DAVIS EXPEDITION FUND

REPORT ON EXPEDITION / PROJECT

Expedition/Project Title:	A Survey of the Lepidoptera of Eastern Limpopo, South Africa			
Travel Dates: Location: Group Members:	7 October – 9 November 2024 Greater Kruger National Park, South Africa Jamie C. Weir, PhD BSc(Hons) FLS MemRES			
Aims:	 Extend and enhance our understanding of the biodiversity, distribution, and ecology of the Lepidoptera of the bushveld ecosystems of NE South Africa. Systematically sample Lepidoptera in these areas, with particular emphasis on neglected or previously ignored taxonomic groups. Make systematic observations on the ecological, habitat, and hostplant associations of all individuals recorded. Where possible, this includes rearing larvae on foliage, assaying host-plant preferences, making observations on development, and recording parasitoids. Retain specimens, where necessary, for morphological analysis and comparison, genitalia dissection, and COI/DNA barcoding where this does not already exist for a given species. Make adventitious records and natural history observations of other taxa, particularly invertebrates and other groups hitherto relatively under-studied and/or under-recorded. 			
Photography consent form att refer to your award letter)	ached: (<i>please</i> 🛛 Yes 🗌 No			

Outcome (a minimum of 300 words):-

See attached report.



A Survey of the Lepidoptera of Eastern Limpopo, South Africa

October – November 2024



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Cover Image: Gonimbrasia zambesina, © Jamie C. Weir.

Summary

The southern tip of Africa is a global biodiversity hot-spot—some 64% of terrestrial species are endemic. Despite this, comparatively little is known about the distribution, abundance, and ecology of many taxa—this is particularly true for the invertebrates, which play a crucial role in ecosystem functioning. The Lepidoptera (butterflies and moths) are ecologically significant as pollinators, herbivores, and prey, but hitherto only the butterflies have received any significant research attention, and these represent a small fraction of the Lepidoptera currently known from southern Africa (approx. 800 of 11,000 spp.). South African ecosystems face urgent threats from climate change, land use modification, and an expanding human population. In order to effectively inform conservation efforts, increasing our understanding of the region's unique biodiversity is a vital and pressing concern. I carried out fieldwork targeted at recording Lepidoptera in the Greater Kruger National Park, South Africa from 7 October – 9 November 2024, overlapping the onset of the spring rains. In total, I gathered 1,891 records of Lepidoptera (596 macro- and 1,287 micro-moths) and 357 records of other insect taxa from 7 sites, in five private nature reserves around the Hoedspruit area. This represents a substantial increase in our understanding of the region's fauna-there are currently only 40,000 records from the entire Kruger National Park area, of which the majority are butterflies or larger, more conspicuous moths. In addition, I accumulated 7,500 photographic records of other animals and plants, and over 400 hours of passively recorded acoustic data targeting birds and bats. I gathered natural history observations on a wide range of Lepidoptera and other taxonomic groups, encompassing host use in Lepidoptera, host associations of oxpeckers Buphagus, and feeding preferences in the Southern Lesser Bushbaby Galago moholi. My recording work demonstrated a clear impact of the onset of the first spring rains on the abundance of Lepidoptera. Understanding this effect is important if we are to adequately forecast the long-term effects of climatic and seasonal change on insect biodiversity. Finally, I made observations on the potentially unusual prevalence of different strategies of adaptive colouration in the Lepidopteran fauna of the region (bird-dropping mimicry and distractive/disruptive markings), which would benefit from further study.

Introduction

Southern Africa is exceedingly biodiverse. The Republic of South Africa (RSA) is consistently high-ranking in global estimates of biodiversity, and contains three of the 36 world biodiversity hot-spots (SANBI, 2018*a*). Some 67,000 species of animal and 20,400 species of plants have hitherto been described from the RSA (SANBI, 2018*a*). Remarkably, 64% of terrestrial taxa are thought to be endemic to the region—including, 53% of reptiles, 50% of amphibians, 16% of mammals, and 5% of birds (SANBI, 2018*b*). Among the plants, around 13,700 (67%) described taxa occur nowhere else in the world (SANBI, 2018*b*), meaning that about 5% of the world's vascular plant flora occurs uniquely in the southern tip of Africa. This is the second highest rate of plant endemism globally (SANBI, 2018*b*), Kier *et al.*, 2009)—

indeed, it is comparable to rates of endemism expected on oceanic islands (Linder, 2003). The geographical isolation of the Cape region seems to have contributed to its rich biodiversity, with the arid lands and deserts to the north separating it from the rest of the continent (Linder, 2003). As well as its isolation, environmental variation and the diversity of habitat types in southern Africa contribute to its concentration of biodiversity. Climate can vary from sub-tropical to temperate to arid and, within these categories, seasonal cycles are influenced by the timing of rains throughout the year (Staude *et al.*, 2023). The terrestrial environment of the RSA has been divided into nine biomes and over 450 distinct ecosystem types, with three quarters of the latter being unique to the country (SANBI, 2018*b*). There are 88 distinct described savannah vegetation types alone in RSA (SANBI, 2021). This complex and rich patchwork of environmental conditions has provided a wealth of niches for diversification and speciation.

