HISTORICAL BIOGEOGRAPHY OF THE TRIBE PLATYPLEURINI SCHMIDT, 1918 (HEMIPTERA: CICADIDAE) WITH A FOCUS ON SOUTHERN AFRICA

A thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

at

RHODES UNIVERSITY

by

Benjamin Wills Price

January 2010

Abstract

With our contemporary biota under increasing threat of extinction, it is of interest to understand where, why and how biological diversity is generated. If focussed on appropriate taxa, phylogeographic and phylogenetic studies can assist in the identification of both places and processes central to the origin and maintenance of biological diversity. It is explained why southern Africa presents a perfect test-bed for exploring such mechanisms of diversification and why cicadas (Hemiptera: Cicadidae) have proved very suitable tools for studies of historical biogeography. This study then exemplifies these points by providing the first large-scale investigation of the historical biogeography of the tribe Platypleurini Schmidt, 1918, with emphasis on the genus *Platypleura* Amyot & Seville, 1843 in southern Africa.

Standard methods of DNA sequencing provided data from portions of the mitochondrial small subunit ribosomal 16S RNA (16S) and cytochrome oxidase subunits I (COI) and II (COII); and the nuclear elongation factor 1 alpha (EF-1α) from 400 ethanol-preserved specimens. These data were analysed using standard phylogenetic methods and a time scale of diversification was estimated using a Bayesian framework and both fossil data and DNA substitution rates.

The results showed that the tribe is too recent to be of Gondwanan origin. The lack of monophyly of the genera represented in both Asia and Africa showed that the tribe needs formal taxonomic revision. Diversification of the African platypleurine genera coincides with aridification in the early Oligocene. Dispersal of Asian platypleurine taxa coincides with the meeting of Africa and Eurasia in the mid-Oligocene. Two radiations within African *Platypleura* are hypothesised; one distributed over most of sub-Saharan Africa and the second restricted to southern Africa, with clades restricted within regional biomes.

Within each of the three focal biomes, cryptic taxonomic diversity was confirmed, suggesting that, even in relatively well understood groups such as the southern African platypleurine cicadas, molecular data can identify further diversity. Although each focal taxon was

restricted to non-overlapping biomes, comparison of the three biomes highlighted interactions between palaeoclimates and fixed landscape features (coastal topography, river catchments and escarpments) as causative agents of vicariance, dispersal, extinction and diversification of these volant insects.

The results of using co-distributed species for comparative study cautions against making inferences based on single-taxon datasets and highlights the need to use many, evolutionarily-independent taxa when identifying mechanisms of diversification. The dating analyses imply that within-species lineage diversification occurred overwhelmingly within the Pleistocene, a trend that is being increasingly recognised in print for other biota. Some caveats about using phylogenetic approaches to estimate ancestral areas are illustrated. Several recommendations are made regarding additional taxa and data sources for understanding the origin and maintenance of biological diversity.

Table of Contents

List of Tables

List of Figures

Preface

This thesis comprises six chapters. The first and last chapters provide a brief introduction and general discussion respectively. The four intervening chapters are written as manuscripts for publication resulting in the unavoidable repetition of certain methodological aspects. The publication status of each data chapter is summarized below.

Chapter 2:

Price BW, Barker NP, Villet MH (in prep.) Out of Africa: a molecular phylogeny of the tribe Platypleurini Schmidt, 1918 (Hemiptera: Cicadidae) with a focus on the genus *Platypleura* (Amyot & Seville, 1843) in Africa.
Intended journal: *Molecular Phylogenetics and Evolution*

Chapter 3:

Price BW, Barker NP, Villet MH (2007) Patterns and processes underlying evolutionary significant units in the *Platypleura stridula* L. species complex (Hemiptera: Cicadidae) in the Cape Floristic Region, South Africa. *Molecular Ecology*, **16**, 2574-2588.

Chapter 4:

Price BW, Barker NP, Villet MH (2010) A watershed study on genetic diversity: Phylogenetic analysis of the *Platypleura plumosa* (Hemiptera: Cicadidae) complex reveals catchment-specific lineages. *Molecular Phylogenetics and Evolution*, **54**, 617-626.

Chapter 5:

Price BW, Barker NP, Villet MH (in prep.) From tree to tree: comparative phylogeography of two forest-dwelling cicada lineages in South Africa.
Intended journal: *Molecular Phylogenetics and Evolution*

Acknowledgements

There are many people I would like to thank for their support and encouragement. Firstly, I would like to thank my supervisors Martin Villet and Nigel Barker for their support and guidance. Martin, the countless hours of discussion, at times regarding the research here presented, have shaped not only what questions I will ask but also how I will approach answering these questions in my future research. Your passion for knowledge is truly inspiring. Nigel, your frenetic pace has inspired me to reach my full potential - thank you. I cannot thank you both enough for the time and effort you have devoted to this work.

Louise, if it were not for your academic brilliance and my competitive streak I would not have strived to be my best and I fear this would have resulted in a far shorter university experience so I cannot thank you enough! Your dedication to proof reading and help with formatting this thesis has been far beyond my expectations and I will be eternally grateful.

I would also like to thank my family for their love and support and for encouraging my love for nature from a young age. I hope that by reading my thesis, which is expected of you, you will come to realize my exploits for the last few years, and no, I will not be naming any cicadas after you!

My thanks go to Prof. Chris Simon, Kathy Hill and Dr. Dave Marshall from the University of Connecticut for hosting both Louise and I during my lab visit, and for your help with samples, sequences, primers, and analyses. The Society of Systematic Biologists (SSB) provided funds to cover part of this lab visit for which I am very thankful. My thanks also go to Prof. Doug Downie for always being available when I had a question regarding analyses and primer selection and to Susan Abraham for the design of some of the published figures.

My sincere gratitude goes to the past and present members of the Molecular Ecology and Systematics Group for their camaraderie. Specific thanks must go to: Seranne Howis, for her patience when teaching me how to set up a PCR and load a gel; Syd Ramdhani for being the driving force behind one of my longer field trips; Lyndall Pereira for assistance with sample

sequencing; Garreth Keevey for help with presenting the forest GIS data and finally Peter Teske and Chris Kelly for their help choosing from the rapid radiation of analytical techniques now available.

I would like to apologise for my continual hounding of anyone going anywhere with interesting cicadas and thank the people who managed to collect and generously donated cicada material for this project. There are almost two hundred contributors listed in the appendix (Table A1) who have helped the cicada database grow to over 1500 ethanol-preserved samples, providing material that will sustain this research for many years.

To my friends, thank you for your continual encouragement and for dragging me out of the lab and into the Rat & Parrot often enough to maintain some semblance of sanity!

I would like to express my sincere gratitude to John Gillam (Postgraduate Financial Aid, RU), your friendliness, unending patience and willingness to go beyond the call of duty has facilitated my postgraduate studies at Rhodes University - thank you!

This research would not have been possible without funds provided by the Rhodes University Joint Research Committee (JRC) and National Research Foundation (NRF Grants: 2069059 to Prof. Villet and 2053645 to Prof. Barker). Furthermore, I would like to acknowledge the funding provided to me by the NRF Prestigious, Deutscher Akademischer Austausch Dienst (DAAD), and Andrew Mellon Mentors Scholarships during the period of my research. I would also like to acknowledge the funding provided by Rhodes University, the American Society of Naturalists (ASN) and the South African Biosystematics Initiative (SABI), which enabled the presentation of various aspects of this research at the Evolution 2007 (Christchurch, New Zealand) and Evolution 2008 (Minneapolis, USA) conferences.

Declaration

The following thesis has not been submitted to a university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author unless otherwise stated.

"I keep six honest serving-men:

(They taught me all I knew)

Their names are What and Where and When

And How and Why and Who."

~

Rudyard Kipling

1 Introduction

With the contemporary animal and plant life of the world under increasing threat of extinction for a variety of causes, it is of interest to understand how biological diversity is generated. It is also crucial to know if particular locations and associated conditions are more predisposed to producing diversity, as they should receive preferential protection.

This study concerns the historical biogeography of the platypleurine cicadas of southern Africa, with an emphasis on the genus *Platypleura* Amyot & Seville, 1843. This chapter introduces the discipline, the focal organisms and the study area. In addition, it provides the reasoning behind the selection of the focal organisms and the study area concluding with an outline of the scope and broad aims of this study.

1.1. A brief outline of historical biogeography

At its simplest, biogeography may be defined as "the study of the geographical distributions of organisms" (Crisci, 2001). The discipline has been divided, largely based on spatial and temporal scale, into two sub-disciplines: "ecological biogeography" and "historical biogeography" (*sensu* Myers & Giller, 1988). Ecological biogeography is concerned with ecological processes, which occur over short temporal and small spatial scales; whereas historical biogeography is concerned with evolutionary processes, which occur over long temporal and often large spatial scales (Myers & Giller, 1988; Crisci, 2001). Historical biogeography therefore aims to explain the current distribution of an organism (or group of organisms), using a combination of their known distribution and inferences about the past environment, including past geological and climatic events (Myers & Giller, 1988).

At its most abstract level, biogeography uses three processes to explain the current spatial distributions of organisms: extinction, dispersal and vicariance (Crisci, 2001). While the influence of extinction is widely accepted, the relative contribution of dispersal (the

movement of organisms across a pre-existing barrier and the subsequent formation of a population in isolation from the parent population) and vicariance (the formation of a barrier, fragmenting a single continuous population into two or more isolated populations) has been a subject of intense debate in the literature (see Nelson, 1978; Wiley, 1988; Zink *et al.*, 2000; de Queiroz, 2005).

Historically, the relative emphasis on dispersal and vicariance in explaining disjunct distributions shifted from an initial 'dispersalist' viewpoint based primarily on the concept of a stable earth, to the almost complete rejection of dispersal in favour of vicariance following the acceptance of plate tectonics (Wegener, 1912) and the development of phylogenetic systematics (Hennig, 1966) in the early 1960's and 1970's (de Queiroz, 2005).

More recently, the incorporation of palaeontological data with molecular phylogenetics has enabled the contributions of dispersal and vicariance to be explored in historical biogeography, by allowing the dating of lineage-splitting events. These dates can then be used in combination with relevant palaeoenvironmental data and knowledge of ecology and population genetics to infer the processes of diversification, and to test between competing hypotheses based on dispersal and vicariance. The fine-scale study of the historical and phylogenetic components of the spatial distribution of gene lineages within and among closely related species is termed phylogeography (Avise *et al.*, 1987; Avise, 2001, 2009).

When studied in a comparative context, the genetic structure of co-distributed, but evolutionarily independent populations may reveal processes by which communities are structured (Knowles, 2009). Indeed, if focussed on appropriate taxa, phylogeographic and phylogenetic studies can lead to the identification of places and processes central to the origin and maintenance of biological diversity (Bermingham & Moritz, 1998; Moritz & Faith, 1998; Soltis *et al.*, 2006).

1.2. Invertebrates as tools for historical biogeography

Not all groups are equally suited as tools for biogeographic studies. Groups selected to study the historical biogeography of a region should have properties in common with potential biological indicator species (*sensu* McGeogh, 1998); which tend to have the following properties: (1) sufficiently well understood taxonomy that the biologies of different genomes

are not conflated; (2) sufficient taxonomic diversity to provide adequate replication and precision; (3) clearly defined distribution; (4) endemicity in the region in question to increase precision; (5) well understood biology in relation to the environmental factors in question to facilitate the interpretation of the organism's occurrence; (6) a clear ecological relationship with their habitat, again to aid interpretation; (7) responsiveness to environmental change over relevant distances and time scales; and (8) an ability to survive in small, isolated populations so that the environmental signal they represent is not lost through extinction.

Within the animal kingdom, invertebrates in general, and insects in particular, provide a good starting point to look for suitable taxa when studying the historical biogeography of a particular region as their diversity far outstrips that of vertebrates. Furthermore many invertebrate groups possess high habitat specificity and thus restricted geographical distributions. Endemism is a particularly important criterion determining the precision gained in historical biogeography studies; more precision, but less geographical coverage, may be gained from narrowly endemic taxa. The ectothermic nature of invertebrates means they are more likely to be responsive to the climate than endotherms are. Finally, the small size and high rate of turnover exhibited by invertebrate populations enables these animals to survive in small, isolated populations, thus enabling the continuity of their phylogenetic signal.

1.3. Southern Africa as a laboratory

The academic study of historical biogeography gains much if the focal land area has a well understood geological and palaeoclimatic history with which the processes of genetic diversification can be correlated. In this regard, southern Africa presents a perfect test-bed for exploring the mechanisms of diversification as the region has a reasonably well understood geological and palaeoclimatic history (Partridge & Maud, 1987; McCarthy & Rubidge, 2005). In addition, southern Africa possesses steep topographic, environmental and climatic gradients, which have resulted in the diversification of plants and the recognition of nine floristic biomes with distributions restricted to southern Africa (Mucina & Rutherford, 2006).

1.4. Previous molecular studies of invertebrates in southern Africa

Despite their apparent suitability to studies of historical biogeography, invertebrates have received little attention in southern Africa. Most animal studies in the region have focused on vertebrates, which are not as speciose, naturally distributed or generally as narrowly endemic

as the region's invertebrates. To date, fifteen studies have been published that focus on southern Africa and use molecular data with either a phylogenetic or phylogeographic approach (Table 1.1).

Table 1.1. Previous molecular studies focussing on invertebrates in southern Africa, shown in chronological order of publication.

Focal taxa	Organism	Study	Reference
Potamonautes spp.	Decapods	Phylogenetic	Daniels *et al.*, 2001
Elporia barnardi	Insect	Phylogeographic	Wishart & Hughes, 2001
Potamonautes spp.	Decapods	Phylogenetic	Daniels *et al.*, 2002
Potamonautes perlatus	Decapod	Phylogenetic	Daniels, 2003
Potamonautes spp.	Decapods	Phylogenetic	Daniels *et al.*, 2003
Mantophasmatodea	Insects	Phylogenetic	Klass *et al*., 2003
Platypleura spp.	Insects	Phylogenetic	Villet *et al*., 2004
Mesamphisopus spp.	Isopods	Phylogenetic	Gouws *et al*., 2004
Mesamphisopus spp.	Isopods	Phylogenetic	Gouws *et al*., 2005
Scarabaeus spp.	Insects	Phylogeographic	Sole *et al.*, 2005
Potamonautes spp.	Decapods	Phylogenetic	Daniels *et al.*, 2006
Scarabaeini	Insects	Phylogenetic	Forgie *et al.*, 2006
Mantophasmatodea	Insects	Phylogenetic	Damgaard *et al.*, 2008
Prestonella spp.	Gastropods	Phylogenetic	Herbert & Mitchell, 2009
Natalina spp.	Gastropods	Phylogenetic	Mousalli *et al.*, 2009

Broad biogeographic inferences from these studies have been overlooked by either focussing on higher-order (i.e. family and tribal) relationships or focussing on the taxa in question and not on the region as a whole. Of the fifteen published studies, eight concern aquatic or semi-aquatic taxa. Furthermore, many of these studies have focussed on groups with little taxonomic diversity and highly restricted distributions that lack many of the previously mentioned characteristics desirable in a good biogeographical tool. Nonetheless, these studies have shown that southern Africa has the potential to be a useful natural laboratory for studies of historical biogeography.

1.5. Use of cicadas as biogeographic tools

Cicadas (Hemiptera: Cicadidae) have proved very suitable for studies of historical biogeography, but almost all previous studies using cicadas have focussed on islands such as New Zealand (Buckley *et al.*, 2001, 2002; Arensburger *et al.*, 2004a, 2004b; Buckley *et al.*, 2006; Buckley & Simon, 2007; Marshall *et al.*, 2008; Hill *et al.*, 2009; Marshall *et al.*, 2009)

and the Pacific (de Boer, 1995; de Boer & Duffels, 1996a, 1996b; de Boer, 1997, 1999; Beuk, 2002; Duffels & Turner, 2002; de Jong & de Boer, 2004) and Greek (Pinto-Juma *et al.*, 2009) archipelagos, with few case studies dealing with continental taxa (Marshall & Cooley, 2000; Cooley *et al.*, 2001; Beuk, 2002; Sueur *et al.*, 2007; Pinto-Juma *et al.*, 2008). Cicadas as a group are generally thought to be poorly vagile (de Boer & Duffels, 1996b) and previous studies have shown that even continental cicada fauna can exhibit relatively high rates of endemism (Villet & van Noort, 1999; Beuk, 2002; Villet *et al*., 2004).

Within Africa, the tribe Platypleurini Schmidt, 1918 consists of 38 currently recognized genera (Duffels & van der Laan, 1985) with an African and Asian distribution and an African centre of diversity (Distant, 1906; Metcalf, 1963; Duffels & van der Laan, 1985). The two most diverse genera within the tribe are *Afzeliada* Boulard, 1972 and *Platypleura* Amyot & Seville, 1843. The taxonomy of *Afzeliada* is not well understood, whereas *Platypleura* has been the subject of more taxonomic research (Boulard, 1972; Villet, 1989, 1997) and currently contains 55 known species in Africa. This is probably an underestimate of the diversity within *Platypleura* as only the southern African taxa have received recent attention. Only one study has looked at the diversification of the genus *Platypleura* in southern Africa (Villet *et al.*, 2004), with the resulting inferences hampered by limited taxon sampling (12 of 55 species) and preliminary data (~ 400 bp of mitochondrial DNA).

The genus *Platypleura* presents a particularly useful group of cicadas to explore the historical biogeography of the southern African region as it fulfils many of the criteria previously identified for good biogeographic tools. Of the cicadas in the region, the taxonomy of *Platypleura* is the best-studied (property 1), and the genus contains sufficient diversity (property 2) to explore the processes of lineage diversification and resultant speciation. The distribution of species within southern Africa is well-studied (property 3), based on over 2000 specimen locality records within the genus *Platypleura*. Selected species groups are known to be endemic to specific biomes (property 4), allowing the possibly divergent histories between biomes to be excluded from the analyses. The biology of platypleurine cicadas (property 5) has been investigated in the region (Villet, 1987, 1988, 1992; Sanborn *et al.*, 2003, 2004; Chawanji *et al.*, 2005) and their relationship with host plants (property 6) is known (Villet *et al.*, 2004). The numerous biogeographical studies of cicadas on islands have shown that the group as a whole is responsive to environmental change (property 7) and thus

should be appropriate for a study of continental biogeography. Finally, as the majority of the cicada lifecycle is subterranean, it may act as a buffer to harsh climates, increasing their ability to survive in small, isolated populations during unfavourable climatic periods (property 8). Thus, the platypleurine cicadas of southern Africa present a good opportunity to explore the processes of diversification in this biodiverse region.

1.6. Scope

This thesis is restricted to the tribe Platypleurini as this represents a coherent evolutionary unit, with a well understood taxonomy and biology. Using molecular (DNA sequence) data, the placement of the most speciose genus, *Platypleura*, is investigated with regard to the majority of the remaining platypleurine genera in Africa and Asia. This is followed by three phylogeographic chapters focusing on three separate biome-restricted groups within southern Africa.

1.7. Aims

This thesis aims to understand some of the mechanisms underlying the diversification of invertebrates in southern Africa by using cicadas in the tribe Platypleurini as heuristic tools.

Generally stated, the aims include:

- Placement of the genus *Platypleura* in the tribe Platypleurini
- Confirmation of the monophyly of *Platypleura*
- Investigation of the timing of, and mechanisms underlying, the diversification of *Platypleura* in Africa
- Detailed phylogeographic investigation and comparison of three biome restricted *Platypleura* species groups in southern Africa

Specific hypotheses are outlined within each of the following chapters.

2 Out of Africa: a molecular phylogeny of the tribe Platypleurini Schmidt, 1918 (Hemiptera: Cicadidae) with a focus on the genus *Platypleura* (Amyot & Seville, 1843) in Africa

2.1. Introduction

Previous studies of taxa with an African and Asian distribution often cite two possible biogeographic scenarios, the commonly cited "Out of Africa" (African origin followed by dispersal into Asia) hypothesis and the less commonly cited "Out of India" (Gondwanan origin with dispersal into Asia mediated by rafting on proto-India) hypothesis. "Out of Africa" dispersal is often followed by diversification in Asia and subsequent dispersal "Out of Asia" back into Africa (Folinsbee & Brooks, 2007; Kodandaramaiah & Wahlberg, 2007). It is postulated that the connection of Africa and the Arabian peninsula to Eurasia in the mid-Oligocene (Willis & McElwain, 2002; Gheerbrant & Rage, 2006) facilitated the "geo-dispersal" (*sensu* Lieberman & Eldridge, 1996) of many vertebrate and invertebrate taxa (Mey, 2005; Bossuyt *et al.*, 2006; Folinsbee & Brooks, 2007; Kodandaramaiah & Wahlberg, 2007; Schaefer & Renner, 2008; Aduse-Poku *et al.*, 2009; Kelly *et al.*, 2009; Rasmussen & Cameron, 2010). Furthermore, the connection of Africa to Eurasia resulted in the closing of the Tethys Sea in the mid-Miocene (Hessami *et al.*, 2001), which reorganised oceanic currents and is thought to have thus caused a drastic drop in global temperatures and the aridification of north Africa and the Arabian Peninsula, resulting in disjunct biological distribution patterns involving Africa and Asia (Zachos *et al.*, 2001; Douady *et al.*, 2003; Teske *et al.*, 2007; Aduse-Poku *et al.*, 2009).

The "Out of India" hypothesis is suggested as an explanation for the Asian distribution of otherwise Gondwanan lineages (Karanth, 2006 and references cited therein). It is generally accepted that India and Madagascar started to move away from Africa in the late Jurassic / early Cretaceous (~ 160 mya) period, and that Madagascar split from India in the mid- to late Cretaceous (~ 90 mya; Karanth, 2006; Yoder & Nowak, 2006). This was followed by India colliding with Asia in the Eocene (50 - 35 mya; Karanth, 2006; Aitchison *et al.*, 2007; Ali & Aitchison, 2008), facilitating the transfer of lineages of Gondwanan origin into Asia

(Karanth, 2006). In addition, although Madagascar has been separated from Africa for 160 million years, its proximity to the East African coast is thought to have allowed faunal interchange for 20 million years (Yoder & Nowak, 2006).

A model taxon for exploring the significance of the "Out of Africa", "Out of Asia" and "Out of India" hypotheses in explaining the diversity of modern animal groups is provided by the platypleurine cicadas, which occur in Africa, Madagascar and Asia. Cicadas (Hemiptera: Cicadidae) are an enigmatic group of insects with a global distribution, widely known for their loud songs used in mate attraction. The taxonomic history of this group has included several major revisions, a summary of which is included in the most recent revision (Moulds, 2005). The family currently consists of 21 tribes in three subfamilies: Cicadettinae, Cicadinae and Tibicininae (Moulds' (2005) "Tettigadinae"). The tribe Platypleurini (Cicadinae) consists of 38 currently recognized genera (Duffels & van der Laan, 1985) with an African and Asian distribution and an African centre of diversity (Distant, 1906; Metcalf, 1963; Duffels & van der Laan, 1985). Current understanding of the origins of the tribe Platypleurini are based on current distribution patterns of species within Platypleurini and its closely related tribes Cryptotympanini and Thophini (Moulds, 2005). Morphological data suggest a sister group relationship between the Platypleurini (currently with an African and Asian distribution) and Thophini (currently with an Australian distribution), with the Cryptotympanini (currently with an Australian and Holarctic distribution) and then the Cyclochilini (currently with Australian distribution) basal to them (Moulds, 2005). This combination of current distributions suggests a Gondwanan origin for these tribes, with subsequent dispersal of the Cryptotympanini into Asia.

Within the Platypleurini, the most widespread (Distant, 1882) and speciose genus, *Platypleura* Amyot & Seville, 1843, was most recently revised by Boulard (1972), who confined his attention to the African taxa. Morphological conservatism and convergence within *Platypleura* has resulted in very few characters with which to infer species' relationships, resulting in the need for additional data sources. The first molecular phylogeny involving the genus (Villet *et al.*, 2004) suggested that the South African *Platypleura* cicadas might have an East African origin with dispersal and speciation in a southward and westward direction. The ancestral plant association was hypothesised to be with *Acacia* species, with speciation sporadically associated with host plant shifts (Villet *et al.*, 2004). However, these

inferences were based on limited taxon sampling (12 [22 %] of 55 taxa) and only a 400 bp portion of one mitochondrial gene.

The aim of this study was to investigate the biogeographic history of the tribe Platypleurini. This enabled several hypotheses to be tested: (1) The tribes Platypleurini and Cryptotympanini are of Gondwanan origin; (2) Asian platypleurine taxa resulted from dispersal from Africa that post-dates the meeting of Africa and Eurasia in the mid-Oligocene ("Out of Africa"; cf. Kodandaramaiah & Wahlberg, 2007); (3) Platypleurine genera containing both Asian and African representatives (*Platypleura*, *Pycna*, *Oxypleura*) do not represent monophyletic assemblages; (4) Forest-associated platypleurine lineages predate the aridification of Africa; (5) Madagascan platypleurine taxa (genus *Yanga*) are a result of recent dispersal from Africa, and not Gondwanan vicariance (cf. Yoder & Nowak, 2006); (6) The African members of *Platypleura* originated in the north-eastern Africa, with a southward radiation (Villet *et al.*, 2004).

2.2. Methods

2.2.1. Sampling and laboratory protocols

A total of 32 (84 %) of the 38 currently recognised genera in the Platypleurini were included, using type species to represent genera where possible (Table A2). The genus *Platypleura* contains 102 known species (55 African; 47 Asian); of these, 38 African species (20 described; 18 awaiting description) and 8 Asian species (all described) were included. Samples were not available for 17 African and 39 Asian species of *Platypleura*. In addition to *Platypleura* five genera of platypleurine cicadas occur within Asia: *Suisha*, *Oxypleura*, *Pycna*, *Kalabita* and *Hainanosemia*, samples of which were available for representative species of the first three (Table A2). The genera *Chremistica*, *Cryptotympana* and *Lyristes* (from the closely related tribe Cryptotympanini: Moulds, 2005), *Orapa* (Orapini), *Hamza* (Hamzini) and *Oncotympana* (Oncotympanini) were included in the analyses to test the monophyly of the Platypleurini. Sequences representing *Oncotympana*, *Chremistica* and *Platypleura takasagona* were provided by Professor Chris Simon (University of Connecticut, USA) and are denoted 'CS' (Table A2). Outgroups were chosen to enable fossil calibrations in the dating analyses using the genera *Meimuna* and *Cicada* (Cicadini; Table A2).

Cicadas were collected and preserved in 95 % ethanol. Total genomic DNA was extracted from the wing or tymbal muscle tissue following the Chelex® 100 protocol (Walsh *et al.*, 1991). Small pieces of tissue (c. 2 mm^3) were homogenised in 300 µl 5 % Chelex® 100 solution and incubated in a heating block at 105 °C for 15 minutes, vortexing the samples every five minutes. Following the incubation period, the supernatant was removed for subsequent use in PCR amplifications.

Portions of three mitochondrial and one nuclear gene were sequenced from each sample. The mitochondrial genes used were the small subunit ribosomal 16S RNA (16S) and the Cytochrome Oxidase subunits I (COI) and II (COII). The nuclear data derived from a portion of elongation factor 1 alpha (EF-1α) encoding exons 3, 4 and 5 and the two associated introns (Table A2). Primers used for amplification and sequencing reactions were 16S: 16Sar with 16Sbr (Palumbi *et al.*, 1991); COI: COI-F2 (Price *et al.*, 2010) with C1-N-2568 (Brady *et al.*, 2000); COII: TL2-J-3033 with TK-N-3786 (Simon *et al.*, 1994); EF-1α: EF1-F650-mod (Arensburger *et al.*, 2004a) with EF1-N-1419 (Sueur *et al.*, 2007).

PCR amplifications were performed in 25 - 50 µl reactions using the following protocol: 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 48 °C (16S, COI), 51 °C (COII) or 58 °C (EF-1α) for 45 seconds and extension at 72 °C for two minutes. PCR products were confirmed by electrophoresis of 5 µl PCR product stained with ethidium bromide and 5µl tracking dye in a 1 % agarose gel, and visualized using a UV trans-illuminator.

PCR products were purified using the Invitek Invisorb MSB® Spin PCRapace purification kit (Invitek, Germany) and sequenced in both directions. The sequencing reactions were carried out using the ABI Big Dye Sequencing kit v.3.1, according to the manufacturer's instructions. Sequence trace files were generated using an ABI 3100 genetic analyzer sited at Rhodes University. Trace files were checked and edited using Genestudio Pro v.1.0 (GeneStudio, Inc.). The sequence data were imported into MEGA v.4 (Tamura *et al.*, 2007), aligned using the Clustal W algorithm (Thompson *et al.*, 1994) and the alignments were then checked manually.

2.2.2. Phylogenetic analysis

Homogeneity in the different genes was assessed using the Incongruence Length Difference (partition homogeneity) test (Farris *et al.*, 1994) with 1000 replicates, conducted in PAUP* v.4.0b10 (Swofford, 2003) with invariant characters removed (Cunningham, 1997) before being combined into one dataset. In addition the combined mtDNA data were analysed separately from the nDNA data and the resulting trees were inspected visually for (supported) incongruence. The presence of multiple substitutions (saturation) was investigated using transition and transversion plots against F84 distance in DAMBE v.5.1 (Xia & Xie, 2001).

Maximum Parsimony analysis (MP) was conducted using PAUP* as follows: a simple heuristic search with TBR branch-swapping enforced was conducted to approximate the length of the shortest tree. Following this a heuristic search with 1000 random addition replicates was conducted, starting from random trees and keeping a single tree less than or equal to the shortest tree, for each replicate. If the second search found shorter trees the process was repeated until no shorter trees were found. A strict consensus tree was then generated from the resulting parsimony trees. Confidence in each node was assessed with 1000 full heuristic bootstrap replicates in PAUP*.

The most appropriate model of sequence evolution for the Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were selected for each of the data partitions using the AIC test (Akaike, 1974) as implemented in Modeltest v3.7 (Posada & Crandall, 1998; ML - one partition) and MrModeltest v.2.2 (Nylander, 2004; MB - 11 partitions); models selected are summarised in Table 2.1.

Partitioned Bayesian Inference analyses were conducted using MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001) carried out on the University of Oslo Bioportal facility (www.bioportal.uio.no). Although there is no definitive method for partitioning a dataset, the datasets were partitioned first by gene, then by coding properties (introns or exons). In addition, all protein-coding regions were partitioned by codon position to incorporate rate variation (Marshall *et al.*, 2006). This resulted in three partitioning strategies: no partition; each gene (5 partitions); each gene and each codon (11 partitions). The effect of partitioning strategy was estimated using pairwise comparison of Bayes Factors (*sensu* Nylander *et al.*, 2004, Brandley *et al.*, 2005) with $2\ln BF_{A\text{-}B} \geq 10$ indicative of strong support for partitioning

strategy A over strategy B, following Kass and Raftery (1995). Each analysis comprised four independent runs of 10 million generations each, using random starting trees with four chains (one cold, three hot), sampling every 1000 generations. Model parameters for each partition as selected by MrModeltest (Table 2.1) were achieved using the "Lset" and "Prset" commands. An initial branch length prior was set using the "brlenspr = Unconstrained : Exponential (500.0)" command. All other parameters (excluding branch lengths and topology) were unlinked across partitions using the "unlink" command. Stationarity in each analysis was assessed using the potential scale reduction factor (PSRF) data and plots of likelihood scores, tree length and average standard deviation of split frequencies against generation. These plots showed that the analyses reached stationarity well within the first 1 million generations. Thus the first 1000 trees generated in each analysis were discarded as "burn-in", ensuring that only trees generated at stationarity were used to calculate the posterior probabilities.

Maximum Likelihood (ML) analysis was undertaken using the model parameters highlighted in Modeltest (Table 2.1) by using the "Lset" command in PAUP*. Confidence in each node was obtained using RAxML v.7.0.4 (Stamatakis, 2006) with 100 bootstrap replicates using the computational resources of the CIPRES portal (http://8ball.sdsc.edu:8889/cipres-web/Bootstrap.do). To ascertain whether the current taxonomic understanding of *Platypleura* accurately reflected the most likely topology separate likelihood analyses were performed in PAUP* with constraint trees enforced corresponding to the monophyly of all sampled members of *Platypleura* and the monophyly of the sampled African members of *Platypleura*. To remove the confounding effect of *Azanicada zuluensis* being nested within the African *Platypleura* as indicated by the preliminary analyses, the two constraint analyses were repeated including *A. zuluensis* within the genus *Platypleura*. The effect of constraining monophyly was examined by comparing the most likely topology with no constraints against the most likely constrained topology using the Shimodaira-Hasegawa (SH) test (Shimodaira & Hasegawa, 1999) with RELL optimization and 1000 bootstrap replicates. The results of the SH test were then confirmed with a likelihood ratio test (LRT) using a Chi-squared test of likelihood scores (Felsenstein, 1981).

2.2.3. Molecular dating

Although there are no fossil data present for the tribe Platypleurini, there are fossils in the tribe Cicadini ascribed to the genera *Meimuna* [23.8 - 7.1 mya] (Zhang & Zhang, 1990; Zhang *et al.*, 1994) and *Cicada* [23.8 - 16.4 mya] (Heer, 1853). The minimum age for these two genera was then set to the more precise of these two dates (23.8 - 16.4 mya) using both a uniform prior (upper bound = 23.8 mya; lower bound = 16.4 mya) and a normal prior (mean = 20.1 mya; sd = 2.25 myr).

The age of each node and its corresponding 95 % confidence interval were then estimated under the gene-specific models (Table 2.1) using an uncorrelated lognormal (UCLN) relaxed clock in BEAST v.1.5.2 (Drummond & Rambaut, 2007). The analysis used a Yule speciation prior and random starting trees. Each of the two analyses were run using the resources of the Computational Biology Service Unit, Cornell University (http://cbsuapps.tc.cornell.edu), with one run of 20 million generations, following a discarded burn-in of 0.2 million generations. Stationarity and the estimation of the effective sample size (ESS) in the analysis were confirmed by inspection of the MCMC samples using Tracer v.1.5 (Rambaut & Drummond, 2007). Each run was summarized in TreeAnnotater v.1.5.2 and viewed using FigTree v.1.2.3, both part of the BEAST package.

2.2.4. Rates of diversification within the African Platypleura

To visualise the temporal pattern of diversification within the African members of *Platypleura*, a lineage-through-time (LTT) plot, corresponding to the logarithm of the number of lineages plotted against time, was constructed using Tracer.

2.3. Results

2.3.1. Data characteristics

The final molecular data sets comprised 2610 bp, of which 1921 bp are mitochondrial data. The EF-1α data (689 bp) were made up of portions of both exons (3, 4 and 5) and associated introns. All coding portions of the gene datasets aligned readily, while gaps corresponding to insertion or deletion events were included in the 16S and EF-1α intron datasets. The initial 16S alignment was reduced from 497 bp to 350 bp to exclude A/T rich regions which could not be aligned unambiguously. Data characteristics and model selection for each partition are

summarised in Table 2.1. The ILD tests indicated that the data sets were not significantly incongruent (p = 0.24), so the individual gene datasets were combined for analysis.

Table 2.1. Data characteristics showing data partitioning and model choice.

Partition	No. Sites	No. Variable	No. Pars Info	% Pars Info	Model
16S	350	137	101	28.8	GTR+I+G
CO1	938	447	383	40.8	GTR+I+G
CO1_1	313	102	78	24.9	GTR+I+G
CO1_2	313	46	23	7.3	GTR+I+G
CO1_3	312	301	282	90.3	GTR+I+G
CO2	633	367	314	49.6	GTR+I+G
CO2_1	211	104	90	42.6	GTR+I+G
CO2_2	211	59	34	16.1	GTR+I+G
CO2_3	211	198	190	90	GTR+G
EF-1α	689	334	207	30	GTR+I+G
EF-1α_EX	422	128	61	14.4	GTR+I+G
EF-1α_EX_1	141	18	3	2.1	GTR
EF-1α_EX_2	141	15	2	1.4	HKY
EF-1α_EX_3	140	89	55	39.2	HKY+G
EF-1α_IN	267	206	146	54.6	GTR+I+G
TOTAL	2610	1285	1005	38.5	GTR+I+G

2.3.2. Phylogenetic analysis

Parsimony analysis of the data yielded 225 most-parsimonious trees with a tree length of 6585 steps (CI = 0.265; RI = 0.460). Likelihood analysis of the data resulted in one most-likely tree. Bayes Factor comparison of the three partitioning strategies suggested that partitioning data by gene provided a significantly better log-likelihood than not partitioning them ($2\ln BF_{1\text{-}5}$ = 13.4). However, partitioning the data by gene and codon provided a significantly better log-likelihood than either not partitioning ($2\ln BF_{1\text{-}11}$ = 14.9) or partitioning by gene alone ($2\ln BF_{5\text{-}11}$ = 13.7), so the BI analysis using data partitioned by both gene and codon is presented (Figure 2.1). As all three analyses returned highly congruent topologies, only the BI topology with corresponding BI and ML nodal support values is shown (Figure 2.1). Although many nodes lack substantial support, the congruent topologies of the MP, ML and BI analyses suggest that long-branch attraction is not prevalent in this dataset, providing a first estimate of the relationships within the Platypleurini.

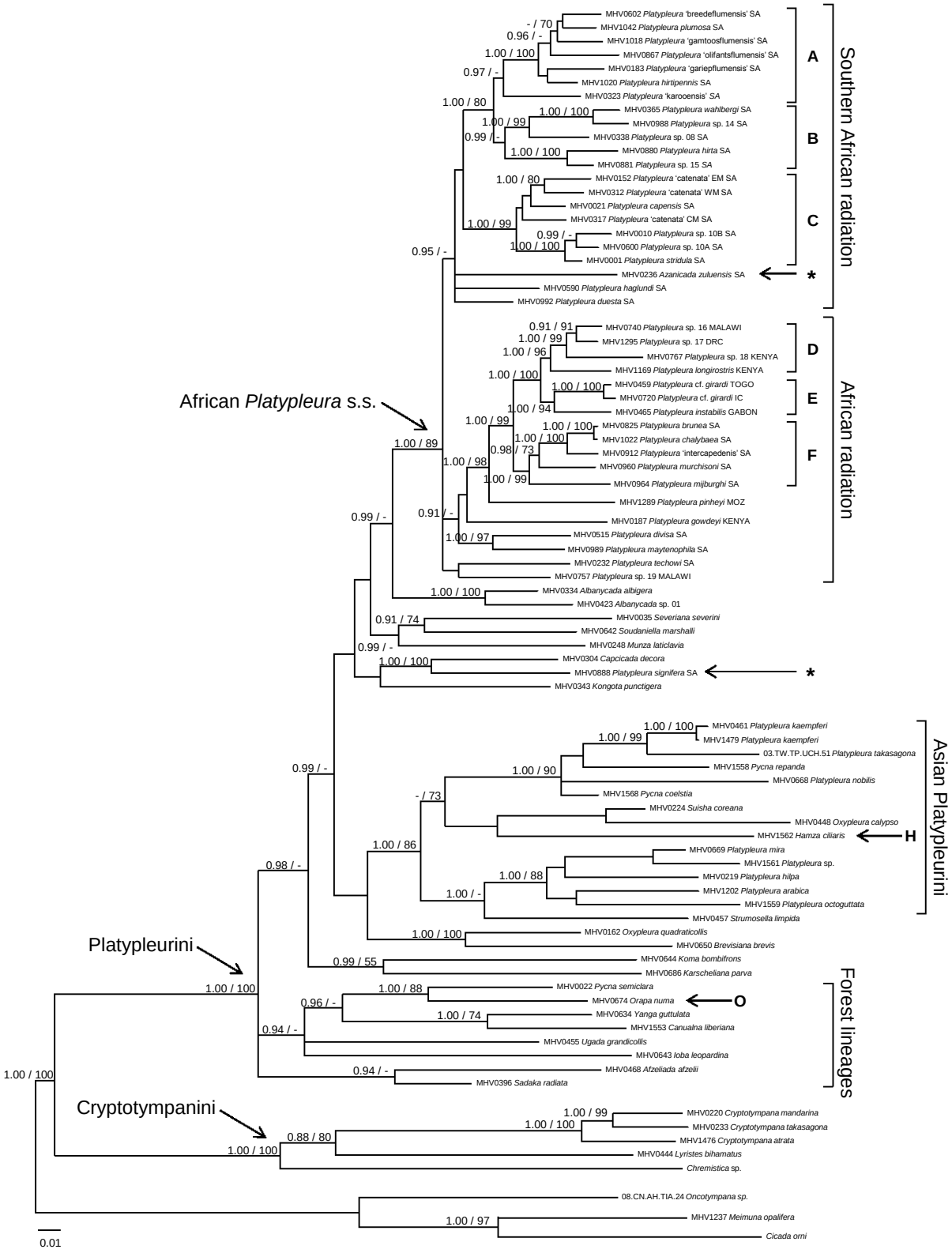

Figure 2.1. Majority rule Bayesian phylogram with posterior probability (≥ 0.95) and ML bootstrap (≥ 70 %) indicated on nodes. Discrepancies in the placement of *Azanicada* and *P. signifera* with regard to *Platypleura* s.s. are highlighted using an asterisk. The localities of African representatives of *Platypleura* are coded by country (DRC: Democratic Republic of the Congo; IC: Ivory Coast; MOZ: Mozambique; SA: South Africa). Labelled clades (A - F) are discussed in the text. The position of *Hamza* (H) and *Orapa* (O) are indicated.

Plots of transition and transversion showed substitution saturation was not present in 16S or EF-1α gene, but codon-based plots suggested substitution saturation of 3rd base positions in COI and COII genes and EF-1α exons (data not shown). This suggests a slower evolving gene, less prone to saturation at the tribal level, may be required to strengthen support for deeper nodes. The tribes Platypleurini, Orapini and Hamzini form a well supported clade, sister to the Cryptotympanini, although this relationship cannot be confirmed without inclusion of the remainder of the recognised tribes in the Cicadinae *sensu* Moulds (2005). *Orapa numa* (Distant, 1907), representing the African tribe Orapini Boulard, is sister to *Pycna semiclara* (Germar, 1834) and nested within the Platypleurini. *Hamza ciliaris* (Linneaus, 1758), representing the tribe Hamzini is nested within the Asian Platypleurini (Figure 2.1).

The Asian members of the Platypleurini form a monophyletic assemblage with the inclusion of the African taxon *Strumosella limpida* (Karsch, 1890) with moderate support (Figure 2.1). The Asian taxa are deeply nested within the African genera, and the African and Asian species of the genera *Pycna*, *Oxypleura* and *Platypleura* do not form monophyletic clades (Figure 2.1).

The African members of *Platypleura* do not form a monophyletic assemblage due to the nesting of *Azanicada zuluensis* (Villet, 1987) within *Platypleura*, and the strongly supported placement of *P. signifera* (Walker, 1850) as sister to *Capcicada decora* (Germar, 1834). The likelihood constraint analyses (Table 2.2) show that constraining either all members of the genus *Platypleura* or only the African members of the genus *Platypleura* to be monophyletic had a significantly negative effect on the log-likelihood of the likelihood constraint analyses.

Including *A. zuluensis* as a member of the genus *Platypleura* improved the log-likelihood of the constraint analyses; however these constraint analyses were still significantly worse than the most likely topology (Table 2.2). This is a result of *P. signifera* and the Asian members of *Platypleura* being more closely related to platypleurine genera other than *Platypleura* s.s. (Figure 2.1). The likelihood ratio tests confirmed the results of the SH tests and are not presented.

Table 2.2. Summary of likelihood constraint analyses and corresponding probability values derived from the SH test. Significant values are in bold.

Monophyly Constraint	No. trees	-ln L	probability (p)
ML (no constraints)	1	35188.49992	-
African *Platypleura*	1	35254.77937	**0.00**
African *Platypleura* and *A. zuluensis*	1	35217.69545	**0.02**
All *Platypleura*	1	35459.10517	**0.00**
All *Platypleura* and *A. zuluensis*	1	35411.34583	**0.00**

Within the African members of *Platypleura* s.s. there are two major lineages, one exclusively southern African and the second distributed throughout the savannah and forest regions of Africa (Figure 2.1). The southern African lineage is subdivided into three supported clades corresponding to the Karoo (clade A), Grassland (clade B) and Fynbos (clade C) biomes (Figure 2.1). The African lineage is subdivided into three well supported clades corresponding to a central and eastern African distribution (clade D), sister to the west African group (clade E). Basal to these northern clades is a clade restricted to the forest biome of South Africa (clade F; Figure 2.1).

