Ecology and conservation of the butterfly *Thestor brachycerus brachycerus* (Trimen, 1883) from the Western Cape

EA Bazin

22424563

Dissertation submitted in fulfilment of the requirements for the degree *Magister Scientiae* in Environmental Sciences at the Potchefstroom Campus of the North-West University

Supervisor:	Dr D Edge
Co-supervisor:	Prof H van Hamburg

May 2015

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Acknowledgements

I wish to acknowledge and extend my sincere thanks and appreciation to the following people:

Dr. Dave Edge, my dissertation supervisor for guiding the research and providing invaluable assistance and comments throughout the project. This research project would not have been possible without his continued encouragement and guidance.

To my co-supervisor, Professor Huib van Hamburg from North-West University for his support, guidance and assistance both academically and in administrative matters.

The Lepidopterists' Society of Africa and the Brenton Blue Trust for initiating the COREL programme which has provided the wherewithal and institutional support for my research activities.

Di Turner, and the other members of the CREW Outramps (a SANBI programme), as well as SANBI's Compton Herbarium in Cape Town, all of which provided much assistance in plant identification.

Nicolas Cole from SANParks for providing data for the vegetation map.

Ant identification relied upon the skills of Peter Hawkes (owner of Afribugs CC), who also provided valuable insights into the behaviour of *Anoplolepis custodiens*.

Professor Pieter Theron (North-West University) for the kind loan of his personal stereo microscope. Professor Christo Fabricus and Professor Josh Louw (Nelson Mandela Metropolitan University, George Campus) for organising the loan of a soil auger.

The owners of the Pezula Golf Estate for allowing us access to the Pezula sites and for carrying out the recommended management actions.

Steven Seiler and Melody van Aardt's photography as well as their invaluable assistance in searching the Coney Glen coastline for further colonies.

Lida Leggatt who translated the abstract into Afrikaans, assisted by Hanna du Toit.

My mother, Roz Bazin for proof reading.

Finally, my partner, Chris Leggatt, for his support, encouragement and understanding for my long hours of work, his assistance with data collection, photography, vegetation mapping and proof reading.

Abstract

The 1883 discovery, research into generic life histories and taxonomic investigations into the aphytophagous butterfly *Thestor brachycerus brachycerus* (the Knysna Skolly) from Knysna, South Africa are described. Its slide towards extinction through loss and degradation of habitat in the late 20th century left only one known colony by 2009 leading to it being assessed as Critically Endangered in the 2013 South African Red List and Atlas. During searches for more populations in 2011, a second *T. b. brachycerus* colony was found which differed markedly with respect to aspect, altitude and vegetation. Study areas were defined around the original colony on the Pezula Golf Estate (Pezula) and the newly discovered coastal colony east of Coney Glen (Coastal site). An assessment is made of the ecological differences and similarities between the two sites.

The extent and size of the two known populations was measured; the Extent of Occupancy was found to be 7.5 km^2 and the Area of Occupancy as 1.05×10^{-3} km^2. During observations over 5 seasons in December and January 2009 – 2014 the average number of butterflies observed at the original Pezula colony varied from 1 – 8 males and 0 – 3 females per survey, compared with 1 – 6 males and 1 – 3 females observed over 3 seasons from 2011 to 2014 at the Coastal colony.

The mating, territoriality, oviposition and predation avoidance behaviour of the adult *T. b. brachycerus* butterflies was studied by direct observation. Males were found to exhibit territorial behaviour, spending long periods of time perched in one spot, particularly in areas where the vegetation was open or low. Oviposition was rapid and no preference was shown by females for any particular plant. Between the two colonies, oviposition was observed on a total of 16 different plants.

The vegetation composition at the two study sites was analysed using Braun–Blanquet methodology. Only one plant, *Tarchonanthus littoralis*, was found to be common to both. There appeared to be no correlation between floristic composition and the occurrence of *T. b. brachycerus*. All the *Thestor* species so far studied by other workers have been found to be myrmecophilous, having an association with the pugnacious ant *Anoplolepis custodiens*. Ant communities were sampled by employing pitfall traps and direct sampling by hand. At both the Pezula and Coastal sites *A. custodiens* was the most ubiquitous ant species.

The insights gained have enabled implementation of a habitat management plan. Experimental management methods are recommended at the Pezula site which include small scale burning and selective cutting of vegetation. Search for more colonies is ongoing, using the improved ecological knowledge of the butterfly.

Opsomming

Die ontdekking in 1883, en die navorsing oor die generiese lewensgeskiedenis en taksonomiese ondersoeke van die afitofage skoenlapper, *Thestor brachycerus brachycerus* (die Knysna Skolly) van Knysna, Suid-Afrika, word beskryf. Die verlies en degradasie van die natuurlike habitat van hierdie skoenlapper in die laat 20ste eeu, wat teen 2009 slegs een bekende kolonie oorgelaat het, het gelei tot die skoenlapper se klassifikasie as 'n Krities Bedreigde Spesie in die 2013 Suid Afrikaanse Rooiedatalys en Atlas. Gedurende soektogte na meer kolonies in 2001, is 'n tweede *T. b. brachycerus* kolonie gevind, wat merkbaar verskil het t.o.v. aspek, hoogte bo seespieël en plantegroei. Studiegebiede rondom die oorspronklike kolonie op die Pezula-Golflandgoed (Pezula) en die nuutgevonde kuslynkolonie oos van Coney Glen (Kuslyngebied) is gedefinieer. 'n Evaluasie van die ekologiese verskille en ooreenkomste tussen die twee gebiede is gemaak.

Die omvang en grootte van die twee bekende populasies is gemeet; die Omvang van Okkupasie was bereken as 7.5 km^2 en die Gebied van Okkupasie 1.05 x 10-3 km^2. Gedurende waarnemings oor periodes van 5 seisoene gedurende Desember en Januarie 2009 – 2014, het die gemiddelde aantal skoenlappers wat per opname waargeneem is by die Pezula kolonie, gevarieer van 1 – 8 manlike en 0 – 3 vroulike skoenlappers, in vergelyking met 1 – 6 manlike en 1 – 3 vroulike skoenlappers wat waargeneem is oor 3 seisoene vanaf 2011 – 2014 by die Kuslynkolonie.

Die paring, grondgebied, eier posisionering en predasie vermydingsgedrag van die volwasse *T. b. brachycerus* skoenlappers, is bestudeer deur direkte waarneming. Daar is bevind dat manlike skoenlappers territoriale gedrag vertoon, deur lang periodes op een spesifieke plek te spandeer, spesifiek in gebiede waar die plantegroei laag en oop is. Eier posisionering was vinnig en geen voorkeur vir 'n spesifieke plant is deur die vroulike skoenlappers getoon nie. Eier posisionering is waargeneem op 16 verskillende plantsoorte in die twee kolonies saam.

Die plantegroeisamestelling in die twee studiegebiede is ge-analiseer deur die gebruik van die Braun-Blanquet-metodologie. Slegs een plant, *Tarchonanthus littoralis*, is gevind om gemeenskaplik aan albei gebiede te wees. Dit blyk of daar geen korrelasie tussen die floristiese samestelling en die verspreiding en voorkoms van die *T. b. brachycerus* is nie. Al die *Thestor* spesies wat sover deur ander navorsers bestudeer is, was myrmecophilies, met 'n assosiasie tussen die skoenlapper en die aggressiewe mier *Anoplolepis custodiens*. Steekproewe van miergemeenskappe is gedoen deur van vanggate gebruik te maak, asook direk per hand. By beide die Pezula en Kuslyngebiede was die *A. custodiens* die mees alomteenwoordige mier spesie.

Die insigte wat verkry is het die implementasie van ’n habitatbestuursplan moontlik gemaak. Eksperimentele bestuursmetodes, wat kleinskaalse brande en selektiewe snoei van die plantegroei insluit, word aanbeveel vir die Pezula gebied. Soektogte na meer kolonies, wat die verbeterde ekologiese kennis van die skoenlapper, in ag neem word voortgesit.

Table of Contents

List of Tables

List of Figures

List of Appendices

Appendix 1: Trophic resources known to be used by members of the genus *Thestor*

Appendix 2: Adult *T. b. brachycerus* counts made between during the emergence period between December 2009 and January 2014 at the Pezula colony, P1 and between January 2012 and January 2014 at the Coastal site colony, C1

Appendix 3: Adult *T. b. brachycerus* counts made between during the emergence period between December 2009 and January 2014 at the Pezula colony, P1 and between January 2012 and January 2014 at the Coastal site colony, C1, clustered into seasons

Appendix 4: Soil and rock analysis results showing macro and micro element composition, pH and electrical conductivity (EC) of the soil samples. Samples 1-5 are from the Pezula site – sample 2 lies within the P1 colony. Sample 6 relates to the Coastal site colony, C1. Differences are highlighted in red. The pH and quartz and muscovite content of the rock samples taken from P1 (sample 2) and C1 (sample 6) are also given

Appendix 5: Plant family abbreviations

Appendix 6: Vegetation abundance matrices showing the Braun-Blanquet cover abundance scale recorded for each of the plant species sampled at the Pezula site relevés PEZ01-PEZ15

Appendix 7: Vegetation abundance matrices showing the Braun-Blanquet cover abundance scale recorded for each of the plant species sampled at the Coastal site relevés CS01 – CS08

Abbreviations and Key Terms

AOO	Area of Occupancy
C1	First coastal *T. b. brachycerus* colony site (within the Coastal study site)
C2	Second coastal *T. b. brachycerus* colony site
Coastal site	Coastal study site
COREL	Custodians of Rare and Endangered Lepidoptera
EOO	Extent of Occupancy
LepSoc	Lepidopterists' Society of Africa
P1	Pezula *T. b. brachycerus* colony site (within the Pezula study site)
Pezula	Pezula study site
PGE	Pezula Golf Estate

Key Terms

Thestor brachycerus brachycerus, aphytophagous larvae, myrmecophily, trophallaxis, butterfly-ant relationships, butterfly conservation, butterfly habitat management.

Chapter 1 General introduction

1.1 Butterfly conservation in South Africa

The Lepidopterists' Society of Africa (LepSoc) was established in 1983 and since then there have been many efforts to alert authorities and the public at large to the plight of South African butterflies (Edge, 2011). The first red data book on southern African butterflies was published 25 years ago (Henning & Henning, 1989). Following publication of the third update (Henning *et al.*, 2009), LepSoc decided to launch the COREL (Custodians of Rare and Endangered Lepidoptera) programme to promote and ensure the conservation of all butterflies and moths Red Listed as threatened in South Africa (Edge, 2011). This programme is currently focused on the 15 Critically Endangered butterfly and moth taxa, which includes *Thestor brachycerus brachycerus* (Trimen, 1883), the Knysna Skolly (Fig. 1-1).

Figure 1-1: Male *T. b. brachycerus*, underside (Photo: Reinier Terblanche)

1.2 Literature review of *Thestor brachycerus brachycerus* and research motivation

Lycaenidae is the second-largest family of butterflies worldwide (Fiedler, 1996), comprising between 30 and 40% of all butterfly species (New, 1993). South Africa has an abundance of lycaenid butterflies (Ball, 2006) and nearly 30% of these are taxa of conservation concern (Edge *et al.*, 2013). The majority of lycaenids have associations with ants that are either facultative or obligate and range from mutualism to parasitism, (Pierce *et al.*, 2002). A large proportion of the South African endangered butterflies are habitat specialists with myrmecophilous lifestyles and complex trophic associations.

The lycaenid genus *Thestor* Hübner is endemic to southern Africa and belongs to the subfamily Miletinae. Other genera of Miletine butterflies occur elsewhere in Africa (*Aslauga* Kirby; *Lachnocnema* Trimen; *Megalopalpus* Röber and *Spalgis* Moore); in the Indian subcontinent and across south-east Asia (*Allotinus* C & R. Felder; *Miletus* Hübner), with one species in Australia (*Liphyra brassolis* Westwood, 1864). There is also one species in North America (*Feniseca tarquinius* [Fabricius, 1793]).

According to Pierce (1995), all the known members of the Miletinae are aphytophagous (meaning that they do not feed on plants) and there is great diversity of different feeding strategies. Many studied miletine larvae have been found to be specialised to feed on hemipterans including coccids, jassids, psyllids, membracids and aphids (Pierce, 1995). Larvae of Southeast Asian miletine (tribe: Miletini) have been found to be predators of greenideid aphids (Hemiptera: Greenideidae) and leafhoppers (Hemiptera: Cicadellidae) and several instances have been reported in which larvae of a single lepidopteran species fed on different prey species at the same site (Lohman & Samarita, 2009).

Adult *Thestor* butterflies are morphologically distinct, principally because of their short antennae and the reduced, non-functional proboscis in the adults (Williams & Joannou, 1996; Heath & Pringle, 2004). This makes it impossible for them to feed on nectar or on hemipteran honeydew secretions as has been observed in adults of the closely related genus *Lachnocnema* (Clark & Dickson, 1971).

The type species of *Thestor* is *Thestor protumnus* (Linnaeus, 1764). *T. b. brachycerus*, also known as the Knysna Skolly butterfly, was discovered at Knysna by Roland Trimen (Trimen, 1883; Trimen & Bowker, 1887), and was the first so-called “black” *Thestor* to be described. In their review of the *Thestor* genus, Heath and Pringle (2004) proposed a provisional phylogeny based on morphological characteristics, describing how the genus as a whole has diversified into 27 currently recognised species and 7 additional subspecies, which they allocated into eight species groups. The *brachycerus* species group is the largest, containing 12 univoltine taxa, with adult emergences recorded from November to February. Significantly, Heath and Pringle (2004) treated the more widely distributed *T. dukei* van Son, 1951 as a subspecies of *T. brachycerus*, basing their diagnosis on morphological characteristics (genitalia and facies). This resulted in *T. brachycerus* being renamed *T. brachycerus brachycerus.*

The subspecies concept has lost many adherents of late (see Braby *et al*., 2012) and can have adverse effects on butterfly conservation in some circumstances (e.g. Gompert *et al*., 2006; Frankham *et al*., 2012) as the more endangered subspecies often gets ignored due to there being a more prevalent alternative subspecies.

Although d'Abrera (2009) informally treats *T. dukei* as a valid species, it officially remains a subspecies of *T. b. brachycerus* (Heath & Pringle, 2011; Collins *et al*., 2013). Recent DNA work being conducted at Harvard University has indicated that there might be sufficient divergence in the molecular markers examined to separate *T. b. brachycerus* at species level from its closely related montane cousin, *T. b. dukei* (Kaliszewka, pers. comm., 2014). However it is too early to confirm a *T. brachycerus/T. dukei* species revision at this point. Accordingly, the Knysna Skolly will be referred to as *T. b. brachycerus* for the remainder of this dissertation.

There are also significant ecological differences between *T. b. dukei,* which is found high up in the mountains of the Western Cape along a wide belt of Karoo and Fynbos habitat (Woodhall, 2005), and the coastal insect *T. b. brachycerus* which is a relatively isolated taxon geographically. *T. b. brachycerus* is the furthest east of its species group, with its closest relative, with regards to distance, being *T. barbatus* Henning and Henning, 1997, which occurs some 80 km north-west of Knysna.

More than 99% of butterflies and moths consume plants, and comparatively little research has focused on the ecology and evolution of predatory or parasitic taxa (Pierce, 1995). Similarly, there is very little published information available on the *Thestor* genus and recent research is particularly lacking.

All *Thestor* larvae studied thus far appear to be completely aphytophagous. According to Clark and Dickson (1971), females of the genus *Thestor* lay their eggs close to ants' nests or near colonies of scale insects or other similar hemipterans and the larvae have a mutualistic or parasitic relationship with these insects. However, larvae of the *Thestor* genus do not all have the same trophic strategy (see Table 1-1 for a species group summary and Appendix 1).

The early instars of some species have been found in association with, and preying on, coccids and psyllids (Hemiptera: Sternorrhyncha) – *T. protumnus aridus* van Son, 1941 and *T. basutus basutus* (Wallengren, 1857) (Clark & Dickson 1960, 1971) and *T. basutus capeneri* Dickson, 1972

(Williams & Joannou 1996). The later instar larvae of *T. yildizae* Koçak, 1983 have been observed being fed by trophallaxis from ants (Claassens & Heath, 1997). In further studies on *T. basutus basutus,* Claassens and Heath (2003) found that ants were feeding the final instar larvae by trophallaxis, and the larvae were preying on host-ant eggs or larvae and feeding upon organic detritus within the nest.

Table 1-1: Trophic resources of the larvae of the 8 *Thestor* species groups (source: Heath & Pringle, 2004)

***Thestor* Species Group**	**Number of Species**	**1st- 3rd Instar Larvae**	**Final Instar Larvae**
basutus	2	Psyllids, coccids	Trophallaxis from ants, detritus, ant brood
protumnus	4	Coccids	Unknown
brachycerus	12	Unknown	Trophallaxis from ants
braunsi	5	Unknown	Unknown
montanus	5	Unknown	Unknown
compassbergae	4	Unknown	Unknown
rossouwi	1	Unknown	Unknown
murrayai	1	Unknown	Unknown

In terms of previous research specific to *T. b. brachycerus* there is very little available literature; the life history has not yet been fully described. Clark and Dickson (1971) described the egg and first instar larva of *T. b. brachycerus*, from laboratory observations, which died fairly soon after hatching. The adult butterflies have been recorded between the end of November and the beginning of February (Clark & Dickson, 1971), although Heath and Pringle (2004) state the flight period is from mid-December to early January. It is not known for how many days an individual adult survives. There is only one brood per year.

Thestor larvae are thought to be obligate myrmecophiles. The only ant associate of *Thestor* larvae so far recorded is the common pugnacious ant *Anopolepsis custodiens* Smith (Formicinae) (Heath & Claassens, 2000; Heath *et al*., 2008; Williams, 1994). Pierce *et al*. (2002) considered it unlikely that all members of such a large genus would associate with the same species of ant and suggested that *A. custodiens* might consist of a "constellation of sibling species." Preliminary molecular analysis at Harvard (The Pierce Lab) has revealed that this is very likely to be the case (Heath & Pringle, 2004).

Clark and Dickson (1971) recorded *T. b. brachycerus* “in numbers” near the Knysna Eastern Heads during the height of its emergence period. In the first red data book on South African butterflies Henning and Henning (1989), assessed its status as “indeterminate” since there was some uncertainty about whether the Still Bay populations 160km to the west were the same species. This has proved not to be the case since Heath and Pringle (2004) described the Still Bay populations as a separate species *T. claassensi* Heath & Pringle, 2004.

Edge (2005a) reported on the decline of the Knysna populations, as a result loss of habitat to property development and degradation due to agricultural activities, which has accelerated in the last 20 years. Between 1995 and 2005, Edge (2005a) reported that he had located eight *T. b. brachycerus* colonies on the Knysna Eastern Heads, which by 2009 had been reduced to one remaining extant colony on the Pezula Golf Estate (PGE) (labelled P1 – see Figure 2-1 below). Consequently Henning *et al*. (2009) raised *T. b. brachycerus* to Critically Endangered status. In 2012 a second colony was located on the coast east of Coney Glen beach (C1 on Figure 2-1), but the Critically Endangered status was upheld by Mecenero *et al*. (2013), who estimated the Extent of Occupancy as <15 km^2 and the Area of Occupancy as <2 km^2.

Under the auspices of COREL a comprehensive research project was launched in 2013, with the aims of discovering the critical ecological factors influencing the survival of this butterfly in order to develop conservation strategies. The owners of the PGE have agreed to conserve a 20 hectare site, between the golf course and the northern boundary, where *T. b. brachycerus* still occurs, and to manage the site in accordance with the recommendations of butterfly experts.

1.3 Physical description of *T. b. brachycerus*

The adult *T. b. brachycerus* is inconspicuous, well-camouflaged and easily over-looked. The upper side of the butterfly (Fig. 1-2) is pale greyish-brown with prominent black discal spots and a small whitish apical patch (Heath & Pringle, 2004) which is more prominent in the female. The cilia on the wing margins are chequered black and white (Williams, 1994). The underside of the wings (Fig. 1-1) is a cryptic mosaic of grey, brown and black markings. There are few morphological differences between the male and female of the species and the best way to distinguish them in the field is by the difference in flight behaviour (see 3-2). The wings of the female are more rounded than the male (Woodhall, 2005) and the female’s abdomen is larger than that of the male. Males tend to have a smaller wingspan, 27-34 mm, than females, 29-37 mm (Clark & Dickson, 1971). Occasionally two colour forms occur in the species: lighter and darker (Heath & Pringle, 2004).

Figure 1-2: **Male *T. b. brachycerus*, upperside (Photo: Steven Seiler)**

1.4 Research question

The research question postulated is as follows:

What factors can be identified relevant to the conservation and management of the existing *T. b. brachycerus* colonies?

1.5 Aims and objectives

The research aims to identify factors relevant to the conservation and management of the *T. b. brachycerus* colonies.

The specific objectives are to:

1. Explore the history of past *T. b. brachycerus* populations and describe the searches for new colonies.
2. Establish the extent and size of the extant populations.
3. Describe the present remaining colony sites.
4. Investigate the adult behaviour of *T. b. brachycerus* that affects its conservation, namely territoriality, mating, oviposition, predation, and predator avoidance.
5. Describe the vegetation characteristics at the colony sites.
6. Sample and identify the ant assemblages at the colony sites.
7. Formulate conservation and management recommendations.

These objectives are addressed in the following chapters, each dealing with the relevant materials and methods, results and discussions.

Chapter 2 Distribution of *T. b. brachycerus*, past and present and searches for other populations

2.1 Introduction

The first description of *T. b. brachycerus* was by Trimen in 1883, and the type locality was listed merely as “Knysna”. A few years later Trimen & Bowker (1887) described the butterfly as exceeding local but “numerous” at Knysna on the southern coast. In 1953 Swanepoel defined the butterfly’s location more specifically as the Eastern Heads at Knysna. In subsequent years the distribution of the species received little attention until Edge (pers. comm., 2010) started searching for *T. b. brachycerus* colonies in the late 1990s, and by 2010 had established that it had been present in eight localities. The location of the eight previously known colonies can be seen in Figure 2-1, with a description of when each one was found, and its current status in Table 2-1.

According to Edge (2005a) the Eastern Heads colonies (EH1 & EH2) were destroyed by building activities in 1989 and 1996 respectively, and the colony on the Woodbourne farm was eliminated in the early 2000s due to a change to sheep farming (Edge, 2005a).

Despite efforts to preserve the colonies on the PGE, through an EIA scoping report drawn up by Dave Edge, the developer chose to only partially implement the recommendations, resulting in the loss of the *T. b. brachycerus* colonies PE1 and PE2 when the PGE was built in 1998. The developers at Fernwood were persuaded to set aside an area for the butterflies after a strong colony was identified there (see FW on Fig. 2-1). However no *T. b. brachycerus* activity has been seen at FW since around 2006 (Edge, 2005a).

A small colony of *T. b. brachycerus* butterflies was discovered in 2004 by Edge (2005a) whilst he was completing a scoping study for a proposed development in the south-west corner of Pezula, but that colony has not been seen since 2009.

Consequently when this research commenced in 2009, only one *T. b. brachycerus* colony was still known to exist (Edge, 2005a), which was situated on the north-west facing slopes close to the 6th fairway of the PGE (see P1 on Fig. 2-1). The importance of searching for other colonies was therefore recognised.

Figure 2-1: Map of The Heads and the Pezula Golf Estate, Knysna showing previously known colonies of *T. b. brachycerus* (adapted from Figure 2 in Edge 2005a, with permission), and extant colonies (in red). EH1 and EH2 – Eastern Knysna Heads; WB – Woodbourne Farm; PE1, PE2, P1 and HH – Pezula Golf Estate; FW – Fernwood Property; C1 and C2 – Coastal.

Table 2-1: ***T. b. brachycerus*** **colonies with codes, location and coordinates/altitude. GPS coordinates and information of extinct colonies provided by D. Edge (pers. comm., 2010); Edge 2005a.**

Discovered	Code	Property	Description	Population	Extinct	Coordinates		Alt.	Reason for demise
						S	E	m amsl	
1883.12	EH1	Eastern Knysna Heads	Unknown - type locality	Large	c. 1989	34°04'35"	23°03'39"	20	Housing development
1993.12.25	EH2	Eastern Knysna Heads	North-east of Emu Crescent	10♂2♀	1996	34°04'35"	23°04'04"	125	House built on locality
1995.01.05	WB	Woodbourne Farm	Near top of north facing slope of small hill	3♂1♀	c. 2002	34°04'35"	23°04'37"	135	Change from cattle to sheep grazing
1997.12.20	PE1	Pezula Golf Estate	North facing slope below proposed hotel site	10♂3♀	1998	34°04'14"	23°05'17"	165-180	Building of road and houses
1997.12.27	PE2	Pezula Golf Estate	South-east of proposed 7th green	2♂	1998	34°04'02"	23°05'33"	170	Vegetation change - no cattle grazing
1998.01.04	P1	Pezula Golf Estate	North of and below 6th fairway	12♂2♀	Extant	34°03'54"	23°06'02"	170-180	
1999.12.23	FW	Fernwood Property	On north-east facing slope of hill above valley	21♂5♀	c. 2006	34°04'16"	23°05'07"	145-170	Change from cattle to sheep grazing
2004.12.29	HH	Pezula Golf Estate	East of Pezula at Hunters Home development	3♂1♀	c. 2009	34°03'45"	23°05'33"	80	Vegetation change - no cattle grazing
2012.01.20	C1	Coastal	East of Coney Glen beach	8♂3♀	Extant	34°04'58"	23°03'59"	5-20	
2014.01.06	C2	Coastal	Further east of Coney Glen beach	5♂2♀	Extant	34°04'58"	23°04'10"	15	

2.2 Materials and methods

Prior to 2009, Edge (pers. comm., 2010) conducted searches for further *T. b. brachycerus* populations during the flight period of the butterfly elsewhere on the conserved 20 hectare site on the PGE. They were specifically carried out along the lower (northern) boundary and in an altitude band east and west of the known locality. Several south-north traverses were also conducted. Similar searches were made at other places where the butterfly used to occur on the PGE (see Fig. 2-1), the neighbouring Fernwood property, and parts of the Woodbourne farm (Edge, pers. comm., 2010). The extreme difficulty of the terrain (steep and rocky with dense thicket patches) and the cryptic colouration of the butterfly made these searches arduous and unproductive.

In 2011, enquiries were made with other lepidopterists about a possibly extant coastal colony, and following information received, it was also decided to search along the coastline to the east of Coney Glen beach. From studying adult behaviour in 2009/2010 it was ascertained that the peak of adult *T. b. brachycerus* emergence was mid-December to mid-January (see 3.3) therefore searches were concentrated during this period.

2.3 Results

Searches at the inland sites EH2, WB, PE1, PE2, FW and HH (Fig. 2-1 & Table 2-1) during the 2011 to 2014 flight periods did not reveal any adult *T. b. brachycerus*, except for a few adult sightings to the east of HH and at the same altitude, inside the PGE boundary. The site at EH1 was also visited without success, but in December 2011 lepidopterist Harald Selb reported that, in the past, he had seen a colony of *T. b. brachycerus* on the coast to the east of Coney Glen, Knysna (C1 on Fig. 2-1). The area was visited and the presence of the butterfly was confirmed. This site was radically different from the Pezula site with respect to aspect, altitude and vegetation.

