A morphological analysis of weevils from sub-Antarctic Prince Edward Islands: an assessment of ecological influences

by

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Abstract The ecologically sensitive, but relatively simple sub-Antarctic mouse-colonised Marion and mouse-free Prince Edward Islands represent ideal ecosystems for addressing questions relating to the partitioning of potential influences of anthropogenic changes, such as climate change and alien species on ecosystem functioning. Consequently, weevil species were used in the present investigation to address questions of morphological change over time with reference to climate change and mouse predation.

As a prelude to the assessment of morphological changes over time, the six currently recognised weevil species, namely, *Bothrometopus randi*, *B. parvulus*, *B. elongatus*, *Ectemnorhinus similis*, *E. marioni* and *Palirhoeus eatoni* from both Marion and Prince Edward Islands were confirmed by morphometric analyses. However, the taxonomic status of *Ectemnorhinus* weevil species on both islands is much more complex than previously considered. A multi-faceted approach based on both morphometric and molecular (COI gene) data suggest the presence of a single species, comprising diverse, genetically discrete populations on Marion Island and two genetically distinct species on Prince Edward Island.

The assessment of morphological changes over time included the four remaining weevil species (*B. elongatus*, *B. randi*, *B. parvulus* and *P. eatoni*) occurring on both Marion and Prince Edward Islands, collected non-consecutively over five decades. These analyses suggest morphometric size differences between samples from both Marion and Prince Edward Islands collected in the mid-1960s and 1970s and those collected recently. Similarly, samples from both islands collected in the early- to mid-1980s also showed morphometric shape differences with those collected recently for both Prince Edward and Marion Islands, respectively. Generalized Linear Models (GLZ) suggested year of sampling to contribute more, to both, morphometric size and shape in all species sampled on both islands, while temperature contributed more to shape for species collected on Marion Island. Given the consistent pattern of morphological change over time for both the mouse-infested Marion Island and the mouse-free Prince Edward Island, it is possible that climate change rather than mouse predation may primarily influence weevil morphological changes on the two islands.

Subsequent analyses extended the question of weevil morphological changes over time with reference to climate change and mouse-predation, and included sub-fossil weevil elytra and head capsule remains collected on Marion Island mire habitats. As a prelude to these analyses, an attempt was made to first identify the sub-fossil samples (using head capsules) with reference to recent samples of the currently recognised weevil species on both islands. While species-level identifications were not possible, morphometric analyses suggest that the sub-fossil remains belong to the *Ectemnorhinus* group of weevils. Sub-fossil weevil remains recovered in mire sediments from 2 m and 2.5 m depths, considered to represent different dimensions in space and time, were dated at 789 BC and 2331 BC, respectively. Subsequent analyses showed no significant morphometric changes in sub-fossil material between the various depths, predating the effects of climate change on both islands and the introduction of the house mouse (*Mus musculus*) on Marion Island in the early 1800s. However, a comparison between the sub-fossil remains and recently collected material considered to represent a period characterised by climate change and mouse-predation showed significant morphometric differences over time. However, the questions investigated in this study need to be investigated further because the potential ecological influences driving ecosystem functioning on the islands may be much more complex than currently understood.

Keywords: *Ectemnorhinus* weevils, sub-fossils, systematics, morphometrics, COI gene, morphological change over time, ecosystem functioning, anthropogenic influences, climate change, mouse-predation, sub-Antarctic Marion and Prince Edward Islands

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"Challenge is a dragon with a gift in its mouth. Tame the dragon and the gift is yours."

Noela Evans

Disclaimer

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Chapter 1

A morphological analysis of weevils from sub-Antarctic Prince Edward Islands: an assessment of ecological influences

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Introduction

Anthropogenically-induced global environmental modifications, such as green-house gas-driven climate change and biological invasions and their effect on species biodiversity and species changes, are of great conservation concern (Dukes and Mooney 1999; Pimm et al. 2001; Luck et al. 2004). The effects of human-induced change on mainland as well as island ecosystems, influencing physiology, distribution, life cycle events (phenology) and adaptation of organisms may have serious consequences for the future survival of organisms (Hughes 2000). A diverse range of studies show species are already exhibiting changes with regard to population densities, distributional ranges and morphological change as a result of the anomalous global climate (Huyser et al. 2000).

There is increasing concern that higher CO_2 production due to human activities is affecting global climate (Dukes and Mooney 1999). Long-term climatic monitoring programmes indicate that the earth is warming up and continual temperature increases in future are anticipated (Mitchell et al. 1990; Schneider 1992; Hughes 2000). It has been predicted that over the next century, the earth's average temperature will increase by between 1 – 3.5°C, and precipitation regimes will change continually (Dukes and Mooney 1999). Such climatic changes have far-reaching consequences on both ecosystems and species (McCarty 2001).

Anomalous climate changes have already been reported to be affecting species' distributions, abundance, physiology and phenology (Hill and Hodkinson 1992; Lawton 1995; Parmesan 1996; Kuchlein and Ellis 1997; Parmesan et al. 1999; Hughes 2000). For example, a 90% decline in population numbers of sooty shearwaters (*Puffinus griseus*) occurred in North America between 1987 and 1994 (Veit et al. 1996, 1997). Similarly, 75% of 65 bird species in the United Kingdom showed a tendency towards earlier breeding between 1971 and 1995 (Crick et al. 1997; for more examples, see McCarty 2001). Invertebrates are also affected by temperature increases that are most likely to influence insect distribution and abundance (Whittaker 2001).

Both temperature and precipitation play an important role in the life cycles of many organisms (Block 1990; Erelli et al. 1998; Karan et al. 1998), therefore, important conservation issues for species' survival (either extinction, genetic, or morphological change) are highlighted as a result of a continuously changing environment, due to, for example, global warming. Temperature is an important determinant of body size in insects, with warmer environments favouring smaller body size, and vice-versa (Atkinson 1994; David et al. 1994; Chown and Gaston 1999). Increasing temperatures as a result of global warming are considered to decrease developmental time in organisms, particularly in insects, shortening larval stages and allowing these organisms to reach adult stage much faster (Hughes 2000).

Long-term morphological changes, including body size changes in various organisms resulting from human activities have already been reported (see Bas and Sarda 1998; Roy et al. 2003). Body size is closely related to insect life history traits, such as fecundity, mating success and dispersal ability (see Peters 1983; Partridge 1988; Prout and Barker 1989; Reiss 1989; Harvey and Pagel 1991). Thus, temperature-induced modifications in body size may change insect life history traits (Karan et al. 1998; Bitner-Mathé and Klaczko 1999; Hodkinson et al. 1999). However, not only are changes evident in individual organisms, but global temperature change currently poses a real threat to ecosystems (McCarty 2001).

Removal of barriers preventing long-distance dispersal of both plants and animals, as a result of human activities, leads to an increase in the number of ecosystems altered in their composition and functioning (Vitousek et al. 1997; Dukes and Mooney 1999). Dukes and Mooney (1999) reported that most aspects of global change, especially climate change and habitat fragmentation, will favour invasive species, therefore, increasing the severity of their impact on ecosystems. For example, the majority of endangered vertebrate species in south Florida are threatened by habitat fragmentation and destruction (Humphrey 1992; Moler 1992), and are considered to favour the establishment of non-indigenous invasive species (Forys and Allen 1998), such as the Cuban tree frog (*Osteopilus septentrionalis*) and is linked to the decline of some native hylids (Wilson and Porras 1983). Invasive species tend to be 'generalists', making them successful in a wide range of habitats, thus, mainly 'specialist' species dependent on specific habitats will be greatly influenced by environmental change (Dukes and Mooney 1999). Consequently, the prevalence of invasive species is most likely to increase as a result of global anthropogenic changes (Dukes and Mooney 1999).

The increase in human-facilitated biological invasions constitutes one of the most important conservation threats to the functioning of ecologically sensitive ecosystems, particularly in the isolated sub-Antarctic South Indian Ocean Province Islands (Bonner 1984; Chown et al. 1998; Bergstrom and Chown 1999). A large number of both plant and animal species are endemic to the Southern Ocean Islands (Gremmen 1981; Crafford et al. 1986; Kuschel and Chown 1995; Huyser et al. 2000), highlighting the critical need for the biological conservation of these isolated sub-Antarctic land masses (Chown et al. 1998). So far, the relatively small surface area and the isolation of sub-Antarctic islands, together with limited human activities, have facilitated the continuous monitoring of the arrival, establishment, and the impact of alien species on island ecosystem functioning (Bergstrom and Chown 1999). These ecologically sensitive, but relatively simple ecosystems are, therefore, ideal for addressing questions relating to the partitioning of the potential influences of anthropogenic changes, such as climate change and alien species invasions on ecosystem functioning (Smith 1991).

Since the late 1960s, mean annual temperature on various sub-Antarctic islands has increased by approximately 1°C, while mean annual precipitation has declined (Adamson et al. 1988; Smith and Steenkamp 1990; Weimerskirch et al. 2003). Not only are indigenous island fauna and flora directly influenced by these climatic changes, but in many instances, alien invasive species are responding favourably, in for example, increasing their reproductive success and in broadening their distributional ranges (Ernsting et al. 1995; Dukes and Mooney 1999; Jones et al. 2002; Smith 2002).

The sub-Antarctic Prince Edward archipelago has two islands (Marion and Prince Edward Islands) with long-term climatic records since the 1950s for Marion Island (South African Weather Bureau) and regular vegetation surveys to monitor the number of alien invasive species and their establishment for both Marion and Prince Edward Islands (Gremmen 1981, 1997; Crafford et al. 1986; Watkins and Cooper 1986). Marion and Prince Edward Islands are located 22 km apart and the latter is house mouse-free (see below for more details). These two islands are essentially similar in their species composition, vegetation and insect fauna, and have fairly similar weather regimes. Both islands have a cool oceanic climate, with precipitation in excess of 2000 mm per annum (Crafford et al. 1986; Smith 1987). While Marion Island exhibits a mean annual air temperature of about 5.5°C (Smith and Steenkamp 1990), Prince Edward Island is thought to have a milder local climate with a tendency for drier weather conditions (Verwoerd 1971).

The ecosystem functioning of both islands is to a large extent, dependent on arthropods and other invertebrates that act as herbivores and detritivores (Gremmen et al. 1998). These macro-invertebrates play an important role in nutrient cycling, where for example, moth and weevil larvae, and earthworms have been reported to play a key role in enhancing rates of nitrogen, phosphorus and potassium mineralisation from peat on Marion Island (Smith and Steenkamp 1992a, 1993). Instead of nutrient flow through a grazing cycle, most of the energy and nutrients trapped during primary production go through a detritus cycle (Smith 1977; Smith and Steenkamp 1992b).

The increase in temperature and elevated CO_2 concentration as a result of global warming, and its drying effect, are expected to increase vegetation productivity and hence nutrient demand (Smith 1991). Consequently, the detritus cycle remains a main source of nutrient cycling, highlighting the importance of invertebrate detritivores on the ecosystem functioning of both Marion and Prince Edward Islands (Klok and Chown 1997).

Since the introduction of the house mouse (*Mus musculus*) on Marion Island more than 180 years ago (Watkins and Cooper 1986), populations of this species have increased in association with elevated temperatures, and appear to have an increasing impact on Marion Island's invertebrate fauna and its flora through seed consumption (Smith and Steenkamp 1990; Chown and Smith 1993; Bergstrom and Chown 1999; Smith 2002). For example, it has been reported that weevil species contribute even more to the diet of *M. musculus* than previously observed (Crafford and Scholtz 1987; Smith et al. 2002). In addition, pronounced population declines have been observed for *P. marioni* and *Ectemnorhinus* weevil species between Burger's (1978) study, and a study conducted in similar vegetation types in 1996 (Hänel 1999).

While mouse predation has been implicated in the decline of insect population numbers, it is also considered to be responsible for changes in body size distributions of various macro-invertebrates important in nutrient cycling on Marion Island through size-selective predation (Crafford 1990; Chown and Smith 1993; Smith et al. 2002). For example, it has been reported that size-selective mouse predation may be contributing significantly to body size changes in especially *Bothrometopus randi* and *Ectemnorhinus similis* (Chown and Smith 1993; Smith et al. 2002), and that it may also be responsible for accelerating speciation in *Ectemnorhinus* weevil species on Marion Island (Chown and Smith 1993). The continual and extensive feeding of mice on especially *Ectemnorhinus* weevil species and *P. marioni* has led to an overall concern for the survival of these species on Marion Island (Chown and Smith 1993; van Aarde et al. 1996).

Indirectly, the reduction of insect prey populations through mouse predation is also considered to have a significant impact on indigenous predator species such as the lesser sheathbill (*Chionis minor*) (Huyser et al. 2000). Traditionally, predation by the lesser sheathbill, a natural predator on the islands, has been considered to impact on weevil and flightless moth populations, with weevils and *P. marioni* larvae contributing 16.8% and 21.4% to its diet, respectively (Burger 1978). However, Huyser (2000) considers that the contribution of macro-invertebrates to the diet of the lesser sheathbill has declined dramatically, leading to a decrease in lesser sheathbill population numbers.

With limited and highly restricted human visits to the mouse-free Prince Edward Island (Cooper and Avery 1986), the introduction of invasive species has been kept to a minimum. In contrast, Marion Island has been exposed to a relatively higher rate of invasions by alien species, which includes the introduction and establishment of mice due to frequent human visits and a high rate of human activities (Cooper and Condy 1988; Hänel and Chown 1999). Consequently, both Marion and Prince Edward Islands provide ideal terrestrial ecosystem models to investigate the potential influences of climate change and alien invasive species on macro-invertebrate morphology.

The present study, therefore, attempts to partition the potential influences of climate change and/or alien invasive species on body size and/or shape changes by using six currently recognised weevil species endemic to the Prince Edward archipelago. These species include: 1) the coastal *Palirhoeus eatoni*; 2) *Bothrometopus elongatus*, *B. parvulus*, and the mainly coastal *B. randi*; and 3) *Ectemnorhinus similis* and *E. marioni* that jointly occur on *Azorella selago*-dominated habitat communities.

The *Ectemnorhinus*-group of weevils comprises 36 species, representing 80% of the Coleoptera on all of the sub-Antarctic islands and is the most diverse taxon on the South Indian Ocean Province Islands (Chown and Scholtz 1989; Chown 1993; Kuschel and Chown 1995). The 36 species comprise 6 genera with two major feeding habits that include angiosperm and cryptogam herbivory (Chown 1994).

Feeding habits of the *Ectemnorhinus*-group of genera determine their distributions on the islands (Chown 1994). Present data indicate the *Ectemnorhinus*-group of genera to be polyphagous, except for the supra-littoral *Palirhoeus eatoni,* an oligophagous species feeding on three species of marine alga (Chown 1994). Although the majority of weevils are polyphagous cryptogam-feeding species, members of the genera *Canonopsis, Christensenia* and *Ectemnorhinus* are the only species known to feed on angiosperms, but also incorporate bryophytes and other cryptogams in their diets (Chown 1989).

The feeding adaptations of the *Ectemnorhinus*-group gives them the ability to feed on a wide range of food plants and make them one of the most successful groups on the sub-Antarctic islands (Kuschel 1971). Species belonging to the genera *Palirhoeus*, *Bothrometopus* and *Ectemnorhinus* (*vanhoeffenianus* group - four species restricted to Îles Crozet) are restricted to the cryptogam-dominated epilithic biotope, comprising fellfield, shoreline rocks and outcrops, where they feed on lichens, cyanobacteria, algae and bryophytes (see Chown 1994). The remainder of the *Ectemnorhinus* weevils (*viridis* group - cosmopolitan within the South Indian Ocean Province Islands) are mainly associated with the vegetated biotope or with angiosperms at high altitudes, although some species such as *E. marioni* are always found in association with bryophytes irrespective of altitude (see Chown 1994). Smith (1977) reported that the largest and the most conspicuous herbivorous arthropod species is *Ectemnorhinus similis* (C.O. Waterhouse), the only weevil species on the Prince Edward Islands that feeds on angiosperms (Chown 1989; Chown and Scholtz 1989). Despite the different climatic histories of Marion and Prince Edward Islands (Verwoerd 1971), no morphological or ecological differences were found between weevil assemblages (Chown 1992).

Palirhoeus eatoni (C.O. Waterhouse 1876) are restricted to the upper- to supra-littoral zone, regularly inundated and exposed to sea spray, where they feed on marine algae (Kuschel 1971; Crafford et al. 1986). To facilitate a firm grip on rocks, *P. eatoni* adults have small hooks on the lower angle of the tibiae, elongated claw segments and long claws (Kuschel 1971; Crafford et al. 1986). Adults vary in length between 4.0 – 5.5 mm, and are easily distinguishable from other weevil species by the presence of a tarsal claw segment that is longer than the first three tarsal segments. In addition, the species lacks any vestiges of meta-thoracic wings or a lateral flange on the inner surface of the elytra (Crafford et al. 1986). Larvae associate with debris and vegetation in both intertidal and supra-littoral zone and are distinguished on the presence of a darkly sclerotised peritreme on the thoracic spiracle (Crafford et al. 1986).

Adults of *B. elongatus* (Jeannel 1953) are restricted to the central highlands (300 – 1000 m a.s.l.) of Marion and Prince Edward Islands, where they feed on lichens and epilitic moss (*Andreaea acuminata*) (Crafford et al. 1986). It is the smallest weevil species present on Marion and Prince Edward Islands, ranging between 2.5 mm and 4.0 mm. The species is distinguished from other species, based on the presence of long transverse hairs on the elytra and pronotum (Crafford et al. 1986). Larvae feed on both moss and detritus, with larvae and pupae occurring inside clumps and balls of moss (*Andreaea* and *Ditrichum* spp.) (Crafford et al. 1986).

The distribution of *B. parvulus* (C.O. Waterhouse 1885) ranges from the supra-littoral zone, overlapping with the distribution of *P. eatoni*, up to 900 m a.s.l. (Crafford et al. 1986). Adults mainly feed on lichens, algae, mosses and certain vascular plants (e.g., *A. selago*; Kuschel 1971) (Crafford et al. 1986). Adults range between 3.0 mm and 5.0 mm and are characterised by a compressed and carinate humeral area. Larvae are white with labial sclerotisation with lateral arms broad at the base as well as air-tubes of abdominal spiracles directed dorso-caudad (Crafford et al. 1986).

Bothrometopus randi (Jeannel 1953) occurs from sea level up to approximately 750 m a.s.l. (Chown 1992), and rarely up to 1000 m a.s.l. (L. Janse van Rensburg and G.C. Grobler pers. obs.), and are distinguished by a distinctive green elytral scale pattern. Large adults (5.0 – 7.5 mm) occur abundantly on lichen-covered rocks and both adults and larvae appear to be specialised algae- or lichen-feeders. Larvae are large and have a grey-green colour (Crafford et al. 1986).

The taxonomic status of the currently recognised *E. marioni* (Jeannel 1940) and *E. similis* (Waterhouse 1885) has been the subject of considerable debate (Kuschel 1971; Crafford et al. 1986; Chown 1989; Chown and Scholtz 1989; Chown 1990). Waterhouse (1885) first described *E. similis* from Marion Island followed by Jeannel's description of *E. marioni* in 1940 and is distinguished from *E. similis* on the basis of humeri, interstriae and striae morphology. Kuschel (1971), however, synonymised the two species due to lack of consistent differences in internal or external characteristics and ecological preferences. Later, Dreux and Voisin (1978) again recognised *E. marioni*. Thereafter, Crafford et al. (1986) recognised three distinct ecotypes within the *Ectemnorhinus* species complex, considered to be correlated with three morphs distinguished on body size and colour.

The brown morph ranged between 6.5 mm and 9.0 mm, covered by dense brown scales, interspersed with sparse, erect setae. Adults associated mainly with *A. selago* and *Poa cookii* grassland but also fed on *Acaena magellanica* and mosses (Crafford et al. 1986). The brown morph larvae were characterised as detritus-feeders, associated predominantly with *A. selago* litter, but also with albatross nests and well-manured vegetation.

An intermediate-sized group (5.0 – 7.0 mm) labelled as the green morph, were characterised by a brown integument covered with simple rows of dense green scales, interspersed with sparse, erect setae. Adults occur in a wide range of habitats, from the supra-littoral up to 800 m a.s.l. associated mainly with *Poa cookii* grassland and drainage line communities on the coastal plain. Main food sources included *Acaena magellanica* and the moss, *Brachythecium rutabulum*. The larvae were primarily detritivores, but may also feed facultatively on mosses and lichens (Crafford et al. 1986).

The black morph, smallest of the three morphs (4.0 – 5.5 mm), were distinguished from the other morphs by the virtual absence of body scales and a black integument. This morph was chiefly associated with *Agrostis magellanica* mire vegetation, but has also been found on *A. selago* and drainage line communities adjacent to mires. Adults, larvae and pupae were often found submerged in the saturated litter of mire communities, with larvae feeding predominantly on detritus, but also on fresh fragments of the mire-associated mosses *Jamesoniella* and *Drepanocladus* (Crafford et al. 1986).

However, Chown (1990) noted that the use of vestiture colour and body length to distinguish between ecotypes was not justified, and the species complex was instead separated into two morphologically similar but ecologically distinct species. Bryophyte-feeding individuals (3.77 – 7.79 mm) associated with *Azorella selago*, *Agrostis*, *Campylopus* and *Ptychomnion* mires as well as diverse bryophytes including *Ditrichum strictum*, were designated *E. marioni* (Chown 1990). The larger (4.51 - 8.69 mm) angiosperm-feeding individuals associated with *Acaena* herbfields, *Callitriche antarctica*, *Pringlea antiscorbutica* and *A. selago* were designated *E. similis*. Although *E. similis* feeds mainly on angiosperms, at the end of the growing season when vascular plant foliage deteriorates, bryophytes and other cryptogams are incorporated into their diet (Chown 1989; Chown and Scholtz 1989; Chown 1990). In addition, Chown (1990) noted that *E. marioni* and *E. similis* also differ in the length of their life cycles and time of emergence. *Ectemnorhinus marioni* adults are present throughout the year and are characterised by shorter life cycle with fewer instars, while *E. similis* adults emerge during summer months, synchronised with the first flushes of angiosperm growth and flowering. Apart from body size, no consistent differences in the male genitalia of the two taxa are present (Chown 1990). Similarly, females show no differences in either the ovipositor or the spermatheca (Chown 1990). It has been suggested that *E. marioni* and *E. similis* evolved sympatrically, as a result of differences in food preference and reproductive isolation induced by individual body size differences (Chown 1990; Crafford and Chown 1991).

If the *Ectemnorhinus* species complex is considered as two separate species, their body length and dietary variability becomes considerably reduced, bringing them within the range found in other weevil species occurring on other sub-Antarctic Islands (see Chown 1991). Chown (1991) reported that the variation in both diet and morphology of the *Ectemnorhinus* complex on Marion Island may be a result of the between-phenotype component and suggested that the radiation of *Ectemnorhinus* species on Marion Island is relatively far advanced (see Roughgarden 1972; explanation in Chown 1991). Therefore, Chown (1991) suggested that taxonomic decisions concerning species present on each island should be based on intensive studies of morphology, biology, ecology and island history, rather than morphology based on museum material.

The present study uses a morphometric approach as well as molecular (COI gene) data from a parallel study (G.C. Grobler) in an attempt to assess the taxonomic status of *Ectemnorhinus* weevil species from the Prince Edward archipelago. In addition, long-term morphometric size/shape changes in weevil species sampled on both Marion and Prince Edward Islands over the last five decades are assessed. This analysis of the long-term data includes data from up to approximately 4000 year-old sub-fossil weevil remains recovered from mire core samples on Marion Island. Given that both Marion and Prince Edward Islands are thought to have fairly similar temperature regimes, the latter island being mouse-free, would serve as a control in the partitioning of the potential effects of climate change and mouse predation on weevil morphology. Furthermore, altitude above 750 m a.s.l. was initially considered as an additional control because of previous reports that, unlike weevils, mice only occur up to 750 m a.s.l. in the summer months on Marion Island (Chown and Klok 2003). However, mice and/or mice pellets have since been observed to occur from 800 m up to 1000 m a.s.l. on Marion Island (L. Janse van Rensburg and G.C. Grobler, pers. obs.). Body size changes together with increased mice predation may eventually alter fundamental processes of ecosystem functioning, which may lead to weevil species extinctions (Chown and Smith 1993). The problem in body size changes is exacerbated further by reports of elevation-linked declines in size for some weevils from Marion Island (Chown 1992; Chown and Klok 2003).

Given the background above, the objectives of the present investigation are:

1) To clarify the taxonomic status of the *Ectemnorhinus* weevil species occurring on both Marion and Prince Edward Islands using morphometric data complemented by molecular data from a parallel study (G.C. Grobler).

2) To assess weevil morphological changes on both Marion and Prince Edward Islands over time, with reference to climate change and mouse predation.

Relevance of Study

Both Marion and Prince Edward Islands are under the jurisdiction of the South African government, with their management being vested under the administration of its Department of Environmental Affairs and Tourism (DEA&T). As two of its major management objectives, the Prince Edward Islands Management Plan by the department's Prince Edward Islands Management Committee seeks to:

1) "… *maintain biological diversity, including genetic diversity, species diversity and the diversity of ecological processes*"; and
2) "… *minimise interference with natural processes and the destruction or degradation of natural features resulting from human interference*" (Anonymous 1996).

Invasive species pose one of the greatest threats to indigenous biodiversity on both Marion and Prince Edward Islands (Chown et al. 1998; Bergstrom and Chown 1999). The need to prevent further introductions and mitigate the impacts of alien species already present is entrenched into the current Management Plan for the Islands. One aspect of concern in the Prince Edward Islands Management Plan is the ecological impact arising from the introduction of the alien, invasive house-mouse (*Mus musculus*, sensu lato) on Marion Island because mice arguably constitute one of the main threats to biodiversity and ecosystem functioning on the Prince Edward and other Southern Ocean Islands (Jones et al. 2003). Similarly, climate on Marion and Prince Edward Islands is changing (Smith and Steenkamp 1990) and could also have an effect on the ecosystem functioning of the islands. Therefore, there is a critical need to partition potential ecological influences that could affect weevil morphology, that in turn impact on the islands' ecosystem functioning.

To exacerbate these potential problems further, the taxonomic status of some of the affected weevil species is largely uncertain. It is, therefore, not clear which species occur on Marion Island and/or Prince Edward Island, and whether the populations/species which also appear to be in the process of speciation are sufficiently indistinct to be managed as single conservation units (Moritz and Faith 1998). Similarly, it is not clear whether the weevil populations occurring on the mouse-free Prince Edward Island are sufficient to ensure the conservation of weevil species diversity on Marion Island.