2.3.3. Molecular dating - Platypleurini

Using a normal or a uniform prior on the dating analysis estimates did not noticeably affect the mean TMRCA values of each node; as a result only the chronogram estimated using the normal prior is shown (Figure 2.2). Although the wide 95 % confidence intervals inherent with this dating analysis preclude decisive dating of divergence, the fossil-calibrated results provide a plausible first estimate.

The common ancestor for all sampled cicadas implies a post-KT origin of the subfamily Cicadinae (~ 60 mya; Figure 2.2), although the wide error bars and numerous excluded tribes (particularly the Thophini) prevent further analysis. The divergence between the tribes Platypleurini and Cryptotympanini is estimated to the early Eocene (~ 51 mya) with subsequent diversification within both tribes at the start of the Oligocene (~ 34 mya; Figure 2.2).

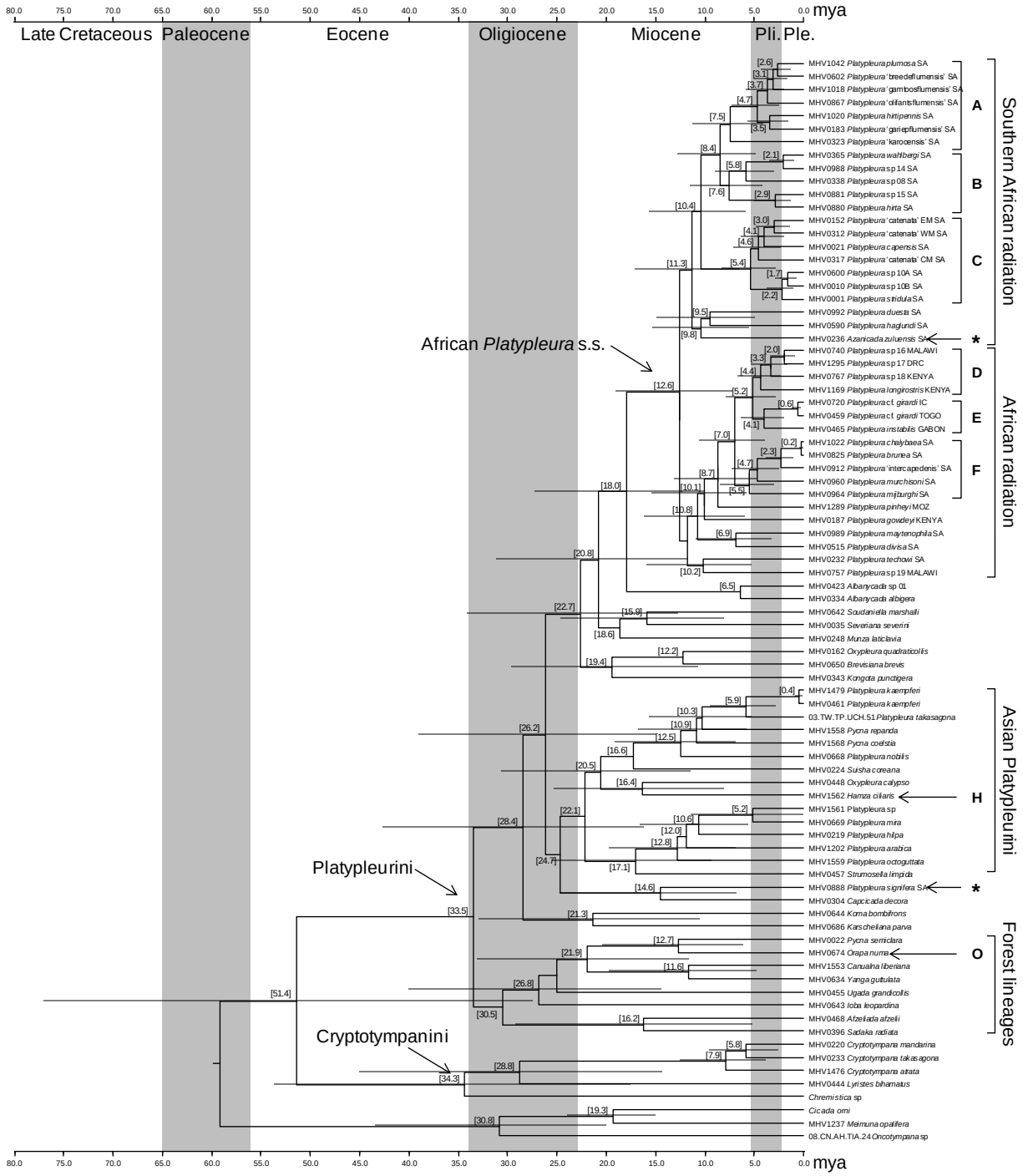

Figure 2.2. Majority rule Bayesian chronogram generated in BEAST. Numbers above nodes indicate [age in myr]. Nodes are centred on the mean TMRCA with shaded bars indicating the distribution of the 95 % HPD for each estimate, only those discussed in the text are shown. Discrepancies in the placement of *Azanicada* and *P. signifera* with regard to *Platypleura* s.s. are highlighted using an asterisk. The localities of African representatives of *Platypleura* are coded by country country (DRC: Democratic Republic of the Congo; IC: Ivory Coast; MOZ: Mozambique; SA: South Africa). Labelled clades (A - F) are discussed in the text. The position of *Hamza* (H) and *Orapa* (O) are indicated.

The forest-associated clade within the Platypleurini, consisting of large-bodied cicadas, widely distributed over sub-Saharan Africa, associated with forest and woodland habitats, diverged from a common ancestor in the early Oligocene (~ 31 mya). The Asian taxa apparently diverged from the main body of African taxa in the late Oligocene (~ 25 mya) with subsequent mid-Miocene (~ 17 mya) "out of Asia" dispersal of the ancestor to *Strumosella* back into Africa. The genus *Platypleura* s.s. is estimated to have diverged from its sister genus, *Albanycada*, in the early Miocene (~ 18 mya), with subsequent diversification of *Platypleura* in the mid-Miocene (~ 12 - 9 mya; Figures 2.2 & 2.3 B). The Madagascan cicada *Yanga gutullata* shares a common ancestor with the monotypic West African genus *Canualna* dated to the mid-Miocene (~ 11 mya).

2.3.4. Molecular dating and rates of diversification within African Platypleura

That the diversification of the genus *Platypleura* s.s. does not follow a constant rate for diversification is shown by the discrepancy between the LTT plot of log-lineages against time and a hypothetical constant rate (dashed line; Figure 2.3 A). This result is due to rapid initial diversification in the mid-Miocene (~ 12 - 9 mya; dotted line in Figure 2.3 A), followed by an extended period of lineage accumulation at a reduced but consistant rate into the mid-Pliocene (~ 4 mya). The rate of lineage diversification decreases substantially from the mid-Pliocene onwards (Figure 2.3 A). This may be due to a combination of three factors: reduced rates of speciation, high rates of lineage extinction, and poor taxon sampling. Although the vast majority of South African taxa have been included in the analysis, the true number of species within *Platypleura* in the remainder of Africa is unknown, thus insufficient taxon sampling cannot be discounted.

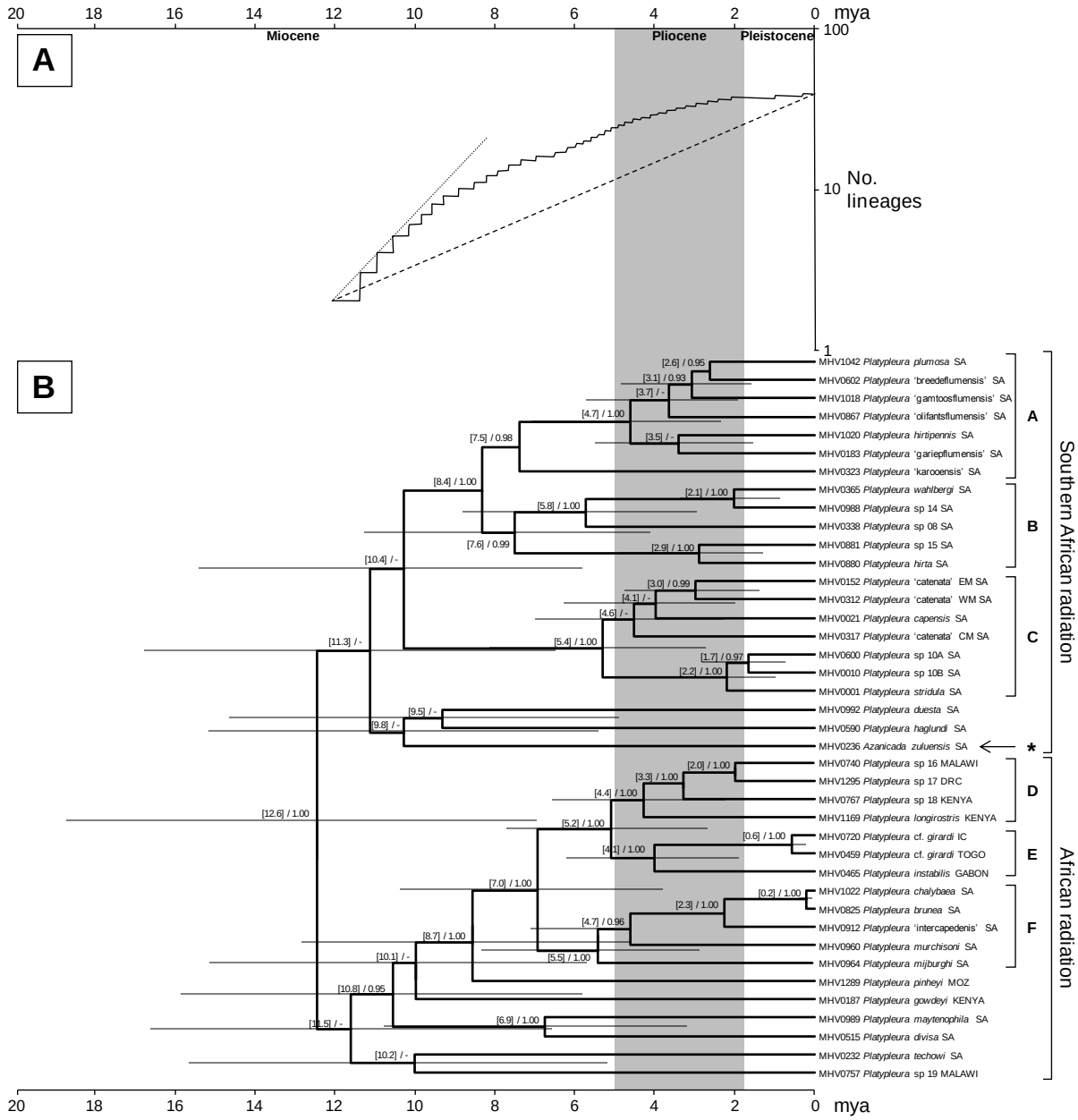

Figure 2.3. (A) Lineage-through-time plot showing theoretical constant rate (dashed line) and initial rate (dotted line) of diversification; (B) Majority rule Bayesian chronogram of African representatives of *Platypleura* s.s generated in BEAST. Numbers above nodes indicate [age in myr] and posterior probability support. Nodes are centred on the mean TMRCA with shaded bars indicating the distribution of the 95 % HPD for each estimate. Discrepancies in the placement of *Azanicada* with regard to *Platypleura* s.s. is highlighted using an asterisk. The localities of African representatives of *Platypleura* are coded by country country (DRC: Democratic Republic of the Congo; IC: Ivory Coast; MOZ: Mozambique; SA: South Africa). Labelled clades (A - F) are discussed in the text.

2.4. Discussion

The comprehensive representation of platypleurine taxa in this analysis allows a robust assessment of the monophyly of the Platypleurini (Figure 2.1), which is upheld provided that the tribes Orapini and Hamzini are subsumed within it. The general appearance of *Orapa* (five species) and *Hamza* (one species) is essentially platypleurine, with extensively pigmented, camouflaged wings, the abdomen as long as the head and thorax together, and the proximal profemoral spine inclined against the femur. Because the type species of all three tribes are included in the analysis, the formal publication of the following synonyms is suggested:

Hamzini Distant 1905

= Platypleurini Schmidt 1918 **syn. nov.**

= Orapini Boulard 1985 **syn. nov.**

For the purposes of this thesis the tribe Platypleurini is still recognised, pending formal publication. Although less well sampled, the Cryptotympanini were also found to be monophyletic and sister to Platypleurini as suggested by cladistic analysis of their morphology (Moulds, 2005).

Hypothesis 1: The tribes Platypleurini and Cryptotympanini are of Gondwanan origin.

Although based on a single calibration involving two fossils, the results of the preliminary dating analyses suggest that the tribe Platypleurini diverged from the Cryptotympanini in the early Eocene (51 mya), with rapid diversification of both tribes at the Eocene - Oligocene boundary (~ 34 mya; Figure 2.2). The break up of Gondwana began in the middle Jurassic (~ 170 mya; Ali & Aitchison, 2008), thus the divergence between the Platypleurini and Cryptotympanini is too recent to be suggestive of Gondwanan vicariance driving differentiation between these two tribes.

Hypothesis 2: Asian platypleurine taxa resulted from dispersal from Africa that post-dates the meeting of Africa and Eurasia in the mid-Oligocene ("Out of Africa").

The Asian fauna are well supported as being nested between the African genera (Figure 2.1), confirming an African origin of the tribe, with an estimated divergence of the African and Asian fauna from a common ancestor in the late Oligocene (~ 25 mya; Figure 2.2). This period was characterised by the closure of the Tethys Sea and the meeting of the African and Eurasian land masses (Hessami *et al.*, 2001; Willis & McElwain, 2002; Gheerbrant & Rage,

2006), so it is likely that the Asian platypleurine fauna arose from dispersal into Eurasia post-dating the meeting of Africa and Eurasia, and is yet another example of "Out of Africa" geo-dispersal *sensu* Lieberman & Eldridge (1996). The monophyletic origin of the Asian taxa (Figure 2.1) suggests a single dispersal event from Africa resulted in the Asian platypleurine cicada fauna, however this inference is based on only four of the six Asian genera and excludes a large proportion of Asian species within *Platypleura*, and is necessarily tentative.

It has been further suggested that "Out of Africa" dispersal is often followed by diversification in Asia and subsequent dispersal "Out of Asia" back into Africa (Folinsbee & Brooks, 2007; Kodandaramaiah & Wahlberg, 2007); this view is supported in the Platypleurini by the nested position of *Strumosella limpida* (Karsch, 1890), a West African species, within the distinctly Asian clade, with a estimated date of divergence from the Asian species in the early Miocene (~ 17 mya). This relationship is present in all three analyses but is only well supported in the BI analyses. This suggests that cicada faunal exchange between Asia and Africa occurred in both directions within the late Oligocene and early Miocene (~ 25 - 17 mya), after the connection of the two landmasses (Willis & McElwain, 2002; Gheerbrant & Rage, 2006), but before the aridification of the Sahara and Arabian peninsula (~ 17 mya: Douady *et al.*, 2003).

Hypothesis 3: Platypleurine genera containing both Asian and African representatives (Platypleura, Pycna, Oxypleura) do not represent monophyletic assemblages.

This study represents a first estimate of the phylogeny of the tribe Platypleurini and the results suggest that the taxonomy of the Asian Platypleurini requires review. The genera *Platypleura*, *Oxypleura* and *Pycna* do not form reciprocally monophyletic lineages (Figure 2.1). With regard to *Platypleura*, the type species, *P. stridula*, is included in this study and falls within the clade of [African *Platypleura* + *Azanicada*], so that the Asian taxa currently assigned to *Platypleura* are misplaced and require removal. Several (currently synonymised) candidate genera and sub-genera based on Asian species are available to receive these cicadas, but this is beyond the scope of this study and requires a more complete sample of Asian species and further molecular and morphological attention. The African species *P. signifera* Walker, 1850 forms a well supported sister relationship with *Capcicada decora* Germar, 1834, which is confirmed by gross morphology and suggests that the former should be excluded from *Platypleura* s.s.

The type of *Oxypleura* is *O. clara* Amyot & Serville, 1843, an African species clearly related to *O. quadraticollis*. Although confident that the results presented warrant the removal of *O. calypso* Kirby, 1889 from *Oxypleura*, the results are necessarily tentative regarding where it should be placed.

The type species of *Pycna* is *P. strix* Amyot & Serville, 1843. This Madagascan cicada is physically distinctive and, based on morphology alone, cannot be confidently grouped with the described African or Asian *Pycna* species, suggesting that both the African and Asian cicadas in *Pycna* will need to be taxonomically revised once a molecular and morphological analysis of the type species is completed.

Within *Platypleura* s.s. the following taxonomic changes require formal publication:

Platypleura Amyot & Serville, 1843

= *Azanicada* Villet, 1989 **syn. nov.**

Platypleura zuluensis Villet, 1987

= *Azanicada zuluensis* (Villet, 1989)

Capcicada Villet, 1989

Capcicada signifera (Walker, 1850) **comb. nov.**

= *Platypleura signifera* (Walker, 1850)

Hypothesis 4: Forest-associated platypleurine lineages predate the aridification of Africa.

The forest-associated lineages (*Ugada*, *Yanga*, *Pycna*, *Orapa*, *Canualna* and *Sadaka*) within the tribe are distinct from the remainder of the tribe and when combined with the forest habitat of cryptotympanine cicadas, it is not unreasonable to assume that the ancestral association of platypleurine cicadas was with forest habitats, which are understood to have been the dominant African habitat in the early Eocene (Jacobs, 2004; Aduse-Poku *et al.*, 2009). The diversification of the Platypleurini from the early Oligocene can thus be correlated with the fragmentation of widespread forest habitat in Africa, primarily due to dramatic global cooling associated with the formation of a permanent ice sheet in Antarctica (Couvreur *et al.*, 2008; Wahlberg *et al.*, 2009). Aridification and the dynamic nature of forests has been suggested as a possible cause for a number of lineage diversification events in the Oligocene and Miocene periods in Africa, including trees (Couvreur *et al.*, 2008), ctenoplectrine bees (Schaefer & Renner, 2008), nymphalid butterflies (Wahlberg *et al.*,

2009), rhytidid snails (Moussalli *et al.*, 2009), *Hyperolius* frogs (Wieczorek *et al.*, 2000), birds (Roy *et al.*, 2001) and various mammal lineages (Moritz *et al.*, 2000; Mayaux *et al.*, 2004; Willows-Munro & Matthee, 2009).

Hypothesis 5: Madagascan platypleurine taxa (genus Yanga) are a result of recent dispersal from Africa, and not Gondwanan vicariance (cf. Yoder & Nowak, 2006).

Although only represented by a single species in this dataset, the genus *Yanga* Distant is sister to *Canualna* Boulard, a monotypic genus found in West Africa and Annobon island. This relationship is well supported (Figure 2.1), suggesting a common ancestor in the mid-Miocene (~ 11 mya; Figure 2.2). Although the limitations of the dating analysis are recognised, the estimated date of divergence is an order of magnitude smaller than required if this divergence is to be suggestive of Gondwanan vicariance, thus there is confidence in the suggestion of mid-Miocene dispersal of the ancestor of this species to Madagascar. Trans-oceanic dispersal in *Yanga* is not surprising when considering that the distribution of all of the 14 species in this genus are restricted to the African islands of the Seychelles, Comores, Pemba and Madagascar. Indeed many groups found on Madagascar and Africa are now thought to have dispersed between these landmasses relatively recently (Raxworthy *et al.*, 2002; Vences, 2004; Yoder & Nowak, 2006; Vences *et al.*, 2009).

Hypothesis 6: The African members of Platypleura originated in the north-eastern Africa, with a southward radiation (Villet et al., 2004).

The ancestral lineage of *Platypleura* s.s. is proposed to have diverged from a common ancestor with *Albanycada* Villet in the early Miocene (~ 18 mya). *Albanycada* is currently restricted to the thicket biome in the Eastern Cape of South Africa, suggesting that *Platypleura* and *Albanycada* diverged from a common ancestor in southern Africa, not East Africa as previously suggested (Villet *et al.*, 2004). However, this hypothesis requires further attention as southern African thicket is part of a global thicket biome which was once widespread during the Palaeogene (65 - 23 mya; Cowling *et al.*, 2005; Schrire *et al.*, 2005). Furthermore the sister relationship between the tettigomyiine cicada genera *Xosopsaltria* Kirkaldy in the thicket biome in South Africa and *Paectira* Karsch in savannah in East Africa (unpublished data) and the disjunct distributions of multiple vertebrate taxa (e.g. Matthee & Robinson, 1996, 1997; Muwanika, 2003) suggests a link between East and southern Africa that requires further attention.

Two main radiations of *Platypleura* within Africa are hypothesised; the first distributed throughout the forest and savannah biomes of sub-Saharan Africa (including clades D & E), and the second restricted to southern Africa (clades A-C & F; Figures 2.3 B & 2.4). These radiations appear to have occurred concurrently and to have begun in the mid-Miocene (~ 12 mya) with rapid lineage accumulation in the period of the late Miocene (Figure 2.3 B).

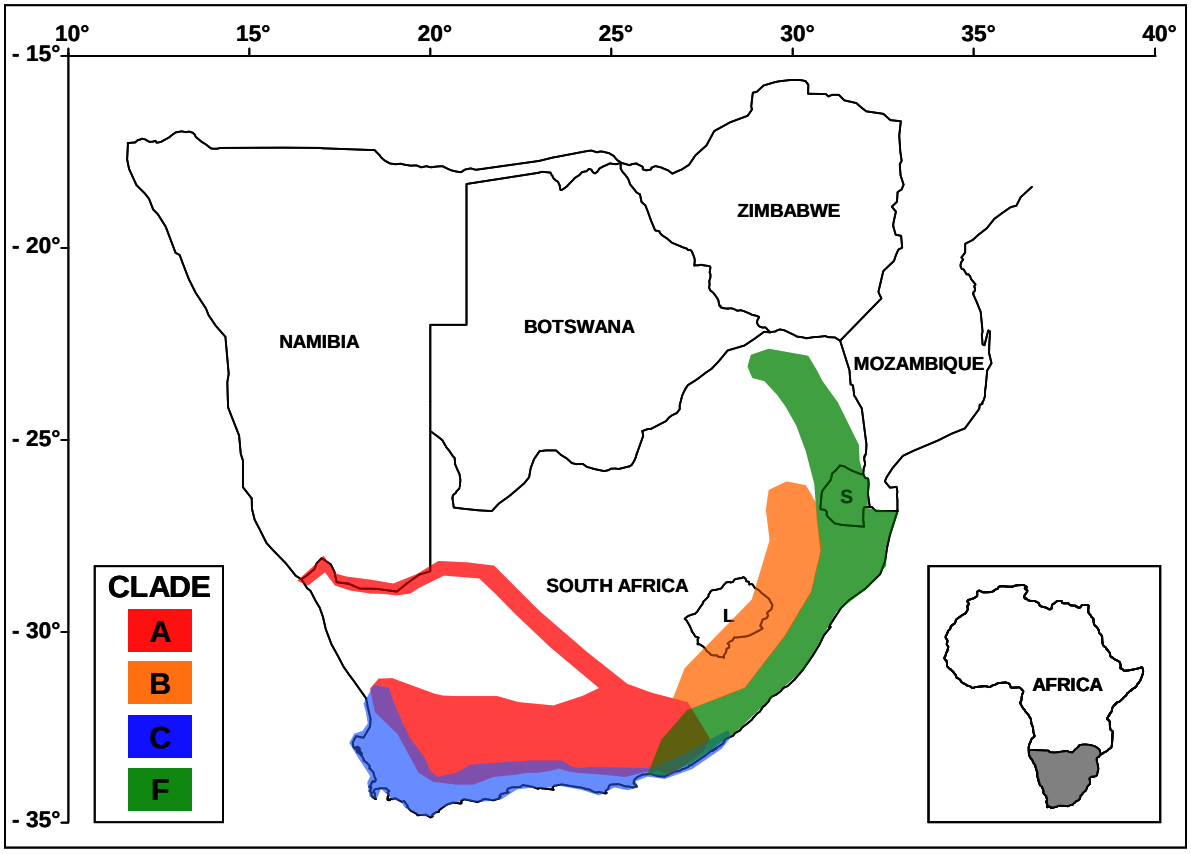

Figure 2.4. Approximate distributions of selected species groups of *Platypleura* in southern Africa. Colours correspond to clade labels of Figures 2.1 - 2.3 and denote Karoo (A), Grassland (B), Fynbos (C) and Forest (F) clades. The clades inhabiting East Africa (clade D) and West Africa (clade E) are not shown. Within South Africa letters denote Lesotho (L) and Swaziland (S).

The African radiation does not follow a pattern of regional monophyly (Figure 2.3 B) which is indicative of diversification in response to large scale climatic shifts, however an absence of samples of species with a central African distribution precludes further analysis in this group. The pattern of initial high diversification in the late Miocene (~ 12 - 10 mya) is highly congruent with a study of the chameleon fauna of southern Africa (Tolley *et al.*, 2008) and is suggestive of a common process in this region. One plausible mechanism in southern Africa is the development of the Benguela current in the late Miocene (Siesser, 1980; Dupont *et al.*,

2005) and the associated onset of cooler, more arid conditions (Van Zinderen Bakker, 1975), which would result in habitat fragmentation. Indeed this has been suggested as a stimulus for lineage diversification in a number of southern African taxa (Lamb & Bauer, 2003; Lamb *et al.*, 2003; Bauer & Lamb, 2005; Makokha *et al.*, 2007). Within the late Miocene (~ 7 mya) the rate of lineage accumulation in *Platypleura* decreases rapidly, indicative of increased extinction rates although the effect of incomplete taxon sampling cannot be completely discounted. This period is characterised by large-scale changes in the vegetation of southern Africa in response to climatic shifts (Linder, 2003). Within southern Africa, four well supported clades correspond to the Karoo (clade A), Grassland (clade B), Fynbos (clade C) and Forest (clade F) biomes suggesting biome-specific radiations in this region (Figures 2.3 B & 2.4).

2.5. Conclusion

As this is the first estimate of the relationships within the Platypleurini, the resulting inferences are necessarily tentative on the origin and diversification of this tribe in Africa. The dating analysis confirms that the current distribution of the cryptotympanine and platypleurine cicadas is not a result of Gondwanan vicariance followed by dispersal into Asia. The Asian taxa are shown to be of a single origin post-dating the meeting of the African and Eurasian landmasses, the taxonomic implications of which highlight the need for a formal review of the platypleurine genera. The combination of older forest-associated lineages and diversification coincident with the Oligocene onset of aridification in Africa suggests that the fragmentation of forests and expansion of savanna may have lead to the diversification of this tribe in Africa. Although based on one representative the platypleurine cicada fauna of Madagascar are most likely not the result of ancient vicariance, but of recent dispersal, as is now suggested for many Madagascan fauna. Finally the origin of the genus *Platypleura* in Africa is not definitively placed within eastern or southern Africa and awaits a more complete sample, specifically from central and eastern African species (e.g. *P. machadoi*, *P. kabindana* and *P. makaga*) not available for this study. Within South Africa the majority of representatives of *Platypleura* form four distinct clades, each restricted to separate biomes: Fynbos, Karoo, Forest and Grassland respectively. Based on preliminary assessment of morphology and acoustic data, the cicada fauna within three of these biomes (Fynbos, Karoo and Forest) are sufficiently diverse to warrant further investigation into the processes responsible for their diversification.

3 Patterns and processes underlying evolutionary significant units in the *Platypleura stridula* L. species complex (Hemiptera: Cicadidae) in the Cape Floristic Region, South Africa

3.1. Introduction

Phytophagous insects are diverse, in many cases possessing specialized plant associations as a result of plant chemistry and assortative mating (Feder *et al*., 1994; Percy *et al*., 2004). These insects provide tools to test models of speciation (Cooley *et al*., 2001; Despres *et al*., 2002), dietary specialization (Morse & Farrell, 2005) and host race formation in the absence of geographic isolation (Simon *et al*., 2003). Phylogeographic studies can lead to the identification of places and processes central to the origin and maintenance of biological diversity (Bermingham & Moritz, 1998; Moritz & Faith, 1998; Soltis *et al.*, 2006), factors that are essential for conservation (Kremen *et al*., 1993; Moritz, 2002). Furthermore, phylogeographic and phylogenetic studies of phytophagous insects, such as cicadas, can illuminate the effects of past climate change (Buckley *et al*., 2001) and the origins of extant taxa (Arensburger *et al*., 2004a).

The Cape Floristic Region (CFR) houses a large proportion of southern Africa's unique flora and fauna and is one of the most biodiverse regions known (Goldblatt & Manning, 2002; Galley & Linder, 2006). Many factors, not all mutually exclusive, have been cited as explanations for the unusually high biodiversity of the CFR. The role of climate change during the Pliocene and Pleistocene has been particularly debated, with support for conflicting hypotheses. Tyson (1999), Richardson *et al*. (2001) and Cowling *et al*. (2005) postulate rapid, dramatic climate shifts in Southern Africa, while Weaver *et al*. (1998), Meadows & Baxter (1999), Dynesius & Jansson (2000) and Barraclough (2006) suggest that there was relative stability in the Cape region. The topographical complexity of the region, with an abundance of altitudinal gradients, may have allowed organisms to migrate altitudinally, acting as a buffer to the effects of climate change (Linder & Vlok, 1991; Midgley *et al*., 2001). Furthermore, the region has undergone neotectonic crustal uplift during the Miocene and Pliocene by as much as 900 m in the east (Artyushkov & Hofmann,

1998), resulting in a westward tilt of the sub-continent. The combination of uplift and rapid sea level change associated with the Pleistocene glacial cycles would alternately expose and inundate much of the wide continental shelf, especially south of Cape Agulhas (Dingle & Rogers, 1972). These geological and climatic perturbations may underlie speciation in many of the region's plants and animals (Goldblatt, 1978; Daniels *et al*., 2001; Richardson *et al*., 2001; Gouws *et al*., 2004; Cowling *et al*., 2005; Linder, 2005; Tolley *et al*., 2006), and the CFR thus serves as a natural laboratory for studies on speciation. Here the results of the first phylogeographic study on a hemipteran from the CFR: the *Platypleura stridula* L. complex of cicada species, are presented.

Cicadas are peculiar amongst phytophagous insects in that most of their life cycle is subterranean (Moulds, 1990), where they are obligate plant xylem feeders (Cryan, 2005). In general, cicadas are not considered to be vagile (de Boer & Duffels, 1996b) and commonly possess relatively high rates of endemism and plant specificity (Villet & van Noort, 1999). Furthermore, they rely on long-range mate attraction, using loud, species-specific acoustic signals (Claridge, 1985; Villet, 1995), which would tend to lessen selection pressures leading to the diversification of short-range cues, including those of male genital morphology (Quartau *et al*., 2000). When coupled with the strong selection for predator avoidance exhibited by cicadas (Villet & van Noort, 1999), it is not unusual that there is a propensity to form morphologically cryptic species within the family (Quartau *et al*., 2000). Thus morphology alone is often insufficient to reveal the relationship between closely related species, and molecular characters provide a useful additional source of data. Although the relative rate of character evolution between nuclear and mitochondrial DNA is gene-specific, mitochondrial DNA data are especially useful when working with recently diverged taxa, due to their higher rate of character evolution relative to nuclear DNA (Moore, 1995; Lin & Danforth, 2004).

The tribe Platypleurini is distributed from the Cape of South Africa northwards throughout the Afrotropics, via Arabia, India and South East Asia, to Korea and Japan (Distant, 1906; Duffels & van der Laan, 1985; Lee & Hayashi, 2003). It is absent from Australia (Moulds, 1990), Europe and the New World, implying that the group is probably not Gondwanan. Previous phylogenetic work on South African species within the genus *Platypleura* Amyot

and Serville, 1843 implied that these cicadas show an ancestral plant association with *Acacia* species, with speciation sporadically associated with host plant shifts (Villet *et al*., 2004).
Four species were described in the *P. stridula* species complex, two of which are currently recognised: *Platypleura stridula* (Linnaeus, 1758) and *P. capensis* (Linnaeus, 1764). *Cicada catenata* (Drury, 1773) and *C. nigrolinea* (De Geer, 1773) are currently treated as synonyms of *P. stridula* (Distant, 1906; Duffels & van der Laan, 1985; Villet, 1989), but the taxonomy of this group is still unresolved. A putative, localised species, *Platypleura* sp. 10, was recently discovered in the Western Cape, based primarily on songs. These species are abundant and posses a relatively linear north - south (in the case of *P. stridula* and *Platypleura* sp. 10) or east - west (in the case of *P. capensis*) distribution within the CFR (Villet, 1989; Villet *et al*., 2004). All species have moved onto a variety of non-leguminous hosts, and appear to have become oligophagous (Villet *et al*., 2004). Their dietary breadth seems unusual, considering the narrow plant associations of other closely related species within the genus (Villet *et al*., 2004).

Given its checkered taxonomic history, a previously observed morphological intractability and a distribution restricted to the CFR, this taxon is a good candidate for examining the effects of environmental processes, plant association and geographic barriers to gene flow within a phylum that has been relatively neglected by scientists studying the CFR biota (e.g. Wishart & Hughes, 2001; Klass *et al*., 2003). The hypothesis that there are more than two species in this complex was tested using independent molecular (mtDNA sequence), morphological and ethological data, and the phylogenetic and phylogeographic relationships are assessed to infer the causes of this taxonomic diversity.

3.2. Methods

3.2.1. Sampling and laboratory protocols

Tymbal muscles were collected in 96 % alcohol from one specimen of *Platypleura* sp. 10, one specimen of *Platypleura capensis* from each of 66 sites (Table A3), and one specimen of *Platypleura stridula* from each of 15 sites (Table A4), covering most of their respective geographical ranges. In addition three other southern African species of *Platypleura* (Villet *et al.*, 2004) were included as outgroups for the analysis (Table A4). All voucher specimens are housed at the Albany Museum, Grahamstown. Plant association data were collected with almost every specimen, and supplemented with museum data.

Total genomic DNA was extracted from the muscles following the Chelex® 100 protocol (Walsh *et al*., 1991). Small pieces of tissue (c. 2 mm^3) were sliced finely using a sterile scalpel blade, and placed in 5 % Chelex extraction buffer (150 µl of 20 % Chelex 100 solution, 450 µl TE buffer (10 mM Tris, 1 mM EDTA)) and incubated at 60 °C for two hours, and then at 100 °C for 15 minutes in a heating block. Samples were then centrifuged at 13 000 rpm for one minute and the supernatant removed for subsequent use in PCR amplifications.

A 525 base pair region of the mitochondrial small subunit ribosomal 16S RNA gene was amplified and sequenced using the primers 16Sar and 16Sbr (Palumbi *et al.*, 1991), and an 816 base pair portion of the 3' end of the Cytochrome Oxidase I (COI) gene was amplified using the primers C1-J-1718 (Simon *et al.*, 1994) and C1-N-2568 (Brady *et al*., 2000). The difficulty of amplifying some taxa resulted in the development of an internal primer: (5' – GTATCATGYAARACAATATCAAT – 3'), being designed to replace C1-N-2568 for PCR. PCR amplifications were confirmed by electrophoresis of 5 µl PCR product and 5 µl tracking dye in a 1 % agarose gel, stained with ethidium bromide and visualized using a UV trans-illuminator.

PCR products were purified using the Wizard® SV quick purification kit (Promega Corp.) and sequenced in both directions using the flanking primers for each region and the two internal primers C1-N-2191 and C1-J-2183 (Simon *et al*., 1994) for the COI region. The sequencing reaction was carried out using the ABI Big Dye Sequencing kit v.3.1, according to the manufacturer's instructions. Sequence trace files were generated using an ABI 3100 genetic analyzer sited at Rhodes University. Trace files were checked and edited using Sequencher v.3.01 (Gene Codes Corporation). The sequence data were imported into McClade v.4.06 (Maddison & Maddison, 2000) and aligned manually.

3.2.2. Phylogenetic analysis

To ascertain whether the data from each gene region could be combined into a single data set the Incongruence Length Difference (partition homogeneity) test (Farris *et al.*, 1994) was conducted in PAUP* v.4.0b10 (Swofford, 2003) with invariant characters removed (Cunningham, 1997) because the number of variable characters differed between the 16S and COI data sets.

Three different methods of phylogenetic analysis were performed: Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI). An unweighted MP analysis was conducted using PAUP* as follows: 100 random addition replicates were conducted keeping a single shortest tree for each replicate (TKEEP = 1). All trees retained in memory from this process were then swapped to completion using a heuristic search with TBR branch swapping. Confidence in each node was assessed with 1000 full heuristic bootstrap replicates.

The most appropriate model of sequence evolution was selected using the AIC test (Akaike, 1974) as implemented in Modeltest v.3.7 (Posada & Crandall, 1998). ML analysis was conducted under this model in PAUP*.

The BI analysis was conducted using MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001) under the best fitting model as selected by the AIC test in MrModeltest v.2.2 (Nylander, 2004). The MrBayes analysis comprised four independent runs of 2 000 000 generations using random starting trees with four chains (one cold, three hot), sampling every 100 generations. Plots of likelihood scores, tree length and average standard deviation of split frequencies against number of generations showed that the analysis reached stationarity well within the first 10 % of trees generated. Thus the first 10 % of trees generated were discarded, ensuring that only trees generated at stationarity were used to calculate the BI posterior probabilities. The mean intra- and net inter-clade percent sequence divergence was calculated in MEGA v. 3.1 (Kumar *et al.*, 2004) using the Kimura 2-parameter model (Kimura, 1980).

3.2.3. Molecular dating

Although the application of a global molecular clock is problematic (Buckley *et al*., 2001; Heads, 2005), the COI region has been shown to be the most reliable gene when enforcing a molecular clock for insects because it possesses the most consistent rates of sequence evolution between lineages (Gaunt & Miles, 2002). The data were tested for equal substitution rates across the tree using RRTree v.1.1 (Robinson-Rechavi & Huchon, 2000) and the topology of the BI analysis. Furthermore they were tested for the applicability of a molecular clock using two separate ML analyses, first with and then without the molecular clock enforced. The likelihood ratio test (LRT) was then used in combination with a Chi-

squared test to assess whether or not the molecular clock significantly influenced the ML analysis (Felsenstein, 1981).

Without fossil evidence or an adequate geological vicariance event to calibrate the molecular clock, the dating analysis resorted to a bounded estimate of the timing of divergence using both the 16S and COI gene regions independently. The mean rate of sequence evolution was obtained for each region from previously published rates, using hemimetabolous insect taxa calibrated to within the last 10 million years. The mean rates used in this study were COI = 2.25 % per MY (Brower, 1994; Buckley *et al*., 2001) and 16S = 1.4 % per MY (Brower, 1994). The time to the most recent common ancestor (TMRCA) between each clade was then estimated under the model parameters highlighted in MrModeltest using a Bayesian approach in BEAST 1.4 (Drummond & Rambaut, 2003) with 5 000 000 steps, following a discarded burn-in of 50 000 steps. Convergence to stationarity and the estimation of effective sample size (ESS) in each analysis was confirmed by inspection of the MCMC samples using Tracer 1.2 (Rambaut & Drummond, 2003).

3.2.4. Acoustic analysis

Cicada calls were recorded at multiple localities for each major clade resolved by the phylogenetic analysis using a Sony MZ-NH1 Minidisc Recorder and Sony ECM-719 stereo microphone. Sonograms were produced for each call using Raven v.1.2 (Charif *et al*., 2004) with default settings. Both within- and between-clade variability in the frequency range and length of each call echeme were analysed with separate two-way ANOVAs as implemented in Statistica v.7 (StatSoft Inc.); significant pairwise differences were analysed using Scheffe's test.

3.2.5. Morphological analysis

Samples from each clade were pinned as voucher specimens. The morphology of specimens in each clade within the group is very similar, and the most morphologically variable body part is the males' 10th abdominal segment, the urite of their genitalia. A preliminary analysis was conducted using measurements from both the forewing and the urite of specimens from each clade except *Platypleura* sp. 10, for which too few specimens were available for meaningful analysis. Wing measurements were obtained at 6x magnification, using a WILD M5A stereo microscope (Leica, Switzerland) and eye-piece graticule. Urite measurements

were obtained at 35x magnification using a Leica EZ4D stereo microscope (Leica, Switzerland) and the Leica Application Suite v.2.3 (Leica Microsystems, Switzerland) image analysis software. The data were log-transformed to linearize allometric covariation and analysed using a correlation matrix-based principle component analysis (PCA) in Statistica.

3.3. Results

3.3.1. Phylogenetic analysis

The final molecular data set comprised 1341 base pairs, comprising 816 base pairs of COI data that aligned readily and 525 base pairs of 16S data that aligned readily with some gaps corresponding to insertion or deletion events. The ILD test indicated that the data sets were not significantly different ($p = 0.41$), so the data were combined. Of the 1341 base pairs, 258 characters were variable (19 %); of these 176 characters (13 %) were parsimony-informative. All analyses resolved six well-supported clades within the *P. stridula* complex, but the topological arrangement of some of the clades lacked support (Figure 3.1).

Parsimony analysis yielded 32 most-parsimonious trees with a tree length of 340 steps (CI = 0.653; RI = 0.951). The TrN+I+G model was selected as the best model of sequence evolution for the combined data set using the Akaike information criteria (Akaike, 1974) in Modeltest. Maximum Likelihood analysis under this model yielded one most likely tree (lnL = -4076.2019 and lnL = -4111.9915 clock enforced). Bayesian analysis under the most similar model implemented in MrBayes 3.1.2 (GTR+I+G) also yielded one tree (Figure 3.1), on which the MP bootstrap and the BI posterior probability values for each of the nodes are shown.

The clades found in all analyses include a monophyletic *P. stridula* clade (sister to *Platypleura* sp. 10) and four other clades, comprising specimens all identified as *P. capensis* following Villet (1989). For the purposes of this paper the clades are labelled as the *P. stridula* clade (P), the Coastal clade (C) and the East (EM), West (WM) and Central Montane (CM) clades (Figure 3.1). The three Montane clades lack well-supported substructure, but closer inspection of the BI tree shows that the Coastal clade can be further subdivided into well-supported Eastern [EC], Central [CC] and Western [WC] lineages and the *P. stridula* clade can be further subdivided into well-supported Northern [N], Southern [S] and Eastern [E] lineages (Figure 3.1).

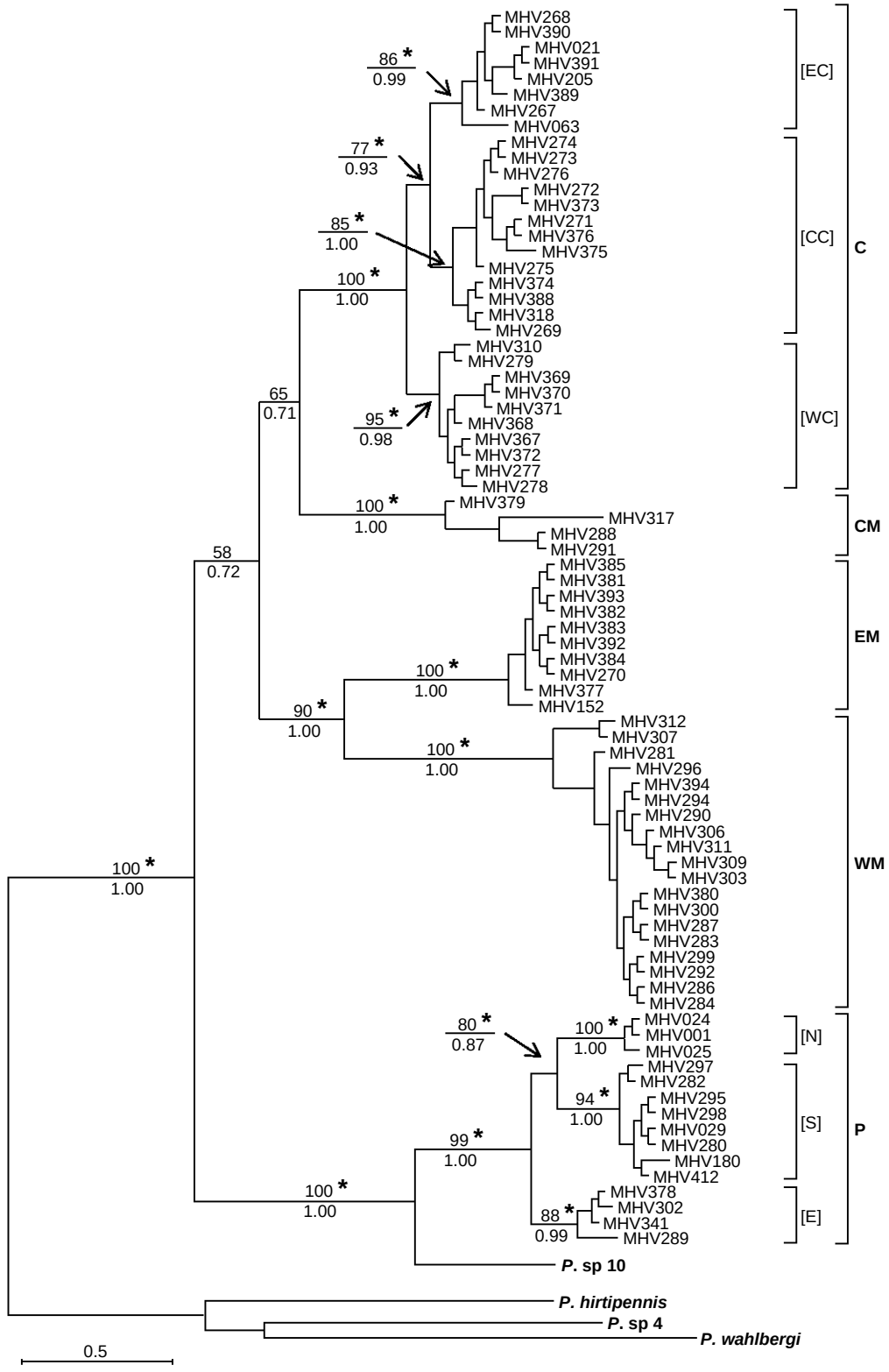

Figure 3.1. Majority rule Bayesian Inference phylogeny highlighting five major clades: *P. stridula* (P), Coastal (C) and the Western (WM), Central (CM) and Eastern (EM) Montane clades in addition to three sub-clades within *P. stridula*: Northern [N], Southern [S] and Eastern [E] and Coastal: Eastern [EC], Central [CC] and Western [WC] clades. Statistical support generated in the analyses for each node is shown as MP bootstrap above and BI posterior probability below each line, each node present in the ML tree is indicated with (*).

