An investigation of the aquatic macroinvertebrate fauna of the southern Great Escarpment (South Africa): Insights from ecological and genetic studies

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Abstract

Biological diversity in freshwater biomes is vital to maintain healthy, functioning ecosystems with resilience to disturbance and the impacts of climate change. Freshwater ecosystems provide essential resources to life on Earth. However, as increasing pressure is being placed on the environment by human population growth, the quality of freshwater resources and the health of these ecosystems are at risk. Mountain streams provide an important source of water and are usually less affected by anthropogenic stressors, compared to lowland freshwaters. These montane streams are therefore of important conservation value and due to their untransformed nature serve as ideal ecosystems for biodiversity studies and as reference sites for studies on environmental change.

This study explores aquatic macroinvertebrate biodiversity of the southern Great Escarpment in South Africa. Species assemblages and the environmental variables of each site were sampled from first order streams across five different mountain blocks along the Great Escarpment. Additionally, mitochondrial DNA of three mayfly species (*Afroptilum sudafricanum, Demoreptus natalensis* and *Demoreptus capensis*), commonly occurring in the study area, was analysed to compare the genetic diversity between habitat specialist and habitat generalist species.

A total of 2 595 macroinvertebrate specimens from 47 families and 86 species were collected with several interesting and potentially new species being discovered. Partitioned diversity analyses indicate that macroinvertebrate diversity varies across mountain blocks. Multivariate analyses indicate that differences in assemblages could be attributed to differences in environmental variables between sites, particularly water velocity, total dissolved solids and salinity. As these environmental variables reflect of the topography of the sites, differences in species assemblages was attributed to difference in topography and therefore biotopes present.

Habitat-restricted mayfly species (*D. natalensis* and *D. capensis*), occur in distinct populations confined to mountains blocks. Isolation-by-distance analyses further emphasis that these species are genetically restricted by their habitat preference for mountain streams. In contrast, *A. sudafricanum,* a habitat generalist, showed no indication of genetic structure due to location or distance. Possible cryptic taxa and new species were identified within *A. sudafricanum* and *Dermoreptus* respectively.

This study provides an important contribution to the baseline data of freshwater macroinvertebrate diversity for the southern Great Escarpment region and provides insights into the considerable genetic diversity of selected aquatic taxa across this region.

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List of Abbreviations

16S	Small subunit ribosomal 16S
AIC	Akaike information criterion
AMOVA	Analysis of Molecular Variance
ANOSIM	Analysis of similarities
BI	Bayesian Inference
CCA	Canonical Correspondence Analysis
COI	Cytochrome c oxidase subunit I
DEAT	Department of Environmental Affairs and Tourism
DO	Dissolved oxygen
EC	Eastern Cape
ENPAT	Environmental Potential Atlas for South Africa
FEPA	Freshwater Ecosystem Priority Area
GARLI	Genetic Algorithm for Rapid Likelihood Inference
GIS	Geographic Information System
GTR	Generalised time-reversible
GPS	Global Positioning System
GWA	Great Winterberg-Amathole
IBD	Isolation-by-Distance
IBT	Inter-basin [water] transfers
KZN	KwaZulu-Natal
m.a.s.l.	metres above sea level
MYA	million years ago
NEMA	National Environmental Management Act
NFEPA	National Freshwater Ecosystem Priority Areas project
NMDS	Non-metric multidimensional scaling
NWA	National Water Act
PCA	Principle Component Analysis
PCR	Polymerase chain reaction
pers. comm.	Personal communication
PSU `	Practical salinity units
RIVPACS	River Invertebrate Prediction and Classification System
SASS	South African Scoring System
SIC	Stones in current
SIMPER	Similarity percentages
SOOC	Stones out of current
TBR	Tree bisection and reconnection
TDS	Total dissolved solids

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I am grateful for the continued support of many people who made this thesis possible. Firstly to my supervisors: Prof Nigel Barker for his endless advice in the lab, support and planning for fieldwork and everything else involved in writing up; Dr Helen Barber-James, whose expertise in aquatic entomology was invaluable and who always encouraged me; and Prof Martin Villet, who always pushed me that little bit further to better my work and gave extensive advice on various draft versions. I appreciate all the input you have all given throughout my research.

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Declaration

This thesis is the result of the author’s original work except where acknowledged or specifically stated in the text. It has not been submitted for any other degree of examination at any other university or academic institution.

Taylor

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C.L. Taylor

CHAPTER 1

General Introduction: Freshwater ecosystems

Mountain catchments

Mountains and highlands have immense hydrological importance for downstream lowlands and are often referred to as 'water towers' because of the essential source of freshwater they provide to people, animals and plants (Viviroli et al., 2007, 2003). The origins of many of the Earth's rivers are in mountain regions, due to the general correlation between altitude and precipitation and the negative correlation between altitude and evapotranspiration, resulting in large amounts of runoff (Weingartner et al., 2009). The runoff from mountains is often used extensively downstream by humans (Viviroli and Weingartner, 2004).

A few studies have tried to quantify the significance of mountain regions as a water resource for lowland areas (López and Justribó, 2010; Viviroli and Weingartner, 2004; Viviroli et al., 2007, 2003). The most recent global study by Viviroli et al. (2007) investigated the role of the world's mountains in supplying the Earth's surface with freshwater. The study showed that outside the humid tropics, mountains cover only 40% of total land surface but contribute as much as 56% of total runoff. Additionally, in arid zones where mountains are the major source of water, the contribution of mountains to total runoff can be as much as 70%. Viviroli et al. (2003) ranked the world's mountain regions in terms of their hydrological and ecological significance, taking into account the aridity of the lowlands. In Southern Africa, the Orange River is considered extremely important due to the amount of water it supplies to the arid lowlands (Viviroli et al., 2003, 2004). The Orange River originates in the highlands of Lesotho, the Drakensberg mountains and the Stormberg mountains, then flows west through the Northern Cape Province to the Atlantic Ocean.

Ecosystem services

The processes through which natural ecosystems, and the species within them, sustain human life are termed 'ecosystem services' (Daily et al., 1997). Freshwater ecosystems occupy only 0.8% of Earth's surface, yet they provide humans with essential natural resources, in economic, cultural, aesthetic, scientific and educational terms (Dudgeon et al., 2006). In South Africa, agricultural irrigation accounts for the highest percentage (62%) of the country's total water requirement, this is followed by urban requirements, mining and bulk industry, rural requirements, afforestation and power generation (Department of Environmental Affairs and Tourism [DEAT], 2006).Water and its associated biodiversity also provides livelihoods for fishermen, farmers, foresters and people who use reeds for thatching and basket-making (DEAT, 2006).

It is not only the services we receive directly from the use of water that are important, but also the ecological infrastructure that freshwater ecosystems provide. Intact freshwater ecosystems support communities of plants and animals that are able to remove excess nutrients and toxic substances from water, keeping it cleaner for drinking, irrigation and recreation. Natural vegetation along river banks helps to trap sediments, prevent erosion and break down pollutants draining from the surrounding catchment (Fenessey and Cronk, 1997). These ecosystems also provide regulating services such as preventing floods, easing the impacts of droughts and buffering the effects of storms (Allan and Castillo, 2007). These services are often overlooked due to the difficulties in quantifying their economic value. The biodiversity of freshwater ecosystems play an important role in the functioning and quality of the ecosystem as a whole. Even though only a very small proportion of the Earth's surface is occupied by freshwater ecosystems, they are estimated to support at least 100 000 species, which accounts for nearly 6% of all described species (Dudgeon et al., 2006).

Aquatic invertebrates are involved in many different processes in freshwater ecosystems. They contribute significantly to nutrient cycling and the turnover of organic material, whether produced within the system or when entering from the riparian zone (Malmqvist, 2002). Macroinvertebrates are consumers at intermediate trophic levels and are therefore influenced by both bottom-up and top-down forces in streams (Wallace and Webster, 1996). The physical environment of streams is heterogeneous and invertebrates have evolved a diverse array of morphological and behavioural mechanisms for acquiring food resources (Wallace and Webster, 1996). Cummins and Klug (1979) characterised the following functional feeding groups based on these mechanisms: scrapers, grazers, shredders, gatherers, filterers and predators, reflecting how the varied physical characteristics of a stream results in a highly diverse assemblage of macroinvertebrates. Individual species may be of little unique use in the sense of ecosystem functioning, but the macroinvertebrates as a group perform essential functions and are critical to stream health, maintenance and integrity (Angermier and Karr, 1994). This illustrates the importance of biodiversity and its role for healthy, functioning ecosystems. The three levels at which biological diversity[1] is considered are genetic diversity, species diversity and ecosystem diversity. The value of biodiversity is not only in the provision of ecosystem services such as through cleaning water; it also has direct benefits to economic productivity by

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[1] Biological diversity, or biodiversity, is defined by the Convention on Biological Diversity (United Nations, 1992) and the National Biodiversity Act (Act No. 10 of 2004) (Republic of South Africa, 2004) as the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.

providing a food source to fish and as a storehouse of genetic information (Dudgeon et al., 2006). Genetic diversity allows plasticity of phenotypes and is of fundamental importance for the adaptation of species to future environmental changes (Jump et al., 2009).

The diversity of habitats and species is a result of local to global evolution over millions of years. This process of evolution is dynamic, whereby new genetic variation within a species or population, creation of a new species or the formation of novel ecosystems would cause an increase of biodiversity. Similarly, decline of genetic variation, extinction of species or loss of ecosystems would result in a decrease in biodiversity.

Aquatic macroinvertebrates as biological indicators

Macroinvertebrates serve as valuable indicators of stream degradation and have been used extensively to evaluate water quality through the rapid bioassessment of rivers. In recent years, interest in this area has grown with the proliferation of numerous techniques, such as those reviewed by Rosenberg and Resh (1993). The most widely used methods are based on tolerance values, where specific taxa are given a numerical score according to the ability of the taxon to inhabit streams differing in water quality (Chutter, 1972, 1994; Lenat, 1993; Plafkin et al., 1989).

Regulatory authorities have recognised the value of bioassessment data in the management of freshwater resources and countries such as the United Kingdom and Australia have since implemented the RIVPACS (River Invertebrate Prediction and Classification System) method developed by Wright et al. (1984). In South Africa there is a large amount of support for this type of river assessment. Chutter first developed the Biotic Index in 1972, but it was excessively labour-intensive and in the 1990s he set out to develop an index that would be faster and easier to use. Through a forum hosted by the South African Water Research Commission, input was received from a large group of practitioners and finally SASS (South African Scoring System) evolved. This method was published, in brief, in 1994 and more fully in 1998 (Chutter, 1994, 1998). Over recent years the method has become the standard for the rapid bioassessment of rivers in southern Africa and now forms the backbone of the National River Health Programme (Uys et al., 1996). SASS has since been further developed, refined and the SASS version 5 (Dickens and Graham, 2002) is now extensively used.

The many roles performed by macroinvertebrates in streams are clearly evident, from the direct benefits of maintaining healthy freshwater habitats, to their indirect value as vital indicator organisms in biomonitoring. These roles underpin the importance of aquatic macroinvertebrate conservation and, when considering the lack of data available, the need for further research is very evident.

Threats

Due to the relentless demands on freshwater ecosystems to sustain human needs, this valuable natural resource is in crisis. The biodiversity and biological resources of inland waters are facing unprecedented and growing threats from human activities with recorded rates of biodiversity loss being far greater in freshwater ecosystems compared to terrestrial ecosystems (Sala et al., 2000; Nel et al., 2011a). More than half of the river and wetland ecosystems in South Africa are now classified as threatened (Nel et al., 2011b). If trends in human demands for water remain unaltered and species losses continue at current rates, the opportunity to conserve much of the remaining freshwater biodiversity will be lost (Dudgeon et al., 2006). This will mean that the current natural functioning of the ecosystems will be destroyed, along with the benefits to humans that, ironically, created the initial demand. Ultimately, knowledge of the total diversity of freshwaters is hugely incomplete, particularly among invertebrates and microbes, with data relating to fish fauna being more comprehensive (Wishart et al., 2003; Dudgeon et al., 2006). Of the world’s 10 000 described freshwater fish species it is estimated that approximately 20% are extinct, threatened or endangered (Moyle and Leidy, 1992), with many more aquatic species such as crabs, macroinvertebrates and molluscs recorded as threatened (Darwell et al., 2009). A number of factors contribute to the decline of freshwater ecosystems including flow alteration, water pollution, habitat degradation and alien invasive species.

South Africa is considered a water-stressed country, bordering on water-scarce, according to the United Nations’ definition of terms (UN Water, 2013). Since water demand in many areas of South Africa exceeds the natural supply, water transfer schemes are widespread and large reservoirs are used as storage. Such transfers are termed inter-basin water transfers (IBTs); water is relocated from basins with perceived surpluses to basins and human populations with perceived water deficits (Wishart et al., 2003). This results in altered flow regimes (e.g. timing, frequency, speed or volume of flow) which changes river channel characteristics and habitats and has profound negative effects on freshwater plants and animals (Nel et al., 2011a).

Water quality in South Africa has declined due to increased pollution from industry, urban expansion, mining, power generation, agriculture, forestry and inadequate sewage treatment (DEAT, 2006). Pollution occurs when an excess of an unwanted or harmful substance is introduced into the environment by humans (Holdgate, 1979). Salinisation and eutrophication, resulting from an increase in salinity and nutrient loads in water resources, have a huge impact on human health, the utility of water resources and the structure and functioning of freshwater ecosystems (Nel et al., 2011a).

River (and especially wetland) ecosystems are frequently transformed for planting crops or for building infrastructure resulting in habitat destruction and degradation. This often causes irreversible damage to freshwater ecosystems and reduces the positive ecosystem services they provide (Nel et al., 2011a). Alien invasive plants also degrade riparian habitats and in 2001 were estimated to consume 7% of South Africa’s annual runoff (Binns et al., 2001). With the continued spread of alien invasive species this figure was predicted to increase significantly over time (Van Wilgen et al., 2008). Encroachment by alien invasive riverine trees such as black wattle (*Acacia mearnsii*) and long-leaved wattle (*A. longifolia*) along water courses threaten endemic dragonfly species, with 11 of the 31 South African species under threat (Samways and Taylor, 2004). Invasive alien fish are also a threat to aquatic ecosystems through altering habitats, competing for resources and eating indigenous plants and animals; they have also been associated with loss of invertebrate species (Nel et al., 2011a). Since these invasive alien species currently occur extensively in most large rivers in South Africa, uninvaded streams (often the smaller tributaries of large rivers) now serve as the last remaining refuges for indigenous freshwater species.

Studies indicate that climate change is expected to alter hydrological systems, with predictions showing changes in the amount and distribution of rainfall and alterations in evaporation rates (Mukheiber and Sparks, 2003; Schulze, 2005; DEAT, 2006). It is estimated that parts of South Africa will experience increases in annual streamflows by 20% to 30%, with the exception of the southwestern Cape which is expected to experience reduced streamflows (Schulze, 2011). In addition, an increase in occurrence of extreme events such as flooding and drought is predicted (Dallas and Rivers-Moore, 2014). If freshwater ecosystems are kept healthy and intact, they will help to buffer the effects of climate change, thereby mitigating the disruption to ecosystem services (Nel et al., 2011c). It is also likely that climate change will have an impact on freshwater species. As temperatures increase, organisms may move upstream to higher altitudes where water temperatures are cooler (Sauer et al., 2011; Isaak and Rieman, 2013) and phenology of many freshwater insects has been shown to be affected by changing temperatures (e.g. Owen, 2011; Everall et al., 2015).

South Africa is facing widespread degradation of freshwater ecosystems that will continue to compromise ecosystem service delivery and result in the loss of resilience to changing circumstances (Nel et al., 2011a). With the demand for water predicted to increase dramatically (DEAT, 2006), the current situation is even more disturbing, especially considering that pressures on freshwater resources will almost certainly intensify.

Nonetheless, there are still many examples of freshwater ecosystems that are in good condition; these are often the smaller tributaries and streams further away from human activities. These healthy ecosystems are considered to be the vital resources that replenish and sustain the larger, heavily-impacted rivers, which underpin South Africa’s economy (Nel et al., 2011a). The focus of this study is on the aquatic macroinvertebrate diversity of pristine montane streams, these organisms contribute to the essential ecosystem functioning of all freshwater systems (Malmqvist, 2002). In addition these macroinvertebrates serve as valuable indicators of stream condition and can also be used to monitor changes, whether human induced or natural, in the area. As society becomes increasingly aware of the risks to the future sustainability of our natural resource base so calls for conservation measures are made and legislation is developed.

Legislation and conservation

Water-related legislation in South Africa has the overarching aim of achieving the sustainable management of water resources to ensure that a sufficient amount of water is available, of good quality, so as to meet the needs of people and the environment into the future (Kidd, 2008).

The Constitution of the Republic of South Africa stipulates that, among other things, everyone has the right to:

- an environment that is not harmful to their health or well-being; and
- have the environment protected, for the benefit of present and future generations.

Through the foundation provided by the Constitution, certain legislative acts give effect to these constitutional rights. The National Environmental Management Act (NEMA) (Act No. 107 of 1998) (Republic of South Africa, 1998a) provides legislation that aims to prevent pollution and ecological degradation, promotes conservation, and secures ecologically sustainable development and the wise use of natural resources, while promoting justifiable economic and social development. This balance between protecting the environment, so that it can continue to support life, while at the same time transforming it in the interests of human development, provides a major challenge to the future well-being of life on Earth.

The National Water Act (NWA) (Act No. 36 of 1998) (Republic of South Africa, 1998b) was promulgated to ensure that that South Africa’s water resources are protected. According to this legislation these resources should be used, developed, conserved, managed and controlled in a manner that accounts for the following factors, among others:

- protecting aquatic and associated ecosystems and their biological diversity; and
- reducing and preventing pollution and the degradation of water resources.

The conservation and management of freshwater resources is critical to the interests of all humans, governments and nations (Dudgeon et al., 2006). In South Africa, the National Freshwater Ecosystem Priority Areas project (NFEPA) was developed in 2011 to respond to the high levels of threat reported for freshwater ecosystems. The project provides strategic spatial priorities for conserving South Africa’s freshwater ecosystems and supporting sustainable use of water resources (Nel et al., 2011a). The strategic spatial priorities, known as Freshwater Ecosystem Priority Areas (FEPAs) were developed using well-known principles of systematic biodiversity planning. Nearly all of the study sites for this research project fall within a FEPA.

The mandate for conserving freshwater ecosystems is currently shared between the Department of Water and Sanitation and the Department of Environment Affairs. Water is also a key strategic resource to a number of other departments most notably the Department of Agriculture, Forestry and Fisheries the Department of Energy and the Department of Mineral Resources. It is therefore crucial that water is dealt with in an integrated and cooperative manner across these key departments (Nel et al., 2011a).

The Mountain Catchment Areas Act (Act 63 of 1970) (Republic of South Africa, 1970) provides the only legislation that recognises mountains and arises from the vital role that water plays in the South African economy (Browne et al., 2004). The act was promulgated to provide for the conservation, use, management and control of land situated in mountain catchment areas. Unfortunately, not many Mountain Catchment Areas have been declared in terms of the Mountain Catchment Areas Act (Nel et al., 2011a). However, due to the large contribution that these areas make to conservation and the supply and management of freshwater ecosystems, Nel et al. (2011a) proposed that all high water yield areas should be declared and protected in terms of the Mountain Catchment Areas Act.

Aims

In an effort to maintain and restore biodiversity there is need to document patterns in aquatic biodiversity and identify the major environmental factors controlling these patterns (Vinson and Hawkins, 1998). Given the enormous risks to the production and sustainable management of water in South Africa this study aims to research the freshwater streams further. In particular the aim of the thesis is to explore the aquatic macroinvertebrate biodiversity of the southern Great Escarpment both at an ecological and at a genetic level. The different levels of diversity were considered to provide a thorough assessment of biodiversity with species/taxon diversity giving an overall impression of each area and the genetic studies providing deeper insight into particular species phylogenetics and phylogeography. More detailed aims are given in each chapter while the following points offer a general overview:

- Chapter 2 provides an introduction and detailed description of the study site;
- Chapter 3 assesses the aquatic macroinvertebrate diversity in headwater systems across the southern Great Escarpment and explores differences in environmental variables across sites;
- Chapter 4 investigates genetic diversity within three species of Ephemeroptera, *Afroptilum sudafricanum*, *Demoreptus natalensis* and *Demoreptus capensis*, all of which occur abundantly in the southern Great Escarpment and;
- Chapter 5 provides a discussion and synthesis of the findings of this research.

CHAPTER 2

Description of the study area

The Great Escarpment in southern Africa forms an arc of mountain ranges and scarps between the coastal lowlands and the inland plateau highlands (Birkenhauer, 1991; Partridge and Maud, 2000). It extends from northern Angola in the north-west, south through Namibia and into South Africa; in the Western Cape province it turns and continues through the eastern provinces and Lesotho, and then north into the highlands of northern Mozambique and Zimbabwe (Moore et al., 2009) (Figure 2.1).

Elevated, passive continental margins such as the Great Escarpment in southern Africa are found around the Earth and much debate surrounds the formation and topography of these continental margins (Green et al., 2013). However, the traditional view is that the southern African landscape formed at the time of the break-up of Gondwana, around 120 million years ago (MYA) (Partridge and Maud 1987; Partridge and Maud 2000; McCarthy and Rubidge, 2005; Moore et al., 2009). Rifting and the formation of the Atlantic and Indian Oceans resulted in a marginal escarpment, a step in the landscape from the elevated interior (McCarthy and Rubidge, 2005). The Great Escarpment was accentuated by continental uplift events, raising it slightly relative to the interior – particularly in the east of South Africa, forming the Drakensberg Mountain Range (Partridge and Maud, 1987; McCarthy and Rubidge, 2005; Moore and Blenkinsop, 2006). Although there is debate around the rate of erosion (Moore and Blenkinsop, 2006), essentially the Great Escarpment has been in its present position since the end of the Cretaceous, with a broad, gently sloping coastal plain cut at its base (McCarthy and Rubige, 2005; Moore and Blenkinsop, 2006).