For the Prince Edward Islands Management Committee to take informed decisions regarding threats to indigenous biodiversity and ecosystem functioning, it clearly needs information on the distinctiveness of the weevil species and the extent to which their roles are changing in the Marion Island ecosystem. The present investigation has as its major rationale, the provision of information that will be of direct relevance to the management strategies for both Marion and Prince Edward Islands.

Key Questions and Thesis Outline

To this end, the following key questions (and their associated parts of the thesis) will be addressed in the present study:

- **Key Question I: Can the currently recognised weevil species be distinguished using a selected set of morphometric characters?**

The first part of this study (Chapter 2) is directed towards selecting meaningful morphometric characters for use in the assessment of the taxonomic status and morphological changes over time in weevils from Marion and Prince Edward Islands. This includes a morphometric assessment of functional units of weevil morphology that attempts to conform to the morphological integration concept advocated by Olson and Miller (1958). This part of the study will also include a series of univariate and multivariate procedures undertaken with the primary objective of establishing whether sexes should be treated separately or pooled in all subsequent analyses.

- **Key Question II: Do the two currently recognised *Ectemnorhinus* species on Marion and Prince Edward Islands show sufficient morphological and/ or genetic differences for them to be considered separate species?**

Chapter 3 addresses questions relating to the taxonomic status of *Ectemnorhinus* species on both Marion and Prince Edward Islands using multivariate morphometric and molecular analyses. The molecular aspect forms part of a parallel study (G.C. Grobler) that attempts to genetically characterise weevil species from Marion and Prince Edward Islands. Both morphometric and molecular analyses will be included in the assessment of the taxonomic status of *Ectemnorhinus* weevil species occurring on both islands.

- **Key Question III: Have there been morphological changes in weevil populations on both Marion and Prince Edward Islands over time with reference to potential influence, particularly climate change and mouse predation?**

Chapter 4 addresses the question of morphological change over time using *B. elongatus*, *B. parvulus*, *B. randi* and *P. eatoni* from both Marion Island and Prince Edward Island and attempts to partition its potential influence particularly with reference to climate change and mouse predation. Potential influential factors examined include temperature, rainfall, altitude, year of sampling, gender and mouse predation.

- **Key Question IV: Have there been morphological changes in weevil populations on Marion Island over time during a period that predates the potential influence of climate change and mouse predation, and with reference to recent times?**

Chapter 5 extends the question of weevil morphometric changes over time by examining sub-fossil weevil remains recovered from mire core samples on Marion Island. This part of the study includes the identification of sub-fossil remains with reference to the currently recognised weevil species on both Marion and Prince Edward Islands, followed by a comparison between sub-fossil and recently collected material in Marion Island mire habitats.

The final chapter (Chapter 6) provides a synthesis and general discussion of the major findings of the study and includes recommendations to the Department of Environmental Affairs and Tourism's Prince Edward Islands Management Committee.

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“I’ve never failed at anything in life. I was simply given another opportunity to get it right”

Winston Churchill

Chapter 2[*]

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.

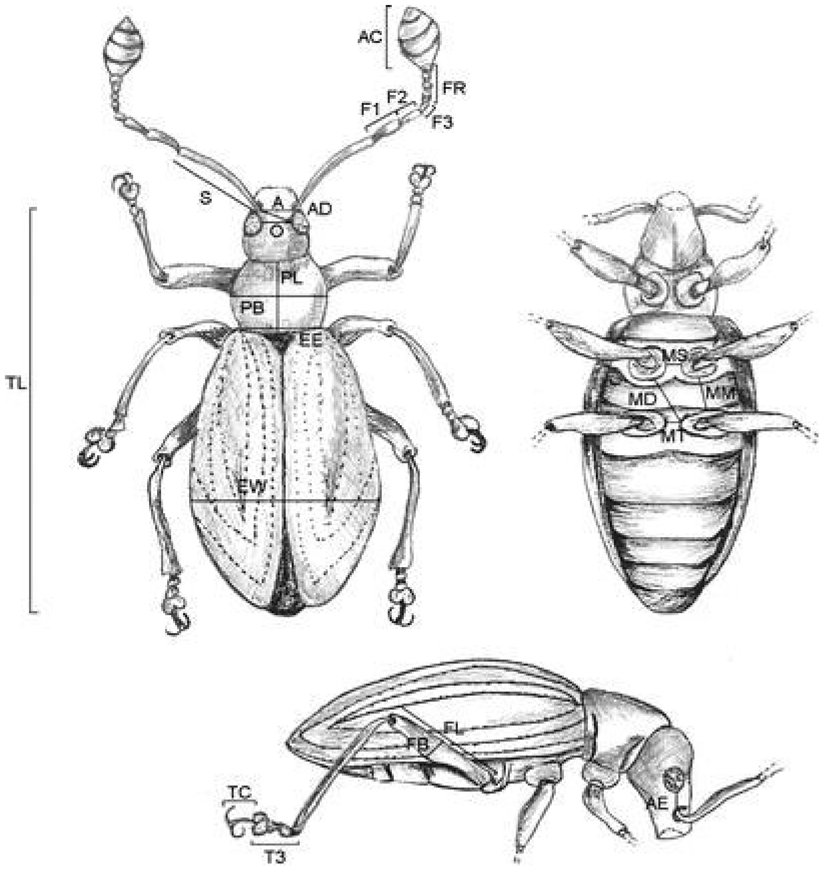


Fig. 1 Morphometric measurements assessed in the present paper as defined by Chown (1989a) (*A* inter-antennal distance, *AC* antennal club length, *AD* antenna/eye diagonal, *AE* antenna/eye distance, *EE* breadth of elytra, leading edge, *EW* maximum breadth of elytra, *F1* funicle segment 1, *F2* 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 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	*P .eatoni* *F*-value	*B. randi* *F*-value	*B. parvulus* *F*-value	*B. elongatus* *F*-value	*E. marioni* *F*-value	*E. similis* *F*-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: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

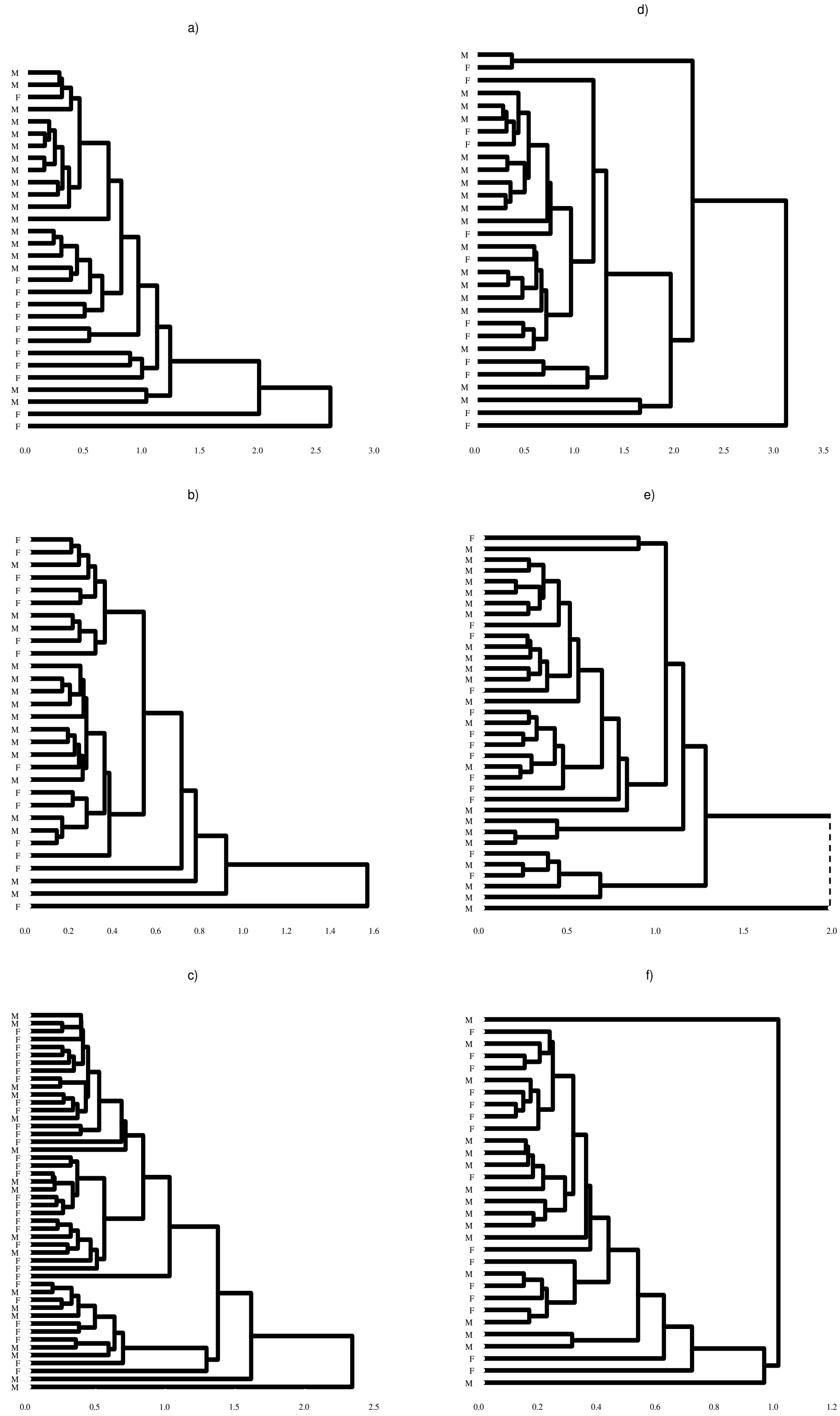


Fig. 2a–f Distance phenograms from a UPGMA cluster analysis of **a** *Palirhoeus eatoni*, **b** *Ectemnorhinus marioni*, **c** *E. similis*, **d** *Bothrometopus randi*, **e** *B. parvulus*, and **f** *B. elongatus* from sub-Antarctic Marion Island showing the absence of sexual dimorphism in overall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

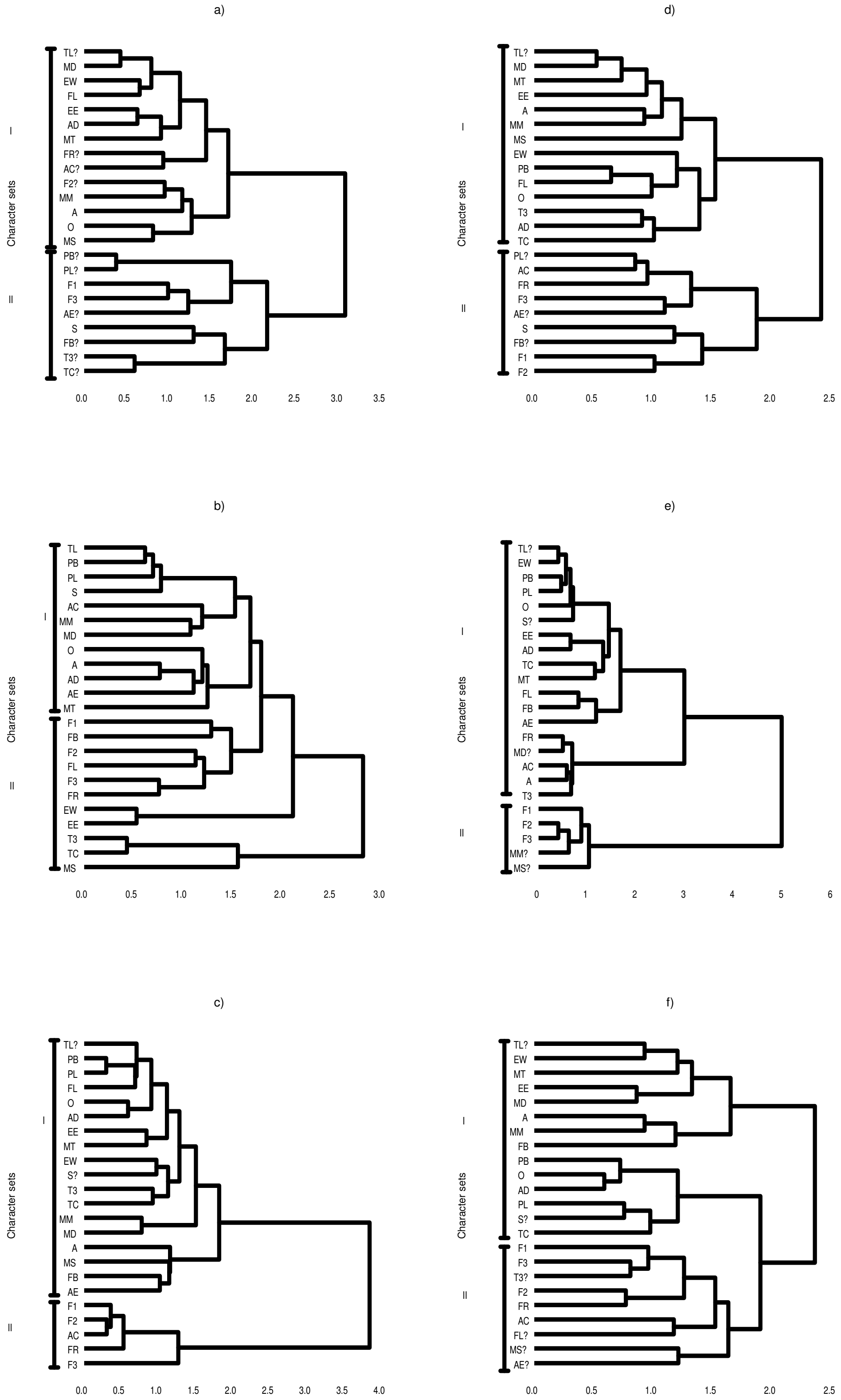


Fig. 3a–f Phenograms derived from Ward's (1963) cluster analysis of **a** *Palirhoeus eatoni*, **b** *Ectemnorhinus marioni*, **c** *E. similis*, **d** *Bothrometopus randi*, **e** *B. parvulus*, and **f** *B. elongatus* from sub-Antarctic Marion Island, depicting relationships between 23 morphometric 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

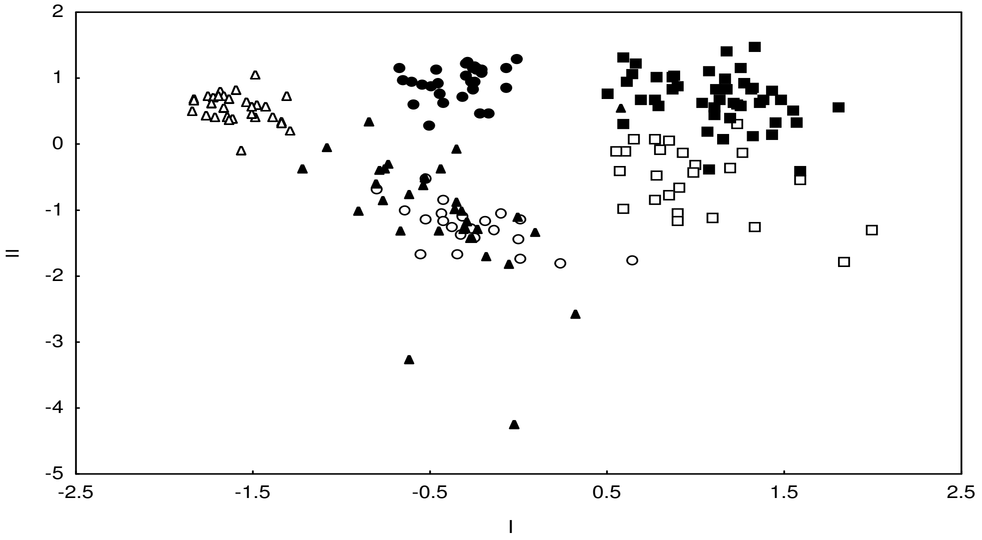
Table 2 Representative principal component (PCA) loadings for the first two axes and coefficients of variation (CV) in *Ectemnorhinus similis*, and percent measurement error (% ME) in *E. marioni* for each character in samples from sub-Antarctic Marion Island. Characters preceded by a question mark are 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.

a



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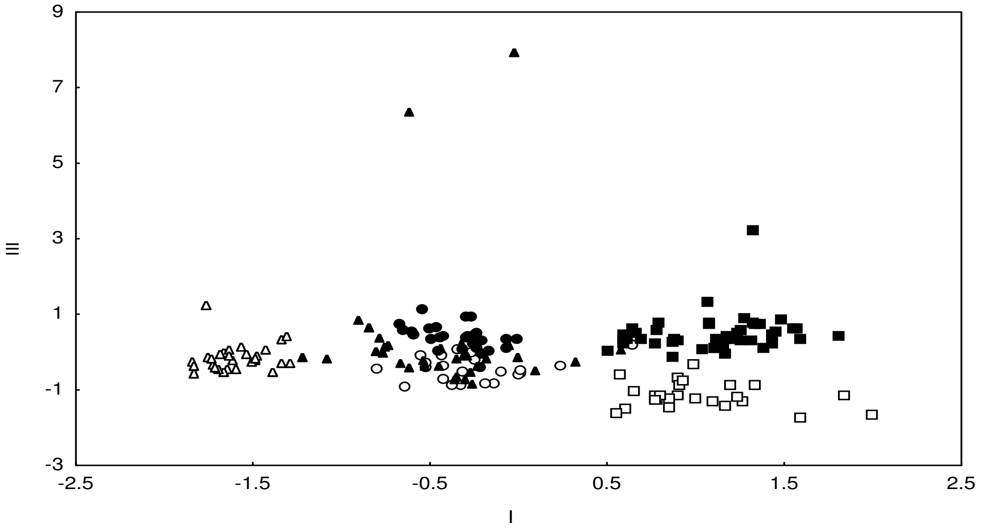

Fig. 4 a The first two components from a principal components analysis (PCA) using 23 measurements of *Palirhoeus eatoni* (*unfilled circle*), *Ectemnorhinus marioni* (*filled circle*), *E. similis* (*filled square*), *Bothrometopus randi* (*unfilled square*), *B. parvulus* (*filled triangle*), and *B. 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

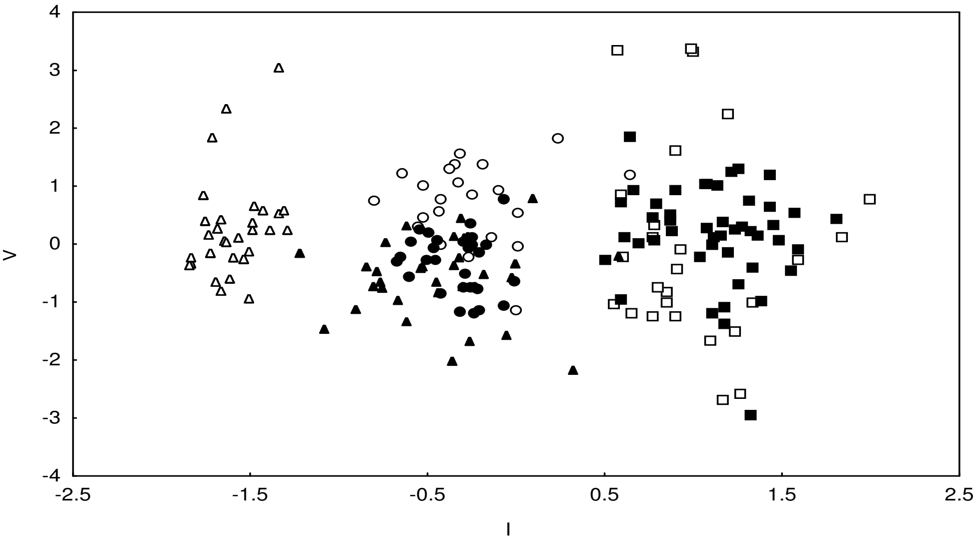

Fig. 4 c The first and fifth principal component axes showing a shape-related separation between *P. eatoni* (*unfilled circle*) and *B. parvulus* (*filled triangle*)

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.

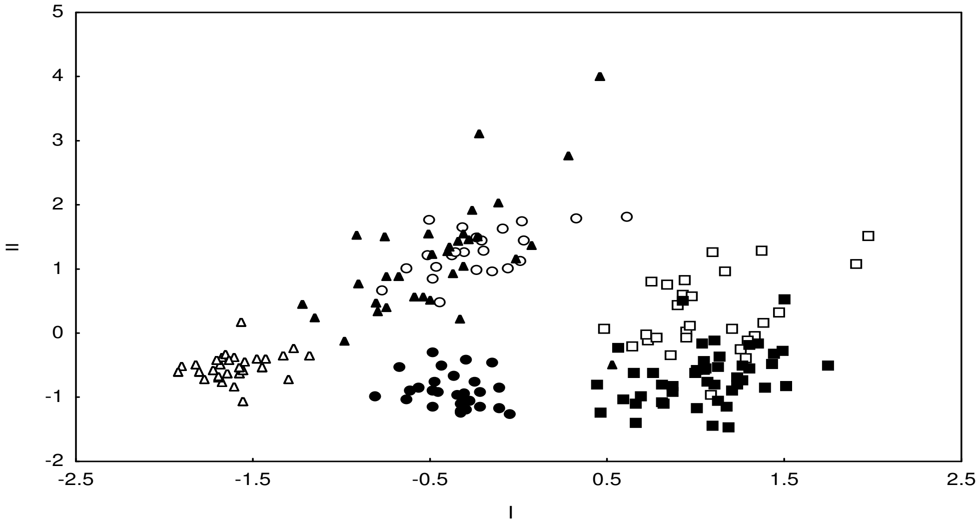

Fig. 5 The first two components from a principal components analysis using 15 measurements of *Palirhoeus eatoni* (*unfilled circle*), *Ectemnorhinus marioni* (*filled circle*), *E. similis* (*filled square*), *Bothrometopus randi* (*unfilled square*), *B. parvulus* (*filled triangle*), and *B. elongatus*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

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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, 346-34.9, 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).

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"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

Chapter 3[*]

Molecular and morphometric assessment of the taxonomic status of *Ectemnorhinus* weevil species (Coleoptera: Curculionidae) from the sub-Antarctic Prince Edward Islands

Abstract There are long-standing controversies on the taxonomic status of *Ectemnorhinus* weevil species occurring on the sub-Antarctic Prince Edward Islands. Since the two islands that constitute the Prince Edward Islands archipelago, Marion Island and Prince Edward Island differ in terms of alien invasive species and conservation management strategies, it is important to consider inter-island dynamics when investigating inter-specific relationships. Using a combined molecular phylogenetic and morphometric approach, we attempted to resolve the taxonomic status of the Prince Edward Islands archipelago *Ectemnorhinus* weevil species. A COI gene phylogeny was inferred following genetic characterisation of 52 *Ectemnorhinus* weevils from both islands, and morphometric assessment using a set of 15 linear, external measurements was used to differentiate between the two currently recognised species, *E. similis* and *E. marioni*. Analyses revealed the presence of two genetically and morphometrically distinct species on Prince Edward Island, whilst evidence for a single species, comprising diverse genetically discrete populations was found on Marion Island.

Key words: Weevils, *Ectemnorhinus*, Prince Edward Islands, COI gene, phylogenetics, morphometrics, conservation

Running title: Species status of *Ectemnorhinus* weevils on Marion and Prince Edward Island

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[*] This chapter forms part of a parallel and complementary molecular and morphological study undertaken jointly with G.C. Grobler and was submitted and is under review with the *Journal of Zoological Systematics and Evolutionary Research* as: Grobler GC, Janse van Rensburg L, Chimimba CT, Bastos AD, Chown SL Molecular and morphometric assessment of the taxonomic status of *Ectemnorhinus* weevils (Coleoptera: Curculionidae) from the sub-Antarctic Prince Edward Islands

Introduction

The weevils of the South Indian Ocean province of the Southern Ocean all belong to a single, monophyletic unit, the *Ectemnorhinus*-group (Kuschel and Chown 1995). There are approximately 36 species in the group, and they have proven to be taxonomically difficult (Brown 1964; Kuschel 1970; Dreux and Voisin 1989; Chown 1991). In particular, the taxonomic status of *Ectemnorhinus marioni* and *E. similis* from the sub-Antarctic Prince Edward Islands has long been controversial. *Ectemnorhinus similis* was the first *Ectemnorhinus* species described from Marion Island by Waterhouse (1885). Subsequently, Jeannel (1940) described *E. marioni*, which was distinguished from *E. similis* on the basis of the form of the humeri, and interstrial and strial morphology. However, Kuschel (1971) synonymised the two species due to lack of consistent differences in either internal or external morphology. Subsequently, Dreux and Voisin (1986) continued to recognise the two species, noting that they differed by virtue of the form of their elytral striae and interstriae, and the elytral punctuation.

Crafford et al. (1986) recognised three distinct ecotypes within *E. similis* based on body size and colour. Following a detailed investigation of habitat use, feeding biology, life history, morphology and mating preferences, Chown (1990), however, noted that the use of vestiture colour and body length to distinguish between ecotypes was not justified. Rather, he argued that the species complex should be separated into two morphologically similar, but ecologically distinct species. Small-sized (3.77 – 7.79 mm) bryophyte-feeding individuals associated with *Azorella selago*, *Agrostis magellanica*, *Campylopus spp.* and *Ptychomnion ringianum* mires as well as diverse bryophytes including *Ditrichum strictum*, were designated *E. marioni* (Chown 1990). The larger (4.51 – 8.69 mm) angiosperm-feeding individuals associated with *Acaena magellanica* herbfields, *Callitriche antarctica*, *Pringlea antiscorbutica* and *A. selago* were designated *E. similis*. Although *E. similis* feeds mainly on angiosperms, bryophytes, and other cryptogams are incorporated into their diet at the end of the growing season when vascular plant foliage deteriorates (Chown 1989; Chown and Scholtz 1989; Chown 1990). Both *E. marioni* and *E. similis* can be found on *A. selago*, but the former species feeds only on epiphytic bryophytes growing on this plant species (Chown and Scholtz 1989), whereas the latter species feeds both on the *A. selago* and on epiphytic species including the grass, *A. magellanica* and bryophytes.

Apart from variation in body size and diet, *E. marioni* and *E. similis* also differ in the length of their life cycles and times of emergence (Chown 1990). *Ectemnorhinus marioni* exhibits a shorter life cycle with fewer instars and adults are present throughout the year, while adults of *E. similis* only emerge during summer months, and their emergence appears to be synchronised with the first flushes of angiosperm growth and flowering. Apart from body size, there are neither consistent differences in the male genitalia (Chown 1990), nor consistent differences in either the ovipositor or the spermatheca in females of the two species. Chown (1990) suggested that *E. marioni* and *E. similis* evolved sympatrically in a manner similar to that proposed by Rice (1984), with reproductive isolation being induced by size-based assortative mating associated with dissimilarities in food preference (Chown 1990; Crafford and Chown 1991). This interpretation was subsequently accepted and a wide range of studies have been based on this taxonomy (reviewed in Chown et al. 2002; Klok and Chown 2003), such that management decisions regarding invertebrates on the Prince Edward Islands have been based on this classification (Anonymous 1996).

