Systematics of Skuas (Aves: Stercorariidae) with Particular Reference to Evidence from their Feather Lice (Insecta: Phthiraptera)

A thesis
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B. Sc. (Hons) (Malaya), 1992

Declaration

I declare that the work recorded in this thesis is entirely my own, unless otherwise stated, and that it is my own composition. No part of this work has been submitted for any other degree to any institution.

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Abstract

Skuas (Aves: Stercorariidae) are large and aggressive seabirds which possess a combination of the features of birds of prey and seabirds. These birds may show two phases (dark and light) of polymorphism in their plumage pattern. They can be found breeding mainly in Arctic and subArctic, or Antarctic and subAntarctic regions.

Based on a morphological study, skuas have been separated into two groups; small (genus *Stercorarius*) and large (genus *Catharacta*) skuas. Small skuas are Arctic (*S. parasiticus*), Long-tailed (*S. longicaudus*) and Pomarine (*S. pomarinus*) skuas while large skuas are Great (*C. skua*), Falkland (*C. antarctica antarctica*), Tristan (*C. a. hamiltoni*), Brown (*C. a. lonnbergi*), Chilean (*C. chilensis*), and South Polar (*C. maccormicki*) skuas. However, evidence from other sources (such as behaviour and molecular analyses) suggests a slightly different classification; they can either be clustered together into one group or separated into two groups with a different composition.

The fact that a single taxon of large skuas (i.e. Great skua) is geographically separated from others raises issues regarding the evolutionary process. Two possibilities have been suggested: first, some Great skuas migrated to the Southern hemisphere and gave rise to other large skuas. Alternatively, some large skuas from a specific taxon arrived in the Northern hemisphere and gave rise to the Great skua. This study examined both possibilities and tried to determine which hypothesis seemed more likely.

Four methods were employed to infer systematic relationships among skuas. These were morphometric analysis, cladistic analysis, a study of the coevolution of skuas and their parasitic lice and examination of mitochondrial DNA.

Morphometric and cladistic analyses of seventy two museum specimens and morphometric analyses of eighteen skeletal specimens indicated that skuas should be separated into two groups: small and large skuas. The degree of separation among large

skuas was difficult to determine and morphological evidence failed to resolve clear relationship among large skuas.

Skuas harbour four genera of feather lice: *Haffneria grandis, Austromenopon fuscofasciatum, Quadraceps normifer, Saemundssonia stresemanni, S. inexspectata,* and *S. cephalus*. *S. inexspectata* is specific to Long-tailed skua and *S. cephalus* is specific to Arctic skuas. *S. stresemanni* is widespread on all skuas, *H. grandis* only occurs on large skuas and *Q. normifer* and *A. fuscofasciatum* are scattered on various skua taxa. The lice do not appear to correspond to any specific morphological features on their skua hosts.

It was intended to compare mitochondrial DNA (mtDNA) of lice with that of the skua hosts in order to ascertain the coevolutionary relationship between the two but this study failed to extract mtDNA from lice. This failure may be due to sample quality or condition (dried lice instead of fresh or frozen) or quantity of samples.

The main conclusions of this study are as follows: first, there are major differences between small and large skuas and skuas should be separated into two groups; small skuas (which consist of Arctic, Long tailed and Pomarine skuas) and large skuas (composed by Great, Tristan, Falkland, South polar, and Brown skuas). Second, there is no indication from morphology and parasitological data that Pomarine skuas arose due to hybridization between members of small and large skuas as suggested by mtDNA data. Third, the evolutionary problems among large skuas are very hard to clarify since evidence relating to this phenomenon is very vague and is difficult to resolve. Therefore, more information from molecular or other approaches is required before this problem can be solved. Finally, while there may be coevolution between skuas and feather lice, there is no evidence for a strict relationship between the two. This may be because skua feather lice possess less host-specific characters or the hosts may have separated from each other too recently to allow time for lice to modify their morphology.

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Chapter 1

General Introduction

1.1. General Introduction to Skuas

Skua is the substantive name for all members of the Family Stercorariidae (Order Charadriiformes, suborder Lari); in the plural, it is the general term for the family (Campbell & Lack 1985). It is believed that skuas separated from their closest relatives, gulls (family Lariidae) during the Miocene, at least 10 millions years ago (Fisher & Lockley 1954). However, the oldest fossil skua (*Stercorarius shufeldti*, from Oregon, USA; Furness 1987) is only around 50,000 years old.

Skuas are predators and pirates, and many display a kleptoparasitic lifestyle (stealing food from other birds). Their legs not only have hard scales but also possess strong hooked claws and a swimming membrane. The bill is strong and hooked. In addition, the presence of large supra-orbital salt glands permits them to excrete excess salt. Thus skuas have a combination of the features seen in birds of prey and seabirds (Furness 1987).

The skua Family consists of nine species or subspecies based on the current classification, divided into two groups - large skuas (genus *Catharacta*), shown in Plates 1-6 are included the Great or Bonxie (*C. skua*), Tristan (*C. antarctica hamiltoni*), Falkland (*C. a. antarctica*), Brown (*C. a. lonnbergi*), Chilean (*C. chilensis*), and South Polar or McCormick (*C. maccormicki*) skuas. The small skuas (genus *Stercorarius*), shown in Plates 7-9 are included the Pomarine (*S. pomarinus*), Arctic (*S. parasiticus*), and Long-tailed (*S. longicaudus*) skuas. Members of the latter group are smaller, and possess two greatly elongated central tail feathers; their wings are narrow and pointed compared with the former group, which are larger and have broad blunt wings. The plumage pattern in large skuas is normally brown, with some rufous or golden markings

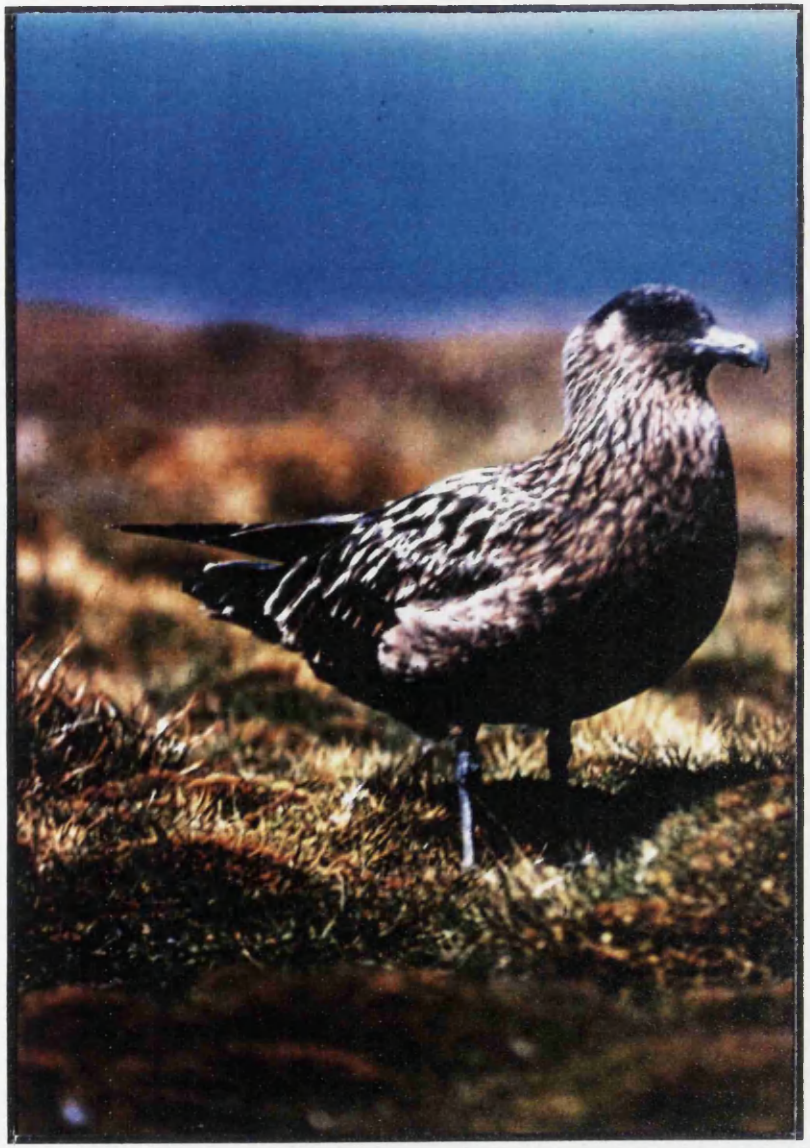

Plate 1. Great skua or bonxie (*Catharacta skua*)

Plate 2. Tristan skua (*Catharacta antarctica hamiltoni*)

Plate 3. Falkland skua (*Catharacta antarctica antarctica*)

Plate 4. Brown skua (*Catharacta a. lonnbergi*)

Plate 5a . Chilean skua (*Catharacta chilensis*), dorsal

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Plate 6. South Polar or McCormick skua (*Catharacta maccormicki*)

Plate 7. Pomarine skua (*Stercorarius pomarinus*)

Plate 8. Arctic skua (*Stercorarius parasiticus*)

Plate 9. Long-tailed skua (*Stercorarius longicaudus*)

and a conspicuous white wing flash consisting of white lower parts of the primaries, obvious on the underwing (Furness 1987).

Small skuas of all three species show polymorphism in the plumage pattern. Two phases occur; dark and light. Pomarine and Long-tailed skuas show the same plumage polymorphism as Arctic skuas. Polymorphism in large skuas is rather different from that in small skuas. In small skuas, polymorphism is only in the ventral plumage of the body (e.g. Arctic skua), and the changes occur gradually from totally sooty brown through many intermediate stages to an extreme pale phase, in which the entire belly, breast and neck are white or cream coloured and the wing, tail, back and crown of the head are dark brown. In the South Polar skua two colour phases occur as dark brown-bodied or light brown-bodied individuals. The proportions of colour phases vary geographically. In the South Polar skua, the frequency of light birds increases towards the Ross Sea but is low in the Antarctic Peninsula, while in the Arctic skua the proportion of light birds tends to increase with latitude. In Pomarine skua populations, about 5-10% of birds are dark phase in all areas, while the dark phase of the Long-tailed skua is extremely rare (Furness 1987).

1.2. Breeding Distributions of Skuas

There is little doubt that the gulls originated and evolved in the northern hemisphere. Since skuas evolved from the same stock as gulls, they probably also originated from the northern hemisphere. This supposition is supported by the northern distribution of the small skuas and the fossil remains found in Oregon, USA which show that a skua was present during the Pleistocene period, 50,000 years ago (Furness 1987).

The breeding ranges of small skuas (genus *Stercorarius*) covered Arctic areas whereas large skuas (genus *Catharacta*) are restricted mainly to Antarctica and the subAntarctic region, except for the Great skua which occurs in both the Northern and Southern hemisphere. The small skuas each have circumpolar Arctic breeding distributions, penetrating variable distances into the subArctic. The Arctic skua has the

widest distribution range while the Pomarine skua has a very limited breeding area. The Arctic skua often breeds in the same area as other seabirds, from which they steal food. For example, they breed near tern colonies in Europe, and are associated with puffins (*Fratercula arctica*), terns, or kittiwakes (*Rissa tridactyla*) in north Scotland and the Faeroes. Arctic skuas also inhabit tundra-like habitat in Iceland, and sometimes interact with coastal seabird populations living in the tundra habitat in Canada, Alaska, and northern regions of the former USSR (Figure 1.1a). Another representative of the small skuas, the Long-tailed skua, has larger populations in the former USSR and Alaska, and sometimes in Canada, Norway, Greenland, and Sweden (Figure 1.1b). The other member of this group, the Pomarine skua, nests in a slightly smaller area of the former USSR, Alaska and Canada, and does not breed in Europe (Figure 1.1c). Their breeding population density varies depending on local food sources, predominantly lemming (small rodent) populations. The total population of the Pomarine skua is certainly less than that of the Arctic skua (Furness 1987).

Genus *Catharacta* has a very restricted breeding range in the northern hemisphere. The Great skua (*C. skua*) breeds in Shetland, Orkney, northern Scotland, Western Isles, the Faeroes, and Iceland (Figure 1.1d). The closest neighbour to this species from the same group is the Tristan skua (*C. a. hamiltoni*) which breeds in Tristan da Cunha and Gough Island. Another subspecies, the Falkland skua (*C. a. antarctica*) breeds throughout the Falkland Islands and on the South American continent at Punta Tombo, Camarones, Bahia Bustamante, and Puerto Deseado (Figure 1.2). In Patagonia, the distribution of the Falkland skua overlaps with that of the Chilean skua (*C. chilensis*). In addition to this area, the Chilean skua also breeds at Lee Bay, on the north coast of Tierra del Fuego, Magdalena Island, and in the Strait of Magellan. They also breed at numerous sites along the Western coast of Chile, as well as in smaller numbers on the eastern side of the continent. The Brown skua (*C. a. lonnbergi*) nests on most subAntarctic islands, the northern parts of the South Polar skua (*C. maccormicki*) breeding sites on the Antarctic peninsula, and on the islands south of New Zealand. The South Polar skua breeds in the Theron mountains, and on

Figure 1.1. Maps showing breeding distributions of small skuas (top and bottom left) and Great skua (bottom right) around the world, after Furness (1987). A = Arctic skua; B = Long-tailed skua; and C = Pomarine skua.

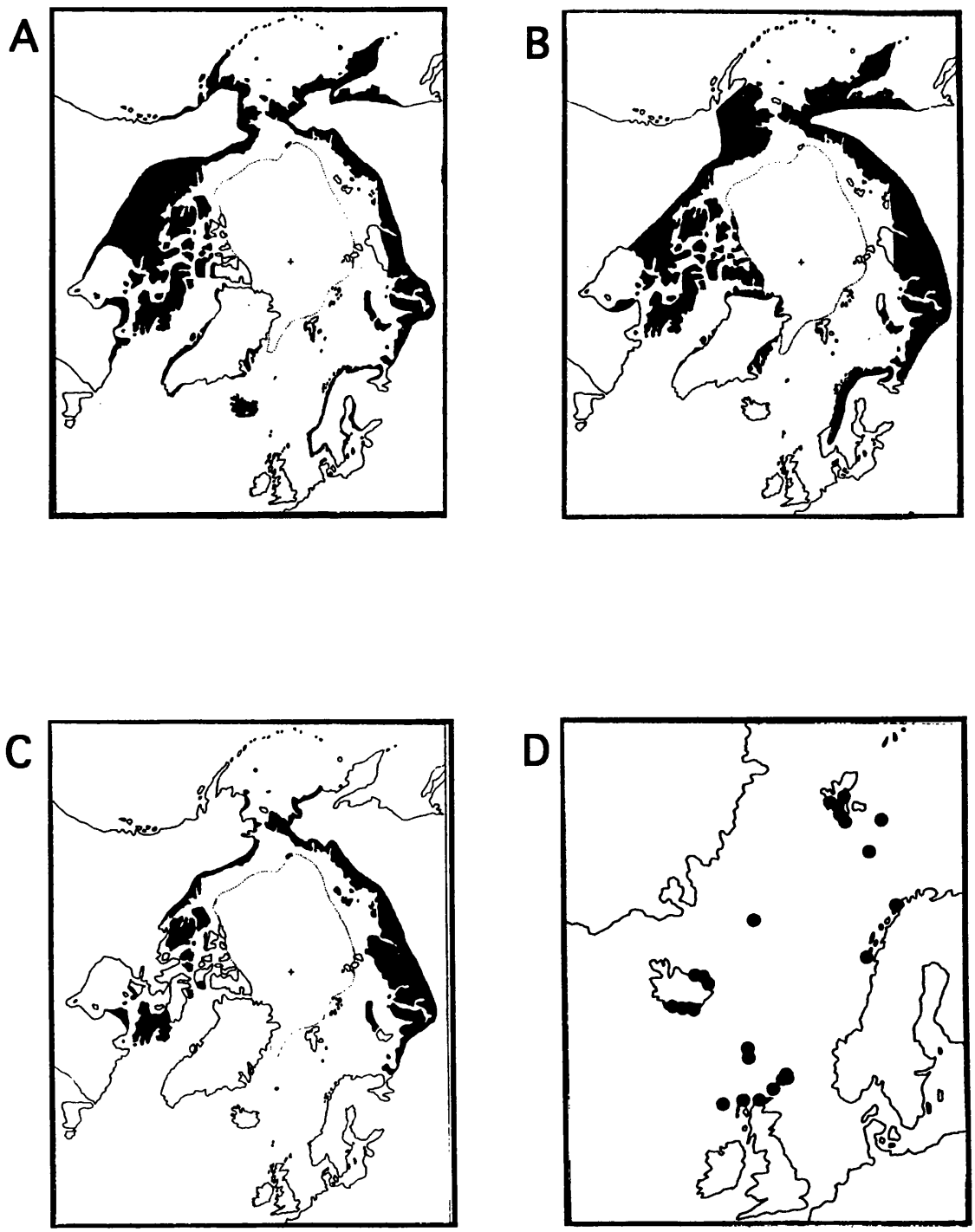
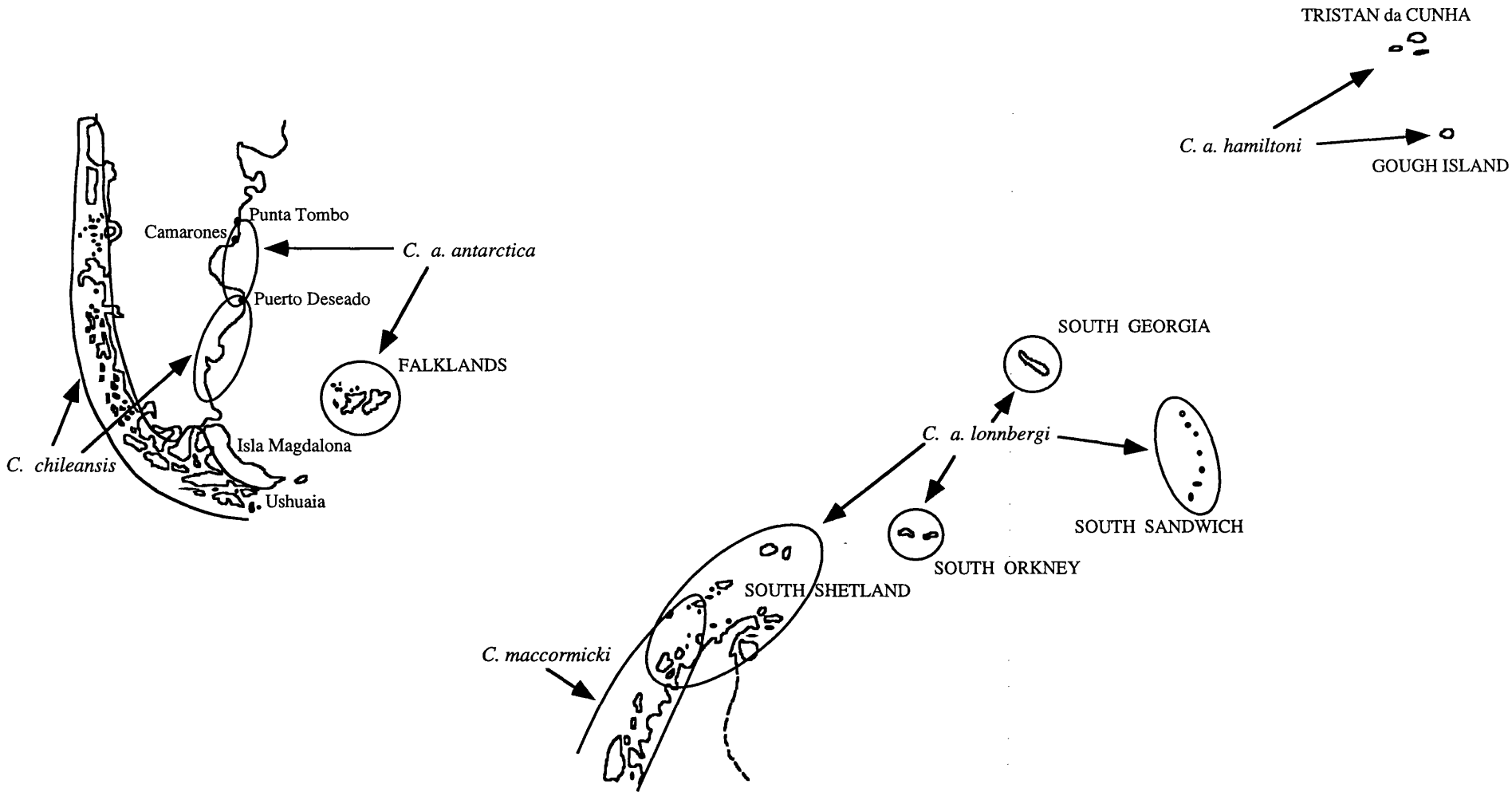


Figure 1.2. Map showing breeding distributions of Southern hemisphere large skuas (extracted from Devillers 1978).



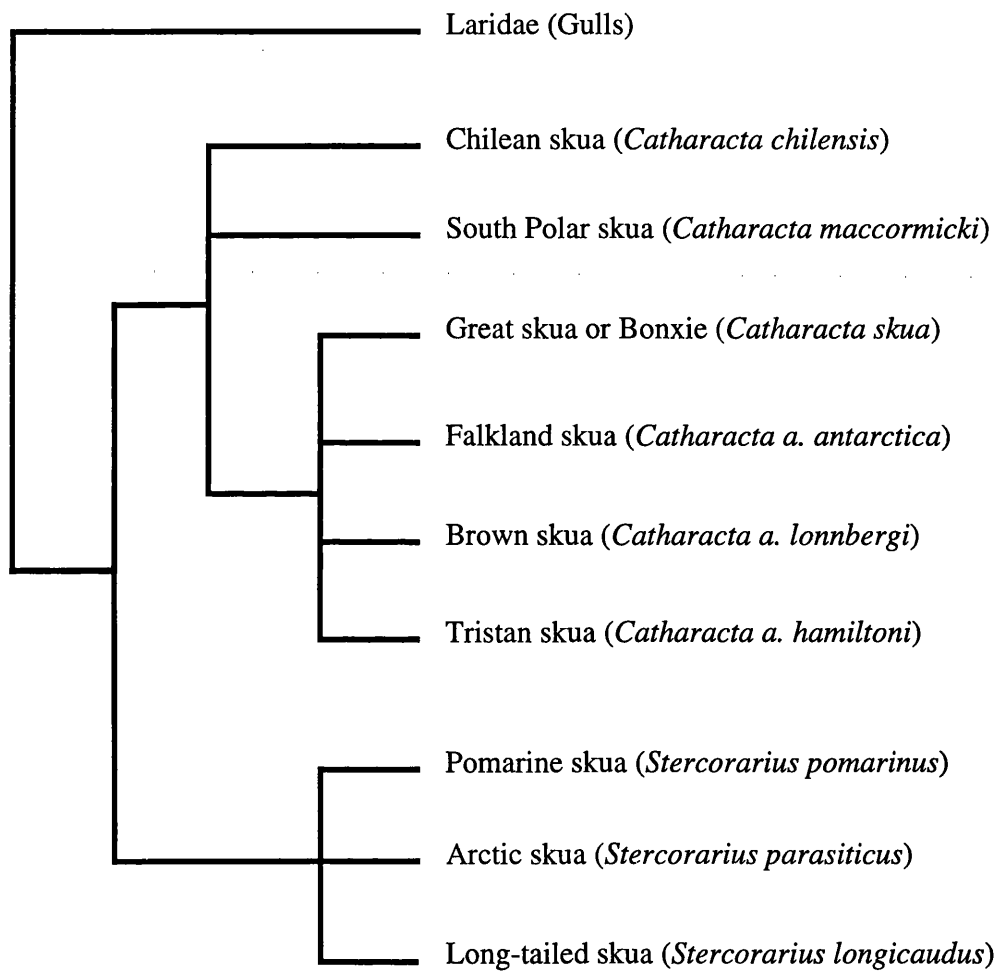
most ice-free areas of the Antarctic continent. The Breeding range of the South Polar skua appears to have spread north into the range of the Brown skua, and this may be a relatively recent event (Furness 1987).

1.3. Classification of Skuas

Systematic relationships among skuas are still unresolved. Members of the family Stercorariidae are morphologically very homogenous within each of the two genera. However, low congruence between molecular data (mitochondrial cytochrome b and 12S rRNA sequences) and classical taxonomy (based on qualitative morphology) confuse this situation. Traditional classifications divided the Stercorariidae into two genera, i.e. *Stercorarius* and *Catharacta* (Figure 1.3). This classification was proposed by Wynne-Edwards (1935), Bannerman (1963), Howard & Moore (1980) and many others, and is strongly supported by evidence from size, adult external morphology and plumage, and breeding distribution. All members of the *Stercorarius* genus are smaller, possess similar adult plumage, but show marked differences from the large skuas. Moreover, members of the genus *Stercorarius* breed in Arctic areas, and are therefore distinguished from large skuas which normally have breeding distributions in the southern hemisphere with an outliner in the North Atlantic (Furness 1987). Considering plumage variations, all juvenile small skuas shows barred underparts, in contrast to juvenile large skuas which do not possess any barring (Brooke 1978). It is suggested that this character must have evolved in *Stercorarius* after they separated from *Catharacta* but before the small skuas had separated from each other (Furness 1987).

Contrary to this morphological classification, behavioural data suggest lumping all skuas into one or two groups with different composition. Behavioural criteria such as long call display and wing raising posture suggest a different pattern of skua classification (Hartert 1912 in Furness 1987; Moynihan 1959; Andersson 1973). Arctic and Long-tailed skua share the same long call displays, which is rather different from that of the Pomarine skua which possess similar displays to the Great skua and other

Figure 1.3. Classification of skuas, suggested by Furness (1987) and supported by the evidence from size, adult plumage, and breeding distribution.



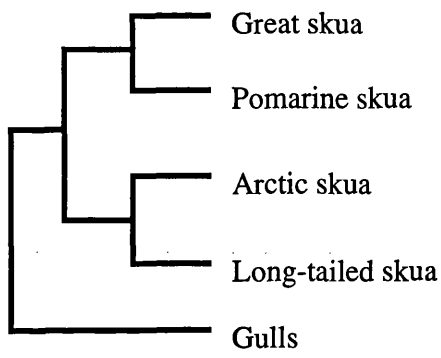
large skuas. Furthermore, the Pomarine skua also shows wing raising posture during the long call display like those of other large skuas but Arctic and Long-tailed skuas never display this behaviour.

Examination of skeletal structure (Schnell 1970) gave a mixed perception about skuas classification. The phenogram produced depend on which method was used and the suite of characters included in the analysis. Great skua (all *Catharacta* taxa combined) was classified as closely related to Pomarine skua when an analysis was carried out based on fifty-one characters, but all small skuas were lumped together with the larger characters (Figure 1.4). Schnell (1970) concluded that correlation matrices gave a more robust classification than distance matrices since the former are less affected by transformations or the use of different character suites. These lines of evidence (behaviour and skeletal structure) give quite distinct skua classifications; either lumping all skuas together into a single genus or separating them into two genera with Pomarine skuas placed in the large skuas group.

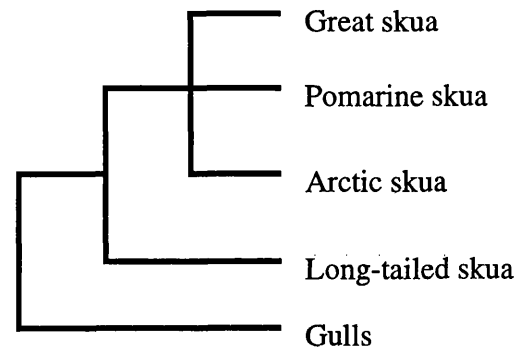
Study of skua mitochondrial cytochrome b and 12S rRNA base sequences, however, has shown that the hierarchical relationship inferred by molecular data is not congruent with trees suggested by the phylogenetic analyses of morphology, but agrees with the behavioural and skeletal classification. Mitochondrial DNA sequences provide strong evidence that the Pomarine skua is more closely related to the Great skua than to other small skuas (Blechschmidt *et al.* 1993; Figure 1.5.).

According to Furness (1987), skuas probably originated in the northern hemisphere before colonising the Antarctic and neighbouring area and evolving into the large (*Catharacta* sp.) skuas. Recently (in a geological time-scale) they migrated back to the north and recolonised the northern hemisphere and, as a result, the Great skua was successfully established in this area. It is still unclear which species of large skua was the ancestor of the north Atlantic Great skua. Several hypotheses have been presented. Data from breeding distribution strongly suggests that the Tristan skua or Falkland skua may be the candidate (Fisher & Lockley 1954). Unfortunately, these two species are short distance migrants and it is unlikely that they would reach the North

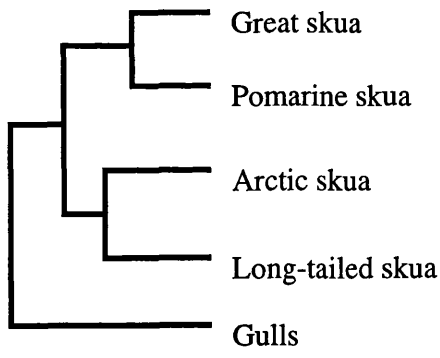
Figure 1.4. Phenograms show mixed relationships among small skuas and Great skua. These phenograms were based on correlation matrices from various data of Schnell (1970).



