)	KM577153
B5	*Gromphadorhina portentosa*	*Gromphadorhin aportentosa* (100)	KM577153

Specimen ID	Specimen names	COI (Blast Match %)	Accession number
B6	*Gromphadorhina portentosa*	*Gromphadorhina portentosa* (99)	AY165661
C13	*Hermetia illucens*	*Hermetia illucens* (100)	GBMIN61491
C10	*Naupheota cinerea*	*Naupheota cinerea* (100)	KF372526
C11	*Naupheota cinera*	*Naupheota cinera* (100)	KF372526
C12	*Naupheota cinera*	*Naupheota cinera* (*100*)	KF372526
B7	*Pandinus imperator*	*Pandinus viatoris* (94)	AY156583
B8	*Pandinus imperator*	*Pandinus viatoris* (95)	AY156583
B9	*Pandinus imperator*	*Pandinus viatoris* (95)	AY156583
C6	*Tenebrio molitor*	*Tenebrio molitor* (99)	KC407739
C7	*Tenebrio molitor*	*Tenebrio molitor* (99)	KF692052
C8	*Tenebrio molitor*	*Tenebrio molitor* (100)	MG062464
C9	*Zophobas morio*	*Zophobas atratus* (100)	MG057841
B10	*Zophobas morio*	*Zophobas atratus* (100)	MG057841
B11	*Zophobas morio*	*Zophobas atratus* (100)	MG057841

Table S2. The total number of terrestrial invertebrate records obtained from three sources (websites, pet stores and breeders) in 2016 and 2017, including duplicates. *Source* is the source where invertebrates were obtained. *All terrestrial invertebrate names* are all the species collected including recognized and unrecognized names including the duplicates. *All trade names* are all unrecognized species including the duplicates. *All common names* are all common names obtained including duplicates. *Trade names* and *unrecognized* species are the species whose names were not recognized when searched on GBIF. *Recognized species* are species whose scientific names are recognized by GBIF.

Source	**All terrestrial invertebrate names**	**All trade names**	**All common names**	**Recognized**	**Unrecognized**
Online store	342	41	293	36	21
Physical store	98	5	117	11	5
Breeders	51	14	48	18	13
Total	491	95	458	65	39

Table S3. Reference sequences acquired from NCBI, GenBank and BOLD. *Specimen name (ID)* is the scientific name of the specimen DNA sequence. *GenBank Accession Number* is the number of the specimen on the GenBank. *Sampling sites* refers to country where the specimen has been collected. Notes highlight the environment in which the specimens have been collected.

Specimens name (ID)	GenBank Accession Number	Sampling sites	Notes	Reference
Achatina fulica	KM114610	China	Wild caught	Unpublished (deposited by Zhang *et al*. 2014)
Achatina fulica	KJ631336	India	Wild caught	Unpublished (deposited by Prakash *et al*. 2015)
Acheta domesticus	KR919237	Canada	Wild caught	Hebert *et al*. 2016
Acheta domesticus	JF419327	Austria	Captive bred	Sint *et al*. 2011
Blatta lateralis	KM577065	U.S.A., Argentina, Australia, Belize, Guyana, Spain and Venezuela	Wild caught	Beeren *et al*. 2014
Blatta lateralis	MF458822	France	-	Corse *et al*. 2017
Extatosoma tiaratum	KJ202052	Australia	Wild caught	Velona *et al*. 2015
Gryllus assimilis	GBUPS010	Germany (Munich)	Captive bred specimen	Unpublished (Deposited in SNSB, Zoologische Staatssammlung Muenchen)

Specimens name (ID)	GenBank Accession Number	Sampling sites	Notes	Reference
Gryllus assimilis	GBUPS011	Germany (Munich)	Captive bred specimen	Unpublished (Deposited in SNSB, Zoologische Staatssammlung Muenchen)
Gromphadorhina portentosa	KM577153	U.S.A., Argentina, Australia, Belize, Guyana, Spain and Venezuela	Wild caught	von Beeren *et al*. 2015
Gromphadorhina portentosa	AY165661	Canada	Captive bred specimen	Hebert *et al*. 2003
Hermetia illucens	GBMIN61491	Russia	NA	Unpublished (Deposited by Ushakova *et al*. 2017)
Hermetia illucens	KY817115	Russia (Moscow)	Wild caught	Unpublished (Deposited by Ushakova NA, 2017)
Naupheota cinerea	KF372526	Canada	Captive bred specimen	Hebert *et al*. 2002
Naupheota cinerea	KY212743	Brazil	Captive bred specimen	Dumans *et al*. 2017
Pandinus imperator	KF548115	Portugal	Wild caught and captive bred	van der Meijden *et al*. 2013
Pandinus imperator	JQ514251	Portugal	Wild caught	Meijden *et al*. 2012

Specimens name (ID)	GenBank Accession Number	Sampling sites	Notes	Reference
Tenebrio molitor	KC407731	South Korea	Wild caught	Cho *et al*. 2013
Tenebrio molitor	MG062464	Canada	Wild caught	Unpublished (Submitted by Dewaar JR, 2017)
Zophobas atratus	KF692056	Korea	Wild caught	Park *et al*. 2013
Zophobas atratus	KF692058	Korea	Wild caught	Park *et al*. 2013

Table S4. Recognized terrestrial invertebrate species names per class obtained from the South African pet trade (online, pet stores and breeders) in 2016 and 2017. *Common names* are the names commonly used and recognized by scientific literature, GBIF and ITIS. *Source* is the source where invertebrates were obtained. *Use* refers to what these terrestrial invertebrates are used for. *Availability index* was calculated by counting the number of times each species was available either independently or in the same source when visiting the pet stores, websites and breeders.

Class	**Order**	**Scientific name**	**Common name**	**Availability index**	**Use**
Arachnida	Scorpiones	*Pandinus imperator* Koch, 1843	Emperor scorpion	19	Pets
Arachnida	Scorpiones	*Pandinus cavimanus* Pocock, 1888	Tanzanian red clawed scorpion	6	Pets
Arachnida	Scorpiones	*Hottentota saulcyi* Simon, 1880	None	3	Pets
Arachnida	Scorpiones	*Heterometrus longimanus* Herbst, 1800	Black emperor scorp; Asian forest scorpion	2	Pets
Arachnida	Scorpiones	*Hottentotta tamulus* Fabricius, 1798	Indian red scorpion; eastern Indian scorpion	2	Pets
Arachnida	Scorpiones	*Leiurusquin questriatus* Hemprich & Ehrenberg, 1829	Deathstalker scorpion	2	Pets
Arachnida	Scorpiones	*Mesobuthus eupeus* Koch, 1839	Lesser Asian scorpion; mottled scorpion	2	Pets
Arachnida	Scorpiones	*Pandinus viatoris* Pocock, 1890	None	1	Pets
Chilopoda	Scolopendromorpha	*Scolopendra subspinipes* Leach, 1815	Vietnamese centipede	1	Pets
Clitellata	Haplotaxida	*Eisenia fetida* Savigny, 1826	Brandling worm; common dung-worm	2	Pet food

Class	Order	Scientific name	Common name	Availability index	Use
Gastropoda	Eupulmonata	*Achatina immaculata* Lamarck, 1822	None	4	Pet food
Gastropoda	Eupulmonata	*Cornu aspersum* O.F. Muller, 1774	Brown garden snail; European brown snail	2	Pet food
Insecta	Coleoptera	*Tenebrio molitor* Linnaeus, 1760	Yellow mealworm; mealworm	87	Pet food
Insecta	Coleoptera	*Zophobas morio* Fabricius, 1776	Superworm; zophobas	76	Pet food
Insecta	Blattodea	*Blaptica dubia* Serville, 1838	Dubia roach; orange-spotted cockroach	49	Pet food
Insecta	Blattodea	*Gromphadorhina portentosa* van Herrewege, 1973	Madagascar hissing cockroach	40	Pets; Pet food
Insecta	Orthoptera	*Acheta domestica* Linnaeus, 1758	House crickets	32	Pet food
Insecta	Blattodea	*Naupheota cinerea* Olivier, 1790	Lobster; speckled roach	30	Pet food
Insecta	Blattodea	*Blatta lateralis* Walker, 1868	Turkestan cockroach; rusty red cockroach	29	Pet food
Insecta	Orthoptera	*Gryllus bimaculatus* De Geer, 1773	Field crickets; two-spotted cricket	14	Pet food
Insecta	Blattodea	*Periplaneta americana* Linnaeus, 1758	American cockroach; waterbug	14	Pet food
Insecta	Orthoptera	*Gryllus assimilis* Fabricius, 1775	Jamaican field cricket	12	Pet food
Insecta	Lepidoptera	*Bombyx mori* Linnaeus, 1761	Silkworms	11	Pets; Pet food

Class	Order	Scientific name	Common name	Availability index	Use
Insecta	Blattodea	*Phoetalia pallida* Brunner von Wattenwyl, 1865	Palid roach	9	Pet food
Insecta	Diptera	*Hermetia illucens* Linnaeus	Black soldier fly	9	Pet food
Insecta	Diptera	*Drosophila hydei* Sturtevant, 1921	Flightless fruit fly	6	Pet food
Insecta	Coleoptera	*Alphitobius diaperinus* Panzer, 1797	Lesser mealworm; litter beetle	6	Cleaners
Insecta	Blattodea	*Rhyparobia maderae* Fabricius, 1781	Madeiran cockroach	4	Pet food
Insecta	Coleoptera	*Dermestes maculatus* De Geer, 1774	Hide beetle	3	Cleaners
Insecta	Phasmatodea	*Extatosoma tiaratum* Macleay, 1826	Macleay's spectre; spiny Leaf Insect	3	Pets
Insecta	Lepidoptera	*Achroia grisella* Latreille, 1802	Lesser wax moth	2	Pet food
Insecta	Lepidoptera	*Manduca sexta* Linnaeus, 1763	Tobacco hornworm; goliath worm	2	Pet food
Insecta	Diptera	*Drosophila melanogaster* Meigen, 1830	Common fruit fly; vinegar fly	2	Pet food
Insecta	Blattodea	*Lucihormetica verrucosa* Brunner von Wattenwyl, 1865	Warty glowspot cockroach	1	Pets; Pet food
Insecta	Lepidoptera	*Samia cynthia* Drury, 1773	Ailanthus silkmoth	1	Pet food
Insecta	Blattodea	*Blaberus discoidalis* Audinet-Serville, 1839	False death's head cockroach	1	Pet food

Table S5. Unrecognized terrestrial invertebrate species obtained from South African pet trade (online, pet stores and breeders) in 2016 and 2017.

