Systematics of southern African Anostostomatidae (Orthoptera) based on morphological and molecular data

by

Helene Brettschneider

Supervisor: Prof. C.H. Scholtz

Co-Supervisors: Dr. P. W. Bateman

Prof. C.T. Chimimba

Dr. A.D. S. Bastos

Submitted in partial fulfillment of the requirements for the degree

Magister Scientiae

(Entomology)

In the

Faculty of Natural and Agricultural Sciences

University of Pretoria

PRETORIA

June 2006

This work is dedicated to my parents Albert & Marie who, through love, acceptance and eternal belief in me have motivated, encouraged and fueled my education.

I who was given my big brain by mistake cannot deny the foolishness in the chances that I take but for dumb luck I have survived and moreover have even thrived for I was built to laugh.

Anonymous

Acknowledgements

I would like to thank my supervisors for their guidance, patience and support during this project. The study was funded by the University of Pretoria and the South African National Research Foundation (NRF) under grant number GUN 2053665 and is gratefully acknowledged. The authors wish to thank the following curators and collections who loaned material for study: Dr. K.A. Johansson (NHRS); Prof. U. Aspöck (NMW); S. van Noort & M. Cochrane (SAMC); R. Toms, B. Dombrowsky, K. Manamela (TM); Mrs. R. Urban (NCI); Dr. G. Beccaloni (BMNH); Dr. P. J. Schwendinger (MHNG); Dr. A. Angulo (Universidad de Concepción, Chile). We are grateful to G. Malherbe for valuable advice, guidance and motivation with the molecular work. Y. de Beer, L. Rotherham, L. Hart, D. Saccaggi, N. Maputla, G. Grobler and Dr. C. Sole provided encouragement, entertainment and valuable comments on analyses and manuscripts. The study was funded by the University of Pretoria and the South African National Research Foundation (NRF) under grant GUN 2053665 and is gratefully acknowledged.

Table of Contents

List of Figures

List of Tables

Chapter 1

General Introduction

Taxonomy & Systematics

The history of the classification of the Stenopelmatoidea between 1910 and 1937 includes contributions by Karny, whose last classification in 1937 included all the then known Stenopelmatoidea in one family, the Gryllacrididae, which consisted of seven subfamilies and eight tribes (Gorochov 2001). During this period, the first phylogenetic studies of the Stenopelmatoidea emerged but they either proved to be too complex or oversimplified (Gorochov 2001). Taxonomic reviews that focused on higher taxonomic ranks and their origin were subsequently undertaken; the most recent being that by Johns (1997) that mainly involved nomenclatural changes (Johns 1997, Gorochov 2001). The Anostostomatidae has many primitive characteristics, making its phylogenetic position complex (Gorochov 2001). Nevertheless, the phylogeny of three families within the Stenopelmatoidea, namely the Anostostomatidae, the Rhaphidophoridae and the Stenopelmatidae, seems to be largely resolved (Gorochov 2001).

The family name Anostostomatidae is the emended form of Anostostomidae Stål (1876), which is based on the Anostostomii tribe Saussure (1859) and contains along with the *Mimnermis* (Mimnermidae) Stål (1876) several African genera and species (Johns 1997). Along with the Cratomelini Brunner (1888), these three taxa were all included in the Gryllacrididae Stål 1876, the priority of Stenopelmatidae Burmeister 1838 being neglected (Johns 1997). Karny 1932 created a replacement name for *Anostostoma* namely *Australosoma*, and placed it in the Deinacridinae subfamily, in spite of the Henicinae (genus *Henicus*) and

Mimnermidae (*Mimnermis*) already existing (Johns 1997). Kevan (1977) noted that Mimnermidae had priority over Henicinae, but failed to recognise that *Anostostoma* is a valid genus name and its family-group name has date priority over all other names and is most commonly used in literature (Johns 1997). Following Johns (1997) the Anostostomatidae now contains, although not exclusively, all the southern African genera.

The bulk of taxonomic work on the Anostostomatidae occurred between 1803 and 1943, the latest revision being by P.M. Johns in 1997, after a long period of deficiency of taxonomic work on this group. These works, however, are replete with synonymies, misspellings and incorrect identifications, not to mention the continuous reshuffling of taxa, which makes studies on this group difficult (see Karny 1930, Ander 1943, Toms 1986a & b). No recent reviews that focus on taxonomic levels lower than superfamilial level are presently available. Unfortunately, molecular studies using mitochondrial genes such as the ribosomal subunits 12S, 16S and 18S to investigate the higher phylogeny of the orthopteran order only concentrate at superfamily level (Flook & Rowell 1997, Flook *et al*. 1999). Further investigation into the generic and specific levels of anostostomatid phylogeny and taxonomy, using molecular and morphological tools has not been attempted (Toms 2001). Regrettably, most fossils are difficult to correctly place into the higher taxa of this superfamily, and are generally not dealt with, even in higher classifications (Gorochov 2001). Morphologically, however, the Anostostomatidae can be distinguished on the features of the subtriangular fastigium on the vertex, spinous lobes on the coxae, spination and tympanum of the fore tibia, the shape of the metasternum and the chevron ridges with aligned musculature of the hind femur (Johns 1997).

Southern African anostostomatids include *Bochus* Péringuey, 1918, *Borborothis* Brunner von Wattenwyl, 1888, *Henicus* Gray, 1837, *Libanasidus* Péringuey, 1918, *Nasidius* Stål, 1878, *Onosandrus* Stål, 1878 and *Onosandridus* Péringuey, 1918, belonging to the tribe Anostostomatini Saussure, 1859. The genus *Libanasa* Walker, 1869 within the tribe Lutosini tribe Walker, 1869 also shows a southern African distribution (Otte *et al*. 2005). Together, these taxa represent 51 species, described since the early 1800s (Otte *et al*. 2005). Although the most basic nomenclatural problems are resolved, much work is necessary at the lower taxonomic levels, especially in these African fauna (Chopard 1943, Toms 2001). The many synonymies created in the past add to the current confusion and lack of concrete information available about this group of insects (Toms 2001). Table 1.1 illustrates some of the disparity and nomenclatural confusion at subgeneric level pertaining to these eight southern African genera.

Table 1.1. List of currently and previously recognised species of eight southern African genera according to Otte *et al*. 2005.

Genus	**Species**	**Notes**
Bochus Péringuey, 1918	Type: *Bochus contemnendus* Péringuey (= *puncticeps*)	
	puncticeps (Pictet & Saussure, 1891)	Original: *Onosandrus puncticeps*
	Synonym: *contemnendus* Péringuey, 1918	
Borborothis Brunner, 1888	Type: *Borborothis opaca* Brunner	
	brunneri Bolivar, 1890	
	opaca Brunner, 1888	
Henicus Gray, 1837	Type: *Henicus stolli* Gray (= *pattersoni*)	
Synonym: *Mimnermis* Stål, 1878	Type: *Mimnermis prodigiosus* Stål	
	bechuanus (Péringuey, 1918)	Original: *Nasidius bechuanus*
	brevimucronatus Griffini, 1911	
	cephalotes (Bolivar, 1890)	Original: *Mimnermis cephalotes*
	costulatus (Brunner, 1888)	Original: *Mimnermis costulatus*
	ferox (Péringuey, 1918)	Original: *Nasidius ferox*

	monstrosus (Herbst, 1803)	Original: *Locusta monstrosus*
	Synonym: *herbstii* (Gray, 1837)	Original: *Anostostoma herbstii*
	Synonym: *portentosus* (Burmeister, 1838)	Original: *Stenopelmatus portentosus*
	pattersoni (Stoll, 1813)	Original: *Gryllus pattersoni*
	Synonym: *stolli* Gray, 1837	
	prodigiosus (Stål, 1878)	Original: *Mimnermis prodigiosus*
	promontori Péringuey, 1918	
Libanasidus Péringuey, 1918	Type: *Carcinopsis vittatus* Kirby	
Synonym: *Nasaliba* Karny, 1937		
Preoccupied: *Libanasa* Griffini, 1914	Type: *Libanasa impicta* Stål	
	impicta (Stål, 1878)	Original: *Libanasa impicta*
	vittatus (Kirby, 1899)	Original: *Carcinopsis vittatus*
Nasidius Stål, 1878	Type: *Nasidius truncatifrons* Stål	
Misspelling: *Fakua* Johns, 1997		Correct spelling of name is *Faku*
Synonym: *Dyscapna* Brunner, 1888	Type: *Dyscapna atra* Brunner	Original: *Dyscapna atra*
Synonym: *Faku* Péringuey, 1918	Type: *Faku minax* Péringuey	
	atra (Brunner, 1888)	Original: *Dyscapna atra*
	auditor (Karny, 1935)	Original: *Faku auditor*
	brunneri (Karny, 1929)	Original: *Faku brunneri*
	Synonym: *truncatifrons* Brunner, 1888	Not *truncatifrons* of Stål
	dregii (Burmeister, 1838)	Original: *Stenopelmatus dregii*
	karnyi Chopard, 1950	
	longicauda Karny, 1929	
	mimus Péringuey, 1918	
	minax (Péringuey, 1918)	Original: *Faku minax*
	minotaurus Karny, 1929	
	Synonym: *infuscata* Ander, 1929	
	monachus Péringuey, 1918	
	pulchriventris (Griffini, 1914)	Original: *Dyscapna pulchriventris*
	punctulata (Kirby, 1898)	Original: *Carcinopsis punctulata*
	truncatifrons Stål, 1878	
	Synonym: *nigrifrons* Karny, 1929	
	whellani (Chopard, 1950)	Original: *Henicus whellani*
Onosandridus Péringuey, 1918	Type: *Onosandrus deceptor* Péringuey	
	bolivari Chopard, 1962	
	calcaratus Karny, 1929	
	deceptor (Péringuey, 1918)	Original: *Onosandrus deceptor*
	larvatus Karny, 1929	

	pictifrons (Péringuey, 1918)	Original: *Onosandrus pictifrons*
	plebeius (Péringuey, 1918)	Original: *Onosandrus plebeius*
	sakaniensis Ander, 1938	
	simplex Karny, 1929	
Onosandrus Stål, 1878	Type: *Onosandrus fasciatus* Stål	
	bipinnatus Karny, 1929	
	Synonym: *mediocris* Péringuey, 1918	
	crassipes Brunner, 1888	
	fasciatus Stål, 1878	
	Misidentified: *femoralis* Karny, 1930	
	fuscodorsalis Sjöstedt, 1913	
	natalensis Karny, 1929	
	Primary homonym: *fasciatus* Brunner, 1888	Not *fasciatus* of Stål
	opacus Brunner, 1888	
	saussurei Brunner, 1888	
	splendens Sjöstedt, 1913	
	tigrinus Karny, 1929	
Libanasa Walker, 1869	Type: *Libanasa incisa* Walker	
Synonym: *Platysiagon* Brunner, 1888	Type: *Platysiagon signatus* Brunner	
	brachyura Karny, 1928	
	capicola (Péringuey, 1918)	Original: *Platysiagon capicola*
	femoralis (Brunner, 1888)	Original: *Carcinopsis femoralis*
	incisa Walker, 1869	
	Synonym: *fusca* (Brunner, 1888)	Original: *Carcinopsis fusca*
	parvula Karny, 1929	
	signatus (Brunner, 1888)	Original: *Platysiagon signatus*

Nomenclatural uncertainties at the specific level are also problematic as can be exemplified by the famed genus *Libanasidus*. The genus contains two species: *L. vittatus* and *L. impicta*. The latter, described by Stål (1876) is widely regarded a synonymy, as it was described from a single female and is very rarely mentioned in literature (Johns 1997). *Libanasidus vittatus* is the only formally recognised species in the genus, but may itself contain a species complex, as implied by the large amount of genetic and morphological variation present in the species (P.W. Bateman, unpubl. data).

Geographic Distribution and Study Group

The Anostostomatidae Saussure, 1859 (Orthoptera: Ensifera: Stenopelmatoidea), shows a discontinuous distribution across Africa, Australasia, South and Central America and Asia, represented by three subfamilies primarily in the southern hemisphere (Karny 1931, Johns 1997, Gorochov 2001, Otte *et al*. 2005). The Deinacridinae Karny, 1932 and the Leiomelinae Gorochov, 2001 are restricted to New Zealand and South America respectively (Gorochov 2001, Otte *et al*. 2005). The subfamily Anostostomatinae Saussure, 1859 is the focus of this study and is composed of six tribes according to Otte *et al*. 2005, showing the global distribution discussed below. The tribes Anabropsini Rehn, 1901, Anostostomatini Saussure, 1859 and Glaphyrosomini Rentz & Weissman, 1973 have been recorded from Central America, specifically Ecuador, Mexico, Guatemala and Costa Rica. The Glaphyrosomini have been recorded to occur in California and Texas in south-western North America (Otte *et al*. 2005). The tribe Cratomelini Brunner von Wattenwyl, 1888 occurring mainly in Chile and the tribe Lutosini Gorochov, 1988 recorded from Brazil and Venezuela are the dominant groups in South America (Otte *et al*. 2005). The tribes Anabropsini, Anostostomatini, Lutosini and Brachyporini Gorochov, 2001 occur in Australia, New Zealand and New Caledonia (Otte *et al*. 2005). Species from the tribes Anabropsini, Anostostomatini and Lutosini also occur in China and Indo-Malaysia, specifically Nepal, Vietnam, Philippines and India (Otte *et al*. 2005).

In Africa, the tribes Brachyporini and Lutosini occur in Madagascar, while members from the Anabropsini have also been recorded from the Democratic Republic of Congo (= former Zaire) (Otte *et al*. 2005). The tribe Lutosini occurs in southern Africa represented by one genus occurring in South Africa while other

genera are found as far north as Tanzania (Otte *et al*. 2005). Seven of the eight African genera are placed within the tribe Anostostomatini and have been recorded from South Africa, Angola, Mozambique, Zimbabwe, Democratic Republic of Congo and Tanzania (Johns 1997, Otte *et al*. 2005). Of these eight southern African genera, *Onosandrus* is also found in New Zealand and south Australia while all the others are restricted to Africa (Karny 1931).

Within this geographic range, anostostomatids have inhabited a variety of habitats where they live in self-constructed burrows or rotten wood (Toms 2001, Picker *et al.* 2002) (Figure 1). *Bochus* is a monotypic genus known from the Free State in South Africa only, while *Borborothis* can be found along the southern mountainous regions of South Africa (Toms 2001, Karny 1929). *Henicus* and *Libanasidus* are the more familiar genera in South Africa due to their prevalence in suburbia and frequent encounters with people. Males of both genera have highly modified mandibles, used for stridulation and plugging of burrow entrances in the former and for male-male conflict in the latter (Toms 2001). *Henicus* occurs in the mountainous region of the Cape, where *Libanasidus vittatus* occurs naturally in the indigenous and coastal forests of eastern South Africa, reaching to Zimbabwe (Chapter 2). The second species in this genus, *L. impicta* is rarely encountered in literature and known only from dryer areas in the Northern Cape (Johns 1997). *Libanasa* species have been found in dryer thornveld areas in Mpumalanga, as well as in rotting logs in indigenous forest along the coast and KwaZulu Natal (Toms 2001). The fourteen species of *Nasidius* are distributed in open grassland and indigenous forest areas in South Africa. Biological information on *Onosandridus* species is not available, but Péringuey (1916) propose that they

occur in a habitat similar to *Onosandrus*, which are abundant in indigenous forests of the Mpumalanga, KwaZulu-Natal and Limpopo provinces.

Locally, these insects are known as King Crickets (Weta in New Zealand) (Orthoptera: Anostostomatidae) and are large, mainly flightless crickets (Field 2001). Nocturnal behaviour within the group of 'monstrous' crickets is the norm, which has possibly contributed to the lack of available biological information on them (Field 2001). They are omnivorous, feeding on plant and decaying material and the species prevalent in suburban gardens in Johannesburg (*L. vittatus* or 'Parktown Prawn') is famed for wandering into homes (Toms 1985). Sexual dimorphism is widespread in five of the eight genera (*Bochus*, *Henicus*, *Libanasa*, *Libanasidus* and *Nasidius*), often resulting in grossly enlarged mandibles and facial features of males, which they use in male-male conflict (*Libanasidus*), or plugging of burrow entrances (*Henicus*) (Toms 2001). The African species also show fascinating defence mechanisms, including abdominal-femoral stridulation and squirting of foul-smelling faeces at offenders, which is absent in related taxa from New Zealand (Wolf *et al.* 2006).

Study Objective

The present investigation will add knowledge to this poorly understood group of insects in southern Africa. Considering the extensive African invertebrate fauna and their uncertain taxonomic status, it is possible that many anostostomatid species and higher taxonomic hierarchies are yet to be discovered (Toms 2001). While anostostomatids in southern Africa, Madagascar, and Central America are diverse, most species are poorly known, therefore precluding general biological research of this ancient group of insects (Johns 1997).

Justification

This study aims to revise the current generic classification of southern African anostostomatids that are available for study, concentrating in specific detail on the genus *Libanasidus*. The investigation follows a multi-disciplinary approach that includes classical taxonomy, advanced morphological techniques and molecular analysis.

Thesis Outline

What is the status of the current generic classification of the Anostostomatidae in southern Africa with reference to current philosophical positions of classifications based on modern systematic techniques?

Chapter 2 revises the current extent of anostostomatid fauna in southern Africa. The eight prevalent genera are redescribed from type species, and diagnostics updated to include all the available specimens from South African museums. An identification key is provided to distinguish between the eight genera. Specifically, the status of *Libanasidus impicta* is investigated and species characteristics confirmed in construction of an identification key to the two *Libanasidus* species. In addition to promoting descriptive constancy and ease of comparison, this section serves as a compilation of available taxonomic literature on these taxa.

Following the nomenclatural revision of anostostomatids occurring in southern Africa, Chapter 3 investigates the relationships between these eight genera. Molecular techniques, utilising sequence data from the large mitochondrial ribosomal subunit (16S) and morphological analysis focussing on discrete external morphological measurements used in identification, are employed. Phylogenetic

reconstruction using phenetic, cladistic and Bayesian methods will serve to evaluate the authenticity of the genera and investigate relationships between the genera. This also allows assessment of the morphological characters used in identification for their phylogenetic importance.

How many species are within the genus Libanasidus and what is the nature and extent of the geographic variation within the species of Libanasidus delineated by multi-disciplinary techniques?

Chapter 4 deals specifically with taxonomic problems within the famous Parktown Prawn, *Libanasidus vittatus*, thereby elucidating the genetic and morphological variation present in this species. Preliminary morphometric data suggests two distinct groups of *L. vittatus*, based on their occurrence on either side of the prominent escarpment in eastern South Africa (P.W. Bateman unpubl. data). *Libanasidus* specimens, predominantly from four provinces in South Africa, are sampled from museum material and freshly captured individuals. A portion of the mitochondrial Cytochrome Oxidase I gene is amplified to investigate genetic variation within the *Libanasidus* sample. Similarly, the use of morphometric techniques explores the phenotypic diversity within the species.

Chapter 2

Review of southern African Anostostomatidae (Orthoptera: Ensifera), with a key to genera[†]

H. Brettschneider, C.T. Chimimba, C.H. Scholtz and P.W. Bateman

Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002

South Africa

[†] Submitted to the Journal of African Entomology

Review of southern African Anostostomatidae (Orthoptera: Ensifera), with a key to genera

H. Brettschneider, C.T. Chimimba, C.H. Scholtz, & P.W. Bateman

Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002 South Africa

ABSTRACT The eight southern African King Cricket genera, namely *Bochus*, *Borborothis*, *Henicus*, *Libanasa*, *Libanasidus*, *Nasidius*, *Onosandridus*, and *Onosandrus* are redescribed from type and museum material. A key to the genera is provided. The status of *Libanasidus impicta* is investigated and species characteristics confirmed. A key to the two *Libanasidus* species is also provided.

KEY WORDS Anostostomatini, systematics, southern Africa, identification key

INTRODUCTION

The family Anostostomatidae Saussure, 1859 (Orthoptera, Ensifera, Stenopelmatoidea) shows a discontinuous distribution across Africa, Australasia, South and Central America and Asia and is represented by six tribes (Anabropsini Rehn, 1901; Anostostomatini Saussure, 1859; Brachyporini Gorochov, 2001; Cratomelini Brunner von Wattenwyl, 1888; Glaphyrosomini Rentz & Weissman, 1973 and Lutosini Gorochov, 1988) primarily in the southern hemisphere (Karny 1931, Johns 1997, Gorochov 2001). The majority of these king crickets are nocturnal omnivores occurring in a variety of habitats ranging from montane forest to open grassland and dry thornveld where they live in burrows or rotten wood (Toms 2001, Picker *et al.* 2002). The tribes Anabropsini, Anostostomatini and Glaphyrosomini have been recorded from Central America, specifically Ecuador, Mexico, Guatemala and Costa Rica. The Glaphyrosomini have been recorded in California and Texas in south-western North America (Otte *et al.* 2005). The dominant tribes in South America are the Cratomelini, occurring mainly in Chile, and the Lutosini, recorded from Brazil and Venezuela (Otte *et al.* 2005). The tribes Anabropsini, Anostostomatini, Lutosini and Brachyporini occur in Australia, New Zealand and New Caledonia (Otte *et al*. 2005). Anabropsini, Anostostomatini and Lutosini are present in China and Indo-Malaysia, specifically Nepal, Vietnam, Philippines and India (Otte *et al*. 2005).

The tribes Brachyporini and Lutosini occur in Madagascar, while members of the Anabropsini have also been recorded from the Democratic Republic of Congo (= former Zaire) (Otte *et al*. 2005). The

tribe Lutosini occurs in southern Africa where it is represented by one genus in South Africa and other genera that occur as far north as Tanzania (Otte *et al.* 2005). Seven of the eight African genera are placed in the tribe Anostostomatini and have been recorded from South Africa, Angola, Mozambique, Zimbabwe, Democratic Republic of Congo and Tanzania (Johns 1997, Otte *et al*. 2005). These genera include *Bochus* Péringuey, 1916, *Borborothis* Brunner von Wattenwyl, 1888, *Henicus* Gray, 1837, *Libanasidus* Péringuey, 1916, *Nasidius* Stål, 1878, *Onosandrus* Stål, 1878, *Onosandridus* Péringuey, 1916 and *Libanasa* Walker, 1869 of the tribe Lutosini. *Onosandrus* is also found in New Zealand and South Australia while all the other genera are restricted to Africa (Karny 1931).

Most of the taxonomic studies on the family Anostostomatidae Saussure, 1859 were undertaken between 1803 and 1943. This family name is emended from Anostostomidae Stål, 1876, which is derived from the tribe Anostostomii Saussure, 1859 (Johns 1997). Along with the Cratomelini, it was originally classified under the family Gryllacrididae Stål, 1876 but the family name Stenopelmatidae Burmeister 1838 had priority and, therefore, became the appropriate family name for this group of king crickets (Johns 1997). Karny (1932) created a replacement name for the type genus *Anostostoma* Gray, G. R., 1837, namely *Australosoma*, and placed it in the subfamily Deinacridinae Karny, 1932, despite the existence of the families Henicinae Karny, 1928 and Mimnermidae Brunner von Wattenwyl, 1888 (Johns 1997). Kevan (1982) noted that the family name Mimnermidae had priority over family Henicidae Karny, 1928 and the two

taxa were combined under the Mimnermidae. The genus *Anostostoma* is a valid genus name and its family-group name has date priority over all other names (Johns 1997). The Anostostomatidae now contains, although not exclusively, all the southern African genera (Johns 1997).

Johns (1997) conducted the most recent taxonomic study of the group, paying attention to species diversity within the family. He also synonymised the genus *Platysiagon* Brunner von Wattenwyl, 1888 with the genus *Libanasa*. Prior to 1997, studies focussed on higher taxonomic ranks and their relationships, except for Ander (1943) who revised some nomenclatural disparities and synonymies in anostostomatids and sister taxa. Ander (1943) dealt specifically with the genus *Nasidius* and consequently synonymised the genus *Faku* Péringuey, 1916 with *Nasidius*. Lastly, Ander (1943) also considered misidentifications of species within the genus *Henicus*. No recent reviews that focus on taxonomic levels lower than superfamily level are presently available.

A review of the original descriptions of the southern African anostostomatids revealed a general lack of detail and uniformity among the various authors. The numbers of undescribed species listed by Johns (1997), the many synonymies created in the past and the difficulty in identifying genera pose problems for further taxonomic studies on this group of king crickets (Toms 2001). This generic review aims to improve the identification of specimens, since many species are cryptic, the females and immatures often appearing indistinguishable (see Chopard 1943, Toms 2001).

MATERIALS AND METHODS

The type species of each genus is redescribed from type material and specimens obtained from South African museums to delineate generic characteristics. This allowed for an updated identification key to the genera. The possible *nomen dubium*, *Libanasidus impicta* (Stål 1878) is reviewed, redescribed and compared with *Libanasidus vittatus* (Kirby 1899) and an identification key to the species provided. Specimens were obtained from the following museums as indicated by their standard abbreviations: Muséum d'Histoire Naturelle (MHNG), Geneva, Switzerland; Naturhistorisches Museum Wien (NMW), Vienna, Austria; Naturhistoriska Riksmuseet (NHRS) Stockholm, Sweden; The Natural History Museum, London (BMNH), United Kingdom; South African Museum (SAMC), Cape Town, South Africa; Transvaal Museum (TM), Pretoria, South Africa.

Descriptions of the specimens focus on morphological characteristics frequently mentioned in the taxonomic literature of this group, thus promoting consistency (see Johns 1997 for familial characteristics). The distinguishing morphological characteristics include the presence/absence of the tibial tympanum, the number and position of spines on the tibiae, armature of the femora and genicular lobes, modification of the male head and the shape and length of the female ovipositor. The identification key below distinguishes the eight southern African anostostomatid genera based, however, not only on the characteristics of the type species, but also on morphological data available in the literature from all the known species of each genus. This allowed identification of specimens to generic

level, avoiding an exhaustive review of all 51 currently known species in this group. Further taxonomic revision at specific levels is crucial, but falls beyond the scope of this synthesis. Knowledge on the biology and behaviour of these crickets is sparse and, for the most part, limited to the genera *Libanasidus* and *Henicus*. Nevertheless, the available information is summarised in the respective remarks section for each genus.

Measurements of head width (HW) and length (HL), hind femur length (HFL), hind femur base width (HFW), body length (BL) and ovipositor length (OL) in females, were made with a pair of Mitutuyo digital callipers to the nearest 0.05 mm, for general comparison. Appendix 1 contains the coordinates of collection localities of specimens included in the review. Distribution maps were generated with ArcView GIS 3.3 software.

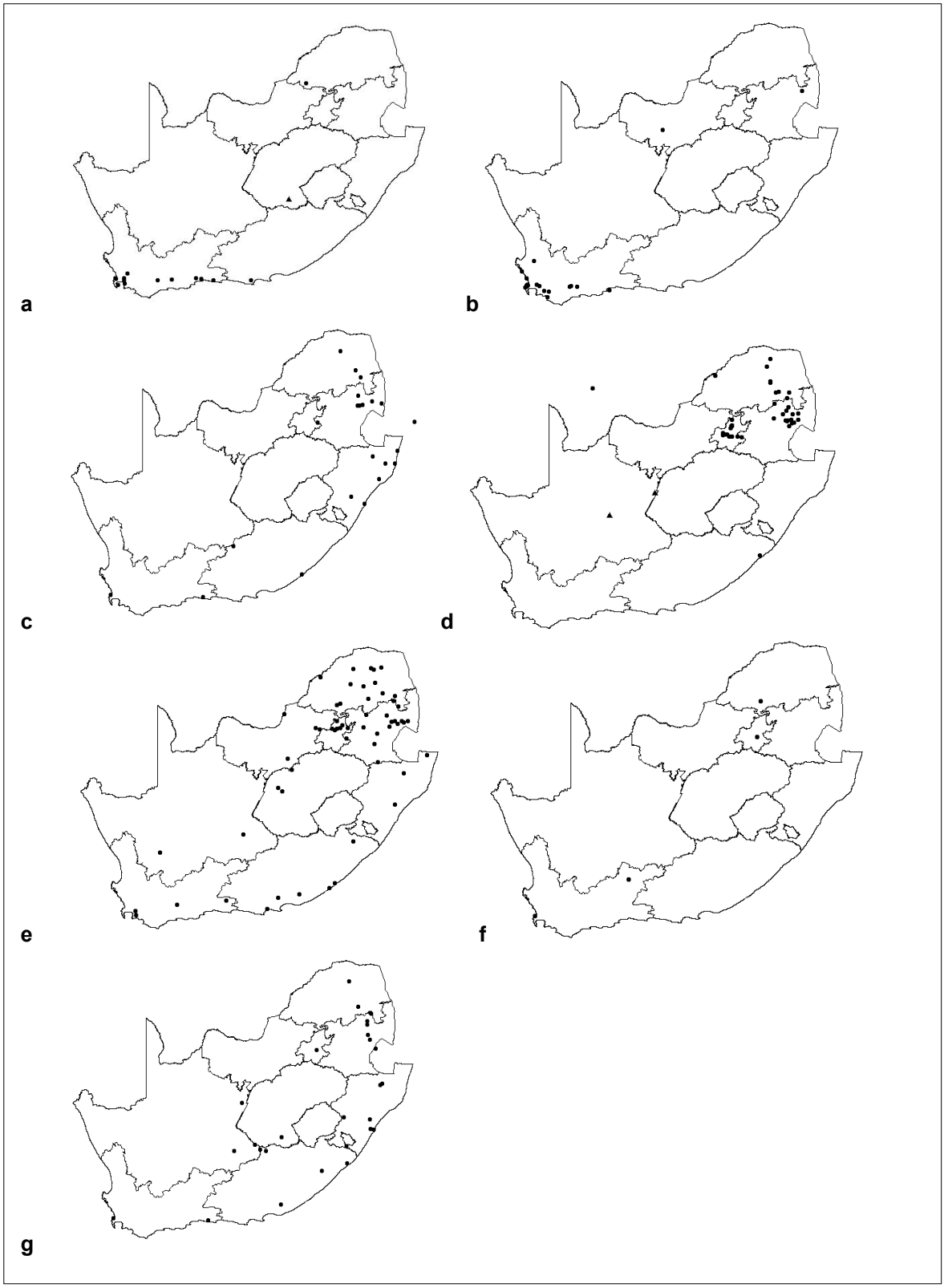


Figure 2.1. Known South African geographic distributions of the genera *Bochus* (▲) and *Borborothis* (●) (a), *Henicus* (b), *Libanasa* (c), *Libanasidus impicta* (▲) *L. vittatus* (●) (d), *Nasidius* (e), *Onosandridus* (f) and *Onosandrus* (g).

Key to eight anostostomatid genera from southern Africa

1. Tympanum absent from fore tibia or only present as indented area on either side of tibia…..……..……..…….**2**

– Tympanum clearly visible, perforated on both sides of fore tibia…..…..……....**6**

2. Two spines on inner margin of fore tibia. Hind femur never armed with spikes/hooks…….....…….**3**

– One spine on inner margin of the tibia ……..……..…**9**

3. Dimorphism between sexes – ♂♂ with some modification or enlargement of the head. ♀ ovipositor long, at least ⅓ as long as hind femur……..……..……..…..…..…**4**

– ♂♂ with no modification of the head or mandibles, ♀♀ with either very short or long ovipositor. ……..……**5**

4. In ♂, upper part of frons produced into blunt, obtuse cone, which is slightly indicated in ♀…….....*Nasidius* Stål, 1878

– ♂♂ with elongated mandibles and usually horn-like extensions from frons or mandibles, no modification to ♀ head.....*Henicus* Gray, 1837

5. ♀ with extremely short ovipositor – only about 3 mm long, almost vertical and stout. Face roughened with punctures…….....*Bochus* Péringuey, 1916

– Ovipositor at least one-third of hind femur length. Face smooth and shiny…….....*Onosandridus* Péringuey, 1916

6. Hind femur unarmed above……..…….……**7**

– Hind femur furnished with teeth on top margin. Body uniformly dark colour. Body size 30-35 mm…….……*Borborothis* Brunner von Wattenwyl, 1888

7. Spines on hind margin of hind tibia less than 4. Body without strikingly black-banded tergites. Cerci curled, thin. Body size 10-40 mm……..……**8**

– Hind femur armed with small teeth on lower margin. Hind margin of hind tibia with 4 spines. Body ferruginous to pale ferruginous with black bands on hind margins of tergites. Body size 30-60 mm……..……*Libanasidus* Péringuey, 1916

8. Mandibles simple in both sexes, if not, a strikingly pale ring is present proximal to hind knee and any projections from ♂ face is situated on the mandible base ….....*Libanasa* Walker, 1869

– ♂ mandibles enlarged, usually with horn-like process projecting from frons. No pale ring present proximal to hind knee……*Henicus* Gray, 1837

9. Middle or hind genicular lobes spined on either inner or outer margin……..**10**

– Genicular lobes unspined……....*Nasidius* Stål, 1878

10. Hind femur unarmed……..…….**8**

– Lower margin of hind femur denticulate……..…*Onosandrus* Stål, 1878

Genus ***Bochus*** Péringuey, 1916 (Fig. 2.1a, Fig. 2.2)

Bochus Péringuey, 1916: 297. Type species by subsequent designation: authority, Karny, 1937: *Bochus puncticeps* (Pictet & Saussure, 1891).

***Bochus puncticeps* (Pictet & Saussure, 1891)**

Bochus contemnendus Péringuey, 1916: 419. Karny 1937: 210 > *Bochus puncticeps*.

Material examined: Free State, Smithfield, 1891, Pictet & Saussure (♂ holotype, MHNG); O.R.C. Kannemeyer, *Bochus puncticeps* det. Karny (1♀ paratype, 1♂ SAMC). South Africa, 1875, Hughel, det. Karny (1♂ paratype, SAMC).