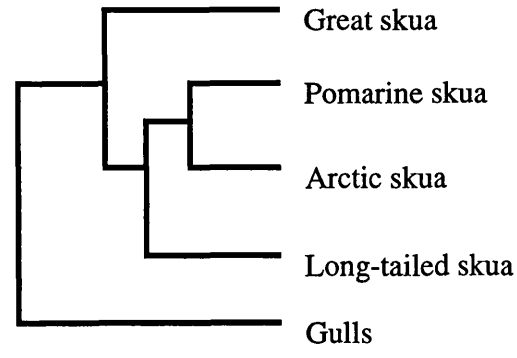
51 skeletal characters



PCA scores on skeletal characters

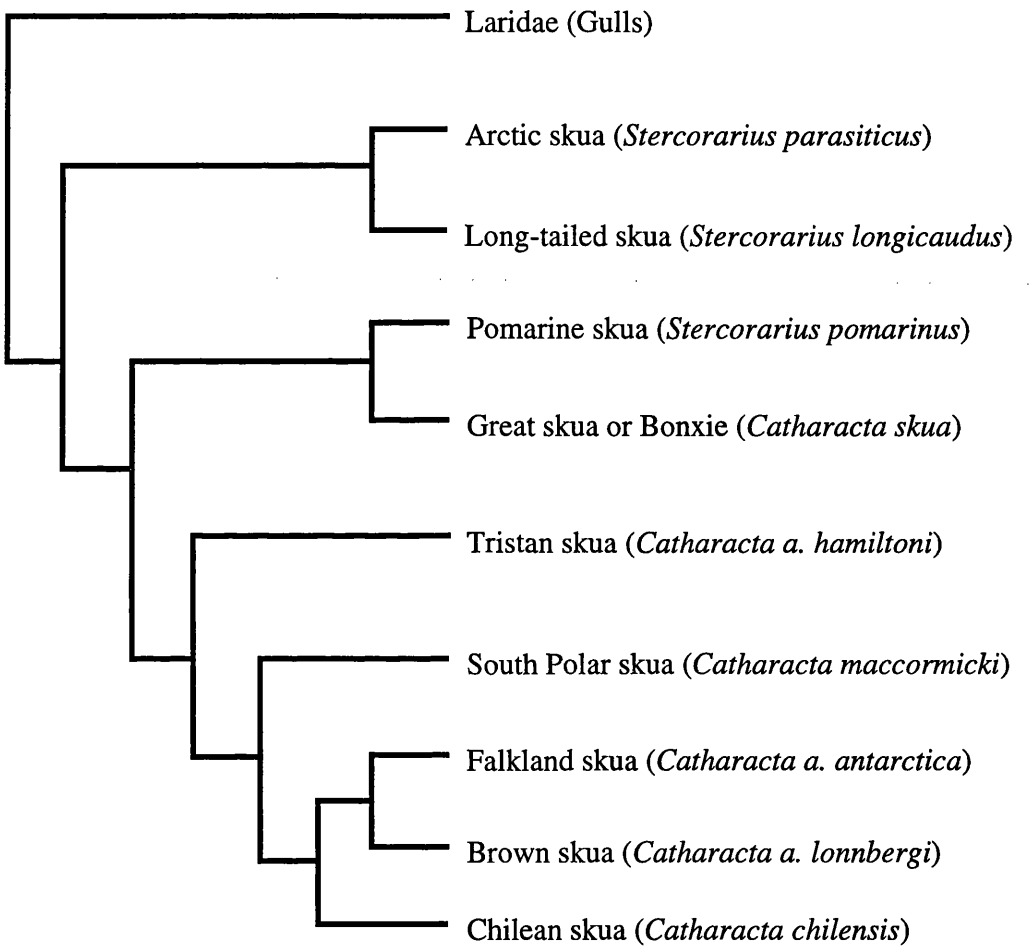


50 skeletal characters / sternum length



72 external and 51 skeletal characters

Figure 1.5. Cladogram based on mitochondrial DNA cytochrome b (964 base-pairs) shows Great skua more closely related to Pomarine skua rather than others large skua (extracted from Blechschmidt *et al.* 1993).



Atlantic region. It is possible that they reached the North Atlantic by freak events, as suggested by Fisher & Lockley (1954). The ability to migrate long distances give more credence to the South Polar skua as a candidate for the ancestor of the Great skua. However, Furness (1987) rejected this idea on the basis of differences in their plumage and smaller size (among large skuas). Considering its rufous colouration, Swales (1965) suggested that the Chilean skua could be the true ancestor, but again Furness (1987) concluded that although the Chilean skua has good similarity with the Great skua in this feature, the majority of plumage characters are different from the Great skua. Furthermore, besides breeding in a limited area, some Chilean skua behaviour is totally different from that of the Great skua.

1.4. Research Overview

There are many questions about skua evolution and taxonomy still unanswered. Two main questions arise when considering the evolutionary history of skuas. First, should skuas be separated into two or more groups, and if so, what species or subspecies belong to which group? Second, did great skuas evolve from a southern form of large skua, and if so, which taxon of large skuas led to the northern Great skua? This study has been designed with those questions in mind and attempts to obtain more knowledge about skua systematics.

As mentioned earlier, skua systematics involved both controversies and uncertainties. In this study, these problems have been tackled using two approaches. First, by gaining as much data as possible from skuas and second, by looking at indirect evidence from their ectoparasites. In the first part of this study, emphasis is placed on examining skuas and then deducing their relationship by using morphometric and cladistic analyses. Both of these methods have proved very useful in solving systematic problems.

In the second part of this study attempts were made to obtain indirect evidence for skua classification from bird ectoparasites i.e. feather lice (Insecta: Phthiraptera).

This ectoparasite group, previously known as Mallophaga, has been used in several systematic studies especially when morphological and molecular data have been unable to provide solutions. Feather lice have been shown to be very host specific, and only to transfer from host to host by direct contact. They have to adapt to the available sources of food and habitat provided by their host. Therefore, any evolutionary changes in their host will affect them too, leading to host-parasite co-evolution. When host ancestors were spreading and faced different kinds of geographical barrier in a new habitat, they will have evolved to adapt to new conditions. With time, host developed new characters and slowly differ from their ancestors. However, drastic change in host lifestyle will not affect parasites very much. This is because the microhabitats provided by the host such as body temperature or feathers as food remain almost uniform (Hopkins 1942). Any modifications in parasites, if they occur at all, will take place at a different rate compared to the evolution of the hosts (Emerson & Price 1985). This feature, together with the high host specificity indicates that feather lice can provide information about their host relationships. This can be inferred from feather lice based on the degree of resemblance between the parasites from two groups of hosts (Hopkins 1942). However, before any conclusion can be drawn, the degree of host specificity between these two life-systems, and the extent they can be used as a taxonomic indicator has to be established. This can be done by looking at the diversity of lice inhabiting skua feathers.

In acquiring more data to provide a strong base for skua phylogeny, a comparison between the phylogenetical tree from skuas and their feather lice should be possible. If co-speciation really exists, phylograms deduced from similar evidence (e.g. same region of mitochondrial DNA (mtDNA) sequences) of host and its ectoparasites should show congruence, and it can be determined whether ectoparasites are coevolved with hosts at higher, similar, or lower rates. Microcomputers facilitate this study by providing easy access to analyses such as the neighbour-joining method, UPGMA, and cladistic which can be executed by several programs like SPSS (Norusis & SPSS Inc. 1994), PAUP (Swofford 1993) and MacCLADE (Maddison & Maddison 1992).

This thesis has been divided into several chapters and each section will emphasise different aspects of systematics. In this introductory section, I provide a basic review of the systematic problems and the object of study; skuas and their feather lice. Different opinions on skua classification and their supporting facts have been presented.

Chapter two deals with obtaining more data from skua specimens using morphometric analysis. In this analysis, twenty five (for exploring size variations) and fifteen characters (for examining shape differences) have been chosen for morphological investigation and eight museum specimens for each taxon for both sexes were measured. In addition, fifty-one measurements have been carried out (whenever possible) on each of eighteen skua skeletons available for study from the Natural History Museum, Tring. Multivariate morphometric analyses have been carried out by well-known software package such as SAS/STAT (SAS Inc. 1990), MINITAB (Minitab Inc. 1993), and SPSS (Norusis & SPSS Inc. 1993).

In addition to morphometric analysis, cladistic analysis of skuas was done on forty multistate characters. Explanation of these characters and how cladistic analysis has been carried out is given in Chapter three. Later, all these data are processed by computer analysis using specific programs such as MacCLADE (Maddison & Maddison 1992) and PAUP (Swofford 1993).

Chapter four investigates host-parasite coevolution and the relationships between these two life-systems. This chapter concentrates on ecological relationships between skuas and their lice, and the degree of host influence on their ectoparasite community. In this section, several methods pertaining to lice extraction from various forms of host including live, frozen, and museum specimens are described. This chapter also deals with the distribution of lice on their hosts, their diversities on each bird and infestation rates. The taxonomically-useful characteristics of feather lice are reviewed, along with their role as an alternative model or to provide supplementary evidence to other taxonomic methods such as molecular taxonomy or classical techniques whenever

these later approaches are unable to provide adequate data to resolve a taxonomic problem.

In Chapter five, the existence of convergent evolution between lice and host is discussed. Some well-preserved lice have been chosen for morphometric analysis and seventeen characters have been selected for this purpose. This chapter intends to reveal any morphological changes within a species of ectoparasite in different environments (hosts), and if this modification does occur, examines if they present any information about host relationships. Further study attempt to discover the degree of modification and whether it is presented in their morphology.

The progress of molecular studies on skuas and feather lice regarding coevolutionary research is discussed in Chapter six. This chapter also examines the mitochondrial DNA (mtDNA) characteristics and their advantages and disadvantages in taxonomic studies of birds and insects. Problems in deducing phylogenetical relationships based on molecular data are discussed in this section.

Finally, Chapter seven presents a general discussion about this research. Here I present a conclusive discussion based on various data available from current and previous studies. Suggestions about the most appropriate classification of skuas are also included, along with appropriate reasons.

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Chapter 2

Taxonomic Allocation of Skuas (Aves: Stercorariidae) by Multivariate Morphometric Analysis

2.1. Introduction

Some groups of animals, such as sibling species, are almost indistinguishable in general appearance. Rodents in the Family Echimyidae, *Proechimys dimidiatus* and *P. iheringi*, provide a good example of this predicament (Pessoa & Dosreis 1992). Homospecies like these are very difficult to identify using conventional keys based on physical expression. Identification of these homospecies therefore demands more evidence which ideally originates from various approaches. However, if only limited data are available, efforts have to be concentrated on extracting more information from a particular approach.

In morpho-taxonomy, increasing the number of characters in the analysis will provide more information and this can be done by scrutinising the study objects. The use of several morphological characters in a single analysis (i.e. multivariate morphometric analysis) permits morphological information to be summarised in numerical and graphical form and at the same time can express the hypothetical relationships in the data in a more appropriate fashion than univariate analysis (Daly 1985). As a result, multivariate morphometric analysis is a powerful tool in biological studies, especially in systematics. It successfully separated some conspecific or closely related birds such as American woodnymphs, *Thalurania* sp. (Escalante-Pliego & Paterson 1992), goshawks, *Accipiter* sp. (Burton & Alford 1994), subspecies of African bustards, *Eupodotis afra* (Crowe *et al.* 1994) and subspecies of falconet, *Microhierax fringillarius* (Kemp & Crowe 1994). Several studies determining the sex of birds have also applied discriminant analysis to morphometric data. Examples of these include Great skuas, *Catharacta skua* (Hamer & Furness 1991), Spanish imperial eagle, *Aquila*

adalberti (Ferrer & Delecourt 1992), fulmarine petrels, *Fulmarus* sp. (Van Franeker & Terbraak 1993), mynas, *Acridotheres tristis* and *A. javanicus* (Counsilman *et al.* 1994) and Parasitic jaegers, *Stercorarius parasiticus* (Phillips & Furness, in press). Some of the results of morphometric analysis have been used in strengthening findings from other methods. A combination of morphometric data and evidence from plumage development, for example, successfully determined the age and growth pattern of francolin, *Francolinus africanus* (Little & Crowe 1992). In another example, a concordance between morphometric analysis and Restriction Fragment Length Polymorphism (RFLP) analysis in the study of galliforms has also been obtained (Crowe *et al.* 1994).

Morphological variations among taxa have been used to allocate animals to appropriate groups. This is based on the assumption that all characters will vary over time and space. Any characters which evolve at a reasonably constant rate over a given period of time can be regarded as a useful phylogenetic tracer at the time scale considered (Sbordoni *et al.* 1991). The most common features used in morphometric studies of birds are variations in beak, tarsus and wing morphologies. Variation in these characters, normally size and shape differences, are very important for taxonomic comparison. These characters generally display a slight divergence even among closely related species (Leslie & Grant 1994). Variations in size, however, are more pronounced than those of shape (Wiens 1991). This may be because size modification involves a smaller genetic component than shape. Schluter (1984) in his study on darwin finches (*Geospiza fortis*) concluded that changes in shape will require more time (more selection) than size (less selection).

Variations in morphology are caused by several inter-related factors. The majority of morphological variations are influenced by evolutionary history or phylogenetical effects. Through natural selection, the most successful forms will survive. This 'good character' will be transmitted to offspring through inheritance to ensure a high fitness in the next generation. This process therefore, will lead to a slightly distinct feature in each species.

Geographical differences also play an important role in the physical appearance of birds. Every locality possesses a wide range of habitats which is particular to each area. These habitats present various environmental pressures (e.g. temperature, humidity and habitat condition) to their inhabitants. Differences in response to each parameter by various birds will result in specific adaptation and consequently particular modification in their morphology such as alteration in their size or shape of some characters or variation in their plumage colours (Aldrich & James 1991; Price 1991; Escalante-Pliego & Paterson 1992). An example of this modification is clearly shown in studies of the association of bird with habitat and altitudinal gradients (Barker 1980, James 1982, Johnston 1994).

Schluter (1982) suggested that changes in bird morphology may be due to the distribution of favoured food in the habitats as shown by Galapagos finches (*Geospiza sp.*). Birds have to enhance their foraging techniques to ensure higher success in extracting important resources such as food. To achieve this, modification of behaviour and some morphological characters is required especially for those which are related to the feeding mechanism such as bill, tarsus and wings. Birds living in open woodland (e.g. *Accipiter fasciatus*) possess a longer and narrower wing and longer tail (for greater speed and better control) to facilitate them in catching prey whereas their congener (such as *Accipiter novaehollandiae*), which lives in dense forest, has broad and short and concave wing shape for increasing manoeuvrability (Burton & Alford 1994).

Specific adaptation to variation in temperature and humidity can be found in the American robin (*Turdus migratorius*). Inhabiting hot areas such as Mexico, this bird has shorter legs and tarsus and larger legs where present in cool, humid forest along the Pacific coast (Aldrich & James 1991). This finding led the authors to postulate that smaller size would be an advantage in a warm, humid environment because a large ratio of respiratory surface area to body size would facilitate heat loss, whereas a larger bird would be expected in higher, cooler, or drier environments.

Mode of life also influences bird morphology. Predators, for instance, will generally have massive claws together with thicker and shorter tarsi to capture prey.

Whereas frugivorous birds in tropical forest normally will have a heavy bill for cracking seeds (Wattel 1973). In ground-foraging species, tarsus and toe are modified to improve foraging efficiency (Fitpatrick 1985). This effect can be clearly seen in warblers where ground-foraging species have longer tarsi than arboreal species (Miles & Ricklefs 1984). In addition to the foraging substrate, perching on different substrates will also result in modification of foot and leg structure such as in *Parus sp.* (Partridge 1976).

Interactions among animals such as species richness and species packing (Gatz 1980) and community organisation (Herrera 1978; Ricklefs & Travis 1980) also affect morphology. Comparison between skeletal morphologies of introduced North American and ancestral German populations of Eurasian tree sparrow (*Passer montanus*) showed that a smaller body size in the North American population. This is thought to result either from interspecific interactions and/or flight habit differences from their ancestral counterparts (St. Louis & Barlow 1991).

Finally, minor differences (microevolutionary changes) in bird or animal morphology may be due to several causes such as sexual dimorphism (Penzhorn *et al.* 1991; Van Wynsberghe *et al.* 1992; Seutin *et al.* 1993), migration (Aldrich & James 1991; Price 1991), parasitism (Titmus & Babcock 1981) or physiological demand (Ryan *et al.* 1989).

The degree of influence of the above-mentioned factors on morphological alteration depends on the quality and quantity of factors and the life stage of the animal. Some stages of animal development, especially early life, are crucial and highly affected by environmental conditions. Any changes in environmental condition at this time will significantly influence animal morphology. Adult great tits (*Parus major*) for instance are slightly smaller when limited food supply (less than average) was available during the chicks' growing stage (Garnett 1981). The morphology of adult birds is also influenced by the diet of their nestlings (James 1983; Boag 1987; Larsson & Forslund 1991).

Studies on various birds such as finches, *Geospiza sp.* (Boag & Grant 1978; Boag 1983; Grant 1983; Grant & Grant 1995), song-sparrow, *Melospiza melodia* (Smith & Dhondt 1980), great tits, *Parus major* (Garnett 1981; Gebhard-Henrich & van Noordwijk 1991) and blue tits, *Parus caeruleus* (Dhondt 1982) show that variation in birds' external morphology are highly heritable. The degree of heritability of particular morphological traits varies among birds. Two major factors which normally influence heritability are genetics and the environment. The genetical factor is accountable for 75% of total phenotypic variation in body size of great tits (Garnett 1981). Smith & Dhondt (1980) showed that environmental or ecological pressures are responsible for morphological variations in song sparrow.

The fact that size and shape variations are highly heritable allow one to use these morphological differences as supplementary evidence for taxonomic description and, therefore, could play a meaningful role in systematics. The results of morphometric analysis have already been used by taxonomists to justify synonymy as well as to recognise new taxa at the species level (Daly 1985). Morphological evolution may dissociate from molecular evolution and therefore, morphological similarities between taxa should reflect a similar response to selective forces (Cherry *et al.* 1982). A few studies on the morphology of southern hemisphere large skuas have been carried out (Hamilton 1934, Peter *et al.* 1990), but none of these morphometric studies were designed to answer taxonomic questions. By making a morphometric study of all taxa of skuas, it is hoped that it will be possible to derive an accurate classification of these birds.

2.2. Materials and Methods

In total, 72 skin specimens of skuas (listed in Appendix 2.1) obtained from the Natural History Museum at Tring were examined. Eight specimens (four of each sex) were chosen arbitrarily from museum collection (after excluding incomplete specimens) to represent each taxon (refer to section 1.1, chapter 1 for taxon definition). Only adult

birds were measured and different sexes were analysed separately. This precaution was taken to minimise the effects of ontogenic and sexually dimorphic characters present in skuas, though only very few are involved. Twenty five characters were used to investigate size variation. All these characters were adopted from Schnell (1970) and are listed in Appendix 2.2 and schematically illustrated in Appendix 2.3. For examining shape differences, fifteen characters were used. These characters are ratios of one character to another and some of these characters were used in measuring size differences. Characters used for studying shape differences are listed in Appendix 2.4.

In addition to skin specimens, measurement on skuas skeletons were also included. For this study, eighteen skua skeletons were available for examination (Appendix 2.5). All specimens were provided by the Natural History Museum at Tring. Unfortunately not all samples possess complete skeletal structure. For some samples, only skull or sternum was available. For the complete specimens fifty one characters were measured by using vernier callipers. Which ever of these fifty one characters were available in incomplete specimens were measured. Most of measurement characters, except two (width and length of narial of skull) were adopted from Schnell (1970). Explanation of these characters is presented in Appendix 2.6.

Due to the small number of samples, each specimen was treated separately as a single taxonomic unit rather than presenting all four samples as a mean for the respective taxon. This precautionary step was designed to check whether small sample number contributes any significant effects to the result of this study. It is expected that these taxonomic units shall be classified together (if they belong to same taxon) if variations within taxa are small relative to variations between taxa.

Morphometric variation between study birds can be presented graphically as bivariate graphs. The differences between observations can be deduced from the patterns of individuals clusters produced on bivariate plots. The dimension of the multivariate data therefore, has to be reduced into two dimensions before it can be plotted on bivariate graph. This can be done by using principal component analysis (PCA). The capability in presenting data in an appropriate dimension without reducing

the information contained in the data-sets is a major advantage of this approach. Principal component analysis is a one group method, therefore it will be computed for all variables individually for each group (Airoldi & Flury 1988). The assumption of this study is that all characters contribute equally to morphological variation, leading to the standardisation of data-sets and the use of correlation matrices.

Later, data are processed by two different methods of multivariate analysis, cluster analysis and canonical discriminant analysis (CANDISC). These analyses are provided by common statistical packages such as MINITAB (Minitab Inc. 1993), SPSS (Norusis & SPSS Inc. 1992), and SAS (SAS Inc. 1990). The advantage of these methods is their capability of investigating groups based on the evidence from all variables simultaneously. Selection of informative variables for extracting morphological information has been made through stepwise analysis. Selective variables are then processed by the same multivariate analyses.

Three distance indices have been used in this study to determine the morphological distances in the above multivariate analyses. Euclidean distance (Manly 1994) and Manhattan distance (Cherry *et al.* 1982) are used in cluster analysis, whereas Mahalanobis distance (Mahalanobis 1948 in Manly 1994) is used in canonical discriminant analysis. All these indices are represented by formulae below;

(i) *Euclidean distance* : $\mathbf{d_{ij} = \sqrt{\{(x_{i1} - x_{j1})^2 + (x_{i2} - x_{j2})^2\}}}$

where *i* and *j* are two respective taxa.

(ii) *Manhattan distance* : $d(i,k) = \sum_j |X_{ij} - X_{kj}|$

where d(i,j) is the mean value of the relative length of particular trait in row *i* and column *j* is the distance between observations *i* and *j*.

(iii) *Mahalanobis distance* : $\mathbf{D_{ij}^2 = (\mu_i - \mu_j)' \; V^{-1} \; (\mu_i - \mu_j)}$

where μ_i and μ_j are the vectors of means for the *i*th and *j*th population respectively and **V** is the covariance matrix.

2.3. Results

Descriptive statistics of 25 characters used in this study reveals that there is a major size difference between small and large skuas groups (ANOVA: F>96.31; df=168.90, 21.11; p<0.0001 for female and F>139.46; df=179.22, 22.4; p<0.0001 for male). As defined by their name, large skuas should dominate maximum values for the majority of measured characters. However, this is not true for all characters studied. Some characters such as the distance from the tip of outer primary to adjacent primaries (MC14 for female; ANOVA: F=11.12, df=216.80, 27.10; p<0.0001 and MC15 for both sexes, ANOVA: F=4.19; df=129.70, 16.20; p=0.002 for female and F=29.87; df=245.1, 30.6; p<0.0001 for male) indicate a reverse effect by showing that small skuas have greater measurement than large skuas.

Surprisingly, the controversial taxon, Pomarine skua indicates that some of their features (six for female; MC4, MC11, MC12, MC14, MC18, MC20 and three for male; MC11, MC14, MC18) are similar to or greater than those of some large skuas. Female Pomarine skuas show a slightly similar measurement to female Falkland skuas in characters of gony length (MC4, ANOVA: F=31.20; df=284.22, 35.53; p<0.0001) and wing length (MC12, ANOVA: F=148.20; df=601.15, 48.09; p<0.0001). Whereas on measurement of width of the outer vane (MC20) and body length (MC11), female Pomarine skuas show higher measurements than female South Polar skua (ANOVA: F=33.58; df=664.22, 83.03; p<0.0001 and F=96.31; df=21.10, 16.80; p<0.0001 respectively). Female Pomarine skuas also show higher measurements than Chilean and Tristan skuas for the latter character (MC11, ANOVA: F=96.31; df=21.11, 16.89, p<0.0001). Both sexes of Pomarine skuas show domination in two characters; MC14 (distance from tip of ninth to eight primary feather, ANOVA: F=11.12; df=216.88,

27.11; $p<0.0001$ for female and $F=9.07$; $df=141.93, 17.74$; $p<0.0001$ for male) and MC18 (rectrix length, ANOVA: $F=124.99$; $df=112.49, 14.06$; $p=0.0001$ for female and $F=59.45$; $df=234.61, 29.32$; $p<0.0001$ for male), by showing a highest measurement values even when compared to large skuas. Male Pomarine skuas are not as successful in dominating measurement values as their female counterpart. Contrary to their female counterpart, male Pomarine skuas are smaller than male Chilean and Tristan skuas but are similar in size to male South Polar skuas (ANOVA: $F=139.46$; $df=179.22, 22.40$; $p<0.0001$).

In comparing morphological variations within a group, it is clear that only a particular species dominates maximum or minimum values for each measured characters (Table 2.1). Within the small skua group for example, Pomarine skua shows maximum values in most characters (23 for female and 19 for male), followed by Arctic skua (one for female, MC15 and five for male) and Long-tailed skua (both sexes dominate one character each, MC09 for female and MC22 for male). Since the small skua group only comprises a small number of birds (i.e. only three taxa), taxa which failed to dominate maximum values will shows more minimum or intermediate values. Long-tailed skuas for example shows more minimum value for measured characters compare to Arctic and Pomarine skuas.

Morphological variations among the large skua group are more complicated than small skuas. In this group, each taxon except female Falkland skua possesses a highest measurement value for at least a single character. Brown skua shows their domination in morphological measurements by having a maximum value for seventeen and thirteen characters for male and female respectively (Table 2.2.). The other skua taxon which possesses a larger maximum value for studying characters is Tristan skua. Male skuas of this taxon have a maximum value in six characters whereas their female counterpart have two (MC07 and MC25, shared with Brown skua). Other skuas normally have a maximum value in a smaller number of characters. These were female Great (4 characters), Chilean (3 characters) and female South Polar skuas (2 characters).

Table 2.1. Morphometric analysis of small skuas. Minimum and maximum values for within group comparisons. AS, Arctic skua; LT, Long-tailed skua; PS, Pomarine skua.

	minimum values		maximum values	
Characters	female	male	female	male
MC01	LT	AS	PS	PS
MC02	AS	LT	PS	PS
MC03	LT	LT	PS	PS
MC04	AS	LT	PS	PS
MC05	LT	LT	PS	PS
MC06	AS	LT	PS	PS
MC07	AS	LT	PS	PS
MC08	LT	LT	PS	PS
MC09	PS	LT	LT	AS
MC10	AS	PS	PS	AS
MC11	LT	LT	PS	PS
MC12	LT	LT	PS	PS
MC13	LT	LT	PS	PS
MC14	AS	AS	PS	PS
MC15	PS	PS	AS	AS
MC16	LT	LT	PS	PS
MC17	AS	LT	PS	PS
MC18	LT	AS	PS	PS
MC19	LT	LT	PS	AS
MC20	LT	LT	PS	AS
MC21	LT	LT	PS	PS
MC22	LT	AS, PS	PS	LT
MC23	LT	LT	PS	PS
MC24	LT	LT	PS	PS
MC25	LT	LT	PS	PS

Table 2.2. Morphometric analysis of large skuas. Minimum and maximum values for each characters were presented by the taxon which championed in that aspect. SP, South polar skua; FS, Falkland skua; CS, Chilean skua; GS, Great skua; BS, Brown skua; TS, Tristan skua.

	minimum values		maximum values	
Characters	female	male	female	male
MC01	CS	FS	BS	BS
MC02	CS	FS	BS	TS
MC03	SP,FS	SP	BS	TS
MC04	FS	SP	CS	TS
MC05	SP	CS	BS	BS
MC06	SP,GS,TS	SP	BS	FS
MC07	FS	SP	TS	BS
MC08	CS	SP, TS	GS	GS
MC09	CS	FS	BS	BS
MC10	SP	GS	BS	CS
MC11	SP	SP	BS	BS
MC12	FS	SP	SP	BS
MC13	GS	TS	BS	BS
MC14	TS	BS	GS	CS
MC15	BS	SP	GS	CS
MC16	CS	CS	BS	TS
MC17	FS,CS,TS	GS	BS	SP
MC18	CS	CS	BS	BS
MC19	GS	CS	BS	BS
MC20	SP	CS	GS	BS
MC21	FS	SP	BS	BS
MC22	SP	SP	BS	BS
MC23	SP	SP	BS	BS
MC24	TS	SP	SP	TS
MC25	SP	SP	BS,TS	TS

Four skuas have a maximum value in a single character only. These were male South Polar, male Falkland, male Great and female Chilean skuas.

As for small skuas, large skuas which possess less characters with maximum values will have more chance of possessing a character with a minimum value (Table 2.2). This was proved by South Polar and Chilean skuas. Female South Polar skua for instance show a minimum value in thirteen characters whereas their male counterpart shows a minimum value in nine characters. Chilean skua on the other hand possess less character with minimum value than South polar skua. They only have five (for male) and seven characters (for female) which show a minimum values. It is clear that Falkland skua have more characters with minimum values than characters with maximum values. This skua has a minimum value in three and six characters for male and female respectively. Other female skuas only have four (Tristan skua) and three characters (Great skua) with minimum values. While male Great and Tristan skuas show a minimum value in two characters each. The only taxon which has a single character with minimum value is the Brown skua. This taxon has a minimum value for character of MC14 (male) and MC15 (female).

