The tusked king cricket (*Libanasidus vittatus* (Kirby, 1899), Anostostomatidae) from South Africa: morphological and molecular evidence suggest two cryptic species

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Abstract. An evaluation of genetic structuring and morphometric variation within tusked king crickets, *Libanasidus vittatus* (Kirby, 1899) from South Africa suggests two main population assemblages. Maximum likelihood (ML), Parsimony, Bayesian and phenetic analyses of mitochondrial cytochrome oxidase I (COI) sequence data recovered two well-supported clades corresponding to two biogeographically distinct populations. Canonical variates (discriminant) analysis (CVA) also showed evidence of two phenetic assemblages that correspond to the genetically delineated groups. *Libanasidus vittatus* is the recognized species occurring within an eastern population in South Africa (Mpumalanga and Eastern Cape Provinces), while a possible novel species occurs within a north–western population in South Africa (Limpopo and Gauteng Provinces). Using a molecular clock estimate of 2.0 % divergence per million years, suggests isolation of the two populations at ~1.65 MYA, possibly due to the formation of isolating forest pockets during the dry Pleistocene Epoch. The average genetic divergence of 3.3% between the two populations, and low migration rate estimates corresponding to less than one female migration per generation further support the presence of two cryptic tusked king cricket species in South Africa.

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Introduction

Libanasidus vittatus (Kirby, 1899). (Orthoptera: Ensifera: Anostostomatidae), colloquially known as the 'Parktown prawn', is a common and legendary cricket in suburban gardens in the cities of 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 its offenders (Wolf *et al.* 2006). The species is sexually dimorphic, the males possessing large curved 'tusks' on their mandibles that are used in male-male competition (Bateman 2000; Brettschneider *et al.* 2007). The species is nocturnal, remaining in self-constructed burrows in soft moist soil during daylight, which may partly account for the general lack of knowledge of their distributional range.

It has been reported that *L. vittatus* may originally have been localised in the indigenous forests of the eastern parts of South Africa, especially along the Soutpansberg and Drakensberg mountain ranges (Toms 1985). 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 three South African provinces where *L. vittatus* occurs, Gauteng Province is characterised by Moist Highveld Grassland centrally and Central Bushveld in the north; Limpopo Province consists of Northern Arid Bushveld in the north, Central Bushveld in the centre and the west, Lowveld Mountain Bushveld and Lowveld Bushveld to the south-east; Mpumalanga Province consists of Lowveld Bushveld in the east, Lowveld Mountain Bushveld in the centre, and Eastern Mountain Grassland on the western border and stretching south along the Drakensberg mountain range (Kruger 2004). The Lowveld Mountain Bushveld still contains pockets of Afromontane forest inhabited by *Libanasidus* (Brettschneider *et al*. 2007).

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 (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 and Tanzania (Johns 1997). 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 southern Australia while all the other genera are restricted to the African continent (Karny 1931).

The bulk of taxonomic studies on the Anostostomatidae were undertaken between 1803 and 1943. The latest taxonomic revision (Brettschneider *et al.* 2007) focused on the generic classification within the subfamily Anostostomatinae. The earlier taxonomic studies are, however, replete with synonymies, misspellings and incorrect identifications, not to mention the continuous taxonomic reallocation of taxa, resulting in more taxonomic confusion for the group (see Karny 1930; Ander 1943). These earlier studies largely focused 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.

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). Kirby (1899) provisionally placed the species within the family Stenopelmatidae under the genus *Carcinopsis* Brunner von Wattenwyl, 1888 and referred to it as *C. vittata* (Péringuey 1916). This species was subsequently included in the new genus *Libanasidus* by Péringuey (1916) as the type species. Currently, the genus is considered to contain two species, *L. vittatus* and *L. impicta*. The latter, described by Stål (1878) was, until recently, regarded as a *nomen dubium*, as it was described from a single and possibly immature female from the Northern Cape ("Cape Colony") and is rarely mentioned in the taxonomic literature (Johns 1997). Descriptive revision of the genus conducted in 2007, however, revealed distinct differences between the two species (Brettschneider *et al.* 2007).

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 and to gain some insight into the biogeography of the species in southern Africa.

