Chapter 4

Morphometric changes over time: an analysis based on weevils (Coleoptera: Curculionidae) from the sub-Antarctic Marion and Prince Edward Islands

Abstract The objective of the present study was to assess morphometric changes over time using four weevil species (*Bothrometopus elongatus*, *B. randi*, *B. parvulus* and *Palirhoeus eatoni*) occurring on the sub-Antarctic Marion and Prince Edward Islands. Data analysed included material collected non-consecutively over five decades, and was based on both univariate and multivariate statistical analyses of 15 linear measurements. Results suggest multivariate morphometric size differences between samples collected in the mid-1960s and 1970s and those collected recently (2001-2003) from both Marion and Prince Edward Islands. Samples from the early- to mid-1980s show multivariate morphometric shape differences (principal component III) when compared with recently (2001-2003) collected samples from both Marion and Prince Edward Islands. Similarly, regression analysis revealed statistically significant correlations between the morphometric shape-related principal component III and sampling year for all species collected on Marion Island, while the morphometric size-related principal component I revealed positive and statistically significant correlations with sampling year for *B. parvulus* and *P. eatoni* on Prince Edward Island. Generalized Linear Models (GLZs) indicated year of sampling to contribute most significantly to both multivariate size (principal component I) and shape (principal component III) changes in all species sampled on both Islands, while temperature contributed highly significantly to shape changes (principal component III) on Marion Island. Mice predation contributed less to the overall percent deviance explained by the GLZs. All these results are discussed with reference to the potential effect of climate change and predation by the invasive house mouse (*Mus musculus*, sensu lato) that was introduced on Marion Island in the early 1800s but not on Prince Edward Island.

Key words: Weevils, morphometric change, time, climate change, mouse predation

Running title: Morphometric changes over time in weevils from Marion and Prince

Edward Islands

Introduction

Anthropogenic changes may have serious implications for island ecosystem functioning (Vitousek 1986; Atkinson 1989; Chapuis et al. 1994; Frenot et al. 2005). The Southern Ocean Province Islands, occupied by various endemic fauna and flora, are of considerable biological and conservation importance (Chown et al. 1998). Human-induced impact, either through climate change or introduced invasive species are important contributors to changes in island fauna and flora (see Smith and Steenkamp 1990; Chown et al. 1998; Bergstrom and Chown 1999; Smith et al. 2002; Weimerskirch et al. 2003). Pronounced changes in fauna and flora and consequences thereof on two of the Southern Ocean Islands, Marion and Prince Edward Islands, are well-documented (see Chown and Smith 1993; Hänel and Chown 1998; Huyser et al. 2000; Smith 2002).

Although both islands are considered to share similar geological and paleo-climatic histories, only Marion Island shows evidence of extensive glaciation, while local climate on Prince Edward Island is generally considered to be milder and drier than on Marion Island (Verwoerd 1971). It has been suggested that these local climatic differences may be due to differences in island size, topography, elevation and the absence of a permanent ice cap on Prince Edward Island (Crafford and Scholtz 1987).

An additional difference between the two islands is the accidental introduction of the house mouse (*Mus musculus*, sensu lato) on Marion Island by sealers in the early 1800s, but not on Prince Edward Island (Watkins and Cooper 1986). The house mouse is considered to have a significant impact, either directly or indirectly, on both plant and invertebrate communities (Bonner 1984; Chapuis et al. 1994), therefore, influencing ecosystem functioning (Smith and Steenkamp 1990; Chown and Smith 1993; Hänel and Chown 1998; Bergstrom and Chown 1999; Huyser et al. 2000). For example, Chown and Smith (1993) showed a decrease in the body lengths of *Bothrometopus randi* and *Ectemnorhinus similis* on Marion Island from the mid-1980s to 1992 and suggested that this may be due to increasing size-selective predation by mice.

In addition, invertebrate population densities are also considered to be influenced by mouse predation. For example, the low densities of some invertebrates, such as *Pringleophaga marioni* on Marion Island, but not on Prince Edward Island, have been attributed to mouse predation (Burger 1978; Crafford and Scholtz 1987; Hänel and Chown 1998). The presence of mice and their diet preference has similarly been of great concern on both Macquarie Island and the Kerguelen archipelago (Copson 1986; Le Roux et al. 2002), suggesting that mice represent a significant conservation threat to many of the indigenous Southern Ocean Island

fauna (Jones et al. 2003). On Marion Island, mice are considered to be responsible for the declining population numbers of weevils and flightless moths (Gleeson and van Rensburg 1982; Smith and Steenkamp 1990; Hänel and Chown 1998). These invertebrates represent important prey items for various endemic predators, such as the lesser sheathbill (Huyser et al. 2000) and are also considered to contribute significantly to nutrient cycling on both Marion and Prince Edward Islands (Smith and Steenkamp 1993).

Of particular concern is that mice are not only preying on endemic species of conservation concern, but have also been reported to be selecting certain prey sizes (Smith et al. 2002) where bigger individuals are targeted, thereby influencing the ecologically active component of the population (Crafford 1990; Smith et al. 2002). On Marion Island, size-selective predation by mice has been reported for two mouse-preferred weevil species, *Bothrometopus randi* and *Ectemnorhinus similis* (Chown and Smith 1993). The reduction in relative abundance and decline in body length between 1986 and 1992 in both species has been attributed to an increased intensity in size-selective predation by mice (Chown and Smith 1993). In addition, marked differences in species composition, population size and structure of invertebrate fauna attributed to mice predation, have been reported between Marion Island and the mouse-free Prince Edward Island (Crafford and Scholtz 1987).

Smith and Steenkamp (1990) suggested that an increase in mouse population numbers attributed to global warming may lead to enhanced predation on soil invertebrates and reduce the overall nutrient mineralisation on Marion Island. Consequently, an imbalance between primary production and decomposition may change rates of peat accumulation that is important in controlling vegetation succession on the island (Smith and Steenkamp 1990, 1993). In addition, increased temperature may allow mice to occupy habitats at higher altitudes, therefore, allowing predation on a previously inaccessible invertebrate prey base (Klok and Chown 1997).

It has been reported that since the late 1960s, the annual mean surface air temperature on Marion Island has increased by an average of 0.04^{o}C (Smith 1991) and that mire habitats that cover most of the lowlands are consistently drying out (Chown and Smith 1993). The increase in temperature, elevated CO_2 concentration and the drying effect are expected to increase vegetation productivity and nutrient demand (Smith 1991). Microbiological processes, however, are not expected to increase significantly as they are mainly influenced by water-logging rather than temperature (Smith 1991). Consequently, the main source of nutrient cycling on Marion and Prince Edward Islands remains a detritus cycle that is largely influenced by invertebrate detritivores (Klok and Chown 1997).

Smith and Steenkamp (1990) suggested the effect of increased temperature on macro-invertebrate activity to be greater than the effect of changing temperature and moisture levels

on microbe-mediated nutrient cycling. The activities of soil macro-invertebrates are strongly temperature-dependent, for example, ingestion rates of *Pringleophaga marioni* larvae increase with increasing temperature but assimilation efficiencies decrease (Smith and Steenkamp 1990). In addition, weevil larvae have been shown to stimulate nutrient release from plant litter and peat, therefore, playing an important role in certain vegetation communities (Smith and Steenkamp 1990). Thus, not only temperature but also the added effect of increased mouse predation on preferred weevil species may lead to a decrease in population densities, in turn, having an influence on the Marion Island ecosystem (Smith and Steenkamp 1990).
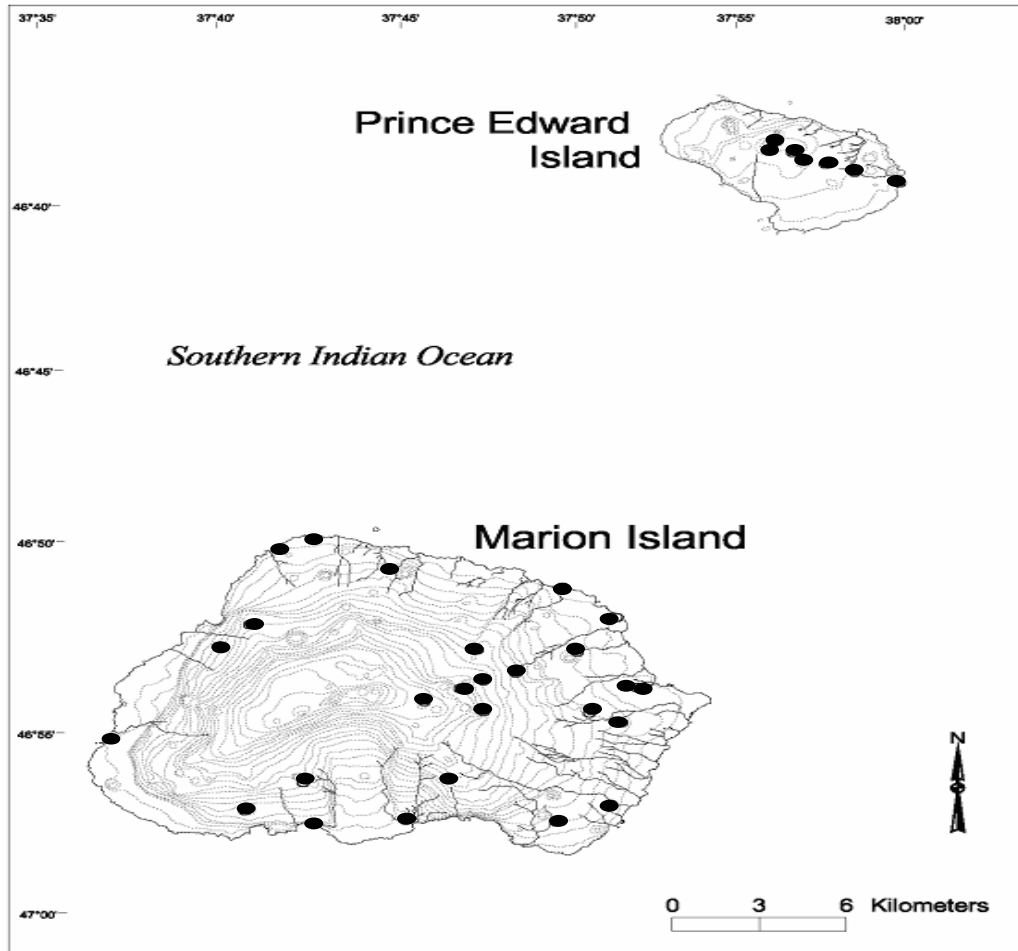
Marion and Prince Edward Islands offer ideal ecosystems to study the responses of weevil species to changing global climate and/or biological invasions through the analysis of morphometric size and shape changes. As the two islands are separated by a 22 km distance, similar temperature increases are assumed to occur on both islands, with the mouse-free Prince Edward Island serving as an ideal control in an assessment to partition the potential influence of mouse predation. The present study, therefore, attempts to assess: 1) morphometric changes over time in four weevil species, namely *Bothrometopus elongatus, B. parvulus, B. randi* and *Palirhoeus eatoni* using multivariate analyses and Generalized Linear Models; and 2) the potential influence(s) of climate change and mouse predation.

Materials and methods

Study area and samples

The study is based on four weevil species, namely, *Bothrometopus elongatus* (Jeannel 1940), *B. parvulus* (C.O. Waterhouse 1885), *B. randi* (Jeannel 1940) and *Palirhoeus eatoni* (C.O. Waterhouse 1885) collected in 1986, 1998 and 2000 by S.L. Chown, between 2001 and 2003 by L. Janse van Rensburg, and previously collected material (1965, 1975 and 1980-1984) housed in the collection of the Transvaal Museum (TM) and the National Insect Collection (NIC), Pretoria, South Africa. Sampling was conducted over the entire Marion Island and along a 200 m interval altitudinal gradient on the eastern side of Prince Edward Island (Fig. 1). Sample size, sampling localities, mean annual temperature (Marion Island) and mean annual rainfall (Marion Island) are presented in Appendix I. For information on the natural history of the islands see Smith (1987), Hänel and Chown (1999) and Chown et al. (2002).

Fig. 1a Collection localities (*filled black circles*) indicated for Marion and Prince Edward Islands

Preliminary molecular analyses in a parallel study by G.C. Grobler and morphometric analysis of *Bothrometopus* samples from 0 m a.s.l. on Marion Island, strongly suggest the traditionally recognised *B. parvulus* may include another undescribed species. Consequently, pending a resolution of this taxonomic uncertainty, all *B. parvulus* samples collected at 0 m a.s.l. on Marion and Prince Edward Islands are herein excluded from analysis. In addition, due to the uncertainty of the taxonomic status of the *Ectemnorhinus* species on both Marion and Prince Edward Islands (see Chapter 3), these species were herein also excluded from analysis.

