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Unveiling South African insect diversity: DNA barcoding's contribution to biodiversity data

Insects are one of the most species-rich groups on Earth. They comprise much of animal diversity and play vital roles in ecosystems, including pollination, pest control, and decomposition. However, only a fraction of this diversity has been formally described. South Africa is recognised as one of the most biologically diverse countries globally, with an estimated 44 000 insect species. Many crops rely on insect pollinators, including canola, apples, oranges, and sunflowers. A shortage of wild pollinators currently threatens crop yields, yet our knowledge of insect diversity within South Africa is sparse. There are few taxonomic specialists relative to South Africa's biodiversity, and the methods used for insect identification can be time-consuming and expensive. DNA barcoding provides an important research tool to accelerate insect biodiversity research. In this review, we queried the public DNA barcoding BOLD (Barcode of Life Data System) database for records of "Insecta" within South Africa, and 416 211 published records assigned to 28 239 unique BINs (Barcode Index Numbers) were returned. We identified five taxonomic orders with more BINs than known species in southern Africa (Hymenoptera, Diptera, Thysanoptera, Plecoptera, and Strepsiptera). Most of the barcoded records were derived from Malaise trap sampling in Gauteng, Mpumalanga and Limpopo, while the rest of South Africa remains poorly sampled. We suggest that there is a need for a comprehensive national sampling effort alongside increased investment in taxonomic expertise to generate critical baseline data on insect biodiversity before species are lost to extinction.

Significance:

Insects provide critical ecosystem services, but our knowledge of insect biodiversity is limited. DNA barcoding can help fill biodiversity knowledge gaps; however, within South Africa, sampling has been uneven. Well-sampled provinces include Gauteng, Mpumalanga and Limpopo, while the Eastern Cape, Free State, KwaZulu-Natal, Northern Cape, North-West and Western Cape remain under-sampled. We argue for a broad national Malaise trapping effort to generate crucial baseline data on insect biodiversity. Without urgent investment in taxonomic expertise and biomonitoring, we risk losing much of our biodiversity before it is even described.

Introduction

Insects comprise around 70% of animal diversity¹, with over 1 million species described and a lower estimate of 5 million more to be discovered². The most species-diverse orders are Coleoptera (~387 000 currently described species), Lepidoptera (~157 000), Diptera (~155 000), and Hymenoptera (~117 000).² Insects play vital roles in ecosystems, including in pollination, pest control, decomposition of organic materials, and nutrient cycling.³ They deliver many of nature's contributions to people, including pollination by bees of agricultural crops^{4,5} such as sunflowers, tomatoes, canola, cowpeas, and coffee. Crop yields are currently threatened by a shortage of wild pollinators⁶ as agricultural landscapes become isolated from the natural and semi-natural habitats that support pollinating insects⁷. Smith et al.⁸ linked pollination decline to half a million premature human deaths annually due to a shortage of affordable, nutritious foods such as nuts, legumes, fruits, and vegetables.

Insect predators and parasitoids can help control pests and insect vector abundance. Spiders, beetles, ants, and specific true bugs are considered to be the main groups of invertebrate predators^{9,10}, and some pests are also suppressed by parasitoids. Natural biological control can thus help limit the population size and reduce the impact of pests in agricultural settings.¹¹ Insect scavengers, detritivores, or filter-feeders on microbes also contribute importantly to decomposition.¹² Thus many insects additionally help with nutrient cycling and transformation of living biomass into frass, cycling the carbon and nitrogen back into the soil.¹³ This process of breaking down the dead organic matter accounts for about 29% of forest deadwood decomposition.¹⁴

While the benefits of insect diversity are now becoming more widely appreciated, some insects can have negative impacts. Millions of lives are lost annually to human diseases carried by insect vectors¹⁵, and insect crop pests can significantly reduce agricultural yield¹⁶. African sleeping sickness (*African trypanosomiasis*) and malaria (*Plasmodium falciparum*), for example, are carried by the tsetse fly (*Glossina* sp.) and the female *Anopheles* mosquito, respectively, which infect their human hosts through blood feeding. It has been suggested that malaria-endemic countries have lower economic growth because of the negative impact that the disease has on worker productivity and population growth.¹⁷ Non-native pests can be particularly damaging to crops, wild plants, and our natural environment; this is because they may escape the natural predators from their native ranges, which would normally limit their abundance.¹⁸ These non-native insect pests can have significant economic impacts.¹⁹

South Africa is recognised as being one of the most biologically diverse countries in the world. However, there is currently no comprehensive modern classification of higher insect taxa available for South Africa.²⁰ Records for southern Africa suggest there are about 44 000 insect species, encompassing 7750 genera, 569 families, and 25 orders²¹, but these numbers are now more than 35 years old and have shown to be underestimates²²⁻²⁴. For example, new barcoding efforts with hoverflies have identified species that were not previously included on countrywide checklists.²⁵