Despite their global importance for biodiversity, many South African ecosystems are under threat and 13% of terrestrial taxa have been classed as threatened (SANBI, 2018b). Habitat loss, climate change, invasive species, land degradation, and natural resource exploitation have all been identified as key pressures facing these terrestrial ecosystems (SANBI, 2018a; 2018b). With a steadily increasing population, and sprawling development, increasing effort devoted to ecosystem mapping and evaluating ecosystem health has been identified as a priority. Crucially, understanding species distributions at a fine scale is key to informing the expansion of protected areas (SANBI, 2018b). Although the larger and more charismatic species of southern Africa have been rather well studied, more obscure groups such as the invertebrates are far less well understood, despite the pivotal part they play in ecosystem functioning. New species, across a range of taxa, continue to be described from the region (e.g. Smit et al., 2008; Theron et al., 2012; Moteetee et al., 2014). Even large and conspicuous invertebrates (e.g. butterflies) are poorly understood relative to birds and mammals (e.g. for species classified as threatened; see SANBI, 2018b). In order to adequately conserve the biodiversity of this region, therefore, taxonomic sampling and research focussed on invertebrate groups is a vital and pressing concern.

The Lepidoptera (butterflies and moths) are one of the most species-rich insect orders in southern Africa, with 11,000 known species—exceeding, for example, the 8,300 species found in the whole of Europe (Karsholt and Razowski, 1996). Lepidoptera are important components of food-webs globally, exerting top-down pressure on plants via herbivory and providing an abundant but often ephemeral food source for insectivorous predators, such as birds. However, the butterflies (~800 species) are often the only group of terrestrial invertebrates mentioned in reports such as the National Biodiversity Assessment (Edge and Mecenero, 2015; SANBI, 2018*a*; 2018*b*; Staude *et al.*, 2023). This is largely due to a lack of data on other groups of Lepidoptera (Staude *et al.*, 2023), and insects generally. Of this narrow subset of Lepidoptera, we find that some 52% of butterfly species recorded in the RSA are endemic— one of the highest rates of endemism in any taxonomic group (SANBI, 2018*b*). Agriculture, habitat loss, and land use modification have been identified as the main threats to butterfly populations in southern Africa, and 36% of threatened butterfly taxa have only been recorded *outside* of areas with formal environmental protection (SANBI, 2018*b*).

There are three conspicuous knowledge-gaps in our understanding of the Lepidoptera of southern Africa. First, new species continue to be regularly discovered, even in the butterflies and the larger moth families (for example: Kruger, 2005; Kovtunovich and Ustjuzhanin, 2009; Ustjuzhanin et al., 2018; Laszlo et al., 2021). In tropical and sub-tropical climates, insect diversity is closely linked to plant diversity and Lepidopteran host-plant specialisation increases towards the equator (Forister et al., 2014). There is almost certainly much Lepidopteran diversity that remains undescribed in southern Africa, particularly among the smaller, more inconspicuous moths—and many may be unique, if the moths and butterflies have comparable rates of endemism (see above). Indeed, many globally species-rich families are relatively species-poor in southern Africa, which could be due to differences in recording effort and perhaps suggests substantial undiscovered diversity. Second, the distribution of most species of Lepidoptera in the RSA is very poorly understood (Staude *et al.*, 2023). Existing records of Lepidoptera tend to be adventitious, and are confined to butterflies or large and distinctive moths. Many other records in existing databases are based on older museum specimens, and are not necessarily reflective of modern distributions (e.g. see GBIF, 2024). Compared to the United Kingdom, a country one fifth of its size, there are many fewer records of a much larger fauna (Table 1). Third, for the majority of described African Lepidoptera even very basic details of their life history, ecology, and development are unknown (Staude et al., 2023)—despite recent efforts to prioritise the collection of such information (for example, the Lepidopterists' Society of Africa Caterpillar Rearing Group). If the rich biodiversity in southern Africa is to be effectively conserved, these substantial gaps in our basic biological and ecological understanding of the region's Lepidopteran fauna must be remedied.

Objectives

- Extend and enhance our understanding of the biodiversity, distribution, and ecology of the Lepidoptera of the bushveld ecosystems of NE South Africa.
- Systematically sample Lepidoptera in these areas, with particular emphasis on neglected or previously ignored taxonomic groups.
- Make systematic observations on the ecological, habitat, and host-plant associations of all individuals recorded. Where possible, this includes rearing larvae on foliage, assaying host-plant preferences, making observations on development, and recording parasitoids.
- Retain specimens, where necessary, for morphological analysis and comparison, genitalia dissection, and COI/DNA barcoding where this does not already exist for a given species.
- Make adventitious records and natural history observations of other taxa, particularly invertebrates and other groups hitherto relatively under-studied and/or under-recorded.

For more details, see Weir (2024).

Table 1. Comparison of faunal size and recording effort of the Lepidoptera of South Africa and the United Kingdom. All UK records are taken from GBIF. South African records are a combination of GBIF (292,783) and LepiMAP (230,380) records.