Results from PCA analysis clearly indicates a separation between small and large skuas (Figure 2.1 and 2.2). Study of size differences among skuas by using twenty five characters shows that small skuas do possess totally different size measurements from large skuas (ANOVA: $F>96.31$; $df=168.96, 21.11$; $p<0.0001$ for female and $F=139.46$; $df=179.22, 22.4$; $p<0.0001$ for male). Both sexes of small skuas differ from their respective sex of large skuas in three morphological characters. These were MC14 (ANOVA: $F=11.12$; $df=216.88, 27.11$; $p<0.0001$ for female and $F=9.07$; $df=141.93, 17.74$; $p<0.0001$ for male), MC15 (the distances between tip of outer primary to adjacent primary feather, ANOVA: $F=4.19$; $df=129.72, 16.22$; $p=0.002$ for female and $F=29.87$; $df=245.06, 30.63$, $p<0.0001$ for male) and rectrix length (MC18, ANOVA: $F=124.99$; $df=112.49, 14.06$; $p<0.0001$ for female and $F=59.45$; $df=234.61, 29.32$; $p<0.0001$ for male). All taxa of small skuas show a larger values in these characters (Table 2.3, Figure 2.1 and 2.2). These variations are shown by the first principal

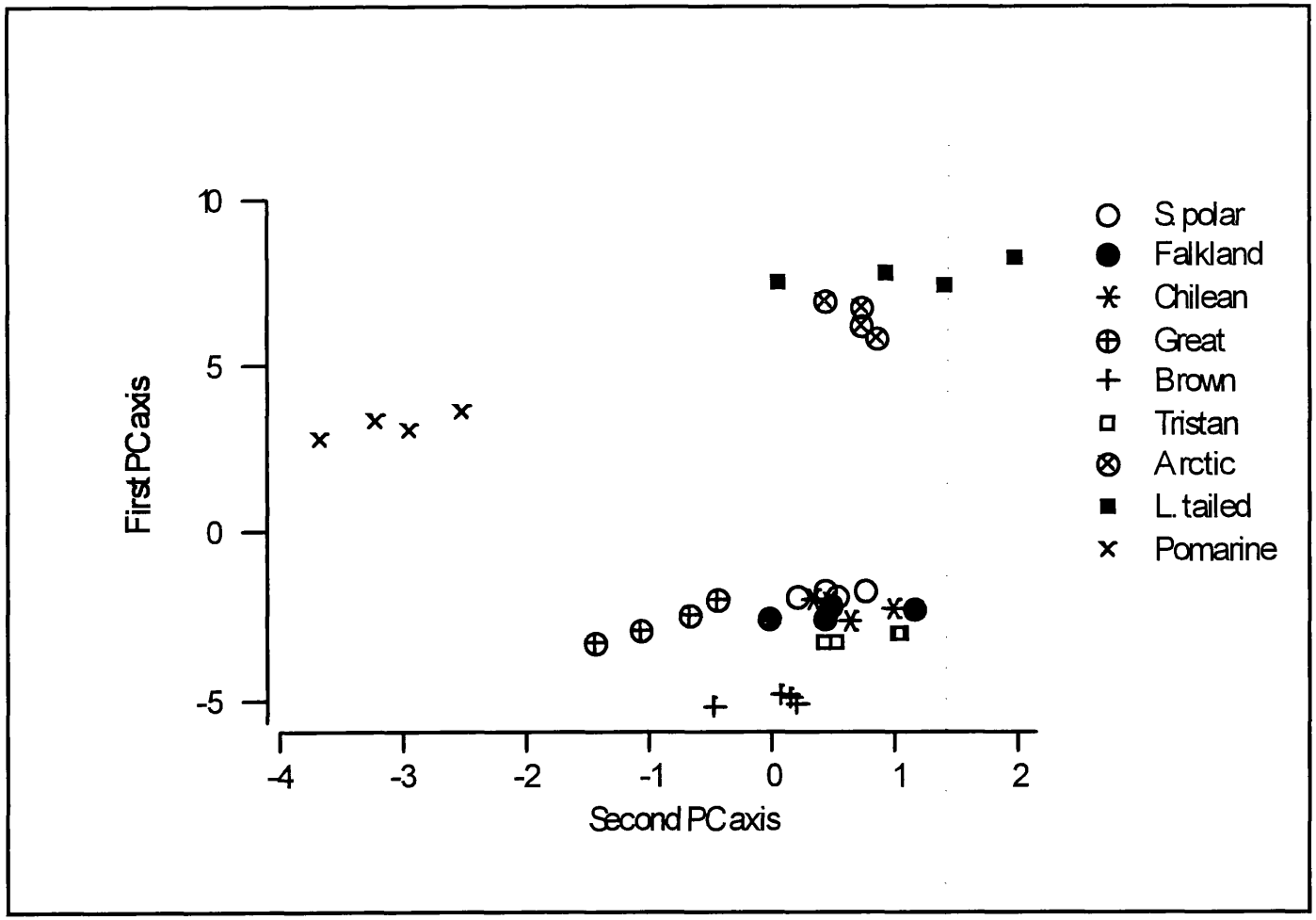

Figure 2.1. Scatter plot depicts size differences among female skuas.

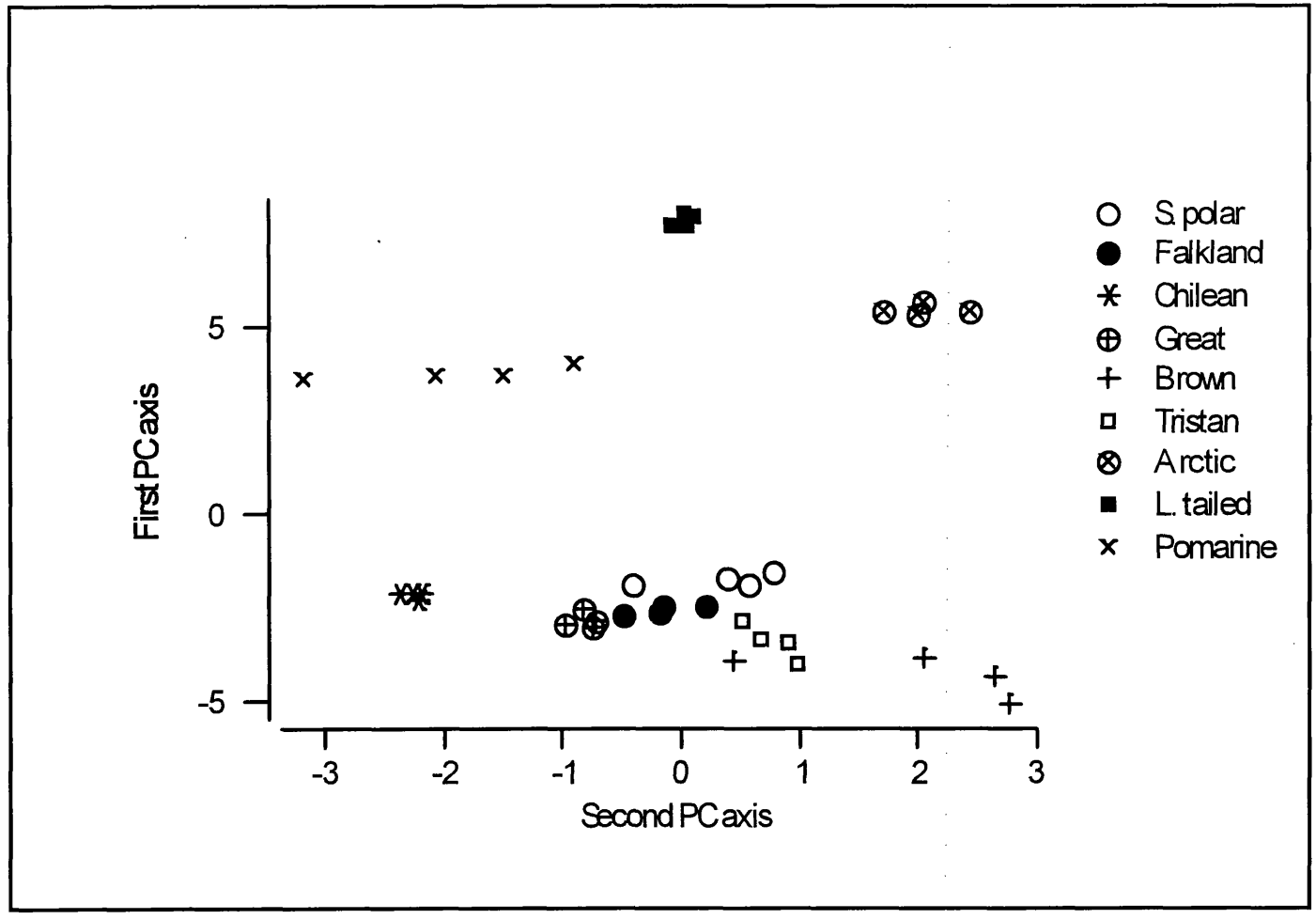

Figure 2.2. Scatter plot of PCA scores shows size separation among male skuas.

Table 2.3. Morphometric analysis of size variation of skua skins. Results of first three principal components values and the distribution of components loading for each measurement characters.

Components	PC1		PC2		PC3	
Sex	male	female	male	female	male	female
Eigenvalue	18.291	19.569	2.335	1.682	1.387	0.864
Cumulative	0.732	0.783	0.825	0.850	0.881	0.885
	component loadings for each characters					
MC01	-0.230	-0.222	-0.037	0.063	0.059	0.012
MC02	-0.230	-0.223	-0.064	0.057	0.067	0.005
MC03	-0.222	-0.221	-0.152	0.009	0.060	0.043
MC04	-0.212	-0.195	-0.160	-0.071	-0.029	0.275
MC05	-0.210	-0.219	0.073	0.035	0.021	-0.093
MC06	-0.223	-0.216	-0.121	0.046	0.066	-0.060
MC07	-0.231	-0.214	-0.018	0.029	-0.034	0.087
MC08	-0.204	-0.177	-0.105	-0.202	-0.014	-0.191
MC09	-0.220	-0.201	0.065	0.212	0.162	-0.067
MC10	-0.217	-0.213	0.020	0.065	0.247	-0.149
MC11	-0.210	-0.201	-0.043	-0.309	-0.248	0.021
MC12	-0.223	-0.205	-0.077	-0.204	-0.181	0.182
MC13	-0.227	-0.218	0.017	-0.028	-0.043	0.170
MC14	0.058	0.114	-0.511	-0.478	-0.206	-0.476
MC15	0.114	0.149	-0.172	0.039	0.293	0.063
MC16	-0.217	-0.201	-0.020	-0.128	-0.214	0.198
MC17	-0.221	-0.194	-0.088	0.076	-0.017	-0.054
MC18	0.088	0.031	-0.242	-0.699	-0.690	0.308
MC19	-0.120	-0.203	0.449	0.050	-0.255	0.030
MC20	-0.108	-0.177	0.525	-0.100	-0.181	-0.561
MC21	-0.230	-0.223	0.035	0.025	-0.036	0.109
MC22	-0.198	-0.204	0.120	-0.034	0.045	-0.282
MC23	-0.165	-0.223	-0.218	0.017	0.215	0.008
MC24	-0.230	-0.222	-0.041	-0.009	-0.025	0.044
MC25	-0.230	-0.223	-0.050	-0.006	-0.001	-0.008

component (PC) axis which generally depicts size differences among skuas. This axis represents 78.3% (for female) and 73.2% (for male) of variation characters examined while the second PC axis represents another 6.7% (for female) and 9.3% (for male) of total variations. Variation from second PC axis is useful in separating Pomarine skua from other small skuas, and it also uses similar characters such as MC14 (ANOVA: $F=11.12$; $df=216.88, 27.11$; $p<0.0001$ for female and $F=9.07$; $df=141.93, 17.74$; $p<0.0001$ for male) and MC18 (ANOVA: $F=124.99$; $df=112.49, 14.06$; $p<0.0001$ for female and $F=59.45$; $df=234.61, 29.32$; $p<0.0001$ for male) to achieve this.

Analysis of shape variation favours the clustering of all skuas into three groups (Figure 2.3 and 2.4). The first group composed of all small skuas, the second group containing South Polar, Tristan and Chilean skuas, whereas the third group is characterised by Falkland and Brown skuas. Great skuas show instability in their group allocation. Based on shape differences among female skuas, it is placed together with Falkland and Brown skuas in the third group but evidence from shape variation of male skuas allocates them in the second group. All small skuas which compose first group are separated from each other by the first PC axis (represents 34.3% and 29.2% of total variation for male and female respectively). Skuas in this group possess a higher ratio of nostril length (relative to culmen) and more elongated culmen. The second group of skuas was separated from the third group by second PC axis. This axis which represents 16.1% and 17.8% of total variation (for male and female respectively) shows that skuas from the second group are different from those of the third group in terms of having a relatively longer wing (to body length) and elongated but compressed upper mandible (Table 2.4 and Appendix 2.4).

Although there is a lot of variation between members of small and large skua groups, the distinctions among taxa within each group (especially in large skuas) are small and hardly visualised by PCA scores. Further study on all members of large skuas (small skuas were excluded from data-sets) reveals that although the degree of variation between taxa is small, but it is sometimes sufficient for allocating some taxon. These results were clearly presented in Figure 2.5 to 2.8. Evidence from size variation provide

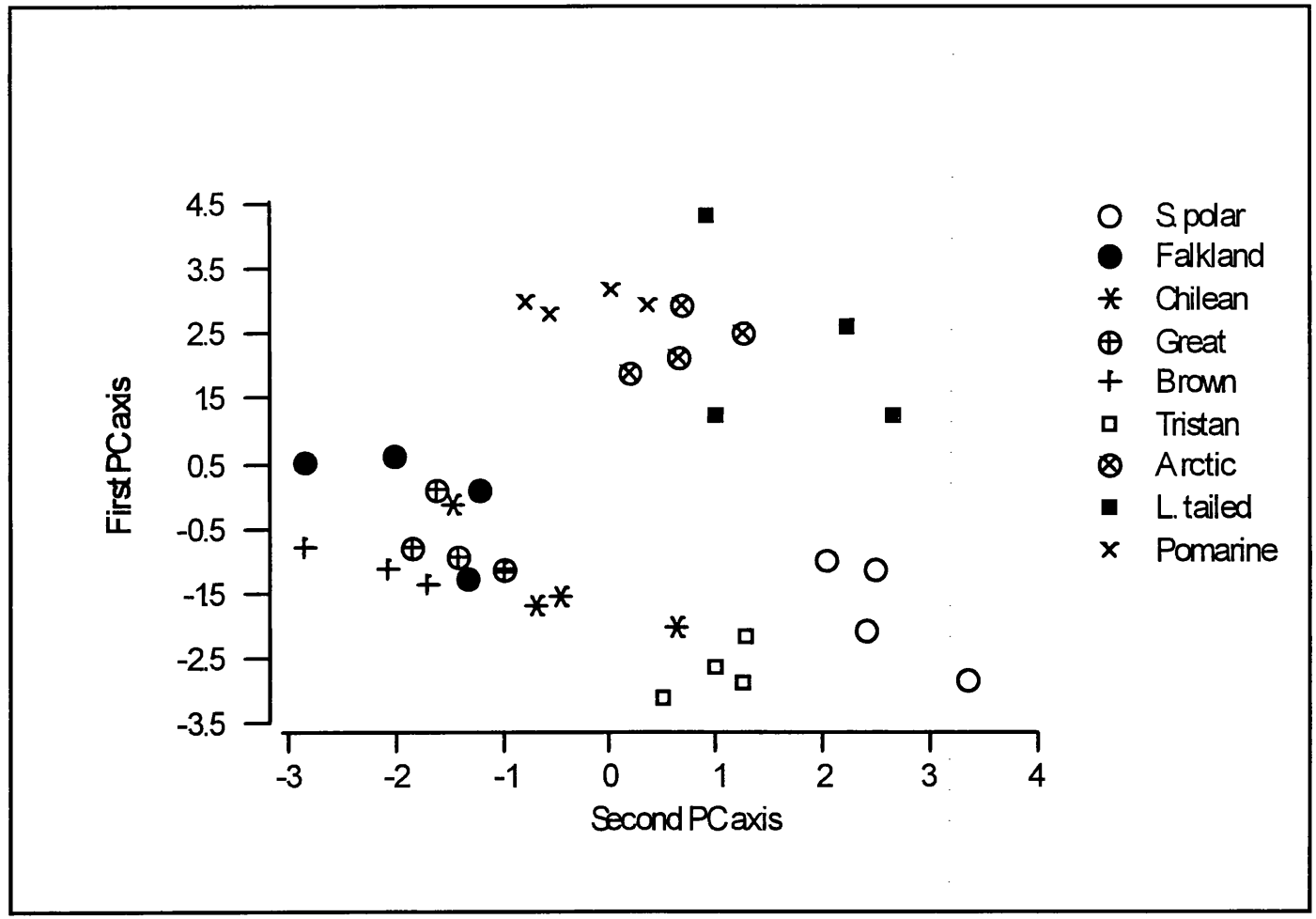

Figure 2.3. Scatter plot of PCA scores depicts shape differences among female skuas.

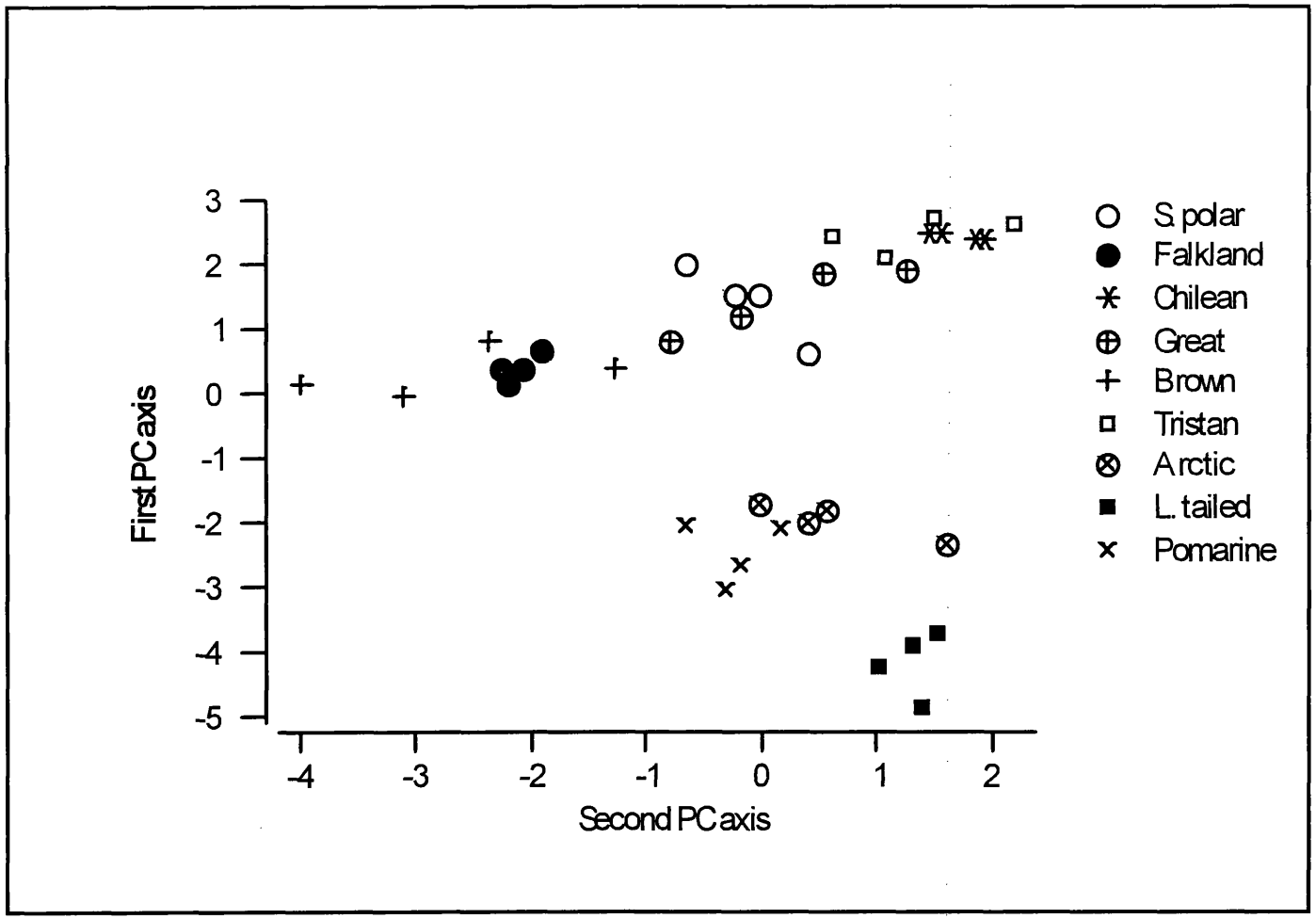

Figure 2.4. Scatter plot of PCA scores shows the differences in shape morphology among male skuas.

Table 2.4. Morphometric analysis of shape variation of skua skins. Results of first three principal component values and the distribution of component loadings for each measurement characters.

Components	PC1		PC2		PC3	
Sex	male	female	male	female	male	female
Eigenvalue	5.143	4.377	2.423	2.673	2.087	1.751
Cumulative	0.343	0.292	0.504	0.470	0.644	0.587
			component loadings for each characters			
MC1/MC5	-0.171	0.027	0.416	0.406	0.094	0.291
MC2/MC6	-0.103	-0.252	0.279	0.108	-0.174	-0.021
MC3/MC7	-0.242	0.261	0.324	0.105	0.282	0.405
MC3/MC11	0.397	-0.437	0.148	-0.030	0.132	0.055
MC4/MC11	0.348	-0.261	0.272	0.293	0.192	-0.232
MC8/MC3	-0.362	0.414	0.076	0.075	-0.295	-0.117
MC8/MC11	0.091	0.100	0.268	0.061	-0.276	-0.043
MC9/MC3	0.206	-0.257	-0.244	-0.085	-0.360	0.336
MC10/MC4	0.231	-0.261	-0.182	-0.309	-0.336	0.343
MC12/MC11	-0.025	0.036	0.478	0.429	-0.374	-0.204
MC20/MC19	-0.223	0.105	-0.002	-0.414	0.303	-0.056
MC21/MC11	0.398	-0.450	0.048	-0.010	-0.091	-0.091
MC26	0.315	-0.192	0.029	0.461	0.378	-0.019
MC27	-0.128	-0.033	-0.273	0.154	0.188	0.553
MC28	-0.252	0.201	-0.271	0.147	-0.065	0.298

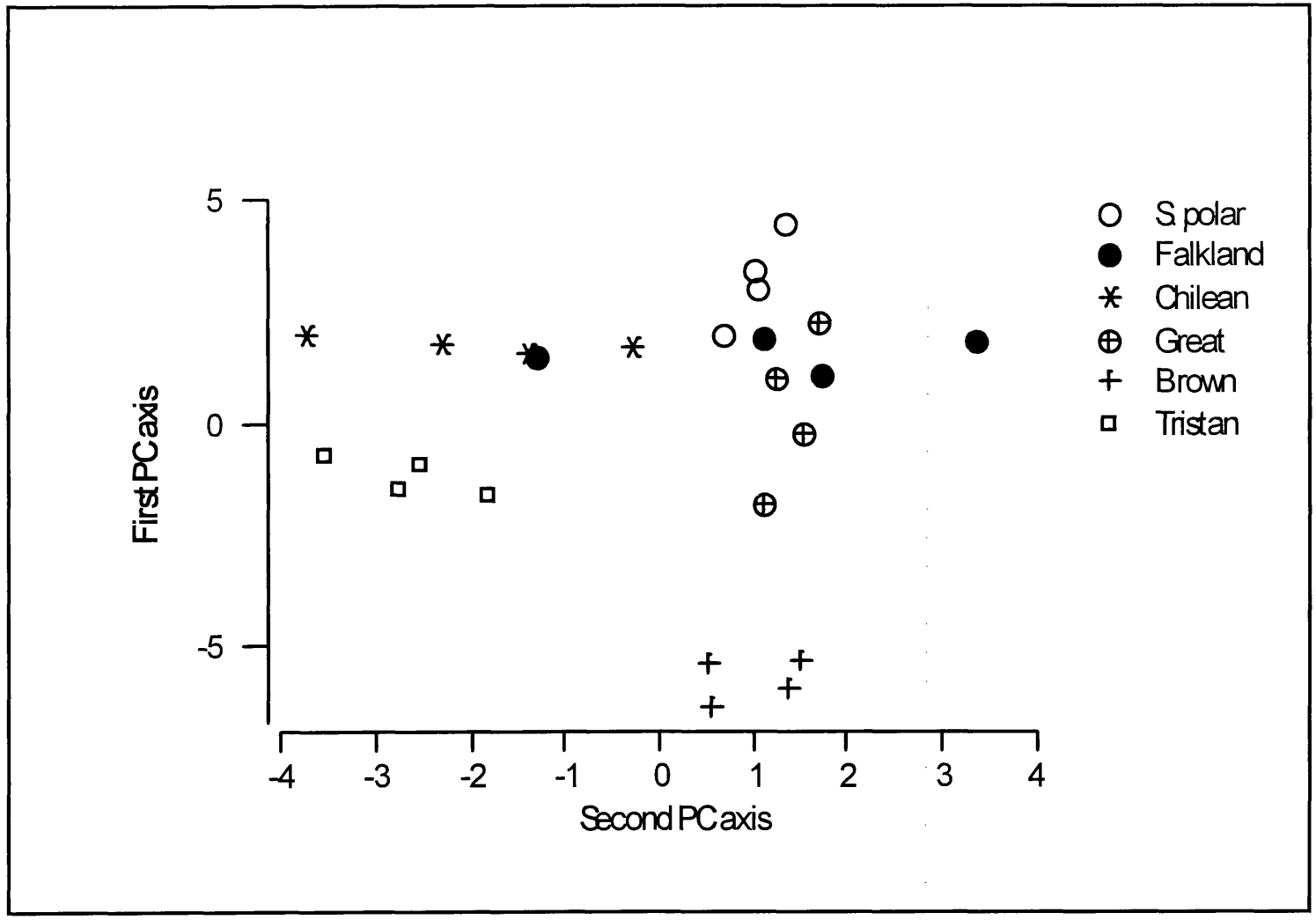

Figure 2.5. Scatter plot showing size variation among female large skuas. Analysis was based on 25 measurement of morphological characters.

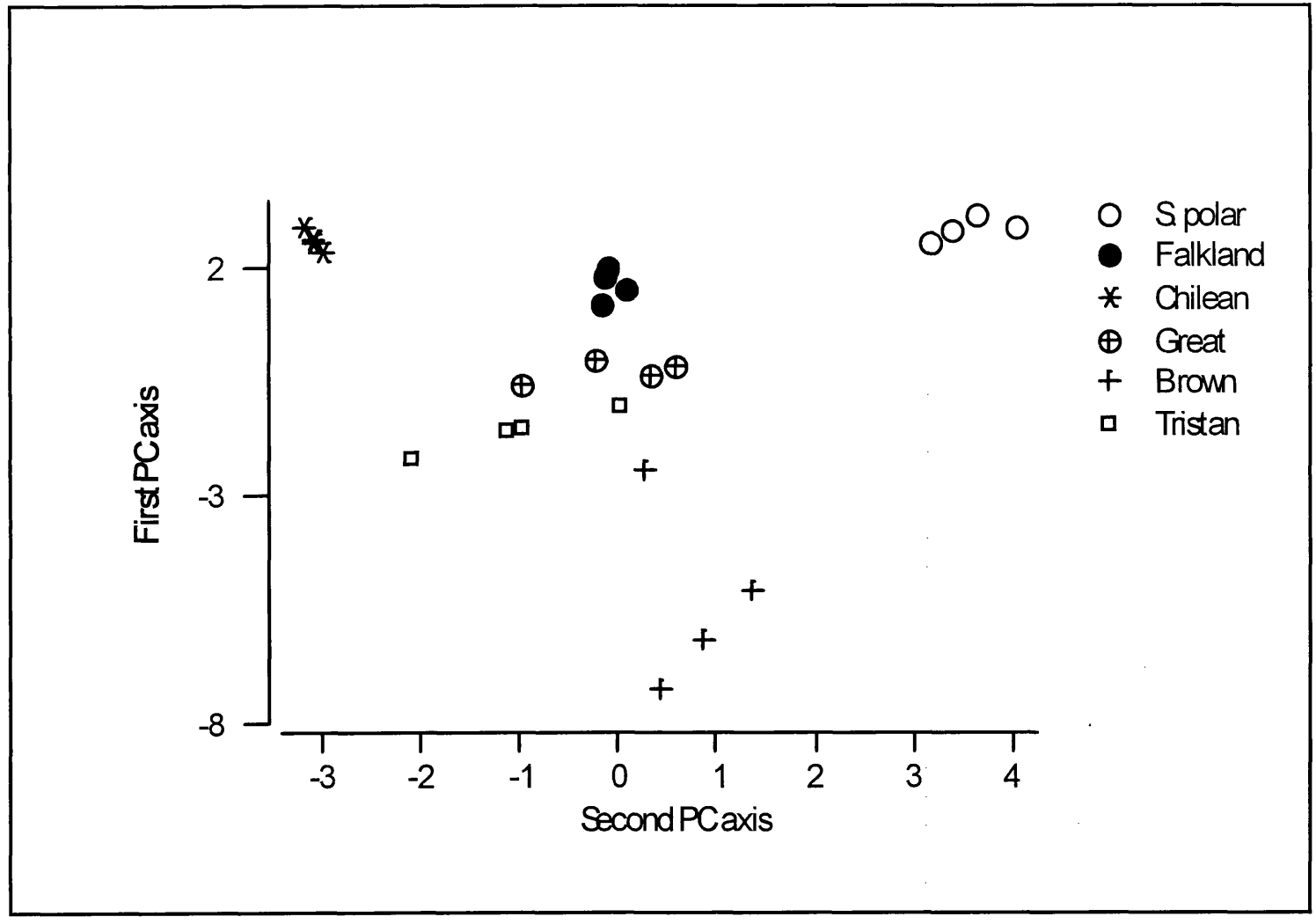

Figure 2.6. Scatter plot showing size variation among male large skuas. Analysis was based on 25 measurement of morphological characters.