In addition to a variable taxonomic history, factors influencing both size and population density of *Ectemnorhinus* species on the Prince Edward Islands have also been documented. While the house mouse (*Mus musculus*, sensu lato) was introduced by sealers on Marion Island, the larger of the two Prince Edward Islands, more than 180 years ago (Watkins and Cooper 1986), the smaller Prince Edward Island has remained mouse-free. Mice feed on a variety of invertebrates and plants on the islands, and especially weevils (Gleeson and van Rensburg 1982; Smith et al. 2002). Moreover, temperature in the sub-Antarctic has increased by approximately 1°C in the last 50 years (Smith and Steenkamp 1990), and is believed to have led to an increase in the survival rate of mice during winter months, resulting in an overall population increase (Smith and Steenkamp 1990; Smith 2002). The mean volume contribution of weevil adults found in the guts of mice increased from 7% in 1979/1980 (Gleeson and van Rensburg 1982) to 11% in 1992/1993 (Smith et al. 2002). House mice are thus considered to be responsible for the significant change in the populations of *Ectemnorhinus* species on Marion Island, amounting to almost an order of magnitude decline in biomass between 1976 and 1996 (Chown et al. 2002), and a pronounced difference between population densities on Marion and Prince Edward Islands (Crafford and Scholtz 1987).

Mice are also thought to have caused a reduction in body size of weevil species on Marion Island relative to Prince Edward Island (Chown and Smith 1993). As part of their investigation, the authors noted that the frequency distributions of the size of the *Ectemnorhinus* species differed considerably between the islands. They noted that the situation across the two islands seemed to reflect predation by mice, but also concluded that further investigations were necessary, especially because the genus is taxonomically difficult (see also Chown 1991).

Given the severity of weevil predation on Marion Island and uncertainties on the taxonomic status of the *Ectemnorhinus* species on both islands (Chown 1990, 1991), it is not clear whether the Prince Edward Island populations alone are sufficient to ensure conservation of the two *Ectemnorhinus* weevil species, especially if predictions that mice predation is likely to continue escalating (Chown et al. 2002) are realised. If the species occur on both islands and if the populations are not so dissimilar that they should be considered different management units, then the current management regime (Anonymous 1996) will suffice. An essential assumption of this regime is that Prince Edward Island serves largely as a mouse-free haven for both islands (but see contrary views in Gremmen and Smith 1999; Chown et al. 2002). However, if the species or populations differ, then management practices would have to change and serious consideration would have to be given to eradication of the mice on Marion Island. Thus, it is clear that resolving the status of the two currently recognised *Ectemnorhinus* species is important from a conservation perspective.

The aim of the present study was, therefore, to evaluate the current taxonomic status of the *Ectemnorhinus* weevil species occurring on the Prince Edward Islands using both molecular and morphometric techniques. The COI gene, that has been successfully used to differentiate between island-bound Coleopteran species (Emerson et al. 1999; Sequeira et al. 2000; Trewick 2000; Caccone and Sbordoni 2001), was selected for genetic characterisation, whilst a set of 15 linear external measurements (Janse van Rensburg et al. 2003) was used for the morphometric assessment.

Materials and methods

Study area and samples

Ectemnorhinus weevil specimens were collected over three consecutive years (April 2001 – April 2003) from 28 localities (Fig. 1) on Marion Island. Samples from Prince Edward Island which has restricted access were collected along an altitudinal gradient (0 – 675 m a.s.l.) at 200 m intervals and from an additional locality consisting largely of *Ditrichum strictum*, in April 2003. Coordinates for all the sampling localities are summarised in Table 1. All specimens were collected by hand and preserved in absolute ethanol.

For the morphometric component of the study, between five and 30 *Ectemnorhinus* specimens per locality were measured. Due to uncertainty about the taxonomic status of *Ectemnorhinus* species on both Marion Island and Prince Edward Island, individuals were only identified as belonging to the genus, based on the generic descriptions provided by Kuschel and Chown (1995), with *a priori* rather than *a posteriori* multivariate morphometric analyses being used to define phenetic groupings.

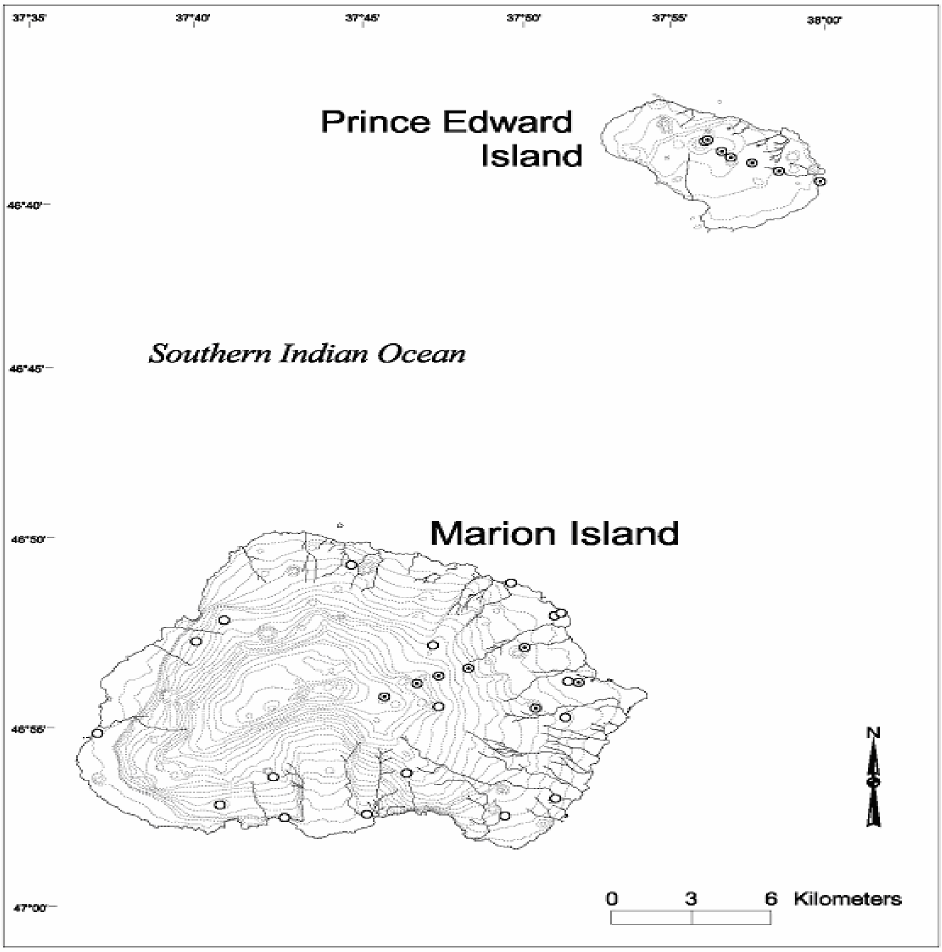

Fig. 1 Map indicating *Ectemnorhinus* weevil sampling localities on Marion Island and Prince Edward Island that correspond to the coordinates summarised in Table 1. Samples collected from all localities were included in the morphometric analyses while only samples from the dotted localities were included in the genetic analyses

Table 1 Summary of sampling locality coordinates for Marion Island (MI) and Prince Edward Island (PE)

Sampling Locality	Coordinates
MI 200 m Junior's Kop	S 46°52.794’ E 37°50.083’
MI 400 m First Red Hill	S 46°53.412’ E 37°48.21’
MI 600 m First Red Hill	S 46°53.647’ E 37°47.208’
MI 800 m Katedraalkrans	S 46°53.896’ E 37°46.482’
MI 1000 m	S 46°54.29’ E 37°45.375’
MI Tate's Hill *Pringlea antiscorbutica*	S 46°54.6’ E 37°50.478’
MI Albatross Lakes	S 46°53.82’ E 37°51.916’
PE Cave Bay 0 m	S 46°38.752’ E 37°59.780’
PE 200 m	S 46°38.457’ E 37°58.396’
PE 400 m	S 46°38.211’ E 37°57.482’
PE 600 m	S 46°37.533’ E 37°55.985’
PE TvZB 672 m	S 46°37.590’ E 37°55.891’
PE *Ditrichum strictum*	S 46°38.057’ E 37°56.771’

*TvZB indicates samples collected at the top of Van Zinderen Bakker Peak

Fifty-two *Ectemnorhinus* individuals from seven localities on both Marion and Prince Edward Islands (Fig. 1) were analysed for the molecular component. In an attempt to ensure adequate representation of species-associated feeding preferences as indicated by Chown (1990), *Ectemnorhinus* individuals were collected from *D. strictum*, *A. selago*, and *P. antiscorbutica*. Individuals collected from *D. strictum* on Marion Island were collected from an *A. selago*-free polar desert site near Albatross Lakes while those collected from *D. strictum* on Prince Edward Island were collected from a site comprising mainly of *D. strictum* (Coordinates given Table 1). As gut contents were not evaluated, we were, however mindful that sampling from a particular plant species did not necessarily imply feeding preference for that plant species. Body size variation, another criterion used by Chown (1990) to distinguish between *E. marioni* and *E. similis*, was accommodated by including the extreme size classes (largest two and the smallest two individuals) per locality. *A priori* assignment into different species was not taken into account in subsequent molecular analyses. *Ectemnorhinus viridis* (Waterhouse 1853) from Heard Island was selected as an outgroup, since it is reasonably closely related to *E. marioni* (Kuschel and Chown 1995).

Molecular characterisation

Following rehydration of ethanol-stored weevils with water, one to two weevil legs per specimen were frozen in liquid nitrogen before being ground and mixed with phosphate-buffered saline (PBS). DNA was extracted using a modified guanidinium thiocyanate (GuSCN)/silica-based method (Boom et al. 1990).

Published primers C1-J-1718 and TL2-N-3014 (Simon et al. 1994) were initially used to generate partial sequence data for representatives of all six weevil species occurring on Marion Island namely, *Bothrometopus elongatus* (Jeannel 1953), *Bothrometopus parvulus* (Waterhouse 1885), *Bothrometopus randi* (Jeannel 1953), *E. marioni* (Jeannel 1940), *E. similis* (Waterhouse 1885) and *Palirhoeus eatoni* (Waterhouse 1879). As these primers generally delivered poor quality sequences, two Marion Island weevil-specific COI primers were designed from the aligned partial sequences, following the guidelines of Rychlik (1993). These Marion Island weevil-specific COI primers termed GF and GR1 (Table 2) amplified a 1059 bp PCR product under the following conditions: 1×Buffer, 0.2 mM dNTP, 0.4 µM of each primer and 1 U *Taq* polymerase in a final volume of 50 µl containing 200 ng of template DNA. A typical temperature profile consisted of an initial denaturation step at 94ºC for 90 s, followed by 40 cycles at 94ºC for 22 s, 46ºC for 30 s and 72ºC for 1 min. PCR products were purified and DNA sequences were determined by automated cycle sequencing on an ABI PRISM^{TM} 3100 Analyser using the ABI PRISM Big Dye^{TM} Terminator version 3.0 sequencing standard.

Internal primers termed GF3, GF4, GF5 and GR5 (Table 2) were designed from the sequences initially generated with the Marion Island weevil-specific primers. The latter two primers were used in all subsequent cycle-sequencing reactions to generate a homologous 885 bp region of sequence data. The sequences were viewed and edited in Chromas version 1.43 (McCarthy 1996-1997) and aligned with DAPSA version 4.9 (Harley 2000).

Table 2 List of primers used in this study

Name	Orientation	Sequence	Tm
C1-J-1718	Forward	5'GGAGGATTTGGAAATTGATTAGTTCC 3'	608C
TL2-N-3014	Reverse	5'ATTATACCGTCTAATCACGTAACCT 3'	588C
GF-1858	Forward	5' GGGACAGGTTGAACAGTTTATC 3'	588C
GR1-2938	Reverse	5' ATGTTGTTATTCTTGAAGATGAAAG 3'	548C
GF3-2206	Forward	5'GGTCACCCAGAAGTATATAT3'	538C
GF4-2662	Forward	5'GCTGGAATAGTACAATGATT3'	538C
GF5-1940	Forward	5' TACATATAGCAGGTGTATCATC 3'	548C
GR5-2935	Reverse	5' GTTATTCTTGAAGATGAAAGATT 3'	518C

Tm: Melting temperature, calculated using the formula:
Tm = [69.3+(0.41*%GC)]-650/primer length

Phylogenetic analyses

Three sequence datasets were compiled, a Marion Island dataset, a Prince Edward Island dataset and a combined Prince Edward Islands archipelago dataset. Neighbor Joining (NJ; Saitou and Nei 1987) and Minimum Evolution (ME; Rzhetsky and Nei 1992) algorithms in MEGA version 2 (Kumar et al. 2001) were used to construct phylogenies with nodal support being assessed by 10 000 bootstrap replications.

Model Test version 3.06 (Posada and Crandall 1998) was used to identify the model of evolution that best fits the data with parameters identified under the Akaike Information Criterion (Akaike 1974) being used for subsequent Maximum Likelihood analyses (Felsenstein 1981). In each case, the TrN + I model with equal rates for all sites that correspond to the General time–reversible model, GTR + I (Rodriguez et al. 1990) was selected. The proportion of invariable sites (I) and three different substitution types estimated for each dataset was as follows: 1) Combined data set: I = 0.7935, rate [A-G] = 48.19, rate [C-T] = 11.65, and other rates = 1.00; 2) Prince Edward Island dataset: I = 0.8018, rate [A-G] = 40.61, rate [C-T] = 9.03, and other rates = 1.00; and 3) Marion Island dataset: I = 0.7985, rate [A-G] = 50.85, rate [C-T] = 12.11, and other rates = 1.00.

Maximum Likelihood analyses were performed in PAUP* version 4.0b10 for Macintosh (Swofford 1999) assuming the above mentioned model parameters prior to bootstrap re-sampling. Bayesian phylogenetic analyses using MrBayes version 3.0B4 (Huelsenbeck and Ronquist 2001) were performed based on the same models and parameters recovered for each of the respective datasets. Analyses were initiated with random starting trees and run for 500 000 generations with Markov chains sampled every 100 generations. Of the 5000 trees obtained, 2000 were discarded as “burn-in”.

Parsimony analyses performed with PAUP* version 4.0b10 included equal weighting and differential weighting schemes such as character weighting where third base positions were down-weighted to 1, and first base positions were up-weighted to 9.76923; successive weighting (Farris 1969); 6 parameter parsimony on its own and combined with both character weighting and successive weighting (Williams and Fitch 1990).

The equality of evolutionary rates between lineages was tested using the relative rate test (Li and Bousquet 1992) in PHYLTEST version 2.0 (Kumar 1996). In addition, the likelihood ratio test (Felsenstein 1981, 1988) was performed and log likelihood scores obtained with and without the molecular clock enforced, were compared. Divergence times were calculated from uncorrected pairwise values and calibrated using 2.3% nucleotide sequence divergence per million years based on the arthropod mtDNA survey of Brower (1994). Haplotype diversity (h) and nucleotide diversity (π) were estimated for each island individually in DNASP 3.51 (Rozas and Rozas 1999). Differences in total body lengths of individuals between clades were determined using analysis of variance (ANOVA; Zar 1996) for Prince Edward Island.

Morphometric analyses

Fifteen morphometric measurements were recorded by a single observer (L.J.v.R.) using a stereomicroscope fitted with a calibrated eyepiece micrometer. Measurements, defined and selected based on a morphometric character selection procedure followed by Janse van Rensburg et al. (2003) included: Total body length (TL), pronotum breadth (PB), femur length (FL), interocular distance (O), metacoxal distance (MT), maximum breadth of elytra (EW), length of first three tarsal segments (T3), meso/metacoxal distance (MM), interantennal distance (A), mesocoxal distance (MS), femur breadth (FB), funicle segments 1, 2, and 3 (F1, F2 and F3), and rest of funicle (FR). Measurements were recorded to the nearest 0.05 mm (TL and EW), 0.03 mm (PB and FL), and 0.01 mm (O, A, F1, F2, F3, FR, T3, MS, MT, MM and FB).

For multivariate morphometric analyses, the absence of multivariate sexual dimorphism (Janse van Rensburg et al. 2003) permitted pooling of sexes for subsequent analyses. For Marion Island, a total of 807 individuals from 28 localities, which provided adequate geographical coverage of *Ectemnorhinus* species, were used for morphometric analysis. A total of 240 *Ectemnorhinus* specimens from six localities on Prince Edward Island were analysed. Data screening revealed five outlier specimens, not considered representative of the populations. A re-examination of these specimens revealed outlier values arising from damaged parts, and to avoid the introduction of bias in the sample, they were excluded from subsequent analyses. After determining the absence of multivariate sexual dimorphism using principal components analysis, these datasets were subjected to a randomisation procedure (Manly 1991), where a new dataset with an equal number of individuals as the original dataset were randomly sampled with replacement for each island to assess whether the absence of multivariate sexual dimorphism in the original dataset was significantly different from the randomly selected dataset.

Following Chimimba et al. (1999), sampling localities on Marion and Prince Edward Islands were grouped into a number of computationally manageable geographical subsets to accommodate for the unweighted pair-group arithmetic average (UPGMA) cluster analyses for the Marion Island (n = 807) and the combined island (n = 1047) data matrices, since the data matrices were too large for simultaneous specimen-level analyses. The results of the individual-level analyses of the geographic subsets facilitated the grouping of locality mean values in subsequent analyses that accommodated entire island data that were similar to the results of the individual-level analyses. The 28 and 24 genetically identified *Ectemnorhinus* individuals from Marion and Prince Edward Islands, respectively, were included in all morphometric analyses to assist in defining phenetically-derived groupings.

Multivariate analyses included principal components analysis (PCA) and unweighted pair-group arithmetic average (UPGMA) cluster analysis to assess whether species could be identified based on morphometric characters (Sneath and Sokal 1973). Canonical variates analysis (CVA) of genetically defined groupings was also undertaken (Pimentel and Smith 1986) in order to define phenetic groups *a posteriori*. The CVA was followed by a multivariate analysis of variance (MANOVA; Zar 1996) in order to test for statistically significant differences between pre-defined groups. UPGMA cluster analysis was based on both Euclidean distances and product-moment correlation coefficients among Operational Taxonomic Units (OTUs; Sneath and Sokal 1973), while PCA was computed from product-moment coefficients among variables (Sneath and Sokal 1973). All statistical procedures were performed using Statistica version 5.5 (Statsoft 1995).

Results

Molecular analyses

A homologous region of 885 bp corresponding to the 5’ end of the COI gene was generated for 52 *Ectemnorhinus* individuals and two *E. viridis* outgroup specimens. All sequences have been deposited in the Genbank database under accession numbers AY762267 - AY762320. For the combined dataset, 775 of the 885 sites were conserved across all 54 sequences and 98 of the 142 variable sites were parsimony informative. The % A + T was 68.6% and the transition (ti)/transversion (tv) ratio was 6. Third base position substitutions accounted for 88.2% of the variation and the remaining 11.8% was due to first base substitutions. Mutations at nucleotide level gave rise to five non-synonymous amino acid substitutions at codons 19, 85, 241, 279, and 289. Of the 52 Prince Edward Island archipelago *Ectemnorhinus* individuals sequenced, 35 had unique haplotypes.

When analysing the combined Prince Edward Island archipelago dataset, all major clades (numbered 1-7; Fig. 2a) were consistently recovered across all methods of phylogenetic analysis. Clades 6 and 7 consisted solely of individuals collected on Prince Edward Island, while clades 1 and 2 incorporated individuals from both islands. In clade 1, Marion Island individuals ranging in size from 4.23 mm to 8.08 mm, and collected on *A. selago*, *P. antiscorbutica*, and *D. strictum*, grouped together. Similarly in clade 7, individuals collected on both *A. selago* and *D. strictum* from Prince Edward Island grouped together.

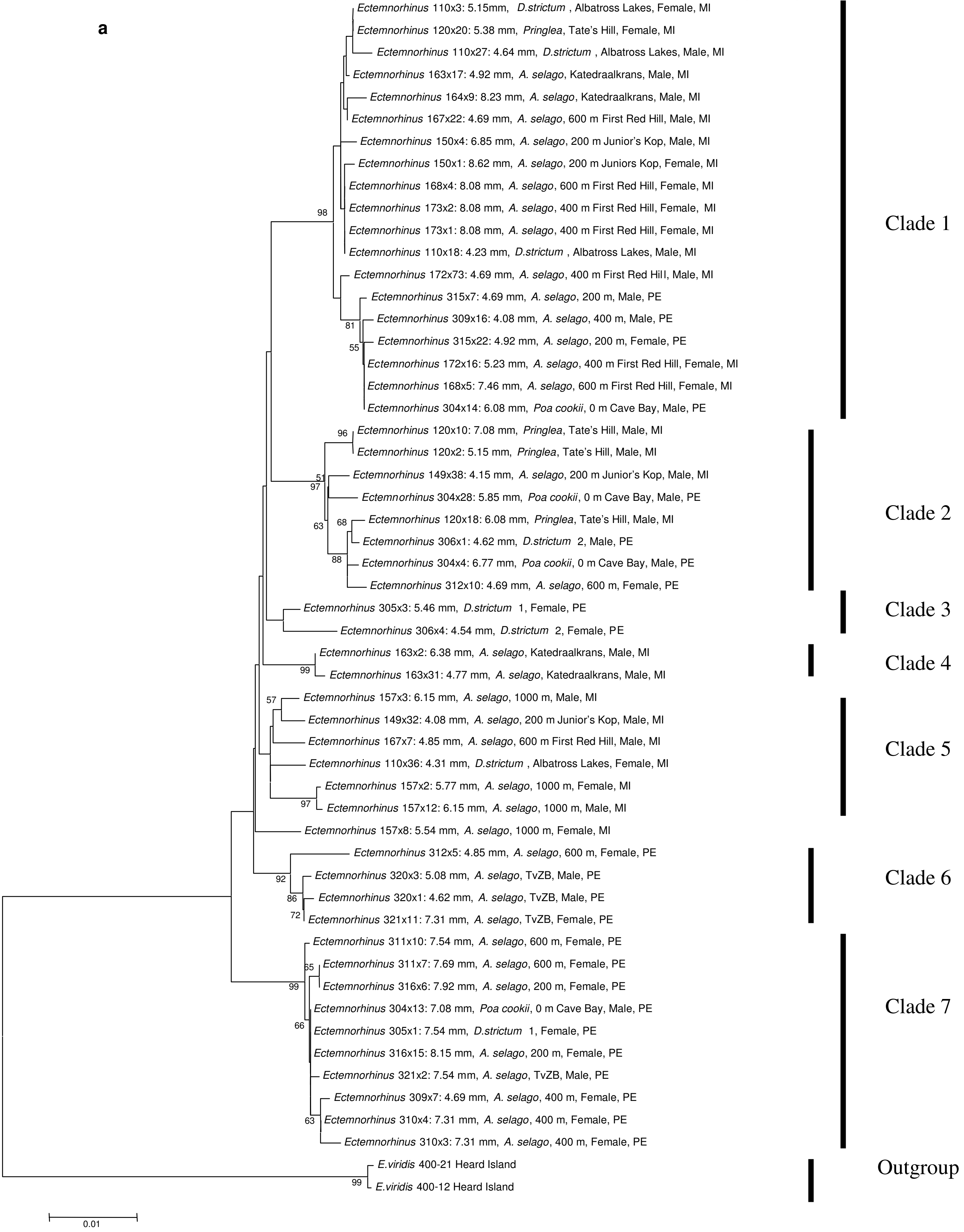

Fig. 2a Minimum Evolution (ME) trees inferred using the Tamura-Nei distance correction algorithm for the combined data set. For each specimen, the sample number is indicated followed by the body length measurement, plant species it was collected from, locality, sex and the island of origin (where 'MI'

denotes Marion Island and 'PE' indicates Prince Edward Island). Nodal support was assessed by 10 000 bootstrap replications, with only those bootstrap values ≥ 50 being indicated next to the relevant nodes. TvZB indicates samples collected at the top of Van Zinderen Bakker Peak at an elevation of 672 m above sea level

When the island datasets were analysed separately, the Marion Island phylogeny revealed that there were 22 unique haplotypes from 28 sequences. Nucleotide diversity π was 0.01217 and haplotype diversity h was 0.976. Four well-supported clades (1, 2, 4, 5) were recovered, but individuals within these clades did not group according to either body size or vegetation type from which individuals were collected (Fig. 2b). The largest between-clade Kimura-2-parameter genetic distance for Marion Island was estimated to be 1.9%.