This study focuses on the southern Great Escarpment mountain complexes, namely the Eastern Cape (EC) Drakensberg, Stormberg, Great Winterberg-Amathole (GWA), Sneeuberg and Nuweveldberge. The EC Drakensberg, Stormberg and Lesotho highlands form the catchment for the Orange River, which flows west to the Atlantic Ocean. The Kei River flows south from these mountains towards the Indian Ocean. Continuing west of the Kei River are the GWA mountains, separated from the Sneeuberg to their west by the Great Fish River. The Sneeuberg form the headwaters of the Sundays River. West of the Sneeuberg lie the Nuweveldberge, which fall within the Western Cape Province.

Several disjunctions or intervals occur along the southern Escarpment (Figure 2.1), the widest being the Nelspoort Interval between the Nuweveldberge and the Sneeuberg. This interval has generally been assumed to be the most significant biogeographical interval on the southern Escarpment

(Nordenstam, 1969; Clark et al., 2009), while the other intervals are much smaller and occur in the Sneeuberg (Sundays River Interval), or separate the Sneeuberg, GWA and Stormberg from each other (Great Fish River and Queenstown Intervals). This study focuses on primary streams along the Southern Great Escarpment. See Figures 2.2 and 2.3 for a photographic overview of streams sampled in this project.

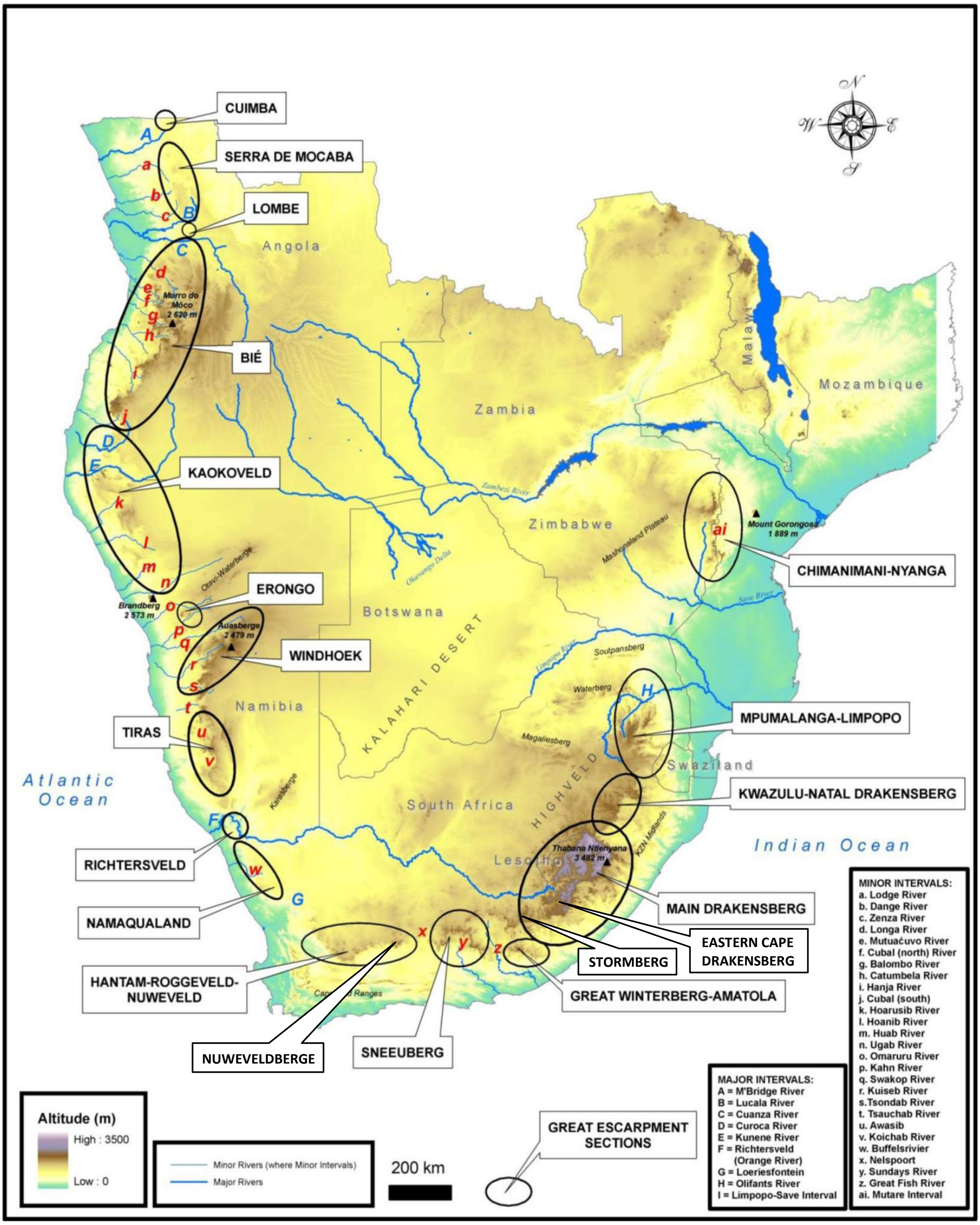

Figure 2.1.The Great Escarpment in southern Africa, indicating Great Escarpment sections, relief, principal drainage, and both major and minor intervals (Clark, 2010)

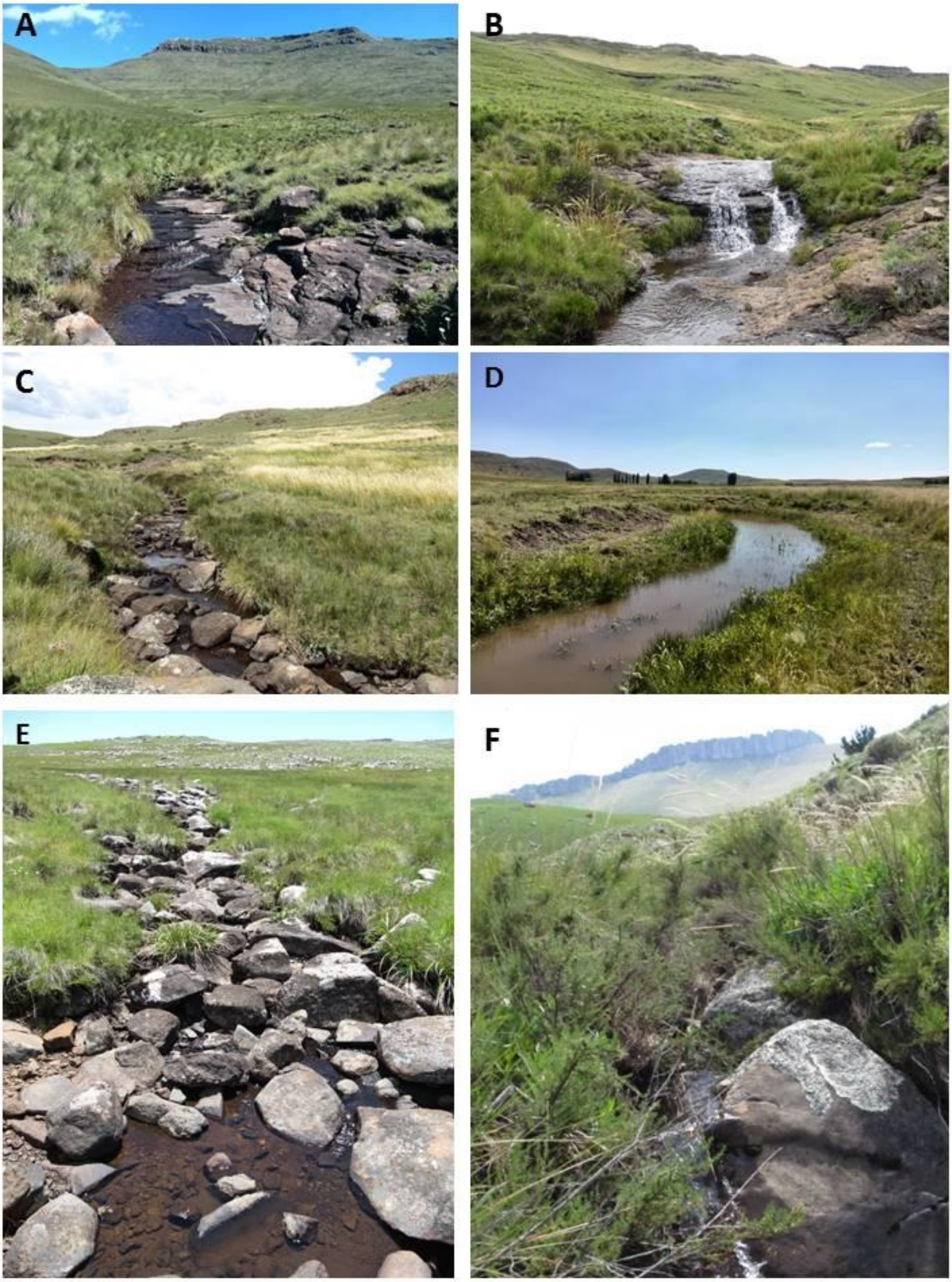

Figure 2.2. Streams along the Southern Great Escarpment: (A) A stream near Bastervoetpad Pass in the EC Drakensberg; (B) One of the sources of the Diepspruit, EC Drakensberg; (C) Beginnings of the Kraai River near Naude's Nek Pass, EC Drakensberg; (D) Holspruit, Stormberg; (E) Elandsberg peak in the GWA mountains; (F) A river starts on the slopes of the Great Winterberg peak in the GWA mountains

Figure 2.3. Streams along the Southern Great Escarpment: (G) Stream flowing towards a waterfall in the Kamdebooberg, Sneeuberg block; (H) Waterkloof stream that joins the Melk River in the Sneeuberg; (I) Source of the Spitskop, Nuweveldberge

Geology

The geology of the southern Great Escarpment is fairly uniform, composed primarily of horizontal sedimentary sequences such as Beaufort Series shales and sandstones of the Karoo Supergroup and heavily intruded by dolerites (Johnson et al., 2006; Moore and Blenkinsop, 2006). The Great Escarpment is highest in the Drakensberg reaching elevations of 3299 m.a.s.l. (metres above sea level). The rocks are generally horizontal with the upper part consisting of 1500 m of Triassic basalt (Ollier, 2004). Sections of the southern and eastern Great Escarpment that are not protected by resistant cap-rock form a gentler, stepped Escarpment (Gilchrist et al., 1994), while sections protected by harder rocks have maintained spectacular drop-offs e.g. sections of the Drakensberg Escarpment and the Sneeuberg (McCarthy and Rubidge, 2005; Clark et al., 2009). These high-altitude dolerite outcrops are broken up by mechanical and chemical weathering into a mass of irregular and angular boulders (Watkeys, 1999; Clark et al., 2009).

Climate

The Great Escarpment defines a dramatic change in altitude between the coastal lowlands and the interior, thereby causing a sharp change in weather patterns between the warm, humid coastal areas and the drier uplands (Birkenhauer, 1991). The southern Great Escarpment falls within the transition zone between the arid west and the more humid east (Kopke, 1988; Boardman et al., 2003; Clark, 2010). This is clearly evident where the Nuweveldberge receives 300-400 mm of rainfall a year (Clark, 2010) in comparison to the main Drakensberg which receives as much as 1600 mm annually (Sene et al., 1998).

The EC Drakensberg and the Stormberg both fall within the summer rainfall region of South Africa (Dollar and Rowntree, 1995; Hoare and Bredenkamp, 2001). The EC Drakensberg receives 700-1 300 mm per annum (Dollar and Rowntree, 1995) with the Stormberg receiving from 400-700 mm across a west-to-east gradient (Hoare and Bredenkamp, 2001).

The GWA is located in an area of climatic transition. Maximum rainfall is received in autumn and the GWA area as a whole receives 800-1 000 mm per annum (Clark et al., 2011b). The Sneeuberg is also located between the winter and summer rainfall regions with the mean annual rainfall being evenly distributed between spring, summer and autumn (Cannon, 1924; Clark et al., 2009). The area is affected marginally by all major weather systems in southern Africa and can receive 400-600 mm per annum (Desmet and Cowling, 1999; Holmes, 1998).

The Nuweveldberge falls into the ‘all-year rainfall’ region in southern Africa (Chase and Meadows, 2007) which is part of the transition zone between the winter rainfall regime in the west and the summer rainfall regime in the east. This results in rainfall that is unreliable and the area experiences regular drought (Esler et al., 2006; Mucina and Rutherford, 2006).

It is important to note that high peaks can have their own micro-climates which are typically slope- and aspect-dependent; they also experience periodic spells of very low temperatures during cold fronts and in winter (Kopke, 1988; Watkeys, 1999; Clark et al., 2009). In winter months snowfall provides an important form of precipitation (Jackson and Bernard, 2005; Clark et al., 2009).

Land use

Across the majority of the southern Great Escarpment the primary land-use is stock grazing, mainly cattle and sheep, especially across the higher altitude grasslands. A few of the more fertile upland valleys are under agricultural crop production. The Sneeuberg and Nuweveldberge have seen a recent shift towards game farming and it is likely that most of the original game species are now represented in the numerous game farms in the region (Clark, 2010).

Overgrazing and invasive plants threaten the natural heritage across the whole of the southern Great Escarpment. The majority of invasive plants in these areas, such as *Populus* x *canescens* and *Salix babylonica* var. *babylonica,* disrupt the hydrological cycle along watercourses (Henderson, 2001). Rangeland management will remain an important concern across the whole southern Great Escarpment for the management and restoration of natural vegetation cover (Clark, 2010).

Biodiversity research

With the Great Escarpment being host to numerous centres of floral endemism (Van Wyk and Smith, 2001; Clark et al., 2009) and biological diversity, there is no limit to its research potential. However, most of the Great Escarpment has yet to be adequately explored in terms of biodiversity (Krüger, 2007). Clark (2010) noted that research efforts have been fragmented and sporadic, resulting in a patchy distribution of studies. Reasons for limited exploration and study are mainly due to limited accessibility, rugged terrain, adverse climatic conditions, and often the distance from main urban centres (Krüger, 2007). Furthermore, the limited number of scientists in particular research fields are also a contributing factor.

Since 2009 a number of extensive studies on the flora of the Great Escarpment have been published (Clark et al., 2009, 2011a, 2011b, 2011c, 2012, 2014) as well as a recent floristic study of wetlands (Janks, 2015). Research has also been undertaken on the fauna of these mountain areas including work that is published (Barker et al., 2013; Kok et al., 2012, 2013) and unpublished. This research covers a number of different organisms including birds, snakes, rodents, terrestrial insects and snails. However, to date there has not been any research relating to aquatic macroinvertebrates across the southern Great Escarpment.

CHAPTER 3

Aquatic invertebrate assemblages in the southern Great Escarpment

INTRODUCTION

Mountains, as sources for rivers, provide an essential source of freshwater for the world and with increased pressure being placed on the environment by human population growth, this water resource is at risk (Viviroli et al., 2011). Most of South Africa is water-limited and therefore rivers are vulnerable to over-exploitation and modification (Darwall et al., 2008; Nel et al., 2011). The country's main rivers are heavily used, with multiple demands from urban settlements, agriculture, and industry (DEAT, 2006).

To maintain healthy functional ecosystems and ecosystem services, mountain catchment ecosystems depend on the biodiversity of the fauna and flora inhabiting the catchment areas (Körner, 2004). In view of the profound influence of human activity on ecosystems and the services humans receive from them, understanding biodiversity loss is becoming increasingly important (Vitousek et al., 1997; Daily et al., 2000; Giller et al., 2004) and every effort should be made to preserve this valuable resource (Rudmann-Maurer et al., 2014). Furthermore, mountain streams are also of particular conservation relevance for lowland stream species. These montane streams are less affected by anthropogenic stressors, such as river degradation, land use, and invasive species, compared to lowland freshwaters. Thus, montane streams form natural refuges for a wide range of freshwater invertebrates as potential source populations for the recolonisation of tributaries of similar order (Sauer et al., 2011).

It is well documented that biodiversity is important in maintaining ecosystem resilience to withstand disturbance and the impacts of climate change (Tilman, 1996; Peterson et al., 1998; Vinson and Hawkins, 1998; Driver et al., 2011). Climate change poses a huge threat to global ecosystems and the threat to water resources could be significant (Viviroli et al., 2011). The South African Country Studies Programme identified water resources as one of the most vulnerable areas to climate change (DEAT, 2006). There is a growing concern regarding establishing climate change impacts on mountain streams and the implications for aquatic macroinvertebrates (e.g. Durance and Ormerod, 2007; Chessman, 2009; Lawrence et al., 2010; Muhlfeld et al., 2011; Giersch et al., 2015). Altitude and the associated water temperatures affect macroinvertebrate diversity, community structure and survival (e.g. Jacobsen et al., 1997; Cox and Rutherford, 2000; Finn and Poff, 2005; Grab, 2014). Climate warming would result in the upward shift of thermal habitats and may have ecological implications which cannot yet be accurately projected into the future (Grab, 2014; Rudmann-Maurer, 2014). In an effort to maintain and restore biodiversity of stream ecosystems, Vinson and Hawkins (1998) recognise the need to document patterns in aquatic insect biodiversity and identify the major environmental factors controlling these patterns.

The physical heterogeneity of streams and rivers has important implications for the distribution of invertebrates (Malmqvist, 2002). Lotic environments have a characteristically patchy distribution of benthic habitats and this is reflected in the patchy spatial distribution of macroinvertebrates (Dallas, 2007). Factors contributing to this patchiness range from geology and climate at a catchment scale to channel type, water velocity and abundance of different biotopes at the habitat or site scale (Dallas, 2007 and references therein). Macroinvertebrate assemblages are influenced by the relative abundance of biotopes at a site, due to the biotope preferences of macroinvertebrates (Dallas, 2007; Curry et al., 2012). The local community structure can therefore be seen as the result of a continuous sorting process through environmental filters ranging from catchment-wide processes, to the small-scale characteristics of individual patches (Malmqvist, 2002).

Aquatic invertebrate studies in South Africa

South Africa has a well-established history of aquatic ecological studies, from the early descriptive approaches (e.g. Barnard, 1927, 1932, 1940; Hutchinson, 1929) towards more applied studies on bioassessments for river health and ecosystem functions (e.g. Oliff, 1960a, 1960b; Chutter, 1972; Dickens and Graham, 2002; Dallas, 2004, 2007; Ollis et al., 2006). However, there are still regions in South Africa where very little is known about the freshwater biota. There are limited studies on aquatic macroinvertebrates in the Eastern Cape Province with most research focusing on large or prominent rivers such as the Buffalo River (Hill and O'Keeffe, 1992), the Great Fish River (Rivers-Moore et al., 2007) and the Wit River (Weyl et al., 2010). An important baseline study was conducted on the Umzimvubu River catchment in the Eastern Cape and provides background information against which environmental threats following regulation may be compared (Madikizela and Dye, 2003). Baseline data are invaluable in providing starting points for monitoring, biodiversity assessments and are an important component of long-term climate change studies.

This study investigates the aquatic macroinvertebrate assemblages in headwater systems along the southern Great Escarpment, and sets out to:

1. Document aquatic macroinvertebrate diversity from primary rivers across the southern Great Escarpment.

2. Compare the macroinvertebrate assemblages between different mountain blocks in the southern Great Escarpment and correlate similarities and differences to environmental drivers to quantify the role of biotope in species assemblages.

This study area spans five different mountain blocks in the southern Great Escarpment across a distance of about 650 km, a range of geology and geomorphology, different biomes and several climatic zones. It is therefore certain that there will be a large variation in habitats across all sites.

The data collected will also serve towards filling in sampling gaps and specimens will be available for further research, housed at the Albany Museum in Grahamstown. Sites can be used for long-term monitoring of climate change and the data collected in this study will serve as baseline data for future studies.

METHODS

Study sites

Twenty-four streams were sampled along the southern Great Escarpment, from the Eastern Cape (EC) Drakensberg in the East to the Nuweveldberge in the West (Table 3.1). All sites were along first order streams; this was to minimise the variability that occurs between small and large rivers and ensure that sites would be as comparable as possible. To clarify terminology used here, a "site" refers to the point along the stream that was sampled and within each site there were a number of collecting events, according to the biotopes identified at each site. Figure 3.1 and Table 3.1 summarise the study area, mountain blocks and sites. The number of sites selected per mountain block largely depended on the total area of the mountain block and the density of perennial rivers in the area. Site selection was also based on accessibility and consent from local landowners. Streams could be located to some extent using 1:50 000 topographical maps; however in certain areas, such as the Stormberg, streams with flowing water were scarce. Land owners were invaluable in contacting neighbours and surrounding farms to help in finding flowing streams. Most sites across the whole sampling range could only be accessed by a 4x4 vehicle and then a fair amount of hiking. Streams from 1 400 m.a.s.l. and above were selected to ensure that only higher altitude/escarpment streams were sampled.

Table 3.1. A basic summary of site information with mountain blocks in bold. Vegetation classification follows Mucina and Rutherford (2006).