DNA barcoding is a molecular method that has gained popularity over the last 20 years; it uses short, standardised genomic sequences to facilitate species identification and discovery.²⁶ In animals, the DNA barcode is a 658 base-pair segment in the gene encoding the mitochondrial cytochrome c oxidase subunit 1 (*COI*); this region has proven effective for species identification in animals.^{27,28} Barcode sequences are uploaded and stored on the Barcode of Life Data (BOLD) System²⁹, which allows for the analysis and sharing of DNA barcode records. Sequences are clustered using the Barcode Index Number (BIN) system.³⁰ This system uses a well-established algorithm that assigns collections of related sequences to an OTU (operational taxonomic unit) that closely corresponds to a species-level identification.

Here, we review the contribution of DNA barcoding to documenting South Africa's rich insect diversity. We queried the public database BOLD for insect records in South Africa and explored their current coverage. Finally, we discuss how national barcoding efforts could be further extended.

Insect decline

Insects are particularly sensitive to environmental change³¹, and thus provide useful bioindicators of ecosystem health^{32–34}. However, their sensitivity to environmental disruption also increases their vulnerability. Recent work has suggested that we may already be experiencing dramatic declines of insects in some regions.³⁵ Hallmann et al.³⁵ reported that the abundance of terrestrial arthropods in Europe has declined by more than 75% over the past three decades, leading to headlines declaring an “insect apocalypse”.³⁶ Given the projected rates of decline, it is possible that some species may go extinct before they can be formally classified.³⁷ However, it is unclear whether such trends generalise more widely, and more data are required to assess large-scale insect trends and determine the causes behind these fluctuations.³⁸ The IUCN RedList (2023) has only evaluated ~1.2% (12 441) of described insects, and of those 25.9% (3222) are “data deficient”. South Africa currently lacks baseline data to evaluate whether our insect diversity is following global trends; there is incidental evidence that some species and insect faunas may be in decline. For example, a survey of South African beekeepers suggested a loss of managed honey-bee colonies³⁹, and the extent of natural vegetation in the Renosterveld ecosystem, a recognised biodiversity hotspot supporting a rich diversity of butterflies⁴⁰, is just about 5% of what it once was. There are, therefore, increasingly urgent calls to better describe and monitor insect diversity globally and regionally.³⁸

The taxonomic impediment, DNA barcoding and dark taxa

The taxonomic impediment refers to the limitations and deficiencies in the resources allocated to the field of taxonomy.⁴¹ This impediment has hindered efforts to generate baseline insect data for conservation and biodiversity monitoring.^{42,43} Currently, there are only a few specialist insect taxonomists relative to the vast amount of biodiversity within South Africa (just 23 insect taxonomists were recognised in 2013²⁴). An analysis by Hamer²⁴ showed that, over a 31-year period, the average (animal) taxonomist in South Africa described less than one new species per year; it would, therefore, take 355 years to describe the remaining undescribed insect species (estimated to number around 80 000).²⁴ The magnitude of the task relative to the capacity of available expertise is such that it is improbable that we will be able to address the challenge using traditional taxonomic methods alone. To describe and identify this wealth of diversity, we will need to develop faster, more high-throughput methods for collecting and studying species diversity; one approach that has proved highly efficient is DNA barcoding.⁴⁴

Importantly, DNA barcoding is not a substitute for good taxonomy but a tool that allows researchers to match samples to previously described barcoded specimens. DNA barcoding has proven its effectiveness in cataloguing Earth's diversity⁴⁵ and can supplement traditional taxonomic approaches where morphological characters are less reliable for delineating species barriers⁴⁶. Globally, barcoding has helped reveal phenotypic plasticity, and identify cryptic, sexually dimorphic, and multiple life-stage species. Importantly, barcoding is still effective when

vouchers are damaged or lack identifying morphological features.^{47–52} For example, Sethusa et al.⁵² demonstrated how DNA barcoding could reliably and rapidly identify economically important scale insects without requiring specialist taxonomic expertise. Kamdem et al.²⁵ similarly showed how DNA barcoding could accurately discriminate between species of Afrotropical hoverflies, although results varied by species delimitation model.

In 2017, Bezeng et al.⁵³ described the accelerating DNA barcoding effort in South Africa. These authors noted that further progress would require addressing several key challenges, including educating the next generation of taxonomists and expanding present efforts to poorly understood taxonomic groupings such as beetles, flies, disease-causing organisms, and pest species.