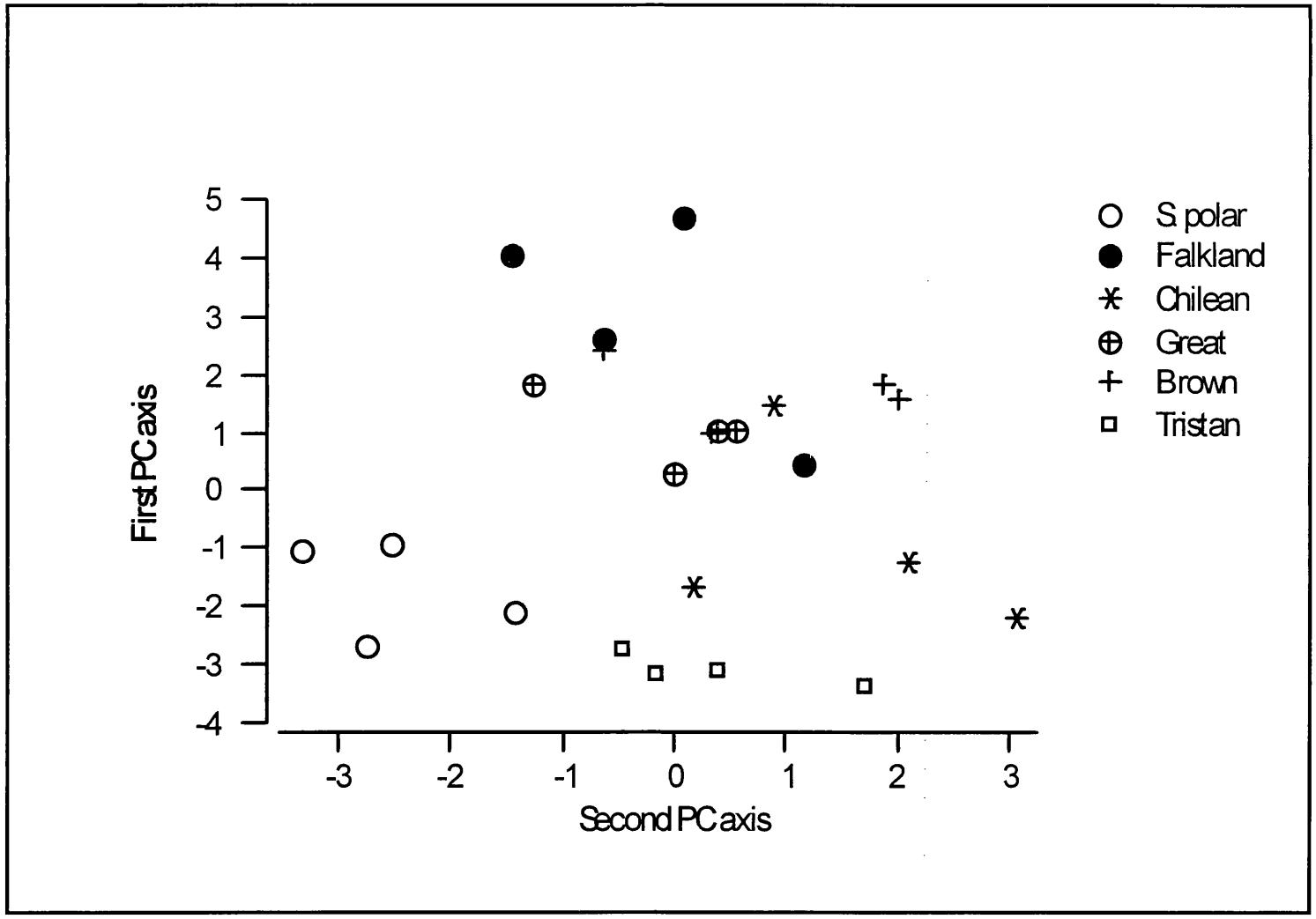

Figure 2.7. Scatter plot showing shape variation among female large skuas. Analysis was based on 25 measurement of morphological characters.

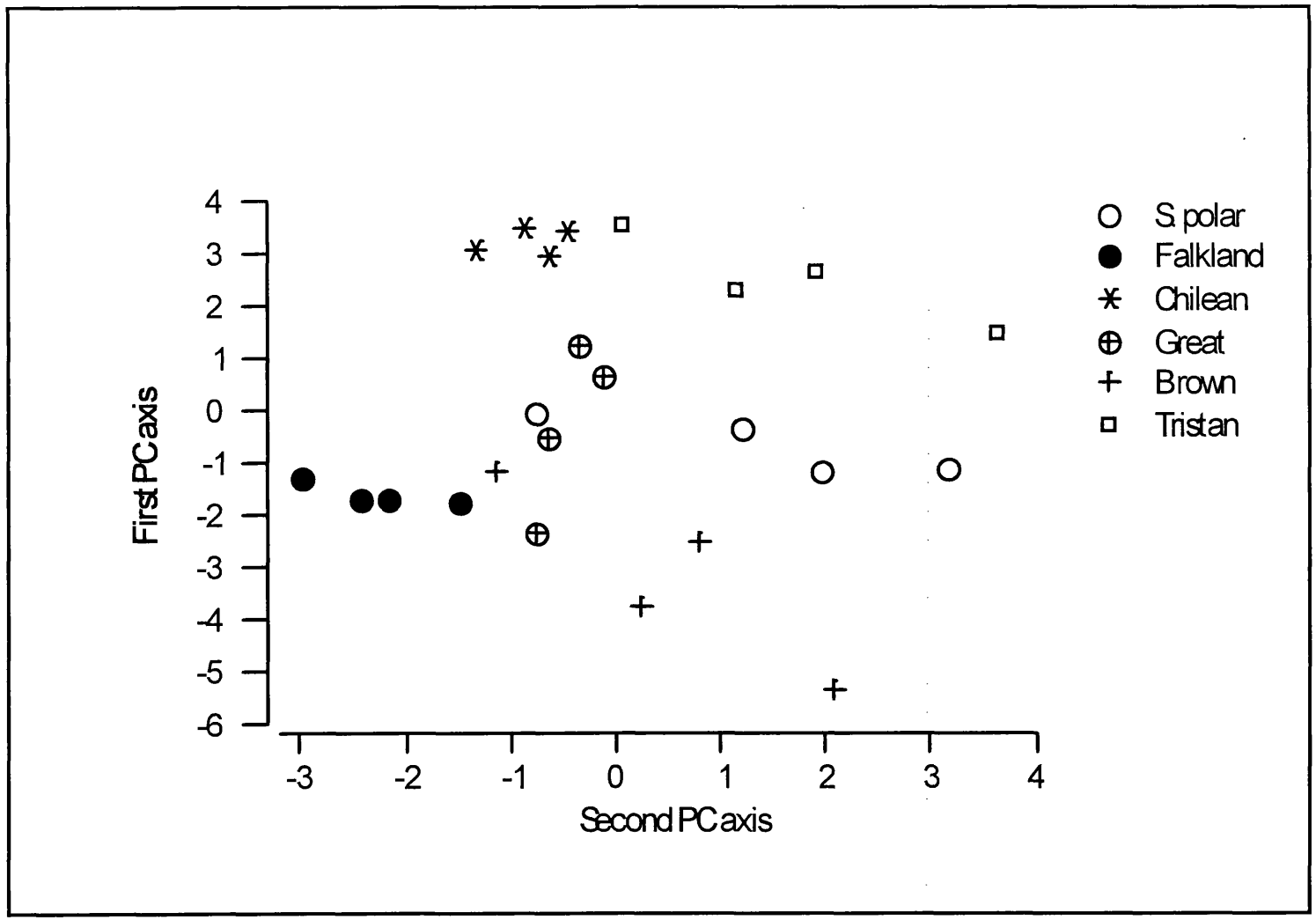

Figure 2.8. Scatter plot showing shape variation among male large skuas. Analysis was based on 25 measurement of morphological characters.

clearer classification than shape. Great skua is always placed at the centre of the scatter plot, suggesting that this taxon may share a higher degree of similarity with other large skua members in several morphological characters. Brown skua is different from other skuas by having a longer tarsus, wider bill and wider inner vane of rectrix for male and longer culmen and lower mandible for female (Table 2.5 and Appendix 2.2). Chilean and Tristan skuas can be distinguished from others based on several features of the bill. Both taxa have a higher measurement in gony length, culmen length and bill width. Male South Polar skuas is easier to be identified from others due to its longer secondary feather (MC13, ANOVA: $F=621.69$; $df=946.23, 118.27$; $p<0.0001$) and rectrix length (ANOVA: $F=59.45$; $df=234.61, 29.32$; $p<0.0001$). Other large skuas, Falkland and Great skuas are difficult to distinguish on the basis of size variation.

Variation in shape morphology successfully separated Falkland and South Polar skuas from other large skuas. Male Falkland skua is distinct from other large skuas by its unique features, wider rectrix (ANOVA: $F=28.81$; $df=535.50, 66.94$; $p<0.0001$) and relatively (to body length) longer nostril (ANOVA: $F=17.22$; $df=41.12, 5.14$; $p<0.0001$). Whereas female South polar skua have longer tarsus (ANOVA: $F=594.70$; $df=51.10, 6.38$; $p<0.0001$) and gonys (ANOVA: $F=31.20$; $df=284.22, 35.53$; $p<0.0001$) than other skuas. Analysis of shape morphology also separated male Chilean and Tristan skuas from other skuas by using relative bill length (ANOVA: $F=32.54$; $df=72.4, 9.01$; $p<0.0001$). Allocation of these skuas were done by first PC axis which represents 41.2% and 37.2% (for male and female respectively) of total variation. Other skuas (Great and Brown skuas) cannot be differentiated from other large skuas by using evidence from shape variation (Figure 2.7 & 2.8 and Table 2.6).

Skeletal data presents similar results to the analysis of morphology of skin specimens. However, only ten characters can be used in comparing eighteen skuas due to imperfect preservation or absence of some section of bone. These characters are SK01, SK04-08, SK10 and SK12-14 (refer to Appendix 2.6 for character explanations). After missing characters were excluded, skeletal data clearly show that the small skuas are distinctly separated from large skuas (Figure 2.9). Among large skuas, Falkland

Table 2.5. Morphometric analysis of size variation of large skuas (all small skuas were excluded). The distribution of principal components values and components loadings for each character are given.

Components	PC1		PC2		PC3	
	male	female	male	female	male	female
Eigenvalue	8.9894	9.4776	4.3634	3.7361	3.3644	3.1193
Cumulative	0.360	0.379	0.534	0.529	0.669	0.653
	components loadings for each characters					
MC1	-0.228	-0.269	0.001	0.101	-0.339	0.253
MC2	-0.176	-0.292	-0.224	0.029	-0.281	0.136
MC3	0.024	-0.281	-0.402	-0.157	0.015	0.056
MC4	-0.078	-0.070	-0.384	-0.298	-0.099	0.259
MC5	-0.108	-0.243	0.138	-0.065	0.368	-0.289
MC6	-0.010	-0.153	-0.208	0.080	0.272	-0.304
MC7	-0.293	-0.166	-0.056	-0.295	0.125	0.234
MC8	-0.036	-0.100	-0.027	0.243	0.381	-0.089
MC9	-0.170	-0.199	-0.021	0.212	-0.346	0.060
MC10	-0.061	-0.179	-0.178	0.299	-0.372	-0.086
MC11	-0.278	-0.272	0.030	0.212	0.110	-0.052
MC12	-0.254	-0.069	-0.035	0.087	0.126	0.483
MC13	-0.199	-0.179	0.234	0.039	0.052	0.164
MC14	0.182	0.052	-0.129	0.187	-0.052	-0.024
MC15	0.061	0.195	-0.309	0.120	0.146	0.125
MC16	-0.211	-0.075	0.019	0.229	0.060	0.145
MC17	0.077	-0.071	0.212	0.300	-0.201	0.140
MC18	-0.239	-0.182	0.251	0.259	0.093	0.286
MC19	-0.230	-0.137	0.238	0.185	-0.100	-0.021
MC20	-0.295	-0.141	0.143	0.166	-0.071	-0.301
MC21	-0.300	-0.270	-0.047	-0.208	-0.097	0.109
MC22	-0.290	-0.257	-0.162	0.080	0.107	-0.225
MC23	0.145	-0.254	-0.190	-0.215	0.037	-0.158
MC24	-0.247	-0.242	-0.268	-0.263	0.051	0.000
MC25	-0.241	-0.264	-0.227	-0.214	0.144	-0.137

Table 2.6. Morphometric analysis of shape variation of large skuas (all small skuas were excluded). The distribution of principal components values and component loadings for each character are given.

Components	PC1		PC2		PC3	
	male	female	male	female	male	female
Eigenvalue	6.180	5.581	2.894	2.553	1.889	1.758
Cumulative	0.412	0.372	0.605	0.542	0.731	0.660
	component loadings for each characters					
MC1/5	0.260	-0.154	0.139	-0.405	0.106	-0.111
MC2/6	0.092	-0.213	0.521	-0.195	0.018	0.299
MC3/7	0.313	0.260	-0.294	-0.027	0.184	-0.153
MC3/11	0.372	-0.347	-0.165	0.137	0.003	0.132
MC4/11	0.379	-0.350	0.031	0.081	0.145	-0.156
MC8/3	-0.245	0.259	-0.126	-0.328	-0.502	0.373
MC8/11	0.139	0.080	-0.305	-0.306	-0.536	0.536
MC9/3	-0.254	0.208	0.331	-0.229	0.125	-0.496
MC10/4	-0.225	0.343	0.096	-0.051	-0.068	0.105
MC12/11	0.295	-0.309	0.076	-0.223	-0.389	0.050
MC20/19	-0.022	0.220	-0.338	0.320	0.428	0.199
MC21/11	0.256	-0.354	0.345	0.083	0.010	0.126
MC26	0.273	-0.331	-0.175	-0.269	0.026	-0.109
MC27	-0.272	0.068	-0.175	-0.418	0.079	-0.274
MC28	-0.219	0.053	-0.259	-0.331	0.179	0.075

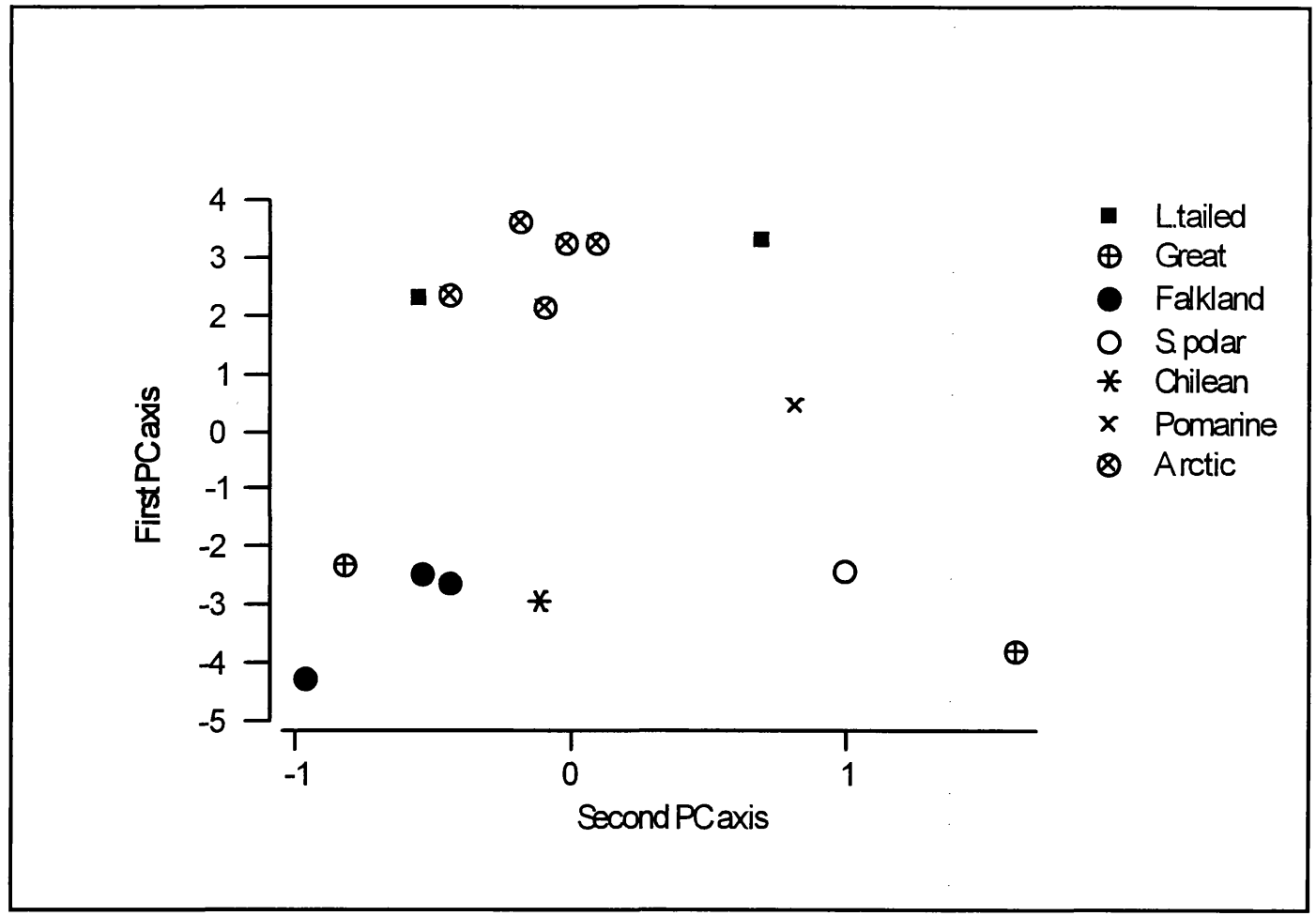

Figure 2.9. Scatter plot of morphological differences in skuas skeletons. Plot is build based on measurement of ten characters (SK01, SK04-08, SK10, SK12-14) from fifteen specimens which possess all these characters.

skua is allocated with Chilean skua but totally separated from South Polar skua. This figure also presents a wide distribution of Great skua which indicates that this taxon may share a higher degree of morphological similarity with other large skuas. Analysis of eigen-values of PCA of these data revealed that the first three PC covered 98.2% (91.3% for first principal component, and 5.6% and 1.6% for second and third PCs respectively) of total variation (Table 2.7). This figure implies a similar conclusion to that drawn with skin specimens; the first three principal components are sufficient in making comparisons between data-sets, and therefore, should present overall patterns for whole data-sets.

In concordance with PCA, a cluster analysis study on skuas has successfully separated small skuas from large skuas. Analysis of standardised data has produced two clusters to indicate the separation between groups. Although results obtained from this approach were greatly affected by indices used in calculating the distances among observations in the analysis, they generally depict similar conclusions; Pomarine skua is allocated together with small skuas. This result has been consistently shown by both sexes (Figure 2.10 and 2.11). In term of within group arrangement, small skua members were nicely clustered but the relationships among members within large skuas group is rather chaotic. As expected, Arctic skua is placed closer to Long-tailed skua and both taxa are related to Pomarine skua. Cluster analysis, however, failed to present a clear separation among members of large skuas.

In addition to distance indices factor, the arrangement of large skua members in the phenogram is also influenced by sex. Although the phenogram derived from both data-sets successfully separated small and large skuas there is a slight variation in the allocation of large skuas members. In the female skuas cluster, South polar skua has been placed as an outgroup, whereas in males, Brown skua has become an outgroup. Female skuas indicates that Chilean, Tristan and Great skuas are closely related and are all connected to Falkland and Brown skuas which behave as an outgroup at different degrees of relationships (Figure 2.10). The situation is slightly different in male skuas;

Table 2.7. Multivariate morphometric analysis on skuas skeletal. Results of PCA using ten characters on eighteen specimens.

Variable	PC1	PC2	PC3
Eigenvalue	9.130	0.531	0.153
Cumulative	0.913	0.966	0.982
	components loadings		
SK01	-0.329	0.026	-0.094
SK04	-0.323	-0.129	0.031
SK05	-0.306	0.287	-0.742
SK06	-0.311	-0.284	0.469
SK07	-0.328	-0.117	-0.063
SK08	-0.329	-0.100	-0.056
SK10	-0.325	-0.177	-0.133
SK12	-0.327	-0.015	0.088
SK13	-0.252	0.861	0.399
SK14	-0.324	-0.153	0.171

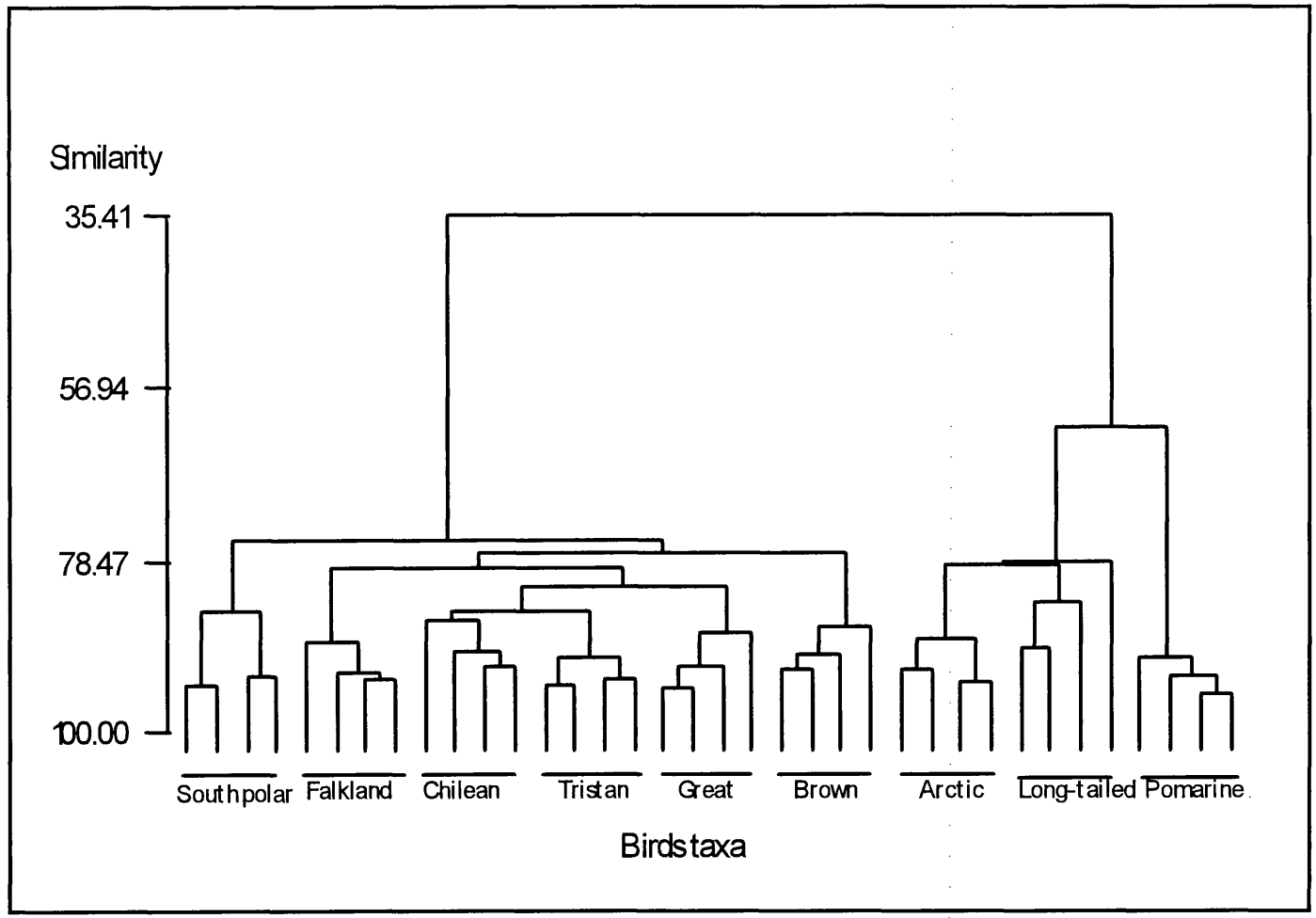

Figure 2.10. Cluster analysis of female skuas, using average method and manhattan distance index.

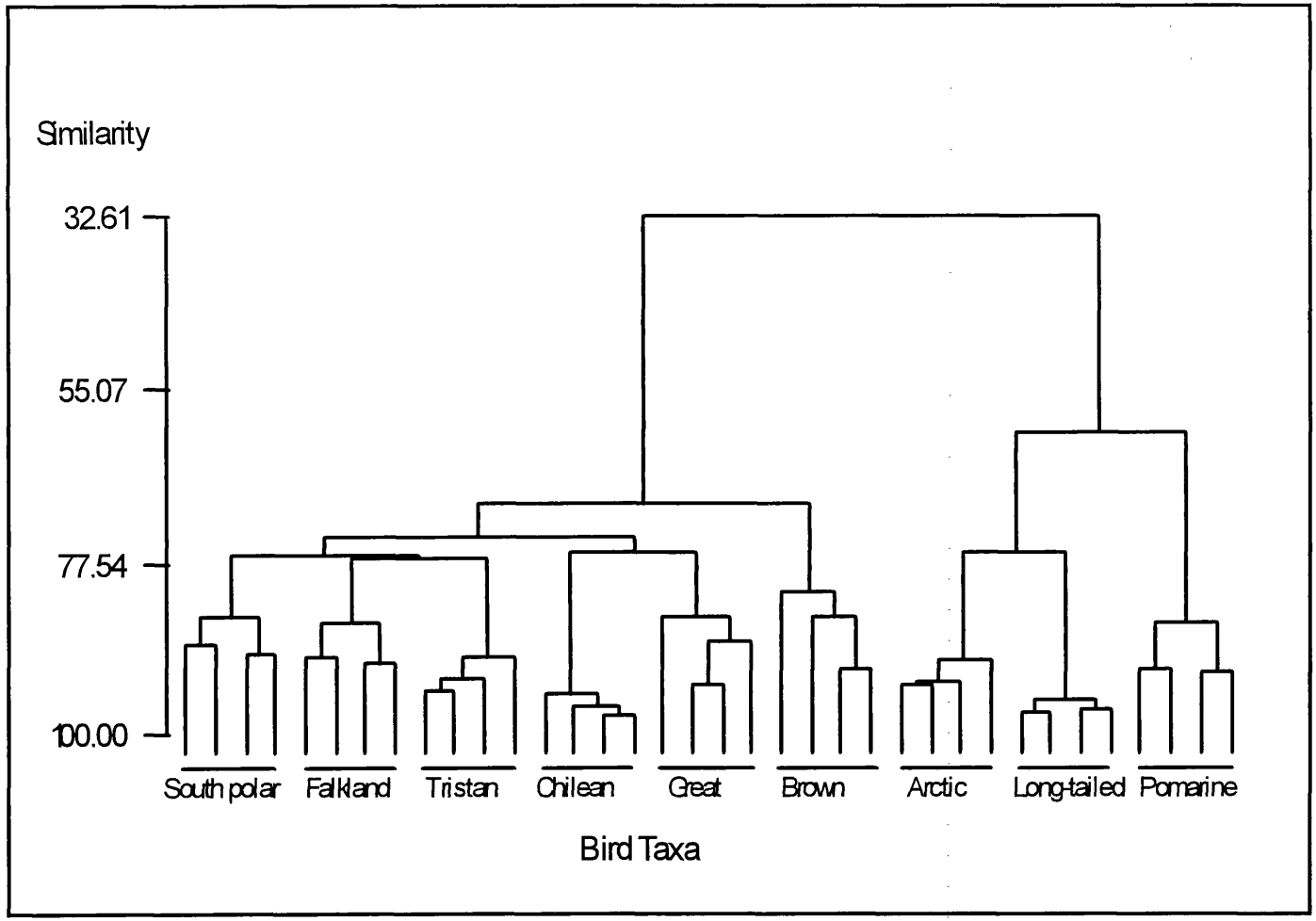

Figure 2.11. Cluster analysis of male skua skins by average method and manhattan distance index.

South Polar, Falkland and Tristan skuas are linked together as a sibling species, but are different from Chilean and Great skuas which compose different groups (Figure 2.11).

Study of skeletal characters present a similar pattern of results by separating small from large skuas (Figure 2.12). Small skuas group has been represented by Arctic and Pomarine skuas, but only Arctic skua specimens were available for comparison due to incomplete preservation of Pomarine skua bones. Results clearly lump together all Arctic skua specimens and separate them from other large skuas. This study also shows that small sample size, together with incomplete specimens greatly disturb the allocation of samples. These problems become more difficult since missing characters are not consistent in all samples. Some specimens for example, may have sternum while others only have skull.

Results from canonical discriminant analysis indicate that due to a high degree of similarity between observations, data-sets are behaving as discrete data rather than continuous data as they should. This is because in the discriminant analysis the amount of characters applied in the analysis should be smaller than the total number of observations. In solving this problem, a stepwise discriminant analysis has been carried out to determine best characters for representing data-sets. As a result, seven characters (variables MC1, MC2, MC9, MC13, MC15, MC18 and MC21) have been chosen to represent female skuas and nine characters (variables MC1, MC5, MC7, MC10, MC20, MC21, MC22, MC24 and MC25) for male skuas.

Canonical values produced by this analysis have been plotted and clearly distinguish skuas into six groups. All small skuas are clearly separated from large skuas but the distinction between large skua taxa is too small. All six taxa of large skuas have been separated into three species, with four taxa as a subspecies (Figure 2.13).