Area	Site	Latitude	Longitude	Altitude (m)	Vegetation type
EC Drakensberg					
Barkly Pass	1	-31,21	27,69	2043	Lesotho Highland Basalt Grassland
	2	-31,17	27,85	2148	Lesotho Highland Basalt Grassland
	3	-31,13	27,81	2049	Southern Drakensberg Highland Grassland
	4	-33.17	27,97	2208	Lesotho Highland Basalt Grassland
Barkly East	1	-30,75	27,55	1834	Southern Drakensberg Highland Grassland
	2	-30,76	27,55	1817	Southern Drakensberg Highland Grassland
	3	-30,72	27,54	2428	Lesotho Highland Basalt Grassland
Rhodes	1	-30,68	27,88	2572	Lesotho Highland Basalt Grassland
	2	-30,67	27,90	2608	Lesotho Highland Basalt Grassland
	3	-30,70	28,15	2545	Lesotho Highland Basalt Grassland
Stormberg					
	1	-31,39	26,86	1775	Southern Drakensberg Highland Grassland
	2	-31,28	26,71	1661	Stormberg Plateau Grassland
	3	-31,16	26,60	1910	Stormberg Plateau Grassland
	4	-31,42	26,84	1801	Stormberg Plateau Grassland
Great Winterberg-Amathole					
Winterberg	1	-32,38	26,39	1783	Amathole Mistbelt Grassland
	2	-32,38	26,38	1694	Amathole Mistbelt Grassland
Elandsberg	1	-32,51	26,90	1843	Amathole Mistbelt Grassland
Sneeuberg					
Nardousberg	1	-32,23	24,95	1818	Karoo Escarpment Grassland
	2	-32,24	24,94	1857	Karoo Escarpment Grassland
Kamdebooberg	1	-32,18	24,02	1967	Karoo Escarpment Grassland
	2	-32,35	23,88	1471	Upper Karoo Hardeveld
	3	-32,35	23,89	1446	Upper Karoo Hardeveld
Nuweveldberge					
Beaufort West	1	-32,11	22,58	1774	Upper Karoo Hardeveld
	2	-32,10	22,64	1670	Upper Karoo Hardeveld

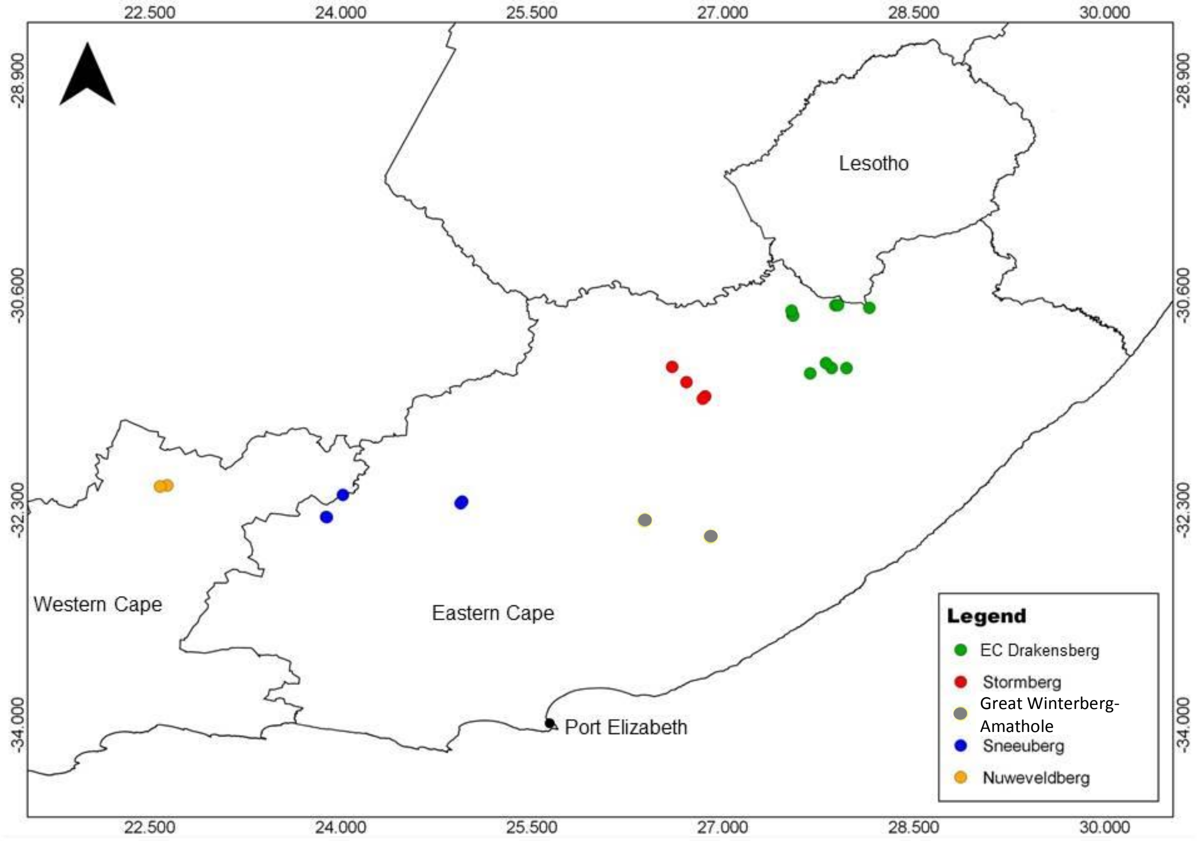

Figure 3.1. Map of study sites along the southern Great Escarpment.

Sampling procedure

Samples were collected in the summer months of 2013 and 2014. All biotopes present at each site were sampled and a catalogue number was assigned to each sample. All samples were placed in 80% ethanol upon collection. The sample containers ranged from 1.5 ml Eppendorf tubes for smaller macroinvertebrates to 50 ml sample tubes for larger macroinvertebrates, all containers had screw-on lids to prevent drying out of samples.

Aquatic sampling was carried out according to the methods outlined in SASS5 (Dickens and Graham, 2002). A standard SASS net with mesh size of 250 µm was positioned downstream of each biotope where invertebrates were removed. For smaller rivers and hygropetric biotopes, a D-shaped hand net (mouth width 350 cm^2, mesh size 80 µm) was used. Everything collected in the net after 1 min of agitating the area was placed into a white tray for sorting. The invertebrates were then placed into sample containers using spring steel fine forceps. All precautions were taken to keep the insects in as immaculate a condition as possible for identification and preservation.

An attempt was made to set up light traps overnight to collect adult invertebrates, especially at dusk and dawn but this proved impractical for logistical reasons, and was considered unfeasible for this study. However, it is an approach that could be employed in future studies where more intensive sampling is needed and to collect adult specimens.

Curation and identification

Each sample was sorted using fine forceps and a dissecting microscope. Specimens where identified using resources at the Albany Museum, which contains the National Collection of Freshwater Invertebrates. Once identified, the specimens were counted and placed into a new vial with a label and an Albany Museum catalogue number. An open-cataloguing system was used where the original catalogue number was retained for referencing the sample and then an additional letter of the alphabet was given to each new species recorded. All individuals from the same species and sample where placed into one vial. All samples are housed in the National Collection of Freshwater Invertebrates at the Albany Museum.

Specimens were identified to the level of species wherever possible, but in some cases identification beyond family level could not be achieved. This was usually due to lack of expert knowledge or identification keys for the nymphal life stages of certain groups. All final taxon names are used at the same level in the analyses, irrespective of whether they were identified to family, genus or species.

Environmental and descriptive parameters

Various environmental data were also collected from each site. Altitude, stream velocity (estimated on a scale of 0-4; see Table 3.2), distance from source, width and depth of the stream, vegetation structure and whether or not there were any disturbances in the area that might affect the stream were recorded (Table 3.2). The biotope of each sample was recorded at all the sites (Table 3.3). In addition, the chemical parameters of each site were measured using a multiparameter water quality meter (model: YSI 556MPS, make: YSI Environmental) and included pH, temperature, salinity, total dissolved solids (TDS) and dissolved oxygen (DO) (Table 3.2). Although electrical conductivity was recorded, it was removed from the analysis due to its direct correlation to TDS (which would pose problems for multivariate statistical analyses). The average annual precipitation was obtained for each site from Bioclim (Hijmans et al., 2005) at a resolution of five minutes (~8 km) and then transformed with DIVA-GIS V7.5.0 (Hijmans et al., 2012) to give site-specific averages.

Table 3.2. List of environmental variables measured at each site with the units of measurement or scale of measurement used

Variable	Units or scale measured
Physical parameters	
Altitude	m.a.s.l.
Distance from source	m
Width of river	cm
Depth of river	cm
Water velocity estimate	0=still, 1=trickle, 2=running, 3=flowing, 4=fast flowing
Temperature	°C
Chemical parameters	
pH	
Salinity	PSU
Total dissolved solids (TDS)	ppm
Dissolved oxygen (DO)	% saturation

Table 3.3. List of biotopes sampled during surveying with key to abbreviations

Biotope	Description
SIC	Stones in current
SOOC	Stones out of current
Bedrock	Bedrock in current
Pool	Pool sample
Surface	Surface of pool
Waterfall	Vertical rock
Sub Veg	Submerged vegetation
Sand	Gravel/sand/mud
Seep	Groundwater seep

Data analysis

Species diversity

Diversity in the strict sense is richness in species, and is measured as the number of species in a sample of standard size (Whittaker, 1972). Species richness is calculated in this study using Microsoft Excel®. To obtain a detailed measure of α-, β- and γ-diversity, Partition 3.0 (Veech and Crist 2009a) was used to analyse data from each of the sites. Diversity partitioning is a means of dividing the total diversity (γ) into its constituent parts α (within site diversity) and β (between site diversity) (Whittaker, 1972). Partition 3.0 calculates expected values of α- and β-diversity using Monte-Carlo permutations thus allowing the significance of the observed diversity to be tested (Veech and Crist 2009b). The sampling design for this study was hierarchical; therefore, the data contained nested levels with the lowest level being that of the biotope from which the sample was collected. Diversity at the lowest level (biotopes within sites) could not be calculated because not all sites contained the same suite of biotopes. The two levels within the data used consisted of the site and the mountain block. The Partition package also allows for an unbalanced sampling design (Veech and Crist 2009b). No sample weighting was used and P-values were based on 1000 individual-based iterations.

Multivariate analyses

Non-metric multidimensional scaling (NMDS) was used to ordinate the macroinvertebrate composition across the sampling sites. NMDS is a commonly used analysis for examining the relationships of macroinvertebrate communities (Faith and Norris, 1989; Downes et al*.,* 2000; Boyero and Bailey, 2001). The NMDS analysis was carried out in PRIMER V6 (Clarke and Gorley, 2006) and data were log-transformed to down-weight abundant species. The abundance data were converted to a similarity matrix composed of Bray-Curtis similarity coefficient values as recommended by Clarke and Gorley (2006). The minimum stress level was left at the default 0.01 with 1000 random restarts. The NMDS was visualised in two ways: firstly, the sample sites were depicted as different colours according to the mountain block where they were collected and secondly, they were depicted according to biotope. This was to explore if variation was only occurring due to differences in biotope or whether it was a reflection of differences between areas.

Differences in macroinvertebrate composition between a priori defined groups (mountain blocks and biotopes) were assessed using a two-way nested ANOSIM in PRIMER V6 (Clarke and Gorley, 2006). These where applied to the same Bray-Curtis similarity matrix as described above. ANOSIM calculates a test statistic, R, which is scaled from +1 to -1. R approaches +1 if all of the most similar samples are in the same groups (Henderson and Seaby, 2008). A SIMPER analysis was then carried out to identify the species contributing most to the discrimination between the a priori groups. This analysis investigates which species account for similarities within groups and the species contributing to the differences between groups. It is therefore a useful analysis in identifying widely occurring species and gives an indication of the endemic faunal composition within the different mountain blocks.

Environmental data

Environmental data and water chemistry results were tabulated in Microsoft Excel and ordinated using Principle Component Analysis (PCA) in PRIMER V6 (Clarke and Gorley, 2006). Unlike species data, environmental data do not have large blocks of zero counts, and therefore a PCA is an appropriate analysis for environmental data (Clarke and Warwick, 2001). The data were first normalised as suggested by Clarke and Warwick (2001), to compensate for the variation in measurement scales (from % to meters to ˚C).

Macroinvertebrate and environmental ordination

To investigate the relationship between species assemblages and environmental variables, a Canonical Correspondence Analysis (CCA) was carried out. The CCA is well suited to limnological data and an overview of the method is given by ter Braak and Verdonschot (1995). The analysis was run as a means of direct gradient analysis using the statistical package CANOCO 4.5 (ter Braak and Šmilauer, 2002) and was carried out using all of the environmental data used for the PCA and species data from all sites. The species data were log-transformed for reasons stated previously.

RESULTS

The macroinvertebrate diversity of the southern Great Escarpment

Across the 24 sites, 9 different biotopes were sampled, resulting in 82 sampling events. A total of 2 595 individual larvae and pupae were collected from 11 orders (Table 3.4) and 47 families. Baetidae (Ephemeroptera) accounted for 42% of the total specimens and a further 14 families together accounted for nearly 90% of specimens collected (Table 3.5). Where possible, specimens were identified to species level, resulting in 86 different species. However, some specimens could only be identified to genus or family level. For a full list of species and abundances see Appendix 1, Table A1.

Table 3.4. Number of individuals collected from each order across all sites and biotopes

Order	**No. of individuals**	**% of total individuals**
Ephemeroptera	1 509	58.17
Diptera	492	18.97
Coleoptera	203	7.83
Trichoptera	140	5.40
Odonata	102	3.93
Hemiptera	67	2.58
Mollusca	42	1.62
Plecoptera	30	1.16
Amphipoda	6	0.23
Lepidoptera	2	0.08
Megaloptera	1	0.04
TOTAL	2594	100

Table 3.5. Macroinvertebrate taxa recorded from the study area comprising more than one percent of the total number of specimens recorded

Taxon occurrence >1%		
Baetidae	Synlestidae	Caenidae
SimuliIdae	Notonemouridae	Elmidae
Leptophlebiidae	Gyrinidae	Scritidae
Hydropsychidae	Corixidae	Libellulidae
Chironomidae	Dytiscidae	Sphaeriidae

The Ephemeroptera contribute by far the most to the total species collected, with the Ephemeropteran families Baetidae and Leptophlebiidae having some of the highest species occurrences. Previous studies in the KwaZulu-Natal Drakensberg have also found that generally the most abundant families were Baetidae and Leptophlebiidae (Grab, 2014; Rivers-Moore et al., 2013).

Species richness and diversity

Species richness was highest in the Sneeuberg and lowest in the Winterberg (Figure 3.2) and there is no trend of decreasing richness along the longitudinal gradient from east to west. Therefore, rainfall patterns along this gradient do not seem to have an effect on species richness.

The partitioning of total diversity (γ) into its constituent parts α and β allows patterns of local, between-local and between-regional diversity to be studied (Lande, 1996; Crist et al., 2003). Originally the relationship between the various components of diversity was considered multiplicative, but it has since been proposed and accepted that the relationship is additive, where $\alpha + \beta = \gamma$ (Lande, 1996; Veech et al., 2002). The partitioned analysis of species diversity revealed no significant difference between observed species diversity and expected species diversity within sites (α) and between sites (β1) (Table 3.6); while a significant difference was found between mountain blocks (β2). The observed diversity in α and β1 is less than that of the expected diversity, but not significantly so (Figure 3.2; Table 3.6). The observed diversity recorded between mountain blocks is significantly higher than expected (Figure 3.2; Table 3.6). This suggests that each mountain block has a unique signature, and that species composition differs between each area. Crist et al. (2003) found similar results in a study on the diversity of forest canopy beetles where α-diversity was lower than expected while β-diversity levels increased with increasing sampling scale. A study reviewing diversity in headwater streams found α-diversity to be low in individual headwater streams and β-diversity to be high among headwater streams (Clarke et al., 2010, 2008) supporting the results found in this study.

The difference between the observed and expected components of diversity is presumed to be due to certain ecological processes leading to non-random dispersion of individuals (Veech et al., 2002). The ecological processes contributing to high β-diversity among headwater streams includes differing ability and opportunity for dispersal of macroinvertebrates, great physical habitat heterogeneity in headwater streams, and a wide range in local environmental conditions (Clarke et al., 2008). These results indicate that endemism may be high in each mountain block.

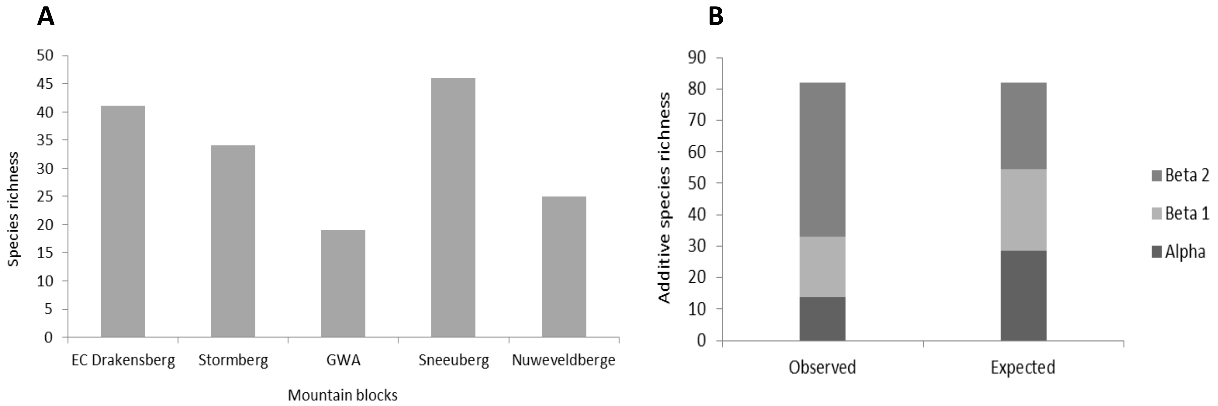

Figure 3.2. A) Species richness in each mountain block. B) Differences in additive species diversity, obtained from the diversity partitioning analysis, between the observed and expected diversity for each level within the hierarchical sampling design. Alpha: within site diversity; Beta 1: between site diversity and; Beta 2: between mountain block diversity.

Table 3.6. Species diversity results from diversity partitioning analysis. P-Values indicate a significant difference between the observed and expected diversity.

	Additive richness (Observed diversity)	**P-Value**
Level 1: Sites		
Alpha 1	13,65	1.00
Beta 1	19,35	1.00
Level 2: Mountain blocks		
Alpha 2 (= alpha 1 + beta 1)	33	1.00
Beta 2	49	0.001
Total	82	

Significant species

A number of significant species were collected including a possible new species of caddisfly (Trichoptera), a non-biting midge (Chironomidae) species showing a range extension, a rare species of beetle (Coleoptera) and an amphipod shrimp that has only ever been recorded once from the Eastern Cape Province. These are described below.

Trichoptera: Leptoceridae

Larval specimens of Trichoptera (Figure 3.3) from the family Leptoceridae where collected and identified to generic level as *Athripsodes* Billberg, Ps group of species. Dr Ferdy de Moor (Albany Museum, Grahamstown) confirmed that no species description matching these specimens exists, and they are thus considered to be a new species. These specimens were collected from two different localities in the Kamdebooberg (within the Sneeuberg block), from submerged vegetation within a pool at one site and from a groundwater seepage at another site. These species make portable cases using extremely small grains of sand (Figure 3.3).

Figure 3.3. Specimens of the cased caddisfly larvae *Athripsodes* Ps group (Trichoptera, Leptoceridae).

Diptera: Podonominae (Chironomidae)

Six larval specimens of *Archaeochlus* Brundin (Figure 3.4) were collected from one site at the top of Barkly Pass in the EC Drakensberg, 140 km from the only known locality of this genus in South Africa. *Archaeochlus* is a plesiomorphic genus of Podonominae with four described species assigned to it (Cranston et al., 1987). The four species resemble one another closely, with the possession of fully developed mandibles in the female adult and metapneustic spiracles in the larva (Cranston et al., 1987). Three of the species occur in Southern Africa: *A. bicirratus* and *A. drakensbergensis* are known only from the Drakensberg Escarpment between South Africa and Lesotho and *A. biko* has been collected only in Namibia (Cranston et al., 1987). The fourth species, *A. brundini,* occurs in southwestern Australia. Specimens collected are therefore either *A. bicirratus* or *A. drakensbergensis* but these species cannot be readily distinguished without adult material (Cranston et al., 1987), which was not collected.

The larvae of *Archaeochlus* have a distinctive habitat and are confined to sources of small temporary streams that flow over rock faces, usually edged with mosses (Cranston et al., 1987). Figure 3.5 shows the site where specimens were collected. The presence of metapneustic spiracles in the larva, shown in Figure 3.4B, are probably beneficial in these hygropetric habitats, where dissolved oxygen may be low (Cranston et al., 1987).