Materials and Methods

Molecular analysis

Twenty individuals of *Libanasidus* from three South African provinces that include eastern Mpumalanga (MP), northern Limpopo (LP) and western Gauteng (GP), and one outgroup sequence were used for the molecular analyses. Unfortunately no genetic material was available from the holotype of *L. vittatus*. Individuals were either collected by hand or obtained from the insect collection of the Transvaal Museum (TM), Northern Flagship Institution (NFI), Pretoria, South Africa. DNA extraction from muscle tissue of the hind femora followed the Roche extraction protocol according to the manufacturer's specifications (Roche Diagnostics, Mannheim, Germany). The 3' half of the mitochondrial cytochrome oxidase I (COI) gene was amplified by the polymerase chain reaction (PCR) and sequenced with the universal insect primers C1-J-2183 and L2-N-3014 (Simon *et al.* 1994), using a BigDye automated cycle sequencing approach (Perkin- Elmer, Foster City, USA). Sequence data for the Chilean red cricket, *Cratomelus armatus* Blanchard, 1851 (Anostostomatidae: Cratomelinae) was generated in this study for use as an outgroup in all phylogenetic analyses. The 21 generated sequences are deposited in GenBank under accession numbers DQ204406-204416, 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) selected under the Akaike Information Criterion (AIC) in Modeltest version 3.06 (Posada & Crandall 1998) was specified for Maximum Likelihood (ML; Felsenstein, 1981) analysis. The ML and maximum parsimony (MP) analyses were performed in PAUP* version 4.0 b10 (Swofford 1999). All characters were initially treated as unordered and of equal weight, with different weighting schemes being considered in subsequent analyses. 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 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 (RC) index (Farris 1969). All distance analyses were carried out with MEGA version 3 (Kumar *et al*. 2004) and employed the Tamura-Nei model of evolution. Between 100 (ML) and 10,000 (distance trees) bootstrap replicates were performed to assess nodal support for the clades recovered (Felsenstein 1985). Bayesian inference was also performed with MrBayes version 3.1 (Huelsenbeck & Ronquist 2001). Priors were guided by the parameter estimates and best-fit model (GTR+I) identified in Modeltest. Three heated chains and one cold chain were started from a random tree, and all four chains ran simultaneously for 100,000 generations, with trees being sampled every 10 generations. The first 2500 trees were discarded as *burn-in*.

In order to infer evolutionary rates, the constancy of a molecular clock was tested between lineages using Phyltest version 2.0 (Kumar 1996). Rate heterogeneity across lineages was also evaluated in PAUP by comparing log likelihood values obtained with and without the constraint of a molecular clock.

Historical estimates of gene flow between the clades obtained from the phylogenetic analyses were estimated with Migrate version 2 (Beerli & Felsenstein 2001). The data were run with 10 short chains (10,000 trees sampled, 500 used) and 5 long chains (10,000 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 from South Africa and Botswana between 1924 and 2003, which represents the largest data set analysed to date for the species from the subregion. Regrettably the holotype of *L. vittatus* was not available for analysis at this time. Due to difficulty in obtaining fresh adult material and amplifying DNA from 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.

Twenty-four male and 25 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 (HB) using a pair

of Mitutuyo® digital callipers (Mitutoyo American Corporation, Aurora, Illinois, U.S.A.). Some measurements, such as tympanal length and width were recorded using an ocular micrometer (Wild 10X/21). All measurements are defined in Table 1 and illustrated in Fig. 1.

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 sexually dimorphic 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 mandibular tusks. In addition, the presence of long, curved, and flexible cerci was assumed to be an adult characteristic in both sexes (see Koning & Jamieson 2001). The obvious presence of sexual dimorphism justified the separate morphometric analysis of the sexes. To facilitate the simultaneous analysis of sexes combined, 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 reference samples in all morphometric analyses to delineate phenetic boundaries in multivariate space.

The phenetic groups obtained *a priori* and with reference to genetic data were further subjected to *a posteriori* canonical variates (discriminant) analysis (CVA; Sneath & Sokal 1973). Statistically significant morphometric 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. All morphometric statistical procedures were accomplished using algorithms in STATISTICA version 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. Transitions occurred almost 3 times more often than transversions (R = 2.8). 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, MP, ML, and Bayesian analyses.