On the basis of distance values, it is obvious that Falkland skua is closer to Chilean skua than other large skuas (ANOVA, $F=4.64$; $df=20,8$; $p<0.002$; Table 2.8 and 2.9). Other large skua taxa are clearly distinct from each other, by possessing distance values from 25.05 (Falkland skua and Chilean skua) to 241.44 (South Polar skua and Tristan skua). Small skuas on the other hand, have higher distance values. Closely

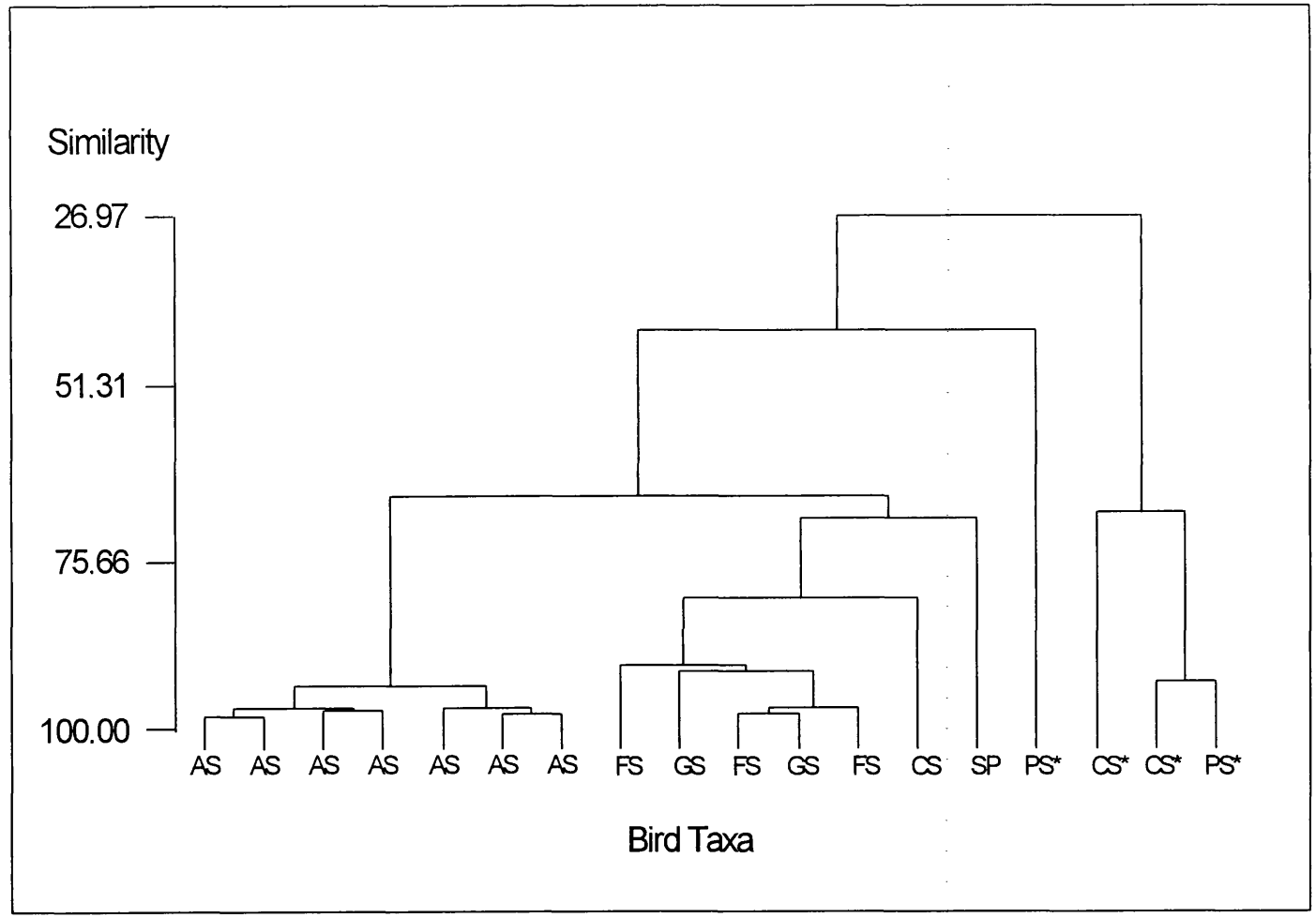

Figure 2.12. Cluster analysis of skua skeletons by average method and manhattan distance index. Allocations were made using ten characters which were available for all specimens involved. AS, Arctic skua; FS, Falkland skua; GS, Great skua; CS, Chilean skua; SP, South Polar skua; PS, Pomarine skua. Observations marked * indicate incomplete specimens.

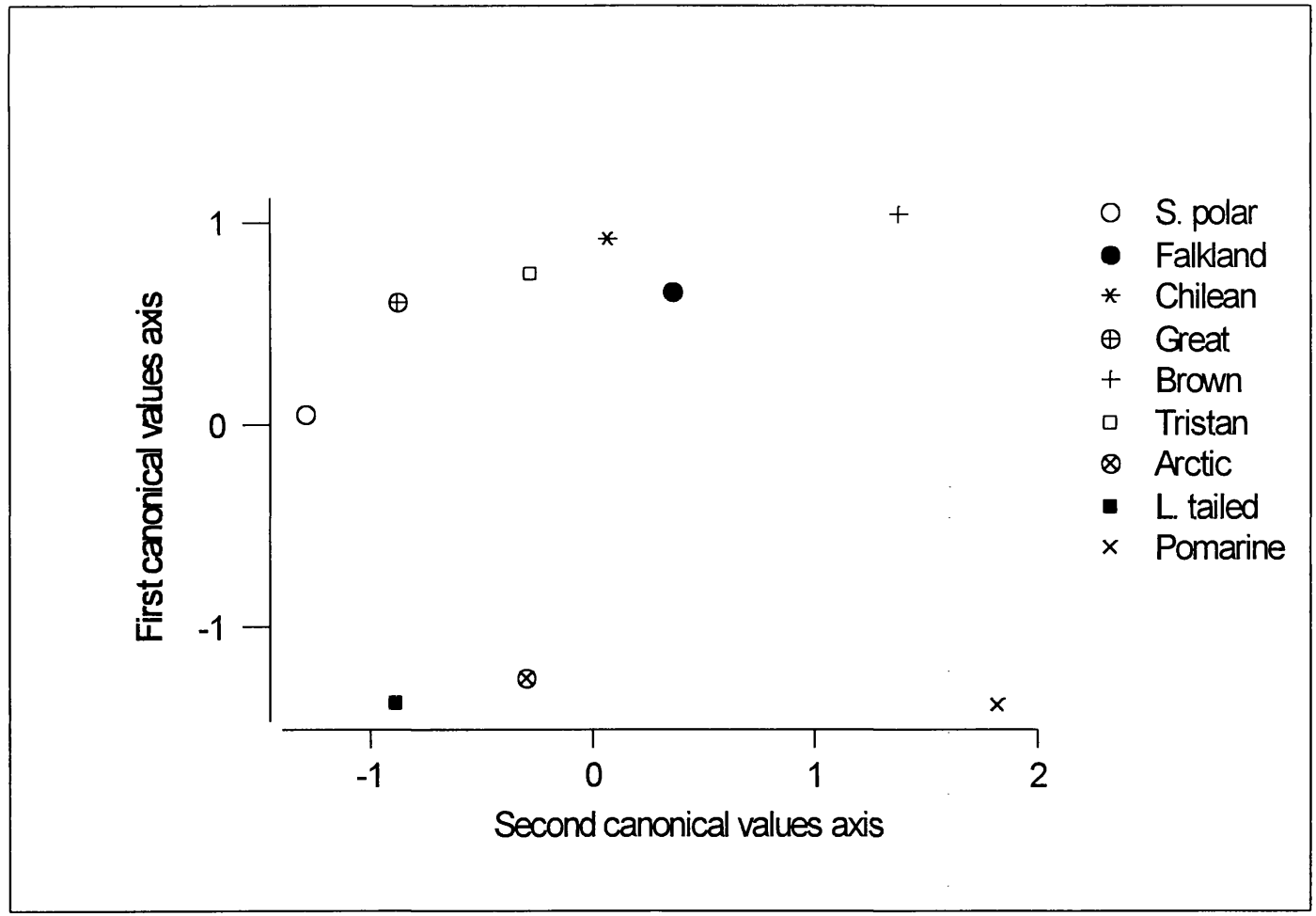

Figure 2.13. Separation of skuas based on distance values obtained from canonical discriminant analysis.

Table 2.8. Distance values between each pair of taxa of female skuas (below diagonal) and F-values (NDF=8, DDF=20, $p < 0.0025$) for respective taxa (above diagonal) obtained from canonical discriminant analysis.

	South Polar	Falkland	Chilean	Great	Brown	Tristan	Arctic	Long-tailed	Pomarine
South Polar	-	8.616	10.314	12.169	29.482	44.710	148.924	176.218	208.713
Falkland	46.530	-	4.638	16.568	23.507	30.166	152.034	181.490	239.430
Chilean	55.697	25.047	-	8.450	15.751	17.702	169.808	198.392	246.499
Great	65.716	89.467	45.633	-	18.066	22.432	180.984	197.994	215.948
Brown	159.204	126.941	85.058	97.557	-	6.655	253.564	290.388	287.396
Tristan	241.436	162.899	95.591	121.133	35.937	-	255.772	291.164	297.095
Arctic	804.190	820.988	916.964	977.313	1369.000	1381.000	-	14.962	94.996
Long-tailed	951.579	980.049	1071.000	1069.000	1568.000	1572.000	80.797	-	122.213
Pomarine	1127.000	1293.000	1331.000	1166.000	1552.000	1604.000	512.979	659.952	-

Table 2.9. Distance values between each pair of taxa of male skuas (below diagonal) and F-values (NDF=9, DDF=19, $p < 0.0001$) for respective taxa (above diagonal) obtained from canonical discriminant analysis

	South Polar	Falkland	Chilean	Great	Brown	Tristan	Arctic	Long-tailed	Pomarine
South Polar	-	34.404	27.452	30.845	81.193	63.533	656.455	924.605	295.565
Falkland	220.007	-	54.047	7.430	22.454	23.166	817.387	1124.000	400.996
Chilean	175.547	345.620	-	32.150	84.882	50.588	753.414	1003.000	351.695
Great	197.250	47.514	205.596	-	17.348	10.428	837.588	1140.000	405.932
Brown	519.213	143.587	542.799	110.938	-	8.821	973.069	1301.000	505.464
Tristan	406.279	148.141	323.502	66.684	56.410	-	882.222	1183.000	431.675
Arctic	4198.000	5227.000	4818.000	5356.000	6223.000	5642.000	-	59.857	90.875
Long-tailed	5913.000	7188.000	6412.000	7291.000	8317.000	7568.000	382.770	-	204.488
Pomarine	1890.000	2564.000	2249.000	2596.000	3232.000	2760.000	581.125	1308.000	-

related taxa among small skuas, Arctic and Long-tailed have a distance value of 80.79, while the maximum value is shown by Long-tailed and Pomarine skua (659.95).

2.4. Discussion

2.4.1. Limitation and Suitability of the Approaches

Several precautionary steps have to be taken when morphological characters are used as taxonomic indicators since various factors can cause errors in measurement and this will lead to unreliable results. Using a museum specimen for example may produce some inaccuracy in morphometric measurement. This is because museum specimens may show either a shorter measurement (due to shrinkage during drying) or slightly longer (due to bone being concealed within skin) than actual measurements of live specimens or cleaned skeletons. However, since only museum specimens were used for measurement and with the assumption that the degree of shrinkage in specimens is uniform among samples, results from this study should yield accurate estimates of relative body proportions of skuas. Moreover, an accurate measurement on live skuas is difficult to carry out due to their aggressive behaviour and large size. The wide distribution of live skuas would also increase research costs if the study were to include all skua taxa. On the other hand, a high accuracy in museum specimens has been obtained in several bird studies such as American Robin, *Turdus migratorius* (Aldrich & James 1991) and Spanish Imperial Eagle, *Aquila adalberti* (Ferrer & Delecourt 1992). Repeated experiments by Ferrer & Delecourt (1992) and Seutin *et al.* (1993) also proved that museum skins produced similar results as those obtained from live birds and cleaned skeletons.

Any inconsistency in the results of morphological measurements for a specific character is normally due to misunderstanding about the definition of a particular character. This will lead one to use different points from others when measuring a similar character. Problems like this has been shown by Seutin *et al.* (1993) in their study on live and museum skins of redpolls (*Carduelis sp.*). In measuring the

differences of hallux length, various values were obtained and these differences were mainly due to where the callipers were positioned rather than affected by the status of the specimen. Therefore, a clear definition about each morphological character together with sufficient illustrations should be provided in every morphometric study. For skua measurements, all characters were properly defined and illustrated in enclosed Appendices. For skuas skeletons, all measurements were adopted from Schnell (1970).

In addition to the above factors, inaccuracies in morphometric analysis can also be caused by improper preparation of skin specimens. Seutin *et al.* (1993) showed that certain characters such as bills may not be tightly closed during the drying process. Estimation of bill depth therefore, will be slightly inaccurate. This problem is not applicable to this study since lower and upper bill were measured separately and no measurement involving bill depth was made. Furthermore, all specimens were originated from one source, and with the assumption that similar methodologies (or possibly the same personnel was involved) were used in treating all specimens and therefore, cancelled out or at least greatly minimised any error.

Numerical taxonomists believe that if enough characters are used, the error from unreliable characters is mostly cancelled out, and the residual errors is the lesser of two evils when compared to the personal bias introduced by any attempt to evaluate characters (Eades 1970). However, it is very difficult to determine a sufficient amount of characters for representing morphological variation because not all of them are taxonomically informative. Skua characters are not excluded. It has been noted that only some of the skuas characters used in this study are useful. These informative characters were selected by using a stepwise analysis. This finding is parallel with several previous studies on various birds. Aldrich & James (1991) for example discovered that wing length can represent general size of American robin (*Turdus migratorius*). In sexing Spanish imperial eagle, Ferrer & Delecourt (1992) suggests that forearm length is the most appropriate character. All these studies suggest that the general pattern of bird size can be represented by a few or even a single character. The number of characters used in each study therefore, should not influence results very

much. However, increasing the amount of characters for investigation will enhance the opportunity for discovering an appropriate character for morphological representation.

Similar environmental pressure can affect morphological characters differently (Eades 1970). Therefore, even though sufficient characters were used in morphological analysis, the selected characters should not be assumed to represent total variation of all characters. Furthermore, morphological variation between related species may not always be proportional to the amount of genetic differentiation between them. Variation in shape for instance is due to an interaction between developmental processes and environmental pressures (Nijhout 1990). Characters which are subject to large directional selection pressures or to other significant environmental effects are not good tracers. Sbordoni *et al.* (1991) provide a good example of this by showing that genetic drift and gene flow are not good predictors of levels of genetic variability in *Dolichopoda*. Therefore, some morphological characters which can be affected by convergent evolution should not be used in making phylogenetical inferences. Additionally, not all characters can provide a correct estimation of time. Anatomical features such as bones and teeth can evolve rapidly in one lineage and slower in another (Lowenstein 1985).

Skuas also show unequal variation in their morphological characters. Some measured characters such as MC14, MC15 and MC18 present higher variation between skuas whereas others only show smaller or no variation at all. Inequality in variation among bird morphological characters not only exists in skuas but it has been presented in several studies. Several factors may play an important role in this inequality in bird morphological variation. Inequality in skuas' morphology, for example, may be influenced by differences in sex, behaviour or environment. In addition, inconsistency in morphological evolutionary rates also produced inequality in bird morphology. In Cardulinae for instance, variation in bill morphology is more conservative than variation in other parts of the body (Bjorklund & Merila 1993), whereas Price (1991) discovered that warblers have retained their ancestral bill morphology and each species chose its environment rather than being moulded by it. Inequality in bird's

morphological variation is normally due to variability in factors which control bird's physical appearances. Some bird morphological characters are affected by only a single factor such as body size whereas other characters may be influenced by various factors which blend together such as sex and behaviour. Wing shape for example is greatly affected by bird size. In skuas, smaller species possess more slender and pointed wings than larger species which have broad and blunt wings (Furness 1987), whereas the American robin shows more pointed wings in larger birds within the species (Aldrich & James 1991). This study also discovered that not all skua characters were affected by size. Some characters such as the distances between tip of outer feather to adjacent feather shows an inverse correlation with skua size. Small skuas possess higher value for this character compared to large skua.

Previous studies have proved that all methods used in this study are capable of presenting accountable results for morphometric analysis. Principal component analysis for example has been used extensively in several studies such as in the separation of closely-related species. PCA successfully separated 103 *Hylomis* skulls based on measurement of 16 cranial variables (Ruedi *et al.* 1994). This classification is concordant with evidence from a behavioural study. PCA also has been used successfully in separating six strains of musk shrews (*Suncus murinus*) which originated from various geographical areas (Ishikawa *et al.* 1995). Canonical discriminant analysis has successfully differentiated between ling (*Genypterus blacodes*) from the west coast of the South Island and from the Chatham Rise and Canterbury waters off New Zealand. The former had longer and narrower heads and thinner otoliths than ling from the latter areas (Colman 1995). Further analysis on musk shrew by discriminant analysis successfully separated NAG strain from other closely related Japanese strains based on 15 log-transformed morphometric characters (Ishikawa *et al.* 1995). These two studies show that discriminant analysis is a better choice when separating closely related taxa, especially when PCA failed to do so. The third approach used in this study, cluster analysis, was used in studying several birds such as Lariidae (Schnell 1970), Black-

thighed falconet, *Microhierax fringillarius* (Kemp & Crow 1994) and Soft-plumaged petrel, *Pterodroma mollis* (Bretagnolle 1995).

The accuracy of the results has also been enhanced by studying male and female adult birds separately. This will reduce the effects of ontogeny and sexual dimorphism. This approach has successfully eliminated the above effects in turtles, *Pseudemys* sp. (Seidel & Palmer 1991) and moles (Corti & Loy 1987).

2.4.2. Allocation of Skuas to Taxa

Analysis of size and shape differences clearly separated small skuas from large skuas but failed to present a clear separation between members of the large skuas group. Due to its larger size among small skuas, the Pomarine skua has been placed closer to members of large skuas group than are Arctic and Long-tailed skuas. This placement however, does not mean that Pomarine skua belong to large skuas group because they are in fact closer to small skuas than large skuas.

Multivariate analyses, PCA and cluster analysis have supported the separation of skuas into two groups; small and large skuas. These analyses also suggest that Pomarine skua should be lumped together with Arctic and Long-tailed skuas in the small skuas group. In terms of body size, it is obvious that the former species is the largest birds among small skuas, and therefore, it is not surprising that this skua has been placed closer to large skuas. However, the degree of similarity between Pomarine skua and large skuas group is not so obvious (relative to Pomarine skua and other small skuas) to allow these birds to be classified together. This result was expected since all these analyses were based on morphology and it was in parallel with previous studies on size variation (Howard & Moore 1980), breeding distribution (Furness 1987) and skeletal data (Schnell 1970).

Various analyses used in this study were also unsuccessful in coherently separating members of large skuas. All members of this group are lumped together and the degree of separation between them is hardly identified either from size or shape

variations. Although some taxa are clearly separated from others, this allocation is not consistent. Therefore, this allocation may be due to other factors (e.g. differences in sex) rather than real variation possessed by a particular taxon. Higher degrees of variation between males and females indicates that certain characters are heavily sex dependent. This character changes more rapidly in one sex. The only indicator that can be used in deducing relationships among large skuas members is the distance value provide by Mahalanobis distance in canonical discriminant analysis.

Failure to separate certain members of large skuas may be due to two reasons. First, the differences between these skuas are too small to be visualised by methods applied in this study. If this were true, it is very hard to conclude whether such small differences are enough for someone to grant subspecies or species title to all these taxa. Therefore, a specific amount of differences should be determined in assigning taxonomic recognition for problematic taxa. Second, various effects may have more pronounced influence on skuas than variation in morphology due to species variation. Some phenomenon such as convergent evolution always presents a serious problem to morphometric analysis of conspecific taxa. This process happens when two unrelated creatures resemble each other because they responded to similar environmental pressure (Lowenstein 1985). This factor has to be taken seriously in morphometric study since its existence can lead to fallacy in judgement. Convergent evolution has influenced skuas in that size (tarsus and wing length) and shape differences are due to ecogenesis or selective pressures (height of ground vegetation and flight performance respectively) (Furness 1987). This unavoidable phenomenon has influenced skuas in their process of adaptation to a new habitat, and it has masked evolutionary relationships especially between members of large skuas. Several authors (Niemi 1985, Miles *et al.* 1987) have suggested that species occupying structurally similar habitats, although from different sites, tend to converge. This suggestion has been supported by Aldrich & James (1991) by proposing that species with different phylogenetic relationships and life histories will shows concordant variation in size and colour when they are facing similar physical adaptation. Price (1991) however, disputes this proposal by stating that two distinct

species with totally different phylogenetic backgrounds (e.g. a finch and a dove) are unlikely to ever converge to the same morphology and behaviour, even if they were exploiting identical resources. If we assume that some taxa of large skuas did converge and possess similar characteristic, the amount of convergence being experienced by them are hardly determined. More study is required to clarify this possibility before relationships between all large skuas can be clarified.

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Appendix 2.1. List of museum specimens used in this study.

Taxon	Origin	Collection date	Label	Sex
South polar skua	Palmer Archipelago	19.12.1945	1949.7.28	Male
	Antarctic area	17.11.1902	1905.12.30.278	Male
	Palmer Archipelago	n/a	1949.7.27	Female
	Antarctic area	09.01.1902	1905.12.30.279	Female
	Palmer Archipelago	23.02.1945	1949.7.6	Female
	Palmer Archipelago	17.02.1945	1949.7.2	Female
	Palmer Archipelago	04.11.1945	1949.7.26	Male
	Antarctic area	26.01.1926	42.12.16.91	Male
Falkland skua	Falkland Island	04.01.1931	1940.12.6.12	Male
	Falkland Island	05.12.1930	1940.12.6.23	Male
	Falkland Island	05.11.1936	1948.78.143	Female
	Falkland Island	26.11.1930	1940.12.6.10	Female
	Cape Dolphin	13.01.1930	1940.12.6.20	Female
	Cape Dolphin	18.12.1930	1940.12.6.18	Female
	Carcass Island	01.03.1932	1940.12.6.19	Male
	Port Stephens	19.01.1932	1940.12.6.8	Male
Chilean skua	South America	n/a	(6)2986	Male
	South America	00.12.1879	80.8.3.38	Male
	South America	05.02.1903	1903.12.30.194	Female
	South America	09.02.1903	1903.12.30.195	Male
	Stanley Harbour	29.10.1936	1949.78.142	Female
	Eagle Point	26.10.1930	1932.7.2.33	Female
	Eagle Point	09.12.1930	1932.7.2.34	Male
	Patagonia	05.02.1903	1903.12.30.194	Female
Great skua	Shetland	30.09.1911	1941.5.30.3073	Male
	Shetland	21.08.1939	1965.M.4081	Male
	Orkney Island	11.08.1938	1965.M.4087	Female
	Shetland	26.11.1939	1965.M.4080	Female
	Shetland	10.10.1934	1950.17.133	Female
	Foula	10.07.1949	1949.22	Male
	Foula	10.10.1912	1991.60.45	Male
	Shetland	20.06.1935	1950.17.132	Female

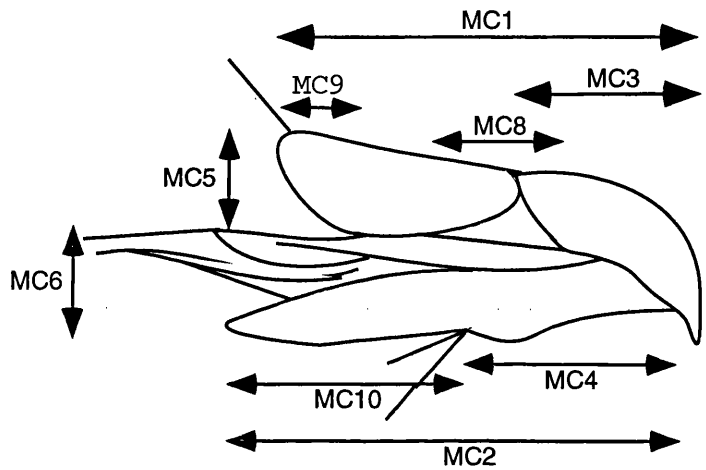
Brown skua	Signy Island	01.04.1934	1940.12.7.6	Male
	Signy Island	28.12.1948	1949.7.58	Male
	South Orkney	12.12.1922	1923.9.10.1	Male
	South Orkney	28.12.1948	1949.7.58	Male
	South Orkney	18.12.1949	1949.7.56	Female
	South Orkney	26.12.1923	1924.5.8.8	Female
	Stanley	05.11.1936	1948.78.143	Female
	Stanley	05.11.1936	1948.78.144	Female
Tristan skua	Tristan da Cunha	09.03.1951	1953.55.4	Male
	Gough Island	21.02.1952	1953.55.5	Male
	Tristan da Cunha	28.05.1952	1953.55.2	Female
	Tristan da Cunha	28.08.1950	1953.55.3	Female
	Inaccessible Island	09.05.1950	1953.55.6	Male
	Tristan da Cunha	25.07.1973	1975.93.142	Male
	Inaccessible Island	20.01.1974	1975.93.149	Female
	Gough Island	27.10.1972	1975.93.150	Female
Arctic skua	Norway	31.05.1898	98.6.24.48	Male
	Bering Sea	02.08.1896	98.7.4.160	Male
	Norway	26.06.1939	1722S	Female
	Bering Sea	02.08.1896	98.7.4.159	Female
	Shetland	03.07.1936	1950.17.136	Female
	Orkney Island	08.09.1908	1934.61.3444	Female
	Fair Isle	06.08.1956	1956.62.2	Male
	Orkney Island	25.08.1909	1934.61.3445	Male
Long-tailed skua	Lapland, Russia	05.08.1899	1934.1.1.2987	Male
	Iceland	09.06.1914	1914.8.24.10	Male
	Alaska	30.08.1979	88.10.10.2922	Female
	Southampton Island	14.07.1936	1947.6.55	Female
	Alaska	21.06.1962	1962.36.21	Female
	Hoeberg Beach	28.06.1876	1898.4.317	Female
	Norfolk	00.00.1910	1952.19.1942	Male
	Tauloppmark	28.07.1906	1934.61.3448	Male

Pomarine skua	Nova Zeuhla	15.07.1903	1941.5.30.3075	Male
	Alaska	09.06.1879	88.10.10.2914	Male
	Greenland	19.06.1917	1918.1.16.1	Female
	Greenland	17.06.1917	1918.1.16.2	Female
	Firth of Forth	00.10.1879	1896.133.1811	Male
	Dunbar	01.10.1912	1912.164	Male
	Cockersauds	26.11.1908	1934.61.3433	Female
	Orkney	05.11.1913	1934.61.3434	Female

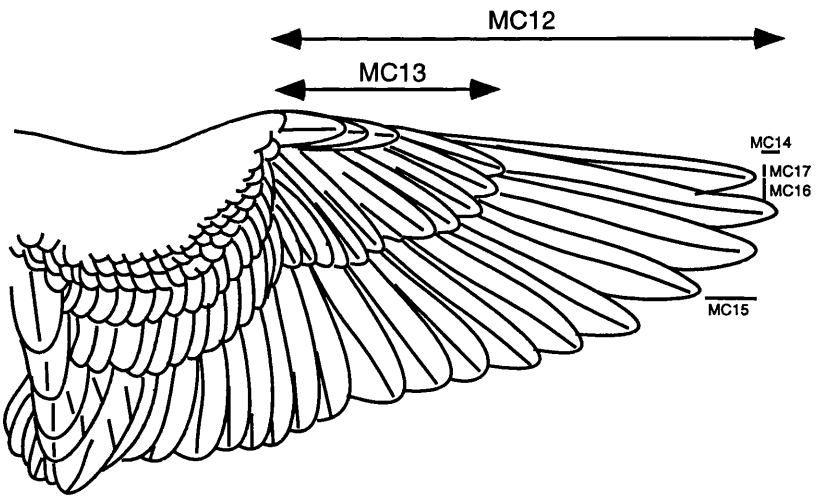
Appendix 2.2. Description of external characters measured and used in this study (adapted from Schnell 1970). Details about how measurement were carried out is illustrated in Appendix 2.3.

