

Morphometric measurement selection: an invertebrate case study based on weevils from sub-Antarctic Marion Island

Abstract A character selection procedure initially applied in vertebrates (viverrid carnivores and murid rodents), but with a potential, more general application, was used to select appropriate characters for a morphometric investigation of weevils on Marion Island. An initial set of 23 linear measurements, adopted from a previous morphometric study, was subjected to cluster and ordination procedures to summarise patterns of correlations between measurements. Criteria were developed for the selection of representative measurements within cluster analysis-generated sub-clusters, after the exclusion of redundant measurements. This reduced the 23 initial variables to a final set of 15 measurements. The general grouping of variables was broadly consistent across all weevil species examined. Apart from economising, by reducing the number of characters that have to be measured for subsequent analyses, the procedure also provides a way to adequately represent the phenotype, and to investigate morphological integration.

Key words: Curculionidae, ecomorphology, morphometrics, phenetics, sub-Antarctic

Running title: Morphometric analysis of weevils from Marion Island

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Introduction

The members of the *Ectemnorhinus* group of genera, a monophyletic unit of weevils (Curculionidae) restricted to South Indian Ocean Province Islands of the Southern Ocean, are amongst the most ecologically significant insects on these islands. They are the most species rich herbivore group, on islands generally dominated by detritivory (Chown and Scholtz 1989), and are the numerically dominant species in several habitat types, especially fellfield (or wind desert) (Davies 1973; Chown and Scholtz 1989; Barendse and Chown 2000). In lowland vegetated habitats they contribute substantially to ecosystem functioning via nutrient recycling (Smith and Steenkamp 1992). As a consequence of their abundance, many of the weevil species form an important prey item of alien, invasive predators such as the house mouse (*Mus musculus*, sensu lato), which has been introduced to many of the South Indian Ocean Province Islands (Gleeson and van Rensburg 1982; Chapuis et al. 1994; Le Roux et al. 2002). Indeed, on sub-Antarctic Marion Island, it appears that introduced mice are having a profound effect on several of the weevil species occurring there (Crafford and Scholtz 1987; Chown and Smith 1993; Chown et al. 2002), and this affect appears to be increasing in severity (Smith et al. 2002).

Two species in the genus *Ectemnorhinus* (*E. similis* C.O. Waterhouse and *E. marioni* Jeannel) are most intensely affected by this predation. Unlike the other species of the group on Marion Island, they are restricted to habitats that are characterised by angiosperm vegetation (Chown 1989a), where mice are particularly abundant (Gleeson and van Rensburg 1982). Because these species are also present on the nearby mouse-free Prince Edward Island (22 km distant) (Crafford and Scholtz 1987; Chown 1989a; Chown and Smith 1993), and because eradication of mice is likely to be costly, though not impracticable (Chown and Cooper 1995), it is generally thought that the weevil species are not at any substantial risk. Indeed, they have not been assigned particular attention in any of the management actions currently implemented at the islands (see for example, Anonymous 1996). This decision is based on the implicit assumption that populations of the two *Ectemnorhinus* species on the two islands can be considered equivalent management units (see Moritz and Faith 1998).

However, not only are species delimitations within the *Ectemnorhinus* group of genera, especially within the genus *Ectemnorhinus*, uncertain (Chown 1991, 1994), but populations within each of these two species on Marion and Prince Edward islands have also been reported to differ in several respects, most notably in general body size and shape (Chown 1989b, 1992; Chown and Smith 1993). Therefore, given the severity of weevil predation by mice on Marion Island, it is not clear whether the Prince Edward Island weevil populations can be considered

sufficient for the conservation of the *Ectemnorhinus* species. Similarly, it is also not clear whether *Ectemnorhinus* populations on the two islands represent either a single species or a complex of species (Chown 1990, 1991).

As a consequence, a detailed population level study, based on an integrated approach involving morphometric, molecular, and ecological data was initiated to investigate the relationships between the *Ectemnorhinus* species on the two islands (see Chown et al. 2002). The present study focuses on the selection of suitable quantitative taxonomic characters for the morphometric part of the study, a critical but often neglected step in morphometric studies (Strauss and Bookstein 1982; Rohlf 1990).

Despite the importance of choosing suitable quantitative taxonomic characters for morphometric analysis, there is no established selection procedure. Studies generally rely either on measurements that have been used previously, occasionally with the ad hoc addition or deletion of measurements after simple correlation analysis (Power 1970; Chapman et al. 1992), or use as many characters as is feasible (Watson and Dippenaar 1987; Chimimba and Kitchener 1991). Although characters so selected can perform well (Sneath and Sokal 1973), it is important to assess character redundancy (Thorpe 1976; Rohlf 1990). The use of unevaluated characters can have an effect that may range from an increase in analysis time (Chimimba and Kitchener 1991) to the distortion of inter-Operational Taxonomic Unit (OTU) (sensu Sneath and Sokal 1973) relationships (Blackith and Reyment 1971). It has been shown that after character screening, a set of many measurements can be substantially reduced, but still contain equivalent information (Mahalanobis et al. 1949; Albrecht and Blackith 1957).

There are a variety of procedures that have been used in the past to screen for reliable characters, and these range from the use of analysis of variance (ANOVA) (Pimentel and Smith 1986), Mahalanobis' (1936) D^2 statistic, to correlations among characters summarised by either principal components analysis (PCA) (Gould et al. 1974), factor analysis (Johnston 1973), or cluster analysis (Taylor and Meester 1993). Although some of these procedures may perform well, others either ignore redundancy or can be unstable as a result of small sample sizes (Thorpe 1976; Pimentel and Smith 1986).

The selection of morphometric characters in the present study is based on a procedure previously applied to small mammals that included a viverrid carnivore, *Cynictis penicillata* (Taylor and Meester 1993) and a murid rodent, *Aethomys namaquensis* (Chimimba and Dippenaar 1995), but with a potential application to other taxa. Consequently, this study demonstrates the broader utility of this character selection protocol using weevils as a case study. Although largely based on cluster analysis, the procedure also incorporates Olson and Miller's (1958) concept of morphological integration in its interpretation of relationships among characters. Based on a concept of evolutionary change that emphasises on the unity of

the genotype, Olson and Miller (1958) demonstrated that developmentally and functionally related traits are highly correlated and that this can be reflected in the statistical degree of association in the phenotype (see also Cheverud 1982; Pigliucci 2003).

The present study uses the above approach to: 1) adequately represent the phenotype in the character selection procedure; 2) economise by removing redundant variables; and 3) summarise patterns of relationships between characters, and by so doing revealing morphological integration (Olson and Miler 1958).