Morphometric measurements

A set of 15 linear measurements, defined and illustrated in Janse van Rensburg et al. (2003) were recorded to the nearest 0.05 mm (TL and EW; see below), 0.03 mm (PB and FL) and 0.01 mm (O, A, F1, F2, F3, FR, T3, MS, MT, MM and FB). All measurements for the entire dataset were recorded by a single observer (L. J. van Rensburg) using a stereo microscope fitted with a calibrated eye-piece micrometer. These variables include: Total body length (TL), elytra width

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(EW), pronotum breadth (PB), inter-ocular distance (O), inter-antennal distance (A), funicle segments 1-3 (F1, F2, F3), rest of funicle (FR), length of first 3 tarsal segments (T3), mesocoxal distance (MS), metacoxal distance (MT), meso/metacoxal distance (MM), femur length (FL) and femur breadth (FB). The absence of multivariate sexual dimorphism (see Janse van Rensburg et al. 2003) justified the pooling of sexes in all subsequent analyses.

Statistical analyses

Exploratory univariate and multivariate data screening revealed some outlier specimens that were considered not to be representative of the population. A re-examination of these outlier specimens revealed outlier values arising from damaged parts, and to avoid the introduction of bias these specimens were excluded from subsequent analyses.

Multivariate morphometric analyses were used to assess morphometric changes over time in samples of *B. elongatus*, *B. parvulus*, *B. randi* and *P. eatoni* collected non-consecutively between 1965 and 2003. While locality information was not available for a number of specimens from museum collections and some localities were represented by very small sample sizes, species-specific data from similar sampling years were pooled for computational easiness. The analyses included principal components analysis (PCA) based on among-character product-moment correlation coefficients (Pimentel and Smith 1986; Rohlf 1986) and unweighted pair-group arithmetic average (UPGMA) cluster analysis computed from among operational taxonomic units (OTU; Sneath and Sokal 1973) euclidean distances and product-moment correlation coefficients. The *a priori*-derived groupings were further examined using the *a posteriori* canonical variates (discriminant) analysis (CVA; Sneath and Sokal 1973) based on data from years with adequate sample sizes (n ≥ 3). The PCA, UPGMA cluster analysis and the CVA were always followed by a multivariate analysis of variance (MANOVA; Zar 1996) to test for statistically significant differences between groups. Patterns of morphometric variation over time were also evaluated by Spearman's rank correlation analysis (Zar 1996) of PCA scores and individual measurements on sampling year.

The relationships between sampling year, gender, mice predation (represented by the island of origin), altitude, and species were examined using Spearman's rank correlation coefficients (Zar 1996) and Type III sum of squares Generalized Linear Models (GLZs; McCullagh and Nelder 1989). These analyses included the full dataset comprising all four species. Some variables were significantly correlated with each other ($r_s > 0.20$) and had very low tolerances (< 0.00001) indicating that co-linearity was likely to influence the GLZs (Quinn and Keough 2002). Therefore, highly correlated variables were excluded as explanatory

variables in the GLZs (Quinn and Keough 2002). Subsequently, species-specific data were analysed independently using GLZs.

Chown and Klok (2003) reported mice on Marion Island to occur up to 750 m a.s.l. during summer months. Consequently, data was initially collected along an altitudinal gradient during 2001-2003 with the aim of using the area above 750 m a.s.l. as a control. However, mice and mice pellets were observed to occur at between 800 m and 1000 m a.s.l. in the field (L. J. van Rensburg and G.C. Grobler, pers. obs.). Given that mice are present on Marion Island but absent from Prince Edward Island, an attempt was made to assess the potential effect of mouse predation on weevil morphology using the latter island as a control.

Previously collected museum material was insufficient and also from a limited number of altitudes and there was a statistically significant correlation between altitude and sampling year in all species on both Marion (r_s = 0.21; P < 0.001) and Prince Edward (r_s = 0.21; P < 0.001) Islands. This necessitated using subsets of altitudinal data with adequate sample sizes across years within each species in GLZs in order to reduce the potential influence of elevation in addressing the question of morphometric size and shape changes over time. Therefore, the final predictors chosen for the independent GLZs for all four species were sampling year, mice predation and gender.

Mean annual temperature ($°C$) and mean annual rainfall (mm) data were only available for Marion Island and were obtained from the South African Weather Bureau that included data from 1950 to 2004 (also see Smith and Steenkamp 1990; Smith 2002). Although temperature and rainfall were significantly correlated with each other (ranging between r_s = -0.56 and r_s = -0.75 for all species), tolerances were high (> 0.80) indicating that co-linearity was unlikely to influence the GLZs (Quinn and Keough 2002). However, a zero pivot error (see STATISTICA version 5.5; Statsoft 1995) was encountered in the GLZs of the Marion Island dataset using temperature, rainfall and gender and while using rainfall and gender only. Therefore, rainfall being highly correlated with most variables, was excluded from the GLZs, resulting in only temperature and gender being included in the final model (Quinn and Keough 2002) to assess the percentage deviance explained by these variables.

GLZs were used to assess the degree of contribution to the total percent deviance of individual independent variables: sampling year, mice predation, gender and temperature to dependent variables that included the overall size-related PCA I and the shape-related PCA II and III scores. Consequently, GLZs were computed for: 1) each species for a combined Marion and Prince Edward Islands dataset; and 2) each species for Marion Island but using only temperature and gender as additional independent variables.

In all Type III sum of squares GLZs, a normal error distribution was specified for scores of principal components I, II and III and linked to predictor variables with an identity

link function (McCullagh and Nelder 1989). The goodness of fit was calculated using the deviance statistic (deviance/df close to 1.0 indicates a good fit) and the proportion of deviance explained by the models (% de) was estimated (McCullagh and Nelder 1989; Dobson 2002). Change in the deviance for single variables in the final model was used to estimate the contribution of individual variables to the total explained deviance by the final models to specified PCA axes (Collet 1991; Lobo et al. 2002).

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

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Results

Multivariate analysis

The results of both the *a priori* PCA and UPGMA cluster analysis were broadly similar and are best illustrated by the results of the former series of analyses.
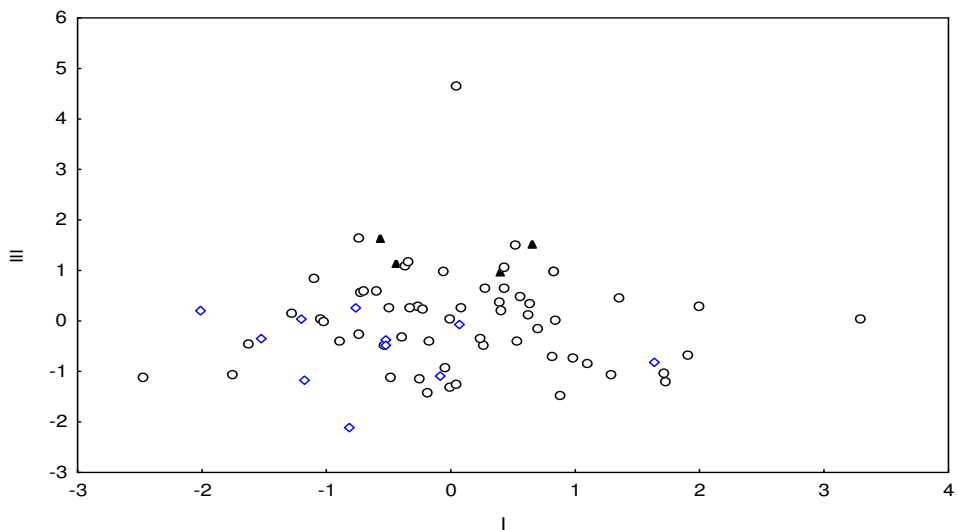
Bothrometopus elongatus

Principal components analyses of *B. elongatus* from both Marion and Prince Edward Islands indicated no size-related (PCA axis I) differences over time (Fig. 2a, b). However, there was evidence of a morphological shape difference on the third PCA axis (7.06% variance for Marion Island and 7.92% for Prince Edward Island) for samples collected in some sampling years on both Marion (1986 *vs* all years examined) (Fig. 2a) and Prince Edward (1983 *vs* 2000) (Fig. 2b) Islands. Measurements that contributed most to the shape-related separation on axis III between 1986 and all other sampling years on Marion Island are: MS, MM, FL and FB (Table 1a). In addition, F1, MS, MM and FB contributed most to the differences between samples collected in 1983 and 2000 on axis III for Prince Edward Island (Table 1a).
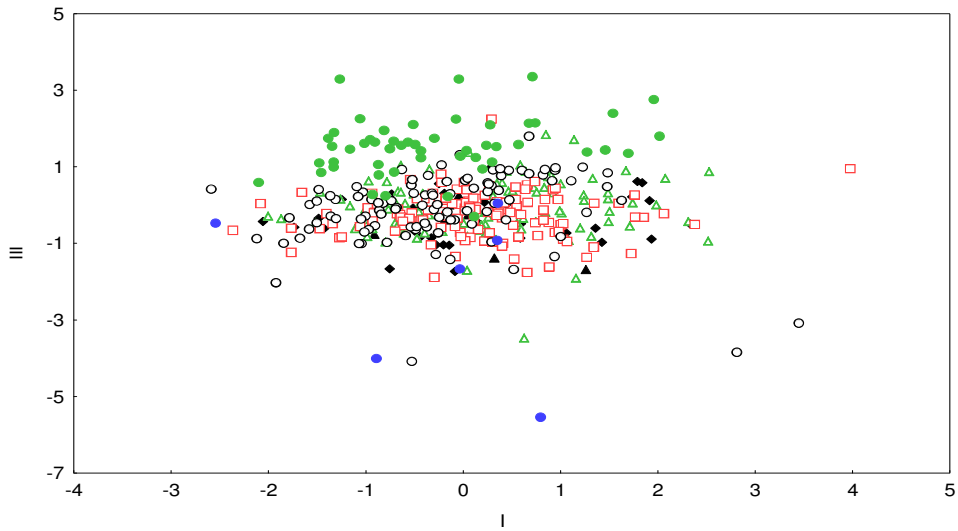
Fig. 2a Components I and III from a principal components analysis (PCA) of *Bothrometopus elongatus* collected on Marion Island during 1975 (*filled blue circle*), 1983 (*filled black triangle*), 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)
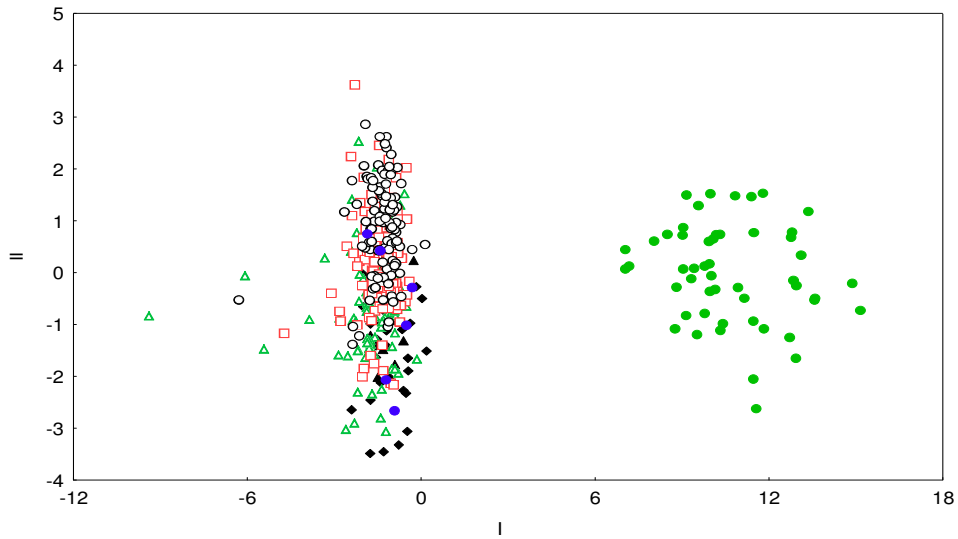

Fig. 2b Components I and III from a principal components analysis (PCA) of *Bothrometopus elongatus* collected on Prince Edward Island during 1983 (*filled black triangle*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)
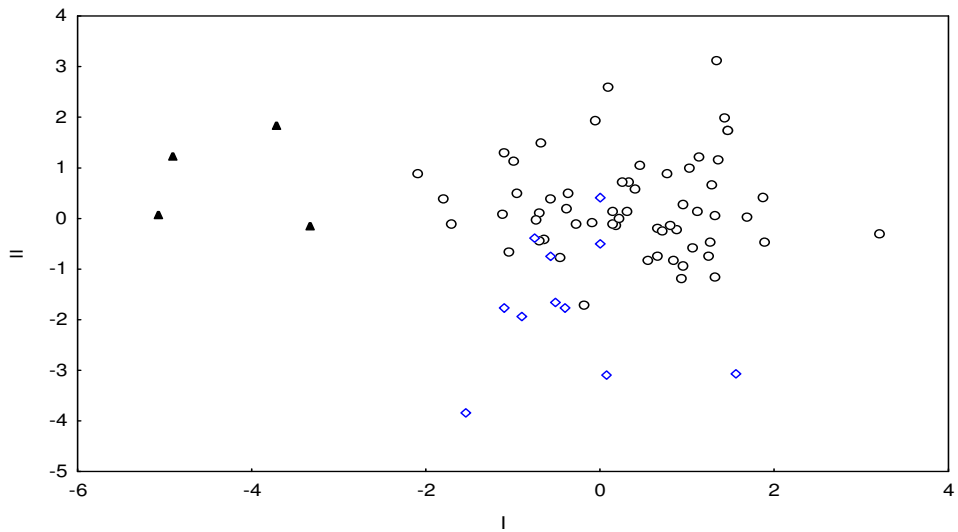
Table 1a Loadings of variables on components I, II and III from a principal components analysis of *Bothrometopus elongatus* collected on Marion and Prince Edward Islands