MC1 : Upper mandible length
MC2 : Lower mandible length
MC3 : Culmen length
MC4 : Gonys length
MC5 : Height of upper mandible
MC6 : Height of lower mandible
MC7 : Bill width (taken at widest point)
MC8 : Nostril length
MC9 : Distance between commisure and mandibular ramus (upper mandible)
MC10 : Distance between commisure and mandibular ramus (lower mandible)
MC11 : Body length
MC12 : Wing length (primary) - distance of unflattened wing from carpal joint bend of wing) to tip of longest primary.
MC13 : Wing length (secondary) - distance of unflattened wing from carpal joint bend of wing) to tip of longest secondary.
MC14 : Distance from tip of ninth to eighth primary - distance from tip of ninth primary to tip of eighth primary. If ninth primary was shorter than eighth, then measurement given as a negative value
MC15 : Distance from tip of eighth to seventh primary - distance from tip of eighth primary to tip of seventh primary. If eighth primary was shorter than seventh, then measurement given as a negative value
MC16 : Primary inner vein width -maximum width of inner vein of tenth primary
MC17 : Primary outer vein width -maximum width of outer vein of tenth primary
MC18 : Rectrix length (excluding tail projection) - maximum length from point where skin joins shaft of middle pair of retrices to tip of longest rectrix when tail closed naturally
MC19 : Rectrix inner vein width - maximum width of inner vein of outer rectrix
MC20 : Retrix outer vein width - maximum width of outer vein of outer retrix
MC21 : Length of tarsus
MC22 : Length of digit 1 (hallux)
MC23 : Length of digit 2 (inner toe)
MC24 : Length of digit 3 (middle toe) - measured from junction of the tarsus to point where nail and skin meet.
MC25 : Length of digit 4 (outer toe)

Appendix 2.3. Schematic illustration of each characters used in this study (refer to Appendix 2.2 for character's explanation)

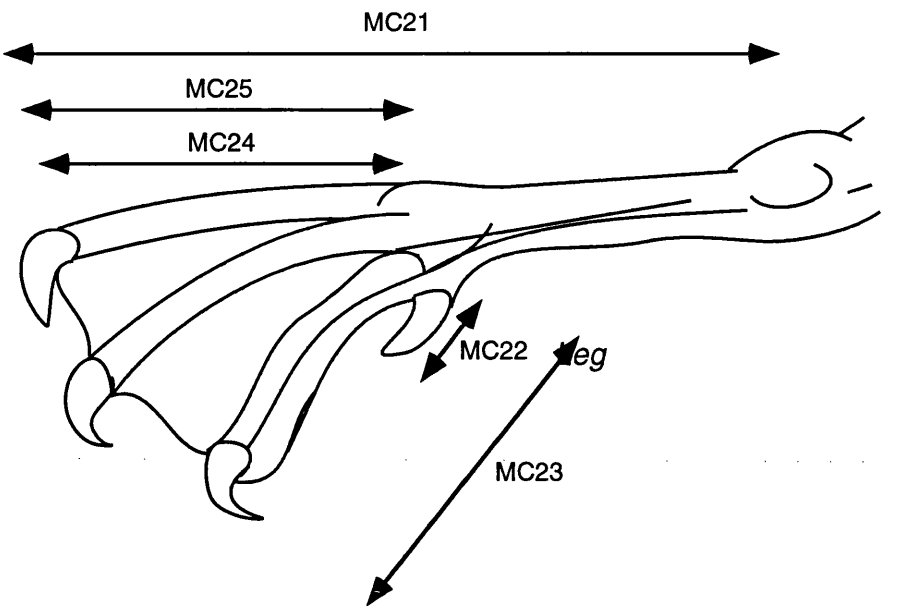


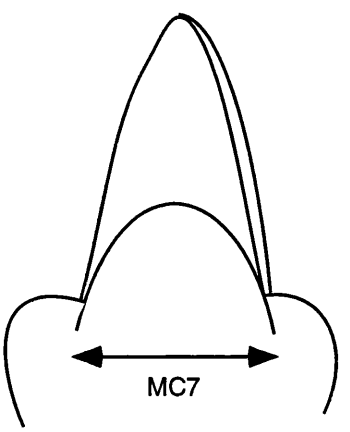
Beak (Lateral view)



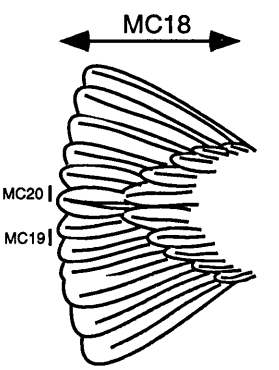
Wing (ventral view)

Appendix 2.3. (Continue..)





Bill (dorsal view)



Tail (dorsal view)

Appendix 2.4. List of characters used in analysis of shape differences in skuas.

MC1/MC5	:	Length over height of upper mandible
MC2/MC6	:	Length over height of lower mandible
MC3/MC7	:	Culmen length over bill width
MC3/MC11	:	Proportion of culmen length in relative to total body length
MC4/MC11	:	Proportion of gony length in relative to total body length
MC8/MC3	:	Ratios of nostril length to culmen
MC8/MC11	:	Proportion of nostril length in relative to body length
MC9/MC3	:	Ratios of the distance between commisure and mandibular ramus of upper mandible to culmen length
MC10/MC4	:	Ratios of the distance between commisure and mandibular ramus of lower mandible to gony length
MC12/MC11	:	Ratios of wing length to total body length
MC20/MC19	:	Ratios of outer vane to inner vane in rextrix
MC21/MC11	:	Ratios of tarsus length to total body length
MC26	:	Ratios of outer vane to inner vane in tenth primary feather
MC27	:	Ratios of outer vane to inner vane in ninth primary feather
MC28	:	Ratios of outer vane to inner vane in eighth primary feather

Appendix 2.5. List of skeletal specimens used in this study. All specimens were obtained from Natural History Museum, Tring, England.

Taxon	Location	Collection date	Label	Sex
1) Long-tailed skua	Devon	02.01.1986	5/1986.64.1	n/a
2) Arctic skua	Spitzbergen	01.08.1894	1895.3.2.6	n/a
3) Great skua	South Iceland	27.05.1959	S/1959.17.25	n/a
4) Falkland skua	Kerguelen Island	n/a	1890.11.3.7	n/a
5) Falkland Island	Australia	n/a	1896.2.16.19	n/a
6) Falkland Island	n/a	n/a	S/1952.2.314	n/a
7) South polar skua	Antarctic area	n/a	1842.12.16.91	Male
8) Chilean skua	St. Catharina	n/a	S/1952.2.213	n/a
9) Chilean skua	Smythes Island	n/a	1903.12.30.229	n/a
10) Pomarine skua	Europe	n/a	1846.5.27.133	n/a
11) Pomarine skua	n/a	20.05.1977	n/a	Male
12) Pomarine skua	Suffolk	03.11.1909	1919.12.10.316	Female
13) Great skua	St. Kilda	00.00.1985	S/1987.42.1	n/a
14) Arctic skua	n/a	n/a	S/1981.6.1	n/a
15) Arctic skua	n/a	n/a	1867.4.5.2	n/a
16) Arctic skua	Spitzbergen	n/a	1892.3.2.1	n/a
17) Arctic skua	n/a	n/a	S/1952.2.318	n/a
18) Arctic skua	Isles of Sicilly	05.10.1963	S/1964.4.1	Female

Appendix 2.6. Description of skeletal characters used in this study (adopted from Schnell 1970 (except SK2 and SK3)).

SK1 : Premaxilla length - medially from posterior edge to anterior tip of premaxilla
SK2 : Length of lower mandible
SK3 :Width of lower mandible
SK4 : Internarial width - transversely, the least dimension of premaxilla between the narial openings
SK5 : Nasal bone width - transversely, minimum dimension between regions of contact between nasal bones and process of maxilla
SK6 : Interorbital width - tranversely, the least dimension of frontal in mid-orbital region
SK7 : Postorbital width - transversely, the maximum dimension between postorbital processes of frontal
SK8 : Skull width - transversely, the maximum dimension between processes of parietals near articulation of main part of skull with quadrates
SK9 : Occipital depth - medially, from dorsal edge of foramen magnum to dorsal edge of supra-occipital
SK10 : Skull depth - medially, from spenoidal rostrum to dorsal region of frontal
SK11 : Skull length - medially, from posterior part of supraoccipital to anterior tip of premaxilla
SK12 : Mandible length - lateromedially, from dentary symphysis to postarticular process
SK13 : Minimum mandible length - medially, from anterior to posterior edge of dentary symphysis
SK14 : Mandible depth - from angular process to dorsal edge of surangular, on line perpendicular to length of lower mandible
SK15 : Coracoid width - minimal width of shaft, taken in the same plane as flattened proximal end of coracoid
SK16 : Coracoid length - from most distal edge of the coraco-humeral surface to proximal edge of sterno-coracoidal process
SK17 : Scapula length - maximum dimension from apex to acromion
SK18 : Scapula width - maximal width from edge of glenoid facet to acromion
SK19 : Furcular process length - medially, maximal dimension from symphysis to furcular process
SK20 : Furcula length - lateromedially, from furcular process to centre of coracoid facet
SK21 : Sternum length - medially, from posterior edge of sternum to anterior edge of ventral manubrial spine
SK22 : Keel length - medially, from posterior edge of sternum to anterior edge of keel
SK23 : Sternum width - transversely, maximal dimension between sterno-coracoidal process
SK24 : Keel depth - medially, from dorsal edge of ventral manubrial spine to ventral edge of keel
SK25 : Costal margin - from posterior edge of first costal process to anterior edge of fifth costal process

SK26 : Synsacrum depth - from region just posterior to pectineal process to juncture of posterior and anterior iliac crests
SK27 : Posterior synsacrum length - from posterior notch to posterior edge of ilio-ischiatric fenestra
SK28 : Anterior synsacrum length - maximal dimension from posterior edge of antitrochanter to anterior edge of ilium
SK29 : Synsacrum width - transversely, minimum dimension between acetabula as seen from dorsal view
SK30 : Synsacrum minimum width - transversely, minimum width between lateral edges of ilia in anterior region of synsacrum
SK31 : Femur proximal end width - transversely, maximum dimension from flattened lateral surface of proximal end of femur
SK32 : Femur minimum width - tranversely, minimum width of femur near midpoint of long axis of femur
SK33 : Femur distal end width - transversely, maximum dimension from flattened lateral surface (adjacent to internal condyle) to lateral edge of fibular condyle
SK34 : Femur length - maximum dimension from trochanter to external condyle
SK35 : Tibiotarsus width - minimum dimension of tibia near end of spine of fibula
SK36 : Tibiotarsus length - maximum dimension from articular surfaces to external condyle
SK37 : Tarsometatarsus length - maximum dimension from proximal end to trochlea for digit 3
SK38 : Tarsometatarsus width - transversely, minimum dimension
SK39 : Tarsometatarsus distal end width - tranversely, maximum dimension from wing of trochlea for digit 2 to lateral edge of trochlea for digit 4
SK40 : Humerus trochanter length - maximum dimension from head of proximal end to distal edge of muscle scar found along deltoid crest
SK41 : Deltoid crest depth - maximum depth of deltoid crest
SK42 : Humerus distal end width - maximum width from ente picondylar prominence to ectepicondylar prominence
SK43 : Humerus length - maximum dimension from head to internal condyle
SK44 : Radius length - maximum length
SK45 : Ulna length - maximum dimension from olecranon to external condyle
SK46 : Ulna width - minimum dimension (on line perpendicular to row of papillae of secondaries) taken approximately one-third from the distal end of the ulna
SK47 : Carpometacarpus length - maximum dimension from carpal trochlea to facet for digit 3
SK48 : Carpometacarpus depth - maximum dimension from metacarpal 3 to metacarpal 2 at midpoint on long axis of carpometacarpus
SK49 : Phalynx length - maximum dimension from digital facet to metacarpal facet
SK50 : Phalynx depth - maximum depth at midpoint on long axis of phalynx
SK51 : Pollex length - maximum dimension from digital facet to distal end

Chapter 3

Phylogenetic Relationships Among Stercorariidae Inferred From Morphological Evidence

3.1. Introduction

The study of phylogenetic relationships among organisms has been carried out extensively since the translation of Willi Hennig's book, *Phylogenetic Systematics* in 1966. In 1969, Mayr coined the terms 'cladist' and 'cladistics' for phylogeneticist and their field of study. Cladistics involves investigation of phylogenetical relationships. In constructing a biological system, cladists normally present their results in a graphical model known as a cladogram. A cladogram is a predominantly bifurcating, asymmetric, nontruncate dendogram, with no defined vertical and horizontal axes.

Cladistic analysis has played an important role in revealing phylogenetical relationships among organisms. In studies of avian taxonomy for instance, cladistic analysis has been used to explain phylogenetic relationships among various birds such as American robin, *Turdus migratorius* (Aldrich & James 1991), sharp-tailed sparrow, *Ammodramus caudacutus* (Greenlaw 1993), modern seaducks (Livezey 1995a), stiff-tailed ducks (Livezey 1995b), albatrosses, penguins and petrels (Paterson *et al.* 1995) and cuckoos (Hughes 1996).

Cladistic analysis has also proved to be an excellent method for determining the evolutionary relationships among birds (such as among hesperornithoform birds, Cracraft 1982). Cladistic analysis is not only suitable for studying evolutionary history of living birds but also for extinct taxa or fossils (Chatterjee 1991). In deducing evolutionary relationships among various entities, cladistic analysis concentrates on three types of information. These are; 1) examining the inter-relationships among organisms, 2) depicting the speciation events, and 3) exposing any evolutionary change

along phyletic lineages for particular taxa. Through the combination of those three sets of information cladistic analysis can act as a powerful method for grouping analysis capable of presenting patterns of relationships among individuals or species, or producing a classification that reflects the phylogeny of the study taxa (Funk 1986). The key to the success of cladistic analysis in performing this task is due to it sensitivity in detecting any changes between two or more entities caused by modification through descent (Platnick 1979).

The ease of cladistic analysis is assisted by the fact that input from various sources can be employed. This information can be extracted from comparative studies of various methods which explain evolutionary processes such as biochemical or genetical evidence, morphological measurements, or evidence from a wide-range of anatomical structures (Lang 1990). By using an *a-priori* hypothesis, cladistic analysis can be used to examine all these data and present appropriate phylogenetical relationships among taxa. In addition to the neccessity of an *a-priori* hypothesis, cladistic analysis also requires an outgroup (other closely related individual or taxon) in order to derive a more appropriate conclusion about systematic relationships.

Only shared derived characters (synapomorphy) are used by cladistic analysis in revealing systematic relationships. Cladists normally ignore shared primitive characters (synplesiomorphies) because they believe that these characters have a high tendency to produce misleading conclusions about phylogenetic relationships (Patterson 1982). In revealing systematic relationships, cladistic analysis normally reduces the information from an organism to a set of discrete characters. Cladistic analysis will assume that the characters used in the analysis are independent (David & Laurin 1996). Analysis begins by selecting a group of taxa which is believed to be monophyletic (those include an ancestral species and all of its decesdants), then characters which reflect evolution are selected although this tends to be subjective (Pankhurst 1995).

Several differences can be summarised when cladistic analysis is being compared with other methods such as traditional systematics and phenetics. Traditional systematics differs from cladistic analysis because the former only uses some selected

characters which are assumed to be more important than others in classifying particular organisms. However, the degree of neccessity of a particular character is different and it depends on personal judgement. The second approach, phenetics, is similar to traditional systematics in the way of determination of the relationship among study organisms. The major advantage of cladistic analysis over traditional systematics and phenetics is the use of morphological variations among study organisms without considering the effects of parallel evolution or convergent evolution. In comparing traditional systematics and phenetic analysis, the latter has more advantages than the former due to the use of various algorithms in deriving its conclusion (Wiley *et al.* 1991). These differences have led cladistics to become superior to traditional systematics and phenetic analysis. Researchers like Pankhurst (1995), believe that classification based on phylogeny is not only superior to phenetic analysis but also much better than molecular approaches. The superiority of cladistic analysis however, depends heavily on its capability to group entities based on monophyletic and alternative phylogenetic hypotheses chosen by parsimony (Crowe 1994).

In addition to the above distinction, cladistic analysis also has several advantages over other methods. Cladograms produced by cladistic analysis for example are easy to compare and a consensus tree for the most parsimonious trees (which assume fewest character changes) can be selected to represent phylogenetical relationships among organisms in the dataset. Furthermore, this analysis is also capable of detecting which character is responsible for each grouping (Funk 1986). All these advantages strongly support cladistic analysis in revealing evolutionary patterns, without refering to any particular assumptions about evolutionary processes (Nelson & Platnick 1981; Janvier 1984).

Results from the studies of morphometric data of several birds show that morphological variations are closely related to phylogenetical relationships. This may be due to the occurrence of homoplastic events (reversal and convergences) in the evolutionary process of particular birds. Gould (1977) suggested that a combination of morphometric approaches with phylogenetic analyses shall provide a wide opportunity

for investigation of particular kinds of evolutionary processes, such as those related to allometries and heterochronies. These sorts of evolutionary processes are important in tracing the morphological implications of phylogenetic patterns (David & Laurin 1996).

In this chapter cladistic analysis was carried out to determine systematic relationships among skuas (Aves: Stercorariidae). These birds are widely known, but have unresolved controversial evolutionary relationships. More information regarding their systematics relationships is discussed in Chapter 1. Members of the large skuas group, for example, provide a very interesting question to tackle. All of them, except the Great skua (*Catharacta skua*), have a southern hemisphere distribution which indicates that they may have originated from the same stock. Separation in large skuas' distribution (Great skua from others) suggests various hypotheses. This separation may be due to some population of Great skuas which may have migrated to the South and gave rise to the rest of large skuas population. Otherwise, some taxon of southern large skuas may have arrived in the Northern hemisphere through freak events and gave rise to the Great skua (Fisher & Lockley 1954).

The systematic relationship between the Pomarine skua and other skuas is also investigated in this study. Some researchers (Furness *et al.* 1995, Cohen *et al.*, in press) believe that this taxon resulted from hybridization between Great skuas and Arctic skuas. This is because Pomarine skuas possess intermediate features (in term of body size and behaviour) between small and large skuas and because mtDNA sequence data indicate unexpectedly close relationships between the Pomarine skua and the Great skua.

3.2. Materials and Methods

Due to the difficulty in obtaining live birds and for the purpose of standardisation, all examinations involved in this study were conducted on museum skins. These specimens originated from various localities and were deposited in the Natural History Museum at Tring, England. The fact that they originated from various areas ensures that they

should present well-diversified data. This will provide an excellent opportunity for investigating the effects of microhabitat variations (i.e. distinction in environmental pressures) on skua morphology.

In total 72 skua specimens were examined in this analysis. Each taxon was arbitrarily represented by eight specimens, four for each sex. Only specimens which were preserved in similar plumage polymorphism phase were used in the analysis. For this study, dark phase skuas were chosen. The contribution of sex variation to skua morphology was determined by studying both sexes separately. In addition to the skuas, three individual gulls (*Larus fuscus fuscus*) were also examined. Gulls are the closest relative to skuas and, therefore, were the best candidate for an outgroup in rooting phylogenetical trees. A cladogram was generated based on forty multistate characters which were coded for all samples. All characters, together with their brief explanation were listed in Appendix 3.1. These characters, including two-state and qualitative or quantitative multistate characters were used in presenting a phylogenetical relationship among skuas. Almost all coding characters were adapted from Schnell (1970) with slight modification of codings for colour. This is because skuas possess a slightly different plumage compared to gulls and a few adjustments had to be carried out to ensure correct codes were used.

An initial examination of the data matrix indicates that there is no obvious variation between different individuals belonging to particular taxa. Therefore, data from individuals of the same taxon were pooled. The data matrix was analysed later by using specific analysis provided by computer software such as MacClade (Maddison & Maddison 1992) and PAUP (Swofford 1993). To facilitate analysis, data were entered into MacClade before being exported into PAUP as a Nexus file. In examining morphological variation among skuas, the dataset was examined as a whole and then separated into three smaller categories and the level of homoplasy for each type measured. These datasets are; 1) head and neck colourations (characters EX1 to EX13), 2) body, wings and tail colourations (characters EX14 to EX29), 3) other quantitative

and qualitative characters (characters EX30 to EX40). All these datasets were examined revealing morphological variations of various regions of the bird body.

Phylogenetical relationships among study taxa can be displayed by cladistic analysis in two ways. First, by using the compatibility method, which obtains maximal numbers of uniquely derived character states (apomorphies) or assumes that the most likely phylogeny is the one that is fully compatible with the largest number (or clique) of individual characters. Second, through parsimony, which will search for the minimal number of character state changes (homology) or attempt to minimize the number of evolutionary changes that must be assumed. Both approaches however, share similar basic methodology in proposing a hypothetical case for the true phylogeny and then demonstrating that a particular method either fails to retrieve it from the hypothetical data or is inconsistent with it if those data are added to it (Panchen 1992). Parsimony analysis was preferable in this analysis because it is concordant with the assumption of this study that convergence and reversals are scattered randomly over characters (Felsenstein 1982). Parsimony analysis has also been chosen because it has several advantages over compatibility analysis. Parsimony analysis, for instance, builds a phylogenetical tree by using a minimum number of character changes. It also gives an equal weight to all characters, in total contrast to compatibility analysis.

Although forty characters were available, not all of them were used in the analysis. Only informative characters were used. Uninformative characters were discarded by parsimony analysis to increase the accuracies in calculating consistency index (DeQueiroz & Wimberger 1993). When multistate characters were included, they were interpreted as uncertainties. Unknown or unclassified characters for particular taxa were coded as missing. All characters were assumed to be independent and therefore, were weighted equally. As Eernisse *et al.* (1992) pointed out, there is always a possibility that some characters are evolving more rapidly than others, but there is no *a priori* reason for differential weighting. An outgroup (gulls) was employed as a monophyletic sister group to the ingroup (skuas). Some characters were ordered whereas others were not. Ordered characters are EX11, EX12, EX23, EX24, EX30,

EX34, EX36 and EX37. All characters were optimised by the ACCTRAN method (accelerated transformation), and they were weighted equally. Searching for the most parsimonious tree was carried out by using exhaustive search (MAXTREES = 1000). This approach tries all possible combinations for shortest trees. Zero-length branches were collapsed and only minimal trees were saved. Whenever more than a single parsimonious tree was produced bootstrap analysis was carried out to determine which tree can be used as a consensus tree for all most parsimonious trees. A consensus tree is treated as a general agreement among parsimonious trees and, therefore, is not interpreted as depicting proper phylogenies (Swofford 1991). This consensus tree is achieved through bootstrap analysis by randomly sampling all data points and replacing the original dataset with a new one until a new dataset containing the original number of observations is obtained. For each replication, the statistic of interest is computed.

Results obtained from this analysis are compared with previous studies through comparison of the trees produced. This includes trees produced by multivariate morphometric analysis of skuas and partial mitochondrial DNA sequences of 12S rRNA and cytochrome b. Morphometric analysis data were extracted from the previous chapter (Chapter 2) while molecular data were obtained from Cohen *et al.* (in press).

3.3. Results

3.3.1. Analysis of Total Characters

Analysis of forty characters available for this study reveals that not all of them are informative. For the dataset for females, six characters (EX6, EX10, EX22, EX26, EX27, and EX 30) failed to contribute any significant information about skua relationships. One of these characters, EX10 has a constant value and, therefore, failed to present any information. This character and another five uninformative characters were excluded from the analysis to avoid any negative influence (such as reducing the accuracy of consistency index measurement) to the dataset.

Searching for the correct phylogenetical tree for female skuas was carried out on the remaining 34 characters by using an exhaustive search. This search produced eight most parsimonious trees with similar characteristics. All trees have same tree length (114 steps), consistency index (0.711) and retention index (0.629). These most parsimonious trees can be represented by a single consensus tree produced by bootstrap analysis. This representative tree, known as the 50% majority-rule consensus tree, has 121 steps tree-length, consistency index (CI) value of 0.669 and retention index (RI) value of 0.551 (Figure 3.1). The consensus tree suggests that female small skuas are clearly distinct from female large skuas. A member of the large skuas, the female Chilean skua, however, has been placed in the same group with small skuas but the former was connected to the latter at a higher level. In other words, this female Chilean skua acts as an outgroup for small skuas. Two members of large skuas, South polar and Falkland skuas were clustered together. Other large skuas, Tristan, Brown and Great skuas were not clearly separated and were connected to the consensus tree as a polytomy. The presence of this polytomy has restrained the revelation of the degree of relationships among large skuas.

Similar analysis was conducted on the male skua dataset. Analysis revealed that eight characters (EX1, EX2, EX3, EX10, EX22, EX26, EX27, and EX30) were uninformative. As with the female dataset, all uninformative characters were ignored in further analysis. Exhaustive search of this dataset produced only a single most parsimonious tree which had 95 steps of tree length, a CI value of 0.674 and an RI value of 0.630. Further analysis using the bootstrap method produced a consensus tree with 121 steps tree length, a CI value of 0.653 and an RI value of 0.500 (Figure 3.2). Although male and female skuas' consensus tree is slightly different, generally they were in agreement, delivering similar conclusions about the relationship between small skuas and Chilean skua. Both trees classified these skuas in the same group. Unfortunately, male's consensus tree failed to reveal any further relationship among large skuas members. All large skuas (except Chilean skua) were presented by the consensus tree as a polytomy.

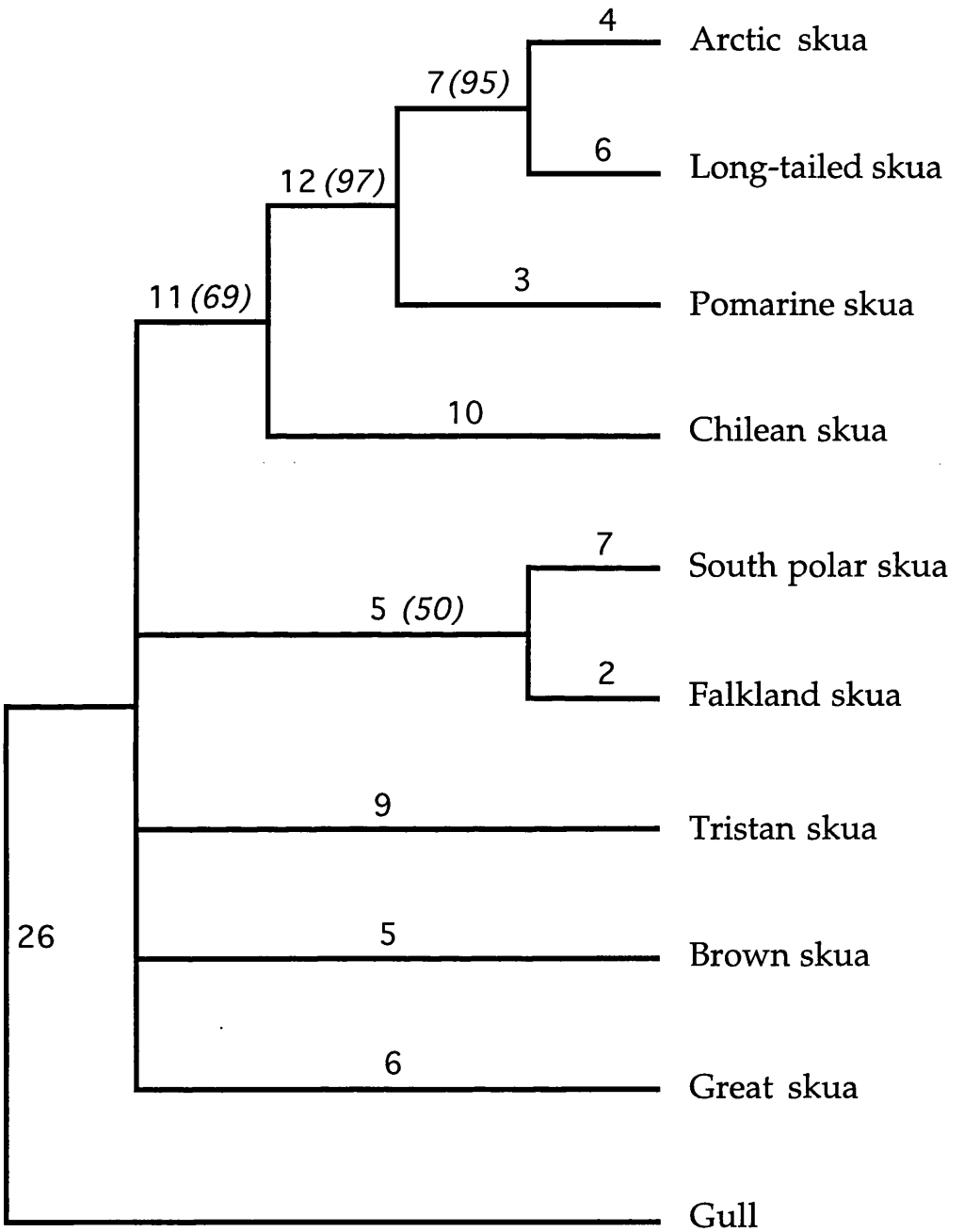


Figure 3.1. Consensus tree for female skuas. Tree was obtained by bootstrap analysis on 34 informative characters contains in whole dataset. Figures at each branch represent branch length whereas italic figures in parentheses represent bootstrap values. (Tree length = 121; CI = 0.669; RI = 0.551).