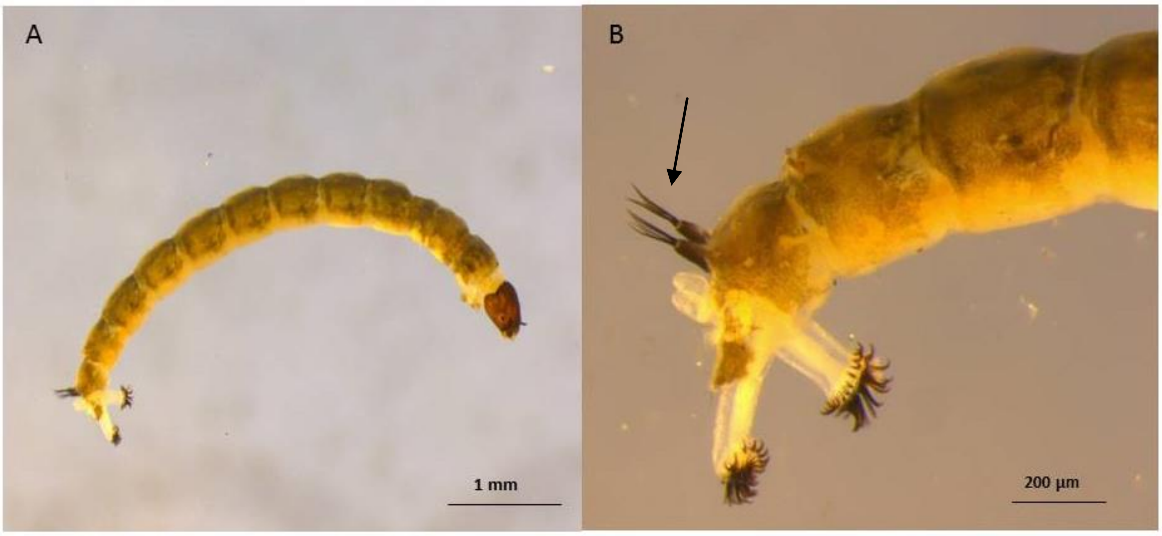

Figure 3.4. Larvae of *Archaeochlus sp.* (Chironomidae: Podonominae). A) A whole larva. B) Detail of the posterior end of the larva with the metapneustic spiracles indicated by an arrow.

Figure 3.5. Hygropetric habitat with mosses where specimens of *Archaeochlus sp*. where collected in the Barkly Pass area (EC Drakensberg).

Coleoptera: Torridincolidae

Torrent beetles (Coleoptera, Torridincolidae) from the genus *Delevea* Reichardt (Figure 3.6), were collected at two different sites, one in the Nuweveldberge and the other in the central Sneeuberg. These beetles are very rarely collected and usually occur in hygropetric habitats (P. Perkins, Harvard University, pers comm). The site in the Nuweveldberge was characteristic of a hygropetric biotope, but in the Sneeuberg the beetle was collected in the conventional “stones in current” biotope. Two genera are known from Southern Africa, *Delevea* and *Torridincola* Steffan. Species of *Delevea* are recorded in the Western Cape and up to Namibia and species of *Torridincola* are recorded from KwaZulu-Natal, Northern Province and Northwestern Province (Endrödy-Younga, 1997). Specimens of *Delevea* sp. collected through this study expand the range of this genus to the Eastern Cape.

Figure 3.6. Torrent beetles (Torridincolidae) collected in the Sneeuberg and Nuweveldberge. Photo credit: Musa Mlambo.

Amphipod: Sternophysingidae

Freshwater amphipods (Figure 3.7) were collected from a spring in the Sneeuberg. Previously there have been no freshwater amphipods reported from anywhere in the Eastern Cape Province except for a single record 250 km away near Knysna (C. Griffiths, University of Cape Town, pers comm). The genus from Knysna, *Mathemelita* Stewart and Griffiths, is still known only from a single report. The genus *Paramelita* Schellenberg, is restricted to the Western Cape and the only other genus *Stenophysinx* Holsinger and Straskraba, is mostly restricted to caves in the North of South Africa, with the exception of one species from a cave in De Hoop Nature Reserve in the Western Cape (C. Griffiths, University of Cape Town, pers comm).

The discovery of these rare specimens contributes to our knowledge of aquatic macroinvertebrates by updating distribution records and adding to biodiversity assessments. The Sternophysingidae specimens have been sent to Musa Mlambo (Albany Museum) and Charles Griffiths for further research that will most likely lead to a new species (or genus) description.

Figure 3.7. Specimen of a Sternophysingidae (Amphipoda).

Multivariate analyses

Mountain blocks

The results from the NMDS analysis are represented in the form of a two-dimensional plot that places the most similar samples closer together and the least similar further apart (Henderson and Seaby, 2008). The NMDS of all samples shows little clustering in terms of mountain block. However, the EC Drakensberg, Sneeuberg and Great Winterberg-Amathole (GWA) samples cluster together and the Nuweveldberge and the majority of Stormberg samples tend to be similar (Figure 3.8), but these groups do overlap; the Stormberg in particular has a range of samples with some that are more similar to EC Drakensberg and Sneeuberg samples (Figure 3.8).

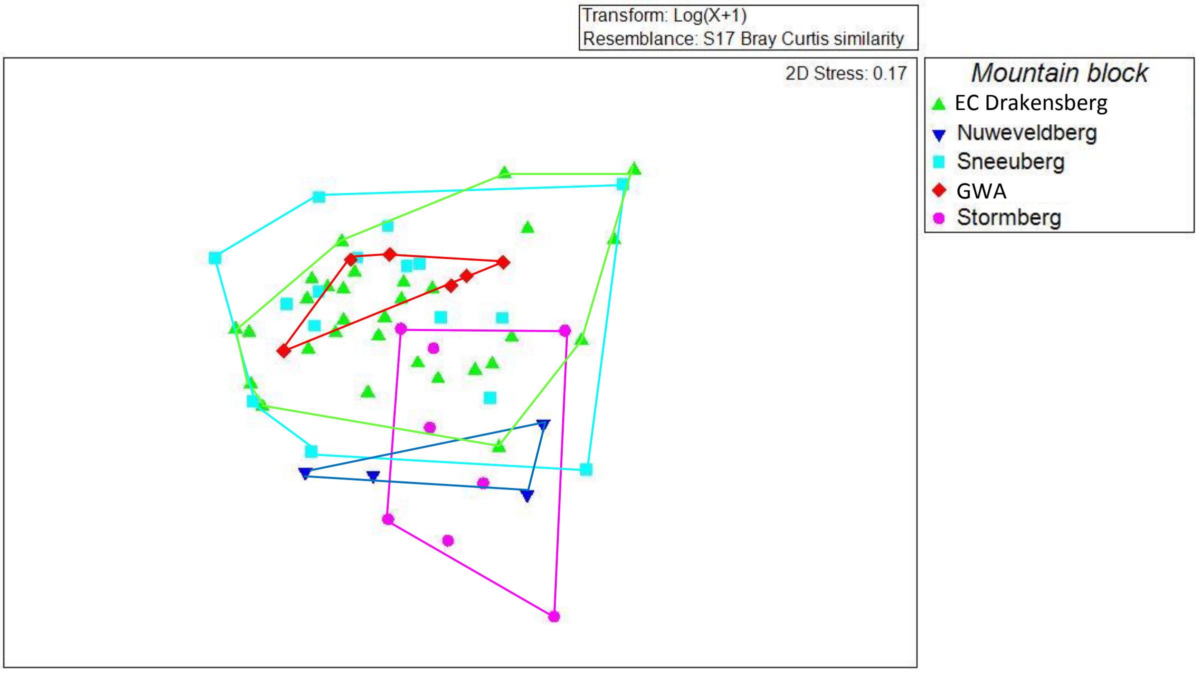

Figure 3.8. NMDS ordination of sample sites colour-coded by geographical origin

The results from the ANOSIM give more rigorous clarity on statistical differences between groups and, with the use of a SIMPER analysis, the species contributing to these differences can be highlighted. A one-way ANOSIM with mountain blocks as a priori defined groups showed significant differences between two or more groups (R = 0.519, P = 0.001). Pair-wise comparisons show that all mountain blocks, except the Stormberg, are significantly different from the Nuweveldberge (Table 3.7, Figure 3.9).The SIMPER analysis indicates that the two species contributing the most to these differences are *Afroptilum sudafricanum* Lestage (Baetidae) and *Simulium medusaeforme* Pomeroy (Simuliidae). This is mainly because these two species occur abundantly, but in areas where *A. sudafricanum* is abundant, *S. medusaeforme* is absent and vice versa.

The Stormberg was also found to be statistically different from the EC Drakensberg and the Sneeuberg (Table 3.7), with the main species responsible for this difference being *Cloeon sp*. (Baetidae) and *A. sudafricanum* (Baetidae).

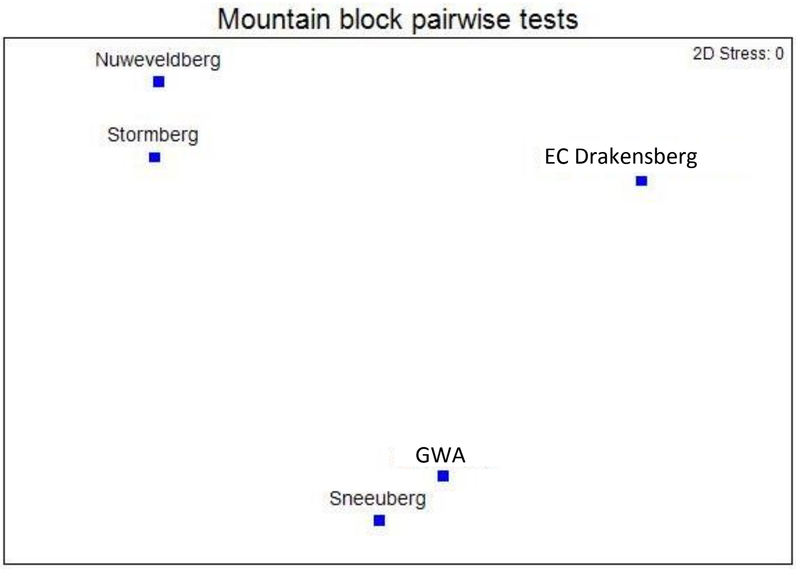

Figure 3.9. NMDS plot of ANOSIM results showing relationships between macroinvertebrate assemblages within each mountain block

Table 3.7. ANOSIM showing results of pair-wise tests. R statistic is given with level of significance in brackets. Numbers in bold indicate a significant difference between the two groups.

	Nuweveldberge	Sneeuberg	GWA	Stormberg	EC Drakensberg
Sneeuberg	**0.667 (0.036)**	-			
GWA	1.000 (0.100)	0.037 (0.417)	-		
Stormberg	0.071 (0.600)	**0.472 (0.019)**	0.352 (0.114)	-	
EC Drakensberg	**0.984 (0.013)**	**0.430 (0.002)**	0.099 (0.283)	**0.771 (0.001)**	-

The SIMPER analysis also shows the species contributing to the similarity of sites within each mountain block. *Afroptilum sudafricanum* is the most common and abundant species across sites in the EC Drakensberg, GWA and Sneeuberg with *Demoreptus sp.* also having a significant contribution (Table 3.8). *Cloeon* sp. and *A. sudafricanum* account for over half of the cumulative similarity in the Stormberg with *S. medusaeforme* and *Cheleocloeon excisum* contributing a similar amount in the Nuweveldberge (Table 3.8). The average similarity between sites within each mountain block was high for the Nuweveldberge (64%), low for the EC Drakensberg (35%), the Sneeuberg (21%) and the GWA (24%) and very low for the Stormberg (12%). These results support those shown in the NMDS of sample sites above in Figure 3.8.

Table 3.8. SIMPER analysis showing species comprising the majority of the faunal composition within each mountain block. The percentage contribution of each species to the overall similarity and the cumulative percentage is shown.

Species	EC Drakensberg	Stormberg	GWA	Sneeuberg	Nuweveldberge
Afroptilum sudafricanum	32	17	44	33	
Demoreptus sp.	23			18	
Simulium nigritarse	8	7			
Cheleocloeon excisum	8				24
Cheumatopsyche sp.	7		7		
Adenophlebia sp.	4		27	8	
Cloeon sp.		36			13
Caenis sp.		9			
Aedes sp.		6			
Ptilodactylidae		5			
Chironomidae		4			
Castanophlebia sp.			7	8	
Elmidae				12	
Athripsodes sp.				3	
Simulium medusaeforme					34
Baetis harrisoni					14
Total %	82	84	85	82	85

Biotope

It is to be expected that different biotopes would host different macroinvertebrate assemblages (e.g. Palmer et al., 1991; Collier, 1995; Humphries, 1996; Kay et al., 1999; Dallas, 2007). Therefore the NMDS plot is depicted with sample sites colour coded according to biotope. This NMDS plot shows slightly more resolution of groups compared to the NMDS depicted according to mountain block, but there is still a large amount of overlap (Figure 3.10). The general trend is that the pool, surface and submerged vegetation biotopes are similar with the bedrock, SIC and waterfall biotopes being more similar to one another (Figure 3.10).

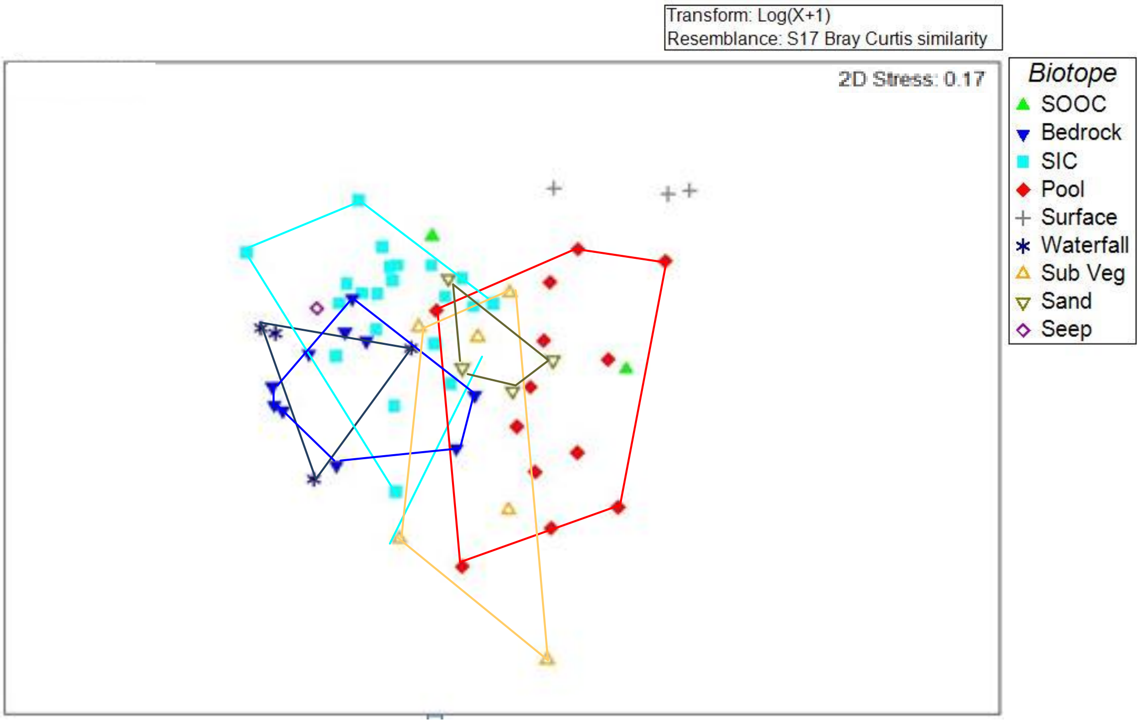

Figure 3.10. NMDS ordination of sample sites showing macroinvertebrate assemblages from different biotopes colour-coded by biotope. SOOC (stones out of current); Bedrock (bedrock in current); SIC (stones in current); Pool (pool sample); Surface (surface of pool); Waterfall (vertical rock); Sub Veg (submerged vegetation); Sand (gravel/sand/mud sample); Seep (groundwater seep).

A two-way ANOSIM was done to explore the influence of biotope on species assemblages. In this analysis the variation is partitioned between biotope and mountain block so that their individual effects can then be tested while taking the other variable into account. There was a significant difference between biotopes (R = 0.562, P = 0.001) but not between mountain blocks (R = 0.118, P = 0.092). This suggests that the differences between mountain blocks shown in the one-way analysis was only a reflection of the differences in the suite of biotopes present in each area (Table 3.9). A different assemblage of species occurs in each biotope (Table 3.10) and these therefore account for the differences across mountain blocks.

Table 3.9. The most commonly occurring biotopes (excluding seep and stones out of current) across all sites showing how many of each biotope were present in the different mountain blocks

Biotope	EC Drakensberg	Stormberg	GWA	Sneeuberg	Nuweveldberge
Stones in current	11	2	3	7	1
Bedrock	4	1	1	4	0
Waterfall	3	0	1	4	0
Submerged Vegetation	2	3	1	2	0
Pool	5	2	1	3	2
Gravel/Sand/Mud	2	0	0	1	1
Surface	2	0	1	1	1

Table 3.10. SIMPER analysis of the most commonly occurring biotopes with the species that contributes to the similarity of each biotope group.

	Number of individuals collected in each mountain block				
Species	EC Drakensberg	Stormberg	GWA	Sneeuberg	Nuweveldberge
Stones in current					
Afroptilum sudafricanum	125	26	46	44	1
Cheumatopsyche sp.	48	13	8	14	
Demoreptus sp.	44		2	12	
Simulium nigritarse	36	7		5	
Castanophlebia sp.	27		17	110	
Adenophlebia sp.	16	14	17	23	
Scritidae sp.	14			11	
Elmidae	7		1	15	
Bedrock					
Demoreptus sp.	34			16	
Chironomidae	10	2		5	4
Afroptilum sudafricanum	39	1	1	66	
Simulium dentulosum	33			2	25
Elmidae				13	
Waterfall					
Demoreptus sp.	54		43	59	
Submerged Vegetation					
Cloeon sp.		27		8	10
Lestes sp.		5		13	
Caenis sp.		2		10	5
Pool					
Cheleocloeon excisum	27			19	24
Afroptilum sudafricanum	13			11	
Adenophlebia sp.	11	1	8	12	
Chlorolestes sp.	9	4	3	8	
Orectogyrus sp.	3	2		4	
Caenis sp.	2	1		5	
Cloeon sp.		55			20
Lestes sp.		1		4	
Aeshna sp.				3	6
Gravel/Sand/Mud					
Cheleocloeon excisum	12			1	14
Adenophlebia sp.	3			8	
Afroptilum sudafricanum	2			29	
Surface					
Orectogyrus sp.	3		3	3	1

Environmental data

A PCA was carried out to investigate the pattern of similarities between sites according to their environmental characteristics (Figure 3.11). Eigenvalues show that the first and second axes account for 45.6% of the variation in the data (Table 3.11). This indicates that the two-dimensional plot illustrates nearly half of the variation observed. The factor coordinate values indicates that velocity, salinity and TDS have a strong overall influence on the first axis, the variables influencing the second axis are all related to oxygen, while the third axis was essentially variables of water depth (Table 3.11). Sites generally clustered according to their areas in terms of mountain block (Figure 3.11). The Sneeuberg sites clustered together around the DO gradient indicating a strong influence of DO. These sites are also characterised by low TDS and high velocity; this high velocity explains the high DO. Nuweveldberge and Stormberg sites cluster around high gradients of TDS and salinity and a low gradient of velocity. The EC Drakensberg sites are very variable but are generally characterised by lower salinity and TDS and are slightly warmer than average.

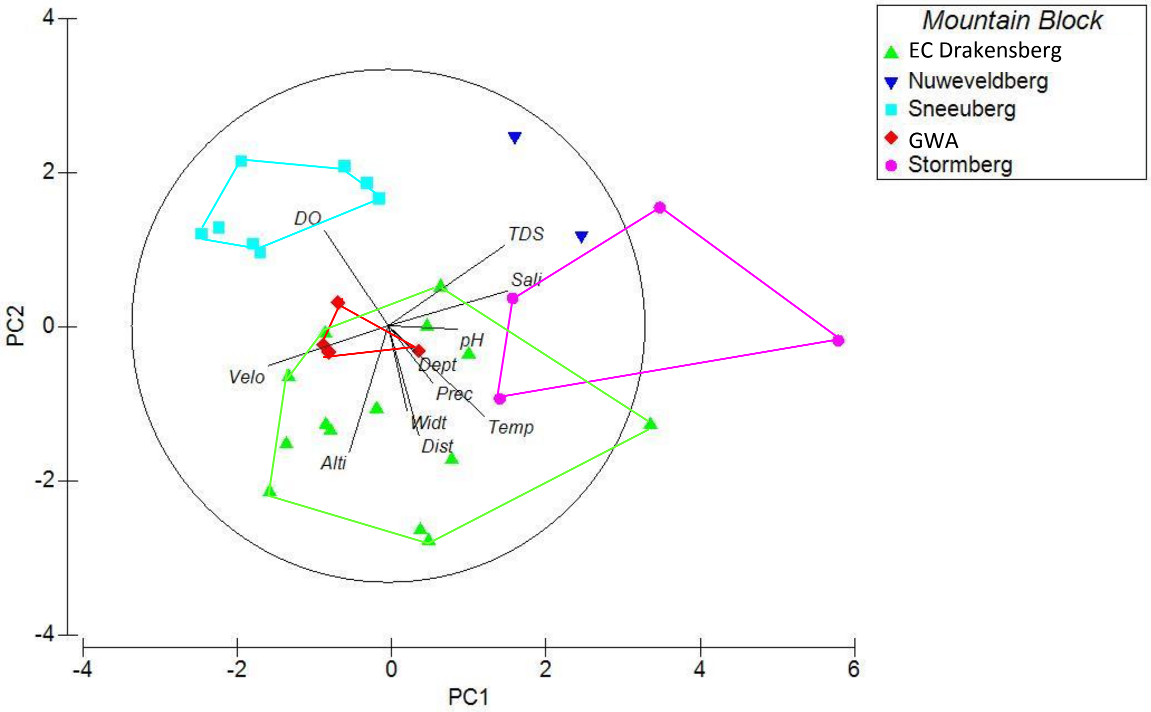

Figure 3.11. PCA plot of environmental variable measure at each site. DO (dissolved oxygen), TDS (total dissolved solids), Sali (salinity), Dept (depth of stream), Prec (annual precipitation), Temp (temperature), Widt (width of stream), Dist (distance from source), Alti (altitude), Velo (water velocity).

Table 3.11. Eigenvalues, percentage variation and Eigen vectors factor coordinates of the first 5 factors generated from the PCA of environmental variables across all sites. Bolded numbers indicate variables with a strong influence of the variability in the data.