Here, we describe how DNA barcoding provides a valuable tool for generating baseline data on insect diversity to inform nationwide biomonitoring efforts for assessing future global change.^{22,54} Currently, however, DNA barcodes are being generated faster than species are being described, giving rise to a growing list of ‘dark taxa’ — species with barcode sequence data but lacking formal taxonomic classification.⁵⁵ Barcoding allows us to cluster samples by sequence similarity, essentially discriminating between OTUs without detailed taxonomic knowledge. However, this lack of taxonomic information limits the insight that might be gained into their ecological functions, and there is not always a one-to-one match between barcode BINs species recognised by trained taxonomists.^{30,56,57} Further developing South Africa's reference libraries would allow unknown sequences to cluster with identified specimens and thus reduce the number of ‘dark taxa’, although the lack of expert taxonomists to identify reference taxa presents a major challenge.²

Progress on insect barcoding in South Africa

Here, we review current progress in barcoding insects of South Africa. In June 2023, we queried the BOLD public database for insect records (“Insecta”) within “South Africa” and downloaded the specimen data. We identified 416 211 published records encompassing 3785 genera and 491 families. These records were assigned to 28 239 unique BINs. This is a massive sevenfold increase in numbers reported by Myburgh et al.³² (56 392 records), who published on South Africa's contribution of insect records in BOLD. However, within the same year, the study by Myburgh et al. became outdated, with D'Souza et al.²² adding 339 193 insect records to BOLD from a deployment of 25 Malaise traps across the Kruger National Park – the Kruger Malaise Programme. South Africa is now ranked 4th in the number of insect records on BOLD, which at the time of query had 7 497 794 publicly available insect records.

The number of unique BINs in BOLD for South Africa is equivalent to 65% (28 239) of the insect fauna estimated (43 565) by Scholtz and Chown⁵⁸. However, we identified five taxonomic orders where the number of BINs from South Africa exceeds the known species count for southern Africa (Hymenoptera = 9259 vs. 5273, Diptera = 7611 vs. 6243, Thysanoptera = 263 vs. 228, Plecoptera = 32 vs. 22, and Strepsiptera = 7 vs. 6; Figure 1). Our analysis thus suggests that the diversity in these species-rich groups may be greatly underestimated. The underestimation of Hymenoptera diversity is notable. South Africa is a centre of bee diversity⁵⁹, and bees are among the best-studied insect groups. Currently, BOLD records capture six of the seven families that should be present in South Africa.⁶⁰ Thus, we still lack representative DNA barcode coverage, even for some of the most well-known groups.

In contrast to results for Hymenoptera, the number of BINs for Coleoptera and Psocoptera is much lower than their estimated number of species. Coleoptera is generally considered the most species-rich order globally and accounts for roughly 25% (about 400 000 species) of all described animal species⁶¹, but we documented only 2983 unique BINs for the group. The current BIN count for Hymenoptera (9259, representing an estimated 5273 species), which includes several poorly known parasitic wasps which are very diverse, is over triple that of Coleoptera. Forbes et al.⁶² proposed that Hymenoptera was possibly 2.5–3.2 times (estimated 1 152 127 species) more diverse than Coleoptera. Although