Class	Order	species	Availability	Use
Arachnida	Scorpiones	Black forest scorpion	3	Pets
Arachnida	Scorpiones	*Hadogenes* sp.	4	Pets
Arachnida	Scorpiones	*Opistachanthus* sp.	2	Pets
Arachnida	Scorpiones	*Opistachanthus wahlbergi*	3	Pets
Arachnida	Scorpiones	*Parabuthus capensis*	4	Pets
Chilopoda	Scolopendromorpha	Blue leg centipede	1	Pets
Chilopoda	Scolopendromorpha	Red leg centipede	2	Pets
Chilopoda	Scolopendromorpha	Yellow leg centipede	1	Pets
Gastropoda	Eupulmonata	Giant African snails	1	Pet food
Insecta	Blattodea	*Blaberus fusca*	1	Pet food
Insecta	Blattodea	Cape red headed roach	1	Pet food
Insecta	Isoptera	Termites	4	Pet food
Insecta	Coleoptera	*Dischista cinta*	1	Pet food
Insecta	Diptera	Maggots	1	Pet food
Insecta	Orthoptera	Crickets	34	Pet food
Oligochaeta	Lubricina	Composting worms	1	Soil improvement
Oligochaeta	Lubricina	Earthworms	2	Pet food

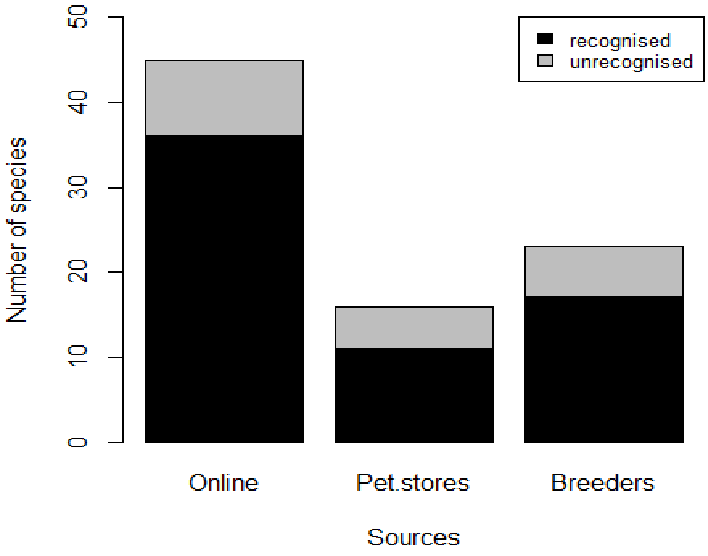


Figure S1. The number of terrestrial invertebrate species obtained from breeders, websites and pet stores.

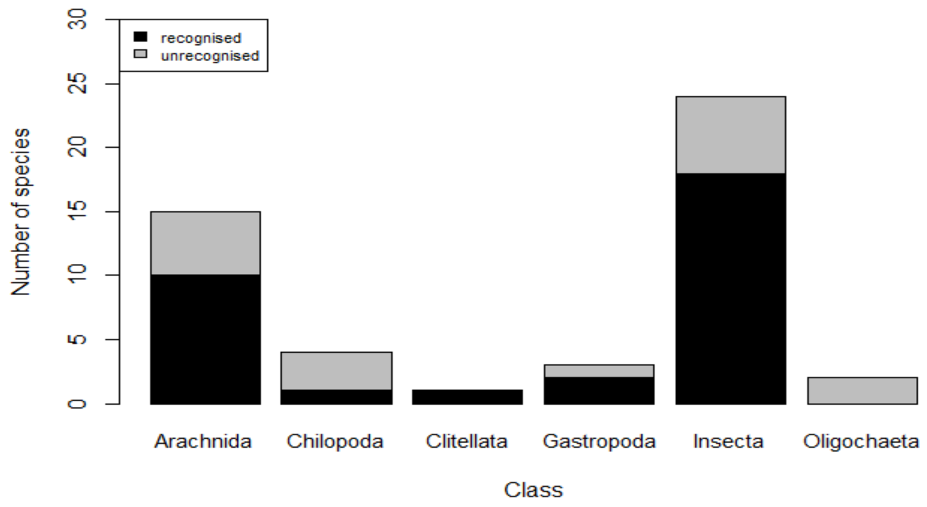


Figure S2. The total number of terrestrial invertebrate species per class obtained from the South African pet trade.

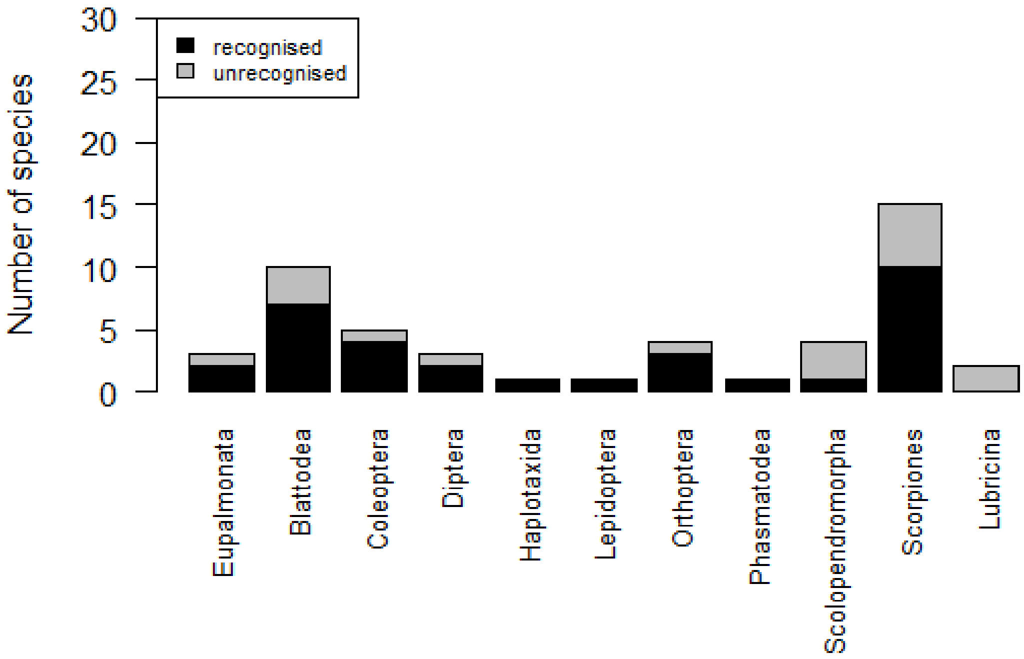


Figure S3. The total number of terrestrial invertebrate species per order obtained from the South African pet trade.

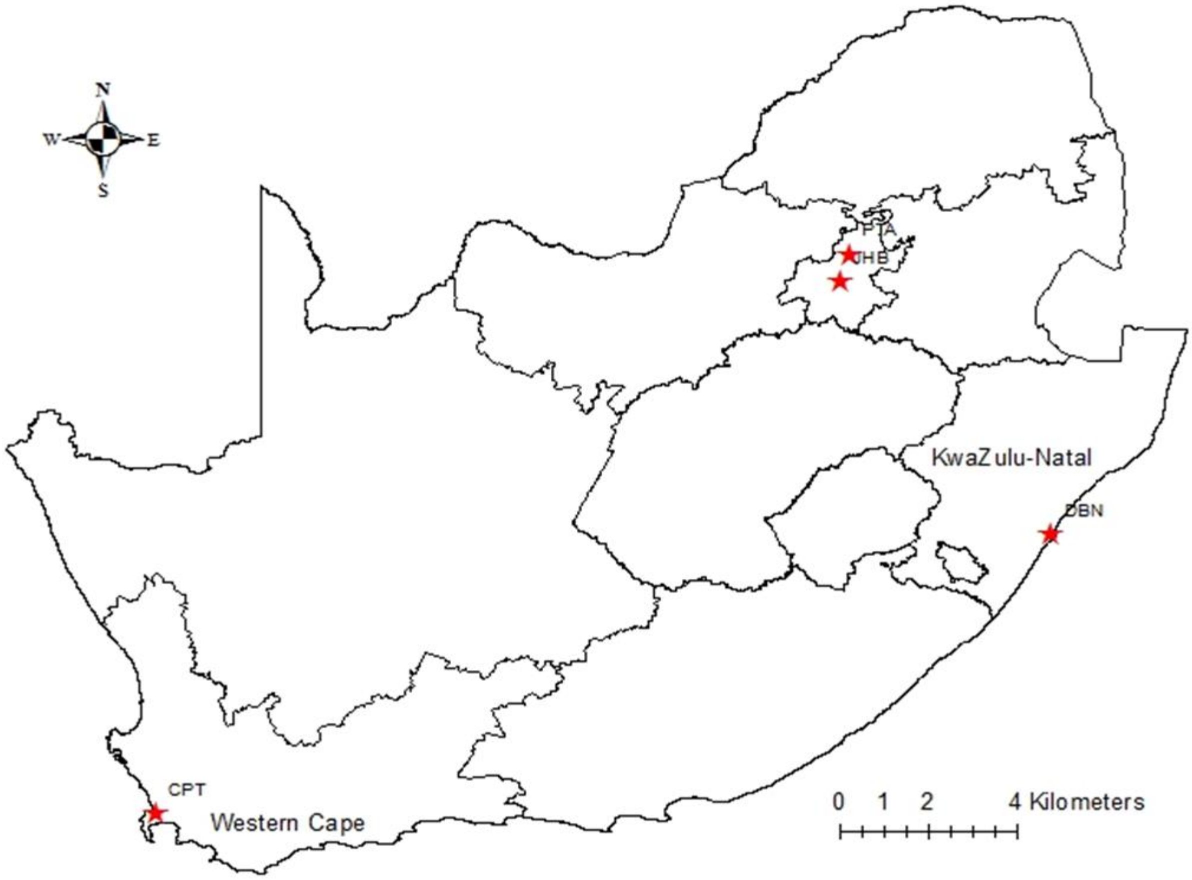


Figure S4. A map of South Africa indicating four major cities from three Provinces visited: DBN = Durban: CPT = Cape Town: JHB and PTA are in Gauteng province.

Chapter 3: The invasion risks of alien terrestrial invertebrates in the South African pet trade

Abstract

Many alien species kept as pets have been reported to be released or escape from captivity and become invasive. In South Africa, it is not known whether terrestrial invertebrate species could pose a risk of becoming invasive should they be released or escape. The occurrence records for each terrestrial invertebrate species from the pet trade were obtained from the native and invaded ranges of these species. To determine if any parts of South Africa would be climatically suitable, climate match and species distribution modelling (Jackknife approach and cross validation approach) was developed for 34 terrestrial invertebrate species. Large towns and major cities were superimposed onto the maps showing areas that are climatically suitable for each species. Species availability index was used as a measure for propagule pressure. Availability index refers to the number of times the species was available when sampling in the pet stores, websites or breeders. The Socio-Economic Impact Classification of Alien Taxa (SEICAT) and Environmental Impact Classification for Alien Taxa (EICAT) were used to assess the recorded impacts of these species. We propose that species with high availability, large area of climate suitability and impact elsewhere should be prioritized for management.The model results agreed and showed that 15 species had suitable climate in South Africa, but only six of these had extensive (over 40%) areas of suitable climate. Some of the species had climate suitability that covered major towns and cities (where availability is likely to be highest) which might lead to the species to find suitable areas should they escape or be released. This study found that species with large areas of climatic suitability had low availability index values. Similarly, several species with high availability index values had low areas of suitable climate in South Africa. Most species studied here were data deficient. In addition, we did not find any species that had large areas that were climatically suitable and high availability that also had high environmental or socioeconomic impacts. Overall, the risk of invasion from terrestrial invertebrates in the pet trade appears to be low based on the species considered in this study. However, the trade is dynamic which could mean that species availability could change in future or new species could be introduced.