Variable	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**
Eigenvalues	3.11	1.91	1.43	1.19	0.914
% Variation	28.2	17.3	13.0	10.8	8.3
Cumulative % Variation	28.2	45.6	58.6	69.4	77.7
Water velocity	**0.467**	0.155	0.253	-0.002	0.112
Salinity	**-0.468**	-0.137	0.278	0.180	-0.082
Total dissolved solids	**-0.453**	-0.313	-0.003	-0.012	-0.087
Altitude	0.152	**0.493**	-0.213	0.165	-0.370
Distance from source	-0.119	**0.427**	**0.503**	-0.181	-0.066
Temperature	-0.373	**0.352**	-0.120	0.117	-0.099
Dissolved oxygen	0.249	**-0.373**	0.264	0.360	0.308
Depth of stream	-0.106	0.108	**0.440**	**-0.564**	0.246
Precipitation	-0.173	0.222	0.348	**0.639**	0.139
Width of stream	-0.073	0.333	-0.276	0.097	**0.727**
pH	-0.269	0.014	-0.292	-0.167	0.344

The environmental data suggest that there are certain abiotic variables that are common to each mountain block. This results from the overall topography of the different mountain blocks which in turn influences the stream morphology and the biotopes present in each area. The EC Drakensberg, GWA and Sneeuberg have similar topography with high peaks and steep slopes (Figures 2.2 and 2.3). This results in faster-flowing rivers with high dissolved oxygen. In contrast, the Stormberg and the Nuweveldberge have a more gently sloping topography and, although high in altitude, these mountain blocks form more of a plateau with relatively small hills and moderate slopes. This results in streams that meander, often with still pools, causing a higher build-up of TDS and salinity, which is clearly evident in factor 1 of the PCA, and lower dissolved oxygen, evident in factor 2 and related to temperature and altitude (Table 3.11).

Any disturbances visible at the collection sites were noted. Due to the pristine nature of the streams sampled and that sites where close to the top of catchments, disturbances were minimal if present at all. As the majority of areas were farm lands used for grazing, cattle and sheep where present in the surrounding landscape, however this had no immediately notable negative effects on the streams. Farm roads crossed the streams close to four of the sites and although this affected the stream in the immediate vicinity of the road the streams recovered quickly to their original state further down. Agricultural crop lands were present close to one of the sites in Barkley Pass; this could affect the natural drainage of the area and if fertilizers and/or herbicides are used on the fields this could have serious consequences for the integrity of the stream (Nel, et al., 2011a). Two farm dams were noted at sites in the Stormberg, these are common in the area and cause some disturbance to the river system (O'Keeffe and Palmer, 1990) although a positive effects of dams has been recorded for Odonata (Samways, 1989). Alien invasive species were only noted on a large scale in the Nuweveldberge where *Populus* x *canescens* grows along many of the water courses. Although sites for this study were situated away from these *Populus* x *canescens* stands studies have shown that the trees negatively affect the hydrological flow of the streams (Henderson, 2001).

Macroinvertebrate and environment ordination

The CCA clarifies the influence of biotope on the macroinvertebrate assemblages occurring across mountain blocks (Figure 3.12). The sum of all canonical eigenvalues is 2.375 with the Total Inertia (the measure of the total variation in the dataset) being 4.567. The total variation explained is therefore 52%, meaning that half of the variation in the dataset is explained by the environmental variables measured. This is well above the expected range of 20% to 50% suggested by Økland (1999). Both the first and all of the canonical axes are significant according to the Monte-Carlo test of significance with p-values of 0.024 and 0.006, respectively. This suggests that the ordination is useful because there is a relationship between the species data and environmental data.

The TDS gradient is the longest axes on the CCA plot (Figure 3.12), indicating that sites clustered at the top end of this gradient are characterised by relatively high levels of TDS. In a similar plane to TDS are the gradients for altitude and velocity. The CCA shows Nuweveldberge and most Stormberg sites at the high end of the TDS gradient (Figure 3.12). This indicates that streams sampled in these areas are naturally characterised by relatively high levels of TDS, pH and salinity. This ties in with the topographical observations made above. The gradient for water velocity, altitude and average annual precipitation is the reverse or opposing that for TDS. EC Drakensberg and GWA sites are arranged along this altitude-velocity-precipitation gradient. The Sneeuberg sites are found along the low end of the temperature and depth of stream gradient, indicating that the Sneeuberg streams sampled are characterised by colder and shallower waters compared to other mountain blocks. These results correlate well with the PCA of environmental data above suggesting that the species assemblages are meaningfully associated with the various environmental variables (Figure 3.11, Figure 3.12).

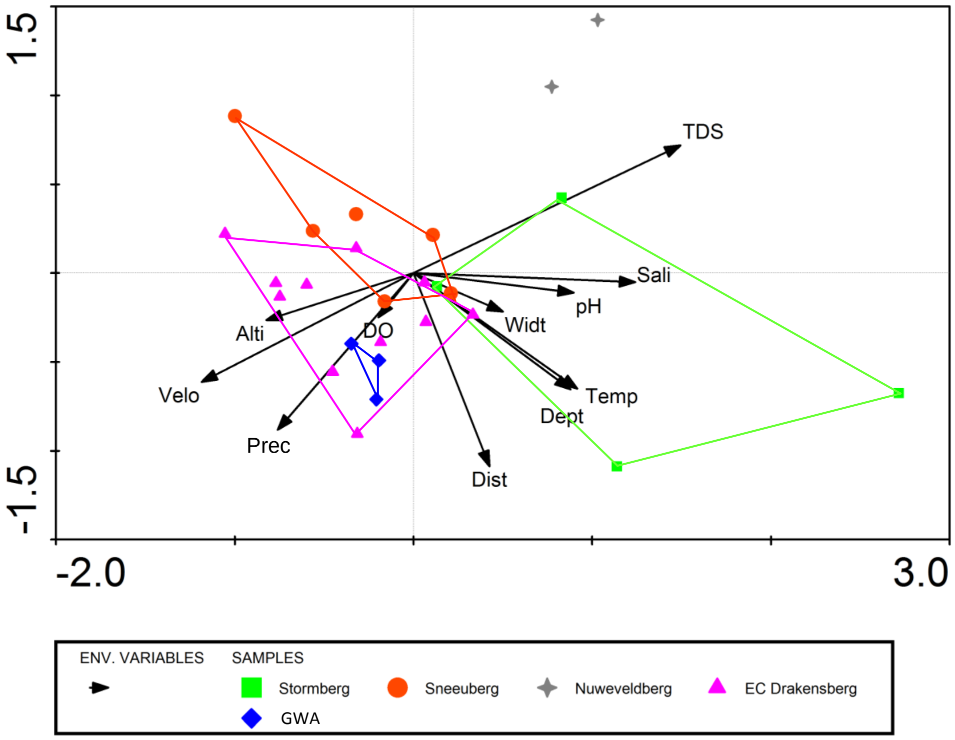

Figure 3.12. CCA ordination plot showing the sites from the different mountain groups in relation to environmental variables measured.

DISCUSSION

Species collected in this study make a considerable contribution to the development of inventories of freshwater species for the areas covered. This is critical for the proper documentation of biodiversity. The range of new and interesting species collected in this study emphasises the invaluable role of baseline data-gathering.

Diversity partitioning into α- and β-diversity was calculated to determine how diversity is distributed within the region. This is important as conservation science moves from protecting single species and isolated protected areas (Poiani et al., 2000) to conserving entire communities within regions (Chandy et al., 2006). Results indicate that although local species richness may be low within headwater streams, at larger scales the overall contribution to regional diversity becomes more apparent. Therefore, β-diversity may be an important component of macroinvertebrate diversity in headwaters. These results and those found elsewhere (Clarke et al., 2008, 2010; Jost et al., 2010) highlight the importance of diversity partitioning for conservation planning. For example, the need to move beyond linear models of stream ecosystems to consider the role of river networks in determining diversity patterns at the landscape scale (Grant et al., 2007).

It must be noted that the sampling design was not balanced, having an unequal number of biotopes and sites sampled in each area. This was mainly due to the availability of biotopes and this sampling effort may have had an effect on the species richness recorded in each area. Further sampling of all streams present in each area will give a better representation of species diversity. As this was not possible for this study the unbalanced sampling design must be considered when interpreting the results.

When considering the environmental factors of these southern Great Escarpment streams, the study found a relationship between the species assemblages and the environmental attributes of a site. This is not surprising due to the fact that aquatic insects collectively have a vast range of habitat preferences, feeding guilds, temperature and other tolerances (Davies and Day, 1998). Previous studies of South African communities have also found differences in the occurrence of taxa among biotopes (Palmer et al., 1991; Dallas, 2007). Minimal disturbances were recorded at some of the sites due to the relatively pristine nature of the sites.

Results indicate that similar species occur in the bedrock, SIC and waterfall biotopes. These biotopes are similar in that they all provide a habitat of water flowing over stones or rock surfaces. It is therefore not surprising that *A. sudafricanum* nymphs were commonly collected as they are found under small to medium-sized stones in slow- and fast-flowing streams at a wide range of elevations (Barber-James and Lugo-Ortiz, 2003). The most abundant species in bedrock and waterfall biotopes were the *Demoreptus* species. These species are restricted to habitats on rock faces in fast-flowing mountain streams (Barber-James and Lugo-Ortiz, 2003).

The pool, surface and submerged vegetation biotopes were also shown to have similar species assemblages. Pools have a more gentle gradient, weaker currents and finer substratum particles; this environment therefore would allow organisms with a burrowing life style to penetrate the sediments for feeding and protection (Malmqvist, 2002), and although no true burrowers were collected during the survey reported on here, some of the taxa are silt dwellers. For example, *Caenis* sp. (Caenidae) nymphs were frequently collected in pools. These nymphs are often associated with conditions where there is little or no flow, such as in pools and ponds. They feed on fine particulate detritus and periphyton among the silty substrates (Barber-James and Lugo-Ortiz, 2003). Submerged vegetation was usually found in pools which would explain the similarity of these two biotopes. *Lestes* sp. (Lestidae) and *Chlorolestes* sp. (Synlestidae) were common to these habitats as damselfly nymphs prefer the well-lit, weedy margins of pools, streams and rivers (Samways et al., 1996). *Cloeon* sp. (Baetidae) were also commonly collected in these biotopes with their preferred habitat among vegetation in ponds, dams, stream-pools and slow-flowing streams (Barber-James and Lugo-Ortiz, 2003). Given that there are pronounced differences between major habitat types, one expects to find quite different macroinvertebrate assemblages (Malmqvist, 2002).

The two-way ANOSIM found that differences in macroinvertebrate assemblages were greater among biotopes than between sites; this result was also found by a study based in the Western Cape and Mpumalunga (Dallas, 2007). Differences in macroinvertebrate assemblages between mountain blocks are attributed to environmental variables and biotopes present. This is because the variation in topography across the different mountain blocks results in differences in the aquatic habitats occurring in each area.

Rising temperatures and increasing variability of rainfall associated with climate change will affect surface waters, increasing drought in some regions and causing floods in others (Dallas and Rivers-Moore, 2014). Furthermore, portions of the Eastern Cape interior may experience an increased rainfall late in summer (DEAT, 2006). Although this study shows that precipitation does not currently have a strong influence on species assemblages, an increase in rainfall or delayed rains could have severe impacts over time, affecting emergence and breeding times, for example. Using climate modelling to make predictions under various climate change scenarios in Europe, a study found extreme negative effects of global climate change on freshwater assemblages within low mountain ranges (Sauer et al., 2011). Further research on climate modelling of macroinvertebrate assemblages along the Great Escarpment would be extremely valuable for conservation planning.

CHAPTER 4

Genetic variation in three mayfly (Ephemeroptera: Baetidae) species with different habitat requirements

INTRODUCTION

Evolutionary processes result from a number of factors, including the effects of natural selection, genetic drift, mutation and the movement of individuals (Allendorf and Phelps, 1981). It is generally accepted that genetic differentiation between populations is, in part, a reflection of their members' dispersal activity through space and time (Bohonak, 1999). Theoretically, if there is widespread intermigration between subpopulations of a species, levels of genetic differentiation will be low, whereas if this dispersal is restricted by physical barriers or limitations to mobility, then genetic differentiation is likely to be higher (Slatkin, 1985). The relationship between a species' dispersal ability and its genetic population structure can provide important insights into the micro-evolutionary processes operating throughout its history (Hanski and Gaggiotti, 2004; Avise, 2009).

Genetic structure of aquatic organisms

Aquatic insects are generally considered to be highly mobile with strong dispersal abilities (Hughes and Mather, 1995; Bunn and Hughes, 1997). This is reflected in the ability of stream organisms to recover from disturbance (Wallace, 1990; Yount and Niemi, 1990) and the widespread geographic distribution of many species across catchments. Consequently, many aquatic insects show low levels of genetic differentiation among populations, both within and between catchments. This result has been attributed to extensive dispersal of adults by flying (Schmidt et al., 1995; Hughes et al., 1998, 2000; Miller et al., 2002; Monaghan et al., 2002; Pereira-da-Conceicoa et al., 2012). However, while genetic variation is related to the ability of a population to disperse, a high degree of genetic structure has been observed among populations of some South African aquatic winged insects (Wishart and Hughes, 2001, 2003) and has also been observed in terrestrial winged insects (Price et al., 2007, 2010). This is often because the species is highly habitat-specific and therefore, despite a winged adult stage, the habitat requirement results in limited potential for dispersal (Price et al., 2007). In addition, the Ephemeroptera (mayflies) in particular are seen to have limited dispersal capacity over long distances due to their fragility and short adult life (Brittain and Sartori, 2003). Aquatic invertebrate species, including Ephemeroptera, show varied degrees of habitat specificity with some species being completely restricted to a certain habitat and others occurring in a range of different habitat types (Barber-James and Lugo-Ortiz, 2003). Specialization on a rare and stable instream habitat type (e.g. hygropetric zone) further increases the risk associated with dispersal (Wishart & Hughes 2001, 2003).

The linear, unidirectional, hierarchical nature of rivers, and the geological structure of the catchments they drain, impose a number of potential barriers to the dispersal of aquatic organisms (Wishart et al., 2003). Aquatic habitats within the terrestrial landscape can therefore be conceptualised as islands for some aquatic organisms. For certain winged insect species, especially in mountainous landscapes with rugged topography, population genetic variance is structured significantly according to drainage basin (Hughes et al., 1999, 2003; Wishart and Hughes, 2001, 2003; Monaghan et al., 2002; Price et al., 2010).

Some aquatic taxa such as mayflies form an important component of bioassessments as they are a key indicator species for the health of river systems (Chutter, 1972; Bauernfeind and Moog, 2000; Beketov, 2004; Pereira-da-Conceicoa et al., 2012). It is evident that sound taxonomy is fundamental to applied biology and with bioassessment methods gaining increasing popularity, a detailed understanding of commonly collected species will aid in further development of assessment methods and clarify species identification (Ball et al., 2005). Previous molecular research on baetid mayfly species that are used in bioassessment, *Baetis rhodani* Pictet (Williams et al., 2006), *Baetis vernus* Curtis (Ståhls and Savolainen, 2008) and *Baetis harrisoni* Barnard (Pereira-da-Conceicoa et al., 2012)*,* found that they represent a complex of cryptic species. These taxa are common and widespread in their respective distribution ranges. These studies highlight the need for accurate taxonomy associated with knowledge of their ecological requirements in widely used indicator species.

Understanding genetic diversity and genetic structure of species can also help inform conservation managers. Studies on genetic population structure of winged aquatic insects has been used to motivate for the use of catchments as units for conservation (Wishart and Davies, 2003; Price et al., 2010).

Three species of mayfly were chosen to investigate the relationship between habitat specialisation and genetic divergence in aquatic insects with flying adults. The southern Great Escarpment was chosen as the study region because of its highly dissected topography, which occurs on a spatial scale commensurate with the anticipated dispersal abilities of the study organisms. The three species are *Afroptilum sudafricanum* (Lestage), *Demoreptus natalensis* (Crass) and *Demoreptus capensis* (Barnard). Using DNA sequence data from these three species, the hypothesis that habitat-restricted taxa (e.g. *D. natalensis* and *D. capensis*) have greater genetic structure than widespread species (e.g. *A. sudafricanum*) is tested.

Species chosen to test hypothesis

Afroptilum sudafricanum

The genus *Afroptilum* Gillies has been the subject of much taxonomic revision. Gillies (1990) transferred all but one Afrotropical species of Baetidae from the Paleartic genus *Centroptilum* Eaton to a new genus *Afroptilum* (for the remaining one, he created the genus *Demoulinia* Gillies). However, Gillies' concept of *Afroptilum* was soon shown to be polyphyletic by McCafferty and de Moor (1995) and new genera have since been created to accommodate the different species groups (Wuillot and Gillies, 1993a, 1993b, 1994; Lugo-Ortiz and McCafferty, 1996a, 1996b, 1996c, 1997a, 1997b, 1998; Barber-James and McCafferty, 1997; McCafferty et al., 1997; Lugo-Ortiz et al., 2001). Consequently the concept of *Afroptilum* has been severely restricted and it is now readily possible to distinguish nymphs of the genus from other Afrotropical genera (Barber-James and Lugo-Ortiz, 2003).

Ten species of *Afroptilum* are currently recognised, of which two inhabit South Africa, *A. parvum* and *A. sudafricanum* Lestage. Specimens of *A. parvum* Crass were not collected during the sampling process and therefore no fresh material was available for genetic analysis. *Afroptilum sudafricanum* is a common, widespread African species, distinguished by having only six pairs of gills and a minute terminal segment on the labial palp (Barber-James and Lugo-Ortiz, 2003). Mature nymphs are about 6.0-8.5 mm long, with cerci 4-6 mm long; they occur in a range of ecological conditions, including different flow regimes and a wide altitude range (Barber-James and Lugo-Ortiz, 2003).The nymphs vary subtly in morphology across their distribution, with populations in the Western Cape being greyer in colour compared with the more eastern populations which are browner (H. Barber-James, Albany Museum, Grahamstown, pers. comm.).

Demoreptus capensis and D. natalensis

The genus *Demoreptus* Lugo-Ortiz and McCafferty is known only from three species endemic to southern Africa, *Demoreptus capensis* Barnard, *Demoreptus monticola* Crass and *Demoreptus natalensis* Crass. These species were originally placed in *Acentrella* by Barnard (1932) and Crass (1947), then transferred to *Baetis* (Demoulin, 1970), returned to *Acentrella* (McCafferty and de Moor, 1995) and finally accommodated in the new genus *Demoreptus* Lugo-Ortiz and McCafferty (1997c), based on morphological characteristics.

The nymphs of *Demoreptus* are distinguished from other African baetids by the following combination of characteristics: relatively narrow-elongate body, elongate and outstretched legs, claws with two rows of denticles and a pair of subapical setae and an undeveloped medial caudal filament. Body lengths of nymphs vary in different species from 5-7mm (Barber-James and Lugo-Ortiz, 2003). Nymphs have very specific habitat requirements, being most commonly found on rock faces associated with waterfalls in fast-flowing mountain streams (Barber-James and Lugo-Ortiz, 2003).

METHODS

Study region

The southern Great Escarpment forms an 800 km long stretch of mountain complexes extending from the Nuweveldberge in the west to the Eastern Cape Drakensberg in the East (Figure 4.1). Various intervals divide the five main mountains blocks that range in altitude from 1 600 - 3 000 m.a.s.l. The spatial scale of the study region is therefore large enough, relative to the dispersal abilities of mayflies, to validly test the hypothesis. The nature of mountain streams also provides suitable habitat for numerous samples of *Demoreptus* species to be collected.

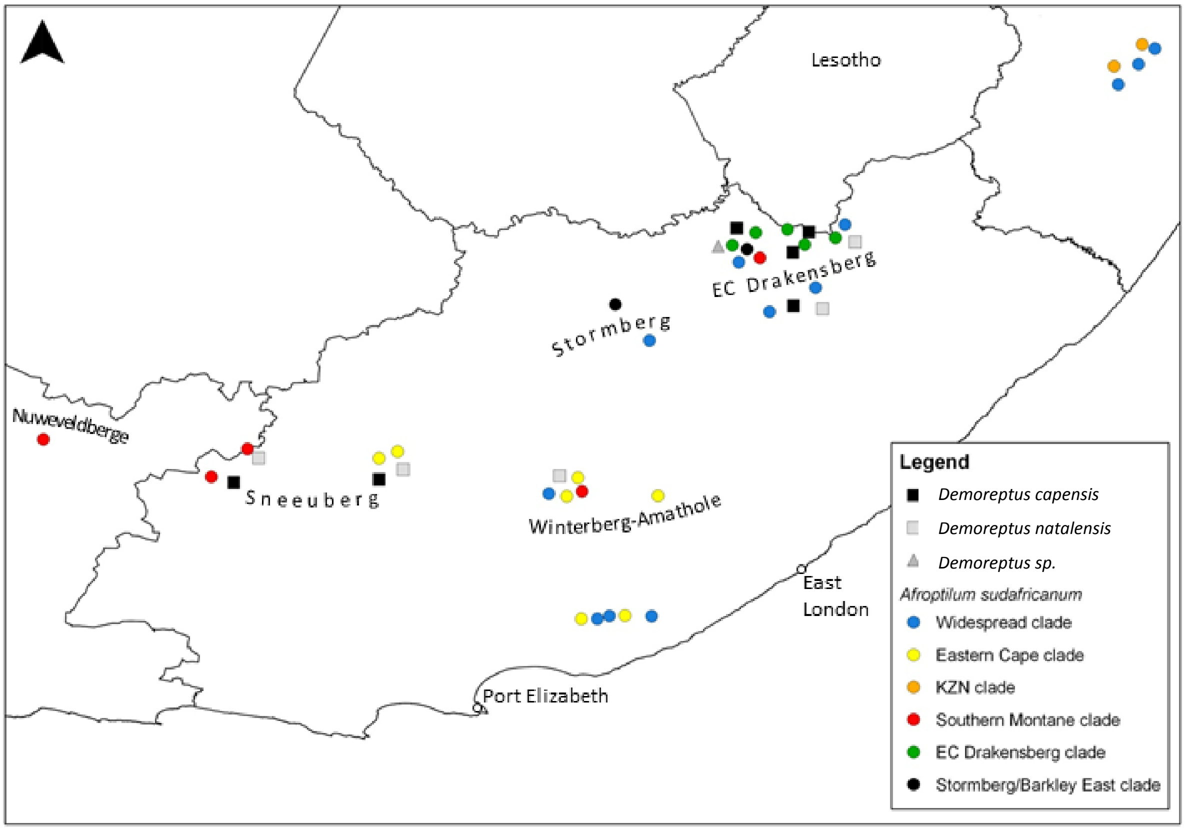

Figure 4.1. Map of samples collected in the southern Great Escarpment and low-lying areas of the Eastern Cape and Kwa-Zulu Natal. Habitat-restricted *Demoreptus* species indicated by squares and the widespread *Afroptilum sudafricanum* indicated with circles. *Afroptilum sudafricanum* clade colours are derived from the Bayesian analyses.