The Kimura 2-parameter distance of the ingroup samples showed no overlap between the within-clade mean (COI: 0.1 - 1.4 %; 16S: 0.0 - 0.5 %) and the net between-clade (COI: 2.3 - 6.3 %; 16S: 1.4 - 4.6 %) divergence.

3.3.2. Molecular dating

Both the LRT (p = 0.76) and the relative rate tests (p = 0.19) showed that the rate of sequence evolution did not differ significantly between major clades. Dating estimates on the sequence data indicate that radiation within the in-group occurred within the last 2.4 - 5.5 million years. The vicariance of the three *P. stridula* lineages and the three Coastal lineages is estimated to have occurred more recently, within the last 0.11 - 0.56 and 0.13 - 0.65 million years, respectively (Figure 3.2).

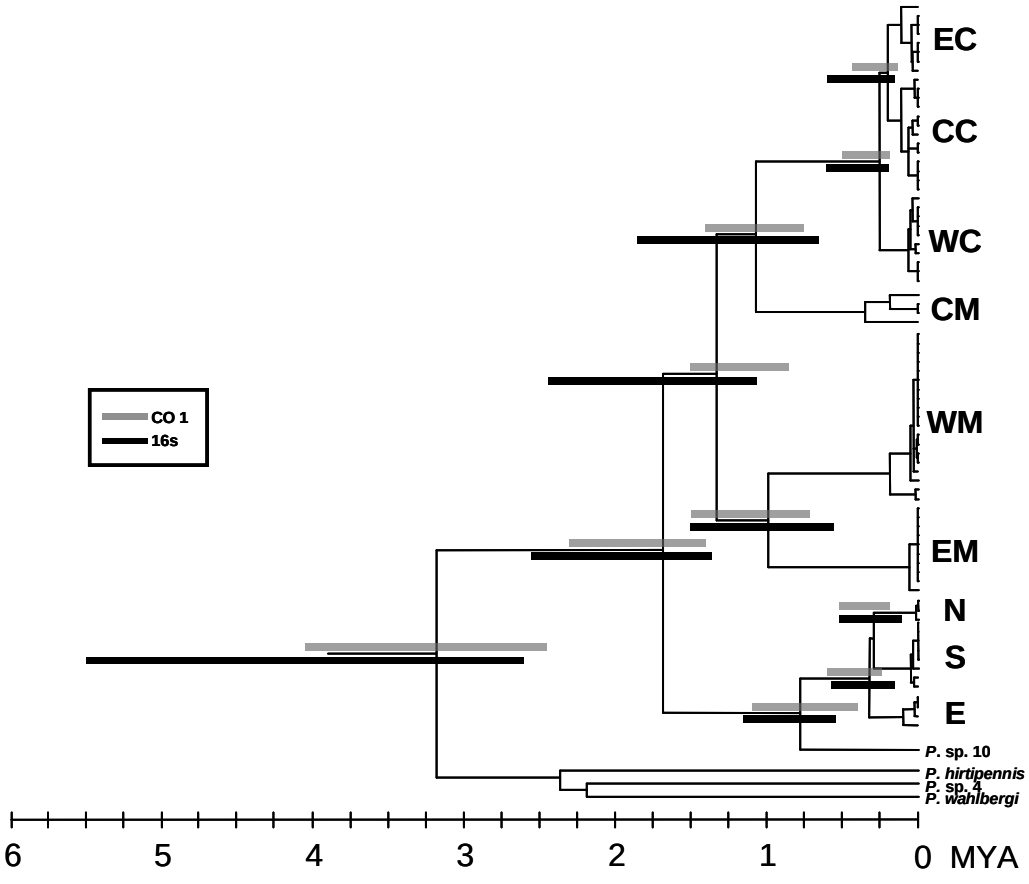

Figure 3.2. Clock-enforced Maximum Likelihood tree showing the estimated time to the most recent common ancestor (TMRCA) between each clade. Bars denote the upper and lower bounds of the 95 % HPD interval as estimated in BEAST, using COI (grey) and 16S (black) regions independently. Clade labels correspond to Figure 3.1.

3.3.3. Acoustic analysis

Sonograms of the calls recorded at one site for each of the major clades show that the emphasised frequencies differ between clades. Lower frequencies are utilized by *P. stridula* (6 - 8 kHz), *Platypleura* sp. 10 (4 - 8 kHz) and the Coastal clade (6 - 13 kHz), while the Montane clades emphasise a higher frequency range (9 - 14 kHz). In addition the Coastal and Montane clades and *Platypleura* sp. 10 show frequency modulation, a trait absent in *P. stridula*. ANOVA of the Coastal and three Montane clade call echemes showed high within-clade variability and no significant difference ($p > 0.05$) in the length of the call echeme between clades.

3.3.4. Morphological analysis

The first two principal components of the PCA of wing and urite measurements (Figure 3.3 A & B) describe 80 % of the variation in the samples (Table 3.1), while the remaining factors did not significantly describe variation between the specimens. The plot of the first two factors shows three distinct groupings: *P. stridula*, Coastal and the combined montane specimens. Specimens of the three montane clades were distinctly smaller than those of the other clades, which were slightly different in size. All clades overlapped on the second axis and are not readily distinguishable.

Table 3.1. Eigenvectors and Eigenvalues of a Principal Component Analysis of the body measurements defined in Figure 3.3 A. Factor 1 may be interpreted as an expression of overall body size. Factor 2 summarises variation in two of the processes of the urite. The species are differentiated fairly well by body size, and poorly by genitalia.

Variable		Factors			
		1	**2**	**3**	**4**
Wing	1	**-0.353**	0.041	0.135	-0.084
	2	**-0.348**	0.090	0.084	-0.047
	3	**-0.352**	0.042	0.174	-0.048
	4	**-0.346**	0.044	0.125	0.022
	5	**-0.344**	0.149	0.078	-0.101
	6	**-0.351**	0.101	0.096	-0.101
Urite	1	**-0.273**	-0.121	-0.212	-0.602
	2	-0.103	**-0.794**	0.520	0.206
	3	**-0.275**	0.079	-0.117	0.382
	4	**-0.270**	0.104	-0.290	0.641
	5	-0.181	**-0.538**	-0.706	-0.050
Eigenvalue		7.729	1.064	0.772	0.619
Cumulative variance (%)		70.27	79.95	86.97	92.60

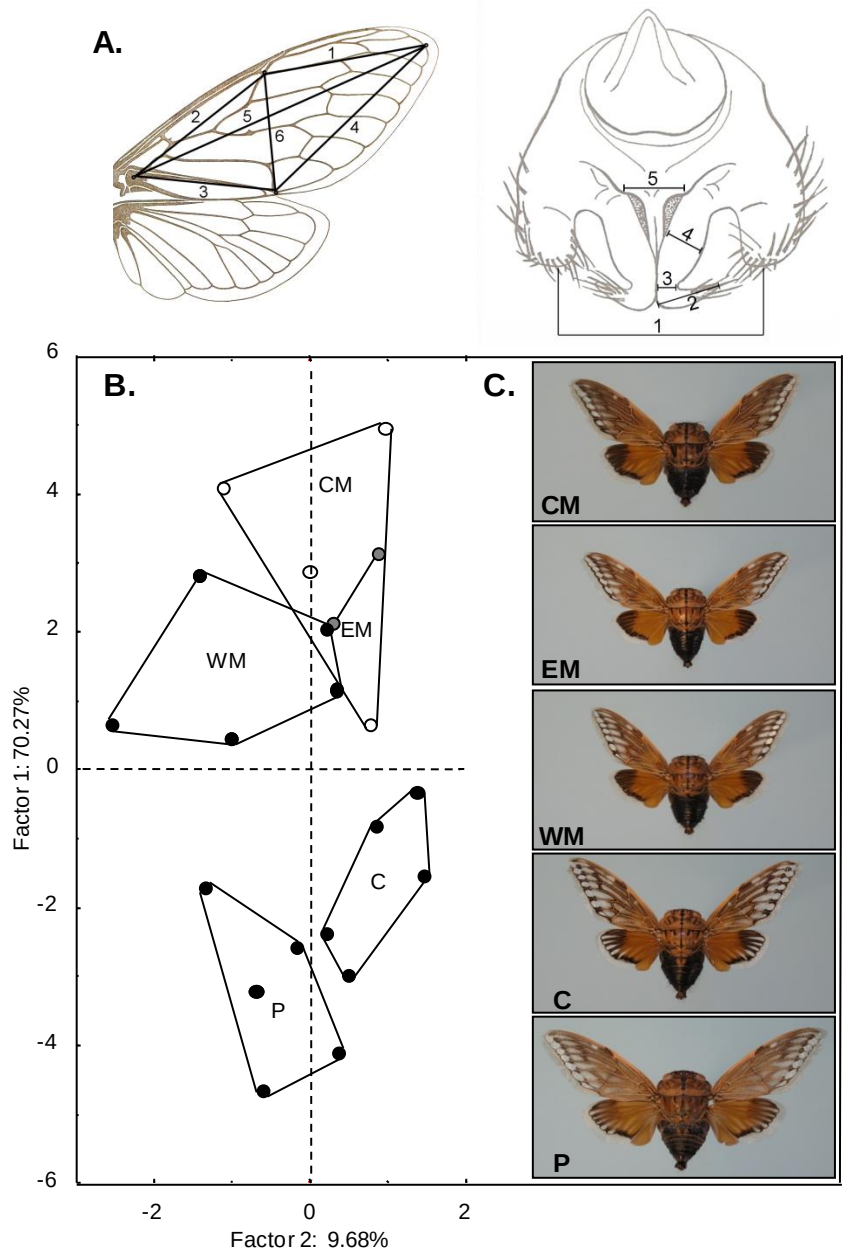

Figure 3.3. (A) Diagram of right wings and urite showing measurements used in the PCA; (B) Plot of the first two principal components of the PCA, with some horizontal exaggeration of the second component; convex hulls enclose samples of each of the major clades; (C) Dorsal view of a specimen characteristic of each clade.

3.4. Discussion

3.4.1. Taxonomy

The recovery of six lineages from samples of what were previously two nominal species was surprising. Should each clade receive species status? Assigning species status to organisms occurring in allopatry has proven to be more difficult than with organisms occurring in sympatry, especially when the taxa concerned are morphologically cryptic (Daniels *et al*.,

2003). In particular the Central and Western Montane clades occur in such close proximity (within 10 km in two pairs of samples) that they are apparently parapatric, yet this has not been rigorously established. As a result the more holistically biological approach as emphasised by Sites & Marshall (2004), utilizing genetic, morphological and courtship data, was used when approaching this question. The mtDNA data distinguish six clades with high levels of statistical support, suggesting the applicability of a species concept akin to the Phylogenetic Species Concept (Mishler & Donoghue, 1982; Cracraft, 1983; Zink & McKitrick, 1995). The clades show levels of sequence divergence that warrant species rank using previously cited thresholds of DNA variability (Hebert *et al*., 2003; Kartavtsev & Lee, 2006; Smith *et al*., 2006). Furthermore the comparable divergence in the mtDNA COI data between these clades and between recognized species within the genus (data from Villet *et al*., 2004) indicate that all six clades warrant species status. The lack of overlap between the COI intra-clade (< 1.4 %) and inter-clade (> 2.3 %) divergence may argue the usefulness of this region in species delineation (Kartavtsev & Lee, 2006). That said, there are hazards in interpreting genetic distance as criterion for species delineation (Ferguson, 2002; Meyer & Paulay, 2005; Brower, 2006), and it is prudent to favour the more holistic and biological approach of species delineation involving morphological and acoustic data.

The overall size of the individuals within the complex (Figure 3.3) enables the identification of three distinct morpho-species represented by the *P. stridula*, Coastal and combined Montane clades. *Platypleura* sp. 10 is similar in size to *P. stridula*. Morphology alone does not separate the three Montane clades and has proven to be a generally problematic basis for a species concept within the group. This is expected given the crypsis and sexual signalling channels exhibited in the group (Villet, 1989) and the proposed recent diversification of the Montane taxa. Platypleurine cicadas show only rudimentary courtship (Villet *et al*., 2003), so that most of the interaction between the sexes occurs through long-range acoustic signals (Villet, 1992), which are therefore the major determinant of gene flow through mating (cf. Paterson, 1985; Villet, 1995; Quartau *et al.*, 2000). In this regard the acoustic data indicate at least four species: *P. stridula*, *Platypleura* sp. 10, Coastal and combined Montane, based on frequency modulation and the peak frequency of the call. No difference in length of call echemes between clades was observed due to within-clade variability in echeme duration. Although some cicadas have been shown to exhibit fine-scale pitch discrimination (Fonseca *et al*., 2000), the present acoustic data are insufficiently divergent between the three Montane

clades to interpret each of them as distinct biological species under the Specific Mate Recognition System species concept (Paterson, 1985; Villet, 1995). However, these data are preliminary, and suggest a worthwhile avenue for further investigation. Samples of the Coastal and Eastern Montane clades were collected within 3 km of one another at Jeffreys Bay (Table A3), and probably occur within 1 km of one another at the southern extreme of the distribution of the Western Montane clade, so that the Coastal and Montane taxa are practically sympatric in places. This provides strong support to the morphological and acoustic data for interpreting the Coastal clade as a distinct species from the Montane clades. As a result of a lack of similar evidence regarding the taxa within the Montane group, it is suggested that at present they should be considered three distinct evolutionary significant units (Ryder, 1986; Moritz, 1994) for conservation and management purposes, pending further evidence (both acoustic and morphological) that these three clades warrant species status.

Preliminary comparisons of photographs of the putative type specimens of *P. stridula* and *P. capensis*, held in the Uppsala University Museum collection confirm the identity of *P. stridula*. However the identity of the putative type specimen of *P. capensis* is ambiguous, requiring physical scrutiny. The nomenclature of the Coastal and three Montane clades with regard to *P. capensis*, *C. nigrolinea* and *C. catenata* will be addressed at a later date once all types have been scrutinized.

3.4.2. Plant association

The host plant data (Table A3 & A4) suggest that the species within the complex show a wide range of plant-specificity with strict plant association in the Montane clades, oligophagy in the Coastal clade and polyphagy in *P. stridula*. *Platypleura* sp. 10 is tentatively associated with *Cliffortia* plants (unpublished data), but current samples are not definitive. These differences are most likely a result of the plant associations being mediated via long-range visual cues, like overall plant structure (tree vs. shrub) and gross leaf morphology (broad-leaved vs. fine-leaved), rather than close-range chemical cues. Although the original host plant of *P. stridula* was most likely the indigenous *Salix mucronata* Thunberg (unpublished records), it can now be found on many large trees, most of which are recently introduced exotics (Table A4), lending support to the hypothesis of long-range visual mediation of plant associations within this species. The large taxonomic discrepancy in the plant associations of

the taxa within this group indicate shifts in plant associations as a mechanism for speciation within this group, based on the premise that recently-diverged sister species are most likely to differ most in traits linked to speciation (Barraclough, 2006). This result is not surprising as the majority of speciation events in phytophagous insects are accompanied by host plant shifts (Futuyma, 1991).

3.4.3. Biogeography

Although preliminary, the dating analyses are plausible first estimates of the date of divergence within this group, highlighting initial diversification at the Pliocene - Pleistocene boundary. The common association of *Platypleura* sp. 10 and the Montane clades with *Cliffortia* plants (unpublished data) suggests that they represent models for the ancestral taxon of the species complex. This ancestor was therefore probably also associated with montane habitats, implying that vicariance of such habitats into northern and southern blocks was an important initial step in the diversification of the group, but the mechanism is not obvious. Recent evidence (Weaver *et al*., 1998; Meadows & Baxter, 1999) implies only a small impact of the last glacial maximum (a recent but severe glaciation) on the temperatures of the South Western Cape, probably because it is a winter rainfall region, so that climate is less likely to be a consideration. A more likely scenario involves events consequent on neotectonic uplift at about 2.5 mya driving initial diversification in this complex at about 1.8 mya (Figure 3.2). Subsequent diversification within both the northern and southern blocks produced all of the major extant clades at about 1.2 - 0.8 mya (Figure 3.2).

In all analyses, little support is generated for any particular topology of relationships between the Coastal and Montane clades. This result may be interpreted in one of two ways. Firstly, rapid evolution of mtDNA can reduce the resolution of deeper branches (Avise, 2001). This is unlikely here because there is no evidence of saturation in either gene. Another interpretation is that cladogensis occurred either simultaneously or in rapid succession (Hoelzer & Menlick, 1994), resulting in a polychotomy where practically no opportunity existed for character state changes to occur, or in which there is no clade structure to resolve (Page & Phelps, 1994). However it has been argued that explanations involving simultaneous differentiation should be considered only as a last resort (DeSalle *et al*., 1994). That said, situations abound where simultaneous or near-simultaneous isolation may have occurred in the Cape Floristic Region, including rapid changes in sea levels (generally in step with ice

ages) throughout the Plio-Pliestocene, a prominent factor in theories of the origins of the subtropical thicket vegetation (Cowling *et al*., 2005) and the rapid diversification of the Cape flora (Richardson *et al*., 2001; Linder, 2003). Additionally, neotectonic crustal uplift in the Pliocene at about 2.5 mya (Artyushkov & Hofmann, 1998) may have been a great stimulus for the diversification of plant lineages (Cowling & Proches, 2005) by altering altitudes and accentuating a rain shadow in the western parts of southern Africa. The concomitant increase in erosion (Partridge & Maud, 1987) also fragmented the substantially older Cape fold mountains with which the Montane clades are associated. Recent studies have shown similar disjunctions in both invertebrate and vertebrate taxa in the region, highlighting the complex topography of the region (Stevens & Picker, 1999) and rapid sea level changes in the late Pliocene (Hendey, 1983) as the most plausible explanations (Daniels *et al*., 2001; Wishart & Hughes, 2001; Gouws *et al*., 2004).

What seems clear is that *P. stridula* and the Coastal clade both arose and developed new plant and habitat associations during this period. *Platypleura stridula* became associated with trees in drainage lines in areas west of the distribution of *Platypleura* sp. 10, while the Coastal clade moved to broad-leafed woody daisies generally growing within 200 m of the sea south of the distributions of the Montane clades. The coincidence of these changes may be fortuitous, since although they may be explained by altered plant-recognition cues, no common environmental cause is obvious.

The three distinct lineages of *P. stridula* originated almost simultaneously within the late Pleistocene. They show a similar geographical pattern to the tentative distribution of the species of "heelwalkers" (Insecta: Mantophasmatodea) within the region (Klass *et al*., 2003). The *P. stridula* clade as a whole shows relatively large genetic distances between lineages that are each contained within a major watershed within the Western Cape (Figure 3.4 A), suggesting that the mountains within this region provide significant barriers to gene flow in this species. Although catchments have been found to define the distributions of invertebrates with aquatic stages within the region (Wishart & Hughes, 2001) it is unusual that *P. stridula*, which lacks an aquatic stage, is similarly circumscribed. In this case habitat philopatry may interact with the mountain barriers to reduce gene flow between catchments. This may be a consequence of the fact that *P. stridula* is primarily associated with an endemic willow (*Salix mucronata* ssp. *hirsuta* (Thunberg, Immelman) that grow along river courses in the Olifants

and Berg River catchments of the Western Cape region. River capture in the region (Hendey, 1983) may allow the colonisation of new catchments, but it is unlikely to produce three clades almost simultaneously. An additional evolutionary scenario is that the willows spread high into the headwaters in wetter periods, allowing the cicadas relatively easy migration between catchments, while range contraction of both willows and associated *P. stridula* populations in the cooler, drier Pleistocene glacial periods resulted in vicariance between catchments. Such a vicariance model needs to be validated by palaeoclimatic data.

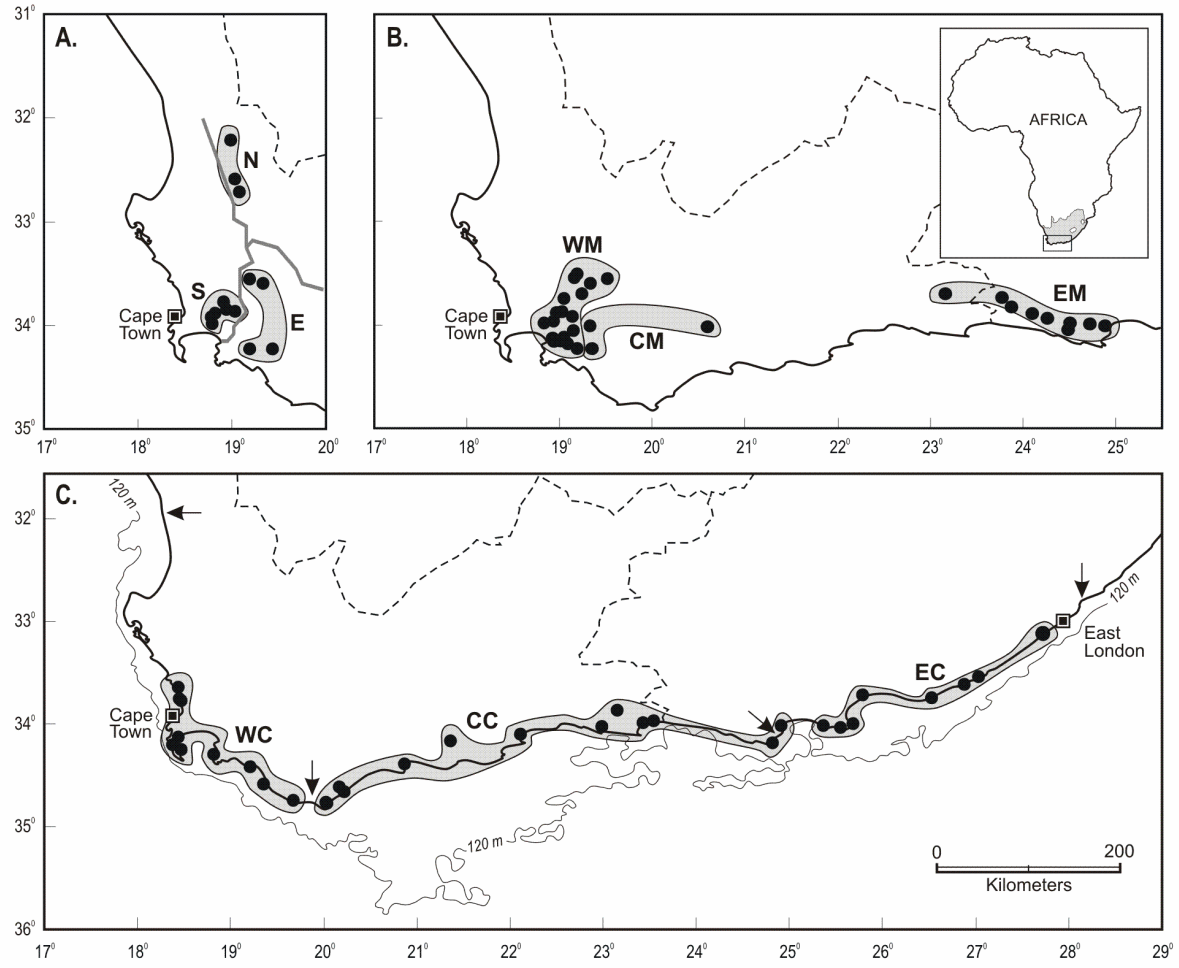

Figure 3.4. Geographical distribution of clades found in the analyses (A) *P. stridula*, (B) combined Montane clades and (C) Coastal clade. Sample localities within each clade are shown as dots. Within the *P. stridula* clade (A) solid lines indicate the position of the major watersheds. Within the Coastal clade (C) the solid line offshore indicates the -120 m isobath redrawn from the ETOPO 2' Global Bathymetry Grid (National Geophysical Data Center, 2001). Arrows show boundaries between the three supratidal zones within the region defined by Tinley (1985).

The Coastal clade shows three distinct lineages that originated at approximately the same time as those of *P. stridula* (Figure 3.2) and conform to the supratidal biogeographic zones defined by Tinley (1985; Figure 3.4 C). The boundary between the Western and the Central Coastal clades is centred on Cape Agulhas, whereas the boundary between the Central and Eastern Coastal clades is centred on the Gamtoos River valley (Figure 3.4 C). No obvious geographical barriers are present at clade boundaries that would impede recent migration within this species. Preliminary dating of the split between the Western and Central Coastal clades indicates one or more vicariance events within the Pleistocene (662 - 197 kya) as the most likely explanation. Within the Pleistocene, lower sea levels associated with glaciation (c. -120 m) would expose the majority of the marine Agulhas plain (Dingle & Rogers, 1972), pushing the coastline over a degree of latitude further south of the present Cape Agulhas (Figure 3.4 C). The colder climate of this exposed region as a result of the glacial maximum, associated with the drop in sea levels, could have excluded the Coastal clade from the more southerly Agulhas region.

The apparent disjunction between the Central and Eastern Coastal clades mirrors that found in its associated plant *Chrysanthemoides monilifera* (L.) T. Norl. subsp. *rotundata* (DC.) T. Norl. (Scott, 1996) and could involve the Gamtoos River system, which might have acted as a physical barrier to gene flow by forming a large marine bay when inundated by higher sea levels than present. Preliminary dating between clades indicates the vicariance event leading to the separation of the two clades to have occurred in the recent Pleistocene (655 - 136 kya). Indeed, marine transgressions that might result in division of the coastal population have been recorded in the Pliocene (Siesser & Dingle, 1981), but no inundations above 6m have been recorded in the Pleistocene (Hendey, 1983). A more likely explanation is the exposure of unsuitable habitat offshore of the present position of the Gamtoos River during Pleistocene glacial periods as a result of the close proximity of the 0 m and -120 m isobaths (Figure 3.4 C), forming steep shores when sea levels dropped. The secondary effects of climate changes, such as sea level change and the associated exposure of unsuitable habitat, seem the most likely causes of the modern pattern. Thus, although there is evidence of only limited local climate change in the South Western Cape (Weaver *et al*., 1998; Meadows & Baxter, 1999), the effects of glaciation elsewhere could still indirectly affect this buffered environment. In addition, because of peculiarities in the submerged topography of the Gamtoos valley and St. Francis Bay region, changes in sea level cause very substantial

changes in the length of the local coastline (Figure 3.4 C), as they do on the Agulhas plain. This would result in the alternating dispersion and compression of Coastal populations, which have an essentially one-dimensional distribution. The effects of such processes on population genetics need to be explored further, since they potentially affect all species restricted to narrowly coastal distributions.

3.5. Conclusion

This study has highlighted the effect of different mechanisms as drivers of cicada diversification in the CFR, and highlights the importance of using multiple sources of data when differentiating cryptic sister taxa. The combination of phylogenetic and ecological perspectives provides the timeframe and possible scenarios driving diversification and speciation in this group of CFR insects, which is most likely a result of the combination of Pliocene - Pleistocene uplift, climate change and associated diversification and radiation of the fynbos flora, as well as Pleistocene vicariance associated with the interaction of sea level changes and local coastal topography.

4 A watershed study on genetic diversity: phylogenetic analysis of the *Platypleura plumosa* (Hemiptera: Cicadidae) complex reveals catchment-specific lineages

4.1. Introduction

Planetary motion has been suggested to drive cycles of climate change that cause the fragmentation or extinction of lineages of organisms ("Orbitally-Forced Range Dynamics" *sensu* Dynesius & Jansson, 2000; Jansson & Dynesius, 2002; Jansson, 2003), leading to changes in populations and communities that drive biodiversification (Green & Sadedin, 2005). The severity of such climate changes varies predictably with latitude (Jansson, 2003). Local anomalies in this pattern are referred to the historical biogeographical processes of dispersal and vicariance. Dispersal processes are generally contingent on the characteristics of the organism, while vicariance is generally attributable to changing environmental features. Historical biogeographers have at their disposal a suite of vicariance models to explain genetic differentiation of species that includes continental drift, sea level change, and landscape features as barriers or refugia.

The generation of new landscape features such as seas, rivers, valleys and mountains are essentially events in the histories of catchments that, while they direct the movement of water, also act as basins of biological diversity. Although the role of catchments and their corresponding watersheds in structuring the genetic history of organisms such as plants through hydrochory (e.g. Levin *et al.*, 2003) and aquatic organisms (e.g. redfin barbs: Swartz *et al.*, 2007, 2009; atyid shrimp: Hughes *et al.*, 1995, 1996) is documented, the impact of different catchments on invertebrates with aquatic stages is varied, with evidence both for (e.g. Jackson & Resh, 1992; Wishart & Hughes, 2001) and against (e.g. Hughes *et al.*, 1998, 1999) a catchment effect, dependent primarily on the dispersal ability of the organism (Wishart, 2000). The effect of catchments on terrestrial organisms has only been considered recently (e.g. Measey *et al.*, 2007), with some studies being limited in scope, focussing on either the organism (e.g. Garrick *et al.*, 2007; Price *et al.*, 2007) or the habitat (Pearson & Raxworthy, 2009), rather than the landscape. Recognising that climate change in the near

future is likely to alter the environment, and particularly precipitation patterns, insight into recent patterns of population change related to landscape, and particularly catchments, may be useful in a conservation context.

If catchments and watersheds are important in structuring populations during periods of climatic oscillations, several hypotheses should be fulfilled: (1) different (primary or secondary) catchments should contain genetically distinct lineages; (2) sister clades should occur in adjacent (primary or secondary) catchments; (3) the proportion of genetic structure that can be explained using Isolation By Distance (IBD) should be small in comparison to that explained by catchment association; (4) the time to most recent common ancestor (TMRCA) should correspond to periods of increased aridity if vicariance is involved in cladogenesis as catchments are fixed landscape features, notwithstanding river capture and orogeny.

South Africa presents a perfect test-bed for this mechanism of diversification due to its (a) high plant and animal diversity of the region, amongst the highest in the world; (b) numerous catchments that span various environmental gradients and topographic features; and (c) reasonably well understood climatic and geological history.

Almost a third of South Africa's vegetation (28.2 % of land area) can be classified as semi-arid, including the Succulent-Karoo (SK), Nama-Karoo (NK) and Albany Thicket (AT) biomes (Mucina & Rutherford, 2006). The extent of these three biomes varies from widespread (NK: 19.5 %) to restricted (SK: 6.5 %; AT: 2.2 %; Mucina & Rutherford, 2006). Albany Thicket is considered the oldest of these three biomes, being of putatively Eocene origin (Mucina & Rutherford, 2006). The Nama- and Succulent-Karoo biomes are thought to have developed as a result of increased aridity in the late Miocene, coincident with the formation of the Benguela current (Siesser, 1980), continental uplift (Partridge & Maud, 1987; Artyushkov & Hofmann, 1998) and the rainshadow effects associated with these two phenomena (Levyns, 1964; Scott *et al.*, 1997; Mucina & Rutherford, 2006; Verboom *et al.*, 2009).

Within the Plio-Pleistocene (5 mya - present), aridity and seasonality have increased with the intensification of the Benguela current (Siesser, 1980) and global climate fluctuations in

response to Milankovitch oscillations. Evidence for the dramatic changes in South Africa's climate in response to global glacial cycles throughout the Pleistocene can best be illustrated by comparing the Last Glacial Maximum (LGM; 21 ± 2 kya; Mix *et al.*, 2001; Gasse *et al.*, 2008) and the Holocene Altithermal (7 ± 1 kya; Partridge *et al.*, 1999). In the LGM, temperatures have been estimated at 5 °C cooler than present (Heaton *et al.*, 1983; Talma & Vogel, 1992) with the region experiencing only 50 - 70 % of its current annual rainfall (Partridge *et al.*, 1999).

Sedentary invertebrates with short generation times are good tools to capture the geographic partitioning of genetic structure that results from processes such as climate cycling (Garrick *et al.*, 2007). In addition they are likely to survive in small, isolated populations, thus reducing the chances of local extinctions and preserving the continuity of their phylogeographical signal. As a result, these organisms may be better suited to studies of historical biogeography than most vertebrates, which have received the majority of attention in the region.

Although cicadas (Hemiptera: Cicadidae) are capable of flight, they may be considered sedentary organisms. Their unusual lifecycle consists of 11 months - 17 years of sedentary development that occurs underground on the roots of a single plant, followed by about three weeks of mobile adult life above ground. Furthermore, estimates of dispersal ability in cicadas have ranged from less than 150 m (Simões & Quartau, 2007) to 300 m from their place of emergence (Karban, 1981; Williams & Simon, 1995). Thus it seems that long-range dispersal may take many generations in cicadas (Maier, 1982; Arensburger *et al.*, 2004a).

The *Platypleura plumosa* (Germar) group provides a model clade for exploring the effect of catchments and watersheds on the historical biogeography of the semi-arid region of South Africa because of their (1) distribution across several catchments occupying a climatic gradient (arid west - moist east); (2) short life cycles (like many insects); (3) relatively low vagility (partly because adults are only active for a few weeks each year); and (4) identical small range of host plant associations, primarily on *Acacia karroo*, which removes the confounding effects of speciation due to host plant shifts.

Although the group is currently composed of two nominal species, *P. plumosa* Germar and *P. hirtipennis* Germar (Villet, 1997), morphological and acoustic variation suggests that five other cryptic species may be present in this group, referred to here as *Platypleura* 'karooensis', *P.* 'gariepflumensis', *P.* 'olifantsflumensis', *P.* 'breedeflumensis', *P.* 'gamtoosflumensis'; species names await formal description.

The aim of this study was to test the effect of catchments and watersheds in shaping the genetic structure of the *Platypleura plumosa* complex both at the population level and between the species.

4.2. Methods

4.2.1. Sampling and laboratory protocols

Cicadas belonging to the *P. plumosa* group were collected into 95 % ethanol from a total of 119 sites, covering their known geographical range (Table A5; Figure 4.1). In addition, two closely related southern African species of *Platypleura* (Chapter 2; unpublished data) were included as outgroups for the analysis (Table A5).

Total genomic DNA was extracted from the muscles following the Chelex® 100 protocol (Walsh *et al.*, 1991). Small pieces of wing or tymbal muscle tissue (c. 2 mm^3) were homogenised in 300 µl 5 % Chelex® 100 solution and incubated in a heating block at 105 °C for 15 minutes, vortexing the samples every five minutes. Following the incubation period, the supernatant was removed for subsequent use in PCR amplifications.

Portions of mitochondrial Cytochrome Oxidase I (COI) and small subunit ribosomal 16S RNA (16S) were amplified from each sample. In addition portions of two nuclear genes: Elongation Factor 1 alpha (EF-1α) and Calmodulin (CAL) were amplified for two representatives of each of the major mitochondrial clades (Table A6). Primers used for amplification and sequencing reactions were COI: either COI-F1 (5'-TAATATAAACT-ATTAACCTTCAAAGT-3') or COI-F2 (5'-TCTACTAATCACAAAGATATYGGAAC-3'; designed for this study) with C1-N-2568 (Brady *et al.*, 2000); 16S: 16Sar with 16Sbr (Palumbi *et al.*, 1991); EF-1α: EF1-F650-mod (Arensburger *et al.*, 2004a) with EF1-N-1419 (Sueur *et al.*, 2007); CAL: Cal-60-For (Buckley *et al.*, 2006) with Cal-2-Rev (UBC insect primer kit).

PCR amplifications were performed in 25 - 50 µl reactions using the following protocol: 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 48 °C (16S and COI) or 58 °C (EF-1α and CAL) for 45 seconds and extension at 72 °C for two minutes. PCR products were confirmed by electrophoresis of 5 µl PCR product stained with ethidium bromide and 5 µl tracking dye in a 1 % agarose gel, and visualized using a UV trans-illuminator.

PCR products were purified using the Invitek Invisorb MSB® Spin PCRapace purification kit (Invitek, Germany) and sequenced in both directions. The sequencing reactions were carried out using the ABI Big Dye Sequencing kit v.3.1, according to the manufacturer's instructions. Sequence trace files were generated using an ABI 3100 genetic analyzer sited at Rhodes University. Trace files were checked and edited using Genestudio Pro v.1.0 (GeneStudio, Inc.). The sequence data were imported into Mega v.4 (Tamura *et al.*, 2007), aligned using the Clustal W algorithm (Thompson *et al.*, 1994) and checked manually.

4.2.2. Phylogenetic analysis

Two sets of analyses were undertaken, the first on the mitochondrial gene dataset (MT) for each sample and the second on two representative taxa for each of the major mitochondrial clades with the combined nuclear and mitochondrial datasets (ALL).

To ascertain whether the data from each gene region could be combined into a single data set, the Incongruence Length Difference (partition homogeneity) test (Farris *et al.*, 1994) with 1000 replicates was conducted in PAUP* v.4.0b10 (Swofford, 2003) with invariant characters removed (Cunningham, 1997) as the number of variable characters differed between each gene region.

Parsimony analysis was conducted using PAUP* as follows: a simple heuristic search with TBR branch swapping enforced was conducted to approximate the length of the shortest tree. Following this a heuristic search with 1000 random addition replicates was conducted, starting from random trees and keeping a single tree less than or equal to the shortest tree, for each replicate. If the second search found shorter trees the process was repeated until no shorter trees were found. A strict consensus tree was then generated from the resulting parsimony trees. Confidence in each node was assessed with 100 full heuristic bootstrap replicates in PAUP*.

The most appropriate model of sequence evolution for the Bayesian analysis was selected for each of the partitions in the two datasets (MT and ALL) using the AIC test (Akaike, 1974) as implemented in MrModeltest v.2.2 (Nylander, 2004), models selected are summarised in Table 4.1.

Partitioned Bayesian analyses were conducted using MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001) carried out on the University of Oslo Bioportal (www.bioportal.uio.no). Although there is no definitive method for partitioning a dataset, the datasets were partitioned first by gene, then by coding properties (introns or exons). In addition all protein coding regions were partitioned by codon position to incorporate rate variation (Marshall *et al.*, 2006). This resulted in three partitioning strategies (no partition; each gene; each gene and each codon) for both the MT and ALL datasets The effect of partitioning strategy was estimated using pairwise comparison of Bayes Factors (*sensu* Nylander *et al.*, 2004; Brandley *et al.*, 2005) with $2\ln BF_{A\text{-}B} \geq 10$ indicative of strong support for partitioning strategy A vs B, following Kass & Raftery (1995). Each analysis comprised four independent runs of 10 million generations each, using random starting trees with four chains (one cold, three hot), sampling every 1000 generations. Model parameters for each partition as selected by MrModeltest (Table 4.1) were achieved using the "Lset nst= rates=; Prset statefreqpr=" commands. A branch length prior was set using "brlenspr = Unconstrained : Exponential (500.0)". All other parameters (excluding branch lengths and topology) were unlinked across partitions using the "unlink" command. Stationarity in each analysis was assessed using the potential scale reduction factor (PSRF) data and plots of likelihood scores, tree length and average standard deviation of split frequencies against generation. These plots showed that the analyses reached stationarity well within the first 1 million generations. Thus the first 1000 trees generated in each analysis were discarded as "burn-in", ensuring that only trees generated at stationarity were used to calculate the posterior probabilities.

4.2.3. Landscape genetic analysis

To assess the spatial component of the genetic structure three alternatives were tested: a) Genetic structure present due to isolation by distance; b) Genetic structure present due to catchments and associated watersheds; c) Genetic structure present due to other undefined barriers. The COI dataset was used for all analyses as it is the most informative. Primary and secondary catchments were identified based on South Africa's Water Research

Commission's river region classification (Midgley *et al.*, 1994). The contribution of isolation by distance (IBD) was assessed using the Mantel test (Mantel, 1967) as implemented in GeneAlEx 6.1 (Peakall & Smouse, 2006) with 999 permutations. While the identification of barriers was carried out using Barrier v.2.2 (Manni *et al.*, 2004), which highlights areas of pronounced genetic discontinuity. For this analysis, samples were connected using Delauney triangulation; barriers were then identified using Monmonier's maximum distance algorithm. The number of barriers to be identified was stipulated *a priori* in Barrier, based on the number of secondary catchments occupied by each of the species concerned (# catchments - 1). To confirm the relative contribution of catchments to population structure, samples were then grouped according to either primary or secondary catchments and AMOVA was conducted on these predefined groups using GeneAlEx with 999 permutations followed by pairwise comparisons of Φ_{pt}.

4.2.4. Molecular dating

The lack of fossil data representative of this group and the absence of an adequate geological vicariance event to calibrate the molecular clock resulted in the use of previously estimated rates of sequence evolution for the COI region. Although the application of a global molecular clock is problematic (Heads, 2005), the COI region is suggested to be the most reliable gene when enforcing a molecular clock for insects because it possesses the most consistent rates of sequence evolution between lineages (Gaunt & Miles, 2002). The COI data were tested for the applicability of a molecular clock using the likelihood ratio test (LRT). Two separate Maximum Likelihood analyses were conducted in PAUP* using the model settings as selected in Modeltest v.3.7 (Posada & Crandall, 1998) with or without the clock enforced. A Chi-squared test of the difference between the two likelihood values ($\alpha = 0.05$) was then used to assess whether or not the molecular clock significantly influenced the ML analysis (Felsenstein, 1981).

The mean rate of sequence evolution was set at 2.3 % per MY using the estimate of Brower (1994). The age of each node and its corresponding 95 % confidence interval were then estimated under the GTR+I+G model as suggested by MrModeltest using an uncorrelated lognormal (UCLN) relaxed clock in BEAST v.1.4.8 (Drummond & Rambaut, 2007). The data was partitioned into three codon positions with the substitution model, rate heterogeneity model and base frequencies unlinked; in addition a Yule speciation prior and a UPGMA

starting tree were used. This analysis was run using the resources of the Computational Biology Service Unit, Cornell University (http://cbsuapps.tc.cornell.edu/beast.aspx), with two independent runs of 20 million generations, following a discarded burn-in of 200 000 generations. Stationarity and the estimation of the effective sample size (ESS) in the analysis were confirmed by inspection of the MCMC samples using Tracer v.1.4 (Rambaut & Drummond, 2007). The two runs were combined using LogCombiner and then summarized in TreeAnnotater, part of the BEAST package.

4.3. Results

4.3.1. Data characteristics

The final molecular data set comprised 2842 base pairs, of which 1524 comprised mitochondrial COI and 16S data. The EF1α data was made up of portions of both exons (3, 4 & 5) and introns, and the CAL data comprised one intron. All coding portions of the gene datasets aligned readily while gaps corresponding to insertion or deletion events were included in the 16S, EF1α and CAL datasets. As indels were parsimony informative, they were coded as binary characters and used in all analyses; multiple-base indels were treated as one character when recoded. Sequence characteristics and models selected for each partition are summarised in Table 4.1.