Keywords: Climatic match, Jackknife, impact assessement, invasion, propagule pressure, species distribution modelling (SDM), risk assessment, terrestrial invertebrates.

Introduction

The pet trade has recently become an important pathway for introducing alien species into new areas (van Wilgen et al. 2009; Chucholl and Wendler 2017). Some of these species that have been released or have escaped, have found suitable areas and then established in new regions (Marr et al. 2017; Mori et al. 2017). The growth in the pet trade is facilitating the increase in the establishment rate of species globally (Kopecký et al. 2013; Mori et al. 2017). In South Africa, the risks associated with species introduced for the pet trade have not been comprehensively assessed. For example, some of the species which have been recorded to be invasive are available in the pet stores, including rose ringed parakeet, *Psittacula krameri* (Hart and Downs 2015).

Invertebrates are generally understudied when it comes to biological invasions (Pyšek et al. 2008). Until recently little work had been done on terrestrial invertebrate species in the pet trade in South Africa. However, almost 200 tarantula species (Theraphosidae) were reported to be in the South African pet trade (Shivambu 2018), and many other terrestrial invertebrates are offered for sale for various uses such as pets, sources of food for other pets and for cleaning pet cages (Chapter 2). This study therefore focuses on assessing whether any of the terrestrial invertebrate species in the pet trade pose an invasion risk to South Africa, which is an important first step to preventing invasions (Keller et al. 2007).

Risk assessments for alien species require predictions of whether species may become invasive and have negative impacts on ecosystems and humans (Keller et al. 2011). They are therefore a valuable tool that can be used to prioritise efforts to prevent introductions of alien and potentially invasive species (Keller and Kumschick 2017). In many risk assessments, climate suitability, invasion history and impact are considered (Hulme 2012). Faulkner et al. (2014) developed a watch list approach using history of invasion, environmental suitability and propagule pressure. Their contention was that species which have been shown to be invasive and/or have impacts elsewhere, are from climatically suitable areas and which are introduced in high numbers pose a high risk.

As propagule pressure plays a major role in the establishment success of a species (Lockwood et al. 2009), it is important to determine the number of individuals introduced for different species. It is also essential to understand what influences the popularity of the species in the trade and to identify which species are more likely to become highly abundant (van Wilgen et al. 2009). Studies have attempted to quantify the availability of species in the pet trade as a way to quantify the likely propagule pressure (Patoka et al. 2014; Chucholl and Wendler 2017). For example, availability scores were calculated for the red-eared slider turtle (*Trachemys scripta elegans*) using pet shop density, distance to urban areas and human density (Banha et al. 2017). Species with high availability scores are assumed to have high propagule pressure because it is more likely that these species will be moved around in large numbers.

History of invasion has been used for predicting likely impacts of species in their introduced ranges (Kulhanek et al. 2011). Impact elsewhere has often also been used as a predictor for impact in a new region, and is often the strongest predictor of risk (Weber et al. 2009). Alien species can cause devastating negative impacts on the environment and socio-economic well being (Cadi and Joly 2003). In order to manage or minise these impacts, Aichi target 9 has suggested several approaches that could be followed, one of which includes identification of damaging alien species and their prioritisation (CBD 2013). Various impact scoring schemes have been developed in order to reach these goals (Hawkins et al. 2015; Nentwig et al. 2016; Bacher et al. 2017).

As with other taxa, terrestrial invertebrates depend upon suitable climate in order to survive (Sincage and Hardin 2015). Climate plays a major role in determining which species can establish and become invasive in a region, as they depend on suitable environments in order to reproduce and establish (Kitzberger et al. 2000; Gaston 2003; Jiménez-Valverde et al. 2011). Climatic zones from the species' native range have been used as a tool to predict the likelihood of invasion of introduced species (Hill and Terblanche 2014). Therefore, in order to assess the likelihood of an invasion, it is important to know which areas are climatically suitable before introducing a new species (Thuiller et al. 2005; Lockwood et al. 2009; Banha et al. 2017). This can be achieved through assessment protocols such as climate matching and species distribution models. This approach has been used successfully in many studies to determine areas at risk of invasion, for example in reptiles and amphibians in the pet trade (Bomford et al. 2009). Correlative species distribution models have also been used globally in many risk assessment studies to determine which areas are climatically suitable for introduced species and likely to be at risk of being invaded by these species (Ficetola et al. 2007; Jiménez-Valverde et al. 2011). These models use environmental variables and occurrence records to predict the likelihood of the area being suitable for the survival of a species (Elith and Leathwick 2009). Distribution models are usually preferable to climate matching approaches for identifying areas that are climatically suitable, but these correlative distribution models are only possible if sufficient occurrence records are available with which to calibrate the models (Pearson et al. 2007).

This study assesses various aspects of risks associated with terrestrial invertebrates in the South African pet trade based on the watch list approach by Faulkner et al. (2014). The aims of the study are to 1) identify areas of South Africa that are climatically suitable, 2) determine likely environmental and socio-econmic impacts, and 3) estimate propagule pressure for a selection of terrestrial invertebrate species in the pet trade in South Africa.

Materials and methods

A list of 36 recognized species in the South African pet trade was compiled from visits to pet stores, websites and breeders (Chapter 2). We only focused on the species that are alien to South Africa. For the climate suitability, occurrence records were obtained from the Global Biodiversity Information Facility (GBIF 2017). These occurrence records were available for 34 species. To assess the history of invasion, impact records were obtained from scientific literature (available for 18 species, note details below). Finally, we used availability index scores from chapter 2 as an index of propagule pressure. We classified the species into three groups based on the availability index values. Those with availability index scores of more than 39 as classified as high availability, those with availability index scores of 20 to 39 as medium availability and those with scores from 1 to 19 as low availability. This was doned based on the upper and lower quartile.

Propagule pressure

The availability index was calculated by counting the number of times a species was available when visiting the pet stores, websites and via prvate breeders (Chapter 2). Species with high availability index values are assumed to have high propagule pressure because it is more likely that these species will be moved around in large numbers. It is assumed that propagule pressure of pets in general is likely to be higher in large towns and cities, due to the presence of pet stores and expected large human populations in the city that are likely to be using terrestrial invertebrates as food for pets and keeping them as pets (Banha et al. 2017).

Large towns and cities were used as a measure of propagule pressure. A map layer of large towns and cities was superimposed onto the maps showing areas that are climatically suitable for each species. Species for which several cities or large towns fell into climatically suitable areas were considered to have a greater likely propagule pressure than species with few large towns or cities.

Impact assessment

Literature search

To assess the impacts of alien terrestrial invertebrates, a global literature review was conducted. The ISI Web of Knowledge, Google Scholar as well as biological invasion websites and databases such as the Global Invasive Species Database (www.iucngisd.org/gisd) were used to search for scientific publications and grey literature with reported environmental and socio-economic impacts for the selected species. Common names and scientific names were used as a search string, as well as other relevant search terms (i.e. mealworms; *Cornu aspersum*). Publications were manually filtered based on the information provided in the title and abstracts. The references cited within publications found were screened with only the primary references included.

The Socio-Economic Impact Classification of Alien Taxa (SEICAT) and Environmental Impact Classification for Alien Taxa (EICAT) were used to assess the recorded impacts in this study (Bacher et al. 2017; Hawkins et al. 2015). These two schemes complement each other, as SEICAT only considers impacts on socio-economy, whilst EICAT considers only environmental impacts.

EICAT was developed by Blackburn et al. (2014) and it was adopted by the IUCN (https://portals.iucn.org/congress/motion/014) to ensure the classification of all alien species globally (Hawkins et al. 2015, Evans et al. 2016). EICAT categorizes environmental impacts into 12 mechanisms (competition; predation; hybridisation; transmission of disease; parasitism; poisoning/toxicity; bio-fouling; grazing/herbivory/browsing; chemical, physical or structural impact on ecosystem and interaction with other alien species) (Hawkins et al. 2015).

SEICAT assesses changes to human well-being and livelihoods through impacts on different constituents of well-being, including safety, material and immaterial assets, health and social, spiritual and cultural relations (Bacher et al. 2017).

Both schemes rely solely on published evidence and consider five categories of impact; Minimal Concern (MC); Minor (MN); Moderate (MO); Major (MR); and Massive (MV) (Bacher et al. 2017; Hawkins et al. 2015). They allow different taxa with diverse levels of impact to be compared, thus providing schemes for comparing impacts among different taxa, mechanisms, introduction events and regions. These schemes can also be used to highlight the species with high potential impacts in areas where they are not yet introduced. Both schemes have been described in full elsewhere (Bacher et al. 2017; Blackburn et al. 2014; Hawkins et al. 2015).

For each assessed species, a confidence score of high, medium or low was attached according to the quality of data acquired during the assessment (Hawkins et al. 2015). For analysis, the maximum impact score per species found on any constituent of human well-being and through any mechanism for SEICAT and EICAT was used respectively (Blackburn et al. 2014; Hawkins et al. 2015). All data deficient (DD) entries in the dataset were excluded from analysis. We allocated a numeric value from one for minimal concern (MC) to five for massive (MV) for both schemes following Kumschick et al. (2017).

Occurrence records

Lists of occurrence records were compiled for all species on the list using scientific literature and online databases including GBIF. Occurrence records were taken from the native and invaded ranges of these species and cleaned using the R package Biogeo (Robertson et al. 2016). Furthermore, we converted all coordinates to decimal degrees and removed all the duplicate records per 10-minute grid cell to avoid pseudoreplication. Each species was assigned to one of three groups based on the number of occurrence records available for that species (Table 1). These groups included 1) those species with insufficient numbers of records for producing reliable correlative distribution models (fewer than 10 records), 2) those with small numbers of records (10 to 29), and 3) those with a large number of records (30 or more). A climate matching approach was used for species in the first group (< 10 records), while distribution models were produced for species in the other two groups. For the distribution models, two different approaches were followed based on the type of model evaluation approach that could be used (referred to as SDM 1 and SDM 2). For species where there were small numbers of records (10 to 29) the Jackknife approach was used (Pearson et al. 2007) and where there were large numbers of records (> 30) a cross-validation approach was used (Stockwell 1992). In both cases the Maximum entropy (Maxent) modelling software was used to develop the models (Philips et al. 2006).

Climate match

A world map of Metzger climate zones (Metzger et al. 2013) was used to identify those climatic zones that contained occurrence records for a species. Where the same climate zones were present in South Africa, climatic suitability maps were shown for each species.

Distribution models

For the distribution models, bioclimatic variables available from Worldclim were used as predictor variables (www.worldclim.org, Fick and Hijmans 2017). For each species, a subset of six out of the 19 available bioclimatic variables (Bio 1-19) that contributed the most in predicting the distribution of species was selected. The bioclimatic variables used included: annual precipitation; precipitation of warmest quarter; minimum temperature of coldest month; precipitation of wettest quarter; temperature seasonality; maximum temperature of warmest month; precipitation seasonality; and mean temperature of warmest month. The environmental variables were at 10 minute spatial resolution.