In January 2014, a third *T. b. brachycerus* colony (C2) was discovered during a search along the coast to the east of the known C1 colony (see Table 2-1). The straight line distance between the C1 and C2 colonies is approximately 300m, separated by rocky outcrops (Fig. 2-2).

Figure 2-2: **Map showing the new coastal colonies, C1 (found January 2012) and C2 (found January 2014).**

2.4 Discussion

Prior to this research project suitable *T. b. brachycerus* habitat was known to be in fynbos on north to north-west facing hillsides at an altitude of 120–180 m, with some degree of disturbance that created relatively sparse vegetation patches (Edge, 2005a). Searches for new populations of the butterfly were therefore focused on such biotopes. The recent discovery and description of the coastal sites, which differ so radically from the inland sites, both in abiotic conditions and vegetation, has shown that the biotope concept may be of limited value in defining habitat, particularly for an aphytophagous insect such as *T. b. brachycerus*. There may need to be more focus on determining butterfly behaviour and the resource requirements both of the butterfly and its ant associates in order to better define suitable habitat (Dennis *et al.,* 2006; Dennis, 2010).

The current coastal sites and the inland sites would probably have been linked along the coast to the west (Coney Glen beach) and then round the eastern headland to the known former site EH1 and up the rocky slopes to EH2. The Woodbourne Farm site WB was a remnant of a former wider distribution on this farm (perhaps reduced by habitat destructive farming practices such as too regular burning and overgrazing by sheep), as was the Fernwood site FW. The PGE sites (PE1 and PE2) and Pezula (P1 and HH) sites further east were never so degraded because only low intensity cattle grazing ever took place there. Loss and degradation of these sites only took place more recently (since 2000) during and subsequent to the golf course and housing development being built , when the cattle were removed and the vegetation became much denser, except at P1 which underwent habitat management to keep the vegetation more open.

According to Edge (pers. comm.), before the advent of modern man, conditions were quite different, and this would have promoted a patchy distribution of the butterfly, forming a classical metapopulation structure and essentially a single large gene pool. This patchy distribution would have been a result of free roaming megaherbivores, both grazers and browsers, which would have influenced the vegetation composition and structure, making it more heterogeneous and with less dominance of shrubs and grassy ground cover. Fire would also have been naturally intermittent and patchy as opposed to the current artificial fire regime, which is either too frequent to promote livestock grazing, or no fire at all to protect human properties from fire.

It is clear that both *T. b. brachycerus* and its host ant are able to adapt to a broad range of abiotic conditions and vegetation types. Regarding the coastal sites, it should be noted that there is another *Thestor* taxon that inhabits sites close to the sea – *T. dicksoni malagas* Dickson 1971, found near Langebaan on the West Coast of South Africa, but which has not been recorded as occurring further inland (Heath & Pringle, 2004).

Chapter 3 The extent and size of the extant populations

3.1 Introduction

At the beginning of this research, only one extant *T. b. brachycerus* colony was known, and later a second one was discovered. In view of this it was considered to be important to estimate population size in terms of number of butterflies and extent in terms of area. According to Edge, (2005b), an estimate of absolute population size and assessment of the degree of year-to-year fluctuation are essential data for assessing extinction risk, threat from loss of genetic variability and the relative success or failure of management techniques.

The mark-release-recapture method first used in Africa on the Karkloof Blue butterfly (*Orachrysops ariadne* [Butler, 1898]) by Lu, (2003) was ruled out due to the risk of butterfly trauma or possible death which would further undermine the *T. b. brachycerus* numbers, as was also decided by Edge (2002) in studying the Brenton Blue (*O. niobe* [Trimen, 1858]). Other recognised methods such as larvae or egg counts, described by Plant *et al*. (2005), were also ruled out. Counting larvae was impossible due to larvae never having been found on a plant. Egg counts were also not practical because females did not show any preference for laying eggs on any one plant species (see 5.4.3); small egg size - about 0.5 mm in diameter making them difficult to find; and lack of knowledge of fecundity of the *T. b. brachycerus* females.

In butterflies with phytophagous larvae, examining the host plant for eggs and egg shell remains can prove useful in mapping the local distribution of breeding sites and may also be used to locate new colonies (Lu & Samways, 2001); however this is not possible with *T. b. brachycerus* since the larvae appear to be aphytophagous. As a result, survey methods were limited to adult butterfly observation during the flight period.

3.2 Materials and methods

Records were kept of all sightings of male and female butterflies observed during site visits. Female butterflies were distinguished from males by their larger abdomen size and their slow fluttering flight. Males take somewhat faster, and seemingly more purposeful flights. All observations made at the extant colonies were GPS referenced to enable the extent of each colony to be determined accurately and mapped. The GPS points were entered into ESRI® ArcGIS® Explorer Desktop software and the outer points were used to calculate overall Extent of Occupancy (EOO), and the Area of Occupancy (AOO) in each colony using the “measure” function.

Adult butterfly numbers were recorded over 5 seasons between December 2009 and January 2014 at the Pezula colony (P1), and over 3 seasons between January 2011 and January 2014 at the Coastal colony (C1). The sites were surveyed between 10:30 and 13:00 on warm and sunny days with little wind. Each day that the sites were surveyed a butterfly count was conducted. The transect method was employed, where paths through the colony were walked at a uniform pace and all *T. b. brachycerus* adults recorded; gender and behaviour were also noted.

Thestor species are often inconspicuous and live in isolated colonies often not more than a hectare in size (Williams, 1994). Although there was a chance that the same butterfly was counted more than once, it was more probable that some butterflies were missed due to their cryptic colouration (see Fig. 3-1). It was extremely easy to overlook a *T. b. brachycerus* butterfly when perched, particularly on dead plant material. To minimise this, a stick was used to gently tap the vegetation as the area was patrolled to disturb any perching butterflies. Where known perching sites occurred this method was avoided and only sight used instead. It was noted that disturbing the butterflies affected their natural behaviour for a short time. Therefore, in order to do the butterfly count, this disturbing was kept to a minimum and only done once during each survey session.

Figure 3-1: Male *T. b. brachycerus*, camouflaged on dead plant material (Photo: Chris Leggatt)

The number of butterflies observed each day was collated into tables (Appendix 2) and displayed in bar charts (Figs. 3-2 & 3-3) using Microsoft® Excel. The mean number of adult butterflies counted at P1 and C1 was calculated and Student's independent two-sample t-test was used to test whether the population means were significantly different from each other.

Pearson's *chi*-squared test (χ^2) was carried out on the data to test if the ratio of observed males to females of *T. b. brachycerus* was significantly different from the expected ratio of 1:1. This test assumes that males and females are equally easy to find. Pearson's *chi*-squared test was applied using Microsoft® Excel.

3.3 Results

The *T. b. brachycerus* Pezula colony (P1 – see Fig. 2-1) covers an area of approximately 500 m^2. During observations of adults over 5 seasons in December and January 2009 – 2014 the average number of butterflies observed in the colony in a day at the peak varied from 1-8 males and 0-3 females (Fig 3-2). The greatest number of individuals seen on one day was 11 (on 6th January, 2010) and the mean number of individuals seen per day was 5.08 butterflies.

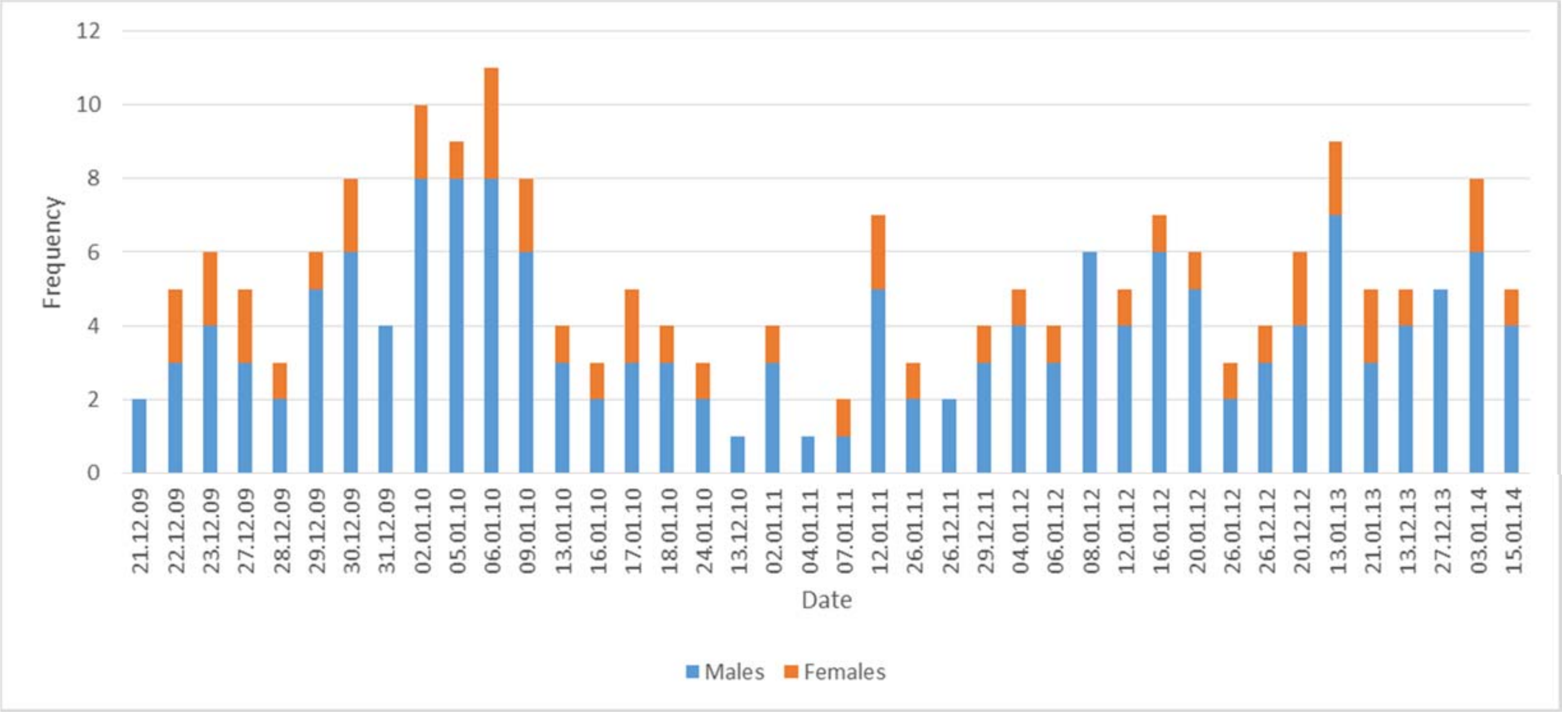

Figure 3-2: **Frequency of *T. b. brachycerus* sightings at the Pezula colony (P1) during the five Dec/Jan emergence periods between December 2009 and January 2014**

The first coastal *T. b. brachycerus* colony (C1) covers an area of approximately 550 m^2. Adult butterflies were observed during December and January over 3 seasons from 2011 to 2014 and the average number of butterflies observed in the C1 colony in a day at the peak varied from 1-6 males and 1-3 females (Fig 3-3). The greatest number of individuals seen on one day was 9 (on 12th December, 2012) and the mean number of individuals seen each day was 5.67 butterflies. On 31st January 2012, although the weather conditions were suitable, no *T. b. brachycerus* adults were seen. The second coastal colony (C2) was discovered late in the 2014 season, but the initial impression is that this colony is of a similar strength to the C1 colony. The Student's t-test result of t = 0.44734 (Appendix 2) indicates that there is not enough evidence to suggest that the mean observed frequencies at P1 and C1 are significantly different.

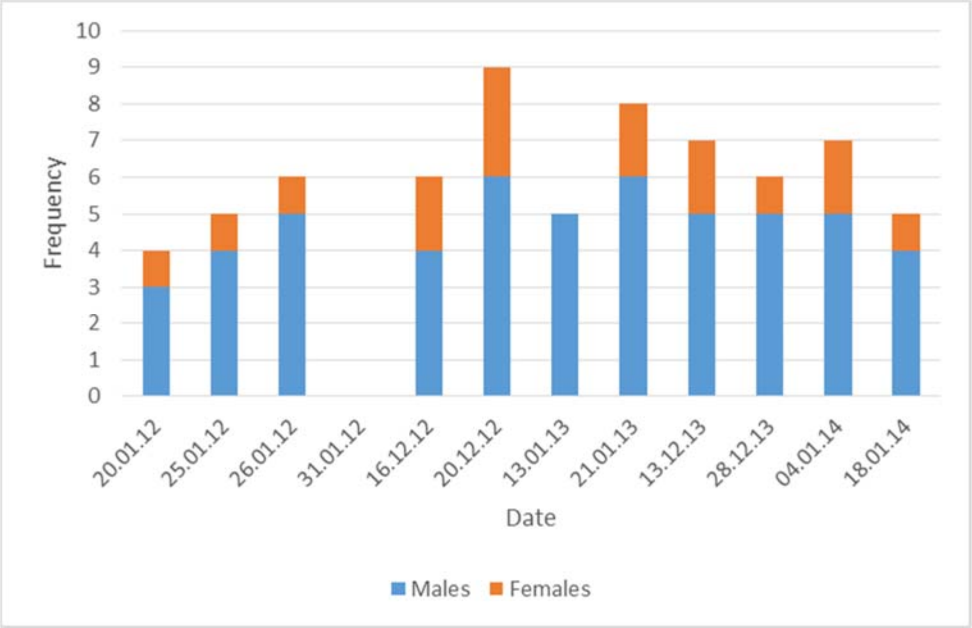

Figure 3-3: **Frequency of *T. b. brachycerus* sightings at the coastal colony (C1), during the three Dec/Jan emergence periods between January 2012 and January 2014**

In total, 76.8 % of the butterflies at P1 were identified as male while only 23.2 % were female giving a male biased sex ratio of slightly over 3:1 (Table 3-1). Similarly, 76.5 % of the butterflies at C1 were identified as male and 23.5 % were female giving a male biased sex ratio of approximately 3:1 (Table 3-1). Pearson's *chi*-squared test results show p-values of 0.00256 for P1 and 0.01993 for C1 (Appendix 2). The results indicate that, in both populations, more males than females were observed, with a male to female ratio of 3.56 in the P1 population, and 3.39 at the C1 colony (Table 3-1). Pearson's *chi*-squared test results indicate that the greater number of males over females appear to be significant in both the P1 and C1 colonies (Appendix 2), although with a cut off of 0.05, the C1 p-value of 0.01993 is much less significant than the P1 p-value of 0.00256.

These results should be viewed with caution due to the small size of the expected frequencies (E*i*). Some statisticians suggest that the minimum size of E*i* should be greater than 10, whilst others accept E*i* > 5 (Haberman, 1988). Clustering the data into seasons which increases E*i*, results in considerably smaller p-values for both colonies (Appendix 3) implying that the differences in observed male female numbers are even more significant.

Table 3-1: Observed adult *T. b. brachycerus* average numbers per day and male: female ratios at the P1 colony between December 2009 and January 2013 and C1 colony between January 2012 and January 2014

	PEZULA COLONY P1			**COASTAL COLONY C1**		
	Average		**Male: female**	**Average**		**Male: female**
Season	**Males**	**Females**	**ratio**	**Males**	**Females**	**ratio**
Dec 09 - Jan 10	4.24	1.41	3.00			
Dec 10 - Jan 11	2.17	0.83	2.60			
Dec 11 - Jan 12	3.89	0.78	5.00	3.00	0.75	4.00
Dec 12 - Jan 13	4.25	1.75	2.43	5.25	1.75	3.00
Dec 13 - Jan 14	4.75	1.00	4.75	4.75	1.50	3.17
Mean	**3.86**	**1.15**	**3.56**	**4.33**	**1.33**	**3.39**

3.4 Discussion

According to Clark & Dickson, (1971) the emergence period of *T. b. brachycerus* is from late November to early February, however no butterflies were noted in November nor February at either P1 or C1. Heath & Pringle (2004), reported that *T. b. brachycerus* flew from mid-December to early January. Over the whole study period, the earliest emergence noted was 13th December, and the last butterfly seen for the season was 26th January. When the Coastal colony was visited on 31st January 2012 (which was warm and sunny), no *T. b. brachycerus* were seen and the brood was probably over. Currently it can be stated that *T. b. brachycerus* flies in December and January.

The daily counts (not including days when no butterflies were seen), at P1 resulted in numbers ranging between 1 and 11 *T. b. brachycerus* butterflies and between 2 and 9 butterflies at C1. These population numbers appear to be very low and there is a good chance that some butterflies were missed due to their cryptic colouration (see Fig. 3-6) and behaviour (see Chapter 5) meaning the actual population is expected to be larger. From the current data the mean number of individuals observed per day was higher at C1 than P1 (5.67 vs. 5.08 respectively), but the t-test result of 0.44724 indicated there was no significant difference between these observations.

There is some evidence of a highly male biased sex ratio amongst lycaenid butterflies. According to Hughes *et al*. (2000), the mating system of the Australian lycaenid butterfly, *Jalmenus evagoras* Hübner, 1818 is characterised by, *inter alia*, a highly male biased operational sex ratio; its physical sex ratio however is unbiased. Male sex ratio bias has also been reported in overwintering monarch butterflies (*Danaus plexippus*, Linnaeus, 1758) by Frey & Leong (1993), where males accounted for over 60% of the population. Frey & Leong (1993) proposed four hypotheses to explain the male-biased sex ratio often seen in butterflies: male-biased primary sex ratio; differential pre-adult mortality; lag in emergence of females relative to males and differences in 'catchability' due to sex-related behavioural differences.

There is no literature suggesting that the primary sex ratio of *Thestor* butterflies should be anything other than 1:1. Whilst it has been reported in some butterflies (e.g. *Iolaus mimosae* Trimen, 1874) that earlier pupating larvae yield predominately males and later pupating larvae mostly females (Edge, 1991) this has not been observed in *T. b. brachycerus* as counts have consistently revealed more males throughout the emergence period. It is possible that there are less females because the female larvae are more resource constricted than males, requiring more larval food sources to develop ova since they do not feed as adults. Alternatively, the counting methodology is flawed in a way that females are underestimated. Females are no better camouflaged than the males but their behaviour might make them less conspicuous, especially if they do not move when flushed. Williams, (1994) describes *T. b. brachycerus* females as having a slow fluttering flight pattern and resting on the ground for long periods. If this is so they would be easily missed, particularly if they perch in the undergrowth rather than in the open as the males do. The males are easier to find as they usually return to the same perching site. If the butterfly is not in flight (see 3.2), the size and shape of the abdomen and wings determine whether the observed butterfly is male or female when perched, but because these differences can sometimes be subtle, occasional miss-identification of females may have occurred.

Figure 3-4: **Male *T. b. brachycerus*, camouflaged on dead plant material (Photo: Chris Leggatt)**

No systematic counting method for the entire population of *T. b. brachycerus* has yet been devised (such as was done for *O. niobe* – see Edge 2005b), however an index of abundance has been established which can form a basis for future population monitoring. It seems probable that the overall areal extent of a population is a function of the number of males and the size of the average male territory, and consequently colony area is a meaningful surrogate for population size. It appears that the putative host ants are quite widespread on both coastal and inland sites (see Chapter 7) so this should not be a constraint to an increase in AOO and thus population growth.

From Figure 2-1 it has been possible to recalculate the EOO previously determined in Mecenero *et al.* (2013) as <15 km^2, and it has more accurately calculated as 7.5 km^2. Similarly the AOO of <2 km^2 has been calculated as 1.05 x 10^{-3} km^2. This does not result in a reassessment of *T. b. brachycerus* from its current Critically Endangered (CR) conservation status, but as more colonies are discovered and populations are stabilised by good management, this may eventually be possible.

Chapter 4 Description of the study sites

4.1 Introduction

The study areas, Pezula and the Coastal site, are situated to the east of the Eastern Heads at Knysna (Fig. 4-1).

Figure 4-1: Arial view of the two study areas, Pezula and the Coastal site and the two extant *T. b. brachycerus* colonies within those areas, C1 and P1.

The Pezula study site (Pezula) refers to an area of approximately 130,000 m^2 which was surveyed with regards to vegetation and ant assemblages. The *T. b. brachycerus* colony at Pezula (denoted P1 in Fig. 2-1) lies within Pezula and covers an area of about 500 m^2 (Fig. 4-2).

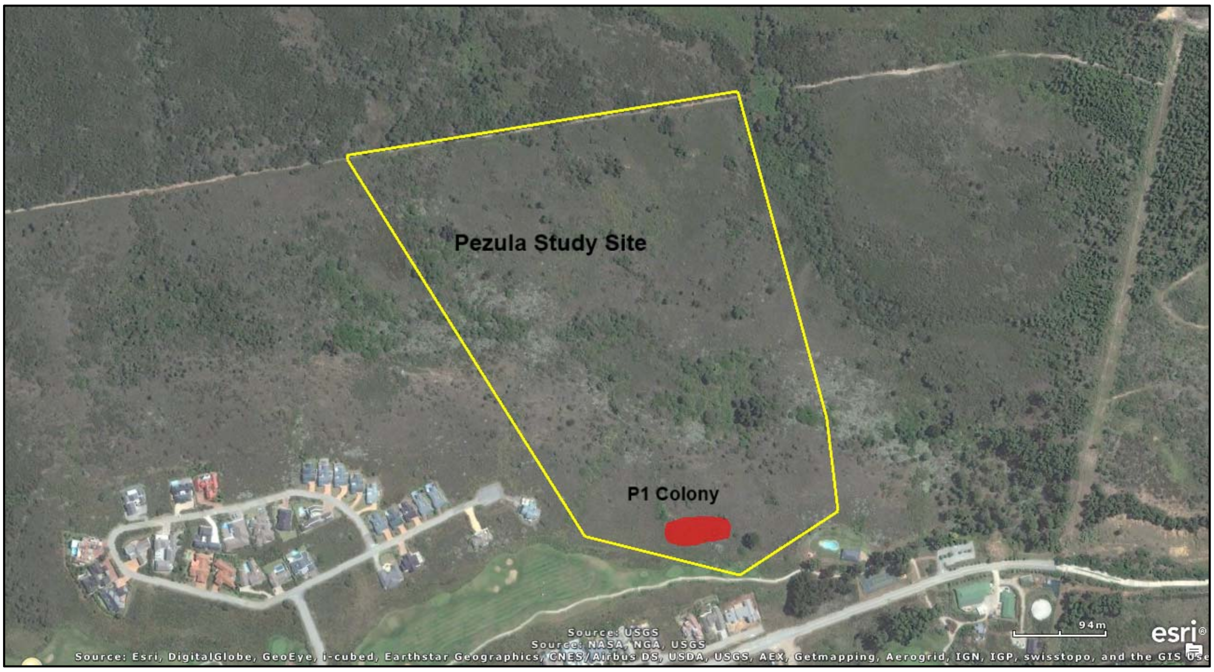

Figure 4-2: **The Pezula study site outlined in yellow with the *T. b. brachycerus* colony, P1 shaded red.**

Similarly C1 refers to the *T. b. brachycerus* colony at the first coastal study site (Coastal site). The area of the Coastal site is approximately 2,000 m^2 and the C1 colony covers approximately 550 m^2 of this (Fig. 4-3).

Figure 4-3: **The Coastal study site outlined in yellow with the *T. b. brachycerus* colony, C1 shaded red.**

The second coastal colony is referred to in the text as C2, and has not as yet been studied in the same depth as the other sites (see Fig. 2-2).

Prior to the discovery of the colony at Coney Glen it was thought that *T. b. brachycerus* inhabited fynbos covered north, north-west and north-east facing slopes at an altitude of 10 – 180 m above sea level (Edge, 2005a). The Pezula site on the PGE (Figs. 4-2, 4-4 & 4-5), fits this description and covers an area of 130,000 m^2 (0.13 km^2). The initial study was focused on surveying the vegetation, ant communities and abiotic factors along this slope to examine why, out of the whole Pezula area, the butterfly colony only covered a small (~500 m^2) area at P1.

When the C1 colony was discovered in January 2012 east of Coney Glen beach, the Coastal study site was selected. Due to the area being bound to the north by a steep cliff, the south by the ocean and the west and east by rocky outcrops and a stony beach respectively (see Figs. 4-3, 4-6 & 4-7), the Coastal site, at around 2,000 m^2, was considerably smaller than Pezula. The C1 colony covers some 550 m^2 of this area.

The straight line distance between the two study sites at Pezula and the coast, is only about 4 km, however the two sites are markedly different in terms of altitude, aspect, slope, microclimate, soil composition, vegetation, floristic composition and structure (see Figs. 4-4 to 4-7).

Figure 4-4: **View of the *T. b. brachycerus* colony site P1 on the Pezula Golf Estate facing north-west. (Photo: Dave Edge)**

Figure 4-5: **View from the *T. b. brachycerus* colony site P1 on the Pezula Golf Estate facing north. (Photo: Dave Edge)**

Figure 4-6: View from above the *T. b. brachycerus* colony site C1, east of Coney Glen beach facing south. (Photo: Chris Leggatt)

Figure 4-7: View of the *T. b. brachycerus* colony site C1, east of Coney Glen beach facing east. (Photo: Mark Williams)

4.2 Materials and Methods

A general description of the habitats in which *T. b. brachycerus* populations occurred and are currently extant was obtained by researching the literature pertaining to the underlying geology, soils, climate (rainfall and temperatures), and vegetation types. These parameters were further defined by measurement and observations of the specific local conditions such as slope, aspect, local substrate conditions, and the extent of disturbance and transformation (if any) from natural conditions for all the sites studied.

The slopes at Pezula and the Coastal site were measured by using an improvised inclinometer described by Edge (2005b). A spirit level was attached to a straight stick and one end placed on the ground, the other end was raised until the spirit level showed it was horizontal. The distance from the ground was measured and used, with the stick length, to calculate the angle of the slope.

Soil samples were taken from both study sites in September 2014. Due to the different areas of the study sites, 5 soil and 1 rock samples were taken at Pezula (three at the top of the slope, one further down and one at the bottom) while one soil and rock sample was taken at the Costal site. The soil samples, of approximately 0.5 kg each, were taken with a soil auger down to a depth of 0.3 m and analysed at Eco Analytica. Soil was tested for the amount of various macro and micro elements, pH and electrical conductivity (EC). A particle size distribution analysis was also carried out on the soil samples. The rock samples were tested for pH, and the amount of quartz and muscovite was measured.

4.3 Results

4.3.1 Geology and soils

The underlying geology of the area is the Peninsula Formation of the Table Mountain Group - quartz sandstones of Ordovician age, overlain at the Pezula site by aeolian sands of late Pleistocene age (Toerien 1979). For location and altitude of the study sites see Figure 2-1 and Table 2-1. The Pezula site is on a north-west facing slope with an inclination varying between 10 and 15 degrees (12.5° average). The substrate is medium grained acidic to neutral regic sands (Mucina and Rutherford, 2006; Fey, 2010) with occasional rocky outcrops.