Variable	Marion Island			Prince Edward Island		
	Principal components			Principal components		
	I	II	III	I	II	III
TL	0.845	0.306	-0.036	0.851	0.266	0.111
EW	0.485	0.471	-0.189	0.691	0.330	0.055
PB	0.879	0.106	0.109	0.893	0.087	-0.151
O	0.727	0.223	-0.139	0.702	0.079	0.034
A	0.540	0.459	-0.134	0.583	0.099	0.324
F1	0.729	-0.319	0.153	0.564	-0.451	0.431
F2	0.754	-0.335	0.154	0.598	-0.292	0.091
F3	0.629	-0.397	-0.093	0.515	-0.581	-0.027
FR	0.675	-0.404	0.024	0.567	-0.421	-0.174
T3	0.789	-0.161	0.009	0.822	-0.030	-0.184
MS	0.206	0.352	0.365	0.241	0.436	-0.499
MT	0.739	0.136	-0.085	0.509	0.538	-0.220
MM	0.505	0.113	-0.568	0.314	0.472	0.593
FL	0.112	0.467	0.585	0.831	-0.006	0.010
FB	0.645	-0.213	0.333	0.565	-0.310	-0.366
% trace	42.59%	10.44%	7.06%	41.31%	12.03%	7.92%

PCA groupings were further analysed using a CVA which indicated pronounced differences between samples collected in 1986 from all other sampling years on Marion Island (Fig 2c: MANOVA: $F_{90,22}$ = 22.94; n = 420; $P < 0.001$) as well as between 1983 and 2000 from Prince Edward Island on the first CVA axis (Fig. 2d: MANOVA: $F_{30,12}$ = 3.08; n = 75; $P < 0.001$). However, there is a tendency for 2000 and 2003 samples to separate on axis II in the Prince Edward Island CVA (Fig. 2d). For both Marion and Prince Edward Islands, TL (axis I and II) contributed highly to the separation of sampling years indicated above. FR, T3, FL (axis I); A, F3, FR (axis II) contributed highly to the variation on Marion Island, while EW, A, F3 (axis I); EW, PB, O, F1 (axis II) were additional variables contributing to the variation in Prince Edward Island samples (Table 1b). Collation of all the multivariate results suggest a tendency for both Marion and Prince Edward Islands samples from the early- and mid-1980s to be morphometrically different in terms of shape from recently collected samples.

Fig. 2c First two axes from a canonical variates analysis (CVA) of *Bothrometopus elongatus* collected on Marion Island during 1975 (*filled blue circle*), 1983 (*filled black triangle*), 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)


Fig. 2d First two axes from a canonical variates analysis (CVA) of *Bothrometopus elongatus* collected on Prince Edward Island during 1983 (*filled black triangle*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

Table 1b Loadings of variables on canonical variate axes from canonical variates analyses of *Bothrometopus elongatus* collected on Marion and Prince Edward Islands

Variable	Marion Island		Prince Edward Island	
	Canonical variate		Canonical variate	
	I	II	I	II
TL	-0.171	-0.430	-1.870	-0.603
EW	0.002	0.065	1.167	0.788
PB	-0.115	-0.188	-0.025	-1.298
O	0.010	0.040	0.291	1.060
A	-0.010	-0.491	-0.691	0.451
F1	-0.031	0.067	-0.278	0.744
F2	-0.038	0.020	0.121	-0.081
F3	0.057	0.458	0.674	0.019
FR	-0.282	0.600	0.248	0.186
T3	-0.277	-0.202	-0.070	-0.462
MS	0.071	-0.268	-0.095	0.220
MT	-0.179	-0.265	0.438	-0.264
MM	-0.069	-0.032	-0.059	-0.347
FL	1.341	0.065	0.578	0.496
FB	-0.099	0.234	0.005	-0.022

Bothrometopus parvulus

Neither Marion Island nor Prince Edward Island samples showed multivariate size-related differences over time. Similar to *B. elongatus*, *B. parvulus* exhibited distinct shape-related differences between samples from the early- and mid-1980s and recently collected samples from both Marion Island (1986 samples; axis III; 6.61% variance; Fig. 3a) and Prince Edward Island (1980/2000 samples; axis IX; 2.35% variance; Fig. 3b). Measurements that contributed to the differences in the Marion Island samples were, F1, MS, MM and FL and for Prince Edward Island samples were, PB, F2, F3 and FR (Table 2a).
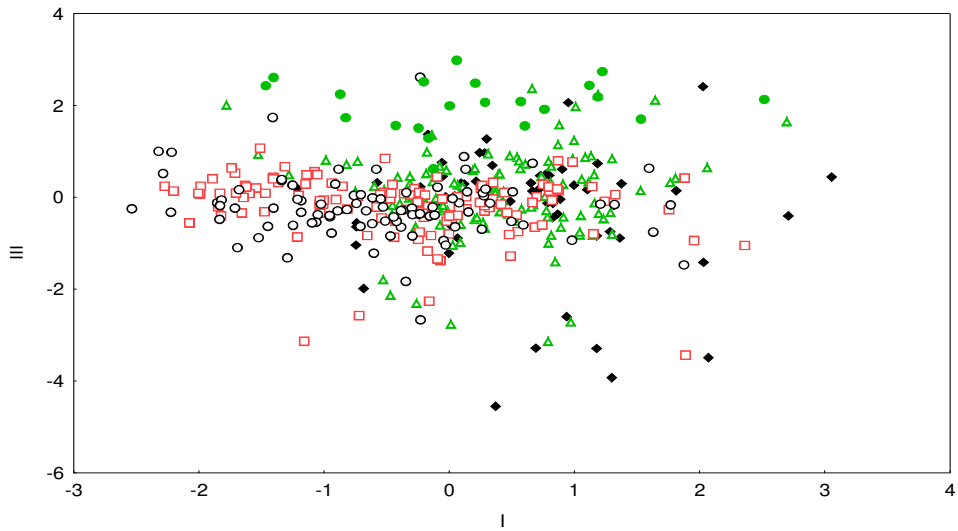
Fig. 3a Components I and III from a principal components analysis (PCA) of *Bothrometopus parvulus* collected on Marion Island during 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)
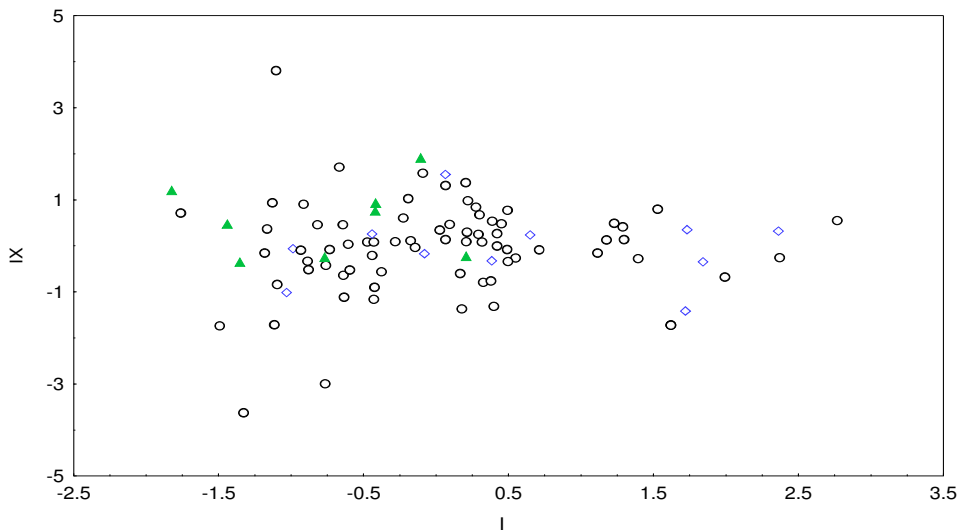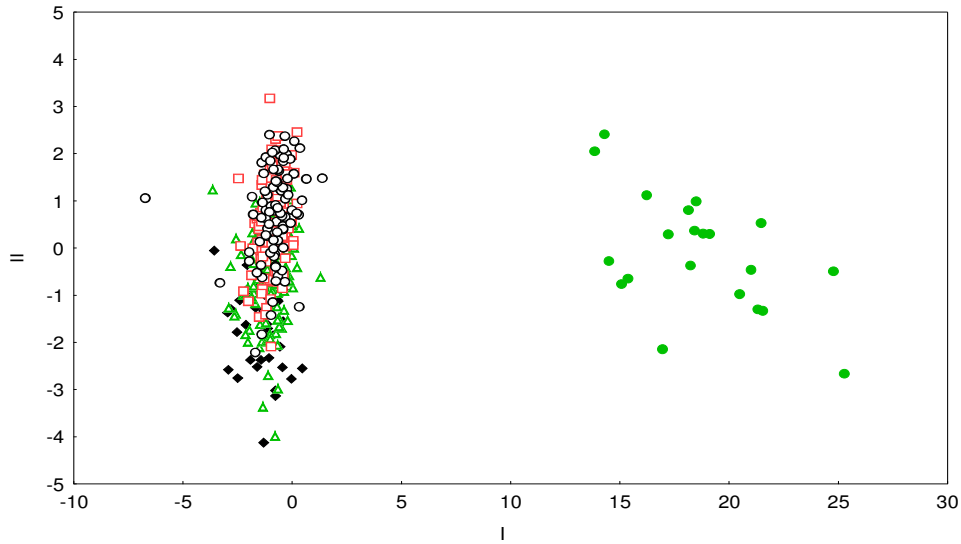

Fig. 3b Components I and IX from a principal components analysis (PCA) of *Bothrometopus parvulus* collected on Prince Edward Island during 1980 (*filled green triangle*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

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Table 2a Loadings of variables on components I, II, III, IV (Marion Island) and IX (Prince Edward Island) from a principal components analysis of *Bothrometopus parvulus* collected on Marion and Prince Edward Islands

	Marion Island				Prince Edward Island			
Variable	Principal components				Principal components			
	I	II	III	IV	I	II	III	IX
TL	0.905	-0.032	-0.020	-0.001	0.885	-0.117	-0.183	0.129
EW	0.828	-0.077	0.030	-0.074	0.513	-0.193	-0.636	-0.085
PB	0.919	-0.046	0.031	0.039	0.757	-0.328	0.038	-0.225
O	0.833	-0.152	0.010	0.101	0.869	-0.197	-0.072	0.154
A	0.750	-0.422	0.010	0.099	0.695	-0.442	-0.087	0.074
F1	0.528	0.331	-0.448	-0.064	0.668	0.387	0.084	0.015
F2	0.703	0.454	-0.014	0.070	0.756	0.279	0.157	-0.188
F3	0.363	0.651	-0.051	-0.128	0.740	0.364	0.197	-0.335
FR	0.645	-0.359	-0.289	-0.078	0.733	0.328	0.224	0.297
T3	0.697	-0.433	-0.239	-0.056	0.865	0.180	0.000	0.041
MS	0.538	0.282	0.573	0.252	0.347	-0.793	0.246	-0.043
MT	0.753	-0.123	0.266	0.275	0.593	-0.528	0.210	-0.014
MM	0.501	0.407	-0.304	-0.074	0.487	0.369	-0.381	-0.022
FL	0.391	-0.081	0.382	-0.815	0.904	0.123	-0.149	0.027
FB	0.755	0.198	0.081	0.002	0.573	0.162	0.363	0.089
% trace	48.30%	10.48%	6.61%	5.81%	50.42%	13.20%	6.50%	2.35%

Differences observed in the PCA are supported by the CVA on the first axis for both Marion Island (Fig. 3c: MANOVA: $F_{60,15} = 40.77$; $n = 424$; $P < 0.001$) and Prince Edward Island (Fig. 3d: MANOVA: $F_{30,150} = 1.43$; $n = 92$; $P > 0.05$). TL and FL (axis I) exhibited high loadings (regardless of sign) on both islands, while F3 and O were of additional importance in the differences between the Prince Edward Island samples (Table 2b). Collation of all these results also suggest a tendency for *B. parvulus* collected during the early- and mid-1980s on both Marion and Prince Edward Islands to differ from those samples collected in recent years based on shape.