Redescription

Legs. Fore tibiae and femora robust, broad. Fore tibia in ♀ rounded, ♂♂ rectangular. ♀ light brown, ♂♂ dark brown (knees paler, tibia and base of femora dark again) to black, holotype body uniform dark brown, shiny. Fore tibia, inner margin with 2 spines, outer margin unarmed, hind margin with 8 spines. No tympanal organ present. Spines on fore and middle tibia robust, dark brown. Middle tibia with 2 spines on inner margin, 3 on outer margin, hind margin with 2 spines on inner and 3 on outer margin. Hind tibia with 6 small tooth-like spines on inner margin, 4 very small spines on outer margin. Hind margin has 3 similarly small spines in holotype and 2 in other specimens. 2 apical spines on fore margin and 2 on side margins equal in length, 1 - 1 ½ times the tibial width. Spines on inner margin slightly longer than those on outer margin. 2 apical spines on hind margin smaller than first four, and much thinner. All legs are flattened laterally. All femora and genicular lobes unarmed. 6 apical spines present on all legs, of which last 2 in each case are very small. Apical spines on fore legs of holotype are damaged - only 3 visible. Hind femur base hardly dilated and only slightly broader than knee. Hind femur same length as hind tibia. Chevron ridges on femur flank hardly visible. Fore and middle coxae have small, weak spines.

Genitalia. Holotype: Subgenital plate small, elongated, light brown to black. Posterior margin greatly indented to form 2 rounded lobes with a small stylus at tip of each lobe. Paraprocts have styli as long as cerci, crossing in centre. Cerci moderately long, conical, thin. ♂♂ (SAMC): cerci black-tipped and slightly curved to outside. Cerci situated next to

paraprocts, not above as usual. ♀ (SAMC): subgenital plate, pale yellow, triangular with clear indentation in centre of posterior margin, resulting in 2 sharp points. Ovipositor dark brown, very short, robust, lower valves curved strongly upwards, upper valves rather straight. Ovipositor as long as wide. Cerci small, conical, straight.

Head. Rounded from frontal view, light brown to black (holotype) and broader than pronotum, impresso-punctate (dot-like impressions over whole surface) from vertex, covering most of facial area. Face pronounced (rounded) outwards in profile. Clypeus vaulted (posterior margin over-hanging upper margin of labrum). Labrum rounded and impresso-punctate in all specimens except holotype. Clypeus and labrum dark brown to black, holotype and ♀ labrum edged in yellow below. ♂ mandibles dark brown to black, robust, indented on outer margin below anterior tentorial pits, forming a hollow from which rounded flanks ('wings') point outward from face. Below these flanks, mandibles curve inwards normally. Dente black. ♀: mandibles normal, light brown, punctured, dente black. Lighter ♂, with two pale areas on anterior tentorial pits and upper inside of mandibles. Fastigium of frons rotund and indented (not pronounced as usual), with frons bulging outwards lightly below fastigium. Fastigium pale yellow in ♀, in ♂♂ coloured as face. 3 ocelli small, yellow (holotype), only median ocellus visible in ♀, none in SAMC ♂♂. Eyes unusually far apart, oval, bulging. Antennal scape black, normal, with white spot in centre in frontal view in ♀. Frons shiny, genae matt.

Thorax & Abdomen. Pronotum and body slightly roughened in SAMC ♂♂, shiny in SAMC ♀. Pronotum of ♀ lighter coffee-brown, dark brown in holotype, posterior margin punctured and two lateral keels pronounced. Fore and hind margin black banded. Tergite flanks yellow, creating a row of pale spots on each side of body in all specimens, above dark grey, edged in black posteriorly, smooth or faintly wrinkled. Meso- and metabasisternum light brown, 2 spikes at their extremities. Metabasisternum posterior margin straight, spikes on either side reaching middle coxae. Sternites pale yellow to light brown, dark brown hind margins (SAMC ♀).

Holotype. BL 25.99 mm**,** HW 8.92 mm**,** HL 11.02 mm**,** HFL 16.38 mm**,** HFW 3.50 mm.

Other. BL 30-35 mm, HFL 16-19 mm, OL 3 mm (ovipositor diameter at base 2 mm).

Remarks: *Bochus*, along with *Borborothis* are often mentioned as most the primitive of the 8 southern African Anostostomatid genera due to the lack of sexual dimorphism (Toms 2001), which I disagree with considering the modified flanks on the male mandibles. *Bochus* is a monotypic genus and *B. puncticeps* is known only from the Free State in South Africa.

The holotype has no legs on the left side of the body; the fore and middle legs on the right are without tarsi, and the hind leg is with an incomplete tarsus. The antennae are damaged.

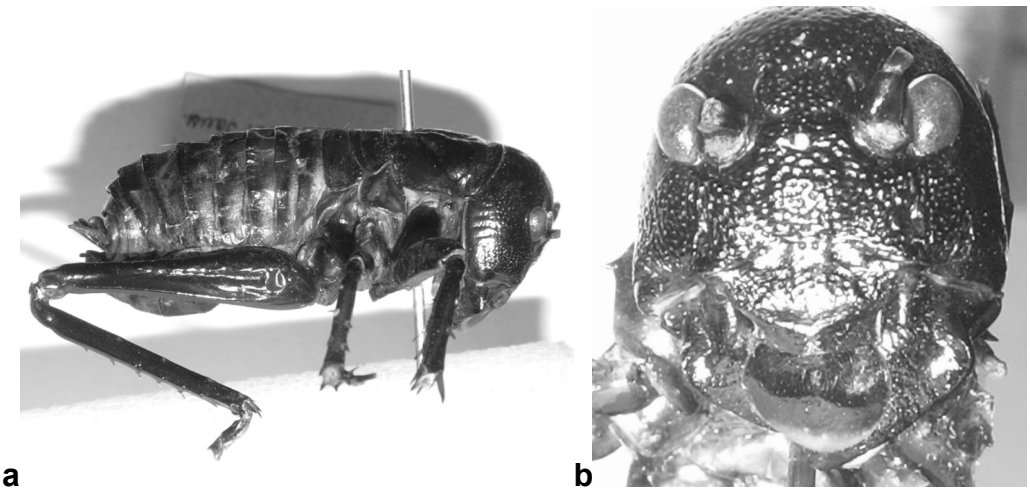


Figure 1.2. *Bochus puncticeps* holotype lateral (a) and frontal (b) views.

Genus ***Borborothis*** Brunner von Wattenwyl, 1888 (Fig. 2.1a, Fig. 2.3)

Borborothis, Brunner von Wattenwyl, 1888: 280. Type species by subsequent designation, authority Karny, 1937: *Borborothis opaca* Brunner von Wattenwyl, 1888.

Borborothis opaca Brunner von Wattenwyl, 1888

Material examined: Cape Province, 1888, Brunner von Wattenwyl (♂ holotype NMW). Western Cape, Cape Town, 1878, R. Trimen, "*B. punctulata*" det. Karny (1♀ SAMC). Western Cape, Gordon's Bay, xi.1878, P.C. Smith, det. Johns (1♂ SAMC). Western Cape, George, Le Leup (2♂ TM). Western Cape, Jonkersberg, G. van Son (2♀ TM). Western Cape, Knysna, det. Johns (1♀ SAMC). Western Cape, Oakley, Newlands, det. Brunner von Wattenwyl (1♂ SAMC). Western Cape, Oudebosch, Caledon, xii.1920, det. Johns (4♂ SAMC) det. Karny (1♀ SAMC). Western Cape, Paarl, det. Johns, (1♂ SAMC). Western Cape, Rondebosch, det. Brunner von Wattenwyl (1♀ SAMC) det. Karny (1♂ SAMC). Western Cape, Somerset West, Morning Star, det. Johns (3♀, 2♂ SAMC). Western Cape, Stellenbosch, "*O. saussurei*" det. Brunner von Wattenwyl, det. Johns (1♂

SAMC); 1913, "*O._opacus*" det Karny, det. Johns (2♂ SAMC). Western Cape, Tradouw pass, Swellendam district, det. Johns (1♂ SAMC). Western Cape, Zonde einde, 1921, R.W. Bucker, det. Johns (1♂, 1♀ SAMC). Locality unknown, xi.1922, R. Lawrence, det. Johns (1♀ SAMC), ii.1929, det. Johns (2♂ SAMC), x.1926, K.H. Barnard det. Johns (1♂ SAMC).

Redescription

Legs: Holotype – uniform ferruginous, top margin of hind femur darkened, chevron markings paler. ♀♀ ferruginous, lighter than body, ♂♂ uniform light brown. Fore tibia with 1 spine on inner margin, unarmed on outer margin. Tympanal organ large, clearly visible. Middle tibia with 2 spines on inner margin, 3 on outer margin, 8 on hind margin, 4 apical spines. Hind tibia bears 4-5 small spines on hind margin, 8-9 larger spines on inner margin, 7-9 on outer (last 2 small). Spines on fore margin robust, twice tibial width in length. Holotype – hind tibia on fore margin 14 (7 on inner margin and outer margin) robust spines, twice tibial width in length (last 2 very small), hind margin bears 4 small spines, ¼ length of tibial width, 8 apical spines. 2 apical spines on fore margin 2 ½ times (holotype) – 4 (others) times tibial width in length, 2 spines tibial flanks - shorter, 2 apical spines on hind margin – ½ length of apical spurs on tibial flank, final 2 apical spines - ¼ of tibial width in length. Femora slightly compressed laterally, fore and middle femora unarmed, hind femur long, greatly dilated at base, longitudinal keel running length-wise below pronounced chevron markings, top margin toothed. Hind knee same brown colour as femora.

All genicular lobes unarmed, fore and middle coxae normally spined. Femora of immatures yellow, knees and femur above darkened.

Genitalia: ♂: subgenital plate trapezoidal, uplifted in centre, descending to apex, fold visible before 2 styli at apical points (appears cube-like). Paraprocts with 2 small styli, cerci very long, rounded, epiproct posterior margin indented in centre. ♀♀: ovipositor brown, slender, upcurved, base not dilated – ovipositor diameter constant throughout, as long as hind femur. Subgenital plate small, trapezoidal, rounded at apex, cerci conical, straight or upcurved in ♀♀, curved outwards in ♂♂- very thin tips. Ovipositors of immatures shorter.

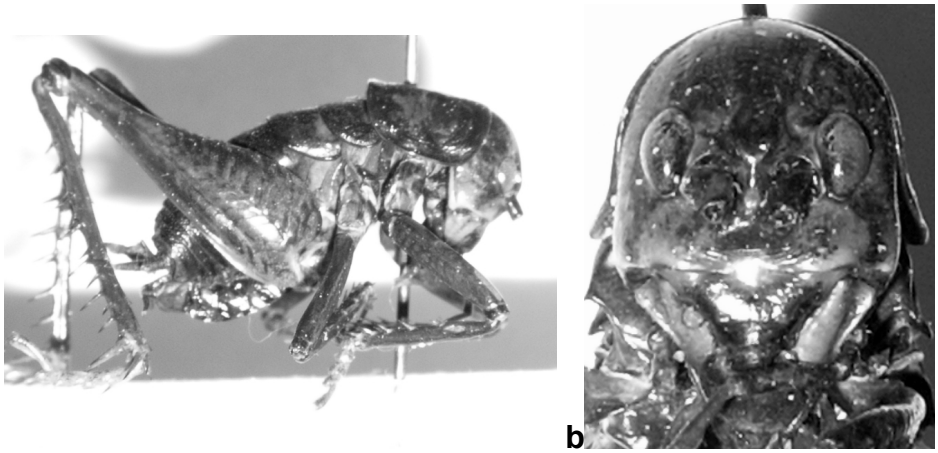
Head: uniform dark brown, holotype with faint yellow marbling, with pale patch over each gena. Face simple, impresso-punctate but shiny, only median ocellus visible. Mandibles not enlarged or armoured, yellowish, triangular indentation on side darker. Labrum brown to light brown, always same colour as frons, holotype - labrum and clypeus dark brown, lateral ocelli small, yellow and small, median ocellus darker yellow, hardly noticeable. Clypeus with 3 pale spots, 1 on bottom margin, 2 above on either side of middle. Frontoclypeal suture yellowish, genae and subgenae finely wrinkled; holotype – genae, subgenae, area above frontoclypeal suture, anterior tentorial pits - blue-green. Antennal base normal, brown, situated very low on frons – near lower edge of eyes. Fastigium small, elongated, slightly indented in centre, angles rounded. 3 ocelli clearly visible in immatures.

Thorax & Abdomen: Body dark brown to black, vaguely arched downwards in profile, pronotum broader than head. Pronotum margins dark brown, mottled with yellow or completely black, roughened with punctures, especially along fore and hind margins in ♂♂, faintly roughened in ♀. Tergites dark brown to black or ferruginous with black margins, hind margin - impresso-punctate and dark, last tergites wrinkled on hind margin. Holotype – tergites 1-3 mottled with yellow on anterior margin, last tergites completely black, meso- and metabasisternum, 2 spined, dark ferruginous. Venter pale, sternites with darkened band on hind margin. Immatures black dorsally, pale yellow ventrally.

Holotype: BL 21.44 mm, HW 6.29 mm, HL 8.06 mm, HFL 16.59 mm, HFW 5.59 mm.

Other: BL 18-25 mm, HFL 14-16 mm, OL 11-12 mm.

Remarks: The genus currently contains two species, the second being *B. brunneri* (see Karny 1930 for key to the species). The genus is considered primitive due to the absence of sexual dimorphism, and can be found along the southern mountainous regions of South Africa (Toms 2001). Meso- and metabasisternum of holotype damaged.



a **b**

Figure 2.3. *Borborothis opaca* holotype lateral (a) and frontal (b) views.

Genus ***Henicus*** Gray, 1837 (Fig. 2.1b, Fig. 2.4)

Henicus Gray, 1837: 144. Type species *Henicus pattersoni* (Stoll, 1813).

Mimnermis Stål, 1878: 52. Johns 1997: 128 > *Henicus*.

Henicus pattersoni Stoll, 1813

Originally described as *Gryllus pattersoni* Stoll, 1813.

Henicus stolli Gray, 1837: 144. Kirby 1906: 122 > *Henicus pattersoni*.

Material examined: Western Cape, Marloth Nature Reserve (Duiwelsbos & Koloniebos), 8-9.x.1997 P.M. Johns (1♀ SAMC). Western Cape, Swellendam, 1813, C. Stoll (♂ holotype, NMW); 1907, L.E. Taylor (1♀, 1♂, SAMC).

Redescription

Legs: Dark brown to black or yellow with light brown upper margins (holotype), knees pale. Tibia above dark in lighter specimens. Fore tibia – fore inner margin with 1 spine, fore outer margin unarmed, hind margin with 8 spines, 4 apical spines. Tympanal organ visible on both sides of tibia. Middle tibia with 2 spines on fore inner margin, 3 on fore outer, 8 on hind margin, 4 apical spines. Hind tibia on fore margin with 20 small spines (½ tibial width in length), hind margin with 3 (holotype) to 4 small

spines. 8 apical spines - 2 apical spines on inner margin of tibia (one on fore margin, one on flank) 2 ½ times tibial width in length, 2 on outer margin (one on fore margin, one on flank) twice tibial width in length, 2 on hind margin as long as tibia is thick, lower 2 on hind margin hardly visible. Fore and middle femora laterally compressed, outer genicular lobes of middle femora spined, inner genicular lobes smooth. Hind femur very long, greatly dilated at base, apex much attenuated, both inner and outer genicular lobes spined, longitudinal keel present below darkened chevron ridges on flank. Spines on first four coxae small, yellow. All femora unarmed.

Genitalia: ♂ – subgenital plate large, trapezoidal, 2 small styli at posterior margin, cerci small, yellow, broad base and very thin tips, curled downwards. Paraprocts with small black styli. ♀♀ – ovipositors as long as hind femur, uniform dark brown or pale at base darkening towards apex, subgenital plate pale or dark, triangular with rounded edges.

Head: ♂ head broader than pronotum, considerably elongated, vertex yellow (holotype) or mottled with yellow with dark red colouration on sides extending to gena and area below fastigium (SAMC ♂). Thin, black vein-like lines run from vertex to eyes, eyes far apart, antennae situated below fastigium. Fastigium triangular, darkened on raised surface, with dark lines running from 2 top corners to half of vertex. Head expands towards thin mandibles that are elongated (about three times normal), small gap between labrum and mandibles at clypeolabral suture. Mandibles straight, not wavy or curved as in *H. monstrosus*. 3 ocelli yellow, median ocellus

larger, teardrop-shaped, surrounded by yellow area in SAMC ♂, also with a yellow spot on each anterior tentorial pit. Sides of head roughened, fine wrinkles below eyes. Frons, above anterior tentorial pits with 2 horn-like spines – thin, curved downward and inward, sharp and pale at apex. From median ocellus frons indents towards pale yellow clypeus. Clypeus yellow, raised, with black spot above and 2 brown tubercles just off middle margin, labrum spoon-shaped, greatly elongated, covering dente, with black wrinkled, indented oval area on either side (possible stridulatory function), bottom margin light yellow, side margin with blunt spike at labrum's broadest point. Clypeus and anterior margin of labrum hunched in profile. Occipital suture behind genae with protruding spike. Genae indented, darkened, antennal base yellow. ♀ - face normal, genae to anterior tentorial pits paler, clypeus and labrum uniform black or uniform pale. Fastigium black with grey waved lines (inverted V) below each eye.

Thorax & Abdomen: ♀♀ – very large body size. ♂ - body small compared to head. Pronotum broadens to posterior, fore and hind margins diffusely black, mottled with black and yellow or light brown (butterfly shape) in centre, flanks yellow. Tergites yellow or paler on anterior margin. Tergites 1- 2 yellow on sides, rest completely dark brown, shiny. Meso- and metabasisternum small, 2 pointed - not distinctly spined. Sternites dark, with thin black line (not banded) on hind margin, first three sternites paler, black posterior margins more distinct. ♀ tergite flanks faintly mottled with light brown.

Holotype: BL 22.64 mm, HW 10.76 mm, HL 16.32 mm, HFL 21.99 mm, HFW 5.72 mm.

Other: BL 21-27 mm, HFL 20-24 mm, OL 16-20 mm, ♂ facial length 15 mm.

Remarks: The genus contains 9 species. Males may use modified mandibles and enlarged labrum to plug burrow entrances. Mandibles are also used for stridulation in *H. monstrosus*, in contrast with most anostostomatids, which use abdominal-femoral stridulation (Toms 2001). The genus occurs in the mountainous region of the Cape. The right fore tibia of the holotype has three spines on the fore inner margin, 1 on the outer and 8 on the hind margin, with no tympanum present. This tibia may be a regenerated limb, as 3 spines on the fore inner margin are unusual for these crickets (see McDonald & Hanrahan 1993). Meso- and metabasisternum damaged in holotype.

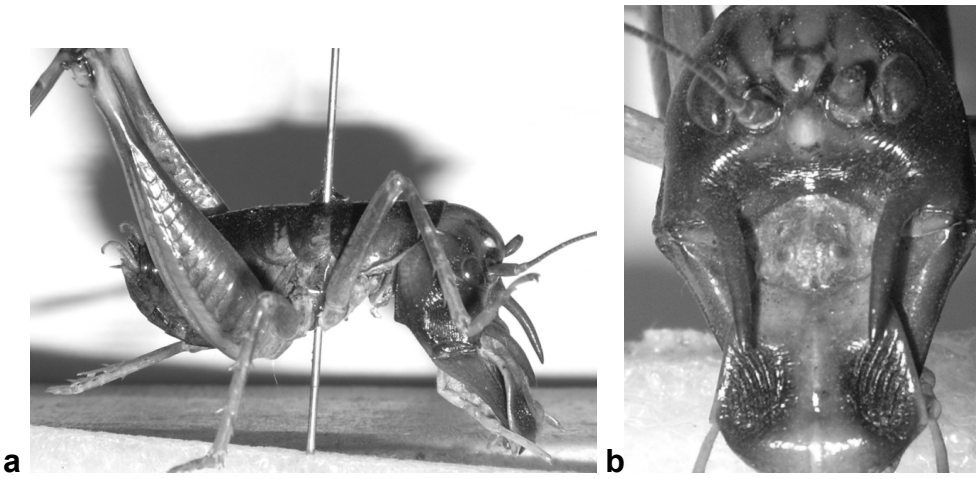


Figure 2.2. *Henicus pattersoni* lateral (a) and frontal (b) views.

Genus ***Libanasa*** Walker, 1869 (Fig. 2.1c, Fig. 2.5)

Libanasa Walker, 1869: 208. Type species by original description, *Libanasa incisa* Walker, 1869.

Platysiagon Brunner von Wattenwyl, 1888: 292. Karny 1937: 181 > *Libanasa*.

Libanasa incisa Walker, 1869

Carcinopsis fusca Brunner von Wattenwyl, 1888: 278. Karny 1937: 202 > *Libanasa incisa*.

Material examined: KwaZulu-Natal, 1969, Walker, (♀ holotype, BMNH).

Redescription

Legs: Fore tibia as long as femur, dark brown, inner margin black with 1 spine, none on outer margin, 8 spines on hind margin, 4 apical spines. Tympana oval, perforated on both sides of tibia. Fore femur light coffee-brown with dark brown blotches near knee. All femora compressed laterally. Middle tibia with 2 spines spaced abnormally far apart on inner margin, upper spine near knee, lower spine situated just beyond middle of tibia, outer margin bears 3 spines, hind margin 8, 4 apical spines. Hind tibia dark brown below and near knee, light coffee-brown in-between, 10 short tooth-like spines on fore inner margin, 11 on outer, hind margin with 2 even slimmer spines. 8 apical spines, 2 spines on fore margin twice the length of tibial width, 2 on sides extraordinarily long – four times length of tibial width, upper 2 on hind margin – as long as tibia is thick, lower 2 on hind margin – ½ as long as tibia is thick. Hind tibia elongated and slender - longer than hind femur. All femora unarmed. Hind femur - light coffee-brown, mottled with black, lengthy, greatly dilated basally, black pinnate markings on flank, 2 broken black lines run longitudinally from pale ring

near knee to base - 1 in centre, 1 on lower margin, originating at pale ring near knee, top margin black with light brown spots, knee black. Genicular lobes of middle femur spined on outer margin, hind genicular lobes spined on inner margin above and outer margin below. Fore coxa armed with sharp spine curved downward and forwards, middle coxae with sharp, curved, outwardly pointing spine.

Genitalia: Subgenital plate sharply triangular, ovipositor sword shaped, sharply pointed, up-curved, base is light brown, three times broader than darker tip. Cerci long, thin, curled to outside.

Head: Pronotum and vertex dark brown - forming two broad dark bands towards eyes, genae and frons light coffee-brown. Fastigium triangular, raised, light coffee-brown, with heart-shaped dark patch in centre, two brown patches run from vertex to corners fastigium. Light coffee-brown ridge runs from lower angle of fastigium to frontoclypeal suture, sides of ridge and fastigium darkened. Eyes teardrop-shaped, light brown, antennal scape light brown with dark patch on inner margin. Pre-clypeus rectangular, light brown with two horizontal black bars lying on margins, post-clypeus light brown, triangular, separated from pre-clypeus by constriction. Labrum darker brown, with darker lower margin, mandibles robust, reddish brown, the triangular indented region at the base clearly visible.

Thorax & Abdomen: Body glabrous, black above light coffee-brown on flanks. Pronotum with conspicuous dark brown spot in middle near bottom

margin of flanks, a light median line runs the length of pronotum above. Sternites light coffee-brown, the second last sternite with median dark patch. Mesobasisternum with two sharp, robust spines pointing upwards and outwards, metabasisternum triangular, sharply pointed - the two angles not divergent but joined.

Holotype: BL 23.59 mm, HW 6.36 mm, HL 9.42 mm, HFL 20.09 mm, HFW 5.59 mm, OL 12.63 mm.

Remarks: There are five other species currently in this genus. Species have been found in dryer thornveld areas in Mpumalanga, as well as in rotting logs in indigenous forest along the coast and KwaZulu Natal (Toms 2001). The holotype is in good condition, with only the antennae damaged and one hind leg broken from the body.

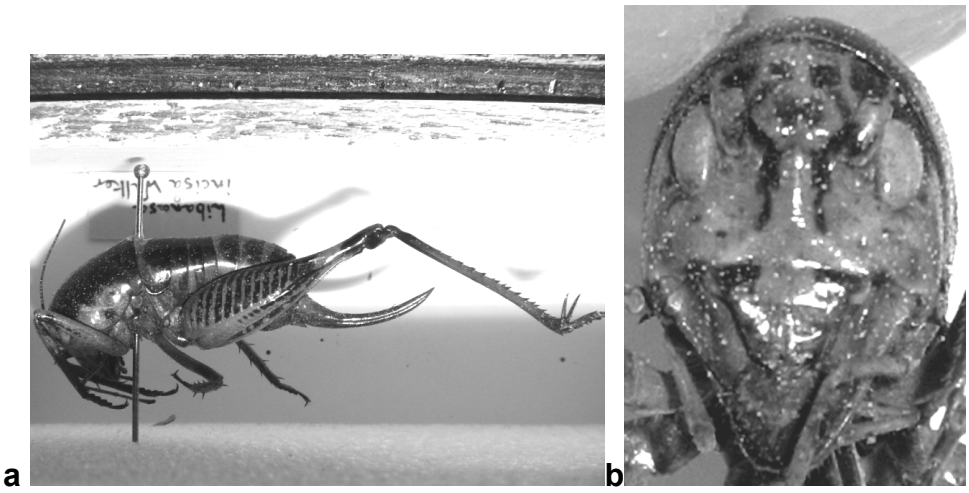


Figure 2.5. *Libanasa incisa* holotype lateral (a) and frontal (b) views.

Genus ***Libanasidus*** Péringuey, 1916 (Fig. 2.1d, Fig. 2.6)

Libanasidus Péringuey, 1916: 424. Type species *Libanasidus vittatus* (Kirby, 1899) by subsequent designation authority: Johns (1997).

Nasaliba Karny, 1937: 197.

Libanasidus vittatus (Kirby, 1899)

Originally described as *Carcinopsis vittatus* Kirby, 1899: 478. Johns 1997: 128 > *Libanasidus vittatus*.

Material examined: Gauteng, Johannesburg, 9-i-1978, G. van Son (1♂ TM); 1-ii-1962, R. du Preez (1♀ TM); 9-xi-1993 (1♀ TM). Gauteng, Pretoria, 15-ii-1996, Bateman & Prinsloo (1♀ TM); 21-i-1995, G. van Son (1♀ TM); xi-1995, A. Matlou (1♀ TM). Mpumalanga, Badplaas Mondi Forest, 11-xi-2002, TMSA expedition (1♀ TM). Mpumalanga, Barberton, 1899, Kirby (♀ holotype, BMNH). Limpopo, Entabeni, xi-1931, G. van Son (1♀, 3♂ TM). Limpopo, Malta Forest, 9-x-1978, M. Keeping (1♀ TM). Limpopo, Moordrift, 1-x-1924, G. van Dam (1♀ TM). Limpopo, Woodbush, xi-1942, G. van Son (2♂, 5♀ TM).

Redescription

Legs: Fore tibia compressed laterally, same length as fore femur, light yellow, with 1 spine on inner margin, unspined on outer margin, hind margin with 8 spines, 4 apical spines. Tympanal organ oval, large, perforated on either side of tibia. Middle tibia with 2 spines on inner margin, 3 on outer margin, hind margin bears 8 spines, 4 apical spines. Hind tibia light yellow, as long as hind femur bearing 8 strong black-tipped spines (uniform dark brown in darker specimens) as long as tibia is thick on inner and outer margin, lower 2 spines small, hardly visible, hind margin with 4 small, black-tipped, spines ⅓ tibial width in length. Apical

spines: 2 on fore margin twice as long as tibia is thick, 2 on side shorter, upper 2 spines on hind margin as long as tibia is thick, lower 2 spines are ⅓ as long as tibia is thick. Fore femur light yellow, compressed laterally, upper surface roughened, indented. Middle femur light yellow, shorter than tibia, compressed laterally, slightly indented and sculptured on sides. Holotype: hind femur long, robust, base greatly dilated, dark brown, knee pale. Other: legs uniform ferruginous or yellow, to ferruginous with pale knees. Hind femur laterally compressed, excavated pinnate markings on upper half of femur flank, lower margin faintly denticulate. Middle genicular lobes spined on outer margin, hind genicular lobes spined on outer margin below, on inner margin above. Fore coxae with strong, forward and downward pointing spine, middle coxae with smaller, backwards and downward pointing spine.

Genitalia: ♀ subgenital plate trapezoidal, brown to dark brown with black markings, hind margin incurved. Ovipositor dark ferruginous, up-curved, very long, base five times wider than sharp tip. Cerci conical, upcurved, long, yellow. ♂ subgenital plate trapezoidal, 2 styli at posterior edges, dark brown, paraprocts with styli, epiproct simple.

Head: Uniform coffee- to ferruginous or dark-brown; vertex, fastigium, genae and area around the eyes dark ferruginous. Fastigium raised, triangular, dark, punctuate, occasionally edged in yellow. Holotype: fastigium with 2 small teardrop-shaped yellow markings on bottom angle, 2 top angles with round, large yellow ocelli. Frons below fastigium with diamond-shaped yellow blotch flowing over frontoclypeal suture to pre-

clypeus; median ocellus as darkened spot on yellow. Eyes large, black, teardrop-shaped, antennae light yellow-brown, area below scape paler, reaching to light brown subgenae. Preclypeus yellow, postclypeus yellowish with two dark brown spots on margin, pre- and post-clypeus separated by shallow cylindrical groove. Labrum light brown to pale yellow, round, mottled with yellow, dark longitudinal lines medially (holotype). Mandibles light brown, strong, robust, strongly pronounced triangular indentations near base. ♂: tusks on mandibles brown, tips darkened and sharp, small (as long and broad as mandible itself) to enormous (immensely thickened bases), base of tusk wrinkled where they extend upwards at almost 90° angle.

Thorax & Abdomen: Body glabrous, large, pronotum and head dark ferruginous. Tergites pale yellow with distinct black bands posteriorly or uniform brown. Sternites ferruginous or pale yellow. Holotype: tergites light brown on fore margin, distinct black bands on hind margin. Pronotum uniform light coffee-brown to ferruginous, with darkened band on posterior margin, broader towards hind margin with forward pointing grooves on side. Holotype: pronotum without black band, yellow markings on side margins and above on anterior margin, slightly roughened. Meso-, metabasisternum and sternites 1-3 light yellow, 4th dark brown on hind margin, yellow on fore margin, last 2 sternites darker. Mesobasisternum with 2 triangular spikes pointing outwards, metabasisternum consists of 2 trapezoidal lobes orientated towards coxae.

Holotype: BL 41.70 mm, HW 11.09 mm, HL 16.20 mm, HFL 27.63 mm, HFW 8.83 mm, OL 23.33 mm.

Other: BL 22-40 mm, HFL 16-31 mm, OL 10 -24 mm.

Remarks: The genus occurs naturally in the indigenous and coastal forests of eastern South Africa, reaching to Zimbabwe. *Libanasidus vittatus* invaded suburban gardens of Gauteng in the 1960's, and is commonly known as the 'Parktown Prawn'. Since then they have featured in newspaper articles, cartoons and is the first insect to have its own website! They are nocturnal, hiding is self-constructed burrows during the day (see McDonald & Hanrahan 1993) and are equipped with fascinating defence behaviours (Wolf *et al.* 2006). Holotype generally in good condition, apart from the antennae and left fore leg that are damaged, and the ovipositor tip that is broken.

Libanasidus impicta (Stål, 1878)

Originally described as *Libanasa impicta* Stål, 1878: 51. Johns 1997: 128 > *Libanasidus impicta*.

Material examined: Northern Cape, "Cape Colony", det. Karny (1♂, SAMC). Northern Cape, Kimberly, J. H. Power, "*Libanasa impicta*" (1♀, SAMC).

Redescription

Legs: Fore tibia with 1 spine on inner margin, outer margin unarmed, hind margin with 8 spines. Tympanum perforated on either side of tibia. Middle tibia with 2 spines on inner margin - situated far apart, 3 spines on outer margin. Hind tibiae arched outwards in profile, with 7 spines on inner

and outer margin, 2 spines on hind margin. Apical spines – black-tipped, 2 on inner margin 1 ½ times tibial width in length, 2 on outer margin shorter, upper 2 apical spines on hind margin ⅔ tibial width in length, lower 2 on hind margin minute, thin. Hind femur abnormally large and long, denticulate on upper and lower margin. Tibiae as long as femora, uniform light brown, knees of hind femur light brown. Genicular lobes spined on outer margin (middle femur), inner and outer margin (hind femur) with sharp, robust spinelet. ♀ fore legs yellow, femur dark brown mottled on flank, tibia uniform yellow, middle legs missing, ♂♀ hind femur - very robust, greatly dilated at base, golden brown, top margin dark brown, chevron markings on flank darker, knees lighter, inner and outer genicular lobes spined. Tibia as long as femur, 7 spines on inner and outer margin, 2 on hind margin. Hind femur denticulate on bottom margin, top margin less denticulate.

Genitalia: ♂ subgenital plate trapezoidal with styli at apices, paraprocts with black styli, cerci long, dark brown, conical, slightly curved to outside. ♀ subgenital plate trapezoidal, fold in centre with clear dark brown longitudinal band, ovipositor long, up-curved, slender, slightly broadened at base, cerci long, conical, pale.

Head: Vertex and frons uniform pale yellow, fastigium dark, 3 ocelli yellow. ♂ without indication of tusks or bumps on mandibles (♂ clearly adult). ♀: occiput yellow brown, brown vein-like lines run from eyes and upper fastigium corners to pronotum, fastigium and eyes dark brown,

below each eye dark C-shaped patch facing genae, from median ocellus pale band reaching to frontoclypeal suture, rest of face yellow brown.

Thorax & Abdomen: Body ferruginous, fore and hind margins of pronotum black (band on hind margin broader than one on fore margin, both diffuse into yellow-brown of rest of pronotum), hind margins of tergites 1-3 with black band posteriorly, rest without black bands, last tergite indented in centre on posterior margin appearing bilobed. Pronotum mottled with yellow, lower margins of flanks yellow. Meta- and mesobasisternum consist of 2 sharp, light brown triangles. Sternites uniform dark brown with thin, darker brown band on hind margin. ♀ venter yellow-brown.

♂: BL 38.30 mm, HW 9.11 mm, HL 12.23 mm, HFL 31.05 mm, HFW 9.00 mm.

♀: BL 38.27 mm, HFL 22.45 mm, OL 20.1 mm.