Country	Area (km²)	No. Species Occurring (approx.)	No. Records
South Africa	1,221,037	11,000	523,163
United Kingdom	243,610	2,500	45,911,460

Methods

Itinerary—Fieldwork took place from 7 October 2024 to 9 November 2024, deliberately aiming to overlap the end of the winter dry period and the onset of the spring rains. Rainfall is known to be an important stimulus for insect emergence after the long winter drought. In finalising the localities to be visited, I aimed to strike a balance between covering as much ground as possible (in terms of sites and habitats) and gathering sufficient material from each locality to form a meaningful and representative sample. Biodiversity sampling techniques typically yield a characteristic 'species accumulation curve', such that continuous recording at a single site using the same method is likely to lead to an initial steep rate of new species records followed by steadily diminishing returns. However, it is also important to have built-in flexibility in case of poor weather conditions which make trapping impossible or unproductive. I prioritised increasing our knowledge of Lepidopteran biodiversity and distribution, and therefore sought to maximise the number of species recorded overall. I targeted a series of sites in private game reserves around Hoedspruit (Limpopo Province), in the Greater Kruger National Park area. I trapped at seven sites in total across five private game reserves, each from a minimum of three nights to a maximum of six nights (Fig. 1).

Collecting and Recording Methods—Lepidoptera were collected and recorded at all sites through a combination of systematic light trapping (using MV and actinic light in traps or suspended in front of a sheet to allow inspection of incoming insects; see Figs. 2-3) and manual observation/sampling during the day (Fig. 4). Meta-data associated with each specimen was recorded (e.g. locality, GPS co-ordinates, habitat associations, general natural history observations). Specimens were retained where necessary—larger Lepidoptera were stored dried in envelopes and smaller species were micro-pinned in the field and stored in foam-lined cassette boxes (Fig. 5). All specimen collection was conducted in compliance with the Code of Conduct for Collecting Insects and other Invertebrates (Second edition, 1987) produced by the Joint Committee for the Conservation of British Invertebrates. However, outside of known protected species (e.g. some Sphingidae and Saturniidae), I tried to collect as many series of distinct morphotypes as possible for future examination, the chance of new species being very high—particularly in the 'micro-moth' families. Environmental conditions such as temperature and humidity can significantly impact insect abundance and activity. I used a continuous monitor ('SwitchBot IP65') to record temperature and humidity data, kept



c)	Site	Reserve	Dates
	1. Parsons Hilltop	Parsons (Balule)	7 – 11 October
	2. 'Africa on Foot' Camp	Klaserie	12 – 14
	 Mbizi Lodge 	Grietjie (Balule)	15 – 20
	 Bundox Lodge 	Guernsey	21-26
	 Baluleni Safari Lodge 	Grietjie (Balule)	27 – 30
	 Emhosheni Lodge 	Parsons (Balule)	31 October – 4 November
	7. Pezulu Camp	Guernsey	5 – 7

Figure 1. (*a*) Geographical situation of the reserves surveyed, (*b*) location of field sites surveyed within each reserve, (*c*) and the duration spent based at each locality. Larger private nature reserves (e.g. Balule) contain serval contiguous smaller concessions (e.g. Parsons, Grietjie).



Figure 2. 'EntoNets' Ranger (a, b, d) and Safari (c) moth traps with actinic lights, set up at various sites. Note the dry state of the background vegetation. © Jamie C. Weir.



Figure 3. Sheet trapping, using a light suspended in front of a sheet/white surface to attract moths which can then be inspected and recorded. Shown are (a, c, d) actinic and (b) MBT bulbs. © Jamie C. Weir.



Figure 4. Butterflies observed in Greater Kruger. (*a*) Pieridae sp. in Guernsey Reserve, (*b*) Papilionidae sp. in Balule Reserve, and (*c*) Nymphalidae sp. in Balule Reserve. © Jamie C. Weir.



Visible on the table are cassette boxes for micro moth pinning. (*b*) Noctuid moth specimen, showing distractive wing colour patterns. (*c*) Longhorn beetle Cerambycidae specimens taken at actinic lights. (*d*) Smaller moths prepared and micro-pinned in foam-lined cassette boxes. © Jamie C. Weir.

kept in the shade and at a standard height of between 1.25 and 2m from ground level.

General Natural History—In addition to my main goal of recording Lepidoptera, I made adventitious records of other animal and plant groups, retaining specimens or documenting occurrences and behaviours through photography where appropriate. At each site, I set up acoustic loggers (Open Acoustic Devices, 'AudioMoth') in weatherproof housings (IPX7 Waterproof Case) to passively sample bird (low/med frequency, diurnal and crepuscular) and bat biodiversity (high frequency, nocturnal and crepuscular) through sound recording of calls and chirps.