Fig. 3c First two axes from a canonical variates analysis (CVA) of *Bothrometopus parvulus* collected on Marion Island during 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)
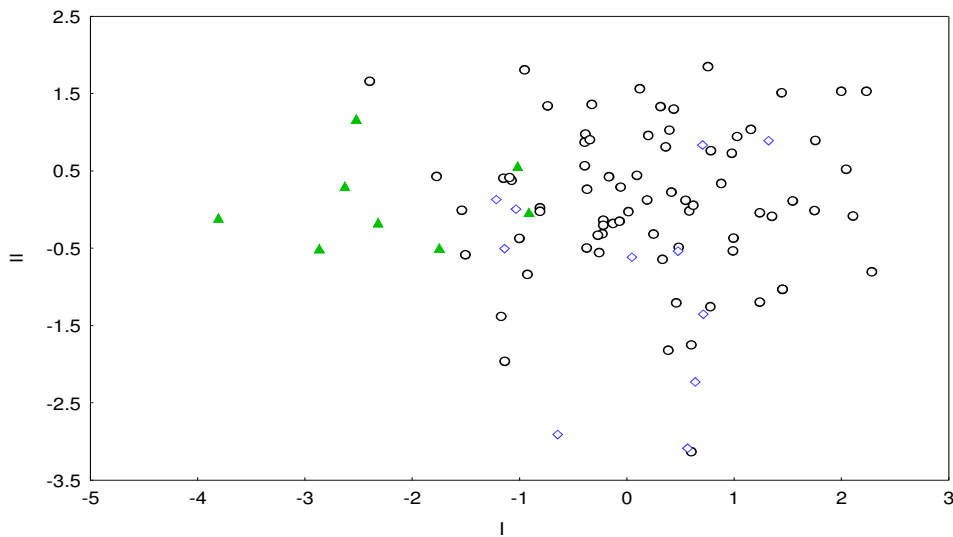
Fig. 3d First two axes from a canonical variates analysis (CVA) of *Bothrometopus parvulus* collected on Prince Edward Island during 1980 (*filled green triangle*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

Table 2b Loadings of variables on canonical variate axes from canonical variates analyses of *Bothrometopus parvulus* collected on Marion and Prince Edward Islands

	Marion Island		Prince Edward Island	
Variable	Canonical variate		Canonical variate	
	I	II	I	II
TL	-0.642	-1.132	-0.915	0.265
EW	-0.160	0.451	0.741	0.193
PB	-0.248	-0.067	0.471	-0.329
O	-0.036	-0.228	0.612	-0.242
A	0.076	-0.297	0.210	-0.578
F1	-0.064	-0.127	-0.028	0.210
F2	-0.265	-0.125	-0.032	0.280
F3	-0.055	-0.082	0.726	0.363
FR	-0.229	0.025	0.270	0.086
T3	-0.167	0.108	-0.024	0.175
MS	0.223	-0.327	0.117	-0.318
MT	-0.080	0.209	-0.314	-0.396
MM	-0.039	0.241	0.432	-0.230
FL	1.865	-0.013	-1.145	-1.088
FB	-0.154	0.509	0.171	-0.077

Bothrometopus randi

On Marion Island, *B. randi* sampled in 1986 showed a shape-related difference with other sampling years on the third PCA axis (8.07% of variance) (Fig. 4a). For Prince Edward Island PCA, however, the 1965 sample separated from the other sampling years on the first size-related PCA axis (50.07% variance) (Fig. 4b). FL was important in the size-related separation on both islands, while MS and MM contributed to additional differences on Marion Island (axis III), with TL, EW, PB, O and A (axis I) being of additional importance in the separation of the Prince Edward Island samples (Table 3a).
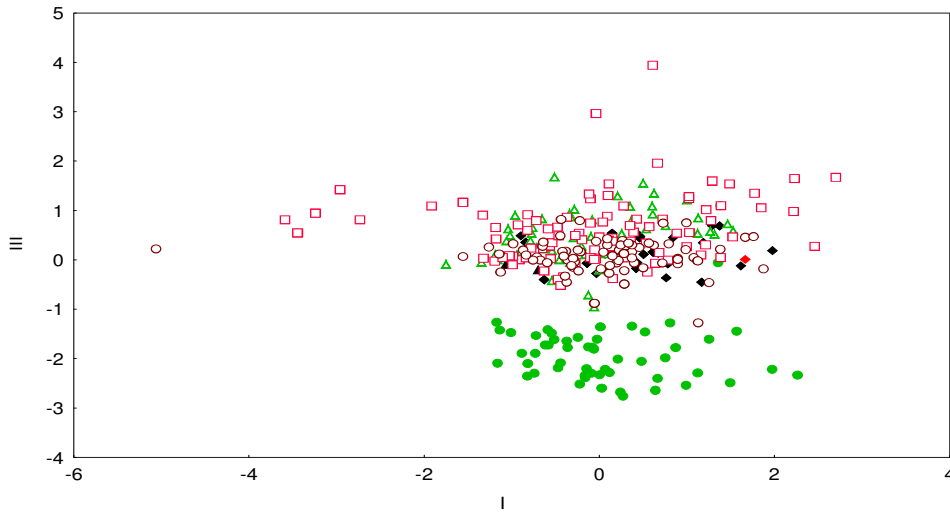
Fig. 4a Components I and III from a principal components analysis (PCA) of *Bothrometopus randi* collected on Marion Island during 1981 (*filled red diamond*), 1983 (*filled black triangle*), 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)
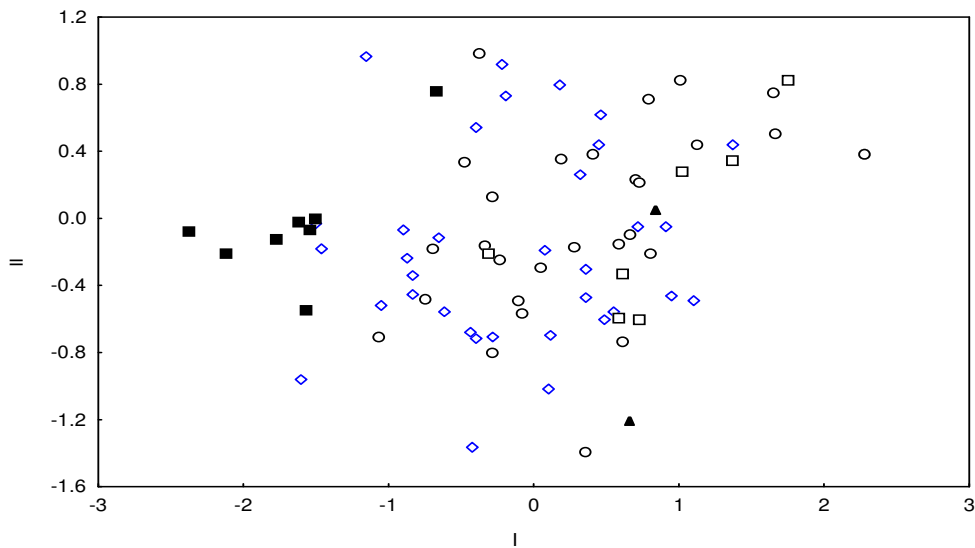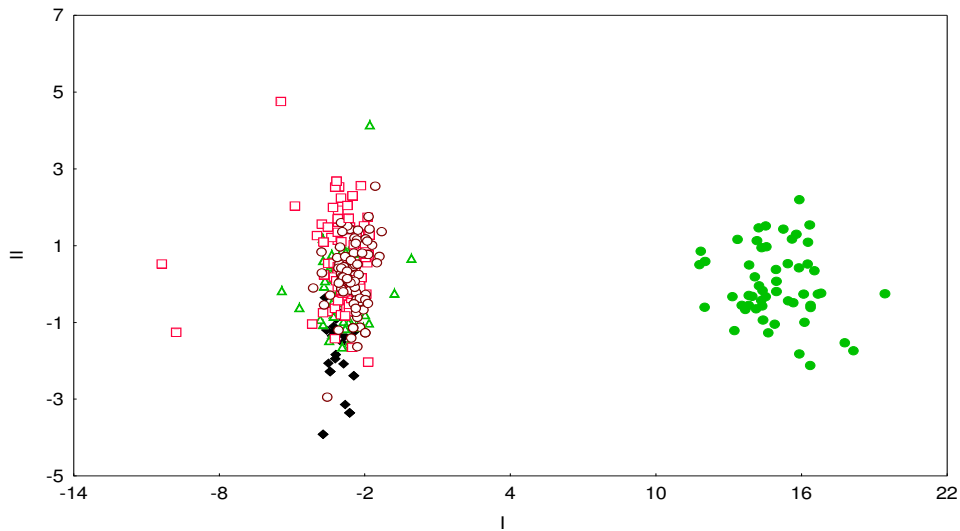

Fig. 4b Components I and II from a principal components analysis (PCA) of *Bothrometopus randi* collected on Prince Edward Island during 1965 (*filled black square*), 1983 (*filled black triangle*), 1984 (*unfilled black square*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)
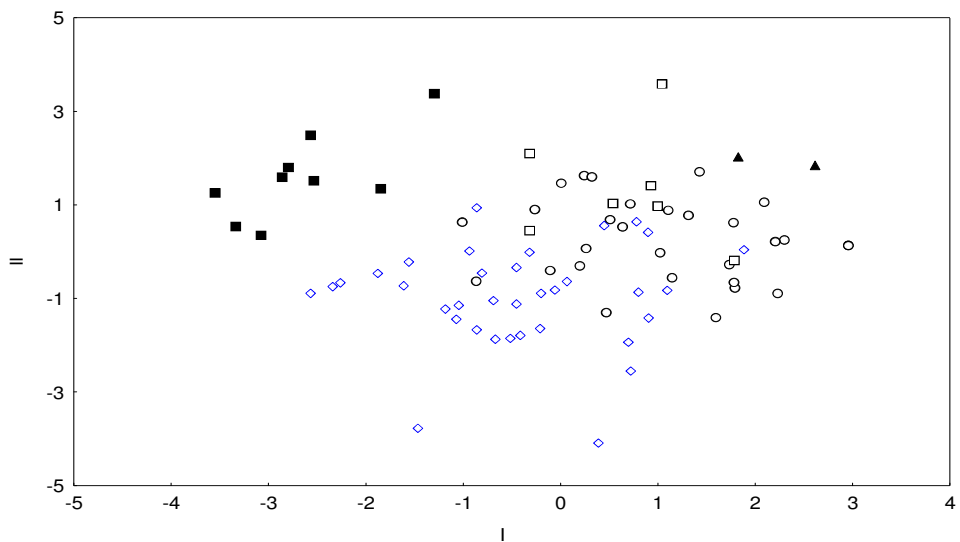
Table 3a Loadings of variables on components I, II and III from a principal components analysis (PCA) of *Bothrometopus randi* collected on Marion and Prince Edward Islands

	Marion Island			Prince Edward Island		
Variable	Principal components			Principal components		
	I	II	III	I	II	III
TL	0.910	-0.253	-0.049	0.891	0.157	-0.241
EW	0.781	-0.379	-0.264	0.825	0.334	-0.220
PB	0.916	-0.029	-0.030	0.882	0.170	0.059
O	0.829	-0.114	-0.028	0.819	-0.147	-0.194
A	0.779	-0.238	-0.017	0.849	-0.225	-0.173
F1	0.701	0.353	-0.019	0.541	-0.627	0.234
F2	0.753	0.279	0.092	0.382	0.637	0.560
F3	0.582	0.480	-0.048	0.389	0.513	0.615
FR	0.609	0.508	0.078	0.471	-0.666	0.161
T3	0.804	0.283	-0.030	0.771	-0.239	0.221
MS	0.223	-0.489	0.572	0.306	0.264	-0.480
MT	0.695	-0.346	0.144	0.767	0.050	-0.272
MM	0.624	-0.167	0.336	0.589	0.484	-0.253
FL	0.238	-0.290	-0.810	0.926	-0.065	0.182
FB	0.717	0.115	0.025	0.756	-0.267	0.213
% trace	49.86%	10.23%	8.07%	50.07%	14.55%	9.64%

The PCA groupings are complemented by the CVA of both Marion (Fig. 4c: MANOVA: $F_{75,15}$ = 32.54; n = 339; $P < 0.001$) and Prince Edward Islands (Fig. 4d: MANOVA: $F_{60,25}$ = 3.04, n = 82, $P < 0.001$) samples. In addition, the Prince Edward Island CVA shows a separation between samples from the early- and mid-1980s with recently collected material (Fig. 4d). Similar to the PCA, FL contributed to the differences in samples from both Marion and Prince Edward Islands (Table 3b). Additional measurements that showed high loadings included: TL, PB (axis I) and PB, O, A (axis II) for Marion Island and O, FR, MS, MT (axis I) and TL, EW, MS (axis II) for Prince Edward Island (Table 3b). All these multivariate results correspond to those of the other *Bothrometopus* species examined from both Marion and Prince Edward Islands. There is a general trend of shape-related differences between recently collected samples and those collected during the early- and mid-1980s. In addition, samples collected much earlier in the mid-1960s also showed a distinct size-related difference in the Prince Edward Island samples.