Materials and methods

Study area and samples

The development of a character selection procedure in this study is based on homogeneous samples of six currently recognised weevil species collected in April 2001 from two localities on sub-Antarctic Marion Island (see Smith 1987; Hänel and Chown 1999; Chown et al. 2002 for information on the natural history of the island). These species are: *Palirhoeus eatoni* (C.O. Waterhouse 1885) (19 males, 11 females) and *Bothrometopus randi* Jeannel, 1940 (17 males, 13 females) from Trypot Beach (46°53.052'S; 37°52.060'E), *B. parvulus* (C.O. Waterhouse 1885) (22 males, 13 females), *B. elongatus* (Jeannel 1940) (16 males, 15 females), *Ectemnorhinus marioni* (Jeannel 1940) (16 males, 14 females), and *E. similis* (C.O. Waterhouse 1885) (17 males, 31 females) from Tafelberg (46°53.135'S; 37°48.201'E) (Appendix I). To reduce the potential effect of geographic and altitudinal variation, inland species were all collected at Tafelberg, while coastal species were collected at Trypot Beach. The taxonomic status of both *E. similis* and *E. marioni* has been the subject of debate (Kuschel 1971; Chown and Scholtz 1989; Chown 1991, 1992). While current taxonomic authorities treat both as valid species (Kuschel and Chown 1995), others have considered them synonymous. Pending a final resolution of the taxonomic status of the two species, we follow taxonomic convention and treat *E. similis* and *E. marioni* as two separate species.

Morphometric measurements

All analyses were based on adult weevils. An initial set of 23 linear measurements adopted from Chown (1989a), selected to adequately represent the weevil phenotype (Fig. 1), were recorded to the nearest 0.05 mm (TL and EW), 0.03mm (PB and FL) and 0.01mm (O, A, F1,

F2, F3, FR, T3, MS, MT, MM and FB). All measurements were recorded by a single observer (L. J. van Rensburg) using a stereo microscope fitted with a calibrated eye-piece micrometer.

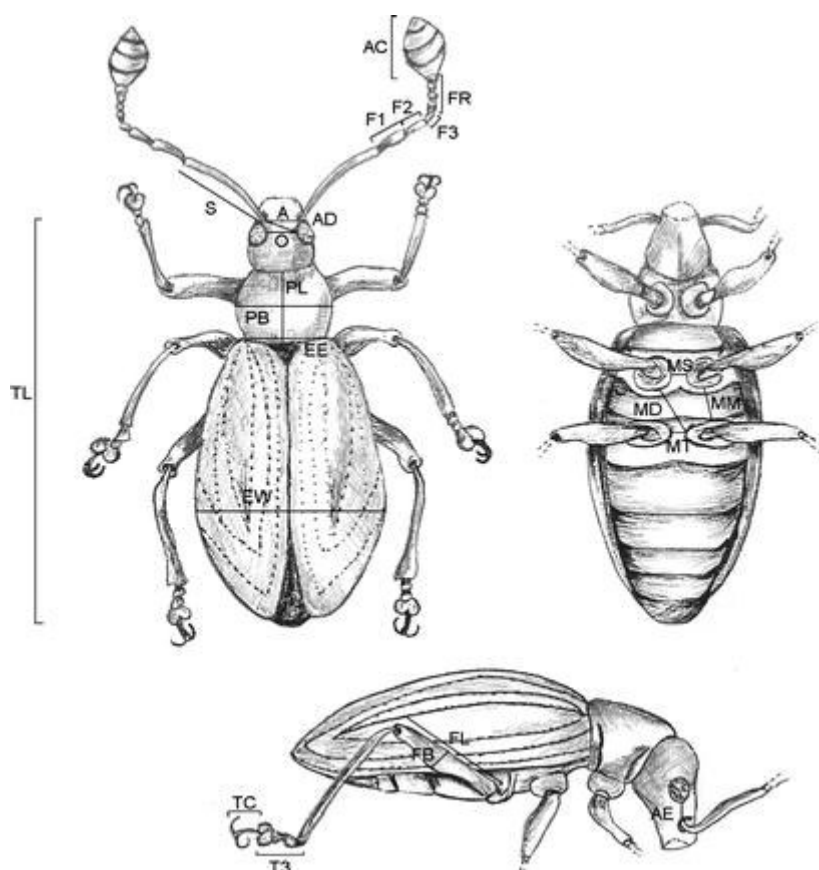


Fig. 1 Morphometric measurements assessed in the present paper as defined by Chown (1989a) (A inter-antennal distance, AC antennal club length, AD antenna/eye diagonal, AE antenna/eye distance, EE breadth of elytra, leading edge, EW maximum breadth of elytra, F1 funicle segment 1, F2 funicle segment 2, F3 funicle segment 3, FB femur breadth, FL femur length, FR rest of funicle, MD coxal diagonal, MM meso/metacoxal distance, MS mesocoxal distance, MT metacoxal distance, O inter-ocular distance, PB pronotum breadth, PL pronotum length, S scape length, T3 length of first three tarsal segments, TC tarsal claw segment length, TL total body length)

Statistical analyses

Exploratory univariate and multivariate data screening revealed two female outlier specimens of *P. eatoni* and *B. randi* that were not considered representative of the population. A re-examination of these specimens revealed that they had outlier values as a consequence of injury. To avoid biasing the sample, they were excluded from subsequent analyses. One-way analysis of variance (ANOVA), Euclidean distance-based unweighted pair-group arithmetic average (UPGMA) cluster analysis, and R-mode principal components analysis (PCA) (Sneath and Sokal 1973) were used to evaluate sexual dimorphism.

Character associations within each of the six weevil species were assessed by Euclidean distance-based cluster analysis of Q-mode PCA scores derived from standardised

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measurements (Sneath and Sokal 1973). Euclidean distances of the 23 generated PCA components, which explained 100% of the sample variance within each of the six species, were subjected to Ward's (1963) hierarchical cluster analysis to assess character associations within each species. Ward's clustering algorithm minimises the positional variation of elements in a cluster independently at each step to produce homogeneous clusters (Cheverud 1982).