Preliminary Project Outcomes

1. Records of Lepidoptera

In total, over the course of this survey, I gathered a total of 1,891 specimens and records of butterflies and moths. I made 596 records of macro-moths (the larger Lepidopteran families, such as Noctuidae, Geometridae, Saturniidae, Sphingidae) and 1287 of micro-moths (the remaining families of smaller-sized Lepidoptera). Comparatively few butterflies were observed in the field (several dozen) and most were too active to catch or photograph in order to make a positive identification. I collected eight specimens of various smaller, more difficult to identify butterfly species, spread across several sites. Specimens collected are currently being gradually sorted into family groups, as the first stage to identification. Of the more readily identified families, I collected 15 specimens of the Saturniidae and 25 Sphingidae. Overall, the observations made in this project represent a significant increase in the number of records of Lepidoptera within these reserves (Table 2), and with emphasis on taxa that have largely been ignored previously. In addition to collecting records and specimens, I gathered extensive notes on behaviour, ecology, and habitat associations for a range of the Lepidopteran and other insects encountered, as well as general natural history observations.

Table 2. Total Lepidoptera records contributed per reserve surveyed in this project, including estimates of previous records on Global Biodiversity Information Facility (GBIF.org, Dec 2024).

Site	Macro #	Micro #	Total #	Prior Records (approx. to 2025)
Balule Private Reserve	366	902	1268	320
Klaserie Private Reserve	36	64	100	300
Guernsey Reserve	194	321	515	150

2. Seasonal change and its impact on Lepidopteran abundance

The timeframe of this project was intended to overlap the end of the winter dry season and the onset of the spring rains, which are known to stimulate the mass emergence of insects. In so doing, I also aimed to record both winter- and spring-season specialist species and quantify the impact of the seasonal change on Lepidopteran abundance. There were no marked or consistent directional trends in temperature or humidity across the duration of the project (Fig. 6). However, there were several heatwaves during which daytime temperatures peaked in excess of 35°C or 40°C degrees for a few days. My recording fieldwork successfully overlapped the first rainfall for months, which began with light showers on the 14th and 15th of October and continued intermittently and with heavier rainfall on 21st, 22nd, 27th October, and the 5th and 6th November. I used a variety of methods to sample Lepidoptera using light, including the use of light traps left in place overnight or bulbs suspended in front of a white sheet or other surface. In order to quantitatively compare the catch made on different occasions, I estimated the number of specimens recorded per hour trapped. Over the course of the project duration, I observed a rapid increase in the rate of moths trapped. This impression may be due to a linear increase occurring after the first rains (Fig. 7). Rainfall appears to have a more substantial and immediate impact on the abundance of small moth species recorded, compared with taxonomic families containing on average larger individuals. There is also a clear linear increase in micro-moth diversity across the study period, quantified as the number of distinct morphotypes measured in a sample, per hour trapped. Given the morphological similarity of many species of smaller Lepidoptera, these estimates based on superficial appearance are likely a significant underestimate of the diversity represented in the samples.

3. Acoustic data

I collected 184 hours of high frequency acoustic data (nocturnal) and 238 hours of low frequency data (all-day or strictly diurnal), with samples from each target site. Although birds (particularly more charismatic/conspicuous species) are typically well-recorded in the Greater Kruger reserves, the bat recordings represent potentially valuable data of obscure and difficult to sample taxa.

4. Records and natural history of other taxa

Where possible, and in addition to my focus on Lepidoptera, I gathered records, specimens, and documented the natural history of a wide range of other animal and plant taxa. I recorded and collected approximately 357 specimens of invertebrate species other than Lepidoptera. Most of these remain to be identified but represent obscure or under-recorded families of Hemiptera, Coleoptera, and Diptera and therefore are very likely to be important first records for the reserve, province, or nationally. As well as invertebrates, I gathered over 7500 photographic records of other animal and plant taxa, documenting their distribution and natural history. Specific small scale observational and experimental dataset generated include: (i) Over 100 observational records documenting host associations and preferences in



Figure 6. Continuous temperature (°C) and relative humidity (%) records taken over the course of the project. Moves between sites are not indicated.



Figure 7. Effects of winter/spring seasonal change on moth abundance in Southern African bushveld. Rate of moths trapped estimated per hour. Moth trap rate increases clearly after the first rains (14th October 2024). Included are all sites visited and moths collected/recorded through trapping, sheet collection, and several types of bulb.

oxpeckers *Buphagus*, and how this varied among reserves with differences in their game animal populations. Surprisingly few efforts have been made to document host preferences in these birds (e.g. Koenig, 1997). These data are novel for this part of Africa and could make an important contribution to our understanding of geographical variation in host associations in this genus. (ii) Detailed behavioural notes on the Southern Lesser Bushbaby *Galago moholi* made while observing an individual hunt insects drawn to my light trapping equipment. Insects are known to be a highly important and seasonally transient food source for this species, but we have little understanding of the kinds of insects taken as prey (size, species, order). I was able to assess dietary preferences at the level of individual insect taxa and test the relative importance of prey appearance, movement, and sound as stimuli for prey attractiveness in *G. moholi*.