Maxent estimates the species probability distribution across the entire study area based on the principle that the estimated environmental variables must not differ with the known variables. Maxent was selected as it uses only presence records, has been shown to perform well when compared to other modelling methods (Elith et al. 2006) and it produces good results with relatively few occurrence records (Pearson et al. 2007).

Maxent was set to the default values to produce models: convergence threshold at 10^{-5}, random test percentage at zero, regularization multiplier at 1, maximum iterations at 500 and replicates at 5 for cross validation. The logistic output format in ArcGIS version 10.4 was used to produce the potential distribution maps of the species (Phillips and Dudick 2008). Data were prepared in R using Biogeo and raster packages (R Core Team, 2014; Robertson et al. 2016).

Jackknife models

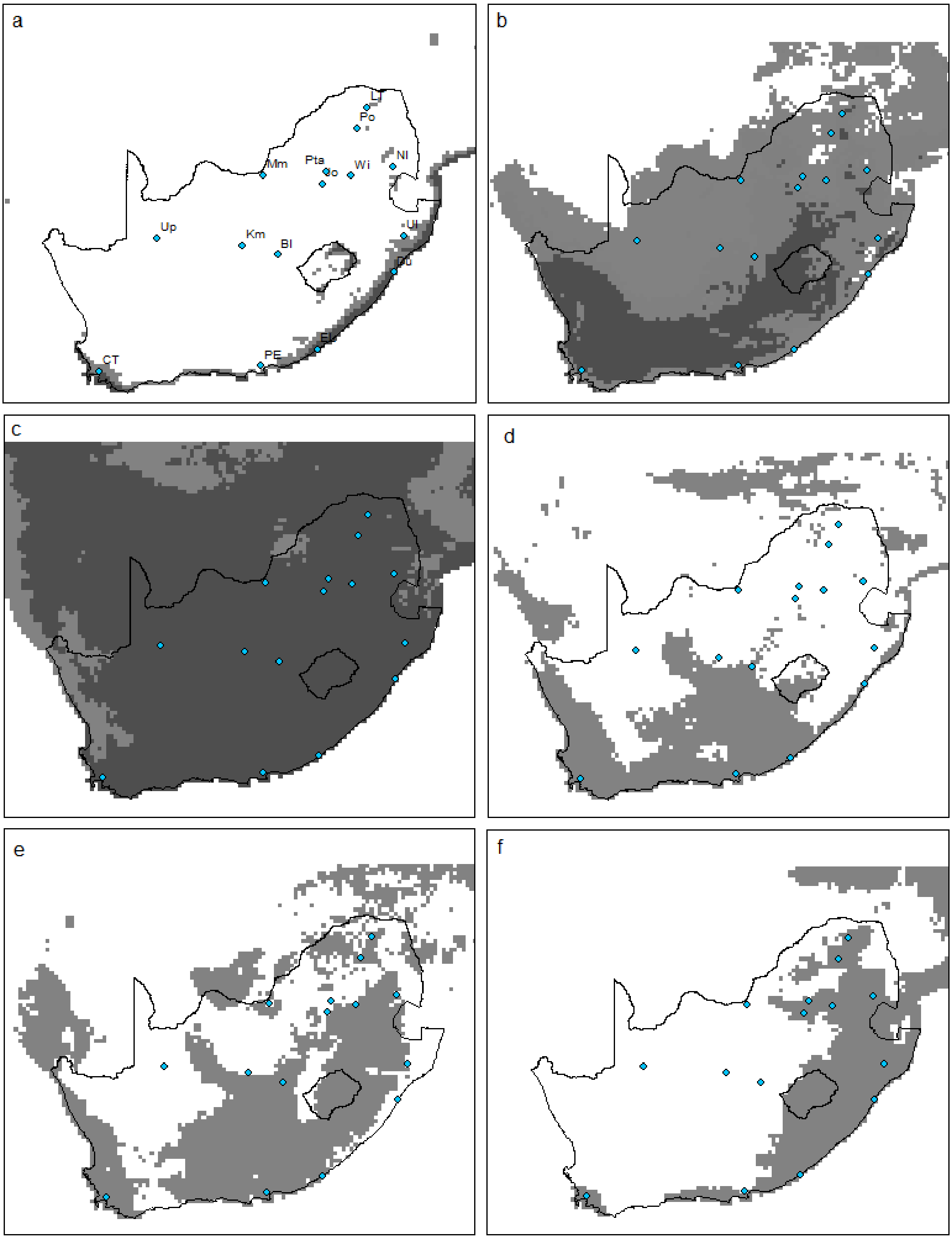
For the SMD1 species, the jackknife approach developed by Pearson et al. (2007) was used to calibrate and evaluate the models. The models were calibrated using n-1 of the occurrence records. Model testing was performed using one record and then repeated until all the records had been used for model evaluation. The significance of the proportion of presences correctly predicted was assessed by calculating a p-value using a software program developed by Pearson et al. (2007) called pValueCompute. The p-value of jackknife predictions and the proportions of records predicted correctly were reported. Due to the small sample size used here, we considered models where the proportion of records predicted correctly of 0.5 and above to be suitable (following Pearson et al. 2007). Models with proportion of records predicted correctly of >0.7 were considered to be good, 0.6 to 0.7 moderate and <0.6 poor. Models where the jackknife was not significant ($p > 0.05$) were considered to be unreliable and were discarded. All species with unrealible species distribution models were subjected to climate matching.

Cross-validation

A five-fold cross validation was performed for SMD2 species. The performance of the model was evaluated using the Area Under Curve (AUC) statistic (Fielding and Bell 1997). We took an average Area Under Curve (AUC) value across the five models. The final model was generated by taking the average across the five separate models. Models with an AUC value of >0.9 are regarded to be excellent, while those with AUC values ranging between 0.7- 0.9 very good and models with values of below 0.7 considered to be poor (Swets 1988).

Results

Of 36 alien terrestrial invertebrate species assessed, occurrence records were obtained for 34 species, of which 26 species occurred in areas that are climatically suitable in South Africa (Table 1). Out of seven species selected for SMD1, the jackknife analysis indicated that the models performed well for only two species. Of the 16 species selected for SMD2, the models successfully projected climatically suitable areas in South Africa except for one species (Fig. 1; S1). The AUC values indicated that model performance was excellent (AUC > 0.9) for five species, while performance was very good (0.7 to 0.9) for 10 species (Table S2). Only eight species did not have areas of climatic suitability in South Africa.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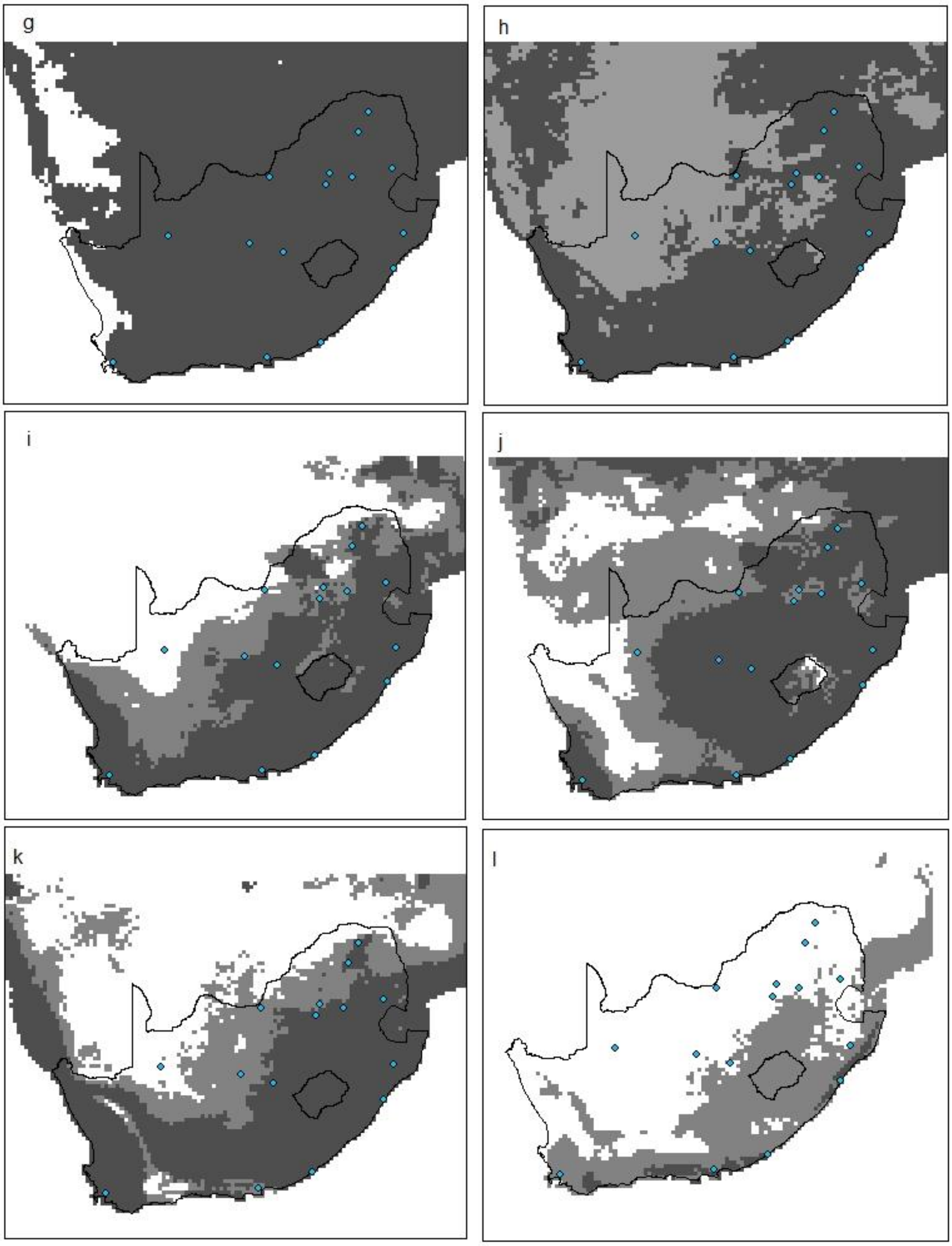
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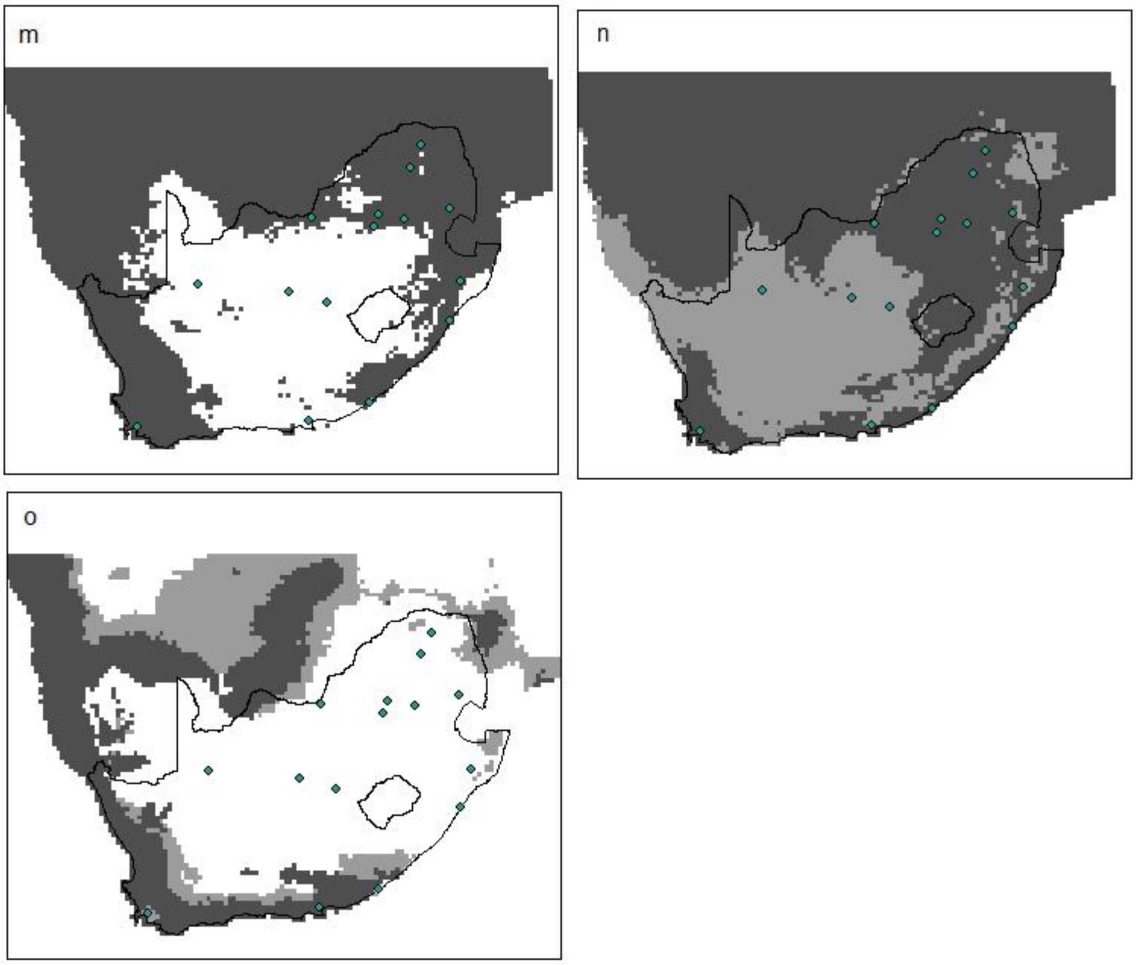