All phylogenetic analyses derived two major clades. The distance tree inferred with the Tamura-Nei model of sequence evolution and summarising bootstrap support values from all methods of analysis, is illustrated in Fig. 2. High bootstrap values for clade A support the clustering of all specimens from the east and the north (Limpopo and Gauteng Provinces), in the neighbor-joining (94%), MP (90%) and Bayesian (1.00) analyses. Bootstrap values for clade B, encompassing all specimens collected from the west (Mpumalanga Province) are lower (58-77%), but the clade is recovered consistently in all analyses. Resolution within clade A was not optimal, but revealed a close association between specimens from Alberton (GP) and Louis Trichardt (LP) (84-100% support), while relationships between individuals from Pretoria (GP), Klipreviersberg Nature Reserve (GP), Magoebaskloof (LP) and Tzaneen (LP) were unresolved. Relationships within the eastern (B) clade 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 cluster together with 89-100% bootstrap support. Trees constructed from weighted characters did improve within-clade resolution of relationships.

Uncorrected p-distances calculated in MEGA of the mitochondrial COI gene region sequenced revealed 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%. Pairwise genetic distances within clade B ranged from 0-2.4% (average = 2.3%) (Barberton ↔ Pretorius Kop and Graskop), and from 0-3.7% (average = 3.1%) (Pretoria ↔ Alberton), within clade A. The molecular clock hypothesis was not rejected under the 95% ($P < 0.05$) confidence interval in either Phyltest or PAUP* where likelihood values obtained for trees with and without a molecular clock enforced, in the latter program, were –log 1837.90955 and –log 1742.02460, respectively.

Population parameters estimated with ML in Migrate version 2 (Beerli & Felsenstein 2001) showed levels of past gene flow (as represented by female migrations per generation) was highest from the north-western group (Clade A) to the eastern group where $2N_2\ m_{21} = 0.311$ (95% confidence intervals = 0.146-7.258). 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 ($\theta = 2N_e\ u$) suggest that the north-western population (Clade A) is slightly larger with $\theta = 0.01800$ (95% confidence intervals = 0.010609-0.032396) compared to Clade B where θ was 0.01223 (95% confidence intervals = 0.005726-0.28540).

Morphometric assessment

Neither the PCA nor the distance and correlation phenograms showed any geographically discernible phenetic pattern in all three combinations of data analysed (males, females, males and females combined) and with reference to the molecular data (not illustrated). A two-group CVA that specified the eastern and north-western specimens as *a posteriori* groups based on the results of the molecular data did, however, suggest the presence of two phenetic groups (Fig. 3) that are congruent with the molecular data.

A MANOVA of the group 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 2 shows that pronotum length (C1), head width (C5), mandible length (C7), front leg femur width (C13), front tibia length (C17), front tibia width (C18), tusk base thickness (C21) and tusk length (C22) underlie the separation between males, while pronotum length (C1), eye length (C3), hind femur length (C14) and hind femur width (C15) are responsible for the separation in females. Standard statistics on these groups are presented in Table 1 for males and females. All these descriptive data indicate that the phenetic differences between groups within the sexes are subtle, suggesting the presence of cryptic taxa.

Discussion

Congruence between genetic structuring and morphometric variation within *Libanasidus* from southern Africa suggests two main population assemblages. The morphological and genetic similarity of *Libanasidus* individuals collected from localities in Limpopo and Gauteng Provinces suggest a continuous population with a north-south distribution, while those collected from Mpumalanga Province represent an eastern population. Individuals collected from the Eastern Cape Province grouped morphometrically with the eastern group and although this study could not confirm this grouping with molecular data, suggests that the geographic distribution of this group extends as far south as Port St. Johns (Eastern Cape Province).

Mitochondrial sequence analysis supported the two populations (two clades), with Clade A corresponding to the north-western population and Clade B representing the eastern population. These clades are separated by an average sequence divergence of 3.3% (max. 5%), and historical migration rates of less than one female per generation ($2N_1\ m_{12} = 0.311$). The constancy in tree topology across the different methods of analysis together with the high posterior probabilities and bootstrap support values confirms the validity of the two COI gene lineages recovered. 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 supports historical isolation and diversification. Morphometrically-distinguishing characters between the north-western and eastern populations as revealed by canonical variates (discriminant) analysis (CVA) (Table 2) 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 male data, and pronotum length (C1), eye length (C3), hind femur length (C14), and hind femur width (C15) for females.

