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DNA barcoding of alien invertebrates and biological control agents in South Africa: a review

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The rate of human-induced spread of organisms is increasing with globalisation. In addition, climate change is altering ecosystems, enabling species to invade new environments. Invertebrates are particularly well-suited to invasion due to their generally small size and short generation time and their impacts can be extreme. Therefore, reliable species identification is a fundamental requirement for intercepting such alien organisms at borders and managing their populations, but traditional taxonomic identifications can be time-consuming and often require expertise. DNA barcoding is a molecular technique that is rapid, cost-effective and does not require taxonomic expertise. In this study, we compiled an updated checklist of all known alien invertebrate species in South Africa and their status on the Barcode of Life Data System (BOLD) using previous published records and literature. In total, 1013 alien invertebrate species, including 132 biocontrol agents, were found. Insects, predominantly hemipterans, comprised most of the alien species. Overall, 66.8% of alien species in the dataset were accessioned on BOLD. However, few of these alien invertebrate records were South African specimens (24.3%). This study marks the first comprehensive DNA barcoding checklist of alien and biocontrol agent invertebrates in South Africa. The findings are promising because many alien species can be identified to a Molecular Operational Taxonomic Unit (MOTU) or Barcode Index Number (BIN) on BOLD using their COI barcode. However, there is a gap in available barcodes for alien invertebrates. As climate change alters the biogeography of alien species, baseline molecular data such as COI barcodes will be invaluable in monitoring and limiting their spread.

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INTRODUCTION

The era of globalisation has seen an increase in the rate and range of human-induced spread of species due to an increase in international travel and trade (Lockwood et al. 2007a; Gariepy et al. 2014). Invasion pathways, such as intercontinental shipping, international road networks and overseas flights, are ubiquitous and far-reaching, allowing species to spread into non-native regions (Lockwood et al. 2007a; Faulkner et al. 2016). Developing countries with growing trade markets are particularly at risk of new invasions (Faulkner et al. 2016). Points of entry, such as harbours and airports, are where alien species should be intercepted to limit their invasion.

Once established, alien species can disrupt communities and ecosystem functions in their invaded territories (van Wilgen et al. 2022). In natural ecosystems, alien species disrupt native communities by competing with native species for resources or by preying upon them (Armstrong and Ball 2005; Fortuna et al. 2022). As in many other countries globally, agroecosystems in South Africa are threatened by pests, many of them alien (Janion-Scheepers and Griffiths 2020). Here, alien species can be direct pests by consuming crop plants or acting as vectors for plant diseases. This is concerning as South Africa has a highly valuable agricultural export market, totalling US$13.2 billion in 2023 (SARS 2023; Sihlobo 2024). Although it is unlikely that the spread can be halted entirely, prevention strategies can minimise the future spread of alien species (Hill et al. 2020).

A basic prerequisite for managing an alien population is the correct species identification of a representative specimen (Pyšek et al. 2008; Hanner et al. 2009). Species identification traditionally relies on morphology, often requiring an expert in a particular taxon, which is time-consuming, especially for very diverse taxa such as insects (Myburgh et al. 2021). Yet, the detection and management of an alien species requires rapid species identification (Hanner et al. 2009). Moreover, there is a global lack of taxonomic expertise across many taxa (Coleman 2015; Ge et al. 2021). Molecular techniques can provide a time-effective means to identify a specimen, though baseline genetic data is required for efficiency and accuracy (Hanner et al. 2009).

DNA barcoding is a broad term for using short nucleic acid sequences for taxonomic identification (Ratnasingham and Hebert 2007, 2013). In animals, the mitochondrial *COI* region is the standard barcode gene (Armstrong and Ball 2005; Ratnasingham and Hebert 2007). However, *COI* is not the most effective marker for all animal taxa, and other genetic markers have been used for certain taxa where *COI* is less effective in delineating species (Guo et al. 2022; Liu et al. 2023). Different genes are used as barcodes in other taxa, such as *rbcL* and *matK* in plants and ITS and the large subunit ribosomal rRNA in fungi (Schoch et al. 2012; Batley 2015). A public database containing *COI* sequences from confirmed species is required to use the sequence data to identify a specimen. The Barcode of Life Data System (BOLD) is an open-access DNA barcode database with ~1.3 million specimen records, comprising 623,442 Barcode Index Numbers (BINs), the molecular operational taxonomic units (MOTUs) used to represent putative species in BOLD (BOLD 2023). DNA barcoding is a baseline tool that can facilitate integrative taxonomic approaches to early detection of alien species (Armstrong and Ball 2005; Hanner et al. 2009; Madden et al. 2019). Ad hoc detection is achieved by individually sampling suspected alien specimens (e.g. Niemann et al. 2022). However, metabarcoding can also detect known alien species in taxonomically complex samples containing many specimens (Singh et al. 2021). The efficacy of metabarcoding taxonomically complex zooplankton samples was tested in South Africa, comparing the technique to traditional morphological sorting and identification. Singh et al. (2021) found that metabarcoding provided a higher taxonomic resolution of the samples than morphological analysis, identifying more species. However, the small number of DNA barcodes retrieved from BOLD limited the power of metabarcoding (Singh et al. 2021). Metabarcoding is increasingly being suggested as an approach for the detection and management of alien species in terrestrial, freshwater and marine environments (Comtet et al. 2015), including for insects (Piper et al. 2019; Kaczmarek et al. 2022). However, this method is only effective if there are sufficient baseline barcode data available for comparison.