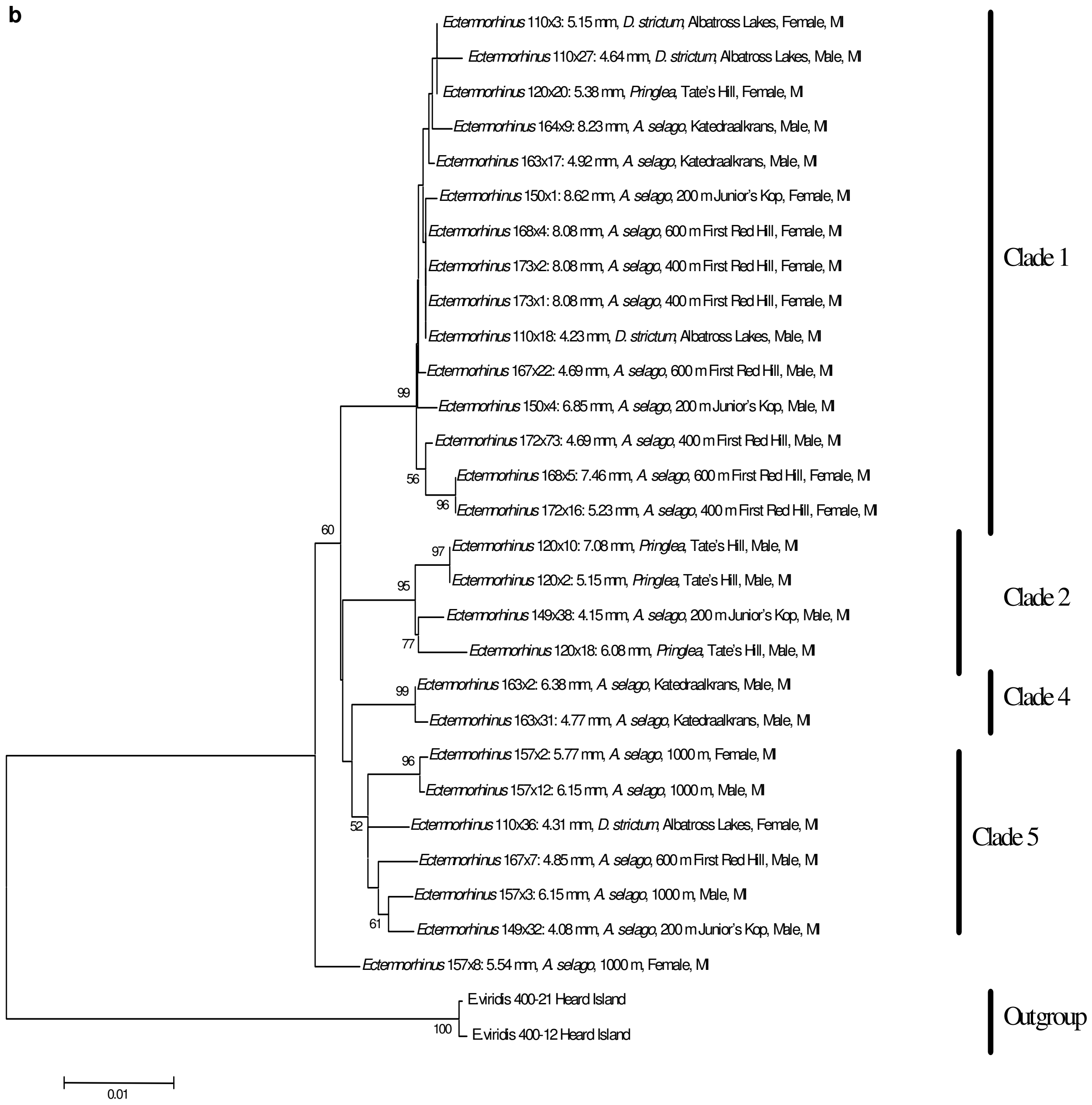

Fig. 2b Minimum Evolution (ME) trees inferred using the Tamura-Nei distance correction algorithm for Marion Island only samples. For each specimen, the sample number is indicated followed by the body length measurement, plant species it was collected from, locality, sex and the island of origin (where 'MI' denotes Marion Island and 'PE' indicates Prince Edward Island). Clades are numbered according to the clades initially identified following analysis of the combined dataset. Nodal support was assessed

by 10 000 bootstrap replications, with only those bootstrap values ≥ 50 being indicated next to the relevant nodes. TvZB indicates samples collected at the top of Van Zinderen Bakker Peak at an elevation of 672 m above sea level

For Prince Edward Island, nucleotide (π) and haplotype diversity (h) was estimated to be = 0.01687 and 0.986, respectively. A large number of unique haplotypes, 21 from 24 sequences, were recovered. Individuals from Prince Edward Island clustered within five distinct clades, of which clades 1, 2, 6, and 7 had high levels (> 85%) of bootstrap support (Fig. 2c).

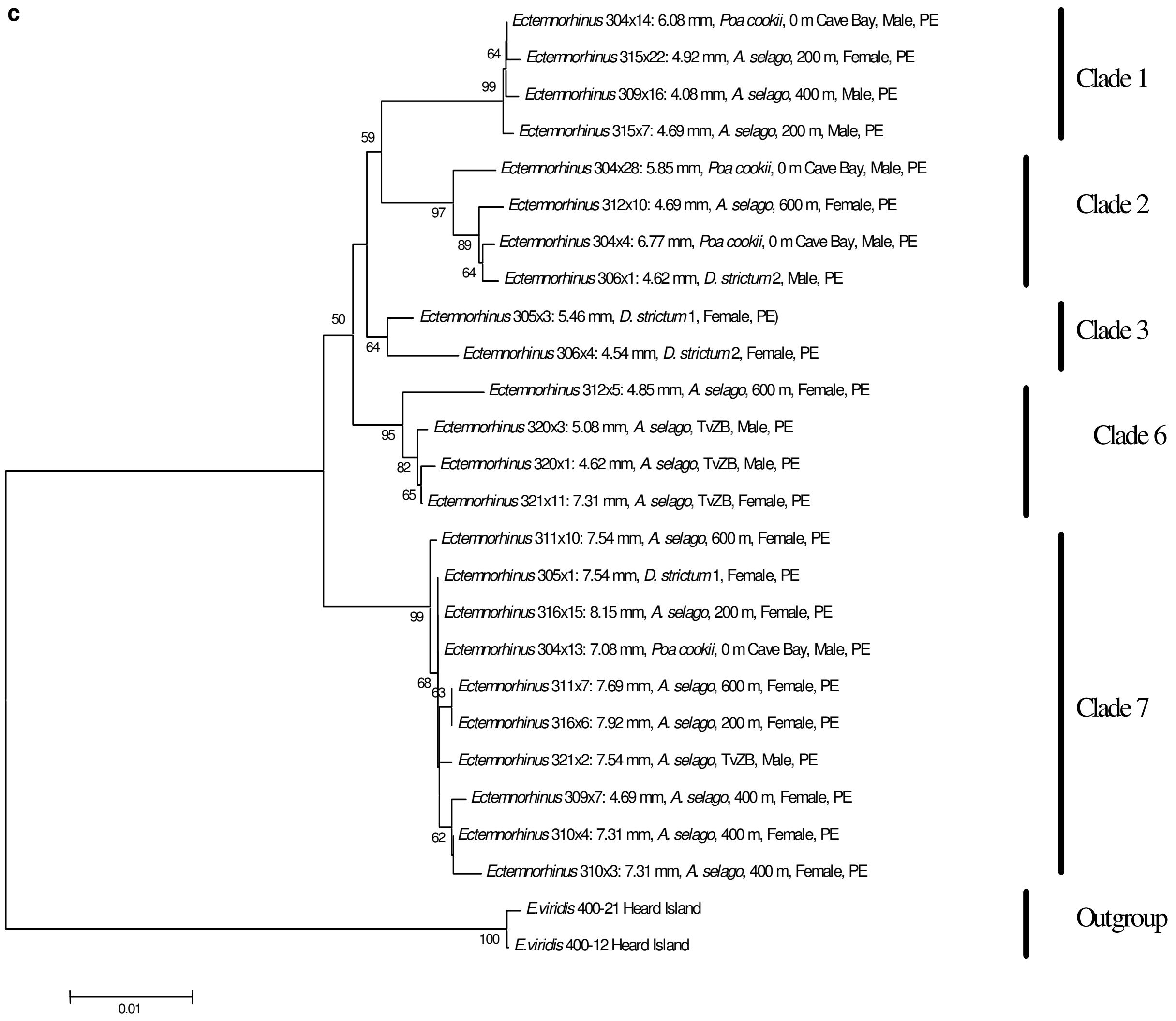

Fig. 2c Minimum Evolution (ME) trees inferred using the Tamura-Nei distance correction algorithm for Prince Edward Island only samples. For each specimen, the sample number is indicated followed by the body length measurement, plant species it was collected from, locality, sex and the island of origin (where 'MI' denotes Marion Island and 'PE' indicates Prince Edward Island). Clades are numbered according to the clades initially identified following analysis of the combined dataset **a**. Nodal support was assessed by 10 000 bootstrap replications, with only those bootstrap values ≥ 50 being indicated next to the relevant nodes. TvZB indicates samples collected at the top of Van Zinderen Bakker Peak at an elevation of 672 m above sea level

Clades 6 and 7 retained their autonomy, even in the combined island analysis (Fig. 2a). The largest between-clade distance was 2.6% and in contrast to Marion Island, clade 7 individuals were significantly larger than those of the other Prince Edward Island clades (ANOVA: $F = 9.38$, $P < 0.05$). Clade 7 consisted of individuals collected from both *A. selago* and *D. strictum* which indicates that although there is structuring according to body size on Prince Edward Island, there is none according to plant species from which they were collected.

No significant rate heterogeneity was found among the substitution rates at $P < 0.05$ according to both relative rate and likelihood ratio tests. Therefore, it was concluded that *Ectemnorhinus* individuals from the Prince Edward Islands do not evolve at markedly different rates and a molecular clock based on the mtDNA clock for arthropods (Brower 1994) could therefore, be imposed. In consequence, it was estimated that these *Ectemnorhinus* weevils arose approximately 0.49 million years ago (MYA) and 0.33 MYA on Prince Edward and Marion Islands, respectively.

Morphometric analyses

Principal component analyses based on both original and randomly selected data for both Marion and Prince Edward Islands, showed no grouping of the sexes indicating the absence of multivariate morphometric sexual dimorphism within datasets.

All multivariate morphometric analyses of both individual and combined island datasets as well as individual-level analyses and those based on mean values were similar, and are best illustrated by PCA results. The PCA of the combined Prince Edward Island archipelago dataset (encompassing all size classes) showed neither a distinct separation with reference to the two currently recognised *Ectemnorhinus* species nor with regard to island of origin (Fig. 3).

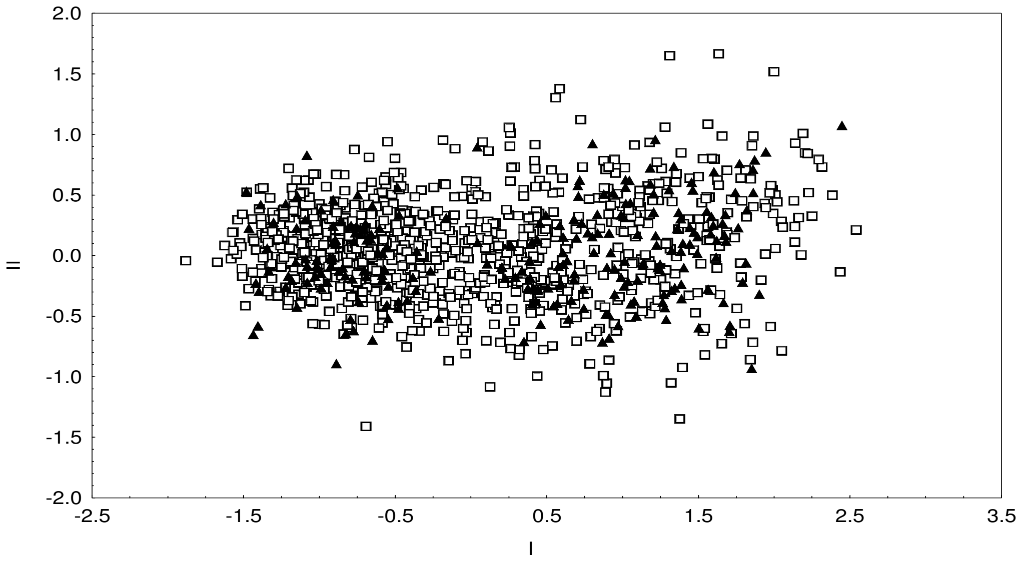

Fig. 3 Components I and II from a principal components analysis of *Ectemnorhinus* species collected from both Marion Island (*open squares*) and Prince Edward Island (*closed triangles*)

Lack of separation, however, seems to be largely confounded by a large degree of body size variation among individuals from the two islands. This is reflected by the high positive loadings of the measurements on the first PCA axis that accounted for 86.08% of the total variance (Table 3).

Table 3 Loadings of variables on components I and II from principal components analyses of pooled samples of *Ectemnorhinus marioni* and *E. similis* from a) Marion Island b) Prince Edward Island and c) Marion and Prince Edward Islands combined

	a) Marion Island		b) Prince Edward Island		c) Marion and Prince Edward Island	
	Principal components		Principal components		Principal components	
Variable	I	II	I	II	I	II
TL	0.974	-0.029	0.989	0.057	0.976	-0.012
EW	0.956	-0.036	0.976	0.041	0.960	-0.038
PB	0.946	-0.042	0.986	0.080	0.952	-0.066
O	0.952	-0.009	0.977	-0.006	0.952	-0.058
A	0.897	-0.012	0.957	0.090	0.906	-0.065
F1	0.940	-0.047	0.959	-0.020	0.944	-0.041
F2	0.938	-0.059	0.961	-0.051	0.941	-0.062
F3	0.884	-0.052	0.921	-0.115	0.890	-0.093
FR	0.931	-0.054	0.938	-0.064	0.930	-0.013
T3	0.956	-0.043	0.807	-0.517	0.949	-0.010
MS	0.555	0.830	0.833	0.258	0.712	0.698
MT	0.936	0.044	0.945	0.071	0.937	0.082
MM	0.875	-0.071	0.950	0.089	0.889	-0.045
FL	0.986	-0.061	0.988	0.020	0.986	-0.054
FB	0.958	-0.023	0.973	0.011	0.960	-0.055
% trace	84.23	4.78	89.37	2.58	86.08	3.54

* Total body length (TL), pronotum breadth (PB), femur length (FL), interocular distance (O), metacoxal distance (MT), maximum breadth of elytra (EW), length of first three tarsal segments (T3), meso/metacoxal distance (MM), interantennal distance (A), mesocoxal distance (MS), femur breadth (FB), funicle segments (F1, F2 and F3) and rest of funicle (FR)

A PCA restricted to the 52 genetically identified specimens from both Marion and Prince Edward Islands indicated no separation based on either body size or shape in the Marion Island sample. However, two groups are observed for Prince Edward Island *Ectemnorhinus* species, with only a single *Ectemnorhinus* individual from group A (clades 1, 2, 3 and 6: Fig. 2c) falling within *Ectemnorhinus* group B (clade 7: Fig. 2c) (Fig. 4a).

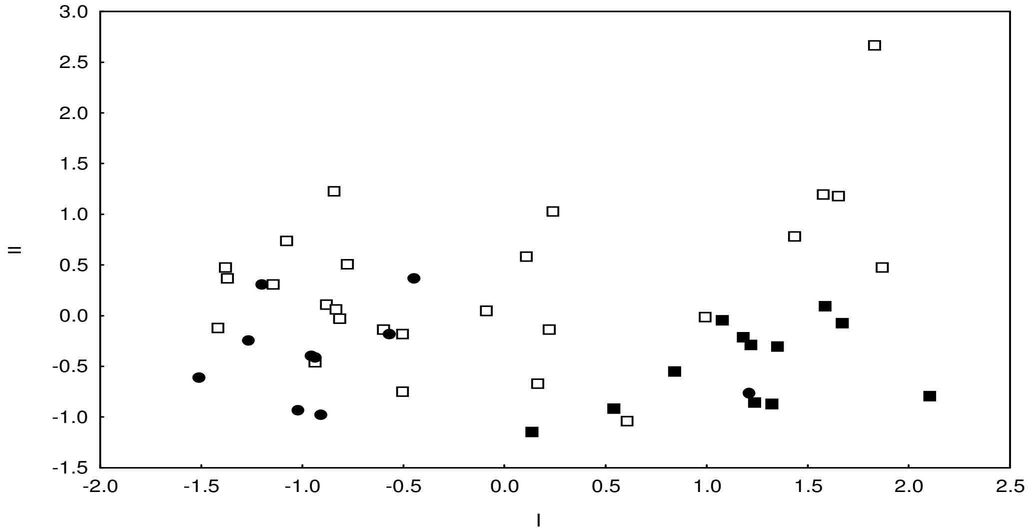

Fig. 4a Components I and II from a principal components analysis of genetically identified individuals from Marion Island (*open squares*) and Prince Edward Island, *Ectemnorhinus* group A (*closed circles*) and *Ectemnorhinus* group B (*closed squares*)

The 52 genetically identified specimens showed a more pronounced and statistically significant separation between *Ectemnorhinus* group A, B and the Marion Island *Ectemnorhinus* specimens on the first CVA axis. A shape related separation was observed on the second CVA axis between the Marion Island *Ectemnorhinus* species and *Ectemnorhinus* group A from Prince Edward Island (Fig. 4b). A MANOVA showed a statistically significant phenetic differences between these pre-defined groupings ($F_{(30,62)} = 4.34$, $P < 0.0001$).

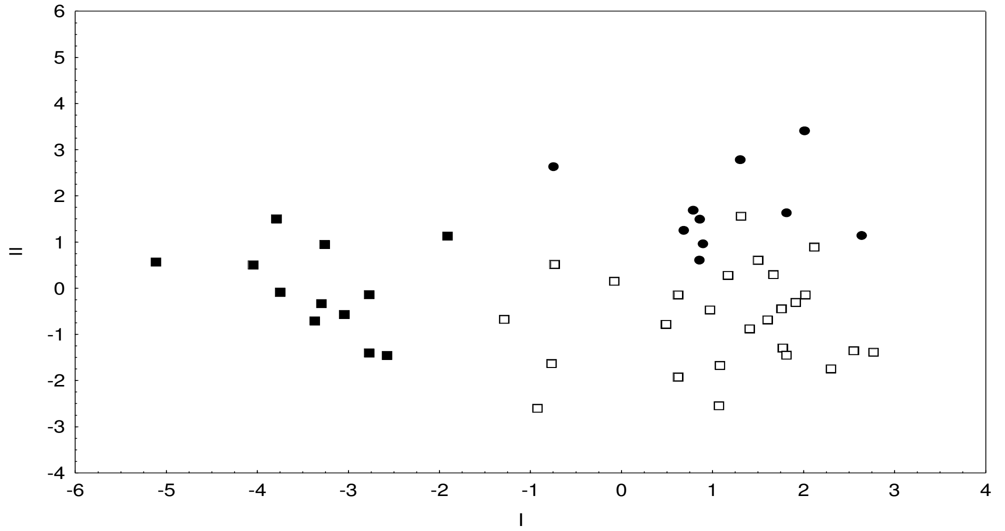

Fig. 4b Components I and II from a canonical variates analysis (CVA) of genetically identified individuals from Marion Island (*open squares*) and Prince Edward Island, *Ectemnorhinus* group A (*closed circles*) and *Ectemnorhinus* group B (*closed squares*)

A PCA of Marion Island samples (represented by all size classes) showed a considerable degree of phenetic variation, but no separation based on either size or shape variables (Fig. 5a).

Principal component I (84.23% of the total variance) had high positive loadings on all measurements (Table 3), highlighting the importance of size variation. No separation was observed on the second (Table 3) and subsequent PCA axes.

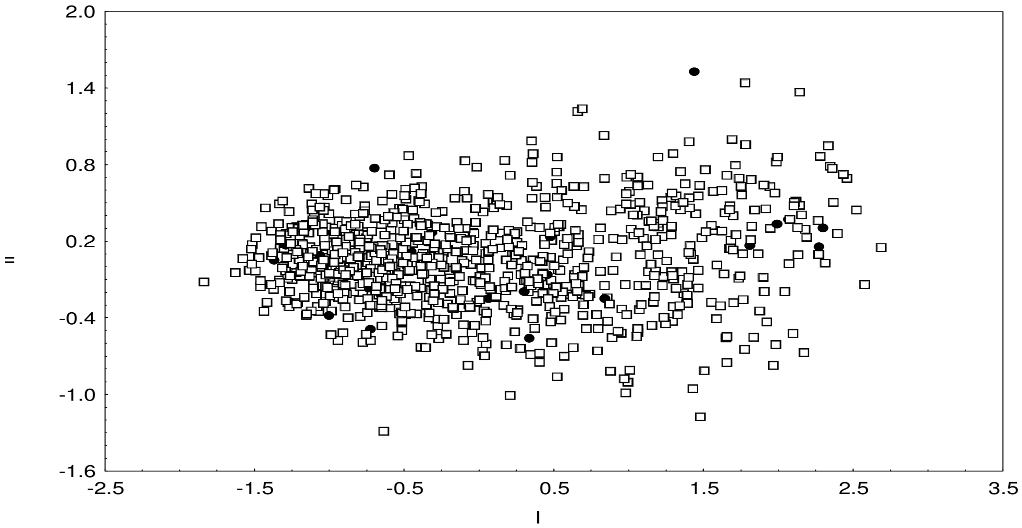

Fig. 5a Components I and II from a principal components analysis of *Ectemnorhinus* species collected from Marion Island. Genetically identified individuals are indicated with black circles

A PCA, comprising all size classes and including genetically identified Prince Edward Island samples (Fig 5b), however, showed two phenetic groupings for the *Ectemnorhinus* species. Only a single genetically-identified individual of *Ectemnorhinus* group A clustered with individuals of *Ectemnorhinus* group B. Separation between the two groups is based on a size- rather than a shape-related variation, as is shown by PCA axis I (89.37%), which has high positive loadings on the measurements (Table 3).

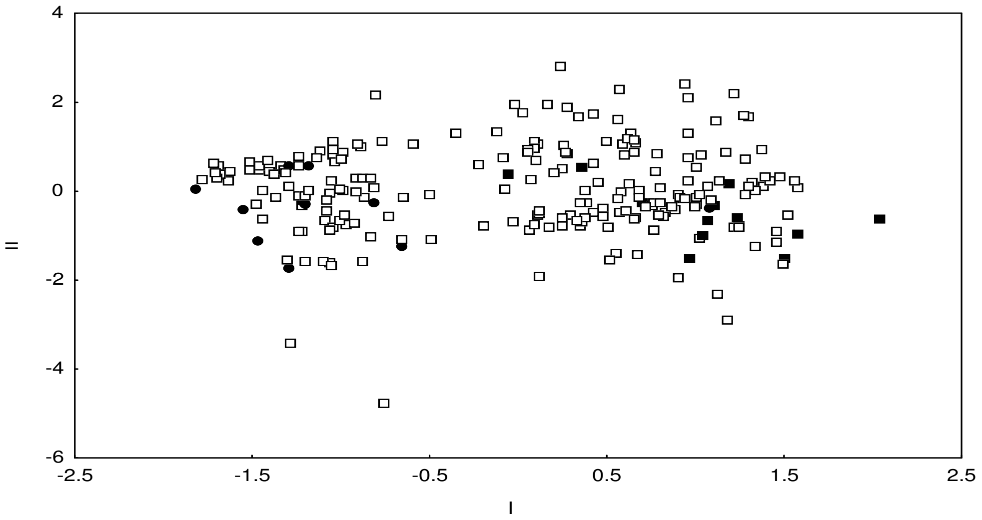

Fig. 5b Components I and II from a principal components analysis of *Ectemnorhinus* species collected from Prince Edward Island. Genetically identified *Ectemnorhinus* group A (*closed circles*) and *Ectemnorhinus* group B (*closed squares*) are indicated

Discussion

Assessment of the taxonomic status of weevil species originally described from Marion Island was undertaken using a combined molecular and morphometric approaches. The COI gene phylogeny identified four recently diverged, well-supported clades on Marion Island, but gave no support for the presence of the two species, designated *E. marioni* and *E. similis* by Chown (1990). None of the clades in the molecular phylogeny displayed clustering on the basis of body size or according to plant species from which they were collected. In addition, multivariate analyses of the Marion Island sample showed no separation of *Ectemnorhinus* individuals according to either body size or body shape variation. The molecular analyses, therefore, suggest that previous morphologically- and ecologically-defined distinguishing characteristics (Crafford et al. 1986; Chown and Scholtz 1989; Chown 1990) do not correspond to the clades identified using genetic markers. Moreover, the current morphological analysis once again confirms that size variation is confounding the difference between individuals within the genus (see Brown 1964; Kuschel 1970, 1971; Crafford et al. 1986; Chown and Scholtz 1989; Chown 1990, 1991). In addition, because the morphological analysis was undertaken with individuals that could be assigned to clades based on molecular data, the present analysis also suggests that morphological evolution is much more complex than has previously been considered.

In contrast to the results obtained from Marion Island, two major size-distinct clades were discernible for Prince Edward Island from both the genetic and the morphometric analyses. Sequence divergence values of 2.6% were observed between the two major Prince Edward Island clades that are greater than the intra-generic Kimura-2-parameter genetic distances of 1.5% and 2.1% reported for arthropods from other island systems (Trewick 2000). This, together with the high levels of bootstrap support for the two size- and genetically-distinct clades, suggests that the present recognition of two species distinguishable on the basis of size is supported on Prince Edward Island. The presence of two size-discrete groupings of *Ectemnorhinus* individuals on Prince Edward Island was also confirmed by the morphometric analyses, where all multivariate analyses indicated the presence of two size-related phenetic groupings. The single *Ectemnorhinus* individual from group A, that clustered within the *Ectemnorhinus* group B assemblage may be indicative of the extent of body size variation within *Ectemnorhinus* species.

While our data suggest the presence of two species on Prince Edward Island and only one on Marion Island, both *E. marioni* and *E. similis* were originally described from Marion Island. As *Ectemnorhinus* individuals found on Prince Edward Island were morphologically and ecologically similar to Marion Island individuals, it was concluded that both species, therefore, also occurred on Prince Edward Island (Dreux 1971). The critical question that may now be posed relates to the apparent disappearance of one species of *Ectemnorhinus* on Marion Island. Was there only one *Ectemnorhinus* species to begin with on Marion Island or is it possible there were indeed two species 65 years ago when Jeannel (1940) first described *E. marioni* as a second species distinct from *E. similis*?

The first hypothesis for the observed difference in weevil assemblage between the two islands is the loss of one of the originally described *Ectemnorhinus* species from Marion Island. One possible cause for the loss could be that the reduction in body size of *E. similis* through size-selective predation by mice (Chown and Smith 1993; Smith et al. 2002) which would have removed the size-induced reproductive barrier that was proposed on grounds of the significant relationship between female and male body size in *in-copula* pairs observed by Chown (1990) on Marion Island. This scenario would allow the two previously recognised species to interbreed. In addition to mice predation, it is also possible that climate change may play a role as temperature on the Prince Edward Islands has increased by an average of 0.04°C per year since the late 1960s (Smith 1991). It is well-known that in arthropods, and indeed most invertebrates, increasing developmental temperatures lead to a decline in body size (Atkinson 1994). Moreover, if temperatures increase to such an extent that generation time is much shorter than season length, then additional declines in body size with increasing temperature can be expected (Kozłowski et al. 2004). Investigations of weevil species on Marion Island have shown that long-term warming (at least since the 1960s) may well have led to on-going declines in body size, accompanied by a secondary, significant influence of mouse predation (L. Janse van Rensburg, unpublished data). These changes in the environment may select for smaller individuals within the populations, leading eventually to introgression.

The second hypothesis is that there was originally only one *Ectemnorhinus* species on Marion Island that was erroneously identified as two species. The original descriptions of *E. similis* by Waterhouse (1885) and *E. marioni* by Jeannel (1940) indicate that there are distinct differences between the two species. The controversial taxonomic status of these two species is, however, a clear indication of the difficulty taxonomists were faced with in the past when using morphological characteristics alone. One possible reason for the different status of *Ectemnorhinus* species between the two islands may also be due to differences in glaciation histories, as Marion Island was extensively glaciated whilst Prince Edward Island was not (Verwoerd 1971). As a result, weevils on Prince Edward Island would have had longer exposure to vascular plants as an additional, more nutritious food source to bryophytes than those on Marion Island giving rise to two species, a smaller one with a preference for bryophytes and a larger one with a preference for angiosperms, as suggested by Chown (1990).