Taxon sampling

Nymphs of *A. sudafricanum, D. capensis* and *D. natalensis* were sampled from 21 rivers in the Eastern Cape Great Escarpment, flowing both north and south from the Escarpment (Figure 4.1). One additional *Demoreptus* species could not be identified, and did not fit the criteria of *D. monticola*. Nymphs of *A. sudafricanum* were also collected from six rivers in lower-altitude areas in the Eastern Cape and KwaZulu-Natal (Figure 4.1). All specimens were preserved in 80% ethanol for less than 2 years. The list of specimens and their locations of collection is given in Appendix 2, Table A2.

A related Baetidae species, *Baetis rhodani* Pictet, was used as the outgroup for phylogenetic analyses. Sequences were obtained through Genbank (Benson et al., 2008) for both cytochrome c oxidase subunit I (COI) (KP438135 and KP438160) and 16S (KP438109 and KP438119) gene regions (Rutschmann et al., 2014).

Molecular data

DNA was extracted using the Invisorb® Spin Tissue Mini Kit following manufacturer's protocol (Invitek, Berlin, Germany). Extraction was non-destructive, and ensured the preservation of the exoskeleton (housed in the Albany Museum, Grahamstown) for future morphological analysis. Two mitochondrial gene regions were amplified, cytochrome c oxidase subunit I (COI) and small subunit ribosomal RNA (16S). The COI regions of *D. natalensis* and *D. capensis* were successfully amplified with the standard 'universal' primer pair (Folmer et al., 1994), which worked with only limited initial success with *A. sudafricanum*, so a new forward primer (5'-GGTGGATGGGCAGGAATGGTAGGA-3') was designed and used successfully to sequence the rest of the samples of *A. sudafricanum*. The 16S region was amplified with the primer pair 16Sar and 16Sbr (Palumbi, 1996), which did not produce adequate sequences for the *Demoreptus* samples and time constraints limited the optimisation process.

The polymerase chain reaction (PCR) was performed in a 50 µl volume using the following thermal regime: 95°C for 5 minutes, 35 cycles of 95°C for 45 seconds, 50°C for 45 seconds and 72°C for 1.5 minutes, followed by a final extension period of 72°C for 5 minutes. PCR amplifications were checked for the presence of amplified PCR products by gel electrophoresis (0.5% agarose gel stained with SYBR green) and viewed with a UV-transluminator. Successful PCR products were cleaned up using the Invisorb PCRapace® Quick purification kit (Invitek, Berlin, Germany). Cleaned PCR products were then cycle-sequenced using the ABI Big Dye Sequencing kit v.3.1, according to manufacturer's instructions (Applied Biosystems). PCR products were sequenced with the ABI Genetic Analyzer 3500 (Applied Biosystems) in both directions using the same primers as for amplification.

Sequence trace files were assembled and edited using Sequencher 3.0 (Gene Code Corporations). The sequences where then aligned in MEGA v.6 (Tamura et al., 2013) using the ClustalW algorithm, with default settings. Amino acid sequences of the protein-coding gene were examined and each mutation was cross-checked in the original trace files. The messy ends of each sequence were trimmed and short sequences were filled with question marks to the end to indicate missing data.

Phylogenetic analyses

Each aligned gene dataset was tested for the most appropriate model of sequence evolution indicated in jModelTest 2.1.6 (Darriba et al., 2012) using akaike information criteria (AIC).

A Bayesian Inference (BI) analysis was conducted using MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001) using the Generalised time-reversible (GTR) model. This model was selected because it is the most complex parameterised model that MrBayes can accommodate. Two runs of 20 million generations, sampling every 200 generations with four chains (3 heated and 1 cold), were plotted against the likelihood scores and tree length, to ascertain when the analysis reached stationarity. The first 10% of the trees were discarded as burn-in, which represents 20 000 of the 200 000 trees sampled.

Parsimony analyses were performed in PAUP* version 4.0b10 (Swofford, 2011) using the heuristic search option with 100 random addition replicates. A search with Tree Bisection and Reconnection (TBR) branch-swapping was used to find the approximate length of the shortest trees, with 1 tree kept with each random addition. All the trees from this search were used as starting trees for a second heuristic search with MAXTREES set to 5 000. The *Demoreptus* analysis used FASTBOOTSTRAP with 10 000 replicates.

Maximum likelihood analysis was conducted with 2 000 bootstrap replicates using the GARLI (Genetic Algorithm for Rapid Likelihood Inference) web service (Bazinet et al., 2014) running the best-fitting substitution model selected by jModeltest (Table 4.1).

Population genetics and genetic variation

Population structure was investigated using the COI datasets as these where the most complete datasets. Haplotype networks were constructed using the software program TCS ver. 1.21 (Clement et al., 2000), with default settings. Separate haplotype networks were created for each of the species.

An Analysis of Molecular Variance (AMOVA) was conducted using Arlequin ver. 3.5.2 (Excoffier and Lischer, 2010) to compare genetic variation within and between populations. AMOVA was also performed for the largest haplotype network representing the widespread clade of *A. sudafricanum.* This was to ensure that there was an analysis that did not include possible cryptic species. F_{ST} (fixation index) values were calculated between localities to determine whether populations differed significantly in their genetic composition. For both AMOVA analyses a priori groups where defined by each site where the insects were collected.

Isolation-by-Distance (IBD) analyses were conducted using the Isolation by Distance Web Service version 3.23 (Jensen et al., 2005). A data matrix of the genetic distances and the geographical distances between all specimens was generated. Genetic distances were calculated from the COI data, as this was the most complete data set, using the Maximum Composite Likelihood model in

MEGA v.6 (Tamura et al., 2013). The geographical distances were calculated in R (R Development Core Team, 2008) with a precision of 1 m using the latitude and longitude recorded at each site on a GPS (Garmin, eTrex 10). The genetic and geographical data were log-transformed following Slatkin (1993) and the analysis was run with 10 000 randomisations as part of the Mantel test.

RESULTS

Data characteristics

Afroptilum sudafricanum

The COI region was successfully amplified in all 88 samples, but 16S amplified for only 59 of these. Therefore two data sets were analysed: all 88 COI sequences only, and then a partitioned dataset of 88 specimens including both COI and 16S with missing 16S data coded as missing. The COI + 16S molecular dataset consisted of 88 specimens and 1191 nucleotides (COI = 649 base pairs; 16S = 542 base pairs) including the outgroup (Alignment in digital Appendix 3). Missing data accounted for only 16.4% of the total dataset (Table 4.1).

Demoreptus spp.

The COI dataset consisted of 26 sequences (*D. natalensis* = 12; *D. capensis* = 11; unidentified = 1), including the outgroups, and 528 base pairs (Alignment in digital Appendix 3).

Table 4.1. Data characteristics and summary of the parsimony analysis. The number of specimens with sequence data (ntax), percent missing data (% ?), total number of base pairs (bp), parsimony informative (# Pi), percent parsimony informative (% Pi) is reported. The results of the parsimony are summarised with the number of trees retained (# trees), tree length (score), Consistence Index (CI) and Retention Index (RI). The summary of the models for the Maximum Likelihood analysis (ML) selected by jModeltest.

Species	**Dataset**	**ntax**	**Characters**				**Parsimony analysis**				**ML analysis**
			% ?	**bp**	**# Pi**	**% Pi**	**# trees**	**Score**	**CI**	**RI**	**Model (AIC)**
A. sudafricanum	COI	88	0.78	649	192	29.6	5 000	421	0.601	0.932	GTR+I+G
	COI+16S	88	16.4	1191	336	28.2	5 000	645	0.662	0.939	TIM3+I+G
Demoreptus spp.	COI	26	0.09	528	159	30.1	8	302	0.745	0.922	TVM+G

Phylogenetic analyses

The results of the parsimony analyses are summarised in Table 4.1. Abbreviations used in phylogenetic trees are summarised in Table 4.2.

Genetic diversity of the widespread A. sudafricanum

The phylogenetic analyses indicate six distinct clades in all analyses except the Bayesian tree with the COI+16S dataset, which shows paraphyly in the widespread clade (blue) (Figure 4.2 and 4.3). This widespread clade occurs across a range of locations in the Eastern Cape and KwaZulu-Natal, in both high and low altitude regions (Figure 4.1). The KZN clade (orange) and all three montane clades (green, red and black) and are strongly supported by Bayesian posterior probability, maximum likelihood and bootstrap values for both the COI-only and COI+16S datasets, while the Eastern Cape clade (yellow) occurring in both montane and lowland areas has limited support (Figure 4.2 and 4.3). In some locations more than one clade is found at a site, such as Barkly East in the EC Drakensberg and the Winterberg-Amathole.

Table 4.2. Key to abbreviations used in Figures 4.2, 4.3 and 4.4

Eastern Cape Drakensberg
RH: Rhodes
BE: Barkly East
BP: Barkly Pass
MC: Maclear

Stormberg
SB: Stormberg

Winterberg-Amathole
EL: Elandsberg
WB: Winterberg

Sneeuberg
SN: Sneeuberg
KM: Kamdebooberg

Nuweveldberge
NV: Nuweveldberge

Non Escarpment sites
GHT: Grahamstown
  -PM: Palmiet River
  -KP: Kap River
  -KW: Kowie River
  -CR: Coleridge
KZN: KwaZulu-Natal
  -KK: Karkloof
  -LR: Lions River
  -UM: Umgeni Valley

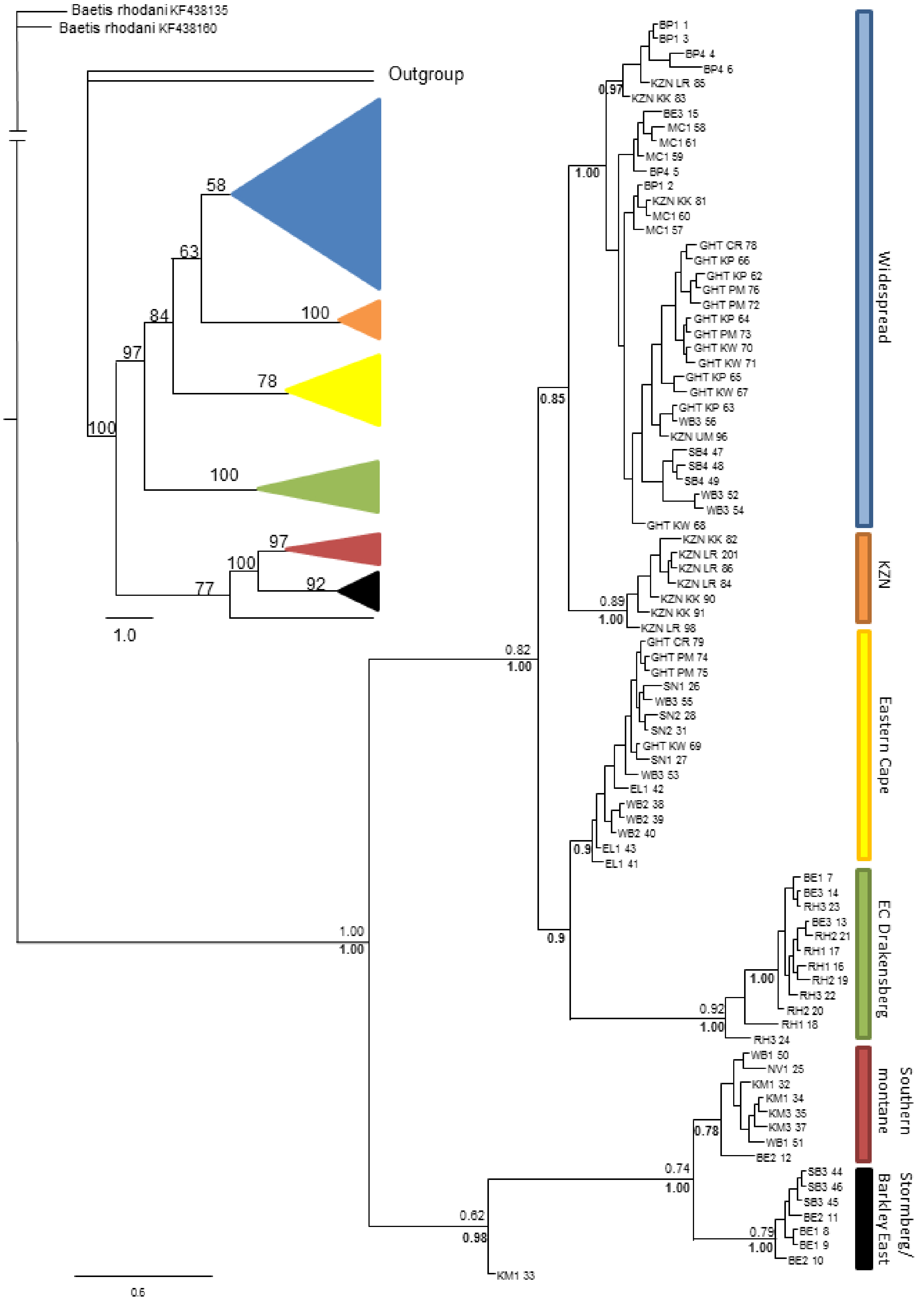

Figure 4.2. Bayesian Inference phylogram of *Afroptilum sudafricanum* from the COI-only dataset. Support for major nodes shown above (Maximum Likelihood) and below (Bayesian posterior probabilities) each branch. The columns to the right of the tree indicate the five different clades, colour-coded as blue, orange, yellow, green and red to match the map in Figure 4.1. Inset diagram is the Parsimony phylogram showing the relationship of each node with bootstrap support above the branch. Key to abbreviations in Table 4.2.

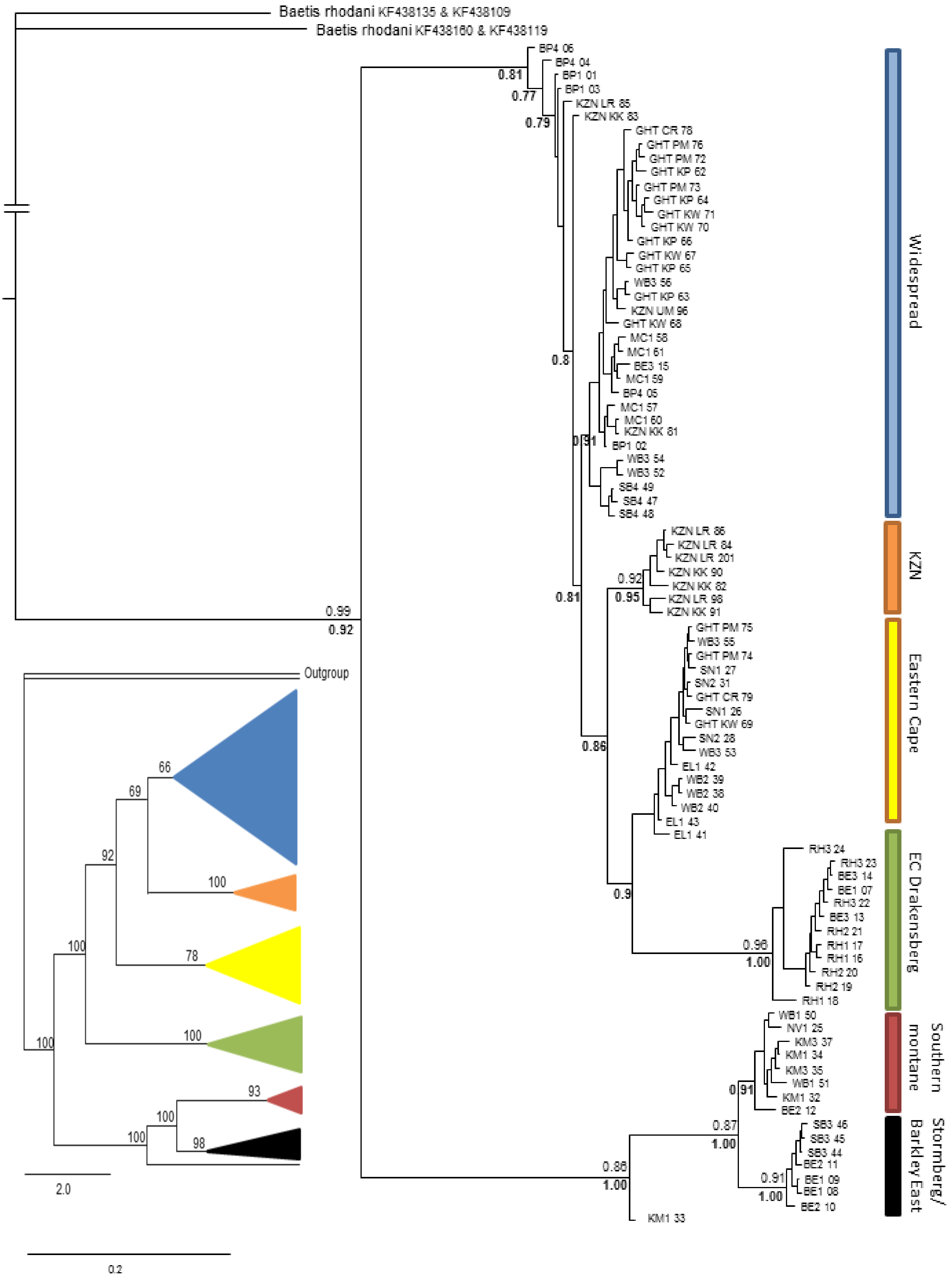

Figure 4.3. Bayesian Inference phylogram of *Afroptilum sudafricanum* from the COI+16S dataset. Support for major nodes shown above (Maximum Likelihood) and below (Bayesian posterior probabilities) each branch. The columns to the right of the tree indicate the five different clades, colour coded as blue, orange, yellow, green and red to match the map in Figure 4.1. Inserted diagram is the Parsimony phylogram showing the relationship of each node with bootstrap support above the branch. Key to abbreviations in Table 4.2.

Genetic diversity of the habitat-restricted Demoreptus spp.

The phylogram of *D. capensis* and *D. natalensis* clearly indicates strong genetic structure correlated with location (Figure 4.4). Both species have genetically distinct populations with strong support from parsimony, Bayesian and maximum likelihood analyses.

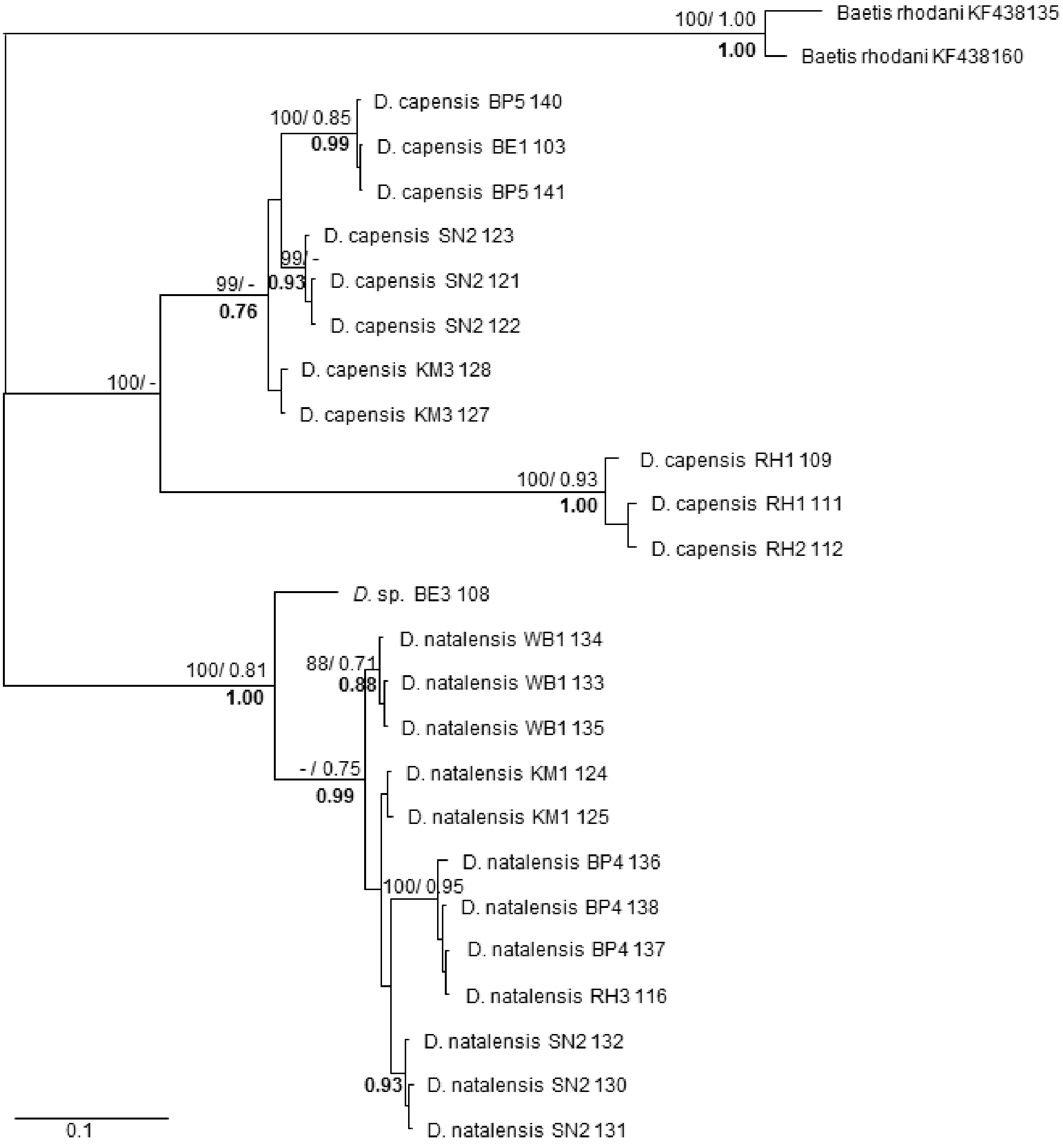

Figure 4.4. Bayesian Inference phylogram of *Demoreptus natalensis* and *D. capensis.* Support for major nodes shown above branch (Parsimony bootstrap/Maximum Likelihood) and below branch (Bayesian posterior probabilities). Key to abbreviation in Table 4.2

Population genetics

Network analyses

The TCS analysis collapsed the 85 *A. sudafricanum* COI sequences into 66 haplotypes and six haplotype networks (Figure 4.5). The six networks compare to the clades retrieved by the Bayesian analyses (Figure 4.2 and 4.3).