Table 4.1. Mitochondrial and nuclear DNA data characteristics showing data partitioning and model choice.

Partition	No. Sites	No. Variable	No. Pars Info	% Pars Info	Model
MT	1526	337	248	16.3	GTR + I + G
COI	1022	260	191	18.7	GTR + I + G
COI_1	341	46	28	8.2	HKY + G
COI_2	341	31	12	3.5	F81 + I
COI_3	340	183	151	44.4	GTR + I + G
16S	504	77	57	11.3	HKY + I
16S_gene	497	72	52	10.5	HKY + I + G
16S_indel	7	7	7	100.0	Mk
EF1a_exon	419	13	7	1.7	HKY + I
EF1a_intron	235	21	13	5.5	HKY
EF1a_indel	17	17	14	82.4	Mk
CAL_intron	637	30	23	3.6	GTR + G
CAL_indel	10	10	8	80.0	Mk
TOTAL	2842	428	313	11.0	-

Of the 2842 base pairs, 428 characters were variable (15 %); of these 313 (11 %) were parsimony-informative including outgroups. The ILD tests indicated that the data sets were

not significantly different so the individual gene datasets were combined into two datasets (MT: p = 0.72; ALL: p = 0.93). All analyses of the MT and ALL data sets resolved seven well-supported clades within the group, but the topological arrangement of some of the clades lacked support in the analyses (Figures 4.1 & 4.2).

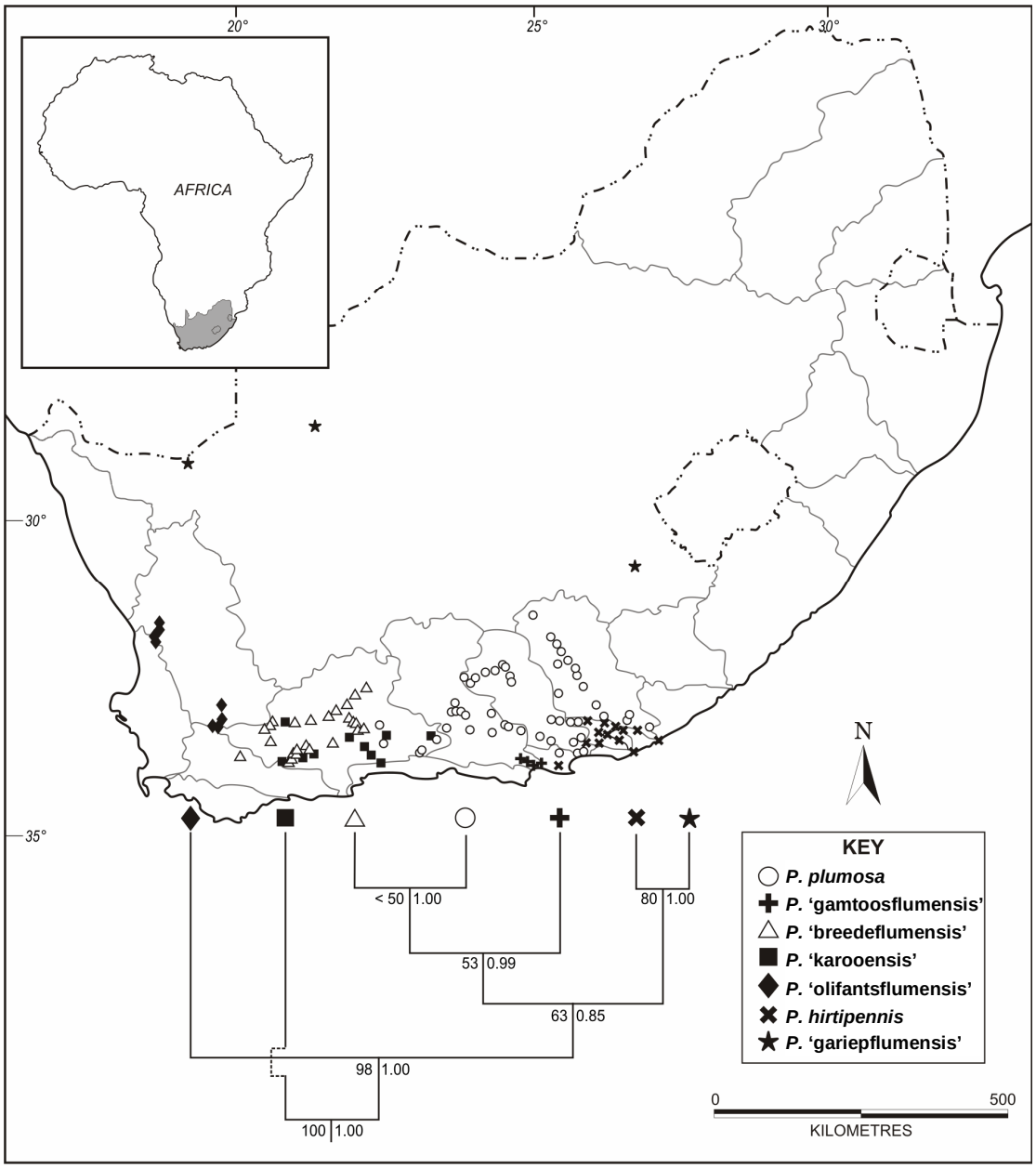

Figure 4.1. Sample localities with primary catchments delimited and cladogram representing the most parsimonious tree and majority rule Bayesian cladogram of all data combined. Bootstrap (left) and posterior probability (right) support are indicated at each node.

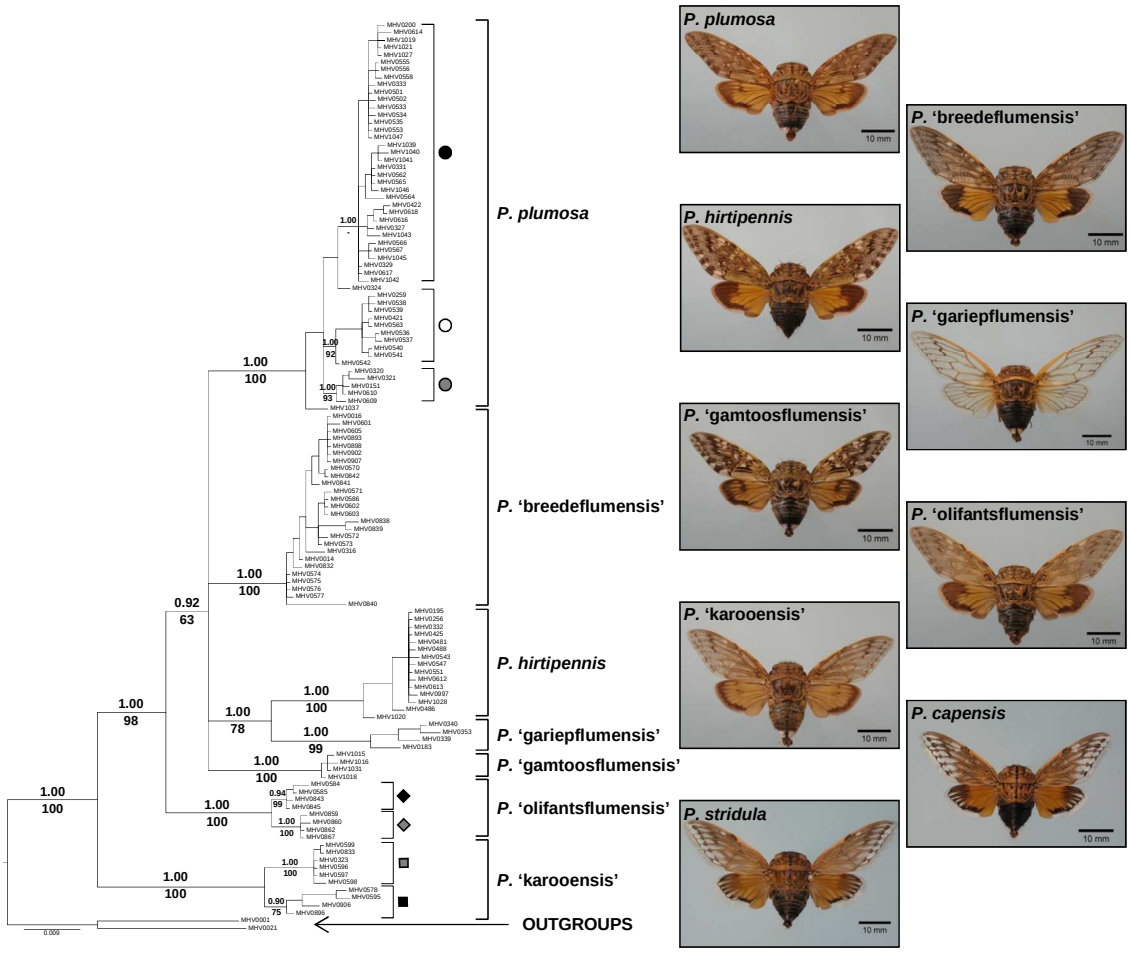

Figure 4.2. Majority rule Bayesian phylogram based on the mitochondrial (MT) dataset, images depict the gross morphology of the species within the group. Symbols represent well supported clades and correspond to Figure 4.4.

4.3.2. Phylogenetic analysis - mitochondrial dataset

Parsimony analysis of the mitochondrial data yielded 19 most-parsimonious trees with a tree length of 496 steps (CI = 0.591; RI = 0.949). Comparison of the three partitioning strategies suggested that partitioning data by gene provided a slightly better log likelihood than not partitioning the data ($2\ln BF_{1\text{-}2} = 7.8$). However, partitioning the data by gene and codon provided a significantly better log likelihood than either not partitioning the data ($2\ln BF_{1\text{-}4} = 11.4$) or partitioning the data by gene alone ($2\ln BF_{2\text{-}4} = 11.0$), thus the Bayesian analysis that utilized the data partitioned by both gene and codon is presented. The majority rule Bayesian phylogeny (Figure 4.2) recovered the monophyly of each proposed species, yet failed to support many of the ingroup relationships, resulting in a polytomous phylogram for the group (Figure 4.2).

4.3.3. Phylogenetic analysis - combined dataset

Parsimony analysis of the combined mitochondrial and nuclear data recovered the monophyly of the proposed seven species, yielding one most-parsimonious tree with a tree length of 406 steps (CI = 0.685; RI = 0.763). The Bayesian analysis recovered the monophyly of each species with the same topology as the parsimony analysis; however the level of support of some nodes differed from the parsimony analysis (Figure 4.1).

4.3.4. Landscape genetic analysis

Catchment association was strong at the species level, with five of the seven species each being restricted to a single primary catchment (Hypothesis 1, Figure 4.1) with only one example of primary catchment sympatry involving *P. plumosa*, *P.* 'karooensis' and *P.* 'breedeflumensis'. *Platypleura plumosa* and *P. hirtipennis* were distributed over multiple primary catchments (Figure 4.1).

At the population level, isolation by distance was not a significant factor in the population structure of any species (Table 4.2), except for *P.* 'olifantsflumensis' ($R^2 = 0.8272$; $p = 0.008$) and to a much lesser extent *P. plumosa* ($R^2 = 0.0115$; $p = 0.037$).

Table 4.2. AMOVA analysis showing contribution of catchments to population structure (Φ_{pt}) and corresponding probability values (p). The contribution of isolation by distance (R^2) and corresponding probability values (p) are shown for comparison. An asterisk (*) indicates a reduction in the number of populations tested due to inadequate sample sizes in some catchments (catchments not numbered in Figure 4.4). Pairwise Φ_{pt} and corresponding probability values for analyses with more than two populations below are shown in the supplementary material. Bold values indicate a significant effect ($\alpha < 0.05$).

			% Variation				IBD	
Species	**catchment**	**# populations**	**Between**	**Within**	**Φ_{pt}**	**p**	**R^2**	**p**
P. plumosa	1°	5*	31	69	**0.313**	**0.009**	**0.0115**	**0.037**
	2°	11*	57	43	**0.575**	**0.001**		
P. hirtipennis	1°	3*	0	100	-0.010	0.476	0.0043	0.321
	2°	0*	-	-	-	-		
P. 'karooensis'	1°	1	-	-	-	-	0.0004	0.422
	2°	2	80	20	**0.800**	**0.003**		
P. 'gariepflumensis'	1°	1	-	-	-	-	0.0045	0.650
	2°	0*	-	-	-	-		
P. 'olifantsflumensis'	1°	1	-	-	-	-	**0.8272**	**0.008**
	2°	2	81	19	**0.815**	**0.032**		
P. 'breedeflumensis'	1°	1*	-	-	-	-	0.0027	0.240
	2°	2*	15	85	**0.149**	**0.001**		
P. 'gamtoosflumensis'	1°	1	-	-	-	-	0.0314	0.415
	2°	0*	-	-	-	-		

The Barrier analyses (data not shown) highlighted barriers to gene flow which corresponded to catchment watersheds in *P.* 'olifantsflumensis' (one barrier, corresponds with secondary catchment watershed), *P.* 'karooensis' (one barrier, corresponds with secondary catchment watershed) and *P. plumosa* (ten barriers, one corresponds to a primary catchment watershed and four correspond to secondary catchment watersheds). The barriers highlighted by Barrier showed little correspondence with primary or secondary catchments for *P. hirtipennis* (four barriers, one corresponds to a primary catchment watershed) and did not correspond to any secondary catchments for *P.* 'breedeflumensis' (two barriers, no correspondence to the secondary catchment watersheds).

Grouping samples by catchment resulted in the identification of groups that were significantly different using AMOVA for *P. plumosa* (primary and secondary catchments), *P.* 'karooensis' (secondary catchments), *P.* 'olifantsflumensis' (secondary catchments) and *P.* 'breedeflumensis' (secondary catchments), although this effect was weak in *P.* 'breedeflumensis' (Table 4.2); no significant variation was found for *P. hirtipennis*, calculations were not performed for *P.* 'gariepflumensis' and *P.* 'gamtoosflumensis' due the restriction of these samples to one secondary catchment each (Table 4.2; Figure 4.1). Population parameters and Φ_{pt} values are summarised in Table 4.2; pairwise Φ_{pt} values for species inhabiting more than two catchments can be found in the supplementary data (*P. plumosa*: Tables A7 & A8; *P. hirtipennis*: Table A9).

4.3.5. Molecular dating

Maximum Likelihood analysis of the COI data under the GTR+I+G model with and without a clock resulted in two likelihood values (clock: lnL = -4314.9564; no clock: lnL = -4230.8537). A chi-squared test of the difference in likelihood values was significant (2lnL = 168, df = 119, p = 0.02), so the dating analysis used a relaxed clock in BEAST. The dating estimates suggest that cladogenesis in the group began with the split between *P.* 'karooensis' and the remaining species approximately 2.3 mya, followed by a radiation with rapid cladogenesis occurring at about 1.9 mya that produced all of the major primary catchment-associated lineages except for *P. hirtipennis* and *P.* 'gariepflumensis', which are tentatively estimated to have diverged at 1.2 mya. Thus, by 1.2 mya all of the putative species had been formed (Figure 4.3). Within each putative species the TMRCA ranges between 0.3 mya (*P.* 'gamtoosflumensis') and 1.1 million years ago (*P.* 'karooensis'; Figure 4.3).

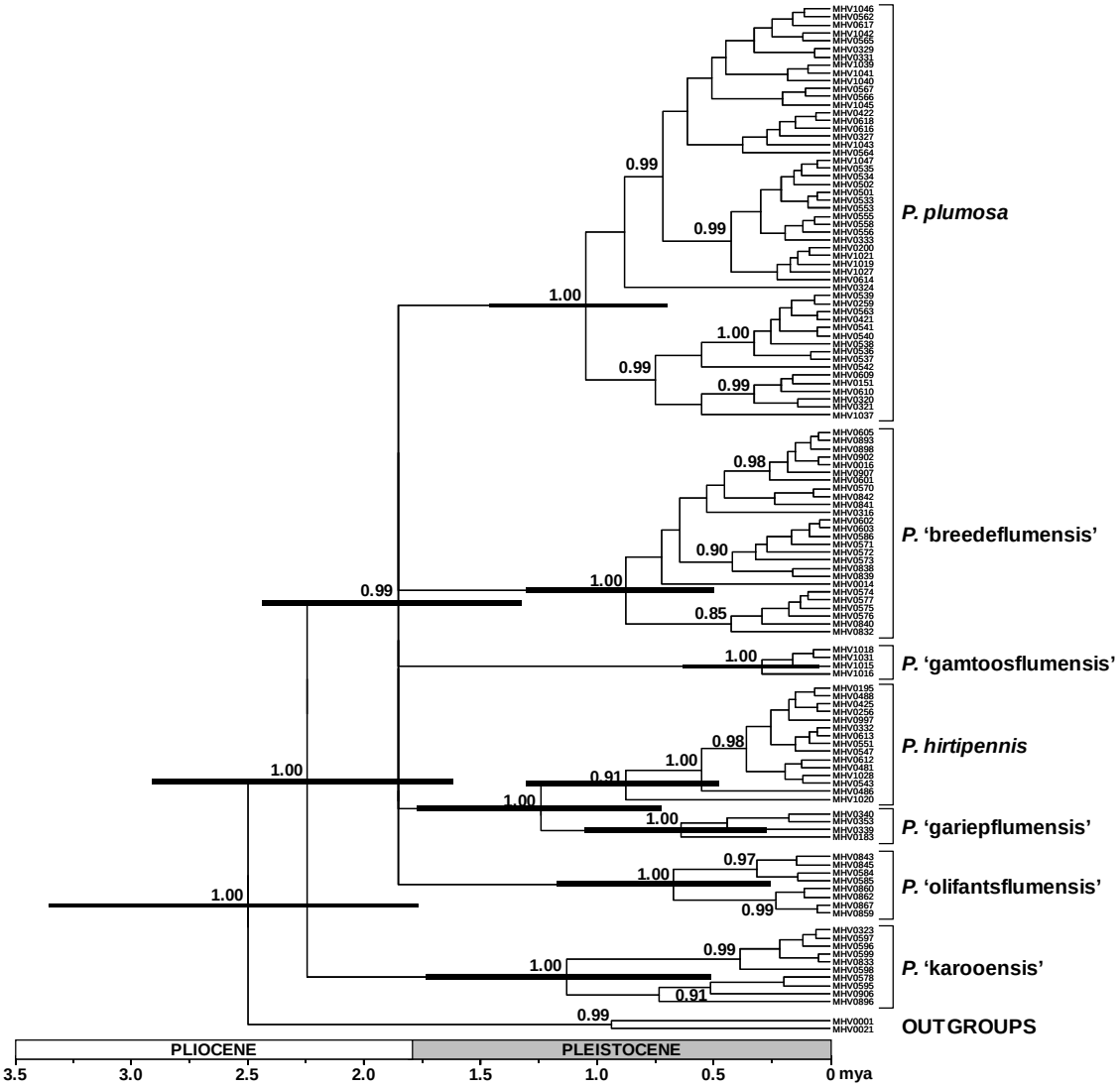

Figure 4.3. Majority rule Bayesian chronogram generated in BEAST using the COI data. Posterior probabilities of major nodes greater than 0.85 are shown. Nodes are centred on the mean TMRCA with shaded bars indicating the distribution of the 95 % HPD for each estimate, only those discussed in the text are shown.

4.4. Discussion

4.4.1. Catchments and watersheds as drivers of diversification

At the population level, isolation by distance was found to be significant only in *P. plumosa* and *P.* 'olifantsflumensis', but the contribution of IBD is small in *P. plumosa* and the samples of *P.* 'olifantsflumensis' are essentially from two distinct geographic points and thus the apparent IBD is not surprising (Hypothesis 3). At least some of this may be explained by the habits of the males, which are mutually antagonistic. Calling males of *P. plumosa* and

P. 'breedeflumensis' have been observed flying between branches to confront conspecific males, and were often so engrossed in fighting that these normally alert and wary insects could be hand-picked during fights. Fights often result in a male flying to another tree, which would spread genes across the landscape and promote oligomixis. It is not known how mobile females are, but they search for males (Villet & van Noort, 1999) and possibly travel a greater distance than the males in their lifetimes. When pursued by predators such as birds, cicadas of the *P. plumosa* group will often fly tens of metres from tree to tree to avoid capture.

Previous studies that have found an effect of catchment on population structure have focussed on riverine plants (e.g. hydrochory: Levin *et al.*, 2003), aquatic organisms (e.g. fiery redfin barbs: Swartz *et al.*, 2007) or organisms that are small and very habitat-specific (e.g. springtails: Garrick *et al.*, 2007; leaf litter frogs: Measey *et al.*, 2007). Although the effect of catchments on cicadas has been mentioned in the Cape Floristic Region (Chapter 3; Price *et al.*, 2007), the catchments in this case were circumscribed by the high Cape Fold mountains. This study highlights the effect that catchments that are not as divided by such dramatic geological features may yet have on a large, volant terrestrial invertebrate.

Evidence for catchment-driven biodiversification can be seen in the pattern of most species being restricted to one (*P.* 'karooensis', *P.* 'gariepflumensis', *P.* 'olifantsflumensis', *P.* 'gamtoosflumensis') or two (*P.* 'breedeflumensis') primary catchments with *P. hirtipennis* being distributed across five (very small) primary catchments and *P. plumosa* being distributed across four (large) primary catchments (Hypothesis 1; Figure 4.4). In addition, the predominantly allopatric distribution of species in the group, with only two examples of sympatry (*P.* 'karooensis' and *P. plumosa*; *P.* 'karooensis' and *P.* 'breedeflumensis') suggests vicariant processes resulting in speciation within the group (Figure 4.4). At the population level the effect of catchment cannot be examined for *P.* 'gariepflumensis' and *P.* 'gamtoosflumensis' as their distributions are encompassed by one secondary catchment each. With the remaining species the correspondence between genetic structure and catchment type (either primary or secondary) is more interesting and is dependent on the lineage examined, with examples of strong correspondence (*P.* 'karooensis', *P.* 'olifantsflumensis' and *P. plumosa*) and examples of little correspondence (*P. hirtipennis* and *P.* 'breedeflumensis'; Hypothesis 1 & 2; Figure 4.4). The catchment effect is more

pronounced in species inhabiting the more arid western part of South Africa, most likely as a result of the restriction of host plants, specifically *Acacia karroo*, to drainage lines. If host plants are restricted to drainage lines, the opportunities for short range dispersal between catchments is reduced in these areas. This influence of present day aridity on host plant restriction points to the mechanism of fragmentation whereby arid periods would likely have resulted in a contraction of host plant ranges to drainage lines, restricting gene flow between catchments and promoting divergence.

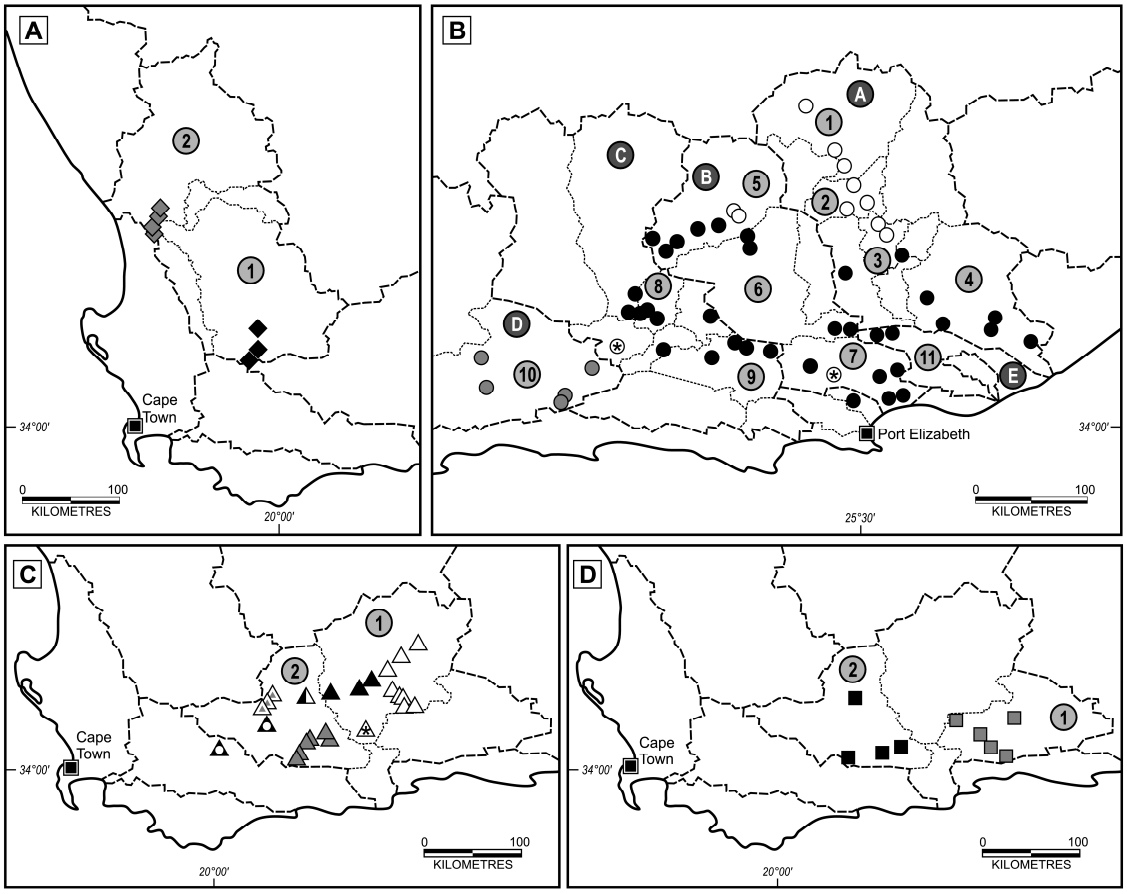

Figure 4.4. Primary (bold) and secondary (faint) catchment delimitation of species showing distinct genetic structure. (A) *P.* 'olifantsflumensis', (B) *P. plumosa*, (C) *P.* 'breedeflumensis', (D) *P. karooensis*. In all maps circled numbers denote secondary catchments. In map (B) circled letters denote primary catchments: (A) Fish, (B) Sundays, (C) Gamtoos, (D) Gouritz and (E) Albany. Samples were assigned separately to primary and secondary catchments for the AMOVA analyses. Symbol shading corresponds to supported clades in the MT analyses as highlighted in Figure 4.2, sites marked with (*) are not found in supported clades.

The contrasting patterns shown by *P.* 'karooensis' and *P.* 'breedeflumensis' are especially interesting because they inhabit the same primary catchment in the arid western Karoo and would be exposed to identical climatic processes as they are of similar ages (Figure 4.3). Thus, this is an example of a common process with two different results. One factor that may explain the contrasting pattern is that *P.* 'karooensis' is found restricted to the "Little Karoo" bounded by the Swartberg (North) and Langeberg (South) mountain ranges whereas *P.* 'breedeflumensis' primarily inhabits the "Great Karoo" region to the north of the Swartberg and is restricted in distribution in the "Little Karoo" to the western half (Figure 4.4). This emphasizes the effect that the Swartberg mountains may have in this catchment, as the host plants are restricted to drainage lines, resulting in the mountains (which are secondary watersheds) acting as barriers to dispersal.

Within the most widely distributed taxon, *P. plumosa*, the molecular data show three distinct groups (Figures 4.2 & 4.4 B), the first consisting of samples from the Gouritz catchment, the second from the Gamtoos, Sundays and lower Great Fish catchments, and the third from the "Cradock Gap" above the escarpment (Figure 4.4 B), suggesting an interaction between catchments and the escarpment in population structuring. Although all pairwise comparisons of Φ_{pt} for primary catchments were significant except comparisons involving catchment E (Figure 4.4 B), the reduced affect of catchments in the eastern part of the distribution is most likely due to the moister climate resulting in the host plants, particularly *A. karroo*, spreading from the drainage lines and thus enabling dispersal between catchments.

The sister relationship between *P. hirtipennis* and *P.* 'gariepflumensis' does not fit Hypothesis 2 because they are separated by a large catchment which is inhabited by *P. plumosa*. This relationship between species inhabiting the Orange river system (e.g. *P.* 'gariepflumensis') and species inhabiting the Eastern Cape (e.g. *P. hirtipennis*) has been indicated in the phylogeny of fish (redfin barbs: Swartz *et al.*, 2009) which suggests a palaeoriver linkage between these regions. The headwaters of the Fish and Orange rivers are in close proximity (< 50 km) and, when coupled with regional Pliocene uplift (~ 2.5 mya, ~ 900 m: Partridge & Maud, 1987; Artyushkov & Hofmann, 1998), it is plausible that these rivers were connected before the uplift altered the course of the upper Orange onto its present course; this hypothesis however requires independent geological evidence.

Within *P. hirtipennis* primary catchments do not influence population structure (Table A9). This is most likely due to the widespread nature of *A. karroo* in these catchments, a result of the combination of a moister climate relative to the arid western catchments, and the small nature of catchments in this region (Figure 4.1) promoting host-enabled dispersal between catchments.

The dating of the *P. plumosa* group indicates rapid cladogenesis at the Plio-Pleistocene boundary (~ 1.9 mya) resulting in the seven major lineages in the group. Thus this arid-adapted group radiated well after the three biomes they inhabit originated (in the Eocene and late Miocene: Mucina & Rutherford, 2006). This pattern of rapid radiation is broadly congruent with the *P. stridula* group (Chapter 3; Price *et al.*, 2007) in the Cape Floristic Region and indicates that common processes may be responsible for shaping the history of these two groups of cicadas even though they inhabit vastly different biomes.

The population structuring within each putative species can be dated to the recent Pleistocene (1.1 mya - 0.3 mya). Within South Africa the effects of Pleistocene climate oscillations in general and aridification in particular were substantial, and have been cited as the cause of genetic structuring in a range of taxa in the fauna of the Succulent and Nama-Karoo: mammals (Prinsloo & Robinson, 1992; Matthee & Robinson, 1996, 1997; deMenocal, 2004; Kryger *et al.*, 2004; Smit *et al.*, 2007); birds (Freitag & Robinson, 1993; Ryan & Bloomer, 1997, 1999); reptiles (Daniels *et al.*, 2002; Matthee & Flemming, 2002; Tolley *et al.*, 2004, 2006; Makokha *et al.*, 2007; Swart *et al.*, 2008; Tolley *et al.*, 2008); insects (Sole *et al.*, 2005; Damgaard *et al.*, 2008) and plants (Bergh *et al.*, 2007; Howis *et al.*, 2009). Thus it is plausible that the aridification in response to climatic oscillations resulted in the speciation events and to some extent also the pattern that is visible today at the population level.

Although the dating analyses lack sufficient resolution to show conclusively whether aridification was responsible for cladogenesis within the group (Hypothesis 4), evidence that this may be a factor includes the restriction of host plants to drainage lines in the more arid western ranges. It is thus plausible that the host plants would be restricted in distribution closer to drainage lines in arid glacial periods of the Pleistocene, resulting in population fragmentation and the current population structure. A more rigorous test of Hypothesis 4 must await better chronometric tools or a more tractable case study.

4.4.2. Conservation implications

Although the effect of catchments in structuring the populations of cicadas in this complex is unexpected, the interaction of poor dispersal ability, arid landscapes and Pleistocene climate cycling has resulted in clear examples of terrestrial species being bounded by catchments and their associated watersheds. The use of catchments as management units for conservation has been proposed for riverine organisms (South African National Water Act, 1998). Although it is evident that catchments do not describe every species' genetic variation (e.g. Hughes *et al.*, 1998, 1999; Wishart, 2000), it would be appropriate to incorporate catchment structure should a conservation plan for the *Platypleura plumosa* complex be developed. Given that the immediate future seems likely to be dominated by fluctuations in temperature and rainfall, and that the biota will respond to these on a catchment basis, catchment-based conservation has an obvious strategic appeal.

4.5. Conclusion

This study highlights the important role that catchments may play in shaping genetic structure, not only in aquatic organisms as previously thought, but also of terrestrial organisms capable of flight and suggests the incorporation of catchment data into conservation plans. Furthermore the study has confirmed the cryptic diversity within this complex and suggests further study of the invertebrates in these semi-arid and arid regions of South Africa for comparative purposes.

5 From tree to tree: comparative phylogeography of two forest-dwelling cicada lineages in South Africa

5.1. Introduction

Forests are considered the second most botanically rich of South Africa's nine biomes (Mucina & Rutherford, 2006), containing an estimated 7 % of South Africa's vascular plant diversity (Hughes *et al.*, 2005). Incredibly, this diversity is contained in the smallest biome, covering less than 3000 km^2 or 0.1 % of South Africa (Mucina & Rutherford, 2006). Besides their small area and highly fragmented distribution, forests face many threats including overutilization, encroachment by alien vegetation, increased fire regimes, and competition with agriculture and sylviculture. Thus the forest biome is of high priority for conservation, and understanding forest dynamics is crucial in the support of that goal.

Forests are thought to have dominated southern Africa prior to the mid-Miocene, with a reduction in distribution into the Pliocene in response to a cooling global climate (Linder *et al.*, 1992; Scott, 1995; Linder, 2003). However, within the more recent Pleistocene epoch (1.8 mya - present) dramatic changes in South Africa's climate in response to global glacial cycles (Dynesius & Jansson, 2000; Jansson & Dynesius, 2002), in particular the alternation between cool-dry glacial or interpluvial and warm-wet interglacial or pluvial periods, had a dramatic effect on the distribution and abundance of the region's forests (Meadows & Linder, 1989; deMenocal, 1995; Eeley *et al.*, 1999; Partridge *et al.*, 1999; Fattorini, 2007) and provided an impetus for allopatric speciation (Jansson, 2003; Bellsted & Edwards, 2004). Indeed, large-scale historical processes are thought to have shaped the floral and faunal assemblages of South African forests (van Wyk, 1989, 1990; Lawes, 1990; Bellstedt & Edwards, 2004; Hughes *et al.*, 2005; Lawes *et al.*, 2007). The influence of these changes on the evolution of South African plant and animal taxa is thought to be dramatic (Ewer & Cooke, 1964; Grant & Leslie, 1993; Kryger *et al.*, 2004), but to date no studies on invertebrate taxa have been undertaken.

The twelve types of forest currently recognised within the South African forest biome are partitioned into nine zonal: Scarp, Afrotemperate (Northern and Southern), Mistbelt (Northern and Southern), Coastal (Northern and Southern), Sand and Ironwood; and three azonal: Lowveld Riverine, Swamp and Mangrove elements (Mucina & Rutherford, 2006). Of these forest types, Scarp forest contains the highest plant diversity and the most species, sharing species with both Coastal and Afrotemperate forest types (Eeley *et al.*, 1999) and containing many paleo-endemic lineages that suggest an earlier origin of this forest type (Mucina & Rutherford, 2006). Mistbelt forests are not as species-rich as Scarp forests but share floristic elements with Afrotemperate and extralimital subtropical forests; the Northern Mistbelt forests are thought to have been drastically affected by the LGM and have only re-established approximately 6 kya (Deacon & Lancaster, 1988; Scott *et al.*, 1997). Afrotemperate forests are generally thought of as species-poor in comparison to Scarp and Mistbelt forests (Mucina & Rutherford, 2006). Within the coastal forests, the Northern Coastal forests contain many tropical species and are more species-rich than the impoverished Southern Coastal forests, suggesting that these latter coastal forests are evolutionarily very young (Mucina & Rutherford, 2006).

Lawes *et al.* (2007) suggest that the effects of the Last Glacial Maximum (LGM: 21 ± 2 kya; Partridge *et al.*, 1999; Mix *et al.*, 2001; Gasse *et al.*, 2008) were more severe on the Afrotemperate forests than on Scarp forests because the latter were able to persist in refugia. This is in contrast to those taxa occupying the Coastal forest belt, which are thought to be recent colonizers since the LGM due to the relatively young age of Coastal forests (Martin, 1968; Hobday, 1976; Tinley, 1985; MacDevette *et al.*, 1989; Lawes, 1990). However, the Coastal forests of the Eastern Cape are thought to have been important refugia during the LGM (Stuckenberg, 1969; Lawes, 1990; Lawes *et al.*, 2007). Thus two processes are thought to be responsible for the current patterns of plant diversity in South Africa's forests: (1) filtering of the community by extinction due to climatic change and (2) recolonization after the LGM (Lawes *et al.*, 2007).

Cicadas have previously been shown to be useful tools when examining the geographic partitioning of genetic structure that may result from processes such as climate cycling (Buckley *et al.*, 2001; Buckley & Simon, 2007; Price *et al.*, 2007, 2010). Their ability to survive in small, isolated populations, thus reducing the chances of local extinctions and

preserving the continuity of their phylogeographical signal, makes them good candidates for inferring the history of forests in South Africa. Comparative phylogeography of phylogenetically independent sympatric taxa aims to infer the local processes underlying their diversification from their current geographic patterns of genetic diversity (Feldman & Spicer, 2006). It can assist in evaluating the regions important to the maintenance of biodiversity (Tolley *et al.*, 2008), yet studies of this nature are limited in South Africa (Desmet *et al.*, 2002).

Within South Africa, two groups of large-bodied cicadas (tribe: Platypleurini) inhabit forests: the *Platypleura chalybaea* group and *Pycna semiclara* Germar. The most recent common ancestor between these two groups is estimated at ~ 33 mya (Chapter 2, unpublished data). The use of these phylogenetically independent taxa will greatly enhance our understanding of the origin and evolution of South Africa's forest taxa.

Within the *Pl. chalybaea* group there are two nominal species, *Pl. chalybaea* Villet and *Pl. brunea* Villet, and one species awaiting formal description, *Pl.* 'intercapedinis'. *Platypleura chalybaea* and *Pl. brunea* are both restricted in distribution to the Eastern Cape and are differentiated primarily by host plant association, with *Pl. chalybaea* found almost exclusively on *Euphorbia triangularis* and *E. tetragona* and *Pl. brunea* found on various thick-trunked tree species including the introduced *Salix babylonica* and *Platanus* sp. In contrast to this restricted distribution, *Pl.* 'intercapedinis' is found in coastal and inland forests extending from the Eastern Cape to the borders of Mozambique and Zimbabwe and is associated with a variety of tree species, including *Bridelia microantha*, *Dichrostachys cinerea*, *Harpephylum caffrum*, *Zanthoxylum davyi* and *Trichilia emetica*. *Pycna semiclara* is a large-bodied cicada with a similar distribution to *Pl.* 'intercapedinis' and is found associated with a wide variety of tree species (Villet & Reavell, 1989), including the indigenous *Ficus sur* and *Olinia emarginata*, as well as the introduced *Eucalyptus* sp., *Pinus* sp., *Quercus robur*, *Platanus* sp., and *Salix* sp.

Given the small size and highly fragmented nature of forests in South Africa (Figure 5.1; Mucina & Rutherford, 2006), the platypleurine cicadas provide a useful tool to examine the effects of habitat changes on the population dynamics of forest invertebrates using two independent case studies. Thus the aim of this study was to compare and contrast the

biogeographic history of these two forest-associated cicada lineages. This enabled three hypotheses to be tested: (1) currently recognised forest types adequately delimit the population structuring in both cicada groups; (2) the timing of population fragmentation is primarily concordant with Pleistocene climatic oscillation; (3) the phylogenetic signal of taxa occupying older Afrotemperate and Scarp forests is greater than that of taxa occupying the younger Coastal forests.

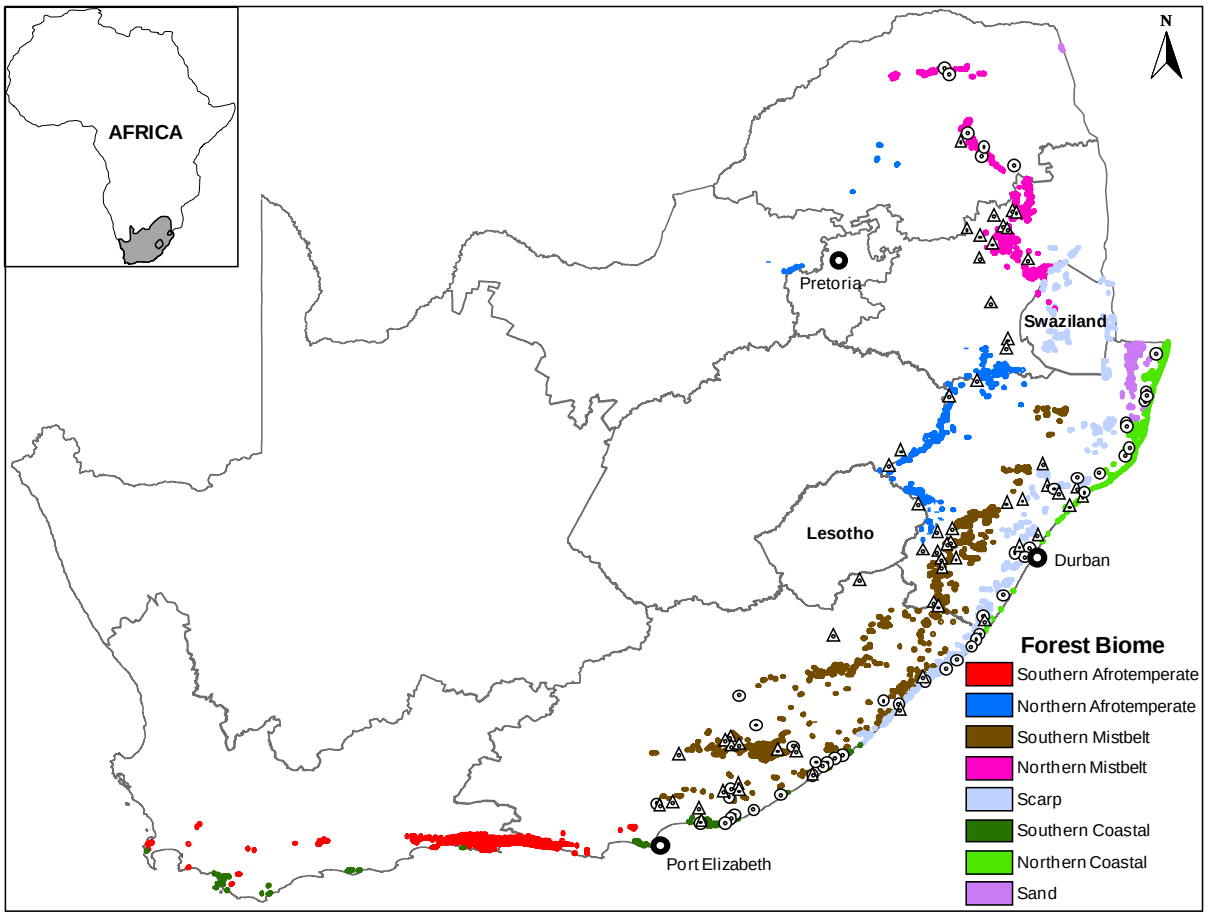

Figure 5.1. Map of South Africa showing the distribution of forest types. Ironwood forest and azonal elements are excluded (redrawn from Mucina & Rutherford, 2006). Sample localities are shown for *Pl. chalybaea* group (circles) and *Py. semiclara* (triangles).

5.2. Methods

5.2.1. Sampling and laboratory protocols

Two cicada species were selected for this study, both of which occur widely in South African forest habitats: *Platypleura* 'intercapedinis' (a species still to be formally described) and *Pycna semiclara* Germar (Figure 5.2). Cicadas were collected into 95 % ethanol from a

total of 118 sites, covering their known geographical ranges (Tables A10 & A11; Figure 5.1). Outgroups for the analyses were chosen from closely related, non-forest inhabiting taxa, based on a phylogenetic analysis of the tribe Platypleurini using both mitochondrial and nuclear genes (Chapter 2, unpublished data; Tables A10 & A11). Samples of *Pl. chalybaea* Villet, *Pl. brunea* Villet and *Pl.* cf. *argentata* Villet (Table A10) were included to test species limits due to their morphological similarity to *Pl.* 'intercapedinis' (Figure 5.2).