Figure 1. Maps showing areas that are potentially climatically suitable as determined by Maximum entropy following the SMD1 approach for 15 terrestrial invertebrate species in the South African pet trade, namely a) *Scolopendra subspinipes*, b) *Acheta domestica*, c) *Alphitobius diaperinus*, d) *Cornu aspersum*, e) *Drosophila melanogaster*, f) *Extatosoma tiaratum*, g) *Gryllus assimilis*, h) *Grylus bimaculatus*, i) *Periplaneta americana*, j) *Manduca sexta*, k) *Nauphoeta cinerea*, l) *Tenebrio molitor*, m) *Hermetia illucens* , n) *Achroia grisella*, o) *Drosophila hydei*. Darker shades represent greater climatic suitability. The blue dots are the major cities and towns in South Africa, each represented by abbreviation Th -Thohoyandou, LT - Louis Trichardt, Pl - Polokwane, Nl - Nelspruit, Wi - Witbank, Pta - Pretoria, Jo - Johannesburg, Mm - Mmabatho, Ul - Ulundi, Du - Durban, Up - Upington, Km - Kimberly, Bl - Bloemfontein, El - East London, PE - Port Elizabeth, and CT - Cape Town.

Of the resulting 36 species, impact records were obtained for 18 species, while 18 species were data deficient (with no record of impact). A total of 48 published literature sources were used with an average of three publications per species. Socio-economic impact records were assigned for 11 species whereas environmental impacts were assigned for eight species (Table 1).

Table 1. Terrestrial invertebrate species with respective availability index values, SEICAT and EICAT scores. *Availability index* represents the number of times the species was available when visiting the physical pet stores, online pet stores and breeders. MC = Minimal concern, MN = minor, MO = moderate, MR = major, and MV = massive. DD refers to Data Deficient.

Species	EICAT	SEICAT	Availabilty index
Tenebrio molitor	MN	MN	89
Zophobas morio	DD	MN	78
Blaptica dubia	DD	DD	49
Gromphadorhina portentosa	MN	MC	40
Acheta domestica	DD	DD	32
Nauphoeta cinerea	DD	DD	31
Blatta lateralis	DD	MO	30
Pandinus imperator	DD	DD	19
Gryllus bimaculatus	DD	MC	14
Periplaneta americana	DD	MN	14
Gryllus assimilis	MN	DD	12
Bombyx mori	DD	DD	11
Hermetia illucens	DD	DD	9
Phoetalia pallida	MN	DD	9
Alphitobius diaperinus	MN	MO	6
Drosophila hydei	MN	DD	6
Pandinus cavimanus	DD	DD	6
Achatina immaculata	DD	DD	4
Rhyparobia maderae	DD	DD	4
Extatosoma tiaratum	DD	DD	3
Dermestes maculatus	DD	MN	3
Hottentotta tamulus	DD	MV	3
Cornu aspersum	MN	MO	2
Achroia grisella	MN	DD	2
Leiurus quinquestriatus	DD	MV	2
Mesobuthus eupeus	DD	MV	2
Hottentotta saulcyi	DD	MV	2

Species	EICAT	SEICAT	Availabilty index
Manduca sexta	MN	DD	2
Drosophila melanogaster	DD	DD	2
Heterometrus longimanus	DD	DD	2
Eisenia fetida	DD	DD	2
Scolopendra subspinipes	DD	MO	1
Blaberus discoidalis	DD	DD	1
Samia cynthia	DD	DD	1
Pandinurus viatoris	DD	DD	1
Lucihormetica verrucosa	DD	DD	1

We did not find any species that had large areas of suitable climate as well as high availability index values (Fig. 2). Six species used as pet food had low availability index values, while only one species (*Nauphoeta cinerea*), also used as pet food, had a medium availability index value (Table 1). Of these seven species, only four species were recorded as having environmental or socio-economic impacts. *Gryllus assimilis* had minor environmental impacts (transmission of diseases to native species), *Periplaneta americana* had minor socio-economic impacts affecting human health and material and immaterial goods while *Alphitobius diaperinus* has moderate socio-economic impacts affecting material and immaterial goods (Fig 2; Table 1). *Gryllus bimaculatus* has minimum concern socio-economic impacts affecting human health (Fig 2; Table 1).

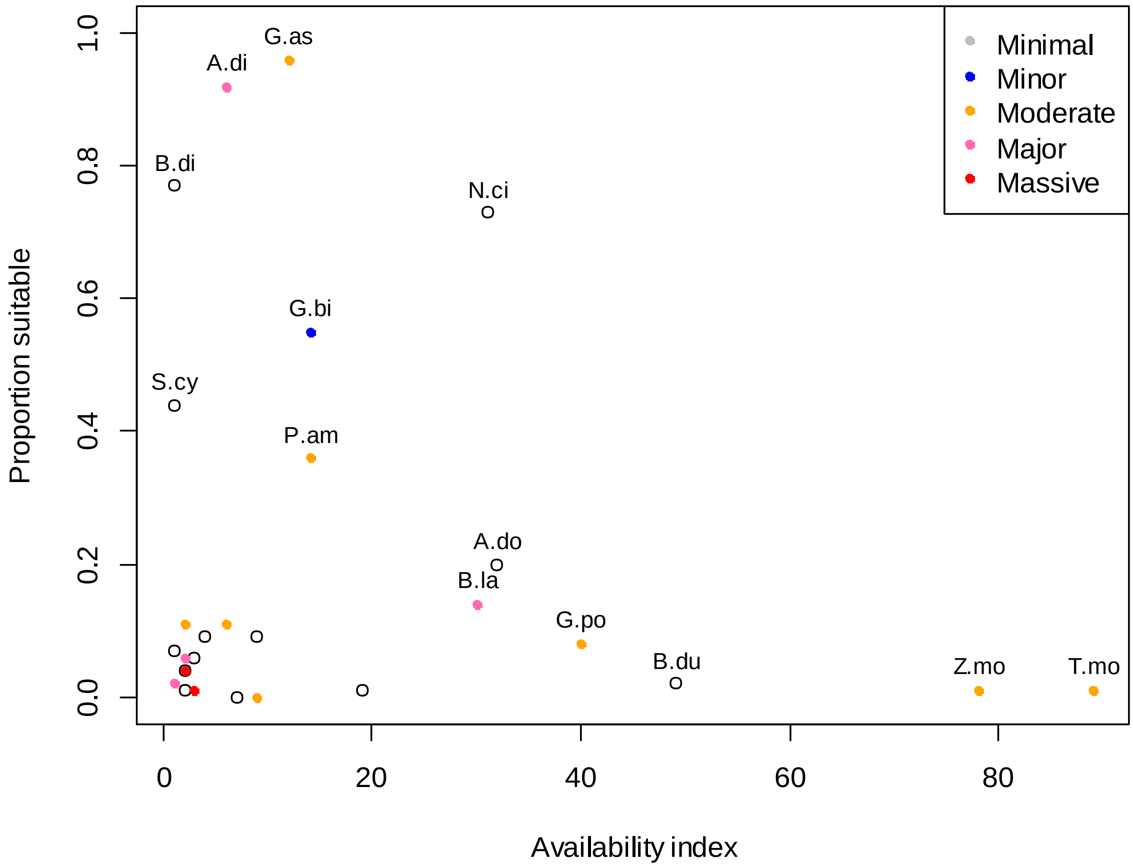

Figure 2. Terrestrial invertebrate availability index values and areas of the map region that are predicted to be climatically suitable. The species are: A.do = *Acheta domestica*: A.di = *Alphitobius diaperinus*: B.di = *Blaberus discoidalis*: B.du = *Blaptica dubia*: B.la = *Blatta lateralis*: G.po = *Gromphadorhina portentosa*: G.as = *Gryllus assimilis*: G.bi = *Gryllus bimaculatus*: N.ci = *Nauphoeta cinerea*: P.am = *Periplaneta americana*: S.cy = *Samia cynthia*: T. mo = *Tenebrio molitor*: Z.mo = *Zophobas morio*. Species that were data deficient for impacts are indicated by open circles .Where species have both SEICAT and EICAT scores, a maximum score was chosen between the two schemes.