The coastal sites are on fairly level ground with the highest spring tide mark only 5m to the south. The study site is situated on a littoral terrace (the Swartkops horizon) formed during the Pleistocene around 40–45 000 years ago (Butzer & Helgren, 1972). The extant substrate consists of a jumble of rocky boulders and stones with thin soil cover in places.

The analysis of the soil samples taken (Appendix 4) shows that the macro- and micro- element composition and the pH of the Pezula soil samples does not differ much, except for the high amount of manganese (Mn) and copper (Cu) at the P1 butterfly colony site (sample point PEZ02). Copper was also high at the sample from the bottom of the slope (PEZ13) where individual *T. b. brachycerus* males have been seen in the past (See Fig. 2-1 and Table 2-1). The only real difference between the Pezula samples was that the sample taken at the bottom of the slope (PEZ13) contained significantly higher silt and clay, as would be expected.

The Coastal site soil sample was radically different from the Pezula samples with much higher quantities of all macro-elements apart from phosphate (PO_4) and ammonium (NH_4). Notable differences were the presence of salt minerals originating from sea spray, a much higher pH and electrical conductivity, and a predominance of coarser size fractions, probably because the finer fractions would have been washed deeper into the substrate by percolating sea spray.

With regards to the rock samples, quartz is the main mineral present at both Pezula and the Coastal study site. Unlike the Pezula rock sample there is a significant muscovite content in the Coastal site rock. Muscovite is quite a common mineral and is indicative of either a different sedimentary layer, or possibly of some weathering process by sea water spray having taken place on the coastal site. The higher pH of the coastal rock is a result of the higher muscovite content.

4.3.2 Climate

The climate (Edge, 2005b) is Köppen classification Cfa (Schultze & McGee, 1978), with mean annual precipitation of 750mm, and rainfall in all months, peaking in spring and autumn. Daily maximum and minimum temperatures average 23.3°C and 12.5°C respectively, and the relative humidity averages a high 88%. Prevailing winds are south-easterly during summer and south-westerly during winter, with occasional northerly winds which cause a severe lowering of humidity and increased fire hazard.

At the Coastal site the abiotic conditions are severe with high salt deposition (exposure to salt spray and seawater splash), extremely high insolation, and high levels of UV radiation. Nutrient input by birds, and constant deposition and erosion of fine soil by relentless winds are ecologically critical (Mucina and Rutherford 2006).

4.3.3 Vegetation

The vegetation at Pezula was classified by Mucina and Rutherford (2006) as Knysna Sand Fynbos (FFd 10) (Fig. 4-8), a Critically Endangered vegetation type with almost 70% already transformed and only 5% conserved (Rouget *et al*., 2006). Important taxa mentioned by Mucina and Rutherford (2006) that have been recorded on the site are *Metalasia densa*, *Anthospermum aethiopicum*, *Leucadendron salignum*, *Seriphium plumosum*, *Eragrostis capensis*, *Ficinia bulbosa*, *Heteropogon contortus*, *Ischyrolepis eleocharis*, and *Tristachya leucothrix*. The vegetation has been transformed to some extent by past agricultural practices (e.g. low intensity cattle grazing) and artificial fire regimes (too regular burns to promote grazing in earlier years and then exclusion of fire since the mid-1990s) (Edge, 2005a).

The vegetation at the Coastal site is Cape Seashore Vegetation (AZd 3) (Mucina & Rutherford, 2006). A small patch of this vegetation type is found at the study site, between the ocean to the south and the South Outeniqua Sandstone Fynbos (FFs 19) to the north. The study site is the south coast variant (Lubke, 1998) with rocky marine shelves below rocky cliffs. Important taxa mentioned by Mucina and Rutherford (2006) that have been recorded on the site are *Pelargonium capitatum*, *Tetragonia decumbens*, *Gazania rigens*, *Carpobrotus edulis*, *Limonium* sp. nov. and *Lobelia anceps*. The site has been traversed regularly by fishermen and this has caused some littering; otherwise it is in natural condition.

4.4 Discussion

From the description of the study sites it is evident that the two regions where *T. b. brachycerus* occurs are markedly different in many respects, indicating that the butterfly can adapt to various biotic and abiotic conditions. There is therefore no clear indication from the description of the two study sites as to why the colonies do not expand.

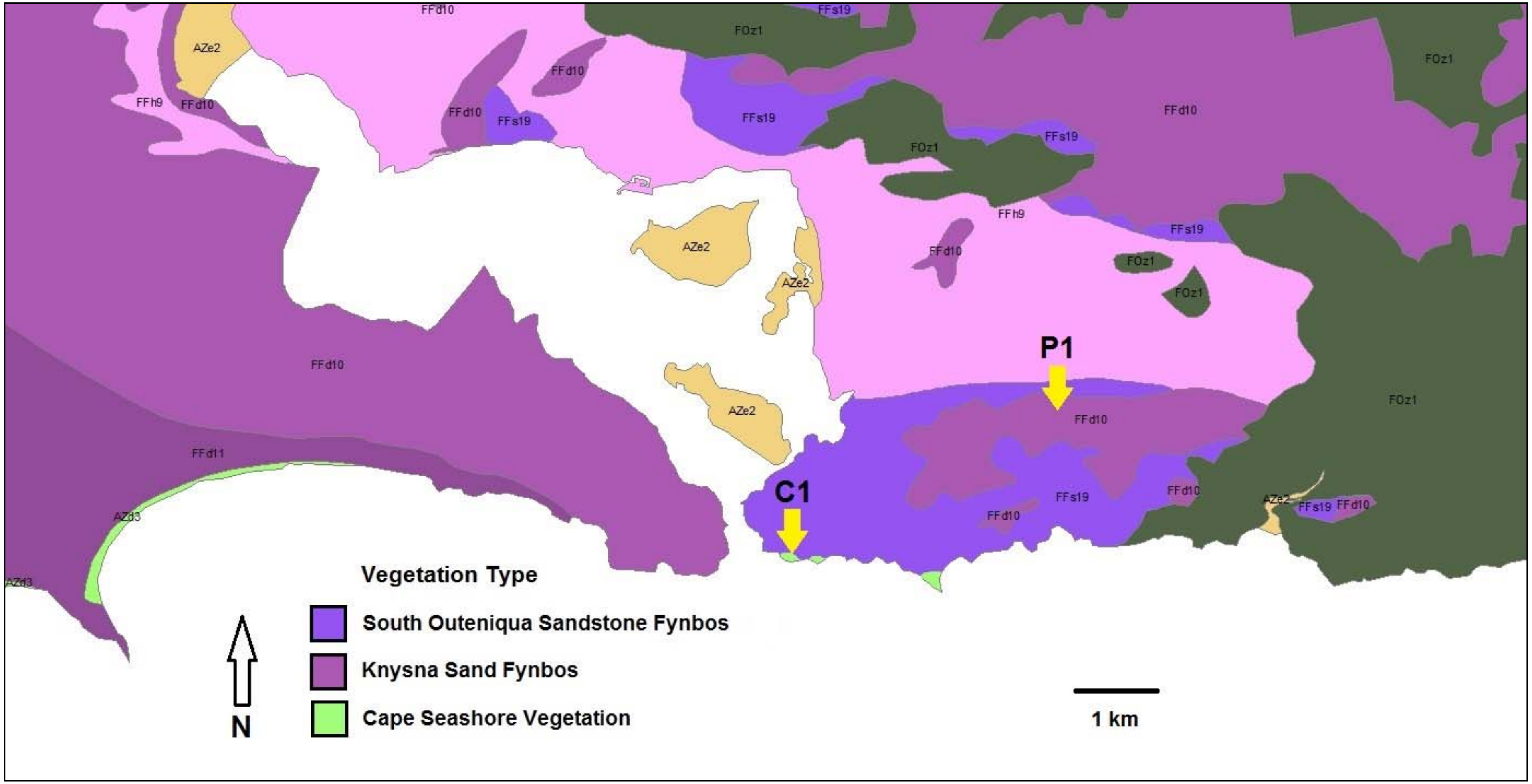

Figure 4-8: Vegetation map of the Knysna Heads area with Pezula (P1) and Coastal (C1) *T. b. brachycerus* colonies. The Pezula colony, P1 is situated on Knysna Sand Fynbos (FFd 10) and the Coastal site colony C1 is on a small patch of Cape Seashore Vegetation (AZd 3). Adapted from Mucina & Rutherford, (2006).

Chapter 5 Adult behaviour of *T. b. brachycerus*

5.1 Introduction

There has been much debate as to the relevance of including behavioural studies in research aimed at conservation; historically, few journals have included papers combining both subject matters (Sutherland, 1998: Angeloni *et al*., 2008). According to Buchholz (2007), it is obvious that species-typical patterns of animal movement, feeding and mating should be important in conservation planning, but that many conservation biologists believe animal behaviourists work at scales of minor value to protecting entire landscapes.

Sutherland (1998) suggests that animal behaviour research should be used to contribute to solving conservation problems and suggests, *inter alia*, that although small populations decline and go extinct for many reasons, some of these are behavioural. This may be true for individual *T. b. brachycerus* populations which, although not accurately measured (see 3.3), appear to be small. However Caro, (2007) argues that advances in understanding animal behaviour have made little practical contribution to conserving animal populations in the decade prior to 2007. Conversely there are incidences of descriptive behavioural information augmenting solutions to specific conservation problems (Caro, 2007).

Dennis *et al*. (2006) stress the importance of paying more attention to butterfly behaviour and resource use as the keys to understanding how landscape is exploited, leading to successful conservation at the landscape scale. They criticise current vegetation-based definitions of habitat and suggest that habitat is better understood in terms of resource distributions which lies at the root of life history strategies. This is particularly relevant for completely aphytophagous butterflies such as *T. b. brachycerus*. Since the life cycle of *T. b. brachycerus* has not yet been fully described, adult behaviour observations are considered to be an important initial step in determining the habitat and resource requirements.

5.2 Materials and methods

The behaviour of adult *T. b. brachycerus* was observed during 40 site visits to P1 from December 2009 to January 2014, and 12 visits to C1 from January 2012 to January 2014, and recorded on data sheets (Fig. 5-1). A butterfly count (see 3.2), butterfly gender, male territorial behaviour, mating, female oviposition, and other behaviour were all recorded. The sightings were abandoned if the weather became too cloudy, cold and windy or if it started to rain, since the butterflies then disappeared or became inactive. The behavioural aspect was concentrated on during the initial stages of the research, particularly the first season of study at P1. In the following seasons the focus shifted to searching for more populations. No attempt was made to statistically analyse the behavioural events between P1 and C1 due to the relative lack of observations at C1.

OBSERVATIONS – *Thestor brachycerus brachycerus* Trimen					
DATE			OBSERVER		
TEMP °C			WIND		
TIME	LOCATION	♂/♀	BEHAVIOUR		

Figure 5-1: Data sheet for observations of *T. b. brachycerus* adults

After a few visits to observe the colony, it became apparent that male *T. b. brachycerus* butterflies were found to perch in one place for long periods of time, and would return to the same spot day after day. When a male butterfly was found to return regularly to the same specific “perching site” that point was marked with a bamboo cane, labelled and coordinates taken with a Garmin® eTrek Legend GPS. The next time the study site was visited, in addition to systematically patrolling the colony area for new perching sites, the existing perching sites were visited and the presence or absence of a male butterfly noted. The plant the butterfly perched on was recorded and the height above ground estimated to the nearest 5cm.

Behavioural observations were enhanced by the use of Olympus® 8 x 21 DPCA binoculars. The duration of mating events was recorded with a Polar® digital watch with a stopwatch function. Females that were laying eggs were followed until sight of the butterfly was lost. Due to the speed at which the female laid her eggs, oviposition sites were initially marked with pre-numbered and dated masking tape which was wrapped around the stem of the plant close to the oviposition site. This process was much easier with two researchers (one to mark the previous site and the other to watch the female for her next site), although this was not always possible. After the female had finished laying or flew out of sight, the marked plants were examined and the specific plant, the egg position, and the height above ground noted. GPS coordinates of the oviposition sites were recorded.

During the Dec 2009/Jan 2010 season 33 eggs were collected from P1 and examined every day until they hatched, in order to estimate the incubation period and attempt to record first instar larvae. Photographs of an ovum were taken with a FEI Quanta 250 environmental scanning electron microscope, operating at 10kV under high vacuum. In later seasons at both the P1 and C1 colony sites the ova were left *in situ* and studied on the plant.

During the course of the first season (Dec 2009 – Jan 2010), paths were inadvertently trampled in the vegetation by walking the same route within the P1 colony area during observations.

5.3 Results

5.3.1 Adult male territorial behaviour

Male *T. b. brachycerus* butterflies exhibited territorial behaviour and spent long periods of time perched in one spot, which they returned to over a number of subsequent days. At P1 the perching sites selected by males were usually low, dead plant material whereas at the C1 site perches were mostly on *Stenotaphrum secundatum* or bare rock. During the study period, over five seasons, a maximum of seven perching sites at any one time, each containing one male, were identified within the P1 colony. At the C1 site a maximum of 5 perching sites were observed on a single day.

When perching sites were marked with a stake, a male could be found at these spots for a number of subsequent days. It was hypothesized that the same individuals returned to the same sites each day. There was no way of confirming that it was the same males at each perching site, although evidence supporting this came from observing a male with a distinctive tear in its wing several days running. The majority of P1 males (85%) chose dead branches over green live plants, to perch on, which afforded them better camouflage. The maximum perch height was 50cm, while the minimum was ground level and the mean height male butterflies perched at was 17.3 cm. The two active perching sites with the closest proximity to each other were 2.6 m apart.

At the less visited C1 site, perches were on *S. secundatum* (64%), bare rock (21%) or other plants (14%) and were lower to the ground compared to P1 with an average height of 12.1 cm above the ground.

Territorial males were observed leaving their perching sites to engage in aerial interactions with conspecific male and female butterflies. These interactions involved the butterflies circling around one another often whilst moving away from the perching site. These non-contact interactions resulted in one butterfly returning to the perching site after chasing the other one off. It was not possible to identify which butterfly returned to the perching site, the original territory holder or the challenger. Territorial battles where the two males would fly in circles around each other were observed on 21 occasions at P1 (out of 40 site visits) and 6 times (out of 12 site visits) at C1.

The butterfly's behaviour may have been affected by the paths made at the P1 colony, as males appeared to select perch sites in the areas that were trampled.

5.3.2 Adult mating behaviour

When a female approached a territorial male a courtship dance would ensue, during which the butterflies would whirl around each other for a few minutes before settling together on a plant. The courtship dance was not always followed by mating. Copulation was observed on seven occasions (five times at P1 out of 40 site visits and twice at C1 during 12 site visits). During copulation the male and female positioned themselves alongside each other; the male curved his abdomen to the female's abdomen so that they were joined (Fig. 5-2).

Figure 5-2: ***T. b. brachycerus*** **adults mating (female on the left) (Photo: Steven Seiler)**

Out of the seven mating events, from the two that were observed from initiation, copulation lasted between three and four minutes. After copulation, the male flew away while the female remained motionless. On one occasion the female was watched until, after 63 minutes, she flew to a plant 40 cm away and appeared to be preparing to oviposit. It was thought that oviposition took place immediately, but this could not be confirmed as the female disappeared from sight.

5.3.3 Adult female oviposition behaviour and sites

T. b. brachycerus females were observed laying a total of 87 eggs on 17 different occasions at P1 and 31 eggs on 5 occasions at C1. All the observations of oviposition occurred on plants, but the species, part of the plant and whether it was dead or alive varied. Oviposition was very rapid, eggs were laid singly (n = 118) and after a second or two the female would fly a short distance to another plant and lay again. On average a female laid 5 eggs before she was lost sight of. Attempts to relocate the female were unsuccessful, and although on two occasions a laying female was found during the same survey session, it was impossible to ascertain whether it was the same female that had been laying earlier or another one.

A list of plant species where oviposition was confirmed at P1 and C1 can be seethe n in Table 5-1 and Table 5-2. There is no overlap in plant species on which eggs were observed at sites P1 and C1.

Table 5-1: Frequency of *T. b. brachycerus* oviposition events per plant taxon showing the average heights laid and the total number of eggs laid at the Pezula colony (P1) between December 2009 and January 2013.

Plant taxon	Family	Average height laid (cm)	No. Eggs laid	Proportion (%)
Leucadendron salignum P.J. Bergius	Proteaceae	75	25	28.7
Searsia pyroides var. *pyroides* (Burch.) Moffett	Anacardiaceae	50	18	20.7
Seriphium plumosum (L.) Thunb.	Asteraceae	55	18	20.7
Erica canaliculata Andrews	Ericaceae	60	10	11.5
Helichrysum cymosum cymosum (L.) D.Don	Asteraceae	50	10	11.5
Anthospermum aethiopicum L.	Rubiaceae	40	3	3.4
Conyza scarbrida DC.	Asteraceae	30	1	1.1
Helichrysum petiolare Hilliard & B.L. Burtt	Asteraceae	5	1	1.1
Restio triticeus Rottb.	Restionaceae	10	1	1.1
Total			**87**	**100**

Table 5-2: Frequency of *T. b. brachycerus* oviposition events per plant taxon showing the average heights laid and the total number of eggs laid at the coastal colony (C1) between December 2011 and January 2014.

Plant taxon	Family	Average height laid (cm)	Eggs laid	Proportion
Stenotaphrum secundatum (Walter) Kuntze	Poaceae	5	11	35.5
Indigofera porrecta var. *porrecta* Eckl. & Zeyh.	Fabaceae	5	7	22.6
Limonium scabrum var. *scabrum* (Thunb.) Kuntze	Plumbaginaceae	10	5	16.1
Delosperma patersoniae (L.Bolus) L.Bolus	Aizoaceae	5	3	9.7
Gazania rigens var. *uniflora* (L.f) Roessler	Asteraceae	5	2	6.5
Maytenus procumbens (L.f.) Loes.	Celastraceae	30	2	6.5
Hypoestes aristata var. *aristata* (Vahl) Sol. Ex Roem. & Schult.	Acanthaceae	15	1	3.2
Total			**31**	**100**

The majority of eggs were laid on the underside of plant leaves, however oviposition did also occur on the plant floret or the stem (Fig. 5-3). Up to three eggs were laid on the same plant but usually the female would move to another plant after having laid only one egg. The eggs were laid between 10cm and 130cm from the ground at P1 and between 5cm and 75cm at C1.

From 33 ova collected from the P1 colony at Pezula during the Dec 2009/Jan 2010 season, 63.6% failed to hatch under artificial conditions. Of the remaining 12 ova the mean incubation period was 12.75 days. During the Dec 2011/Jan 2012 season attempts were made to calculate the incubation period from ova left in position on the plant. This was unsuccessful due to the time it took to relocate the ova on the plant and the fact that it wasn't possible to visit the study site every day. Out of a total of 31 ova, 22 hatched, 3 failed to hatch, while 6 could not be found. The mean incubation time (where it was calculable) was 11.7 days (n=8).

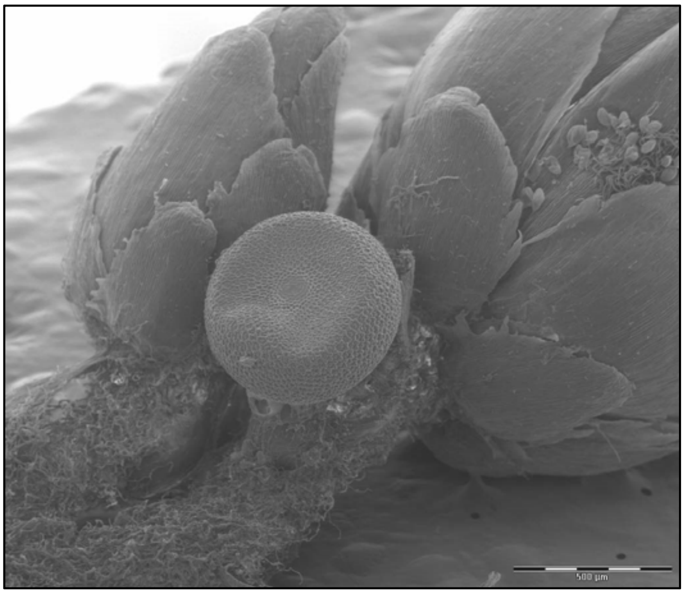

Figure 5-3: ***T. b. brachycerus* ovum at the base of a floret. Image produced with a FEI QUANTA 250 ESEM™, operating at 10 kV under vacuum (Photo: Dr. L.R. Tiedt)**

On five separate occasions an adult female *T. b. brachycerus* was observed climbing to the top of a plant stem, pausing regularly along the way for a few seconds to drum its foremost pair of legs against the stem. Once it reached the top it flew to midway up another stem and repeated the process. On one occasion this was repeated on four different plant stalks within a 1 m radius for between 2 and 11 seconds before flying to another plant and repeating. Over a period of 7 minutes this behaviour was observed on 25 plant stems: *Restio triticus* (4), *Helichrysum* spp. (11), *Struthiola hirsuta* (4) and *Erica canaliculata* (4), dead plant stems (2). After this the female perched at the top of an *Erica* stem with its back to the sun with closed wings. Ants were observed moving up and down the plant at the same time on four of the stems, although the specific ant species was not ascertained by taking a sample. This behaviour was only observed in females and although it did not occur during egg laying, it is thought to be related to the oviposition process.

5.3.4 Predation and predator avoidance behaviour

Predation on *T. b. brachycerus* adults was observed on two occasions out of 48 site visits. At the P1 colony several attempts at predation by Karoo Prinia [*Prinia maculosa* (Boddaert)] (Aves: Passeriformes) were witnessed but only one of these was successful. The Karoo Prinia were very vocal, calling intermittently while foraging in the lower strata of fynbos and on the ground. *T. b. brachycerus* adults became motionless when Karoo Prinia were calling nearby. The only successful attempt at predation occurred when the Prinia remained still and silent on the ground watching the movement of the butterfly and then caught it as it flew past.

At the C2 colony an adult *T. b. brachycerus* butterfly was captured by a Robber fly (Diptera: Asilidae). The Robber fly caught its prey while it was perched on a bare rock and then carried it to a number of plants before flying off with it (Fig. 5-4).

Figure 5-4: Predation of *T. b. brachycerus* by Robber fly (Diptera: Asilidae (Photo: Steven Seiler)

5.4 Discussion

5.4.1 Territorial behaviour

Male butterflies display a diversity of mate-locating strategies (Rutowski, 1991). The two commonly used methods used by male butterflies for mate location are patrolling and perching (Bergman *et al*., 2007). The evidence from this study indicates *T. b. brachycerus* males employ the latter method, as individual males at both the P1 and C1 colonies were seen consistently returning to their respective perching sites within the colony. When disturbed, the male would fly a short distance (~ <10 m) before returning to the perching site. Little information has been published on mating systems in *Thestor* species although male *T. basutus capeneri* butterflies have been reported as establishing and defending territories and exhibiting perching behaviour

(Williams & Joannou, 1996). Williams (1994) describes *T. b. brachycerus* as being territorial. The perching behaviour and defence of those perches displayed by *T. b. brachycerus* males supports this view.

Butterflies that adopt perching as a strategy have been shown to have higher mating success than patrolling ones (Wickman, 1986). Given that the adult *T. b. brachycerus* butterflies do not feed, energy conservation and speedy reproduction might be important factors determining breeding success. In experiments on the effects of distance and perch local on visual mate detection in the territorial male butterfly *Asterocampa leilia* Edwards, 1874 (Nymphalidae family) Rutowski *et al*. (2001) found that at distances of under 2 m males perched on the ground were more likely to detect conspecifics than males perched off the ground. In this study, male butterflies were seen perching at ground level on five occasions at P1 and three times at C1, although the mean heights of the male *T. b. brachycerus* perch site were 17.3 cm and 12.1 cm above the ground respectively. This might explain why the paths trampled at the P1 colony site seemed to attract perching males. Temperature and thermoregulation also play a role in optimal perch height, (Wiklund, 2003) which changes throughout the day so prevailing conditions should be considered in any future behavioural studies.

The two active perching sites with the closest proximity to each other were 2.6 m apart at the P1 colony. Rutowski *et al*. (2001) determined that *A. leilia* males, using the sit-and-wait strategy, did not detect conspecifics from more than 3m away. It is possible that the *T. b. brachycerus* males were unaware of each other at 2.6 m apart or this distance was great enough for them to tolerate the presence of each other.

The male territorial behaviour observed for *T. b. brachycerus* is very similar to that observed by Takeuchi & Imafuku (2005) with another lycaenid *Chrysozephyrus smaragdinus* (Bremer, 1861), a Green Hairstreak. They also recorded mating between territorial males and visiting females, but reported that such males usually lost their territory after mating. In a follow-up study Takeuchi & Honda (2009) found that ownership of a territory conferred an advantage to the owner during territorial contests, and they inferred that males appearing earlier in the season had an advantage over later emerging males.

From the results it was not apparent for how long a territory was held, and the life span of the adult *T. b. brachycerus* is as yet unknown. It was also not apparent whether freshly emerged males selected new perching sites or took over those of deceased males. Territorial battles where the two males would fly in circles around each other were observed on 21 occasions at P1 and 6 times at C1, after which one male would invariably return to the original perching site. Previous studies on the lycaenid, *C. smaragdinus*, have indicated that the male that ceased the circling flight first was the loser (Takeuchi & Imafuku, 2005). The view that the resident usually wins these contests is commonly held (Stutt & Willmer, 1998; Bergman *et al*., 2007). In experiments it was found that in the lycaenid butterfly *C. smaragdinus* residency period played a significant role, as males that had occupied a contested territory for a day or longer always defeated those that had held territories for only 15 minutes (Takeuchi & Honda, 2009). Additionally, Bergman *et al*. (2007) found that resident males of the Speckled Wood butterfly [*Pararge aegeria* (Linnaeus, 1758)] successfully mated almost twice as many times as non-residents in a territorial mating system. There might also be a clear advantage for *T. b. brachycerus* males that hold territories. The majority of males at P1 chose dead branches, rather than live plants to perch on, which afforded them better camouflage. Additionally by basking at perch sites, thereby raising body temperature, males might have more success defending their territories than challenging males that might have cooled down flying around the colony, as seen by Stutt and Willmer (1998) with *P. aegeria*.

5.4.2 Mating

In their territorial, courtship and coupling study of the miletine butterfly *Spalgis epius* (Westwood, 1851), Dinesh & Venkatesha (2013) claim that no information is available on the mating behaviour of any other carnivorous lycaenid species. The mating behaviour of *T. b. brachycerus* has not been previously described, and specific details have not been published for any other *Thestor* species. The two occasions that copulation was observed from initiation it lasted for 3-4 minutes before the butterflies separated and the male took flight. The male left shortly after the other five mating events that were discovered after mating had started. According to Wiklund (2003) it is normal for one of the pair to fly away after copulation and the sex of that partner is species specific. The copulation duration of 4 minutes is relatively short; a low of 10 minutes was reported by Wickman (1985) for *Coenonympha pamphilus* (Linnaeus, 1758) (Nymphalidae: Satyrinae) and it has been reported as lasting for up to a week in *Gonepteryx rhamni* (Linnaeus, 1758) (Pieridae) (Labitte, 1919).