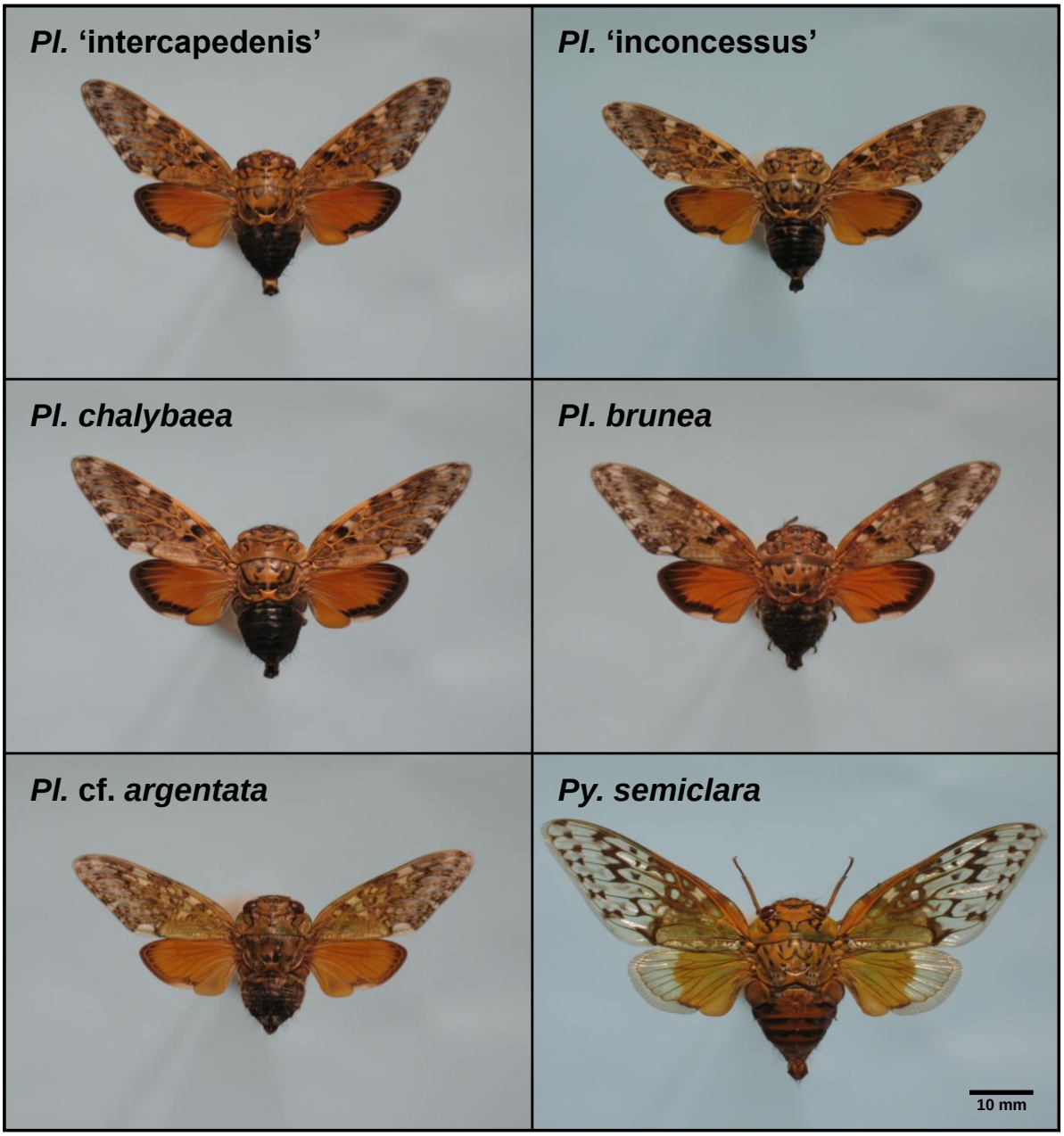

Figure 5.2. Images depicting the gross morphology of the forest associated cicada species used in this study.

Total genomic DNA was extracted from muscle tissues following the Chelex® 100 protocol (Walsh *et al.*, 1991). Small pieces of wing or tymbal muscle tissue (c. 2 mm^3) were homogenised in 300 µl 5 % Chelex® 100 solution and incubated in a heating block at 105 °C for 15 minutes, vortexing the samples every five minutes. Following the incubation period, the supernatant was removed for subsequent use in PCR amplifications.

Portions of mitochondrial Cytochrome Oxidase subunit I (COI) and II (COII) were amplified from each sample (Table A10 & A11). Primers used for amplification and sequencing reactions were COI: COI-F2 (Price *et al.*, 2010) with C1-N-2568 (Brady *et al.*, 2000) and COII: TL2-J-3033 with TK-N-3786 (Simon *et al.*, 1994).

PCR amplifications were performed in 25 - 50 µl reactions using the following protocol: 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 48 °C (COI) or 51 °C (COII) for 45 seconds and extension at 72 °C for two minutes. PCR products were confirmed by electrophoresis of 5 µl PCR product stained with ethidium bromide and 5 µl tracking dye in a 1 % agarose gel, and visualized using a UV trans-illuminator.

PCR products were purified using the Invitek Invisorb MSB® Spin PCRapace purification kit (Invitek, Germany) and sequenced in both directions. The sequencing reactions were carried out using the ABI Big Dye Sequencing kit v.3.1, according to the manufacturer's instructions. Sequence trace files were generated using an ABI 3100 genetic analyzer sited at Rhodes University. Trace files were checked and edited using Genestudio Pro v.1.0 (GeneStudio, Inc.). The sequence data were imported into MEGA v.4 (Tamura *et al.*, 2007), aligned using the Clustal W algorithm (Thompson *et al.*, 1994) and checked manually.

5.2.2. Phylogenetic analysis

Although the mitochondrion is inherited as a single unit, the two gene regions were tested for incongruence using the Incongruence Length Difference (partition homogeneity) test (Farris *et al.*, 1994) with 1000 replicates, conducted in PAUP* v.4.0b10 (Swofford, 2003) with invariant characters removed (Cunningham, 1997) before being combined into each lineage dataset. Each lineage data set (*Pl. chalybaea* group and *Py. semiclara*) was then analysed separately.

Parsimony (MP) analysis was conducted using PAUP* as follows: a simple heuristic search with TBR branch swapping enforced was conducted to approximate the length of the shortest tree. Following this a heuristic search with 1000 random addition replicates was conducted, starting from random trees and keeping a single tree less than or equal to the shortest tree, for each replicate. If the second search found shorter trees the process was repeated until no shorter trees were found. A strict consensus tree was then generated from the resulting parsimony trees. Confidence in each node was assessed with 1000 full heuristic bootstrap replicates in PAUP*.

The most appropriate model of sequence evolution for the Bayesian Inference (BI) analysis was selected for each of the two gene partitions (COI and COII) in the datasets using the AIC test (Akaike, 1974) as implemented in MrModeltest v.2.2 (Nylander, 2004), models selected are summarised in Table 5.1.

Bayesian Inference analyses with the data partitioned by gene were conducted using MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001) carried out on the University of Oslo Bioportal (www.bioportal.uio.no). Each analysis comprised four independent runs of 10 million generations each, using random starting trees with four chains (one cold, three hot), sampling every 1000 generations. Model parameters for each partition as selected by MrModeltest (Table 5.1) were achieved using the "Lset" and "Prset" commands. A branch length prior was set using "brlenspr = Unconstrained : Exponential (100.0)". All other parameters (excluding branch lengths and topology) were unlinked across partitions using the "unlink" command. Stationarity in each analysis was assessed using the potential scale reduction factor (PSRF) data and plots of likelihood scores, tree length and average standard deviation of split frequencies against generation. Based on these plots the first 1000 trees generated in each analysis were discarded as "burn-in", ensuring that only trees generated at stationarity were used to calculate the posterior probabilities.

5.2.3. Landscape genetic analysis

To assess the spatial component of the genetic structure, three alternatives were tested: (a) genetic structure present due to isolation by distance (IBD); (b) genetic structure present due to association with different forest types (*sensu* Mucina & Rutherford, 2006); and (c) genetic structure present due to other undefined barriers to gene flow between forests.

The contribution of IBD was assessed using the Mantel test (Mantel, 1967) in GeneAlEx 6.3 (Peakall & Smouse, 2006) with 999 permutations. To ascertain whether the clades found in the analyses of the molecular data corresponded to the forest types as defined by Mucina & Rutherford (2006), samples were classified according to the forest type from which they were collected (Table A10 & A11; Figure 5.1). Forest types which contained only one sample per lineage were excluded from subsequent analysis. To ascertain the relative genetic variation within forest types, the mean within-group *p*-distance was calculated in Mega v.4 (Tamura *et al.*, 2007); the standard error was estimated using 1000 bootstrap replicates in Mega. The relative contribution of forest types to population structure was estimated using AMOVA of pre-defined forest groups, using GeneAlEx with 999 permutations followed by pairwise comparisons of Φ_{pt}.

Maximum Likelihood (ML) analysis was undertaken using the model parameters highlighted in MrModeltest (Table 5.1) by using the "Lset" command in PAUP*. For each forest type separate likelihood analyses were performed with constraint trees enforced corresponding to the monophyly of samples from that particular forest type only. A further combined analysis constraining all forest types as monophyletic was performed. The effect of constraining the monophyly of forest types was examined by comparing the most likely topology with no constraints against the most likely topology in each constraint analysis using the Shimodaira-Hasegawa (SH) test (Shimodaira & Hasegawa, 1999) with RELL optimization and 1000 bootstrap replicates. The results of the SH test were then confirmed with a likelihood ratio test (LRT) using a Chi-squared test of likelihood scores (Felsenstein, 1981).

5.2.4. Molecular dating

Although there are no fossil data appropriate to constrain nodes in the present analyses, the root height of each tree was constrained using secondary calibrations based on an unpublished fossil-constrained phylogeny of the tribe (Chapter 2).

The data were tested for the applicability of a molecular clock using the likelihood ratio test (LRT). Two separate Maximum Likelihood analyses were conducted in PAUP* using the model settings as selected in MrModeltest with or without the clock enforced. A Chi-squared test of the difference between the two likelihood values ($\alpha = 0.05$) was then used to assess

whether or not the molecular clock significantly influenced the ML analysis (Felsenstein, 1981).

Consequently, the age of each node and its corresponding 95 % confidence interval were then estimated under the HKY model using an uncorrelated lognormal (UCLN) relaxed clock in BEAST v.1.5.2 (Drummond & Rambaut, 2007). The gene regions were combined into one data partition with a Yule speciation prior and random starting trees. The age of the root of each dataset was constrained based on the results of a fossil constrained dating analysis of the tribe Platypleurini (Chapter 2, unpublished data) using the "treeModel.rootHeight" prior with a normal distribution (*Pl. chalybaea* group: mean = 7 mya, SD = 0.5; *Py. semiclara*: mean = 12 mya, SD = 0.5) and a corresponding value for the rate of sequence evolution with the "ucld.mean" prior (*Pl. chalybaea* group = 0.00665 sub/site/my; *Py. semiclara* = 0.00524 sub/site/my). The combination of these two priors was used to ensure adequate ESS values (Simon Ho pers. comm.). Each analysis was run using the resources of the Computational Biology Service Unit, Cornell University (http://cbsuapps.tc.cornell.edu/beast.aspx), with one run of 10 million generations, following a discarded burn-in of 100 000 generations. Stationarity and the estimation of the effective sample size (ESS) in the analysis were confirmed by inspection of the MCMC samples using Tracer v.1.5 (Rambaut & Drummond, 2007). Each run was summarized in TreeAnnotater v.1.5.2 and viewed using FigTree v.1.2.3, both part of the BEAST package.

5.2.5. Rates of diversification within forest lineages

To visualise the temporal pattern of diversification within the two groups, lineage-through-time (LTT) plots, corresponding to the logarithm of the number of lineages plotted against time, were constructed for both the *Pl. chalybaea* group and *Py. semiclara* using Tracer.

5.3. Results

5.3.1. Data characteristics

The final molecular data sets comprised mitochondrial COI and COII data (*Pl. chalybaea* group: 1584 bp; *Py. semiclara*: 1453 bp). Sequence characteristics and model selection for each partition are summarised in Table 5.1. The ILD tests indicated that the data sets were not significantly different (*Pl. chalybaea* group: p = 0.37; *Py. semiclara*: p = 0.93), so the individual gene datasets were combined into the two species datasets for analysis.

Table 5.1. Data characteristics, summarised by data partition, and showing model choice.

Dataset	Partition	No. Sites	No. Variable	No. Pars Info	% Pars Info	Model
Pl. chalybaea group	COI	895	176	97	10.8	GTR+G
	COII	689	131	67	9.7	GTR+I+G
	TOTAL	1584	307	164	10.3	-
Py. semiclara	COI	764	196	84	10.9	HKY+G
	COII	689	138	60	8.7	GTR+G
	TOTAL	1453	334	144	9.9	-

5.3.2. Phylogenetic analysis - Pl. chalybaea group

Parsimony analysis of the *Pl. chalybaea* group's data yielded 65 most-parsimonious trees with a tree length of 316 steps (CI = 0.589; RI = 0.929). Both the strict consensus of the MP analysis (tree not shown) and the majority rule BI phylogeny recovered three well-supported major lineages (Figure 5.3).

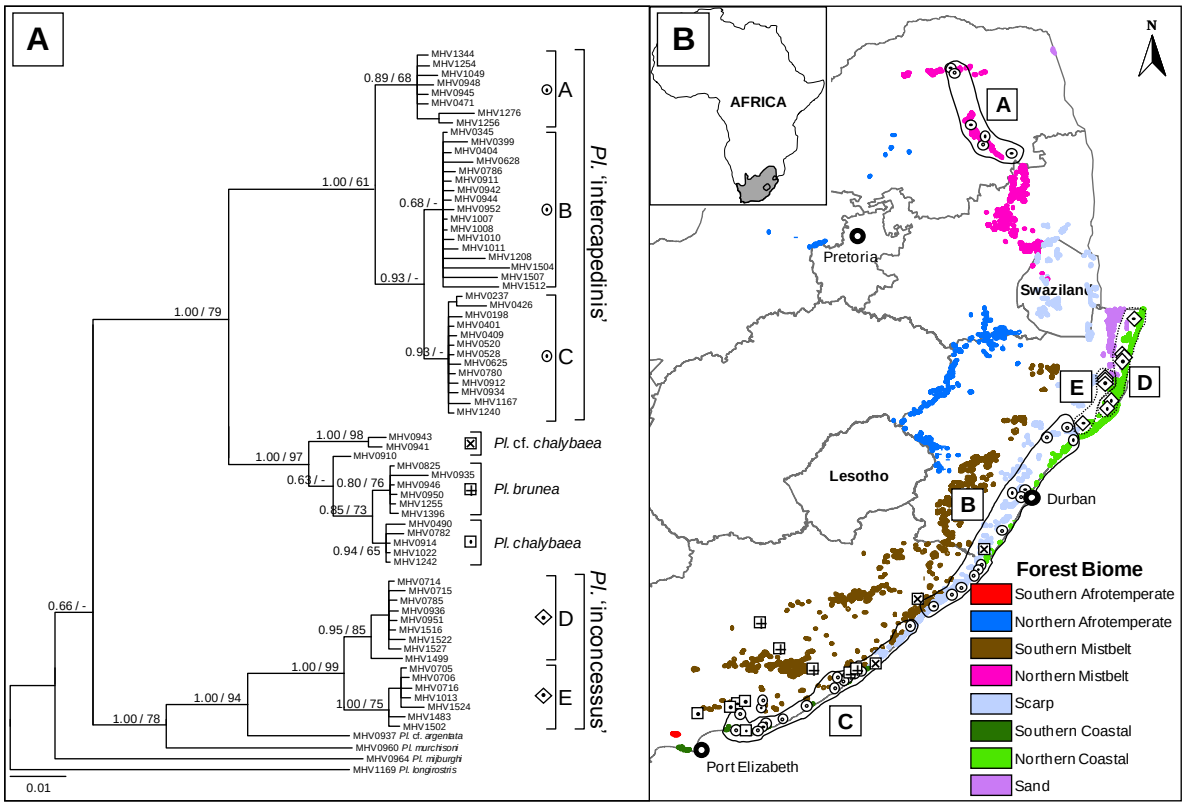

Figure 5.3. (A) Majority rule Bayesian Inference phylogram highlighting major lineages within *Pl. chalybaea* group. Support is indicated above each node (Bayesian posterior probability / Parsimony bootstrap). (B) Distribution of lineages mapped onto corresponding forest types, symbols correspond to part (A).

The remainder of the samples identified initially as *Pl.* 'intercapedinis' fell into two well-supported lineages: the first sister to *Pl.* cf. *argentata* is hereafter informally termed

Pl. 'inconcessus' (species awaiting formal description), and is split into two well supported clades, corresponding to coastal (clade D) and inland (clade E) forest samples from northern KwaZulu-Natal (Figure 5.3). The second lineage *Pl.* 'intercapedinis', sister to the [*Pl. brunea* + *Pl. chalybaea* + *Pl.* cf. *chalybaea*] clade is split into three well-supported clades which correspond to a northern escarpment clade (clade A) and two coastal lineages (clades B and C; Figure 5.3). Within each of the three clades of *Pl.* 'intercapedinis' there is little genetic structure (Figure 5.3).

5.3.3. Phylogenetic analysis - Py. semiclara

Parsimony analysis of the *Py. semiclara* data yielded 78 most-parsimonious trees with a tree length of 358 steps (CI = 0.603; RI = 0.905). Both the strict consensus of the parsimony analysis (tree not shown) and the majority rule BI phylogeny recovered six well supported lineages (Figure 5.4).

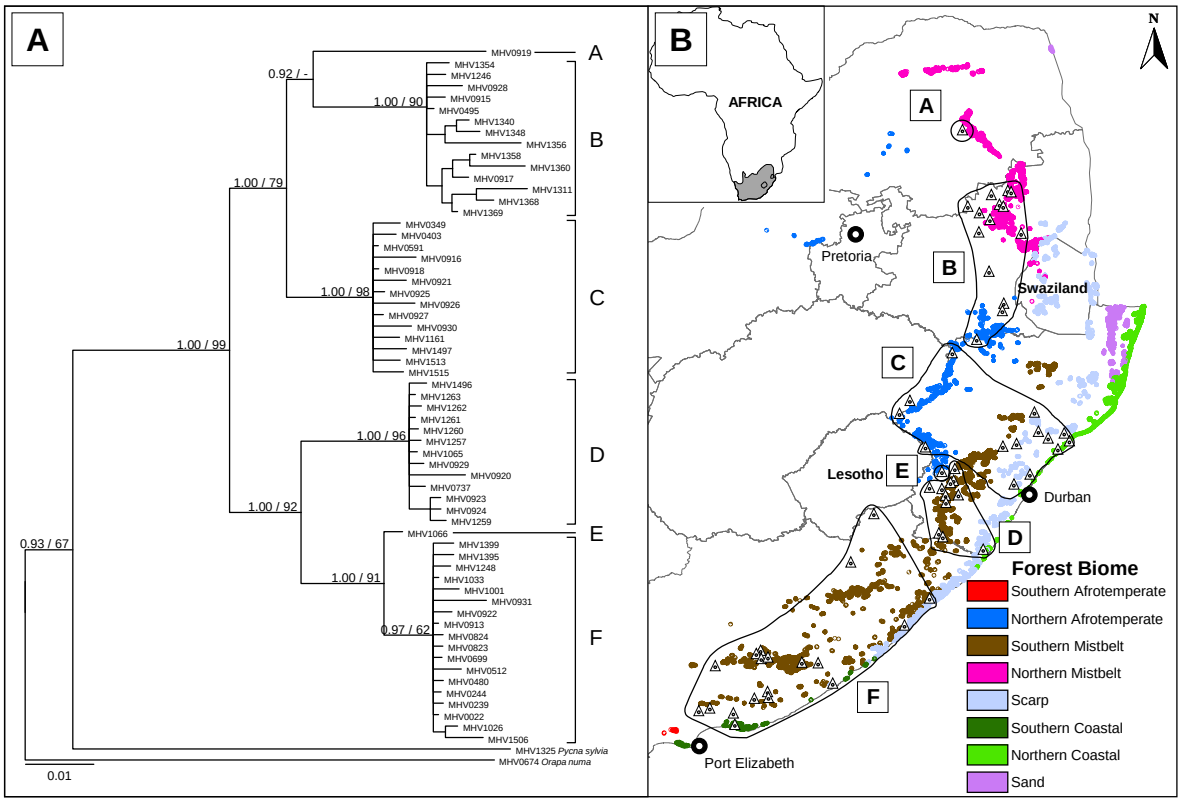

Figure 5.4. (A) Majority rule Bayesian Inference phylogram highlighting major lineages within *Py. semiclara*. Support is indicated above each node (Bayesian posterior probability / Parsimony bootstrap). (B) Distribution of lineages mapped onto corresponding forest types.

The basal split divided the samples into two groups, corresponding to a northern (Figure 5.4: A, B & C) and southern (Figure 5.4: D, E & F) lineage. Each of these groups was further subdivided into two well-defined clades and a lineage comprising of one specimen (Figure 5.4). Within each of the four clades there is little genetic or geographical structure (Figure 5.4).

5.3.4. Landscape genetic analysis

The effect of forest types as estimated with AMOVA and isolation by distance on population structure varied for *Pl.* 'intercapedenis', *Pl.* 'inconcessus' and *Py. semiclara* (Tables 5.2, A12 & A13). Both forest type and IBD had a small but significant effect within *Pl.* 'intercapedinis' (Table 5.2 & A12), whereas only IBD had a significant effect within *Py. semiclara* (Table 5.2 & A13). Within *Pl.* 'inconcessus' neither forest type nor IBD significantly affected population structure (Table 5.2). The mean within-group *p*-distance was greatest for Scarp forest, followed by Mistbelt and Afrotemperate forests, with the least genetic variation present in the two Coastal forest types for both *Pl.* 'intercapedenis' and *Py. semiclara* (Figure 5.5). The within-group *p*-distance was not compared for *Pl.* 'inconcessus' because samples from Sand forest are essentially from one site, which would bias this analysis.

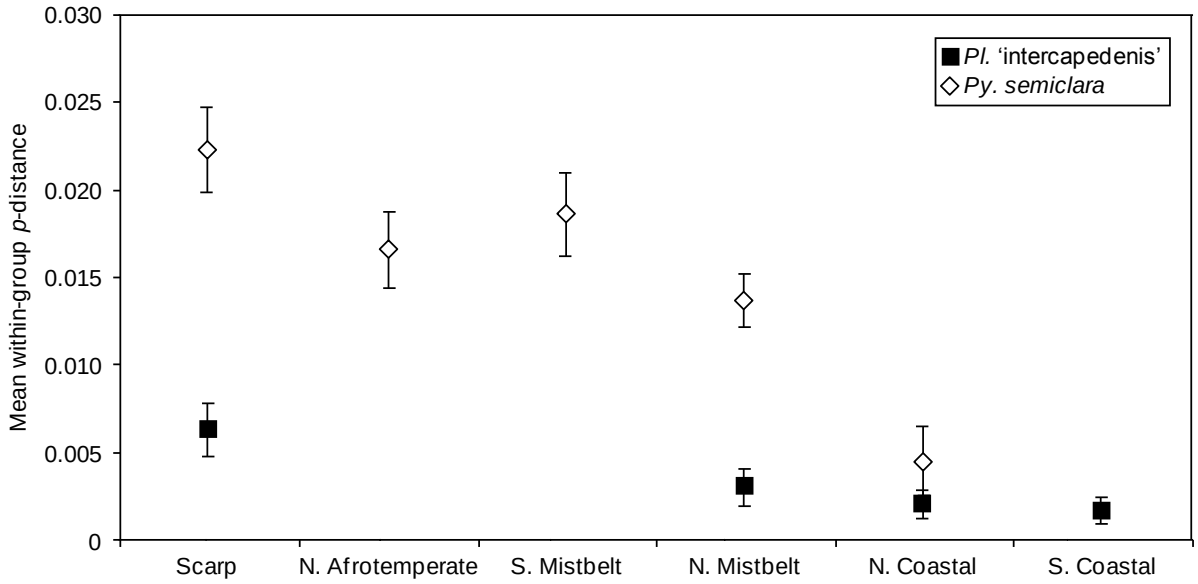

Figure 5.5. Mean within-group *p*-distance of *Pl.* 'intercapedenis' (dark squares) and *Py. semiclara* (light circles) for each different forest type inhabited. Forest types are arranged in order of putative age (oldest: Scarp - newest: Southern Coastal). Error bars indicate the standard error estimated using 1000 bootstrap replicates.

Table 5.2. AMOVA analysis of contribution of forest types to population structure (Φ_{pt}) and corresponding probability values (p). The contribution of isolation by distance (R^2) and corresponding probability values (p) are shown for comparison. An asterisk (*) indicates a reduction in the number of populations tested due to inadequate sample sizes in some forest types. Pairwise Φ_{pt} and corresponding probability values for analyses with more than two populations below are shown in Table A12 & A13. Significant values are in bold ($\alpha < 0.05$).

		% Variation				IBD	
Species	**# populations**	**Between**	**Within**	**Φ_{pt}**	**p**	**R^2**	**p**
Pl. 'intercapedenis'	4*	2	98	**0.023**	**0.023**	**0.058**	**0.001**
Pl. 'inconcessus'	2	0	100	0.000	1.000	0.029	0.110
Py. semiclara	5*	0	100	0.001	0.320	**0.416**	**0.001**

The effect of constraining the ML analyses to monophyletic lineages associated with each forest type on the number of trees and tree likelihood scores are summarised in Table 5.3. The likelihood ratio tests confirmed the results of the SH tests and are not presented. Within *Pl.* 'intercapedinis', constraining samples originating from Scarp forest (FOz 5) to be monophyletic had a significant effect (p = 0.003), whereas constraining samples originating from Northern Mistbelt (FOz 4), Southern Coastal (FOz 6) and Northern Coastal (FOz 7) forests did not significantly influence the analysis.

Table 5.3. Summary of likelihood constraint analyses and corresponding probability values derived from SH test. Significant values are in bold.

Constraint analysis	**Number of trees**	**-ln L**	**probability (p)**
<u>*Pl. chalybaea* group:</u>			
ML	5	4713.94	-
FOz 4 (*Pl.* 'intercapedinis')	17	4719.44	0.239
FOz 5 (*Pl.* 'intercapedinis')	2	4748.63	**0.003**
FOz 6 (*Pl.* 'intercapedinis')	3	4713.94	0.406
FOz 7 (*Pl.* 'intercapedinis')	2	4714.09	0.219
FOz 7 (*Pl.* 'inconcessus')	8	4728.48	**0.000**
FOz 8 (*Pl.* 'inconcessus')	11	4721.48	0.110
FOz ALL	12	4790.94	**0.000**
<u>*Py. semiclara*:</u>			
ML	5	4772.07	-
FOz 2	3	4898.65	**0.000**
FOz 3	1	5028.15	**0.000**
FOz 4	9	4822.10	**0.001**
FOz 5	4	5023.71	**0.000**
FOz 7	2	4777.04	0.199
FOz ALL	8	5340.70	**0.000**

Within *Pl.* 'inconcessus', constraining Northern Coastal forest (FOz 7) to be monophyletic had a significant effect (p = 0.000), whereas constraining Sand forest (FOz 8) did not

significantly influence the analysis. The analysis constraining all forest types to be monophyletic was significantly different from the most likely (unconstrained) topology (p = 0.000). Within *Py. semiclara*, all analyses constraining monophyly except the Northern Coastal forest (FOz 7) constraint were significantly different from the most likely topology (Table 5.3).

5.3.5. Molecular dating

A Chi-squared test of the likelihood scores of the two datasets data under the models specified by MrModeltest (Table 5.1) with or without enforcing a clock suggested that enforcing a molecular clock did not significantly influence the analysis of the *Pl. chalybaea* group's data (*Pl. chalybaea* group: clock lnL = 4760.1602; no clock: lnL = 4713.94896, df = 69, p = 0.03) but significantly affected the *Py. semiclara* analysis (*Py. semiclara*: clock lnL = 4822.9489; no clock: lnL = 4772.0729, df = 61, p = 0.001). As a result the dating analyses used a relaxed clock in BEAST.

Within the *Pl.* chalybaea group dataset, the dating estimates suggest that cladogenesis in the group began with the split between the [*P. murchisoni* + *P.* cf. *argentata* + *Pl.* 'inconcessus'] and [*Pl. chalybaea* + *Pl. brunea* + *Pl.* 'intercapedinis'.] lineages at 5.6 mya; with the split occurring between *P. murchisoni* and [*P.* cf. *argentata* + *Pl.* 'inconcessus'] at approximately 4.2 mya and the split between [*Pl. chalybaea* + *Pl. brunea*] and *Pl.* 'intercapedinis' at approximately 3.4 mya (Figure 5.6 B). The oldest of the putative focal species is *Pl.* 'intercapedinis' (TMRCA = 1.5 mya), followed by *Pl.* 'inconcessus' (TMRCA = 1.2 mya; Figure 5.6 B). Diversification within each putative species lineage is estimated to have occurred in the recent Pleistocene; within *Pl.* 'inconcessus' the two clades are estimated to have diverged 0.4 - 0.6 mya, whereas the three clades of *Pl.* 'intercapedinis' seem to have diverged 0.4 - 0.7 mya (Figure 5.6 B). The dating analysis suggests that *Pl. chalybaea* and *Pl. brunea* moved out of the forests in the recent Pleistocene, within each species the TMRCA is estimated at 0.3 mya (Figure 5.6 B).

The dating estimate suggests that *Py. semiclara* is relatively old compared to members of the *Pl. chalybaea* group, with a TMRCA of ~ 6.1 mya (Figure 5.6 C). The six major lineages of *Py. semiclara* are estimated to have arisen between 4.2 and 4.7 mya with the most recent common ancestor within each clade estimated at between 0.9 and 1.7 mya (Figure 5.6 C).

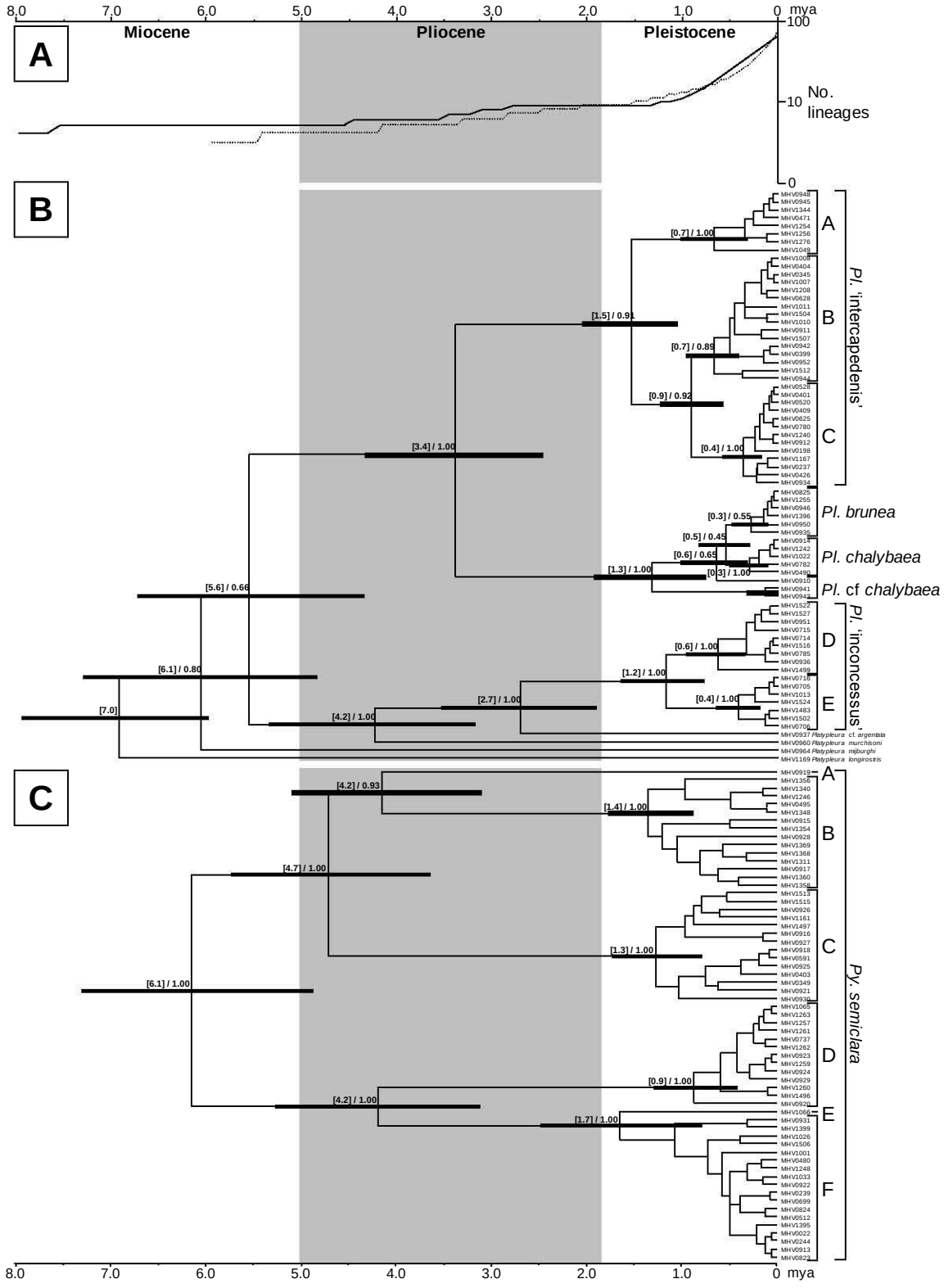

Figure 5.6. (A) Lineage-through-time plot of *Pl. chalybaea* group (dotted line) and *Py. semiclara* (solid line). Majority rule Bayesian chronogram of (B) the *Pl. chalybaea* group and (C) *Py. semiclara* (outgroups not shown) generated in BEAST. Numbers above nodes indicate [age in myr] and posterior probability support. Nodes are centred on the mean TMRCA with shaded bars indicating the distribution of the 95 % HPD for each estimate, only those discussed in the text are shown.

5.3.6. Rates of diversification within forest lineages

The plot of log-lineages through time shows a similar pattern for both the *Pl. chalybaea* group (dotted line) and *Py. semiclara* (solid line) deviating from a hypothetical constant rate of diversification. Very little diversification is shown within the Miocene and Pliocene followed by a significant increase in the rate of lineage accumulation in the early Pleistocene (~ 1.2 mya) which continues throughout the remainder of the Pleistocene for both groups (Figure 5.6 A).

5.4. Discussion

5.4.1. Taxonomic status of Pl. chalybaea group

Surprising diversity was found within the *Pl. chalybaea* group. The samples preliminarily identified as *Pl.* 'intercapedinis' were separated into three well supported lineages, with one lineage, here termed *Pl.* 'inconcesssus' consisting of samples from the KZN north coast, sister to *Pl.* cf. *argentata* (Figure 5.3). The second aberrant lineage groups with *Pl. chalybaea* and *Pl. brunea*, suggesting that the specific status of this clade requires attention. Although *Pl. chalybaea* and *Pl. brunea* form monophyletic, allopatric clades, the level of genetic variation between them is low and suggests very recent divergence. These two species use distinctly different host plants, which occur predominantly in different habitats, suggesting host plant switching as a possible isolating mechanism (Chapter 3; Price *et al.*, 2007). It is unclear whether this would be sufficient to act as an isolating mechanism should the taxa occur in para- or sympatry, suggesting that a fine-scale study of these two species is required to assess the status of possible sympatric populations. The genetic data from this study, combined with preliminary acoustic evidence (not presented), suggest that *Pl.* 'intercapedinis' and *Pl.* 'inconcesssus' require further attention, particularly a morphological and acoustic diagnosis to confirm whether specific status should be afforded to these two clades.

5.4.2. Comparative biogeography

This study represents the first molecular study of the population genetic structure of invertebrates from the South African forest biome. From Figures 5.3 & 5.4 it is clear that *Py. semiclara* and the *Pl. chalybaea* group do not show the same pattern of geographic partitioning of genetic variation.

Hypothesis 1: Currently recognised forest types adequately delimit the population structuring in both cicada groups.

Within *Pl.* 'intercapendinis', IBD accounts for a small but significant proportion of genetic variation (Table 5.2). In addition the AMOVA and likelihood constraint analyses suggest a small but significant portion of genetic variation can be explained by forest association, with the likelihood constraint analysis showing that samples collected in Scarp forest do not form a monophyletic clade (Table 5.2). Within *Pl.* 'inconcessus' there is no evidence of IBD (Table 5.2) and the AMOVA and likelihood constraint analyses suggest all of the variation is within forest types. Sand forest samples form a monophyletic clade, which is not surprising as all samples from Sand forest derive from essentially one locality. This is not the case for samples collected in Northern Coastal forest that do not form a monophyletic clade as one sample from Northern Coastal forest falls within the predominantly Sand forest clade (Table 5.2). Within *Py. semiclara*, IBD accounts for a significant proportion of genetic variation (Table 5.2). In addition, AMOVA and likelihood constraint analyses suggest that all of the variation is unrelated to forest types as none of the different forest types, with the exception of Northern Coastal forest, house monophyletic lineages of *Py. semiclara* (Table 5.2; Figure 5.4).

Contrary to the hypothesis, the overall pattern suggests that forest type is not an adequate surrogate to describe (let alone explain) population fragmentation. This suggests that either each forest type has a single origin and the cicadas disperse between forest types (analogous to horizontal gene transfer), or that the cicadas do not disperse, but each forest type does not share a single origin (equivalent to monophyly), i.e. forests of different types may converge to a common community over time at localities where conducive climate change occurs. (This latter process in fact represents dispersal of the plants defining the forest type). The geographical pattern of mismatches between cicada lineages and forest types does not provide sufficient evidence to decide which process predominates, and both processes (genetic dispersal and environmental convergence) are likely to have shaped the genetic structure of these forest-associated cicada lineages.

This result also highlights that phylogenetic approaches to tracing the history of environments may be founded on erroneous assumptions about the nature of the characteristics of the environments, e.g. that species are analogous to characters, that an

environment's community is equivalent to a genome, and that species are 'inherited' by an environment in a monophyletic manner. Indeed the effects of differential survival and the ephemeral nature of forest communities have long been recognised for both northern and southern hemisphere forests (e.g. Livingstone, 1967; Davis, 1983; Schoonmaker & Foster, 1991). The recent, post LGM, origin of the coastal forests suggests the speed at which these communities may form and that the different forest communities as currently defined (by different forest types) are only a 'snapshot' of what may be a loose assemblage of species, responding on an individualistic basis to a changing environment.

Hypothesis 2: The timing of population fragmentation is primarily concordant with Pleistocene climatic oscillation.

The eight lineages within the *Pl. chalybaea* group are estimated to have diverged from a common ancestor in the late Miocene (~ 5.6 mya; Figure 5.6 B), predating the onset of Pleistocene climatic oscillations and regional uplift of ~ 900 m in the late Pliocene (~ 2.5 mya; Partridge & Maud, 1987; Artyushkov & Hofmann, 1998). Further diversification is estimated to have occurred in the mid- to late Pliocene (~ 3.4 - 2.7 mya) with the divergence between *Pl. chalybaea*, *Pl. brunea* and *Pl.* 'intercapedinis' and between *Pl.* 'inconcessus' and *Pl.* cf. *argentata* (Figure 5.6 B). This period was characterised by regional uplift, global cooling and a reduction in forest cover (Partridge & Maud, 1987; Linder *et al.*, 1992; Scott, 1995; Artyushkov & Hofmann, 1998; Linder, 2003), which have all been suggested as drivers of diversification in a number of southern African taxa (Ewer & Cooke, 1964; Grant & Leslie, 1993; Kryger *et al.*, 2004).

The six lineages within *Py. semiclara* are separated along a latitudinal gradient and show a higher level of divergence than the *Pl. chalybaea* group (Figures 5.4 & 5.6 C). The dating analysis implies that they have been separated for at least 4 myr predating the late Pliocene regional uplift (Partridge & Maud, 1987; Artyushkov & Hofmann, 1998). The low level of variation within each major clade (Figure 5.6 C) is suggestive of a recent expansion from six separate refugia, a process that would reduce any previous genetic variation as a result of bottlenecking within each population. The dating analysis of *Py. semiclara* suggest that each clade has a common ancestor within the early to mid-Pleistocene (0.7 - 1.7 mya), a pattern that can be related to the geographical contraction of forest habitats during cold, dry

interpluvial periods (Meadows & Linder, 1989; deMenocal, 1995; Eeley *et al.*, 1999; Partridge *et al.*, 1999; Fattorini, 2007).

The shape of the lineage-through-time plots (Figure 5.6 A) suggest an initial period of reduced lineage diversification (or increased lineage extinction) in the Pliocene, followed by a rapid increase in the accumulation of lineages in the Pleistocene for both forest associated lineages (Figure 5.6 A).

Shifting attention from lineage diversification to the other focus of the hypothesis, the effects of climatic oscillations (Dynesius & Jansson, 2000; Jansson & Dynesius, 2002; Jansson, 2003), the low level of modern within-clade genetic divergence within the group implies recent expansion from interpluvial refugia within the Pleistocene (Figure 5.6 C). In addition, the relatively long internode lengths in the Pliocene (Figure 5.6 C) suggest that these clades have been subjected to repeated 'climatic extinction filtering events' (*sensu* Balmford, 1996) that have repeatedly pruned lineages from the stem.

Although no previous comparative phylogenetic studies on South African forest fauna are available, a comparative study in West Africa (Nicolas *et al.*, 2008) found similar variation in the response to forest fragmentation between two species of forest rodents, attributed to differing resilience to fragmentation (Nicolas *et al.*, 2008). In this case, the varied pattern of diversification may stem from the relative ages and dispersal capabilities of *Py. semiclara* and *Pl. chalybaea* which would tend to reflect different historical processes. Although the pattern of diversification is varied between the *Pl. chalybaea* group and *Py. semiclara* (Figures 5.3 & 5.4) the timing and rates of diversification are very similar (Figure 5.6 A). In terms of Hypothesis 2, the results confirm that the timing of fragmentation within the forest-associated lineages corresponds with the Pleistocene, a period of climatic oscillation and repeated habitat fragmentation.

Hypothesis 3: The phylogenetic signal of taxa occupying older Afrotemperate and Scarp forests is greater than that of taxa occupying the younger Coastal forests.

Both *Pl.* 'intercapedenis' and *Py. semiclara* show a similar pattern of genetic diversity within forest types (Figure 5.5). Scarp forests house the greatest mean within-clade genetic distances, supporting the view that Scarp forests have survived in isolated refugia during the

Pleistocene climatic oscillations (Lawes *et al.*, 2007). Samples inhabiting Afrotemperate and Mistbelt forests show a lower mean within-clade genetic distance than those within Scarp forest. This supports the suggestion by Lawes *et al.*, (2007) that these forests were more affected by interpluvial climates in the Pleistocene and that Northern Mistbelt forests re-established very recently (Deacon & Lancaster, 1988; Scott *et al.*, 1997). The lowest mean within-clade genetic distance was recorded for samples originating from Coastal forests, supporting their proposed recent age (Martin, 1968; Hobday, 1976; Tinley, 1985; MacDevette *et al.*, 1989; Lawes, 1990). The hypothesis that Scarp and Afrotemperate forests house older lineages than the younger Coastal forests is therefore supported.

5.5. Conclusion

These results add to the growing body of literature about the mechanisms generating biological diversity in southern Africa. Forest types have been shown to not be correlated with population substructure of these two cicada lineages. In addition the two forest-associated lineages show different patterns of genetic structure in response to forest fragmentation, most likely as a result of a combination of the different ages and dispersal habits of each lineage. However within each lineage it is clear that within-species diversification is primarily restricted to the Pleistocene, where glacial / inter-glacial cycles have probably repeatedly fragmented the cicada populations. The different pattern observed from two seemingly similar cicada lineages cautions against the use of a single organism to identify population genetic boundaries applicable to a wider range of organisms and suggests that further comparative work is needed to gain a better understanding of the population histories of taxa inhabiting forests in southern Africa.

6 General Discussion

In this chapter, the findings of the previous chapters are reviewed and placed into the greater context of the thesis, as outlined in the introductory chapter.

6.1. Summary of findings

This study provides the first large-scale investigation of the historical biogeography of the tribe Platypleurini and some of the first fine-scale studies of the geographic partitioning of genetic diversity of the terrestrial invertebrates in southern Africa. The results here presented show that the platypleurine cicadas provide useful tools for the inference of processes responsible for the diversification of invertebrates, not only in archipelagos as previously studied, but also on continents.