Work Remaining

I anticipate that all the remaining lab- and analysis-based work outlined in the expedition proposal document (Weir, 2024), along with the preparation and submission of a paper based on these results, will be completed by year-end 2025 to mid-2026. See Table 3 for an approximate timeline. Tasks that remain to be completed are:

- (a) Specimen and Photograph Identification—Specimens are currently being sorted to family level, as a precursor to specific identification. Of the 1,882 voucher specimens of Lepidoptera collected on this expedition, 1,567 remain to be identified to family level (281 macro-moths and all 1,287 micro-moths). The macro-moths remaining will be grouped across a period of several weeks at family level, then identified to species level. The micro-moths are more challenging to identify and often require dissection/genitalia examination—as such, identification will likely be more protracted. However, because of their inconspicuousness and small size, these species are likely to contain the highest proportion of new-to-site records at the reserve, province and perhaps country level. Indeed, there is also a high chance of species new to science, which continue to be discovered and named in southern Africa (e.g. example: Kruger, 2005; Kovtunovich and Ustjuzhanin, 2009; Ustjuzhanin *et al.*, 2018; Laszlo *et al.*, 2021). Lepidoptera are being prioritised over the identification of other insect material collected. In addition to voucher specimens, the 7,762 photographs of vertebrate animals and plants collected need to be sorted, identified, and databased alongside associated GPS tag information.
- (b) Acoustic Data Analysis—Analysis of the recordings made on this trip can be automated using specialist software. 'Kaleidoscope Pro' (Wildlife Acoustics) contains sound libraries of birds and bats for southern Africa, and will be used to generate species lists for each site and recording session. There are over 130 species of bat in southern Africa, and mapping their distribution is more challenging than that of birds because of their nocturnal habits. I will therefore prioritise extracting the bat data from my recordings in the coming months. If automated software is not sufficiently comprehensive or fails to match available species I will seek to manually isolate and identify calls (e.g. using 'Audacity'), bringing on board appropriate collaborators.

- (c) *Publication*—The main publication outcome from this project will be a paper focused on the advances made in our understanding of the distribution of Lepidoptera across the region. As well as reporting and describing the records made, this paper will document and synthesise field observations made, such as novel habitat and host-plant associations where appropriate. This will incorporate data and analyses quantifying abundance and diversity changes at the onset of spring, particularly the impact of the first rains. Additional analyses based on geographic variation in certain taxa, or specific isolated examples of host associations or behaviours, will be published separately as papers or short communications (Appendix 1). I also anticipate that data on the observed host associations of oxpeckers *Buphagus* and dietary preferences in the Southern Lesser Bushbaby *G. moholi* will be published as separate short communications.
- (d) Deposition of Data—A comprehensive database of records made during the expedition will be made publicly available. I will generate 'reserve lists' and share these directly with local lodge and reserve management teams and, where appropriate, make site specific recommendations where rare species occur, or where particular habitats appear to be of special importance for invertebrate biodiversity. The records will also be formatted and uploaded to GBIF, making them widely accessible to researchers. All datasets and publications will be shared with the Limpopo Department of Economic Development, Tourism and Environment.

	Task		J	F	м	A	м	J	J	Α	S	0	N	D
(a)	Identification of remaining specimens	'Macro-moths' 'Micro-moths'												
(b) (mammals, birds, etc.)														
(c)	(c) Statistical analysis of seasonal change in Lepidopteran abudadance													
(d) Analysis of acoustic data														
(e)	Publications	Main Lep. Paper – Prep.												
		Main Lep. Paper – Submission												
		Diet in <i>G. moholi</i>												
		Host preference in oxpeckers Buphagus												
(f)	(f) Deposition of species records													

Table 3. Schedule for completion of remaining work.

Expenditure

Total project expenditure was £10,644.01, with £8500 contributed from the Davis Expedition Fund and personal contributions of £2,144.01. *COI barcoding work has not yet been conducted, and the £730 contribution from the Davis Fund will be used to finance this once specimen sorting and identification has been completed.

Item		Cost (£)
Accommodation		6250
Subsistence (additional to full board rate)		150 (estimate)
Flights (Edinburgh to Hoedspruit, return)		1382.32
Literature		155.87
Site transfers		686.44
Collecting/Recording Equipment and Materials		336.87
Field clothing		181.70
LEDET Permit (application processing fee)		3.14
LepSoc Africa membership		17.67
Travel insurance		121.55
COI barcoding *		730
Post-trip stipend		750
	Total	£10,644.01

Future Research

The main aim of this project was to contribute as much to our knowledge of the Lepidoptera of the Greater Kruger area of eastern Limpopo as possible in a relatively short time frame. Even at this early stage of analysis and write up, it is clear that the project succeeded in producing considerable scientific return for a comparatively small financial investment. As an example, the rapid survey approach adopted here succeeded in increasing the number of records in the Balule Nature Reserve from ~320 to ~1600 (5x). When fully identified and collated, about 5% of all records of Lepidoptera (approx. 40,000 records listed on GBIF, Jan 2025) in the whole Greater Kruger National Park—and area the size of the Netherlands—will have been contributed as a direct result of this project.

There are several notable areas where future research would likely be very productive:

First, although the rapid assessment approach was effective, the short time-frame of this project necessarily placed limits on the extent of local biodiversity that could be sampled. In order to represent the abundance and richness of species in these habitats, surveys would need to be conducted across the seasons, and at minimum in mid-spring and mid-summer, when we would expect quite distinct suites of species. While I observed a short-term turnover in species as the rains arrived, these were not as substantial as I had anticipated and a longer-term assessment might be required to quantify this change. A complete picture of the

biodiversity of these habitats and their seasonal changes is important in order to inform conservation efforts, and is a next step building on this project. In addition, the careful study of insect groups that are less well known than the butterflies and moths would almost certainly yield new species and a range of hitherto unrecorded taxa. As is so often the case with remoter locations which lack in-depth biological recording, a matter of pressing concern is engaging researchers with niche taxonomic expertise to conduct surveys such as this one. South Africa's private game reserves present a unique confluence of business, tourism, and conservation interests and a deeper understanding of the invertebrate biodiversity they host could provide compelling evidence for their continued preservation and expansion.