Remarks: The species is not well known, rarely encountered in literature and known only from the Northern Cape. Holotype not available for examination. SAMC ♀ face damaged. Fold in ♀ subgenital plate is possibly an artefact of the preservation process. Final instar ♂♂ of *L. vittatus* may show bumps on the mandibles where the tusks will develop. The colouration of the ♀ is the same as the ♂, being only shinier and more metallic. The black band on the fore and hind margin of the pronotum is clear, and is a distinguishing characteristic of *L. impicta*.

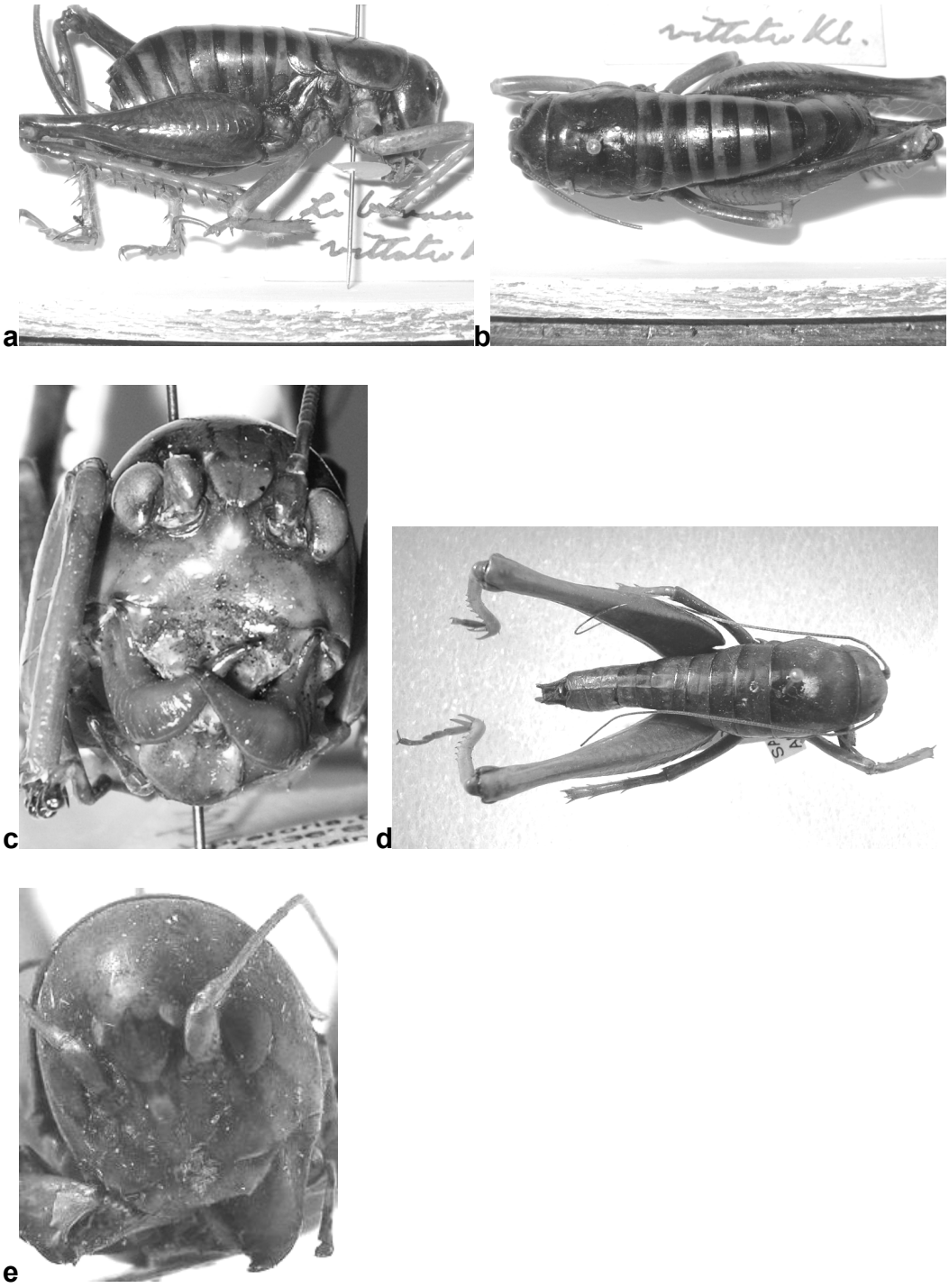


Figure 2.6. *Libanasidus vittatus* holotype from lateral (a) and dorsal (b) views and the sexually dimorphic tusks on male mandibles (c). *Libanasidus impicta* male (d) dorsal view and frontal view (e) showing lack of mandibular tusks.

Key to species of *Libanasidus*

1. Mandibles in both sexes simple. Tergites darkened diffusely along hind margins or only in first 3 or 4 tergites, pronotum with black band on fore and hind margins. Hind femur denticulate above and below beyond middle. In profile: hind tibiae decidedly curved outwards in ♂, straight in ♀, their spines < ½ tibial thickness in length……..*L. impicta* (Stål, 1878)

– ♂ Mandibles with long, up-curved process (tusks) before end, crossing before tips. Pronotum with black cross-band only along hind margin, thoracic and abdominal tergites with well-defined black cross-band along hind margin. Hind femur unarmed, hind tibia straight in ♂, slightly curved in ♀, spines as long as tibia is thick.....*L. vittatus* (Kirby, 1899)

Genus ***Nasidius*** Stål, 1878 (Fig. 2.1g, Fig. 2.7)

Nasidius Stål, 1878: 51. Type species by subsequent designation; authority Karny, 1937: *Nasidius truncatifrons* Stål, 1878.

Dyscapna Brunner von Wattenwyl, 1888: 279. Johns 1997: 129 > *Nasidius*.

Faku Péringuey, 1916: 419. Johns 1997: 129 > *Nasidius*.

Fakua Johns, 1997: 129. Otte *et al*. 2005 > misspelling of *Faku*.

Nasidius truncatifrons Stål, 1878

Nasidius nigrifrons Karny, 1930: 107. Ander 1943: 199 > *Nasidius truncatifrons*.

Material examined: Eastern Cape, Mount Frase, "*Faku nigrifrons*" det. Karny (1♂, SAMC). Prakkloof Farm Ecovq2, G. White, "*Faku nigrifrons*" det. Karny (1♀, SAMC). South Africa "Caffraria", 1878, Stål (♂ holotype, NHRS).

Redescription

Legs: Femora light brown on sides, black on top. ♂, ♀ (SAMC) – tibiae brown, slightly mottled with light brown, knees black, area near knee light brown. Holotype – legs uniform black-brown. Fore tibia, inner margin 1 spine, outer margin unarmed, 8 spines on hind margin, 4 apical spines. Tibia compressed laterally, no tympanal organ visible. Middle tibia bears 2 spines on inner margin, 3 on outer, 8 on hind margin, 4 apical spines. Holotype – hind tibia bears 8 small spines (½ tibial width) on inner margin, 8 similar ones on outer margin, 2 spines on hind margin smaller and thinner. ♂, ♀ (SAMC) – hind tibia with 6 spines on inner and outer margins, 3 on hind margin. Apical spines: two on fore margin and two on side 1 ½ times the width of tibia in length, two larger apical spines on hind margin are ½ the length of those on fore margin, two smallest are ½ as long as tibia is thick. Sides of tibia with longitudinal groove. Fore femur compressed laterally, surface shiny, although ridged. Middle femur similar to fore femur, unarmed, genicular lobes smooth. Hind femur unarmed, base hardly broader than knee, completely black, laterally compressed. Chevron markings on flank clearly pronounced. Prominent length-wise furrow runs below middle of femur. Fore and middle coxae armed with small black spine directed downwards.

Genitalia: ♂ subgenital plate large, trapezoidal, uniform black-brown, bearing 2 small, black, rounded styli on posterior corners. Subgenital plate descends from anterior margin to middle and sides, peaking in centre where it connects with last sternite, from there two lengthwise ‘ridges’ run to apical styli. Paraprocts black, with long, dark styli crossing at tips. Cerci

black, conical, straight. Epiproct large, rounded, shiny. ♀ subgenital plate trapezoidal, dark brown, anteriorly broadened, indented at posterior apex. Ovipositor short, thick basally, sharply pointed, up-curved. Ovipositor base yellow on margins.

Head: Head broader than pronotum. Holotype – black-brown, vertex and mandibles dark ferruginous. ♂ (SAMC) – occiput dark grey-brown. Vertex large, rounded, protruding above pronotum, shiny, punctuated. Eyes teardrop-shaped, dark brown. Fastigium depressed, roughened, punctuated, rounded on vertex. Antennal base normal, dark brown, situated at lower edge of eye, black wrinkled crosswise band runs below eyes and fastigium. Below fastigium – a prominent abrupt elevation to ridge running vertically over frons, forming elevated 'ramp' before frontoclypeal suture, surface declines abruptly towards clypeus. Clypeus as large as labrum, grey in SAMC ♂, rounded. Frontal ridge and genae along frontoclypeal suture punctuated and roughened. Declining area of frontal ridge and clypeus, wrinkled, roughened. Frontal view: frontoclypeal suture slants downwards towards mandibles from ridge. Labrum black on margins, dark brown in centre, smooth, mandibles elongated, slender throughout, curving outwards around labrum leaving gap between labrum and mandibles. Mandible broader at apex, dark brown. Lateral ocelli pale, median ocellus not visible. ♀ – genae dark brown, rest of face black. Post-clypeus with 2 yellow rectangles on lower half, labrum black on margins, dark brown in centre. Median ocellus clear. Fastigium indented, fastigium and frons punctuated, ridge on frons visible in profile, not as pronounced as in ♂. ♀ occiput not enlarged.

Thorax & Abdomen: Body uniform black-brown, ♂♂ darker than ♀. Pronotum faintly wrinkled along fore and hind margin. ♀: black, above with mottled yellow area (butterfly-shaped). Holotype – tergites with elevated roughened band above along posterior margin and on flank below. ♂, ♀ (SAMC) – bottom margin of flanks with small yellow blotch, tergites smooth, shiny without elevated roughened band, diffusely sprinkled with yellow in ♀. Last 2 tergites triangular, pointed posteriorly, furrow running along centre in SAMC ♂. Meso-and metabasisternum black, 2 spiked, pointing to coxae, spikes of mesobasisternum sharp, longer. Sternites dark brown to black, slightly punctured, shiny, faint black band visible on posterior margins. SAMC ♂ sternites black with faint yellow spots on sides, creating longitudinal row of yellow spots on ventral abdomen. ♀ sternites sprinkled with yellow on black, shiny.

Holotype: BL 29.63 mm, HW 10.01 mm, HL 16.76 mm, HFL 20.23 mm, HFW 4.86 mm.

Other: BL 24-30 mm, HL 17-19 mm, ♂HFL 15 mm, OL 10 mm.

Remarks: This genus currently contains 14 species, occurring in open grassland and indigenous forest. Holotype in very good condition. With the holotype, the fore tibia on the right hand side bears 2 spines on the fore inner margin, while the left bears only 1 spine. The presence of 2 fore tibial spines on one side of the body and only 1 on the other in some specimens leads to confusion in Karny's (1929) generic identification key (see Ander 1943). This condition could be the result of regeneration of a lost limb,

resulting in 2 spines on the fore margin, instead of the normal 1 (see McDonald & Hanrahan 1993).

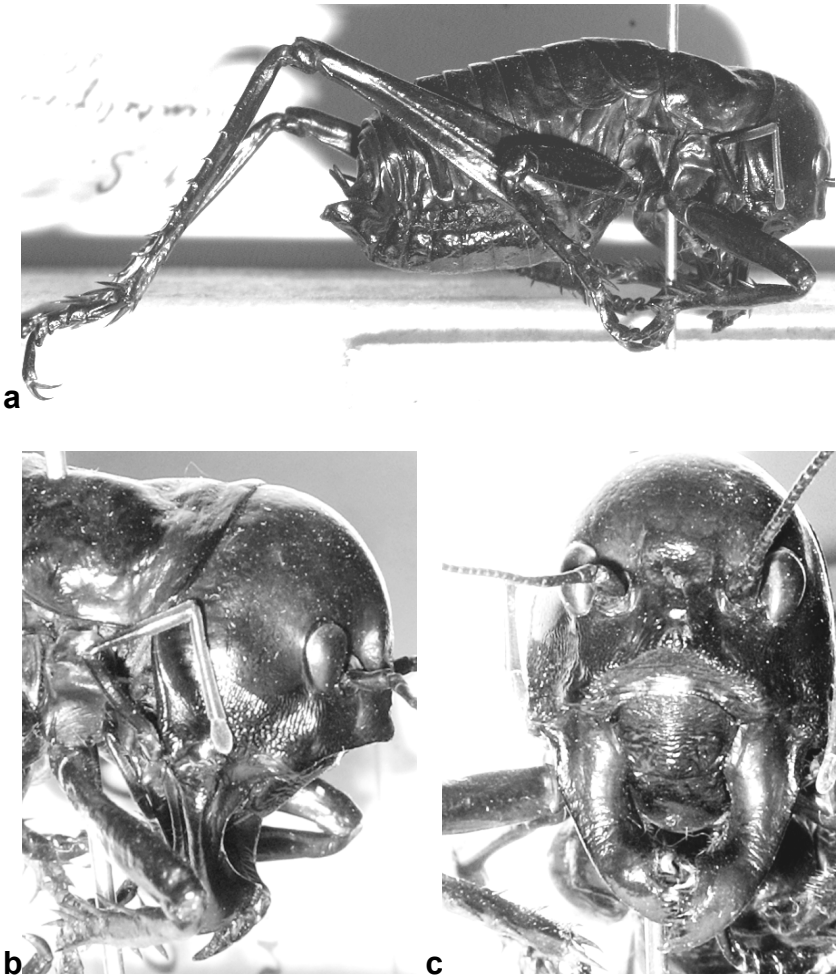


Figure 2.7. *Nasidius truncatifrons* holotype lateral view (a) and frontal: profile (b), frontal view (c).

Genus ***Onosandridus*** Péringuey, 1916 (Fig. 2.1e, Fig. 2.8)

Onosandridus Péringuey, 1916: 421. Type species by subsequent designation: authority Karny, 1937: *Onosandridus deceptor* (Péringuey, 1916).

Onosandridus deceptor (Péringuey, 1916)

Originally described as *Onosandrus deceptor* Péringuey, 1916. Karny 1937: 208 > *Onosandridus deceptor*.

Material examined: Zimbabwe, Umtali (=Mutare), 1918, Péringuey (♀ holotype, SAMC).

Redescription

Legs: Fore tibia's inner margin with 2 spines, unarmed on outer, hind margin with 8 spines, 6 apical spines, tibia keeled along side. Tympanal area indented, not perforated. Middle tibia with 2 spines on inner margin, 3 on fore outer, hind margin with 8 spines, 4 apical spines. Hind tibia with broadened centre in profile, front surface bears 13 large (as long as the tibia is thick) and 2 small spines, hind surface bears 3 small spines (½ as long as tibia is thick) - lower one longer. 6 apical spines: 2 on inner fore and side margin twice as long as tibial diameter, 2 spines on outer fore and side margin as long as tibia is thick, upper 2 spines on hind margin as long as tibia is thick, lower 2 apical spines on hind margin smaller. Fore femur laterally compressed with impressed lines on outer flank, unarmed. Middle femur compressed in middle, broader at knee, unarmed. Hind femur base thickened, strongly attenuated towards knee, chevron ridges sculptured upper half of femur base with longitudinal keel running below, longitudinal sulcus on top margin, unarmed. Fore and middle coxae with sharp short spike pointing forwards, genicular lobes unspined. Legs dark, mottled with yellow brown, hind femur and knees light brown.

Genitalia: Ovipositor base broad, pointed, up-curved, outer valves covering inner valves, subgenital plate large, triangular, dark ferruginous, cerci moderately long, yellow.

Head: Vertex dark, sides of face, eye area, below fastigium to post-clypeus – yellow, pre-clypeus dark brown, labrum yellow colour, mandibles dark ferruginous, strong with triangular yellow indented region on sides, dente black. Fastigium raised, rounded, 3 small white ocelli on corners, fastigium separated from raised bar on frons reaching to post-clypeus, by suture.

Thorax & Abdomen: Head paler than pronotum, abdomen dark brown - tergites 1-2 with pale band at apical margin (visible as spot only on flank of second tergite). Pronotum ferruginous; mottled with yellow (moulting suture visible). Meta- and mesobasisternum with 2 outwardly pointing spikes, sternites yellow with dark ferruginous bands posteriorly to uniform ferruginous.

Holotype: BL 23.87 mm, HW 7.74 mm, HL 11.69 mm, HFL 17.13 mm, HFW 5.41 mm and OL 13.85 mm.

Remarks: Six other species belong to this genus. The biology of the species is not known, but Péringuey (1918) suggested that they occur in a habitat similar to *Onosandrus*. The holotype is generally in good condition, with only one cercus, both antennae and the maxillary palps damaged.

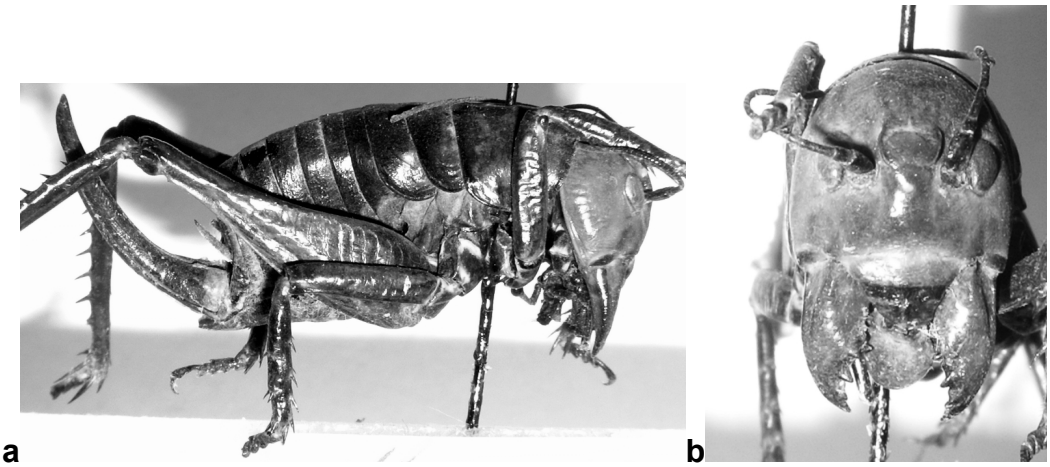


Figure 2.8. *Onosandridus deceptor* holotype lateral (a) and frontal (b) views.

Genus ***Onosandrus*** Stål, 1878 (Fig. 2.1f, Fig. 2.9)

Onosandrus Stål, 1878: 51. Type species by monotypy: *Onosandrus fasciatus* Stål, 1878.

Onosandrus fasciatus Stål, 1878

Onosandrus femoralis, Karny, 1930: 166. Otte *et al.* 2005 > *Onosandrus fasciatus*.

Material examined: South Africa "Caffraria", Stål, 1878 (♀ holotype, NHRS).

Redescription

Legs: uniform pale yellow. Fore tibia with 1 spine on inner margin, none on outer, hind margin with 8 spines, 4 apical spines. No tympanum visible. Middle tibia with 2 spines on inner margin, 3 on outer, hind margin with 8 spines, 4 apical spines. Hind tibia with 7 spines on inner and outer margins – ½ the length of tibial width, hind margin with 3 small spines, 4 apical spines on inner and outer margin 1 ½ times width of tibia in length, upper 2 on hind margin shorter, lower 2 on hind margin hardly visible. Fore femur as long as tibia, laterally compressed, groove visible on outer flank.

Middle femur unarmed, laterally compressed, keeled on outer side, inner and outer genicular lobes armed with small spine. Hind femur base greatly dilated, laterally compressed, bottom margin bluntly denticulate, outer genicular lobe with small spine, inner smooth, longitudinal groove runs below middle, chevron markings clear, top margin at base darkened – fading to yellow towards middle of femur, knee pale. Fore coxa armed with forward pointing yellow spine, middle coxa with yellow outward pointing spine.

Genitalia: ♀: subgenital plate trapezoidal, light brown, apex cupped to cover ovipositor, appearing rounded, ovipositor sword shaped, up-curved, tip dark brown, ¾ of hind femur length. Cerci conical, straight, pale yellow.

Head: simple, not broader than pronotum, vertex light brown, rest of facial area pale yellow, dark brown fastigium rounded, indented, punctuated. Below fastigium runs an elevated vertical ridge to frontoclypeal suture (ridge is more elevated than fastigium ascending from area between eyes). Flanking to ridge are 2 indented oval areas below antennal base, reaching to frontoclypeal suture, area towards gena wrinkled. Eyes teardrop-shaped, pale yellow, antennal scape oval parallel with eyes, ocelli not visible. Clypeus and labrum pale yellow, constricted in-between. Pre- and post-clypeus separated by thin brown horizontal line, anterior tentorial pits prominently dark. Mandibles pale yellow, normal, dente black, triangular indented area on base of mandible paler.

Thorax & Abdomen: Body arched in profile, shiny, light brown, no sculpturing, pronotum banded with dark brown on fore and hind margin, usual vertical keel visible on pale flanks of pronotum. Tergites banded on posterior margin with dark brown, last two uniform pale yellow, smooth - last three slightly wrinkled on posterior margin. Meso- and metabasisternum pale yellow, with 2 black triangles pointing outwards. Sternites pale yellow, smooth, 1-5 has irregular black spot in centre, last two sternites with irregular black band on posterior margin.

Holotype: BL 20.98 mm, HW 5.72 mm, HL 9.07 mm, HFL 16.43 mm, HFW 5.49 mm, OL 10.23 mm.

Remarks: This genus comprises nine species. Species are abundant in indigenous forests of the Mpumalanga, KwaZulu-Natal and Limpopo provinces.

a **b**

Figure 2.9. *Onosandrus fasciatus* holotype lateral (a) and frontal (b) views.

ACKNOWLEDGEMENTS

The authors wish to thank the following curators and collections who loaned material for study: Dr. Kjell Arne Johansson (NHRS); Prof. Ulrike Aspöck (NMW); Simon van Noort & Margie Cochrane (SAMC); Barbara Dombrowsky (TM); Dr. George Beccaloni (BMNH) and Dr. Peter J. Schwendinger (MHNG).

REFERENCES

Ander, K. 1943. Uber die von Stål beschriebenen Stenopelmatiden (Saltatoria). *Entomologisk Tidskrift* **64**: 198-201.

Brunner von Wattenwyl, C. 1888. Monographie der Stenopelmatiden und Gryllacriden. *Verhandlungen der Kaiserlich Koniglichen Zoologisch-Botanischen Gesellschaft in Wien* **38**: 247-394.

Chopard, L. 1943. Two new species of Gryllacridoidea from southern Africa. *Entomologisk Tidskrift* **14**: 1446-452.

Gorochov, A.V. 2001. The higher classification, phylogeny and evolution of the superfamily Stenopelmatoidea. In: *The biology of the Wetas, King Crickets and their allies* (Ed) Field, L.H. Pp 3-33. Cabi publishing, Wallingford UK.

Gray, G.R. 1837. Description of some singularly formed orthopterous insects. *Annals and Magazine of Natural History, London* **2**: 141-145.

Johns, P.M. 1997. The Gondwanaland Weta: Family Anostostomatidae (formerly in Stenopelmatidae, Henicidae or Mimnermidae): Nomenclatural problems, world checklist, new genera and species. *Journal of Orthoptera Research* **6**: 125-138.

Karny, H.H. 1930. A revision of the South African Gryllacrididae (Orthoptera: Saltatoria). *Annals of the South African Museum* **29**: 138-45.

Karny, H.H. 1931. On the geographical distribution of the Indo-African and Mediterranean Gryllacrids. *Transactions of the Royal Society of South Africa* **19**: 99-105.

Karny, H.H. 1932. Uber zwei Brunnersche Gryllacrididen-typen im Stettiner Museum (Orthoptera). *Stettiner Entomologische Zeitung* **93**: 147-151.

Karny, H.H. 1937. Orthoptera Family Gryllacrididae Subfamily Omnes In: *Genera Insectorum de Wytsman, P.* 206 pp 1-317. Bruxelles: Desmet-Verteneuil.

Kevan, D.K.McE. 1982. Orthoptera In: *Synopsis and Classification of Living Organisms* (Ed.) Parker, S.P. pp 352-355. McGraw-Hill Book Company, Inc. New York.

Kirby, W.F. 1906. *Synoptic Catalogue of Orthoptera (Orthoptera Saltatoria, Locustidae vel Acridiidae)*. British Museum, London.

Kirby, W.F. 1899. Notes on the collection of Gryllidae, Stenopelmatidae, Grylacrididae and Hetrodidae formed by Mr. W.L. Distant in the Transvaal and other South- and East-African Localities. *Annals and Magazine of Natural History* **3**: 33-35, 478-481.

McDonald, I.R. & Hanrahan, S.A. 1993. Aspects of foraging and behaviour of the king cricket, *Libanasidus vittatus* (Kirby) (Orthoptera: Mimnermidae). *South African Journal of Science* **89**:354-357.

Otte, D., Eades, D.C. & Naskrecki, P. 2005. *Orthoptera Species File Online*. Version 2.2. <http://osf2.orthoptera.org>.

Péringuey, L. 1916. Descriptions of new or little known Orthoptera: Family Locustidae, Sub-Fam. Stenopelmatinae. *Annals of the South African Museum* **15**: 414-425.

Picker, M., Griffiths, C. & Weaving A. 2002. *Field guide to insects of southern Africa*. Struik publishers. South Africa.

Rentz D. C. F. & Weissman, D. B. 1973. The origins and affinities of the Orthoptera of the Channel Islands and adjacent mainland California. *Proceedings of the Academy of Sciences Philadelphia* **125**: 94.

Stål. C. 1878. Systema Acridiodeorum. Essai d'une Systématisation des Acridiodées. *Bihang till Kungliga Svenska Vetenskaps-Akademiens Handlingar.* **5**:1-100.

Toms, R.B. 2001. South African King Crickets (Anostostomatidae) In: *The biology of the Wetas, King Crickets and their Allies*. (Ed) Field, L.H. pp 73-78. Cabi publishing, Wallingford UK.

Walker, F. 1869. *Catalogue of the Specimens of Dermaptera Saltatoria in the Collection of the British Museum.* **1**:208.

Wolf, S. Brettschneider, H. and Bateman, P.W. 2006. The predator defence system of an African King Cricket (Orthoptera; Anostostomatidae): does it help to stink? *African Zoology* **41**:75-80.

Appendix I Geographic coordinates to collection localities of all Anostostomatids studied.

Locality	**Coordinates**
Eastern Cape, Mount Frase	No coordinates available
Free State, Smithfield	30.21°S 26.53°E
Gauteng, Johannesburg	26°12'S 28°02'E
Gauteng, Pretoria	25°12'S 28°13'E
Limpopo, Entabeni	22°15'S 30°00'E
Limpopo, Malta Forest	24°15'S 30°15'E
Limpopo, Moordrift	23°30'S 27°30'E
Limpopo, Woodbush	23°45'S 30°00'E
Mpumalanga, Badplaas Mondi Forest	25°43'S 30°39'E
Mpumalanga, Barberton	25°37'S 31°03'E
Northern cape, Kimberly	28°44'S 24°46'E
Prakkloof Farm 'Ecovq2'	No coordinates available
South Africa "Caffraria"	No coordinates available
Western Cape, Cape Town	33°55'S 18°25'E
Western Cape, George	33°57'S 22°28'E
Western Cape, Gordon's Bay	34°10'S 18°52'E
Western Cape, Jonkersberg	33°55'S 22°13'E
Western Cape, Knysna	34°01'S 23°01'E
Western Cape, Marloth Nature Reserve (Duiwelsbos & Koloniebos)	33°59'S 20°27'E
Western Cape, Oakley, Newlands	33°58'S 18°28'E
Western Cape, Oudebosch, Caledon	33°58'S 21°04'E
Western Cape, Paarl	33°42'S 18°58'E
Western Cape, Rondebosch	33°58'S 18°28'E
Western Cape, Somerset West, Morning Star	34°03'S 18°50'E
Western Cape, Stellenbosch	33°56'S 18°51'E
Western Cape, Swellendam	34°01'S 20°25'E
Western Cape, Tradouw pass, Swellendam	34°01'S 20°25'E
Western Cape, Zonde einde	24°49'S 27°21'E
Zimbabwe, Umtali (=Mutare)	18°58'S 32°40'E

CHAPTER 3

A morphological and molecular approach to resolving the phylogeny and classification of southern African anostostomatids (Orthoptera: Ensifera)

H. Brettschneider, C.T. Chimimba, A.D.S. Bastos, C.H. Scholtz and P.W. Bateman

Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002 South Africa

A morphological and molecular approach to resolving the phylogeny and classification of southern African anostostomatids (Orthoptera: Ensifera)

H. Brettschneider, C.T. Chimimba, A.D.S. Bastos, C.H. Scholtz and P.W. Bateman

Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002 South Africa

ABSTRACT Thirty-six morphological attributes characteristic of the eight southern African anostostomatid genera were selected for a cladistic morphological analysis. A dataset comprising 264 individuals and inclusive of type specimens for each species revealed no morphological support for the eight presently recognized southern African anostostomatid genera. High levels of homoplasy and possible incorrect species identification resulted in character ambiguity within genera, rendering many of the diagnostic characters of this group ineffective for resolving generic relationships using morphological data. Nucleotide sequences corresponding to the large ribosomal subunit (16S) were generated for 18 individuals representative of six of the eight known genera. Phylogenetic analysis resulted in a highly resolved and well-supported tree the results of which confirm the ancestral nature of *Bochus* and *Borborothis* to most other genera within the Anostostomatini, as well as the placement of *Libanasa* within a separate tribe, the Lutosini. The recent merger of the genus *Platysiagon* with *Libanasa* is also provisionally supported. A close association was obtained between *Libanasidus* and *Nasidius*, with *Onosandrus* being more closely related to *Bochus* and *Borborothis*. The latter association

corresponds well with the lack of sexual dimorphism in *Onosandrus* and *Borborothis*. The phylogenetic position of the genera *Henicus* and *Onosandridus* remain unresolved due to a lack of available molecular data. It is suggested that the designation of six of the eight anostostomatid genera in southern Africa are valid, but that species placement within these genera requires revision and further investigation.

KEY WORDS Anostostomatidae, molecular phylogeny, cladistic morphology, 16S gene, southern Africa, systematics, taxonomy

INTRODUCTION

The insects commonly known as King crickets or Weta (Orthoptera: Anostostomatidae) are large (body length up to 40 mm), mainly flightless crickets that radiated greatly in the southern hemisphere after the split of Gondwanaland (Field 2001). Nocturnal behaviour within the group of 'monstrous' crickets is the norm, which has possibly contributed to the lack of available biological information on them (Field 2001). They are omnivorous, feeding on plant and decaying material and the species prevalent in suburban gardens in Johannesburg, South Africa are famed for wandering into houses at night (Toms 1985). Specific to southern Africa, eight anostostomatid genera have been identified since the early 1800s, encompassing 51 species (Otte *et al*. 2005). These include, from within the Anostostomatini, the genera *Bochus* Péringuey, 1916, *Borborothis* Brunner von Wattenwyl, 1888, *Henicus* Gray, 1837, *Libanasidus* Péringuey, 1916, *Nasidius* Stål, 1878, *Onosandrus* Stål, 1878, *Onosandridus* Péringuey, 1916 and from within the Lutosini, the genus *Libanasa* Walker, 1869.

Within this geographic range, anostostomatids inhabit a variety of habitats where they live in self-constructed burrows or rotten wood (Toms 2001, Picker *et al.* 2002) (Figure 3.1). *Bochus* is a monotypic genus known only from the Free State Province in South Africa, while the two *Borborothis* species can be found in the southern mountainous regions of South Africa (Toms 2001; Karny 1929). *Henicus* and *Libanasidus*, with nine and two species respectively, are the more familiar genera in South

Africa due to their prevalence in suburbia and their frequent encounters with people. Males of both genera have highly modified mandibles, used for stridulation and for plugging burrow entrances in the former, and for male-male conflict in the latter (Toms 2001). *Henicus* occurs in mountainous parts of the Cape region, and *Libanasidus vittatus* occurs naturally in the indigenous and coastal forests of eastern South Africa, probably extending into Zimbabwe (Chapter 2). The second species in this genus, *L. impicta,* is known conclusively only from drier areas in the Northern Cape Province (Johns 1997). The six *Libanasa* species have been found in drier thornveld areas in Mpumalanga Province, as well as in rotting logs in indigenous forest along the coast and in KwaZulu-Natal Province (Toms 2001). The fourteen species of *Nasidius* are distributed in open grassland and indigenous forest areas in South Africa. The biology of the seven *Onosandridus* species is not known, but Péringuey (1916) proposed that they occur in a habitat similar to *Onosandrus*, which contains nine species abundant in the indigenous forests of the Mpumalanga, KwaZulu-Natal and Limpopo Provinces.