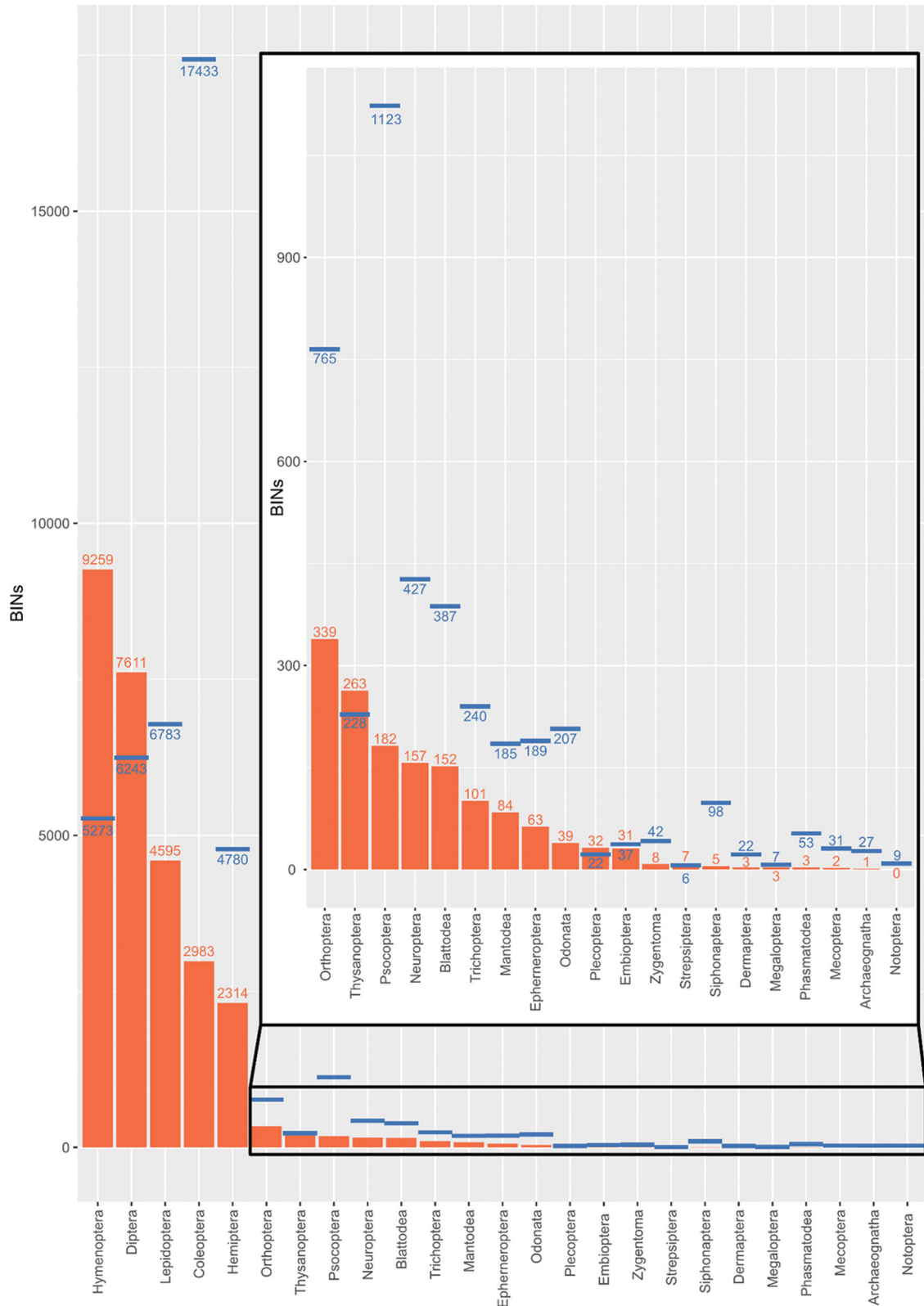


Figure 1: Number of Barcode Index Numbers (BINs) from South Africa on the Barcode of Life Data (BOLD) system (orange) across taxonomic orders and the estimated number of southern Africa species in each order from Scholtz and Chown⁵⁸ (blue). The graph is sorted from most to least number of BINs. The data were collected on 22 June 2023 from BOLD systems (<http://www.boldsystems.org/>).

the BOLD records for South Africa provide only a biased snapshot of the true diversity in both groups, they provide some support for Forbes et al.⁶² Thus, we still cannot answer fundamental questions on insect biodiversity, such as which taxonomic group is more diverse in South Africa, highlighting the significant gaps in our biodiversity knowledge.

Diptera contributes 49% (121 513 records) of the records that have been identified to family level (248 845), and, based on the number of BINs, it is the second most diverse insect group. Scholtz²⁰ noted that within Diptera there are approximately 150 families found in southern Africa; of these we could find only 77. Therefore, despite its good representation

on BOLD, Diptera remains considerably undersampled, although the true number of families in South Africa is unclear. Of all the orders, Plecoptera has the most records identified to genus and species level – 88% (113 records out of 129 total Plecoptera records) – due to a recent phylogenetic analysis of the African stoneflies.⁶³ This group is not one of the most taxonomically diverse (around 3500 species globally⁶⁴), and its well-resolved representation in BOLD indicates the value of targeted sampling to fill taxonomic data gaps, but such approaches require skilled taxonomic experts.

To date, most of the South African records on BOLD (343 166) have been collected in the northeast (Ehlanzeni, Mopani, and Vhembe) – where the Kruger National Park is located (Figure 2). South Africa's most significant contribution in a single submission to BOLD was the 339 193 specimens and about 260 000 sequences generated from a single project, the Kruger Malaise Programme.²² The majority of sequences represented Diptera (130 628), Hymenoptera (57 212), Hemiptera (28 060), Lepidoptera (22 534), and Coleoptera (14 750). Remarkably, the number of contributed BINs for Hymenoptera (6867) exceeded the known species count for southern Africa (5273).⁵⁸ The second and third most notable contributions were the ca. 29 400 and 15 884 specimens collected using Malaise traps in the West Rand district and the City of Johannesburg, respectively (H. Staude in 2012 and R. Stewart in 2017).

It is notable that winter rainfall areas, such as the Cape Floristic region and Succulent Karoo hot spots (Western Cape and Northern Cape), have a rich bee diversity⁵⁹, but Hymenoptera within these two provinces remain poorly sampled (926 and 556 specimens, respectively)

compared to regions that have been sampled with Malaise traps (Figure 3; Table 1). It is likely that many Hymenoptera within these areas have not been described or barcoded.^{59,65} Other areas that remain undersampled include the Free State and North-West Province, which have a total of only 137 and 156 specimens sampled, respectively (Figure 3; Table 1). Recent collecting efforts using Malaise traps have thus provided much of the gains in barcode data for insects over the last few years. Malaise traps offer a passive sampling technique that requires little work from the collection team, but they are efficient at gathering a variety of insects including both flying and ground-dwelling species. Combined with DNA barcoding, this technique can provide a comprehensive overview of the insect community in an area.⁶⁶ Such data allow us to study population changes over time, compare diversity gradients among regions, and assess the impact of human activities on insect populations.