It was not determined whether *T. b. brachycerus* females mate with more than one male during her lifetime. Wang *et al*. (2005) found that in the Diamondback moth [*Plutella xylostella* (Linnaeus, 1758)], there was no significant correlation between copulation duration and number of times a female mated.

The life expectancy of *T. b. brachycerus* is unknown but undoubtedly short given that the adults do not appear to feed at all. A brief copulation duration would be expected in a butterfly that does not feed, since time is of a premium. Females have been recorded to become unreceptive for a number of days after mating (Wiklund *et al*., 2003), and it might follow that there is not enough time for the *T. b. brachycerus* females to mate more than once. The frequency of mating events observed (7 occasions during a combined total of 52 site visits to P1 and C1) is not sufficient to suggest that multiple female mating takes place, however further data should be collected to verify this. There are advantages to multiple mating in that it provides better genetic mixing in small populations and females may receive nutrients from the male sperm which enhances their fecundity and longevity (Wicklund *et al*., 1993, 1998).

5.4.3 Oviposition behaviour and sites

Oviposition site has a significant influence on the survival of the caterpillars, particularly in butterflies whose larvae are specialist parasites, so it is likely that choice of oviposition site reflects larval requirements (Wynhoff *et al*., 2008). This view is supported by Doak *et al.* (2006) who found larval survivorship to be significantly influenced by ovipositing females, since larvae do not usually travel far from their natal locations.

Most butterfly larvae are phytophagous and vision as well as plant chemical signals are thought to play an important role in adult females finding oviposition substrates (Rutowski, 2003). In addition to vision, olfactory, gustatory and tactile senses play a part and these stimuli have been reviewed and summarised by Otto (2014). Larvae of the sub-family Miletinae are entirely aphytophagous (Pierce *et al*., 2002), so it is unlikely that the adult female butterflies use plant derived cues. Females of a number of myrmecophilous lycaenids, many of which have obligate ant associations, use ants as oviposition cues, but ant-independent oviposition also seems to occur in one obligatory associated species of *Maculinea* (Atsatt, 1981), and in *O. niobe* (Edge, 2005b).

Many genera of Miletinae have been recorded laying eggs among or near hemipteran prey, but little is known of the cues that lead to such oviposition. Adult lycaenids have been known to feed on hemipteran honeydew (Hinton, 1951; Pierce, 1995) and it has been suggested that some adults of the aphytophagous Miletinae subfamily may derive nutrients and moisture from ant-guarded hemipteran honeydew or extra-floral nectary sources (Pierce, 1995; Lohman & Samarita, 2009).

Cottrell (1984) suggested that hemipterophagy in the subfamily Miletinae, could have had its origins in the adult habit of feeding on hemipteran honeydew. If this is the case, it might then be expected that oviposition is dependent upon hemipteran cues rather than ant-derived cues. Alternatively, ant-derived cues in *Thestor* species may represent an evolutionary advance from the hemipteran derived cues used in other genera (Williams & Joannou, 1996).

Other *Thestor* species females have been reported to lay their eggs among or near colonies of hemipterans (including scale insects) that are tended by the aggressive pugnacious ant, *A. custodiens* (Williams, 1994). From field observations it was concluded by Williams & Joannou

(1996) that the cue for oviposition of female *T. basutus capeneri* appeared to be ant-derived. Furthermore Williams (2006) observed oviposition in females of *Thestor murrayi* Swanepoel, 1953 and reported that *A. custodiens* ants were invariably present within 1 metre of all oviposition sites. However, captive rearing of 17 *Thestor* species has demonstrated that *Thestor* females will oviposit on almost any surface without plant material, ants or Hemipterans being present (Williams & Joannou, 1996; Heath & Claassens, 2000).

Female *T. b. brachycerus* were observed laying on a wide variety of plants (both living and dead), and at varying heights above ground level (Tables 5-1 and 5-2). There do not seem to be any obvious oviposition cues – the laying of eggs is swift, with apparently no single plant preference for chosen sites, and no evidence of hemipteran presence at any of these sites. This behaviour is similar to observations on oviposition of *T. murrayi*, where females searched for oviposition sites in a seemingly aimless manner and laid eggs singly on a diverse number of plants, small rocks and stones (Williams, 2006).

The speed of oviposition may well enhance fitness by rendering the females less vulnerable to predation while ovipositing (Doak *et al*., 2006). Indeed, Williams and Joanou (1996) observed *T. b. capeneri* females being attacked by *A. custodiens* ants as they attempted to lay an egg. Although *T. b. brachycerus* was observed laying eggs very swiftly, no evidence of attack from ants was witnessed.

Otto (2014) suggests that a butterfly will oviposit on an alternative plant or object, other than its true larval host plant (LHP), under certain circumstances and that repeat oviposition observations are required to determine the LHP. Tables 5-1 and 5-2 show that there is no preference for one single plant species, (neither within each colony, nor between the two colonies), which supports the view that the larvae of *T. b. brachycerus* are completely aphytophagous.

From Table 5-1 it seems that there might be a positive correlation between the number of oviposition events and the height of oviposition site on the vegetation. 93.3% of ova were seen being laid 50-75 cm from the ground, compared with only 6.7% laid below 50 cm above ground. This could demonstrate a preference for taller vegetation or simply be observational bias. Lower oviposition events may have been less visible due to lower strata dense vegetation. It is difficult to see how larval fitness for survival is promoted by laying eggs far above the ground if the larvae need to be quickly adopted by ants and taken to their nest in order to survive. It is possible that the larvae can drop safely to the ground, or they may be detected by ants soon after hatching and carried down.

The data in Table 5-2 suggests that there is no correlation with frequency of oviposition events and plant heights at the coastal colony, C1. The average height of oviposition site was substantially lower (~ 10 cm) compared to the average height at the Pezula colony, P1 (~ 40 cm). This is no surprise since the vegetation at the coastal colony is substantially shorter than that at the Pezula colony (see Fig. 6-3 *vs*. Fig. 6-4).

Females were observed on five separate occasions possibly trying to detect ants (or Hemipterans) but no oviposition took place on these occasions. Behaviour similar to that noted, involving a female landing midway up a plant stem and climbing to the top moving its foretarsi, has been described before in neotropical euptychiines. When searching for oviposition sites *Euptychia hermes* Fabricius, 1775 (Nymphalidae: Satyrinae) alights on various plant species, frequently "drumming" them with their fortarsi (Singer, 1984). Furthermore, it has been demonstrated by Calvert & Hanson (1983), that chemoreceptors in setae located on the foretarsi have been used by drumming for host verification prior to oviposition in the nymphalid, *Chlosyne lacinia* (Geyer, 1837).

However, since the females observed carrying out this behaviour were not observed to oviposit directly afterwards, it might be unrelated to oviposition and to do with nutrition. Scott (1983) suggested that although the Miletinae adult, *Feniseca tarquinius* (Fabricius, 1793) possesses a proboscis too short to feed on nectar it is well adapted to imbibing aphid honeydew, dung and sap. Whether this is the case for members of the *Thestor* genus is as yet unknown, but there are no substantiated field observations of *Thestor* species adults feeding (Williams and Joanou, 1996).

All these observations collectively reveal a complex suite of oviposition behaviours which we need to understand better in order to achieve conservation success, as was the case with the Mardon Skipper in Washington State (Henry & Schultz, 2013). Since the ovipositon data came from observations of females who were in the middle of laying it was not possible to calculate fecundity. More workers would be required to follow an ovipositing female to determine how many ova she laid in total.

Current knowledge regarding the trophic resources utilised by the members of this genus is summarised in Table 1-1 and Appendix 1. *T. b. brachycerus* belongs to the *brachycerus* species group of the genus (Heath & Pringle 2004), as do *T. b. dukei*, *T. rileyi* and *T. yildizae*. Whilst there is no evidence as to the food source of the 1st instar larvae in this species group, in two species trophallaxis has been recorded between *A. custodiens* ants and the final instar larvae (Claassens & Heath, 1997). Searches at the *T. b. brachycerus* oviposition sites for Hemipterans such as coccids and psyllids have so far been unsuccessful, but this is not surprising given that the exploitation of this trophic resource has not been recorded in this species group. Further research is urgently required since the conservation of *T. b. brachycerus* cannot succeed until its larval food resource is discovered (Heath *et al*., 2008). Attempts to observe the larvae hatched from the ova collected were unsuccessful. Of the 36.4 % that hatched the first instar larvae appeared to disappear. It is possible that they hatched at night, became rapidly dehydrated and died in the egg but no obvious trace was found.

5.4.4 Predation and predator avoidance behaviour

Predation events and anti-predator behaviour may prove important to the survival of *T. b. brachycerus* since removing one or two individuals from a small population could potentially impact on the breeding success.

The man-made paths within the colony appeared to be utilised by the male butterflies as perching sites and they appeared to prefer perching in areas close to where the vegetation had been trampled and the majority (78%) choose dead plant material as a perch. *T. b. brachycerus* was extremely well camouflaged when perched with closed wings on dead plants and crypsis is often the first line of defence against predation (Vallin *et al*., 2006). The effectiveness of this strategy is supported by the observations that the Karoo Prinia appeared to forage mainly amongst the live plants rather than the dead branches.

The observations at Pezula that the butterflies seem to be avoiding predation by ceasing to fly when the predators are detected has no precedent. It is uncertain whether detection is by sound or sight, but it would be interesting to test this experimentally by playing sound recordings of the Karoo Prinia and seeing whether this triggers the predator avoidance behaviour. Previous work has been carried out regarding insect hearing, particularly in moths, and there is some evidence that nocturnally active butterflies of the Hedylidae family have ultrasound-sensitive ears on their wings to help them avoid bats (Yack & Fullard, 2000). It is possible that *Thestor* butterflies have some type of sensory organ that allows them to detect the bird’s call and recognise it as a threat. Since most butterfly species are diurnal and mute, bat detection can be ruled out as a role for hearing but Lane *et al*. 2008 hypothesize that Vogel's organs in butterflies have evolved to detect flight sounds of predatory birds.

Chapter 6 Vegetation communities at the study sites

6.1 Introduction

In the past vegetation community analysis techniques have been successfully used to define and understand butterfly habitats in South Africa (Deutschländer & Bredenkamp, 1999; Terblanche *et al*., 2003; Edge, Cilliers, *et al*., 2008). These methods can evaluate associations between vegetation types and the occurrence of butterflies or their host ant species. However the butterflies in these studies were phytophagous for some stage of their life-cycle. Since it is hypothesized that *T. b. brachycerus* adults and larvae are aphytophagous, this approach has been slightly modified with additional focus being placed on floristic community structure.

There is no published detailed information regarding vegetation communities associated with the habitats of *Thestor* species.

6.2 Materials and methods

The well-established Braun–Blanquet methodology was used to sample vegetation by establishing relevés (Mueller-Dombois & Ellenberg, 1974; Westhoff & van der Maarel, 1980). At the Pezula site a stratified random sampling design was employed, with the stratification based on slope position and steepness (top – gentle slope; middle – steep slope; bottom – flat). At each slope position five 5 m x 5 m square relevés were placed along an east – west axis (PEZ01–05 – top; PEZ06–10 – middle; PEZ11–15 – bottom) (Fig. 6-1 and Table 6-1). At the Pezula site, the dense vegetation and steepness was a limiting factor in selecting the mid-slope axis. Two relevés (PEZ02 and PEZ03) were situated within the Pezula butterfly colony, P1.

On the smaller coastal site eight relevés (CS01-CS08) of the same size were placed randomly to sample the habitat (Fig. 6-2 and Table 6-1). Four relevés (CS02, CS03, CS04 and CS05) were situated within the Coastal butterfly colony, C1 (Figs. 6-3 and 6-4).

At the Coastal study site the relevés were closer to each other than at the larger Pezula study site.

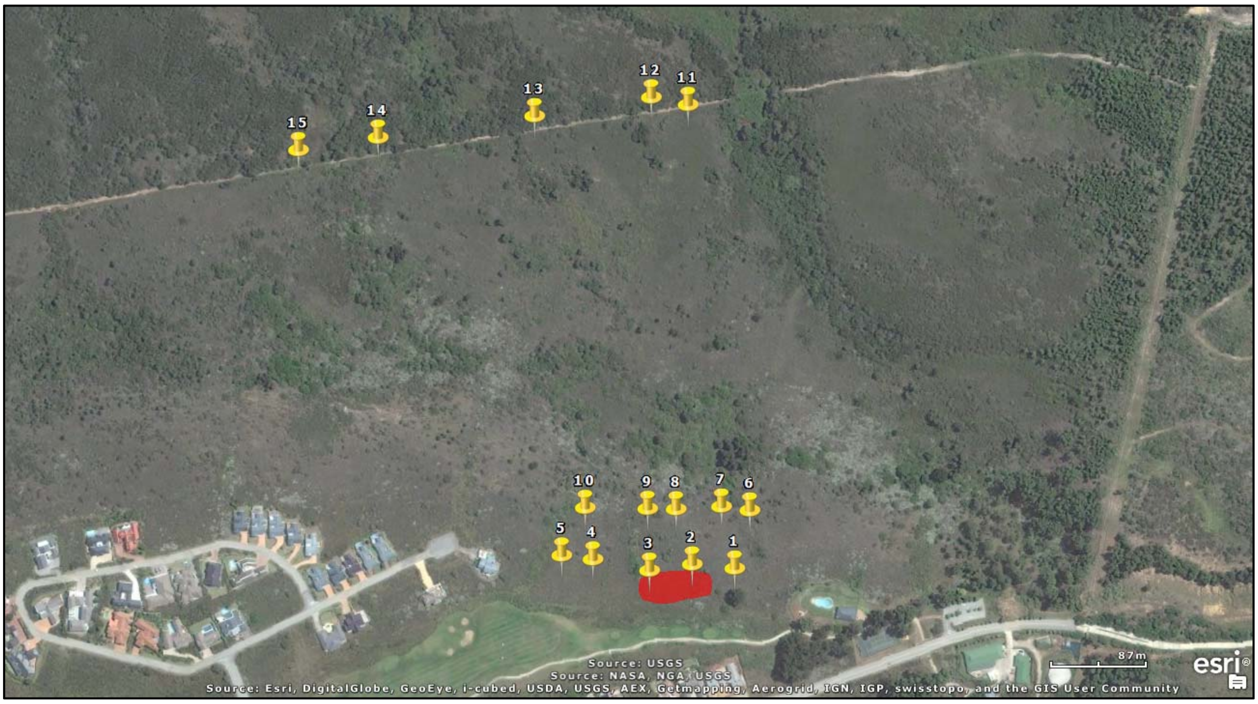

Figure 6-1: **Diagram of the Pezula study size with 15 relevés (PEZ01 – PEZ15) denoted 1-15 on map, with *T. b. brachycerus* colony area in red. Refer to Table 6-1 for relevé GPS coordinates and altitudes.**

Figure 6-2: **Diagram of the Coastal study site with 8 relevés (CS01 – CS08), with *T. b. brachycerus* colony area in red. Refer to Table 6-1 for relevé GPS coordinates and altitudes.**

Table 6-1: Relevés at the Pezula and Coastal study sites with codes, names and coordinates/altitude

PEZ Study Site Coordinates				CS1 Study Site Coordinates			
Relevé	(S)	(E)	Altitude (meters amsl)	Relevé	(S)	(E)	Altitude (meters amsl)
PEZ01	34° 03' 54.3"	23° 06' 04.2"	177	CS01	34° 04' 57.6"	23° 03' 57.3"	21
PEZ02	34° 03' 54.2"	23° 06' 02.7"	173	CS02	34° 04' 58.0"	23° 03' 59.0"	21
PEZ03	34° 03' 54.4"	23° 06' 01.2"	175	CS03	34° 04' 58.0"	23° 03' 59.7"	23
PEZ04	34° 03' 54.1"	23° 05' 59.2"	174	CS04	34° 04' 58.2"	23° 03' 59.5"	17
PEZ05	34° 03' 54.0"	23° 05' 58.1"	175	CS05	34° 04' 58.5"	23° 03' 59.6"	17
PEZ06	34° 03' 52.6"	23° 06' 04.7"	160	CS06	34° 04' 57.7"	23° 03' 59.9"	25
PEZ07	34° 03' 52.5"	23° 06' 03.7"	156	CS07	34° 04' 58.4"	23° 04' 00.3"	20
PEZ08	34° 03' 52.6"	23° 06' 02.1"	155	CS08	34° 04' 58.4"	23° 04' 00.6"	21
PEZ09	34° 03' 52.6"	23° 06' 01.1"	157				
PEZ10	34° 03' 52.6"	23° 05' 58.9"	161				
PEZ11	34° 03' 40.8"	23° 06' 02.3"	78				
PEZ12	34° 03' 40.6"	23° 06' 01.0"	79				
PEZ13	34° 03' 41.2"	23° 05' 56.9"	78				
PEZ14	34° 03' 41.9"	23° 05' 51.4"	77				
PEZ15	34° 03' 42.3"	23° 05' 48.6"	76				

Figure 6-3: Relevé CS03 at the Coastal study site, within the *T. b. brachycerus* colony (C1), looking south (Photo: Chris Leggatt)

Figure 6-4: Relevé CS08 at the Coastal study site, outside the *T. b. brachycerus* colony (C1), looking south-east (Photo: Chris Leggatt)

Vegetation surveys were carried out in December 2011 at Pezula and in March 2014 at the Coastal study site. All plant species present were recorded in each relevé, and if necessary samples were taken for identification. In February 2014 lists were compiled of all the plant species within the *T. b. brachycerus* colonies, P1 and C1. Photographs were taken of each plant species. Plant species identification was accomplished with reference to relevant publications (Manning, 2003; Manning, 2007) and the assistance of botanical experts in the area (see acknowledgements). In difficult cases samples were sent to the Compton Herbarium of the South African National Biodiversity Institute in Cape Town. Valid names were established with reference to Germishuizen & Meyer (2003).

The height of the smallest and largest example of each species of shrub or tree was recorded, and it was classified into a life-form category (fern, graminoid, monocot, herb, climber, shrub or tree). The occurrence and amount of moribund vegetation, bare ground or rock was noted. Abundance of each plant species in the relevé was estimated using the Braun–Blanquet cover-abundance scale, with midpoint values being used for input to the quantitative vegetation analysis.

Vegetation data and relevé characteristics were entered into data sheets using Microsoft® Excel. A separate two-way data matrix for each of the two sites surveyed (Pezula and the Coastal study site) was constructed with plant species as rows and relevés as columns, showing species abundance data (Appendices 6 and 7). Midpoint values were input into the spreadsheets which were uploaded into the Primer® 6 software programme and a number of statistical procedures were carried out for each of the two sites. The α-diversity of each relevé was assessed through the use of the Primer® 6 DIVERSE module – species richness or number of species (*S*), the Shannon-Wiener index (*H'*) and equitability (*J'*) were calculated according the following formulae:

Shannon-Wiener diversity index $H' = - \sum_{i=1}^{S} p_i \, . \ln p_i$

Where s = total number of species and

p_i = the relative abundance of the ith species in the relevé.

H' gives more weight to scarce species and was preferred to the Simpson diversity index, which gives more weight to abundant species (Begon, *et al.*, 1996).

Equitability $J' = \frac{H'}{H'_{max}} = \frac{H'}{\ln S}$

Where H' = the Shannon -Wiener diversity index and H'_{max} = is the maximum value of H'

The one-way analysis of variance (ANOVA) tool from the Analysis ToolPak in Microsoft® Excel, was used to determine if there was any significant difference in α-diversity, H' between the relevés at the top (PEZ01 – PEZ05), middle (PEZ06 – PEZ10) or bottom (PEZ11 – PEZ15) of the slope. A similar one-way ANOVA was carried out to test the difference between the relevés in the P1 colony (PEZ02 & PEZ03) and the relevés outside the colony (PEZ01, PEZ04 – PEZ15). ANOVA was also applied to the Coastal site relevés to see if there was a difference in H' of the four relevés within the colony (CS02 - CS05) compared with the four outside the colony (CS01, CS06 – CS08).

The Bray-Curtis coefficient was used to create a similarity matrix. Following the reasoning of Clarke & Warwick (2001), in order to down-weight the importance of the highly abundant species and take the less abundant species into account, a 4th root transformation (√√) was applied to the species abundance data matrix before the similarities were calculated. Hierarchical clustering with group-average linking based on the Bray-Curtis similarities was carried out to produce dendrograms to show the similarities between the relevés within each study site diagrammatically.

Two ordination methods were used in Primer® 6 to enable depiction of the degree of similarity between relevés and plant species graphically. Non-metric multi-dimensional scaling (MDS) was applied to the √√-transformed resemblance matrices, which graphically represents dissimilarities between the relevés. The classic principal component analysis (PCA) was applied to the original abundance data matrices. In addition to plant species, the abundance of dead plant material, bare ground and rocks was included in the MDS and PCA analysis.

Further analysis was done on the vertical stratification of the vegetation at the Pezula study site. The actual heights measured were used to decide the height class boundaries. From survey information the standardised relative abundances of the plant species in each relevé were classified into height classes (<0.4m, 0.4-0.8m, 0.8-1.2m, 1.2-1.6m and <1.6m) and placed in Microsoft® Excel spreadsheet along with the abundance of bare ground and rocks. This was not repeated at the Coastal study site due to the majority of the vegetation being < 0.4 m in height (see Fig. 6-3 *vs*. Fig. 6-5).

Figure 6-5: Vegetation at the Pezula study site, between relevés PEZ04 & PEZ05, outside the *T. b. brachycerus* colony (P1), looking south-west (Photo: Reinier Terblanche)

6.3 Results

6.3.1 Floristic composition

The most abundant plant taxa found at the Pezula relevés and at the Coastal relevés are detailed in Table 6-2. The only plant taxon common to both sites is *Tarchonanthus littoralis* (Coastal camphor bush) which is not in the top twenty at Pezula, but otherwise the sites are remarkably dissimilar.

Table 6-2: **Top twenty most abundant plant taxa, with family and average height, recorded during vegetation surveys carried out at the Pezula site in December 2011 and at the Coastal site in March 2014. Taxa are listed in order of abundance (greatest to smallest). For plant family abbreviations see Appendix 5.**

Pezula Study Site			Coastal Study Site		
Plant Taxon	**Family**	**Height range (m)**	**Plant Taxon**	**Family**	**Height range (m)**
Seriphium plumosum	Aster.	0.4–1.6	*Stenotaphrum secundatum*	Poa.	0.1–0.3
Erica canaliculata	Eric.	0.3–1.1	*Gazania rigens* var. *uniflora*	Aster.	0.1–0.2
Anthospermum aethiopicum	Rubi.	0.3–1.4	*Delosperma patersoniae*	Aizo.	0.05–0.1
Helichrysum cymosum cymosum	Aster.	0.2–0.9	*Hypoestes aristata* var. *aristata*	Acan.	0.3–0.5
Restio leptoclados	Rest.	0.2–0.4	*Asparagus aethiopicus*	Aspar.	0.2–0.4
Tristachya leucothrix	Poa.	0.2–0.5	*Eriocephalus* species	Aster.	0.2–0.5
Restio triticeus	Rest.	0.4–1.0	*Tarchonanthus littoralis*	Aster.	0.5–0.8
Leucadendron salignum	Prot.	0.4–1.6	*Carpobrotus deliciosus*	Aizo.	0.1–0.2
Searsia lucida var. *lucida*	Anac.	1.4–1.8	*Drosanthemum* species	Aizo.	0.1–0.4
Phylica axillaris	Rham.	0.4–1.2	*Limonium scabrum* var. *scabrum*	Plumb.	0.2–0.6
Cymbopogon marginatus	Poa.	0.4–1.2	*Tetragonia fruticosa*	Aizo.	0.1–0.5
Searsia pyroides var. *pyroides*	Anac.	0.2–1.1	*Chironia baccifera*	Gent.	0.2–0.4
Conyza scrabida	Aster.	0.4–1.7	*Indigofera porrecta* var. *porrecta*	Fab.	0.1–0.3
Pteridium aquilinium	Denn.	0.6–0.8	*Maytenus procumbens*	Celast.	0.3–0.6
Ficinia hirsuta	Cyper.	0.2–0.3	*Passerina rigida*	Thym.	0.4–0.9
Struthiola hirsuta	Thym.	0.4–0.9	*Rhoicissuss digitata*	Vit.	0.05–0.1
Digitaria eriantha	Poa.	0.4–1.3	*Cotula turbinata*	Aster.	0.2–0.3
Helichrysum petiolare	Aster.	0.3–0.5	*Helichrysum teretifolium*	Aster.	0.3–0.6
Senecio rigidus	Aster.	0.5–1.0	*Pelargonium capitatum*	Geran.	0.4–0.5
Heteropogon contortus	Poa.	0.2–0.8	*Pterocelastrus tricuspidatus*	Celast.	0.6–1.2

6.3.2 Species richness and diversity

The species richness (*S*), the Shannon-Wiener index (*H'*) and equitability (*J'*) for the Pezula and Coastal study site relevés can be seen in Table 6-3.

Table 6-3: Plant species richness (*S*), the Shannon-Wiener index (*H'*) and equitability (*J'*) for the Pezula study site relevés (PEZ01 – PEZ15) and the Coastal study site relevés (CS01 – CS08). PEZ02 and PEZ03 (in red) lie within the *T. b. brachycerus* colony, P1 while CS02, CS03, CS04 and CS05 (in red) fall within the *T. b. brachycerus* colony, C1

Pezula Study Site				Coastal Study Site			
Relevé	**S**	**J'**	**H'**	**Relevé**	**S**	**J'**	**H'**
PEZ01	19	0.8176	2.407	CS01	17	0.9664	2.738
PEZ02	15	0.7567	2.049	CS02	14	0.7387	1.95
PEZ03	11	0.6189	1.484	CS03	13	0.6395	1.64
PEZ04	10	0.7601	1.75	CS04	4	0.7822	1.084
PEZ05	9	0.6626	1.456	CS05	8	0.4771	0.9921
PEZ06	12	0.7603	1.889	CS06	12	0.7621	1.894
PEZ07	8	0.5607	1.166	CS07	13	0.8315	2.133
PEZ08	11	0.7162	1.717	CS08	12	0.7657	1.903
PEZ09	9	0.2238	0.4918				
PEZ10	11	0.7104	1.704				
PEZ11	8	0.6303	1.311				
PEZ12	10	0.6803	1.566				
PEZ13	10	0.7988	1.839				
PEZ14	6	0.4875	0.8735				
PEZ15	12	0.6473	1.608				

The results of the one-way ANOVA on the H' indices of the Pezula relevés are shown in Tables 6-4 and 6-5. Since F= 1.375506 is less than the critical F value (F-crit) of 3.885294, the null hypothesis that there is no significant difference between the average Shannon-Wiener (*H'*) values of the relevés at the top of the slope, towards the middle, or at the bottom of the slope should be accepted. There is no significant difference between the plant species in α-diversity in the relevés within the P1 colony and those outside of the P1 colony at the Pezula site, since F= 0.456066 is less than the F-crit value of 4.667193 (Table 6-5).