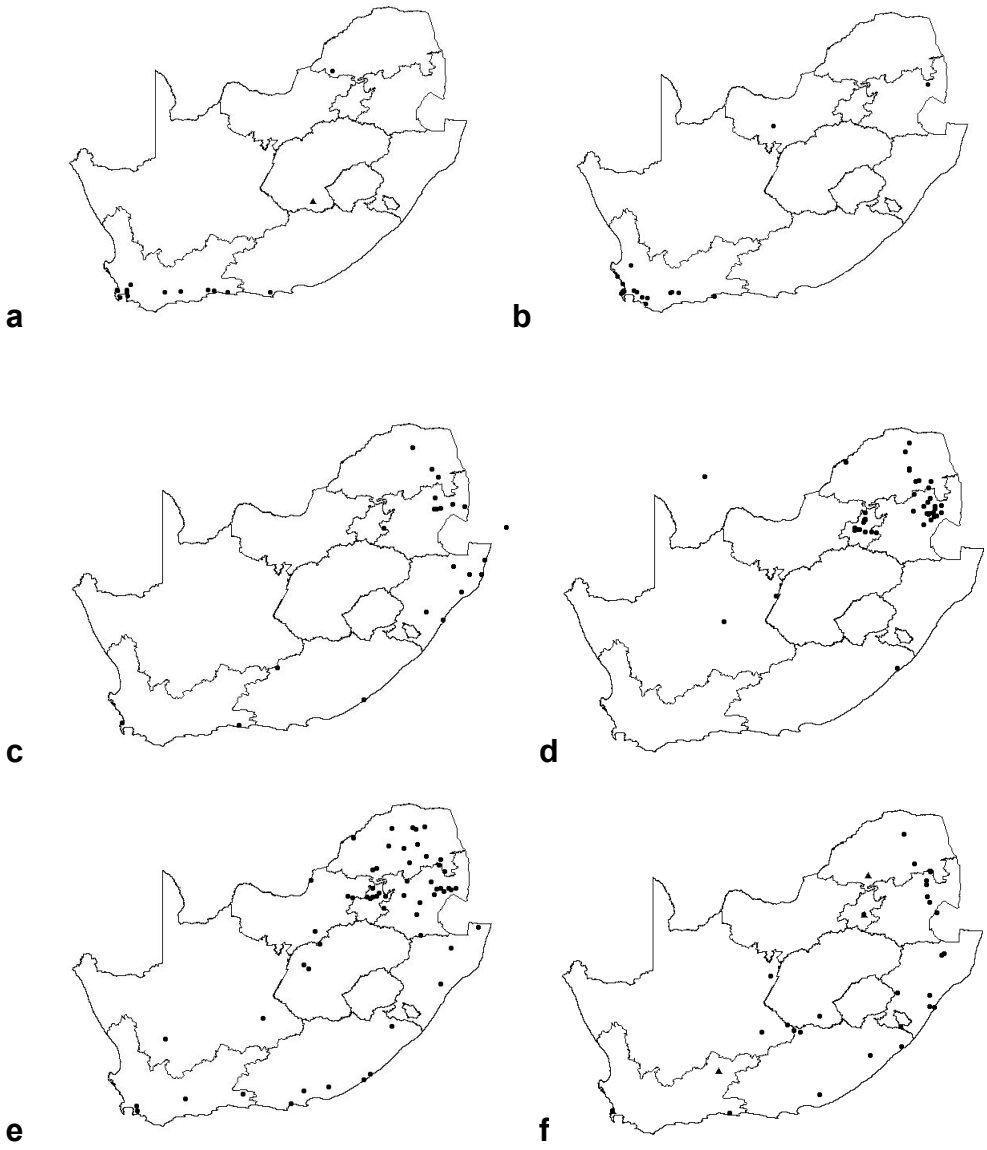


Figure 3.1. Known South African geographic distributions of the genera *Bochus* (▲) and *Borborothis* (●) (a), *Henicus* (b), *Libanasa* (c), *Libanasidus* (d), *Nasidius* (e), *Onosandridus* (▲) and *Onosandrus* (●) (f).

Sexual dimorphism is prevalent in five of the eight genera (*Bochus*, *Henicus*, *Libanasa*, *Libanasidus* and *Nasidius*), in the form of grossly enlarged mandibles and facial features of males, which they can use in male-male conflict (H. Brettschneider pers. obs.). The African species also show fascinating defence mechanisms, including abdominal-femoral stridulation and squirting of foul-smelling faeces at offenders, which is absent in related taxa from New Zealand (Wolf *et al.* 2006).

Historically, anostostomatid taxa were variously placed in different families and subfamilies including the Mimnermidae Stål (1878), the Gryllacrididae Stål 1876, the Deinacridinae Karny 1932 and the Henicinae Karny 1928. Johns (1997) conducted the most recent taxonomic study of the group, paying attention to species diversity within the family and combining the genus *Platysiagon* Brunner von Wattenwyl, 1888 with the genus *Libanasa*. Prior to 1997, studies focussed on higher taxonomic ranks and their relationships, except for Ander (1943) who revised some nomenclatural disparities and synonymies in anostostomatids and sister taxa. Ander (1943) specifically dealt with the genus *Nasidius* and consequently synonymised the genus *Faku* Péringuey, 1916 with *Nasidius*. Lastly, Ander (1943) also considered misidentifications of species within the genus *Henicus*.

Phylogenetic studies at superfamilial level were initiated in the 1930s, with many being either too simple or too complex (Gorochov 2001). The most significant thus far are the works of Sharov (1968) and Gorochov (2001). Currently, phylogenetic relationships between the Anostostomatidae and its two sister taxa, the Rhaphidophoridae and the Stenopelmatidae, appear to be resolved (Gorochov 2001). A phylogenetic study focussing on the lower taxa, however, is critically needed. Similarly, relationships between the southern African genera are uncertain. Although it has been speculated that the *Bochus* and *Borborothis* genera are primitive due to the lack of sexual dimorphism (Toms 2001), a recent

review (Chapter 2) suggests that males of *Bochus* do have modified mandibles compared to females.

This study will attempt to resolve the phylogeny of the eight recognised southern African anostostomatids using molecular and cladistic morphological techniques.

MATERIALS AND METHODS

Morphology

A comparative analysis (Chapter 2) and review of the original descriptive literature allowed the identification of 36 characteristic morphological attributes of the southern African anostostomatid genera (Appendix I), and were selected for the morphological analysis. The eight genera delineated in the revised key (Chapter 2) represented members of the ingroup. Specimens examined comprised 264 individuals, representative of all eight genera, housed in the insect collections of the Transvaal Museum, Pretoria, South Africa (TM), the South African Museum, Cape Town, South Africa (SAMC), and specimens privately collected (deposited in TM and National insect collection, Pretoria, South Africa (NCI)) (Appendix II). In addition, the specimens of each type species were included in the analysis (Chapter 2). All other specimens were identified to generic level only due to lack of adequate specific identification keys being available. Numbers of individuals for each genus are as follows: *Bochus* 4, *Borborothis* 8, *Henicus* 18, *Libanasa* 54, *Libanasidus* 22, *Nasidius* 64, *Onosandridus* 10 and *Onosandrus* 92.

Character states were recorded from all available adult specimens (Chapter 4) (Appendix III). Outgroup characters were scored as 0 using the Chilean red cricket (*Cratomelus armatus* Blanchard, 1851, Anostostomatidae), while missing characters were coded 9. Sexually dimorphic characters were included in the analysis due to the importance of these traits in classification, which justified the separate analysis of male and female data. This resulted in four subsets of data, organised as follows: Male specimens only, female specimens only, males and females combined (sexual characteristics excluded) and adult male and female individuals that formed part of the molecular study (sexual characteristics excluded).

These matrices (Appendix III) were subjected to cladistic analysis using PAUP* version 4.0b10 (Swofford 1999), employing parsimony methods (MP) with branch and bound searches, based on parsimony informative characters only. All characters were treated as unordered with equal weights and 200 bootstrap replicates were used to estimate confidence intervals (Page & Holmes 1998). Character optimisation by accelerated (ACCTRAN) and delayed (DELTRAN) transformation options in PAUP* was also explored to improve retrieval of the most parsimonious tree.

Genetics

Where possible, four specimens from six genera were characterised genetically by sequencing part of the mitochondrial large ribosomal

subunit (16S). Due to difficulty of identifying specimens to specific level, individuals were classified to generic level only, and numbered sequentially. Museum material from some specimens proved degraded and difficult to sequence, resulting in the following 18 ingroup individuals distributed as follows: *Bochus* (1), *Borborothis* (1), *Libanasa* (4), *Libanasidus* (4), *Nasidius* (4), and *Onosandrus* (4). No genetic material was available from the *Henicus* and *Onosandridus* genera. DNA extraction from muscle tissue of the hind femora of fresh specimens followed the Roche extraction protocol (Roche Diagnostics, Mannheim, Germany). DNA from museum material was extracted from the tarsus of one leg, using either the Roche extraction protocol or the salting-out method, as previously implemented successfully on museum specimens (Sunnucks & Hales 1996; Trewick 2000). The primer 16Sb2 was used to amplify the short (531 bp) 16S fragment, and the reverse LRN13398 was only employed with museum samples (Simon *et al*. 1994). Sequences were determined using an automated cycle-sequencing approach employing the BigDye 3.1 Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, USA).

Sequences were viewed and edited with BioEdit version 5.0.6 (Hall 1999) and aligned in ClustalX (Thompson 1997). Aligned sequences were subsequently manually adjusted with reference to *Locusta migratoria* (Orthoptera, Acrididae) (GenBank accession no. NC_001712). The predicted 16S secondary structure was determined with *mfold* (Zuker 2003) and viewed with RNAdraw version b2 (Matzura & Wennborg 1996). Aligned sequences were used to infer a phylogeny in PAUP* version

4.0b10 (Swofford 1999) using maximum parsimony (MP) and maximum likelihood (ML) analyses and MEGA version 3 (Kumar *et al.* 2004) to generate neighbor-joining (clustering) and minimum evolution (optimality) trees. The optimal model of evolution was selected as TRN+G with gamma parameter α = 0.4246 under the Akaike Information Criterion (AIC) in Modeltest version 3.7 (Posada & Crandall 1998). Confidence intervals were assessed by bootstrap resampling (Felsenstein 1985). Bayesian analysis of the same data set was performed in MrBayes version 3.1 (Huelsenbeck & Ronquist 2001). The outgroup used for all phylogenetic analysis is the Chilean red cricket (*Cratomelus armatus* Blanchard, 1851). All sequences were deposited in GenBank and can be obtained under the accession numbers DQ314841 - DQ314859.

RESULTS

Morphology

Cladistic characters analysed for the four separate morphological data sets did not prove useful in resolving relationships between ingroup taxa. All data sets revealed the presence of a single clade representing a polytomy that included all individuals. Due to the identical unresolved topology, only the tree for the combined dataset is illustrated in Figure 3.2. The single most parsimonious tree obtained for each of the three datasets revealed the following tree statistics: Combined dataset TL = 79, CI = 0.59; Male-only dataset TL = 93, CI = 0.61; Female-only dataset TL = 84, CI = 0.58; Genetically-characterised individuals-only dataset TL = 62, CI = 0.47. Bootstrap support was very poor, and did not exceed 50%.

Character optimisation provided no further resolution in the trees obtained for each of the four data sets.

Characters 5, 6, 8 and 9 were constant in all datasets, while C7, 10 and 11 were constant in the sequenced dataset. The measure of fit of a character to the tree can be assessed by CI levels, where high levels indicate a good fit and less homoplasy (Forey *et al.* 1992). In this case C3, C7, C10, C11, C12, C23 and C37 for all datasets, while C18, C19, C25 for males, C31 for females and sequenced individuals showed high CI values (>0.7). C32, C34, C36 and C39 showed high CI values for all datasets except the sequenced individuals (Table 3.1). All other characters showed high homoplasy (low CI) levels.

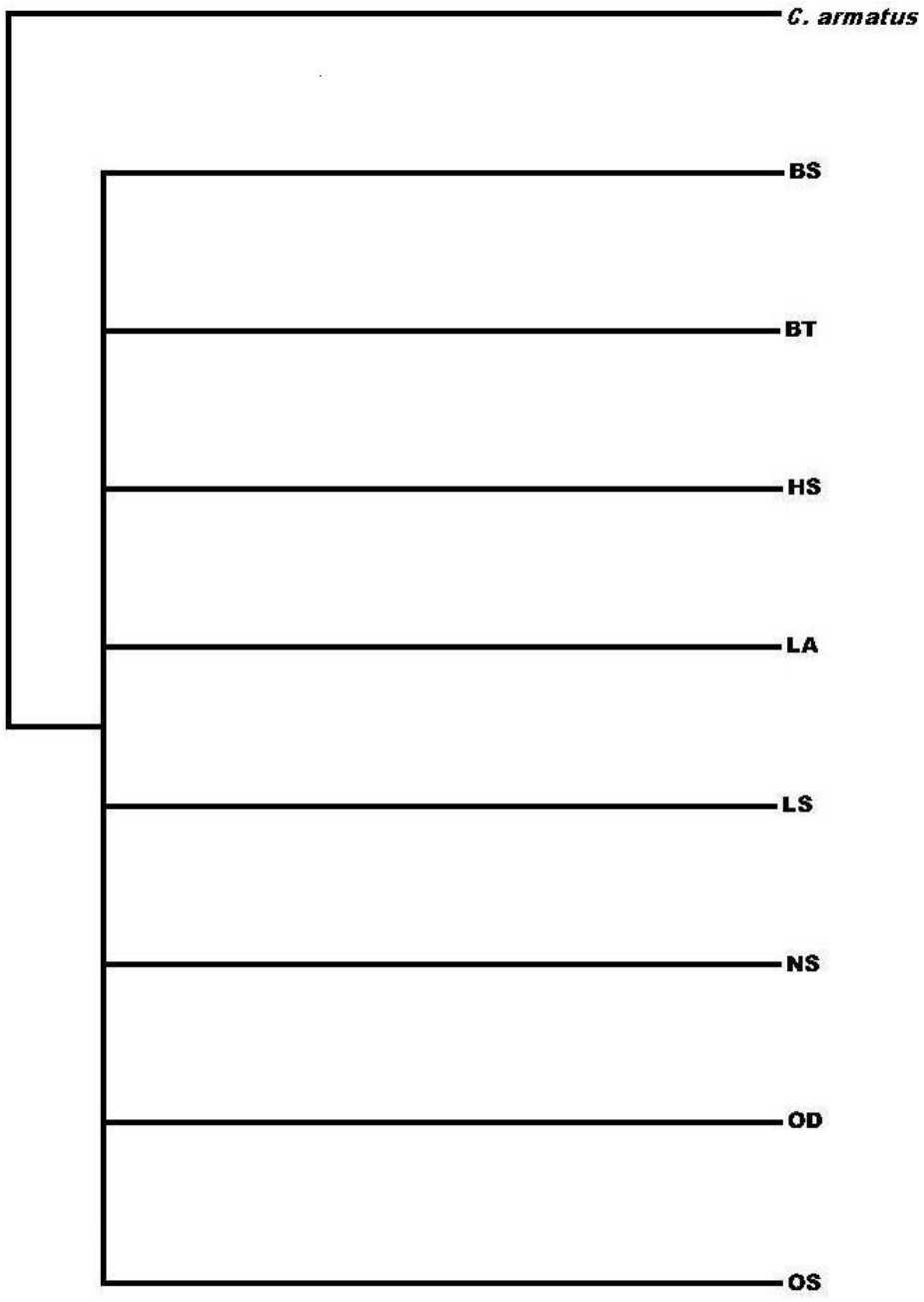


Figure 3.2. Most parsimonious tree obtained with PAUP* from morphological cladistic analyses of eight anostostomatid genera showing results from the combined dataset including males and females (TL=79, CI=0.59). BS = *Bochus*, BT = *Borborothis*, HS = *Henicus*, LA = *Libanasa*, LS = *Libanasidus*, NS = *Nasidius*, OD = *Onosandridus*, OS = *Onosandrus*.

Table 3.1. Consistency indices (CI) for morphological characters analysed for each of three datasets where Comb = the combined male and female dataset, Mal = Males, Fem = females and Seq. = sequenced individuals. Characters that were not parsimony informative are indicated by -.

	Character	Comb.	Mal.	Fem.	Seq.
C1	Head width	0.50	0.50	0.50	0.20
C2	Hind femur base width	0.40	0.40	0.40	0.40
C3	Femoral compression	1.00	1.00	1.00	1.00
C4	Fore tibial spines: fore internal	0.50	0.50	0.50	0.50
C7	Middle tibial spines: fore internal	1.00	1.00	1.00	-
C10	Hind tibial spines: fore internal	1.00	1.00	1.00	-
C11	Hind tibial spines: fore external	1.00	1.00	1.00	-
C12	Hind tibial spines: hind	0.75	0.75	0.75	0.40
C13	Middle genicular lobes armature	0.25	0.25	0.25	0.50
C14	Hind genicular lobes armature	0.60	0.60	0.60	0.40
C15	Tergite texture	0.67	0.67	0.67	0.50
C16	Head & pronotal texture	0.67	0.67	0.67	0.25
C17	Mandibular-labral gap	-	0.33	-	-
C18	Mandible armature	-	0.80	-	-
C19	Facial armature	-	1.00	-	-
C20	Ovipositor curvature	-	-	-	-
C21	Body profile	0.50	0.50	0.50	-
C22	Hind femur armature	0.60	0.60	0.60	1.00
C23	Middle femur armature	1.00	1.00	1.00	-
C24	Cercal structure	0.67	0.67	0.67	-
C25	Male subgenital plate shape	-	1.00	-	-
C26	Female subgenital plate shape	-	-	0.25	-
C28	Male genital styli	-	0.67	-	-
C29	Body colour	0.40	0.40	0.40	0.33
C30	Tympanum presence	0.40	0.40	0.40	0.67
C31	Ovipositor length	-	-	1.00	-
C32	White ring on hind femur	1.00	1.00	1.00	0.50
C33	Female subgenital plate notched	0.67	0.67	0.67	0.20
C34	Fastigium elevation	0.67	0.67	0.67	1.00
C36	Clypeus vaulted	0.04	0.40	0.40	1.00
C37	Ovipositor shape	1.00	1.00	1.00	1.00
C38	Stridulatory structure on hind femur	0.50	0.50	0.50	0.60
C39	Stridulatory structure on pleura	1.00	1.00	1.00	0.25

Genetics

Sequence data from the mitochondrial large ribosomal subunit comprised 464 bp, of which 172 sites were variable and 101 phylogenetically informative. As expected from insect mitochondrial sequences, data showed a high A-T bias (70.7 %) compared to the C and G composition, which was 18.1% and 11.2%, respectively. The transition transversion ratio was biased to transversions with R = 0.6.

Phylogenetic reconstruction of relationships between six of the eight anostostomatid genera in South Africa using distance, cladistic and Bayesian methods revealed identical tree topologies. The single most parsimonious tree obtained via a maximum parsimony search in PAUP*, along with bootstrap support values from four different analysis methods, and Bayesian confidence estimates is presented in Figure 3.3. Three clades (I-III) were consistently recovered comprising primarily of (I) *Nasidius* and *Libanasidus*, (II) *Onosandrus*, *Borborothis* and *Bochus* and (III) *Libanasa*. The placement of *Libanasa* species was variable with individuals (LA1 & 2) clustering within clades I and II, in addition to the *Libanasa*-specific clade (III).

Clade I revealed the presence of two well-supported lineages, of which one comprised several *Libanasidus* specimens and a single *Libanasa* individual (94-95 % with distance and parsimony methods), whilst the other lineage grouped *Nasidius* representatives together (>80 % bootstrap support with distance and ML methods and 1.00 with Bayesian analysis). Bootstrap support for the *Libanasidus* - *Nasidius* clade (I) was however

generally low across all methods of analysis (50-56 % for distance, maximum parsimony and ML) and only 0.5 for Bayesian analysis.

Clade II includes all the *Onosandrus* along with the *Bochus* and *Borborothis* individuals, with support values above 60%. The inclusion of two *Libanasa* (LA1 & LA2) and one *Nasidius* individual (NS4) within this clade is possibly due to misidentification as these were both immature insects, which are difficult to classify. The misidentification is supported by the fact that individuals NS4 and OS3 were caught from the same locality (Appendix II). Similarly, individuals LA1 and OS1 were both collected from the same site in Mpumalanga Province. The inclusion of individual LA2 within the *Libanasidus* cluster is relatively well-supported (bootstrap support >50%), suggesting incorrect identification although this was an adult specimen. Hereafter, individuals NS4 and LA1 are treated as specimens of *Onosandrus*, while individual LA2 is treated as a *Libanasidus*. The association of the genus *Bochus* with *Borborothis* is strongly supported by bootstrap values above 90% as suggested previously (Toms 2001). Collectively, Clade II incorporates all the genera in the analysis that display no sexual dimorphism, and together with clade I represents members of the tribe Anostostomatini.

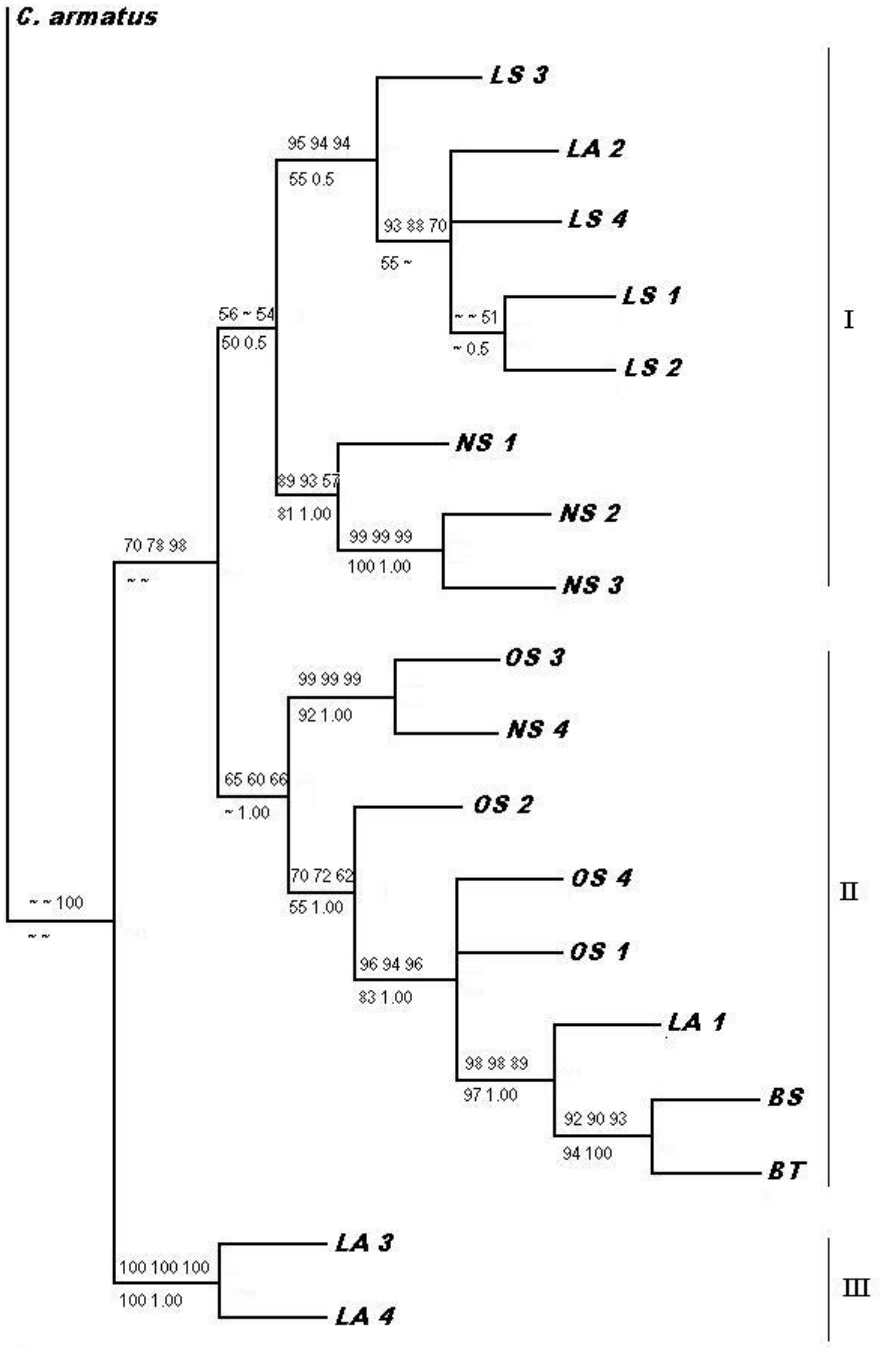


Figure 3.3. Consensus tree resulting from unweighted parsimony analysis of 16S sequence data for 6 anostostomatid genera, showing bootstrap support from NJ, ME and MP analyses above branches, respectively and ML and Bayesian branch support below. (Tree length 296, CI=0.74, RI=0.76). BS = *Bochus*, BT = *Borborothis*, LA = *Libanasa*, LS = *Libanasidus*, NS = *Nasidius*, OS = *Onosandrus* with each unique number behind the genus code corresponding to a different individual. Only those bootstrap and Bayesian support values above 50 % and 0.5, respectively are indicated next to the relevant nodes. Values below these cut-offs are denoted with a '~'.

The separate grouping of Clade III basally to Clades I and II is well-supported across all methods of analysis (100 % bootstrap support for MP, distance and ML and 1.00 from the Bayesian analysis), and is consistent with *Libanasa* currently being classified within the tribe Lutosini. *Libanasa* individual LA4 was formerly classified within the genus *Platysiagon*, but has recently been merged with *Libanasa* (Johns 1997). The strong grouping with individual LA3, which was originally classified within *Libanasa*, supports this merger.

Genetic divergence as calculated from the uncorrected 'p' distances between taxa is shown in Appendix IV. Genetic divergence within Clade I ranges from 0.1-1.3 % with an average of 0.66 %, while Clade II was less variable with a range of 0.00 % to 0.8 %, and an average of 0.46 %. The sequence divergence within Clade III is small, with individuals LA3 and LA4, the only two representatives of this lineage showing 99.9 % sequence identity across the gene region sequenced. Maximum between-clade sequence divergence ranged from 1.5 % between Clade I and II (LS4 and BS), to > 2 % (clades II and III was 2.1% between LA4 and BT - LA1, and Clades I and III was 2.2% between individuals LA3 and LS4).

DISCUSSION

Attempts at resolving phylogenetic relationships between taxa have often made use of molecular and morphological evidence (Chimimba 2005; Chapco *et al*. 2001; Flook & Rowell 1997; Cigliano *et al*. 1996). This approach provides a useful and reliable way of evaluating existing classification, and the morphological characters that have laid the basis for

these (Flook *et al.* 2000). The nomenclatural history of southern African anostostomatids is complicated, with frequent reshuffling of taxa, as evaluated morphologically by different taxonomists (Johns 1997; Kevan 1982; Ander 1943). Assessment of the eight genera in this region, using morphology in conjunction with genetics was the next plausible step in trying to gain insight, not only on the validity of existing genera, but also on the morphological characters that can effectively be used to distinguish between them.

The results from phylogenetic analyses based on 16S sequence data for six genera strongly support the legitimacy of these genera. Five genera currently group under the tribe Anostostomatini, separating into two clades (I and II). Consequently, it can be concluded that sequence data support the current generic classification of *Libanasidus*, *Nasidius*, *Onosandrus*, *Bochus* and *Borborothis* individuals (Figure 3.3). It has been suggested that the lack of sexual dimorphism is ancestral, and being found in *Borborothis* and *Onosandrus*, supports the clustering of these genera (see Toms 2001). *Bochus*, also grouping with the above two genera, has been regarded as ancestral based on the same assumption, but males show modification of the mandibles that is absent in females (Chapter 2). Nevertheless, genetic distances as derived from 16S sequence data suggests ancient divergence between *Borborothis* and *Libanasa* (2 %), and *Bochus* and *Libanasa* (1.9 %), supporting the notion that *Borborothis* and *Bochus* are ancestral to the other genera within the tribe Anostostomatini (Toms 2001).

The higher degree of relatedness of *Onosandrus* to the *Bochus-Borborothis* clade (0.45-0.6 %) is also suggested based on morphology (Chapter 2). The validity of using only one sequence for *Bochus* and *Borborothis* is debatable, but was unavoidable. The previously postulated ancestral nature of *Bochus*, *Borborothis* and *Onosandrus* (Toms 2001), however, does support the conclusions drawn here. In addition, *Bochus* is a monotypic genus, and the two species of *Borborothis* are clearly distinguishable, verifying the stability of these genera. *Nasidius* and *Libanasidus* appear as sister taxa within Clade I showing a divergence of 0.85 %, where relatedness of these two genera to *Onosandrus* is equally high (0.86-0.87 %). These three genera are morphologically easily confused, especially where they coexist in Afromontane forests of eastern South Africa. The third clade recovered supports the classification of the genus *Libanasa* in a separate tribe, the Lutosini, and is supported by the high sequence divergence values and individuals from Clades I and II (1.8-2 %). The merge of *Libanasa* and *Platysiagon* (Johns 1997) is also supported by this genetic study as is seen by the grouping of LA3 and LA4, where LA3 represents the former genus *Platysiagon*, and LA4 the original *Libanasa* genus.

Within Clades I and II, however, morphological discrepancy has resulted in what appears to be uncertainty in generic placement of the individuals LA1 (*Libanasa*), LA2 (*Libanasa*) and NS4 (*Nasidius*). Clustering of individual LA2 with *Libanasidus* is supported by high bootstrap values and sequence divergence of only 0.7 % between individuals LS4 and LA2. This is consistent with recognised intra-specific

genetic divergences reported in the literature (Trewick & Wallis 2001), suggesting that individual LA2 belongs to *Libanasidus*. Similarly, clustering of individuals NS4 and LA1 with *Onosandrus* is well-supported and these individuals are genetically very similar to neighbouring specimens of *Onosandrus* (0.0 % and 0.4 %, respectively). This strongly suggests generic misidentification of these three specimens. These misidentifications can be attributed to the inconsistency and inapplicability of diagnostic characters to these three specimens. Both female and sub-adult specimens, such as individuals LA1 and NS4, are often impossible to distinguish (see Chopard 1943; Toms 2001). Misidentification of the adult individual LA2 illustrates the difficulty in defining characters that distinguish between these taxa.

Morphological ambiguity in this taxonomic group has created many conflicting opinions among taxonomists. This is very clearly shown by the inability to resolve taxonomic relationships between eight genera, based on 37 morphological characters (Figure 3.2). Character ambiguity and homoplasy within the morphological dataset could be responsible for the lack of resolution between taxa provided by the morphological cladistic analysis. Less than half of the characters (16 of 33) showed low levels of homoplasy (CI > 0.7). These characters include: femoral compression (C3), middle tibial spines on the fore margin (C7), spines of the hind tibia (C10, C11 & C12), male armature of the head and mandibles (C17, C18 & C19), armature of the middle and hind femora (C22 & 23), the shape of the male subgenital plate (C25), ovipositor length (C31), the presence of a white ring before the knee of the hind femur (C32, characteristic of former

genus *Platysiagon* now merged with *Libanasa*), the elevation of the fastigium (C34), the clypeal shape (C36), the ovipositor shape (C27) as well as the stridulatory structure on the pleura (C39). Analyses performed with exclusion of homoplasious characters, however, did not improve the resolution of relationships between the eight genera.

The secondary sexual characteristics of the males have been used extensively in the classification of these crickets; yet prove redundant in resolving relationships between taxa. These, along with features of the ovipositor and subgenital plate (see Field & Bigelow 2001), were expected to provide useful insight into the distinction between these insects, but did not. Evaluating the remaining homoplasious characters, showed several characteristics that have been used extensively in previous classifications. Specifically, these include: the number of spines on the fore margin of the fore tibia (C4), the armature of the genicular lobes (C13 & C14), the texture of the body and facial area (C15 & C16), the presence of a gap between the mandibles and labrum in males (C17), the shape of the female subgenital plate (C26), the presence of tympanal structures (C30), body colour (C29), and the stridulatory structure of the hind femur (C38) (see Péringuey 1916; Karny 1929).

High levels of homoplasy have been documented in similar morphological characters for other Ensiferans (Jost & Shaw 2005), and this seems to be a common problem in Ensiferan taxonomy. Novel diagnostic characters tested in this study include the body profile (C21), the shape of the cerci (C24), and the position of styli on the male genitalia

(C21) obtained low CI values in this study, dismissing their use as diagnostic characters within this group of insects.

Although no morphological support for the eight existing genera was attained using the most noteworthy characters, genetic data allows evaluation of relationships between six genera, confirming the speculation by previous authors as to the ancestral nature of *Bochus* and *Borborothis*. The placement of *Libanasa* within a separate tribe, the Lutosini, is also validated. Furthermore, *Libanasidus* and *Nasidius* appear to be sister taxa, with *Onosandrus* being more closely related to *Bochus* and *Borborothis*, while sharing the lack of sexual dimorphism with these two genera. The taxonomic review in Chapter 2 suggests that the designation of the eight anostostomatid genera in southern Africa are valid, but that species placement within these genera needs to be revised, with the possible erection of new genera to accommodate all the currently recognised species. Molecular evidence in this study confirms this notion for six of these genera, with the phylogenetic position of the genera *Henicus* and *Onosandridus* remaining unresolved.

Unfortunately, many of the diagnostic morphological characteristics for southern African anostostomatids are subject to damage and deformation, not only in nature but also during the preservation of museum specimens. Together with high levels of homoplasy and incorrect species placement, resulting in character ambiguity within genera, these problems render many of the diagnostic characters of this group ineffective for resolving generic relationships. Given these problems, it is suggested that an extensive morphological review of all 51 species be conducted to evaluate

the taxonomic position of each species individually, which would eliminate the current morphological ambiguity within genera. Generation of functional identification keys to each of the species is vital to this cause. This should be supplemented with molecular revisions to confirm the identification of immatures and females.

ACKNOWLEDGEMENTS

The authors wish to thank the following curators and colleagues: B. Dombrowsky and K. Manamela (TM); and Dr. S. van Noort and M. Cochrane (SAMC); Dr. Kjell Arne Johansson (NHRS); Prof. Ulrike Aspöck (NMW); Dr. George Beccaloni (BMNH); and Dr. Peter J. Schwendinger (MHNG) for the loan of specimens for both morphological and genetic analysis. We thank G. Malherbe for technical assistance and guidance with molecular work. Andrés Angulo (Universidad de Concepción, Chile) is thanked for specimen loans. The study was funded by the University of Pretoria and the South African National Research Foundation (NRF) under grant GUN 2053665 and is gratefully acknowledged.

REFERENCES

Ander, K. 1943. Uber die von Stål beschriebenen Stenopelmatiden (Saltatoria). *Entomologisk Tidskrift* **64**: 198-201.

Chapco, W. Litzenberger, G. & Kuperus, W.R. 2001. A molecular biogeographic analysis of the relationship between North American melanoploid grasshoppers and their Eurasian and South American relatives. *Molecular Phylogenetics and Evolution* **18**:460-466.

Chimimba, C.T. 2005. Phylogenetic relationships in the genus *Aethomys* (Rodentia: Muridae). *African Zoology* **40**: 271-284.

Chopard, L. 1943. Two new species of Gryllacridoidea from southern Africa. *Entomologisk Tidskrift* **14**: 446-452.

Cigliano, M.M., Ronderos, R.A. & Kemp, W.P. 1996. Phylogenetic relationships of *Scotussa* and *Leiotettix* (Orthoptera: Acrididae). *Cladistics* **12**: 125-138.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Field, L.H. 2001. Introduction In: *The Biology of Wetas, King Crickets and their Allies.* (Ed.) L.H Field. Cabi publishing, Wallingford UK.