Discussion

It was not possible to obtain data on the three criteria (availability, impact and climatic suitability) for all species considered in this study, but we were able to obtain data for 14 species. Some species had large areas of South Africa that were predicted to be climatically suitable for them, although these species all had low availability (i.e. *Gryllus assimilis*, *Gryllus bimaculatus, Alphitobius diaperinus, Blaberus discoidalis, Nauphoeta cinerea*, and *Periplaneta americana*). However, *Periplaneta americana* has been widely established in South Africa for a long time predating pet trade. Conversely, species that had high availability scores had relatively small regions of suitable climate (i.e. *Zophobas morio, Tenebrio molitor, Blaptica dubia* and *Gromphadorhina portentosa*). We did not find any species that had both high availability and large areas of suitable climate. Despite some species having a history of impact elsewhere and large areas of climate suitability, the results indicate that the terrestrial invertebrates studied here currently pose low invasion risks due to low propagule pressure (Fig. 2). However, as the market for terrestrial invertebrate species is dynamic, these species could suddenly pose high invasion risk due to changes in availability. Species such as *Alphitobius diaperinus*, *Gryllus assimilis*, and *Gryllus bimaculatus* have high climate match and impact elsewhere and if many of these species are introduced into the wild they could establish populations and spread into new areas. *Brachypelma vagans* (a tarantula) has been reported to be traded as a pet in Florida where it has been released or escaped into the wild and become invasive (Edward and Hibbard 1999). In South Africa, many terrestrial invertebrate species have been reported to have established sustaining populations (Picker and Griffiths 2011). This could indicate that many alien terrestrial invertebrate species can survive here. Species of greatest concern are those that have large areas of suitable climate, high availability and high impact. However, none of the terrestrial invertebrate species found meet all of these criteria at once (Fig. 2).

Species for which several cities or large towns fell into climatically suitable areas are considered to have a greater likely propagule pressure than species with few large towns or cities as it is expected that large towns and cities have high human density with many pet stores through which many species could be introduced in large numbers (Duarte-Quiroga and Estrada 2003; Daszak et al. 2003; Ceballos and Fitzgerald 2004). Several terrestrial invertebrate species including *Achroia grisella*, *Nauphoeta cinerea, Periplaneta americana, Manduca sexta, Gryllus assimilis, Gryllus bimaculatus, Alphitobius diaperinus, Acheta domestica* and *Blaberus discoidalis* had large areas of climate suitability, which covers major towns and cities (Fig. S1). Large towns and cities where pet stores are found could be at risk of invasion should these species be released intentionally or unintentionally in large numbers (Fig. 1; S1 and S2). Banha et al. (2017) incorporated human variables such as pet store density, distance to urban and human density in species distribution modelling to determine suitable areas for the red-eared slider turtle, *Trachemys scripta elegans* to establish populations. They found that human variables and environmental variables could assist in predicting areas at risk of invasion at a regional scale. We did not have data on some of the human variables used i.e. pet store density, but we used large cities and towns as a proxy for human density. Large cities tend to have slightly higher overall temperatures due to the abundance of human made structures and they tend to be warmer than the surrounding rural areas, which is known as the heat island effect (Asimakopoulos et al. 2001). It is likely that species can survive better in the city than the country side or in the wild because of the heat island effect. It was not clear to us how we could account for the heat island effect in our models. This is a possible limitation of this study but it is most likely to be an important factor for species with distributions that are limited by temperature.

Terrestrial invertebrates identified in this study can cause environmental impact through three mechanisms (e.g. parasitism, transmission of dieseases to native species and competition) and socio-economic impacts through four constituents of human well-being (safety; material and immaterial assets; health; social, spiritual and cultural relations). This study indicated that recorded socio-economic impacts were generally higher than the recorded environmental impacts. Similarly, other terrestrial invertebrates such as gastropods scored more impacts on the socio-economic scale than the environmental scale (Kesner and Kumschick 2018). Two scorpions, *Hottentotta saulcyi* and *Mesobuthus eupeus* could have massive impacts on human health but have small areas of climate suitability and low availability index values (Fig 2; Table 1). These species could have impacts on human health by stinging people and causing severe pain, erythema, renal failure, blurred vision and death (Bacher et al. 2017). According to SEICAT, these species cause massive impacts on human health because it is likely that there will be a permanent change to human health due to fundamental structural changes of socio-economic community due to a loss of life. Although these impacts are massive it appears that the risk of invasion for these species is low as they have low availability indeces and do not have large areas of climate suitabilty which could indicte that they are unlikely to escape in large numbers and establish a population.

The lack of recorded impacts could be due to a lack of recording rather than a lack of impact. All environmental impact scores were low (minimum concern and minor) which were caused via parasitism (*Achroia grisella, Cornu aspersum, Tenebrio molitor, Drosophila hydei)*, transmission of diseases to native species (*Drosophila hydei, Phoetalia pallida, Cornu aspersum, Gryllus assimillis,* and *Gromphadorhina portentosa*), and competition (C*ornu aspersum*) (Table 1). In contrast, other alien taxa have been reported to cause massive environmental impacts (Blackburn et al. 2014;

Karatayev et al. 2009; Pimentel et al. 2005). As the terrestrial invertebrate market is dynamic, there is a need to monitor the trade of terrestrial invertebrate species in order to identify and prevent the trade of species that could become problematic.

Conclusion

This study found that species with large areas of climatic suitability had low availability index values. Similarly, several species with high availability index values had low areas of suitable climate in South Africa. Most species studied here were data deficient. In addition, we did not find any species that had large areas that were climatically suitable and high availability that also had high environmental or socioeconomic impacts. Finally, there were some species with high impacts but these are likely to have a low invasion risk. Overall, the risk of invasion from alien terrestrial species in the pet trade appears to be low based on the species considered in this study. However, the trade is dynamic which could mean that species availability could change in future or new species could be introduced.

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Appendix 2

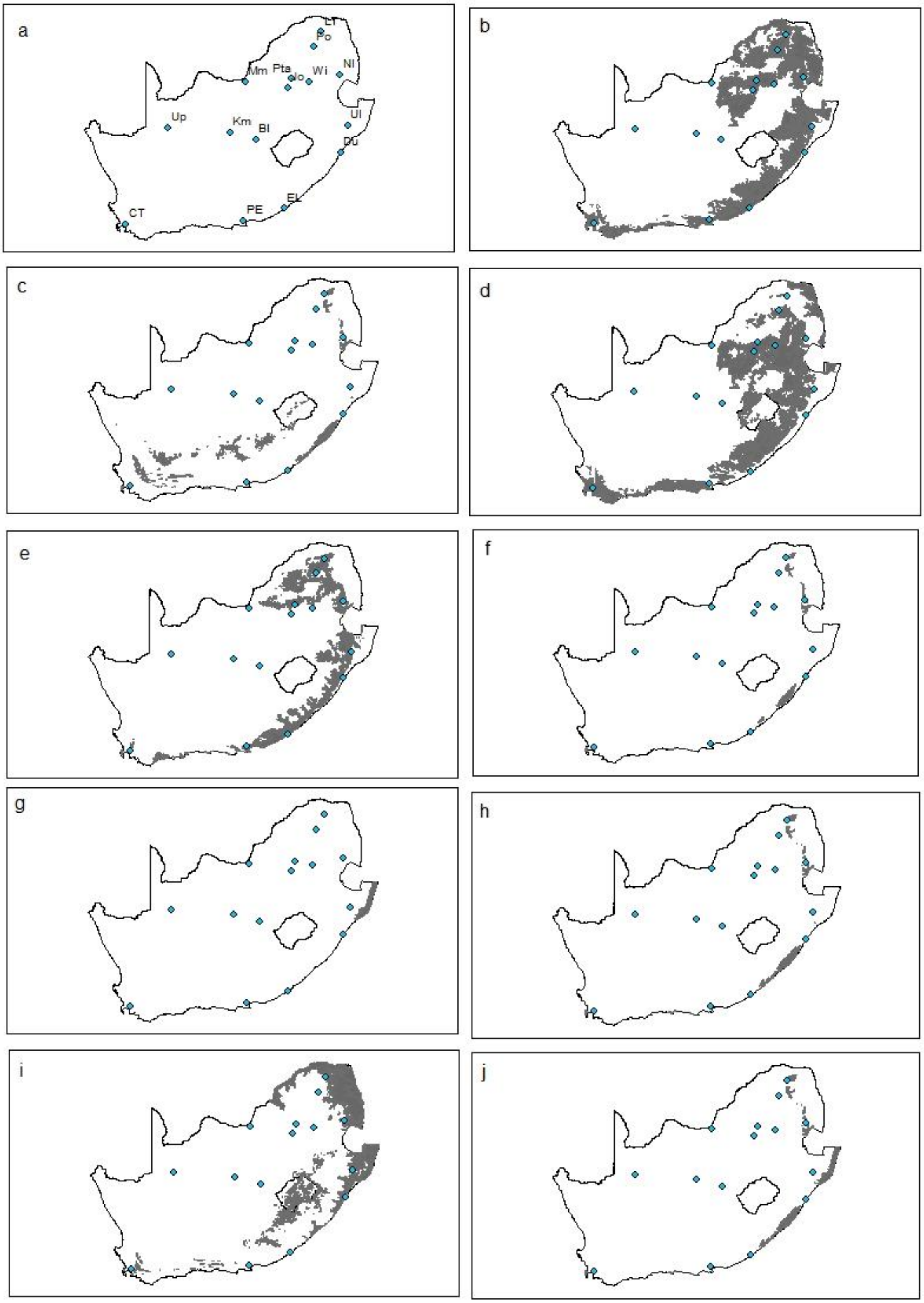
Table S1: Terrestrial invertebrate species assessed for potential climatic suitabiltility in South Africa using one climate matching technique and two species distribution modelling approaches. *Total number of records available* is the number of occurence records after removal of duplicates using a 10 minute spatial resolution. *Method used* represents the three approached followed in this study.

Family	**Species**	**Total number of records available**	**Method used**
Blaberidae	*Gromphadorhina portentosa*	5	Climate match
Scorpionidae	*Pandinus cavimanus*	5	Climate match
Scorpionidae	*Pandinurus viatoris*	6	Climate match
Blaberidae	*Phoetalia pallida*	9	Climate match
Scorpionidae	*Heterometrus longimanus*	6	Climate match
Blaberidae	*Lucihormetica verrucosa*	7	Climate match
Blaberidae	*Blaptica dubia*	8	Climate match
Achatinidae	*Achatina immaculata*	8	Climate match
Buthidae	*Hottentotta tamulus*	8	Climate match
Tenebrionidae	*Zophobas morio*	8	Climate match
Buthidae	*Hottentotta saulcyi*	9	Clmate match
Blaberidae	*Rhyparobia maderae*	10	SMD1
Scorpionidae	*Pandinus imperator*	11	SMD1
Buthidae	*Mesobuthus eupeus*	12	SMD1
Blattidae	*Blatta lateralis*	13	SMD1
Bombycidae	*Bombyx mori*	13	SMD1
Blaberiae	*Blaberus discoidalis*	14	SMD1
Saturniidae	*Samia cynthia*	24	SMD1
Phasmatidae	*Extatosoma tiaratum*	33	SMD2
Buthidae	*Leiurus quinquestriatus*	36	SMD2
Scolopendridae	*Scolopendra subspinipes*	43	SMD2
Blaberidae	*Nauphoeta cinerea*	48	SMD2
Gryllidae	*Gryllus assimilis*	50	SMD2
Sphingidae	*Manduca sexta*	53	SMD2

Family	**Species**	**Total number of records available**	**Method used**
Gryllidae	*Gryllus bimaculatus*	82	SMD2
Blattidae	*Periplaneta americana*	87	SMD2
Gryllidae	*Acheta domestica*	106	SMD2
Drosophilidae	*Drosophila melanogaster*	116	SMD2
Drosophilidae	*Drosophila hydei*	34	SMD2
Pyralidae	*Achroia grisella*	289	SMD2
Stratiomyidae	*Hermetia illucens*	207	SMD2
Helicidae	*Cornu aspersum*	131	SMD2
Tenebrionidae	*Alphitobius diaperinus*	187	SMD2
Tenebrionidae	*Tenebrio molitor*	876	SMD2

Table S2. Terrestrial invertebrate species used in assessing the potential climatic suitabiltility in South Africa following the SMD1 and SMD 2 approach. The SMD1 performance testing was evaluated with pValueCompute software to determine the best performing model in predicting climatic suitability for terrestrial invertebrate species. *Occurence records* are the number of samples obtained per species after removal of duplicates using a 10 minute spatial resolution. *AUC value* is the Area Under Curve as described in the methods section. *Proportion predicted* are the values calculated using pValueCompute by as described in the methods. *P-value* are the values obtained from pValueCompute as described in the methods.