DNA barcoding in South Africa began in earnest in 2011 when an International Barcode of Life Project (iBOL) node was established in South Africa (Table 1), indicating South Africa's commitment to the iBOL's goals for barcoding global biodiversity. That same year, the Urban DNA Barcode Project was launched in KwaZulu-Natal (da Silva and Willows-Munro 2016). In 2016, the first review of DNA barcoding of animals in South Africa was published (da Silva and Willows-Munro 2016). At the time, BOLD contained approximately 48,000 animal records from South Africa, grouped into 10,526 BINs and representing 1,487 species (da Silva and Willows-Munro 2016). Since there are over 65,000 known South African animal species (Hamer 2013), only 2.3% of the country's species diversity was represented on BOLD at the time. Insects were underrepresented, with only 1% (37,105 records in 8,223 BINs, representing 513 species) of native insect species represented on BOLD (da Silva and Willows-Munro 2016). The low barcoding rate was attributed to a lack of taxonomists, with only 28 vertebrate taxonomists and 23 insect taxonomists working in South Africa at the time (da Silva and Willows-Munro 2016). However, due to focused funding initiatives such as the NRF-FBIP (National Research Foundation Foundational Biodiversity Information Programme) and other endeavours, a subsequent review of South Africa's contribution to BOLD found the representation of insects to have improved considerably, with over 56,000 records in 10,492 BINs representing 9,504 species (Myburgh et al. 2021) — an 18-fold increase in insect representation. Estuarine macroinvertebrates have also received greater attention recently, with Fagg et al. (2021) barcoding 15 species associated with seagrass meadows, eight of which were not previously barcoded. Despite these important achievements, more focused barcoding needs to be done to increase the country's biosecurity since roughly half of all alien animal species in South Africa are insects (Picker and Griffiths 2017).

The representation of South African invertebrate species on BOLD has substantially increased in the last decade (Myburgh et al. 2021; Stewart et al. 2024). Between 2014 and 2023, there has been a roughly 20-fold increase in the number of South African records accessioned on BOLD and a 30-fold increase in the number of South African species represented on the database (Supplementary Table S1) (da Silva and Willows-Munro 2016). Platyhelminths have had a 30-fold increase in the number of records accessioned on BOLD, while arachnids have had a 110-fold increase in the number of species represented on the database. This reflects the efforts of researchers and institutions to catalogue the country's biodiversity on the database.

To help facilitate these efforts, this study aimed to collate progress made in DNA barcoding of alien invertebrates in South Africa to highlight gaps which could inform future projects. The most recent summary of alien invertebrates focused on terrestrial species (Janion-Scheepers and Griffiths 2020), while this study will include marine and freshwater species, as well as those on South Africa's sub-Antarctic island territories, the Prince Edward Islands.

METHODS

Data collection

A dataset of terrestrial, marine and aquatic alien invertebrates in South Africa was initially compiled using published articles and reports, including Picker and Griffiths (2011, 2017), Prinsloo and Uys (2015), Robinson et al. (2020), Skowno et al. (2019), Zachariades (2021), Janion-Scheepers et al. (2015) and Janion-Scheepers and Griffiths (2020). In cases where data were lacking from these sources or when a species' alien status was uncertain, the Global Biodiversity Information Facility (GBIF), Barcode of Life Data System (BOLD) and the National Centre for Biotechnology Information's (NCBI) GenBank were searched, particularly for nucleotide sequences in the latter two. Species names were also added from the species list provided in 'The status of biological invasions and their management in South Africa in 2019' (Zengeya and Wilson 2020).

Table 1. A brief history of DNA barcoding globally and in South Africa.