Another possible explanation for the differential island species status may be due to differences in colonisation times. Results from this study indicate that *Ectemnorhinus* weevils colonised Prince Edward Island approximately 0.49 million years ago, soon after the islands emerged, while Marion Island was colonised approximately 0.16 million years later.

Whatever the underlying cause(s), there is no doubt that presently a marked difference in the species between the islands exists. The two islands also differ considerably with respect to the genetic composition of the populations, with each island having numerous island-unique haplotypes and only one haplotype being shared between the islands. Prince Edward Island can thus not be considered as a safe haven for *Ectemnorhinus* populations that are on Marion Island. The *Ectemnorhinus* weevils populations from the two islands are simply too distinct and should be considered different management units. Consequently, the current management regime at the Prince Edward islands should be revised. Moreover, there is an urgent need to explore possibilities of either eradicating or drastically controlling mice on Marion Island as a long-term goal to preserve the genetic variation unique to Marion Island. In the interim, the current policy of restricting human visits to Prince Edward Island should be strictly maintained.

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"If we knew what it was we were doing, it would not be called research, would it?"

Albert Einstein

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°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).

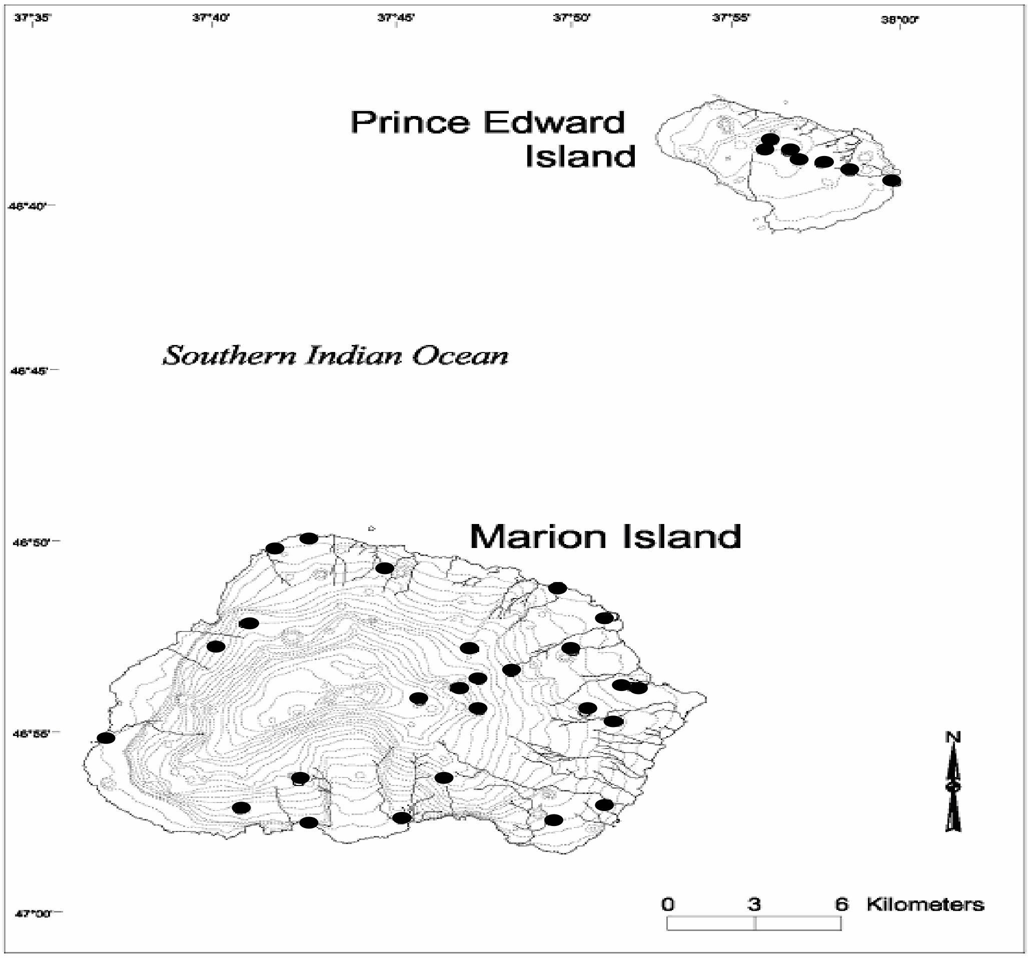


Fig. 1a Collection localities (*filled black circles*) indicated for Marion and Prince Edward Islands

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

(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 ($n \geq 3$). 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).

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).

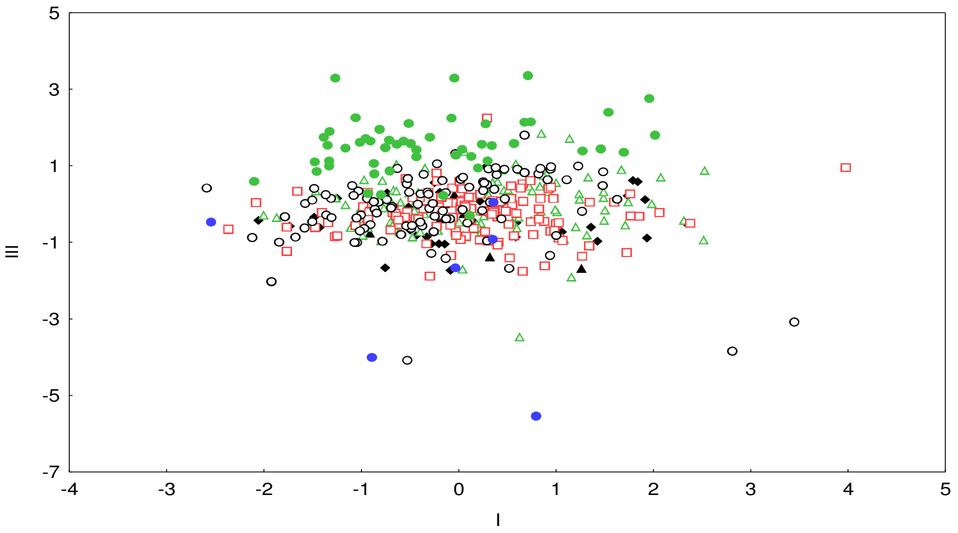


Fig. 2a Components I and III from a principal components analysis (PCA) of *Bothrometopus elongatus* collected on Marion Island during 1975 (*filled blue circle*), 1983 (*filled black triangle*), 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)

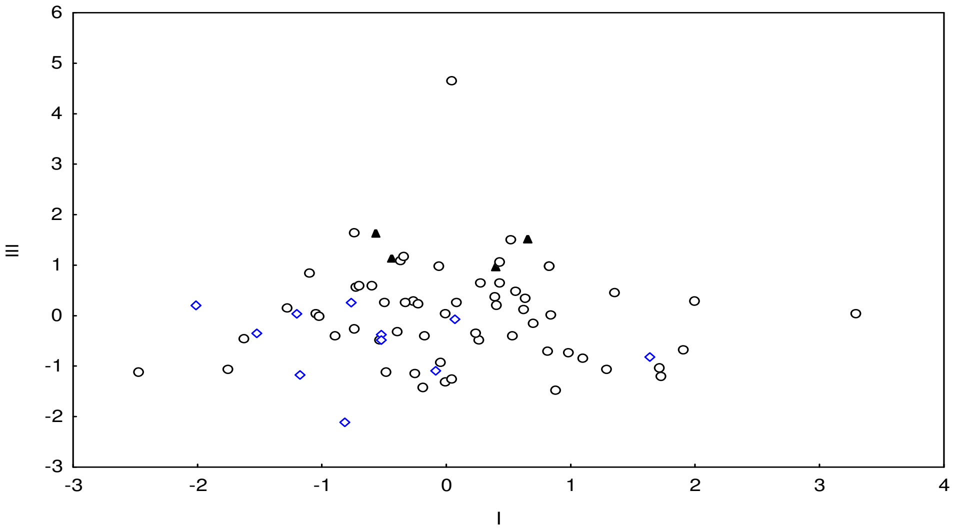


Fig. 2b Components I and III from a principal components analysis (PCA) of *Bothrometopus elongatus* collected on Prince Edward Island during 1983 (*filled black triangle*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

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

	Marion Island			Prince Edward Island		
Variable	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.

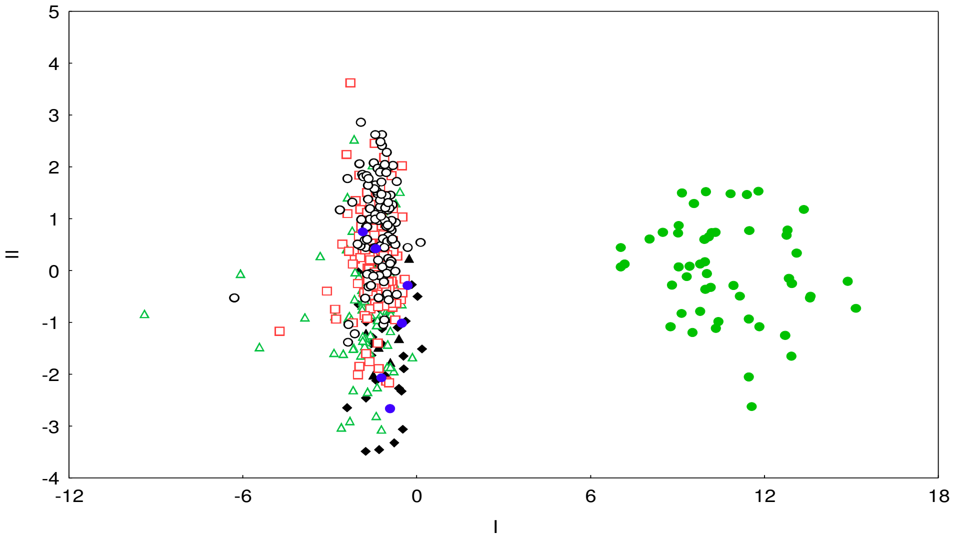


Fig. 2c First two axes from a canonical variates analysis (CVA) of *Bothrometopus elongatus* collected on Marion Island during 1975 (*filled blue circle*), 1983 (*filled black triangle*), 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red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

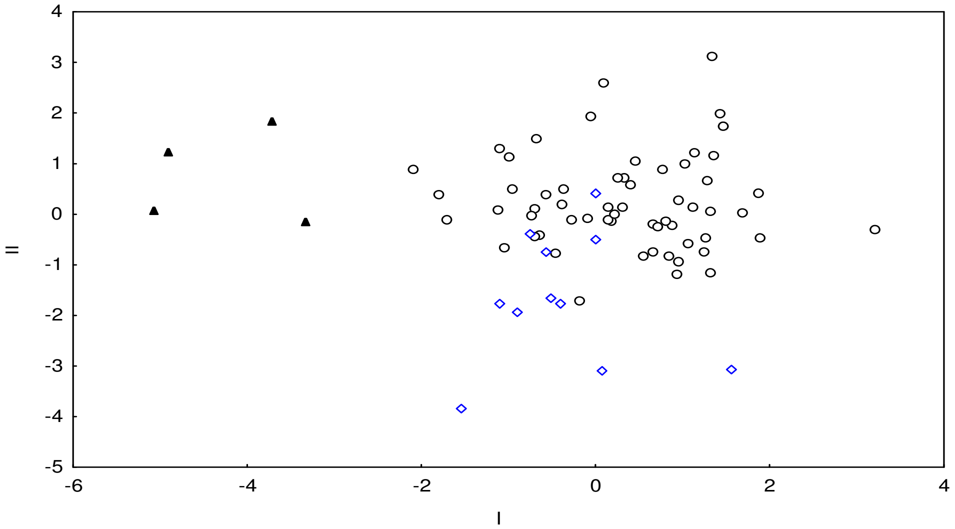


Fig. 2d First two axes from a canonical variates analysis (CVA) of *Bothrometopus elongatus* collected on Prince Edward Island during 1983 (*filled black triangle*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

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

	Marion Island		Prince Edward Island	
Variable	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).

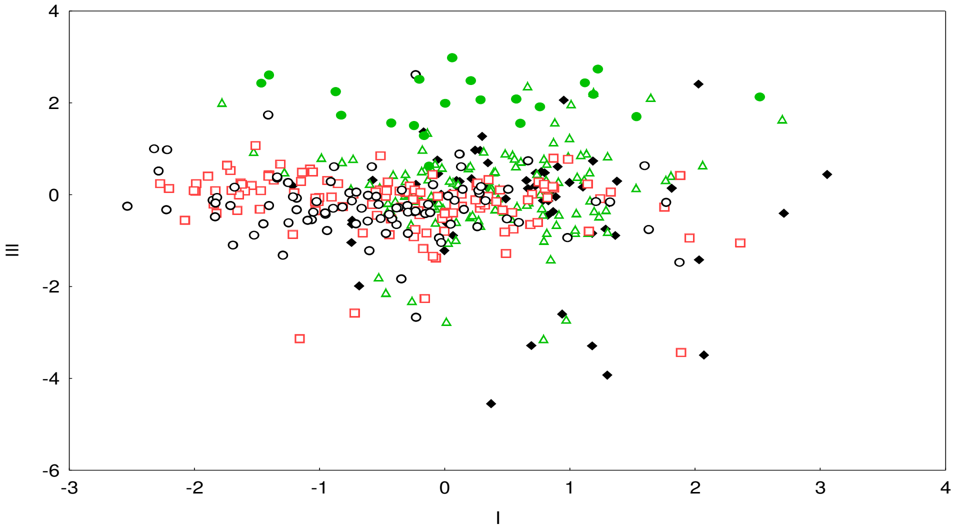


Fig. 3a Components I and III from a principal components analysis (PCA) of *Bothrometopus parvulus* collected on Marion Island during 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)

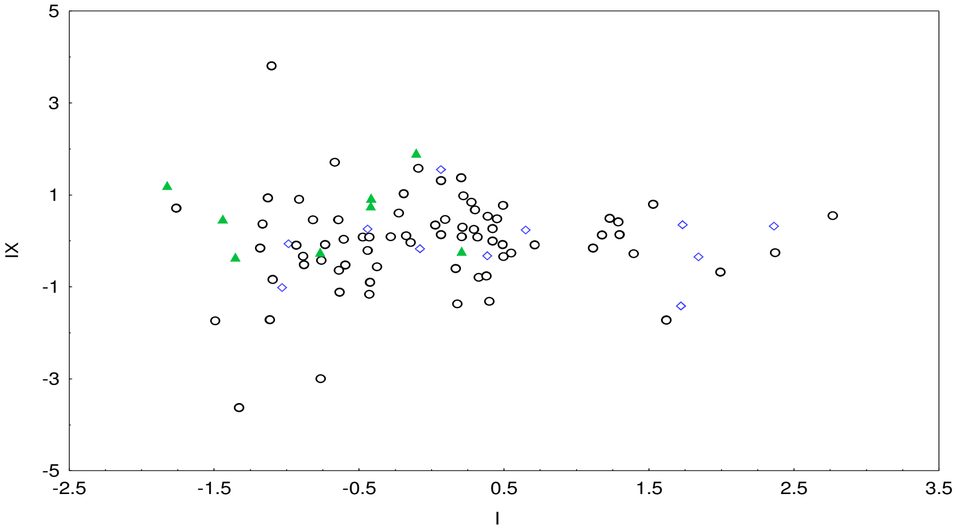


Fig. 3b Components I and IX from a principal components analysis (PCA) of *Bothrometopus parvulus* collected on Prince Edward Island during 1980 (*filled green triangle*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

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.

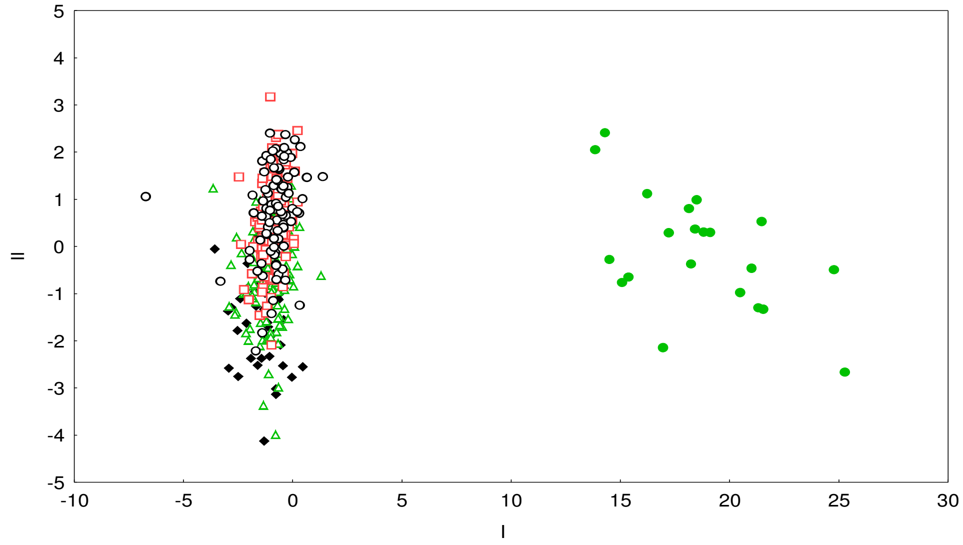


Fig. 3c First two axes from a canonical variates analysis (CVA) of *Bothrometopus parvulus* collected on Marion Island during 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)

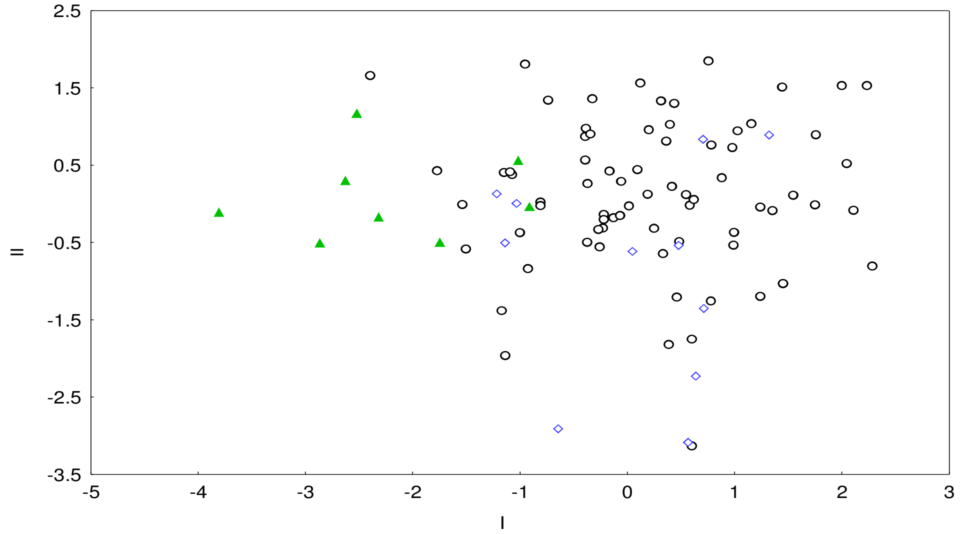


Fig. 3d First two axes from a canonical variates analysis (CVA) of *Bothrometopus parvulus* collected on Prince Edward Island during 1980 (*filled green triangle*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

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).

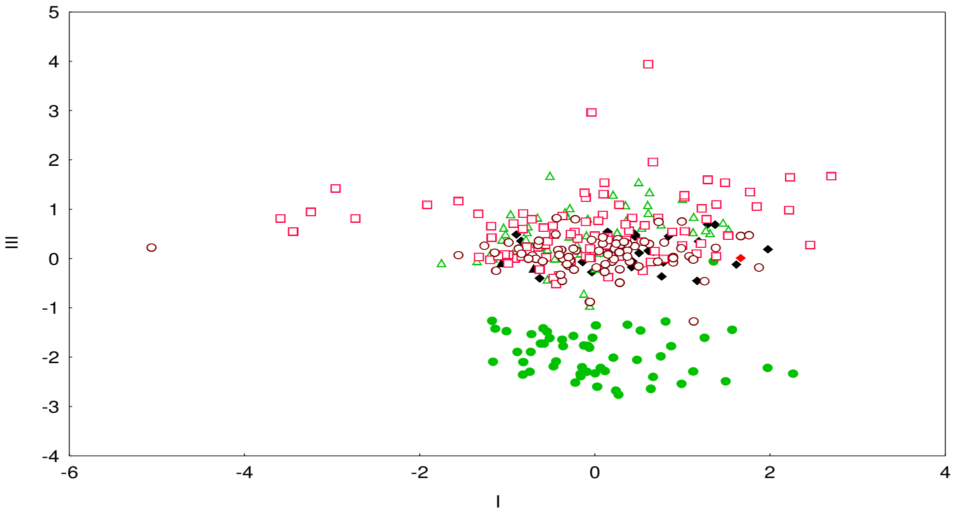


Fig. 4a Components I and III from a principal components analysis (PCA) of *Bothrometopus randi* collected on Marion Island during 1981 (*filled red diamond*), 1983 (*filled black triangle*), 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)

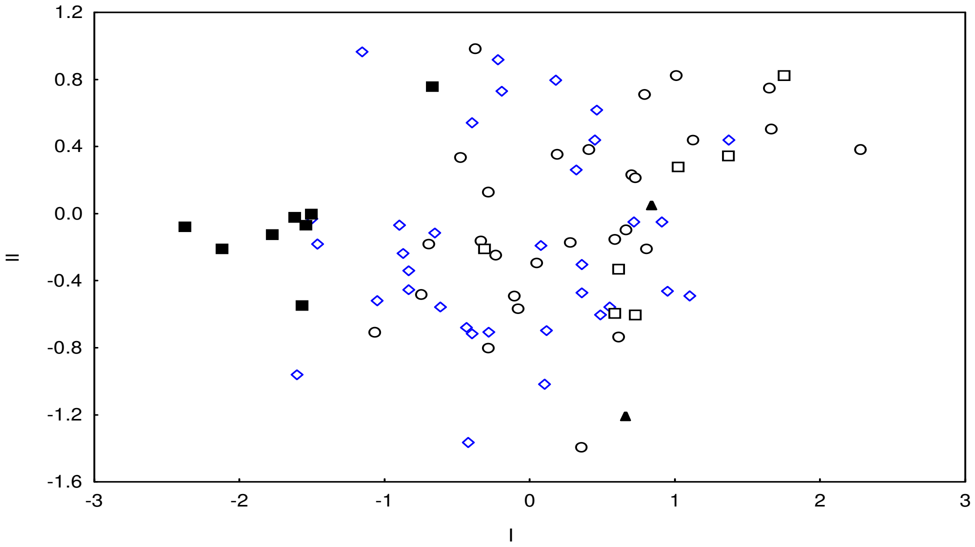


Fig. 4b Components I and II from a principal components analysis (PCA) of *Bothrometopus randi* collected on Prince Edward Island during 1965 (*filled black square*), 1983 (*filled black triangle*), 1984 (*unfilled black square*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

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.

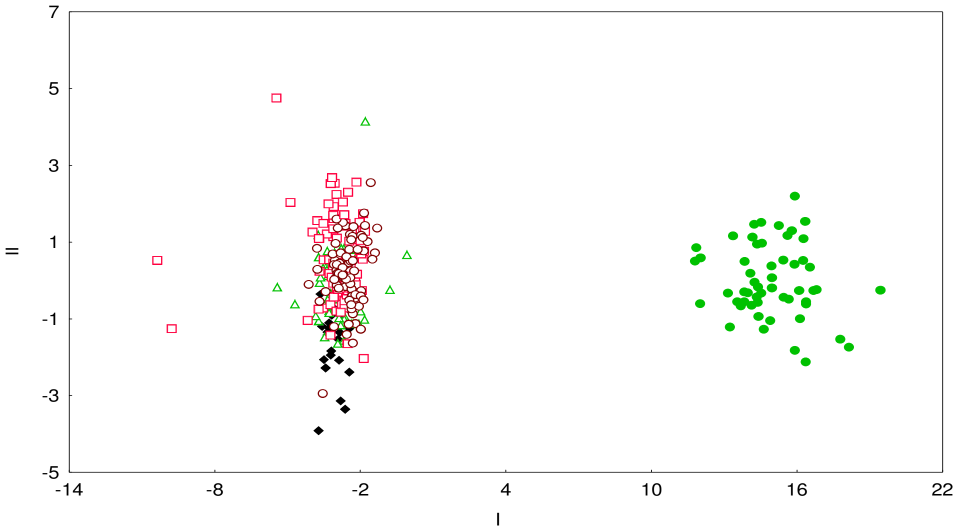


Fig. 4c First two axes from a canonical variates analysis (CVA) of *Bothrometopus randi* collected on Marion Island during 1981 (*filled red diamond*), 1983 (*filled black triangle*), 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*),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

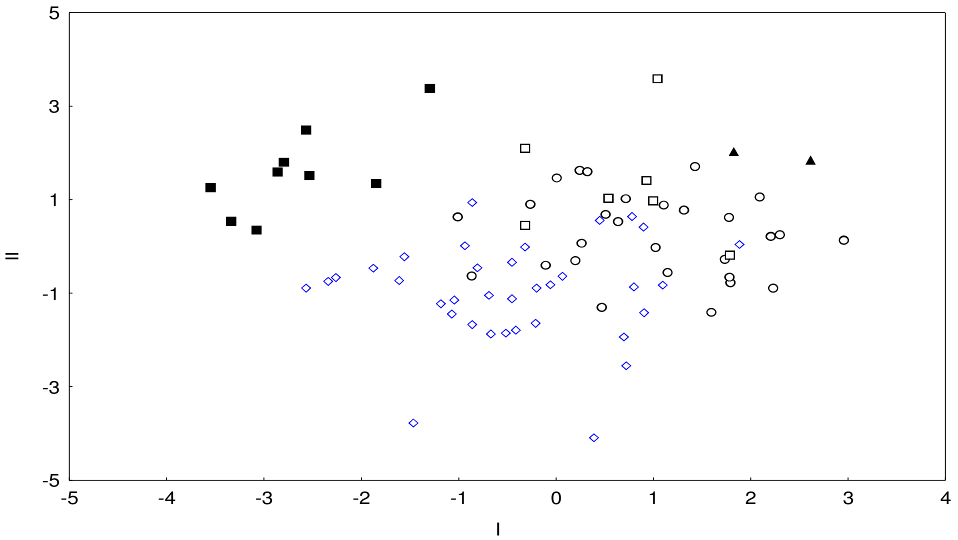


Fig. 4d First two axes from a canonical variates analysis (CVA) of *Bothrometopus randi* collected on Prince Edward Island during 1965 (*filled black square*), 1983 (*filled black triangle*), 1984 (*unfilled black square*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

Table 3b Loadings of variables on canonical variate axes from canonical variates analyses of *Bothrometopus randi* collected on Marion and Prince Edward Islands

	Marion Island		Prince Edward Island	
Variable	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).