In interpreting isolation events, invertebrate studies generally use divergence rates of 1.5-2.3% per million years (Trewick & Wallis 2001). As rate heterogeneity between lineages was

not significant, a molecular clock could be imposed for the COI sequence data using the rate calibrated for related weta species (Anostostomatidae; 2.0% per my; Trewick & Morgan-Richards, 2005). Despite the small sample size of the genetic data set, it falls within the optimal sample and amplicon size suggested in the population genetic studies of Pluzhnikov & Donnelly (1996). Consequently, we can assume that based on the average genetic divergence between the two groups of 3.3% that these populations diverged approximately 1.65 million years ago.

Correlation of the proposed separation of the two populations with Paleoclimatic 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 a subsequent recession of- and decrease in vegetation abundance (Eeley *et al.* 1999). Alternating hyper- and hypo-thermal 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, as is found along the Drakensberg mountain range (Stuckenberg 1969). At lower elevations, forests occurred in pockets, probably much as in the present. Consequently, due to unfavourable terrain between populations and slow apteral dispersal, the two populations in this study could have become permanently separated during the marked dry era between 1.8-1.6 mya due to the fragmentation of their habitat (deMenocal 2004; Eeley *et al.* 1999). Reliance on moist habitats by *Libanasidus* probably also currently restricts movement across unfavourable terrain between neighbouring forest clumps.

The recognition of subgroups within *Libanasidus* has gone unnoticed until now due to the subtle phenetic differences between the two populations as revealed by the canonical variates (discriminant) (CVA) analysis. This may suggest 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 morphometric characters included in this study (tympanal size, primary and secondary sexually dimorphic characteristics), there is plasticity in the development of many structures since these crickets regenerate lost limbs and mouthparts, or develop malformed legs (Flook *et al.* 2000). Regenerated appendages also often differ in armature and structure from the original morphology (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 a parallel cladistic study based on qualitative morphology within the Anostostomatidae (H. Brettschneider unpubl. data). Resultantly, recognition of either population would have to be confirmed with molecular data due to the lack of discrete morphological characteristics for identification.

In conclusion, this study represents the first extensive morphological and molecular study of this group of insects in South Africa. With genetic divergence similar to levels reported for other anostostomatid sibling species (Trewick 2001), the data suggests that individuals of

Libanasidus found in the north and west of South Africa (Gauteng and Limpopo Provinces) may represent a novel cryptic species, currently identifiable with molecular techniques only, that is distinct from the more easterly distributed clade (Barberton, Mpumalanga Province), within which the type locality of *Libanasidus vittatus* falls. Congruence between the morphometric and genetic analysis also supports an extended north-south distributional range of this new species. Low gene flow between the two populations can be attributed to climatic changes during the Pleistocene, and in addition to the organisms' apterous nature, current migrational events may be restricted due to habitat fragmentation.

Additional sampling effort for both populations, combined with an extension of the techniques used in this study, comprising multiple genetic loci, cytogenetics, and geometric morphometrics is required to obtain further insight into the systematics of *Libanasidus* in southern Africa and for the proper diagnosis of separate species. Possible differences in chromosome number and morphology along with behavioural differences may increase our understanding of speciation events in this group of crickets. In addition, extensive sampling of areas between the collection localities used in this study would be useful in delineating geographic boundaries between populations and identifying possible paleoclimatic factors responsible for these boundaries.