Date	Milestone	Reference
2003	Paper published showing that the *COI* region in eukaryotes can identify specimens with satisfactory accuracy and proposes this gene be adopted as the standard DNA barcode region for animals.	Hebert et al. (2003)
	Three meetings concerning DNA barcoding, sponsored by the Sloan Foundation, are held at the Banbury Centre in Cold Spring, USA.	DeSalle and Goldstein (2019)
2005	The African Centre for DNA Barcoding is founded in Johannesburg.	(Bezeng et al. 2017)
2010	The International Barcode of Life Project (iBOL) is launched.	da Silva and Willows-Munro (2016)
2011	An iBOL node is opened in South Africa to facilitate the country's commitment to the iBOL.	da Silva and Willows-Munro (2016)
2011	The Urban DNA Barcode Project, a part of the eThekwini Municipality-University of KwaZulu-Natal Joint Research Partnership, is launched to establish a biodiversity inventory for eThekwini.	da Silva and Willows-Munro (2016)
2016	Review published of DNA barcoding in South Africa. This article remains one of few on the topic and outlines the state of barcoding in South Africa at the time.	da Silva and Willows-Munro (2016)
2019	About 3 700 articles with "DNA barcoding" in their titles have been published.	DeSalle and Goldstein (2019)
2021	A review of the contributions of insect DNA barcodes to BOLD made by South Africa.	Myburgh et al. (2021)

An advanced literature search was performed on the Web of Science using the following search terms: (alien OR invasive OR non-native OR "biological control agent" OR "biocontrol agent" OR biocontrol) AND (invertebrate OR arthropod OR insect OR arachnid OR crustacea OR myriapod OR collembola OR mollusc OR platyhelminth OR annelid OR porifera OR sponge OR cnidaria OR echinoderm) AND ("South Africa"). Three relevant review articles were Dittrich-Schröder et al. (2020), Musundire et al. (2011) and Stokwe and Malan (2016). The final dataset contained South African alien species published up until July 2023.

Alien taxa were divided into either phyla or groups, as used by da Silva and Willows-Munro (2016). The latter was chosen to compare this study's results with previous literature on South African barcoding. The distribution of species in terrestrial, marine and freshwater realms was analysed using the list from Zengeya and Wilson (2020). Provincial distribution of species was determined from the collection locality from BOLD records.

Data analysis

The dataset was analysed using R v.4.3.1 (R Core Team 2023). The *ggplot* package was used to produce all the figures. The *rgbif* package was used to retrieve taxon names for each species, namely phylum, class, order, and family (Chamberlain et al. 2024). The *bold* package was used to retrieve DNA barcode metadata for each species from BOLD using species names only on 16 December 2023 (Dubois and Chamberlain 2023). These data were 1) the number of available records, 2) the number of associated BINs, 3) the number of available images and 4) the country where specimens were collected. The R code used is available in the Supplementary Material.

RESULTS

The final dataset comprised 1013 alien species, including 132 biocontrol agents, of which 96.9% were insects and 3.1% were arachnids (see Supplementary Dataset). Of these species, 677 were represented on BOLD, including 56 biocontrol agents. Arthropods made up the majority of the barcoded alien species (461), followed by molluscs (61), annelids (46), nematodes (15) and cnidarians (11), followed by other taxa (Supplementary Table S2). Within the arthropods, insects were the largest taxon (Figure 1) and made up 49.7% (329) of the barcoded alien arthropod species in the dataset. Of the insect orders, the bugs (Hemiptera) accounted for the majority (40.4%) of barcoded species (Figure 2).

Most alien species in the dataset, including biocontrol agents, were terrestrial, with 855 species, while 103 were marine and 55 were from freshwater environments (Table 2). The Western Cape had the highest number of alien species (214), of which insects and molluscs were the most barcoded, at 72 and 32 species respectively (Figure 3; Supplementary Table S3). The Prince Edward Islands had the least with 26 species, mostly insects, of which 16 were accessioned on BOLD. Gauteng had the highest number of barcoded alien insect species at 81, of which 74 were barcoded.

Of the alien species in the dataset, 66.8% are represented by records on BOLD (Figure 4). Of these records, 92.6% were associated with BINs (meaning these records are assigned to MOTUs), while 55.4% had specimen images (meaning record specimens can be compared to other specimens morphologically). Of the biocontrol agents, 42.4% were represented by BOLD records. Of these, 86.6% were associated with BINs, while 59.7% had specimen images. Most of all BOLD records were from specimens collected outside of South Africa, with 17.1% of alien