Field, L.H. & Bigelow, S. 2001. Morphometric analysis of *Hemideina* spp. in New Zealand. In: *The Biology of Wetas, King Crickets and their Allies,* (Ed.) L.H. Field, pp. 163-177. Cabi publishing, Wallingford UK.

Flook, P.K. & Rowell, C.H.F. 1997. The effectiveness of mitochondrial rRNA gene sequences for the reconstruction of the phylogeny of an insect order (Orthoptera). *Molecular Phylogenetics and Evolution* **8**: 177-192.

Flook, P.K., Klee, S. & Rowell, C. H. F. 2000. Molecular Phylogenetic analysis of the Pneumoroidea (Orthoptera, Caelifera): Molecular data resolve morphological character conflicts in the basal Acridomorpha. *Molecular Phylogenetics and Evolution* **15**: 345-354.

Forey, P.L., Humphries, C.J., Kitching, I.L., Scotland, R.W., Siebert, D.J. & Williams, D.M. 1992. Cladistics: A practical course in systematics. Oxford University Press, New York.

Gorochov, A.V. 2001. The higher classification, phylogeny and evolution of the superfamily Stenopelmatoidea. *In: The Biology of the Wetas, King Crickets and their Allies* (Ed.) L.H. Field, Pp 3-33. Cabi publishing, Wallingford UK.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**:95-98.

Huelsenbeck, J.P. & Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**:754-755.

Johns, P.M. 1997. The Gondwanaland Weta: Family Anostostomatidae (formerly in Stenopelmatidae, Henicidae or Mimnermidae): Nomenclatural problems, world checklist, new genera and species. *Journal of Orthoptera Research* **6**: 125-138.

Jost, M.C. & Shaw, K.L. 2005. Phylogeny of Ensifera (Hexapoda: Orthoptera) using three ribosomal loci, with implications for the evolution of acoustic communication. *Molecular Phylogenetics and Evolution*, In Press.

Karny, H.H. 1929. A revision of the South African Gryllacrididae (Orthoptera: Saltatoria). *Annals of the South African Museum* **29**: 138-45.

Kevan, D.K.McE. 1982. Orthoptera. In: *Synopsis and Classification of Living Organisms* (Ed.) Parker, S.P. pp 352-355. McGraw-Hill Book Company, New York.

Kumar, S., Tamura, K. & Nei, M. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics* **5**: 150-163.

Matzura, O. & Wennborg, A. 1996. RNAdraw: an integrated program for RNA secondary structure calculation and analysis under 32-bit Microsoft Windows. *Computer Applications in the Biosciences* **12**: 247-249

Otte, D., Eades, D.C. & Naskrecki, P. 2005. *Orthoptera Species File Online*. Version 2.2. <http://osf2.orthoptera.org>.

Péringuey, L. 1916. Descriptions of new or little known Orthoptera: Family Locustidae, Sub-Fam. Stenopelmatinae. *Annals of the South African Museum* **15**: 414-425.

Picker, M., Griffiths, C. & Weaving A. 2002. *Field Guide to Insects of Southern Africa*. Struik publishers, Johannesburg, South Africa.

Posada, D. & Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics Applications Note* **14**: 817-818.

Sharov, A.G. 1968. Phylogeny of the Orthopteroidea. *Transactions of the Palaeontological Institute, Academy of Sciences USSR* **118**: 1-216.

Simon, C. Frati, F. Beckenbach, A., Crespi, B., Liu, H. & Flook, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a combination of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87**: 686-701.

Stål, C. 1878. Systema Acridiodeorum. Essai d'une Systématisation des Acridiodées. *Bihang till Kungliga Svenska Vetenskaps-Akademiens Handlingar.* **5**:1-100.

Sunnucks, P. & Hale, D.F. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution* **13**: 510-524.

Swofford, D.L. 1999. PAUP*. *Phylogenetic analysis using parsimony (*and other methods)*. Version 4.0b10 Sunderland, Massachusetts: Sinnauer Associates.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**:4876-4882

Toms, R.B. 1985. Johannesburg's cricket invader: The 'Parkmore Prawn'. *African Wildlife* **39**: 200-202.

Toms, R.B. 2001. South African King Crickets (Anostostomatidae) In: *The Biology of the Wetas, King Crickets and their Allies*. (Ed) Field, L.H. pp 73-78. Cabi publishing, Wallingford UK.

Trewick, S.A. 2000. Molecular evidence for dispersal rather than vicariance as the origin of flightless insect species on the Chatham Islands, New Zealand. *Journal of Biogeography* **27**: 1189-1200.

Trewick, S.A. & Wallis, G.P. 2001. Bridging the “Beech Gap”: New Zealand invertebrate phylogeography implicates Pleistocene glaciation and Pliocene isolation. *Evolution* **55**: 2170-2180.

Wolf, S., Brettschneider, H. & Bateman, P.W. 2006. The predator defence system of an African King Cricket (Orthoptera; Anostostomatidae): does it help to stink? *African Zoology* **41**:75-80.

Zuker, M. 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research.* **31:** 3406-15.

Appendix I. Description of morphological characters used in phylogenetic analysis of southern African Anostostomatidae. Characters are graded from 1-5, outgroup state is recorded as 0.

1. Head broader than pronotum: **0:** No **1:** Yes **2:** Variable
2. Hind femur base: **0:** Dilated **1:** Very dilated **2:** Hardly dilated
3. Femora laterally compressed: **0:** Fore & middle **1:** Fore **2:** Mid **3:** All **4:** None **5:** Variable
4. Fore tibia: no. of spines on fore internal margin: **0:**1 **1:** 2 **2:**3 **3:** 1 or 2
5. Fore tibia: no. of spines on fore external margin: **0**: 0
6. Fore tibia: no. of spines on hind margin: **0:** 8
7. Middle tibia: no. of spines fore internal: **0:** 2 **1:** 3 **2:** 3
8. Middle tibia: no. of spines fore external: **0:** 3 **1:** 2
9. Middle tibia: no. of spines on hind margin: **0:** 8 **1:** 7
10. Hind tibia: no. of spines fore internal: **0:** 5-10 **1:** 11-15 **2:** 16-19 **3:** 20+
11. Hind tibia: no. of spines fore external: **0:** 5-10 **1:** 11-15 **2:** 16-19 **3:** 20+
12. Hind tibia: no. of spines hind: **0:** 3 **1:** 2 **2:** 4 **3:** 5
13. Middle genicular lobes spined: **0**: None **1:** Inner **2:** Outer **3:** Both **4:** Variable
14. Hind genicular lobes spined: **0:** Both **1:** Inner **2:** Outer **3:** None **4:** Variable
15. Tergites: **0:** Smooth **1:** Rough **2:** Smooth/roughened
16. Head & pronotum: **0:** Smooth **1:** Roughened **2:** Smooth/roughened
17. Male: gap between mandibles & labrum: **0:** No **1:** Yes
18. Male armature of mandibles: **0:** None **1:** Spined/Knobbed **2:** Elongated **3:** Elongated & spined **4:** Angled to side **5:** Tusked
19. Male head armature: **0:** None **1:** Frons **2:** Side **3:** Front & Side **4:** Frontal Cone
20. Ovipositor curvature: **0:** Upcurved **1:** Perpendicular **2:** Straight
21. Body profile: **0:** Straight **1:** Arched 2: Straight/Arched

22. Hind femur armature: **0:** Toothed above & below **1:** Toothed above **2:** Toothed below **3:** None
23. Middle femur armature: **0:** Toothed below **1:** Toothed above **2:** Toothed above & below **3:** None
24. Cerci: **0:** Straight **1:** Curled **2:** Curved **3:** Hooked 4: Variable
25. Male subgenital plate: **0:** Forked with styli **1:** Triangular **2:** Trapezoidal **3:** Trapezoidal with 2 styli at apex **4:** Elongated (rodlike) with styli at apex
26. Female subgenital plate: **0:** Triangular **1:** Trapezoidal **2:** Arrowhead shaped
28. Male genitalia with styli: **0:** None **1:** Epiproct **2:** Paraprocts **3:** Both epiproct & paraprocts
29. Body colour: **0:** Yellow/Light **1:** Black/Dark **2:** Mottled with yellow **3:** Mottled with black **4:** Brick red, black banded **5:** Pale, black banded
30. Tympanum presence: **0:** No **1:** Indentation only **2:** Perforated either side **3:** Perforated both sides **4:** Indented either side **5:** Variable
31. Ovipositor length: **0:** > half of hind femur length **1:** < half of hind femur length **2:** Longer than hind femur
32. White ring on hind femur: **0:** No **1:** Yes
33. Female subgenital plate indented at apex: **0:** No **1:** Yes **2:** Variable
34. Fastigium elevation: **0:** Raised **1:** Indented/ flat **2:** Variable
36. Clypeus vaulted: **0:** No **1:** Yes
37. Ovipositor shape: **0:** Broad at base, thin at tip **1:** Thin throughout **2:** Broadest in middle
38. Stridulatory structure, hind femur: **0:** Spikes **1:** Roughened **2:** Small knobs **3:** Ridges
39. Stridulatory structure, abdomen: **0:** Knobs & bristles **1:** Rounded spikes **2:** Hollow knobs **3:** Hooks **4:** Roughened

Appendix II. List of specimens from selected museums used for the cladistic morphological analysis of eight southern African Anostostomatid genera. * indicate specimens included in the morphological and genetic analysis. † indicates individuals only included in the genetic study. Museums are indicated by standard abbreviation: Muséum d'Histoire Naturelle (MHNG), Geneva, Switzerland; Naturhistorisches Museum Wien (NMW), Vienna, Austria; Naturhistoriska Riksmuseet (NHRS) Stockholm, Sweden; The Natural History Museum, London (BMNH), United Kingdom; South African Museum (SAMC, S. Afr.), Cape Town, South Africa; Transvaal Museum (TM), Pretoria, South Africa, National Insect Collection (NCI), Pretoria, South Africa.

Code	Locality	Museum Ref
Bochus		
TBS184	Free State, Smithfield	MHNG
BS251	Free State, Smithfield	SAM-OR-A-000025
BS253	Free State, Smithfield	SAM-OR-A-000027
*BS252 (BS)	Free State, Smithfield	SAM-OR-A-000026
Borborothis		
TBT186	Cape Province	NMW
BO	Locality unknown	TMOR6184
BT249	Locality unknown	TMOR6187
BT250	Locality unknown	TMOR6189
*BT254 (BT)	Locality unknown	SAM-OR-A-000028
BT255	Locality unknown	SAM-OR-A-000029
BT256	Locality unknown	SAM-OR-A-000030
BT257	Locality unknown	SAM-OR-A-000032
Henicus		
TH187	Western Cape, Swellendam	NMW
HM	Locality unknown	TMOR6152
H104	Western Cape, Sneeukop	TM
H105	Western Cape, Sneeukop	TM
H106	Western Cape, Sneeukop	TM
H107	Western Cape, Sneeukop	TM
H108	Western Cape, Sneeukop	TM
H109	Western Cape, Sneeukop	TM
H110	Western Cape, Sneeukop	TM
H111	Western Cape, Sneeukop	TM
H147	Northern Cape, Versveldpan	TM
H148	Western Cape, Grootvadersbosch	TM
H149	Western Cape, Grootbrak Valley	TMOR6292

H150	Western Cape, Botrevier	TM
H151	Western Cape, Yzerfontein	TM
H18	Limpopo, Pietersburg	TMOR6297
H88	Limpopo, Pietersburg	TMOR6225
H94	Limpopo, Nylsvlei Nature Reserve	TMOR6281
Libanasa		
TLA188	KwaZulu Natal	BMNH
*LA1990 (LA4)	KwaZulu Natal, Cape Vidal	NCI
LA300	KwaZulu Natal, Ngome Forest Reserve	TM
LA306	KwaZulu Natal, Ngome Forest Reserve	TM
LA1	Limpopo, Gravelotte	TMOR6140
LA10	Zimbabawe, Mnt. Selinda	TMOR17226
LA11	Zimbabawe, Mnt. Selinda	TMOR17226
LA12	Mpumalanga, Clanor	TM
LA13	Mpumalanga, Clanor	TM
LA133	Mpumalanga, Nelshoogte, Devil's Knuckles	S.Afr.No. 2352B
LA14	Zimbabawe, Mutare	TMOR17225
LA15	Zimbabawe, Mutare	TMOR17225
LA153	Limpopo, Tzaneen, Malta Forest	TM
LA16	Mpumalanga, Ngwenya Lodge	TMOR17224
LA17	Mpumalanga, Clanor Lisbon Citrus Estate	TMOR6284
LA2	Mpumalanga, Ngwenya Lodge	TMOR6147
LA20	Mpumalanga, Clanor Lisbon Citrus Estate	TMOR6301
LA21	Mpumalanga, Clanor Lisbon Citrus Estate	TMOR6302
LA22	KwaZulu Natal, Ngome Forest Reserve	TM
LA221	Mpumalanga, Berlin Forest Station	S.Afr.No. 2301B
LA23	KwaZulu Natal, Ngome Forest Reserve	TM
LA24	KwaZulu Natal, Ngome Forest Reserve	TM
LA25	KwaZulu Natal, Ngome Forest Reserve	TM
LA26	KwaZulu Natal, Ngome Forest Reserve	TM
LA27	KwaZulu Natal, Ngome Forest Reserve	TM
LA28	Mozambique, Inhaca Island	TM
LA29	Mozambique, Inhaca Island	TM
LA3	Mpumalanga, Kruger National Park, Stolznek	TMOR6141
LA30	KwaZulu Natal, Mntuzini	TMOR6303
LA31	Mpumalanga, Berlin Forest Station 1500	SAFR2362G
LA32	Mpumalanga, Nelspruit Nature Reserve	TM
LA33	Limpopo, Ben Lavin Nature Reserve	TMOR6283
LA34	Mpumalanga, Clanor Lisbon Citrus Estate	TMOR6333
LA35	Zimbabwe, Mnt. Selinda	TMOR17223
LA36	Zimbabwe, Mnt. Selinda	TMOR17223
LA37	Zimbabwe, Mnt. Selinda	TMOR17223
LA38	KwaZulu Natal, Hluhluwe Game Reserve	TMOR2829
LA4	KwaZulu Natal, Ferncliffe	TM
LA40	Mpumalanga, Berlin Forest Station, Karst Plateau	TM
LA42	Mpumalanga, Berlin Forest Station 1500	TMOR 2358B
LA43	Limpopo, Ben Lavin Nature Reserve	TMOR6295
LA44	Limpopo, Ben Lavin Nature Reserve	TMOR6296
LA48	Western Cape, Grootvadersbosch	TMOR6337
LA5	KwaZulu Natal, Ferncliffe	TM
LA51	Locality unknown	TMOR2372G
LA52	Gauteng, Moreletta Nature Trail	TMOR17207

LA6	KwaZulu Natal, Sordwana Bay	TMOR6190
LA7	Zimbabwe, Mnt. Selinda	TMOR17228
LA77	Mpumalanga, Blyde Rivier Canyon	TM
LA78	Mpumalanga, Blyde Rivier Canyon	TM
LA8	Mpumalanga, Kruger National Park, Berg & Dal	TMOR6286
LA9	Zimbabawe, Mnt. Selinda	TMOR17226
*G276 (LA2)	Gauteng, Klipreviersberg Nature Reserve	TM
†LA1	Mpumalanga, Bridal Veil Falls	Donated to S.Trewick
†LA3	KwaZulu Natal, Ngome Forest	Donated to S. Trewick
Libanasidus		
TLS189	Mpumalanga, Barberton	BMNH
LSiM	Cape Colony	SAM-OR-A-000111
LSiF	Free State, Kimberly	SAM-OR-A-000939
*G264LS (LS2)	Gauteng, Klipreviersberg Nature Reserve	TM
*L251LS (LS1)	Limpopo, Tzaneen	TM
*M255LS (LS3)	Mpumalanga, Bridal veil falls	TM
UP1LS	Gauteng, Johannesburg	TM
LS190	Gauteng, Irene	TM
LS191	Limpopo, Louis Trichardt Hanglip Forest Station	TMOR6211
LS227	Gauteng, Pretoria	TM
LS228	Gauteng, Klipreviersberg Nature Reserve	TM
LS229	Gauteng, Pretoria	TM
LS231	Gauteng, Klipreviersberg Nature Reserve	TM
LS232	Gauteng, Pretoria	TM
LS233	Gauteng, Pretoria	TM
LS234	Gauteng, Pretoria	TM
LS235	Gauteng, Pretoria	TM
LS236	Gauteng, Johannesburg	TM
LS237	Gauteng, Klipreviersberg Nature Reserve	TM
LS238	Gauteng, Klipreviersberg Nature Reserve	TM
LS239	Gauteng, Pretoria	TM
LS240	Gauteng, Randburg	TM
†LS4	Gauteng, Johannesburg	NCI
Nasidius		
TN248	South Africa, Caffraria	NHRS
*N241 (NS1)	Western Cape, Bosbokstrand	TM
NS322	Gauteng, Bronkhortspruit	TM
NS323	Gauteng, Bronkhortspruit	TM
*G278NS (NS2)	Gauteng, Klipreviersberg Nature Reserve	TM
N100	Mpumalanga, Berlin Forest Station, Karst Plateau	TM
N101	Mpumalanga, Berlin Forest Station, Karst Plateau	TM
N102	Mpumalanga, Uitsoek Forest Station	TM
N103	Mpumalanga, Barberton	S.Afr.No.2331E
N114	Western Cape, Newlands	TM
N19	Limpopo, Pietersburg	TMOR6298
N193	Locality unknown	TMOR6307
N194	Locality unknown	TMOR6312
N195	Locality unknown	TMOR6310
N196	Locality unknown	TMOR6309
N198	Locality unknown	TMOR6308

N199	Locality unknown	TMOR6311
N200	Gauteng, Cullinan, Premier Game Farm	TM
N330	Mpumalanga, Kruger National Park	TM
N45	Mpumalanga, Nelspruit	TM
N50	Mpumalanga, Pretorius Kop	TMOR6290
N53	Mpumalanga, Maleoskop	TM
N54	Gauteng, Gerhardsville	TMOR17202
N55	Limpopo, Nylstroom, Farm Makapan	TMOR17204
N56	Limpopo, Nylstroom, Farm Makapan	TMOR17204
N57	Limpopo, Lekgalameetse Nature Reserve	TMOR17193
N58	Limpopo, Lekgalameetse Nature Reserve	TMOR17193
N59	Locality unknown	S. Afr. No 2315G
N60	Limpopo, Pietersburg	TMOR6227
N61	Limpopo, Pietersburg	TMOR6228
N62	Limpopo, Pietersburg	TMOR6229
N63	Locality unknown	TMOR6329
N64	Gauteng, Rooihuiskraal	TMOR6200
N65	Limpopo, Ben Lavin Nature Reserve	TMOR6198
N66	Limpopo, Ben Lavin Nature Reserve	TMOR6197
N67	Mpumalanga, KNP Pretorius Kop	TMOR6291
N68	Gauteng, Gerhardsville	TMOR17201
N69	Mpumalanga, Nelspruit Nature Reserve	S. Afr. No 2315B
N70	Limpopo, Hanglip Plateau	TMOR6304
N71	Gauteng, Pierre van Reyneveld	TM
N72	Locality unknown	S. Afr. No. 2332E
N73	Limpopo, Lekgalameetse Nature Reserve	TMOR17194
N74	Mpumalanga, Hoedspruit	TMOR6332
N75	KwaZulu Natal, Ingwavuma, Tembe	TM
N76	Zimbabwe, Mnt. Selinda	TMOR17227
N79	Limpopo, Nylsvlei, Naboomspruit	TMOR6282
N80	Gauteng, Boekenhoutkloof	TMOR6285
N81	Limpopo, Pietersburg	TMOR6223
N82	Limpopo, Ben Lavin Nature Reserve	TMOR17198
N83	Limpopo, Peitersburg	TMOR6223
N84	Gauteng, Gerhardsville	TMOR17197
N85	KwaZulu Natal, Ingwavuma, Tembe	TMOR17191
N86	Limpopo, Pietersburg	TMOR6224
N87	Mpumalanga, Middelburg	TMOR17206
N89	Mpumalanga, Berlin Forest Station, Karst Plateau	S.Afr.No.2411E
N90	Mpumalanga, Uitsoek Forest Station	S.Afr.No.2392G
N91	Mpumalanga, Uitsoek Forest Station	S.Afr.No.2392G
N92	Limpopo, Ben Lavin Nature Reserve	TMOR6305
N93	Limpopo, Ben Lavin Nature Reserve	TMOR6306
N95	Mpumalanga, Maleoskop	TM
N96	Gauteng, Gerhardsville	TM
N97	Mpumalanga, Wits Pullen Farm	TMOR6288
N98	Limpopo, Lekgalameetse Nature Reserve	TM
N99	Mpumalanga, Maleoskop	TM
†NS3	Gauteng, Klipreviersberg Nature Reserve	Donated to S. Trewick
†NS4	KwaZulu Natal, Ngome Forest	TM
Onosandridus		
TOD185	Zimbabwe, Umtali	SAMC
OD263	Locality unknown	SAM-OR-A-000145
OD192	KwaZulu Natal, Ngome Forest	TM

OD230	KwaZulu Natal, Ngome Forest	TM
OD258	Locality unknown	SAM-OR-A-000139
OD259	Locality unknown	SAM-OR-A-000141
OD260	Locality unknown	SAM-OR-A-000143
OD261	Locality unknown	SAM-OR-A-000144
OD262	Locality unknown	SAM-OR-A-000142
OD49	Gauteng, Randburg	TMOR6208
Onosandrus		
TOS247	South Africa, Caffraria	NHRS
OS 122	Mpumalanga, Uitsoek Forest Station	TM
OS 124	Mpumalanga, Berlin Forest Station	S. Afr. No. 2280B
OS112	KwaZulu Natal, Mntuzini	TM
OS113	KwaZulu Natal, Mntuzini	TM
OS115	Mpumalanga, Berlin Forest Station	S.Afr.No. 22764
OS116	Locality unknown	TMOR6336
OS117	Mpumalanga, Nelshoogte Forest Station	S.Afr. No.v2312E
OS118	Mpumalanga, Uitsoek Forest Station	S.Afr.No. 2317E
OS119	Limpopo, Mariepskop Office	TM
OS121	Locality unknown	TMOR6338
OS125	Northern Cape, Noemeesberg	S.Afr.No. 1285
OS126	Northern Cape, Noemeesberg	S.Afr.No. 1285
OS127	Mpumalanga, Berlin Forest Station	S.Afr.No. 2366
OS128	Mpumalanga, Berlin Forest Station	S.Afr.No. 2366
OS129	KwaZulu Natal, Mntuzini, Twin Streams	TMOR6293
OS130	KwaZulu Natal, Mntuzini, Twin Streams	TMOR6294
OS131	Mpumalanga, Nelshoogte, Devil's Knuckles	S.Afr.No. 2352B
OS132	Mpumalanga, Nelshoogte, Devil's Knuckles	S.Afr.No. 2352B
OS134	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS135	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS136	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS137	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS138	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS139	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS140	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS141	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS142	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS143	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS144	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS145	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS146	Eastern Cape, Impetyena Forest	S.Afr.No. 2681
OS152	Mpumalanga, Berlin Forest Station, Karst Plateau	S.Afr.No. 2375G
OS154	Mpumalanga, Wits Pullen Farm	TMOR17212
OS155	Locality unknown	TMOR6254
OS156	Locality unknown	TMOR6244
OS157	Limpopo, Mnt. Sheba Forest	TMOR6265
OS158	Limpopo, Mnt. Sheba Forest	TMOR6366
OS159	Limpopo, Mnt. Sheba Forest	TMOR6364
OS160	Gauteng, Alberton	TMOR17213
OS161	Limpopo, Tzaneen, Malta Forest	TMOR6215
OS162	Limpopo, Mnt. Sheba Forest	TMOR6206
OS163	Limpopo, Louis Trichardt, Hanglip Forest	TMOR6212
OS164	KwaZulu Natal, Mntuzini, Twin Streams	TMOR6350

OS165	KwaZulu Natal, Mntuzini, Twin Streams	TMOR6349
OS166	Limpopo, Mnt. Sheba Forest	TMOR6339
OS167	Mpumalanga, Nelshoogte Forest Station	S.Afr.No. 2310B
OS168	Limpopo, Louis Trichardt, Hanglip Forest	TMOR6213
OS169	Limpopo, Mnt. Sheba Forest	TMOR6342
OS170	Limpopo, Mnt. Sheba Forest	TMOR6342
OS171	Limpopo, Tzaneen, Malta Forest	TMOR6328
OS172	Western Cape, Tsitsikamma Forest	TMOR6358
OS173	Western Cape, Tsitsikamma Forest	TMOR6357
OS174	KwaZulu Natal, Mntuzini, Twin Streams	TMOR6343
OS175	KwaZulu Natal, Mntuzini, Twin Streams	TMOR6344
OS176	Western Cape, Knysna Forest	TMOR6353
OS177	Western Cape, Knysna Forest	TMOR6352
OS178	Western Cape, Knysna Forest	TMOR6355
OS179	Locality unknown	TMOR6374
OS180	Locality unknown	TMOR6372
OS181	Locality unknown	TMOR6374
OS182	Locality unknown	TMOR6370
OS183	Locality unknown	TMOR6331
OS197	Locality unknown	TM
OS201	Mpumalanga, Karst Plateau Valley	TM
OS202	Mpumalanga, Karst Plateau Valley	TM
OS203	Limpopo, Mariepskop Office	TM
OS204	Limpopo, Mariepskop Office	TM
OS205	Mpumalanga, Nelshoogte Forest Station	S.Afr.No.2310B
OS206	Mpumalanga, Nelshoogte Forest Station	S.Afr.No.2311B
OS207	Mpumalanga, Nelshoogte Forest Station	TM
OS208	Eastern Cape, Impetyena Forest	S.Afr.No. 2679G
OS209	Locality unknown	TMOR6346
OS210	Mpumalanga, Blyde Revier Canyon	S.Afr.No. 1285
OS211	Eastern Cape, Farzer Gorge	TM
OS212	Northern Cape, Torino Ranch	S.Afr.No.2773
OS213	Northern Cape, Torino Ranch	S.Afr.No.2773
OS214	KwaZulu Natal, Ngome Forest Reserve	OR17210
OS215	Mpumalanga, Nelshoogte Forest Station	S.Afr.No. 2292G
OS216	Mpumalanga, Nelshoogte Forest Station	S.Afr.No. 2292G
OS217	Mpumalanga, Nelshoogte Forest Station	S.Afr.No. 2292G
OS218	Mpumalanga, Nelshoogte Forest Station	S.Afr.No. 2292G
OS219	Mpumalanga, Nelshoogte Forest Station	S.Afr.No. 2292G
OS220	Mpumalanga, Nelshoogte Forest Station	S.Afr.No. 2292G
OS222	Mpumalanga, Berlin Forest Station	S.Afr.No. 2301B
OS223	Mpumalanga, Berlin Forest Station	S.Afr.No. 2301B
OS224	Mpumalanga, Berlin Forest Station	S.Afr.No. 2301B
OS225	Mpumalanga, Berlin Forest Station	S.Afr.No. 2301B
OS226	Mpumalanga, Berlin Forest Station	S.Afr.No. 2301B
OS41	Mpumalanga, Berlin Forest Station, Karst Plateau	TM
OS46	Western Cape, Grootvadersbosch	TM
OS47	Western Cape, Grootvadersbosch	TM
†OS1	Mpumalanga, Bridal Veil Falls	TM
†OS2	Mpumalanga, Sani Top	Donated to S. Trewick
†OS3	KwaZulu Natal, Ngome Forest	TM
†OS4	Mpumalanga, Bridal Veil Falls	Donated to S. Trewick

Appendix III. Morphological data matrices for eight southern African anostostomatid genera, including females, males, females and males combined excluding sexual characteristics and sequenced individuals excluding sexual characteristics. BS = *Bochus*, BT = *Borborothis*, H = *Henicus,* LA = *Libanasa*, LS = *Libanasidus*, NS = *Nasidius*, OD = *Onosandridus*, OS = *Onosandrus*.

Females									
	BS	**BT**	**HS**	**LA**	**LS**	**NS**	**OD**	**OS**	**OUT**
C1	1	0	2	2	2	2	2	0	0
C2	2	1	0	0	1	2	1	1	0
C3	3	3	3	5	3	3	3	3	0
C4	1	0	1	0	0	2	1	0	0
C5	0	0	0	0	0	0	0	0	0
C6	0	0	0	0	0	0	0	0	0
C7	0	0	2	0	0	0	0	0	0
C8	0	0	0	0	0	0	0	0	0
C9	0	0	0	0	0	0	0	0	0
C10	0	0	0	2	0	0	0	0	0
C11	0	0	0	2	0	0	0	0	0
C12	0	3	2	1	2	2	2	2	0
C13	0	4	0	4	4	0	4	4	0
C14	3	4	2	0	0	4	4	4	0
C15	1	1	0	0	0	2	0	0	0
C16	1	1	0	0	0	2	0	0	0
C20	0	0	0	0	0	0	0	0	0
C21	0	2	2	0	0	0	0	0	0
C22	3	1	3	3	2	2	3	2	0
C23	3	3	3	3	3	3	3	3	0
C24	0	4	4	4	4	4	4	2	0

Males									
	BS	**BT**	**HS**	**LA**	**LS**	**NS**	**OD**	**OS**	**OUT**
C1	1	0	2	2	2	2	2	0	0
C2	2	1	0	0	1	2	1	1	0
C3	3	3	3	5	3	3	3	3	0
C4	1	0	1	0	0	2	1	0	0
C5	0	0	0	0	0	0	0	0	0
C6	0	0	0	0	0	0	0	0	0
C7	0	0	2	0	0	0	0	0	0
C8	0	0	0	0	0	0	0	0	0
C9	0	0	0	0	0	0	0	0	0
C10	0	0	0	2	0	0	0	0	0
C11	0	0	0	2	0	0	0	0	0
C12	0	3	2	1	2	2	2	2	0
C13	0	4	0	4	4	0	4	4	0
C14	3	4	2	0	0	4	4	4	0
C15	1	1	0	0	0	2	0	0	0
C16	1	1	0	0	0	2	0	0	0
C17	0	0	1	1	0	1	0	0	0
C18	4	0	3	3	5	2	0	0	0
C19	0	0	3	0	0	4	0	0	0
C21	0	2	2	0	0	0	0	0	0
C22	3	1	3	3	2	2	3	2	0

C26	0	1	0	0	1	1	1	1	0
C29	1	1	0	3	3	1	1	0	0
C30	0	3	0	3	3	5	5	0	0
C31	1	0	0	0	0	0	0	0	0
C32	0	0	0	1	0	0	0	0	0
C33	1	0	0	0	1	2	0	0	0
C34	1	0	2	0	0	2	0	0	0
C36	1	0	2	2	0	2	0	2	0
C37	0	2	0	0	0	0	0	0	0
C38	2	2	1	1	0	0	0	0	0
C39	1	1	4	1	3	2	1	1	0

C23	3	3	3	3	3	3	3	3	0
C24	0	4	4	4	4	4	4	2	0
C25	3	3	3	3	3	3	3	3	0
C28	2	2	3	3	2	2	2	2	0
C29	1	1	0	3	3	1	1	0	0
C30	0	3	0	3	3	5	5	0	0
C32	0	0	0	1	0	0	0	0	0
C33	1	0	0	0	1	2	0	0	0
C34	1	0	2	0	0	2	0	0	0
C36	1	0	2	2	0	2	0	2	0
C37	0	2	0	0	0	0	0	0	0
C38	2	2	1	1	0	0	0	0	0
C39	1	1	4	1	3	2	1	1	0

	Combined								
	BS	**BT**	**HS**	**LA**	**LS**	**NS**	**OD**	**OS**	**OUT**
C1	1	0	2	2	2	2	2	0	0
C2	2	1	0	0	1	2	1	1	0
C3	3	3	3	5	3	3	3	3	0
C4	1	0	1	0	0	2	1	0	0
C5	0	0	0	0	0	0	0	0	0
C6	0	0	0	0	0	0	0	0	0
C7	0	0	2	0	0	0	0	0	0
C8	0	0	0	0	0	0	0	0	0
C9	0	0	0	0	0	0	0	0	0
C10	0	0	0	2	0	0	0	0	0
C11	0	0	0	2	0	0	0	0	0
C12	0	3	2	1	2	2	2	2	0
C13	0	4	0	4	4	0	4	4	0
C14	3	4	2	0	0	4	4	4	0
C15	1	1	0	0	0	2	0	0	0

	Sequenced									
	BS	**BT**	**LA**	**LA**	**LS**	**LS**	**LS**	**NS**	**NS**	**OUT**
C1	1	0	1	0	1	0	1	0	1	0
C2	2	1	1	0	1	1	1	2	0	0
C3	3	3	3	3	3	3	3	3	3	0
C4	1	0	0	0	0	0	0	0	1	0
C5	0	0	0	0	0	0	0	0	0	0
C6	0	0	0	0	0	0	0	0	0	0
C7	0	0	0	0	0	0	0	0	0	0
C8	0	0	0	0	0	0	0	0	0	0
C9	0	0	0	0	0	0	0	0	0	0
C10	0	0	0	0	0	0	0	0	0	0
C11	0	0	0	0	0	0	0	0	0	0
C12	0	3	0	2	2	2	2	0	2	0
C13	0	0	0	3	2	3	3	0	0	0
C14	3	3	2	0	0	0	0	3	3	0
C15	1	1	0	0	0	0	0	0	0	0

C16	1	1	0	0	0	2	0	0	0
C21	0	2	2	0	0	0	0	0	0
C22	3	1	3	3	2	2	3	2	0
C23	3	3	3	3	3	3	3	3	0
C24	0	4	4	4	4	4	4	2	0
C29	1	1	0	3	3	1	1	0	0
C30	0	3	0	3	3	5	5	0	0
C32	0	0	0	1	0	0	0	0	0
C33	1	0	0	0	1	2	0	0	0
C34	1	0	2	0	0	2	0	0	0
C36	1	0	2	2	0	2	0	2	0
C37	0	2	0	0	0	0	0	0	0
C38	2	2	1	1	0	0	0	0	0
C39	1	1	4	1	3	2	1	1	0

C16	1	1	0	0	0	0	0	1	1	0
C21	0	9	0	0	9	0	0	9	9	0
C22	4	9	0	0	9	1	5	9	9	0
C23	0	9	0	0	9	0	0	9	9	0
C24	9	0	9	9	0	9	9	0	0	0
C29	0	0	1	1	0	0	0	1	0	0
C30	3	1	3	3	3	0	3	3	3	0
C32	3	3	3	3	3	0	3	3	3	0
C33	0	0	0	2	2	2	2	2	0	0
C34	3	9	2	3	9	3	3	9	9	0
C36	9	1	9	9	1	9	9	1	1	0
C37	2	9	2	2	9	2	2	9	9	0
C38	1	1	3	1	4	4	4	1	1	0
C39	0	3	3	3	3	3	3	0	0	0

Appendix IV. Uncorrected 'p' distances calculated for 19 individuals representing the genus *Bochus* (BS), *Borborothis* (BT), *Nasidius* (NS), *Libanasa* (LA), *Libanasidus* (LS), *Onosandrus* (OS). OUT = outgroup, *Cratomelus armatus*.