Fig. 4c First two axes from a canonical variates analysis (CVA) of *Bothrometopus randi* collected on Marion Island during 1981 (*filled red diamond*), 1983 (*filled black triangle*), 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)

Fig. 4d First two axes from a canonical variates analysis (CVA) of *Bothrometopus randi* collected on Prince Edward Island during 1965 (*filled black square*), 1983 (*filled black triangle*), 1984 (*unfilled black square*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

Table 3b Loadings of variables on canonical variate axes from canonical variates analyses of *Bothrometopus randi* collected on Marion and Prince Edward Islands
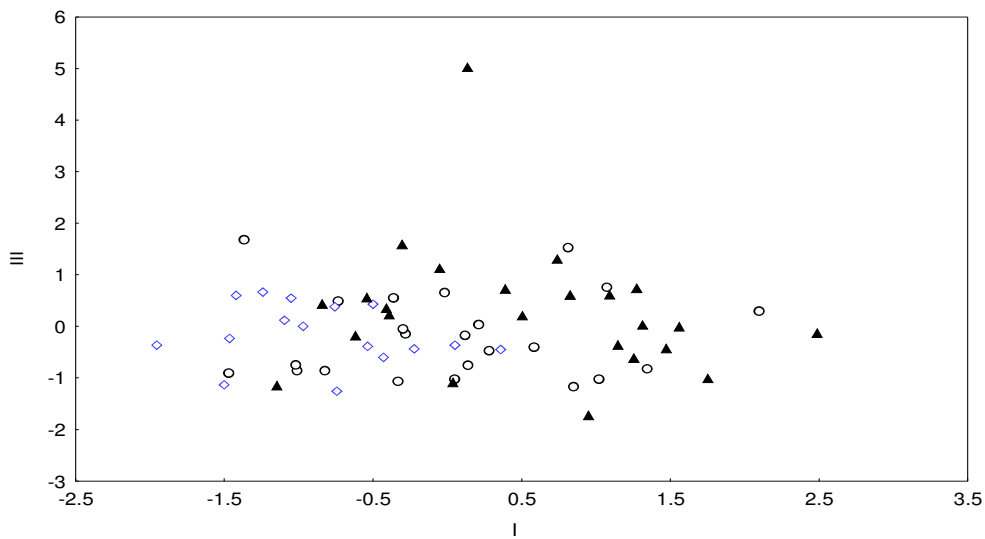
Variable	Marion Island		Prince Edward Island	
	Canonical variate		Canonical variate	
	I	II	I	II
TL	-0.384	-0.006	-0.181	2.162
EW	-0.054	0.543	0.043	-1.086
PB	-0.589	-1.135	-0.010	-0.081
O	-0.091	0.634	-0.814	0.227
A	0.026	-0.868	0.491	0.151
F1	-0.007	-0.349	0.078	-0.384
F2	-0.234	0.058	0.108	-0.054
F3	-0.197	-0.020	0.311	-0.366
FR	-0.028	0.302	0.565	-0.396
T3	-0.173	0.065	-0.036	-0.326
MS	-0.059	0.448	-0.595	-0.841
MT	0.129	0.195	0.547	-0.188
MM	-0.153	-0.085	0.492	-0.323
FL	1.711	-0.019	0.555	0.053
FB	0.176	0.377	-0.247	-0.036

Palirhoeus eatoni

Similar to all other weevil species examined in this study, *P. eatoni* exhibited a shape-related difference between the 1986 sample and more recently collected material from Marion Island on the third PCA axis (7.48% variance) (Fig. 5a). The 1975 sample collected on Marion Island showed separation from subsequently collected samples on the first size-related PCA axis (Fig. 5a), and is broadly similar to the size-related separation in the 1965 sample of *B. randi* from Prince Edward Island (see Fig. 4b). There is a tendency for the 1983 sample to separate from the 2000 sample from Prince Edward Island on the first PCA axis (52.81% variance) (Fig. 5b). FL contributed to the separation on both the third (Marion Island) and first (Prince Edward Island) axes. Additional measurements contributing to the separation included: TL, O, F2, T3, FB (Marion Island; axis I); EW, A (Marion Island; axis III) and TL, EW, PB, MT, FB (Prince Edward Island; axis I) (Table 4a).
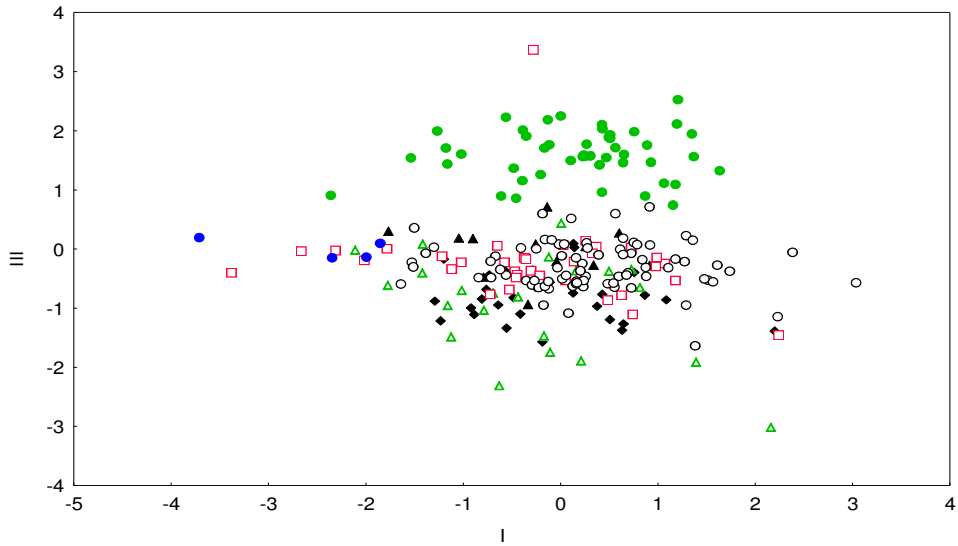
Fig. 5a Components I and III from a principal components analysis (PCA) of *Palirhoeus eatoni* collected on Marion Island during 1975 (*filled blue circle*), 1982 (*filled red square*), 1983 (*filled black triangle*), 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)
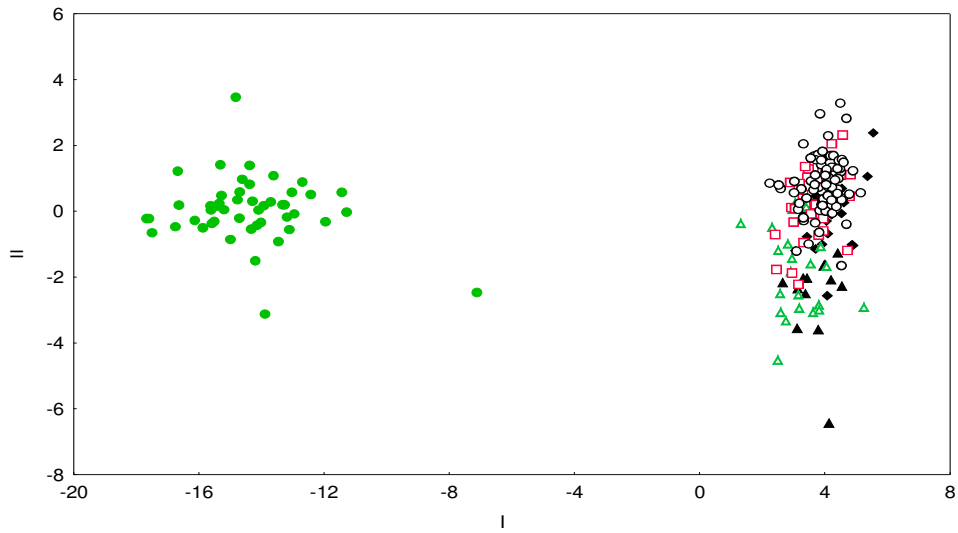

Fig. 5b Components I and III from a principal components analysis (PCA) of *Palirhoeus eatoni* collected on Prince Edward Island 1983 (*filled black triangle*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)
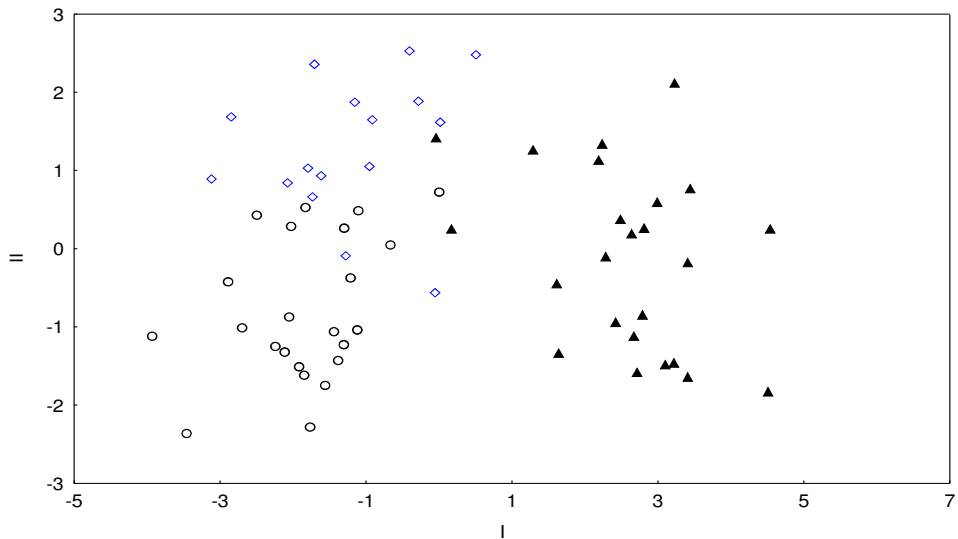
Table 4a Loadings of variables on components, I, II and III from a principal components analysis of *Palirhoeus eatoni* collected on Marion and Prince Edward Islands

| Variable | Marion Island | | | Prince Edward Island | | |
| | Principal components | | | Principal components | | |
	I	II	III	I	II	III
TL	0.782	0.245	0.246	0.908	0.004	0.059
EW	0.519	0.206	0.316	0.800	-0.106	-0.290
PB	0.565	0.080	-0.262	0.863	0.022	-0.061
O	0.816	0.225	-0.067	0.699	0.006	-0.332
A	0.538	0.386	-0.354	0.631	-0.448	-0.255
F1	0.381	-0.590	0.134	0.663	-0.401	0.392
F2	0.734	-0.279	-0.055	0.527	0.306	0.636
F3	0.541	-0.613	-0.060	0.550	-0.554	0.357
FR	0.670	-0.428	-0.096	0.547	0.724	0.026
T3	0.758	-0.054	0.081	0.776	0.449	0.064
MS	0.379	0.420	-0.247	0.234	-0.334	0.126
MT	0.661	0.247	-0.067	0.864	-0.081	-0.222
MM	0.568	0.134	-0.039	0.744	0.055	-0.275
FL	0.236	0.209	0.809	0.943	0.038	0.073
FB	0.665	-0.140	0.044	0.813	0.093	0.081
% trace	37.03%	10.77%	7.48%	52.81%	10.87%	7.40%

Similar patterns are evident in the CVA, where differences in morphology were exhibited in groups with adequate sample size. Marion Island 1986 sample (Fig. 5c: MANOVA: $F_{75,10} = 30.98$; $n = 235$; $P < 0.001$) as well as Prince Edward Island 1983 sample (Fig. 5d: MANOVA: $F_{30,92} = 6.15$; $n = 63$; $P < 0.001$) tended to separate from more recently collected material. FL and F2 were important in the separation on axes I of samples from both Marion and Prince Edward Islands (Table 4b). In addition, Marion Island data showed T3 as an important variable, while Prince Edward Island data exhibited high loadings for TL, EW, PB, FR and MM (axis I) and EW and MS (axis II) (Table 4b). Similar to all other weevil species examined in this study, *P. eatoni* showed a shape-related separation of samples from the early- and mid-1980s from the more recently collected material on both Marion and Prince Edward Islands, as well as size-related separation of 1975 samples with those from the 1980s to the 2000s on Marion Island.