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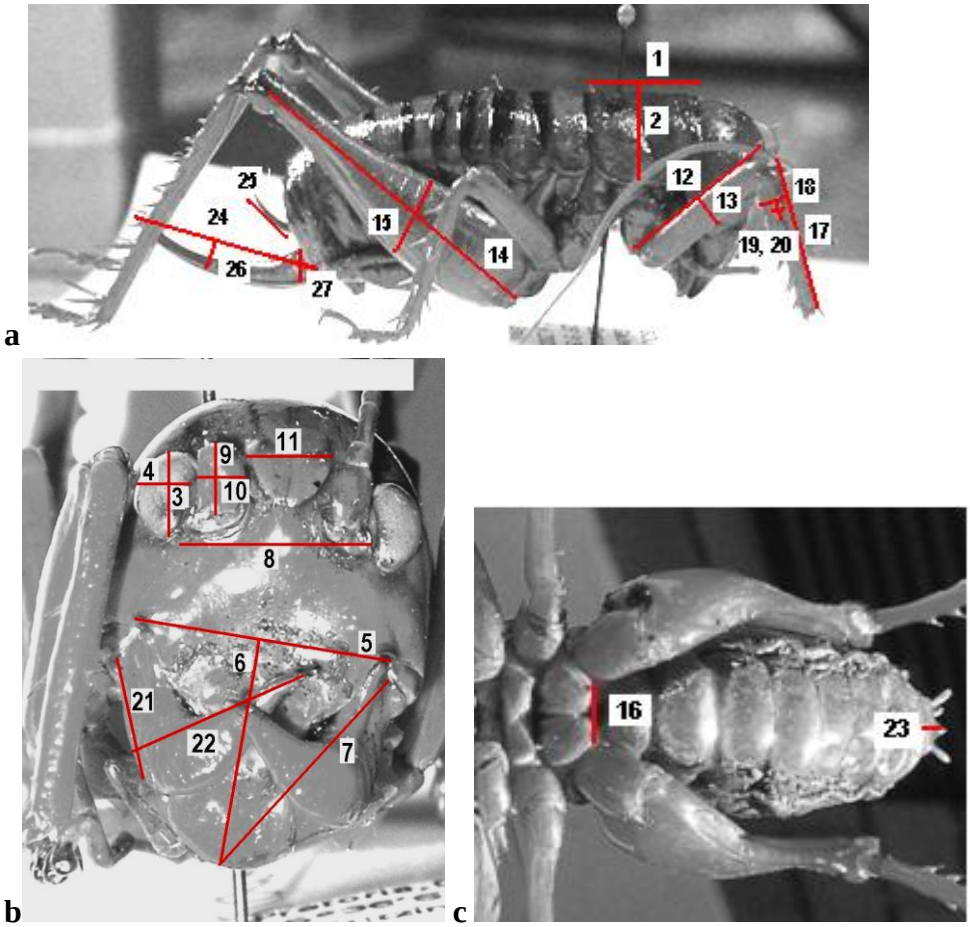


Fig. 1. Lateral (a), frontal (b) and ventral (c) views of *Libanasidus* from southern Africa illustrating 27 external morphological characters used for morphometric analysis. Characters are defined in Table 2.