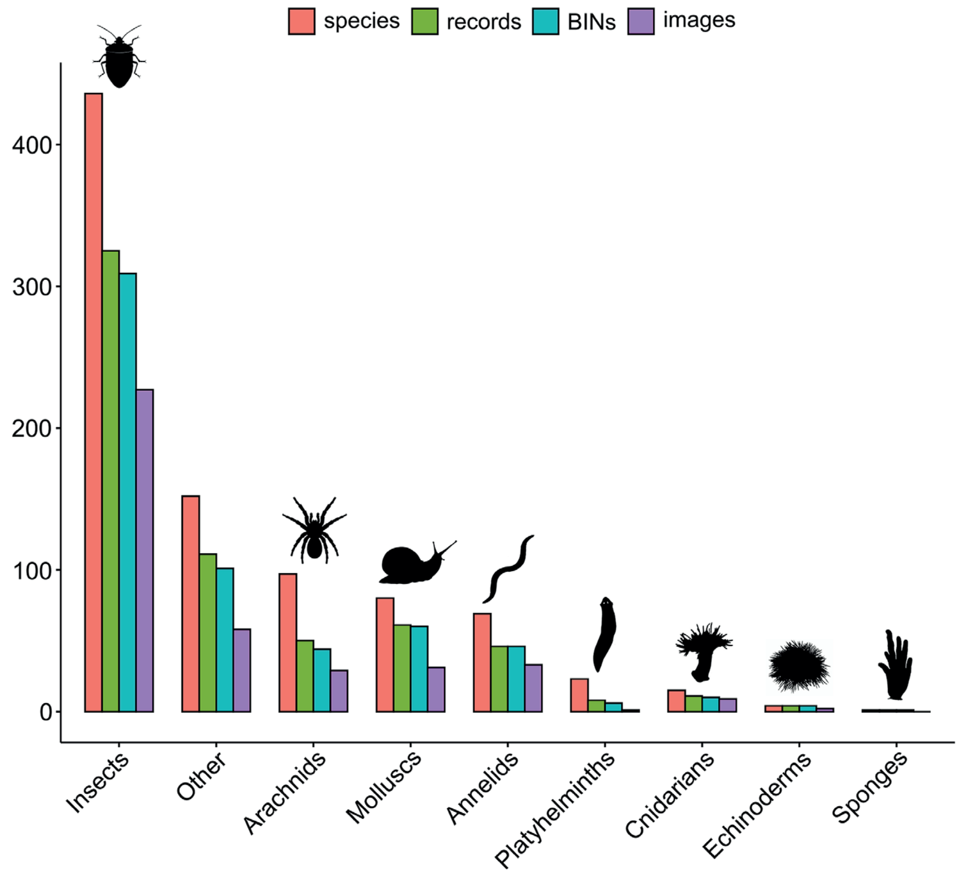

Figure 1. The number of alien species of different invertebrate groups in South Africa (red), the number of those species represented by records on BOLD (green), the number of species with associated BINs (blue) and the number of species with images available on BOLD (purple). 'Other' includes the phyla Nematoda, Chordata, Bryozoa, Brachiopoda, Ctenophora, Euglenozoa and Myzozoa, and the arthropod classes Malacostraca, Collembola, Diplopoda, Maxillopoda, Chilopoda, Copepoda, Branchiopoda, Pycnogonida and Thecostraca.