6.1.1. Tribal Phylogeny

The molecular data generated have enabled a first estimate of the relationships between the majority of the platypleurine genera. The tribe is shown to be too recent to be of Gondwanan origin. The nested positions of the tribes Hamzini and Orapini, and the paraphyly of the genera represented in both Asia and Africa confirm that the tribe is in need of taxonomic review. The diversification of the platypleurine genera within Africa is coincident with the aridification of Africa in the early Oligocene, suggesting that habitat fragmentation drives diversification in this group. The dispersal of Asian platypleurine taxa is coincident with the meeting of Africa and Eurasia in the mid-Oligocene and is yet another example of "Out of Africa" geo-dispersal *sensu* Lieberman & Eldridge (1996). Gondwanan vicariance is rejected in favour of mid-Miocene dispersal from Africa for the Madagascan cicada genus *Yanga*. Within *Platypleura* two radiations are hypothesised, one distributed over most of sub-Saharan Africa and the second restricted to southern Africa, with clades restricted to regional biomes.

6.1.2. Fynbos Clade

Within the Fynbos clade at least four taxa are identified: *P. stridula*, *P. capensis*, *P.* sp 10 and the three montane clades (currently recognised as Evolutionary Significant Units within a single taxon pending further investigation). The widely different host plant associations within the group implicate host plant changes as a possible isolating mechanism. Dating analyses of the group suggest that the interaction of Pleistocene climate oscillations with river catchments (in the case of *P. stridula*), mountains (in the case of the montane clades) and palaeocoastal topography (in the case of *P. capensis*) are important in the genetic structuring of populations.

6.1.3. Karoo Clade

Seven taxa are recognised within the Karoo clade, five warranting formal description as species. The broad host association exemplified by these taxa, primarily with *Acacia karroo*, removes the potentially confounding factor of host plant switching as a mechanism of speciation within this group. The pattern observed of species restricted to specific primary catchments and populations restricted to secondary catchments is suggestive of an iterative process as a primary cause of cladogenesis. The dating analyses suggest diversification within the Pleistocene, a period of marked cyclic climatic oscillation. The pattern of catchment-structured populations is more apparent for taxa distributed in the more arid western region, further suggesting aridification in response to Pleistocene glacial cycles as a mechanism of population fragmentation and allopatric speciation.

6.1.4. Forest Clade

Within the Forest clade the *Pl. chalybaea* group is comprised of four recognised taxa (two requiring formal description as species to alleviate paraphyly) and three aberrant specimens that require further investigation. Speciation related to very recent host plant switching onto *Euphorbia* tree species is implicated for *Pl. chalybaea*. The combination of the *Pl. chalybaea* group with samples of the co-distributed cicada *Pycna semiclara* highlights the need for comparative studies to elucidate processes responsible for lineage diversification within a region. The results confirm that forest types do not adequately describe the population structure of either cicada lineage, although there is some indication that the mean genetic divergence of cicada populations is related to the proposed age of the main forest types they

inhabit. The dating analyses suggest coincident diversification of the two lineages in the Plio-Pleistocene.

In addition to the specific aims of each of the preceding chapters, the broad aims of the thesis as stated in the introductory chapter have been achieved. The placement of the genus *Platypleura* in the tribe Platypleurini using molecular data has enabled the rejection of the proposed monophyly of *Platypleura* as currently understood and highlighted the need for taxonomic review in this group. Furthermore, the molecular data have enabled investigation of the timing of, and mechanisms underlying, the diversification of the platypleurine cicadas in Africa and facilitated the detailed phylogeographic investigation and comparison of three biome restricted *Platypleura* species groups in southern Africa.

6.2. Comparative analysis

The study of three separate biomes within southern Africa allows for the comparative analysis of processes leading to diversification. By comparing the response of groups in independent habitats, the overarching processes may be identified (Knowles, 2009). Additionally this comparison will allow identification of processes that may be biome-dependent.

6.2.1 Common patterns

Within each of the three biomes studied, the presence of suspected cryptic cicada diversity was confirmed. This result suggests that, even in relatively taxonomically well-understood groups such as the southern African platypleurine cicadas, the incorporation of molecular data into taxonomic assessments has the power to identify further cryptic diversity. The ability to recognise cryptic diversity is paramount in the identification of Evolutionary Significant Units (ESUs) for conservation purposes (Manel *et al.*, 2003).

Although each cicada group studied was restricted in distribution to non-overlapping biomes, a comparison between the three biomes highlights the interaction between palaeoclimates and fixed landscape features such as coastal topography (Chapter 3), watersheds (Chapters 3 & 4), escarpments (Chapter 5) and mountains (Chapter 3) as causative agents in the diversification of these volant insects. These landscape features should be taken into consideration when formulating conservation plans for rare but ecologically similar species.

Furthermore the comparative study of the forest platypleurines (Chapter 5) cautions against making inferences based on one species alone and highlights the need to incorporate data from multiple, evolutionarily-independent taxa when generalizing mechanisms of diversification.

The use of fossil-calibrated (Chapter 2) and rate-calibrated (Chapters 3 & 4) dating analyses resulted in widely different age estimates for the Fynbos (Chapter 3: fossil-calibrated = 5.4 mya; rate-calibrated = 1.8 mya) and Karoo (Chapter 4: fossil-calibrated = 7.5 mya; rate-calibrated = 2.3 mya) clades. These disparities caution against the over-reliance of dating analyses based on either a single (distantly related) fossil or an estimated average substitution rate for insects. That said, the dating analyses of the three biome-restricted groups whether based on fossil (Chapter 5) or rate (Chapters 3 & 4) calibration imply that within-species lineage diversification was overwhelmingly within the Pleistocene. Indeed the global climatic oscillations of the Pleistocene are recognised as important in the diversification of extant biota (Hewitt, 2004).

6.2.1 Unique processes

Within the biome-restricted clades in southern Africa, the development of new host plant associations is implicated in the initiation of lineages in the Fynbos and Forest clades, but is absent from the Karoo clade. Although topography is highlighted in all three biome studies, the effect is less pronounced in the Forest clade lineages. Indeed, it would seem that the widespread movement of forests in response to palaeoclimatic causes has facilitated the dispersal of the forest-restricted cicada lineages, whereas dispersal has been more limited in the Fynbos and Karoo clades.

6.3. Cicadas as tools for continental historical biogeography

This study confirms that cicadas are suitable as tools to elucidate the mechanisms of diversification in continental studies, thus as a tool of historical biogeography their use need not be restricted to studies focussed on islands.

Although the taxonomy of platypleurine cicadas has been the focus of previous morphological research (e.g. Boulard, 1972; Villet, 1989, 1997), the use of molecular data has uncovered cryptic diversity resulting in the recognition of new species in the region. It

would seem that the self-validating characteristic of molecular phylogenetic studies alleviates the need for a complete taxonomic understanding of a focal group for historical biogeography (property 1 of a good phylogeographic tool).

The replication provided by using species-rich clades restricted to specific biomes has enabled a comparison of the individual taxon responses to environmental change, allowing common responses to infer important scenarios (property 2 of a good phylogeographic tool).

The clearly defined distributions of southern African species, based on a database of approximately 2000 locality records and a landscape genetic approach to sampling, has enabled sampling from the entire range of the focal taxa (property 3 of a good phylogeographic tool). These distributional data, in combination with the molecular data have confirmed that *Platypleura* is endemic to Africa and that the focal clades are endemic to the biomes they inhabit (property 4 of a good phylogeographic tool).

The combination of a well understood biology (property 5 of a good phylogeographic tool) and a clear ecological relationship with their habitat in the form of host plant and biome information (property 6 of a good phylogeographic tool) has enabled the identification of host plant changes as contributing factors to lineage diversification in both the Fynbos and Forest clades.

The group has shown responsiveness to environmental change over relevant spatial (biome) and time (Plio-Pleistocene) scales, further highlighting their value as a tool for studies of continental historical biogeography (property 7 of a good phylogeographic tool). The fine-scale geographic partitioning of genetic variation exhibited in the focal taxa further confirms the ability of platypleurine cicadas to survive in small, isolated populations (property 8 of a good phylogeographic tool), although the long stems of some groups imply that there have been periods of extinction, erasing older phylogeographic signals.

6.4. Southern Africa as a laboratory

The apparent concentration of *Platypleura* species in southern Africa that have restricted distributions when compared to the widespread distributions of more northern species implies that the southern African fauna has either (1) experienced more environmental change than the northern fauna, or (2) has not been able to migrate in response to environmental change to the same extent as the northern fauna; instead the southern African cicada fauna has diversified *in situ*. This result may be suggestive of a regional characteristic, needing comparison with other similarly distributed invertebrate groups.

The overwhelming plant and animal diversity of southern Africa is well recognised (Branch, 1998; Linder, 2003; Mucina & Rutherford, 2006; Cowling *et al.*, 2009), with the vast majority of molecular based studies uncovering cryptic species in each group examined. Despite this diversity, few studies on animal taxa have resulted in fine-scale examination of the genetic structure of populations; the resulting inferences of causative processes leading to diversification have thus been limited. Although the use of molecular techniques in assessing the genetic structure of populations is a young discipline, a recent estimate by Knowles (2009) estimates that 4730 papers using "phylogeography" in the title (and thus a conservative estimate of the discipline) have been published within the last twelve years (1997 - 2008). In light of this estimate the southern African studies using invertebrates (including the four here presented) account for less than 0.4 % of this published phylogeographic literature. This highlights that the invertebrates of southern Africa, which based on estimates of the regions plant and vertebrate diversity, are in all likelihood inordinately diverse, are not receiving the attention they deserve.

6.5. Future prospects

At the level of the tribe, the results presented provide a first estimate of their phylogenetic history, requiring the corroboration of morphological and further molecular data to provide an even more robust phylogeny. Although the Asian taxa are shown to be of monophyletic origin this was based on a very limited sample, suggesting that the Asian platypleurine fauna are misplaced in otherwise African genera and require formal review. Within the genus *Platypleura* the addition of species not available to this study, specifically those with distributions in central Africa, is required to gain a better understanding of the processes leading to broad-scale patterns in the region. Although southern Africa has a relatively well

studied palaeoenvironment, a review of these data is required to synthesise the available information and allow for inferences of regional patterns.

This study has shown that incorporating fine-scale sampling over the entire distribution of widely distributed invertebrate groups has the ability to identify and distinguish between possible mechanisms of diversification within southern Africa. The most obvious target group for future phylogeographic study within the southern African *Platypleura* is the Grassland clade (Chapter 2: clade B). This clade is especially valuable as it forms a well supported monophyletic group, with a restricted distribution. The grassland and forest biomes are hypothesised to have supplanted one another in response to Pleistocene climatic oscillations (Mucina & Rutherford, 2006), thus a fine-scale study of the cicadas within the Grassland clade may provide independent validation of the inferences made from the Forest clade.

Further comparative phylogeographic studies of invertebrates from distinct taxonomic groups are strongly encouraged as these studies would help to provide a clearer picture of the processes generating biodiversity in the region (Knowles, 2009). Future studies should shift in focus from an organismal approach to a more landscape orientated comparative approach. This landscape approach will be more able to identify the causative mechanisms of lineage diversification and the locations in which these mechanisms are focussed, enabling the long-term management of processes contributing to the generation of our rich biodiversity.

"The wrong view of science betrays itself in the craving to be right;
for it is not his possession of knowledge, of irrefutable truth,
that makes the man of science,
but his persistent and recklessly critical quest for truth."

~

Sir Karl Popper

References

Aduse-Poku K, Vingerhoedt E, Wahlberg N (2009) Out-of-Africa again: A phylogenetic hypothesis of the genus *Charaxes* (Lepidoptera: Nymphalidae) based on five gene regions. *Molecular Phylogenetics and Evolution*, **53**, 463-478.

Aitchison JC, Ali JR, Davis AM (2007) When and where did India and Asia collide? *Journal of Geophysical Research*, **112**, B05423.

Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, **19**, 716-723.

Ali JR, Aitchison JC (2008) Gondwana to Asia: plate tectonics, paleogeography and the biological connectivity of the Indian sub-continent from the Middle Jurassic through latest Eocene (166-35 Ma). *Earth-Science Reviews*, **88**, 145-166.

Arensburger P, Buckley TR, Simon C, Moulds M, Holsinger KE (2004a) Biogeography and phylogeny of the New Zealand cicada genera (Hemiptera: Cicadidae) based on nuclear and mitochondrial DNA data. *Journal of Biogeography*, **31**, 557-569.

Arensburger P, Simon C, Holsinger K (2004b) Evolution and phylogeny of the New Zealand cicada genus *Kikihia* Dugdale (Homoptera: Auchenorrhyncha: Cicadidae) with special reference to the origin of the Kermadec and Norfolk Islands' species. *Journal of Biogeography*, **31**, 1769-1783.

Artyushkov EV, Hofmann AW (1998) Neotectonic crustal uplift on the continents and its possible mechanisms. The case of southern Africa. *Surveys in Geophysics*, **19**, 369-415.

Avise JC (2001) *Phylogeography: the History and Formation of Species*. Harvard University Press, Cambridge.

Avise JC (2009) Phylogeography: retrospect and prospect. *Journal of Biogeography*, **36**, 3-15.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489-522.

Balmford A (1996) Extinction filters and current resilience: the significance of past selection pressures for conservation biology. *Trends in Ecology & Evolution*, **11**, 193-196.

Barraclough TG (2006) What can phylogenetics tell us about speciation in the Cape flora? *Diversity and Distributions*, **12**, 21-26.

Bauer AM, Lamb T (2005) Phylogenetic relationships of southern African geckos in the *Pachydactylus* group (Squamata: Gekkonidae). *African Journal of Herpetology*, **54**, 105-129.

Bellstedt DU, Edwards TJ (2004) A new species of *Streptocarpus* (Gesneriaceae) from the Pondoland Coast, South Africa. *Edinburgh Journal of Botany*, **60**, 409-414.

Bergh NG, Hedderson TA, Linder HP, Bond WJ (2007) Palaeoclimate-induced range shifts may explain current patterns of spatial genetic variation in renosterbos (*Elytropappus rhinocerotis*, Asteraceae). *Taxon*, **56**, 393-408.

Bermingham E, Moritz C (1998) Comparative phylogeography: concepts and applications. *Molecular Ecology*, **7**, 367-369.

Beuk PLT (2002) *Cicadas spreading by island or by spreading the wings? Historic biogeography of dundubiine cicadas of the Southeast Asian continent and archipelagos*. PhD thesis, University of Amsterdam, Amsterdam.

Bossuyt F, Brown RM, Hillis DM, Cannatella DC, Milinkovitch MC (2006) Phylogeny and biogeography of a cosmopolitan frog radiation: Late Cretaceous diversification resulted in continent-scale endemism in the family Ranidae. *Systematic Biology*, **55**, 579-594.

Boulard M (1972) Classification raisonnee des Platypleures africaines (Homoptera-Cicadidae). *Bulletin du Museum National d'Histoire Naturelle (Zoologie)*, **69**, 1161-1188.

Brady SG, Gadau J, Ward PS (2000) Systematics of the ant genus *Camponotus*: a preliminary analysis using data from the mitochondrial gene cytochrome oxidase 1. In: *Hymenoptera: evolution, biodiversity, and biological control* (eds. Austin AD, Dowton M), pp. 131-139. CSIRO Publishing, Canberra.

Branch WR (1998) *Field guide to the snakes and other reptiles of southern Africa*. Struik Publishers, Cape Town.

Brandley MC, Schmitz A, Reeder TW (2005) Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. *Systematic Biology*, **54**, 373-390.

Brower AVZ (2006) Problems with DNA barcodes for species delimitation: 'ten species' of *Astraptes fulgerator* reassessed (Lepidoptera: Hesperiidae). *Systematics and Biodiversity*, **4**, 127-132.

Buckley TR, Simon C (2007) Evolutionary radiation of the cicada genus *Maoricicada* Dugdale (Hemiptera: Cicadoidea) and the origins of the New Zealand alpine biota. *Biological Journal of the Linnean Society*, **91**, 419-435.

Buckley TR, Arensburger P, Simon C, Chambers GK (2002) Combined data, Bayesian phylogenetics, and the origin of the New Zealand cicada genera. *Systematic Biology*, 51, 4-18.

Buckley TR, Cordeiro M, Marshall DC, Simon C (2006) Differentiating between hypotheses of lineage sorting and introgression in New Zealand alpine cicadas (*Maoricicada* Dugdale). *Systematic Biology*, **55**, 411-425.

Buckley TR, Simon C, Chambers GK (2001) Phylogeography of the New Zealand cicada *Maoricicada campbelli* based on mitochondrial DNA sequences: Ancient clades associated with Cenozoic environmental change. *Evolution*, **55**, 1395-1407.

Charif RA, Clark CW, Fristrup KM (2004) *Raven 1.2 User's Manual*. Cornell Laboratory of Ornithology, Ithaca, New York.

Chawanji AS, Hodgson AN, Villet MH (2005) Sperm morphology in four species of African platypleurine cicadas (Hemiptera: Cicadomorpha: Cicadidae). *Tissue and Cell*, **37**, 257-267.

Claridge MF (1985) Acoustic signals in the Homoptera: behaviour, taxonomy and evolution. *Annual Review of Entomology*, **30**, 297-317.

Cooley JR, Simon C, Marshall DC, Slon K, Ehrhardt C (2001) Allochronic speciation, secondary contact, and reproductive character displacement in periodical cicadas (Hemiptera: *Magicicada* spp.): genetic, morphological, and behavioural evidence. *Molecular Ecology*, **10**, 661-671.

Couvreur TL, Chatrou LW, Sosef MSM, Richardson JE (2008) Molecular phylogenetics reveal multiple tertiary vicariance origins of the African rain forest trees. *BMC Biology*, **6**, 54.

Cowling RM, Proches S (2005) Patterns and evolution of plant diversity in the Cape Floristic Region. *Biologiske Skrifter*, **55**, 273-288.

Cowling RM, Proches S, Partridge TC (2009) Explaining the uniqueness of the Cape flora: Incorporating geomorphic evolution as a factor for explaining its diversification. *Molecular Phylogenetics and Evolution*, **51**, 64-74.

Cowling RM, Proches S, Vlok JHJ (2005) On the origin of southern African subtropical thicket vegetation. *South African Journal of Botany*, **71**, 1-23.

Cracraft J (1983) Species concepts and speciation analysis. *Current Ornithology*, **1**, 159-187.

Crisci JV (2001) The voice of historical biogeography. *Journal of Biogeography*, **28**, 157-168.

Cryan JR (2005) Molecular phylogeny of Cicadomorpha (Insecta: Hemiptera: Cicadoidea, Cercopoidea and Membracoidea): adding evidence to the controversy. *Systematic Entomology*, **30**, 563-574.

Cunningham CW (1997) Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution*, **14**, 733-740.

Damgaard J, Klass KD, Picker MD, Buder G (2008) Phylogeny of the heelwalkers (Insecta: Mantophasmatodea) based on mtDNA sequences, with evidence for additional taxa in South Africa. *Molecular Phylogenetics and Evolution*, **47**, 443-462.

Daniels SR (2003) Examining the genetic structure among populations of the common Cape River crab, *Potamonautes perlatus* from river systems in South Africa reveals hydrographic boundaries. *Journal of Crustacean Biology*, **24**, 936-950.

Daniels SR, Gouws G, Crandall KA (2006) Phylogeographic patterning in a freshwater crab species (Decapoda: Potamonautidae: *Potamonautes*) reveals the signature of historic climatic oscillations. *Journal of Biogeography*, **33**, 1538-1549.

Daniels SR, Gouws G, Stewart BA, Coke M (2003) Molecular and morphometric data demonstrate the presence of cryptic lineages among freshwater crabs (Decapoda: Potamonautidae: *Potamonautes*) from the Drakensberg Mountains, South Africa. *Biological Journal of the Linnean Society*, **78**, 129-147.

Daniels SR, Heideman N, Hendricks MGJ, Willson B (2002) A molecular phylogeny for the South African limbless lizard taxa of the subfamily Acontinae (Sauria: Scincidae) with special emphasis on relationships within *Acontias*. *Molecular Phylogenetics and Evolution*, **24**, 315-323.

Daniels SR, Stewart BA, Burmeister L (2001) Geographic patterns of genetic and morphological divergence amongst populations of a river crab (Decapoda, Potamonautidae) with the description of a new species from mountain streams in the Western Cape, South Africa. *Zoologica Scripta*, **30**, 181-197.

Davis MB (1983) Quaternary history of deciduous forests of eastern North America and Europe. *Annals of the Missouri Botanical Garden*, **70**, 550-563.

Deacon J, Lancaster N (1988) *Late Quaternary palaeoenvironments of southern Africa*. Clarendon Press, Oxford.

de Boer AJ (1995) Islands and cicadas adrift in the West-Pacific: biogeographic patterns related to plate tectonics. *Tijdschrift voor Entomologie*, **138**, 169-244.

de Boer AJ (1997) Phylogeny and biogeography of Australian genera of Chlorocystini (Insecta: Homoptera: Tibicinidae). *Memoirs of the Museum of Victoria*, **56**, 91-123.

de Boer AJ (1999) Taxonomy and biogeography of the New Guinean Cicadettini (Hemiptera, Tibicinidae). *Deutsch Entomologische Zeitschrift*, **46**, 115-147.

de Boer AJ, Duffels JP (1996a) Biogeography of Indo-Pacific cicadas east of Wallace's Line. In: *The origin and evolution of Pacific Island biotas, New Guinea to Eastern Polynesia: patterns and processes* (eds. Keast A, Miller S), pp. 297-330. SPB Academic Publishing, Amsterdam.

de Boer AJ, Duffels JP (1996b) Historical biogeography of the cicadas of Wallacea, New Guinea and the West Pacific: a geotectonic explanation. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **124**, 153-177.

de Jong MR, de Boer AJ (2004) Taxonomy and biogeography of the Oriental Prasiini. 4. The genus *Mariekea* gen. n. (Homptera, Tibicinidae). *Tijdschrift voor Entomologie*, **147**, 265-281.

deMenocal PB (1995) Plio-Pleistocene African climate. *Science*, **270**, 53-59.

deMenocal PB (2004) African climate change and faunal evolution during the Pliocene-Pleistocene. *Earth and Planetary Science Letters*, **220**, 3-24.

de Queiroz A (2005) The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology and Evolution*, **20**, 68-73.

DeSalle R, Absher R, Amato G (1994) Speciation and phylogenetic resolution. *Trends in Ecology & Evolution*, **9**, 297-298.

Desmet PG, Cowling RM, Ellis AG, Pressey RL (2002) Integrating biosystematic data into conservation planning: perspectives from southern Africa's Succulent Karoo. *Systematic Biology*, **51**, 317-330.

Despres L, Pettex E, Plaisance V, Pompanon F (2002) Speciation in the globeflower fly *Chiastocheta* spp. (Diptera: Anthomyiidae) in relation to host plant species, biogeography, and morphology. *Molecular Phylogenetics and Evolution*, **22**, 258-268.

Dingle RV, Rogers J (1972) Effects of sea-level changes on the Pleistocene palaeoecology of the Agulhas Bank. *Palaeocology of Africa*, **6**, 55-58.

Distant WL (1882) Distribution of the genus *Platypleura*: a correction. *The Entomologist's Monthly Magazine*, **19**, 67.

Distant WL (1906) *A synonymic catalogue of Homoptera, Part 1: Cicadidae.* British Museum, London.

Douady CJ, Catzeflis F, Raman J, Springer MS, Stanhope MJ (2003) The Sahara as a vicariant agent, and the role of Miocene climatic events, in the diversification of the mammalian order Macroscelidea (elephant shrews). *Proceedings of the National Academy of Sciences of the USA*, **100**, 8325-8330.

Drummond AJ, Rambaut A (2003) BEAST version 1.4 (http://evolve.zoo.ox.ac.uk/beast). Accessed November 2006.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Duffels JP, Turner H (2002) Cladistic analysis and biogeography of the cicadas of the Indo-Pacific subtribe Cosmopsaltriina. *Systematic Entomology*, **27**, 235-261.

Duffels JP, van der Laan PA (1985) *Catalogue of the Cicadoidea (Homoptera: Auchenorrhyncha) 1956-1980. Series Entomologia, 34* (ed. Spencer KA). Junk, The Hague.

Dynesius M, Jansson R (2000) Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 9115-9120.

Eeley HAC, Lawes MJ, Piper SE (1999) The influence of climate change on the distribution of indigenous forest in KwaZulu-Natal, South Africa. *Journal of Biogeography,* **26**, 595-617.

Ewer RF, Cooke HBS (1964) The Pleistocene mammals of southern Africa. In: *Ecological studies in southern Africa* (eds. Davis DHS, DeMeillon B, Harington JS, Kalk M), pp. 35-48. Junk, The Hague.

Farris JS, Kallersjo M, Kluge AG, Bult C (1994) Testing significance of incongruence. *Cladistics*, **10**, 315-319.

Fattorini S (2007) Historical relationships of African mountains based on cladistic analysis of distributions and endemism of flightless insects. *African Entomology*, **15**, 340-355.

Feder JL, Opp SB, Wlazlo B, Reynolds K, Go W, Spisak S (1994) Host fidelity is an effective premating barrier between sympatric races of the apple maggot fly. *Proceedings of the National Academy of Sciences of the USA*, **91**, 7990-7994.

Feldman CR, Spicer GS (2006) Comparative phylogeography of woodland reptiles in California: repeated patterns of cladogenesis and population expansion. *Molecular Ecology*, **15**, 2201-2222.

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, **17**, 368-376.

Ferguson JWH (2002) On the use of genetic divergence for identifying species. *Biological Journal of the Linnean Society*, **75**, 509-516.

Folinsbee KE, Brooks DR (2007) Miocene hominoid biogeography: Pulses of dispersal and differentiation. *Journal of Biogeography*, **34**, 383-397.

Fonseca PJ, Munch D, Hennig RM (2000) How cicadas interpret acoustic signals. *Nature*, **405**, 297-298.

Forgie SA, Kryger U, Bloomer P, Scholtz CH (2006) Evolutionary relationships among the Scarabaeini (Coleoptera: Scarabaeidae) based on combined molecular and morphological data. *Molecular Phylogenetics and Evolution*, **40**, 662-678.

Freitag S, Robinson TJ (1993) Phylogeographic patterns in mitochondrial-DNA of the ostrich (*Struthio camelus*). *Auk*, **110**, 614-622.

Futuyma DJ (1991) Evolution of host specificity in herbivorous insects: genetic, ecological, and phylogenetic aspects. In: *Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions* (eds. Price PW, Lewinsohn TM, Fernandes GW, Benson WW), pp. 431-454. John Wiley and Sons, New York.

Galley C, Linder HP (2006) Geographical affinities of the Cape flora, South Africa. *Journal of Biogeography*, **33**, 236-250.

Garrick RC, Sands CJ, Rowell DM, Hillis M, Sunnucks P (2007) Catchments catch all: long-term population history of a giant springtail from the southeast Australian highlands - a multigene approach. *Molecular Ecology*, **16**, 1865-1882.

Gasse F, Chalie F, Vincens A, Williams MAJ, Williamson D (2008) Climatic patterns in equatorial and southern Africa from 30,000 to 10,000 years ago reconstructed from terrestrial and near-shore proxy data. *Quaternary Science Reviews*, **27**, 2316-2340.

Gaunt MW, Miles MA (2002) An insect molecular clock dates the origin of the insects and accords with palaeontological and biogeographic landmarks. *Molecular Biology and Evolution*, **19**, 748-761.

Gheerbrant E, Rage JC (2006) Paleobiogeography of Africa: How distinct from Gondwana and Laurasia? *Palaeogeography Palaeoclimatology Palaeoecology*, **241**, 224-246.

Goldblatt P (1978) An analysis of the flora of southern Africa: its characteristics, relationships, and origins. *Annals of the Missouri Botanical Garden*, **65**, 369-436.

Goldblatt P, Manning JC (2002) Plant diversity of the Cape Region of southern Africa. *Annals of the Missouri Botanical Garden*, **89**, 281-302.

Gouws G, Stewart BA, Daniels SR (2004) Cryptic species within the freshwater isopod *Mesamphisopus capensis* (Phreatoicidea: Amphisopodidae) in the Western Cape, South Africa: allozyme and 12S rRNA sequence data and morphometric evidence. *Biological Journal of the Linnean Society*, **81**, 235-253.

Gouws G, Stewart BA, Matthee CA (2005) Lack of taxonomic differentiation in an apparently widespread freshwater isopod morphotype (Phreatoicidea: Mesamphisopidae: *Mesamphisopus*) from South Africa. *Molecular Phylogenetics and Evolution*, **37**, 289-305.

Grant WS, Leslie RW (1993) Effect of metapopulation structure on nuclear and organellar DNA variability in semi-arid environments of southern Africa. *South African Journal of Science*, **89**, 287-293.

Green DG, Sadedin S (2005) Interactions matter - complexity in landscapes and ecosystems. *Ecological Complexity*, **2**, 117-130.

Heads M (2005) Dating nodes on molecular phylogenies: a critique of molecular biogeography. *Cladistics*, **21**, 62-78.

Heaton T, Talma A, Vogel J (1983) Origin and history of nitrate in confined groundwater in the western Kalahari. *Journal of Hydrology*, **62**, 243-262.

Hebert PDN, Ratnasingham S, deWaard JR (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **270**, S96-S99.

Heer O (1853) *Die Insektenfauna der Tertiärgebilde von Oeningen und von Radoboj in Croatien. Volume 3. Rhynchoten*. Engelmann, Leipzig.

Hendey QB (1983) Cenozoic geology and palaeogeography of the fynbos region. In: *Fynbos Palaeoecology: a Preliminary Synthesis* (eds. Deacon HJ, Hendey QB, Lambrechts NJJ), pp. 35-60. CSIR, Pretoria.

Hennig W (1966) *Phylogenetic Systematics*. University of Illinois Press, Urbana.

Herbert DG, Mitchell A (2009) Phylogenetic relationships of the enigmatic land snail genus *Prestonella*: the missing African element in the Gondwanan superfamily Orthalicoidea (Mollusca: Stylommatophora). *Biological Journal of the Linnean Society*, **96**, 203-221.

Hessami K, Koyim A, Talbot CJ, Tabasi H, Shabanian E (2001) Progressive unconformities within an evolving foreland fold-thrust belt, Zagros mountains. *Journal of the Geological Society*, **158**, 969-981.

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philisophical Transactions of the Royal Society London B - Biological Sciences*, **359**, 183-195.

Hill KBR, Simon C, Marshall DC, Chambers GK (2009) Surviving glacial ages within the Biotic Gap: phylogeography of the New Zealand cicada *Maoricicada campbelli*. *Journal of Biogeography*, **36**, 675-692.

Hobday DK (1976) Quaternary sedimentation and development of the lagoonal complex, Lake St. Lucia, Zululand. *Annals of the South African Museum*, **71**, 93-113.

Hoelzer GA, Melnick DJ (1994) Patterns of speciation and limits to phylogenetic resolution. *Trends in Ecology & Evolution*, **9**, 104-107.

Huelsenbeck J, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754-755.

Hughes JM, Bunn SE, Hurwood DA, Cleary C (1998) Dispersal and recruitment of *Tasiagma ciliata* (Trichoptera: Tasimiidae) in rainforest streams, south-eastern Australia. *Freshwater Biology*, **39**, 117-127.

Hughes JM, Bunn SE, Kingston DM, Hurwood DA (1995) Genetic differentiation and dispersal among populations of *Paratya australiensis* (Atyidae) in rain-forest streams in southeast Queensland, Australia. *Journal of the North American Benthological Society*, **14**, 158-173.

Hughes JM, Mather PB, Sheldon AL, Allendorf FW (1999) Genetic structure of the stonefly, *Yoraperla brevis*, populations: the extent of gene flow among adjacent montane streams. *Freshwater Biology*, **41**, 63-72.

Hughes M, Moller M, Bellstedt DU, Edwards TJ, de Villiers M (2005) Refugia, dispersal and divergence in a forest archipelago: a study of *Streptocarpus* in eastern South Africa. *Molecular Ecology*, **14**, 4415-4426.

Jackson JK, Resh VH (1992) Variation in genetic structure among populations of the caddisfly *Helicopsyche borealis* from three streams in northern California, U.S.A. *Freshwater Biology*, **27**, 29-42.

Jacobs BF (2004) Palaeobotanical studies from tropical Africa: relevance to the evolution of forest, woodland and savannah biomes. *Philisophical Transactions of the Royal Society London B - Biological Sciences*, **359**, 1573-1583.

Jansson R (2003) Global patterns in endemism explained by past climate change. *Proceedings of the Royal Society B - Biological Sciences*, **270**, 583-590.

Jansson R, Dynesius M (2002) The fate of clades in a world of recurrent climatic change: Milankovitch oscillations and evolution. *Annual Review of Ecology and Systematics*, **33**, 741-777.

Karanth KP (2006) Out-of-India Gondwanan origin of some tropical Asian biota. *Current Science*, **90**, 789-792.

Karban R (1981) Flight and dispersal of the periodical cicadas. *Oecologia*, **49**, 385-390.

Kartavtsev YP, Lee JS (2006) Analysis of nucleotide diversity at the cytochrome b and cytochrome oxidase 1 genes at the population, species, and genus levels. *Russian Journal of Genetics*, **42**, 341-362.

Kass RE, Raftery AE (1995) Bayes factors. *Journal of the American Statistical Association*, **90**, 773-795.

Kelly CMR, Barker NP, Villet MH, Broadley DG (2009) Phylogeny, biogeography and classification of the snake superfamily Elapoidea: a rapid radiation in the late Eocene. *Cladistics*, **25**, 38-63.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 111-120.

Klass KD, Picker MD, Damgaard J, van Noort S, Tojo K (2003) The taxonomy, genitalic morphology, and phylogenetic relationships of Southern African Mantophasmatodea (Insecta). *Entomologische Abhandlungen*, **61**, 3-67.

Knowles LL (2009) Statistical Phylogeography. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 593-612.

Kodandaramaiah U, Wahlberg N (2007) Out-of-Africa origin and dispersal-mediated diversification of the butterfly genus *Junonia* (Nymphalidae: Nymphalinae). *Journal of Evolutionary Biology*, **20**, 2181-2191.

Kremen C, Colwell RK, Erwin TL, Murphy DD, Noss RF, Sanjayan MA (1993) Terrestrial arthropod assemblages: their use in conservation planning. *Conservation Biology*, **7**, 796-808.

Kryger U, Robinson TJ, Bloomer P (2004) Population structure and history of southern African scrub hares, *Lepus saxatilis*. *Journal of Zoology*, **263**, 121-133.

Kumar S, Tamura K, Nei M (2004) MEGA 3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 150-163.

Lamb T, Bauer AM (2003) *Meroles* revisited: complementary systematic inference from additional mitochondrial genes and the complete taxon sampling of southern Africa's desert lizards. *Molecular Phylogenetics and Evolution*, **29**, 360-364.

Lamb T, Meeker AM, Bauer AM, Branch WR (2003) On the systematic status of the desert plated lizard (*Angolosaurus skoogi*): phylogenetic inference from DNA sequence analysis of the African Gerrhosauridae. *Biological Journal of the Linnean Society*, **78**, 253-261.

Lawes MJ (1990) The distribution of the samango monkey (*Cercopithecus mitis erythrarchus* Peters, 1852 and *Cercopithecus mitis labiatus* I. Geoffroy, 1843) and forest history in southern Africa. *Journal of Biogeography*, **17**, 669-680.

Lawes MJ, Eeley HAC, Findlay NJ, Forbes D (2007) Resilient forest faunal communities in South Africa: a legacy of palaeoclimatic change and extinction filtering? *Journal of Biogeography*, **34**, 1246-1264.

Lee YJ, Hayashi M (2003) Taxonomic review of Cicadidae (Hemiptera, Auchenorrhyncha) from Taiwan, part 1. Platypleurini, Tibicenini, Polyneurini, and Dundubiini (Dundubiina). *Insecta Koreana*, **20**, 149-185.

Levin SA, Muller-Landau HC, Nathan R, Chave J (2003) The ecology and evolution of seed dispersal: a theoretical perspective. *Annual Review of Ecology, Evolution and Systematics*, **34**, 575-604.

Levyns M (1964) Migrations and origins of the Cape flora. *Transactions of the Royal Society of South Africa*, **37**, 85-105.

Lieberman BS, Eldredge N (1996) Trilobite biogeography in the Middle Devonian: geological processes and analytical methods. *Paleobiology*, **22**, 66-79.

Lin CP, Danforth BN (2004) How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined datasets. *Molecular Phylogenetics and Evolution*, **30**, 686-702.

Linder HP (2003) The radiation of the Cape flora, southern Africa. *Biological Reviews*, **78**, 597-638.

Linder HP (2005) Evolution of diversity: the Cape flora. *Trends in Plant Science*, **10**, 536-541.

Linder HP, Vlok JHJ (1991) The morphology, taxonomy and evolution of *Rhodocoma* (Restionaceae). *Plant Systematics and Evolution*, **175**, 139-160.

Linder HP, Meadows ME, Cowling RM (1992) History of the Cape Flora. In: *The ecology of fynbos: nutrients, fire and diversity* (ed. Cowling RM), pp. 113-134. Oxford University Press, Cape Town.

Livingstone DA (1967) Postglacial vegetation of the Ruwenzori Mountains in equatorial Africa. *Ecological Monographs*, **37**, 25-51.

MacDevette DR, MacDevette DK, Gordon IG, Bartholomew RLC (1989) Floristics of the Natal indigenous forests. In: *Biogeography of the mixed evergreen forests of southern Africa* (ed. Geldenhuys CJ), pp. 124-145. Ecosystems Programmes Occasional Report no. 45. Foundation for Research Development, Pretoria.

Maddison WP, Maddison DR (2000) *MacClade: Analysis of Phylogeny and Character Evolution*, *Version 4.06*. Sinauer Associates, Sunderland, Massachusetts.

Maier CT (1982) Observations on the 17-year periodical cicada, *Magicicada septendecim* (Hemiptera, Homoptera, Cicadidae). *Annals of the Entomological Society of America*, **75**, 14-23.

Makokha JS, Bauer AM, Mayer W, Matthee CA (2007) Nuclear and mtDNA-based phylogeny of southern African sand lizards, *Pedioplanis* (Sauria: Lacertidae). *Molecular Phylogenetics and Evolution*, **44**, 622-633.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution*, **18**, 189-197.

Manni F, Guérard E, Heyer E (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by ‘‘Monmonier’s algorithm”. *Human Biology*, **76**, 173-190.

Mantel N (1967) The detection of disease clustering and the generalized regression approach. *Cancer Research*, **27**, 209-220.

Marshall DC, Cooley JR (2000) Reproductive character displacement and speciation in periodical cicadas, with description of a new species, 13-year *Magicicada neotredecim*. *Evolution*, **54**, 1313-1325.

Marshall DC, Hill KBR, Fontaine KM, Buckley TR, Simon C (2009) Glacial refugia in a maritime temperate climate: cicada (*Kikihia subalpina*) mtDNA phylogeography in New Zealand. *Molecular Ecology*, **18**, 1995-2009.

Marshall DC, Simon C, Buckley TR (2006) Accurate branch length estimation in partitioned Bayesian analyses requires accommodation of among-partition rate variation and attention to branch length priors. *Systematic Biology*, **55**, 993-1003.

Marshall DC, Slon K, Cooley JR, Hill KBR, Simon C (2008) Steady Plio-Pleistocene diversification and a 2-million-year sympatry threshold in a New Zealand cicada radiation. *Molecular Phylogenetics and Evolution*, **48**, 1054-1066.

Martin ARH (1968) Pollen analysis of Groenvlei lake sediments, Kynsna (South Africa). *Review of Palaeobotany and Palynology*, **7**, 107-144.

Matthee CA, Flemming AF (2002) Population fragmentation in the southern rock agama, *Agama atra*: more evidence for vicariance in Southern Africa. *Molecular Ecology*, **11**, 465-471.

Matthee CA, Robinson TJ (1996) Mitochondrial DNA differentiation among geographical populations of *Pronolagus rupestris*, Smith's red rock rabbit (Mammalia: Lagomorpha). *Heredity*, **76**, 514-523.

Matthee CA, Robinson TJ (1997) Mitochondrial DNA phylogeography and comparative cytogenics of the springhare, *Pedetes capensis* (Mammalia: Rodentia). *Journal of Mammalian Evolution*, **4**, 53-73.

Mayaux P, Bartholome E, Fritz S, Belward A (2004) A new land-cover map of Africa for the year 2000. *Journal of Biogeography*, **31**, 861-877.

McCarthy T, Rubidge B (2005) *The story of Earth and life: a southern African perspective on a 4.6-billion-year journey*. Struik, Cape Town.

McGeogh MA (1998) The selection, testing and application of terrestrial insects as bioindicators. *Biological Reviews*, **73**, 181-201.

Meadows ME, Baxter AJ (1999) Late Quaternary palaeoenvironments of the southwestern Cape, South Africa: a regional synthesis. *Quaternary International*, **57-58**, 193-206.

Meadows ME, Linder HP (1989) A reassessment of the biogeography and vegetation history of the southern Afromontane region. In: *Biogeography of the Mixed Evergreen Forests of Southern Africa* (ed. Geldenhuys CJd), pp 15-29. Foundation for Research Development, Pretoria.

Measey GJ, Galbusera P, Breyne P, Matthysen E (2007) Gene flow in a direct-developing, leaf litter frog between isolated mountains in the Taita Hills, Kenya. *Conservation Genetics*, **8**, 1177-1188.

Metcalf ZP (1963) General catalogue of the Homoptera, fascicle VIII, Cicadoidea, part 1. Cicadidae. section I. Tibiceninae. *North Carolina State College Contribution*, **1502**, 587-919.

Mey W (2005) Genuine Africans or tertiary immigrants? - The genus *Hydropsyche* in the afrotropical region (Insecta, Trichoptera: Hydropsychidae). In: *African Biodiversity: Molecules, Organisms, Ecosystems* (eds. Huber BA, Sinclair BJ, Lampe KH), pp. 141-150. Springer, New York.

Meyer CP, Paulay G (2005) DNA barcoding: error rates based on comprehensive sampling. *PLoS Biology*, **3**, e422.

Midgley D, Pitman W, Middleton B (1994) Surface water resources of South Africa 1990. *WRC Report No. 298/1/94, Water Reseach Commission*, Pretoria.

Midgley GF, Hannah L, Robertson R, Mac Donald DJ, Allsopp J (2001) Have Pleistocene climatic cycles influenced species richness patterns in the Greater Cape Mediterranean Region? *Journal of Mediterranean Ecology*, **2**, 137-144.

Mishler BD, Donoghue MJ (1982) Species concepts: a case for pluralism. *Systematic Zoology*, **31**, 491-503.

Mix A, Bard E, Schnieder R (2001) Environmental processes of the ice age: land, oceans, glaciers (EPILOG). *Quaternary Science Reviews*, **20**, 627-657.

Moore WS (1995) Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution*, **49**, 718-726.

Moritz C (1994) Defining 'Evolutionary Significant Units' for conservation. *Trends in Ecology & Evolution*, **9**, 373-375.

Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology*, **51**, 238-254.

Moritz C, Faith DP (1998) Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology*, **7**, 419-429.

Moritz C, Patton JL, Schneider CJ, Smith TB (2000) Diversification of rainforest faunas: an integrated molecular approach. *Annual Review of Ecology, Evolution and Systematics*, **31**, 533-563.

Morse GE, Farrell BD (2005) Interspecific phylogeography of the *Stator limbatus* species complex: the geographic context of speciation and specialization. *Molecular Phylogenetics and Evolution*, **36**, 201-213.

Moulds MS (1990) *Australian cicadas*. New South Wales University Press, Kensington.

Moulds MS (2005) An appraisal of the higher classification of cicadas (Hemiptera: Cicadoidea) with special reference to the Australian fauna. *Records of the Australian Museum*, **57**, 375-446.

Moussalli A, Herbert DG, Stuart-Fox D (2009) A phylogeny of the cannibal snails of southern Africa, genus *Natalina sensu lato* (Pulmonata: Rhytididae): Assessing concordance between morphology and molecular data. *Molecular Phylogenetics and Evolution*, **52**, 167-182.

Mucina L, Rutherford M (2006) *The vegetation of South Africa, Lesotho and Swaziland*. *Strelitzia*, *19*. South African National Biodiversity Institute, Pretoria.

Muwanika VB, Nyakaana S, Siegismund HR, Arctander P (2003) Phylogeography and population structure of the common warthog (*Phacochoerus africanus*) inferred from variation in mitochondrial DNA sequences and microsatellite loci. *Heredity*, **91**, 361-372.

Myers AA, Giller PS (1988) Process, pattern and scale in biogeography. In: *Analytical Biogeography: An integrated approach to the study of animal and plant distributions* (eds. Myers AA, Giller PS), pp. 3-12. Chapman & Hall, London.

National Geophysical Data Center (2001) ETOPO2 Global 2′-Elevations (CD-ROM). U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Boulder.