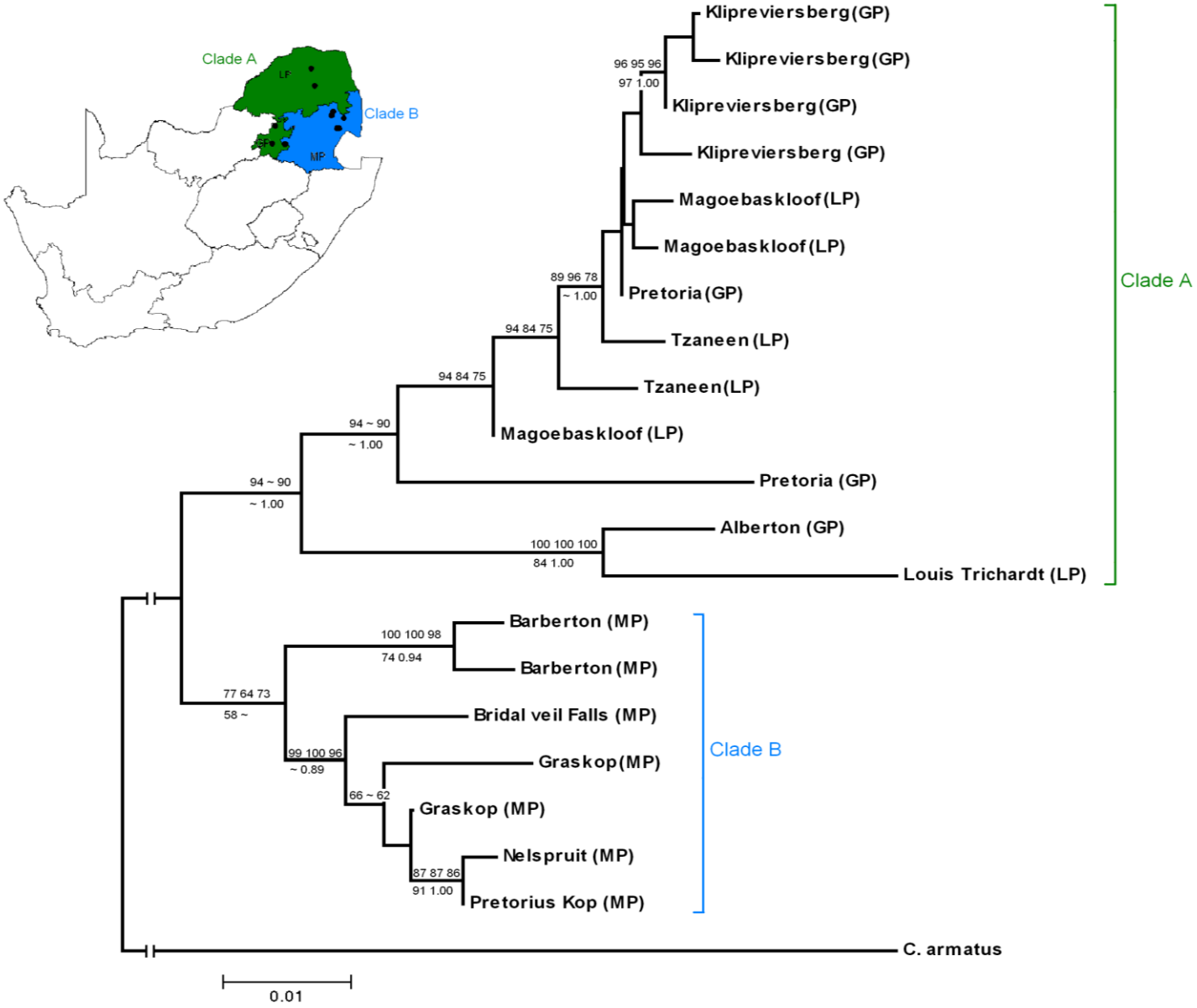
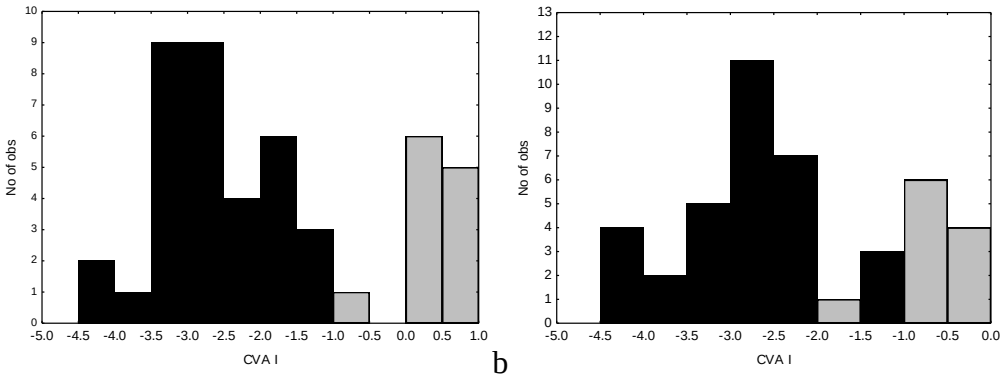


Fig. 2. Neighbor-joining tree depicting mitochondrial cytochrome oxidase I (COI) gene relationships of *Libanasidus* from South Africa. Bootstrap support from neighbor-joining (NJ), minimum evolution (ME) and maximum parsimony (MP) analyses, respectively, is given above each relevant branch, whilst those from maximum likelihood (ML) and Bayesian inference are indicated below the branches. Support values of <50, are denoted by a '-'.Provincial location abbreviated GP = Gauteng LP = Limpopo and MP = Mpumalanga, and is illustrated in the map insert.



a b

Fig. 3. Histograms of the first canonical variates (discriminant) analysis (CVA) axis scores extracted from a CVA of male (a) and female (b) *Libanasidus* from South Africa. Solid bars symbolize the eastern group (Mpumalanga Province (MP), and shaded bars indicate the northwestern group (Gauteng (GP) and Limpopo (LP) Provinces) (see map in Fig. 2). Statistical significance between phenetic group centroids: Males – $F = 11.66$, $P < 0.00$; Females – $F = 10.98$, $P < 0.00$.

Table 1. Standard statistics of external morphometric measurements of two delineated phenetic groups within *Libanasidus* from southern Africa. *n* = sample size; $\bar{x}$ = arithmetic mean; *Range* = observed range; *SD* = standard deviation; *CV* = coefficient of variation. Measurements are illustrated in Fig. 1.