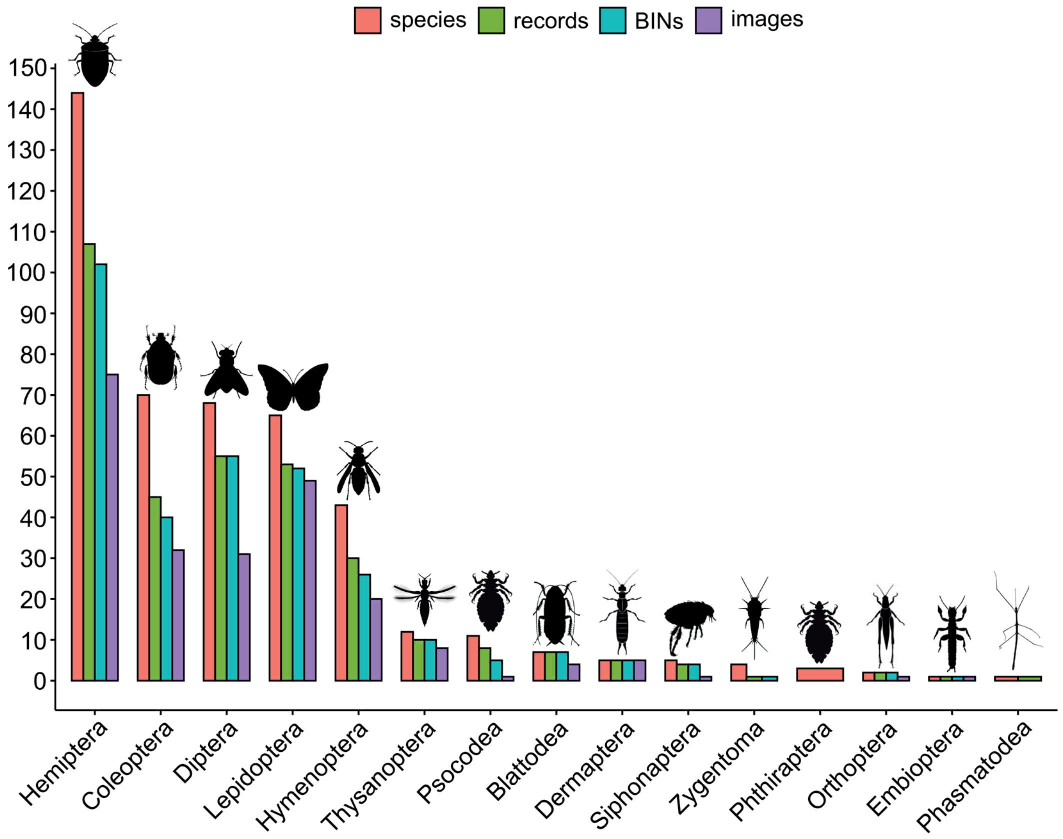

Figure 2. The number of alien insect species in South Africa by order and their representation on BOLD. The y-axis is a count of the number of species (red), species represented by records on BOLD (green), species represented in BINs (blue) and those represented with images on BOLD (purple) per order.

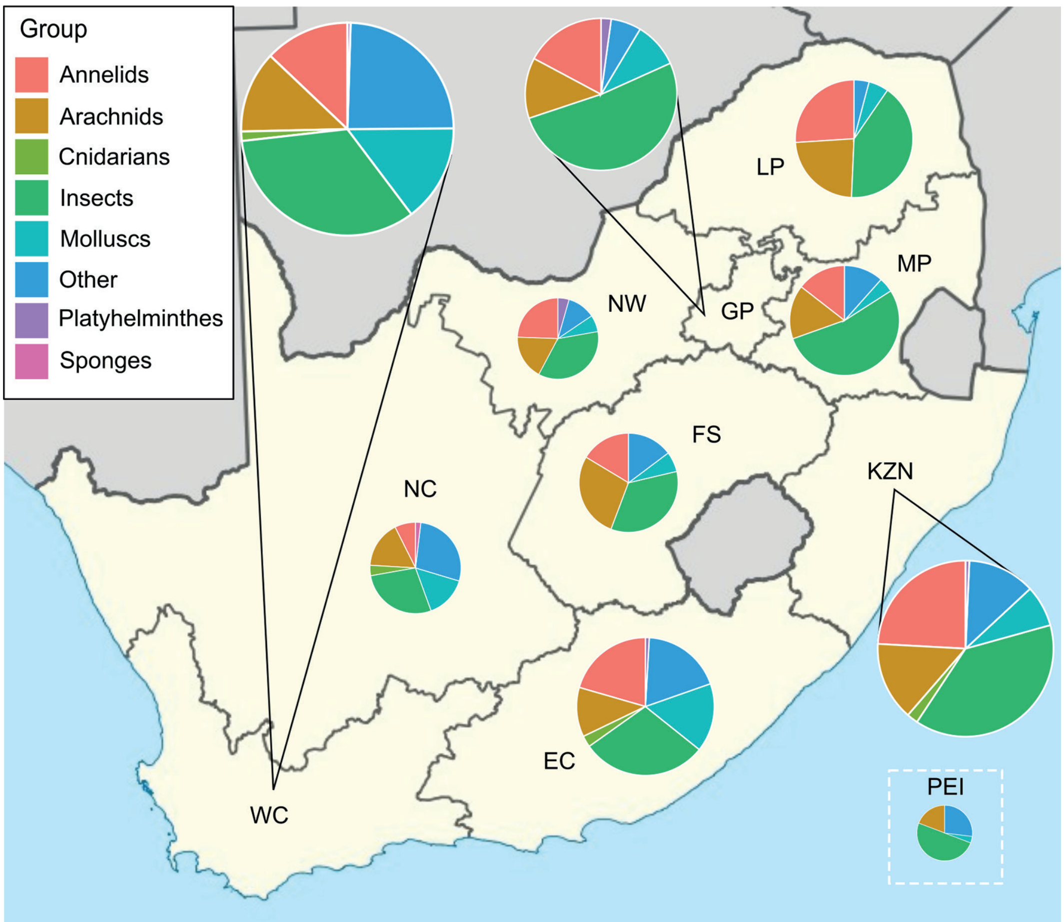

Figure 3. The distribution of barcoded alien invertebrates in South African provinces and island territories (EC = Eastern Cape, FS = Free State, GP = Gauteng, KZN = KwaZulu-Natal, LP = Limpopo, MP = Mpumalanga, NC = Northern Cape, NW = Northwest, PEI = Prince Edward Islands, WC = Western Cape). The groups used here reflect those da Silva and Willows-Munro (2016) used.