Malaise trapping contributed to at least 92% (384 523 records) of South African records on BOLD, with collection methods for the remaining 8% not specified on BOLD. However, out of the total 416 266 published records on BOLD, only 7.7% (31 855) have species names; the remainder represent 'dark taxa', at least at finer taxonomic levels. Our analysis shows that 59.8% (248 845) of records have been identified to family level, 21.8% (90 645) have been identified to order, and 10.8% (44 866) have been identified to genus (Figure 4). This lack of detailed taxonomic information limits the inference we can draw from such data; for example, species level classifications are required to document and understand drivers of taxonomic turnover and species loss.⁶⁷ Advances in DNA barcoding must, therefore, be accompanied by increased investment in alpha

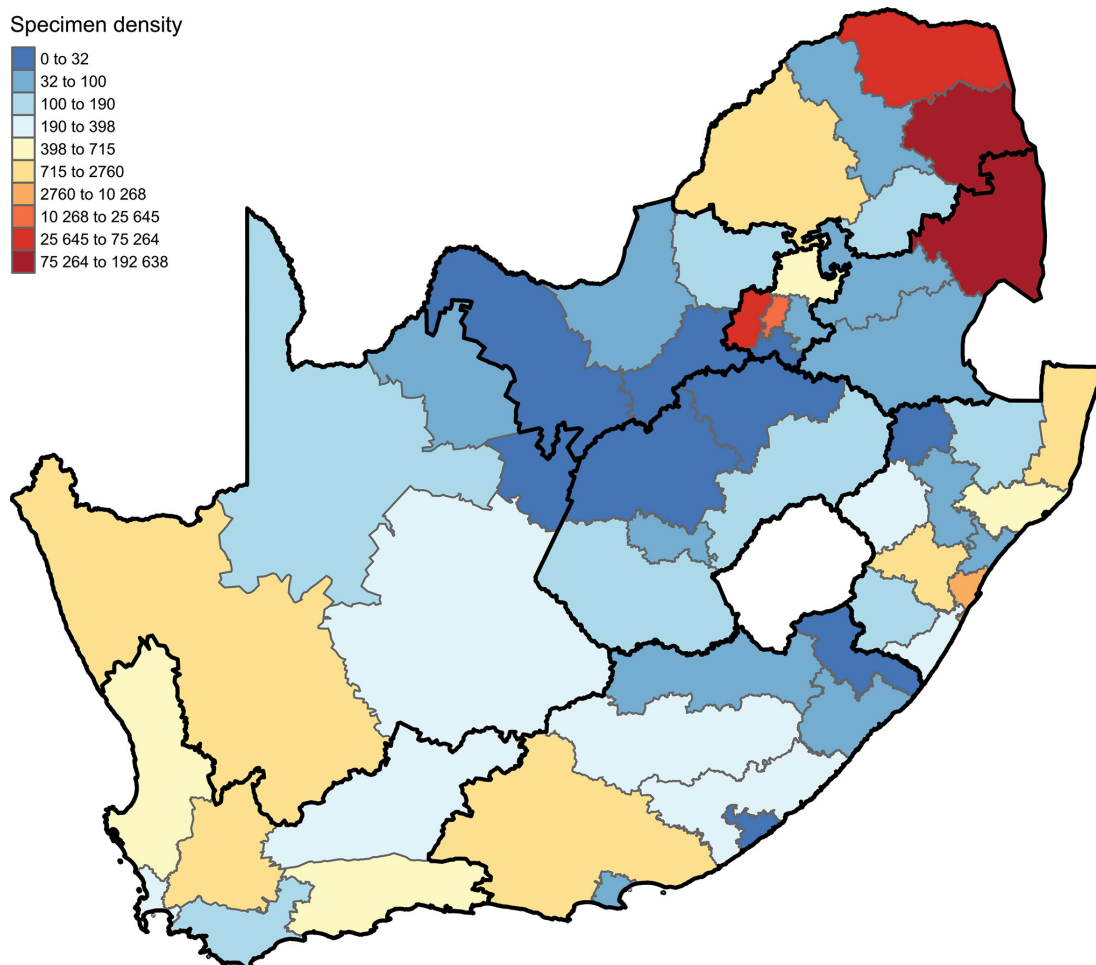


Figure 2: South African district municipalities and the number of South African insect records from the Barcode of Life Data (BOLD) system. The colour of the district municipalities is scaled by the number of BOLD records found within their boundaries. The data were collected on 22 June 2023 from BOLD systems (<http://www.boldsystems.org/>).