Second, relative to the Lepidopteran fauna of Europe, I observed a high incidence of both apparently 'disruptive' or 'distractive' wing colour patterns and bird-dropping mimicry in the specimens and species recorded. These may be particularly effective defensive strategies in these habitats, or particularly in the winter dry season when most vegetation is defoliated or has died back. Bird-dropping mimicry, for example, may offer a one of a small number of cryptic patterns other than simple background colour matching of the savannah environment. Formal phylogenetic comparative analyses aimed at understanding variation in the incidence of these colour strategies relative to other geographical locations would be informative, and an important first step to understanding their function. The idea that conspicuous marks on an otherwise cryptic background can serve to break up the outline of an animal and therefore act as camouflage is frequently evoked, but has proven persistently hard to verify experimentally (e.g. Olofsson et al., 2013; Stevens et al., 2013; Mizuno et al., 2024). Kettlewell (1973) suggested that discontinuous marks on cryptic animals become more sharply defined towards the equator, where the angle of incidence of the sun creates, on average, more sharply defined shadows. Hitherto, experimental work on disruptive colouration has been based in temperate climate and, if Kettlewell's speculation is correct, the fauna of southern Africa may be a more suitable model in which to field test the concept.

Third, among the Lepidoptera, much remains unknown about the host-plant associations and life history of many of the smaller species. Understanding the ecological requirements of taxa is a vital step in effective and sustainable conservation. Although I was able to make extensive behavioural and ecological observations on a number of Lepidoptera, there was very little foliage remaining at the end of the winter dry season, and an unusually long drought period. Caterpillars are most abundant on foliage—particularly newly growing foliage—and so observations of host-plant associations were limited (though see Appendix 1 for observations yielded through ongoing collaborations). In order to gather more comprehensive data on these particular ecological interactions, surveys would need to be conducted in the wet season, likely between December and April. Many species have unknown host-plants, and a project dedicated to making these observations and records could make a considerable contribution to our knowledge. In addition, the mobile nature of this trip, moving frequently between locations, made captive rearing or breeding of larvae from adults logistically challenging. A trip mounted later in the wet season with a more fixed base of study would enable the careful, systematic experiments required on host preference and performance.

Collaborative Links

In designing and planning this project, I built collaborative relationships with staff at the Iziko South African Museum, local South African universities (e.g. University of Limpopo), and the conservation bodies of local and provincial governments (e.g. Limpopo Economic Development, Environment and Tourism). I established working relationships with management staff of private game reserves in the Greater Kruger, management of private lodges, and with local guides, rangers, and wildlife experts. Progressing through the postdoctoral stage of my career with the aim of developing my own research programme, this project provided valuable experience in building relationships with local conservation stakeholders, who have a diverse range of financial, scientific, and cultural interests. These skills are valuable future tools when organising and managing fieldwork or expeditions abroad. The links I established with local interest groups could also form the basis of future work (e.g. see above) on the Lepidoptera of the region and its conservation. Indeed, I continue to maintain these relationships, providing updates to relevant parties as I continue to work on the data gathered in this project. One such link, with a local wildlife guide, has led to the discovery of a new host-plant of the moth Lophonotidia melanoleuca (Lep.: Agaristinae)(See Appendix 1)—demonstrating how the relationships built during this project are continuing to yield important scientific data.

Acknowledgements

Many people contributed to making this project a success—too many to name. I am incredibly grateful to the Davis Expedition Fund for supporting this project, which gave me extremely valuable personal and research experience at this crucial stage of my career development. I would like to thank the Limpopo Economic Development, Environment and Tourism (LEDET) for granting me permission to carry out fieldwork. Specifically, I would like to thank Alder Chimanzi (LEDET), Vincet Egan (LEDET), and Abe Addo-Bediako (University of Limpopo) for their help in coordinating the appropriate fieldwork permits. Jeremy Dobson (LepSoc Africa) provided valuable advice on fieldwork and necessary paperwork in the planning stages of this project. Simon van Noort (Iziko South African Museum) offered support and advice at several key moments in the planning and coordination of this project. Daniella Di Pirro provided valuable assistance in the field and when sorting and processing insect specimens. Ally Phillimore kindly supported this grant application, and Matt Bell provided useful advice on fieldwork in southern Africa. Finally, but perhaps most importantly, I am deeply grateful to all the management, staff, guides, trackers, and local wildlife experts associated with the private reserves I visited during this project—for their invaluable cooperation, interest, enthusiastic support, and constant willingness to help.

References

Edge, D. A., and Mecenero, S. (2015). Butterfly conservation in Southern Africa. *Journal of Insect Conservation* **19**: 325-339.