		Males		Females	
		Eastern	North-western	Eastern	North-western
1	**Pronotum length:**				
	n	49	35	86	34
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	2.17	2.30	2.09	2.25

		Males		Females	
		Eastern	**North-western**	**Eastern**	**North-western**
	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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

		Males		Females	
		Eastern	**North-western**	**Eastern**	**North-western**
19	**Tympanum length:**				
	n	49	35	86	34
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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
	$\bar{x}$	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	-	-
	$\bar{x}$	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	-	-
	$\bar{x}$	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	-	-
	$\bar{x}$	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
	$\bar{x}$	-	-	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
	$\bar{x}$	-	-	2.88	2.66
	Range	-	-	1.47-3.56	1.55-3.72
	SD	-	-	0.50	0.60
	CV	-	-	17.34	22.56

Table 2. External morphological characters used in morphometric analysis of male and female *Libanasidus* from southern Africa and their corresponding loadings on the first canonical variate axis from a canonical variates analysis (CVA). Measurements are illustrated in Fig. 1.

		Males	Females
No.	**Character**	**CVA I**	**CVA I**
1	Pronotum length	-0.82	-0.81
2	Pronotum width	-0.24	0.42
3	Eye length	-0.54	-0.78
4	Eye width	-0.53	-0.03
5	Head width	1.22	0.56
6	Labrum length	-0.18	0.07
7	Mandible length	0.83	0.59
8	Distance between eyes	-0.28	-0.11
9	Antennal scape length	-0.39	-0.53
10	Antennal scape width	-0.19	0.15
11	Fastigium width	0.38	0.06
12	Front leg femur length	-0.68	-0.22
13	Front leg femur width	0.93	-0.04
14	Hind femur length	-0.46	-2.52
15	Hind femur width	0.51	2.21
16	Metabasisternum width	0.00	0.32
17	Front tibia length	-3.81	-0.45
18	Front tibia width	3.48	0.58
19	Tympanum length	0.54	0.00
20	Tympanum width	-0.31	-0.02
21	Tusk base thickness	1.31	-
22	Tusk length	-1.26	-
23	Subgenital plate length	-0.10	-0.23
24	Ovipositor length	-	-0.09
25	Cerci length	-0.09	0.26
26	Ovipositor curvature	-	0.03
27	Ovipositor base thickness	-	0.51

Appendix 1. *Libanasidus* specimens examined and a gazetteer, where * denotes individuals included in the molecular analysis.