Nelson G (1978) From Candolle to Croizat: comments on the history of Biogeography. *Journal of the History of Biology*, **11**, 269-305.

Nicolas V, Bryja J, Akpatou B, Konecny A, Lecompte E, Colyn M, Lalis A, Couloux A, Denys C, Granjon L (2008) Comparitive phylogeography of two sibling species of forest-dwelling rodent (*Praomys rostratus* and *P. tullbergi*) in west Africa: different reactions to past forest fragmentation. *Molecular Ecology*, **17**, 5118-5134.

Nylander JAA (2004) MrModeltest v.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Nylander JAA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL (2004) Bayesian phylogenetic analysis of combined data. *Systematic Biology*, **53**, 47-67.

Page LM, Phelps SM (1994) Speciation and phylogenetic resolution. *Trends in Ecology & Evolution*, **9**, 298.

Palumbi SA, Martin A, Ramano S, McMillan W, Stice L, Grabowski G (1991) *The simple fools guide to PCR*. Department of Zoology, University of Hawaii, Honolulu.

Partridge TC, Maud RR (1987) Geomorphic evolution of southern Africa since the Mesozoic. *South African Journal of Geology*, **90**, 179-208.

Partridge TC, Scott L, Hamilton J (1999) Synthetic reconstructions of southern African environments during the Last Glacial Maximum (21-18kyr) and the Holocene Altithermal (8-6kyr). *Quaternary International*, **57-58**, 207-214.

Paterson HEH (1985) The recognition concept of species. *Species and Speciation*, **4**, 21-29.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288-295.

Pearson RG, Raxworthy CJ (2009) The evolution of local endemism in Madagascar: watershed versus climatic gradient hypotheses evaluated by null biogeographic models. *Evolution*, **63-64**, 959-967.

Percy DM, Page RDM, Cronk QCB (2004) Plant-insect interactions: double-dating associated insect and plant lineages reveals asynchronous radiations. *Systematic Biology*, **53**, 120-127.

Pinto-Juma GA, Quartau JA, Bruford MW (2008) Population structure of *Cicada Barbara* Stål (Hemiptera, Cicadoidea) from the Iberian Peninsula and Morocco based on mitochondrial DNA analysis. *Bulletin of Entomological Research*, **98**, 15-25.

Pinto-Juma G, Quartau JA, Bruford MW (2009) Mitochondrial DNA variation and the evolutionary history of the Mediterranean species of *Cicada* L. (Hemiptera, Cicadoidea). *Zoological Journal of the Linnean Society*, **155**, 266-288.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817-818.

Price BW, Barker NP, Villet MH (2007) Patterns and processes underlying evolutionary significant units in the *Platypleura stridula* L. species complex (Hemiptera: Cicadidae) in the Cape Floristic Region, South Africa. *Molecular Ecology*, **16**, 2574-2588.

Price BW, Barker NP, Villet MH (2010) A watershed study on genetic diversity: Phylogenetic analysis of the *Platypleura plumosa* (Hemiptera: Cicadidae) complex reveals catchment-specific lineages. *Molecular Phylogenetics and Evolution*, **54**, 617-626.

Prinsloo P, Robinson TJ (1992) Geographic mitochondrial-DNA variation in the Rock Hyrax, *Procavia capensis*. *Molecular Biology and Evolution*, **9**, 447-456.

Quartau JA, Ribeiro M, Simoes PC, Crespo A (2000) Taxonomic separation by isozyme electrophoresis of two closely related species of *Cicada* L. (Hemiptera: Cicadoidea) in Portugal. *Journal of Natural History*, **34**, 1677-1684.

Rambaut A, Drummond AJ (2003) Tracer version 1.3 (http://evolve.zoo.ox.ac.uk). Accessed November 2006.

Rambaut A, Drummond AJ (2007) Tracer version 1.4. (http://beast.bio.ed. ac.uk/Tracer). Accessed January 2009.

Rasmussen C, Cameron SA (2010) Global stingless bee phylogeny supports ancient divergence, vicariance, and long distance dispersal. *Biological Journal of the Linnean Society*, **99**, 206-232.

Raxworthy CJ, Forstner MRJ, Nussbaum RA (2002) Chameleon radiation by oceanic dispersal. *Nature*, **415**, 784-787.

Richardson JE, Weitz FM, Fay MF, Cronk QCB, Linder HP, Reeves G, Chase, MW (2001) Rapid and recent origin of species richness in the Cape Flora of South Africa. *Nature*, **421**, 181-183.

Robinson-Rechavi M, Huchon D (2000) RRTree: Relative-Rate Tests between groups of sequences on a phylogenetic tree. *Bioinformatics*, **16**, 296-297.

Roy MS, Sponer R, Fjeldså J (2001) Molecular systematics and evolutionary history of akalats (genus *Sheppardia*): a pre-Pleistocene radiation in a group of African forest birds. *Molecular Phylogenetics and Evolution*, **18**, 74-83.

Ryan PG, Bloomer P (1997) Geographic variation in Red Lark *Certhilauda burra* plumage, morphology, song and mitochondrial DNA haplotypes. *Ostrich*, **68**, 31-36.

Ryan PG, Bloomer P (1999) The long-billed lark complex: a species mosaic in southwestern Africa. *The Auk*, **116**, 194-208.

Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology & Evolution*, **1**, 9-10.

Sanborn AF, Villet MH, Phillips PK (2003) Hot-blooded singers: endothermy facilitates crepuscular signaling in African platypleurine cicadas (Hemiptera: Cicadidae: *Platypleura* spp.). *Naturwissenschaften*, **90**, 305-308.

Sanborn AF, Villet MH, Phillips PK (2004) Endothermy in African platypleurine cicadas: the influence of body size and habitat (Hemiptera: Cicadidae). *Physiological and Biochemical Zoology*, **77**, 816-823.

Schaefer H, Renner SS (2008) A phylogeny of the oil bee tribe Ctenoplectrini (Hymenoptera: Anthophila) based on mitochondrial and nuclear data: Evidence for early Eocene divergence and repeated out-of-Africa dispersal. *Molecular Phylogenetics and Evolution*, **47**, 799-811.

Schoonmaker PK, Foster DR (1991) Some implications of paleoecology for contemporary ecology. *The Botanical Review*, **57**, 204-245.

Schrire BD, Lavin M, Lewis GP (2005) Global distribution patterns of the Leguminosae: insights from recent phylogenies. *Biologiese Skrifter*, **55**, 375-422.

Scott JK (1996) Population ecology of *Chrysanthemiodes monilifera* in South Africa: implications for its control in Australia. *The Journal of Applied Ecology*, **33**, 1496-1508.

Scott L (1995) Pollen evidence for vegetation and climate change in southern Africa during the Neogene and Quaternary. In: *Paleoclimate and evolution with emphasis on human origins* (eds. Vrba ES, Denton GH, Partridge TC, Burckle LH), pp. 56-76. Yale University Press, New Haven.

Scott L, Anderson H, Anderson JM (1997) Vegetation history. In: *Vegetation in Southern Africa* (eds. Cowling RM, Richardson DM, Pierce SM), pp. 62-84. Cambridge University Press, Cambridge.

Shimodaira H, Hasegawa M (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution*, **16**, 1114-1116.

Siesser WG (1980) Late Miocene origin of the Benguela upswelling system off northern Namibia. *Science*, **208**, 283-285.

Siesser WG, Dingle RV (1981) Tertiary sea-level movements around southern Africa. *Journal of Geology*, **89**, 83-96.

Simoes PC, Quartau JA (2007) On the dispersal of males of *Cicada orni* in Portugal (Hemiptera: Cicadidae). *Entomologia Generalis*, **30**, 245-252.

Simon C, Frati F, Beckenbach AT, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, **87**, 651-701.

Simon J-C, Carré S, Boutin M, Prunier-Leterme N, Sabater-Mun B, Latorre A, Bournoville R (2003) Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **270**, 1703-1712.

Sites JW, Marshall JC (2004) Operational criteria for delimiting species. *Annual Review of Ecology Evolution and Systematics*, **35**, 199-227.

Smit HA, Robinson TJ, Van Vuuren BJ (2007) Coalescence methods reveal the impact of vicariance on the spatial genetic structure of *Elephantulus edwardii* (Afrotheria, Macroscelidea). *Molecular Ecology*, **16**, 2680-2692.

Smith MA, Woodley NE, Janzen DH, Hallwachs W, Hebert PDN (2006) DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences of the USA*, **103**, 3657-3662.

Sole CL, Scholtz CH, Bastos ADS (2005) Phylogeography of the Namib Desert dung beetles *Scarabaeus* (*Pachysoma*) MacLeay (Coleoptera: Scarabaeidae). *Journal of Biogeography*, **32**, 75-84.

Soltis DE, Morris AB, McLachlan JS, Manos PS, Soltis PS (2006) Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology*, **15**, 4261-4293.

South African National Water Act (1998) *Government Gazette*, **398**, No. 19182.

Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688-2690.

Stevens DM, Picker MD (1999) A revision of *Aphanicercella* Tillyard (Plecoptera: Notonemouridae) including the *A. barnardi* (Tillyard) species complex. *African Entomology*, **7**, 197-209.

Stukenberg BR (1969) Effective temperature as an ecological factor in southern Africa. *Zoologica Africana*, **4**, 145-197.

Sueur J, Vanderpool D, Simon C, Ouvrard D, Bourgoin T (2007) Molecular phylogeny of the genus *Tibicina* (Hemiptera, Cicadidae): rapid radiation and acoustic behaviour. *Biological Journal of the Linnean Society*, **91**, 611-626.

Swart BL, Tolley KA, Matthee CA (2008) Climate change drives speciation in the southern rock agama (*Agama atra*) in the Cape Floristic Region, South Africa. *Journal of Biogeography*, **36**, 78-87.

Swartz ER, Skelton PH, Bloomer P (2007) Sea-level changes, river capture and the evolution of populations of the Eastern Cape and fiery redfins (*Pseudobarbus afer* and *Pseudobarbus phlegethon*, Cyprinidae) across multiple river systems in South Africa. *Journal of Biogeography*, **34**, 2086-2099.

Swartz ER, Skelton PH, Bloomer P (2009) Phylogeny and biogeography of the genus *Pseudobarbus* (Cyprinidae): Shedding light on the drainage history of rivers associated with the Cape Floristic Region. *Molecular Phylogenetics and Evolution*, **51**, 75-84.

Swofford DL (2003) *PAUP*. Phylogenetic analysis using Parsimony (* and other methods). Version 4*. Sinauer Associates, Sunderland Massachusetts.

Talma AS, Vogel JC (1992) Late Quaternary paleotemperatures derived from a speleothem from Cango Caves, Cape Province, South Africa. *Quaternary Research*, **37**, 203-213.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596-1599.

Teske PR, Hamilton H, Matthee CA, Barker NP (2007) Signatures of seaway closures and founder dispersal in the phylogeny of a circumglobally distributed seahorse lineage. *BMC Evolutionary Biology*, **7**, 138.

Thompson J, Higgins D, Gibson T (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673-4680.

Tinley KL (1985) *Coastal Dunes of South Africa*. South African National Scientific Programmes Report no. 109, Pretoria.

Tolley KA, Burger M, Turner AA, Matthee CA (2006) Biogeographic patterns and phylogeography of dwarf chameleons (*Bradypodion*) in an African biodiversity hotspot. *Molecular Ecology*, **15**, 781-793.

Tolley KA, Chase BM, Forest F (2008) Speciation and radiations track climate transitions since the Miocene Climatic Optimum: a case study of southern African chameleons. *Journal of Biogeography*, **35**, 1402-1414.

Tolley KA, Tilbury CR, Branch WR, Matthee CA (2004) Phylogenetics of the southern African dwarf chameleons, *Bradypodion* (Squamata: Chamaeleonidae). *Molecular Phylogenetics and Evolution*, **30**, 354-365.

Tyson PD (1999) Late-Quaternary and Holocene palaeoclimates of southern Africa: a synthesis. *South African Journal of Geology*, **102**, 335-349.

van Wyk AE (1989) Floristics of the Natal and Pondoland sandstone forests. In: *Biogeography of the Mixed Evergreen Forests of Southern Africa* (ed. Geldenhuys CJ), pp 145-157. Occasional report No. 45. Foundation for Research Development, Pretoria.

van Wyk AE (1990) The sandstone regions of Natal and Pondoland: remarkable centres of endemism. *Palaeoecology of Africa*, **21**, 243-257.

van Zinderen Bakker EM (1975) The origin and palaeoenvironment of the Namib Desert biome. *Journal of Biogeography*, **2**, 65-73.

Vences M (2004) Origin of Madagascar's extant fauna: A perspective from amphibians, reptiles and other non-flying vertebrates. *Italian Journal of Zoology*, **2**, 217-228.

Vences M, Wollenberg KC, Vieites DR, Lees DC (2009) Madagascar as a model region of species diversification. *Trends in Ecology & Evolution*, **24**, 456-465.

Verboom GA, Archibald JK, Bakker FT, Bellstedt DU, Conrad F, Dreyer LL, Forest F, Galley C, Goldblatt P, Henning JF, Mummenhoff K, Linder HP, Muasya AM, Oberlander KC, Savolainen V, Snijman DA, Van der Niet T, Nowell TL (2009) Origin and diversification of the Greater Cape flora: ancient species repository, hot-bed of recent radiation, or both? *Molecular Phylogenetics and Evolution*, **51**, 44-53.

Villet MH (1987) Sound pressure levels of some African cicadas (Homoptera: Cicadoidea). *Journal of the Entomological Society of Southern Africa*, **50**, 269-273.

Villet MH (1988) Calling songs of some South African cicadas (Homoptera: Cicadidae). *South African Journal of Zoology*, **23**, 71-77.

Villet MH (1989) Systemic status of *Platypleura stridula* L. and *Platypleura capensis* L. (Homoptera: Cicadidae). *South African Journal of Zoology*, **24**, 329-332.

Villet MH (1992) Responses of free-living cicadas (Homoptera: Cicadidae) to broadcasts of cicada songs. *Journal of the Entomological Society of Southern Africa*, **55**, 93-97.

Villet MH (1995) Intraspecific variability in SMRS signals: some causes and implications in acoustic signaling systems. In: *Speciation and the Recognition Concept. Theory and Application* (eds. Lambert DM, Spencer HG), pp. 422-439. Johns Hopkins University Press, Baltimore.

Villet MH (1997) Redescription of three species of *Platypleura* Amyot & Serville 1843 (Hemiptera Cicadidae). *Tropical Zoology*, **10**, 321-332.

Villet MH, Reavell P (1989) Annotated provisional checklist and key to the platypleurine cicadas (Homoptera: Cicadidae) of coastal Natal. *South African Journal of Zoology*, **24**, 333-336.

Villet MH, van Noort S (1999) Cicadas (Hemiptera, Homoptera: Cicadoidea) of Mkomazi. In: *Mkomazi: the Ecology, Biodiversity and Conservation of a Tanzania Savanna* (eds Coe MJ, McWilliam NC, Stone GN, Packer MJ), pp. 223-234. Royal Geographical Society, London.

Villet MH, Barker NP, Lunt N (2004) Mechanisms generating biological diversity in the genus *Platypleura* Amyot & Serville, 1843 (Hemiptera: Cicadidae) in southern Africa: implications of a preliminary molecular phylogeny. *South African Journal of Science*, **100**, 589-594.

Villet MH, Sanborn AF, Phillips PK (2003) Endothermy and chorusing behaviour in the African platypleurine cicada *Pycna semiclara* (Germar, 1834) (Hemiptera: Cicadidae). *Canadian Journal of Zoology*, **81**, 1437-1444.

Wahlberg N, Leneveu J, Kodandaramaiah U, Peña C, Nylin S, Freitas AVL, Brower AVZ (2009) Nymphalid butterflies diversify following near demise at the Cretaceous/Tertiary boundary. *Proceedings of the Royal Society B - Biological Sciences*, **276**, 4295-4302.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, **10**, 506-513.

Weaver A, Eby M, Fanning AF, Wiebe EC (1998) Simulated influence of carbon dioxide, orbital forcing and ice sheets on the climate of the Last Glacial Maximum. *Nature*, **394**, 847-853.

Wegener A (1912) Die Entstehung der Kontinente. *Geologische Rundschau*, **3**, 276-292.

Wieczorek AM, Drewes RC, Channing A (2000) Biogeography and evolutionary history of *Hyperolius* species: application of molecular phylogeny. *Journal of Biogeography*, **27**, 1231-1243.

Wiley EO (1988) Vicariance biogeography. *Annual Review of Ecology, Evolution, and Systematics*, **19**, 513-542.

Williams KS, Simon C (1995) The ecology, behaviour and evolution of periodical cicadas. *Annual Review of Entomology*, **40**, 269-295.

Willis KJ, McElwain JC (2002) *The evolution of plants*. Oxford University Press, Oxford.

Willows-Munro S, Matthee CA (2009) The evolution of southern African members of the shrew genus *Myosorex*: Understanding the origin and diversification of a mophologically cryptic group. *Molecular Phylogenetics and Evolution*, **51**, 394-398.

Wishart MJ (2000) Catchments as conservation units for riverine biodiversity. *African Journal of Aquatic Science*, **25**, 169-174.

Wishart MJ, Hughes JM (2001) Exploring patterns of population subdivision in the net-winged midge, *Elporia barnardi* (Diptera: Blephariceridae), in mountain streams of the south-western Cape, South Africa. *Freshwater Biology*, **46**, 479-490.

Xia X, Xie Z (2001) DAMBE: Data analysis in molecular biology and evolution. *Journal of Heredity*, **92**, 371-373.

Yoder AD, Nowak MD (2006) Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. *Annual Review of Ecology, Evolution and Systematics*, **37**, 405-431.

Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science*, **292**, 686-693.

Zhang JF, Zhang XY (1990) Fossil insects of cicada (Homoptera) and true bugs (Heteroptera) from Shanwang, Shandong. *Acta Palaeontologica Sinica*, **29**, 337-348.

Zhang JF, Sun B, Zhang XY (1994) *Miocene insects and spiders from Shanwang, Shandong*. Science Press, Beijing.

Zink RM, McKitrick MC (1995) The debate over species concepts and its implications for ornithology. *Auk*, **112**, 701-719.

Zink RM, Blackwell-Rago RC, Ronquist F (2000) The shifting roles of dispersal and vicariance in biogeography. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **267**, 497-503.

Appendix

Table A1. Collectors acknowledged for their valuable contribution.

Sample Collectors			
L. Allan	D. Emery	L. Knott	T. Richardson
K. Amevoin	F. Erii	F. Koch	N. Riddin
A. Amin	G. Ferreira	D. Kroon	C. Robertson
A. Armstrong	M. Ferreira	M. Kruger	M. Robertson
J. Ball	H. Ficq	S. Kuria	J. Rochat
J. Barker	B. Filter	Y. Lee	Y. Saisho
N. Barker	B. Fisher	J. Legwai	A. Sanborn
P. Barratt	A. Foulis	H. Lewis	P. Sawatpon
T. Bellingan	J. Francis	M. Lorentz	D. Schlange
A. Bernard	H. Geertsema	N. Lunt	S. Shiyake
I. Bills	K. Gendall	A. Marais	E. Sieben
J. Boersma	S. Gess	D. Marshall	T. Smith
J. Booth	F. Gess	A. McClure	W. Snow
M. Boulard	T. Goble	C. Meyer	A. St. Quintin
A. Brassine	H. Gosling	J. Midgley	H. Staude
E. Brassine	R. Grimm	R. Mijburgh	R. Stephen
M. Brassine	M. Hamer	S. Mitchell	J. Sterley
E. Breytenbach	C. Hänel	A. Monadjem	R. Steven
A. Brinkman	R. Harin'Hala	B. Mostert	S. Sudnan
M. Bruce-Miller	P. Hawkes	M. Moulds	D. Swanepoel
E. Bruce-Miller	G. Henning	B. Moulds	L. Tello
B. Bytebier	C. Hepburn	T. Moulds	K. Tolley
K. Canavan	H. Hepburn	T. Muggerwa-Mukasa	C. Turnbull
A. Chawanji	E. Heyns	W. Muller	A. Turner
J. Chen	K. Hill	R. Murphy	A. van Harten
J. Chitimela	K. Hoelmer	M. Musgrave	S. van Noort
L. Clennell	D. Hood	T. Mwadima	K. Venter
I. Cockburn	B. Howells	L. Mwonmwa	I. Vermeulen
J. Cockburn	C. Huchzermeyer	K. Naojee	M. Villet
J. Coetzee	R. Huchzermeyer	T. Ndlovu	N. Voogt
J. Coetzer	M. Humphrey	G. Nicholson	W. Wabeke
S. Collins	D. Hutton	A. Northrop	L. Wagura
J. Cooley	M. Irwin	E. Ohya	N. Wahlberg
C. Coombes	C. Jackson	C. Owen	S. Walton
G. Coombs	D. Jacobs	J. Palmer	C. Warui
J. Coppinger	W. Jacobsz	G. Papenhuijzen	L. Wet
A. Craig	V. Jali	R. Pare	G. Whittington-Jones
K. Crous	L. Jingke	F. Parker	K. Wilkins
J. Cryan	A. Johnson	I. Paterson	G. Williams
M. Cunningham	T. Johnson	C. Pattrick	J. Williams
A. Curle	S. Kaehler	M. Paulsen	S. Wilson
K. Czypionka	S. Kamande	L. Pereira	A. Winkler
R. Daly	H. Kaniba	R. Perrisinotto	S. Wongvilas
F. de Moor	G. Keevey	S. Pierce	N. Woods
M. Denton	C. Kelly	E. Pringle	M-X. Yang
J. Dils	L. Kirkland	H. Purdon	R. Yeadon
B. Dombrowsky	A. Kirk-Spriggs	J. Rapson	R. Young
B. du Preez	J. Klingenberg	C. Richards	G. Youthed

Table A2. Locality and Genbank accession information of samples used for tribal analyses. Sequences donated by the Simon Lab are denoted (CS). Type species of genera in bold.

Species	Locality	Voucher #	Genbank Accession Number			
			16S	COI	COII	EF1a
Platypleurini:						
Afzeliada afzelii	unknown, Gabon	MHV0468	FJ168845	GU344060	GU344016	GU343836
Albanycada albigera	Kieskamma River Pass, South Africa	MHV0334	GU344078	GU344055	GU344006	GU343825
Albanycada sp. 1	nr Willowmore, South Africa	MHV0423	FJ168837	FJ169041	GU344010	GU343829
Azanicada zuluensis	Fish River Mouth, South Africa	MHV0236	GU344077	GU344053	GU344000	-
Brevisiana brevis	Mpumalanga, South Africa	MHV0650	FJ168914	FJ169115	GU344024	GU343843
Canualna liberiana	Annobon Island, Equatorial Guinea	MHV1553	GU344094	-	-	GU343869
Capcicada decora	Bainskloof Pass, South Africa	MHV0304	FJ168810	FJ169014	GU344002	GU343822
Ioba leopardina	Mazabuka, Zambia	MHV0643	FJ168912	FJ169114	-	GU343841
Karscheliana parva	Mpala Ranch, Kenya	MHV0686	GU344083	GU344062	GU344027	GU343846
Koma bombifrons	Mazabuka, Zambia	MHV0644	FJ168913	FJ169081	GU344023	GU343842
Kongota punctigera	Ilfracoombe, South Africa	MHV0343	FJ168825	FJ169030	GU344008	GU343827
Munza laticlavia	Upington, South Africa	MHV0248	FJ168801	FJ169005	GU344001	GU343821
Oxypleura calypso	Christmas Island, Australia	MHV0448	-	GU344058	-	GU343831
Oxypleura quadraticollis	Malilangwe GR, Zimbabwe	MHV0162	FJ168788	FJ168994	GU343993	-
Platypleura arabica	Wadi Bih dam, United Arab Emirates	MHV1202	GU344086	GU344064	GU344041	-
Platypleura 'breedeflumensis'	nr Prince Albert, South Africa	MHV0602	FJ168898	FJ169101	GU344020	FJ215654
Platypleura brunea	Cathcart, South Africa	MHV0825	FJ168923	FJ169127	GU343888	GU343852
Platypleura capensis	Cannon Rocks, South Africa	MHV0021	EF134521	FJ168988	GU343990	FJ215646
Platypleura 'catenata' isolate CM	nr Swellendam, South Africa	MHV0317	EF134579	GU344054	GU344004	-
Platypleura 'catenata' isolate EM	Uniondale, South Africa	MHV0152	EF134560	FJ168993	GU343992	GU343815
Platypleura 'catenata' isolate WM	nr Worcester, South Africa	MHV0312	EF134578	FJ169015	GU344003	GU343823
Platypleura cf. *girardi*	unknown, Togo	MHV0459	FJ168841	FJ169045	GU344013	-
Platypleura cf. *girardi*	Comoe, Ivory Coast	MHV0720	-	FJ169120	GU344028	GU343847
Platypleura chalybaea	Zuurberg Pass, South Africa	MHV1022	FJ168966	FJ169169	GU343912	GU343862
Platypleura divisa	Whittlesea, South Africa	MHV0515	FJ168853	FJ169055	GU344017	GU343837
Platypleura duesta	nr Grasskop, South Africa	MHV0992	FJ168958	FJ169161	GU344037	GU343861
Platypleura 'gamtoosflumensis'	nr Loerie, South Africa	MHV1018	FJ168962	FJ169165	GU344038	FJ215657
Platypleura 'gariepflumensis'	Pella, South Africa	MHV0183	FJ168791	FJ168996	GU343994	FJ215647
Platypleura gowdeyi	Mpala Ranch, Kenya	MHV0187	FJ168792	FJ168997	GU343995	GU343816
Platypleura haglundi	Weenen Nature Reserve, South Africa	MHV0590	FJ168890	FJ169093	GU344018	GU343838
Platypleura hilpa	Ban Sen Island, Vietnam	MHV0219	FJ168796	FJ169000	GU343996	GU343817
Platypleura hirta	Renosterpoort, South Africa	MHV0880	FJ168937	FJ169141	GU344032	GU343854

Table A2. (cont.)

Species	Locality	Voucher #	Genbank Accession Number			
			16S	COI	COII	EF1a
Platypleura hirtipennis	Port Elizabeth, South Africa	MHV1020	FJ168964	FJ169167	GU344039	FJ215659
Platypleura 'intercapedinis'	Alexandria Forest, South Africa	MHV0912	FJ168946	FJ169150	GU343891	GU343857
Platypleura kaempferi	Miuami, Ashikara City, Japan	MHV0461	FJ168842	FJ169046	GU344014	GU343834
Platypleura kaempferi	Zhongjiang, China	MHV1479	GU344093	GU344070	GU344047	GU343868
Platypleura 'karooensis'	Ghwarriepoort River, South Africa	MHV0323	EF134552	FJ169020	GU344005	GU343824
Platypleura longirostris	Mbololo Forest, Kenya	MHV1169	FJ168981	FJ169184	GU343915	GU343863
Platypleura maytenophila	nr Jozini, South Africa	MHV0989	FJ168957	FJ169160	GU344036	GU343860
Platypleura mijburghi	Pongolapoort Pass, South Africa	MHV0964	FJ168953	GU343741	GU343907	-
Platypleura mira	Mae Ho, Thailand	MHV0669	-	-	GU344026	-
Platypleura murchisoni	nr Makhado, South Africa	MHV0960	FJ168952	FJ169156	GU343906	GU343858
Platypleura nobilis	Toungka Forest, Thailand	MHV0668	FJ168915	FJ169116	GU344025	GU343844
Platypleura octoguttata	Nagar Parker, Pakistan	MHV1559	GU344096	-	GU344049	-
Platypleura 'olifantsflumensis'	Vanrhynsdorp, South Africa	MHV0867	FJ168936	FJ169140	GU344031	GU343853
Platypleura pinheyi	Braganza South, Mozambique	MHV1289	GU344088	GU344066	GU344043	-
Platypleura pinheyi	Perekezi Forest, Malawi	MHV1410	GU344090	GU344068	GU344045	GU343866
Platypleura plumosa	Klipplaat, South Africa	MHV1042	FJ168974	FJ169177	GU344040	-
Platypleura signifera	nr Groenriviermond, South Africa	MHV0888	FJ168939	FJ169143	GU344034	GU343856
Platypleura fulvigera	Mindoro, Philippines	MHV1561	GU344097	GU344072	-	-
Platypleura sp. 08	Collingham Ridge, South Africa	MHV0338	FJ168822	FJ169027	GU344007	GU343826
Platypleura sp. 10	Van Rhynsdorp Pass, South Africa	MHV0010	EF134551	FJ168984	GU343989	GU343812
Platypleura sp. 10	nr Montague, South Africa	MHV0600	FJ168896	FJ169099	GU344019	-
Platypleura sp. 14	nr Maclear, South Africa	MHV0988	FJ168956	FJ169159	GU344035	GU343859
Platypleura sp. 15	Wakkerstroom, South Africa	MHV0881	FJ168938	FJ169142	GU344033	GU343855
Platypleura sp. 16	Nichisi Forest Reserve, Malawi	MHV0740	GU344084	GU344063	GU344029	GU343848
Platypleura sp. 17	Swan Mine, Democratic Republic of the Congo	MHV1295	GU344089	GU344067	GU344044	GU343865
Platypleura sp. 18	Kakamega Forest, Kenya	MHV0767	-	FJ169124	GU344030	GU343850
Platypleura sp. 19	Mughese Forest Reserve, Malawi	MHV0757	FJ168921	FJ169123	-	GU343849
Platypleura stridula	Citrusdal, South Africa	MHV0001	EF134568	FJ168982	GU343988	-
Platypleura takasagona	unknown, Taiwan	03.TW.TP.UCH.51	-	CS	CS	CS
Platypleura techowi	Kimberley, South Africa	MHV0232	FJ168797	FJ169001	GU343998	GU343819
Platypleura wahlbergi	Beacon Bay, South Africa	MHV0365	EF134550	GU344056	GU344009	GU343828
Pycna coelstia	Fa-Mu-Dui, Tibet	MHV1568	GU344099	-	-	-
Pycna repanda	Mari, Pakistan	MHV1558	GU344095	GU344071	GU344048	-
Pycna semiclara	Grahamstown, South Africa	MHV0022	FJ168784	FJ168989	GU343932	GU343813

Table A2. (cont.)

Species	Locality	Voucher #	Genbank Accession Number			
			16S	COI	COII	EF1a
Platypleura hirtipennis	Port Elizabeth, South Africa	MHV1020	FJ168964	FJ169167	GU344039	FJ215659
Sadaka radiata	Gola National Forest , Liberia	MHV0396	GU344079	-	-	-
Severiana severini	Ongongo camp site, Namibia	MHV0035	FJ168785	FJ168990	GU343991	GU343814
Soudaniella marshalli	Mazabuka, Zambia	MHV0642	FJ168911	FJ169113	GU344022	GU343840
Strumosella limpida	unknown, Ghana	MHV0457	GU344081	GU344059	GU344012	GU343833
Suisha coreana	Ilsanseo-gu, South Korea	MHV0224	GU344075	-	-	-
Ugada grandicollis	nr Takoradi, Ghana	MHV0455	-	-	-	GU343832
Yanga guttulata	nr Antsohihy, Madagascar	MHV0634	GU344082	GU344061	GU344021	GU343839
Orapini						
Orapa numa	Ngangao Forest, Taita Hills, Kenya	MHV0674	FJ168916	FJ169117	GU343941	GU343845
Hamzini						
Hamza ciliaris	Talilabu, Indonesia	MHV1562	GU344098	GU344073	GU344050	-
Cryptotympanini:						
Chremistica moultoni	Huai Kaeo, Thailand	MHV0771	GU344085	CS	-	GU343851
Cryptotympana atrata	Zhongjiang, China	MHV1476	GU344091	GU344069	GU344046	-
Cryptotympana mandarina	Ban Sen Island, Vietnam	MHV0220	GU344074	GU344051	GU343997	GU343818
Cryptotympana takasagona	Kueitan Hot Spring, Taiwan	MHV0233	GU344076	GU344052	GU343999	GU343820
Lyristes bihamatus	Iwaizumi, Japan	MHV0444	GU344080	GU344057	GU344011	GU343830
Oncotympanini:						
Oncotympana maculicollis	Zhongjiang, China	MHV1477	GU344092	CS	-	GU343867
Cicadini:						
Cicada orni	Bouches-du-Rhône, France	07.FR.BD.CLP.06	-	GQ527099	EU401955	GQ527139
Meimuna opalifera	Anyang, South Korea	MHV1237	GU344087	GU344065	GU344042	GU343864

Table A3. Locality, host plant, voucher number and Genbank accession number of samples within each clade of the *P. stridula* complex.

Clade	Locality	Latitude	Longitude	Collection #	Host plants	Genbank # (COI)	Genbank # (16S)
EC	Gulu River	33° 07' 04.2" S	27° 43' 31.7" E	MHV0063	*B. discolor*	DQ912415	EF134549
EC	Kleinemonde	33° 32' 33.7" S	27° 01' 56.7" E	MHV0205	*C. monilifera*	DQ912416	EF134520
EC	Cannon Rocks	33° 44' 53.9" S	26° 32' 03.2" E	MHV0021	*B. discolor / C. monilifera*	DQ912418	EF134521
EC	Cannon Rocks	33° 45' 07.1" S	26° 31' 54.5" E	MHV0391	*B. discolor*	DQ912417	EF134530
EC	Sundays River	33° 42' 54.7" S	25° 47' 12.7" E	MHV0390	*C. monilifera*	DQ912419	EF134529
EC	Cape Receiffe	33° 59' 51.2" S	25° 40' 55.9" E	MHV0389	*C. monilifera*	DQ912420	EF134527
EC	Schoenmakerskop	34° 02' 30.1" S	25° 33' 01.0" E	MHV0267	*C. monilifera*	DQ912421	EF134531
EC	Sea View	34° 01' 06.1" S	25° 22' 11.2" E	MHV0268	*C. monilifera*	DQ912422	EF134535
CC	Jeffreys Bay	34° 00' 59.6" S	24° 54' 48.3" E	MHV0269	*C. monilifera*	DQ912423	EF134534
CC	Cape St Francis	34° 11' 09.3" S	24° 49' 16.6" E	MHV0388	*C. monilifera*	DQ912424	EF134533
CC	Prince Albert Pass	33° 51' 53.1" S	23° 09' 10.2" E	MHV0318	*M. muricata*	DQ912427	EF134532
CC	Nature's Valley	33° 58' 23.2" S	23° 32' 53.3" E	MHV0376	*C. monilifera*	DQ912425	EF134541
CC	nr Plettenberg Bay	33° 59' 31.6" S	23° 26' 10.5" E	MHV0375	*M. muricata*	DQ912426	EF134540
CC	nr Mosselbay	34° 06' 03.6" S	22° 07' 02.6" E	MHV0374	*C. monilifera*	DQ912429	EF134526
CC	Knysna	34° 01' 56.8" S	22° 59' 20.6" E	MHV0271	*T. camphoratus*	DQ912428	EF134542
CC	nr Albertinia	34° 10' 19.1" S	21° 21' 37.1" E	MHV0373	*C. monilifera*	DQ912431	EF134539
CC	Witsand	34° 23' 22.9" S	20° 52' 02.2" E	MHV0272	*C. monilifera*	DQ912435	EF134545
CC	Arniston	34° 39' 38.1" S	20° 13' 13.5" E	MHV0274	*C. monilifera / M. muricata*	DQ912434	EF134537
CC	Bredasdorp	34° 37' 04.5" S	20° 10' 25.4" E	MHV0273	*C. monilifera*	DQ912430	EF134538
CC	Struisbaai	34° 46' 44.9" S	20° 01' 53.3" E	MHV0275	*C. monilifera / M. muricata*	DQ912433	EF134543
CC	nr Struisbaai	34° 45' 46.8" S	20° 01' 47.0" E	MHV0276	*C. monilifera / M. muricata*	DQ912432	EF134544
WC	Die Dam	34° 45' 00.6" S	19° 40' 40.3" E	MHV0277	*C. monilifera / M. muricata*	DQ912437	EF134546
WC	nr Gansbaai	34° 35' 30.0" S	19° 21' 08.4" E	MHV0278	*C. monilifera*	DQ912436	EF134547
WC	Hermanus	34° 25' 01.0" S	19° 12' 57.9" E	MHV0279	*C. monilifera*	DQ912438	EF134548
WC	Pringle Bay	34° 17' 47.8" S	18° 49' 20.2" E	MHV0310	*M. muricata*	DQ912439	EF134528
WC	nr Melkboschstrand	33° 46' 39.6" S	18° 28' 04.3" E	MHV0371	*C. monilifera*	DQ912443	EF134525
WC	Cape Point	34° 14' 51.8" S	18° 28' 29.0" E	MHV0372	*T. camphoratus*	DQ912440	EF134536
WC	nr Melkboschstrand	33° 45' 24.4" S	18° 27' 22.8" E	MHV0370	*M. muricata*	DQ912444	EF134522
WC	Koeberg	33° 38' 26.0" S	18° 26' 42.1" E	MHV0369	*C. monilifera*	DQ912445	EF134524
WC	Fish Hoek	34° 07' 53.3" S	18° 26' 17.0" E	MHV0368	*C. monilifera*	DQ912441	EF134523
WC	Scarborough	34° 12' 06.7" S	18° 22' 55.8" E	MHV0367	*C. monilifera*	DQ912442	EF134519
EM	nr Uniondale	33° 42' 21.8" S	23° 09' 49.4" E	MHV0152	*C. graminea*	DQ912477	EF134560
EM	Xagodi San	33° 44' 22.9" S	23° 46' 30.4" E	MHV0382	*M. muricata*	DQ912470	EF134563
EM	Joubertina	33° 49' 37.6" S	23° 52' 36.9" E	MHV0381	*M. muricata*	DQ912475	EF134598

Table A3. (cont.)

Clade	Locality	Latitude	Longitude	Collection #	*Host plants*	Genbank # (COI)	Genbank # (16S)
EM	nr Joubertina	33° 53' 34.3" S	24° 06' 08.5" E	MHV0383	*M. muricata*	DQ912474	EF134557
EM	Kareedow	33° 56' 33.0" S	24° 16' 04.4" E	MHV0384	*M. muricata*	DQ912473	EF134564
EM	nr Kareedow	33° 58' 58.8" S	24° 30' 39.9" E	MHV0385	*M. muricata*	DQ912476	EF134565
EM	nr Humansdorp	33° 59' 41.7" S	24° 43' 19.5" E	MHV0377	*M. muricata*	DQ912478	EF134553
EM	nr Humansdorp	33° 59' 42.4" S	24° 43' 09.4" E	MHV0270	*M. muricata*	DQ912469	EF134570
EM	Jeffreys Bay	34° 00' 49.7" S	24° 53' 04.3" E	MHV0392	*M. muricata*	DQ912472	EF134566
EM	nr Humansdorp	34° 03' 14.0" S	24° 29' 33.5" E	MHV0393	*M. muricata*	DQ912471	EF134567
CM	nr Barrydale	34° 01' 26.9" S	20° 35' 46.4" E	MHV0317	*C. graminea*	DQ912466	EF134579
CM	Houwhoek Pass	34° 13' 37.6" S	19° 11' 15.3" E	MHV0379	*M. muricata*	DQ912468	EF134554
CM	Houwhoek Pass	34° 13' 44.0" S	19° 11' 15.2" E	MHV0288	*C. ruscifolia*	DQ912465	EF134572
CM	Theewaterskloof Dam	34° 03' 23.0" S	19° 08' 44.3" E	MHV0291	*C. graminea*	DQ912467	EF134587
WM	nr Worcester	33° 33' 20.0" S	19° 30' 55.0" E	MHV0312	*C. graminea*	DQ912451	EF134578
WM	Bainskloof Pass	33° 33' 07.8" S	19° 09' 31.3" E	MHV0303	*M. muricata*	DQ912462	EF134573
WM	nr Bainskloof Pass	33° 30' 49.4" S	19° 11' 29.2" E	MHV0306	*C. ruscifolia*	DQ912457	EF134574
WM	nr Worcester	33° 36' 10.6" S	19° 20' 12.3" E	MHV0307	*C. ruscifolia*	DQ912448	EF134575
WM	Dutoitskloof Pass	33° 42' 15.2" S	19° 14' 25.3" E	MHV0309	*C. ruscifolia*	DQ912454	EF134576
WM	Dutoitskloof Pass	33° 44' 42.8" S	19° 03' 04.6" E	MHV0311	*C. ruscifolia*	DQ912461	EF134577
WM	Franschhoek	33° 52' 42.7" S	19° 01' 48.6" E	MHV0296	*M. muricata*	DQ912458	EF134591
WM	Boschendal	33° 53' 03.3" S	18° 58' 02.6" E	MHV0299	*C. polygonifolia*	DQ912455	EF134594
WM	Franschhoek Pass	33° 55' 17.8" S	19° 08' 17.9" E	MHV0294	*Eucalyptus sp.*	DQ912456	EF134589
WM	Jonkershoek	33° 58' 07.0" S	18° 56' 04.1" E	MHV0300	*C. ruscifolia*	DQ912453	EF134595
WM	Stellenbosch	33° 59' 01.2" S	18° 50' 10.9" E	MHV0281	*C. ruscifolia*	DQ912449	EF134581
WM	Theewaterskloof Dam	34° 00' 51.7" S	19° 12' 18.5" E	MHV0292	*M. muricata*	DQ912460	EF134588
WM	nr Elgin	34° 11' 15.0" S	19° 05' 39.8" E	MHV0287	*M. muricata*	DQ912450	EF134584
WM	nr Grabow	34° 07' 09.8" S	19° 03' 03.3" E	MHV0290	*C. ruscifolia*	DQ912452	EF134586
WM	Sir Lowry's Pass	34° 08' 04.1" S	18° 55' 31.1" E	MHV0394	*C. ruscifolia*	DQ912446	EF134558
WM	Sir Lowry's Pass	34° 08' 04.2" S	18° 55' 30.6" E	MHV0283	*C. ruscifolia*	DQ912464	EF134600
WM	Sir Lowry's Pass	34° 08' 59.7" S	18° 56' 04.4" E	MHV0284	*M. muricata*	DQ912463	EF134583
WM	Grabow	34° 09' 42.0" S	19° 00' 37.9" E	MHV0380	*Platanus sp.*	DQ912447	EF134556
WM	Grabow	34° 09' 42.2" S	19° 00' 37.9" E	MHV0286	*C. ruscifolia / M. muricata*	DQ912459	EF134571

Table A4. Locality, host plant, voucher number and Genbank accession number of *P. stridula*, *Platypleura* sp. 10 and outgroup samples.

Clade	Locality	Latitude	Longitude	Collection #	Host plants	Genbank # (COI)	Genbank # (16S)
P. stridula	Franschhoek	33° 54' 50.2" S	19° 07' 25.3" E	MHV0180	unidentified	DQ912482	EF134561
P. stridula	Dwarsrivier Farm	32° 13' 14.4" S	18° 59' 19.8" E	MHV0024	*Salix babylonica*	DQ912488	EF134555
P. stridula	Citrusdal	32° 35' 48.0" S	19° 00' 30.0" E	MHV0001	unidentified	DQ912487	EF134568
P. stridula	Algeria	32° 43' 00.0" S	19° 03' 00.0" E	MHV0025	unidentified	DQ912489	EF134559
P. stridula	Bainskloof Pass	33° 33' 21.4" S	19° 08' 59.1" E	MHV0302	*Salix mucronata*	DQ912490	EF134596
P. stridula	nr Worcester	33° 36' 10.6" S	19° 20' 12.4" E	MHV0341	*C. graminea*	DQ912492	EF134597
P. stridula	nr Paarl	33° 47' 35.2" S	18° 57' 09.1" E	MHV0297	*Eucalyptus sp.*	DQ912483	EF134592
P. stridula	nr Pniel	33° 52' 04.3" S	18° 58' 36.0" E	MHV0298	*Platanus x hispanica*	DQ912485	EF134593
P. stridula	Franscheshoek	33° 53' 00.4" S	19° 04' 15.7" E	MHV0295	*Platanus x hispanica*	DQ912486	EF134590
P. stridula	Cloetiesville	33° 54' 18.0" S	18° 51' 32.3" E	MHV0280	*Leucas sp.*	DQ912480	EF134580
P. stridula	Cloetiesville	33° 54' 09.0" S	18° 51' 25.8" E	MHV0029	unidentified	DQ912481	EF134569
P. stridula	Onderpapagaaiberg	33° 56' 27.0" S	18° 49' 11.0" E	MHV0412	unidentified	DQ912479	EF134599
P. stridula	Stellenbosch	34° 00' 10.4" S	18° 49' 28.3" E	MHV0282	*C. ruscifolia*	DQ912484	EF134582
P. stridula	Caledon	34° 13' 41.9" S	19° 25' 48.3" E	MHV0289	*S. babylonica*	DQ912493	EF134585
P. stridula	nr Elgin	34° 14' 05.3" S	19° 11' 30.8" E	MHV0378	*Eucalyptus sp.*	DQ912491	EF134562
P. sp 10	Van Rhynsdorp Pass	31° 23' 00.0" S	19° 01' 00.0" E	MHV0010	unidentified	EF134605	EF134551
P. sp 4	Ghwarriepoort River	33° 23' 18.0" S	23° 23' 15.0" E	MHV0323	*Acacia karroo*	EF134604	EF134552
P. hirtipennis	Brentwood Farm	33° 29' 37.0" S	26° 09' 15.0" E	MHV0195	unidentified	EF134602	EF134601
P. walhbergi	East London	32° 57' 27.0" S	27° 56' 03.0" E	MHV0365	*Berkheya heterophylla*	EF134603	EF134550

Table A5. Locality and Genbank accession information of samples collected in the *P. plumosa* group (mitochondrial DNA data).