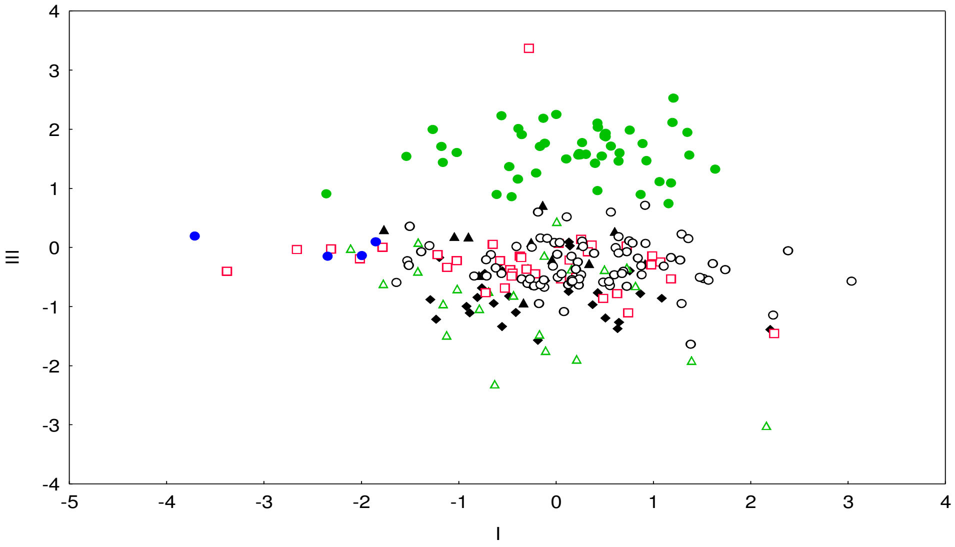


Fig. 5a Components I and III from a principal components analysis (PCA) of *Palirhoeus eatoni* collected on Marion Island during 1975 (*filled blue circle*), 1982 (*filled red square*), 1983 (*filled black triangle*), 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black circle*)

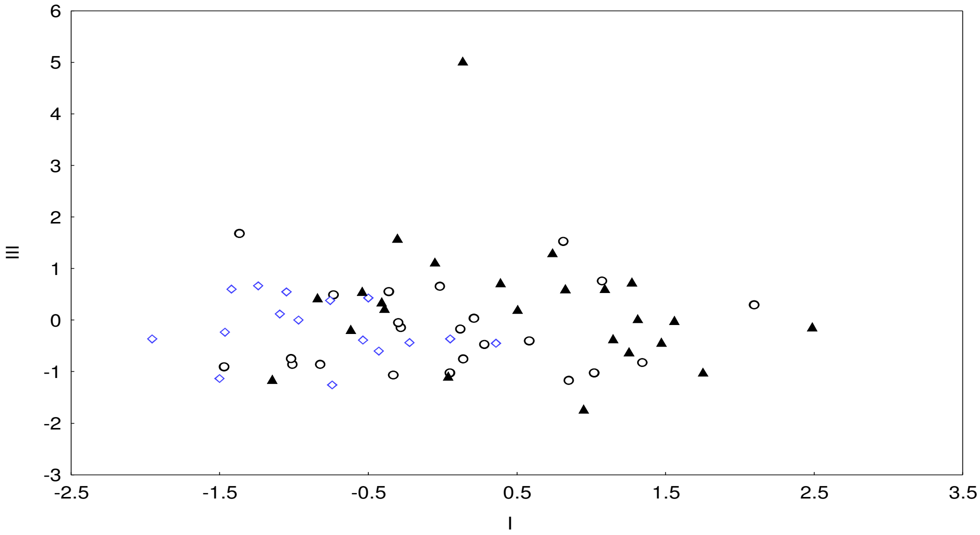


Fig. 5b Components I and III from a principal components analysis (PCA) of *Palirhoeus eatoni* collected on Prince Edward Island 1983 (*filled black triangle*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

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

	Marion Island			Prince Edward Island		
Variable	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.

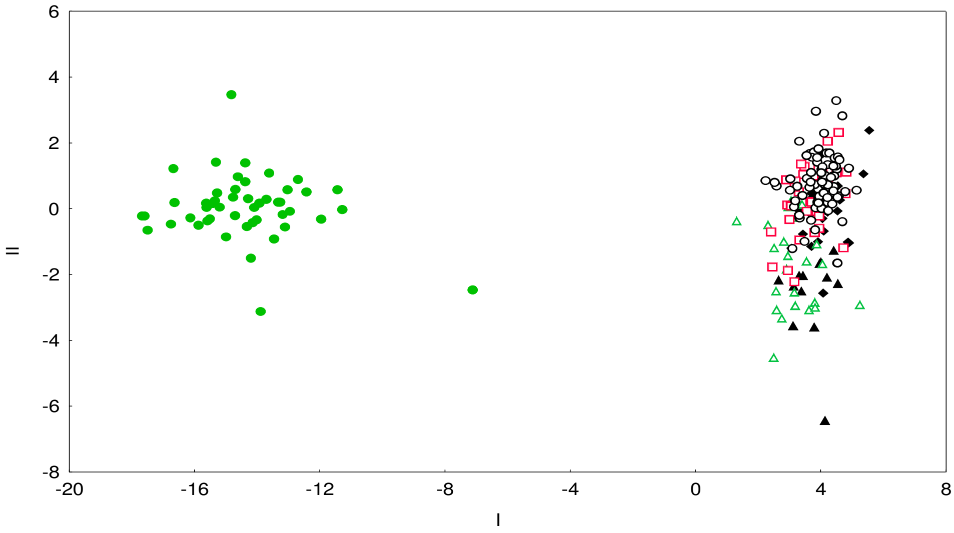


Fig. 5c First two axes from a canonical variates analysis (CVA) of *Palirhoeus eatoni* collected on Marion Island during 1983 (*filled black triangle*), 1986 (*filled green circle*), 1998 (*filled black diamond*), 2001 (*unfilled green triangle*), 2002 (*unfilled red square*), and 2003 (*unfilled black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

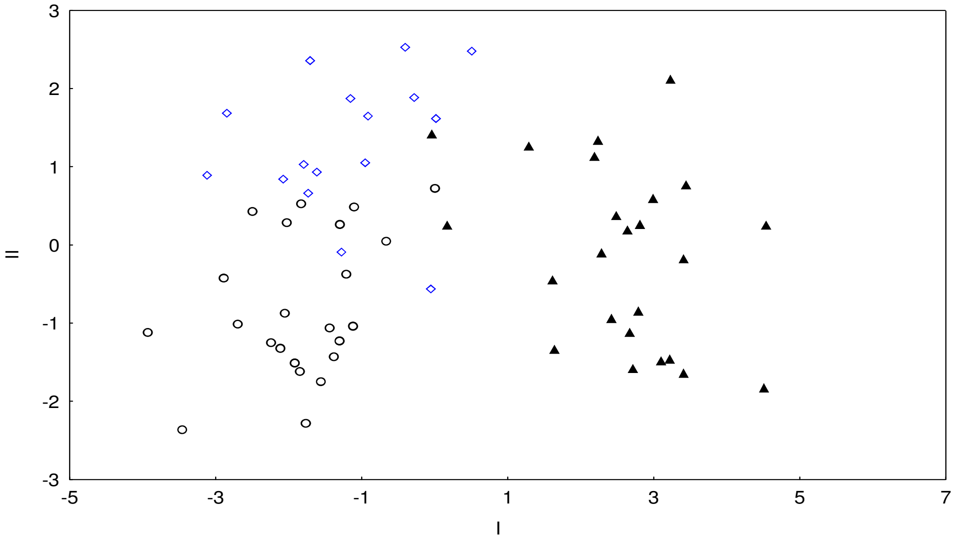


Fig. 5d First two axes from a canonical variates analysis (CVA) of *Palirhoeus eatoni* collected on Prince Edward Island 1983 (*filled black triangle*), 2000 (*unfilled blue diamond*), and 2003 (*unfilled black circle*)

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

	Marion Island		Prince Edward Island	
Variable	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.

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 *n* = 423	*B. parvulus* *n* = 419	*B. randi* *n* = 339	*P. eatoni* *n* = 234	*B. elongatus* *n* = 74	*B. parvulus* *n* = 92	*B. randi* *n* = 73	*P. eatoni* *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 **

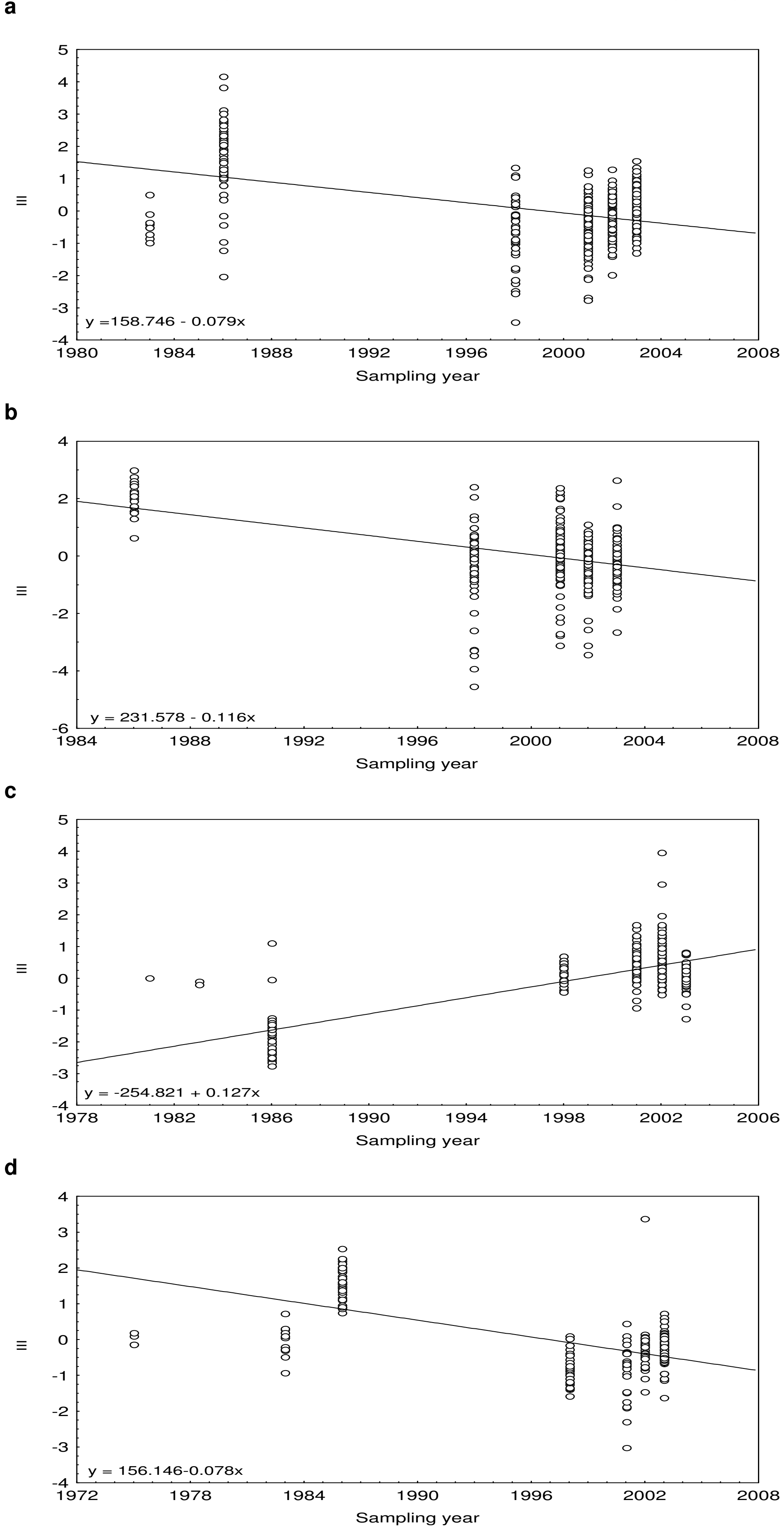
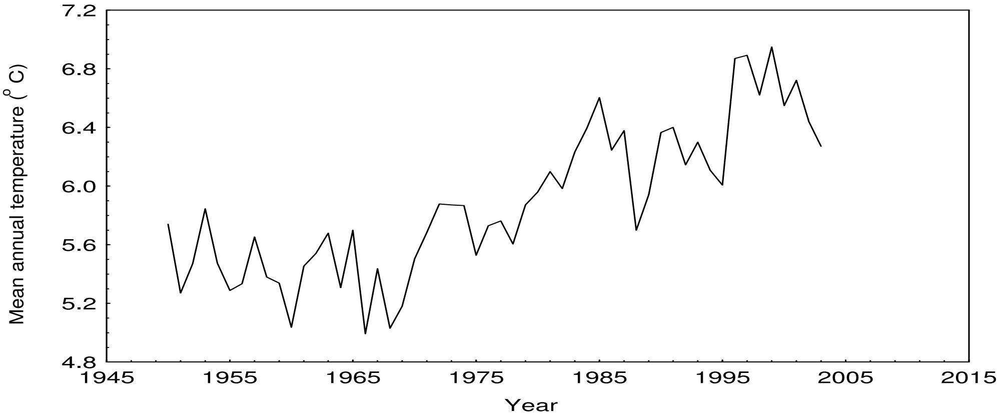


Fig. 6 Relationship between principal component III scores and sampling year for, **a** *Bothrometopus elongatus*, **b** *Bothrometopus parvulus*, **c** *Bothrometopus randi*, and **d** *Palirhoeus eatoni*, collected on Marion Island

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

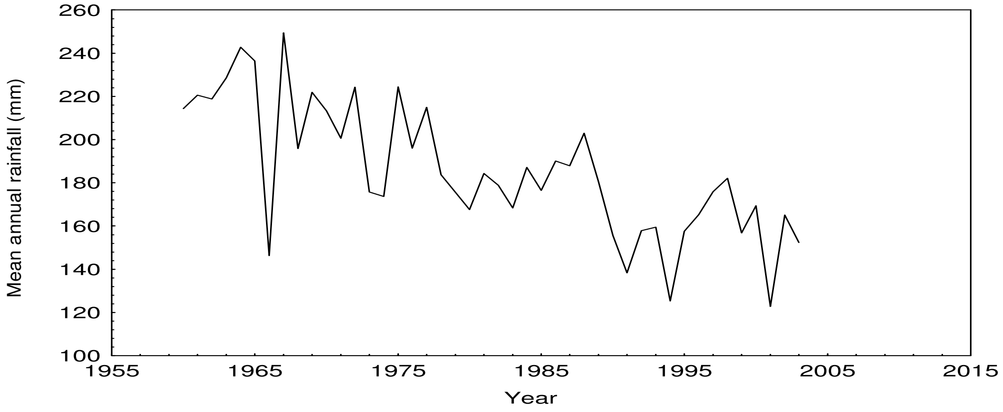


Fig. 7 Mean annual temperatures recorded (ºC) for Marion Island from **a** 1950 up to 2004 and mean annual rainfall measured (mm) for Marion Island from **b** 1960 up to 2004

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

	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
c) Bothrometopus randi	1981	2	Annex Cave	0	6.10	184.37
	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
d) Palirhoeus eatoni	1975	4	-	0	5.53	224.42
	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

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

b) Prince Edward Island

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

Chapter 5

Morphometric changes over time in weevils (Coleoptera: Curculionidae) from the sub-Antarctic Marion Island: An analysis based on mire sediment sub-fossil remains and recent samples

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Abstract Sub-fossil weevil elytra and head capsule remains were recovered in mire sediments from depths ranging between 2.5 m to 2.0 m on the sub-Antarctic Marion Island. The weevil remains from 2.5 m and 2.0 m were carbon-dated to be from 2331 BC and 789 BC, respectively, representing a period considered to pre-date the potential effects of global warming from the green-house effect and predation by house mice (*Mus musculus*, sensu lato) that were introduced on Marion Island in the early 1800s. Head capsules, being the best preserved sub-fossil components, formed the basis of the analyses in this study. Three head capsule measurements were used to: 1) distinguish between the six currently recognised weevil species on Marion Island; 2) identify sub-fossil material; and 3) assess morphometric changes over time. Based on the head capsule data, sub-fossil weevil remains were identified as belonging to the genus *Ectemnorhinus* and were compared to recent samples collected in 1986/87 and 2001 from *Blepharidophyllum* mire communities. The analyses showed no statistically significant morphometric size and shape changes in sub-fossil material examined. However, head capsule measurements exhibited significant differences between sub-fossil material collected at different depths (2.0 – 2.5 m) and recently collected material (0 m), spanning a period of 4332 years. In addition, the overall size-related principal components (PCA) I and a single head capsule measurement decreased significantly from 2331 BC to the present (1986/87 and 2001). These results are interpreted with reference to the potential effect of climate change and size-selective predation by the house mouse.

Key words: Weevils, sub-fossils, morphometric changes over time, climate change, mouse predation, sub-Antarctic Marion Island

Running title: Morphometric changes over time in sub-fossil weevil remains and recent samples from sub-Antarctic Marion Island

Introduction

Terrestrial environments are facing significant threats due to climate change, invasive species and habitat fragmentation (Dukes and Mooney 1999; Mack et al. 2000; Luck et al. 2004). For example, biological invasions constitute one of the most important conservation threats to the functioning of ecologically sensitive ecosystems, such as the isolated sub-Antarctic South Indian Ocean Province Islands (Bonner 1984; Chown et al. 1998; Bergstrom and Chown 1999). In addition, various studies have demonstrated that species are already exhibiting changes in population densities, distributional ranges and morphology as a result of global climate change (for examples, see Lawton 1995; Parmesan 1996; Kuchlein and Ellis 1997; Parmesan et al. 1999; Hughes 2000; Huyser et al. 2000).

These include studies that have alluded to potential anthropogenic factors that may cause changes in weevil morphology on the sub-Antarctic Marion and Prince Edward Islands that in turn, affect their ecosystem functioning (Smith 1987; Chown and Smith 1993; Smith and Steenkamp 1993; Smith et al. 2002; van Aarde et al. 2004). The suggested anthropogenic factors include the potential effect of climate change from the green-house effect and predation by mice that were introduced on Marion Island in the early 1800s, but not on the neighbouring Prince Edward Island that is located 22 km to the north-east of Marion Island (Watkins and Cooper 1986).

This led to the investigation of the potential impact of climate change and mice predation on two mouse-preferred weevil prey species, *Ectemnorhinus marioni* and *E. similis* from *Azorella selago*-dominated habitats on both Marion and the mouse-free Prince Edward Islands (Chown and Smith 1993). The results of this study suggest a tendency towards a reduced body length in samples collected over a six-year period between 1986 and 1992 (Chown and Smith 1993). Chown and Smith (1993) and subsequently, Smith et al. (2002) suggested that this body size reduction in *Ectemnorhinus* species may be attributed to size-selective predation of these mouse-preferred prey species. These studies reported that mice prefer large-sized weevils to such an extent that mouse-predation pressure may be responsible for changes in mean body length and size-class distributions in *Ectemnorhinus* weevil prey species (Chown and Smith 1993; Smith et al. 2002).

Mouse populations on Marion Island have been reported to be temperature-dependent, such that increased island temperature may escalate mouse populations (Smith and Steenkamp 1990; Smith 2002), which in turn would lead to a decrease in weevil populations via predation (Crafford and Scholtz 1987; Chown and Smith 1993). Decreased weevil populations will in turn lead to a decrease in overall rates of nutrient cycling, leading to imbalances between primary production and decomposition (Smith and Steenkamp 1990). It has been documented that mice numbers have increased since 1979 (Matthewson et al. 1994; van Aarde et al. 1996) and the contribution of weevil adults to mice diet have also increased considerably (Smith et al. 2002), posing a significant threat to weevil species, more especially *E. similis*, *E. marioni*, *Bothrometopus randi* and *B. parvulus* (Chown and Smith 1993).

However, other studies suggest that the effect of ameliorating temperatures on Marion and Prince Edward Islands as a result of global warming may have a significant impact on weevil morphometric size and shape (see Chapter 4). The two islands offer ideal ecosystems to study the responses of weevil species to changing global climate and/or biological invasions, through morphometric size and shape changes. The two islands, separated by 22 km and assuming similar temperature increases on both islands, and that only Marion Island is colonised by mice (Watkins and Cooper 1986; Crafford and Scholtz 1987), excludes mice predation as a potential influential factor on morphometric changes on Prince Edward Island weevil species. Similar morphometric size and shape changes on other weevil species (*B. randi*, *B. parvulus*, *B. elongatus* and *Palirhoeus eatoni*) on both Marion Island and Prince Edward Island, suggest that climate change may play a primary role in weevil morphological changes over time (see Chapter 4).

Over the last 50 years, mean surface air temperature on Marion Island has increased by 0.025°C year^{-1} while precipitation decreased (Smith and Steenkamp 1990; Smith 1991, 2002; Weimerskirch et al. 2003). As a result, Marion Island's ecosystem functioning is in the process of changing, with elevated temperatures expected to increase productivity as well as nutrient demand (Smith 1988; Smith 2002). Energy flow and nutrient cycling are mainly dependent on soil macro-invertebrates (Crafford 1990; Smith and Steenkamp 1992). The activities of these organisms are strongly temperature-dependent (Chown et al. 1997; Klok and Chown 1997) and the effect of temperature on insect body size, with increasing temperature may promote smaller-sized individuals to be produced (Atkinson 1994; James et al. 1997; Karan et al. 1998). While these potential influences may be valid, the partitioning of potential causal effects of morphological change over time has been complicated further by the problematic taxonomic status of the two *Ectemnorhinus* species that have traditionally been considered to occur on both islands. An attempt was made to resolve this taxonomic problem using parallel and complementary molecular and morphometric techniques. In contrast to the previous taxonomy, both molecular and morphometric analyses suggest the presence of two genetically distinct species on Prince Edward Island, but only a single species that comprises diverse genetically discrete populations on Marion Island (see Chapter 3). This has led to the *Ectemnorhinus* species occurring on Marion Island to be referred to as an *Ectemnorhinus* species complex.

In the present study, the identity of sub-fossil remains that were recently sampled from mire habitats on Marion Island are first determined with reference to the current insight into the taxonomy of weevil species occurring on both Marion and Prince Edward Islands. These sub-fossil weevil remains, sampled from different depths (2.0 – 2.5 m) considered to represent different dimensions in time and space, together with recently collected *Ectemnorhinus* samples from mire habitats on Marion Island, are in turn used to assess if morphometric changes have occurred in these weevils over time.

By so doing, the hypothesis is that should sub-fossil weevil remains represent the same currently recognised species in mire habitats, then sub-fossil remains from the two sampled depths should not show significant morphological changes in remains from this period that pre-dates the potential effect of mouse-predation and/or global warming. The prediction is that sub-fossil remains from Marion Island should differ morphologically from material collected recently from a period characterised by the potential effect of mouse-predation and/or global warming.

Materials and methods

Study area and samples

Two core samples (50 cm in length) were collected in April 2002 from a mire community, mire samples A (46˚52.730'S; 37˚50.916'E) and B (46˚52.676'S; 37˚51.078'E) on Marion Island using a 7 cm x 50 cm Russian corer. Each core was 2.5 m deep.

Twenty-five 2 cm pieces were recovered from each core sample at depths between 2.0 and 2.5 m, separately washed and sieved to remove excess organic matter, fine-grained mud and stones. Weevil body parts were removed and stored in 100% ethanol. A total of 41 and 51 elytra were recovered from mire samples A and B, respectively. However, the recovered elytra could not be used for morphometric analyses because of their crumpled and soft nature. Thirty-two and 31 weevil head capsules were recovered from mire samples A and B, respectively. Due to damage, a total of 57 sub-fossil head capsules were ultimately measured from both mire samples A and B.

Currently recognised *Ectemnorhinus* weevil species were collected by hand (L. Janse van Rensburg) from *Blepharidophyllum densifolium* (Hook.) Angstr. (Scapaniaceae) mires on Marion Island during 2001. A total of 34 individuals were sampled, stored in absolute ethanol, and head measurements recorded. Additional *Ectemnorhinus* specimens (n = 33) collected from *Blepharidophyllum densifolium* (Hook.) Angstr. (Scapaniaceae) mires on Marion Island during 1986/87 (S.L. Chown) were included in the analyses to assess morphometric changes over time.

Morphometric measurements

Head capsule measurements were recorded to the nearest 0.01 mm (O, A and AD). All measurements were recorded by a single observer (L. J. van Rensburg) using a stereo microscope fitted with a calibrated eye-piece micrometer. These variables defined and illustrated by Janse van Rensburg et al. (2003) include: inter-ocular distance (O), inter-antennal distance (A) and antenna/eye diagonal (AD).

Carbon-dating

The age of weevil elytra sampled in the mires were determined through the services of Quadru – Environmentek, Centre for Industrial Research (CSIR), Pretoria, South Africa in collaboration with the radio-carbon station at Groningen University, Netherlands, Salisbury and Isotopes Inc., USA. Analysis included AMS Date based on 5 mg samples, and was conducted in collaboration with CIO, Groningen, Netherlands. Dating was based on years Before Present (BP) (i.e., before AD 1950) in conventional radio-carbon years based on a half-life of 5568 years for C^{14}. Ageing was corrected for variations in isotope fractionation, and was calibrated for the southern hemisphere using a 2000 up-dated version of the PRETORIA computer programme (Talma and Vogel 1993).

Statistical analyses

Delineation of currently recognised weevil species from Marion Island based on head capsule measurements

Janse van Rensburg et al. (2003) showed that a selected set of 15 measurements is sufficient to distinguish between recent samples of currently recognised weevil species from Marion Island. In the present study, however, only three measurements could be recorded on weevil head capsules collected in core samples. Consequently, the original morphometric dataset used by Janse van Rensburg et al. (2003) was re-analysed using the three recordable head capsule measurements to assess if they were sufficient to distinguish recent samples of all currently recognised weevil species from Marion Island.

The three head capsule measurements were subjected to the same series of multivariate analyses used by Janse van Rensburg et al. (2003). These analyses included: 1) principal components analysis (PCA) based on among-character product-moment correlation coefficients; 2) unweighted pair-group arithmetic average (UPGMA) cluster analysis computed from both, among-operational taxonomic units (OTUs; Sneath and Sokal 1973) euclidean distances and product-moment correlation coefficients (Pimentel and Smith 1986; Rohlf 1986); and 3) canonical variates (discriminant) analysis (CVA; Sneath and Sokal 1973) based on data from years with adequate sample sizes followed by a multivariate analysis of variance (MANOVA; Zar 1996) to test for statistically significant differences between groups.

Identification of sub-fossil samples from Marion Island

The identification of sub-fossil material was based on both PCA and UPGMA cluster analysis using the three head capsule measurements. These analyses, however, included a subset of the data from the original dataset used by Janse van Rensburg et al. (2003) to distinguish recent samples of all currently recognised weevil species from Marion Island, but only focussed on the three head capsule variables.