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Fig. 5c First two axes from a canonical variates analysis (CVA) of *Palirhoeus eatoni* collected on Marion Island during 1983 (*filled black triangle*), 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)

Fig. 5d First two axes from a canonical variates analysis (CVA) of *Palirhoeus eatoni* collected on Prince Edward Island 1983 (*filled black triangle*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

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Table 4b Loadings of variables on canonical variate axes from
canonical variates analyses of *Palirhoeus eatoni* collected on
Marion and Prince Edward Islands

Variable	Marion Island		Prince Edward Island	
	Canonical variate		Canonical variate	
	I	II	I	II
TL	-0.026	-1.142	2.957	0.058
EW	0.148	0.329	-0.738	-0.454
PB	0.022	-0.256	-0.698	-0.313
O	0.121	0.739	-0.117	0.184
A	0.114	-0.643	-0.196	-0.210
F1	0.068	0.133	-0.116	-0.171
F2	0.374	-0.182	-0.563	-0.423
F3	-0.086	0.249	0.327	-0.101
FR	0.065	0.211	0.482	0.085
T3	0.374	0.405	-0.112	-0.139
MS	0.000	-0.032	0.097	0.809
MT	0.313	0.324	-0.362	-0.111
MM	0.072	0.183	-0.674	-0.169
FL	-1.529	0.028	-0.434	0.273
FB	0.121	0.341	-0.050	0.070

Collation of all multivariate results (also see Appendix II) in this study suggest a trend toward shape-related differences between samples collected in early- to mid-1980s and recently collected material for all species on both Marion and Prince Edward Islands. On the other hand, for species for which earlier samples were available for study (mid-1960s to mid-1970s), multivariate size-related differences were observed in samples collected on both Marion and Prince Edward Islands.

Turner (2004) reported that the end of 1986 was the start of an El niño period. The shape-related separation of 1986 samples from all other sampling years on Marion Island for all species, were therefore investigated further. Re-analyses of the Marion Island data for all species, excluding the 1986 sampling year, indicated no separation between the early-1980s data and recently collected material (not illustrated). It is possible that this delineated pattern may be a function of sample size.

Correlations

To assess morphological change over time in weevils from both Marion and Prince Edward Islands, correlations were also performed with PCA axes I – III scores and the 15 individual morphometric measurements, on sampling year for islands and species separately.

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The correlation analysis of *B. elongatus* PCA I scores over time was not statistically significant (Table 5a). However, correlation of PCA III scores revealed a negative and highly significant sampling year effect (Table 5a; Fig. 6a). These results indicate a significant shape relationship between samples collected in the early- 1980s and more recently collected material for *B. elongatus* on Marion Island. Correlations of single measurements revealed significant relationships with sampling year in nine of the 15 measurements for *B. elongatus* (Table 5b). The two measurements with the highest, positive correlation coefficients were, F3 and FR, while FL showed a significant negative relationship between samples from early- 1980s to 2003. These measurements also contributed to the separation of *B. elongatus* on the third PCA axis in the principal components analysis (see Table 1a).

In contrast, correlation analysis of *B. elongatus* sampled from Prince Edward Island showed no significant correlations for PCA I – III scores (Table 5a). This analysis, however, may be constrained by a limited number of sampling years (*n* = 3) for analysis, and although there was separation between sampling years in the PCA axis (Fig. 2b), the separation is not unequivocal (see Fig. 2b). Two of the measurements (F3 and A) that contributed most to the separation in the PCA (see Table 1a), also revealed significant correlations (Table 5b).   In addition, FR indicated a positive and significant correlation coefficient (Table 5b).

Correlations of both PCA I (not illustrated) and III (Fig. 6b) scores of *B. parvulus* collected on Marion Island exhibited negative and significant sampling year effects (Table 5a). In addition, PCA II scores revealed a positive and significant correlation with sampling year (Table 5a). Although the PCA only showed separation on PCA III (see Fig. 3a), two of the measurements, MS and FL, responsible for this separation together with TL also exhibited a negative and significant relationship over time (Table 2a). These results suggest an overall size reduction in a morphometric character complex, with samples collected in the mid- 1980s being smaller than those collected in 2003. In contrast, *B. parvulus* collected on Prince Edward Island revealed a positive and significant correlation between PCA I and sampling year (Table 5a), similar to the separation in *B. parvulus* on the first CVA axis (see Fig. 3d), suggesting an overall size increase of the morphometric character complex in samples collected between the early-1980s and 2003. F3 and PB showed positive and significant correlations with sampling year (Table 5b). Again, the analyses may be constrained by a limited number (*n* = 3) of sampling years.

Correlation analysis of PCA II and III showed positive and highly significant sampling year effects for *B. randi* collected on Marion Island (Table 5a; Fig. 6c). PCA results also showed morphological differences over time (Fig. 4a). Morphological patterns in individual measurements over time are best illustrated by FL, MS and EW (Table 5b). MS and FL also represent two of the measurements with the highest loadings on the third PCA axis in the PCA

(see Table 3a). Correlations of PCA I – III revealed no significant relationship with sampling year for *B. randi* from Prince Edward Island (Table 5a). However, correlation of a single measurement, PB was negative and statistically significant (Table 5b). PB also contributed to the separation over time in *B. randi* on the first PCA axis (see Fig. 4b). However, these analyses may also be constrained by limited (*n* = 3) sampling years.
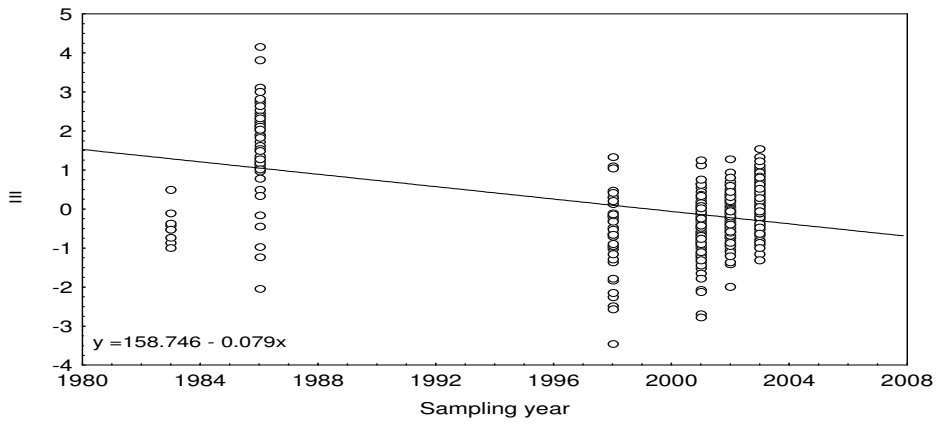
Both PCA II and PCA III of *P. eatoni* collected from Marion Island revealed negative and significant sampling year effects (Table 5a; PCA III: Fig. 6d) corresponding with the separation observed in the third PCA axis (see Fig. 5a). FL, which contributed highly in the PCA (Table 4a), also showed a significant negative relationship from the mid- 1970s to 2003 (Table 5b). FR and F3 exhibited positive and significant correlations (Table 5b). In contrast, *P. eatoni* collected from Prince Edward Island which showed a size-related separation (PCA I) over time in the PCA (see Fig. 5b), exhibited a negative and significant correlation (Table 5a). These morphological changes over time are best illustrated by individual measurements: TL, FL and FB that also loaded highly on the first PCA axis (see Table 4a).

Table 5 Results of correlations of a) the first, second and third principal components (PCA) scores and b) single measurements, with sampling year of I) *Bothrometopus elongatus*, II) *B. parvulus*, III) *B. randi* and IV) *Palirhoeus eatoni* from Marion and Prince Edward Island.
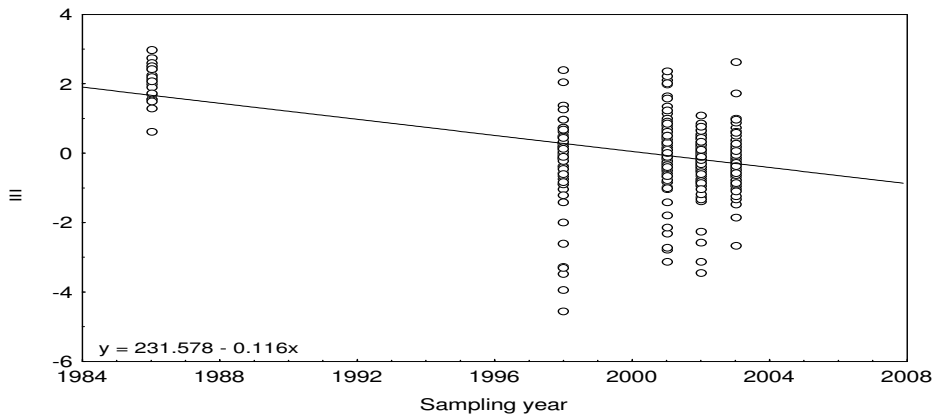* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; *ns* = statistically not significant

Correlation coefficients (*r*)

Dependent variable	Sampling year							
	Marion Island				Prince Edward Island			
	B. elongatus	*B. parvulus*	*B. randi*	*P. eatoni*	*B. elongatus*	*B. parvulus*	*B. randi*	*P. eatoni*
	n = 423	*n* = 419	*n* = 339	*n* = 234	*n* = 74	*n* = 92	*n* = 73	*n* = 63
a)								
Principal component I	0.06 ns	-0.21 ***	-0.07 ns	0.01 ns	0.07 ns	0.22 *	-0.20 ns	-0.37 **
Principal component II	0.62 **	0.11 *	0.28 ***	-0.18 **	0.16 ns	0.03 ns	0.03 ns	-0.04 ns
Principal component III	-0.47 ***	-0.43 ***	0.77 ***	-0.62 ***	0.16 ns	-0.06 ns	-0.11 ns	-0.23 ns
b)								
TL - Total body length	-0.08 ns	-0.20 ***	-0.13 *	-0.17 *	-0.19 ns	0.08 ns	-0.22 ns	-0.65 ***
EW - Elytra width	-0.11 *	-0.19 ***	-0.27 ***	0.06 ns	0.19 ns	0.20 ns	-0.12 ns	-0.19 ns
PB - Pronotum breadth	0.02 ns	-0.17 **	-0.07 ns	0.15 ns	0.11 ns	0.28 **	-0.27 *	-0.24 ns
O - Inter-ocular distance	0.03 ns	-0.14 **	-0.02 ns	0.14 *	0.07 ns	0.25 *	-0.22 ns	-0.15 ns
A - Inter-antennal distance	-0.10 *	-0.16 ***	-0.11 *	0.01 ns	-0.33 **	0.19 ns	-0.23 ns	-0.21 ns
F1 - Funicle segment 1	0.09 ns	-0.04 ns	-0.01 ns	0.02 ns	-0.14 ns	0.10 ns	-0.20 ns	-0.29 *
F2 - Funicle segment 2	0.14 **	-0.11 *	0.09 ns	0.12 ns	0.02 ns	0.09 ns	-0.04 ns	-0.25 *
F3 - Funicle segment 3	0.22 ***	-0.07 ns	0.02 ns	0.20 **	0.31 **	0.28 **	-0.01 ns	-0.17 ns
FR - Rest of funicle	0.20 ***	-0.05 ns	0.10 ns	0.25 ***	0.28 *	0.15 ns	0.07 ns	-0.31 *
T3 - First 3 tarsal segments	0.12 *	-0.10 *	0.01 ns	0.15 ns	0.06 ns	0.25 *	-0.08 ns	-0.28 *
MS - Mesocoxal distance	-0.11 *	-0.20 ***	0.13 *	0.14 ns	-0.08 ns	0.03 ns	0.13 ns	-0.27 *
MT - Metacoxal distance	0.11 *	-0.09 ns	0.00 ns	0.18 ns	0.05 ns	0.04 ns	-0.05 ns	-0.21 ns
MM -Meso/metacoxal distance	0.08 ns	-0.01 ns	0.10 ns	0.12 ns	-0.06 ns	0.20 ns	0.01 ns	-0.07 ns
FL - Femur length	-0.81 ***	-0.87 ***	-0.90 ***	-0.67 ***	0.09 ns	0.06 ns	-0.22 ns	-0.37 **
FB - Femur breadth	0.05 ns	-0.13 **	-0.01 ns	0.02 ns	0.12 ns	0.18 ns	-0.19 ns	-0.38 **