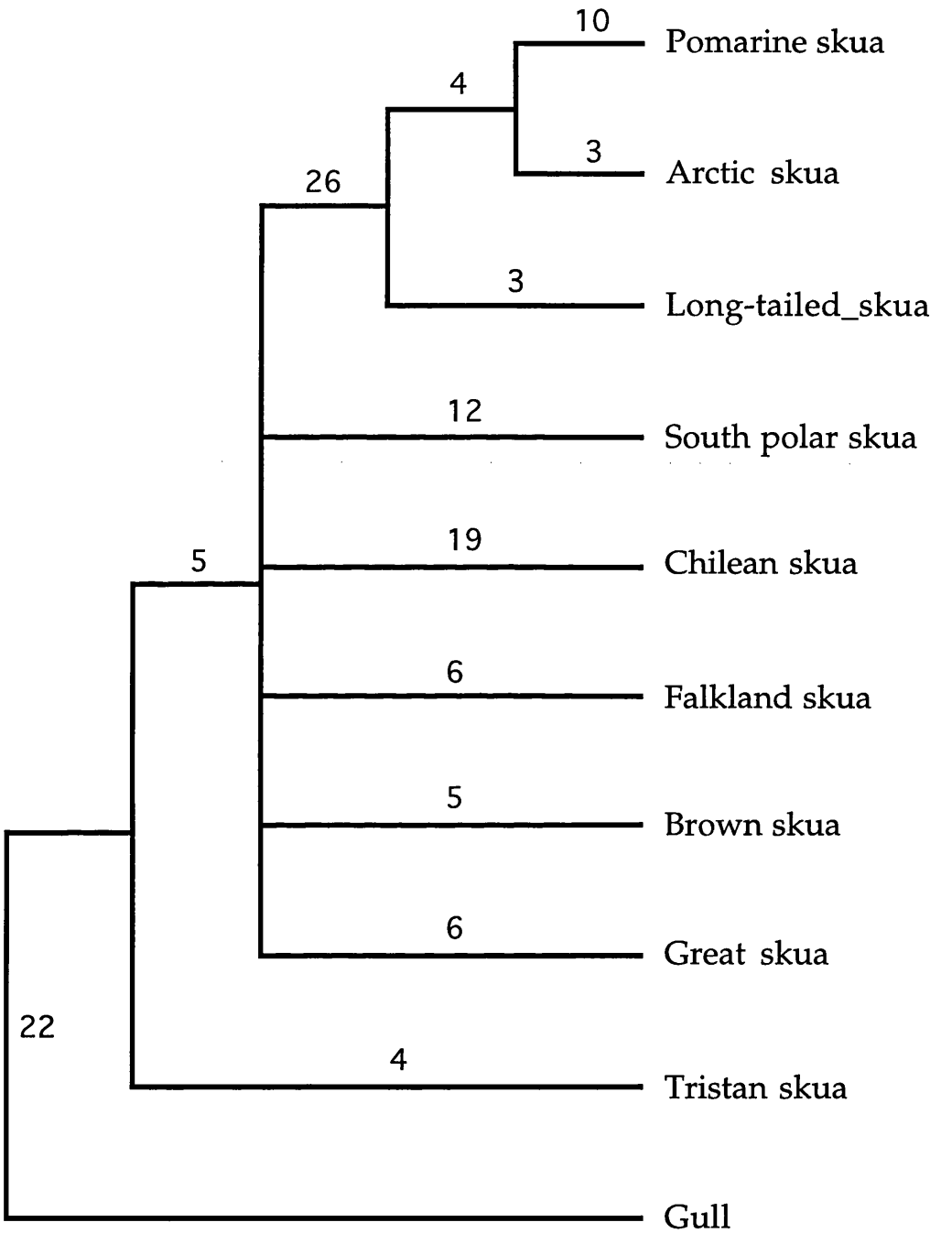


Figure 3.2. Consensus tree for male skuas. Tree is obtained from bootstrap analysis of 32 informative characters in whole dataset. Figures at each branch denotes branch length. (Tree length = 121 steps; CI = 0.653; RI = 0.500).

3.3.2. Variation in Head and Neck Colouration

Thirteen characters have been used to study morphological variations of these regions. Based on informative characters of the female dataset, exhaustive search successfully produced 42 equally most parsimonious trees. All trees have same features with 36 steps tree length, CI value of 0.889 and RI value of 0.818. Further examination by bootstrap analysis had produced a single consensus tree which possesses 39 steps tree length, CI value of 0.821 and RI value of 0.682. This consensus tree also successfully separated small skuas from large skuas (Figure 3.3). Arctic and Long-tailed skuas were clustered together in a single branch and formed a basic entity for this tree. This branch was then joined by Pomarine skua at a higher level. Unfortunately none of the large skuas have been successfully classified. All large skua members were connected to each other as a polytomy.

Four most parsimonious trees were produced when the male dataset was subjected to exhaustive search. All these trees have equal tree length (25 steps), same CI value (0.920) and constant RI value (0.90). Bootstrap analysis of these trees produce a single most parsimonious tree with 25 steps length, CI value of 0.920 and RI value of 0.900. Similar to the female consensus tree, this 50% majority-rule consensus male-tree clearly separated small skuas from large skuas (Figure 3.4). This male's consensus tree presents skuas as two large groups. In one branch all large skuas were clustered together whereas another branch consisted of all small skuas. Although all small skuas were clustered together, they were connected to each other as a polytomy and therefore, the degree of relationship among them cannot be determined. Large skuas branch also shows a similar problem in separating Tristan, Falkland and Great skuas. However, this branch has successfully shown the relationship among South polar, Chilean and Brown skuas. South polar and Chilean skuas were clustered together and this branch was joined later by Brown skua at a higher level.

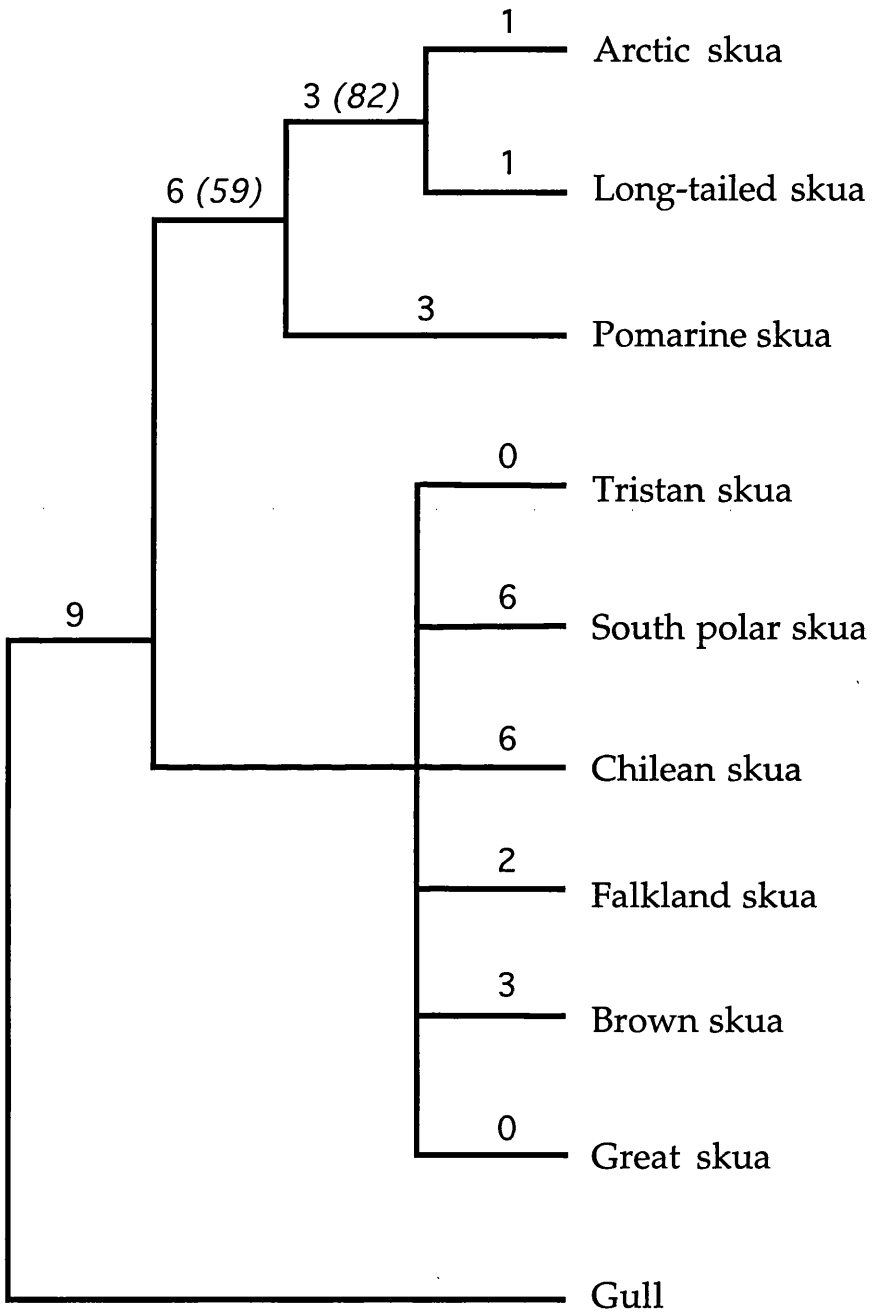


Figure 3.3. Consensus tree for female skuas. Tree was obtained from bootstrap analysis of 11 informative characters in second dataset, variation in head and neck colouration. Figures at each branch denotes branch length while figure in parentheses represent bootstrap value. (Tree length = 39 steps; CI = 0.821; RI = 0.682).

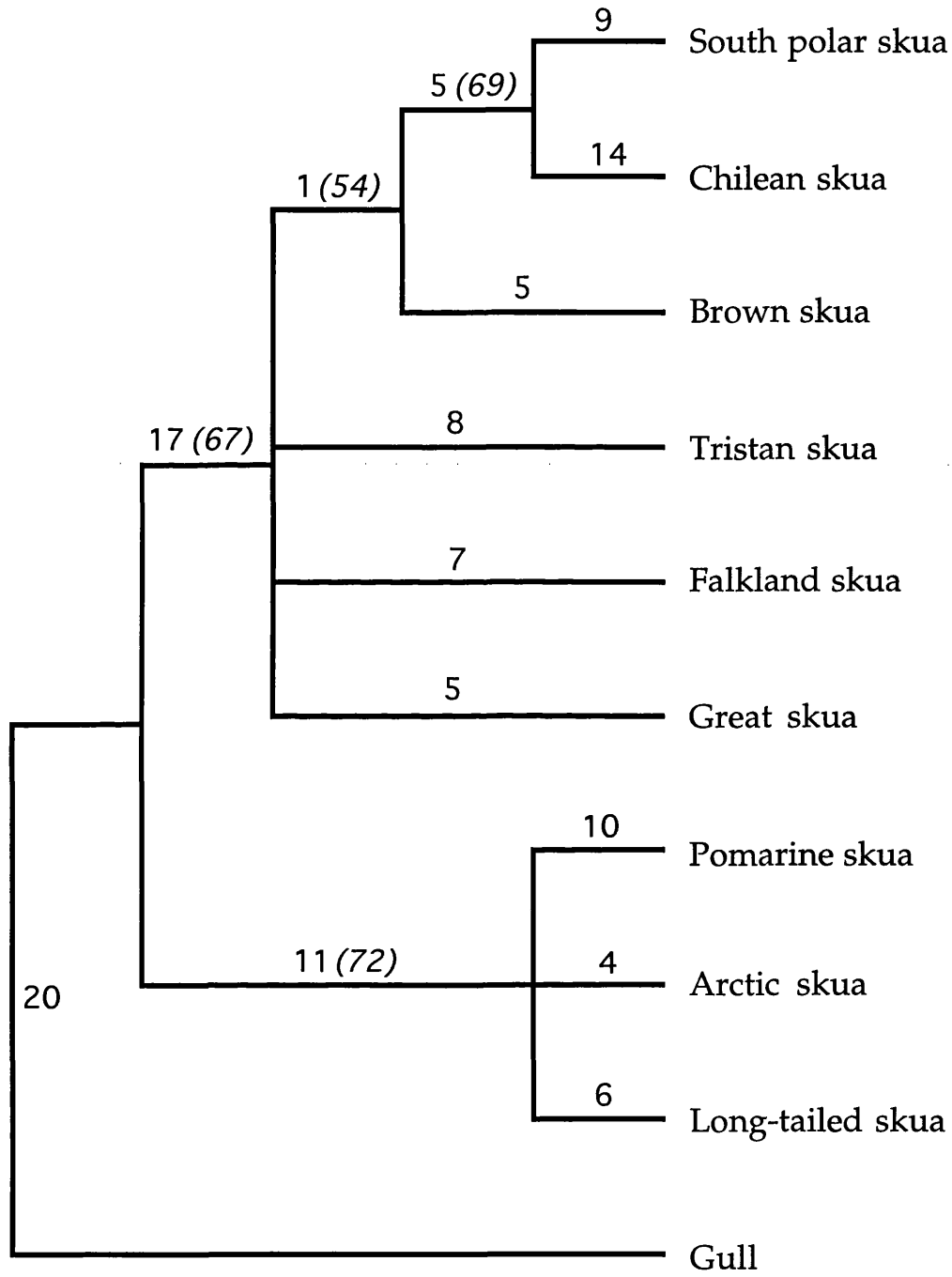


Figure 3.4. Consensus tree for male skuas. Tree was obtained from bootstrap analysis on 11 informative characters in second dataset, variation in head and neck colouration. Figures at each branch represent branch length of trespective branch while figures in parentheses represent bootstrap value for particular branch separation. (Tree length = 25 steps; CI = 0.920; RI = 0.900).

3.3.3. Variation in Body, Wings and Tail Colouration

The relationships among skuas based on morphological evidence of these physical characteristics was examined by using sixteen characters. For the female dataset, exhaustive search successfully revealed two equally most parsimonious trees. These trees have 33 steps length, a CI value of 0.727 and an RI value of 0.735. Both trees presented similar results except the placement of the Falkland skua. Based on informative characters available in this dataset, all skuas were clustered into two major groups. Large skuas (except South polar skua) composed their own branch whereas other skuas (small skuas and South polar skua) form another group. The taxon, in dispute, Falkland skua, was placed either as a polytomy or was grouped together with the large skuas group. A majority-rule consensus tree produced by bootstrap analysis for this dataset clustered all small skuas together in one group whereas Brown, Great, Chilean and Tristan were grouped in another branch. The remaining two large skuas, South polar and Falkland skuas were connected to both groups as a polytomy. This tree which has 36 steps length, a CI value of 0.667 and an RI value of 0.647 indicates that Tristan and Chilean skuas are the most closely related members in the large skuas group (Figure 3.5).

In contrast to the female dataset, exhaustive search of the male dataset only produced a single most parsimonious tree. This tree has 33 steps length, a CI value of 0.727 and an RI value of 0.710. This tree consists of two major branches. The first branch contains all large skuas and the other branch clustered all small skuas. Female's tree also indicates that Pomarine skua is closer to Arctic skua in small skuas branch and Tristan skua is closer to Chilean skua in large skuas cluster. Further examination by bootstrap analysis produced a single most parsimonious tree with tree length of 35 steps, CI value of 0.686 and RI value of 0.645. Basically this consensus tree presents similar results to the exhaustive search tree except that it failed to indicate a proper relationship between South polar and Falkland skua. As a result these two skuas were presented as a polytomy to other large skuas (Figure 3.6). Other arrangements are as in the exhaustive search tree.

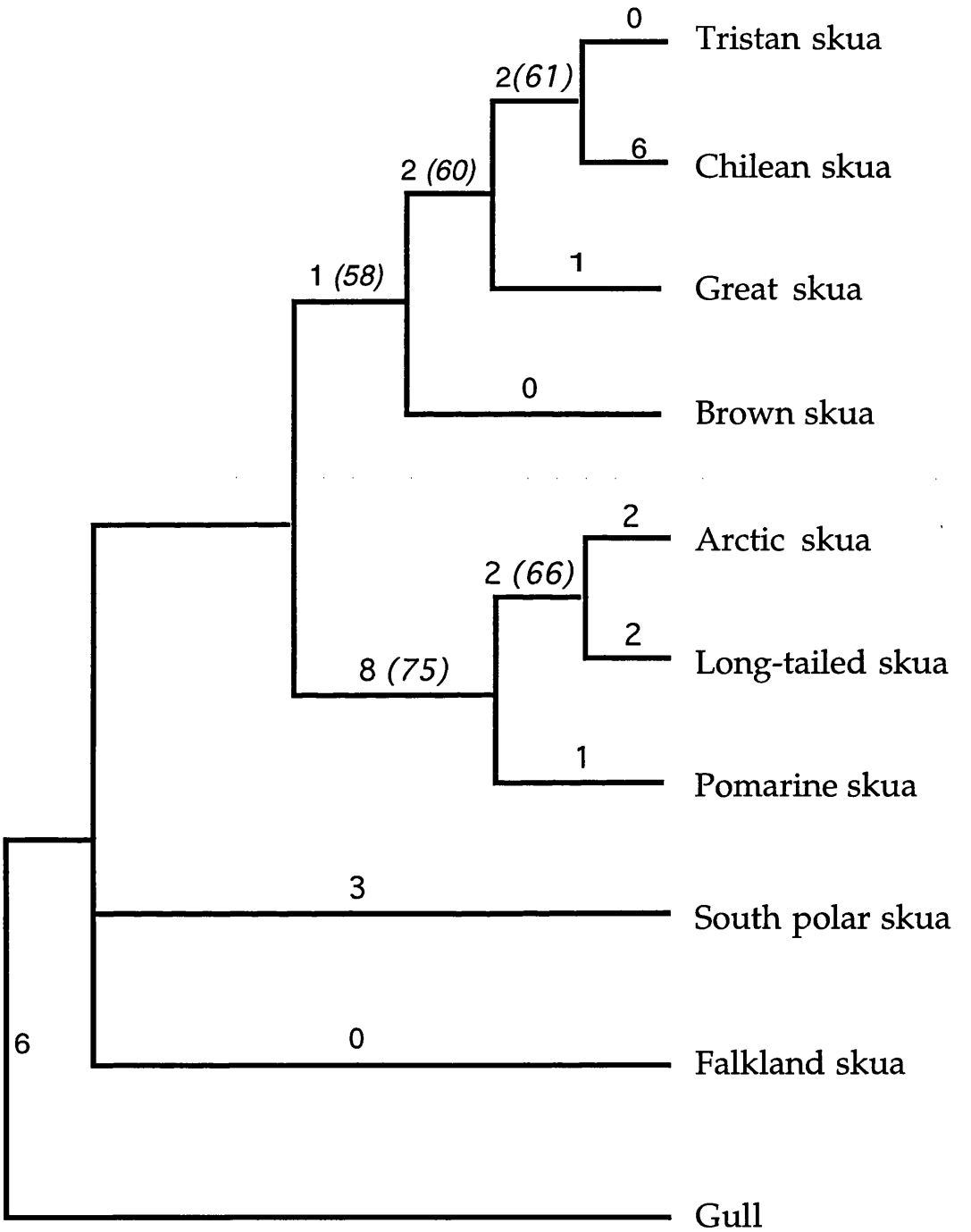


Figure 3.5. Consensus tree for female skuas. Tree was obtained by bootstrap analysis on 13 informative characters in third dataset, variation in body, wings and tail colouration. Figures at each branch denotes branch length while figures in parentheses represent bootstrap value (Tree length = 36 steps; CI = 0.667; RI = 0.647).

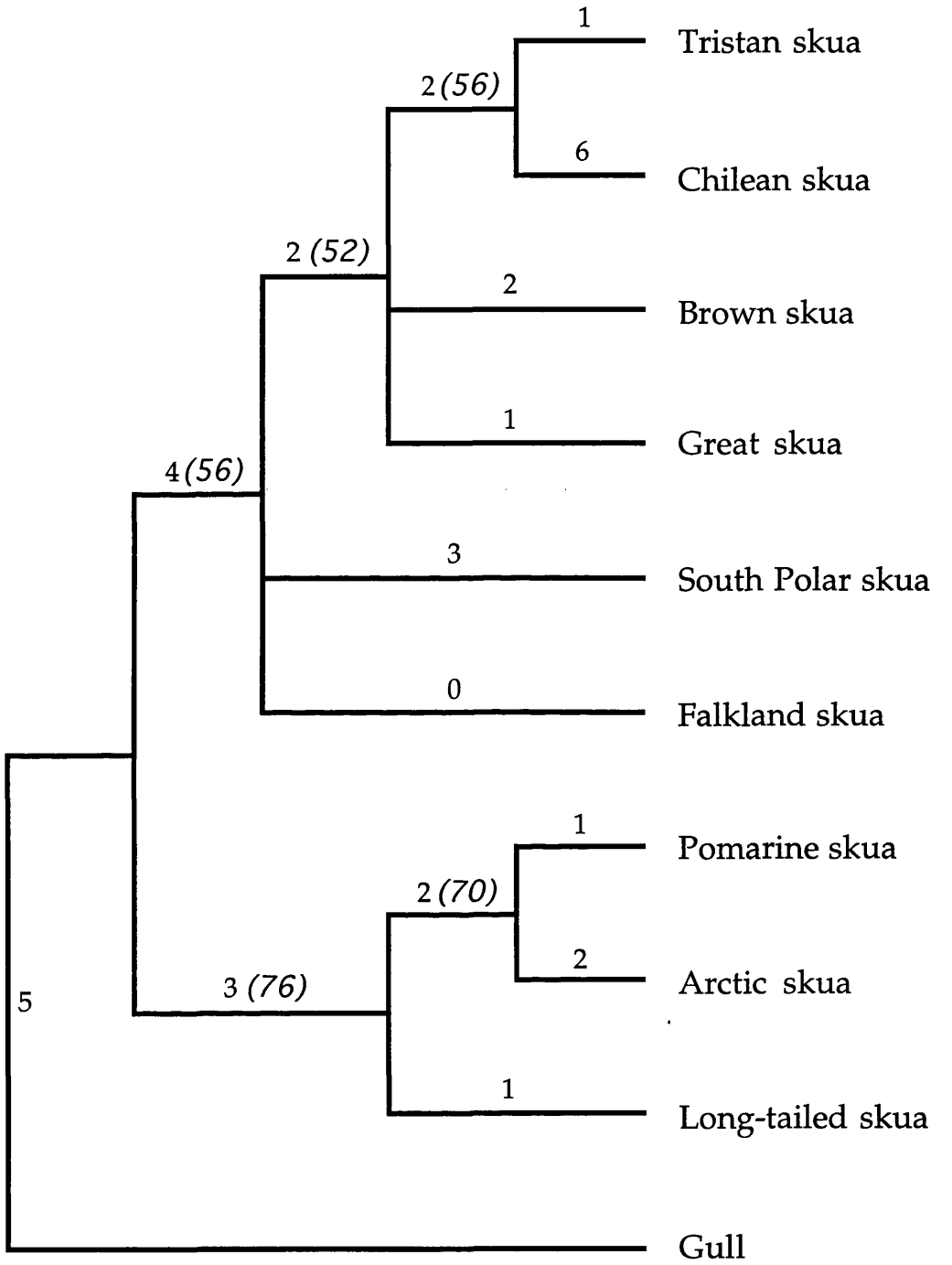


Figure 3.6. Consensus tree for male skua. Tree was obtained from bootstrap analysis on 13 infomative characters in third dataset, variation in body, wings and tail colouration. Figures at each branch denotes branch length while figures in parentheses represent bootstrap value (Tree length = 35 steps; CI = 0.686; RI = 0.645).

3.3.4. Variation in Other Features

For this dataset, both male and female have a similar feature for all coding characters (Table 3.1). Therefore, a similar conclusion was derived by both sexes. A single most parsimonious tree was produced by exhaustive search. This tree has a branch length of 30 steps, a CI value of 0.567 and an RI value of 0.606. This parsimonious tree separated all skuas into two major groups with Tristan skuas connected as a polytomy to all skuas. The Chilean skua however, was clustered together with small skuas instead of connected to large skuas branch. Other large skuas (South polar, Falkland, Great and Brown skuas) were clustered together in a single branch. This tree also shows that Pomarine skua is closer to Arctic skua than Long-tailed skua (Figure 3.7), a conclusion which supports trees produced by the previous dataset (Figure 3.6). A consensus tree produced by bootstrap analysis however, identifies all large skuas as a polytomy. This consensus tree which has 40 steps length, a CI value of 0.425 and an RI value of 0.303 only managed to classify small skuas as closely related taxa and they were allocated together in a single group (Figure 3.8).

3.4. Discussion

Results from this study show that morphology can be used in presenting phylogenetical relationships among skuas. This conclusion is in concordance with several morphometric analyses which used morphological evidence to infer phylogenetic relationships among organisms (Lang 1990, Chatterjee 1991, Greenlaw 1993, Crowe 1994). It is agreed that morphometric data can be used to assess the evolutionary pattern of organisms provided that the shape and size of particular organisms reflect the evolutionary history of that species (Archie 1985).

The most important step in executing cladistic analysis on skuas' morphometric data is selecting an appropriate character which reveals phylogenetic relationships among their members. Choosing and defining suitable characters is very important in this analysis because these two steps can lead to totally different conclusions. Using

Table 3.1. Data matrix of coding multistate characters for female (top) and male (bottom) skuas applied in this study. Missing characters were represented as ?.

characters	1234567891111 0123	1111112222222222 4567890123456789	33333333334 01234567890
Tristan skua	5555555441001	5664446623121200	00134022000
South polar skua	3345555551101	4564445621031200	00126033010
Chilean skua	5555544341211	5564445523221231	00226011110
Falkland skua	5555555441001	5664446621031200	01226033010
Brown skua	665555644?001	6664446622031200	00227023010
Great skua	5555555441001	5664446622131210	00215033110
Pomarine skua	675555223?022	4554145620021211	00215100010
Arctic skua	566645222?022	2552125520021201	00225100011
Long-tailed skua	456644222?022	4552226620023101	00214111011
Gull	212211111?002	6611116600008000	11137020100

characters	1234567891111 0123	1111112222222222 4567890123456789	33333333334 01234567890
Tristan skua	5555555441001	6664446623121200	00134022000
South polar skua	3333444441101	4564446621021200	00126033010
Chilean skua	5555544341211	5564445523221231	00226011110
Falkland skua	555555544?001	5664446621031200	01226033010
Brown skua	5555546441001	6664446622031200	00227023010
Great skua	5555555441001	5664446622131210	00215033110
Pomarine skua	675656222?022	5552155620021211	00215100010
Arctic skua	455655222?022	2552155520021201	00225100011
Long-tailed skua	555645223?022	5554256620023101	00214111011
Gull	111111111?002	6611116600000000	11137020100

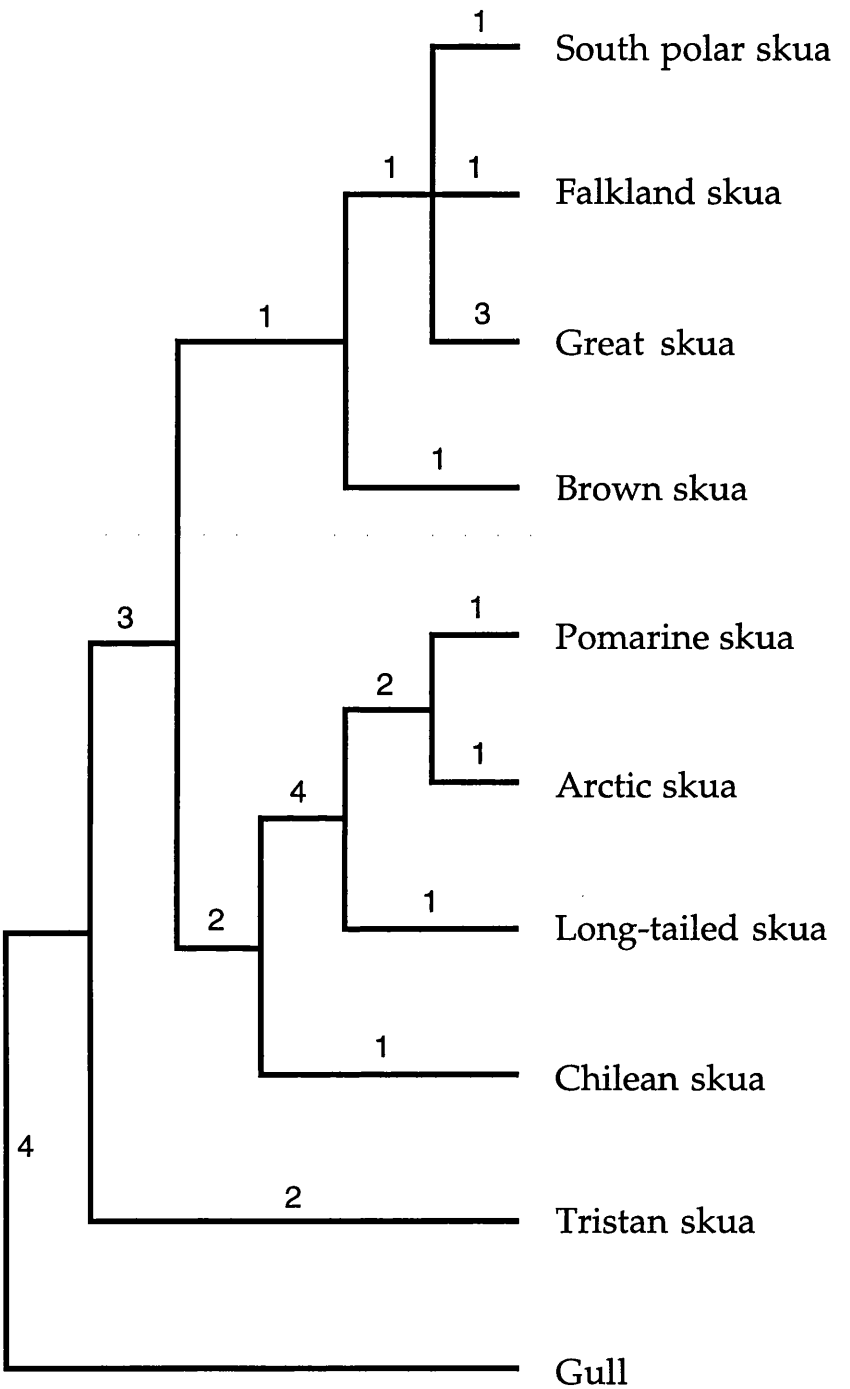


Figure 3.7. Cladogram resulted from exhaustive search of ten informative characters in fourth dataset, variations in other characters of skuas morphology. Figures at each branch denotes branch length (Tree length = 30 steps; CI = 0.567; RI = 0.606).