Analysis of morphometric changes over time

The multivariate PCA, UPGMA cluster analysis and CVA based on the three head capsule measurements were used to assess morphometric size and shape changes over time using both core samples and recently collected mire material (1986/87 and 2001). Kruskal-Wallis analysis of variance (ANOVA; Zar 1996) was used to assess morphometric changes over time using the three head capsule measurements and the overall size-related PCA I independently across all depths and was based only on core depth samples with $n \geq 3$. In addition, a dataset consisting only of the four known sampling times (2331 BC; 789 BC; 1986/87 and 2001) were subjected to Kruskal-Wallis analysis of variance (ANOVA; Zar 1996). Dunn's post hoc analyses (Zar 1996) were used to determine significant differences between sampling years. Mean ± Standard Error (SE) at each depth as well as percentage head capsules recovered in all depths were calculated for each of the three head capsule measurements independently. Patterns of variation were also evaluated by Spearman's rank correlation analysis (Zar 1996) of PCA I scores and individual head capsule measurements (O, A and AD) with depth, representing time.

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

Results

Carbon-dating

Since core samples were collected in similar mire communities, only elytra sampled in mire sample A were used for carbon-dating. Carbon-dating results are presented in Table 1 as 1-sigma range, together with the most probable date (in parentheses). These results indicate that the sediment sequence of core A (2.0 – 2.5 m) spans 1542 years, with elytra sampled at 2.0 m being from 789 years BC, and elytra sampled at 2.5 m being from 2331 years BC (Fig. 1).

Table 1 Radiocarbon analysis results given in years Before Present (BP), i.e. before AD1950. Indicated in superscript are: [1]Laboratory analysis number; [2]Sample number; [3]Dates reported in conventional radiocarbon years (i.e., using a half-life of 5568 years for C^{14} and ages corrected for variations in isotope fractionation); and [4] 1-sigma range, with the most probable date between parentheses

Anal.[1] No. GrA-	Sample [2] designation	$\delta^{13}C$ (‰PDB)	Radiocarbon [3] Age yrs BP	Calibrated [4] Date
24396	A 1:1 (2.0 m)	-25.0	2610 ± 45	801 (789) 774 BC
24394	A 25:2 (2.5 m)	-21.4	3910 ± 40	2447 (2331) 2287 BC

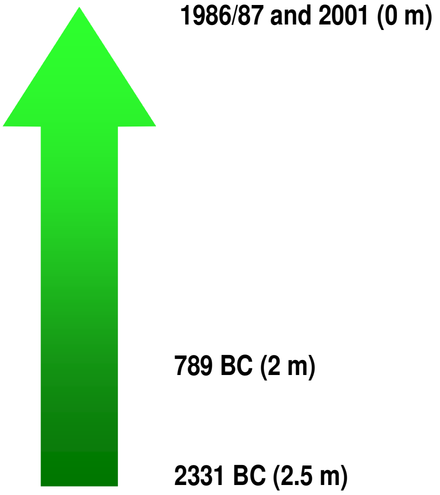

Fig. 1 Sampled mire depths (m) with respective times indicated

Delineation of currently recognised weevil species from Marion Island based on head capsule measurements

The results of both PCA and UPGMA cluster analysis undertaken to assess if the three recordable head capsule measurements were sufficient to distinguish recent samples of currently recognised weevil species, were broadly similar and are best exemplified by the results of the former series of analyses. Separation of all six species, based on three head capsule measurements are best illustrated by the first (92.02% variance) and second (6.33% variance) PCA axes (Fig. 2a; Table 2a). On the first PCA axis *B. elongatus*, *P. eatoni* and *B. randi* are separated, in addition to *E. marioni* and *B. parvulus* separating from *E. similis* (Fig. 2a). All three head measurements showed positive, high loadings on the first PCA axis, with measurement O contributing the most to the separation (Table 2a). Although most species show overlap, the PCA in the present study closely resembles the original PCAs in Janse van Rensburg et al. 2003 (Chapter 2; see Fig 4a-c). Therefore, despite the partial overlaps, these results suggest that the three head capsule measurements may be used to distinguish between the six currently recognised weevil species from Marion Island, particularly when interpreted with reference to the results of the previous analyses by Janse van Rensburg et al. (2003) based on 15 measurements.

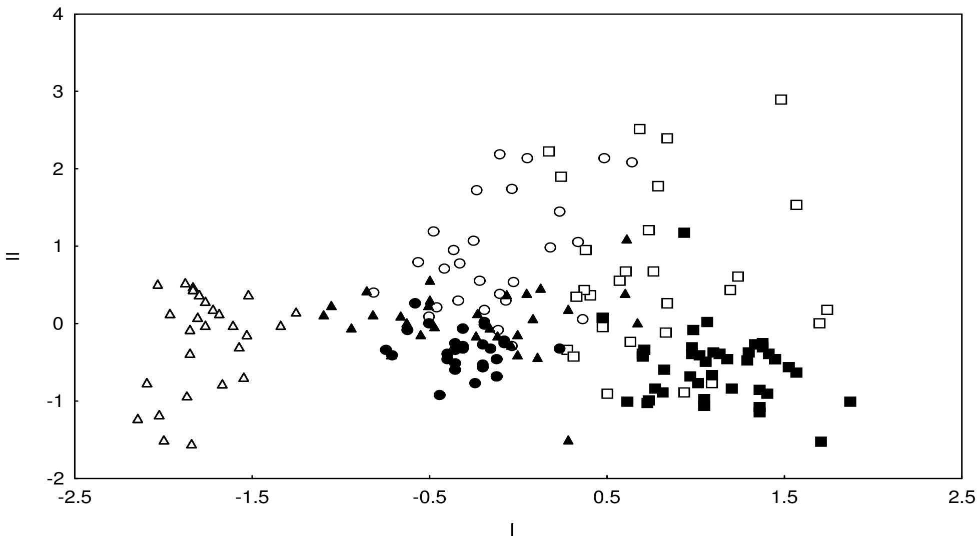

Fig. 2a The first two components from a principal components analysis (PCA) using three head capsule measurements of *Palirhoeus eatoni* (*unfilled circle*), *Ectemnorhinus marioni* (*filled circle*), *E. similis* (*filled square*), *Bothrometopus randi* (*unfilled square*), *B. parvulus* (*filled triangle*), and *B. elongatus* (*unfilled triangle*) from sub-Antarctic Marion Island

Table 2 Loadings of variables on components I, II and III from principal components analyses (PCA) of head capsule measurements to a) distinguish between six currently recognised weevil species on Marion Island using data from Janse van Rensburg et al. (2003) and b) of a combination or the six currently recognised weevil species together with mire core samples from Marion Island

	Measurement	PCA I	PCA II	PCA III
a)	O	0.975	-0.155	-0.162
	A	0.933	0.360	0.011
	AD	0.970	-0.191	0.152
	% trace	92.02	6.33	1.65
b)	O	0.971	-0.185	-0.153
	A	0.922	0.386	-0.001
	AD	0.971	-0.182	0.154
	% trace	91.22	7.21	1.58

Identification of sub-fossil samples from Marion Island

The results of both PCA and UPGMA cluster analysis based on three head capsule variables, undertaken to identify sub-fossil material in the mire core with reference to recent samples of currently recognised weevil species, were broadly similar and are best exemplified by the PCA results. The sub-fossil weevil head capsules sampled in the mire core cluster with the currently recognised *E. similis* and *E. marioni* (Fig 2b). PCA axis I accounted for 91.22% of the total variance with PCA axis II contributing 7.21% of the total variation (Table 2b). High loadings are exhibited by all measurements, with O and AD equally contributing to the separation (Table 2b).

However, both molecular and morphometric analyses suggest the presence of a single *Ectemnorhinus* species that comprises diverse genetically discrete populations on Marion Island (see Chapter 3). Considering that the *Ectemnorhinus* species occurring on Marion Island has been referred to as an *Ectemnorhinus* species complex, these results suggest that the sub-fossil head capsules sampled on Marion Island may belong to the genus *Ectemnorhinus*.

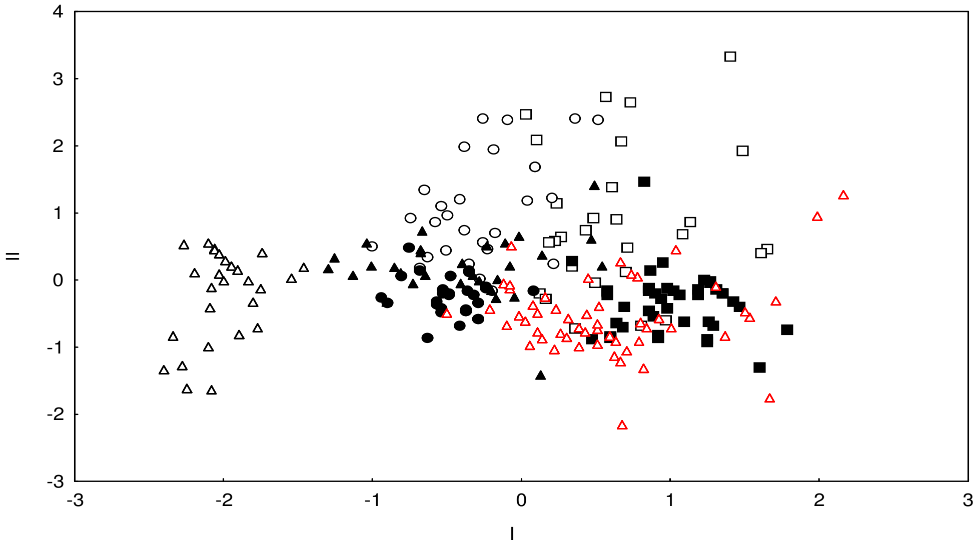

Fig. 2b The first two components from a principal components analysis (PCA) using three head capsule measurements of *Palirhoeus eatoni* (*unfilled circle*), *Ectemnorhinus marioni* (*filled circle*), *E. similis* (*filled square*), *Bothrometopus randi* (*unfilled square*), *B. parvulus* (*filled triangle*), *B. elongatus* (*unfilled triangle*), and sub-fossil mire head capsules (*red triangle*) from sub-Antarctic Marion Island

Analysis of morphometric changes over time

The results of both PCA and UPGMA cluster analysis based on three head capsule variables undertaken to simultaneously assess morphometric size and shape changes over time using both core samples and recently collected mire material were broadly similar and are best exemplified by the former series of analyses. There is no morphological size and shape separation between sub-fossil weevil head capsules and *Ectemnorhinus* species collected in *Blepharidophyllum* mires in 1986/87 and 2001 (Fig. 3a), suggesting no morphological changes over time. Similarly, there is no morphological size and shape separation between sub-fossil material collected at different depths that are considered to represent different dimensions in time and space (results not illustrated).

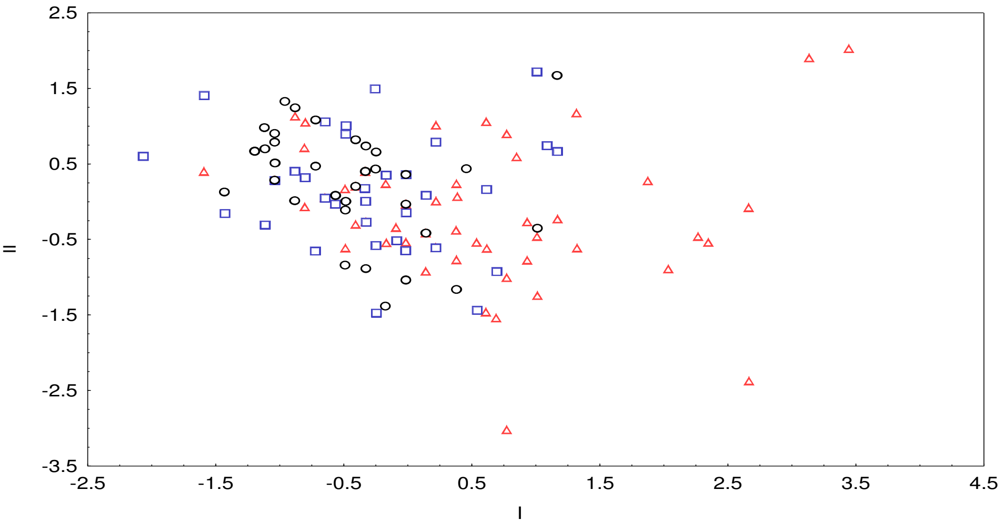

Fig. 3a The first two components from a principal components analysis (PCA) using three head capsule measurements of *Ectemnorhinus* weevil species collected in *Blepharidophyllum* mire communities in 1986/87 (black circles), 2001 (*blue square*), and sub-fossil mire head capsules (*red triangle*) from sub-Antarctic Marion Island

Data that included *Blepharidophyllum* mires in 1986/87, 2001 and carbon-dated sub-fossil core samples, was further analysed using canonical variates analysis (CVA). Ideally, head measurements from both the carbon-dated core samples (2.5 m; 2331 BC and 2.0 m; 789 BC) were considered for analyses. However, sample size from the 2.0 m depth were too small for CVA, thereby only 2.5 m (2331 BC) together with 1986/87 and 2001 samples were analysed using CVA (Fig. 3b). In contrast to the PCA, the 2331 BC sample separates from the recently collected material on axis I (MANOVA: $F_{6,132} = 3.48$; $n = 71$; $P < 0.01$) (Fig. 3b), with O contributing to most of the differences (Table 3).

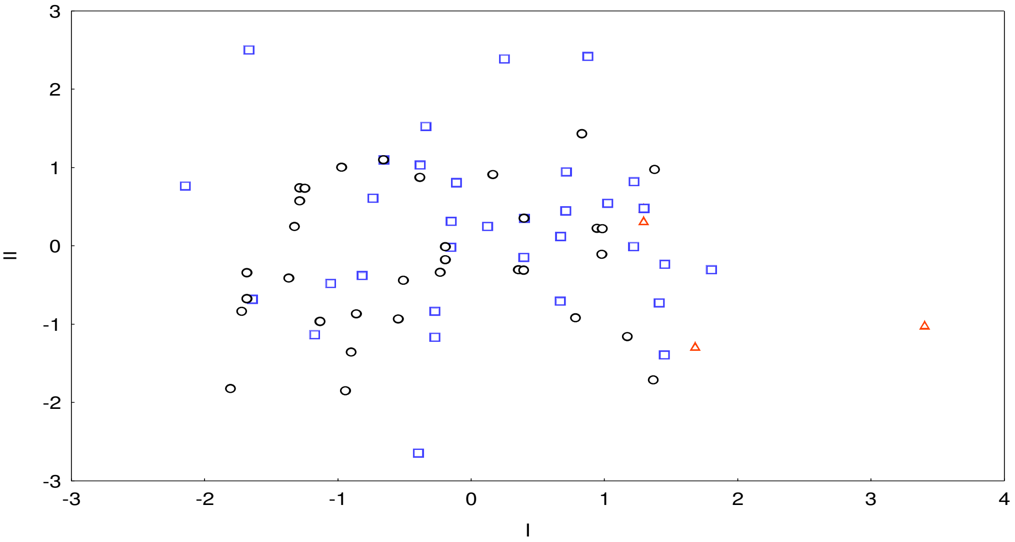

Fig. 3b The first two components from a canonical variates analyses (CVA) using three head capsule measurements of *Ectemnorhinus* weevil species collected in *Blepharidophyllum* mire communities in 1986/87 (black circles), 2001 (*blue square*), and 2331 BC (*red triangle*) from sub-Antarctic Marion Island

Table 3 Loadings of variables on canonical variate axes I and II from a canonical variates analysis (CVA) of head capsule measurements of weevil samples collected in *Blepharidophyllum* mire communities in 1986/87, 2001 and 2331 BC from sub-Antarctic Marion Island

Measurement	CVA I	CVA II
O	1.103	0.924
A	0.002	0.478
AD	-0.143	-1.715

Descriptive statistics (mean ± SE) showed a tendency in all three head measurements (O, A and AD), for samples pre-dating the effects of global warming and the introduction of mice on Marion Island (2.5 m; 2331 BC and 2.0 m; 789 BC), to have larger head capsule measurements than recent samples (1986/87 and 2001) collected at 0 m (Table 4). The complete dataset (n = 110), with different depths representative of different times (i.e., 2.0 m up to 2.5 m representing a time period of 1542 years and 0 m (1986/86 and 2001 representing present time), indicated significant differences for measurement O ($H_{10,115} = 24.71$; $P < 0.01$), A ($H_{10,115} = 17.96$; $P < 0.05$) and AD ($H_{10,115} = 24.29$; $P < 0.01$) between recent and sub-fossil data.

In addition, a dataset consisting only of known times (i.e., 2331 BC: 2.5 m; 789 BC: 2.0 m; 1986/87: 0 m; and 2001: 0 m), showed only measurement O ($H_{4,72} = 13.25$; $P < 0.01$; Dunn's post hoc test: between 2331 BC and 1986/87) and PCA I ($H_{4,72} = 9.54$; $P < 0.05$; Dunn's post hoc test: between 2331 BC and 2001), to be significantly different over time, whereas measurement A ($H_{4,72} = 5.28$; $P = 0.15$) and AD ($H_{4,72} = 7.44$; $P = 0.06$) showed no statistically significant differences between known sampling years. The largest number of weevil head capsules was sampled between 2.14 m and 2.44 m (Table 4). This may be indicative of a higher quality of preserved material at deeper strata within core samples.

Table 4 Mean ± Standard Error (SE) values for inter-antennal distance (A), inter-ocular distance (O) and antenna/eye diagonal (AD) for 57 sub-fossil head capsules sampled in mire communities (Mire samples A and B combined). Percentage heads sampled at different depths are indicated. Head measurements for individuals collected in 1986/87 and 2001 in *Blepharidophyllum* mires are included

Piece number	Depth (m)	Mean O (mm) ± SE	Mean A (mm) ± SE	Mean AD (mm) ± SE	*n*	% occurrence
Blepharidophyllum mire (2001)	0	0.49 ± 0.01	0.32 ± 0.01	0.47 ± 0.01	34	
Blepharidophyllum mire (1986/87)	0	0.47 ± 0.00	0.31 ± 0.00	0.47 ± 0.00	33	
1	2.00-2.02	0.55 ± 0.09	0.41 ± 0.15	0.56 ± 0.13	2	3.51
2	2.02	0.52 ± 0.02	0.38 ± 0.00	0.47 ± 0.02	3	5.26
5	2.08	0.67 ± -	0.38 ± -	0.58 ± -	1	1.75
6	2.1	0.61 ± -	0.29 ± -	0.60 ± -	1	1.75
7	2.12	0.53 ± 0.01	0.35 ± 0.02	0.48 ± 0.02	5	8.77
8	2.14	0.51 ± 0.01	0.33 ± 0.00	0.47 ± 0.01	7	12.28
9	2.16	0.48 ± 0.03	0.33 ± 0.00	0.45 ± 0.05	2	3.51
10	2.18	0.54 ± 0.01	0.36 ± 0.04	0.52 ± 0.01	3	5.26
11	2.2	0.51 ± 0.02	0.33 ± 0.01	0.51 ± 0.02	6	10.53
13	2.24	0.50 ± -	0.31 ± -	0.48 ± -	1	1.75
14	2.26	0.51± -	0.33 ± -	0.49 ± -	1	1.75
15	2.28	0.56 ± -	0.36 ± -	0.52 ± -	1	1.75
16	2.3	0.50 ± -	0.32 ± -	0.52 ± -	1	1.75
19	2.36	0.49 ± 0.04	0.33 ± 0.05	0.47 ± 0.05	2	3.51
20	2.38	0.54 ± -	0.33 ± -	0.51 ± -	1	1.75
21	2.4	0.57 ± 0.04	0.37 ± 0.05	0.52 ± 0.05	3	5.26
22	2.42	0.49 ± -	0.32 ± -	0.47 ± -	1	1.75
23	2.44	0.53 ± 0.00	0.34 ± 0.00	0.49 ± 0.00	8	14.04
24	2.46	0.50 ± 0.02	0.30 ± 0.02	0.41 ± 0.01	4	7.02
25	2.48-2.50	0.57 ± 0.01	0.35 ± 0.02	0.52 ± 0.03	4	7.02

Frequency distributions of sub-fossil (Fig. 4a) and recently collected (1986/87 and 2001) *Blepharidophyllum* mire (Fig. 4b) samples based on the scores of the first, morphometric size-related PCA axis show a tendency for recent samples to be smaller than sub-fossil material. A Kruskal-Wallis ANOVA, showed that differences between sub-fossil and recent sample PCA I scores are statistically significant ($H_{10,115} = 25.52$; $P < 0.01$; $n = 110$).

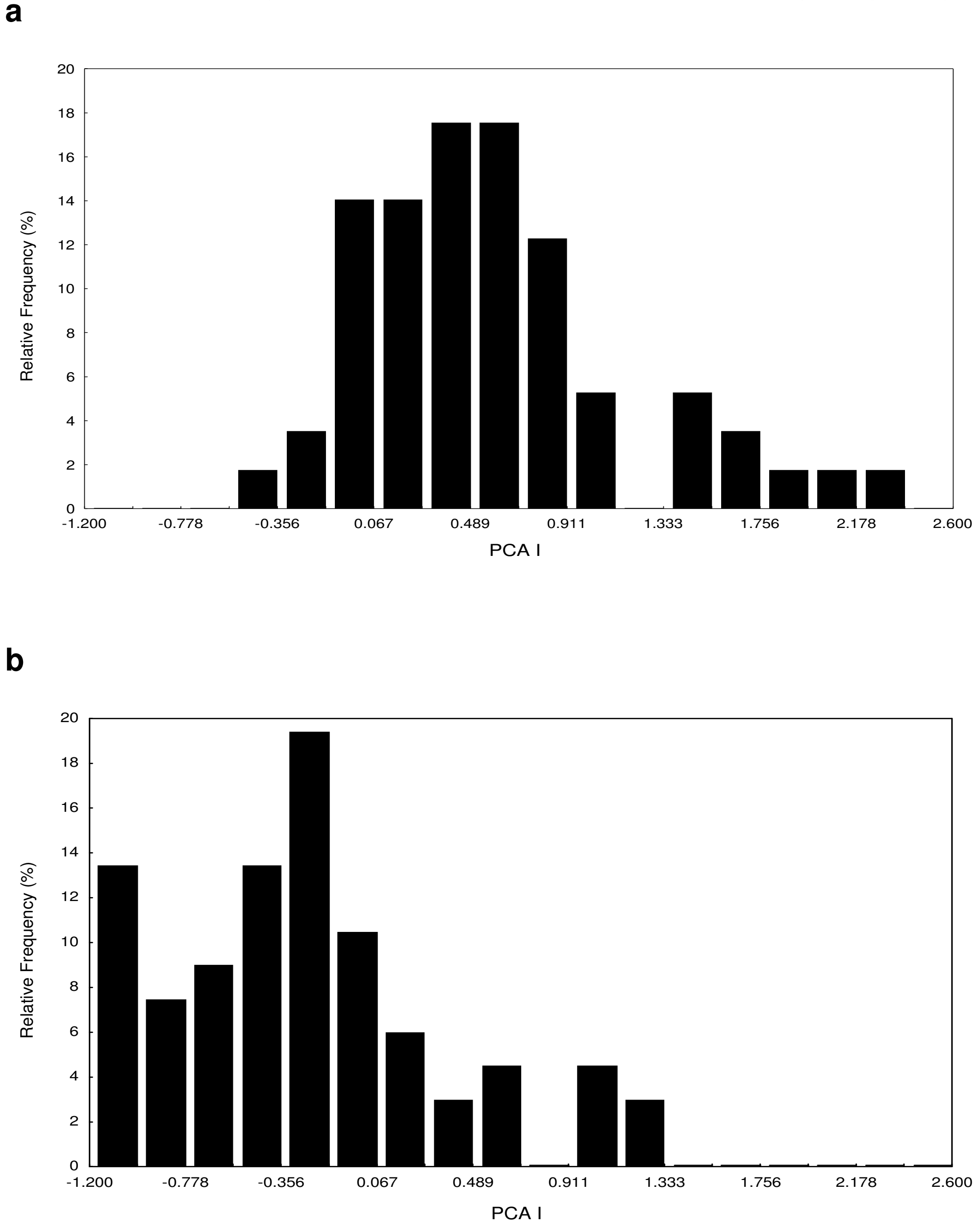

Fig. 4 Percentage relative frequency for principal component I, representing size, of **a** sub-fossil mire head capsules and **b** recently collected *Ectemnorhinus* samples (1986/87 and 2001) from sub-Antarctic Marion Island *Blepharidophyllum* mire communities

Correlations of PCA I scores (Fig. 5a) and individual head capsule measurements (Fig 5b-d) against all depths sampled (Table 5a) and for all depths of known age (Table 5b) indicated a negative and highly significant relationship in size from 2331 BC (2.5 m) to present time (0 m).