Species	**Occurrence records**	**Proportion predicted**	**P-value**	**AUC value**
Rhyparobia maderae	10	0.722	0.0085	NA
Pandinus imperator	11	0.615	0.4764	NA
Mesobuthus eupus	12	0.846	0.1726	NA
Blatta lateralis	13	0.8	0.2566	NA
Bombyx mori	13	0.846	0.5816	NA
Blaberus discoidalis	14	0.6	0.0003	NA
Samia cynthia	24	0.852	0.0069	NA
Extatosoma tiaratum	33	NA	NA	0.867
Scolopendra subspinipes	43	NA	NA	0.813
Nauphoeta cinerea	48	NA	NA	0.741
Gryllus assimilis	50	NA	NA	0.805
Manduca sexta	53	NA	NA	0.787
Grylus bimaculatus	82	NA	NA	0.760
Periplaneta americana	87	NA	NA	0.801
Acheta domestica	106	NA	NA	0.848
Drosophila melanogaster	116	NA	NA	0.791
Hermetia illucens	207	NA	NA	0.807
Drosophila hydei	36	NA	NA	0.849
Achroia grisella	289	NA	NA	0.929
Cornu aspersum	131	NA	NA	0.867
Tenebrio molitor	876	NA	NA	0.916
Alphitobius diaperinus	187	NA	NA	0.850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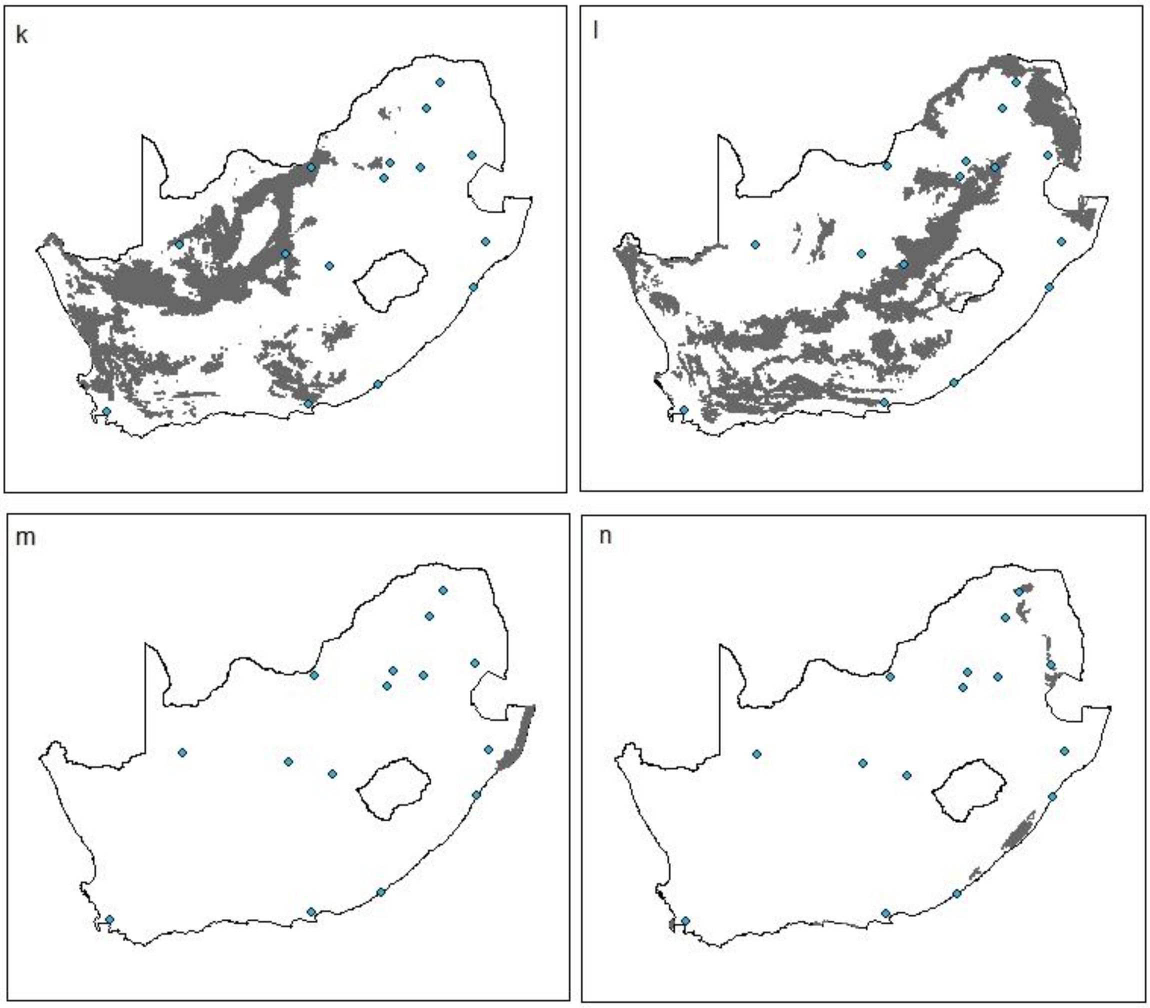

Figure S1. The maps showing areas that are climatically similar as determined by a climate match with Metzger climate zones for 9 of the terrestrial invertebrate species found in the South African pet trade, matched from native distribution ranges to South Africa. The grey shaded areas represent climatically suitable areas while blank areas represent unsuitable areas. The letters in the top left indicate species a) *Pandinus cavimanus*, b) *Achatina immaculata*, c) *Blaptica dubia*, d) *Ethmostigmus trigonopodus*, e) *Gromphadorhina portentosa*, f) *Heterometrus longimanus*, g) *Hottentotta tamulus*, h) *Lucihormetica verrucosa*, i) *Pandinurus viatoris*, j) *Zophobas morio* k) *Blatta latelaris*, l) *Leiurus quinquestriatus*, m) *Pandinus imperator*, n) *Phoetalia pallida*. The blue dots represent the major cities and towns in South Africa with each showed by the following abbreviations, Th - Thohoyandou, LT - Louis Trichardt, Pl - Polokwane, Nl - Nelspruit, Wi - Witbank, Pta - Pretoria, Jo - Johannesburg, Mm - Mmabatho, Ul - Ulundi, Du - Durban, Up - Upington, Km - Kimberly, Bl - Bloemfontein, El - East London, PE - Port Elizabeth, and CT - Cape Town.

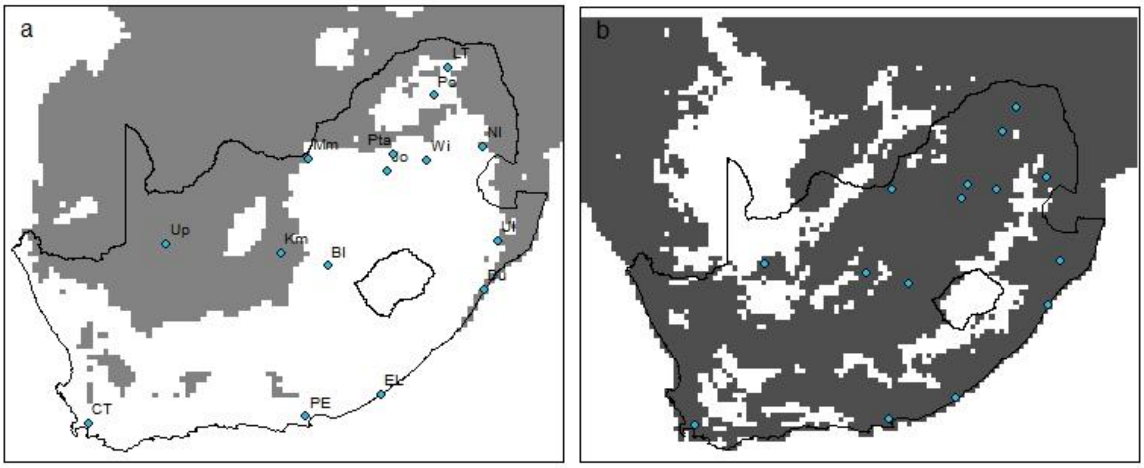

Figure S2. Maps showing areas that are potentially climatically suitable as determined by the Maximum entropy method with Metzger climate zones for two terrestrial invertebrate species evaluated under a Jackknife approach in the South African pet trade, namely a) *Samia cynthia* and b) *Blaberus discoidalis*

Chapter 4: General discussion

What we know about terrestrial invertebrates in the South African pet trade

This study revealed that at least 53 alien terrestrial invertebrate species are offered for sale in the South African pet trade. However, the results show that the number of species collected here is likely to be an underestimate of what is available in trade. These alien terrestrial invertebrates obtained in this study were traded as food for pets, as pets, for cleaning cages and for soil improvement. The total number of species collected here is low compared to tarantulas (n=195) (Shivambu 2018) and reptiles (n=266) (van Wilgen et al. 2008) but higher compared to amphibians (n=3) collected in the South African pet trade (Measey et al. 2017).

There were also clear differences in the availability of terrestrial invertebrate species. Mealworms (*Tenebrio molitor*), superworms (*Zophobas morio*), and dubia roach (*Blaptica dubia*) were all available at over 50% of the outlets we surveyed. This could indicate that highly available terrestrial invertebrate species in the pet trade have many opportunities for release or escape and could subsequently become invasive.

It is important to understand factors that influence availability of species in the pet trade as it can assist in predicting species with high availability (Vall-llosera and Cassey 2017). Other studies have indicated that variables such as rarity of the species, longevity, size, productivity, colour patterns and species that are larger and easier to breed can influence their availability in the pet trade (Wright et al. 2001; Gong et al. 2009; van Wilgen et al. 2010; Su et al. 2015; Chucholl and Wendler 2017; Vall-llosera and Cassey 2017). We found a considerable variation in availability between species. Many terrestrial invertebrates were used for various purposes, but most species were used as food for pets. There appears to be a relationship between the purpose for which terrestrial invertebrates are used and their availability in the South African pet trade. Species with high availability were used as food for pets, which could indicate that the demand for food for pets such as tarantulas and reptiles influences species availability. The trade in these other groups could possibly influence the demand for terrestrial invertebrates that are used as pet food.