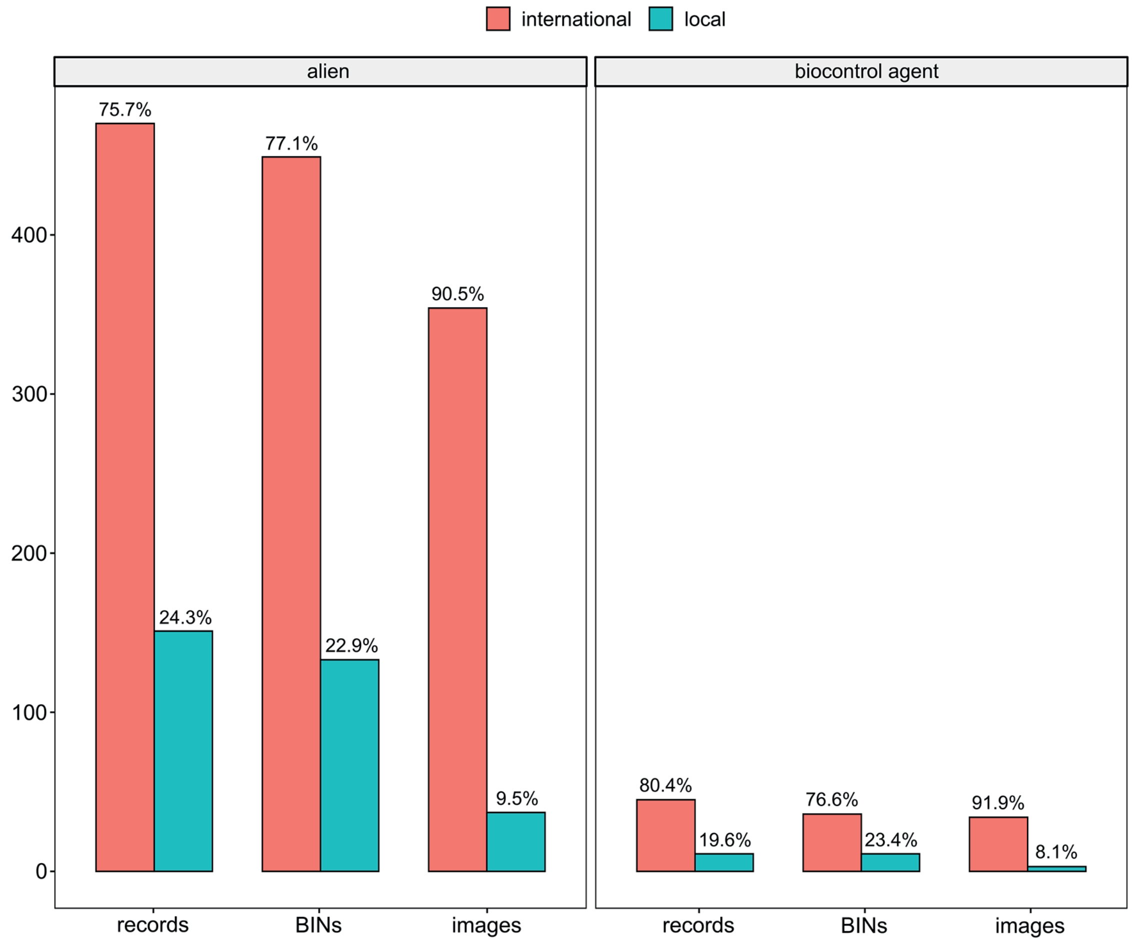

Figure 4. The number of barcoded invertebrate alien (left) and biocontrol agent (right) species in South Africa with records in BOLD, the number of species associated with BINs and the number of species with images on BOLD. The blue bars represent species for which a South African BOLD record is available, while the red bars represent species for which only international specimen records are available. The percentages indicate the proportion of the total alien or biocontrol agent species for each bar.

Table 2. Number of alien and biocontrol agent species from terrestrial, marine and freshwater environments.

Environment	Alien	Biocontrol agent	Total
Terrestrial	726	129	855
Marine	103	0	103
Freshwater	52	3	55
Total	881	132	1013

records and 8.3% of biocontrol agent records barcoded from specimens collected locally.

DISCUSSION

Alien invertebrate species are ubiquitous but poorly documented (Pyšek et al. 2020). The total number of alien invertebrates present in South Africa is substantial and comprises 62% of all known alien species in the country (van Wilgen et al. 2020). Terrestrial invertebrates make up about one-third of the alien species in South Africa (van Wilgen et al. 2020), and insects globally far outnumber other alien taxa (Pyšek et al. 2020). However, invertebrates still receive disproportionately less attention than vertebrates from public and scientific communities (Pyšek et al. 2008; Di Marco et al. 2017; Troudet et al. 2017; Eisenhauer et al. 2019; Eisenhauer and Hines 2021). This study illustrated that despite concerted efforts to barcode diverse taxa (da Silva and Willows-Munro 2016; Myburgh et al. 2021), there remains a gap in the DNA barcoding of invertebrates generally and alien invertebrates and biocontrol agents specifically.