The selection of characters from within sub-clusters generated by Ward's cluster analysis was based on a combination of the following criteria:

1. Relative loadings of measurements as derived from an R-mode PCA (James and McCulloch 1990) of the six weevil species as a measure of a variable's relative importance in delineating species;
2. The magnitude of the coefficient of variation (CV), incorporating Haldane's (1955) correction for small samples (Sokal and Rohlf 1981), as a measure of the relative variability of variables within each of the six weevil species (Zar 1996);
3. The degree of measurement error (ME) expressed as a percentage (% ME) of the total variability due to *within*-individual variation (Pankakoski et al. 1987; Bailey and Byrnes 1990). This analysis was based on one representative weevil sample currently considered to be *E. marioni* ($n = 27$) from which three independent data sets of repeated measurements were derived by L. J. van Rensburg on three separate occasions;
4. Relative ease of measurement;
5. The potential for non-missing values, particularly arising from measuring points associated with frequently damaged parts of the organism in order to accommodate for analyses that require complete data sets (Kim 1975; Klecka 1975); and
6. The potential for a measurement to assist in capturing the overall configuration of the phenotype.

All statistical analyses were performed using various univariate and multivariate algorithms available in STATISTICA version 5.5 (Statsoft 1995).

Results

Univariate screening of measurements

A one-way ANOVA showed that of the 23 initial measurements (see Fig. 1 caption for explanation of coding) only a few of these are sexually dimorphic in the six weevil species.

These include: *P. eatoni* – four characters: PL ($P < 0.05$), TL, EW, EE ($P < 0.001$); *B. randi* - four characters: TL, EE, O ($P < 0.01$), A ($P < 0.001$), *B. parvulus* - two characters: TL, EW ($P < 0.05$), *B. elongatus* – two characters: TL ($P < 0.05$), MT ($P < 0.01$), *E. marioni* – one character: F3 ($P < 0.01$) and *E. similis* – one character: MT ($P < 0.01$) (Table 1). Together with the general lack of a multivariate sexual size dimorphism found in both the UPGMA cluster analysis (Fig. 2) and the PCA (not illustrated), these findings suggested that the sexes could be analysed together in subsequent analyses.

Analysis of character associations

The phenograms derived from Ward's (1963) cluster analysis of the 23 measurements for all six species are presented in Figure 3. Although there are some equivocal placements of measurements (indicated by question marks), all phenograms show two broadly similar, relatively discrete character groupings (designated I and II) among the six weevil species. The degree of equivocal placement of characters seems to be largely influenced by sample size such that the trend of the two discrete character groupings is best illustrated by the results obtained for *E. similis* (Fig. 3c), the species with the largest sample size ($n = 48$). Consequently, the least equivocal trend obtained from the *E. similis* sample is used to illustrate the general pattern of character associations in the six weevil species, and is summarised in Table 2.

Major cluster I consists of antero- and mid-dorsal, and mid-ventral characters with their associated limb measurements. Among this grouping of variables are included measurements such as TL and EW that reflect general body size-related measurements that may potentially span across different developmental and functional units of the phenotype, if not the entire length and width of the weevil. Major cluster II exclusively comprises antennal measurements: F1, F2, F3, FR, and AC. The only exceptional antennal measurement that does not form part of this tight sub-cluster of variables is the antennal scape length (S), which is placed within major cluster I. No other morphologically discernible sub-clusters were evident within each of the two major clusters.

Table 1 *F*-values from a one-way analysis of variance (ANOVA) of six weevil species to determine differences between males and females using 23 morphometric measurements. Measurements are defined in Fig.1

Measurements	<i>P. eatoni</i> <i>F</i> -value	<i>B. randi</i> <i>F</i> -value	<i>B. parvulus</i> <i>F</i> -value	<i>B. elongatus</i> <i>F</i> -value	<i>E. marioni</i> <i>F</i> -value	<i>E. similis</i> <i>F</i> -value
TL	26.84 ***	9.23 **	5.35 *	7.36 *	1.68	1.29
EW	25.96 ***	3.61	4.44 *	1.12	0.58	0.19
EE	37.12 ***	8.63 **	0.38	0.42	0.01	1.28
PB	2.63	3.06	1.82	0.02	0.03	1.04
PL	4.65 *	0.36	3.72	0.16	0.35	1.03
S	0.00	0.10	0.52	1.27	0.35	1.08
F1	0.00	0.06	1.64	2.24	2.22	0.27
F2	1.14	0.26	0.01	1.13	1.60	0.14
F3	0.56	0.02	0.14	0.32	10.12 **	0.06
FR	0.00	0.12	0.69	0.37	6.91	0.14
AC	0.86	0.86	0.06	0.51	0.24	0.00
T3	0.59	1.86	0.01	0.07	0.22	0.11
TC	0.08	0.11	1.35	0.74	0.85	0.01
O	0.00	10.44 **	0.23	0.00	1.56	2.47
A	0.36	16.25 ***	1.39	0.05	3.84	1.23
AD	0.00	1.65	0.94	0.54	0.02	3.06
MS	0.01	0.07	0.22	0.00	0.49	0.17
MT	0.91	0.26	0.63	7.89 **	0.55	11.59 **
MM	0.61	0.14	0.15	0.65	3.75	0.72
MD	1.27	0.10	1.28	0.89	0.01	1.30
FL	0.03	0.48	0.47	0.28	1.60	0.15
FB	2.95	0.56	1.52	0.27	0.67	0.06
AE	0.09	0.67	0.08	0.33	0.20	0.29