Are the names used for terrestrial invertebrate species in the pet trade correctly applied?

A total of 489 species names including duplicates were used for 53 alien terrestrial invertebrate species obtained in the South African pet trade. These 53 species were comprised of 36 recognized species (scientific names) and 17 unrecognized species which were regarded as trade names because their true identity is unknown. The use of different common names for a single species and trade names could lead to species being misidentified and becomes problematic when compiling a list of alien species (Keller and Lodge 2007; Patoka et al. 2014). DNA barcoding and phylogenetic analysis can be used to test the accuracy of identification of terrestrial invertebrates in the pet trade (Hebert et al. 2004; Ng et al. 2016). However, it was not feasible to purchase individuals of all unrecognized species in this study.

The current study used DNA barcoding to confirm whether the names were correctly applied in the pet trade in South Africa. The existing reference sequences were downloaded from NCBI and BOLD then incorporated in phylogenetic analyses for comparison with the sequences generated from the terrestrial invertebrate specimens from the pet trade. This was done in order to construct a phylogenetic tree.

A total of 27 specimens belonging to 11 species with three specimens for each species from different pet stores were selected for DNA barcoding to investigate if the names were correctly applied in the pet trade. Twenty-two out of 27 specimens matched with the barcodes of species from the same proposed species using the COI gene region. It was expected from the phylogenetic tree that all specimens from the same species would form clusters with the reference sequences of the same name. Phylogenetic analysis from this study showed that 22 specimens out of 27 specimens formed sister groups with the reference sequences of species with the same name. The remaining two species *Pandinus imperator* (three specimens of the same species) and *Achatina immaculata* (two specimens of the same species) formed a sister group with reference species of a different species name. This indicates that names used for terrestrial invertebrates in the pet trade are generally correct, however there were several unrecognized species that require further work. This study also shows that identification of species using DNA barcodes can be easily performed when reference sequences are available and the taxonomy of the species is well resolved. This study successfully indicated that DNA barcoding could be used to determine if names are correctly applied in the pet trade (Steinke et al. 2009; Hamilton et al. 2011).

Risk associated with terrestrial invertebrates in South Africa

This study evaluated invasion risks associated with alien terrestrial invertebrate species following three variables, namely; impacts elsewhere, climate suitability and propagule pressure (availability). For most species, much of the data were not available which presents a challenge for understanding risks associated with the pet trade. We were therefore only able to obtain data for all three of these variables for only 14 species (Fig. 1).

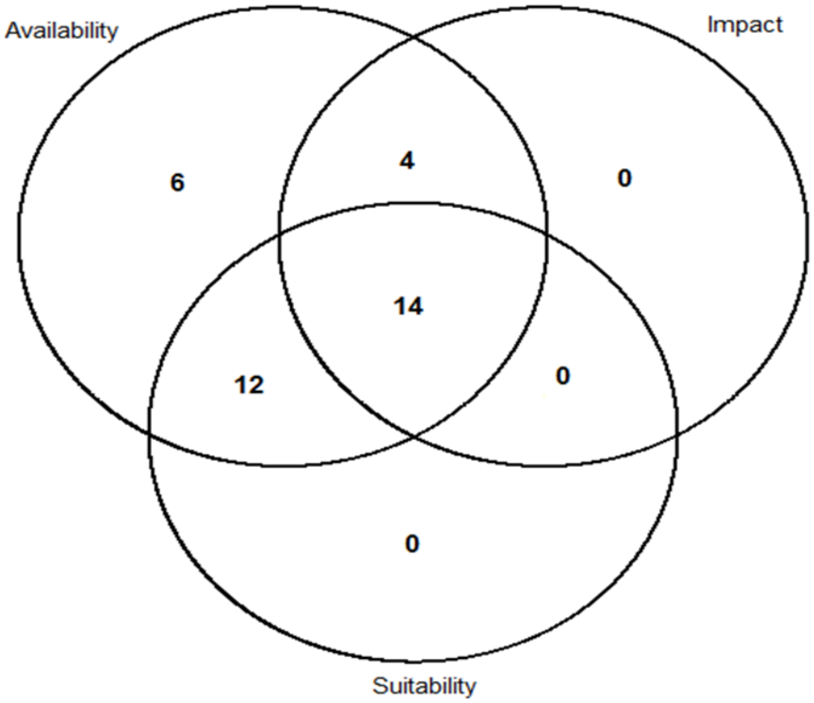

Figure 1. A Venn diagram showing the watch list concept and criteria assessed (availability index, impact elsewhere and climate suitability). The species in the middle (n=14) are species with, availability index, impact elsewhere and climate suitability in South Africa. n=12 are species with both availability and climate suitability. n=4 are the species with both availability index and impact elsewhere. There were no species with both impact elsewhere and climate suitability but no availability index n=0.

Two impact scoring schemes (EICAT and SEICAT) were used to assess recorded impacts (Hawkins et al. 2015; Bacher et al. 2017). This study obtained impacts records for 50% (18 species) of alien terrestrial invertebrate species assessed. The findings of this study indicated that terrestrial invertebrate species could cause high socio-economic impacts (massive) and low environmental impacts (minor). The socio-economic impacts have the potential to affect human life through scorpion stings (*Hottentotta saulcyi* (Scorpiones) and *Mesobuthus eupeus* (Scorpiones, Lesser Asian scorpion) which sometimes results in death. Environmental impacts include the possible transmission of diseases by the introduction of parasites to native species by species such as *Drosophila hydei* (Diptera, Flightless fruit fly), *Phoetalia pallida* (Blattodea, Palid roach) and *Cornu aspersum* (Eupulmonata, Brown garden snail). A more comprehensive risk assessment of terrestrial invertebrates in the pet trade would need to obtain data for all three of the variables considered in this study. Availability of data is a major constraint that has also been reported elsewhere (Shivambu 2018).

The internet has the potential to facilitate the introduction and movement of species within the country as many species are offered for sale over the internet. For example, in New Zealand, 16 alien lizard species were traded over the internet with some species found to be infected with *Hepatozoon* sp. (Protozoa), which is alien to New Zealand (Derraik and Phillips 2009). In the current study more species were obtained from online searches than from pet store visits or from expositions. This demonstrates that the internet is an important means of trade in South Africa.

Species with high availability index scores and impacts elsewhere and large areas of climate suitability including large towns and cities should be regarded as species of greatest concern. Many species had large areas of climate suitability that included large town and cities in South Africa. Terrestrial invertebrate species were traded around major towns and cities assumed to have high human density, which could increase the chances of these species being released (Banha et al. 2017). This could indicate that these areas could be at risk of invasion should these species escape or be released.

Limitations associated with this study

We visited 75 pet stores over a period of 12 months in three provinces (Gauteng = 36, Western Cape = 19 and KwaZulu-Natal Province = 20), which was a substantial amount of work. However, the study was limited in terms of its geographic coverage and in terms of its duration. Additionally, expositions were only visited in Gauteng Province.

This could have affected the number of species obtained as the results from this study indicated that terrestrial invertebrates obtained was an under estimate of what is available in the pet trade. Half of the recognized terrestrial invertebrates (50%, 16 species) had very few occurrence data available on GBIF and subsequently reliable distribution models were produced for only 15 species. The remaining species were considered for climate matching because of the small number of occurrence records. In order to produce more reliable distribution models, it will be useful to gather occurrence records from the native ranges of these terrestrial invertebrates. Occurrence records forms an integral part of the distribution models such as the correlative models used in this study (Robertson et al. 2003; Webber et al. 2011). Mechanistic models could also be used instead of correlative models to get a better understanding of the biology (reproduction and dispersal) for some of the terrestrial invertebrate species with the potential of becoming invasive. To produce a full risk assessment of terrestrial invertebrate species, impact elsewhere and reliable correlative models should be integrated in order to understand the potential risk of each species.

A total of 39 specimens belonging to 13 species with three specimens for each species were selected for DNA barcoding. However, only 27 specimens belonging to 11 species were collected because we could not source the other six specimens, while DNA did not amplify for three specimens. For example, instead of getting three specimens of the same species from different pet stores, we were only able to get one specimen for *Extatosoma tiaratum* and *Hermetia illucens* and two specimens for *Achatina immaculata* and *Gryllus assimilis* respectively. Therefore, due to logistical constraints we were only able to undertake the analysis on 11 species, which is about one third of the known (including recognized and unrecognized species) terrestrial invertebrates in the pet trade. These has hindered effective application of DNA barcoding. Therefore, sampling of these species over a longer period of time will be crucial to understand exactly which species are being traded. To acquire more information on species with trade names, molecular analysis could be used to determine if any of these species are hybrids.

Future directions

This study was able to identify the number of terrestrial invertebrate species available in the South African pet trade, while confirming the identity of some species and determining their invasion risks. An important step to better understanding the trade in terrestrial invertebrates is to establish the identity of the unrecognized species and determine whether they pose a potential invasion risk.

As we found that their use could play a role in availability, it will be important to undertake a study to determine whether there is likely to be a greater risk of release for species kept as pets than for species kept as pet food or cage cleaners. One way to achieve this will be to evaluate if prices could influence availability of terrestrial invertebrate species (Shivambu 2018). In this study, prices were only collected for species sold in low numbers and for species that were sold individually. In order to improve this, species that are sold in large numbers in containers should be bought and counted to accurately record the price per individual.

Visiting pet stores and expositions in other provinces that were not visited would increase the chances of obtaining a larger sample of the species traded in South Africa. This could be done and considered in a follow up study. For an accurate inventory to be made, expositions and pet stores will need to be visited frequently over a long period of time. Two specimens of African land snails, *Achatina immaculata* were identified as *Achatina fulica* albeit with a low percentage match (80.74%) which could indicate that these species could be a hybrid of the invasive *Achatina fulica* or a new species belonging to the genus *Achatina*. To get a better understanding of these species and unrecognized species, these species could be taken to taxonomists for identification. These species could also be sampled again and analysed through DNA barcoding to see if they are hybrids or just a different species.

Because the market is dynamic, certain factors could change in future. For example, species that have low availability now may increase in availability in future, which could increase their invasion risk. Therefore, monitoring of this trade should be implemented in order to identify species that could suddenly pose a risk of invasion.

Conclusion

The study recorded 36 recognized invertebrate species alien to South Africa. However, there were several unrecognized species that require further study. These unrecognized species could include species with the potential to cause negative impacts. Therefore, long term sampling is necessary to obtain a more complete list as the market appears to be dynamic. Although none of the species that were found in the pet trade have been reported to be invasive elsewhere, several species could not be linked to recognized species names. This study found that species with large areas of climatic suitability had a low availability index. We did not find any species that had large areas that were climatically suitable and high availability that also had high environmental or socioeconomic impacts. Based on the species for which appropriate data could be obtained, this study indicated that the risk of invasion from terrestrial invertebrates in the pet trade appears to be low. This study also confirms that the internet is an important means of trade for terrestrial invertebrate species sold as part of the pet trade.

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