Table 6-4: Results of the one-way ANOVA on the Shannon-Wiener index (*H'*) values of plant species sampled in the Pezula relevés at the top (PEZ01 – PEZ05), towards the middle (PEZ06 – PEZ10) and at the bottom (PEZ11 – PEZ15) of the slope

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PEZ01 - PEZ05	5	9.146	1.8292	0.16172
PEZ06 - PEZ10	5	6.9678	1.39356	0.32785
PEZ11 - PEZ15	5	7.1975	1.4395	0.135216

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.572931	2	0.286466	1.375506	0.289839	3.885294
Within Groups	2.499144	12	0.208262			
Total	3.072075	14				

Table 6-5: Results of the one-way ANOVA on the Shannon-Wiener index (*H'*) values of plant species sampled in the Pezula relevés in the P1 colony (PEZ02 & PEZ03) and outside the P1 colony (PEZ01 & PEZ04 – PEZ15)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PEZ01 & PEZ04-PEZ15	13	19.7783	1.521408	0.234028
PEZ02 & PEZ03	2	3.533	1.7665	0.159612

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.104122	1	0.104122	0.456066	0.511302	4.667193
Within Groups	2.967953	13	0.228304			
Total	3.072075	14				

The results of the one-way ANOVA on the H' indices of the Coastal site relevés are shown in Table 6-6. F= 6.162806 is greater than the F-crit value of 5.987378 therefore there is a significant difference between the plant species in α-diversity in the four relevés within the C1 colony and the four outside of the C1 colony at the Coastal site.

Table 6-6: Results of the one-way ANOVA on the Shannon-Wiener index (*H'*) values of plant species sampled in the Coastal site relevés in the C1 colony (CS02 – CS05) and outside the C1 colony (CS01 & CS06 – CS08)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CS02 - CS05	4	5.6661	1.416525	0.208415
CS01 & CS06 - CS08	4	8.668	2.167	0.157141

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.126425	1	1.126425	6.162806	0.047644	5.987378
Within Groups	1.096668	6	0.182778			
Total	2.223094	7				

6.3.3 Hierarchical clustering

Resemblance data matrices using Bray-Curtis similarities after a √√-transformation on the abundance data are shown for Pezula (Table 6-7) and the Coastal site (Table 6-8). This shows similarities between pairs of relevés with the value 100 being identical and 0 having no species in common. A graphical display linking relevés that have mutually high levels of similarity is demonstrated in the hierarchical clustering of the similarity matrices, presented in dendrograms for the Pezula site (Fig. 6-6), and the Coastal site (Fig. 6-7).

Table 6-7: Resemblance data matrix using Bray-Curtis similarities after a √√-transformation on the plant abundance data from the Pezula study site relevés. PEZ02 and PEZ03 (in red) lie within the *T. b. brachycerus* colony, P1.

	PEZ01	PEZ02	PEZ03	PEZ04	PEZ05	PEZ06	PEZ07	PEZ08	PEZ09	PEZ10	PEZ11	PEZ12	PEZ13	PEZ14	PEZ15
PEZ01															
PEZ02	52.26990														
PEZ03	45.48606	62.33456													
PEZ04	47.09688	60.12918	61.24682												
PEZ05	41.59883	54.33636	62.27153	81.27829											
PEZ06	43.37021	31.42121	30.60464	41.82222	45.38815										
PEZ07	42.20274	30.74980	40.91580	50.58859	45.99445	47.25511									
PEZ08	50.46254	56.27271	50.97300	63.03584	54.28636	50.67202	56.84079								
PEZ09	36.30213	57.63664	57.48394	56.90977	58.30817	28.29730	31.47135	57.83744							
PEZ10	53.22056	47.89484	56.57639	58.81947	49.98569	37.18366	70.28197	74.79082	47.71700						
PEZ11	43.07259	37.63543	40.20645	61.14057	51.38382	49.98034	51.02174	48.87204	30.65083	45.93458					
PEZ12	38.11006	29.51822	33.89225	37.56866	34.40608	65.36382	47.35151	45.10213	23.65255	45.43472	39.18957				
PEZ13	35.87907	26.68583	19.66614	29.26870	22.56381	18.26894	28.13838	38.00069	28.94953	32.80584	48.61173	13.69803			
PEZ14	20.11878	19.54393	22.55837	26.92821	26.22368	50.42950	26.34489	37.83811	19.66992	29.18462	32.48110	63.96199	16.90350		
PEZ15	21.54350	21.64763	18.74962	22.16270	21.69506	29.46173	18.87153	31.26500	27.52192	24.51977	18.98518	35.64418	11.11459	45.05506	

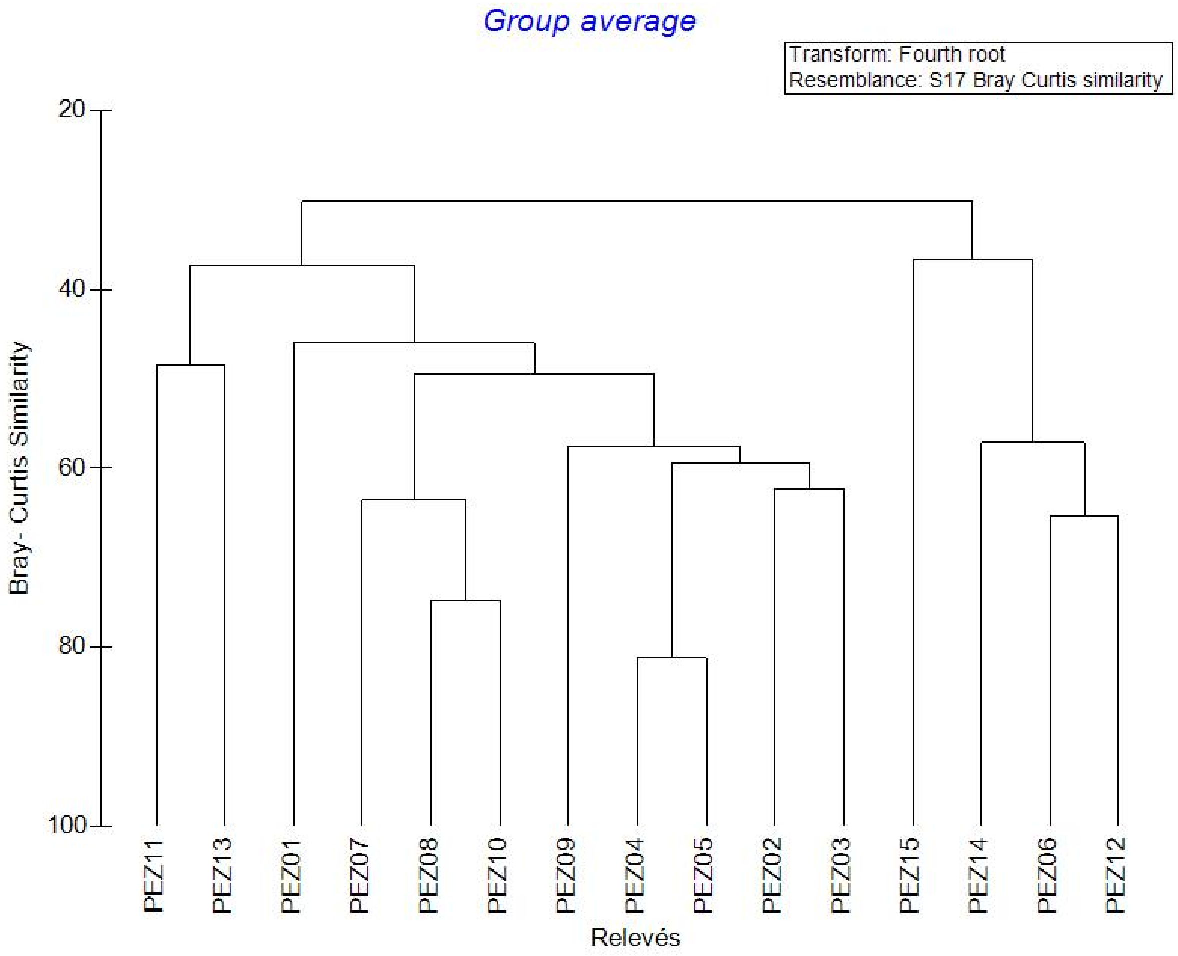

Figure 6-6: **Dendrogram for hierarchical clustering of the 15 relevés at the Pezula site, using group-average linking of Bray-Curtis similarities calculated on √√-transformed plant abundance data. PEZ02 and PEZ03 lie within the *T. b. brachycerus* colony, P1.**

Table 6-8: Resemblance data matrix using Bray-Curtis similarities after a √√-transformation on the plant abundance data from the Coastal study site relevés. The CS02, CS03, CS04 and CS05 (in red) relevés fall within the *T. b. brachycerus* colony, C1

	CS01	CS02	CS03	CS04	CS05	CS06	CS07	CS08
CS01								
CS02	60.32353							
CS03	55.42700	55.55087						
CS04	24.14225	28.77171	26.69756					
CS05	33.12878	40.02131	39.53753	71.67927				
CS06	28.12109	18.43410	28.04432	0.000000	0.000000			
CS07	60.13595	44.73649	53.92385	19.36885	28.88704	41.44080		
CS08	46.56202	53.00750	51.67635	6.625975	11.98273	40.36698	41.76748	

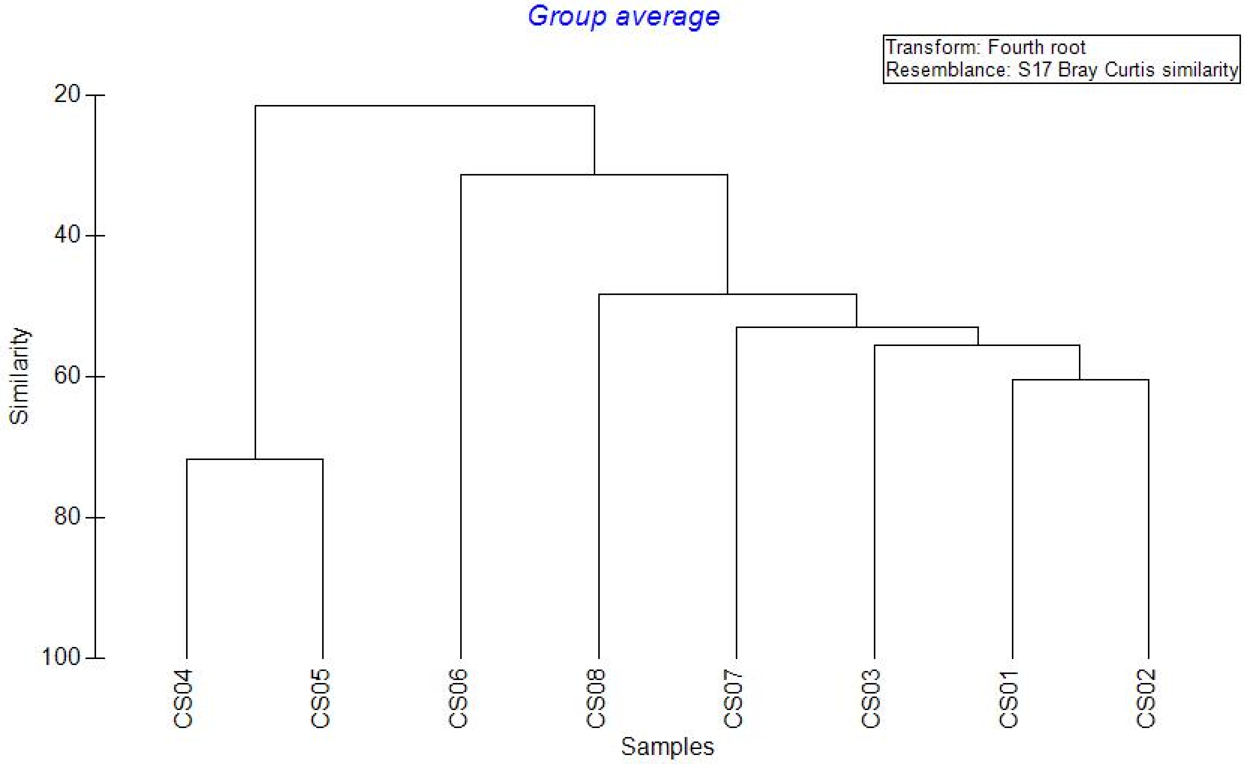

Figure 6-7: Dendrogram for hierarchical clustering of the 8 relevés at the Coastal site, using group-average linking of Bray-Curtis similarities calculated on √√-transformed plant abundance data. CS02, CS03, CS04 and CS05 lie within the *T. b. brachycerus* colony, C1.

6.3.4 Ordinations

The MDS ordinations for the Pezula and coastal study site relevés are presented in Figs. 6-8 and 6-9 respectively, which graphically represent dissimilarities between the relevés in as few dimensions as possible. Clarke (1993) suggests that stress levels > 0.20 is essentially random, < 0.15 is good and < 0.10 is ideal meaning that the configuration is close to the actual dissimilarities.

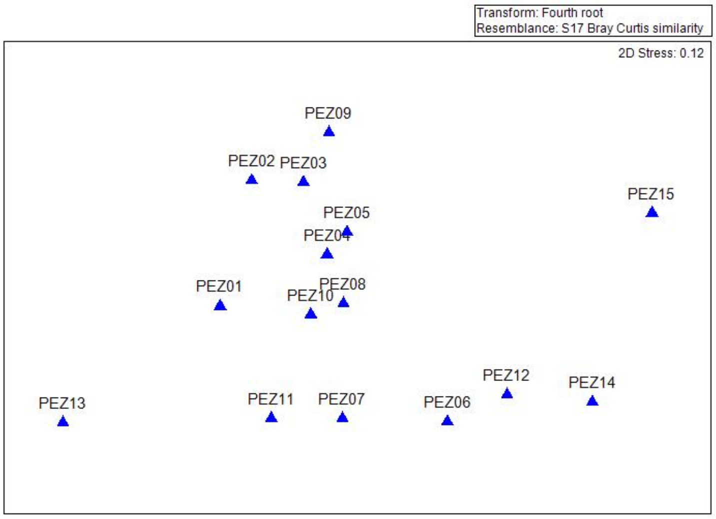

Figure 6-8: **MDS ordination with 15 relevés, 43 plant species and three other variables (bare ground, dead plant material and rocks) at the Pezula study site.**

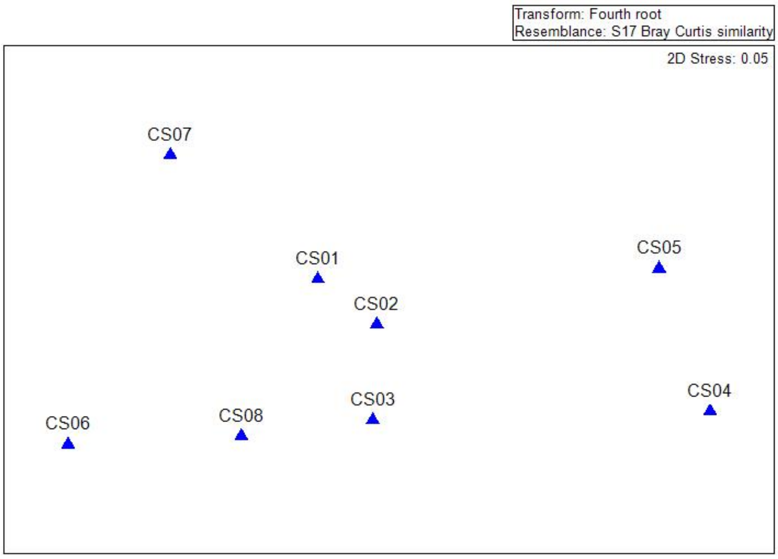

Figure 6-9: **MDS ordination with 8 relevés and 33 plant species at the Coastal study site and three other variables (bare ground, dead plant material and rocks) at the Coastal study site.**

The PCA ordinations for the Pezula study site relevés are presented in Figures 6-10, 6-11 and 6-12. After running the first PCA ordinations for the Pezula relevés (Fig. 6-10) PEZ11 and PEZ14 were considered to be outlier relevés, and were removed from the data set. Both outliers were located at the bottom of the slope. PEZ11 was unique in that it was located in a marshy area which was dominated by *Restio leptoclados* and *Erica canaliculata*, and PEZ14 had *Anthospermum aethiopicum* as the dominant species. After rerunning the ordination (Fig. 6-11) two relevés (PEZ07 and PEZ09) from the middle row were removed from the data set. PEZ07 was dominated by *Restio triticeus* and PEZ09 by *Seriphium plumosum*. The four relevés removed from the analysis each had a dominant plant species with a Braun–Blanquet cover-abundance value of 4 or 5. None of the remaining plots have such high abundance values of any one species. The final PCA ordination for the Pezula relevés is presented in Figure 6-12.

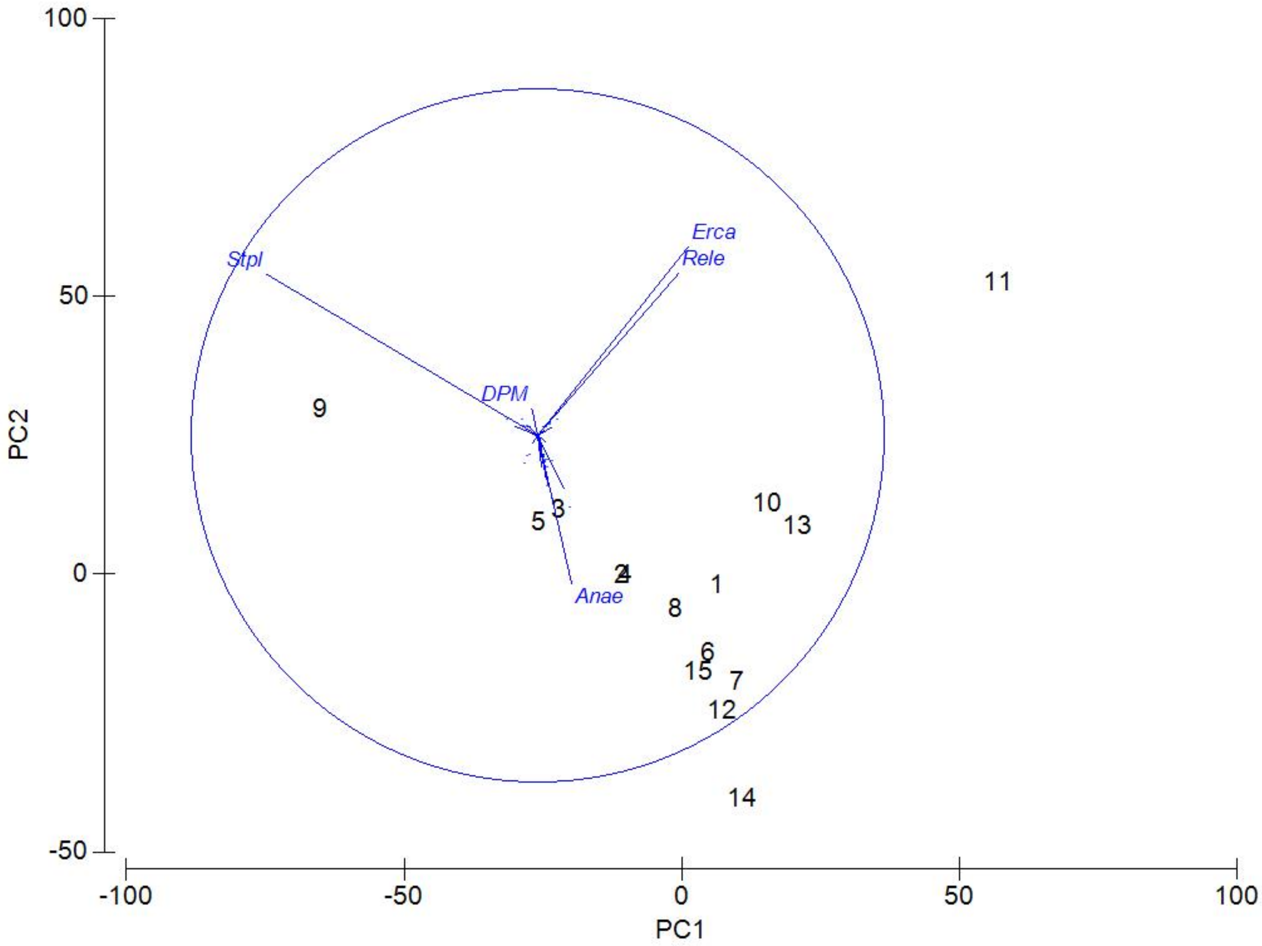

Figure 6-10: **PCA ordination with 15 relevés, 43 plant species and three other variables: bare ground, dead plant material (DPM) and rocks, at the Pezula study site. *Anae - Anthospermum aethiopicum*; *Erca - Erica canaliculata*; *Rele - Restio leptoclados; Stpl - Seriphium plumosum***

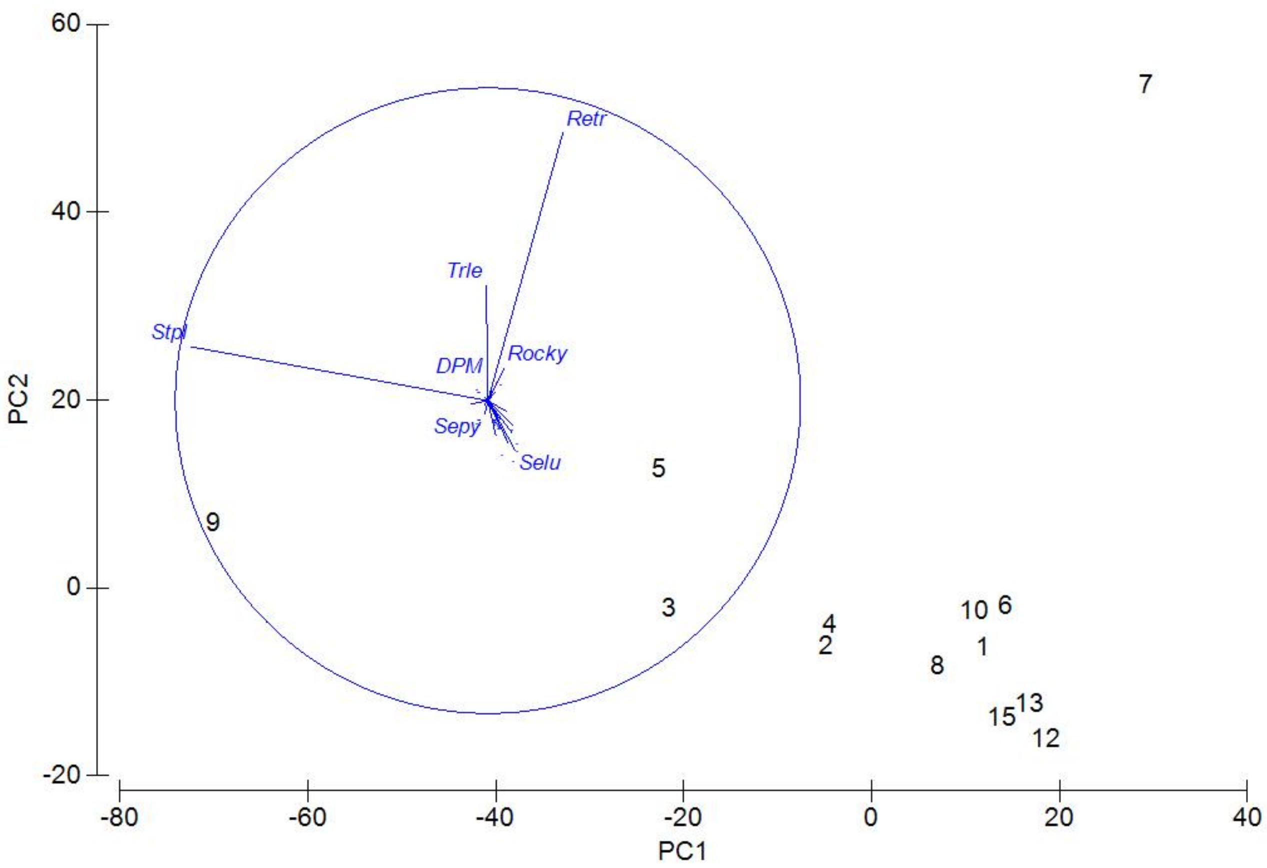

Figure 6-11: **PCA ordination with 13 relevés (PEZ11 and PEZ14 omitted), 43 plant species and three other variables: bare ground, dead plant material (DPM) and rocks (Rocky), at the Pezula study site. *Retr - Restio triticeus*; *Selu - Searsia lucida* forma *lucida*; *Sepy - Searsia pyroides* var. *pyroides; Stpl - Seriphium plumosum; Trle - Tristachya leucothrix***

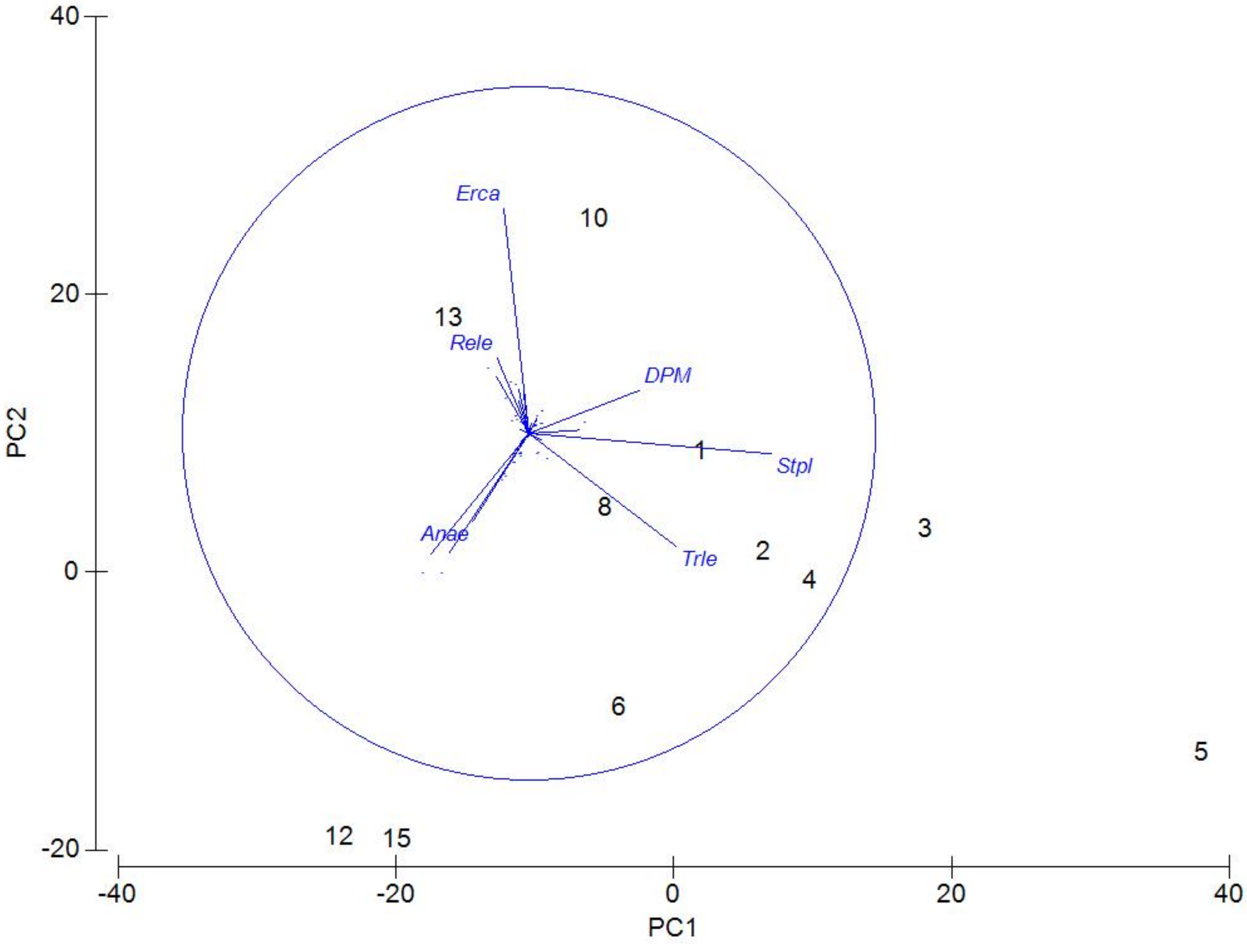

Figure 6-12: **PCA ordination with 11 relevés (PEZ07, PEZ09, PEZ11 and PEZ14 omitted), 43 plant species and three other variables: bare ground, dead plant material (DPM) and rocks, at the Pezula study site. *Anae - Anthospermum aethiopicum*; *Erca - Erica canaliculata*; *Rele - Restio leptoclados; Stpl - Seriphium plumosum; Trle - Tristachya leucothrix***

The PCA ordinations for the Coastal study site relevés are presented in Figs. 6-13 and 6-14. After running the first PCA ordination for the Coastal relevés (Fig. 6-13) CS01 and CS06 were considered to be outlier relevés, and were removed from the data set. CS01 was unique in that it was located to the west of the colony and was dominated by rocks with a Braun–Blanquet cover-abundance value of 4. Similarly CS06 was situated on a slope to the north-east of the colony with a Braun–Blanquet cover-abundance value of 3 for rocks.