	LS1	LA2	LS2	LS4	LS3	NS2	NS3	NS1	OS3	NS4	BS	BT	LA1	OS4	OS1	OS2	OUT	LA3	LA4
LS1	-																		
LA2	0.02	-																	
LS2	0.02	0.01	-																
LS4	0.08	0.07	0.08	-															
LS3	0.03	0.03	0.04	0.08	-														
NS2	0.08	0.08	0.08	0.13	0.07	-													
NS3	0.09	0.08	0.09	0.12	0.08	0.01	-												
NS1	0.06	0.07	0.07	0.12	0.06	0.05	0.06	-											
OS3	0.08	0.07	0.08	0.12	0.06	0.08	0.08	0.06	-										
NS4	0.09	0.07	0.08	0.12	0.07	0.09	0.08	0.07	0.00	-									
BS	0.10	0.09	0.10	0.14	0.09	0.10	0.09	0.08	0.07	0.07	-								
BT	0.11	0.11	0.12	0.15	0.10	0.11	0.11	0.10	0.08	0.08	0.02	-							
LA1	0.11	0.10	0.11	0.14	0.10	0.11	0.10	0.09	0.07	0.07	0.03	0.05	-						
OS4	0.09	0.07	0.08	0.13	0.08	0.09	0.08	0.07	0.04	0.05	0.02	0.04	0.03	-					
OS1	0.08	0.07	0.08	0.12	0.07	0.08	0.08	0.07	0.04	0.05	0.03	0.05	0.04	0.01	-				
OS2	0.09	0.08	0.09	0.13	0.08	0.10	0.09	0.08	0.05	0.05	0.06	0.07	0.07	0.04	0.05	-			
OUT	0.13	0.11	0.12	0.17	0.12	0.15	0.14	0.12	0.11	0.11	0.14	0.15	0.14	0.11	0.12	0.12	-		
LA3	0.18	0.17	0.18	0.22	0.17	0.20	0.19	0.19	0.17	0.17	0.18	0.19	0.19	0.17	0.18	0.17	0.19	-	
LA4	0.18	0.17	0.18	0.21	0.17	0.20	0.18	0.20	0.18	0.18	0.20	0.21	0.21	0.19	0.19	0.19	0.19	0.10	-

Chapter 4

A molecular and morphometric analysis of tusked king crickets (*Libanasidus* Péringuey, Anostostomatidae) from southern Africa

H. Brettschneider, C.T. Chimimba, A.D.S. Bastos, C.H. Scholtz and P.W. Bateman

Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002 South Africa

A molecular and morphometric analysis of tusked king crickets (*Libanasidus* Péringuey, Anostostomatidae) from southern Africa

H. Brettschneider, C.T. Chimimba, A.D.S. Bastos, C.H. Scholtz and P.W. Bateman

Department of Zoology & Entomology, University of Pretoria, Pretoria, 0002 South Africa

ABSTRACT Evaluation of the genetic and morphometric structuring within *Libanasidus vittatus* from southern Africa consistently suggests two main population assemblages. COI sequence data recovered two clades representing these two populations with good bootstrap support in likelihood, parsimony, Bayesian and distance analyses. Genetic divergence between the two clades averaged 3.3 %, while population parameters estimated using maximum likelihood methods show low migration rates corresponding to less that one female migration per generation. Canonical variates (discriminant) analysis (CVA) also showed evidence of two phenetic assemblages that suggest the presence of two cryptic species. *Libanasidus vittatus* is the recognized species occurring within the eastern population of South Africa (Mpumalanga & Eastern Cape Provinces), while a possible novel species occurs to the north and west (Limpopo & Gauteng Provinces). On imposing a molecular clock of 2 % divergence per million years used previously for other Anostostomatidae taxa, time to coalescence between the two major populations is estimated to be approximately 1.65 mya, and corresponds to the presence of isolated forest pockets during the dry Pleistocene era. Further investigation, including cytogenetic, multi-locus sequence analysis, geometric morphometric and behavioural techniques are needed to confirm species status and delineate geographic boundaries.

KEY WORDS *Libanasidus*, molecular phylogeny, morphometrics, COI gene, southern Africa, systematics, taxonomy

INTRODUCTION

Libanasidus vittatus (Orthoptera: Ensifera: Anostostomatidae), colloquially known as the Parktown Prawn, is a common and legendary cricket in suburban gardens in Pretoria and Johannesburg, Gauteng Province, South Africa. This large, omnivorous insect is famous for its fascinating biology and behaviour that includes squirting vile-smelling faeces at offenders (McDonald & Hanrahan 1993; Brettschneider & Bateman 2005; Wolf *et al*. 2006). The species is sexually dimorphic, with males possessing large curved 'tusks' on their mandibles that are used in male-male competition (Bateman 2000). It is nocturnal, remaining in self-constructed burrows in soft moist soil during daylight, which may partly account for the general lack of knowledge on their distributional range.

It is has been reported that *L. vittatus* may originally have been localised to the indigenous forests of the eastern parts of South Africa, especially along the Soutpansberg and Drakensberg mountain ranges (Toms 1985; McDonald & Hanrahan 1993). It is believed that this was followed by an invasion of urban areas in the 1960s that was promoted by artificial, warm, damp habitats with soft, moist soils in gardens and the absence of natural predators (Toms 1985). Of the four South African provinces where *L. vittatus* occurs, Gauteng Province is characterised by Moist Highveld Grassland centrally and Central Bushveld in the north, and Limpopo Province consists of Northern Arid Bushveld in the north, Central Bushveld centrally and westerly, Lowveld Mountain Bushveld and Lowveld

Bushveld to the south-east. Mpumalanga Province shows a continuation of the Lowveld Bushveld (eastern border) and Lowveld Mountain Bushveld (centrally) regions of Limpopo Province, Eastern Mountain Grassland occurs on the western border and stretches south along the Drakensberg mountain range (Kruger 2004). The Lowveld Mountain Bushveld still contains pockets of Afromontane forest that is naturally inhabited by *Libanasidus*. From the Eastern Cape Province, *L. vittatus* has been collected only from Port St. Johns, where the high rainfall South-east Coast grassland dominates.

The subfamily Anostostomatinae Saussure, 1859 to which *L. vittatus* belongs shows a discontinuous distribution across Africa, Australasia, South and Central America and Asia, and is represented by six tribes primarily in the southern hemisphere (Karny 1931, Johns 1997, Gorochov 2001). Seven genera belonging to the tribe Anostostomatini Saussure, 1859 have been recorded from South Africa, Angola, Mozambique and Zimbabwe, with representatives of the tribe also occurring as far north as the Democratic Republic of Congo (formerly Zaire) and Tanzania (Johns 1997; Otte *et al*. 2005). Within this tribe, the sister taxa of *Libanasidus* include: *Bochus* Péringuey, 1916; *Borborothis* Brunner von Wattenwyl, 1888; *Henicus* Gray, 1837; *Nasidius* Stål, 1878; *Onosandrus* Stål, 1878; and *Onosandridus* Péringuey, 1916. The genus *Onosandrus* also occurs in New Zealand and South Australia while all the other genera are restricted to the African continent (Karny 1931).

The bulk of taxonomic work on the Anostostomatidae occurred between 1803 and 1943. The latest taxonomic revision (Johns 1997), after a dormant period, focused on the diversity of the known species within the family. The earlier works, however, are replete with synonymies, misspellings and incorrect identifications, not to mention the continuous reshuffling of taxa, resulting in more taxonomic confusion for the group (see Karny 1930; Ander 1943; Toms 1986a & b). These earlier studies largely focussed on higher taxonomic ranks and their relationships, except for Ander (1943) who revised some nomenclatural disparities and synonymies in anostostomatid genera and their sister taxa. There are no recent reviews that focus on taxonomic levels lower than the superfamily.

Kirby (1899) first described what is presently referred to as *L. vittatus* based on two female specimens from Barberton, Mpumalanga Province, South Africa, of which the type specimens are located in the Natural History Museum (London) (Péringuey 1916). He provisionally placed the species in the family Stenopelmatidae, and referred to it as *Carcinopsis vittata* (Péringuey 1916). The species was subsequently included in the new genus *Libanasidus* by Péringuey (1916). Currently, the genus is considered to contain two species, *L. vittatus* and *L. impicta*. The latter, described by Stål (1878) was, until recently, regarded a *nomen dubium*, as it was described from a single and possibly immature female and is rarely mentioned in the taxonomic literature (Johns 1997; H. Brettschneider unpubl. data).

A preliminary morphometric analysis (P.W. Bateman, unpubl. data) suggested that the 'Parktown Prawn' from South Africa might contain a complex of species. Consequently, the aim of the present study is to assess variation within *Libanasidus* using both morphometric and molecular techniques in an attempt to elucidate the taxonomic status of the species in southern Africa.

MATERIALS AND METHODS

Molecular analysis

Twenty-one individuals of *Libanasidus* from three South African provinces that include the eastern Mpumalanga (MP), as well as the northern Limpopo (LP) and western Gauteng (GP) were used in this analysis (Figure 4.1a). Individuals were either collected by hand or obtained from the Transvaal Museum (TM insect collection). DNA extraction from muscle tissue of the hind femora followed the Roche extraction protocol according to the manufacturer's specifications (Roche Diagnostics, Mannheim, Germany). DNA from the mitochondrial gene, corresponding to the 3' half of the mitochondrial cytochrome oxidase I (COI) was amplified by the polymerase chain reaction (PCR) and sequenced with the universal insect primers C1-J-2183 (Simon *et al*. 1994) and L2-N-3014 (Simon *et al*. 1994) using an automated sequencing approach (Simon *et al*. 1994; Rokas *et al*. 2002). The Chilean red cricket, *Cratomelus armatus* Blanchard, 1851 (Anostostomatidae: Cratomelinae) was used as an outgroup in all phylogenetic analyses. The 21 generated sequences are deposited in GenBank under accession numbers DQ 204406-204416, DQ204418-204423, and DQ204425-204428.

Sequence data were edited using BioEdit version 5.0.6 (Hall 1999) and aligned in DAPSA version 4.91 (Harley 2001). The best-fit model (GTR+I, equal rates) selected under the Akaike Information Criterion (AIC) in Modeltest version 3.06 (Posada & Crandall 1998) was specified for Maximum Likelihood analysis. Maximum Likelihood and parsimony analysis were performed in PAUP* version 4.0 b10 (Swofford 1999). All characters were initially treated as unordered and of equal weight for parsimony analyses, prior to investigating the effect of different weighting schemes. These included *a priori* weighting schemes where: (i) the third codon position was down-weighted to one third of the value of the 1st and 2nd codon positions (due to a higher tendency of becoming saturated) (Chapco *et al.* 2001) and (ii) 6-parameter parsimony weighting (Williams & Fitch 1990); as well as *a posteriori* successive weighting based on a rescaled consistency index (RCI) (Farris 1969; Forey *et al*. 1992). All distance analyses were carried out with MEGA version 3 (Kumar *et al*. 2004) and employed the Tamura-Nei model of sequence evolution. Non-parametric bootstrap resampling was used to assess nodal support for the clades recovered (Felsenstein 1985). Bayesian phylogenetic analyses were performed with MrBayes version 3.1 (Huelsenbeck & Ronquist 2001), using the model parameters identified with Modeltest.

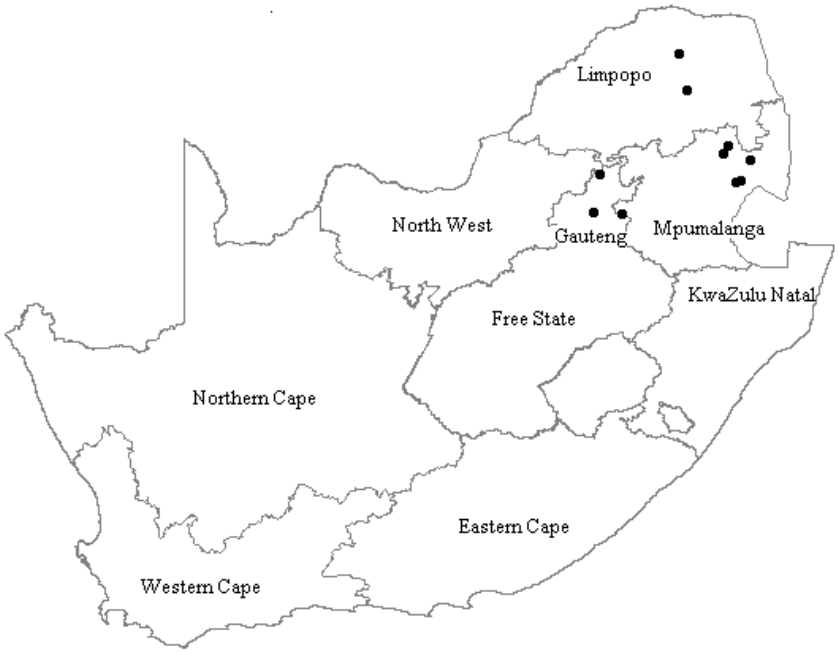
In order to infer evolutionary rates, the constancy of a molecular clock was tested between lineages using Phyltest Version 2.0 (Kumar 1996). The probability of rejecting the molecular clock hypothesis was also tested in PAUP* by comparing log likelihood values and branch lengths of trees constructed with and without the constraint of a molecular clock under the

HKY85 model of sequence evolution (Hasegawa & Kishino 1994; Sole *et al*. 2005).

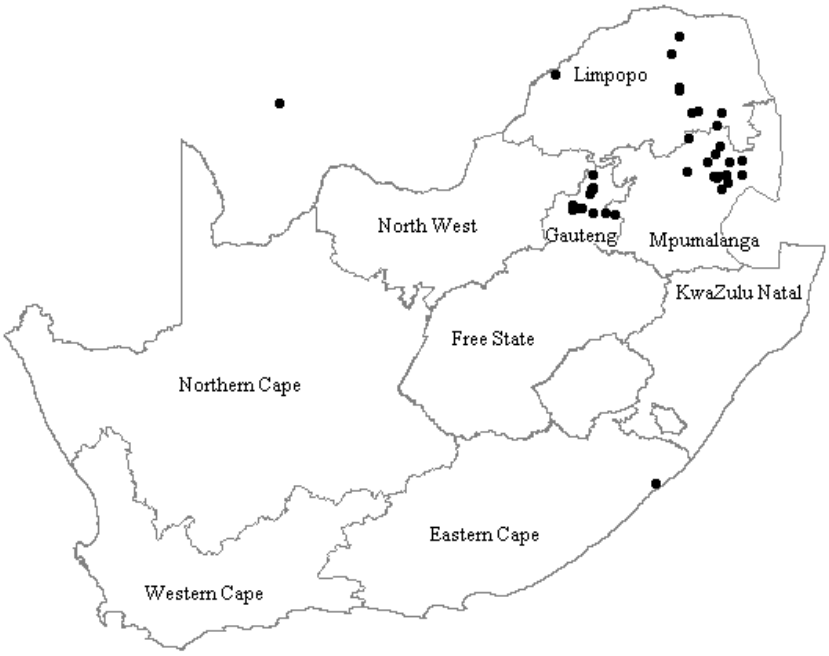
Historical estimates of gene flow between the clades obtained from phylogenetic analysis were estimated with Migrate 2 (Beerli & Felsenstein 1999, 2001). The data were run with 10 short chains (10000 trees sampled, 500 used) and 5 long chains (10000 trees sampled and 5000 used).

Morphometric analysis

The analysis of morphometric variation within *L. vittatus* was based on 206 specimens (85 males; 121 females) collected between 1924 and 2003 from southern Africa, which represents the largest sample analysed for the species in the subregion (Figure 4.1b). Due to difficulties encountered with obtaining fresh adult material and with the sequencing of museum-preserved material, only 10 specimens from the genetic study were available for the morphometric analysis. The material examined and a gazetteer are listed in Appendix I, while geographic coordinates for all localities and their associated climatic regions and annual rainfall are listed in Appendix II (Kruger 2004).



a



b

Figure 4.1. Collection localities of *Libanasidus* in southern Africa used in (a) genetic and (b) traditional morphometric analysis.

Twenty-four male and twenty-five female external morphological measurements were selected and included characters used previously in the identification of species (Field & Bigelow 2001). The measurements

were recorded to the nearest 0.05 mm by one observer (H.B.) using a pair of Mitutuyo® digital callipers. Some measurements, such as tympanal length and width were made with a microscope ocular meter (Wild 10X/21). All measurements are illustrated and described in Figure 4.2 and Appendix III, respectively.

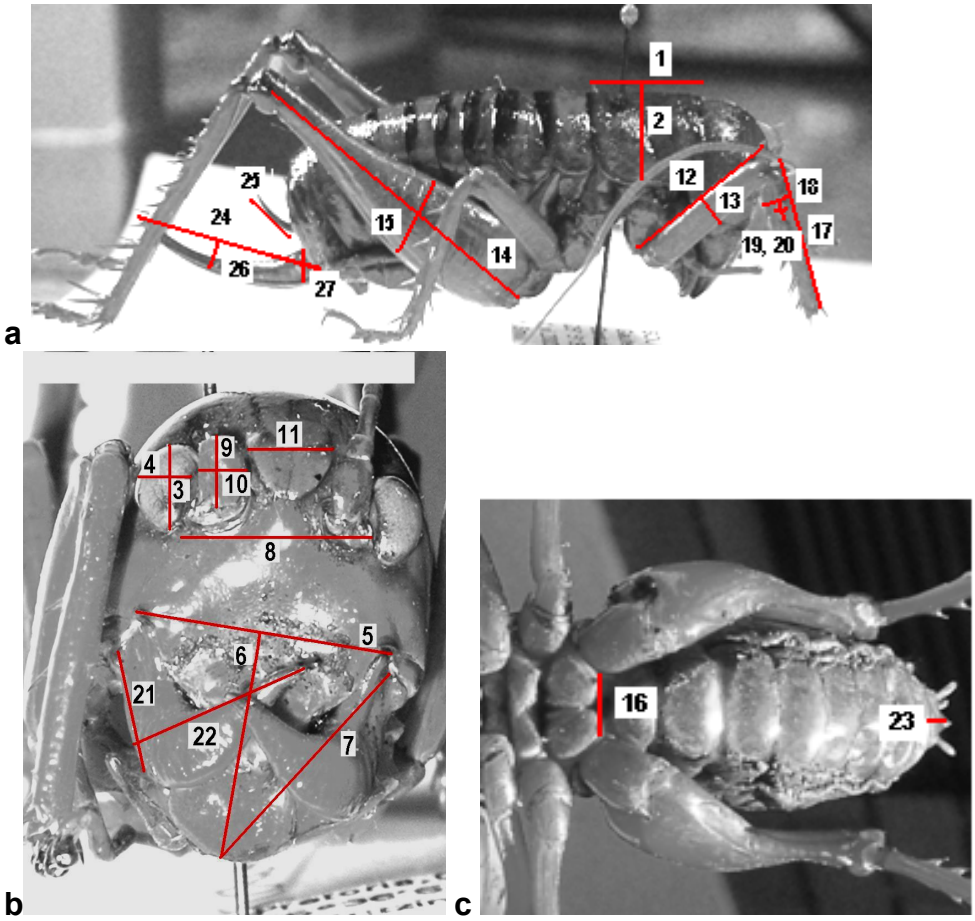


Figure 4.2. Lateral (a), frontal (b) and ventral (c) views of *Libanasidus* from southern Africa illustrating 27 external morphological characters used for morphometric analysis. Characters are described in Appendix III.

To reduce the effect of age variation, all measurements were recorded from adult specimens. Individuals of *L. vittatus* are variable in size and the final instar may show secondary sexual characteristics. Consequently, females were regarded as mature when the ovipositor apex was

sclerotised (see Koning & Jamieson 2001) and adult males were identified based on the presence of the mandibular tusks. In addition, the presence of long, curved, and flexible cerci was assumed an adult characteristic in both sexes (see Koning & Jamieson 2001). The obvious presence of sexual dimorphism justified separate morphometric analysis of males and females. To facilitate the simultaneous analysis of males and females, a third data set that excluded gender-specific characters was also compiled.

Morphometric analyses were based on *a priori* multivariate analyses of standardized measurements that included principal components analysis (PCA) and an unweighted pair group arithmetic average (UPGMA) cluster analysis (Sneath & Sokal 1973) of the 206 specimens. The PCA was performed on product-moment correlation coefficients among variables, whereas the UPGMA cluster analysis was based on both Euclidean distances and correlation coefficients (Sneath & Sokal 1973). Genetically-identified individuals were used as references in all morphometric analyses to delineate phenetic boundaries in multivariate space.

The phenetic groups obtained from the *a priori* multivariate analyses with reference to genetic data were further subjected to *a posteriori* canonical variates (discriminant) analysis (CVA; Sneath & Sokal 1973). Statistically significant differences between group centroids were tested using multivariate analysis of variance (MANOVA; Zar 1996). Additional analyses included the generation of standard descriptive statistics for each delineated phenetic group (Table 4.1). All morphometric statistical

procedures were accomplished using algorithms in STATISTICA 6.1 (Statsoft 2004).

RESULTS

Molecular assessment

The COI sequence data comprised 758 nucleotide sites of which 642 were conserved and 116 were variable. Fifty-three of the latter were parsimony informative. A strong A-T bias (69.6 %) was observed for the dataset with Cs and Gs accounting for 15.4 % and 14.8 % of sites, respectively. Transitional mutations predominated (R = 2.8) and low levels of homoplasy were observed for the dataset as exemplified by a consistency index (CI) of 0.83, a retention index (RI) of 0.89, and a rescaled consistency index (RCI) of 0.74. Tree topology remained constant between distance, parsimony, likelihood and Bayesian analyses.

The strict consensus from 81 equally parsimonious trees (TL = 155, unweighted) derived two clades and is illustrated in Figure 4.3. High bootstrap values (90-100%) for clade A support the clustering of all specimens from the East and North (Limpopo and Gauteng Provinces). Bootstrap values for clade B, encompassing all specimens collected from the West (Mpumalanga Province) are lower (60-77%), but the clade is recovered consistently in all analyses. Resolution within clade A revealed a close association between specimens from Alberton (GP) and Louis Trichardt (LP) (84-100% support), and individuals from Pretoria (GP) and Klipreviersberg Nature Reserve (GP) to those from Magoebaskloof (LP) and Tzaneen (LP) (52-63% support). Relationships within the eastern

clade B were more resolute, with two individuals from Barberton grouping together with 74-100% bootstrap support, while individuals from Bridal Veil Falls, Graskop, Nelspruit and Pretorius Kop clustered together with 89-100% bootstrap support. Trees constructed from weighted characters did not improve resolution or bootstrap support within the two major clades and resulted in trees of increased length.

Uncorrected p-distances calculated in PAUP* and illustrated in Appendix IV reveal a maximum pairwise sequence divergence of 5 % between an Alberton (GP) specimen and a Graskop (MP) specimen. The average sequence divergence between the north-western Clade A and the eastern Clade B was 3.3 %. Genetic distance within Clade B ranges from 0-2.4 % (average = 2.3 %) (Barberton ↔ Pretorius Kop & Graskop), and within Clade A, from 0-3.7 % (average = 3.1 %) (Pretoria ↔ Alberton). The molecular clock hypothesis could not be rejected under the 95 % (P < 0.05) confidence interval in either Phyltest or PAUP* where likelihood values obtained for trees with and without enforcing a molecular clock were –log 1837.90955 and –log 1742.02460, respectively.

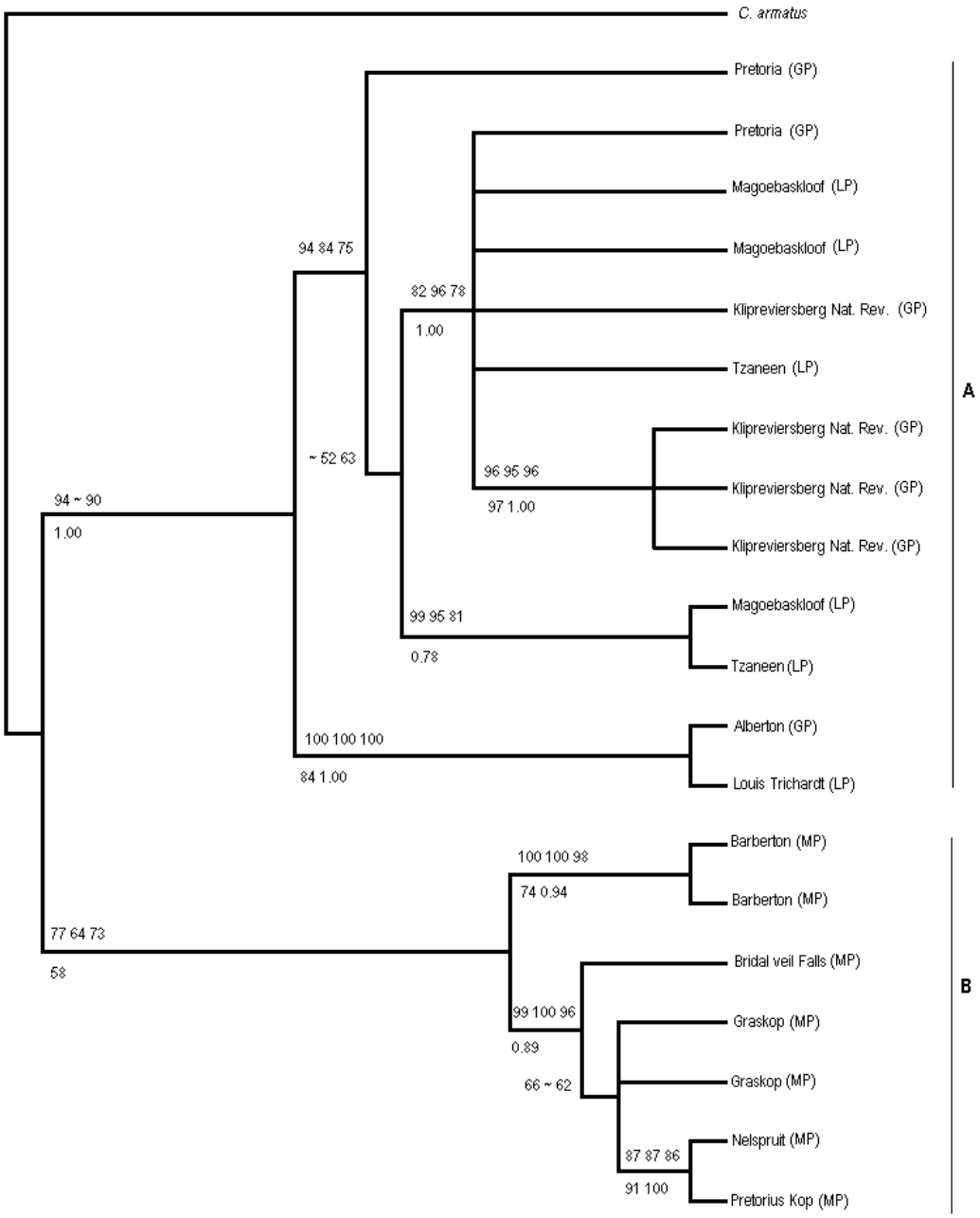


Figure 4.3. Consensus tree resulting from unweighted maximum parsimony analysis of COI sequence data of *Libanasidus* from South Africa, showing bootstrap support from NJ, ME and MP analyses, respectively, above branches and ML and Bayesian branch support below. Tree length = 155, CI = 0.83, RI = 0.89, RCI = 0.74. Provincial location is abbreviated as GP = Gauteng LP = Limpopo and MP = Mpumalanga.

Population parameters estimated with maximum likelihood in Migrate 2 (Beerli & Felsenstein 1999, 2001) are shown in Table 4.1. Levels of past gene flow (as represented by female migrations per generation) was

highest from the north-western group (Clade A) to the western group where $2N_2\ m_{21}$ = 0.311. No migration events were detected from the eastern group to the north-western group ($2N_1\ m_{12}$ = 0.00). Likelihood estimation of present day effective female population size ($\theta = 2N_e\ u$) suggests that the north-western population is slightly larger (Table 4.1).

Table 4.1. Effective female population size ($\theta = 2N_e\ u$) values in brackets correspond to 95% confidence intervals. Migration rates (values in brackets in terms of $2N_e\ m$) estimates for *Libanasidus* from South Africa. Clade A corresponds to individuals from the north-western population (Gauteng and Limpopo Provinces), while Clade B represents individuals from the eastern population (Mpumalanga Province).

	To	**From**	
	Θ	**Clade A**	**Clade B**
Clade A	**0.01800** (0.010609-0.032396)	-	**0.00**
Clade B	**0.01223** (0.005726-0.28540)	**0.311** (0.146-7.258)	-

Morphometric assessment

Neither the PCA nor both distance and correlation phenograms showed any geographically discernable phenetic pattern in all three combinations of data analysed (males, females and males & females combined). No pattern was visible with reference to the molecular data either (results not shown). A two-group CVA that specified the eastern and north-western specimens as *a posteriori* groups based on the results of the molecular data, however, suggest the presence of two phenetic groups that are congruent with the molecular data (Figure 4.4).

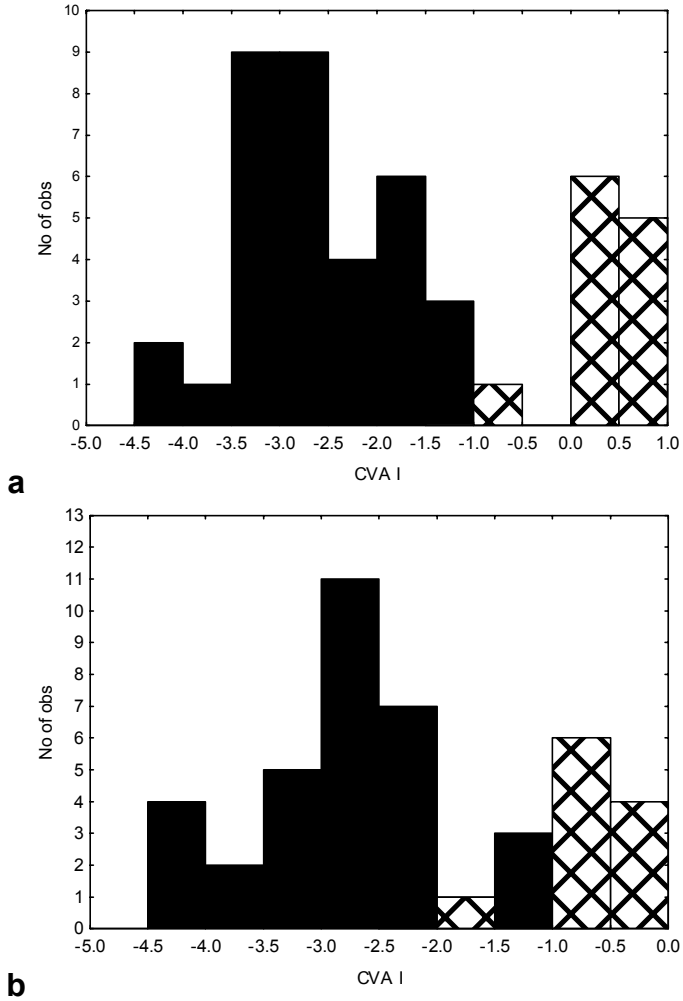


Figure 4.4. Histogram of CVA I scores extracted from canonical variates analysis (CVA) for *Libanasidus* males (a) and females (b). Solid bars symbolize the eastern group (Mpumalanga Province), and shaded bars indicate the north-western (Gauteng and Limpopo province group). $F = 11.66$, $P < 0.00$ (♂) and $F = 10.98$, $P < 0.00$ (♀).

A MANOVA of the centroids of these two groups showed a statistically significant morphological difference between the eastern and north-western phenetic groups (Males: F = 11.66, P < 0.000; Females: F = 10.98, P < 0.000). Table 4.2 shows that pronotum length (C1), head width (C5), mandible length (C7), front leg femur width (C13), front tibia length (17), front tibia width (C18), tusk base thickness (C21) and tusk length (C22) are fundamental in causing the separation between males, while pronotum length (C1), eye length (C3), hind femur length (C14) and hind

femur width (C15) are responsible for separation in the females. Standard statistics on these groups are presented in Table 4.3 for males and females. All the descriptive data indicate that the phenetic differences between groups within the sexes are subtle, suggesting the presence of cryptic taxa.

Table 4.2. Canonical variate loadings from a canonical variates analysis (CVA) of Libanasidus from southern Africa. Measurements are defined and illustrated in Appendix III and Figure 4.3, respectively.