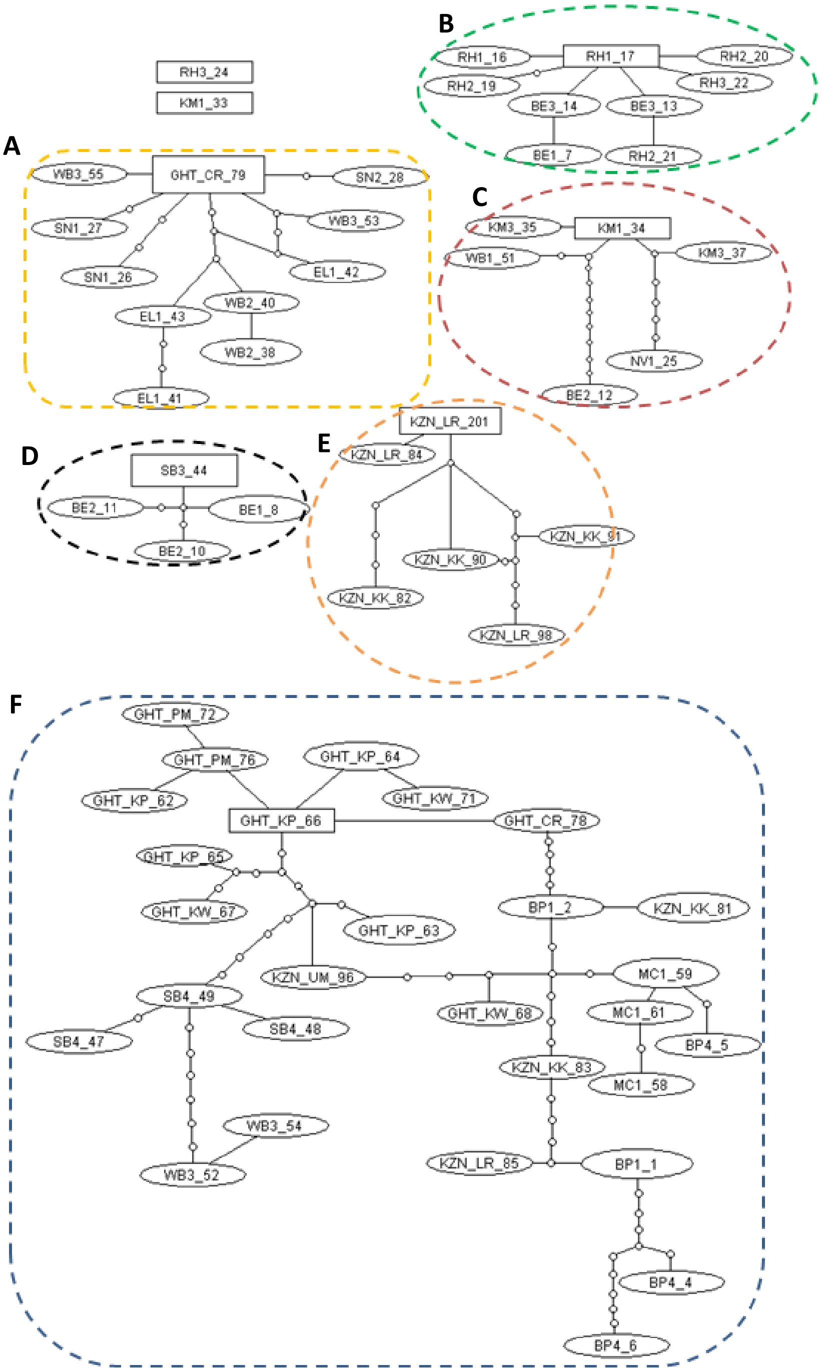

Figure 4.5. Haplotype network produced by TCS for *A. sudafricanum.* A = Eastern Cape clade; B = EC Drakensberg clade; C = southern montane clade; D = Stormberg/Barkly East clade; E = KZN clade; F = widespread clade. Colours correspond to localities in Figure 4.1.

The TCS analyses for *D. capensis* and *D. natalensis* retrieved eight and seven haplotypes respectively. For *D. capensis*, three haplotype networks were generated with a separate network for each locality/population (Figure 4.6). *D. natalensis* has two haplotype networks which are also somewhat separated by locality (Figure 4.6).

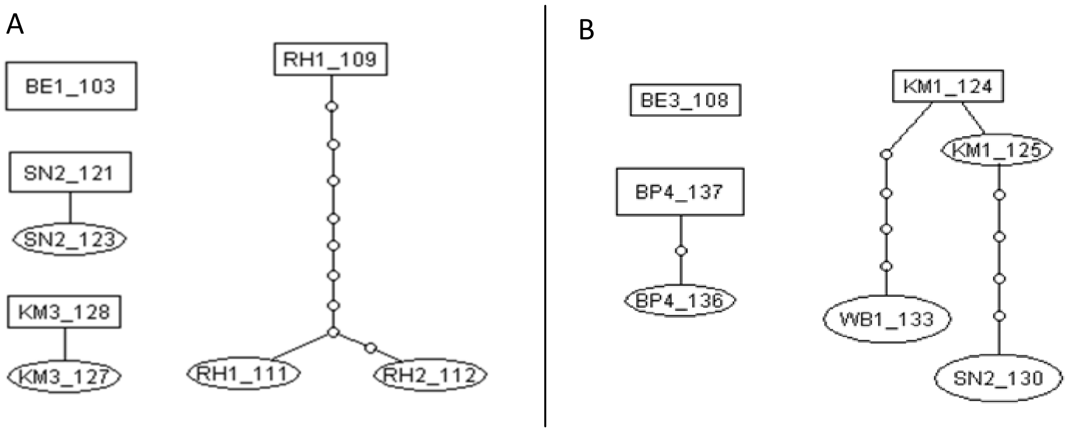

Figure 4.6. Haplotype networks for A) *D. capensis* and B) *D. natalensis*

Analysis of molecular variance

The AMOVA results revealed that over all localities for *A. sudafricanum* 52.33% of the total variance was explained by the variation among populations (df = 10, Va = 12.073) while 47.67% (df = 75, Vb = 10.998) was explained by variation within populations (Table 4.3). A similar result was found with the widespread clade of *A. sudafricanum* with 39.43% of the total variance explained by the variation among populations (df = 5, Va = 2.238) and 60.57% (df = 28, Va = 3.438) explained by variation within populations. In contrast, the AMOVA for the habitat restricted *D. capensis* and *D. natalensis* species indicated a higher percent variation among populations with 94.83% (df = 4, Va = 24.950) and 95.39% (df = 4, Va = 5.423) respectively (Table 4.3). With the total variance explained by variation within populations being 5.17% (df = 6, Vb = 1.361) for *D. capensis* and 4.61% (df = 7, Vb = 0.262) for *D. natalensis.*

The measure of population differentiation due to genetic structure (F_{ST}) was much lower for *A. sudafricanum* compared to *Demoreptus* species (Table 4.3). F_{ST} values range from 0 to 1 with a value close to 0 indicating limited divergence between populations, and an F_{ST} value of 1 indicating complete isolation of populations. The widespread clade for *A. sudafricanum* had a very low F_{ST} value of 0.39 with *D. natalensis* and *D. capensis* having very high F_{ST} values of over 0.94 (Table 4.3).

Table 4.3. AMOVA results for populations of *A. sudafricanum, D. capensis and D. natalensis* showing percentage variation among and within populations, the fixation index (F_{ST}) and corresponding p-value

Species/clade	**% variation**		**F_{ST}**	**P-Value**
	Among	Within		
A. sudafricanum	52.33	47.67	0.52327	< 0.05
A. sudafricanum **widespread clade**	39.43	60.57	0.39426	< 0.05
D. capensis	94.83	5.17	0.94827	< 0.05
D. natalensis	95.39	4.61	0.95393	< 0.05

Isolation by distance

The habitat-restricted species, *D. capensis* and *D. natalensis,* both showed considerable correlation between genetic distance and geographic distance with r-values of 0.709 ($p = 0.01$) and 0.819 ($p < 0.001$) respectively (Figure 4.7). Conversely, the widespread *A. sudafricanum* showed poor correlation ($r = 0.383$; $p < 0.001$) between genetic distance and geographic distance. When samples of *A. sudafricanum* were split according to major clades, there were also no significant correlations (Figure 4.7).

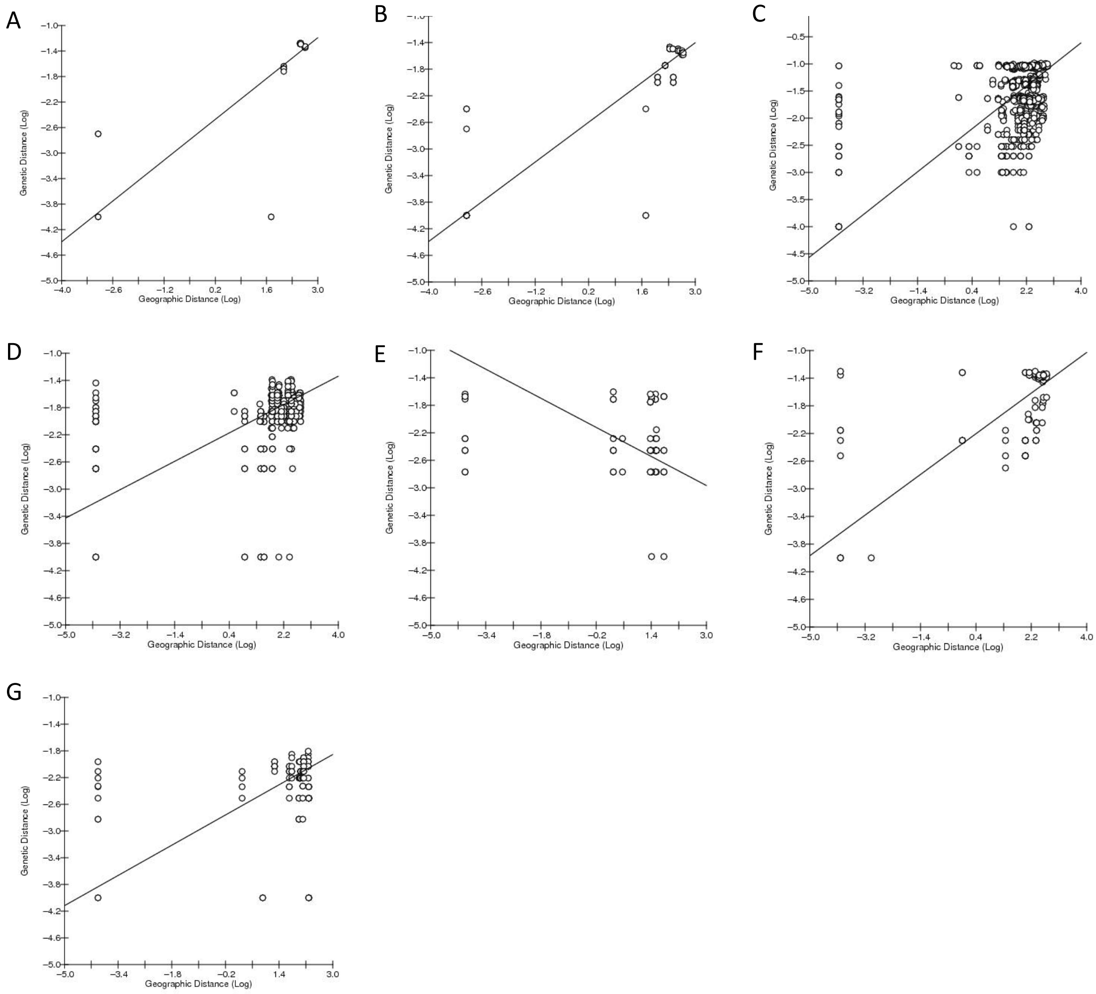

Figure 4.7. Log-transformed Isolation by distance graphs of two habitat-restricted and one widespread species. A) *Demoreptus capensis* (r = 0.709; p = 0.01) B) *Demoreptus natalensis* (r = 0.819; p < 0.001) C) *Afroptilum sudafricanum* (r = 0.383; p < 0.001) D) *A. sudafricanum* blue clade (r = 0.348, p < 0.001) E) *A. sudafricanum* green clade (r = -0.120, p = 0.878) F) *A. sudafricanum* red clade (r = 0.559, p < 0.001) G) *A. sudafricanum* yellow clade (r = 0.259, p = 0.008).

DISCUSSION

Phylogenetic relationships and taxonomy

Afroptilum sudafricanum

This is the first study to consider the phylogenetic structure of the species *A. sudafricanum*, a widespread Afrotropical species. The internal phylogeny indicates six clades with varying levels of support. The two largest clades, the widespread and Eastern Cape clades, have limited support that varies between analyses. The KZN, southern montane, Stormberg/Barkly East and EC Drakensberg clades are well-supported in all analyses. All clades are well defined and each clade forms a separate haplotype network. However, in the phylogenetic analyses some nodes with poor support conflict between analyses. This indicates a shortage of phylogenetic signal in those parts of the phylograms and could possibly be resolved with the addition of sequences chosen specifically to address the shortfall. However, the results may also indicate that the sequence of clade creation was quite rapid, and if so, then any amount of data would be potentially uninformative as rapid radiations are difficult to resolve (Whitfield and Lockhart, 2007). The relationships between clades are generally inconclusive with the only consistent resolution in all analyses being the division of the southern montane clade and Stormberg/Barkly East clade from all other clades.

In terms of distribution, there is a large amount of overlap between clades. All clades occur across relatively large geographic areas, except for the EC Drakensberg and KZN clade (Figure 4.1). The KZN clade may in fact represent samples from throughout KwaZulu-Natal and further sampling will be needed to determine this. The EC Drakensberg clade occurs only in the Eastern Cape Drakensberg. This limited distribution and the long branch length could indicate that this clade underwent a prolonged period of isolation, genetic drift, or a serious genetic bottleneck. However, this clade is no longer geographically isolated as specimens from the southern montane, Stormberg/Barkly East and the widespread clades were also collected in this area, except for in the highest part of the region (Ben Macdhui peak) where only specimens from the EC Drakensberg clade were collected. It would be interesting to sample other areas on the Drakensberg range in KwaZulu-Natal and Lesotho to test whether this clade occurs throughout high altitude, mountainous areas. Four different clades occur in the Eastern Cape Drakensberg with some rivers having specimens from more than one clade at the same site. A similar phenomenon was found by Daniels et al. (2003) where cryptic species of freshwater crabs (*Potamonautes*) occurred sympatrically in the northern Drakensberg.

The high support values for some clades within *A. sudafricanum* and the separate haplotype networks revealed by TCS could indicate the presence of cryptic species. However, because these are mitochondrial clades, these data would not pick up interbreeding with other clades as mitochondrial DNA (mtDNA) only reflects the matrilineal evolutionary history (Zhang and Hewitt, 2003). Cryptic diversity and relationships between putative cryptic taxa within *A. sudafricanum* clearly deserves further investigation including more intensive sampling throughout its geographic range and the use of multiple genetic markers. The discovery of morphologically unrecognised or cryptic species is not uncommon in aquatic insects (Wishart and Hughes, 2003; Pereira-da-Conceicoa et al., 2012). Since the 1980s there has been an exponential increase in the number of studies on cryptic species, partly due to the introduction of the PCR, which resulted in the increasing availability of DNA sequences (Bickford et al., 2007). Molecular (DNA) methods are valuable in resolving cryptic lineages and have been used extensively in discriminating species with little or no morphological differences (Jackson and Resh, 1998; Monaghan and Sartori, 2009). Within the Ephemeroptera, cryptic species have been discovered in numerous families through electrophoretic studies (e.g. Ephemerellidae (Funk and Sweeney, 1994), Heptageniidae (Zurwerra et al., 1987), Isonychiidae (Zloty et al., 1993), and Behningiidae (Sweeney and Funk, 1991)) and, more recently, DNA sequence data (e.g. Baetidae: Williams et al., 2006; Ståhls and Savolainen, 2008; Pereira-da-Conceicoa et al., 2012).

Demoreptus spp.

The branch length for three *Demoreptus "capensis"* samples (RH1 109, RH1 111, RH2 112) collected around the Rhodes area, Eastern Cape, suggests that this may be a new species. These samples were collected from the highest regions of the study area (2600 m.a.s.l) on the slopes of Ben Macdhui. Preliminary morphological observations also indicate these samples have a noticeably shorter segment 3 of the labial palps compared to other *D. capensis* samples. Interestingly, a similar clade was found in *A. sudafricanum*, with samples from this region also being genetically distinct with a long branch length. It is possible that an event isolated both species, resulting in this anomaly.

A second potentially new species was also found in the southern Drakensberg region (BE3 108). This specimen was morphologically unrecognised with the labial palp segment 3 having a small cap-shape and being quite setose (H. Barber-James, Albany Museum Grahamstown, pers. comm.). Genetically this sample was also shown to be distinct (Figure 4.4). Further investigation will be necessary to describe this species, and it would be advantageous to collect adult material too for taxonomic description. It is not unexpected that potentially new species are discovered as mountainous regions are often undersampled (Phiri and Daniels, 2013). Gattolliat et al. (2008) attribute the reported low diversity of Baetidae in most areas of Africa to the lack of data and comprehensive analysis of material collected by taxonomists. Intensive sampling, over large distributions, usually results in the discovery of numerous new taxa and the extension of distribution ranges (Gattolliat et al., 2008).

Habitat specialisation and genetic variation

Results from this study clearly support the hypothesised effect of habitat specialisation on genetic variation and structure of mayfly species. Both habitat restricted species (*D. natalensis* and *D. capensis*) show notable genetic differentiation between geographic localities and catchments. This was evident from the haplotype networks in the TCS analysis, high F_{ST} values from the AMOVA and a strong correlation in the isolation by distance results as well as the structure indicated by the phylogenetic trees. Population analyses are limited by the sample size from each location, therefore results should be further confirmed with the addition of samples. Previous studies on South African species have found genetic differentiation according to catchments in both aquatic insects with limited dispersal ability (net-winged midge: Wishart and Hughes, 2001, 2003) and terrestrial insects with high vagility (cicadas: Price et al., 2007, 2010). The unexpected limited dispersal potential of cicadas was attributed to their habitat philopatry (Price et al., 2010) and host-plant specificity (Price et al., 2007). Similarly, *D. natalensis* and *D. capensis* are restricted by their habitat, only occurring on rock faces associated with waterfalls in fast-flowing mountain streams, and subsequently show high levels of genetic differentiation. Similar limitations to gene flow have been found in various other mountain restricted aquatic insects (Hughes et al., 2003; Wishart and Hughes, 2003; Finn et al., 2006; Lehrian et al., 2010).

In contrast, although the widespread species, *A. sudafricanum*, shows a large amount of genetic differentiation within the species, this is not attributed to geographic location or catchments. Most clades are widespread across numerous catchments with the exception of one of the clades occurring in the Eastern Cape Drakensberg. Even when *A. sudafricanum* is treated as a species complex and the major widespread clade is assessed separately for genetic differentiation, results indicate low divergence between populations suggesting that gene flow is not particularly limited within catchments and across the geographic range. Some clades within *A. sudafricanum* are remarkably widespread, therefore, although mayflies are thought to have limited dispersal because they are weak fliers with short adult lifespans (Brittain and Sartori, 2003); these results support studies indicating that long-distance dispersal is more prevalent in mayflies than previously thought (Monaghan et al., 2005; Pereira-da-Conceicoa et al., 2012).