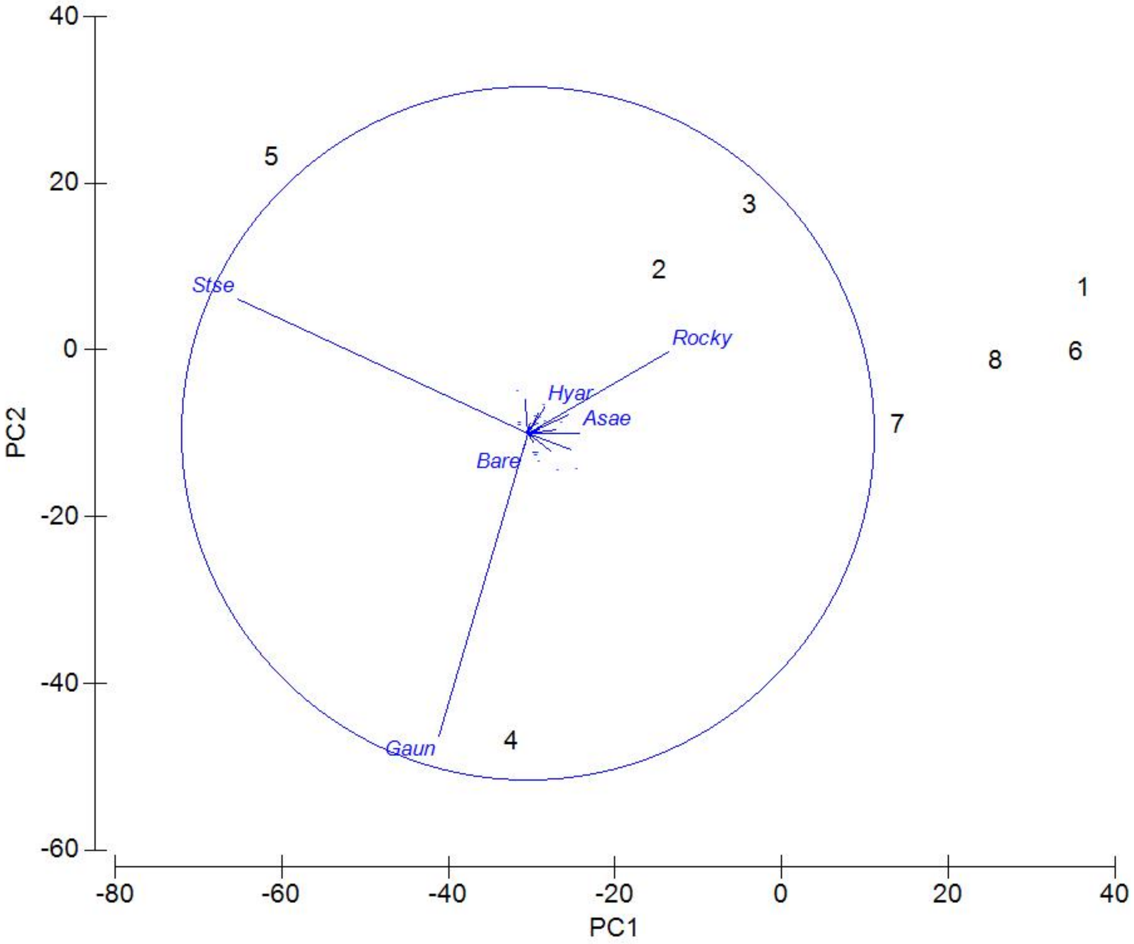

Figure 6-13: **PCA ordination with 8 relevés (CS01 – CS08, labelled 1-8), 33 plant species and three other variables, bare ground (Bare), dead plant material and rocks (Rocky) at the Coastal study site.** ***Asae - Asparagus aethiopicus; Guan - Gazania rigens*** **var.** ***uniflora; Hyar - Hypoestes aristata*** **var.** ***aristata; Stse - Stenotaphrum secundatum***

The results of rerunning the ordination omitting two relevés (CS01 and CS06) can be seen in Fig. 6-14.

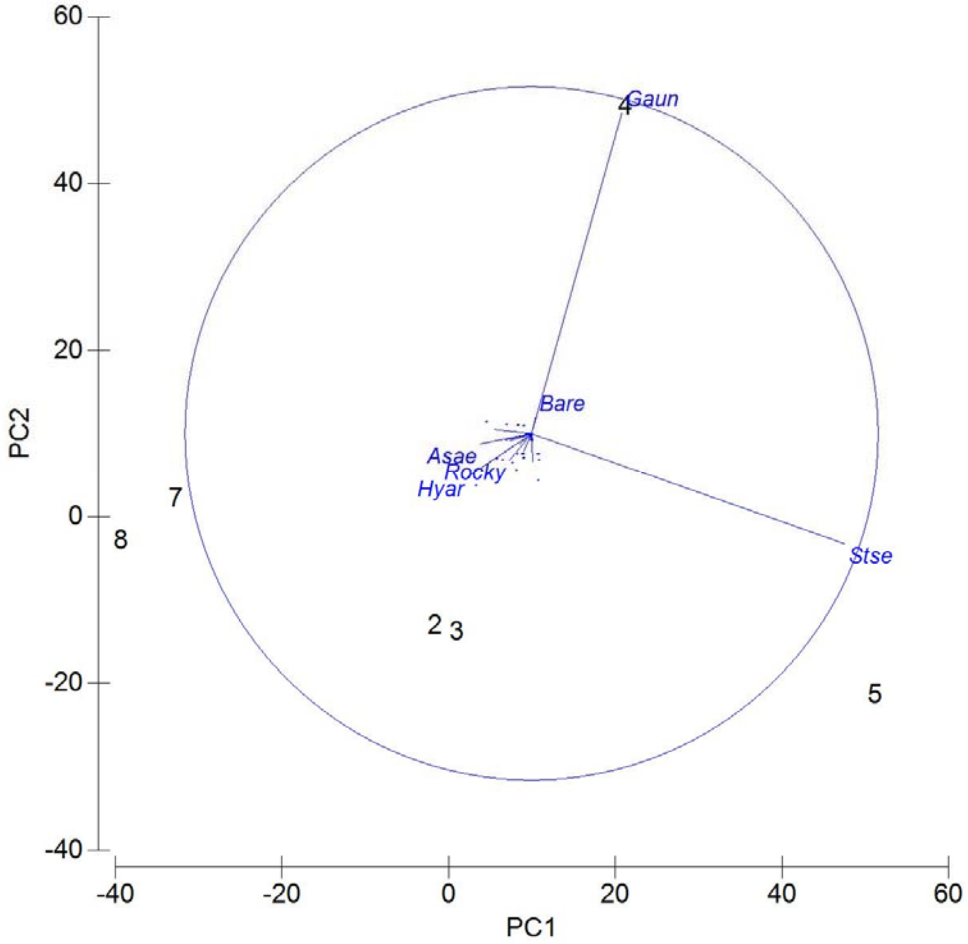

Figure 6-14: **PCA ordination with 6 relevés (CS01 and CS06 omitted), 33 plant species and three other variables, bare ground, dead plant material and rocks (Rocky) at the Coastal study site.** ***Asae - Asparagus aethiopicus; Guan - Gazania rigens*** **var.** ***uniflora; Hyar - Hypoestes aristata*** **var.** ***aristata; Stse - Stenotaphrum secundatum***

6.3.5 Vertical stratification analysis

Vertical stratification of the vegetation at the Pezula site is shown in Table 6-9.

Table 6-9: Abundance of vegetation in height classes, from 15 relevés (PEZ01-PEZ15) at the Pezula study site. PEZ02 and PEZ03 (in red) lie within the *T. b. brachycerus* colony.

	Pezula Study Site Relevés														
Height Class	**PEZ01**	**PEZ02**	**PEZ03**	**PEZ04**	**PEZ05**	**PEZ06**	**PEZ07**	**PEZ08**	**PEZ09**	**PEZ10**	**PEZ11**	**PEZ12**	**PEZ13**	**PEZ14**	**PEZ15**
Bare Ground						5.45		3.59			0.46	3.49		3.28	4.10
Rocks						5.45	7.66	3.59		9.21		0.87			
< 0.4 m	27.19	3.38	7.26	11.99	38.15	65.00	87.09	14.37	4.50	26.05	46.28	15.70	28.53	21.04	23.55
0.4 - 0.8 m	4.09	23.10	88.78	55.43	54.31	1.36	5.25	50.30	7.50	61.05	3.72		27.88	4.10	56.31
0.8 - 1.2 m	67.84	52.39	3.96	32.58	7.54	22.73		28.14	88.00	3.16	49.54	36.34	8.33	68.31	11.95
1.2 - 1.6 m	0.88	21.13								0.53			24.04	3.28	4.10
> 1.6 m												43.60	11.22		
Totals	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

6.4 Discussion

No clear correlation between floristic composition and the occurrence of *T. b. brachycerus* was found. The inland Pezula site and the coastal sites are radically different floristically having none of the top twenty more abundant plant taxa in common. (Table 6-2). The single plant common to the two study sites, *Tarchonanthus littoralis*, was only found in relevés outside of the P1 and C1 colonies. The Pezula site has been sampled over a wide area, and the PEZ01-PEZ15 relevés are broadly similar in floristic composition. However *T. b. brachycerus* only currently occurs in 2 out of the 15 relevés sampled.

In terms of α-diversity, the Shannon-Weiner index of Pezula relevés varies between 0.49 (PEZ09) and 2.41 (PEZ01). The two relevés within the *T. b. brachycerus* P1 colony have *H'* values of 2.05 (PEZ02) and 1.48 (PEZ03). The results of the one-way ANOVA (Table 6-4) show that there is no pattern in α-diversity between the relevés at the top, middle or bottom of the slope, and no significant difference between the α-diversity of the plants species in the relevés outside the P1 colony compared with those within the colony.

Shannon-Weiner (*H'*) values for the Coastal site relevés range between 1.08 (CS04) and 2.74 (CS01) with 3 of the relevés within the C1 colony having similar values (CS02, CS03 & CS05) and CS04 being lower. Unlike Pezula, the Coastal site, although much smaller in size, looks patchy (see Fig. 6-3) with one large area dominated by a single grass species. It is this area that accounts for the relatively low *H'* of CS04. The plant species *H'* values of the four relevés within the C1 colony (CS02 - CS05) are significantly lower than those of the four relevés outside of the C1 colony (CS01, CS06 – CS08) at the Coastal site.

The resemblance data matrix (Table 6-7) and dendrogram (Fig. 6-6) indicate that PEZ02 and PEZ03 are paired with each other with a 62.33% similarity. However PEZ03 is equally similar to PEZ04 and PEZ05 and the fact that *T. b. brachycerus* does not occur there could indicate the presence of other factors determining the presence of *T. b. brachycerus*. Two of the Coastal relevés within the C1 colony (CS04 & CS05) are 71.68% similar (Table 6-8) and are paired with each other in the dendrogram (Fig. 6-7) but they show little similarity with the other two relevés within the colony (CS02 & CS03).

The Pezula site ordinations do show PEZ02 and PEZ03 situated close to each other in both the MDS ordinations (Fig. 6-8) and together in the final PCA in the direction of *Seriphium plumosum* and *Tristachya leucothrix* (Fig. 6-12) which are indicator species for these relevés. The MDS ordinations of the Coastal site relevés (Fig. 6-9) show that CS04 & CS05 are grouped together to some extent and distinct from the other relevés while CS02 & CS03 are also grouped together. However, no clear grouping was shown. The final Coastal PCA ordination (Fig. 6-14) shows no association between the relevés within the *T. b. brachycerus* colony, although it does depict CS04 - *Gazania rigens* var*. uniflora* and CS05 – *Stenotaphrum secundatum* relationships.

The lack of correlation with floristic composition should not be that surprising since the females have been observed laying eggs on sixteen different plant species (Tables 5-1 and 5-2), and all evidence points to the conclusion that *T. b. brachycerus* is aphytophagous, as other workers have found to be the case for its congeners (see Table 1 and Appendix 1). Cottrell (1985) was the first to draw attention to the absence of associations with *Capensis* floral elements in the larval/plant relationships of the majority of endemic Cape butterflies.

Vegetation structure or physiognomy could influence the occurrence of the host ant, because of its effect on the microclimate at ground level (see 7-4). However, it can be inferred from Table 7-2 that no such relationship is detectable from our data. Looking at the abundance of vegetation in height classes at Pezula (Table 6-9), almost 89% of the vegetation in the PEZ03 relevés within the *T. b. brachycerus* P1 colony falls into the 0.4 – 0.8m class. In contrast only 23% of the vegetation in the other relevé within P1 (PEZ02) falls within that range, and just over half (52%) falling into the 0.8 - 1.2 m height class. Additionally, bare ground and rocks do not feature highly in these two sites. The relatively unimportance of bare ground and rocks in the presence of *T. b. brachycerus* is supported by the ordinations. Since most of the vegetation at the Coastal site is under 0.4m the height itself is unlikely to be a significant factor and further investigation into the microclimate should be considered.

Exogenous disturbances (herbivores, fire) are other factors that could render a local habitat patch suitable for *T. b. brachycerus* by altering the vegetation composition or physiognomy (selective grazing or browsing), or by setting in motion a secondary succession process. The dynamic nature of the vegetation has been directly observed during this project, and the Pezula sites where *T. b. brachycerus* currently occurs have become increasingly overgrown with plants such as *Seriphium plumosum* since the 2011 sampling programme. Path cutting through the butterfly habitat has been practised to keep at least some areas open, and this may have assisted in sustaining the butterfly population, since other colony sites that have become overgrown no longer have *T. b. brachycerus* populations (Edge, pers. comm., 2010) (See 2.3). Furthermore, path cutting has proved beneficial in the case of the Brenton Blue butterfly (Edge, Cilliers, *et al.*, 2008).

Chapter 7 Ant Assemblages at the study sites

7.1 Introduction

All the *Thestor* species so far studied by other workers have been found to be myrmecophilous (Heath & Pringle, 2004). However, since no data existed on the life history of *T. b. brachycerus*, and it was deemed to be potentially harmful to the butterfly population at the Pezula site to excavate ant nests, it was decided to indirectly determine potential host ants by sampling at the sites where the butterfly was present. Furthermore, knowledge of ant assemblages can yield important insights into the ecology of the habitats of threatened butterfly species (Thomas, 1995; New & Britton, 1997; van Hamburg *et al*., 2004; Edge, Robertson *et al*., 2008).

7.2 Materials and methods

Ant sampling was done with pitfall traps consisting of 250 ml tapered plastic cups inserted into holes made in the soil so that the top lip of the cup was flush with the surrounding soil. The traps were placed roughly in the centre of each of the 15 vegetation surveying relevés (25 m^2 each) at the Pezula site in December 2011 and May 2012, and at each of the eight similar sized relevés at the Coastal site in December 2012 (see Figs. 6.1 & 6.2 for location and Table 6.1 for GPS coordinates). A preservative of two parts propylene glycol to one part water was poured to a depth of 4cm into the cup. After 5 days the traps were emptied, sieved and the contents were placed in 75% ethanol for later examination. In certain traps the ants were separated from the soil particles using the hot salt water extraction method described by Lattke (2000). In addition ants were collected directly at each site during the time when the *T. b. brachycerus* adults were on the wing. This was done during December 2009 and January 2012 at Pezula, at the Coastal site during November 2012 and February 2014 and at the 3rd and most recent colony in February 2014. The contents of the pitfall traps and the direct samples were examined and the ants were separated into morphospecies using a Leica Zoom 2000 stereomicroscope. Samples of each morphospecies were sent to an ant specialist (Peter Hawkes, Afribugs CC) for identification.

A two-way data matrix was constructed in Microsoft® Excel with ant species as rows and locations as columns, showing the count of each species found at each location and the totals for each site. It is possible to convert these matrices into relative abundance matrices by dividing each species count by the total number of ants of all species for that location (see Edge, Robertson *et al.,* 2008) and it appears to be acceptable practise to use the pitfall trap method to estimate the abundance and species composition of epigaeic ants in an area (Bestelmeyer *et al*., 2000). However it should be recognised that such estimates of abundance may be biased by differences in locomotion among ant species (Andersen, 1983). According to Gotelli, *et al*. (2010) analysing the individual worker ant counts from pitfall traps to estimate diversity should be avoided. Although the number of each ant species are presented and the Shannon-Weiner diversity index was calculated in Microsoft® Excel along with equitability (see 6.2 for formulae), these numbers should be viewed with some caution.

Further analysis was based on incidence of ant species identified in the pitfall traps rather than abundances. Accordingly, tables were compiled, in Microsoft® Excel, of the number of ants found in each pitfall trap and converted into presence/absence of ant species in each relevé for each sampling date.

The quantitative structural data gathered from each of the vegetation sampling relevés are presented in Table 7-7, together with data on the presence or absence of the two principle study animals, *T. b. brachycerus* and *Anoplolepis custodiens*, including the direct sampling at both Pezula and the Coastal site.

7.3 Results

Tables 7-1 and 7-2 show the number of ant species sampled at the 15 plots on the Pezula site in December 2011 and in May 2012 respectively. The *T. b. brachycerus* colony resides in only two of the sampled areas (PEZ02 and PEZ03). Similar information for ants caught in each pitfall trap at the Coastal site in December 2012 is shown in Table 7-3, although here *T. b. brachycerus* can be found at 4 sites (CS02, CS03, CS04, and CS05).

In total 315 ants from 10 different species were caught in the (PEZ01-PEZ15) pitfall traps during sampling at the Pezula site in December 2011 and May 2012 and at the Coastal site in December 2012. A summary of the ants sampled from the pitfall traps at each site is presented in Table 7-4 together with estimates of α-diversity (species richness, Shannon-Wiener index and equitability). The species diversity measured by total species and Shannon-Wiener index was substantially higher at the Pezula site than at the Coastal site. Passive methods such as pitfall trapping, are easy to replicate but rely on ant activity at the sample point to obtain data, and may miss some species due to disparities in behaviour of different ant species (Bestelmeyer *et al*., 2000). At both sites, *Anoplolepis custodiens* was the dominant ant species, comprising 75% of the ants collected in pitfall traps at the Pezula site in December 2011, 58% in May 2012 and 93% of those collected at the Coastal site. It should be noted that 96 of the *A. custodiens* ants found at the Pezula site came from one relevé (PEZ15) implying that the pitfall trap was set very close to an ant's nest. Similarly, 32 *A. custodiens* ants were counted from the CS04 pitfall trap at the Coastal site, which is in the *T. b. brachycerus* colony.

Table 7-1: **Ant taxa caught in pitfall traps over 5 days at the Pezula study site (PEZ01–PEZ15) in December 2011. The PEZ02 and PEZ03 sampling points (in red) fall within the *T. b. brachycerus* P1 colony.**

	Sample Points															
Ant Species	**PEZ01**	**PEZ02**	**PEZ03**	**PEZ04**	**PEZ05**	**PEZ06**	**PEZ07**	**PEZ08**	**PEZ09**	**PEZ10**	**PEZ11**	**PEZ12**	**PEZ13**	**PEZ14**	**PEZ15**	**TOTALS**
Anoplolepis custodiens					1	3	2	5	8	1	8	2	9	17	96	**152**
Camponotus niveosetosus	1										1		1	2		**5**
Pachycondyla [*Bothroponera*] afrc_za15													1			**1**
Pachycondyla [*Mesoponera*] *caffraria*	1		1				1			2						**5**
Leptogenys intermedia																**0**
Tetramorium frigidum			2	1												**3**
Lepisiota capensis		1			1			3			1				2	**8**
Crematogaster (Crematogaster) peringueyi	3	1		1	2	2										**9**
Monomorium australe											11	1	3	6		**21**
TOTALS	**5**	**2**	**3**	**2**	**4**	**5**	**3**	**8**	**8**	**3**	**21**	**3**	**14**	**25**	**98**	**204**

Table 7-2: **Ant taxa caught in pitfall traps over 5 days at the Pezula study site (PEZ01–PEZ15) in May 2012. The PEZ02 and PEZ03 sampling points (in red) fall within the *T. b. brachycerus* P1 colony.**

	Sample Points															
Ant Species	**PEZ01**	**PEZ02**	**PEZ03**	**PEZ04**	**PEZ05**	**PEZ06**	**PEZ07**	**PEZ08**	**PEZ09**	**PEZ10**	**PEZ11**	**PEZ12**	**PEZ13**	**PEZ14**	**PEZ15**	**TOTALS**
Anoplolepis custodiens		2			2		1				19	6	1	2		**33**
Pachycondyla [*Bothroponera*] afrc_za15		1	2	1	1				1						1	**7**
Lepisiota capensis	3		1				1		1							**6**
Monomorium australe			4								1					**5**
Pachycondyla [*Mesoponera*] *caffraria*	2		2													**4**
Crematogaster (Crematogaster) peringueyi	1															**1**
Leptogenys attenuata		1														**1**
TOTALS	**6**	**4**	**9**	**1**	**3**	**0**	**2**	**0**	**2**	**0**	**20**	**6**	**1**	**2**	**1**	**57**

Table 7-3: **Ant taxa caught in pitfall traps over 5 days at the Coastal study site (CS01–CS08) in December 2012. The CS02, CS03, CS04 and CS05 (in red) sampling points fall within the *T. b. brachycerus* C1 colony.**

	Sample Points								
Ant Species	**CS01**	**CS02**	**CS03**	**CS04**	**CS05**	**CS06**	**CS07**	**CS08**	**TOTALS**
Anoplolepis custodiens	1		6	32	2	1	7	1	**50**
Camponotus niveosetosus						1			**1**
Leptogenys intermedia								1	**1**
Tetramorium frigidum		1				1			**2**
TOTALS	**1**	**1**	**6**	**32**	**2**	**3**	**7**	**2**	**54**

Table 7-4: **Summary of ant taxa and subfamilies caught in pitfall traps over 5 days at the Pezula sites (PEZ01–PEZ15) in December 2011 and May 2012 and at the Coastal sites (CS01–CS08) in December 2012.**

Ant Species	**Author**	**Subfamily**	**Pezula (Dec 2011)**	**Pezula (May 2012)**	**Coastal (Dec 2012)**
Anoplolepis custodiens	(Smith, F.)	Formicinae	152	33	50
Camponotus niveosetosus	Mayr	Formicinae	5	0	1
Pachycondyla [*Bothroponera*] afrc_za15	-	Ponerinae	1	7	0
Leptogenys attenuata	(Smith, F.)	Ponerinae	0	1	0
Pachycondyla [*Mesoponera*] *caffraria*	(Smith, F.)	Ponerinae	5	4	0
Leptogenys intermedia	Emery	Ponerinae	0	0	1
Tetramorium frigidum	Arnold	Myrmicinae	3	0	2
Lepisiota capensis	(Mayr)	Formicinae	8	6	0
Crematogaster (Crematogaster) peringueyi	Emery	Myrmicinae	9	1	0
Monomorium australe	Emery	Myrmicinae	21	5	0
TOTALS			**204**	**57**	**54**
Species Richness			8	7	4
Shannon-Wiener Index (H')			0.99	1.35	0.34
Equitability (J)			0.51	0.70	0.25

Table 7-5 shows presence/absence of the various ant species identified from the Pezula sampling points (PEZ01-PEZ5) in December 2011 and May 2012 together. Similarly Table 7-6 shows presence/absence of ant species from the sampling points (CS01 –CS08) in December 2012.

Table 7-5: **Summary of the presence(+) or absence of ant taxa caught in pitfall traps over 5 days at the Pezula sampling points (PEZ01–PEZ15) in December 2011 and May 2012 and direct sampling by hand (h) in December 2009 and January 2012. The PEZ02 and PEZ03 sampling points (in red) fall within the *T. b. brachycerus* P1 colony.**

	Sample Points														
Ant Species	**PEZ01**	**PEZ02**	**PEZ03**	**PEZ04**	**PEZ05**	**PEZ06**	**PEZ07**	**PEZ08**	**PEZ09**	**PEZ10**	**PEZ11**	**PEZ12**	**PEZ13**	**PEZ14**	**PEZ15**
Anoplolepis custodiens		+	h		+	+	+	+	+	+	+	+	+	+	+
Camponotus niveosetosus	+										+		+	+	
Crematogaster (Crematogaster) peringueyi	+	+		+	+	+									
Lepisiota capensis		+			+			+			+				+
Lepisiota capensis	+		+				+		+						
Leptogenys attenuata		+													
Leptogenys intermedia															
Monomorium australe	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pachycondyla [Bothroponera] afrc_za15		+	+	+	+				+				+		+
Pachycondyla [Mesoponera] caffraria	+		+				+			+					
Tetramorium frigidum			+	+											

Table 7-6: **Summary of the presence/absence of ant taxa caught in pitfall traps over 5 days at the Coastal sampling points (CS01–CS08) in December 2012 and direct sampling by hand (h) in November 2012 and February 2014. The CS02, CS03, CS04 and CS05 (in red) sampling points fall within the *T. b. brachycerus* C1 colony.**

	Sample Points							
Ant Species	**CS01**	**CS02**	**CS03**	**CS04**	**CS05**	**CS06**	**CS07**	**CS08**
Anoplolepis custodiens	+	h	+	+	+	+	+	+
Camponotus niveosetosus						+		
Leptogenys intermedia								+
Tetramorium frigidum		+				+		

The quantitative structural data gathered from each of the vegetation sampling relevés is presented in Table 7-7, together with data on the presence or absence of the two principle study animals, *T. b. brachycerus* and *A. custodiens*.