Statistical significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

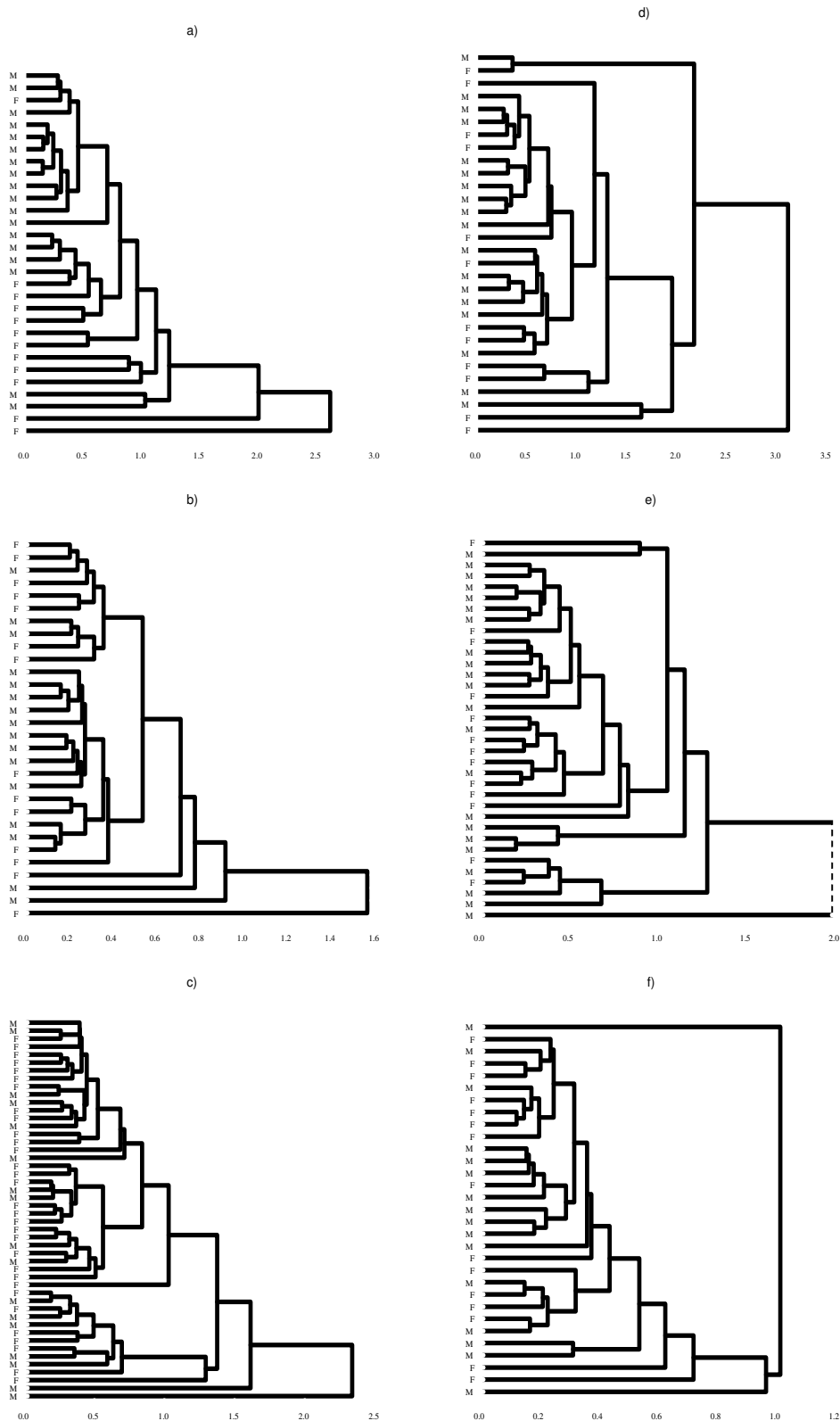


Fig. 2a–f Distance phenograms from a UPGMA cluster analysis of **a** *Palirhoeus eatoni*, **b** *Ectemnorhinus marioni*, **c** *E. similis*, **d** *Bothrometopus randi*, **e** *B. parvulus*, and **f** *B. elongatus* from sub-Antarctic Marion Island showing the absence of sexual dimorphism in overall form

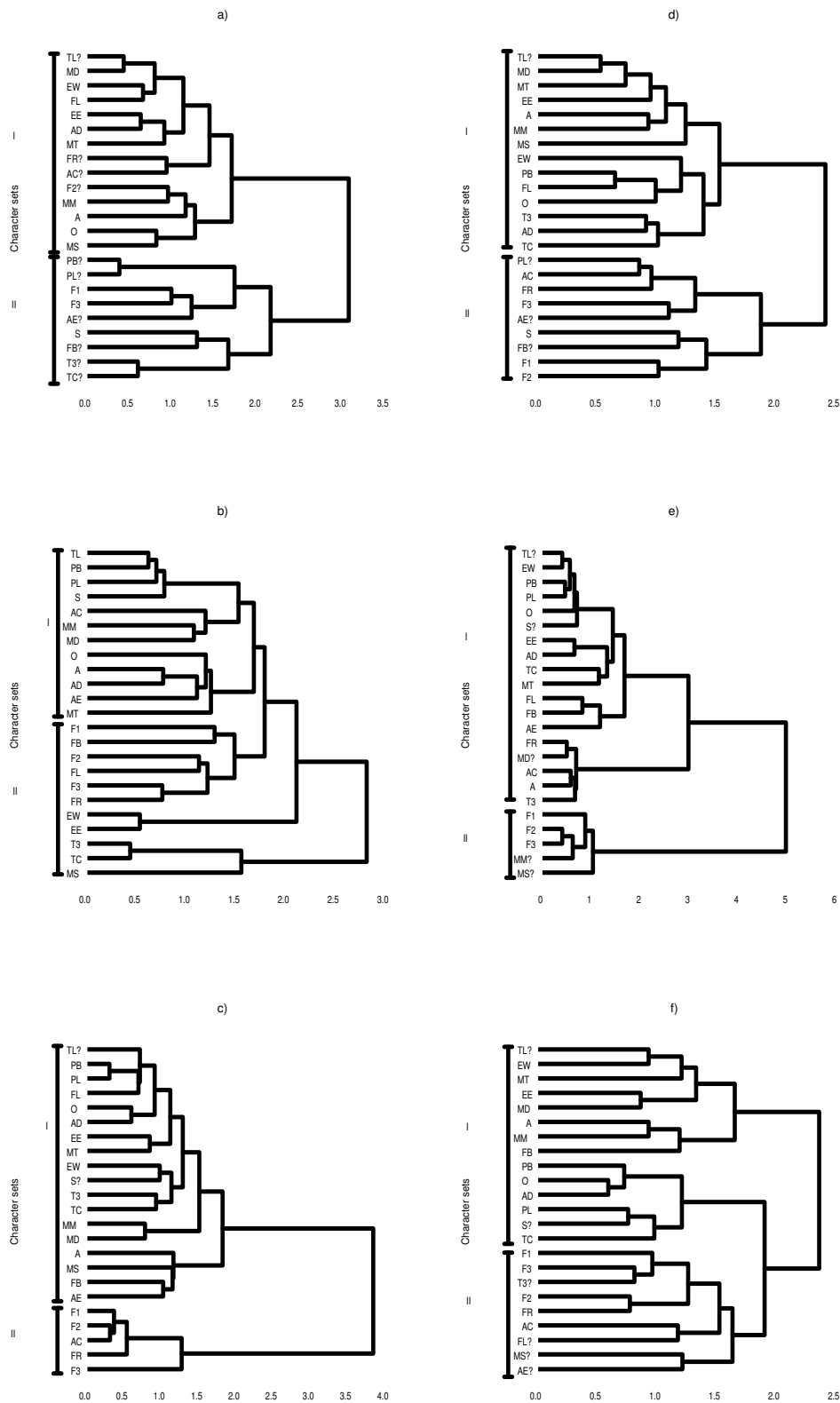


Fig. 3a–f Phenograms derived from Ward's (1963) cluster analysis of **a** *Palirhoeus eatoni*, **b** *Ectemnorhinus marioni*, **c** *E. similis*, **d** *Bothrometopus randi*, **e** *B. parvulus*, and **f** *B. elongatus* from sub-Antarctic Marion Island, depicting relationships between 23 morphometric measurements. Two derived major sub-clusters are indicated by vertical lines. Characters followed by a question mark do not belong in the particular character set. Measurements are defined and illustrated in Fig. 1