	Males	**Females**
Character	**CVA I**	**CVA I**
Pronotum length	-0.82	-0.81
Pronotum width	-0.24	0.42
Eye length	-0.54	-0.78
Eye width	-0.53	-0.03
Head width	1.22	0.56
Labrum length	-0.18	0.07
Mandible length	0.83	0.59
Distance between eyes	-0.28	-0.11
Antennal scape length	-0.39	-0.53
Antennal scape width	-0.19	0.15
Fastigium width	0.38	0.06
Front leg femur length	-0.68	-0.22
Front leg femur width	0.93	-0.04
Hind femur length	-0.46	-2.52
Hind femur width	0.51	2.21
Metabasisternum width	0.00	0.32
Front tibia length	-3.81	-0.45
Front tibia width	3.48	0.58
Tympanum length	0.54	0.00
Tympanum width	-0.31	-0.02
Tusk base thickness	1.31	-
Tusk length	-1.26	-

Subgenital plate length	-0.10	-0.23
Ovipositor length	-	-0.09
Cerci length	-0.09	0.26
Ovipositor curvature	-	0.03
Ovipositor base thickness	-	0.51

Table 4.3. Standard statistics of external morphological measurements of *Libanasidus* from southern Africa. *n* = sample size; *Mean* = arithmetic mean; *Range* = observed range; *SD* = standard deviation; *CV* = coefficient of variation.

	Males		**Females**	
	Eastern	**North-western**	**Eastern**	**North-western**
1 Pronotum length				
n	49	35	86	34
Mean	8.47	8.18	8.39	8.35
Range	6.37-11.08	7.2-10.49	6.17-10.19	6.62-10.79
SD	0.72	1.00	0.69	0.78
CV	8.55	12.22	8.22	9.34
2 Pronotum width				
n	49	35	86	34
Mean	10.79	9.63	10.52	9.37
Range	7.46-14.16	8.53-13.76	7.43-12.65	1.11-13.92
SD	1.18	1.43	1.41	1.30
CV	10.89	14.85	13.40	13.87
3 Eye length				
n	49	35	86	34
Mean	2.95	2.93	2.80	2.89
Range	2.2-3.87	2.31-4.00	2.19-3.47	2.33-3.38
SD	0.31	0.34	0.21	0.26
CV	10.58	11.60	7.50	9.00
4 Eye width				
n	49	35	86	34
Mean	2.17	2.16	2.04	2.08
Range	1.25-2.89	1.38-2.98	1.31-2.54	1.58-2.71
SD	0.34	0.36	0.22	0.23
CV	15.74	16.67	10.78	11.06
5 Head width				
n	49	35	86	34
Mean	8.18	7.37	6.95	6.47
Range	4.84-10.61	5.3-11.49	4.84-7.79	5.7-8.49
SD	1.22	1.32	0.62	0.63
CV	14.90	17.91	8.92	9.74
6 Labrum length				
n	49	35	86	34
Mean	6.69	7.04	6.61	6.67
Range	4.67-9.89	4.76-9.42	4.92-8.02	2.42-8.85

SD	1.77	1.09	1.24	1.32
CV	26.46	15.48	18.76	19.79
7 Mandible length				
n	49	35	86	34
Mean	7.19	6.62	6.51	6.19
Range	3.77-9.09	4.83-9.2	4.5-7.26	5.29-8.69
SD	0.91	1.01	0.59	0.57
CV	12.61	15.26	9.06	9.21
8 Distance between eyes				
n	49	35	86	34
Mean	5.29	4.92	4.69	4.58
Range	3.22-7.66	3.69-7.21	3.54-5.36	3.38-6.29
SD	0.75	0.89	0.72	0.44
CV	14.11	18.09	15.35	9.61
9 Antennal scape length				
n	49	35	86	34
Mean	2.17	2.30	2.09	2.25
Range	1.7-3.12	1.59-2.66	1.63-2.51	1.62-2.54
SD	0.40	0.27	0.21	0.19
CV	18.67	11.74	10.05	8.44
10 Antennal scape width				
n	49	35	86	34
Mean	1.36	1.32	1.24	1.22
Range	0.91-1.88	0.95-1.87	0.98-1.61	0.8-2.12
SD	0.29	0.19	0.17	0.13
CV	21.25	14.39	13.71	10.66
11 Fastigium width				
n	49	35	86	34
Mean	2.60	2.40	2.42	2.18
Range	1.63-3.62	1.88-3.63	1.44-2.75	1.77-5.51
SD	0.35	0.46	0.42	0.26
CV	13.45	19.17	17.36	11.93
12 Front leg femur length				
n	49	35	86	34
Mean	10.68	10.96	10.31	10.71
Range	8.32-14.53	8.36-13.2	8.94-12.45	7.38-12.75
SD	1.93	2.32	1.38	2.05
CV	18.07	21.17	13.39	19.14
13 Front leg femur width				
n	49	35	86	34
Mean	2.22	2.08	2.12	2.04
Range	1.69-3.04	1.89-2.98	1.47-2.49	1.46-3.05
SD	0.43	0.46	0.34	0.22
CV	19.53	22.12	16.04	10.78
14 Hind femur length				
n	49	35	86	34
Mean	22.08	23.26	22.45	25.28
Range	19.26-30.84	19.76-27.73	19.96-29.11	16.41-28.94
SD	6.01	6.31	5.28	2.00
CV	27.23	27.13	23.52	7.91
15 Hind femur width				

n	49	35	86	34
Mean	6.63	6.57	6.69	7.06
Range	5.33-9.57	6.1-8.68	5.44-8.81	5.48-9.45
SD	1.85	1.88	1.64	0.80
CV	27.85	28.61	24.51	11.33
16 Metabasisternum width				
n	49	35	86	34
Mean	3.17	2.87	3.18	2.95
Range	1.13-10.68	2.35-4.88	2.44-3.58	2.27-4.03
SD	0.49	1.45	0.37	0.29
CV	15.44	50.52	11.64	9.83
17 Front tibia length				
n	49	35	86	34
Mean	9.70	11.47	10.39	11.26
Range	8.06-14.88	8.81-14.66	9.14-13.39	2.17-13.92
SD	4.15	2.45	2.21	2.20
CV	42.75	21.36	21.27	19.54
18 Front tibia width				
n	49	35	86	34
Mean	1.33	1.36	1.46	1.33
Range	1.01-2.08	1.14-1.99	1.11-1.78	1.23-2.11
SD	0.58	0.34	0.28	0.28
CV	43.66	25.00	19.18	21.05
19 Tympanum length				
n	49	35	86	34
Mean	1.23	1.26	1.32	1.33
Range	0.93-1.89	0.83-2.31	0.94-1.82	0.94-2.07
SD	0.57	0.38	0.36	0.30
CV	46.10	30.16	27.27	22.56
20 Tympanum width				
n	49	35	86	34
Mean	0.76	0.77	0.81	0.79
Range	0.7-1.11	0.55-1.29	0.6-1.16	0.61-1.33
SD	0.36	0.23	0.22	0.20
CV	46.63	29.87	27.16	25.32
23 Subgenital plate length				
n	49	35	86	34
Mean	2.88	2.92	2.42	2.49
Range	2.08-3.72	2.18-3.8	3.35-5.55	2.73-5.37
SD	0.72	0.66	0.59	0.48
CV	24.92	22.60	24.38	19.28
25 Cerci length				
n	49	35	86	34
Mean	4.53	4.56	4.29	4.16
Range	3.35-5.88	2.89-5.96	1.41-3.65	1.36-3.59
SD	1.12	0.98	1.03	1.15
CV	24.80	21.49	24.01	27.64
21 Tusk base thickness				
n	49	35	-	-
Mean	4.52	3.78	-	-
Range	1.72-5.58	2.36-6.05	-	-

SD	0.89	0.77	-	-
CV	19.73	20.37	-	-
22 Tusk length				
n	49	35	-	-
Mean	6.94	7.30	-	-
Range	0.95-12.68	0.86-13.21	-	-
SD	3.52	2.80	-	-
CV	50.70	38.36	-	-
24 Ovipositor length				
n	86	34	-	-
Mean	17.58	15.44	-	-
Range	6.59-20.28	10.01-19.36	-	-
SD	9.30	4.59	-	-
CV	52.88	29.73	-	-
26 Ovipositor curvature				
n	-	-	86	34
Mean	-	-	2.46	2.35
Range	-	-	1.41-3.65	1.36-3.59
SD	-	-	0.47	0.64
CV	-	-	19.02	27.23
27 Ovipositor base thickness				
n	-	-	86	34
Mean	-	-	2.88	2.66
Range	-	-	1.47-3.56	1.55-3.72
SD	-	-	0.50	0.60
CV	-	-	17.34	22.56

DISCUSSION

Mitochondrial sequence analysis reveals two clades with clear separation between the north-western (Gauteng and Limpopo) group and the eastern (Mpumalanga) group. This finding is strengthened by the congruence between distance, cladistic and Bayesian methods, showing good bootstrap support. COI sequence divergence is maximal at 5 % with an average divergence of 3.3 % between Clade A corresponding to the north-western population and Clade B representing the eastern population. Although variation within other anostostomatid species seems to vary considerably, with values of up to 7.6 % sequence divergence being reported (Trewick *et al*. 2000), investigation of past gene flow

between the two specified populations indicates that these are isolated populations with less that one migrating female per generation from the north-western population to the eastern population ($2N_1\ m_{12} = 0.311$) and no detectable migration from the eastern to the north-western population ($2N_2\ m_{21} = 0.000$).

Morphological assessment of variation between the two prominent populations within *Libanasidus* confirmed the presence of two phenetic groups corresponding to the molecular clades. Distinguishing characters between the north-western and eastern populations revealed by discriminant analysis (CVA) include pronotum length (C1), head width (C5), mandible length (C7), front leg femur width (C13), front tibia length (17), front tibia width (C18), tusk base thickness (C21) and tusk length (C22) for the males and pronotum length (C1), eye length (C3), hind femur length (C14) and hind femur width (C15) for females.

Congruence between genetic and morphometric structuring within *Libanasidus* from southern Africa suggests two main population assemblages. The morphological and genetic similarity of *Libanasidus* individuals collected from areas in Limpopo and Gauteng provinces suggest a continuous North-South distribution of the species, connecting the two collection localities. Within the eastern population, individuals collected from the Eastern Cape grouped morphologically with the eastern group from Mpumalanga, and suggests that the geographic distribution of this group may extend as far south as Port St. Johns (Eastern Cape), although this could not be confirmed with molecular data in this study).

Additionally, the lack of migrational events between these north-western and eastern populations suggests a dispersal barrier. In interpreting isolation events, invertebrate studies generally use divergence rates of 1.5-2.3 % per million years (Trewick & Wallis 2001, Pestano *et al.* 2003). As the molecular clock hypothesis was not rejected for the COI sequence data according to the rate homogeneity tests performed, a molecular clock was imposed using the Anostostomatidae rate of 2.0 % per my used by Trewick (2005) for related weta species. Although the genetic data set appears small, it falls within the optimal sample and amplicon size suggested for the population genetic studies by Pluzhnikov & Donnelly (1996). Consequently, we can assume that gene flow and coalescence estimates obtained in this study are representative and that the dating and migration values obtained will hold.

Correlation of the proposed separation of the two populations with Palaeontological environmental changes revealed recurrent ice ages targeting the Northern Hemisphere during the last 2 million years (Eeley *et al*. 1999). This affected southern Africa with a general increase in aridity due to decreased rainfall and the subsequent decline in vegetation (McCarthy & Rubidge 2005, Eeley *et al*. 1999). Alternating hyper- and hypothermal periods resulted in the expansion and contraction of forests due to the respective wet and dry spells. Forests may even have been eliminated at elevations above 1000 m above sea level, as is found along the Drakensberg mountain range (Stuckenberg 1969). At lower elevations, forests occurred in pockets, probably much as they do today.

Consequently, due to unfavourable terrain between populations and slow apteral dispersal, the two populations in this study most likely became isolated during the marked dry era between 1.8-1.6 mya due to the fragmentation of their habitat (McCarthy & Rubidge 2005; deMenocal 2004; Eeley *et al*. 1999). Reliance on moist habitats of *Libanasidus* probably also restricts movement across unfavourable terrain between neighbouring forest clumps.

This isolation of the eastern population is not morphologically conspicuous, with only subtle differences being identified by discriminant analysis (CVA). This suggests the presence of cryptic species possibly due to adaptation to similar habitats and the plasticity of morphological characters that could limit phenotypic separation between populations. Despite the taxonomic importance of many of the morphological characters included in this study (tympanal size, primary and secondary sexual characteristics) there is plasticity in the development of many structures since these crickets regenerate lost limbs and mouthparts, or develop malformed legs (McDonald & Hanrahan 1993; Flook *et al*. 2000). Regenerated appendages often differ in armature and structure from the original (H. Brettschneider pers. obs). Phenotypic plasticity is not restricted to *Libanasidus*, and high levels of homoplasy are evident in many of the taxonomically important morphological characters used in the generic classification within the Anostostomatidae (Chapter 3). Nevertheless, with genetic divergence similar to levels of divergence reported for anostostomatid sibling species (Trewick 2001), the data suggests that *Libanasidus* individuals found north and west (Gauteng and Limpopo

provinces) most likely belong to a novel cryptic species, which is presently identifiable with molecular techniques only. As the type locality of *Libanasidus vittatus* falls within the eastern population's distributional range (Barberton, Mpumalanga), this species would represent the eastern population.

In conclusion, agreement between the morphometric and genetic analysis supports the close association between *Libanasidus* from the northern province of Limpopo and those from western Gauteng region due to continued gene flow, suggesting an extended north-south range distribution of this new species. Almost no gene flow is apparent between this population and the population in the eastern Mpumalanga Province with molecular clock dating indicating that climatic changes in the Pleistocene era may be the underlying cause. Additional sampling of both *Libanasidus* species/populations throughout the proposed geographic range should be conducted to determine whether *Libanasidus vittatus* extends southwards to include individuals from the Eastern Cape. Elaboration of the techniques used in this study, comprising multiple genetic loci and geometric morphometrics is needed to confirm suggested trends in this study. Possible differences in chromosome number and morphology along with behavioural differences would support the suggested speciation events. Additionally, extensive sampling of areas between the three collection localities in this study would be useful in delineating geographic boundaries and causal paleoclimatic changes.

ACKNOWLEDGEMENTS

The authors wish to thank Rob Toms, Barbara Dombrowsky and Klaas Manamela (Transvaal Museum (TM), Pretoria, South Africa), Ross Urban (Agricultural Research Council (ARC), Pretoria, South Africa) and Andrés Angulo (Universidad de Concepción, Chile) for logistical support and specimen loans. We would like to thank Glen Malherbe for training and advice with molecular laboratory work while G. Grobler, L. Rotherham, L. Hart and N. Maputla assisted with analyses. The study was funded by the University of Pretoria and the South African National Research Foundation (NRF) under grant GUN 2053665 and is gratefully acknowledged.

REFERENCES

Ander, K. 1943. Uber die von Stål beschriebenen Stenopelmatiden (Saltatoria). *Entomologisk Tidskrift* **64**: 198-201.

Bateman, P.W. 2000. The influence of weapon asymmetry on male-male competition success in a sexually dimorphic insect, the African king cricket *Libanasidus vittatus* (Orthoptera: Anostostomatidae). *Journal of Insect Behavior* **13**: 157-163.

Beerli, P. & Felsenstein J. 1999. Maximum likelihood estimation of migration rates and population numbers of two populations using a coalescent approach. *Genetics* **152**: 763-773.

Beerli, P. & Felsenstein, J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Science of the United States of America* **98**: 4563-4568.

Brettschneider, H. & Bateman P.W. 2005. Differential shelter selection in response to predator chemical cues by two orthopterans: *Libanasidus vittatus* (Anostostomatidae) and *Platygryllus primiformis* (Gryllidae). *Journal of Insect Behavior* **18**: 381-387.

Chapco, W., Litzenberger, G. & Kuperus, W.R. 2001. A molecular biogeographic analysis of the relationship between North American melanoploid grasshoppers and their Eurasian and South American relatives. *Molecular Phylogenetics and Evolution* **18**:460-466.

deMenocal, P.B. 2004. African climate change and faunal evolution during the Pliocene-Pleistocene. *Earth and Planetary Science Letters* **220**: 3-24.

Eeley, H.A.C, Lawes, M.J. & Piper, S. E. 1999. The influence of climate change on the distribution of indigenous forest in KwaZulu-Natal, South Africa. *Journal of Biogeography* **26**: 595-617.

Farris, J.S. 1969. A successive approximations approach to character weighting. *Systematic Zoology* **18**: 374-385.

Felsenstein, J. 1985. Confidence estimates on phylogenies: An approach using bootstrap. *Evolution* **39**: 783-791.

Field, L.H. & Bigelow, S. 2001. Morphometric analysis of *Hemideina spp*. in New Zealand. In: *The Biology of Wetas, King Crickets and their Allies,* (Ed.) L.H. Field, pp. 163-177. CAB International, Wallingford.

Flook, P.K., Klee, S. & Rowell, C. H. F. 2000. Molecular phylogenetic analysis of the Pneumoroidea (Orthoptera, Caelifera): molecular data resolve morphological character conflicts in the basal Acridomorpha. *Molecular Phylogenetics and Evolution* **15**: 345-354.

Forey, P.L., Humphries, C.J., Kitching, I.L., Scotland, R.W., Siebert, D.J. & Williams, D.M. 1992. *Cladistics: A practical course in systematics*. Oxford University Press, New York.

Gorochov, A.V. 2001. The higher classification, phylogeny and evolution of the superfamily Stenopelmatoidea. *In: The biology of the Wetas, King Crickets and their Allies* (Ed.) Field, L.H. Pp 3-33. Cabi publishing, Wallingford UK.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**:95-98.

Harley, E.H. 2001. *DAPSA: A program for DNA and Protein Sequence Analysis*. Department of Chemical Pathology, University of Cape Town, South Africa.

Hasegawa, M. & Kishino, H. 1994. Accuracies of the simple methods for estimating the bootstrap probability of a Maximum-Likelihood tree. *Molecular Biology and Evolution* **11**:142-145.

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**:754-755.

Johns, P.M. 1997. The Gondwanaland weta: Family Anostostomatidae (formerly in Stenopelmatidae, Henicidae or Mimnermidae): nomenclatural problems, world checklist, new genera and species. *Journal of Orthoptera Research* **6**: 125-138.

Karny, H.H. 1930. A revision of the South African Gryllacrididae (Orthoptera: Saltatoria). *Annals of the South African Museum* **29**: 138-45.

Karny, H.H. 1931. On the geographical distribution of the Indo-African and Mediterranean Gryllacrids. *Transactions of the Royal Society of South Africa* **19**: 99-105.

Kirby, W.F. 1899. Notes on the collection of Gryllidae, Stenopelmatidae, Grylacrididae and Hetrodidae formed by Mr. W.L. Distant in the Transvaal and other South- and East-African Localities. *Annals and Magazine of Natural History* **3**: 33-35, 478-481.

Koning, J.W. & Jamieson, I.G. 2001. Variation in size of male weaponry in a harem-defence polygynous insect, the mountain stone weta *Hemideina maori* (Orthoptera: Anostostomatidae). *New Zealand Journal of Zoology* **28**: 109-117.

Kruger, A. 2004. Climate of South Africa – WS 45- Climate regions. South African Weather Service. www.weathersa.co.za/Publications/OrderList.jsp.

Kumar, S. 1996. PHYLTEST: phylogenetic hypothesis testing software, version 2.0, Pennsylvania State University, University Park, Pennsylvania.

Kumar, S., Tamura, K. and Nei, M. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics* **5**:150-163.

McCarthy, T. & Rubridge, B. 2005. The story of earth and life: A southern African perspective on a 4.6 billion year journey. Struik Publishers, Cape Town, South Africa.

McDonald, I.R. & Hanrahan, S.A. 1993. Aspects of the foraging and behaviour of the king cricket, *Libanasidus vittatus* (Kirby) (Orthoptera: Mimnermidae). *South African Journal of Science* **89**: 354-357.

Otte, D., Eades, D.C. & Naskrecki, P. 2005. *Orthoptera Species File Online* version 2.2. <http://osf2.orthoptera.org>.

Page, R.D. M. & Holmes, C.E. 1998. *Molecular evolution: a phylogenetic approach.* Blackwell Science Ltd, Oxford, London.

Péringuey, L. 1916. Descriptions of new or little known Orthoptera: Family Locustidae, Sub-Fam. Stenopelmatinae. *Annals of the South African Museum* **15**: 414-425.

Pestano, J., Brown, R.P., Suarez, N. M. & Baez, M. 2003. Diversification of sympatric *Sapromyza* (Diptera: Lauxaniidae) from Madeira: six morphological species but only four mtDNA lineages. *Molecular Phylogenetics and Evolution* **27**: 422-428.

Pluzhnikov, A. & Donnelly, P. 1996. Optimal Sequencing Strategies for Surveying Molecular Genetic Diversity. *Genetics* **144**: 1247-1262.

Posada D. & Crandall K.A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817-818.

Rokas, A., Nylander, J.A.A., Ronquist, F. & Stone, G.N. 2002. A maximum-likelihood analysis of eight phylogenetic markers in Gallwasps (Hymenoptera: Cynipidae): Implications for insect phylogenetic studies. *Molecular Phylogenetics and Evolution* **22**: 206-219.

Simon, C. Frati, F. Beckenbach, A., Crespi, B., Liu, H. & Flook, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a combination of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87**: 686-701.

Sneath, P.H.A. & Sokal, R.R. 1973. Numerical taxonomy: the principles and practice of numerical classifcation. W.H. Freeman & Company, San Francisco.

Sole, L.C., Scholtz, C. H. and Bastos, A.D.S. 2005. Phylogeography of Namib Desert dung beetles *Scarabaeus* (*Pachysoma*) MacLeay (Coleoptera: Scarabaeidae). *Journal of Biogeography* **32**:75-84.

Stål. C., 1878. Systema Acridiodeorum. Essai d'une Systématisation des Acridiodées. *Bihang till Kungliga Svenska Vetenskaps-Akademiens Handlingar* **5**:1-100.

StatSoft, Inc. 2004. STATISTICA. *Data analysis software system version 6*. www.statsoft.com.

Stuckenberg, B.R. 1969. Effective temperature as an ecological factor in southern Africa. *Zoologica Africana* **4**: 145- 197.

Swofford, D.L. 1999. PAUP*. *Phylogenetic analysis using parsimony (*and other methods)*, version. 4.0b10 Sunderland, Massachusetts: Sinnauer Associates.

Toms, R.B. 1985. Johannesburg's cricket invader: The 'Parkmore Prawn'. *African Wildlife* **39**: 200-202.

Toms, R.B. 1986 a. The scientific name of the ‘Parkmore Prawn’. *African Wildlife* **40**: 163.

Toms, R.B. 1986 b. The family name of the ‘Parkmore Prawn’. *African Wildlife* **40**: 263.

Trewick, S.A. & Morgan-Richards, M. 2005. After the deluge: mitochondrial DNA indicates Miocene radiation and Pliocene

adaptation of tree and giant weta (Orthoptera: Anostostomatidae). *Journal of Biogeography* **32**: 295-309.

Trewick, S.A. & Wallis, G.P. 2001. Bridging the "Beech Gap": New Zealand invertebrate phylogeography implicates Pleistocene glaciation and Pliocene isolation. *Evolution* **55**: 2170-2180.

Trewick, S.A. Wallis, G.P. & Morgan-Richards, M. 2000. Phylogeographical pattern correlates with Pliocene mountain building in the alpine scree weta (Orthoptera, Anostostomatidae). *Molecular Ecology* **9**: 657-666.

Williams, F. L. & Fitch, W. M. 1990. Phylogeny determination using a dynamically-weighted parsimony method. Pp. 615-625. In R. E Doolittle, (ed.) *Molecular evolution: computer analysis of protein and nucleic acid sequences. Methods in Enzymology, vol. 183. Academic Press, New York, NY.*

Wolf, S., Brettschneider, H. & Bateman, P.W. 2006. The predator defence system of an African King Cricket (Orthoptera; Anostostomatidae): does it help to stink? *African Zoology* **41:**75-80.

Zar, J.H. 1996. *Biostatistical Analysis*. 3rd edition. Prentice Hall, Inc., Upper Saddle River, New Jersey.

Appendix I. *Libanasidus* specimens examined and gazetteer, where * denotes individuals included in the molecular study.

FEMALES		MALES	
ID	Locality	ID	Location
5	Gauteng, Pretoria Newlands	4	Gauteng, Pretoria Brooklyn
6	Gauteng, Johannesburg Linmeyer	3	Gauteng, Pretoria Brooklyn
7	Gauteng, Johannesburg Parktown North	2	Gauteng, Pretoria Brooklyn
8	Gauteng, Pretoria Brooklyn	1	Gauteng, Johannesburg Linmeyer
*10	Mpumalanga, Pretorius Kop	30	Gauteng, Johannesburg Randburg
18	Gauteng, Pretoria	31	Gauteng, Johannesburg
32	Gauteng, Pretoria	15	Gauteng, Pretoria Arcadia
33	Gauteng, Pretoria	17	Gauteng, Pretoria Brooklyn
35	Gauteng, Pretoria	14	Gauteng, Pretoria Brooklyn
36	Gauteng, Pretoria	16	Gauteng, Johannesburg, Parktown North
37	Mpumalanga, Uitsoek Foreststation 1400	176	Gauteng, Pretoria Arcadia
38	Mpumalanga, Dullstroom	*177	Gauteng, Pretoria Arcadia
39	Mpumalanga, Dwarsrivier Valley	178	Gauteng, Pretoria Arcadia
46	Gauteng, Johannesburg Parktown North	056	Limpopo, Tzaneen Malta Forest
47	Gauteng, Johannesburg Parktown North	179	Mpumalanga, Nelshoogte Devils Knuckles
48	Gauteng, Johannesburg Parktown North	*59	Limpopo, Louis Trichardt Hangklip
49	Gauteng, Johannesburg Parktown North	61	Mpumalanga, Nelshoogte Devils Knuckles
50	Gauteng, Johannesburg Parktown North	73	Mpumalanga, Berlin Foreststation 1500
51	Gauteng, Johannesburg Parktown North	74	Mpumalanga, Berlin Foreststation-Karst Plateau
52	Gauteng, Johannesburg Parktown North	75	Mpumalanga, Nelshoogte, Devils Knuckles
53	Gauteng, Johannesburg Parktown North	76	Mpumalanga, Nelshoogte, Devils Knuckles
54	Gauteng, Johannesburg Parktown North	77	Mpumalanga, Berlin Foreststation-1500
55	Gauteng, Johannesburg Parktown North	78	Mpumalanga, Bourke's Luck
58	Limpopo, Tzaneen Malta Forest	79	Mpumalanga, Nelshoogte, Devils Knuckles
57	Limpopo, Louis Trichardt Hangklip	80	Mpumalanga, Nelshoogte Foreststation
62	Mpumalanga, Berlin Foreststation -1500	81	Mpumalanga, Berlin Foreststation
63	Mpumalanga, Nelshoogte Foreststation	82	Mpumalanga, Berlin Foreststation
64	Mpumalanga, Berlin Foreststation-Karst Plateau	83	Mpumalanga, Berlin Foreststation
65	Mpumalanga, Nelshoogte Devils Knuckles	86	Mpumalanga, Berlin Foreststation
67	Mpumalanga, Nelshoogte Devils Knuckles	88	Mpumalanga, Berlin Foreststation
69	Mpumalanga, Nelshoogte Devils Knuckles	*90	Mpumalanga, Nelspruit
70	Mpumalanga, Nelshoogte Devils Knuckles	91	Botswana Kakwe Pan, Kgalagadi Distr.
71	Mpumalanga, Nelshoogte Devils Knuckles	97	Mpumalanga, Berlin Foreststation
72	Mpumalanga, Nelshoogte Devils Knuckles	103	Mpumalanga, Nelspruit Nature Reserve
84	Mpumalanga, Berlin Foreststation	104	Mpumalanga, Nelspruit Nature Reserve
89	Mpumalanga, Berlin Foreststation	105	Mpumalanga, Nelspruit Nature Reserve
93	Botswana Kakwe Pan, Kgalagadi District	109	Eastern Cape, Port St. Johns, Silaka
94	Limpopo, Blyde River Canyon	108	Eastern Cape, Port St. Johns, Silaka
99	Mpumalanga, Berlin Foreststation 1500	107	Eastern Cape, Port St. Johns, Silaka
100	Mpumalanga, Berlin Foreststation, Karst Plateau	106	Eastern Cape, Port St. Johns, Silaka
101	Mpumalanga, Berlin Foreststation 1500	112	Eastern Cape, Port St. Johns, Silaka
102	Mpumalanga, Pullen Farm Krokodilpoortsberge	119	Eastern Cape, Port St. Johns, Silaka
113	Eastern Cape, Port St. Johns, Silaka	121	Eastern Cape, Port St. Johns, Silaka

114	Eastern Cape, Port St. Johns, Silaka
115	Eastern Cape, Port St. Johns, Silaka
116	Eastern Cape, Port St. Johns, Silaka
117	Eastern Cape, Port St. Johns, Silaka
118	Eastern Cape, Port St. Johns, Silaka
123	Gauteng, Pretoria
124	Gauteng, Irene
126	Gauteng, Roodepoort, Weltevreden Park
128	Limpopo, Lekgalameetse Nature Reserve
129	Limpopo, Lekgalameetse Nature Reserve
130	Limpopo, Lekgalameetse Nature Reserve
138	Gauteng, Pretoria, Brooklyn
139	Gauteng, Pretoria
140	Gauteng, Pretoria
141	Mpumalanga, Berlin Foreststation
143	Gauteng, Johannesburg Parktown North
144	Gauteng, Johannesburg Parktown North
145	Gauteng, Johannesburg Parktown North
146	Gauteng, Johannesburg Parktown North
147	Gauteng, Johannesburg Parktown North
148	Gauteng, Johannesburg Parktown North
149	Gauteng, Johannesburg Parktown North
151	Gauteng, Johannesburg Parktown North
152	Gauteng, Johannesburg Parktown North
154	Gauteng, Johannesburg Victory Park
159	Gauteng, Johannesburg Victory Park
160	Gauteng, Johannesburg Victory Park
161	Gauteng, Johannesburg Victory Park
163	Gauteng, Johannesburg Parktown North
164	Gauteng, Johannesburg Parktown North
163	Gauteng, Johannesburg Parktown North
163	Gauteng, Johannesburg Parktown North
163	Gauteng, Johannesburg Parktown North
126	Mpumalanga, Berlin Foreststation
170	Mpumalanga, Berlin Foreststation
172	Mpumalanga, Berlin Foreststation
173	Mpumalanga, Berlin Foreststation
174	Mpumalanga, Berlin Foreststation
175	Mpumalanga, Berlin Foreststation
182	Limpopo, Woodbush
183	Limpopo, Woodbush
184	Limpopo, Woodbush
185	Limpopo, Woodbush
189	Limpopo, Tzaneen Malta Forest
190	Gauteng, Johannesburg
191	Limpopo, Moordrift
192	Gauteng, Pretoria
193	Gauteng, Pretoria
194	Limpopo, Woodbush
196	Limpopo, Entabeni
122	Eastern Cape, Port St. Johns, Silaka
131	Limpopo, Lekgalameetse Nature Reserve
142	Mpumalanga, Berlin Foreststation
150	Gauteng, Johannesburg Parktown North
153	Gauteng, Johannesburg Lindin/Victory Park
155	Gauteng, Johannesburg Lindin/Victory Park
156	Gauteng, Johannesburg Lindin/Victory Park
157	Gauteng, Johannesburg Lindin/Victory Park
158	Gauteng, Johannesburg Lindin/Victory Park
162	Gauteng, Johannesburg Lindin/Victory Park
171	Mpumalanga, Berlin Foreststation
180	Limpopo, Woodbush
181	Limpopo, Woodbush
186	Limpopo, Entabeni
195	Limpopo, Woodbush
199	Gauteng, Benoni
200	Gauteng, Pretoria
201	Mpumalanga, White River
206	Gauteng, Johannesburg Wits
207	Gauteng, Pretoria
209	Gauteng, Pretoria
211	Gauteng, Pretoria
213	Gauteng, Pretoria
*219	Gauteng, Klipreviersberg Nature Reserve
M255	Mpumalanga, Bridal Veil Falls
G267	Gauteng, Klipreviersberg Nature Reserve
G270	Gauteng, Klipreviersberg Nature Reserve
220	Gauteng, Klipreviersberg Nature Reserve
*G4	Mpumalanga, Graskop
223	Gauteng, Klipreviersberg Nature Reserve
211	Gauteng, Pretoria Arcadia
195	Gauteng, Pretoria
NMW	Gauteng, Kempton Park
nmm4	Gauteng, Pretoria
nmm5	Mpumalanga, Graskop
nmm7	Mpumalanga, Graskop
nmm8	Gauteng, Pretoria
nmm9	Limpopo, Magoebaskloof
*L251	Limpopo, Tzaneen
L253	Limpopo, Magoebaskloof
L293	Northern Cape, Hotazel
LS328	Gauteng, Pretoria

202	Gauteng, Randburg
203	Gauteng, Pretoria
205	Gauteng, Johannesburg, Constantia Kloof
214	Gauteng, Johannesburg, Parktown North
216	Gauteng, Pretoria
217	Gauteng, Pretoria
222	Gauteng, Klipreviersberg Nature Reserve
224	Gauteng, Pretoria Feary Glen
225	Gauteng, Pretoria Feary Glen
226	Gauteng, Randburg
*G264	Gauteng, Klipreviersberg Nature Reserve
G265	Gauteng, Klipreviersberg Nature Reserve
G266	Gauteng, Klipreviersberg Nature Reserve
G269	Gauteng, Klipreviersberg Nature Reserve
G278	Gauteng, Klipreviersberg Nature Reserve
G279	Gauteng, Klipreviersberg Nature Reserve
G280	Gauteng, Klipreviersberg Nature Reserve
G281	Gauteng, Klipreviersberg Nature Reserve
G282	Gauteng, Klipreviersberg Nature Reserve
G283	Gauteng, Klipreviersberg Nature Reserve
G284	Gauteng, Klipreviersberg Nature Reserve
nm5	Gauteng, Pretoria Arcadia
nm8	Gauteng, Pretoria
nm9	Gauteng, Pretoria
nm11	Mpumalanga, Graskop
*L252	Mpumalanga, Graskop
*L254	Limpopo, Magoebaskloof

Appendix II. Geographical coordinates of collecting localities of specimens of *Libanasidus* examined, and their associated climatic regions and annual rainfall (mm per annum) (Kruger 2004).