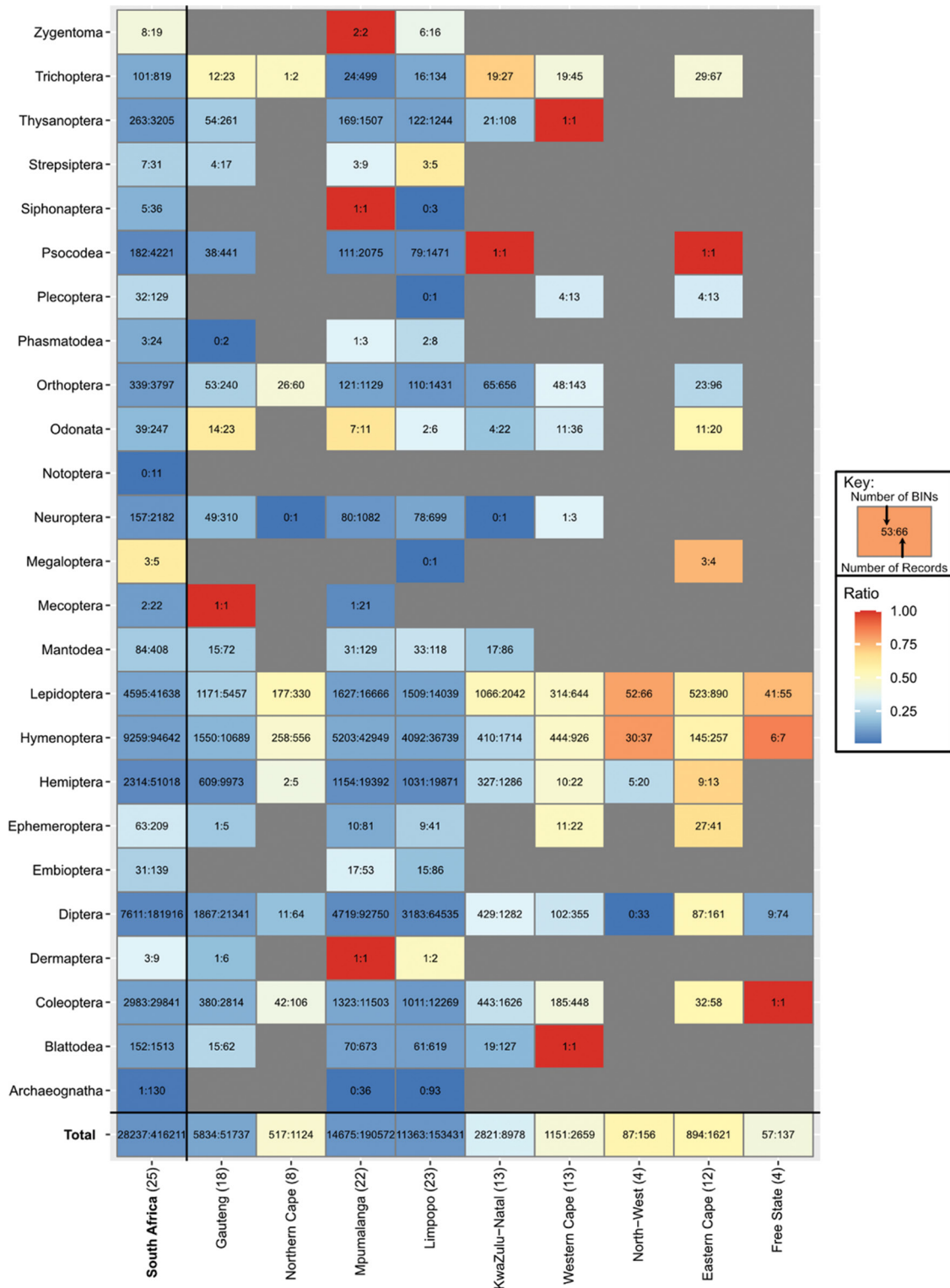


Figure 3: The ratios between the number of Barcode Index Numbers (BINs) and the number of records for a given order and for each South African province. The values within each tile refer to the number of BINs and then the number of records, separated by a colon. The colour of each tile is scaled by the number of BINs divided by the number of records. The data were collected on 22 June 2023 from the Barcode of Life Data (BOLD) systems (<http://www.boldsystems.org/>).

taxonomy and biodiversity science if we are to make sense of the rapidly accumulating barcode data. A first step is to construct robust and comprehensive DNA barcode reference libraries.