Table 2 Representative principal component (PCA) loadings for the first two axes and coefficients of variation (CV) in *Ectemnorhinus similis*, and percent measurement error (% ME) in *E. marioni* for each character in samples from sub-Antarctic Marion Island. Characters preceded by a question mark are considered not to belong in the particular set of measurements. Characters followed by an asterisk were selected for subsequent morphometric analyses. Characters are defined and illustrated in Fig. 1

Character set	PCA I	PCA II	PCA III	PCA V	CV	% ME
I. Anterior, dorsal, and mid-ventral character set						
TL?	0.970	0.082	0.081	-0.003	2.83	0.04
PB	0.905	-0.058	0.046	0.050	3.00	0.00
PL	0.912	0.080	0.096	-0.001	3.27	0.00
FL	0.947	0.017	-0.118	0.010	4.38	0.00
O	0.948	0.025	0.058	-0.004	3.57	0.00
AD	0.940	0.003	0.140	-0.027	3.18	0.01
EE	0.922	0.117	0.164	-0.050	3.44	0.01
MT	0.760	-0.500	-0.156	-0.005	3.53	0.00
EW	0.918	0.095	0.095	0.027	4.03	0.02
S?	0.903	0.094	0.116	0.057	3.43	0.00
T3	0.899	0.195	-0.246	-0.018	3.89	0.00
TC	0.871	-0.119	-0.154	0.081	3.71	0.00
MM	0.800	0.160	0.475	0.111	4.20	0.00
MD	0.930	0.068	-0.081	0.060	6.82	0.00
A	0.855	-0.123	-0.187	0.111	2.90	0.00
MS	0.049	-0.944	0.098	-0.172	3.63	0.00
FB	0.729	-0.299	-0.092	0.400	3.41	0.00
AE	0.840	0.152	-0.084	-0.297	2.49	0.00
II. Antennal character set						
F1	0.880	0.184	0.049	-0.060	4.83	0.00
F2	0.844	-0.278	0.199	-0.019	2.80	0.00
AC	0.752	0.002	-0.525	-0.127	7.39	0.00
FR	0.881	0.094	-0.288	-0.083	3.14	0.00
F3	0.724	-0.106	0.377	-0.192	3.14	0.00
% trace	72.83	6.65	4.55	1.68		

Selection of measurements

The outcome of the PCA of the six weevil species (Fig. 4a) formed one of the criteria that were used to select measurements from within the sub-clusters. The first two axes of the PCA show that apart from total overlap between *P. eatoni* and *B. parvulus*, and partial overlap between *B. randi* and *E. similis*, the weevil species generally separate on both axes. However, *E. similis* and *B. randi* separate on the third PCA axis (Fig. 4b), while *P. eatoni* and *B. parvulus* were

separated on the fifth PCA axis (Fig. 4c). Little or no differentiation was evident between the six weevil species along axes 4, 6 – 23. The first PCA axis, which accounts for 72.83% of the variance, shows generally high and positive loadings for all characters (Table 2), suggesting that species separation along PCA axis I is largely size-related. The second PCA axis, which accounts for 6.65% of the variance (Table 2), suggests a shape-related separation due to the influence of MT and MS, the two measurements with relatively high loadings (regardless of sign) on PCA axis II (Table 2). PCA axis III accounts for 4.55% of the variance, with the highest loading shown by MM and AC. PCA axis V contributes 1.68% of the variance, with FB showing the highest variable loading.