FEMALES		MALES	
ID No.	**Locality**	**ID No.**	**Locality**
5	GP, Pretoria, Newlands	2-4	GP, Pretoria, Brooklyn
6	GP, Johannesburg, Linmeyer	1	GP, Johannesburg, Linmeyer
7	GP, Johannesburg, Parktown	30	GP, Johannesburg, Randburg
8	GP, Pretoria, Brooklyn	31	GP, Johannesburg
*10	MP, Pretorius Kop	15	GP, Pretoria, Arcadia
18	GP, Pretoria	17	GP, Pretoria, Brooklyn
32-33	GP, Pretoria	14	GP, Pretoria, Brooklyn
35-36	GP, Pretoria	16	GP, Johannesburg, Parktown
37	MP, Uitsoek Forest station 1400	176	GP, Pretoria, Arcadia
38	MP, Dullstroom	*177	GP, Pretoria, Arcadia
39	MP, Dwarsrivier Valley	178	GP, Pretoria, Arcadia
46-55	GP, Johannesburg, Parktown	056	LP, Tzaneen, Malta Forest
58	LP, Tzaneen Malta Forest	179	MP, Nelshoogte Devils, Knuckles
57	LP, Louis Trichardt Hangklip	*59	LP, Louis Trichardt, Hangklip
62	MP, Berlin Forest station -1500	61	MP, Nelshoogte Devils, Knuckles
63	MP, Nelshoogte Forest station	73	MP, Berlin Forest station 1500
64	MP, Berlin Forest station, Karst Plateau	74	MP, Berlin Forest station, Karst Plateau
65	MP, Nelshoogte Devils, Knuckles	75-76	MP, Nelshoogte Devils, Knuckles
67	MP, Nelshoogte Devils, Knuckles	77	MP, Berlin Forest station 1500
69	MP, Nelshoogte Devils, Knuckles	78	MP, Bourke's Luck
70-72	MP, Nelshoogte Devils, Knuckles	79	MP, Nelshoogte Devils, Knuckles
84	MP, Berlin Forest station	80	MP, Nelshoogte Forest station
89	MP, Berlin Forest station	81-83	MP, Berlin Forest station
93	Botswana Kakwe Pan, Kgalagadi District	86	MP, Berlin Forest station
94	LP, Blyde River Canyon	88	MP, Berlin Forest station
99	MP, Berlin Forest station 1500	*90	MP, Nelspruit
100	MP, Berlin Forest station, Karst Plateau	91	Botswana Kakwe Pan, Kgalagadi District
101	MP, Berlin Forest station 1500	97	MP, Berlin Forest station
102	MP, Pullenfarm, Krokodilpoortsberge	103-105	MP, Nelspruit Nature Reserve
113-118	EC, Port St. Johns, Silaka	106-109	EC, Port St. Johns Silaka
123	GP, Pretoria	112	EC, Port St. Johns, Silaka
124	GP, Irene	119	EC, Port St. Johns, Silaka
126	GP, Roodepoort, Weltevreden Park	121-122	EC, Port St. Johns, Silaka
128-130	LP, Lekgalameetse Nature Reserve	131	LP, Lekgalameetse Nature Reserve
138	GP, Pretoria, Brooklyn	142	MP, Berlin Forest station
139-140	GP, Pretoria	150	GP, Johannesburg, Parktown
141	MP, Berlin Forest station	153	GP, Johannesburg, Linden/Victory Park
143-149	GP, Johannesburg, Parktown	155-158	GP, Johannesburg, Linden/Victory Park
151-152	GP, Johannesburg, Parktown	162	GP, Johannesburg, Linden/Victory Park
154	GP, Johannesburg, Victory Park	171	MP, Berlin Forest station
159-161	GP, Johannesburg, Victory Park	180-181	LP, Woodbush

FEMALES		MALES	
ID No.	**Locality**	**ID No.**	**Locality**
163-164	GP, Johannesburg, Parktown	186	LP, Entabeni
126	MP, Berlin Forest station	195	LP, Woodbush
170	MP, Berlin Forest station	199	GP, Benoni
172-175	MP, Berlin Forest station	200	GP, Pretoria
182-185	LP, Woodbush	201	MP, White River
189	LP, Tzaneen, Malta Forest	206	GP, Johannesburg, Wits.
190	GP, Johannesburg	207	GP, Pretoria
191	LP, Moordrift	209	GP, Pretoria
192-193	GP, Pretoria	211	GP, Pretoria
194	LP, Woodbush	213	GP, Pretoria
196	LP, Entabeni	*219	GP, Klipreviersberg Nature Reserve
202	GP, Randburg	M255	MP, Bridal Veil Falls
203	GP, Pretoria	G267	GP, Klipriviersberg Nature Reserve
205	GP, Johannesburg, Constantia Kloof	G270	GP, Klipriviersberg Nature Reserve
214	GP, Johannesburg, Parktown	220	GP, Klipreviersberg Nature Reserve
216-217	GP, Pretoria	*G4	MP, Graskop
222	GP, Klipreviersberg Nature Reserve	223	GP, Klipriviersberg Nature Reserve
224-25	GP, Pretoria, Faerie Glen	211	GP, Pretoria, Arcadia
226	GP, Randburg	195	GP, Pretoria
*G264-66	GP, Klipreviersberg Nature Reserve	NMW	GP, Kempton Park
G269	GP, Klipreviersberg Nature Reserve	nmm4, 8	GP, Pretoria
G278-84	GP, Klipreviersberg Nature Reserve	nmm5, 7	MP, Graskop
nm5	GP, Pretoria, Arcadia	nmm9	LP, Magoebaskloof
nm8	GP, Pretoria	*L251	LP, Tzaneen
nm9	GP, Pretoria	L253	LP, Magoebaskloof
nm11	MP, Graskop	L293	Northern Cape, Hotazel
*L252	MP, Graskop	LS328	GP, Pretoria
*L254	LP, Magoebaskloof	L253	LP, Magoebaskloof

GP = Gauteng province, MP = Mpumalanga province, LP = Limpopo Province, EC = Eastern Cape Province.