Table 7-7: **Vegetation structure of all the relevés at the Pezula and Coastal study sites, showing total cover of all plants (sum of midpoints), the percentage bare ground, rocks and dead plant matter, and the presence (+) or absence (-) of *Anoplolepis custodiens* determined from pitfall trap sampling, or detected by hand sampling (h), and presence of *T. b. brachycerus* (+) determined by the relevés within the P1 and C1 colonies. The PEZ02 and PEZ03 sampling points (in red) fall within the *T. b. brachycerus* P1 colony. The CS02, CS03, CS04 and CS05 (in red) sampling points fall within the *T. b. brachycerus* C1 colony.**

Relevés	**Veg. cover**	**% bare ground**	**% rocks**	**% dead matter**	***Thestor b. brachycerus***	***Anoplolepis custodiens***
			PEZULA STUDY SITE			
PEZ01	85.50	-	-	37.50	-	-
PEZ02	88.75	-	-	8.75	+	+
PEZ03	75.75	-	-	8.75	+	h
PEZ04	66.75	-	-	18.75	-	+
PEZ05	116.00	-	-	18.75	-	+
PEZ06	63.75	3.00	3.00	3.00	-	+
PEZ07	114.25	-	8.75	8.75	-	+
PEZ08	83.50	3.00	3.00	3.00	-	+
PEZ09	100.00	-	-	3.00	-	+
PEZ10	95.00	-	8.75	8.75	-	+
PEZ11	161.50	0.75	-	8.75	-	+
PEZ12	86.00	3.00	0.75	3.00	-	+
PEZ13	78.00	-	-	-	-	+
PEZ14	91.50	3.00	-	-	-	+
PEZ15	73.25	3.00	-	-	-	+
			COASTAL STUDY SITE			
CS01	39.00	-	62.50	8.75	-	+
CS02	95.00	-	3.00	8.75	+	h
CS03	77.50	-	37.50	3.00	+	+
CS04	100.00	3.00	8.75	-	+	+
CS05	92.50	-	3.00	-	+	+
CS06	79.50	-	37.50	-	-	+
CS07	74.00	-	-	-	-	+
CS08	82.75	-	18.75	3.00	-	+

The only ant sampling carried out at the recently found third colony, east of the Coastal site (see Fig. 2-2), was by hand in February 2014. All ants (n=4) sampled from this colony were identified as being *A. custodiens*. No specimens of the alien Argentine Ant [*Iridomyrmex humilis*, (Mayr, 1868)] were found at either the Pezula or Coastal study sites. Argentine Ants could adversely affect *T. b. brachycerus* by killing and replacing the indigenous ants (de Kock, 1990; Henning, 2001).

7.4 Discussion

7.4.1 Ant assemblages at Pezula and the Coastal sites.

The only ant known to associate with *Thestor* species is *A. custodiens* (Heath & Claassens 2000; Heath & Pringle, 2004). There is strong circumstantial evidence that the host ant for *T. b. brachycerus* is the *A. custodiens* ant since in all the samples collected by pitfall traps or by hand at virtually all the places sampled this was the most prevalent ant (Table 7-7). However, the observation that very few individuals of *A. custodiens* were trapped at the two sampling sites located in the Pezula *T. b. brachycerus* colony is somewhat puzzling and it is hypothesised this could be put down to some difference in epigaeic foraging behaviour (and thus less chance of them entering the pitfall traps) by the ants in the vicinity of the butterfly colony. Professionals who sample for ants regularly report that it is not unusual for some traps to have a much lower yield of a particular species than other traps nearby (Hawkes – pers. comm., 2014). Generally the number of ants caught in pitfall traps reflects the distance of the pitfall traps from colonies so traps close to a colony will capture multiple worker ants from that colony, those further away will only catch the odd forager.

More sampling will be needed in the vicinity of the Pezula *T. b. brachycerus* colony (P1) to establish whether *A. custodiens* does indeed occur there. Louw (1968) reported that the activity levels of *A. custodiens* were highly seasonal, with peaks in spring and autumn, whereas our samples were taken in high summer and early winter. Steyn (1954) reported that the feeding activities of *A. custodiens* were positively correlated with temperature and negatively correlated with relative humidity. These findings will have to be taken into account when planning more sampling, and may explain some of the more anomalous results.

7.4.2 The biology and ecology of *A. custodiens*

Prins (1982) reviewed the genus *Anoplolepis* and gave a brief description of their biology and ecology. They are extremely aggressive ants and their main food source is honeydew solicited from Hemipterans (aphids and soft scale = coccids), which is supplemented by a whole range of protein obtained by preying on other arthropods (Steyn, 1954). It is not known with certainty whether they are unicolonial, or polydomous (Debout *et al*., 2007; Buczkowski, 2012), but in either case they form large connected colonies with many thousands of workers. They seem to generally prefer more open habitats and become more abundant when the vegetation has been disturbed by grazing or fire (Prins, 1982). They are well adapted to warmer climates, but even in somewhat cooler climates (such as occurs in the Knysna vicinity) they can thrive in areas where the microclimate at ground level is warm. They nest by burrowing into open ground or in the crevices and soil under rocks.

In his review of the host genera of ant-parasitic Lycaenidae butterflies, Fielder (2012) classifies ants of the *Anoplolepis* genus as second-order dominant meaning that they are subordinate to top dominant ants. However in the absence of the dominant ants in the same habitat, they will monopolise resources. None of the other ant species sampled at the study sites are more dominant although according to Fielder, (2012) some genera (*Camponotus, Crematogaster, Lepisiota, Monomorium* and *Tetramorium*) fall into the same second-order dominant category as *Anoplolepis.*

Chapter 8 Conclusions and Recommendations

8.1 Characteristics of the sites where *Thestor brachycerus* occurs

The major breakthrough from this research project has been the discovery of two new populations of *T. b. brachycerus*, which trebles the known extant populations and significantly reduces the risk of imminent extinction. The radically different biotopes represented by the previously known inland sites and the newly researched coastal sites means that the butterfly and its host ants are able to adapt to a variety of biotopes and microclimates. Evidence from the differing vegetation compositions of the sites and records of oviposition on a variety of plants is not entirely surprising, given that research on other butterflies in the *Thestor* genera showed that they are all aphytophagous and not directly plant dependent.

There is some evidence, that vegetation structure plays a role in creating the right microclimate and habitat structure for the butterfly and its host ants (Thomas, 1995; Witt & Giliomee, 1999; Edge, Cilliers *et al*., 2008). Certainly, habitats that do not have a closed canopy, or that have open patches, seem to be favoured by the territorial males as perching sites. No statistical correlation between bare ground and presence of the butterfly in the relevés sampled within the *T. b. brachycerus* colonies was found. It is conceivable that the microclimates resulting from the combination of taller vegetation, increased solar radiation and lower thermal conductivity of coarse soil at the north-west facing P1 colony and shorter grasses with less solar radiation, but higher conductivity from rocks on the south facing C1 colony are similar, allowing both the butterfly larvae and its ant host to utilise these two habitats that appear to be so different.

Thestor dicksoni malagas (from the *braunsi* species group) is also found extremely close to the sea and is entirely endemic to a very small area just north of Tsaarsbank on the Langebaan Peninsula with a couple of sightings to the north of the Langebaan lagoon (Brummer, 2009). *T. d. malagas* has been recorded as flying near and among the rocks at the high water mark (Heath & Pringle, 2004). Not requiring a vegetation type, could explain its distribution so close to the ocean. However apart from *T. b. brachycerus* no other *Thestor* species has thus far been found occupying two such significantly different habitats. It is possible that pressure from human development forced *T. b. brachycerus* towards the coast although they seem to be well adapted to the coastal habitat.

Anoplolepis custodiens is ubiquitous at the sites where *T. b. brachycerus* occurs. The study does not directly associate *T. b. brachycerus* with the ant, and does not claim that the ant is more likely to be seen where the butterfly is also present. However, where *T. b. brachycerus* was found, so were *A. custodiens* ants. It is highly likely that this is the host ant, as has been reported for all

Thestor species investigated by other workers. It is also likely, by inference from other closely related species, that the larvae are cuckoo parasites in *A. custodiens* ant nests and are fed primarily by trophallaxis. No evidence has been found for any hemipteran association, which is consistent with research conducted on other members of the *Thestor brachycerus* species group *sensu* Heath and Pringle (2004). It would be premature to conclude that *T. b. brachycerus* has no hemipteran association, since the ant nests associated with the butterfly have not yet been examined and could contain subterranean hemipterans.

Since *T. b. brachycerus* is so rare and only two colony sites were examined, this assessment of the ecological requirements cannot be regarded as scientifically rigorous. However it increases the knowledge of the butterfly and may be useful as a foundation for further studies or for locating potential colonies, or relocation of populations elsewhere.

8.2 Recommendations and management of the colony sites

An assessment has been made of the ecological differences and similarities between the known *T. b. brachycerus* localities and adjacent areas. The insights thus gained will enable implementation of a habitat management plan for both sites, and for the creation of suitable habitat on adjacent land.

Experimental management methods are recommended at the Pezula site, which should include small scale burning, introduction of grazing animals (bovines or antelopes), and selective cutting of vegetation. Observations on other butterfly species have shown that burning or cutting could be beneficial (e.g. Deutschländer & Bredenkamp, 1999); and that changes of grazing regimes have resulted in loss of colonies (Edge pers. comm.). This will require a high degree of cooperation from the landowners of the Pezula site and a more formalised conservation status for the land would facilitate this.

In August 2013 paths were cut by PGE management. Paths were not cut at C1 due to the vegetation being much shorter overall and there already being a path, made by fishermen, through the colony. In the coming December 2014/January 2015 season, it will be possible to test if *T. b. brachycerus* males are attracted to the paths for their territorial perching sites, as was observed during the behavioural studies.

It is still not fully understood what the specific habitat requirements for *T. b. brachycerus* are and why *T. b. brachycerus* is so rare. The vegetation analysis at the study sites has provided evidence to show that trying to identify habitats suitable for this species in the future, based on vegetation community characteristics is unlikely to be successful, due to its aphytophagous life cycle excluding any direct association with particular plants. It is possible that there could be a significant hemipteran: vegetation relationship which indirectly affects the distribution of the butterfly, but firstly any hemipteran involvement in the *T. b. brachycerus* life cycle must be determined.

This places more importance on establishing the life history of *T. b. brachycerus*. Since *A. custodiens* is the most likely ant associate, careful investigation of *A. custodiens* nests to locate larvae at the coastal sites may provide confirmation that it is indeed the host ant.

If this is the case, the ecology of *A. custodiens* needs to be studied more intensely and understood better (colony structure and size, habitat types and microclimate preferred, nesting habits, and trophic resources of the ant colonies including any hemipteran associations). The establishment of an artificial ants' nest in the laboratory would enable the parasitic/mutualistic interactions between *T. b. brachycerus* and its host ant to be studied.

Although no hemipterans were found during the study, a systematic survey of hemipterans in the known colony locations should be made. This will include searching for hemipterans within the *A. custodiens* ant nests. Once it has been proven that there is an association with the ant *A. custodiens* and / or a hemipteran of the suborder Sternorrhyncha, data bases of the location of the host ant and any other requirements can be compiled and fine-scale GIS mapping used to assist in the search for new colonies or suitable relocation sites.

Future research should also focus on a systematic search for more *T. b. brachycerus* colonies along the coast and inland, taking a broader view of the potential biotopes where the butterfly could occur. Since there is only a six week period from mid-December to late January when the adults are in flight and some of these days are unsuitable for butterfly searches due to rain or wind, the time during which this is possible is limited. The inaccessibility of the coastland adds to the difficulty of searching for further populations, but this gives hope that the species is not threatened by human disturbance since the coastal habitat is unsuitable for development.

Now that it has been established that the overall *T. b. brachycerus* population is larger than initially believed at the start of the research, samples of adult butterflies from the Pezula and Coastal colonies could be taken for mitochondrial DNA barcode sequencing to determine how genetically similar the populations are. With such different habitats and the geological barrier of a steep cliff, the populations may be in the process of separating into sub-species.

References

ANDERSEN, P. R. 1983. A brief survey of ants of Glenaladale National Park, with particular reference to seed-harvesting. *Victorian Naturalist*, 100: 223-237.

ANGELONI, L., SCHLAEPFER, M.A., LAWLER, J.J., & CROOKS, K.R. 2008. A reassessment of the interface between conservation and behaviour. *Animal Behaviour*, 75: 731-737.

ATSATT, P. R. 1981. Lycaenid butterflies and ants: selection for enemy-free space. *American Naturalist*, 118: 638-654.

BALL J.B. 2006. Approaches towards a critical evaluation and update of the Red Data list of South African butterflies. MSc dissertation, University of Stellenbosch, Stellenbosch.

BEGON, M., HARPER, J.L. & TOWNSEND, C.R. 1996. Ecology, individuals, populations and communities. Oxford: Blackwell Science. p. 1068.

BERGMAN, M., GOTTHARD, K., BERGER, D., OLOFSSON, M., KEMP, D.J. & WIKLUND, C. 2007. Mating success of resident versus non-resident males in a territorial butterfly. *Proceedings of the Royal Society B*, 274: 1659-1665.

BESTELMEYER, B.T., AGOSTI, D., ALONSO, L.E., BRANDÃO, C.R., BROWN, W.L., DELABIE, J.H. & SILVESTRE, R. 2000. Field techniques for the study of ground-dwelling ants: an overview, description and evaluation. (*In* Agosti, D., Majer, J.D., Alonso, L.E & Schultz, T.R., *eds.* Ants: Standard methods for measuring and monitoring biodiversity. Washington: Smithsonian Institution Press, Washington, pp. 122–144).

BRABY, M.F., EASTWOOD, R. & MURRAY, N. 2012. The subspecies concept in butterflies: has its application in taxonomy and conservation biology outlived its usefulness? *Biological Journal of the Linnean Society,* 106: 699–716.

BRUMMER, P.D. 2009. A quantitative study of butterfly assemblages from different biotopes at the Langebaan Peninsula. Potchefstroom: NWU. (Unpublished dissertation – MSc).

BUCHHOLZ, R. 2007. Behavioural biology: an effective and relevant conservation tool. *Trends in Ecology and Evolution,* 22(8): 402–407.

BUCZKOWSKI, G. 2012. Colony spatial structure in polydomous ants: complimentary approaches reveal different patterns. *Insectes Sociaux,* 59: 241–250.

BUTZER, K.W. & HELGREN, D.M. 1972. Late Cenozoic evolution of the Cape Coast between Knysna and Cape St. Francis, South Africa. *Quaternary Research,* 2: 143–169.

CALVERT, W.H. & HANSON, F.E. 1983. The role of sensory structures and preoviposition behaviour in oviposition by the patch butterfly, *Chlosyne Lacinia*. *Entomologia Experimentalis et Applicata*, 33: 179-187.

CARO, T. 2007. Behaviour and conservation: a bridge too far? *Trends in Ecology and Evolution*, 22(8): 394–400.

CLAASSENS, A.J.M. & HEATH, A. 1997. Notes on the myrmecophilous early stages of two species of Thestor Hübner (Lepidoptera: Lycaenidae) from South Africa. *Metamorphosis*, 8: 56-61.

CLAASSENS, A.J.M. & HEATH A. 2003. The life history of *Thestor basutus basutus* (Wallengren) (Lepidoptera: Lycaenidae: Miletinae) with new information on its association with pugnacious ants (Anoplolepis sp.: Formicidae) *Metamorphosis,* 14: 48–54.

CLARK, G.C. & DICKSON, C.G.C. 1960. Life histories of two species of *Thestor* (Lepidopt.: Lycaenidae). *Journal of the Entomological Society of Southern Africa*, 23: 278–283.

CLARK, G.C. & DICKSON, C.G.C. 1971. Life histories of the South African lycaenid butterflies. Cape Town: Purnell. 272 p.

CLARKE, K.R. 1993. Non-parametric multivariate analyses of change in community structure. *Australian Journal of Ecology.* 18: 117–143.

CLARKE, K.R. & WARWICK, R.M. 2001. Change in marine communities: an approach to statistical analysis and interpretation. 2nd Edition. Plymouth: Primer-E. 172 p.

COLLINS, S.C., CONGDON, C.E., HENNING, G.A., LARRSEN, T.B. & WILLIAMS, M.C. 2013. A review of d'Abrera's Butterflies of the Afrotropical Region –Part III (second edition) 2009 – Part 2 (Miletinae and Poritiinae) *Metamorphosis,* 24: 44–56.

COTTRELL, C.B. 1984. Aphytophagy in butterflies: its relationship to myrmecophily. *Zoological Journal of the Linnean Society*, 79: 1-57.

COTTRELL, C.B. 1985. The absence of coevolutionary associations with Capensis floral element plants in the larval/plant relationships of south-western Cape butterflies. (*In* Vrba, E.S., *ed.* Species and speciation. Transvaal Museum, Pretoria, pp.115–124).

D'ABRERA, B. 2009. Butterflies of the Afrotropical Region – Part III: Lycaenidae, Riodinidae. Melbourne: Hill House Publishers. 531-880.

DE KOCK, A.E. 1990. Interactions between the introduced Argentine ant, *Iridomyrmex humilis* Mayr, and two indigenous fynbos ant species. *Journal of the Entomological Society of Southern Africa*, 53: 107-108.

DEBOUT, G., SCHATZ, B., ELIAS, M. & MCKEY, D. 2007. Polydomy in ants: what we know, what we think we know and what remains to be done. *Biological Journal of the Linnean Society*, 90: 319-348.

DENNIS, R.L.H. 2010. A resource-based habitat view for conservation. Butterflies in the British landscape. Oxford: Wiley-Blackwell, Oxford 406 p.

DENNIS, R.L.H., SHREEVE, T.G. & VAN DYKE H. 2006. Habitats and resources: the need for a resource-based definition to conserve butterflies. *Biodiversity Conservation,* 15: 1943–1968.

DEUTSCHLÄNDER, M.S. & BREDENKAMP, G.J. 1999. Importance of vegetation analysis in the conservation management of the endangered butterfly *Aloeides dentatis dentatis* (Swierstra) (Lepidoptera: Lycaenidae). *Koedoe,* 42(2): 1–12.

DINESH, A.S., & VENKATESHA, M.G. 2013. Analysis of the territorial, courtship and coupling behaviour of the hemipterophagus butterfly, *Spalgis epius* (Westwood) (Lepidoptera: Lycaenidae). *Journal of Insect Behavior,* 26: 149-164.

DOAK, P., KAREIVA, P. & KINGSOLVER, J. 2006. Fitness consequences of choosy oviposition for a time-limited butterfly: Advancing ecological understanding to develop conservation strategies. *Ecology,* 87(2): 395-408.

EDGE, D.A. 1991. Further comment on the skewed sex ratios of *Iolaus* (*Epamera*) *mimosae* Trimen (Lepidoptera: Lycaenidae). *Metamorphosis*, 2(4): 32-33.

EDGE, D.A. 2002. Some ecological factors influencing the breeding success of the Brenton Blue butterfly, *Orachrysops niobe* (Trimen) (Lepidoptera: Lycaenidae). *Koedoe*, 45(2): 19-34.

EDGE, D.A. 2005a. Butterfly conservation in the southern Cape. *Metamorphosis*, 16(2): 28-46.

EDGE, D.A. 2005b. Ecological factors influencing the survival of the Brenton Blue butterfly, *Orachrysops niobe* (Trimen) (Lepidoptera: Lycaenidae). Potchefstroom Campus: North-West University. (Unpublished thesis - D.Phil.) 265 p.

EDGE, D.A. 2010. Searches for *Thestor brachycerus brachycerus* colonies prior to 2009. [discussion]. 09 Jan, Knysna.

EDGE, D.A. 2011. Custodians of rare and endangered Lepidoptera (COREL). *Metamorphosis*, 22(3 & 4): 81-96.

EDGE, D.A., CILLIERS, S.S. & TERBLANCHE, R.F. 2008. Vegetation associated with the occurrence of the Brenton Blue butterfly. *South African Journal of Science*, 104 (11/12): 505-510.

EDGE, D.A., ROBERTSON, H.G. & VAN HAMBURG H. 2008. Ant assemblages at potential breeding sites for the Brenton Blue butterfly, *Orachrysops niobe* (Trimen) (Lepidoptera: Lycaenidae). *African Entomology,* 16: 253–262.

EDGE, D.A., TERBLANCHE, R.F., HENNING, G.A., MECENERO, S. & NAVARRO, R. 2013. Butterfly conservation in South Africa: Analysis of the Red List and threats. (*In*: Mecenero, S., Ball, J.B., Edge, D.A., Hamer, M.L., Henning, G.A., Krüger. M., Pringle, E.L., Terblanche, R.F. & Williams, M.C., *eds*. 2013. Conservation assessment of butterflies of South Africa, Lesotho and Swaziland: Red List and Atlas. Johannesburg & Cape Town: Saftronics (Pty.) Ltd. & Animal Demography Unit, pp. 13–33).

FEY, M. 2010. Soils of South Africa. Cambridge: Cambridge University Press. 287 p.

FIEDLER, K. 1996. Host-plant relationships of lycaenid butterflies: large-scale pattern interactions with plant chemistry, and mutualism with ants. *Entomologia Experimentalis et Applicata*, 80: 259-267.

FIEDLER, K. 2012. The Host Genera of Ant-Parasitic Lycaenidae Butterflies: A Review. *Psyche*. 2012, Article ID 153975, 10 p. doi:10.1155/2012/153975

FRANKHAM, R., BALLARD, J.D., DUDASH, M.R., ELDRIDGE, M.D.B., FENSTER, C.B., LACY, R.C., MENDELSON, J.R., PORTON, I.J., RALLS, K & RYDER, O.A. 2012. Implications of different species concepts for conserving biodiversity. *Biological Conservation,* 153: 25–31.

FREY, D.F., & LEONG, K.L.H. 1993. Can microhabitat selection of differences in “catchability” explain male biased sex ratios in overwintering populations of monarch butterflies. *Animal Behaviour,* 45: 1025-1027.

GERMISHUIZEN, G. & MEYER, N.L. 2003. Plants of southern Africa: an annotated checklist. Strelitzia 14. Pretoria: National Botanical Institute of South Africa. 1231 p.

GOMPERT, Z., NICE, C.C., FORDYCE, J.A., FORISTER, M.L. & SHAPIRO, A.M. 2006. Identifying units for conservation using molecular systematics: the cautionary tale of the Karner Blue butterfly. *Molecular Ecology,* 15: 1759–1768.

GOTELLI, N.J., ELLISON, A.M., DUNN, R.R. & SANDERS, N.J. 2011. Counting ants (Hymenoptera: Formiciae): Biodiversity sampling and statistical analysis for myrmecologists. *Myrmecological News,* 15: 13–19.

HABERMAN, S.J. 1988. A warning on the use of chi-squared statistics with frequency tables with small expected cell counts. *Journal of the American Statistical Association*, 83(401): 555-560.

HAWKES, P. 2014. Ant sampling and identification [email]. 30 Mar. 2014.

HEATH, A. & CLAASSENS, A.J.M. 2000. New observations of ant associations and life history adaptations (Lepidoptera: Lycaenidae) in South Africa. *Metamorphosis*, 11(1): 3-19.

HEATH, A. & CLAASSENS, A.J.M. 2003. Ant-association among South African Lycaenidae. *Journal of the Lepidopterists' Society*, 57(1): 1-16.

HEATH, A., MCLEOD, L., KALISZEWSKA, Z.A., FISHER, C.W.S. & CORNWALL, M. 2008. Field notes including a summary of trophic and ant associations for the butterfly genera *Chrysoritis* Butler, *Aloeides* Hübner and *Thestor* Hübner (Lepidoptera: Lycaenidae) from South Africa. *Metamorphosis,* 19: 127–148.

HEATH, A. & PRINGLE, E.L. 2004. A review of the southern African genus Thestor Hübner (Lepidoptera: Lycaenidae: Miletinae) in South Africa. Metamorphosis, 15(3): 91-151.

HEATH, A. & PRINGLE, E.L. 2011. Taxonomic notes on the tribe Aphnaeini and genus *Thestor* Hübner (Lepidoptera: Lycaenidae) arising from d'Abrera (2009). *Metamorphosis,* 22: 2–11.

HENNING, G. 2001. Notes on the biology of threatened butterflies in South Africa. *Metamorphosis*, 12(3): 109-122.

HENNING, G.A., TERBLANCHE, R.F. & BALL, J.B., *eds*. 2009. South African Red Data Book - Butterflies. Pretoria: South African National Biodiversity Institute. 158 p.

HENNING, S.F. & HENNING, G.A. 1989. South African red data book: butterflies. South African National Scientific Programmes Report 158. Pretoria: Council for Scientific and Industrial Research.

HENRY, E.H. & SCHULTZ, C.B. 2013. A first step towards successful conservation: understanding local oviposition site selection of an imperilled butterfly, mardon skipper. *Journal of Insect Conservation,* 17: 183–194.

HINTON, H.E. 1951. Myrmecophilous Lycaenidae and other Lepidoptera - a summary. *Proceedings and Transactions of the South London Entomological and Natural History Society*, 1949-1950: 111-175.

HUGHES, L., SIEW-WOON CHANG, B., WAGNER, D. & PIERCE, N.E. 2000. Effects of mating history on ejaculate size, fecundity, longevity, and copulation duration in the ant-tended lycaenid butterfly, *Jalmenus evagoras*. *Behavioral Ecology and Sociobiology,* 47: 119-128.

KALISZEWKA, A. 2014. Phylogeny of the *Thestor* genus [email]. 23 Oct. 2014.

LABITTE, A. 1919. Observations sur *Rhodocera rhamni*. *Bulletin du Museum d'Histoire Naturelle*, 25: 624-625.

LANE, K.A., LUCAS, K.M., & YACK, J.E. 2008. Hearing in the diurnal, mute butterfly, *Morpho peleides*. (Papilionodiea: Nymphalidae). *Journal of Comparative Neurology,* 508(5): 677-686.

LATTKE, J.E. 2000. Specimen processing: Building and curating an ant collection. (*In* Agosti, D., Majer, J.D., Alonso, L.E & Schultz, T.R., *eds.* Ants: Standard methods for measuring and monitoring biodiversity. Washington: Smithsonian Institution Press, Washington, pp. 155–171).

LOHMAN, D.J. & SAMARITA, V.U. 2009. The biology of carnivorous butterfly larvae (Lepidoptera: Lycaenidae: Miletinae: Miletini) and their ant-tended hemipteran prey in Thailand and the Philippines. *Journal of Natural History*, 43 (9&10): 569-581.

LOUW, J.F. 1968. The ecology of the pugnacious ant, *Anoplolepis custodiens* (Smith) (Hymenoptera: Formicidae), in the central Orange Free State – 1: The above-ground activity level of workers. *Journal of the Entomological Society South Africa,* 31: 241–248.