Locality	Coordinates	Climatic region	Rainfall
Botswana Kakwe Pan	24˚04'S; 22˚00'E	----------	-------
Gauteng, Pretoria	25˚30'S; 28˚15'E	Central Bushveld	500-750
Gauteng, Pretoria Brooklyn	25˚46'S; 28˚14'E	Central Bushveld	500-750
Gauteng, Pretoria Newlands	25˚47'S; 28˚16'E	Central Bushveld	500-750
Limpopo, Lekgalameetse Nature Reserve	24˚13'S; 30˚21'E	Eastern Mountain Grassland	500-2000
Limpopo, Louis Trichardt Hangklip	23˚05'S; 29˚50'E	Eastern Mountain Grassland	500-2000
Mpumalanga, Dullstroom	25˚26'S; 30˚08'E	Eastern Mountain Grassland	500-2000
Mpumalanga, Dwarsrivier Valley	24˚45'S; 30˚10'E	Eastern Mountain Grassland	500-2000
Mpumalanga, Graskop	30˚48'S; 24˚55'E	Eastern Mountain Grassland	500-2000
Limpopo, Blyde River Canyon	24˚15'S; 30˚50'E	Lowveld Bushveld	500-700
Mpumalanga, Pretorius Kop	25˚12'S; 31˚15'E	Lowveld Bushveld	500-700
Limpopo, Tzaneen & Magoebaskloof	30˚00'S; 23˚49'E	Lowveld Mountain Bushveld	2000
Limpopo, Tzaneen Malta Forest	24˚15'S; 30˚15'E	Lowveld Mountain Bushveld	2000
Limpopo, Woodbush	23˚45'S; 30˚00'E	Lowveld Mountain Bushveld	2000
Mpumalanga, Berlin Foreststation	25˚33'S; 30˚44'E	Lowveld Mountain Bushveld	2000
Mpumalanga, Berlin Foreststation	25˚33'S; 30˚44'E	Lowveld Mountain Bushveld	2000
Mpumalanga, Berlin Foreststation 1500	25˚32'S; 30˚40'E	Lowveld Mountain Bushveld	2000
Mpumalanga, Berlin Foreststst. -Karst Plateau	25˚31'S; 30˚46'E	Lowveld Mountain Bushveld	2000
Mpumalanga, Bourkes Luck	24˚30'S; 30˚45'E	Lowveld Mountain Bushveld	2000
Mpumalanga, Bridal Veil Falls	30˚43'S; 25˚04'E	Lowveld Mountain Bushveld	2000
Mpumalanga, Nelshoogte Devils Knuckles	25˚47'S; 30˚50'E	Lowveld Mountain Bushveld	2000
Mpumalanga, Nelshoogte Foreststation	25˚47'S; 30˚50'E	Lowveld Mountain Bushveld	2000
Mpumalanga, Nelspruit	25˚38'S; 30˚57'E	Lowveld Mountain Bushveld	2000
Mpumalanga, Nelspruit Nature Reserve	25˚29'S; 30˚55'E	Lowveld Mountain Bushveld	2000
Mpumalanga, Pullen Farm Krokodilpoortsberge	25˚30'S; 31˚15'E	Lowveld Mountain Bushveld	2000
Mpumalanga, Uitsoek Foreststation 1400	25˚15'S; 30˚33'E	Lowveld Mountain Bushveld	2000
Mpumalanga, White River	25˚15'S; 31˚00'E	Lowveld Mountain Bushveld	2000

Gauteng, Benoni	2615'S; 28˚30'E	Moist Highveld Grassland	600-800
Gauteng, Irene	25˚52'S; 28˚12'E	Moist Highveld Grassland	600-800
Gauteng, Johannesburg	26˚15'S; 28˚15'E	Moist Highveld Grassland	600-800
Gauteng, Johannesburg Lindin	26˚08'S; 27˚59'E	Moist Highveld Grassland	600-800
Gauteng, Johannesburg Linmeyer	26˚15'S; 28˚15'E	Moist Highveld Grassland	600-800
Gauteng, Johannesburg Parktown North	26˚08'S; 28˚02'E	Moist Highveld Grassland	600-800
Gauteng, Johannesburg Randburg	26˚05'S; 27˚50'E	Moist Highveld Grassland	600-800
Gauteng, Johannesburg Wits	26˚08'S; 28˚02'E	Moist Highveld Grassland	600-800
Gauteng, Kempton Park	28˚15'S; 25˚45'E	Moist Highveld Grassland	600-800
Gauteng, Klipreviersberg Nature Reserve	28˚41'S; 26˚17'E	Moist Highveld Grassland	600-800
Gauteng, Roodepoort, Weltevreden Park	26˚10'S; 27˚50'E	Moist Highveld Grassland	600-800
Limpopo, Entabeni	22˚45'S; 30˚00'E	Northern Arid Bushveld	300-500
Limpopo, Moordrift	23˚30'S; 27˚30'E	Northern Arid Bushveld	300-500
Eastern Cape Port St. Johns, Silaka	31˚33'S; 29˚30'E	South-east Coast Grassland	>1000

Appendix III. External morphological characters used in morphometric analysis of male and female *Libanasidus.* Measurements are illustrated in Figure 4.3.

EC1	Pronotum length
EC2	Pronotum width
EC3	Eye length
EC4	Eye width
EC5	Head width
EC6	Labrum length
EC7	Mandible length
EC8	Distance between eyes
EC9	Antennal scape length
EC10	Antennal scape width
EC11	Fastigium width
EC12	Front leg femur length
EC13	Front leg femur width
EC14	Hind femur length
EC15	Hind femur width
EC16	Metabasisternum width
EC17	Front tibia length
EC18	Front tibia width
EC19	Tympanum length
EC20	Tympanum width
EC21	Tusk base thickness
EC22	Tusk length
EC23	Subgenital plate length
EC24	Ovipositor length
EC25	Cerci length
EC26	Ovipositor curvature
EC27	Ovipositor base thickness

Appendix IV. Uncorrected 'p' distance calculated in PAUP* for COI sequence analysis of *Libanasidus* from South Africa.

	Individual	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*Cratomelus armatus*	-																				
2	Pretoria 1	0.115	-																			
3	Magoebaskloof 1	0.117	0.003	-																		
4	Magoebaskloof 2	0.116	0.007	0.009	-																	
5	Magoebaskloof 3	0.115	0.000	0.003	0.007	-																
6	Barberton 5	0.106	0.041	0.044	0.045	0.041	-															
7	Barberton 6	0.108	0.042	0.045	0.046	0.042	0.005	-														
8	Nelspruit	0.110	0.040	0.040	0.038	0.040	0.022	0.024	-													
9	Alberton	0.116	0.032	0.034	0.028	0.032	0.046	0.045	0.047	-												
10	Graskop 4	0.108	0.037	0.040	0.036	0.037	0.020	0.021	0.003	0.047	-											
11	Pretorius Kop	0.110	0.040	0.040	0.038	0.040	0.022	0.024	0.000	0.047	0.003	-										
12	Louis Trichardt	0.115	0.033	0.036	0.029	0.033	0.045	0.044	0.046	0.001	0.046	0.046	-									
13	Klipreviersberg 1	0.116	0.004	0.007	0.011	0.004	0.045	0.046	0.044	0.036	0.041	0.044	0.037	-								
14	Pretoria 2	0.119	0.013	0.016	0.015	0.013	0.037	0.038	0.028	0.037	0.025	0.028	0.036	0.017	-							
15	Klipreviersberg 4	0.116	0.001	0.004	0.008	0.001	0.041	0.042	0.040	0.033	0.037	0.040	0.034	0.005	0.012	-						
16	Bridal Veil Falls	0.107	0.037	0.040	0.036	0.037	0.020	0.021	0.005	0.047	0.003	0.005	0.046	0.041	0.028	0.037	-					
17	Graskop 1	0.111	0.040	0.042	0.038	0.040	0.022	0.024	0.005	0.050	0.003	0.005	0.049	0.044	0.028	0.040	0.005	-				
18	Tzaneen 2	0.116	0.001	0.004	0.008	0.001	0.042	0.044	0.041	0.033	0.038	0.041	0.034	0.005	0.015	0.003	0.038	0.041	-			
19	Tzaneen 1	0.119	0.009	0.012	0.003	0.009	0.046	0.049	0.038	0.030	0.036	0.038	0.032	0.013	0.017	0.011	0.036	0.038	0.011	-		
20	Klipreviersberg 3	0.116	0.004	0.007	0.011	0.004	0.045	0.046	0.044	0.036	0.041	0.044	0.037	0.000	0.017	0.005	0.041	0.044	0.005	0.013	-	
21	Klipreviersberg 2	0.116	0.004	0.007	0.011	0.004	0.045	0.046	0.044	0.036	0.041	0.044	0.037	0.000	0.017	0.005	0.041	0.044	0.005	0.013	0.000	-

Chapter 5

General Conclusion

The use of advanced molecular and morphometric techniques in systematic studies have complemented and advanced the value of the existing biological data (Caterino *et al.* 2000). Phylogenetic studies of the suborder Ensifera (Flook & Rowell 1997, Flook *et al*. 1999) and superfamily Stenopelmatoidea (Gorochov 2001) have been undertaken recently. At lower taxonomic levels, however, many problems still plague the taxonomy of King Crickets. This study aimed to resolve the generic problems within southern African anostostomatids, focussing in detail on the genus *Libanasidus* due to its abundance in suburbia. The focus is aimed at a taxonomic group joined by their geographic distribution in southern Africa, including *Bochus* Péringuey, 1918, *Borborothis* Brunner von Wattenwyl, 1888, *Henicus* Gray, 1837, *Libanasidus* Péringuey, 1918, *Nasidius* Stål, 1878, *Onosandrus* Stål, 1878 and *Onosandridus* Péringuey, 1918, belonging to the tribe Anostostomatini Saussure, 1859 and *Libanasa* Walker, 1869 within the tribe Lutosini Walker, 1869 (Otte *et al*. 2005).

Existing taxonomic literature on this group and a thorough review of the morphology of the type species of each genus and museum specimens served to delineate the distinguishing morphological characters of each genus (Chapter 2). A detailed identification key to the eight genera is provided and is accompanied by comprehensive redescriptions of the type species of each genus (Chapter 2).

Identification of museum specimens to generic level based on the revised identification key enabled elaboration of the taxonomic study of this group by the

inclusion of molecular and cladistic morphology to infer phylogenetic relationships between these genera. Thirty-six morphological attributes characteristic of the eight southern African anostostomatid genera were selected for the morphological analysis. A dataset comprising 264 individuals and inclusive of type specimens for each species revealed no morphological support for the eight presently recognized southern African anostostomatid genera (Figure 3.2). High levels of homoplasy and possible incorrect species placement resulted in character ambiguity within genera, rendering many of the diagnostic characters of this group ineffective for resolving the generic relationships. Nucleotide sequences corresponding to the mitochondrial large ribosomal subunit (16S) were generated for 18 individuals representative of six of the eight known genera and subsequent phylogenetic analysis resulted in a highly resolved and well-supported tree (Figure 3.3). These results confirm the ancestral nature of *Bochus* and *Borborothis* to most other genera within the Anostostomatini tribe, as well as the placement of *Libanasa* within a separate tribe, the Lutosini. The recent merge of the genus *Platysiagon* with *Libanasa* (Johns 1997) is also provisionally supported. A close association was obtained between *Libanasidus* and *Nasidius* (0.85 % divergence), with *Onosandrus* being slightly closer related to *Bochus* and *Borborothis* (0.86-0.87 %). Morphologically, *Libanasidus*, *Nasidius* and *Onosandrus* are also easily confused, especially where they coexist in Afromontane forests of eastern South Africa. However, the association of *Onosandrus* with the ancestral *Bochus* and *Borborothis* corresponds well with the lack of sexual dimorphism in *Onosandrus* and *Borborothis*. The phylogenetic position of the genera *Henicus* and *Onosandridus* remain unresolved due to a lack of available molecular data.

Focusing on taxonomic disparities at the specific level, the two recognized *Libanasidus* species, *L. vittatus* and *L. impicta* were redescribed from type and museum material respectively (Chapter 2). The validity of *L. impicta* was confirmed, and an identification key distinguishing between the two species is provided in Chapter 2.

Furthermore, evaluation of the genetic and morphometric structuring within *Libanasidus vittatus*, colloquially known as the Parktown Prawn, consistently suggests two main population assemblages. Mitochondrial Cytochrome Oxidase I sequence data recovered two clades representing these two populations with good bootstrap support in likelihood, parsimony, Bayesian and distance analyses. Genetic divergence between the two clades averaged 3.3 %. This is supported by population parameters showing low migration rates corresponding to less that one female migration per generation. Canonical variates (discriminant) analysis (CVA), based on 27 morphological measurements also shows evidence of two phenetic assemblages. The morphological differences are subtle, however, and suggest the presence of two cryptic species (Table 4.3). *Libanasidus vittatus* was originally described from Barberton in the Mpumalanga province (corresponding to COI Clade B), and is the recognized species occurring within the eastern population of South Africa (Mpumalanga & Eastern Cape Provinces). A possible novel species occurs to the north and west (Limpopo & Gauteng Provinces) (corresponding to COI Clade A). On imposing a molecular clock of 2 % divergence per million years used previously for other Anostostomatidae taxa, time to coalescence between the two major populations is estimated to be approximately 1.65 mya, and corresponds to the presence of isolated forest pockets during the dry Pleistocene epoch. The species status of the north-western population needs to be confirmed

by further investigation, including cytogenetic, multi-locus sequence analysis, geometric morphometric and behavioural techniques. Extended sampling within southern Africa will serve to delineate geographic boundaries of the species.

This systematic study suggests that the designation of the eight anostostomatid genera in southern Africa are valid, but that species placement within these genera needs to be revised, with the possible erection of new genera to accommodate all the currently recognised species. Given these problems, it is suggested that an extensive morphological review of all 51 species is needed to evaluate the taxonomic position of each species individually, which would eliminate the current morphological ambiguity within genera. Generation of functional identification keys to each of the species is vital to this cause. This should be supplemented with molecular revision to confirm the identification of cryptic immatures and females specifically.

General References

Ander, K. 1943. Uber die von Stål beschriebenen Stenopelmatiden (Saltatoria). *Entomologisk Tidskrift* **64**: 198-201.

Brunner von Wattenwyl, C. 1888. Monographie der Stenopelmatiden und Gryllacriden. *Verhandlungen der Kaiserlich Koniglichen Zoologisch-Botanischen Gesellschaft in Wien* **38**: 247-394.

Caterino, M.S., Soowon, C. & Sperling, F.A.H. 2000. The current state of insect molecular systematics: A thriving tower of Babel. *Annual Review of Entomology* **45**: 1-54.

Chopard, L. 1943. Two new species of Gryllacridoidea from southern Africa. *Entomologisk Tidskrift* **14**: 1446-452.

Field, L.H. 2001. Introduction In: *The biology of Wetas, King Crickets and their Allies.* (Ed.) Field, L.H. Cabi publishing, Wallingford UK.

Flook, P.K. & Rowell, C.H.F. 1997. The phylogeny of the Caelifera (Insecta: Orthoptera) as deduced from mtrRNA gene sequences. *Molecular Phylogenetics and Evolution* **8**: 89-103.

Flook, P.K., Klee, S. & Rowell, C.H.F. 1999. Combined Molecular Phylogenetic Analysis of the Orthoptera (Arthropoda, Insecta) and implications for their higher systematics. Systematic Biology 48: 233-253.

Gorochov, A.V. 2001. *The higher classification, phylogeny and evolution of the superfamily Stenopelmatoidea. In: The biology of the Wetas, King Crickets and their allies* (Ed.) Field, L.H. Pp 3-33. Cabi publishing, Wallingford UK.

Gray, G.R. 1837. Description of some singularly formed orthopterous insects. *Annals and Magazine of Natural History (N.S.), London* **2**: 141-145.

Gwynne, D.T. & DeSutter, L. 2004. Ensifera: Crickets, katydids and weta. The tree of life web project. http://tolweb.org/tree?group=Ensifera

Johns, P.M. 1997. The Gondwanaland Weta: Family Anostostomatidae (formerly in Stenopelmatidae, Henicidae or Mimnermidae): Nomenclatural problems, world checklist, new genera and species. *Journal of Orthoptera Research* **6**: 125-138.

Karny, H.H. 1930. A revision of the South African Gryllacrididae (Orthoptera: Saltatoria). *Annals of the South African Museum* **29**: 138-45.

Karny, H.H. 1932. Uber zwei Brunnersche Gryllacrididen-typen im Stettiner Museum (Orth.). *Stettiner Entomologische Zeitung* **93**: 147-151.

Karny, H.H. 1931. On the geographical distribution of the Indo-African and Mediterranean Gryllacrids. *Transactions of the Royal Society of South Africa* **19**: 99-105.

Otte, D., Eades, D.C. & Naskrecki, P. 2005. Orthoptera Species File Online. Version 2.2. <http://osf2.orthoptera.org>.

Page, R.D.M. & Holmes, C.E. 1998. *Molecular evolution: a phylogenetic approach.* Blackwell Science Ltd, Oxford, London.

Péringuey, L. 1916. Descriptions of new or little known Orthoptera: Family Locustidae, Sub-Fam. Stenopelmatinae. *Annals of the South African Museum* **15**: 414-425.

Picker, M., Griffiths, C. & Weaving A. 2002. Field guide to insects of southern Africa. Struik publishers. South Africa.

Toms, R.B. 1986(a). The scientific name of the 'Parkmore Prawn'. *African Wildlife* **40**: 163.

Toms, R.B. 1986 (b). The family name of the 'Parkmore Prawn'. *African Wildlife* **40**: 263.

Toms, R.B. 2001. South African King Crickets (Anostostomatidae) In: *The biology of the Wetas, King Crickets and their Allies*. (Ed) Field, L.H. pp 73-78. Cabi publishing, Wallingford UK.

Publication

Differential Shelter Selection in Response to Predator Chemical Cues by Two orthopterans: *Libanasidus vittatus* (Anostostomatidae) and *Platygryllus primiformis* (Gryllidae)

H. Brettschneider and P. W. Bateman

Department of Zoology & Entomology, University of Pretoria, Pretoria, 0002 South Africa

Journal of Insect Behavior, Vol. 18, No. 3, May 2005 (© 2005)
DOI: 10.1007/s10905-005-3697-z

Short Communication

Differential Shelter Selection in Response to Predator Chemical Cues by Two orthopterans: *Libanasidus vittatus* (Anostostomatidae) and *Platygryllus primiformis* (Gryllidae)

Helene Brettschneider[1] and Philip W. Bateman[1,2]

Accepted October 5, 2004; revised November 10, 2004

KEY WORDS: *Libanasidus vittatus; Platygryllus primiformis*; predator avoidance; secondary chemical cues; olfactory discrimination.

INTRODUCTION

It has been suggested that an early detection of predators may increase the chances of predator avoidance and ultimately increase prey fitness (Lima and Dill, 1990). Of the three major sensory modes, visual and acoustic information may not provide the prey with information on a potential predator's immediate diet history, but olfactory traces of consumed prey from the predator's body and feces can supply qualitative information about the threat status of a predator, by chemically labeling the predator as high risk (Madison *et al.*, 1999).

The identification of potential predators by prey from either direct or indirect signals such as chemicals released from injured conspecifics or connected with diet and waste products has been studied in various vertebrate taxa ranging from amphibians (Laurila *et al.*, 1997; Petranka and Hayes 1998; Madison *et al.*, 1999; Rohr and Madison, 2001), reptiles (Cooper, 1990; Downes, 2002), fish (Brown and Godin, 1999) to mammals (Dickman and Doncaster, 1984; Epple *et al.*, 1993; Ward *et al.*, 1997). Evidence of predator avoidance by invertebrates is less common, but has been reported in

[1]Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa.
[2]To whom correspondence should be addressed at Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa. e-mail: pwbateman@zoology.up.ac.za.

freshwater physid snails (*Physa gyrina*), which reduce activity and seek shelter in response to chemical cues from a predator that has fed on conspecifics (Sih and McCarthy, 2002). One of the only terrestrial arthropod studies, on the wolf spider, *Pardosa milvina*, reported a decrease in their activity when exposed to predatory wolf spider silk and feces (Persons *et al.*, 2001).

Although acoustic communication is recognized as important and widespread amongst orthopterans, intersexual olfactory communication is not unusual in this group (e.g., Bateman and Toms, 1998; Tregenza and Wedell, 1997; Otte and Cade, 1976; Paul, 1976). *Platygryllus primiformis* (Orthoptera: Gryllidae) represents a typical gryllid where males sing to attract silent females (Bateman, 1998; Otte and Cade, 1984), possibly using olfactory communication at close range for sex discrimination (cf., Tregenza and Wedell, 1997). In contrast, *Libanasidus vittatus* (Orthoptera: Anostostomatidae) is wingless and only stridulates by abdominal–femoral contact when disturbed (Bateman and Toms, 1998), but appears to have a well-developed olfactory acuity. It uses chemical cues from its odoriferous feces to find mates, to avoid same-sex rivals, and in initiating male–male competition (Bateman, 2000; Bateman and Toms, 1998) probably using its palps and antennae as chemoreceptors. McDonald and Hanrahan (1993) found that after amputation of palps and antennae, *L. vittatus* retreated to their burrows for up to 4 weeks while these organs regenerated, suggesting their additional importance in general activities.

Both *L. vittatus* and *P. primiformis* are nocturnal omnivores, although *L. vittatus* appears to be more carnivorous than *P. primiformis*, often eating carrion and live snails (McDonald and Hanrahan, 1993; P. W. Bateman, personal observation).

The aim of this study was to investigate whether these two orthopteran species that mainly rely on two different modes of communication (acoustic and olfactory) can distinguish between predators and nonpredators using feces-based chemical cues.

METHODS AND MATERIALS

Platygryllus primiformis used in this experiment were taken from a captive population raised in the Entomology Department of the Transvaal Museum from individuals originally collected along the Crocodile River and in the Kruger National Park (KNP) in the Mpumalanga Province of South Africa. *Libanasidus vittatus* were collected in and around Pretoria in the Gauteng Province of South Africa, during the month preceding the experiment, and maintained in captivity.

Feces from a captive forest shrew, *Myosorex varius*, which had been fed on a variety of cricket species (*P. primiformis, Gryllodes sigillatus, Gryllus bimaculatus*) and mealworms (*Tenebrio molitor*), were used for the predator scent. *Myosorex varius* is a highly predatory nocturnal species (Smithers 1986) and a likely natural predator of crickets. Feces from a captive striped mouse, *Rhabdomys pumilio*, that had eaten only plant material, were used for predator and non-predator fecal-based chemical cue comparisons. *Rhabodomys pumilio* is primarily herbivorous in the wild, and is diurnal and/or crepuscular and unlikely to be a natural predator of crickets. Two controls were used: distilled water and an alcohol-based cologne as an odoriferous unfamiliar control. An equal mass of shrew and mouse feces was taken, and each was mixed with an equal amount of distilled water to produce a liquid, which was frozen for subsequent use.

Fourteen individuals (seven of each sex) of both species were each provided with a glass-topped plastic enclosure (45 cm × 35 cm × 10 cm), with clean vermiculite as a substrate, and a choice of four shelters. The shelters consisted of cardboard boxes (10 cm × 6 cm × 5 cm), open along one short end and inside each one of which on a removable floor of filter paper was placed a small wad of cotton wool into which had been soaked 2 mL of either the shrew feces, mouse feces, water, or cologne.

Each of the study animals was placed in the center of their enclosure and left for an hour under low ambient light. After an hour, each individual was scored as being in one of the boxes or in the open. They were then removed, the position of the boxes randomized, and then the insect was replaced. This was repeated four times. An experimental duration of 1 h was chosen because *L. vittatus*, when placed in the enclosure tended to enter the first shelter encountered, but usually emerged within about 20 min and explored the other alternative shelters.

RESULTS

There was no statistically significant difference in habitat usage between the sexes of either *L. vittatus* or *P. primiformis* ($\chi^2_{13} = 2.51$, $P = 0.99$). The sexes were, therefore, pooled for between-species comparisons.

Differences in shelter selection between species were statistically significant with *P. primiformis* spending more time out in the open or in the shrew feces shelter ($\chi^2_9 = 52.87$, $P < 0.0001$). In contrast, *L. vittatus* preferred the water-treated shelter ($\chi^2_{13} = 109.00$, $P < 0.0001$). Both species totally avoided the cologne-treated shelter, but spent equal amounts of time near the mouse feces-treated shelter (Fig. 1).

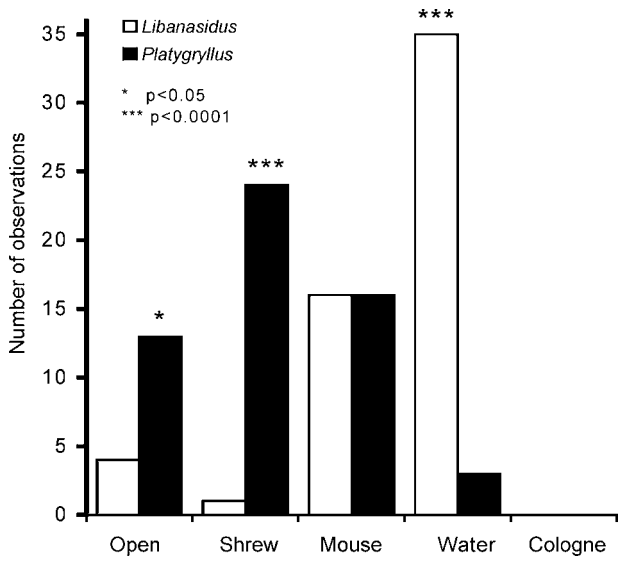


Fig. 1. Frequency of time spent in the open, shrew feces-treated, mouse feces-treated, water-treated, and cologne-treated shelters by *Libanasidus vittatus* and *Platygryllus primiformis*.

DISCUSSION

Time spent in the open presumably poses a risk to the individual as it increases its vulnerability to predation and during foraging bouts, *L. vittatus* periodically takes refuge in burrows (McDonald and Hanrahan, 1993). This species prefers moist habitats found in both indigenous forests and suburban gardens (McDonald and Hanrahan, 1993; Picker *et al.*, 2002). This could explain the preference of *L. vittatus* for the water-treated shelter. *Platygryllus primiformis* on the other hand, occurs in a wide variety of habitats ranging from montane grasslands to coastal dunes (Otte and Cade, 1984) and appears to be less moisture-dependent than other gryllids.

Libanasidus vittatus sometimes feeds on dog feces (McDonald and Hanrahan, 1993), suggesting that both mouse and shrew feces may be possible food sources for this species. There was, however, a significant difference between the numbers of visits to the shrew feces-treated shelter by the two species, with only one *L. vittatus* visiting a shrew feces shelter. Although shrew feces are a potential food source for *L. vittatus*, it appears that shrew feces-treated shelters were avoided as they may signal the presence of a potential predator. According to Petranka and Hayes (1998), nocturnal

animals confronted with "sit-and-wait" predators may benefit from chemical gradients around predators to assess predation risk. Dickman and Doncaster (1984) found that rodents avoided traps tainted with fox odors, a known predator. This, together with rodent avoidance of unfamiliar predator (European badger) tainted traps may be consistent with the theory that there may be chemical signals present in the feces and urine of all carnivorous predators (Ward *et al.*, 1997). Snow geese (*Chen caerulescens*) even avoid fields in which sulfur-producing cabbages have been grown, and herbivore repellents that release sulfurous odors and volatile fatty acids are commercially available (Mason and Clark, 1996).

In contrast to *L. vittatus*, individuals of *P. primiformis* spent most of their time in the shrew feces-treated shelter. Shrews are generalist insectivores and prey on many species of crickets and, although generalist predators may be perceived as a lesser threat to prey, they may selectively search for specific prey even when well fed (Madison *et al.*, 1999), and *L. vittatus* being a much larger cricket, may be a preferred prey. Laurila *et al.* (1997) found that highly palatable frog tadpoles showed strong antipredator behavior, in contrast with less palatable toad tadpoles, which did not alter their behavior according to predator diet.

Acuity in olfaction by our two study species may also influence their predator-avoidance behavior. *Libanasidus vittatus* relies almost entirely on the olfactory mode both for reproduction and foraging, with vision and hearing being of extreme secondary importance. It may be that shrew feces-treated shelters were avoided as they clearly signal the presence of a potential predator. McDonald and Hanrahan (1993) have shown that feces constitute food to *L. vittatus*, which might explain their visits to the mouse feces-treated shelters, but they overwhelmingly preferred the water-treated control shelters, suggesting that indirect cues of potential predators may also be present in the mouse feces. *Platygryllus primiformis* on the other hand, may have a less well-developed olfactory acuity, such that the species may be unable to recognize the feces of a potential predator. The species, however, appears to prefer shrew feces-treated shelters rather than the water-treated control and the mouse feces-treated shelters, suggesting that its olfactory acuity may be able to differentiate between the different fecal treatments. The sulfurous components or volatile fatty acids of the predator feces may act as a signal of protein-rich food to the omnivorous cricket. It would be expected that *L. vittatus* would also recognize the shrew feces as food, but this apparent odoriferous perception may be overridden by its avoidance of a potential predator. Both species completely avoided the odoriferous cologne-treated control shelters, which appear to have been highly repellent, suggesting an equal olfactory acuity to this control experimental treatment.

In conclusion, we can assume that *L. vittatus* recognizes shrew feces-treated shelters as signaling the presence of a predator and, therefore, avoids them. Although *P. primiformis* was able to differentiate between mouse and shrew feces, we cannot however, be certain whether the species (1) was able to recognize the shrew feces as indicating the presence of a predator, (2) is not reacting to the indirect information contained in the feces, or (3) does not recognize shrews as predators.

ACKNOWLEDGMENTS

The authors thank T. Fleming for statistical advice, K. N. P and R. B. Toms for permission and help in collecting crickets, D. Bellars and C. Chimimba for the shrew and the mouse, respectively, and Y. de Beer for comments on the manuscript.

REFERENCES

Bateman, P. W. (1998). Assortative mating by both sexes in the cricket *Platygryllus primiformis* (Orthoptera: Gryllidae; Gryllinae). *Trans. Am. Entomol. Soc.* **124:** 63–68.

Bateman, P. W. (2000). The influence of weapon asymmetry on male-male competition success in a sexually dimorphic insect, the African king cricket *Libanasidus vittatus* (Orthoptera: Anostostomatidae). *J. Insect Behav.* **13:** 157–163

Bateman, P. W., and Toms, R. B. (1998). Olfactory intersexual discrimination in an African King cricket (Orthoptera: Mimnermidae). *J. Insect Behav.* **11:** 159–163.

Brown, G. E., and Godin, J. J. (1999). Who dares, learns: Chemical inspection behaviour and acquired predator recognition in a characin fish. *Anim. Behav.* **57:** 475–481.

Cooper, W. J. (1990). Chemical detection of predators by a lizard, the broad-headed skink (*Eumeces laticeps*). *J. Exp. Biol.* **256:** 162–167.

Dickman, C. R., and Doncaster, C. P. (1984). Responses of small mammals to red fox (*Vulpes vulpes*) odour. *J. Zool. Lond.* **204:** 521–531.

Downes, S. J. (2002). Does responsiveness to predator scents affect lizard survivorship? *Behav. Ecol. Sociobiol.* **52:** 38–42.

Epple, G., Mason, J. R., Nolte, D. L., and Campbell, D. L. (1993). Effects of predator odours on feeding in the mountain beaver (*Aplodontia rufa*). *J. Mammal.* **74:** 715–722.

Laurila, A., Kujasola, J., and Ranta, E. (1997). Different antipredator behaviour in two anuran tadpoles: Effects of predator diet. *Behav. Ecol. Sociobiol.* **40:** 329–336.

Lima, S. L., and Dill, L. M. (1990). Behavioral decisions made under the risk of predation: A review and prospectus. *Can. J. Zool.* **68:** 619–640.

Mcdonald, I. R., and Hanrahan, S. A. (1993). Aspects of foraging and behaviour of the king cricket, *Libanasidus vittatus* (Kirby) (Orthoptera: Mimnermidae). *South African J. Sci.* **89:** 354–357.

Madison, D. M., Maerz, J. C., and Mcdarby, J. H. (1999). Optimisation of predator avoidance by salamanders using chemical cues: Diet and diel effects. *Ethology* **105:** 1073–1086.

Mason, R. J., and Clark, L. (1996). Avoidance of cabbage fields by snow geese. *Wilson Bull.* **108:** 369–371.

Otte, D., and Cade, W. (1976). The role of olfaction in sexual and interspecies recognition in crickets (*Acheta* and *Gryllus*). *Anim. Behav.* **24:** 1–6.

Otte, D., and Cade, W. (1984). African crickets (Gryllidae). 4. The genus *Platygryllus* from Eastern and Southern Africa (Gryllinae, Gryllini). *Proc. Acad. Nat. Sci. Philadelphia* **136:** 45–66.

Paul, R. (1976). Acoustic response to chemical stimuli in ground crickets. *Nature* **263:** 404–405.

Persons, M. H., Walker, S. E., Rypstra, A. L., and Marshall, S. D. (2001). Wolf spider predator avoidance tactics and survival in the presence of diet associated predator cues (Araneae: Lycosidae). *Anim. Behav.* **61:** 43–51.

Petranka, J., and Hayes, L. (1998). Chemically mediated avoidance of a predatory odonate (*Anax junius*) by American toad (*Bufo americanus*) and wood frog (*Rana sylvatica*) tadpoles. *Behav. Ecol. Sociobiol.* **42:** 263–271.

Picker, M., Griffiths, C., and Weaving, A. (2002). *Field Guide to Insects of South Africa,* Struik, Cape Town, South Africa.

Rohr, J. R., and Madison, D. M. (2001). A chemically mediated trade-off between predation risk and mate search in newts. *Anim. Behav.* **62:** 863–869.

Sih, A., and Mccarthy, T. M. (2002). Prey responses to pulses of risk and safety: Testing the risk allocation hypothesis. *Anim. Behav.* **63:** 473–443.

Smithers, R. H. N. (1986). *Land Mammals of Southern Africa: A Field Guide*, Southern Book, Halfway House, Johannesburg.

Tregenza, T., and Wedell, N. (1997). Definitive evidence for cuticular pheromones in a cricket. *Anim. Behav.* **54:** 979–984.

Ward, J. F., Macdonald, D. W., and Doncaster, C. P. (1997). Responses of foraging hedgehogs to badger odour. *Anim. Behav.* **53:** 709–720.