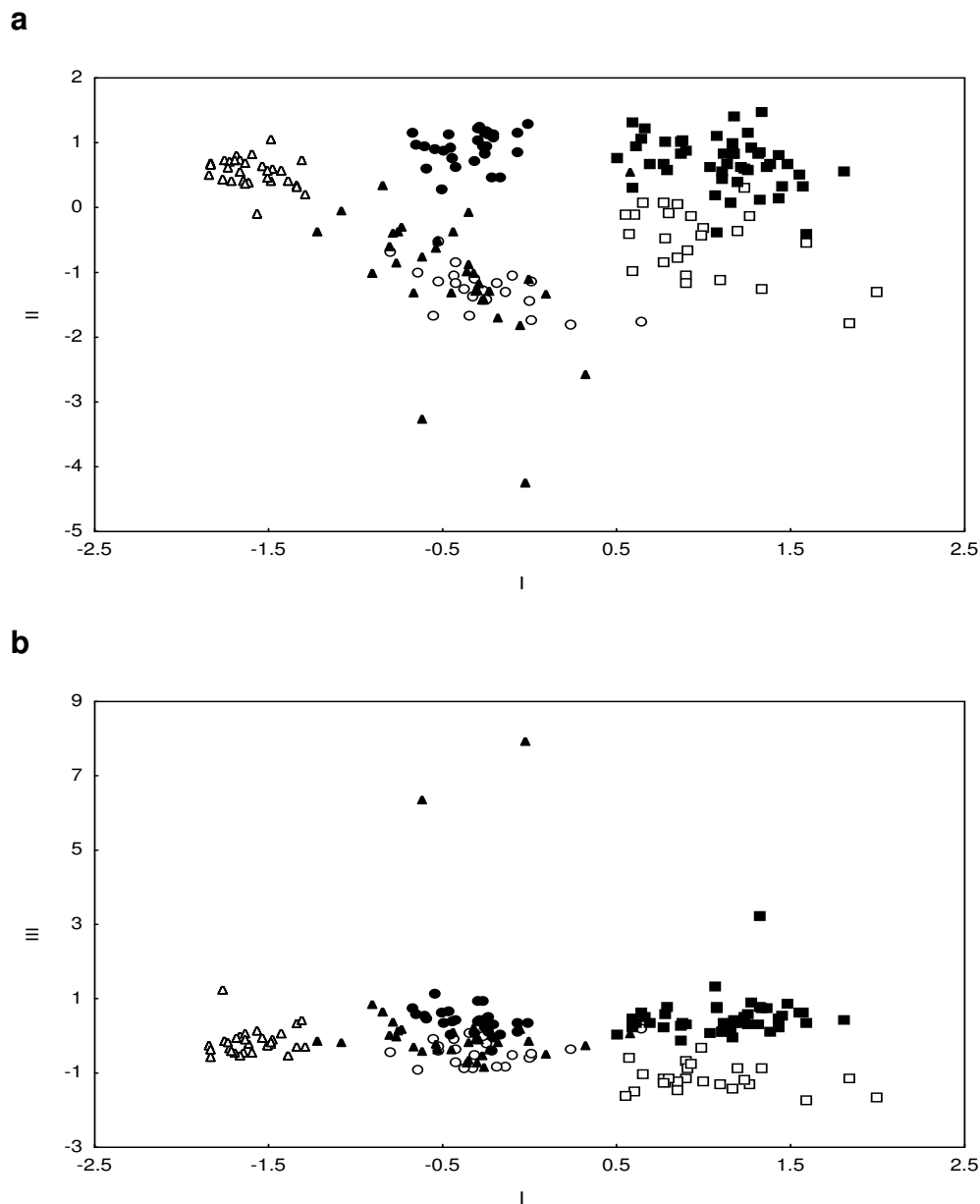


Fig. 4 a The first two components from a principal components analysis (PCA) using 23 measurements of *Palirhoeus eatoni* (unfilled circle), *Ectemnorhinus marioni* (filled circle), *E. similis* (filled square), *Bothrometopus randi* (unfilled square), *B. parvulus* (filled triangle), and *B. elongatus* (unfilled triangle) from sub-Antarctic Marion Island. **b** The first and third principal component axes showing a shape-related separation between *E. similis* (filled square) and *B. randi* (unfilled square)

c

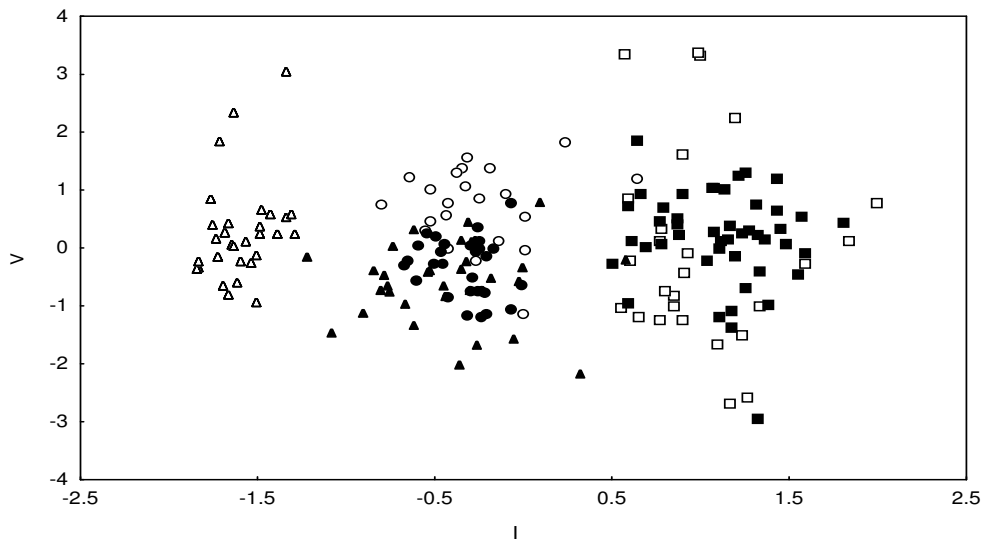


Fig. 4 c The first and fifth principal component axes showing a shape-related separation between *P. eatoni* (unfilled circle) and *B. parvulus* (filled triangle)

Table 2, arranged according to the two major clusters derived from Ward's (1963) cluster analysis of *E. similis*, summarises the criteria used in the character selection procedure in this study. These criteria included loadings of measurements on the first, second, third and fifth PCA axes, magnitude of the coefficient of variation (CV), degree of percentage measurement error (% ME), ease of measurement, potential for non-missing values, and the need to capture the overall configuration of the phenotype. Given that there are only two major discrete clusters, with no further morphologically meaningful sub-clusters within them, one measurement was selected from each extreme terminal sub-cluster as long as it satisfied the aforementioned criteria. In exceptional cases, the selected measurements included characters misplaced with reference to the two major clusters that were delineated. The following section provides the rationale underlying the selection of characters within each of the two major clusters, and their associated extreme terminal sub-clusters (Fig. 3c and Table 2).

Major cluster I: Antero- and mid-dorsal, and mid-ventral measurements

Measurement 1: Total body length (TL)

TL is considered to be in a terminal cluster of its own, was consistently misplaced in the analysis of character associations in all six weevil species, and also represents a measurement that spanned across potential morphological and/or functional units of the phenotype. However, it is selected because of its low CV and % ME as well as its potential in assisting to capture the overall configuration of the phenotype. More importantly, its selection is also influenced by having the highest loading on PCA axis 1 (0.97) that may reflect its importance as a measure of

overall size. In our broader study, the inclusion of TL may assist in addressing questions relating to overall size and shape changes over time, as well as morphometric assessments of potential altitude-related changes.

Measurement 2: Pronotum breadth (PB)

Although both PB and pronotum length (PL) had high loadings on PCA axis I as well as low CVs and % MEs, PB was selected because, being a width measurement, in conjunction with the overall length measurement, TL, it might assist in capturing the overall configuration of the phenotype. In addition, PB is relatively easier to measure than PL.

Measurement 3: Femur length (FL)

FL is placed in a terminal cluster of its own and, being a limb length measurement, it is likely to assist in capturing the overall configuration of the legs. This measurement also had low CV and % ME values.

Measurement 4: Interocular distance (O)

Although both O and antenna/eye diagonal (AD) had high loadings on PCA axis I as well as low CVs and % MEs, the former measurement was selected because of its relative ease of measurement.

Measurement 5: Metacoxal distance (MT)

Although, both MT and elytra breadth leading edge (EE) had high loadings on PCA axis I as well as low CVs and % MEs, MT was selected because of its relative ease of measurement. Being one of the only two measurements with a relatively high loading on PCA axis II, it is important for capturing phenotypic shape information. The character also represents a ventral measurement.

Measurement 6: Maximum elytra breadth (EW)

Although both EW and scape length (S) had high loadings of PCA axis I, low CVs and % MEs, EW was selected. Because it is an overall width measurement it is important for capturing the overall configuration of the phenotype. Furthermore, EW is unlike either TL or S, which are characterised by equivocal placements in the major sub-clusters.