LU, S-S. 2003. Biology and conservation of the threatened Karkloof blue butterfly, *Orachrysops ariadne* (Butler) (Lepidoptera: Lycaenidae). Pietermaritzburg: University of Natal. (Unpublished thesis - D.Phil.) 112 p.

LU, S-S. & SAMWAYS, M.J. 2001. Life history of the threatened Karkloof blue butterfly, *Orachrysops ariadne* (Bulter) (Lepidoptera: Lycaenidae) relevant to its conservation. *African Entomology*, 9: 137-151.

LU, S-S. & SAMWAYS, M.J. 2002. Behavioural ecology of the threatened Karkloof blue butterfly, *Orachrysops ariadne* (Lepidoptera: Lycaenidae) relevant to its conservation. *African Entomology*, 10 (1): 137-147.

LUBKE, R.A. 1998. The coastal environment. (*In*: Lubke, R.A. & de Moore, I., *eds.* Field guide to the eastern and southern Cape coasts. Cape Town: UCT Press. 507 p.).

MANNING, J. 2003. Photographic guide to the wildflowers of South Africa. Pretoria: Briza Publications. 352 p.

MANNING, J. 2007. A field guide to Fynbos. Cape Town: Struik Publishers. 508 p.

MECENERO, S., BALL, J.B., EDGE, D.A., HAMER, M.L., HENNING, G.A., KRÜGER. M., PRINGLE, E.L., TERBLANCHE, R.F. & WILLIAMS, M.C. 2013. Conservation assessment of butterflies of South Africa, Lesotho and Swaziland: Red List and Atlas. Johannesburg & Cape Town: Saftronics (Pty.) Ltd. & Animal Demography Unit. 676 p.

MUCINA, L. & RUTHERFORD, M.C. 2006. The Vegetation of South African, Lesotho and Swaziland. Pretoria: South African National Biodiversity Institute. 807 p.

MUELLER-DOMBOIS, D. & ELLENBERG, H. 1974. Aims and methods of vegetation ecology. New York: John Wiley & Sons. 547 p.

NEW, T.R. 1993. Conservation Biology of Lycaenidae. Switzerland: International Union for Conservation of Nature and Natural Resources. 172 p.

NEW, T.R. & BRITTON, D.R. 1997. Refining a conservation plan for an endangered lycaednid butterfly, *Acrodipsas myrmecophilia,* in Victoria, Australia. *Journal of Insect Conservation*, 1: 65-72.

OTTO, G. 2014. Applying Lepidopteran oviposition science to establish new butterfly larval host plants from Mpumalanga and Limpopo provinces, South Africa. *Metamorphosis*, 25: 82-89.

PIERCE, N.E. 1995. Predatory and parasitic Lepidoptera: Carnivores living on plants. *Journal of the Lepidopterist's Society*, 49(4): 412-453.

PIERCE, N.E., BRABY, M.F., HEATH, A., LOHMAN, D.J., MATHEW, J., RAND, D.B. & TRAVASSOS, M.A. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annual review of Entomology*, 47: 733–771.

PLANT, C., SANDS, R. & FASHAM, M. 2005. Butterflies. (*In* Hill, D., Fasham, M., Tucker, G., Shewry, M. & Shaw, P., *eds.* Handbook of Biodiversity Methods. Survey, Evaluation and Monitoring. Cambridge: Cambridge University Press, pp. 328–334).

PRINS, A.J. 1982. Review of *Anoplolepis* with reference to male genitalia, and notes on *Acropyga* (Hymenoptera, Formicidae) *Annals of the South African Museum,* 89(3): 215–247.

ROUGET, M., JONAS, Z., COWLING, R.M., DESMET, P.G., DRIVER, A., MOHAMED, B., MUCINA, L., RUTHERFORD, M.C. & POWRIE, L.W. 2006. Ecosystem status and protection levels of vegetation types. (*In*: Mucina L. & Rutherford M.C., *eds.* The vegetation of South Africa, Lesotho and Swaziland. Strelitzia 19. Pretoria: South African National Biodiversity Institute, pp. 724–737).

RUTOWSKI, R.L. 1991. The evolution of male mate-locating behaviour in butterflies. *The American Naturalist*, 138: 1121-1139.

RUTOWSKI, R.L. 2003. Visual ecology of adult butterflies. (*In* Boggs, C.L., Watt, W.B., & Ehrlich, P.R., *eds*. Ecology and evolution taking flight. Chicago, London: The University of Chicago Press. pp. 9-25).

RUTOWSKI, R.L., MCCOY, L. & DEMLONG, M. 2001. Visual mate detection in a territorial male butterfly (*Asterocampa leilia*): effects of distance and perch local. *Behaviour*, 138: 31-43.

SCHULZE, R.E. & MCGEE, O.S. 1978. Climatic indices and classifications in relation to the biogeography of southern Africa. (*In* Werger M.J.A., *ed.* Biogeography and ecology of southern Africa, The Hague: W. Junk, pp. 19–55).

SCOTT, J.A. 1986. The butterflies of North America: A natural history and field guide. California: Stanford University Press. 583 p.

SINGER, M. C., 1984. Butterflies-hostplant relationships: Host quality, adult choice and larval success. (*In* Vane-Wright, R.I. & Ackery, P.R., *eds*. The biology of butterflies. London: Academic Press. pp. 81-88).

STEYN, J.J. 1954. The pugnacious ant (*Anoplolepis custodiens* Smith) and its relation to the control of citrus scales at Letaba. *Memoirs of the Entomological Society of Southern Africa,* 3: 1–96.

STUTT, A. & WILLMER, P. 1998. Territorial defence in speckled wood butterflies: do the hottest males always win? *Animal Behaviour*, 55: 1341-1347.

SUTHERLAND, W.J. 1998. The importance of behavioural studies in conservation biology. *Animal Behaviour* 56: 801-809.

SWANEPOEL, D.A. 1953. Butterflies of South Africa. Cape Town: Maskew Miller. 320 p.

TAKEUCHI, T. & HONDA, K. 2009. Early comers become owners: Effect of residency experience on territorial contest dynamics in a Lycaenid butterfly. *Ethology*, 115: 767-773.

TAKEUCHI, T. & IMAFUKU, M. 2005. Territorial behaviour of a Green Hairstreak *Chrysozephyrus smaragdinus* (Lepidoptera: Lycaenidae): Site tenacity and wars of attrition. *Zoological Science*, 22(9): 989-994.

TERBLANCHE, R.F., MORGENTHAL, T.L. & CILLIERS, S.S. 2003. The vegetation of three localities of the threatened butterfly species *Chrysoritis aureus* (Lepidoptera: Lycaenidae) *Koedoe,* 46: 73–90.

THOMAS, J.A. 1995. The ecology and conservation of *Maculinea arion* and other European species of large blue butterfly. (*In*: Pullin, A.S., *ed*. Ecology and conservation of butterflies. London: Chapman & Hall, pp. 180-197).

TOERIEN, D.K. 1979. The geology of the Oudtshoorn area. Explanation to sheet 3322. Government Printer, Pretoria.

TRIMEN, R. 1883. Descriptions of twelve new species of South-African Lepidoptera - Rhopalocera. *Transactions of the Entomological Society London,* 1883: 347-363.

TRIMEN, R. & BOWKER, J.H. 1887. South-African butterflies: A monograph of the Extra-Tropical Species. London: Trübner & Company.

VALLIN, A., JAKOBSSON, S., LIND, J. & WIKLUND, C. 2006. Crypsis versus intimidation: Antipredation defence in three closely related butterflies. *Behavioural Ecology and Sociobiology*. 59: 455-459.

VAN HAMBURG, H., ANDERSEN, A.N., MEYER, W.J. & ROBERTSON, H.G. 2004. Ant community development on rehabilitated ash dams in the South African Highveld. *Restoration Ecology,* 12(4): 552–558.

WANG, X.-P., FANG, Y.-L. & ZHANG, Z.-N. 2005. Effect of male and female multiple mating on the fecundity, fertility, and longevity of diamondback moth, *Plutella xylostella* (L.). *Journal of Applied Entomology*, 129: 39–42.

WESTHOFF, V. & VAN DER MAAREL, E. 1980. The Braun-Blanquet approach. (*In*: Whittaker, R.H. & Junk, W., *eds.* Classification of plant communities. The Hague: Junk Publishers, pp. 287–399).

WICKMAN, P.O. 1985. Territorial defence and mating success in males of the small heath butterfly, *Coenonympha pamphilus* (L.) (Lepidoptera: Satyridae). *Animal Behaviour*, 33: 1162-1168.

WICKMAN, P.O. 1986. Courtship socialisation by females of the small heath butterfly, *Coenonympha pamphilus* (L.) (Lepidoptera: Satyridae) and their behaviour in relation to male territories before and after copulation. *Animal Behaviour*, 34: 153–157.

WIKLUND, C. 2003. Sexual Selection and the Evolution of Butterfly mating systems. (*In* Boggs, C.L., Watt, W.B., & Ehrlich, P.R., *eds*. Ecology and evolution taking flight. Chicago, London: The University of Chicago Press. pp. 67-90).

WIKLUND, C., GOTTHARD, K., & NYLIN, S. 2003. Mating system and the evolution of sex-specific mortality rates in two nymphalid butterflies. *Proceedings of the Royal Society B,* 270: 1823-1828.

WIKLUND, C., KAITALA, A., LINDFORS, V. & ABENIUS, J. 1993. Polyandry and its effect on female reproduction in the green-veined white butterfly (*Pieris napi* L.). *Behavioral Ecology and Sociobiology*, 33: 25-33.

WIKLUND, C., KAITALA, A. & WEDELL, N. 1998. Decoupling of reproductive rates and parental expenditure in a polyandrous butterfly. *Behavioral Ecology,* 9: 20-25.

WILLIAMS, M. 1994. Butterflies of Southern Africa. A field guide. South Africa: Southern Book Publishers. 303 p.

WILLIAMS, M.C. 2006. Notes on oviposition behaviours in three South African lycaenid butterflies (Lepidoptera: Lycaenidae) *Metamorphosis,* 17: 33–38.

WILLIAMS, M.C. & JOANNOU, J.G. 1996. Observations on the oviposition behaviour and caterpillars of *Thestor basutus capeneri* Dickson (Lepidoptera: Lycaenidae) in South Africa. *Metamorphosis,* 7: 12–16.

WITT, A.B.R & GILIOMEE, J.H. 1999. Soil-surface temperature at which six species of ants (Hymenoptera: Formicidae) are active. *African Entomology*, 7: 161-164.

WYNHOFF, I., GRUTTERS, M. & VAN LANGEVELDE, F. 2008. Looking for the ants: selection of oviposition sites by two myrmecophilous butterfly species. *Animal Biology*, 58: 371-388.

WOODHALL, S. 2005. Field Guide to butterflies of Southern Africa. Cape Town: Struik. 400 p.

YACK, J.E. & FULLARD, J.H. 2000. Ultrasonic hearing in nocturnal butterflies. *Nature,* 403: 265-266.

Glossary

aphytophagous – not feeding on plants

coccoids – hemipterans of the superfamily Coccoidea, known as scale insects

epigaeic – dwelling or foraging on the ground

instar – a stage between two periods of moulting in the development of an arthropod larvae

jassids – sap-sucking hemipteran insects known as leap hoppers of the family Cicadellidae

oviposition – laying of eggs

phytophagous – feeding on plant material only

polydomous – ant colonies occupying or maintaining multiple nests

membracids – sap feeding hemipteran insects of the family Membracidae, also known as treehoppers

myrmecophily – a symbiotic relationship between an invertebrate or plant and ants

trophallaxis – exchange of food between adults and young, particularly social insects

unicoloniality – social structure of ants in which the workers can move freely between different nests

univoltine – having only one reproductive cycle per year

Appendices

Appendix 1: Trophic resources known to be used by members of the genus *Thestor*

Thestor Species Group	Number of Species	*Thestor* Species	1^{st}- 3^{rd} Instar Larvae	Final Instar Larvae	Reference
basutus	2	*T. basutus basutus*	Psyllids, coccids	Trophallaxis, detritus, ant brood	Clark and Dickson (1960, 1971) Claassens and Heath (2003)
		T. basutus capeneri	1st – 3rd instars - coccids (*Pulvinaria icyeri*)	Unknown	Williams and Joannou (1996)
protumnus	4	*T. protumnus aridus*	Coccids	Unknown, possibly detritus	Clark and Dickson (1960, 1971)
brachycerus	12	T. brachycerus brachycerus	1st instar larvae died without feeding	Unknown	Clark and Dickson (1971)
		T. brachycerus dukei	1st instar larvae died – offered white scale from bush laid on but would not eat	Unknown	Clark and Dickson (1971)
		T. rileyi	1st instar larvae died in artificial ants' nest	Final instar – trophallaxis (*Anoplolepis custodiens*)	Clark and Dickson (1971) Claassens and Heath (1997)
		T. yildizae	1st instar larvae died in artificial ants' nest	Final instar - trophallaxis (*Anoplolepis custodiens*)	Claassens and Dickson (1980) Claassens and Heath (1997)
braunsi	5		Unknown	Unknown	
montanus	5	*T. pictus*	Unknown	Final instar - trophallaxis (*Anoplolepis custodiens*)	Claassens and Heath (2003)
compassbergae	4		Unknown	Unknown	
rossouwi	1		Unknown	Unknown	
murrayai	1		Unknown	Unknown	

Appendix 2: Adult *T. b. brachycerus* counts made between during the emergence period between December 2009 and January 2014 at the Pezula colony, P1 and between January 2012 and January 2014 at the Coastal site colony, C1

PEZULA GOLF ESTATE COLONY P1

	Observed (O*i*)			Expected (E*i*)	
Date	**Males**	**Females**	**Total**	**Males**	**Females**
21-Dec-09	2	0	2	1	1
22-Dec-09	3	2	5	2.5	2.5
23-Dec-09	4	2	6	3	3
27-Dec-09	3	2	5	2.5	2.5
28-Dec-09	2	1	3	1.5	1.5
29-Dec-09	5	1	6	3	3
30-Dec-09	6	2	8	4	4
31-Dec-09	4	0	4	2	2
02-Jan-10	8	2	10	5	5
05-Jan-10	8	1	9	4.5	4.5
06-Jan-10	8	3	11	5.5	5.5
09-Jan-10	6	2	8	4	4
13-Jan-10	3	1	4	2	2
16-Jan-10	2	1	3	1.5	1.5
17-Jan-10	3	2	5	2.5	2.5
18-Jan-10	3	1	4	2	2
24-Jan-10	2	1	3	1.5	1.5
13-Dec-10	1	0	1	0.5	0.5
02-Jan-11	3	1	4	2	2
04-Jan-11	1	0	1	0.5	0.5
07-Jan-11	1	1	2	1	1
12-Jan-11	5	2	7	3.5	3.5
26-Jan-11	2	1	3	1.5	1.5
26-Dec-11	2	0	2	1	1
29-Dec-11	3	1	4	2	2
04-Jan-12	4	1	5	2.5	2.5
06-Jan-12	3	1	4	2	2
08-Jan-12	6	0	6	3	3
12-Jan-12	4	1	5	2.5	2.5
16-Jan-12	6	1	7	3.5	3.5
20-Jan-12	5	1	6	3	3
26-Jan-12	2	1	3	1.5	1.5
16-Dec-12	3	1	4	2	2
20-Dec-12	4	2	6	3	3
13-Jan-13	7	2	9	4.5	4.5
21-Jan-13	3	2	5	2.5	2.5
13-Dec-13	4	1	5	2.5	2.5
27-Dec-13	5	0	5	2.5	2.5
03-Jan-14	6	2	8	4	4
15-Jan-14	4	1	5	2.5	2.5
Mean	**3.90**	**1.18**	**5.08**		

***Chi*-squared test** **p = 0.00256**

t-test **t = 0.44734**

COASTAL COLONY C1

	Observed (O*i*)			Expected (E*i*)	
Date	**Males**	**Females**	**Total**	**Males**	**Females**
20-Jan-12	3	1	4	2	2
25-Jan-12	4	1	5	2.5	2.5
26-Jan-12	5	1	6	3	3
31-Jan-12	0	0	0	0	0
16-Dec-12	4	2	6	3	3
20-Dec-12	6	3	9	4.5	4.5
13-Jan-13	5	0	5	2.5	2.5
21-Jan-13	6	2	8	4	4
13-Dec-13	5	2	7	3.5	3.5
28-Dec-13	5	1	6	3	3
04-Jan-14	5	2	7	3.5	3.5
18-Jan-14	4	1	5	2.5	2.5
Mean	**4.33**	**1.33**	**5.67**		

***Chi*-squared test** **p = 0.01993**

*** Zero frequency observation not included in *Chi*-squared test**

Appendix 3: Adult *T. b. brachycerus* counts made between during the emergence period between December 2009 and January 2014 at the Pezula colony, P1 and between January 2012 and January 2014 at the Coastal site colony, C1, clustered into seasons

PEZULA COLONY P1

	Observed (O*i*)			**Expected (E*i*)**	
Season	**Males**	**Females**	**Total**	**Males**	**Females**
Dec 09 - Jan 10	72	24	96	48	48
Dec 10 - Jan 11	13	5	18	9	9
Dec 11 - Jan 12	35	7	42	21	21
Dec 12 - Jan 13	17	7	24	12	12
Dec 13 - Jan 14	19	4	23	11.5	11.5

***Chi*-squared test p = 2.67E-12**

COASTAL COLONY C1

	Observed (O*i*)			**Expected (E*i*)**	
Season	**Males**	**Females**	**Total**	**Males**	**Females**
Jan 12	12	3	15	7.5	7.5
Dec 12 - Jan 13	21	7	28	14	14
Dec 13 - Jan 14	19	6	25	12.5	12.5

***Chi*-squared test p = 6.91E-05**

Appendix 4: Soil and rock analysis results showing macro and micro element composition, pH and electrical conductivity (EC) of the soil samples. Samples 1-5 are from the Pezula site – sample 2 lies within the P1 colony. Sample 6 relates to the Coastal site colony, C1. Differences are highlighted in red. The pH and quartz and muscovite content of the rock samples taken from P1 (sample 2) and C1 (sample 6) are also given

Macro-elements													COMMENTS	MINERALOGY OF ROCK		
Sample no.	**Ca**	**Mg**	**K**	**Na**	**PO_4**	**SO_4**	**NO_3**	**NH_4**	**Cl**	**HCO_3**	**SITE**	**LOCATION**		**Quartz**	**Muscovite**	**pH**
	Millimol per litre													SiO_2	$KAl_2(AlSi_3O_{10})(OH)_2$	(H_2O)
1	0.05	0.04	0.07	0.36	0.01	0.12	0.14	0.05	0.23	0.05	PEZ01	East of colony	No significant differences			
2	0.06	0.04	0.07	0.25	0.01	0.07	0.04	0.03	0.29	0.10	PEZ02	P1 colony		99.7	0.3	7.34
3	0.07	0.03	0.31	0.73	0.00	0.12	0.33	0.02	0.64	0.05	PEZ07	Mid-slope				
4	0.01	0.01	0.02	0.34	0.01	0.07	0.09	0.04	0.18	0.05	PEZ04	West of colony				
5	0.05	0.01	0.02	0.52	0.01	0.07	0.14	0.09	0.44	0.05	PEZ13	Bottom of slope				
6	0.39	0.41	0.39	4.59	0.03	0.25	0.22	0.06	4.18	1.70	CS05	C1 colony, near sea	Presence of sea salt elements	94.7	5.2	8.42

Micro-elements and other data												Particle Size Distribution			
Sample no.	**Fe**	**Mn**	**Cu**	**Zn**	**B**	**pH**	**EC**	**P-BRAY 1**				**> 2mm**	**Sand**	**Silt**	**Clay**
	Micromol per litre						*(mS/cm)*	*ppm*							
1	16.43	0.02	0.02	1.39	17	4.90	0.07	< 0.01	PEZ01	East of colony		0.1	77.5	13.6	9.0
2	21.48	2.51	0.67	1.53	1	5.16	0.06	< 0.01	PEZ02	P1 colony	High manganese and copper	0.1	74.3	16.4	9.3
3	17.89	0.46	0.23	0.14	27	5.38	0.13	<0.01	PEZ07	Mid-slope		0.2	74.4	16.4	9.3
4	28.08	1.79	0.31	0.02	15	4.74	0.05	< 0.01	PEZ03	West of colony		0.0	80.3	13.2	6.5
5	25.54	2.86	0.07	0.02	1	4.97	0.08	< 0.01	PEZ13	Bottom of slope	Finer soil	0.0	63.9	25.8	10.2
6	15.55	3.08	0.03	0.02	17	7.96	0.67	< 0.01	CS03	C1 colony	Very high pH and EC; coarse soil	9.3	93.5	4.4	2.1

Appendix 5: Plant family abbreviations

Family	Abbreviation
Acanthaceae	Acant.
Aizoaceae	Aizo.
Anacardiaceae	Anac
Asparagaceae	Aspar.
Asteraceae	Aster.
Celastraceae	Celas.
Cyperaceae	Cyper.
Dennstaedtiaceae	Denn.
Ericaceae	Eric.
Fabaceae	Fab.
Gentianaceae	Gent.
Geraniaceae	Geran.
Plumbaginaceae	Plumb.
Poaceae	Poa.
Proteaceae	Prot.
Restionaceae	Rest.
Rhamnaceae	Rhamn.
Rubiaceae	Rubi.
Thymelaceae	Thym.
Vitaceae	Vit.

Appendix 6: Vegetation abundance matrices showing the Braun-Blanquet cover abundance scale recorded for each of the plant species sampled at the Pezula site relevés PEZ01-PEZ15

		Pezula Study Site Relevés														
Plant Species	**Code**	**PEZ01**	**PEZ02**	**PEZ03**	**PEZ04**	**PEZ05**	**PEZ06**	**PEZ07**	**PEZ08**	**PEZ09**	**PEZ10**	**PEZ11**	**PEZ12**	**PEZ13**	**PEZ14**	**PEZ15**
Pteridium aquilinium	Ptaq													2b		
Ficinia hirsuta	Fihi	2a	r	1												
Cymbopogon marginatus	Cyma						1						1		2b	1
Eragrostis curvula	Ercu	+												1		
Eragrostis capensis	Erca	r														r
Heteropogon contortus	Heco	1					1						1			
Panicum maximum	Pama															2a
Eragrostis obtusa	Erob	+					+									
Setaria sphacelata var. torta	Sesp	r														
Aristida congesta subsp. congesta	Arco	r														
Digitaria eriantha	Dier	+						1			1		1			
Themeda triandra	Thtr	r	r				1									
Tristachya leucothrix	Trle			+	1	3	2b	2b				2a	+			
Restio triticeus	Retr	+	r	r				4	1		2a					
Restio leptoclados	Rele	1			1			1	1		1	4		2b		
Senecio lanifer	Sela	r	r										+			
Dimorphotheca nudicaulis	Dinu			+												
Hermannia decumbens	Hede							1	1	r	+			r		r
Oxalis sp.	Ox01		r		r							r				
Lobelia tomentosa	Loto						+									
Searsia lucida forma *lucida*	Selu												3		1	+
Searsia glauca	Segl															1
Searsia tomentosa	Seto															1
Searsia pyroides var. pyroides	Sepy	r	2b						+	+				1		
Metalasia aurea	Meau			1												
Conyza scrabida	Cosc	2a	+		+	+	1		1	r		1		r		
Helichrysum cymosum cymosum	Hecy	1	2a	2a	2a	1	1	1	3	1	2a	1	2a	1	1	+
Helichrysum foetidum	Hefo					+										
Helichrysum petiolare	Hepe		2a	r						r						
Helichrysum teretifolium	Hete						r									
Hippia frutescens	Hifr									r						
Metalasia muricata	Mede		r													
Tarchonanthus littoralis	Tali						+		+		r		1		+	
Seriphium plumosum	Stpl	1	2b	3	2b	3			2a	5	2a					1
Senecio rigidus	Seri		2a													+
Erica canaliculata	Erca	2a	1	2a	+	1	+	r	1		3	4	+	2b	r	
Erica peltata	Erpe															
Diospyros dichrophylla	Didi													2a		
Leucadendron salignum	Lesa		2a	1	2a	2a			2a	1	1					
Phylica axillaris	Phax															3
Anthospermum aethiopicum	Anae	1	+	r	1	1	2a	1	1	+	1	1	2b		4	2a
Sruthiola hirsuta	Srhi											2a		1		
Watsonia fourcadei	Wafo	+									r					
Commelina africana var. africana	Coaf				+	1										
Dead material	DPM	3	2a	2a	2b	2b	1	2a	1	1	2a	2a	1			
Rocky Ground	Rocky						1	2a	1		2a		+			
Bare Ground	Bare						1		1			+	1		1	1

Appendix 7: Vegetation abundance matrices showing the Braun-Blanquet cover abundance scale recorded for each of the plant species sampled at the Coastal site relevés CS01 – CS08

		Coastal Study Site Relevés							
Plant Species	**Code**	**CS01**	**CS02**	**CS03**	**CS04**	**CS05**	**CS06**	**CS07**	**CS08**
Stenotaphrum secundatum	Stse	1	3	3	3	5		1	r
Gazania rigens var. uniflora	Gaun	+			4	1			
Gazania rigens var. leucolaena	Gale	+							
Silene bellidioides	Sibe								r
Limonium scabrum var. scabrum	Lisc	1	1	1		r		1	1
Solanum africanum	Soaf						r		
Atriplex littoralis	Atli					r			
Indigofera porrecta var. porrecta	Inpo	1	+	1		+		r	
Hypoestes aristata var. aristata	Hyar	1	2b	2a				2a	2b
Asparagus aethiopicus	Asae	1	1	r			2b	1	2b
Helichrysum teretifolium	Heae								1
Eriocephalus sp.	Er01	1					2b	2b	
Passerina rigida	Pari	+	1						
Sideroxylon inerme	Siin	1							
Tarchonanthus littoralis	Tali						2a	2b	
Pelargonium sp.	Pe01						1		
Cotula turbinata	Cotu		1						
Lobelia anceps	Loan		+						
Chironia baccifera	Chba							2a	
Rhoicissus digitata	Rhdi	+						1	
Pterocelastrus tricuspidatus	Pttr							1	
Maytenus procumbens	Mapr	1	1	r					
Capparis sepiaria	Case			+					+
Kedrostis nana var. nana	Kena			r			r		+
Carpobrotus deliciosus	Cade	1		1			2a	1	2a
Delosperma patersoniae	Depa	1	2b	r			2b	r	2b
Erepsia sp.	Er01		r						r
Tetragonia fruticosa	Tefr	1	1						2a
Drosanthemum sp.	Dr01			2b					
Crassula pellucida subsp. marginalis	Crpe			+			r		
Crassula orbicularis	Cror						+		
Cotyledon orbiculata var. orbiculata	Coor						r		
Zygophyllum morgansana	Zymo	1							
Rocks	Rocky	4	1	3	2a	1	3		2b
Dead Plants	DPM	2a	2a	1					1
Bare Ground	Bare				1				