a

$y = 158.746 - 0.079x$

b

$y = 231.578 - 0.116x$

c

$y = -254.821 + 0.127x$

d

$y = 156.146 - 0.078x$
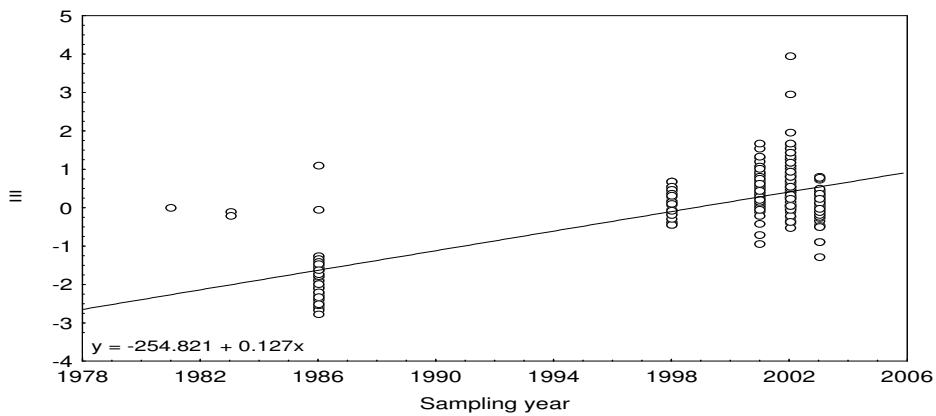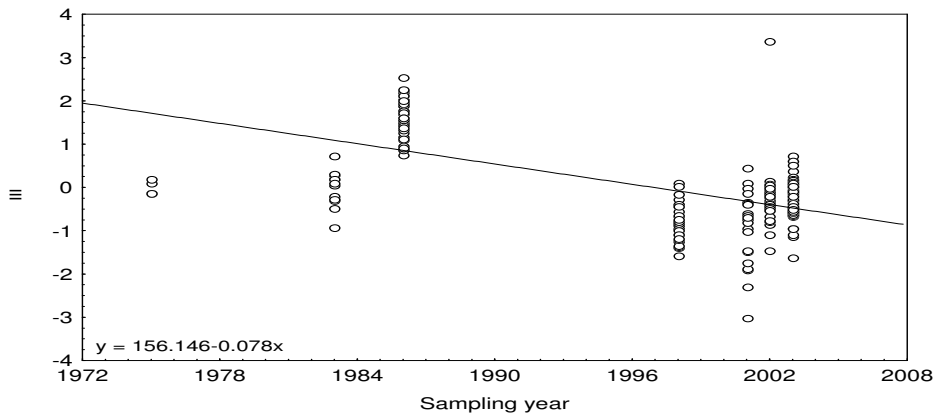
Fig. 6 Relationship between principal component III scores and sampling year for, **a** *Bothrometopus elongatus*, **b** *Bothrometopus parvulus*, **c** *Bothrometopus randi*, and **d** *Palirhoeus eatoni*, collected on Marion Island

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Temperature and rainfall data for Marion Island

Mean annual temperature (˚C) for Marion Island (Fig. 7a) shows an increase from 1950 to 2004. The mean annual rainfall (mm) on Marion Island (Fig. 7b) on the other hand indicates a decrease from the 1960s to 2004.

a
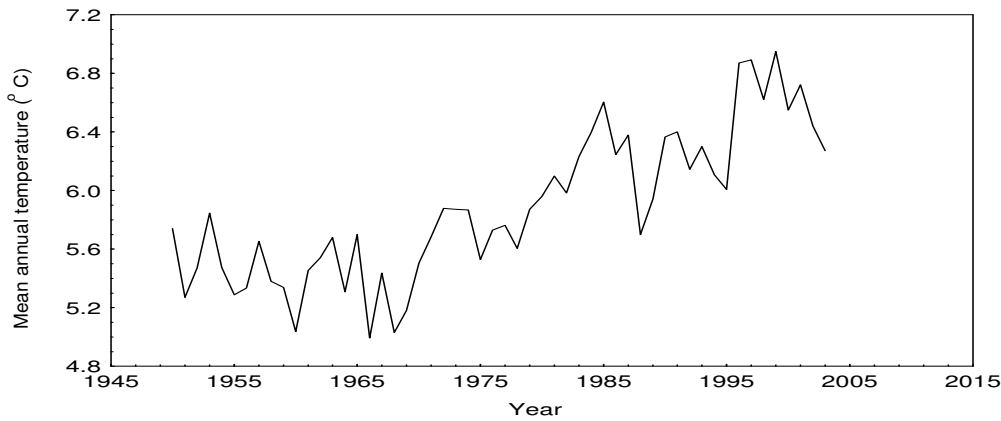
b
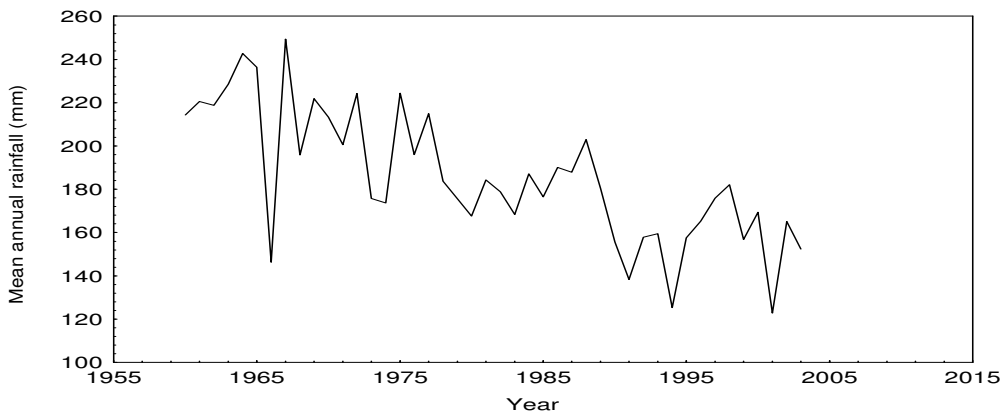
Fig. 7 Mean annual temperatures recorded (ºC) for Marion Island from **a** 1950 up to 2004 and mean annual rainfall measured (mm) for Marion Island from **b** 1960 up to 2004

Generalized Linear Models

a) Marion and Prince Edward Islands

For all species most of the deviance was explained by the full models of PCA III, than PCA I and II (Table 6.1) supporting the morphological differences over time in the PCA and the correlation analyses. Sampling year contributed highly significantly and explained most of the percentage deviance (% de) for the full models (% de (full model)) for all principal components, for all species (Table 6.1). The only exceptions were PCA II of the analysis of *B*.

parvulus, where none of the variables contributed significantly to the full model (Table 6.1b) and PCA I of the analysis of *B. randi*, where gender explained a larger percentage of the deviance than sampling year (Table 6.1c).

Although no overall multivariate sexual dimorphism was found in all species sampled, a few individual measurements (between 1 and 4 measurements of the 15 examined for each species) were sexually dimorphic. These sexually dimorphic measurements were identified in earlier analyses (see Table 1; Janse van Rensburg et al. 2003: Chapter 2). Therefore, gender contributed significantly but to a lesser extent than sampling year, to the deviance explained by the models for both size (PCA I) and shape (PCA II and III) factors (Table 6.1).

Mice predation did not contribute significantly to the deviance in any of the *B. randi* PCA axes, although it is a preferred prey species for mice (Chown and Smith 1993). However, mice predation played a significant role in explaining a small portion of the deviance for other, non-preferred prey species. For example, mice predation explained a significant percentage of the deviance in both PCA I and II for *B. elongatus* (Table 6.1a) and explained 5.40% of the deviance in PCA III of *P. eatoni* (Table 6.1d).

Table 6.1   Type III sum of squares Generalized Linear Model of principal component I (multivariate size variables) and principal components II and III (multivariate shape variables) with 1) sampling year, mice predation and gender for a) *Bothrometopus elongatus*, b) *B. parvulus*, c) *B. randi* and d) *Palirhoeus eatoni* from Marion Island and Prince Edward Island and with 2) temperature and gender for Marion Island. % de = % deviance explained, dev/df (deviance/degrees of freedom) = Goodness of fit * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; *ns* = statistically non-significant

6.1) Marion Island and Prince Edward Island

	Variables	*n*	Wald statistic	% de	% de (full model)	dev/df
a) *Bothrometopus elongatus*						
PCA I	Year	111	43.87	26.30 ***	29.54	0.75
	Mice predation		5.02	1.46 *		
	Gender		0.19	0.21 *ns*		
PCA II	Year		27.83	25.03 ***	44.37	0.59
	Mice predation		8.53	8.63 **		
	Gender		28.04	22.57 ***		
PCA III	Year		330.45	74.34 ***	75.34	0.26
	Mice predation		0.00	1.91 *ns*		
	Gender		4.61	0.00 *		
b) *Bothrometopus parvulus*						
PCA I	Year	209	69.29	18.14 ***	23.76	0.77
	Mice predation		6.54	1.19 *		
	Gender		9.18	2.44 **		
PCA II	Year		6.82	3.81 *ns*	4.22	0.99
	Mice predation		0.82	1.65 *ns*		
	Gender		0.21	0.12 *ns*		
PCA III	Year		25.79	38.43 ***	50.70	0.50
	Mice predation		1.06	1.35 *ns*		
	Gender		5.44	9.66 *		
c) *Bothrometopus randi*						
PCA I	Year	396	31.20	8.80 ***	32.68	0.70
	Mice predation		1.03	1.13 *ns*		
	Gender		137.95	25.64 ***		
PCA II	Year		183.86	25.59 ***	46.96	0.55
	Mice predation		1.96	0.70 *ns*		
	Gender		157.79	22.03 ***		
PCA III	Year		734.98	63.91 ***	65.15	0.36
	Mice predation		0.57	0.15 *ns*		
	Gender		13.44	0.44 ***		
d) *Palirhoeus eatoni*						
PCA I	Year	297	79.06	18.96 ***	33.77	0.69
	Mice predation		3.38	0.03 *ns*		
	Gender		64.29	16.08 ***		
PCA II	Year		95.75	19.84 ***	37.39	0.82
	Mice predation		0.01	1.14 *ns*		
	Gender		83.10	16.77 ***		
PCA III	Year		445.79	61.45 ***	62.34	0.39
	Mice predation		4.39	5.40 *		
	Gender		2.95	0.00 *ns*		

b) Marion Island

More deviance was explained by the full models of PCA III, than PCA I and II for all species (Table 6.2). Temperature contributed most to the percentage deviance explained by the full model for PCA III for all species, except *B. elongatus* (Table 6.2). In addition, temperature contributed significantly to PCA I and II for all species, except *B. elongatus* (Table 6.2a) and *B. parvulus* (PCA II) (Table 6.2b). In general, gender also contributed significantly to the percentage deviance explained for all PCA axes across species (Table 6.2).

Although temperature data are only available for Marion Island, we assume temperature increases to be very similar for the mouse-free Prince Edward Island, 22 km away. Both Marion and Prince Edward Islands exhibit similar PCA results, where mid-1960s and 1970s samples separate from other sampling years based on size, while mid-1980s samples separate from recently collected material on the third PCA axis, representing shape. In support of the latter, sampling year contributed the most to the percentage deviance explained by GLZs for PCA III. The direct link between temperature increase (1950 - 2004) (see Fig. 7a) and sampling year (1965 – 2003) therefore, suggests that temperature plays a significant role in morphometric size and shape changes of the weevil species used in the analyses. GLZs of Marion Island also supported the highly significant contribution of temperature to both PCA I and III, with mice predation contributing to a lesser extent. Therefore, the data suggest that temperature may be a primary contributing factor to size and shape changes in *B. elongatus*, *B. parvulus*, *B. randi* and *P. eatoni*, with mice predation having a secondary effect on these morphometric changes.

Table 6.2 Type III sum of squares Generalized Linear Model of principal component I (multivariate size variables) and principal components II and III (multivariate shape variables) with 2) temperature and gender for a) *Bothrometopus elongatus*, b) *B. parvulus*, c) *B. randi* and d) *Palirhoeus eatoni* from Marion Island. % de = % deviance explained, dev/df (deviance/degrees of freedom) = Goodness of fit * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; *ns* = statistically non-significant

	6.2) Marion Island					
	Variables	*n*	Wald statistic	% de	% de (full model)	dev/df
a) *Bothrometopus elongatus*						
PCA I	Temperature	75	5.51	6.55 [ns]	6.57	0.90
	Gender		0.02	0.05 [ns]		
PCA II	Temperature		5.13	10.73 [ns]	29.98	0.53
	Gender		21.71	25.43 ***		
PCA III	Temperature		332.16	0.00 ***	80.84	0.25
	Gender		2.05	0.29 [ns]		
b) *Bothrometopus parvulus*						
PCA I	Temperature	145	53.34	23.35 ***	28.97	0.80
	Gender		11.47	2.83 ***		
PCA II	Temperature		4.16	2.57 [ns]	3.42	1.36
	Gender		1.28	0.65 [ns]		
PCA III	Temperature		189.61	50.74 *	58.71	0.44
	Gender		27.99	4.73 ***		
c) *Bothrometopus randi*						
PCA I	Temperature	281	13.27	6.60 *	29.87	0.71
	Gender		97.27	26.56 ***		
PCA II	Temperature		154.98	29.45 ***	49.43	0.52
	Gender		110.37	21.32 ***		
PCA III	Temperature		310.28	51.87 ***	52.67	0.49
	Gender		4.71	0.41 *		
d) *Palirhoeus eatoni*						
PCA I	Temperature	233	45.18	17.42 ***	32.63	1.00
	Gender		52.58	19.56 ***		
PCA II	Temperature		102.44	21.26 ***	44.36	0.58
	Gender		96.77	19.90 ***		
PCA III	Temperature		635.93	73.23 ***	73.24	0.38
	Gender		0.14	0.22 [ns]		

Discussion

Climate change and biological invasions pose a serious conservation threat to the Southern Ocean Province Islands (Chown et al. 1998; Bergstrom and Chown 1999). Due to their isolation, specialised and limited biota as well as harsh environments, island ecosystems are ecologically very sensitive (Smith and Steenkamp 1990; Smith 1991). Temperature increase and invasive species, such as mice have been suggested to be important contributors to ecosystem changes on Marion Island (Chown and Smith 1993; Smith 2002; Smith et al. 2002).