Measurement 7: Length of first three tarsal segments (T3)

Out of the terminal sub-cluster consisting of T3 and tarsal claw length (TC), the former measurement was selected. Although both measurements have high loadings on the first PCA axis and low CVs and % MEs, TC is relatively difficult to measure, easily damaged or absent.

Measurement 8: Meso/metacoxal distance (MM)

Despite high PCA axis I loadings and low % ME, MM rather than coxal diagonal (MD) was selected because the latter measurement had a relatively high CV, is difficult to measure, and prone to damage. MM was also one of the two measurements that loaded highly on the third PCA axis, and may assist in capturing gross shape configuration of the phenotype.

Measurement 9: Inter-antennal distance (A)

A is placed in a terminal sub-cluster of its own, and was selected because it represents a measurement associated with the most anterior part of the head, and may assist in capturing a different configuration of the phenotype.

Measurement 10: Mesocoxal distance (MS)

MS is placed in a terminal sub-cluster of its own, and was selected because of its species discriminating properties on PCA axis II. Its choice may assist also in capturing shape characteristics of the phenotype.

Measurement 11: Femur breadth (FB)

Although both FB and antenna/eye distance (AE) had relatively high loadings on PCA axis I, low CVs and % ME, FB was selected. FB together with femur length (FL), capture the configuration of the femur, and FB also had a relatively high loading on the fifth PCA axis.

Major cluster II: Antennal measurements

Measurement 12: Funicle segment 1 (F1)

This antennal measurement is placed in a terminal sub-cluster of its own, and was selected due to its high loading on the first PCA axis as well as low CV and % ME.

Measurement 13: Funicle segment 2 (F2)

Despite both F2 and antennal club length (AC) having high loadings on the first PCA axis and low % MEs, F2 was selected over AC, as a result of the latter's high CV.

Measurement 14: Rest of funicle (FR)

FR is in a terminal sub-cluster of its own, and was selected because of its high loading on PCA axis I, low CV and % ME.

Measurement 15: Funicle segment 3 (F3)

Like the preceding measurement, F3 was placed in a terminal sub-cluster of its own, and was selected due to its high loading on the first PCA axis, low CV and % ME.

To test the information content of the 15 selected measurements with reference to the initial set of 23 measurements, an R-mode PCA based on the 15 measurements was undertaken in an attempt to discriminate the six weevil species. As exemplified by the scores on the first and second PCA axes (Fig. 5), the phenetic pattern shown by the 15-character PCA was essentially similar to the 23-measurement PCA (cf. Fig. 4a) on axes I, II, III, and V.

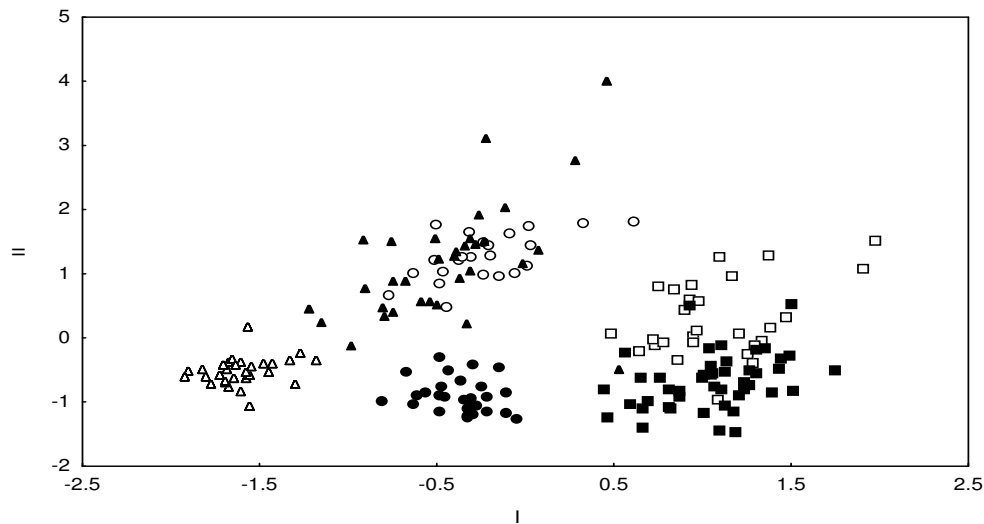


Fig. 5 The first two components from a principal components analysis using 15 measurements of *Palirhoeus eatoni* (unfilled circle), *Ectemnorhinus marioni* (filled circle), *E. similis* (filled square), *Bothrometopus randi* (unfilled square), *B. parvulus* (filled triangle), and *B. elongatus* (unfilled triangle) from sub-Antarctic Marion Island

Discussion

Given the association between developmentally and functionally related traits, Olson and Miller (1958) theoretically placed developmentally and functionally inter-dependent morphological traits in the mammalian cranium into “Functional sets” (*F*-sets) and empirically derived highly correlated characters into “Phenotypic sets” (*P*-sets). Subsequent studies have supported these a priori-defined morphologically integrated functional units (Moss and Young 1960; Cheverud 1982; Cheverud et al. 1989; Cane 1993, see also discussion in Pigliucci 2003),

and were extended into a character selection protocol for small mammals (Taylor and Meester 1993; Chimimba and Dippenaar 1995). However, unlike the mammalian applications of the morphological integration concept, the present study did not have *a priori* defined morphological functional units to use as a frame of reference. Nonetheless, character associations at the two major cluster levels form morphologically logical subsets that broadly relate, first, to the antero- and mid-dorsal, and mid-ventral parts of the phenotype and their associated limbs, and second, to the characteristics of the antenna.

However, caution is required when selecting appropriate characters for recording, particularly with regard to morphological and/or functional units that can be identified within the phenotype. The present investigation was based on previously used characters (see Chown 1989a) to identify a reduced set of measurements, which would summarise the most important variation of the overall phenotype of the weevils. Since most of the previously used measurements may have potentially spanned major morphological and/or functional units of the phenotype, it is perhaps not surprising that the clustering of measurements was equivocal. Nonetheless, the analysis did generate highly correlated subsets of measurements, particularly in the case of the currently recognised *E. similis*, the weevil species represented by the largest sample size. In addition, unlike the case in the mammalian applications, the present study did not show any further sub-clusters (“*P*-set” equivalents of Olson and Miller 1958) beyond the two major cluster levels. It is possible that such phenotypic sets do not exist in the weevils examined, and/or that the measurements that were used did not adequately represent the weevil phenotypes. Alternatively, it is possible that sample sizes were not sufficiently large, because the degree of equivocal character associations among the six weevil species appeared to be a function of sample size. While extensive sampling would have been ideal, our data were constrained by the need to use as homogenous samples as possible to reduce the potential effect of spatial variation in morphology (for discussion see Chimimba and Dippenaar 1995), a feature of these weevil species (e.g., Chown 1992; Chown and Smith 1993; Chown and Klok 2003).