Alien invertebrates and barcoding

In this study, we found that most barcoded alien invertebrate species in South Africa were arthropods. Insects are the largest barcoded arthropod group, most of which are hemipterans. Most of these were of the suborder Sternorrhyncha (aphids and scale insects), which are all sap-sucking insects (Janion-Scheepers and Griffiths 2020). This feeding guild is particularly harmful to crop plants and native plant species (Janion-Scheepers and Griffiths 2020; Huang et al. 2020). Furthermore, Sternorrhyncha are common vectors of plant diseases, which can spread rapidly in monocultures (Huang et al. 2020). The representation of Sternorrhyncha in South Africa's BOLD records is partly due to barcoding done by Sethusa (2014).

The most poorly represented invertebrate groups on BOLD were the arachnids and platyhelminths. Arachnids comprise economically important species such as ticks and mites, the latter including candidate biocontrol agents (Smith Meyer and Craemer 1999; Klein 2011). Since many are predatory, alien arachnids are a concern for local biodiversity. Alien tarantulas (Arachnida: Araneae) are a popular invertebrate pet in South Africa (Shivambu et al. 2020). The pet trade is an important pathway of alien invertebrate introduction in South Africa, but it is poorly studied except in the case of tarantulas (Nelufule et al. 2020; Shivambu et al. 2020). However, rather than being sold as pets, most alien invertebrates are co-introduced as parasites or commensals through the pet trade (Nelufule et al. 2020). This is also the case for platyhelminths, which comprise many parasitic species that have been co-introduced in South Africa along with their fish hosts (Smit et al. 2017). Indeed, nearly all the platyhelminths in the dataset have parasitic life histories (Table S2). Unfortunately, little is known about the distribution of platyhelminths in South Africa (Schockaert et al. 2008). The poorly understood distribution of alien arachnids and platyhelminths, coupled with the deficit of barcoding of these groups, is a major hurdle in the ability to identify and control future invasions in South Africa.

Most alien species in the dataset were terrestrial, while only about 10% were marine and 5% were from freshwater environments. Given that ~75% of the world's invertebrates are insects (Eisenhauer and Hines 2021) and that insects were the largest group in this study's dataset, it is not surprising that most species were terrestrial. In other parts of the world such as Europe, however, the number of terrestrial (~600) and marine (514) alien invertebrates is roughly the same (Keller et al. 2011; European Environment Agency 2023). In addition, marine biological invasions receive less attention globally than terrestrial invasions, suggesting a gap in the cataloguing of marine alien species in South Africa (Giakoumi and Pey 2017).

The Western Cape had the highest number of barcoded alien invertebrates in this study. However, da Silva and Willows-Munro (2016) showed that Gauteng and KwaZulu-Natal superseded the Western Cape in the number of barcoded native and alien species, especially insects, which was confirmed by Stewart et al. (2024) and attributed to intensive malaise trapping in the former provinces. The high number of barcoded alien invertebrates from the Western Cape may be explained by not only a greater abundance of alien species occurring in the province, but also a public interest in cataloguing the biodiversity in the Cape Floristic Region, which includes the famous fynbos biome (Ashwell et al. 2006), or more resources being allocated to barcoding aliens here than in other provinces. The Northwest Province was the least represented province on BOLD, followed by the Northern Cape and the Free State. The ranking of the Free State reflects the findings of Myburgh et al. (2021), who suggested that intense agriculture may be fragmenting ecosystems in the province, reducing the overall biodiversity and making certain species scarcer and more difficult to sample (Statistics South Africa 2023). However, barcoding is important in provinces with high agricultural productivity, as it facilitates the cataloguing of alien species that may have negative impacts on crop yield and natural enemies that could be used to supress pest populations. The provincial occurrence of 153 species recorded in South Africa is unknown from the literature and BOLD. This represents a significant knowledge gap of alien invasions in South Africa. To limit the spread of alien species, they should be intercepted at the earliest point in their invasion, and their distribution data should be made readily accessible to the public so that researchers can establish invasion paths.

Although many alien invertebrates have been accessioned on BOLD, few records were generated from South African specimens. Instead, most of the specimens were collected outside of South Africa. This represents an issue with the validity of the *COI* sequences in those records because genetic drift or hybridisation may cause the South African population to differ from the international population (Lockwood et al. 2007b; Viciriuc et al. 2021). In addition, species native to South Africa may closely resemble alien species collected outside of the country. A small portion of all the alien species in this study had BOLD records which were not associated with BINs. Records that are not associated with BINs represent a lack of sequencing of that species and, thus, limited reliability of the molecular confirmation of the specimen's species identity. Finally, only about half the BOLD records for the alien species had associated images, which prevents a BOLD record from being compared to a physical specimen or the identification being revised based on the morphology. However, there are practical reasons that images are not always feasible, particularly when metabarcoding the contents of traps containing many specimens or when metabarcoding environmental DNA (eDNA) samples. The poor quality of BOLD records for alien invertebrates in South Africa limits the application of DNA barcoding for detection and monitoring and biosecurity programmes in the country.