Since the late 1960s, mean surface air temperature on Marion Island has increased by 0.025°C year^{-1} with a corresponding decrease in precipitation (Smith and Steenkamp 1990; Smith 1991). Increased temperature, accompanied by a consistent drying effect on Marion Island habitats is expected to increase productivity and nutrient demand (Smith 2002). The activities of the macro-invertebrates responsible for the bulk of energy-flow and nutrient cycling on the island are strongly temperature-dependent (Chown et al. 1997; Klok and Chown 1997). Therefore, increasing temperature will result in enhanced rates of litter consumption leading to increased nutrient release (Smith 2002). However, increasing temperatures have led to increased mouse populations, which in turn have resulted in increased predation on macro-invertebrates hence decreasing rates of nutrient cycling and may result in imbalances between primary production and decomposition (Smith 2002). This, together with the direct influence of mice on the vegetation, through for example, granivory (see Chown and Smith 1993), has important implications for island vegetation succession and ecosystem functioning (Smith 2002).

In addition, various invertebrate studies have shown a decrease in size with increasing temperature (Atkinson 1994; Noach et al. 1996; Atkinson and Sibly 1997; James et al. 1997; also see Kozlowski et al. 2004 for explanations of this phenomenon). Thus, due to the importance of temperature on insect body size (Atkinson 1994; James et al. 1997; Karan et al. 1998), it is possible that changes in morphology (multivariate size and shape) of important contributors, such as weevil species to nutrient cycling on both Marion and Prince Edward Islands may also have an influence on island ecosystem functioning.

Despite relatively small sample sizes in the early collections, weevil species on both Marion and Prince Edward Islands showed size-related differences between samples collected during the mid-1960s and 1970s with those collected in more recent years. In addition, all species examined showed a shape-related (PCA axis III) difference between material collected in the mid-1980s and recently collected samples. However, samples from the early-1980s showed no separation with recently collected material and this was confirmed after excluding samples from 1986. Turner (2004) reported that the end of 1986 was the start of an El niño period and may explain this shape-related separation of the 1986 data from all other sampling years. It is also possible that the small sample sizes from the early-1980s may obscure any underlying morphometric patterns.

The trend shown in multivariate analyses was also apparent in correlation analyses that suggested a strong negative relationship between sampling year and body shape on Marion Island. On the other hand, *B. parvulus* from Marion Island and *P. eatoni* from Prince Edward Island showed overall size reduction over time, while *B. parvulus* from Prince Edward Island showed a size-related increase over time.

Most invertebrates exhibit a decline in body size with increasing developmental temperatures (Atkinson 1994). With weevil species from both islands showing similar multivariate size and shape changes for similar time periods, it is possible that climate change may play a primary role in weevil morphological changes. Van der Merwe et al. (1997) reported that the upper lethal temperatures for the six currently recognised weevil species on Marion Island correspond closely to the maximum microclimate temperature in their respective habitats, therefore, suggesting that current temperature increases may be deleterious to the survival of the weevil species (Smith 2002).

Sampling year, directly linked to the temperature increase over time on both Marion and Prince Edward Islands (see Smith and Steenkamp 1990), explained the largest percentage of the deviance in GLZs for PCA III in all species, therefore, supporting weevil morphological changes over time. In addition, sampling year contributed significantly to all PCA-derived data in all species. Furthermore, the significant contribution of temperature to the percentage deviance explained by the GLZs for Marion Island, supports the important contribution of climate change to weevil morphological changes over time. Similarly, Chown and Klok (2003) found temperature to be an important contributor in determining body size clines along an altitudinal gradient.

It is also possible that climate change may have an indirect influence on weevil body size changes through mice predation (Smith and Steenkamp 1990; Chown and Smith 1993). Mice population numbers appear to be increasing as a result of warmer temperatures (Smith and Steenkamp 1990, 1992). Increased mice numbers could impact more profoundly on weevil populations (Crafford and Scholtz 1987; Smith et al. 2002). This seems evident from weevil adults, as a prey item, contributing to a higher percentage of mice stomach contents in recent years than in for example, 1979/1980 (Burger 1978; Smith et al. 2002). In addition, increased predation on weevil adults with the added effect of size-selective predation have been noted (Crafford 1990; Chown and Smith 1993; Smith et al. 2002).

Generalized Linear Models in the present study showed mice predation to contribute significantly although to a lesser extent than sampling year to the first and second PCA – generated data of *B. elongatus*, despite the species not being a preferred prey species of mice (Chown and Smith 1993). In addition, mice predation explained a small but significant percentage of the deviance for *B. parvulus* and *P. eatoni*. Of significance is that mice predation did not contribute significantly to the percentage deviance explained for *B. randi*, a preferred prey species of mice (Chown and Smith 1993). Corresponding patterns in size and shape changes for species on Marion Island and the mouse-free Prince Edward Island (Crafford and Scholtz 1987) may support the primary contribution of temperature change. In addition, the shape-related separation in all species of the 1986 sample, a year that marked the start of an El

niño period, may support the importance of the effect climate change have on weevil morphology.

Although mouse predation was shown to be less important in the present study, it may actually play a significant role since temperature changes directly influences mouse populations. In addition, it is also possible that morphological changes over time may actually be influenced by a complex of factors that include both temperature and mouse predation.

In conclusion, an accurate insight into potentially influential factors that may affect weevil morphological changes over time may be achieved with additional long-term data. Such data could also be examined using "geometric morphometrics" (Rohlf and Marcus 1993) that are more effective in capturing morphological changes in an organism (Marcus et al. 1993a, 1993b).

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"You will notice that a turtle only makes progress when it sticks out its neck"

Anonymous

Appendix I

Sampling year, sample size and sampling localities for species presented in the morphometric analyses for both Marion and Prince Edward Islands. Mean annual temperature (˚C) and mean annual rainfall (mm) obtained from the South African Weather Bureau were only available for Marion Island. A number of museum material (mostly pre-1986) had missing locality/altitude information. Data from similar years were pooled to increase sample size and to comply with multivariate computational requirements

	Marion Island					
	Sampling year	n	Locality	Altitude (m)	Temperature (˚C)	Rainfall (mm)
a) *Bothrometopus elongatus*						
	1975	6	-	-	5.53	224.42
	1983	9	Alphakop	750	6.23	168.36
	1986	20	-	800	6.25	190.11
	1986	2	Long Ridge	500	6.25	190.11
	1986	58	Stony Ridge	150	6.25	190.11
	1998	29	-	1000	6.62	182.18
	1998	9	Bottom first red	400	6.62	182.18
	1998	39	Katedraalkrans	800	6.62	182.18
	2001	31	Tafelberg	450	6.72	122.78
	2001	35	Stony Ridge	150	6.72	122.78
	2001	38	Katdraalkrans	800	6.72	122.78
	2002	27	Halfway	500	6.44	165.08
	2002	29	Above first red	600	6.44	165.08
	2002	11	-	1000	6.44	165.08
	2002	22	Katedraalkrans	800	6.44	165.08
	2002	27	Bottom first red	400	6.44	165.08
	2003	22	Johnny's Hill	400	6.27	152.32
	2003	26	Feldmark	550	6.27	152.32
	2003	20	Pyroxene kop	600	6.27	152.32
	2003	28	Long Ridge	500	6.27	152.32
	2003	6	Sidney	400	6.27	152.32
	1986	43	Kildalkey	150	6.25	190.11
b) *Bothrometopus parvulus*	1986	8	Long Ridge	500	6.25	190.11
	1986	5	Stony Ridge	150	6.25	190.11
	1986	1	Tafelberg	450	6.25	190.11
	1998	27	-	1000	6.62	182.18
	1998	10	-	200	6.62	182.18
	1998	10	-	400	6.62	182.18
	1998	40	-	800	6.62	182.18

	Year		Location			
	1998	6	Deltakop	1000	6.62	182.18
	2001	35	Tafelberg	450	6.72	122.78
	2001	30	Skua	150	6.72	122.78
	2001	34	Stony Ridge	150	6.72	122.78
	2002	12	-	1000	6.44	165.08
	2002	23	Azorellakop	500	6.44	165.08
	2002	42	Halfway	500	6.44	165.08
	2002	18	Juniors kop	200	6.44	165.08
	2002	69	Katedraalkrans	800	6.44	165.08
	2002	30	Repetto's	200	6.44	165.08
	2003	20	-	200	6.27	152.32
	2003	25	Feldmark	550	6.27	152.32
	2003	8	Johnny's Hill	400	6.27	152.32
	2003	23	Long Ridge	500	6.27	152.32
	2003	23	Pyroxene kop	600	6.27	152.32
	2003	15	Sidney	400	6.27	152.32
	1981	2	Annex Cave	0	6.10	184.37
c) Bothrometopus randi	1983	1	Transvaal Cove	0	6.23	168.36
	1983	1	Boulder Cliff	0	6.23	168.36
	1986	12	Trypot	0	6.25	190.11
	1986	38	Kildalkey	0	6.25	190.11
	1998	58	-	0	6.62	182.18
	2001	30	Ship's Cove	0	6.72	122.78
	2001	30	-	0	6.72	122.78
	2002	30	Cape Davis	0	6.44	165.08
	2002	30	Duikers	0	6.44	165.08
	2002	31	Sealer's Beach	0	6.44	165.08
	2003	29	Good Hope	0	6.27	152.32
	2003	13	Kildalkey	0	6.27	152.32
	1975	4	-	0	5.53	224.42
d) Palirhoeus eatoni	1982	2	-	0	5.98	178.91
	1983	13	-	0	6.23	168.36
	1986	38	Kildalkey	0	6.25	190.11
	1986	1	Swartkops	0	6.25	190.11
	1986	9	Trypot	0	6.25	190.11
	1998	31	-	0	6.62	182.18
	2001	24	Trypot	0	6.72	122.78
	2002	28	Cape Davis	0	6.44	165.08
	2002	8	Sealer's Beach	0	6.44	165.08
	2003	28	Good Hope	0	6.27	152.32
	2003	23	Kildalkey	0	6.27	152.32
						152.32
	2003	29	Watertunnel	0	6.27	

Prince Edward Island

a) Bothrometopus elongatus	1983	4	Wolkberg	600
	2000	11	Golden Gate	50
	2000	3	-	200
	2003	30	-	400
	2003	26	-	600
b) Bothrometopus parvulus	1980	8	-	-
	2000	42	Golden gate	50
	2000	1	Kraterkoppie	150
	2000	22	Ditrichum	549
	2000	9	Van Zinderren Bakker	672
c) Bothrometopus randi	1965	9	Van Zinderren Bakker	672
	1983	2	Kent Crater	150
	1984	7	Cave Bay	0
	2000	22	Golden Gate	50
	2000	18	McNish bay	0
	2003	29	-	0
d) Palirhoeus eatoni	1983	25	Kent Crater	150
	2000	30	East Cape	0
	2003	24	-	0

Appendix II

Results of both principal components analyses (PCA) and discriminant analyses (CVA) summarized together with results as presented in
Table 6.1 and 6.2 of the Type III sum of squares Generalized linear Models. The presence (+) or absence (-) of separation between
sampling years are indicated on either principal component I (PCA I – representing size) or principal component II (PCA II – representing shape).
Either a significant (+) or non-significant (-) contribution of year and predation to the separation on the principal components axes are indicated for
a) Marion and b) Prince Edward Islands

a) Marion Island

Species	PCA I			PCA II				
	PCA size	Year	Predation	PCA shape	Year	Predation	CVA size	CVA shape
Bothrometopus elongatus	-	+	+	+	+	+	+	-
Bothrometopus parvulus	-	+	+	+	-	-	+	-
Bothrometopus randi	-	+	-	+	+	-	+	-
Palirhoeus eatoni	-	+	-	+	+	-	+	-

b) Prince Edward Island

Species	PCA I			PCA II				
	PCA size	Year	Predation	PCA shape	Year	Predation	CVA size	CVA shape
Bothrometopus elongatus	-	+	+	+	+	+	+	-
Bothrometopus parvulus	-	+	+	+	-	-	+	-
Bothrometopus randi	+	+	-	-	+	-	+	-
Palirhoeus eatoni	+	+	-	-	+	-	+	-