Several countries, including Ireland, Germany and Canada, have already invested in large-scale DNA barcode reference libraries for insects.⁶⁸⁻⁷⁰ Such investment has facilitated important research and high-profile

studies that have informed our understanding of global insect biodiversity. For example, a survey using DNA barcoding on wild urban bees in France used a robust reference library of 2931 specimens covering 157 bee species and identified 36 bee species in urban environments.⁴⁴ In a separate study⁷¹, a reference library of mosquitoes in Thailand was used to understand the diversity and distribution of these important disease vectors. Similar efforts are needed for South Africa.

Table 1: Comparison of the number of insect specimens for each order and the South African provinces in which they occur

	South Africa	Gauteng	Northern Cape	Mpumalanga	Limpopo	KwaZulu-Natal	Western Cape	North-West	Eastern Cape	Free State
Orders (n=25)	25	17	8	21	23	13	13	4	12	4
Archaeognatha	130	0	0	36	93	0	0	0	0	0
Blattodea	1513	62	0	673	619	127	1	0	0	0
Coleoptera	29 841	2814	106	11 503	12 269	1626	448	0	58	1
Dermoptera	9	6	0	1	2	0	0	0	0	0
Diptera	181 916	21 341	64	92 750	64 535	1282	355	33	161	74
Embioptera	139	0	0	53	86	0	0	0	0	0
Ephemeroptera	209	5	0	81	41	0	22	0	41	0
Hemiptera	51 018	9973	5	19 392	19 871	1286	22	20	13	0
Hymenoptera	94 642	10 689	556	42 949	36 739	1714	926	37	257	7
Lepidoptera	41 638	5457	330	16 666	14 039	2042	644	66	890	55
Mantodea	408	72	0	129	118	86	0	0	0	0
Mecoptera	22	0	0	0	0	0	0	0	0	0
Megaloptera	5	0	0	0	1	0	0	0	4	0
Neuroptera	2182	310	1	1082	699	1	3	0	0	0
Notoptera	11	0	0	0	0	0	0	0	0	0
Odonata	247	23	0	11	6	22	36	0	20	0
Orthoptera	3797	240	60	1129	1431	656	143	0	96	0
Phasmatodea	24	2	0	3	8	0	0	0	0	0
Plecoptera	129	0	0	0	1	0	13	0	13	0
Psocodea	4221	441	0	2075	1471	1	0	0	1	0
Siphonaptera	36	0	0	1	3	0	0	0	0	0
Strepsiptera	31	17	0	9	5	0	0	0	0	0
Thysanoptera	3205	261	0	1507	1244	108	1	0	0	0
Trichoptera	819	23	2	499	134	27	45	0	67	0
Zygentoma	19	0	0	2	16	0	0	0	0	0
Total	416 211	51 736	1124	190 551	153 431	8978	2659	156	1621	137

Biomonitoring in South Africa using DNA barcoding

Biomonitoring involves using organisms sensitive to pollutants, toxins, or other substances to assess environmental conditions. As anthropogenic impacts on the environment are accelerating, there is an urgent need to index these changes and how biodiversity responds to them. DNA barcoding is a powerful tool for biomonitoring as it can rapidly identify and classify organisms (including from partial or degraded samples) without the need for a taxonomic expert. When used in conjunction with mass-sample processing, such as Malaise trapping, barcoding techniques can additionally characterise the full community of organisms in a sample.⁷² For example, by using barcoding to document the diversity of organisms in an area, it is possible to track changes in the environment and identify potential drivers of biodiversity change, such as pollution, climate change and other environmental stressors. D'Souza²² showed insect diversity and abundance in South Africa correlated with rainfall – likely a critical environmental axis that will be impacted by climate change

with consequences for the country's water resources, food security, ecosystem services and, ultimately, biodiversity.^{73,74}

DNA barcoding has already been used to assess environmentally sensitive insect orders (Ephemeroptera, Plecoptera, and Trichoptera) in aquatic environments.⁷⁵ In South Africa, BOLD records for these groups are relatively limited (1157 records with 196 unique BINs, falling within 19 different families), and only 206 of these records are identified to species level. Most of these specimens are from Mpumalanga, with 667 (58%) contributed from the study by D'Souza et al.²² Generating a matching barcode reference library for South Africa that also includes regionally specific taxa would be a valuable tool for rapid environmental assessments and evaluation of aquatic ecosystem restoration efforts.⁷⁴ A next step would be to develop equivalent reference libraries for other habitats of concern.