Biocontrol agents and barcoding

Although many alien species have been accessioned on BOLD, just over half the biocontrol agents in South Africa have not. An important part of the selection process of a biocontrol agent is understanding its host specificity. Host specificity can be better understood if both the host and natural enemy are identified to species, a process aided by barcoding. Furthermore, biocontrol agents are known to hybridise in the field after release (Goldson et al. 2003; Hopper et al. 2019; Viciriuc et al. 2021). Not all hybrids are equally effective against the alien species or pests they were introduced to control (Goldson et al. 2003; Viciriuc et al. 2021). While *COI* barcodes cannot distinguish hybrids, other genetic markers, such as nuclear microsatellites (Abdul-Muneer 2014), can enable hybrid identification and, thus, ideal agent selection and management. Therefore, sequencing DNA barcodes and other genetic markers of biocontrol agents enables hybrid identification and, thus, ideal agent selection and management.

CONCLUSIONS AND FUTURE PERSPECTIVES

Globalisation and climate change will continue to drive species range shifts in complex ways, which may include more rapid invasions of certain species, including pest species (Liebhold et al. 1995; Robinet and Roques 2010; Pyšek et al. 2020; Fortuna et al. 2022). Invertebrates are poorly documented compared to vertebrates, yet they form a significant portion of alien species in South Africa and globally (Pyšek et al. 2020). Furthermore, insects in particular, are damaging to local communities in complex ways (Fortuna et al. 2022) and are more likely to establish at long distances from their natural range in a changing climate (Robinet and Roques 2010). Identification of specimens using morphological taxonomy is slow which means that it is generally inappropriate for monitoring the spread of alien species, so barcoding will be crucial for monitoring and managing alien populations in the future. DNA barcoding enables rapid species cataloguing and identification, though it is limited in its efficacy by the low representation of species on public databases such as BOLD. While BOLD records are helpful, they are most useful once they have all their metadata available (e.g. place of collection, image and collection details) and when they are assigned to a BIN with many other records. Initiatives such as the Urban DNA Barcode Project are important recipients of funding to maximise barcoding (da Silva and Willows-Munro 2016). In addition, a concerted national sampling effort, including malaise trapping for insects (Stewart et al. 2024), could help generate important baseline data on invertebrate diversity. Metabarcoding of bulk trap samples offers a time- and cost-effective approach compared to traditional methods, making it an increasingly valuable tool for detection and monitoring programmes, especially in biosecurity and trade contexts.

Advances in the use of environmental DNA (eDNA) have made it possible to detect the presence of alien species without a specimen (e.g. Larson et al. 2020). eDNA is helpful for elusive species or those inhabiting inaccessible to humans (Larson et al. 2020), for example detecting the spread of invasive silver carp inhabiting difficult-to-sample river systems in the Kruger National Park (Crookes et al. 2020). Given its simple sampling protocol, eDNA can aid in biosecurity monitoring through citizen science, an advantage for countries lacking biosecurity resources, such as South Africa (Larson et al. 2020).

South African biodiversity and agriculture are under threat from invasions. Alien invertebrates make up a significant portion of alien species in South Africa but, despite consistent increases in barcoding of this group in the country, these have not been proportionately barcoded. Alien arachnids and platyhelminths in the country are underrepresented on BOLD. This and underrepresentation of other taxa may be due to difficulties related to the amplification of barcode genes or the ineffectiveness of barcode genes for certain groups or species (Waugh 2007). Reasons for the deficit of alien invertebrate records in Gauteng and KwaZulu-Natal provinces should be addressed through additional investigations, as well as the general gap in occurrence data for alien invertebrate species in the country. Although there is an increased effort to barcode South Africa's alien invertebrate species, some BOLD records are incomplete or represent specimens collected outside South Africa.

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CREDIT CONTRIBUTIONS

Tristan Pitcher was responsible for data curation, formal analysis, and investigation. He also contributed to writing the original draft and was involved in the review and editing process. Abusisiwe Ndaba contributed to the conceptualisation of the study and was involved in the investigation. Adriaana Jacobs participated in the review and editing of the manuscript. Michelle Hamer was involved in the review and editing of the manuscript. Charlene Janion-Scheepers contributed to the conceptualisation of the study, provided supervision, and was involved in the review and editing process.

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