					Genbank Accession	
Species	**Locality**	**Collection #**	**Latitude**	**Longitude**	**16S**	**CO1**
P. hirtipennis	Brentwood Farm	MHV0195	33° 29' 37" S	26° 09' 15" E	EF134601	FJ168998
P. hirtipennis	Grahamstown	MHV0256	33° 18' 28" S	26° 31' 23" E	FJ168805	FJ169009
P. hirtipennis	nr Coombs View	MHV0332	33° 16' 48" S	26° 48' 26" E	FJ168820	FJ169025
P. hirtipennis	Grahamstown	MHV0425	33° 18' 40" S	26° 31' 46" E	FJ168838	FJ169042
P. hirtipennis	Carls' Rust Farm	MHV0481	33° 21' 55" S	26° 17' 49" E	FJ168846	FJ169049
P. hirtipennis	nr Coombs View	MHV0486	33° 16' 50" S	26° 48' 30" E	FJ168847	FJ169050
P. hirtipennis	Fish River Hotel	MHV0488	33° 28' 38" S	27° 08' 28" E	FJ168848	FJ169051
P. hirtipennis	nr Grahamstown	MHV0543	33° 14' 43" S	26° 25' 35" E	FJ168864	FJ169066
P. hirtipennis	nr Riebeck East	MHV0547	33° 11' 39" S	26° 14' 24" E	FJ168865	FJ169067
P. hirtipennis	nr Jansenville	MHV0551	33° 08' 56" S	25° 55' 32" E	FJ168866	FJ169068
P. hirtipennis	Barville Park	MHV0612	33° 38' 42" S	26° 42' 35" E	FJ168904	FJ169107
P. hirtipennis	Salem	MHV0613	33° 28' 10" S	26° 28' 52" E	FJ168905	FJ169108
P. hirtipennis	nr Alicedale	MHV0997	33° 20' 12" S	26° 09' 06" E	FJ168959	FJ169162
P. hirtipennis	Port Elizabeth	MHV1020	33° 51' 42" S	25° 28' 18" E	FJ168964	FJ169167
P. hirtipennis	nr Paterson	MHV1028	33° 29' 09" S	25° 55' 57" E	FJ168968	FJ169171
P. plumosa	nr Uniondale	MHV0151	33° 40' 07" S	23° 07' 45" E	FJ168787	FJ168992
P. plumosa	Sundays River	MHV0200	33° 42' 54" S	25° 47' 13" E	FJ168795	FJ168999
P. plumosa	nr Mountain Zebra N.P.	MHV0259	32° 15' 00" S	25° 27' 00" E	FJ168806	FJ169010
P. plumosa	nr Uniondale	MHV0320	33° 38' 30" S	23° 08' 03" E	FJ168814	FJ169018
P. plumosa	Ghwarriepoort River	MHV0321	33° 23' 13" S	23° 23' 36" E	FJ168815	FJ169019
P. plumosa	nr Willowmore	MHV0324	33° 15' 13" S	23° 33' 56" E	FJ168816	FJ169021
P. plumosa	nr Steydtlerville	MHV0327	33° 17' 16" S	23° 57' 34" E	FJ168817	FJ169022
P. plumosa	Steydtlerville	MHV0329	33° 20' 10" S	24° 20' 16" E	FJ168818	FJ169023
P. plumosa	nr Baroe	MHV0331	33° 14' 27" S	24° 36' 58" E	FJ168819	FJ169024
P. plumosa	Fish River bridge	MHV0333	33° 14' 14" S	26° 59' 49" E	FJ168821	FJ169026
P. plumosa	Graaf Reinet	MHV0421	32° 15' 14" S	24° 31' 47" E	FJ168835	FJ169039
P. plumosa	Willowmore	MHV0422	33° 00' 01" S	23° 45' 11" E	FJ168836	FJ169040
P. plumosa	Fort Brown	MHV0501	33° 07' 54" S	26° 37' 06" E	FJ168849	FJ169052
P. plumosa	Koonap River	MHV0502	33° 02' 55" S	26° 39' 30" E	FJ168850	FJ169053
P. plumosa	Carlisle Bridge	MHV0533	33° 04' 57" S	26° 13' 30" E	FJ168854	FJ169056
P. plumosa	nr Bedford	MHV0534	32° 53' 32" S	26° 05' 51" E	FJ168855	FJ169057
P. plumosa	Baviaans River bridge	MHV0535	32° 36' 14" S	25° 53' 10" E	FJ168856	FJ169058
P. plumosa	Riet River bridge	MHV0536	32° 25' 49" S	25° 46' 11" E	FJ168857	FJ169059
P. plumosa	Tarka River bridge	MHV0537	32° 18' 56" S	25° 44' 29" E	FJ168858	FJ169060
P. plumosa	Cradock	MHV0538	32° 10' 24" S	25° 37' 04" E	FJ168859	FJ169061
P. plumosa	Pauls River bridge	MHV0539	32° 02' 34" S	25° 30' 10" E	FJ168860	FJ169062
P. plumosa	Great Fish River bridge	MHV0540	31° 54' 38" S	25° 24' 46" E	FJ168861	FJ169063
P. plumosa	nr Middleburg	MHV0541	31° 49' 13" S	25° 20' 05" E	FJ168862	FJ169064
P. plumosa	Grootfontein, Middleburg	MHV0542	31° 28' 01" S	25° 01' 02" E	FJ168863	FJ169065
P. plumosa	nr Jansenville	MHV0553	33° 10' 08" S	25° 46' 35" E	FJ168867	FJ169069
P. plumosa	nr Renosterfontein	MHV0555	33° 09' 14" S	25° 28' 49" E	FJ168868	FJ169070
P. plumosa	nr Renosterfontein	MHV0556	33° 10' 39" S	25° 39' 36" E	FJ168869	FJ169071
P. plumosa	nr Renosterfontein	MHV0558	33° 07' 34" S	25° 20' 18" E	FJ168870	FJ169072
P. plumosa	nr Graaff Reinet	MHV0562	32° 31' 11" S	24° 39' 32" E	FJ168871	FJ169073
P. plumosa	nr Graaff Reinet	MHV0563	32° 17' 19" S	24° 33' 33" E	FJ168872	FJ169074
P. plumosa	nr Graaff Reinet	MHV0564	32° 20' 33" S	24° 24' 07" E	FJ168873	FJ169075
P. plumosa	nr Aberdeen	MHV0565	32° 23' 02" S	24° 13' 38" E	FJ168874	FJ169076
P. plumosa	Aberdeen	MHV0566	32° 28' 21" S	24° 03' 10" E	FJ168875	FJ169077

Table A5. (cont.)

Species	Locality	Collection #	Latitude	Longitude	Genbank Accession	
					16S	CO1
P. plumosa	nr Aberdeen	MHV0567	32° 27' 41" S	23° 51' 38" E	FJ168876	FJ169078
P. plumosa	nr De Rust	MHV0609	33° 30' 26" S	22° 29' 54" E	FJ168902	FJ169105
P. plumosa	nr Prince Albert	MHV0610	33° 13' 17" S	22° 25' 33" E	FJ168903	FJ169106
P. plumosa	nr Nanaga	MHV0614	33° 38' 26" S	25° 53' 22" E	FJ168906	FJ169109
P. plumosa	Rietfontein Farm	MHV0616	32° 59' 11" S	23° 44' 16" E	FJ168908	FJ169110
P. plumosa	Rietfontein Farm	MHV0617	32° 59' 40" S	23° 47' 54" E	FJ168909	FJ169111
P. plumosa	Berensleegte	MHV0618	33° 02' 33" S	23° 52' 56" E	FJ168910	FJ169112
P. plumosa	nr Uitenhage	MHV1019	33° 40' 08" S	25° 27' 55" E	FJ168963	FJ169166
P. plumosa	nr Addo N.P.	MHV1021	33° 29' 13" S	25° 42' 25" E	FJ168965	FJ169168
P. plumosa	nr Paterson	MHV1027	33° 25' 31" S	25° 51' 13" E	FJ168967	FJ169170
P. plumosa	Paniel Farm	MHV1037	33° 28' 47" S	25° 19' 51" E	FJ168970	FJ169173
P. plumosa	nr Steydlerville	MHV1039	33° 23' 39" S	25° 08' 59" E	FJ168971	FJ169174
P. plumosa	Wolwefontein	MHV1040	33° 17' 49" S	24° 49' 50" E	FJ168972	FJ169175
P. plumosa	nr Baroe	MHV1041	33° 13' 02" S	24° 33' 16" E	FJ168973	FJ169176
P. plumosa	Klipplaat	MHV1042	33° 01' 15" S	24° 19' 59" E	FJ168974	FJ169177
P. plumosa	Rietfontein Farm	MHV1043	32° 51' 58" S	23° 43' 12" E	FJ168975	FJ169178
P. plumosa	nr Aberdeen	MHV1045	32° 32' 51" S	23° 58' 04" E	FJ168976	FJ169179
P. plumosa	Belmont Farm	MHV1046	32° 26' 01" S	24° 37' 60" E	FJ168977	FJ169180
P. plumosa	nr Somerset East	MHV1047	32° 42' 33" S	25° 27' 09" E	FJ168978	FJ169181
P. 'karooensis'	Ghwarriepoort River	MHV0323	33° 23' 18" S	23° 23' 15" E	EF134552	FJ169020
P. 'karooensis'	Laingsburg	MHV0578	33° 11' 47" S	20° 51' 36" E	FJ168885	FJ169088
P. 'karooensis'	nr Van Wyksdorp	MHV0595	33° 42' 06" S	21° 20' 36" E	FJ168891	FJ169094
P. 'karooensis'	nr De Rust	MHV0596	33° 52' 51" S	22° 27' 33" E	FJ168892	FJ169095
P. 'karooensis'	Merringspoort Pass	MHV0597	33° 24' 01" S	22° 33' 39" E	FJ168893	FJ169096
P. 'karooensis'	nr Kuisrivier	MHV0598	33° 25' 29" S	21° 56' 31" E	FJ168894	FJ169097
P. 'karooensis'	nr Oudtshoorn	MHV0599	33° 42' 41" S	22° 17' 52" E	FJ168895	FJ169098
P. 'karooensis'	Oudshoorn	MHV0833	33° 35' 11" S	22° 11' 43" E	FJ168925	FJ169129
P. 'karooensis'	nr Adamskraal	MHV0896	33° 46' 11" S	21° 08' 51" E	FJ168941	FJ169145
P. 'karooensis'	nr of Barrydale	MHV0906	33° 51' 18" S	20° 48' 16" E	FJ168944	FJ169148
P. 'gariepflumensis'	Pella	MHV0183	28° 57' 39" S	19° 09' 50" E	FJ168791	FJ168996
P. 'gariepflumensis'	Upington	MHV0339	28° 27' 00" S	21° 15' 00" E	FJ168823	FJ169028
P. 'gariepflumensis'	Upington	MHV0340	28° 27' 00" S	21° 15' 00" E	FJ168824	FJ169029
P. 'gariepflumensis'	Aliwal North	MHV0353	30° 40' 58" S	26° 42' 12" E	FJ168826	FJ169031
P. 'olifantsflumensis'	Karoo Kloof	MHV0584	33° 13' 50" S	19° 40' 46" E	FJ168887	FJ169090
P. 'olifantsflumensis'	Doring River	MHV0585	33° 07' 46" S	19° 45' 53" E	FJ168888	FJ169091
P. 'olifantsflumensis'	nr Karookloof	MHV0843	33° 14' 59" S	19° 41' 25" E	FJ168931	FJ169135
P. 'olifantsflumensis'	Leeuwenshof	MHV0845	32° 53' 27" S	19° 45' 58" E	FJ168932	FJ169136
P. 'olifantsflumensis'	Trawal	MHV0859	31° 53' 02" S	18° 38' 13" E	FJ168933	FJ169137
P. 'olifantsflumensis'	N Trawal	MHV0860	31° 50' 13" S	18° 37' 10" E	FJ168934	FJ169138
P. 'olifantsflumensis'	Wiedou River	MHV0862	31° 41' 24" S	18° 41' 49" E	FJ168935	FJ169139
P. 'olifantsflumensis'	Vanrhynsdorp	MHV0867	31° 36' 29" S	18° 44' 09" E	FJ168936	FJ169140
P. 'breedeflumensis'	Anysberg N.R.	MHV0014	33° 28' 06" S	20° 35' 18" E	FJ168782	FJ168986
P. 'breedeflumensis'	nr Lemoenhoek	MHV0016	33° 51' 36" S	20° 46' 48" E	FJ168783	FJ168987
P. 'breedeflumensis'	nr Montgue	MHV0316	33° 42' 54" S	20° 03' 60" E	FJ168812	FJ169016
P. 'breedeflumensis'	nr Matjiesfontein	MHV0570	33° 11' 27" S	20° 35' 02" E	FJ168877	FJ169079
P. 'breedeflumensis'	nr Leeu Gamka	MHV0571	32° 36' 16" S	22° 11' 05" E	FJ168878	FJ169080
P. 'breedeflumensis'	nr Leeu Gamka	MHV0572	32° 44' 20" S	22° 00' 22" E	FJ168879	FJ169082
P. 'breedeflumensis'	nr Leeu Gamka	MHV0573	32° 53' 08" S	21° 52' 22" E	FJ168880	FJ169083
P. 'breedeflumensis'	Prince Albert Road	MHV0574	32° 58' 41" S	21° 41' 28" E	FJ168881	FJ169084

Table A5. (cont.)

Species	Locality	Collection #	Latitude	Longitude	Genbank Accession	
					16S	CO1
P. 'breedeflumensis'	Dwyka River bridge	MHV0575	33° 05' 24" S	21° 34' 38" E	FJ168882	FJ169085
P. 'breedeflumensis'	Koup Station	MHV0576	33° 07' 29" S	21° 16' 14" E	FJ168883	FJ169086
P. 'breedeflumensis'	nr Laingsburg	MHV0577	33° 10' 29" S	20° 59' 20" E	FJ168884	FJ169087
P. 'breedeflumensis'	Swart River bridge	MHV0586	33° 10' 14" S	21° 59' 14" E	FJ168889	FJ169092
P. 'breedeflumensis'	nr Ladismith R327	MHV0601	33° 35' 49" S	21° 14' 33" E	FJ168897	FJ169100
P. 'breedeflumensis'	nr Prince Albert	MHV0602	33° 16' 13" S	22° 09' 30" E	FJ168898	FJ169101
P. 'breedeflumensis'	nr Prince Albert	MHV0603	33° 11' 29" S	22° 00' 45" E	FJ168899	FJ169102
P. 'breedeflumensis'	nr Ladismith	MHV0605	33° 39' 55" S	20° 59' 49" E	FJ168901	FJ169104
P. 'breedeflumensis'	nr Calitzdorp	MHV0832	33° 30' 29" S	21° 39' 32" E	FJ168924	FJ169128
P. 'breedeflumensis'	nr Swartberg Pass	MHV0838	33° 17' 15" S	22° 03' 07" E	FJ168926	FJ169130
P. 'breedeflumensis'	Gamka River	MHV0839	33° 07' 00" S	21° 55' 37" E	FJ168927	FJ169131
P. 'breedeflumensis'	nr Laingsburg	MHV0840	33° 10' 32" S	20° 59' 08" E	FJ168928	FJ169132
P. 'breedeflumensis'	Maitjiesfontein	MHV0841	33° 13' 32" S	20° 34' 35" E	FJ168929	FJ169133
P. 'breedeflumensis'	nr Maitjiesfontein	MHV0842	33° 13' 46" S	20° 32' 53" E	FJ168930	FJ169134
P. 'breedeflumensis'	nr Ladismith	MHV0893	33° 34' 03" S	21° 12' 22" E	FJ168940	FJ169144
P. 'breedeflumensis'	nr Ganskop	MHV0898	33° 38' 55" S	21° 01' 10" E	FJ168942	FJ169146
P. 'breedeflumensis'	nr Barrydale	MHV0902	33° 48' 47" S	20° 53' 51" E	FJ168943	FJ169147
P. 'breedeflumensis'	Lemoenhoek	MHV0907	33° 46' 24" S	20° 56' 08" E	FJ168945	FJ169149
P. 'gamtoosflumensis'	nr Patensie	MHV1015	33° 47' 04" S	24° 50' 41" E	FJ168960	FJ169163
P. 'gamtoosflumensis'	nr Hankey	MHV1016	33° 50' 22" S	24° 56' 17" E	FJ168961	FJ169164
P. 'gamtoosflumensis'	nr Loerie	MHV1018	33° 53' 38" S	25° 04' 45" E	FJ168962	FJ169165
P. 'gamtoosflumensis'	Gamtoos River	MHV1031	33° 56' 04" S	25° 00' 33" E	FJ168969	FJ169172
P. stridula	Citrusdal	MHV0001	32° 36' 00" S	19° 01' 00" E	EF134568	FJ168982
P. capensis	Cannon Rocks	MHV0021	33° 44' 54" S	26° 32' 03" E	EF134521	FJ168988

Table A6. Locality and Genbank accession information of samples collected in the *P. plumosa* group (nuclear DNA data).

					Genbank Accession	
Species	**Locality**	**Collection #**	**Latitude**	**Longitude**	**EF1a**	**CAL**
P. hirtipennis	Brentwood Farm	MHV0195	33° 29' 37" S	26° 09' 15" E	FJ215648	FJ215632
P. hirtipennis	Port Elizabeth	MHV1020	33° 51' 42" S	25° 28' 18" E	FJ215659	FJ215643
P. plumosa	nr Uitenhage	MHV1019	33° 40' 08" S	25° 27' 55" E	FJ215658	FJ215642
P. plumosa	Paniel Farm	MHV1037	33° 28' 47" S	25° 19' 51" E	FJ215661	FJ215645
P. 'karooensis'	nr Van Wyksdorp	MHV0595	33° 42' 06" S	21° 20' 36" E	FJ215652	FJ215636
P. 'karooensis'	nr De Rust	MHV0596	33° 52' 51" S	22° 27' 33" E	FJ215653	FJ215637
P. 'gariepflumensis'	Pella	MHV0183	28° 57' 39" S	19° 09' 50" E	FJ215647	FJ215631
P. 'gariepflumensis'	Aliwal North	MHV0353	30° 40' 58" S	26° 42' 12" E	FJ215650	FJ215634
P. 'olifantsflumensis'	Doring River	MHV0585	33° 07' 46" S	19° 45' 53" E	FJ215651	FJ215635
P. 'olifantsflumensis'	Leeuwenshof	MHV0845	32° 53' 27" S	19° 45' 58" E	FJ215656	FJ215640
P. 'breedeflumensis'	nr Prince Albert	MHV0602	33° 16' 13" S	22° 09' 30" E	FJ215654	FJ215638
P. 'breedeflumensis'	nr Laingsburg	MHV0840	33° 10' 32" S	20° 59' 08" E	FJ215655	FJ215639
P. 'gamtoosflumensis'	nr Loerie	MHV1018	33° 53' 38" S	25° 04' 45" E	FJ215657	FJ215641
P. 'gamtoosflumensis'	Gamtoos River	MHV1031	33° 56' 04" S	25° 00' 33" E	FJ215660	FJ215644
P. stridula	Cloetiesville	MHV0280	33° 54' 18" S	18° 51' 32" E	FJ215649	FJ215633
P. capensis	Cannon Rocks	MHV0021	33° 44' 54" S	26° 32' 03" E	FJ215646	FJ215630

Table A7. *Platypleura plumosa*. Pairwise AMOVA comparisons for primary catchments, Φ_{pt} shown below diagonal, corresponding probability above. Significant values are in bold.

Population	Pop A	Pop B	Pop C	Pop D	Pop E
Pop A		**0.029**	**0.001**	**0.003**	0.311
Pop B	**0.139**		**0.011**	**0.001**	0.413
Pop C	**0.305**	**0.090**		**0.001**	0.050
Pop D	**0.469**	**0.530**	**0.695**		0.051
Pop E	0.154	0.000	0.332	0.819	

Table A8. *Platypleura plumosa.* Pairwise AMOVA comparisons for secondary catchments, Φ_{pt} shown below diagonal, corresponding probability above. Significant values are in bold.

Population	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9	Pop 10	Pop 11
Pop 1		0.382	0.104	**0.025**	0.118	**0.027**	**0.017**	**0.026**	**0.035**	**0.022**	0.088
Pop 2	0.111		0.103	**0.022**	**0.084**	**0.029**	**0.011**	**0.001**	**0.025**	**0.020**	0.116
Pop 3	0.359	0.724		**0.042**	**0.041**	**0.050**	**0.001**	0.071	0.073	**0.050**	0.344
Pop 4	**0.827**	**0.941**	**0.944**		**0.001**	**0.001**	**0.005**	**0.014**	**0.013**	**0.008**	0.718
Pop 5	0.298	**0.404**	**0.447**	**0.385**		0.115	0.098	0.100	0.138	**0.004**	0.060
Pop 6	**0.692**	**0.834**	**0.830**	**0.244**	0.196		0.223	**0.083**	0.410	**0.005**	0.338
Pop 7	**0.531**	**0.637**	**0.637**	**0.138**	0.138	0.074		**0.018**	**0.042**	**0.003**	0.250
Pop 8	**0.672**	**0.806**	0.799	**0.678**	0.227	0.453	**0.268**		0.124	**0.001**	0.142
Pop 9	**0.784**	**0.946**	0.951	**0.748**	0.137	0.222	**0.201**	0.519		**0.007**	0.063
Pop 10	**0.595**	**0.781**	0.789	**0.864**	**0.502**	**0.779**	**0.608**	**0.738**	**0.838**		**0.050**
Pop 11	0.729	0.939	0.933	0.041	0.224	0.000	0.000	0.555	0.769	**0.819**	

Table A9. *Platypleura hirtipennis*. Pairwise AMOVA comparisons for primary catchments, Φ_{pt} shown below diagonal, corresponding probability above. Significant values are in bold.

Population	Pop 1	Pop 2	Pop 3
Pop 1		0.470	0.420
Pop 2	0.000		0.610
Pop 3	0.023	0.014	

Table A10. Locality and Genbank accession information of samples collected for *Pl. chalybaea* group.

Species	Locality	Collection #	forest	Latitude	Longitude	Genbank Accession	
						COI	COII
Pl. cf. *argentata*	Trafalgar	MHV0937	N/A	30° 57’ 33” S	30° 17’ 41” E	GU343735	GU343896
Pl. brunea	Cathcart	MHV0825	N/A	32° 17’ 56” S	27° 08’ 47” E	FJ169127	GU343888
Pl. brunea	Haga Haga	MHV0935	N/A	32° 45’ 32” S	28° 15’ 00” E	GU343733	GU343894
Pl. brunea	Queenstown	MHV0946	N/A	31° 53’ 44” S	26° 53’ 36” E	FJ169152	GU343902
Pl. brunea	Haga Haga	MHV0950	N/A	32° 45’ 33” S	28° 15’ 00” E	GU343738	-
Pl. brunea	Kei Mouth	MHV1255	N/A	32° 40’ 52” S	28° 22’ 53” E	GU343751	-
Pl. brunea	nr King Williams Town	MHV1396	N/A	32° 38’ 10” S	27° 39’ 52” E	GU343752	GU343922
Pl. chalybaea	Salem	MHV0490	N/A	33° 27’ 00” S	26° 29’ 00” E	GU343720	GU343878
Pl. chalybaea	Thomas Baines N.R.	MHV0782	N/A	33° 23’ 32” S	26° 28’ 33” E	GU343729	GU343887
Pl. chalybaea	Kenton	MHV0914	N/A	33° 39’ 10” S	26° 39’ 11” E	FJ169151	GU343892
Pl. chalybaea	Zuurberg Pass	MHV1022	N/A	33° 22’ 53” S	25° 42’ 49” E	FJ169169	GU343912
Pl. chalybaea	Thomas Baines N.R.	MHV1242	N/A	33° 24’ 00” S	26° 30’ 00” E	GU343749	GU343918
Pl. cf. *chalybaea*	Morgan’s Bay	MHV0910	N/A	32° 42’ 26” S	28° 20’ 56” E	-	GU343889
Pl. cf. *chalybaea*	nr Coffee Bay	MHV0941	N/A	31° 54’ 40” S	28° 57’ 38” E	GU343736	GU343897
Pl. cf. *chalybaea*	Port St. Johns	MHV0943	N/A	31° 38’ 24” S	29° 30’ 54” E	-	GU343899
Pl. longirostris	Mbololo Forest (Kenya)	MHV1169	N/A	03° 17’ 04” S	38° 28’ 07” E	FJ169184	GU343915
Pl. mijburghi	Pongolapoort Pass	MHV0964	N/A	27° 29’ 49” S	32° 00’ 44” E	GU343741	GU343907
Pl. murchisoni	Morning Sun Ranch	MHV0960	N/A	22° 56’ 39” S	29° 53’ 26” E	GU343740	GU343906
Pl. 'inconcessus'	False Bay	MHV0705	FOz8	27° 55’ 00” S	32° 22’ 00” E	GU343724	GU343883
Pl. 'inconcessus'	False Bay	MHV0706	FOz8	27° 55’ 00” S	32° 22’ 00” E	GU343725	-
Pl. 'inconcessus'	St. Lucia	MHV0714	FOz7	28° 23’ 00” S	32° 25’ 00” E	GU343726	GU343884
Pl. 'inconcessus'	St. Lucia	MHV0715	FOz7	28° 23’ 00” S	32° 25’ 00” E	GU343727	GU343885
Pl. 'inconcessus'	False Bay	MHV0716	FOz8	27° 55’ 00” S	32° 22’ 00” E	-	GU343886
Pl. 'inconcessus'	Sodwana	MHV0785	FOz7	27° 37’ 00” S	32° 39’ 00” E	GU343730	-
Pl. 'inconcessus'	Mission Rock, St. Lucia	MHV0936	FOz7	28° 16’ 41” S	32° 29’ 06” E	GU343734	GU343895
Pl. 'inconcessus'	Sodwana	MHV0951	FOz7	27° 30’ 52” S	32° 39’ 28” E	GU343739	GU343904
Pl. 'inconcessus'	False Bay	MHV1013	FOz8	27° 59’ 00” S	32° 23’ 00” E	GU343746	GU343911
Pl. 'inconcessus'	False Bay	MHV1483	FOz8	27° 59’ 00” S	32° 23’ 00” E	-	GU343923
Pl. 'inconcessus'	Kosi Bay	MHV1499	FOz7	26° 57’ 19” S	32° 49’ 44” E	GU343753	GU343924
Pl. 'inconcessus'	False Bay	MHV1502	FOz8	27° 57’ 50” S	32° 22’ 11” E	GU343754	GU343925
Pl. 'inconcessus'	St. Lucia	MHV1516	FOz7	28° 23’ 17” S	32° 24’ 32” E	GU343755	-

Table A10. (cont.)

Species	Locality	Collection #	forest	Latitude	Longitude	Genbank Accession	
						COI	COII
Pl. 'inconcessus'	Jesser Point, Sodwana	MHV1522	FOz7	27° 32’ 39” S	32° 40’ 38” E	GU343756	GU343929
Pl. 'inconcessus'	Enseleni N.R.	MHV1524	FOz7	28° 41’ 19” S	32° 00’ 17” E	GU343757	GU343930
Pl. 'inconcessus'	Kosi Bay	MHV1527	FOz7	26° 57’ 38” S	32° 49’ 32” E	-	GU343931
Pl. 'intercapedinis'	Alexandria Forest	MHV0198	FOz6	33° 43’ 15” S	26° 22’ 11” E	GU343712	GU343870
Pl. 'intercapedinis'	Fish River Mouth	MHV0237	FOz6	33° 31’ 11” S	27° 06’ 19” E	GU343713	GU343871
Pl. 'intercapedinis'	Glen Ashleigh	MHV0345	FOz7	29° 45’ 27” S	31° 02’ 54” E	GU343714	GU343872
Pl. 'intercapedinis'	Lupatana River	MHV0399	FOz5	31° 25’ 20” S	29° 51’ 10” E	GU343715	GU343873
Pl. 'intercapedinis'	Cintsa West	MHV0401	FOz6	32° 50’ 40” S	28° 07’ 00” E	GU343716	GU343874
Pl. 'intercapedinis'	Gillitts	MHV0404	FOz5	29° 47’ 00” S	30° 48’ 00” E	GU343717	GU343875
Pl. 'intercapedinis'	Haga Haga	MHV0409	FOz6	32° 45’ 00” S	28° 15’ 00” E	GU343718	-
Pl. 'intercapedinis'	Kayser's Beach	MHV0426	FOz6	33° 12’ 00” S	27° 30’ 00” E	GU343719	GU343876
Pl. 'intercapedinis'	Legalametse N.R.	MHV0471	FOz4	24° 12’ 00” S	30° 21’ 00” E	-	GU343877
Pl. 'intercapedinis'	Waters Meeting N.R.	MHV0520	FOz6	33° 32’ 31” S	26° 47’ 16” E	GU343721	GU343879
Pl. 'intercapedinis'	nr East London	MHV0528	FOz6	32° 48’ 08” S	28° 01’ 16” E	GU343722	GU343880
Pl. 'intercapedinis'	Sebumo Tude	MHV0625	FOz6	33° 35’ 37” S	26° 42’ 56” E	GU343723	GU343881
Pl. 'intercapedinis'	Lupatana River	MHV0628	FOz5	31° 25’ 20” S	29° 51’ 10” E	-	GU343882
Pl. 'intercapedinis'	Thomas Baines N.R.	MHV0780	FOz3	33° 23’ 00” S	26° 29’ 00” E	GU343728	-
Pl. 'intercapedinis'	Umlalazi N.R.	MHV0911	FOz7	28° 57’ 21” S	31° 46’ 31” E	GU343731	GU343890
Pl. 'intercapedinis'	Alexandria Forest	MHV0912	FOz6	33° 42’ 43” S	26° 21’ 56” E	FJ169150	GU343891
Pl. 'intercapedinis'	Coffee Bay	MHV0934	FOz5	31° 59’ 11” S	29° 09’ 07” E	GU343732	GU343893
Pl. 'intercapedinis'	nr Empangeni	MHV0942	FOz5	28° 42’ 22” S	31° 40’ 28” E	-	GU343898
Pl. 'intercapedinis'	Southbroom	MHV0944	unknown	30° 55’ 05” S	30° 18’ 46” E	GU343737	GU343900
Pl. 'intercapedinis'	Makhado	MHV0945	FOz4	22° 57’ 49” S	29° 56’ 07” E	-	GU343901
Pl. 'intercapedinis'	Morning Sun Ranch	MHV0948	FOz4	22° 56’ 39” S	29° 53’ 26” E	FJ169153	GU343903
Pl. 'intercapedinis'	Umlalazi N.R.	MHV0952	FOz7	28° 57’ 23” S	31° 46’ 20” E	-	GU343905
Pl. 'intercapedinis'	Howard College, Durban	MHV1007	FOz7	29° 52’ 05” S	30° 58’ 42” E	GU343742	GU343908
Pl. 'intercapedinis'	Oribi Gorge N.R.	MHV1008	FOz5	30° 42’ 27” S	30° 16’ 14” E	GU343743	GU343909
Pl. 'intercapedinis'	Umlalazi NR	MHV1010	FOz7	28° 57’ 22” S	31° 46’ 20” E	GU343744	-
Pl. 'intercapedinis'	Umthamvuna N.R.	MHV1011	FOz5	31° 03’ 51” S	30° 10’ 11” E	GU343745	GU343910
Pl. 'intercapedinis'	Tzaneen, Letaba	MHV1049	FOz4	23° 50’ 42” S	30° 08’ 07” E	GU343747	GU343913
Pl. 'intercapedinis'	Bosbokstrand	MHV1167	FOz6	32° 46’ 48” S	28° 11’ 01” E	-	GU343914
Pl. 'intercapedinis'	Mkambati N.R.	MHV1208	FOz5	31° 19’ 05” S	29° 58’ 02” E	-	GU343916

Table A10. (cont.)

Species	Locality	Collection #	forest	Latitude	Longitude	Genbank Accession	
						COI	COII
Pl. 'intercapedinis'	East London	MHV1240	unknown	32° 58' 00" S	27° 54' 00" E	GU343748	GU343917
Pl. 'intercapedinis'	Waterval Onder	MHV1254	FOz4	25° 23' 00" S	32° 34' 00" E	GU343750	-
Pl. 'intercapedinis'	Waterval Onder	MHV1256	FOz4	25° 23' 00" S	32° 34' 00" E	-	GU343919
Pl. 'intercapedinis'	Blyolifant NR	MHV1276	FOz4	24° 17' 16" S	30° 50' 33" E	-	GU343920
Pl. 'intercapedinis'	34km SE Tzaneen	MHV1344	FOz4	24° 02' 34" S	30° 22' 23" E	-	GU343921
Pl. 'intercapedinis'	Port St. Johns	MHV1504	FOz5	31° 37' 02" S	29° 30' 11" E	-	GU343926
Pl. 'intercapedinis'	Umdomi Forest	MHV1507	FOz7	30° 23' 12" S	30° 40' 21" E	-	GU343927
Pl. 'intercapedinis'	Dhlinza Forest	MHV1512	FOz5	28° 53' 44" S	31° 26' 55" E	-	GU343928

Table A11. Locality and Genbank accession information of samples collected for *Py. semiclara.*

Species	Locality	Collection #	forest	Latitude	Longitude	Genbank Accession	
						COI	COII
O. numa	Taita Hills (Kenya)	MHV0674	N/A	03° 22’ 29” S	38° 20’ 29” E	GU343766	GU343941
Py. sylvia	nr Bandelierkop	MHV1325	N/A	23° 23’ 10” S	29° 50’ 13” E	GU343798	GU343973
Py. semiclara	Grahamstown	MHV0022	FOz3	33° 18’ 47” S	26° 31’ 16” E	FJ168989	GU343932
Py. semiclara	Thomas Baines N.R.	MHV0239	FOz3	33° 23’ 06” S	26° 30’ 24” E	GU343758	GU343933
Py. semiclara	nr Seymour	MHV0244	FOz3	32° 33’ 48” S	26° 50’ 46” E	GU343759	GU343934
Py. semiclara	Twinstreams Farm	MHV0349	FOz7	28° 58’ 52” S	31° 44’ 07” E	GU343760	GU343935
Py. semiclara	Gillitts	MHV0403	FOz5	29° 47’ 00” S	30° 48’ 00” E	GU343761	GU343936
Py. semiclara	Carls’ Rust Farm	MHV0480	FOz3	33° 21’ 55” S	26° 17’ 49” E	GU343762	GU343937
Py. semiclara	nr Lydenburg	MHV0495	FOz4	25° 09’ 30” S	30° 07’ 48” E	GU343763	GU343938
Py. semiclara	Nico Malan Pass	MHV0512	FOz3	32° 31’ 30” S	26° 48’ 04” E	GU343764	GU343939
Py. semiclara	Greytown	MHV0591	FOz3	29° 04’ 19” S	30° 35’ 35” E	GU343765	GU343940
Py. semiclara	Qachas Nek	MHV0699	FOz3	30° 10’ 00” S	28° 35’ 01” E	GU343767	-
Py. semiclara	nr Himeville	MHV0737	FOz3	29° 46’ 15” S	29° 30’ 04” E	-	GU343942
Py. semiclara	nr Hogsback	MHV0823	FOz3	32° 29’ 04” S	26° 56’ 49” E	GU343768	GU343943
Py. semiclara	Stutterheim	MHV0824	FOz3	32° 34’ 32” S	27° 25’ 36” E	GU343769	-
Py. semiclara	Alexandria Forest	MHV0913	FOz6	33° 42’ 43” S	26° 21’ 56” E	GU343770	GU343944
Py. semiclara	nr Graskop	MHV0915	FOz4	24° 57’ 41” S	30° 51’ 40” E	GU343771	GU343945
Py. semiclara	nr Newcastle	MHV0916	FOz2	27° 33’ 42” S	29° 53’ 06” E	GU343772	GU343946
Py. semiclara	nr Houtkop Farm	MHV0917	unknown	26° 51’ 44” S	30° 44’ 05” E	GU343773	GU343947
Py. semiclara	nr Harrismith	MHV0918	FOz2	28° 19’ 05” S	29° 11’ 13” E	GU343774	GU343948
Py. semiclara	nr Tzaneen	MHV0919	FOz4	23° 56’ 16” S	30° 01’ 26” E	GU343775	GU343949
Py. semiclara	nr Donnybrook	MHV0920	FOz3	29° 54’ 52” S	29° 52’ 15” E	GU343776	GU343950
Py. semiclara	nr Harrismith	MHV0921	FOz2	28° 33’ 46” S	29° 04’ 20” E	GU343777	GU343951
Py. semiclara	nr Maclear	MHV0922	FOz3	30° 59’ 12” S	28° 13’ 48” E	GU343778	GU343952
Py. semiclara	Umkomaas Valley	MHV0923	FOz3	29° 43’ 33” S	29° 56’ 13” E	-	GU343953
Py. semiclara	Fort Nottingham	MHV0924	FOz3	29° 24’ 47” S	29° 55’ 02” E	-	GU343954
Py. semiclara	nr Grasskop	MHV0925	FOz5	29° 02’ 03” S	30° 53’ 22” E	GU343779	GU343955
Py. semiclara	Ongoye Forest	MHV0926	FOz5	28° 51’ 06” S	31° 39’ 03” E	GU343780	GU343956
Py. semiclara	Kataza	MHV0927	FOz5	28° 30’ 39” S	31° 15’ 26” E	-	GU343957
Py. semiclara	Piet Retief	MHV0928	FOz2	27° 17’ 48” S	30° 15’ 44” E	GU343781	GU343958
Py. semiclara	Ngele Forest	MHV0929	FOz3	30° 31’ 29” S	29° 40’ 36” E	GU343782	GU343959

Table A11. (cont.)

Species	Locality	Collection #	forest	Latitude	Longitude	Genbank Accession	
						COI	COII
Py. semiclara	nr Wartberg	MHV0930	FOz5	29° 31’ 38” S	31° 05’ 56” E	GU343783	GU343960
Py. semiclara	Coffee Bay	MHV0931	FOz5	31° 59’ 13” S	29° 09’ 09” E	GU343784	GU343961
Py. semiclara	Olifantshoek Pass	MHV1001	FOz3	33° 19’ 04” S	25° 57’ 00” E	GU343785	GU343962
Py. semiclara	Zuurberg Pass	MHV1026	FOz3	33° 22’ 30” S	25° 43’ 37” E	GU343786	GU343963
Py. semiclara	nr Bedford	MHV1033	FOz3	32° 40’ 20” S	26° 01’ 33” E	GU343787	GU343964
Py. semiclara	Oribi Gorge N.R.	MHV1065	FOz5	30° 43’ 51” S	30° 16’ 27” E	GU343788	GU343965
Py. semiclara	Mkomazi State Forest	MHV1066	FOz3	29° 29’ 14” S	29° 41’ 57” E	-	GU343966
Py. semiclara	Entumeni	MHV1161	FOz5	28° 51’ 21” S	31° 19’ 31” E	GU343789	GU343967
Py. semiclara	Maratang Mountain Creek Farm	MHV1246	FOz4	25° 17’ 43” S	30° 19’ 20” E	GU343790	-
Py. semiclara	East London	MHV1248	unknown	32° 58’ 00” S	27° 54’ 00” E	GU343791	-
Py. semiclara	Creighton	MHV1257	FOz3	30° 02’ 00” S	29° 51’ 00” E	GU343792	GU343968
Py. semiclara	Umkomazi Valley	MHV1259	FOz3	29° 54’ 00” S	30° 05’ 00” E	-	GU343969
Py. semiclara	Underberg	MHV1260	FOz3	29° 46’ 00” S	29° 30’ 00” E	GU343793	-
Py. semiclara	Bulwer	MHV1261	FOz3	29° 48’ 00” S	29° 46’ 00” E	GU343794	GU343970
Py. semiclara	Boston	MHV1262	FOz3	29° 41’ 00” S	30° 00’ 00” E	GU343795	GU343971
Py. semiclara	Xumeni Forest	MHV1263	FOz3	29° 55’ 00” S	29° 51’ 00” E	GU343796	GU343972
Py. semiclara	Noordkaap turnoff	MHV1311	FOz4	25° 36’ 57” S	30° 58’ 34” E	GU343797	-
Py. semiclara	nr Sabie	MHV1340	FOz4	25° 08’ 50” S	30° 44’ 23” E	GU343799	-
Py. semiclara	nr Lydenburg	MHV1348	FOz4	25° 00’ 36” S	30° 29’ 53” E	GU343800	GU343974
Py. semiclara	nr Horseshoe Falls	MHV1354	FOz4	25° 07’ 42” S	30° 41’ 40” E	GU343801	GU343975
Py. semiclara	nr Graskop	MHV1356	FOz4	24° 54’ 46” S	30° 50’ 33” E	GU343802	GU343976
Py. semiclara	Schoemanskloof	MHV1358	FOz4	25° 24’ 27” S	30° 30’ 09” E	GU343803	GU343977
Py. semiclara	nr Machadodorp	MHV1360	FOz4	25° 34’ 47” S	30° 17’ 33” E	GU343804	GU343978
Py. semiclara	nr Ermelo	MHV1368	FOz4	26° 13’ 51” S	30° 26’ 29” E	GU343805	GU343979
Py. semiclara	nr Piet Retief	MHV1369	unknown	26° 46’ 02” S	30° 46’ 20” E	GU343806	GU343980
Py. semiclara	nr King Williams Town	MHV1395	FOz3	32° 38’ 10” S	27° 39’ 52” E	-	GU343981
Py. semiclara	Nico Malan Pass	MHV1399	FOz3	32° 27’ 53” S	26° 50’ 24” E	-	GU343982
Py. semiclara	Ingele Forest	MHV1496	FOz3	30° 37’ 38” S	29° 42’ 29” E	GU343807	GU343983
Py. semiclara	Monk’s Cowl	MHV1497	FOz2	29° 04’ 48” S	29° 20’ 48” E	GU343808	GU343984
Py. semiclara	Port St. Johns	MHV1506	FOz5	31° 35’ 55” S	29° 31’ 55” E	GU343809	GU343985
Py. semiclara	Amatukulu N.R.	MHV1513	FOz7	29° 06’ 44” S	31° 35’ 56” E	GU343810	GU343986
Py. semiclara	Dhlinza Forest	MHV1515	FOz5	28° 53’ 51” S	31° 26’ 57” E	GU343811	GU343987

Table A12. Pairwise AMOVA comparisons of genetic variation in *Platypleura* 'intercapedenis' from forest types, Φ_{pt} shown below diagonal and corresponding probability above. Significant values are in bold.

Forest Type	FOz 4	FOz 5	FOz 6	FOz 7
FOz 4		**0.048**	0.073	0.222
FOz 5	0.052		1.000	0.510
FOz 6	0.052	0.000		1.000
FOz 7	0.056	0.000	0.000	

Table A13. Pairwise AMOVA comparisons of genetic variation in *Pycna semiclara* from forest types, Φ_{pt} shown below diagonal and corresponding probability above. Significant values are in bold.

Forest Type	FOz 2	FOz 3	FOz 4	FOz 5	FOz 7
FOz 2		0.737	1.000	1.000	1.000
FOz 3	0.002		0.564	0.607	0.865
FOz 4	0.000	0.001		1.000	1.000
FOz 5	0.000	0.001	0.000		1.000
FOz 7	0.000	0.002	0.000	0.000	