The above findings support the hypothesis that habitat-restricted *Demoreptus* species have greater genetic structure than widespread *A. sudafricanum*. The restriction in gene flow over distance and across catchments in *D. natalensis* and *D. capensis* is assumed to be a consequence of the isolation caused by being limited to habitats in mountainous areas.

Conclusion

These results help to illustrate some of the evolutionary process occurring in mayfly species and highlight the effect of habitat specificity on a species. Even though all three species have similar levels of dispersal potential in terms of flight ability they have vast differences in gene flow. This study attributes these differences to the differences in habitat requirements between species. These results will contribute to conservation research. For the conservation of riverine organisms, it has been legislated that catchments are used as management units (South African National Water Act, 1998). Previous studies on the genetic population structure of winged aquatic insects in South Africa have further supported the use of catchments as units for conservation (Wishart and Davies, 2003;

Wishart et al., 2003; Price et al., 2010). As with studies by Wishart and Davies (2003) and Price et al. (2010), the results from population analyses of *D. capensis* and *D. natalensis* emphasise the genetic distinctiveness of populations between catchments. These genetically distinct populations form an important component in the evolutionary legacy of a species. Therefore, the development of inter-basin water transfers poses a threat to both *D. capensis* and *D. natalensis* by potentially connecting historically isolated and genetically distinct populations. In addition, dispersal among adjacent catchments has important implications for the recovery of lotic systems following disturbance (Wishart and Davies, 2003) These factors should be considered in the development of strategies for the conservation of aquatic biodiversity (Wishart, 2000).

Although the study also attempts to clarify phylogenetic relationships in *A. sudafricanum*, this was not the aim of this study and analyses reported here are insufficient for species delineation. Further research into the phylogenetics and taxonomy of these species could include nuclear gene sequences to evaluate interbreeding and provide independent loci, detailed analysis of morphology, biology and ecology and a wider sampling area including the whole species' range. Collections over different seasons could also be informative; if the different clades have varying emergence times this may lead to discontinuities in gene flow. The accuracy of species identification is important to studies of community structure, biodiversity and biomonitoring (Ball et al., 2005). Mayflies form a very important component of aquatic research, particularly biomonitoring, and therefore findings from this study form essential baseline data for further development of monitoring methods. A deeper understanding of evolutionary processes and gene flow with regard to commonly occurring mayfly taxa contributes to broader research on ecosystem functioning and environmental processes. The identification of possible cryptic species in *A. sudafricanum* and new species of *Demoreptus* form important advances in the field of aquatic research. This research also highlights the difficulties in the identification of immature specimens of *A. sudafricanum* through morphology, this has also been reported in many other mayfly taxa (McCafferty and Pereira, 1984; Waltz et al., 1996, 1998; Baumgardner and McCafferty, 2000; Jacobus and McCafferty, 2000).

Through this research interesting anomalies were revealed, such as the genetically distinct clades occurring in the Eastern Cape Drakensberg found in both *A. sudafricanum* and *D. capensis*. Further studies could investigate the processes causing the possible isolation event resulting in this finding. It would also be interesting to test whether this anomaly also occurs in other organisms, both aquatic and terrestrial, from this area. The findings from this study set the foundations for more thorough analysis of both *A. sudafricanum* and the *Demoreptus* species.

CHAPTER 5

Synthesis

SIGNIFICANCE OF FINDINGS

A total of 2 595 macroinvertebrate specimens from 47 families and a conservative estimate of 86 species were collected in the southern Great Escarpment. As these areas have not been previously sampled these data have contributed to the biodiversity baseline data of the region. From these data a better understanding of community assemblages was possible. A number of interesting and potentially new species were also discovered.

Genetic analyses of three species of mayflies (Baetidae), occurring abundantly in the southern Great Escarpment, gave a better understanding of evolutionary processes occurring across each mountain block. Additionally possible cryptic taxa and new species where identified in *A. sudafricanum* and *Dermoreptus* respectively.

Biodiversity

In terms of species richness, the Sneeuberg was found to have the highest number of collected species. High species richness and endemism of flora, tetrapod vertebrates and leafhoppers have also been documented from these mountains (Clark et al., 2009, 2012). The unique faunal and floral composition of this region highlights the high conservation importance of these mountains (Clark et al., 2009, 2012).

Species diversity results indicate that rivers from different mountain blocks have a unique signature in terms of species assemblages; this is reflected in the partitioned species diversity analysis which showed β-diversity to be significantly higher than expected. Similar findings regarding high levels of β-diversity are also recorded from Australian headwater systems (Clarke et al., 2008, 2010). From the results of the multivariate analyses carried out, it is clear that discernible patterns exist in the species assemblages of these rivers. These are attributed to differences in various environmental factors which in turn affect the suite of biotopes present. This has consequences for bioassessment studies where the presence or absence of particular biotopes could influence the overall score used to evaluate river health (Dallas, 2007).

It is not only the species assemblages and environmental variables that are unique to each area but in some cases the genetic diversity too. The results from genetic analysis showed species of *D. capensis* and *D. natalensis* as genetically distinct between mountain blocks and therefore between catchments; this has also been shown with aquatic species in other areas of South Africa (Wishart and Hughes, 2001, 2003).

IMPLICATIONS

Climate change

Freshwater ecosystems are considered to be among the most vulnerable ecosystems to global climate change (Bates et al., 2008). Climatic changes are predicted to result in rising temperatures and increasing variability of rainfall with some regions experiencing an increase in drought events and others an increase in flooding events (DEAT, 2006). There is also a predicted increase in the contrast in precipitation between wet and dry regions and between wet and dry seasons (IPCC, 2013). These predictions emphasise the important role headwater streams and ecosystems will play in providing valuable ecosystem services such as water security and mitigating flooding events (Brauman et al., 2007). Portions of the Eastern Cape interior are also predicted to experience increased later summer rainfall (DEAT, 2006) and this could negatively affect emergence and breeding times of macroinvertebrates (Harper and Peckarsky, 2006).

These climatic changes are likely to have an impact on aquatic montane systems and have important implications for macroinvertebrate communities, particularly those occurring at the upper-most reaches (Durance and Ormerod, 2007; Grab, 2014). With an increase in temperature, the ecological consequence of the upward shift of thermal habitats cannot yet be accurately projected (Grab, 2014). This study provides baseline data for research of species distributions and the effects of future climate warming on macroinvertebrates in montane environments. It is important that there is continuous research and monitoring of these areas to enable more accurate modelling of future trends.

Conservation

Since the ecological consequences of climate change for upland streams are anticipated to be substantial (Durance and Ormerod, 2007; Bush et al., 2012; Grab, 2014), planning for the conservation of macroinvertebrates in headwater streams is a task that has become more urgent (Clarke et al., 2008). When planning for aquatic conservation, the two key attributes considered by Linke et al. (2007) are vulnerability (the possibility that future condition will change in a negative direction) and irreplaceability (the extent to which the loss of an area will compromise regional conservation targets). This study indicates that macroinvertebrate assemblages are highly distinctive at both a community and genetic level across mountain blocks and are thus highly irreplaceable. The negligible dispersal among catchments in species such as *D. natalensis* and *D. capensis* and in the headwater-restricted species such as the amphipod collected in the Sneeuberg and *Archaeochlus* sp. from Barkly Pass imply that local extinctions would probably be 'final'. The decline and extinction of species, the loss of genetic diversity, and the degradation of ecosystems will all lead to a reduced ability of the environment to support national development and sustain all life including human well-being in the future (Haines-Young and Potschin, 2010).

In South Africa whole catchments have been proposed as units for conservation (Wishart, 2000; Wishart and Davies, 2003; Price et al., 2010). Results from this study further support the use of catchments as units for conservation. The macroinvertebrate diversity in these southern Great Escarpment streams was low in individual headwater streams. However the study further showed that the overall contribution of headwater streams to regional diversity becomes more apparent at larger scales. These results and those found elsewhere (Clarke et al., 2008, 2010; Jost et al., 2010) highlight the importance of diversity partitioning for conservation planning and the need to move beyond linear models of stream ecosystems to consider the role of river networks in determining diversity patterns (Grant et al., 2007). With the widespread adoption of systems thinking by many biological disciplines (Chong and Ray, 2002; Kitano, 2002), it would be prudent to suggest that catchments represent significant units of unique assemblages of biological diversity (Wishart, 2010).

These results are of particular importance given the increased use of inter-basin water transfer schemes in South Africa. This movement of water between catchments poses a potential threat to the evolutionary legacy of species and community assemblages by connecting historically isolated populations. As suggested by Wishart and Hughes (2003), it seems necessary that these biologically distinct catchments are considered in the development of conservation frame-works.

All study sites, each with unique biodiversity, fit into Freshwater Ecosystem Priority Areas (FEPAs). This study emphasised the importance of these areas and provides a platform for further conservation of our freshwater biodiversity.

OPPORTUNITIES AND FUTURE RESEARCH

Due to budget and time constraints, only a few streams were sampled in each mountain block. This limits the estimation of diversity as it restricts the resolution to which a system can be explored. It also means that the possibility of undiscovered species in each area is still high and many streams of the southern Great Escarpment are yet to be researched. Of the new and interesting species collected, species descriptions are still needed and certain species distributions can be updated.

The genetic studies have opened up a range of further research opportunities. The possible cryptic taxa in *A. sudafricanum* and species discoveries in *D. natalensis* and *D. capensis* are examples that warrant future research. Sequences of *D. natalensis* and *D. capensis* will also be useful for further investigations, for example examining the relationship of *Demoreptus* (a southern African genus) to *Acentrella*, the closely related North American and European genus.

Population level genetic analyses found informative evolutionary processes in all species; however, these analyses would have benefited from having more individuals from each population. Further population-level research of montane species is recommended. Such research will broaden our knowledge of the evolutionary processes taking place. Studies on climate change, where a deeper understanding of mountain restricted species is essential due to their vulnerability, should also be undertaken (Bush et al., 2012). Macroinvertebrate species could also act as indicators of climatic change and further studies on the effects of stream temperature on macroinvertebrates in this study area and others could give insight into the future climate change impacts. Grab (2014) proposed long-term monitoring of stream temperatures and macroinvertebrates through an altitudinal gradient for the Drakensberg/Lesotho mountain region. As climate change impacts are predicted to vary across South Africa, especially across the summer and winter rainfall regions (Schulze, 2005), the inclusion of similar long term monitoring of sites across the southern Great Escarpment would add a further dimension to climate change studies. This study has established sites that can be used as benchmark sites for long-term studies, for instance studies aimed at verifying the predictions relating to climate change discussed in this chapter.

CONCLUSION

Lomolino (2001: pg 11) stated that it is essential that researchers “take up the challenge of describing and understanding patterns of biological diversity of mountain ecosystems” as growing human populations dominate lowland regions throughout the world and mountains become the last refugia for threatened and endangered species. A deeper description and understanding of these isolated, untransformed areas forms a baseline to compare future changes that might take place whether directly human-induced or ‘natural’. This study has led to a more comprehensive understanding of aquatic insect diversity in the southern Great Escarpment. The discovery of undescribed species and species never before collected in this area, have contributed to the documentation of these relatively unresearched mountains, and the work has established a foundation for long-term monitoring.

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APPENDIX 1

Table A1. All species collected and the abundance of each for each mountain block. ECD (Eastern Cape Drakensberg), SB (Stormberg), GWA (Great Winterberg-Amathole), SN (Sneeuberg), NB (Nuweveldberge)

Taxon	ECD	SB	GWA	SN	NV	Total
EXOPTERYGOTA						
EPHEMEROPTERA						
Baetidae						
Baetis harrisoni	4		5	3	26	**38**
Afroptilum sudafricanum	221	200	79	27	1	**528**
Demoreptus sp.	135	97	45			**277**
Cloeodes inzingae	2					**2**
Cheleocloeon excisum	60	20	2	2	43	**127**
Cloeon sp.		8		75	30	**113**
Demoulinia crassi	12			3		**15**
Leptophlebiidae						
Adenophlebia sp.	36	50	37	15		**138**
Castanophlebia sp.	31	126	17			**174**
Caenidae						
Caenis sp.	25	26	2	20	5	**78**
Tricorythidae						
Tricorythus discolor	14		5			**19**
ODONATA						
Libellulidae						
Orthetrum sp.		4		13	12	**29**
Aeshnidae						
Aeshna sp.		7		4	6	**17**
Anax sp.		1	1			**2**
Synlestidae						
Chlorolestes sp.	12	9	6	4		**31**
Lestidae						
Lestes sp.		17		5		**22**
Platycnemididae						
Allocnemis leucosticta				1		**1**
PLECOPTERA						
Notonemouridae						
Aphanicercella sp.	1	9				**10**
Aphanicercopsis sp.		20				**20**
HEMIPTERA						
Naucoridae						
Naucoridae species				3		**3**
Macrocoris sp.		1		2	8	**11**
Notonectidae						
Enrthares sp.					2	**2**
Anisops sp.				5		**5**

Taxon	ECD	SB	GWA	SN	NV	Total
Corixidae						
Corixidae species	3			8	2	**13**
Trichocorixa sp.	19	1				**20**
Sigara sp.				6		**6**
Pleidae						
Plea sp.		1		5		**6**
Hebridae						
Hebrus sp.		1				**1**
ENDOPTERYGOTA						
MEGALOPTERA						
Corydalidae						
Corydalidae species	1					**1**
LEPIDOPTERA						
Crambidae						
Nympula sp.		2				**2**
TRICHOPTERA						
Hydropsychidae						
Cheumatopsyche sp.	51	16	8	13		**88**
Hydoptilidae						
Hydroptila sp.	4					**4**
Leptoceridae						
Leptecho scirpii	9					**9**
Athripsodes (Ps group)		15				**15**
Lepidostomatidae						
Goerodes caffrariae	8	15				**23**
Sericostomatidae						
Cheimacheramus sp.		1				**1**
DIPTERA						
Simulidae						
Simulium nigritarse	57	5	20	55	1	**138**
Simulium medusaeforme	1				69	**70**
Simulium dentulosum	34	3			25	**62**
Simulium vorax					1	**1**
Simulium (Kenyae group)				1		**1**
Simulium (Cervicornutum group)	2					**2**
Simuliidae rutherfoordi		4	4			**8**
Simulium debegene	1					**1**
Simulium adersi				39		**39**
Chironomidae						
Chironomidae species	22	42	1	5	2	**72**
Tanypodinae species	4				2	**6**
Archaeochlus sp.	5					**5**
Muscidae						
Muscidae species	1					**1**
Ceratopogonidae						

Taxon	**ECD**	**SB**	**GWA**	**SN**	**NV**	**Total**
Ceratopogonidae species		1				**1**
Ceratopogoninae species		2				**2**
Atrichopogon sp.	3					**3**
Dixidae						
Dixa sp.	5	4	3	1		**13**
Culicidae						
Culicidae species		6				**6**
Anopheles sp.	3	5		5		**13**
Aedes sp.				15		**15**
Culex sp.				7	1	**8**
Psychodidae						
Pericoma sp.		1				**1**
Athericidae						
Athericidae species		20	1			**21**
Tipulidae						
Tipulidae species			1			**1**
Tabanidae						
Tabanidae species				1		**1**
Empididae						
Hemerodromia sp.	1					**1**
COLEOPTERA						
Coleoptera species	1					**1**
Dytiscidae						
Dytiscidae species	7			2		**9**
Hydaticus sp.		3			1	**4**
Laccophilus sp.				14	4	**18**
Hydrovatus sp.		1				**1**
Yola sp.					1	**1**
Gyrinidae						
Dineutus sp.	2	3				**5**
Orectogyrus sp.	12	7	3	2	1	**25**
Hydrophilidae						
Enochrus sp.		1				**1**
Berosus sp.				2		**2**
Laccobius sp.		2		6	1	**9**
Elmidae						
Elmidae species	8	45	1		3	**57**
Scritidae						
Scritidae spiecies	14	26				**40**
Ptilodactylidae						
Anchytarsinae species	2	13				**15**
Hydraenidae						
Hydraenidae sp.	4	1				**5**
Torridincolidae						
Torridincolidae species		1			3	**4**

Taxon	**ECD**	**SB**	**GWA**	**SN**	**NV**	**Total**
Haliplidae						
Haliplidae species					3	**3**
Curculionidae						
Curculionidae species	1			1		**2**
Dryopidae						
Dryopidae species		1		0		**1**
Rapnus sp.		1				**1**
CRUSTACEA: MALACOSTRACA						
AMPHIPODA						
Sternophysingidae						
Sternophysingidae species		6				**6**
MOLLUSCA: GASTROPODA						
VENEROIDA						
Sphaeriidae						
Pisidium sp.		26				**26**
BASOMMATOPHORA						
Ancylidae						
Ferrissia sp.		7				**7**
Burnupia sp.		6		3		**9**
	838	**890**	**241**	**373**	**253**	**2595**

APPENDIX 2

Table A2: List of specimens used for the genetic analyses and their location.

Afroptilum sudafricanum	Latitude	Longitude	***Afroptilum sudafricanum***	Latitude	Longitude
BP1 1	-31,2149	27,6860	GHT KP 64	-33,3513	26,8576
BP1 2	-31,2149	27,6860	GHT KP 65	-33,3513	26,8576
BP1 3	-31,2149	27,6860	GHT KP 66	-33,3513	26,8576
BP4 4	-31,1733	27,9707	GHT KW 67	-33,3493	26,5601
BP4 5	-31,1733	27,9707	GHT KW 68	-33,3493	26,5601
BP4 6	-31,1733	27,9707	GHT KW 69	-33,3493	26,5601
BE1 7	-30,7508	27,5459	GHT KW 70	-33,3493	26,5601
BE1 8	-30,7508	27,5459	GHT KW 71	-33,3493	26,5601
BE1 9	-30,7508	27,5459	GHT PM 72	-33,3697	26,4758
BE2 10	-30,7573	27,5517	GHT PM 73	-33,3697	26,4758
BE2 11	-30,7573	27,5517	GHT PM 74	-33,3697	26,4758
BE2 12	-30,7573	27,5517	GHT PM 75	-33,3697	26,4758
BE3 13	-30,7573	27,5517	GHT PM 76	-33,3697	26,4758
BE3 14	-30,7573	27,5517	KZN KK 81	-29,3377	30,3066
BE3 15	-30,7573	27,5517	KZN KK 82	-29,3377	30,3066
RH1 16	-30,6757	27,8835	KZN KK 83	-29,3377	30,3066
RH1 17	-30,6757	27,8835	KZN KK 90	-29,3377	30,3066
RH1 18	-30,6757	27,8835	KZN KK 91	-29,3377	30,3066
RH2 19	-30,6744	27,9042	KZN LR 201	-29,4918	30,1084
RH2 20	-30,6744	27,9042	KZN LR 84	-29,4918	30,1084
RH2 21	-30,6744	27,9042	KZN LR 85	-29,4918	30,1084
RH3 22	-30,6959	28,1500	KZN LR 86	-29,4918	30,1084
RH3 23	-30,6959	28,1500	KZN LR 98	-29,4918	30,1084
RH3 24	-30,6959	28,1500	KZN UM 96	-29,4770	30,2612
NV1 25	-32,1103	22,5806	MC1 57	-30,6027	28,2163
SN1 26	-32,2297	24,9531	MC1 58	-30,6027	28,2163
SN1 27	-32,2297	24,9531	MC1 59	-30,6027	28,2163
SN2 28	-32,2432	24,9408	MC1 60	-30,6027	28,2163
SN2 31	-32,2432	24,9408	MC1 61	-30,6027	28,2163
KM1 32	-32,1768	24,0161	***Demoreptus capensis***		
KM1 33	-32,1768	24,0161	BP5 140	-31,1723	27,8541
KM1 34	-32,1768	24,0161	BP5 141	-31,1723	27,8541
KM3 35	-32,3534	23,8899	BE1 103	-30,7508	27,5459
KM3 37	-32,3534	23,8899	RH1 109	-30,6757	27,8835
WB2 38	-32,3797	26,3828	RH1 111	-30,6757	27,8835
WB2 39	-32,3797	26,3828	RH1 112	-30,6757	27,8835
WB2 40	-32,3797	26,3828	SN2 123	-32,2432	24,9408
WB1 50	-32,3759	26,3878	SN2 121	-32,2432	24,9408
WB1 51	-32,3759	26,3878	SN2 122	-32,2432	24,9408
WB3 52	-32,5090	26,2606	KM3 128	-32,3534	23,8899
WB3 53	-32,5090	26,2606	KM3 127	-32,3534	23,8899

Afroptilum sudafricanum	Latitude	Longitude	***Demoreptus natalensis***	Latitude	Longitude
WB3 54	-32,5090	26,2606	BP4 136	-31,1733	27,9707
WB3 55	-32,5090	26,2606	BP4 138	-31,1733	27,9707
WB3 56	-32,5090	26,2606	BP4 137	-31,1733	27,9707
EL1 41	-32,5057	26,9000	RH3 116	-30,6959	28,1500
EL1 42	-32,5057	26,9000	WB1 134	-32,3759	26,3878
EL1 43	-32,5057	26,9000	WB1 133	-32,3759	26,3878
SB3 44	-31,1633	26,6017	WB1 135	-32,3759	26,3878
SB3 45	-31,1633	26,6017	SN2 132	-32,2432	24,9408
SB3 46	-31,1633	26,6017	SN2 130	-32,2432	24,9408
SB4 47	-31,4156	26,8424	SN2 131	-32,2432	24,9408
SB4 48	-31,4156	26,8424	KM1 124	-32,1768	24,0161
SB4 49	-31,4156	26,8424	KM1 125	-32,1768	24,0161
GHT CR 78	-33,3493	26,6177	***Demoreptus sp.***		
GHT CR 79	-33,3493	26,6177	BE3 108	-30,7182	27,5395
GHT KP 62	-33,3513	26,8576			
GHT KP 63	-33,3513	26,8576			