Forister, M. L., Novotny, V., Panorska, A. K., Baje, L., Basset, Y., Butterill, P. T., Cizek, L., Coley, P. D., Dem, F., Diniz, I. R., Drozd, P., *et al.* (2014). The global distribution of diet breadth in insect herbivores. *Proceedings of the National Academy of Science* **112**: 442-447.

GBIF (2024). GBIF Secretariat: GBIF Backbone Taxonomy. <u>https://doi.org/10.15468/39omei</u> Accessed via https://www.gbif.org/species/797 [12 January 2024]

Karsholt, O., and Razowski, J. (1996). *The Lepidoptera of Europe: A Distributional Checklist*. Apollo Books.

Kettlewell, B. (1973). *The Evolution of Melanism.* Clarendon, Oxford University Press. Kier, G., Kreft, H., Lee, T. M., Ibisch, P. L., Nowicki, C., Mutke, J., and Barthlott, W. (2009). A global assessment of endemism and species richness across island and mainland regions. *Proceedings of the National Academy of Science* **106**: 9322-9327.

Koenig, W. D. (1997). Host preferences and behaviour of oxpeckers: co-existence of similar species in a fragmented landscape. *Evolutionary Ecology* **11**: 91-104.

Kovtunovich, V. N., and Ustjuzhanin, P. Y. (2009). New species and records of plume moths of the genus *Agdistis* Hubner, 1825 (Lepidoptera: Pterophoridae, Agdistinae) from southern Africa. *Amurian Zoological Journal* **1**: 37-44.

Kruger, M. (2005). New species of geometrid moths from Lesotho (Lepidoptera: Geometroidea). *Annals of the Transvaal Museum* **42**.

Laszlo, G. M., Schintlmeister, A., Vetina, A. A. (2021). A contribution to the knowledge of the prominent moths (Lepidoptera, Noctuoidea, Notodontidae) of the Maputo Special Reserve with descriptions of four new species. *Zootaxa* **4965**: 201-243.

Linder, H. P. (2003). The radiation of the Cape flora, southern Africa. *Biological Reviews of the Cambridge Philosophical Society* **78**: 597-638.

Moteetee, A. N., Boatwright, J. S., and Jaca, T. P. (2014). A review of Rhynchosia Section Polytropia (Phaseoleae, Fabaceae) and a new species from the Western Cape Province, South Africa. *Systematic Botany* **39**: 1127-1131.

Mizuno, A., Lagisz, M., Pollo, P., Yang, Y., Soma, M., and Nakagawa, S. (2024). A systematic review and meta-analysis of eyespot anti-predator mechanisms. *eLife* doi: 10.7554/eLife.96338.

Olofsso, M., Dimitrova, M., and Wiklund, C. (2013). The white 'comma' as a distractive mark on the wings of comma butterflies. *Animal Behaviour* **86**: 1325-1331.

SANBI. (2018a). National Biodiversity Assessment 2018: The status of South Africa's Ecosystems and Biodiversity. Facts, Findings & Messages. South African National Biodiversity Institute.

SANBI. (2018b). National Biodiversity Assessment 2018: The status of South Africa's Ecosystems and Biodiversity. Synthesis Report. South African National Biodiversity Institute.

SANBI. (2021). *South African National Ecosystem Classification Handbook.* First Edition. South African National Biodiversity Institute.

Smit, H. A., Robinson, T. J., Watson, J., van Vuuren, B. J. (2008). A New Species of Elephant-Shrew (Afrotheria: Macroscelidea: *Elephantulus*) from South Africa. *Journal of Mammalogy* **89**: 1257-1269.

Staude, H., Picker, M., and Griffiths, C. (2023). *Southern African Moths & their Caterpillars*. First Edition. Struik Nature.

Stevens, M., Marshall, K. L. A., Troscianko, J., Finlay, S., Burnand, D., Chadwick, S. L. (2013). Revealed by conspicuousness: distractive markings reduce camouflage. *Behavioural Ecology* **24**: 213-222.

Theron, N., Roets, F., Dreyer, L. L., Esler, K. J., Ueckermann, E. A. (2012). A new genus and eight new species of *Tydeoidea* (Acari: Trombidiformes) from *Protea* species in South Africa. *International Journal of Acarology* **38**, <u>https://doi.org/10.1080/01647954.2011.619576</u>.

Ustjuzhanin, P., Kovtunovich, V., Sáfián, S., Maicher, V., and Tropek, R. (2018). A newly discovered biodiversity hotspot of many-plumed moths in the Mount Cameroon area: first report on species diversity, with description of nine new species (Lepidoptera, Alucitidae). *Zookeys* **777**: 119-139.

Weir, J. C. (2024). *Davis Expedition Fund Project Proposal: A Survey of the Lepidoptera of Eastern Limpopo, South Africa*. University of Edinburgh.

FIGURES



Figure 8. Examples of macro-moths collected at light. © Jamie C. Weir.



Figure 9. Examples of macro-moths collected at light. $\ensuremath{\mathbb{C}}$ Jamie C. Weir.



Figure 10. Examples of macro-moths collected at light. © Jamie C. Weir.