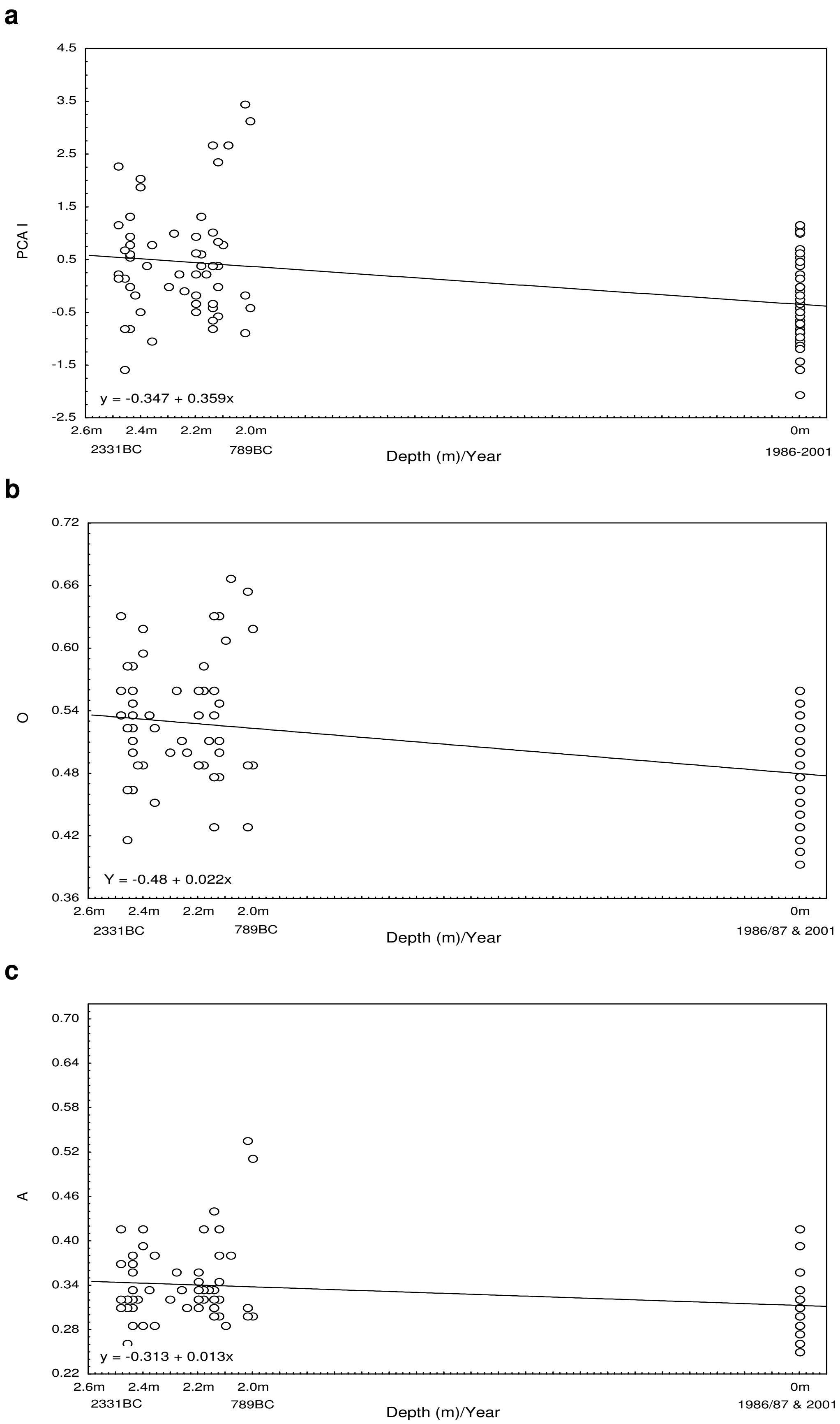

Fig. 5 Relationship between **a** principal component I (PCA I), indicative of overall size **b** measurement O **c** measurement A and depth (m)/year for sub-fossil and recently collected (1986/87 and 2001) *Ectemnorhinus* species in mire habitats. 0 m represents present time (1986/87 and 2001), with core samples collected at 2.0 m representing 789 BC and 2.5 m representative of 2331 BC

d

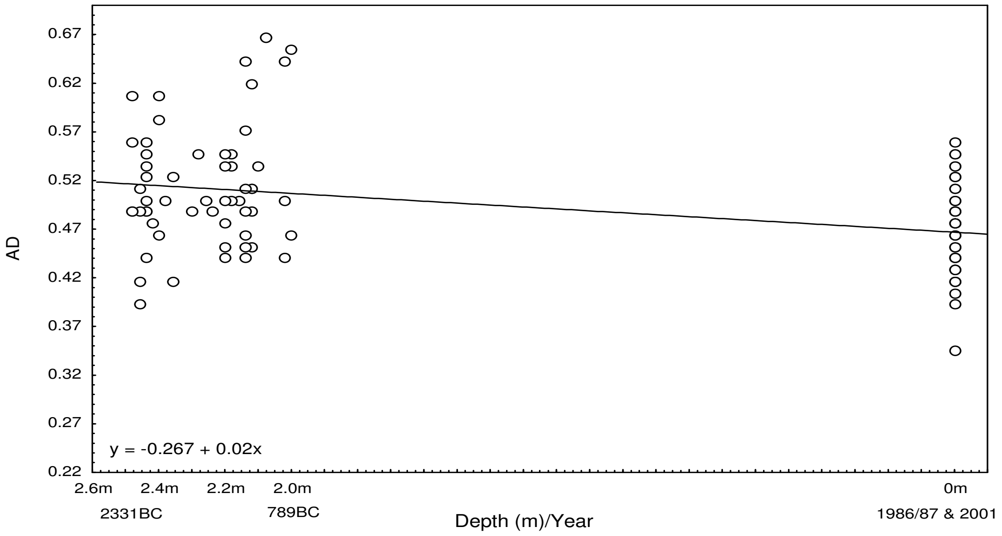

Fig. 5d Relationship between measurement AD and depth (m)/year for sub-fossil and recently collected (1986/87 and 2001) *Ectemnorhinus* species in mire habitats. 0 m represents present time (1986/87 and 2001), with core samples collected at 2.0 m representing 789 BC and 2.5 m representative of 2331 BC

Table 5 Results of correlations of the size-related variable, principal component I and three head capsule measurements (O, A and AD) for a) all depths for which samples were collected, including 0 m (1986/87 and 2001) representing present time (n = 124); and b) for all depths for which time estimates were obtained (2331 BC, 789 BC, 1986/87 and 2001) (n = 73). Sub-fossil and recently collected (1986/87 and 2001) *Ectemnorhinus* species were collected in *Blepharidophyllum* mires. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Variable	Correlation coefficient (r)	
a) Principal component I	-0.42	***
O	-0.44	***
A	-0.31	***
AD	-0.41	***
b) Principal component I	-0.48	***
O	-0.45	***
A	-0.42	***
AD	-0.45	***

Discussion

Body size changes of weevil populations from Marion and Prince Edward Islands may have serious consequences for island ecosystem functioning (Smith 1987; Smith and Steenkamp 1993; Smith et al. 2002). So far, studies suggest that *Bothrometopus randi*, *Palirhoeus eatoni*, *Ectemnorhinus similis* and *E. marioni* from both Marion and Prince Edward Islands are exhibiting morphometric body size changes over time (Chown and Smith 1993; see Chapter 4).

However, the taxonomic status of *Ectemnorhinus* species occurring on both Marion and Prince Edward Islands has largely been uncertain (Kuschel 1971; Crafford et al. 1986; Chown 1990) precluding an insight into the morphological changes in this group of weevils. Nevertheless, a recent multi-faceted genetic and morphometric study (Chapter 3) suggests the presence of a single species of *Ectemnorhinus* species on Marion Island and the presence of two *Ectemnorhinus* species on Prince Edward Island. These *Ectemnorhinus* species also exhibit a wide range of body size variation as is the case with *Ectemnorhinus* species occurring on Heard and Kerguelen Islands (Kuschel 1970).

While the analyses in the present study suggest that the sub-fossil remains recovered from core mire depths on Marion Island are likely to belong to the genus *Ectemnorhinus*, it was not possible to identify them to species level. Having identified the sub-fossil core sample remains, with reference to recently collected *Ectemnorhinus* weevil species in *Blepharidophyllum* mires on Marion Island to the genus level, an attempt was made to evaluate morphometric changes over time.

Both univariate and multivariate statistical analyses suggest a tendency for *Ectemnorhinus* species to decrease in morphometric size over a 4332-year period, whereas no differences were detected between core samples spanning a 1542-year time period. The question that may be posed is: What are the potential influences of these morphological changes over time? The house mouse (*Mus musculus*) was introduced on Marion Island in the late 1800s (Watkins and Cooper 1986) and is considered to be the major contributor to weevil body size reduction (Chown and Smith 1993) and a decrease in weevil population densities (Hänel and Chown 1998; Hänel 1999). With a preference for large-sized individuals (i.e., size-selective predation), mice may influence body size distributions of weevil species (Smith et al. 2002). Chown and Smith (1993) noted that various weevil species, identified as preferred prey species, decreased in body length from 1986 to 1992 on Marion Island. It is possible that similar changes in *Ectemnorhinus* species over time may have consequences for Marion Island ecosystem functioning. This is because of the important relationship that exists between body size and almost all insect life-history, physiological and ecological traits (Peters 1983; Reiss 1989; Wickman and Karlsson 1989; Harvey and Pagel 1991; Davies et al. 1999).

In addition, Marion Island mouse populations are strongly temperature-dependent and ameliorating temperatures may cause an increase in this invasive species (Smith and Steenkamp 1990; Huyser et al. 2000; Smith 2002). Crafford and Scholtz (1987) suggested that this may lead to increased weevil predation, thus decreasing overall rates of nutrient-cycling that would exacerbate the imbalance between primary production and decomposition (Smith 1991).

Furthermore, palynological evidence (Young and Schofield 1973; Scott 1985) suggest a warming trend that began after the last glacial maximum approximately 12000 BP on Marion Island (Chown 1990). However, core peat samples studied by Scott (1985) from depths between 1.65 – 1.80 m (4140 ± 70 y BP) and between 2.80 – 2.95 m (5440 ± 310 y BP) indicated vegetation type and climate to have remained virtually unchanged during these time periods. Mire core samples from depths between 2.5 m (3910 ± 40 y BP) and 2.0 m (2641 ± 45 y BP) in the present study, represent similar time periods to those examined by Scott (1985). It may, therefore, be assumed that these core samples represent a time period of little variation in climate, which may explain the lack of morphometric change observed in head capsules sampled from 2.5 m to 2.0 m in the present study. However, significant morphometric size declines observed between sub-fossil head capsules and recently collected material may, in addition to mice predation, be attributed to climatic changes on Marion Island since the 1960s (Smith and Steenkamp 1990).

Weevil species in their various habitats on Marion Island are functioning at microclimates that closely correspond to their upper lethal temperatures (van der Merwe et al. 1997). It is, therefore, possible that temperature may have an effect on weevils from Marion Island and that a further temperature increase may have negative consequences for Marion Island weevil survival (Smith 2002). In addition, evidence from a parallel morphometric study showed four weevil species (*B. randi*, *B. parvulus*, *B. elongatus* and *P. eatoni*) to exhibit comparable morphometric size and shape changes between the mid-1960s to 2003 on both Marion and Prince Edward Islands (Chapter 4).

On the other hand, it is also possible that the results in this study may have been constrained by the small character suite used, small sample size in some instances and sampling year. These results are only based on three measurements that may have obscured the underlying morphometric pattern. In addition, the availability of only 1986 data that was collected in *Blepharidophyllum* mires for comparison with core samples may also have influenced the results in the present study. Turner (2004) reported that the end of 1986 marked the start of an El niño period, such that the potential influence of temperature on these sub-Antarctic weevils (also see Chapter 4) may be exaggerated by the inclusion of the 1986 data.

However, with an annual mean surface air temperature increase of 0.04°C on Marion Island since the late 1960s (Smith and Steenkamp 1990; Smith 1991, 2002; Weimerskirch et al. 2003), it is expected that productivity as well as nutrient demand on Marion Island will increase (Smith 1988; Smith 2002). It is therefore proposed that on-going monitoring of weevil populations and further investigations are needed to determine factors influencing weevil populations on both Marion and Prince Edward Islands.

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"The test of success is not what you do when you are on top. Success is how high you bounce when you hit bottom"

George Patton

Chapter 6

A morphological analysis of weevils from sub-Antarctic Prince Edward Islands: an assessment of ecological influences

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Synopsis and conclusion

Ecologically sensitive, but relatively simple sub-Antarctic island ecosystems such as Marion and Prince Edward Islands, present unique models of terrestrial ecosystems to study the influence of anthropogenic changes, in particular climate change and species invasion on ecosystem functioning (Smith 1991). Marion and Prince Edward Islands are situated 22 km apart with only Marion Island colonised by mice (Watkins and Cooper 1986; Crafford and Scholtz 1987). Thus, the mouse-free Prince Edward Island represents an ideal natural control system in studies to assess the potential influence of climate change and mouse predation on the ecosystem functioning of the two islands (see Smith 1991; Smith and Steenkamp 1992, 1993; Gremmen et al. 1998).

More specifically, Marion and Prince Edward Islands offer an ideal opportunity to study the responses of macro-invertebrates such as weevils to changing global climate and/or biological invasions, through morphometric size and shape changes over time. The indirect effect of climate change on mice populations as well as the direct effect of size-selective predation by mice have been considered important contributors to weevil body size changes (Chown and Smith 1993; Smith 2002; Smith et al. 2002). In addition, the direct influence of climate change may also affect insect body size, with increasing temperatures leading to a decrease in body size (Atkinson 1994; James et al. 1997; Karan et al. 1998). Therefore, morphological changes in important contributors, such as weevil species, to nutrient cycling, that influence ecosystem functioning on both Marion and Prince Edward Islands (Crafford and Scholtz 1987; Crafford 1990), are of conservation concern. Van der Merwe et al. (1997) have shown that the upper lethal temperatures of the six weevil species that occur on the two islands correspond closely to the maximum microclimatic temperatures in their respective habitats. This suggests that temperature increase on the islands may be deleterious to weevil survival (Smith 2002).

Using a reduced set of 15 morphometric measurements (from an original set of 23 variables) that took into account correlations between variables and the morphological integration concept of Olson and Miller (1958), the present study was able to distinguish between the six currently recognised weevil species on Marion and Prince Edward Islands (Chapter 2). Although sexual size dimorphism is common in virtually all insect species (Helms 1994; Anholt 1997; Fairbairn 1997), multivariate analyses of the weevil species from Marion Island suggested the absence of multivariate sexual dimorphism, leading to the pooling of sexes in all subsequent analyses. Willig et al. (1986) expressed reservations about using a univariate approach in the assessment of non-geographic variation such as sexual dimorphism because significance tests for each variable independently present the dilemma of having to consider the number of variables that must exhibit significance before overall significance is declared. The use of a multivariate approach to identify potential sexual 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).

For the purposes of comparisons with previous studies, the present investigation was based on traditional morphometrics where linear measurements were used to assess multivariate size and shape changes in weevil morphology. While the analyses based on traditional morphometrics may have allowed some insight into aspects of weevil morphology with reference to climate change and mouse-predation, these could be investigated further using geometric morphometric techniques that are superior in partitioning organismal shape differences (Rohlf and Marcus 1993; Rohlf 1999).

The selected reduced set of morphometric measurements (Chapter 2) allowed the assessment of morphometric size and shape changes over time in weevils from Marion and Prince Edward Islands. However, the taxonomic status of both *Ectemnorhinus similis* and *E. marioni* has largely been uncertain (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 as synonymous (see Kuschel 1971).

A multi-faceted approach involving both morphometric and molecular (COI gene) (G.C. Grobler) characterisation provided some insight into the taxonomic status of *Ectemnorhinus* species on both Marion and Prince Edward Islands (Chapter 3). The molecular analyses revealed the presence of two genetically distinct species on Prince Edward Island, while evidence for a single species, comprising diverse genetically discrete populations was found on Marion Island (G. C. Grobler). These results were supported by multivariate analyses that showed neither multivariate size nor shape variation in individuals of *Ectemnorhinus* from Marion Island, but indicated the presence of two multivariate size-related phenetic groupings on Prince Edward Island. The congruence between molecular and morphometric data suggest that previous morphologically- and ecologically-defined groups in *Ectemnorhinus* weevils from Marion and Prince Edward Islands (Crafford et al. 1986; Chown and Scholtz 1989; Chown 1990) need to be re-evaluated.

Currently, the morphological identification of *Ectemnorhinus* species in the field is problematic such that it may have constrained the morphological analyses in the present study. However, the analyses were largely based on *a priori* analyses that made no prior assumptions about group membership, and more importantly included some genetically identified individuals as references to the *a priori*-derived morphometric groupings. Based on the findings in Chapter 3, all *Ectemnorhinus* species on Marion Island may be considered a single species. However, distinguishing between *Ectemnorhinus* species on Prince Edward Island should be investigated further and taxonomic descriptions and the nomenclature for *Ectemnorhinus* species on both Marion and Prince Edward Islands should be revised. In addition to the revised taxonomic descriptions and the nomenclature for these species, it is proposed that the *Ectemnorhinus* species on both Marion and Prince Edward Islands be investigated further using geometric morphometric techniques in order to assess potentially subtle morphological differences between the genetically-distinct groups delineated.

It is also possible that an on-going parallel population genetic study (G.C. Grobler) of *Ectemnorhinus* populations on both Marion and Prince Edward Islands will allow additional taxonomic and evolutionary insight into this group of weevils. It is proposed that an additional further investigation should also consider expanding the cytogenetic study by Chown (1989) to include weevils from both Marion and Prince Edward Islands.

The question of morphological change over time was investigated for the remaining four weevil species which were not of equivocal taxonomic status, namely, *Bothrometopus elongatus*, *B. randi*, *B. parvulus* and *Palirhoeus eatoni* occurring on both Marion and Prince Edward Islands. Morphometric analyses of these weevil species suggest morphometric size differences between samples collected in the mid-1960s and 1970s and recently collected material on both Marion and Prince Edward Islands. Similarly, samples collected in the early- to mid-1980s and recently collected samples for Prince Edward and Marion Islands respectively, suggest morphological changes over time during a period characterised by the green-house effect.

In addition, for all species, most of the deviance in Generalized Linear Models was explained by the full models of PCA III, supporting the separation of species on the third PCA axes in the multivariate analyses. Sampling year contributed highly significantly and explained the largest percentage deviance for the full models for principal components I (representing multivariate size) – III (representing multivariate shape) for all species. The only exceptions were *B. parvulus,* where none of the variables contributed significantly to the full model and *B. randi*, with gender explaining a larger percentage of the deviance than sampling year. Mice predation did not contribute significantly to the deviance in any of the *B. randi* PCA axes analysed, although it is a preferred prey species of mice (Chown and Smith 1993). However, mice predation contributed towards a small portion of the deviance for other, non-preferred prey species, such as *B. elongatus* and *P. eatoni.*

For the Marion Island dataset, temperature contributed most to the percentage deviance explained by the full model for PCA III for all species, except *B. elongatus*. In addition, temperature contributed significantly to PCA I and II for all species, except *B. elongatus* and *B. parvulus*. In general, gender also contributed significantly to the percentage deviance explained for all PCA axes across species.

Although temperature data are only available for Marion Island, it is assumed that temperature increases on the mouse-free Prince Edward Island that is located 22 km away are fairly similar. The suggested multivariate size and shape changes in all weevil species over a similar time period on both Marion and Prince Edward Islands, and the direct link between temperature increase (1950 - 2004) and sampling year (1965 – 2003), with temperature increasing over time, also supports the potential primary effect of climate change on weevil morphological changes.

The effect of climate change on weevil morphometric parameters are further exacerbated by the separation of the 1986 data in all species (Chapter 4). 1986 represents the start of an El niño period (Turner 2004) and may support the argument that climate change plays a major role in weevil size and shape.

Other studies have reported that small increases in temperature or changes in precipitation may influence invertebrates to undergo range expansions or population changes (Masters et al. 1998; Bale et al. 2002; Beaumont and Hughes 2002). Given that adaptation to climate is a physiological response (Addo-Bediako et al. 2000), Bergstrom and Chown (1999) have argued that the short-term effect of a potential predator may be concealed by the effects of climate change.

It is possible that temperature may have an indirect effect on mouse population numbers that in turn may have an added influence on weevil morphological changes through size-selective mouse predation (Crafford 1990; Smith and Steenkamp 1990; Chown and Smith 1993; Smith et al. 2002). Smith and Steenkamp (1990, 1992) suggested that mouse populations may be increasing as a result of warmer temperatures, having an impact on weevil morphology as well as their population densities (Crafford and Scholtz 1987; Hänel and Chown 1998; Hänel 1999; Smith et al. 2002). However, van Aarde et al. (1996) suggested no relationship between minimum temperature and mouse mortality. More recently, van Aarde et al. (2004) reported that mouse numbers on Marion Island between 1991 and 2001 were habitat-dependent, with stable population densities occurring on biotically-influenced areas, but that population numbers increased by approximately 12% per annum on wetlands.

The question of morphological change over time was further investigated by examining sub-fossil weevil remains recovered from mire core samples on Marion Island (Chapter 5). Weevil remains from 2.0 m and 2.5 m depths were carbon-dated and found to be from 789 BC and 2331 BC, respectively. Head capsules, being the best-preserved components, formed the basis of the analyses in this part of the study. These sub-fossil weevil remains were identified as belonging to the *Ectemnorhinus* group of genera and were compared to recent samples collected in 1986/87 and 2001 from *Blepharidophyllum* mire communities on Marion Island.

Previous core peat samples studied by Scott (1985) from between 1.65 – 1.80 m (4140 ± 70 y BP) and 2.80 – 2.95 m (5440 ± 310 y BP) depths indicated vegetation type and climate to have remained virtually unchanged during these time periods. Mire core samples collected at 2.5 m (3910 ± 40 y BP) and 2.0 m (2641 ± 45 y BP) depths in the present study represent samples from similar time periods to those examined by Scott (1985). Consequently, it may be assumed that these core samples represent a time period that exhibited little variation in climate change. Since these core samples predate the onset of global warming due to the green-house effect and the introduction of mice on Marion Island, it was hypothesised that there would be no discernible morphological changes in weevil samples, between 2331 BC and 789 BC, a period spanning 1542 years.

As hypothesised, the analyses of this part of the study did not detect statistically significant morphometric body size and shape changes in sub-fossil material from the two sampled depths that were considered to represent different dimensions in time and space. However, significant differences between sub-fossil and recent material collected in 1986/87 and 2001 were detected in a single head capsule measurement. These results suggest a general lack of morphological change in sub-fossil material from Marion Island over a period of 1542 years.

Given the different views on the potential influence(s) of morphological change with reference to climate change and/or mouse predation, the present study suggests that further investigations based on long-term population density data for both macro-invertebrates and invasive species are required. Based on a five-year (1996 – 2000) mouse-exclusion experiment, van Aarde et al. (2004) recently reported no significant mouse effect on abundance, biomass and community structure based on diversity and composition in any of the mouse-prey species on Marion Island. They reported no significant changes over time in either biomass or abundance in enclosures that were independent of the potential influence of mice, but also indicated that their results may have been affected by a generally low statistical power.

Additional annual weevil and mice sampling on Marion Island and more frequent sampling of weevils on Prince Edward Island may allow the continual monitoring of weevil morphological changes and mice population changes over time. Such long-term datasets may allow the partitioning of potential influences, such as climate change and mouse predation to be investigated. In addition, future core sampling of mires on the mouse-free Prince Edward Island may allow the investigation of weevil morphological changes over time that is independent on the potential influence of mice predation. These potential factors, together with various other factors such as resource limitations may manifest as a result of environmental changes due to global warming. As suggested by Smith et al. (2002) and van Aarde et al. (2004), these future studies may also have to include an assessment of long-term invertebrate population dynamics, the autecology of mouse-prey items, and the proximate controlling factors of invertebrate and mouse populations. For example, Klok and Chown (1997, 2001) have reported that desiccation, and to some extent, temperature may represent population-limiting factors in some invertebrates. In essence, an insight into the potential effect of mouse-predation may only be fully understood with additional data on the biology of both macro-invertebrate prey species and their predators.

Marion and Prince Edward Islands Management Plan Recommendations

With a temperature increase as a result of global warming, the ecosystems of both Marion and Prince Edward Islands will continually be in a process of change. Increased temperature may create a favourable environment for invasive species and facilitate their establishment as well as increasing the severity of their impact on ecosystem functioning (Bergstrom and Chown 1999; Dukes and Mooney 1999). Therefore, additional precautionary measures need to be implemented in order to avoid any further introductions of alien invasive species on both Marion and Prince Edward Islands.

For example, personal and issued gear should be washed and checked more thoroughly to prevent the introduction of seeds, fungus, or invertebrate species before boarding on voyagers to the islands, such as the SA Agulhas. Current methods of cleaning equipment and gear on board opens a potential gap for invasive species to “re-infect” visitors and their equipment before being flown to either Marion or Prince Edward Islands. In addition, the cleaning of voyagers such as the SA Agulhas should be prioritised and strict regulations implemented. For example, rat guards should always be present on anchor ropes to prevent rodents from boarding the ship and augmented by the fumigation of pesticides before each voyage to the islands, to ensure an “alien-free” ship (also see Frenot et al. 2005).

It is important to recognise that an increase in the number of visitors to the islands may also increase the probability of the introduction and establishment of invasive species that would lead to conservation problems (Chown et al. 1998). Already, human influence has increased rapidly, through extensive commercial exploitation, for example whaling and sealing activities (Frenot et al. 2005). In addition, there has been an increase in scientific research on the islands that in turn, may have an impact on the ecosystems of the islands (Frenot et al. 2005). Chown et al. (1998) suggested that the number of human occupants and the interaction between humans and the island area are important determinants of the number of introduced species to the islands. It is, therefore, recommended that commercialisation of the islands through tourism should be strictly regulated or even prohibited, and that both tourism and scientific visits to the islands be limited (Chown et al. 1998). Currently, a strict permitting system exists, where the number of visitors to Prince Edward Island is restricted to six visitors for four days per year, with visitors being issued with new clothing and equipment and flown to Prince Edward Island directly from the ship on arrival. The sustained implementation of this current policy should curtail further introductions of invasive species on Prince Edward Island.

However, Redford (1992) and Terborgh (1999) suggested that even low-intensity human use may have significant effects on natural ecosystems. Therefore, islands not likely to be visited (e.g., Prince Edward Island) that are adjacent to frequently visited islands (e.g., Marion Island) are likely to exhibit conservation problems in future (Gremmen and Smith 1999; Chown et al. 2001). Alien species introduced to Marion Island have been shown to naturally disperse to Prince Edward Island (Gremmen and Smith 1999).

For already established invasive species, such as the house mouse, it is suggested that alternative mouse-control measures be investigated and implemented as soon as possible. Although the present study suggests that climate change may be the main potentially influential factor causing weevil morphological changes, it is possible that further increases in temperature may also facilitate an increase in mouse population numbers (Smith and Steenkamp 1990, 1992; also see Frenot et al. 2005). Indeed, adult weevils are contributing more to mice diet than found in previous studies (Smith et al. 2002). In addition to size-selective predation, increased mouse population numbers may have a devastating effect on the morphology and population densities of invertebrates on both Marion and Prince Edward Islands.

Of major concern is whether it is possible for these invertebrates to successfully maintain viable population densities and still serve as a main food source for predators. Huyser (2000) reported that the contribution of macro-invertebrates to the diet of the lesser sheathbill diet has declined dramatically and suggests that mice, through predation on macro-invertebrates are indirectly responsible for the decreased population numbers of the lesser sheathbill. It is possible that the eradication of mice may ensure sustainable macro-invertebrate population densities for natural predators. It is suggested that intense monitoring of population densities of both macro-invertebrates and invasive species over a similar time period be implemented in order to facilitate the assessment of predator-prey interactions. In addition, Frenot et al. (2005) highlighted the urgent need for the establishment of long-term monitoring programmes on the Southern Ocean Province Islands to ensure appropriate risk management protocols to be implemented.

More importantly, it is further suggested that both Marion and Prince Edward Islands should be managed as separate ecosystems. From the genetic (G.C. Grobler) and morphometric differences between *Ectemnorhinus* species on both islands, it appears that the biodiversity of both islands differ with regards to species composition, such that Prince Edward island, as previously thought, can no longer be considered a potential backup source of biodiversity that may be lost on Marion Island (or vice-versa).

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