Conclusion

This review has shown how DNA barcoding has significantly increased the overall data on insect diversity within South Africa, emphasising

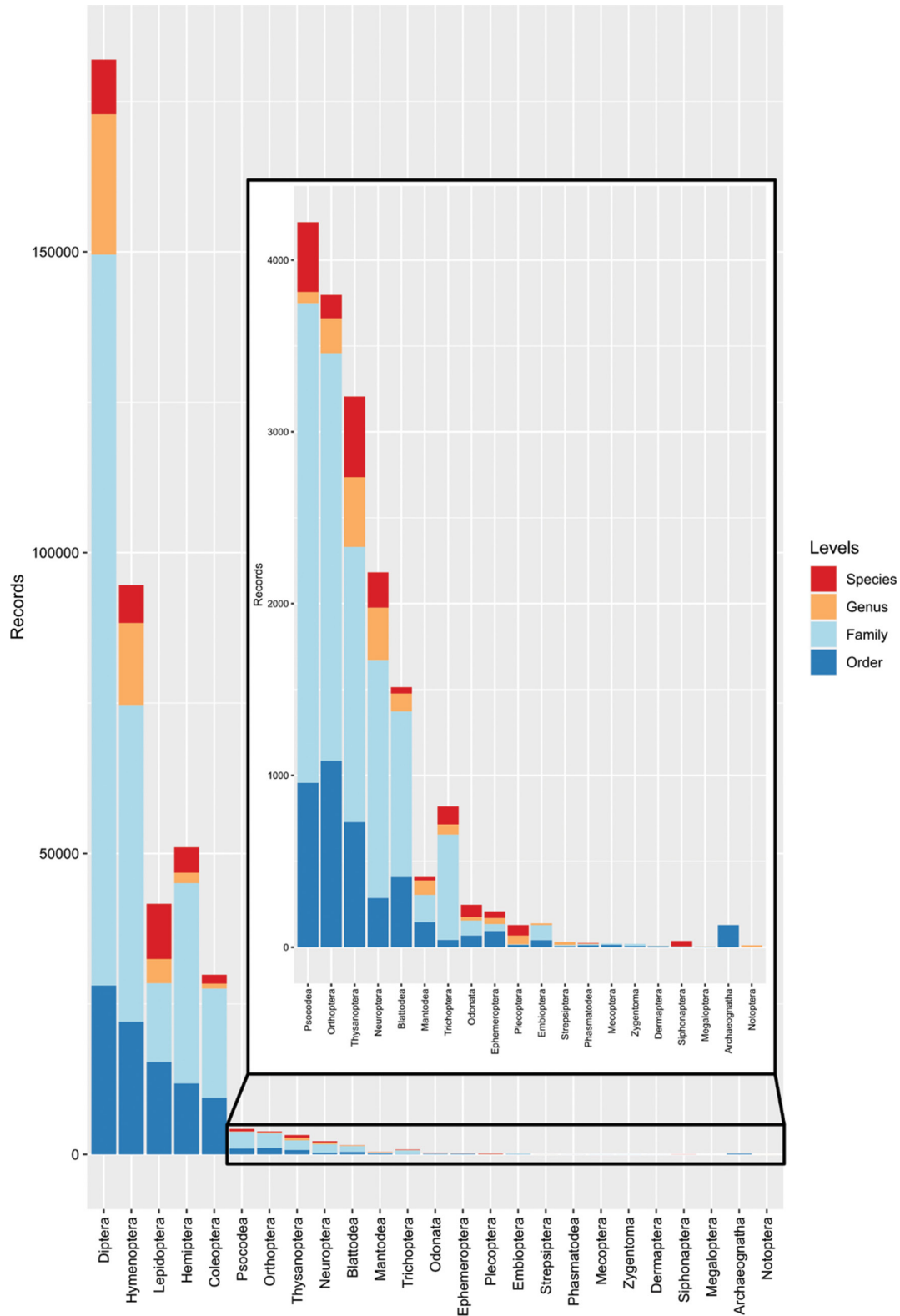


Figure 4: The number of records for each taxonomic level for all 25 South African orders found on the Barcode of Life Data (BOLD) system. The colours within the segmented bars represent the taxonomic level at which the records have been identified. The data were collected on 22 June 2023 from BOLD systems (<http://www.boldsystems.org/>).

underestimates of current taxonomic diversity, and has highlighted barcoding knowledge gaps within provinces and taxonomic orders. The provinces of Gauteng, Mpumalanga and Limpopo have been the best sampled, but some gaps still remain within them, and, in comparison, the rest of South Africa will require a much greater sampling effort. We therefore suggest that there is a need for a comprehensive national

Malaise trapping effort to generate critical baseline data on insect biodiversity. A better knowledge of both the taxonomic and geographic distribution of insect diversity within South Africa would allow for more targeted conservation action to maintain the important ecological functions they provide. Combining DNA barcoding and Malaise trapping would also allow for improved monitoring of agricultural pests



and pollinators, thus supporting food security. However, this effort must be accompanied by an investment in taxonomic expertise and biodiversity science.

Competing interests

We have no competing interests to declare.

Authors' contributions

R.D.S.: Data analysis, writing – the initial draft, writing – revisions.
M.v.d.B.: Conceptualisation, writing – the initial draft, writing – revisions.
T.J.D.: Conceptualisation, writing – the initial draft, writing – revisions.

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