Figure 11. Mammals recorded in Greater Kruger. (*a*) Lion Panthera leo, (*b*) Vervet monkey Chlorocebus aethiops, (*c*) Painted Wild Dogs Lycaon pictus, (*d*) Spotted Hyaena Crocuta crocuta, (*e*) Southern African Ground Squirrel Xerus inauris, (*f*) Black-backed Jackal Canis mesomelas. © Jamie C. Weir.



Figure 12. White-backed Vultures *Gyps africanus* feeding on the body of a juvenile elephant. © Jamie C. Weir.



Figure 13. Red-headed Weaver *Anaplectes rubriceps* preparing and collecting twigs for nest-building. Frames taken via burst shooting and arranged left to right, top to bottom. The individual carried this twig off, tried to weave, but in the end rejected it as construction material. © Jamie C. Weir.



Figure 14. Elephant tracks along a road at sunset in Klaserie Private Nature Reserve. © Jamie C. Weir.



Figure 15. View over the western end of Balule Private Nature Reserve, early October 2024. Trees are almost entirely defoliated after the winter drought. A giraffe is visible feeding in the centre of the frame. © Jamie C. Weir.



Figure 16. View south over the Olifants River from the Balule Private Nature Reserve. © Jamie C. Weir.

APPENDIX 1

SUBMITTED: Metamorphosis (Official Journal of the Lepidopterists' Society of Africa)

Red bush willow *Combretum apiculatum*, a new larval host-plant of *Lophonotidia melanoleuca* (Lep.: Agaristinae)

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From 28-31 Nov 2024, LDW observed around 30 caterpillars of an unknown species feeding on the foliage of a single Red bush willow tree *Combretum apiculatum* in a domestic garden in Hoedspruit (Mopani District, Limpopo, RSA). Photographs of the distinctive larvae were sent to JW for identification, and confirmed as *Lophonotidia melanoleuca* (Figure 1). No morphologically similar species or other members of the genus are found in southern Africa. Adults are active October-February, and caterpillars seem to occur from November (iNaturalist, 2024*a*). Although records for this species are concentrated in the north-eastern provinces of South Africa, scattered observations are known from across Zimbabwe (iNaturalist, 2024*a*).

Among the Agaristinae found in the region, the caterpillars of most species feed on grape foliage, particularly *Cissus* and *Rhoicissus* spp. (see Table 1). For *L. melanoleuca*, Staude *et al.* (2023) give only one known host-plant, Ivy-grape *Cissus cornifolia*. This observation therefore seems to represent the first record of this species feeding on an alternative host-plant taxon, and make it one of the few South African Agaristinae which can feed outside the Vitaceae. Prompted by this sighting, JW re-examined the available photographs of *L. melanoleuca* caterpillars on iNaturalist. In several photos, individuals also appear to be feeding on Bushveld grape *Rhoicissus revoilii* (iNaturalist, 2024*b*; 2024*c*). This could represent another potentially novel host taxon for these caterpillars, though one more in line with those used by other members of the subfamily in the region. There is clearly the possibility that this species may utilise a far wider range of host-plants than has previously been understood, and the distinctive appearance of the caterpillars could facilitate the discovery of further unknown associations. Understanding these ecological interactions forms an essential first step in shedding light on their evolution and facilitating effective conservation efforts.

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LITERATURE CITED

iNaturalist. (2024*a*). URL: <u>https://www.inaturalist.org/taxa/673515-Lophonotidia-melanoleuca</u>. [accessed 18 Dec 2024]

iNaturalist. (2024*b*). URL: <u>https://www.inaturalist.org/observations/19394867</u>. [accessed 18 Dec 2024]

iNaturalist. (2024c). URL: <u>https://www.inaturalist.org/observations/192632683</u>. [accessed 18 Dec 2024]

Staude, H., Picker, M., and Griffiths, C. (2024). *Southern African Moths & their Caterpillars.* Cape Town: Struik Nature.



Figure 1. Caterpillar of *Lophonotidia melanoleuca*, observed feeding in numbers on Red bush willow *Combretum apiculatum* in Hoedspruit, Limpopo. Photo by LDW.



Figure 2. Recorded distribution of *Lophonotidia melanoleuca*, combining observations reported in Staude *et al*. (2023) and iNaturalist (2024*a*).

Table 1. Known host-	-plant associations	of the South Africa	an Agaristinae,	from Staude et al	. (2023)
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Species	Host-plant
Aegocera fervida	Wild grape Rhoicissus spp.
Agoma trimenii	Grape Vitaceae
Brephos festiva	Tremble tops <i>Kohautia amatymbica</i> Wild verbena <i>Pentanisia angustifolia</i>
Brephos decora	Unknown
Paida pulchra	Unknown
Heraclia superba	Wild grape <i>Cissus</i> spp. Cultivated grape <i>Vitis</i> spp.
Heraclia africana	Wild grape <i>Cissus</i> spp.
Heraclia butleri	Wild grape Cissus spp.
Lophonotidia melanoleuca	Ivy-grape Cissus cornifolia
Ovios capensis	Sugarbushes Protea spp.
Syfanoidea schencki	Wild grape Rhoicissus tridentata