Analyses of the available samples from single localities revealed two outliers that were considered unrepresentative of the populations, and were excluded from the data set to avoid the introduction of bias. Similarly, a preliminary univariate assessment of sexual dimorphism found only a few sexually dimorphic measurements. Chown (1989b, 1992) found that total body length (TL) in all of the weevil species on Marion Island, and in most species on other South Indian Ocean Province Islands (e.g., Chown and Klok 2001), is sexually dimorphic, where males are smaller than females. Sexual size dimorphism is common in virtually all insect species (Helms 1994; Anholt 1997; Fairbairn 1997). Although the full nature and extent of this size variation and its biological significance have yet to be explored, it is thought to be of

considerable importance in the group of weevils under investigation (Chown 1990). By contrast, as revealed by multivariate analyses, there appears to be little sexual shape dimorphism in the species examined in the present study. The use of a multivariate approach to identify potential sex shape dimorphism as adopted in this study is recommended since it evaluates overall differences as it utilises rather than ignores correlations among variables (Willig et al. 1986).

To aid the character selection procedure, a 23-variable PCA that included all studied weevil species was undertaken to develop additional criteria for the selection of representative characters within sub-clusters generated by cluster analysis. This step was considered necessary since the analysis of character associations did not produce unequivocal character groupings, and because it is not yet clear how tightly individual morphological units are integrated within the phenotype. The selection of the final set of measurements was also made with reference to the coefficient of variation and measurement error. With regard to the coefficient of variation, and similar to the murid rodent study undertaken by Chimimba and Dippenaar (1995), all characters had low values for this statistic except for coxal diagonal (MD) and antennal club length (AC). Similarly, measurement error was negligible in this investigation, as was the case in Chimimba and Dippenaar's (1995) investigation. In contrast, other studies, such as those on some birds and mussels have revealed substantial measurement error values ($> 50\%$) (Bailey and Byrnes 1990).

The final set of 15 measurements to be used for systematic and population studies of the group include: total body length (TL); pronotum breadth (PB); femur length (FL); inter-ocular distance (O); metacoxal distance (MT); maximum breadth of elytra (EW); length of first three tarsal segments (T3); meso/metacoxal distance (MM); inter-antennal distance (A), mesocoxal distance (MS); femur breadth (FB); funicle segments 1-3 (F1, F2, and F3); and the rest of funicle (FR). Subsequent testing of the usefulness of the 15 measurements using PCA suggests that this data set contains information equivalent to that contained in the 23 initial measurements.

The character selection protocol adopted here is clearly of value for investigations of both vertebrate (Taylor and Meester 1993; Chimimba and Dippenaar 1995) and invertebrate taxa. As part of a broader investigation of the conservation of endemic weevils at the Prince Edward Islands, the present study will make interpretation of long-term morphometric changes in weevil species occurring on the Prince Edward Islands, more straightforward. This is essential if the reasons underlying changes in weevil morphology (which have already been detected in terms of declines in body length, Chown and Smith 1993), are to be fully ascertained. At present, these changes are thought to be a consequence of predation by

introduced mice, but current rapid climate changes at the island (Smith 2002) might also be responsible for morphological change (see e.g., Huey et al. 2000). Determining the causes of these changes is essential for making the appropriate conservation decisions that will be essential for ensuring the long-term persistence of weevils both on the Prince Edward Islands and elsewhere in the region.

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Appendix I

Specimens examined: UP = University of Pretoria, Department of Zoology and Entomology sample acronym.

Palirhoeus eatoni – Trypot Beach (46°53.052'S 037°52.060'E), 19 males (UP 11.1, 11.3, 11.5, 11.7-11.9, 11.11-11.14, 11.6-11.19, 15.4, 15.5, 15.8, 15.10, 15.13) and 11 females (UP 11.2, 11.4, 11.6, 11.10, 11.15, 15.7, 15.9, 15.11, 15.12, 15.14, 15.15).

Bothrometopus randi - Trypot Beach (46°53.052'S 037°52.060'E), 17 males (UP 9.4-9.6, 9.10, 9.12, 9.13, 13.2-13.7, 13.9, 13.13-13.15, 13.20) and 13 females (UP 9.1-9.3, 9.9, 9.11, 13.1, 13.8, 13.10-13.12, 13.16-13.18).

B. parvulus - Tafelberg (46°53.135'S 037°48.201'E), 22 males, (UP 30.2-30.5, 30.7-30.10, 32.1, 32.3, 32.6-32.9, 32.11, 41.7, 41.9-41.11, 41.15, 41.16) and 13 females, (UP 30.1, 30.6, 30.11, 32.2, 32.4, 32.5, 32.10, 41.5, 41.6, 41.8, 41.12-41.14).

B. elongatus – Tafelberg (46°53.135'S 037°48.201'E), 16 males (UP 31.1, 31.4-31.8, 31.10, 34.1, 34.2, 34.5, 34.10, 34.11, 34.13, 36.4, 36.5, 36.8) and 15 females, (UP 31.2, 31.3, 31.9, 34.3, 34.4, 34.6-34.9, 34.12, 36.1-36.3, 36.7, 36.9).

Ectemnorhinus marioni – Tafelberg (46°53.135'S 037°48.201'E), 16 males (UP 23.12, 23.14-23.16, 23.18, 23.19, 35.13-35.15, 35.17, 35.18, 35.20-35.22, 35.28, 35.30) and 14 females (UP 23.11, 23.13, 23.17, 23.20, 35.11, 35.12, 35.16, 35.19, 35.23, 35.24-35.27, 35.29).

E. similis – Tafelberg (46°53.135'S 037°48.201'E), 17 males (UP 24.11, 24.14, 24.15, 24.17, 27.13, 27.15, 27.17, 27.28, 37.13, 37.14, 37.18-37.20, 39.15, 39.16, 39.18, 39.19) and 31 females (UP 24.12, 24.13, 24.16, 24.18-24.20, 27.11, 27.12, 27.14, 27.16, 27.18-27.27, 27.29, 27.30, 37.12, 37.15-37.17, 39.13, 39.14, 39.17, 39.20, 39.21).

...

“That which we persist in doing, becomes easier – not that the nature of the task has changed, but our ability to do has increased”

Ralph Waldo Emerson