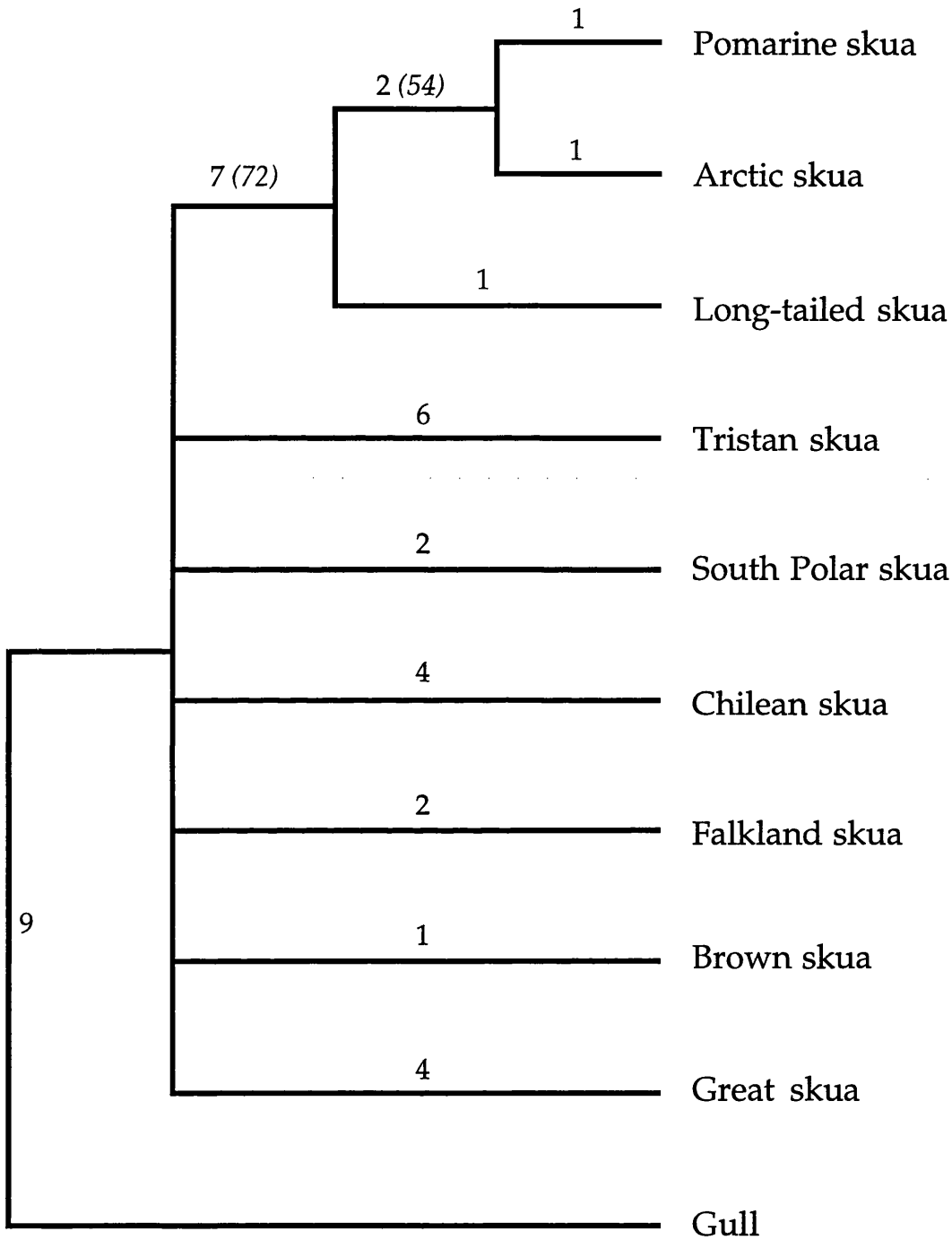


Figure 3.8. Consensus tree for skuas. Tree was obtained by bootstrap analysis based on ten characters in fouth dataset, variation in others coding characters. Figures at each branch denotes branch length while figures in parentheses represent bootstrap value (Tree length = 40 steps; CI = 0.425; RI = 0.303).

unsuitable characters will produce false or inaccurate judgement about study objective. In this study, this effect was tested by dividing the whole dataset into smaller categories and cladistic analysis was applied to each of them. Variation in the final result presented by cladistic analysis confirms the idea that each character has a different degree of contribution to the phylogenetical tree.

The number of characters employed in the analysis also plays an important role in deriving an accurate conclusion about skua phylogeny. Gaffney *et al.* (1995) stated that cladists should use between 20 and 100 characters to decipher reliable evolutionary relationships. However, increasing the number of characters does not promise a good result if those particular characters are uninformative as shown in this study. Although forty characters have been used in the analysis, only 34 characters from females were informative in deriving phylogenetical relationships. For male skuas, only 32 characters were informative. Therefore, a thorough procedure has to be used when selecting characters for cladistic analysis to ensure that only informative characters are used in the analysis. The importance of choosing appropriate characters for cladistic analysis was highlighted by Skala & Zrzavy (1994). They stated that appropriate characters are important in ensuring that a resultant cladogram will be useful in terms of reflecting the characters patterns.

Interactions between characters also play an important roles in revealing skua phylogeny. Each character has the tendency to behave differently due to this interaction. The separation of whole datasets therefore, provides a good opportunity to reveal which characters really contribute significantly toward skua classification. By using all the informative characters, almost all datasets strongly suggest that small and large skuas should be separated. This classification agrees with the evidence gained from other studies of morphological variation of skuas (this study, Chapter 2; Peters 1934, Howard & Moore 1980). Results from this analysis however, are in contrast to the conclusion derived from the evidence of molecular analysis (Figure 3.9, excerpt from Cohen *et al.*, in press) and parasitological evidence (Furness *et al.* 1995). This result is

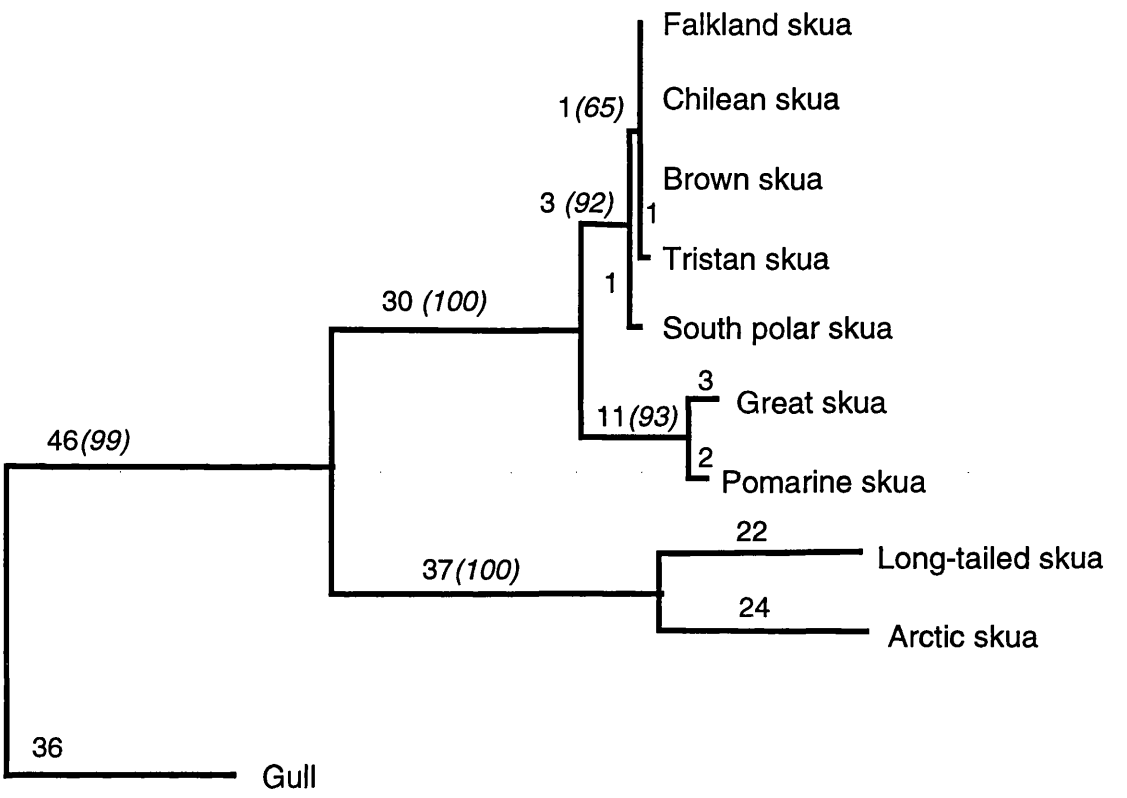


Figure 3.9. The most parsimonious tree obtained by bootstrap analysis based on mitochondrial DNA sequence (12S rRNA and cytochrome b). Figure at each branch denotes branch length while figures in parentheses represent % bootstrap value for respective branch (Tree length = 251 steps; CI = 0.701; RI = 0.665) (Excerpt from Cohen *et. al.* , in press).

understandable since all characters used in this analysis were inferred from coded characters based on morphological variations.

If results from molecular and morphological approaches agree with each other, then the truth is closer and additional comparative studies are welcome to strengthen the hypothesis. Although cladistic analysis presents a different result from molecular analysis in this study, this does not mean that morphological data are unsuitable for inferring phylogenetical relationships. Furthermore, this result is not totally against molecular evidence but only suggests a different hypothesis about the allocation of Pomarine skuas. Incongruencies between molecular and morphological data are not only present in this study but have already been concluded in various previous studies. In a study of Tyrannidae for instance results from a DNA study and morphological analysis suggested different conclusions (McKitrick 1985). This phenomenon occurs when morphology and genetics do not experience similar rates of change. Therefore, there is a possibility that a modification in morphology may occur either faster or slower than genetical or molecular change due to environmental pressures. There is always a possibility that this induced morphological adaptation may revert to the original state when related pressures are removed.

A similar phylogenetic hypothesis will only be generated by both approaches (cladistic and morphological analyses) when animal molecules and their phenotypes are acting to a similar evolutionary pressure. This phenomenon has been suggested by Kimura (1979) in his 'neutral theory of molecular evolution'. Theoretically, for proper comparison, molecular study must concentrate on the DNA section which corresponds to a particular section of morphology under examination. This demanding step needs the information of bird's genomic mapping which is unavailable at present. Although some researchers (such as McKitrick 1985) believe that in the event when different conclusions have been fowarded by various datasets either one must be correct, although they are exposed to different evolutionary pressures, but the frequency of validity of the result varies. A contrasting result which derived from skuas' morphology therefore, may provide some clue about true relationships of skuas.

Results from cladistic analysis do not provide any clues about the possibility of hybridization. The Pomarine skua has been clustered together with other small skuas by all datasets. According to McDade (1992) hybrids possess more extreme characters than either of their parents and therefore behave like apomorphic taxa and are placed cladistically proximate to the most derived parent. Therefore, if Pomarine skuas are derived due to hybridization between Great skua and Arctic skua, it should be placed between them in a cladogram. This did not occur in any of the datasets involved.

Hybrids may actually disrupt the analysis and this cannot be detected. However, McDade (1992) stressed that it is most unlikely that hybrids will disrupt cladistic analysis and this was supported by his study on distance data between parsimonious trees. His dataset presents similar trees before and after the inclusion of the hybrid. Hybrids however, do have the potential to disturb our understanding of phylogenies when they are present in a large number (more than four taxa in a single dataset; McDade 1992) and this may alter an hypothesis of relationship among non-hybrid species. This is because cladistic analysis presupposes divergent evolution and is unable to present a correct phylogeny for hybrids which have a reticulating evolutionary history.

A hybrid is normally very difficult to distinguish from a normal apomorphic or intermediate species. This is untrue for Pomarine skua because it can be recognised easily. If hybridization between small and large skuas did happen, it must have occured a long time ago and according to morphological evidence this occurence is too old to prevent the Pomarine skua from being assigned as a distinct species.

Results from cladistic analysis strongly suggest the separation of skuas into two groups, small and large skuas, but these results are incongruent with molecular data so that cladistic analysis basically deny findings proposed by molecular analysis. Cladistic analysis based on selected characters therefore, failed to contribute clear ideas toward the origin of the Pomarine skua.

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Appendix 3.1. Description of external characters used in cladistic analysis.

For colour; each OTU was coded from one to seven for each character, the numbers corresponding, respectively, to white, the five intermediate steps (pale, light, medium, dark, and blackish gray), and black.

Set 1: *All Characters*

Set 2 : *Head and neck colouration*

EX1 : Forehead colour
EX2 : Crown colour
EX3 : Posterior lores colour - posterior region of lores near eye
EX4 : Anterior lores colour
EX5 : Cheek colour
EX6 : Upper nape colour - anterior region of nape that is most proximal to head
EX7 : Lower nape colour
EX8 : Chin colour
EX9 : Throat colour
EX10 : Iris colour. Coded: light (0); dark (1)
EX11 : Brown in head plumage. Coded: none (0); little (1); moderate amount (2); lot (3)
EX12 : Yellow in neck plumage. Coded: none (0); little (1); lot (2)
EX13 : Bill colour - dominant colour found on bill. Coded: Black (1); brownish (2); grayish (3)

Set 3: *Body, wings and tail colouration*

EX14 : Mantle (includes back & scapulars) colour
EX15 : Rump colour
EX16 : Tail colour
EX17 : Breast colour
EX18 : Belly colour
EX19 : Colour of under tail coverts
EX20 : Wing colour - colour of lesser, middle, and greater coverts of wing
EX21 : Colour of secondaries
EX22 : Leg colour - colour of tarsus. Coded: flesh (0); gray, bluish, or greenish (1); black (3)
EX23 : Brown in body plumage. Coded: none (0); little (1); moderate amount (2); lot (3)
EX24 : Red colour in plumage. Coded: None or little (0); lot (1)
EX25 : Presence of contrasting colour in primaries - primary colour contrasting to that coded for tenth primary outer edge colour in distal two-thirds of length. Coded: none (0); about one-fourth of primary with contrasting colour (1); about one-half (2); about three-fourth (3); most of primary with contrasting colour (4)

EX26 : Contrasting colour in primaries - if present, the score of the contrasting colour on the neutral colour scale described above. Coded: white to black (0 to 7 as above); no compariosn (NC) when contrasting colour not present

EX27 : Contrasting colour at base of primaries - area of contrasting colour at proximal end of webs of primaries. Coded: absent (0); present but indistinct (1); present but distinct (2)

EX28 : Contrasting tail colour - distal end of tail with contrasting colour. Coded: absent (0); contrasting colour present on distal ends of rectices (1); colour present as much as distal one-third of tail (2); colour present as much as distal one-half of tail (3)

EX29 : Lateral colour pattern of primaries. Coded: relatively uniform colouration from outer to inner vein (0); distinct break in feather colouration along long axis of feather (1)

Set 4: Other Features

EX30 : Web shape - degree of indentation of webs or palmations of foot. Coded: incisure shallow (0); incisure moderate (1); incisure deep (2)

EX31 : Outer toe length - recorded relative to length of middle toe. Coded: outer shorter than middle toe (0); outer equal to middle toe (1)

EX32 : Inner toe length - recorded relative to length of middle toe. Coded: inner equal to joint (distal end of second phalynx) of middle toe (0); inner longer than joint of middle toe (1)

EX33 : Relative length of retrices - relative lengths of outer and inner retrices. Coded: outer much longer than inner (1); outer slightly longer than inner (2); outer and inner about equal in length (3); outer slightly shorter than inner (4); outer much shorter than inner (5)

EX34 : Angle of gonys - angle of lower mandible at posterior end of gonys as seen from lateral view. Coded: angle approximately 1 degree (1); 4 degrees (2); 7 degrees (3); 10 degrees (4); 13 degrees (5); 16 degrees (6); 19 degrees or more (7)

EX35 : Tail projection. Coded: absent (0); present (1)

EX36 : Mirrors on tenth primary - mirrors or contrasting spots present near distal end of tenth primary. Coded: absent (0); small mirror present, covering one vein of feather (1); mirror medium in size and covering both webs (2); mirror large and covering both webs (3)

EX37 : Mirrors on ninth primary. Coded: absent (0); small mirror present, covering one vein of feather (1); mirror medium in size and covering both webs (2); mirror large and covering both webs (3)

EX38 : Wing bands - presence of contrasting tipping of distal ends of secondaries and tertials. Coded: absent (0); present (1).

EX39 : Relative length of retrices - if tail feathers of different length, then indication as to which are longest. Coded: same length (0); first (outer) longest (1).

EX40 : Presence or absence or barring sign during juvenile stage. Coded: absent (0); present (1).

Chapter Four

Coevolutionary Relationships Between Skuas (Aves: Stercorariidae) and Their Feather Lice (Insecta: Phthiraptera)

4.1. Introduction

4.1.1. Theory of Coevolution

Close relationships between different species living together in the same environment invite studies to determine the degree of coevolution among these organisms, especially where there is an intimate relationship between host and parasite (Mitter & Brooks 1983; Stone & Hawksworth 1986). Ideas about host-parasite coevolution had already been presented by 1815 (see Mauersberger & Mey 1993) but were explicitly introduced by Ehrlich & Raven in 1964, and properly defined later by Janzen (1980). According to Janzen (1980), coevolution is a process that involves evolutionary change in a trait of the individuals in one population in response to a trait of the individuals of a second population, followed by an evolutionary response by the second population to the change in the first. Coevolution has also been defined as a combination of two processes; coaccommodation between a host and its parasites, and cospeciation (Kim 1985). Coaccommodation is the mutual modification or microevolutionary adaptation in two species, while cospeciation exists when one species speciates at a particular rate in response to another (Hafner & Nadler 1990; Page 1993). Coevolution will produce similar phylogenies in host and parasites. This similarity, referred to as congruence, is evidence that hosts and parasites have cospeciated, so that host and parasite are associated by descent, whereas incongruence is evidence for host switching, or association by colonisation (Brooks & McLennan 1991). Therefore, by comparing phylogenies, the history of cospeciation between host and parasites can be reconstructed. More sophisticated studies involve mapping the parasite's characters

onto host phylogeny and testing goodness of fit to calculate a consistency index have been carried out by various researchers such as Glen & Brooks (1985); Hoberg (1986, 1992); Klassen & Beverly-Burton (1988), and Brooks & McLennan (1991).

Not all coevolutionary processes involve cospeciation. Thompson (1989) divided coevolution into five categories, the first three of which do not involve cospeciation. These are listed below; Type (1) known as gene-for-gene coevolution, normally occurs in pathogens and plants. In this type of coevolution both species have complementary loci for cause (e.g. virulence) and effects (e.g. resistance). Type (2) known as specific coevolution, exists when coadaptation of two species does not specify a gene-for-gene relationship and the association between any two of them may not be strictly reciprocal. Type (3), guild or diffuse coevolution, occurs when reciprocal coevolutionary changes exist among a group of species rather than pair of species. This type of coevolution is important because it emphasises that the evolutionary unit is broader than a species. Type (4), diversifying coevolution, has been defined as reciprocal evolution between species in which the interaction causes at least one of the species to become subdivided into two or more reproductively isolated populations. Previously, this type of coevolution was known as mixed-process coevolution if only one of the species undergoes speciation. The last category of coevolution (Type 5) is known as escape-and-radiation coevolution which differs from guild coevolution in the sense that it may involve both adaptation and speciation and includes periods during which the interaction between the taxa do not occur. It differs from diversifying coevolution in the way in which the interaction is involved in speciation. In Type 5 coevolution, changes occur while the hosts are temporarily free from parasites.

Host-parasite associations have been studied extensively and this relationship has been presented either as phyletic tracking or resource tracking models. All these rules are discussed briefly below.

4.1.1.1. Fahrenholz's Rule

Fahrenholz's rule claims that the natural classification of some groups of parasites correspond with that of their hosts, or 'parasite phylogeny mirrors host phylogeny' (see Eichler 1948). This claim derives from the idea that ancestors of extant parasites must have been parasites of the ancestors of the extant hosts, so that the evolution of hosts and parasites has been in correspondence. Thus, we can obtain knowledge about host systematics by inferring their parasite's classification even in the situation where relationships among hosts have been obscured by the development of extravagant morphological features.

4.1.1.2. Szidat's Rule

This rule implies that host and parasites not only show parallelisms in their systematics but also have resemblance in structural complexity (Brooks 1979; Mitter & Brooks 1983). Primitive hosts tend to harbour simple or primitive parasites whereas specialised parasite can be found on more advance hosts. Szidat develop his rule by showing that Trematodes from the Family Paramphistomidae were more specialised when they lived on a more advanced host (Eichler 1948). This rule is not only valid for Trematodes but is also applicable to other permanent parasites. In Ischnoceran lice for instance, primitive groups tend to possess a circumfasciate head (complete line of thickening around the anterior margin) whereas more specialised genera normally have a non-circumfasciate form. Review of this lice's morphology proved that circumfasciate forms become rare or absent in lice of higher taxonomic rank hosts, for example higher orders or families of birds (Eichler 1948).

4.1.1.3. The Divergence Rule or Eichler's Rule

According to this rule hosts belong to a larger group if they are parasitised by more than one genus of parasite (Brooks 1979; Mitter & Brooks 1983). Isolated hosts normally do not harbour many species of parasites, whereas in comparable groups of hosts, not only

many species of parasites can be found (each host with its louse) but also a differentiation into many genera which may live together on the same host species. Some isolated bird groups (e.g. Burhinidae) do not have more than one or two genera of louse (*Edicnemiceps*), in contrast to Lariidae (for example) which harbour various genera of Mallophaga (*Saemundssonia, Austromenopon*, and *Actornithophilus*) (Eichler 1948).

4.1.1.4. Manter's Rule

This rule expresses three main ideas in host-parasite relationships. First, it is assumed that parasites will evolve at a slower rate compared to their hosts. Second, longer association between host and parasites will produce more specific parasites. Extant parasites such as *Quadraceps* of Charadriiformes will present a clearer and more reliable relationship compared to recent genera such as *Saemundssonia* and *Cummingsiella* (Timmermann 1952a). Finally this rule also suggests that the host will harbour the largest number of parasites in the area it has resided in most, so if the same or two related species of a host exhibit distinct distributions and possess similar parasite faunas, the areas in which the hosts occur must have been contiguous at a previous time (Brooks 1979; Mitter & Brooks 1983).

4.1.1.5. Resource Tracking Hypothesis

In addition to the phyletic tracking models, the resource tracking hypothesis was introduced by Kethley & Johnson (1975), as an alternative interpretation of ectoparasite speciation patterns. This hypothesis does not contradict the above rules because they are based on different assumptions of parasite dispersal (Timm 1983). According to this hypothesis, parasites are adapted to the resources that are, or may be, distributed independently of the taxonomic relationships of the hosts (Lyal 1986). It is also argued that hosts exert strong selective pressures on their ectoparasites through preening and

grooming activities. Therefore, ectoparasites are selected to conform to any given topographic region of the host as protection to avoid host defence (Rozsa 1993).

4.1.2. Feather Lice and Avian Taxonomy

Ectoparasites occur widely on seabirds (Rothschild & Clay 1952). Bird ectoparasites consist of a variety of insects and mites that live on the body of the bird either permanently (such as feather lice), or for a certain part of the life cycle of the parasite (such as fleas), or may just visit the bird at times when they need food (e.g. bed-bugs). Some of these ectoparasites (e.g. feather lice or chewing lice or biting lice) are highly host specific and, therefore, can be used as an indicator of their host phylogeny.

Feather lice (Insecta: Phthiraptera) are divided into four major groups; the first group, the Anoplura also known as sucking lice, whereas others, the Rhynchophthirina, the Ischnocera, and the Amblycera are more popular collectively as chewing or biting or feather lice, depending on which host they affected. Anoplura can normally be found on eutherian mammals whereas Rhynchophthirina parasites occur on elephants and the African wart-hog (Barker 1994). Lice from suborders Ischnocera and Amblycera (normally referred to as Mallophaga) parasitise mammals and birds, and can be easily separated from each other by using the feature of antennae. Amblyceran lice have very short antenna concealed in grooves on the side of the head, whereas Ischnoceran lice possess longer and clearly visible antennae (Chinery 1993).

Generally lice are minute and wingless, have a flattened body (with variation in shape and pigmentation to adapt to host's feathers or hair), spiracles on dorsal surface, poorly developed sensory organs, and small or no eyes. They are obligate parasites, and therefore rarely leave the host except in the transferring process during direct contact between hosts (e.g. between parents and their offspring at the breeding site). This transfer process happens very rapidly because it is very dangerous for the insects. On the completion of sexual reproduction, adult females will lay eggs or nits (around 50-100; Chinery 1993). Eggs are glued to the feathers or hair of the host with a glandular

cement, normally in positions protected from grooming, the primary defence of the host against lice (Waage 1979; Clayton 1991). The nymphs will complete three instar stages before reaching the adult phase with no obvious metamorphosis (Chinery 1993). This process will be completed in 3-4 weeks (Marshall 1981). The adults and nymphs, depending on the species, feed on fur, hair, blood, serum, and secretions of sebaceous glands. Ischnocera feed exclusively on feathers and dermal debris, which they metabolise in the presence of symbiotic bacteria (Eichler *et al.* 1972; Marshall 1981). Ischnoceran lice are morphologically specialised for locomotion on feathers and rarely, if ever, venture onto the skin of the host. In contrast, Amblycera are more agile and occur on the skin as well as the feathers and feed on both feathers and blood (Ash 1960; Marshall 1981). Lice in the latter suborder are capable of abandoning a dying host and may be less vulnerable than Ischnoceran lice (Clayton *et al.* 1992).

Feather lice are highly host specific and nonpathogenic, suggesting the possibility of cospeciation with their host (Marshall 1981). Lice will rapidly die if transferred to an unrelated host (Hopkins 1942). This highly specific character indicates that a certain species of feather lice will only occur on one species of host and related hosts tend to harbour related lice. Generally, differences in feather lice populations indicate the separation between the two hosts (Zonfrillo 1993).

Kellog (1896) proposed the idea of inferring host phylogenies based on parasite distribution. He proposed that the presence of Phthirapteran lice on birds would provide information about the phylogenetic relationships of their hosts. This suggests that host relationships can be deduced from the presence of the same species of parasitic insect on allied hosts. In addition, Harrison (1914) noted that this argument also applied for allied parasites on different hosts. The presence or absence of only a subset of parasite taxa will accurately reflect host phylogeny (Paterson *et al.* 1993). The fact that most species or some genera of feather lice are restricted to one or a few related bird taxa strengthens this finding. *Bedfordiella unica,* for instance, has been found on Kerguelen petrel only, although studies have been done on various samples belonging to all genera of Procellariiformes (Paterson *et al.* 1993).

All previous studies about lice and avian taxonomy can be grouped into four main groups as reviewed by Mauersberger & Mey (1993). The first category contains all general concordance results between feather lice and classical systematics (based mainly on anatomy or morphology) or molecular evidence (primarily by DNA hybridisation). Mallophaga indicated the peculiarity of Galliformes (excluding *Opisthocomus*) by clearly distinguishing between the Megapodiidae and the Numididae. In studying relationships between the Anhimidae and the Anatidae, closer affinity shown by molecular evidence has been supported by feather lice. Both molecular and lice data, therefore, show that they are more closely related to each other than to any other bird group. Lice also successfully proved the isolated position of the mousebirds (six species forming the order Coliiformes). The parrots (Psittaciformes) are clearly separated from other birds by harbouring about 20 genera of Mallophaga that are present almost exclusively in this group. A study of the louse fauna on the Kerguelen petrel by Palma & Pilgrim (1983) shows that this bird should be regarded as a very distinct species, perhaps in a group of its own. Evidence from the Mallophaga (Pilgrim & Palma 1982) disagrees with the current grouping system which is based on the idea of a superspecies (Jounin & Mougin 1979; which positions *Pterodroma brevirostris* together with *P. ultima, P. mollis,* and *P. inexpectata*). However, the Mallophaga data agree with physical taxonomic characters and behavioural data (Harper 1973). A later review by Imber (1985) has classified this petrel as *Lugensa brevirostris* considering the distinctness of its Mallophaga community which is more related to that of fulmar than that of gadfly petrels. Analysis of morphological data of *Quadraceps normifer stellaepolaris* shows that *Stercorarius pomarinus* is farther apart from other two *Stercorarius; longicaudus* and *parasiticus* (Timmermann 1952b). It has also been shown that the hammer-headed stork (*Scopus umbretta*) is more closely related to Charadriiformes than to Ciconiiformes (as classified before) by possessing two species of Mallophaga which are normally present on Charadriiformes only (Hopkins 1942). Three species of lice present on Struthiones are classified as different genera. These are *Degeeriela asymmetrica* found upon the Emu; *Lipeurus asymmetricus* upon two species

of Rhea; and *Lipeurus quadrimaculatus* upon the Ostrich and two species of Rhea. These lice share similar head structure and Harrison (1914) concluded that they are derived from the same ancestor just as their hosts are.

The second group consists of data that show disagreement between feather lice and the classical system, but are supported by DNA evidence. Mallophaga indicate no affiliation of tropic-birds (Phaetontidae) with Steganopodes (or Pelecaniformes), and this was supported by Sibley & Ahlquist (1990) showing that Steganopodes are not monophyletic and can hardly be maintained as a taxonomic unit. Struthionidae and Rheidae share the same lice (genus *Struthiolipeurus*) and therefore, should not be separated as proposed earlier. Designation of an order for Turacos (Musaphagidae) by DNA hybridisation data is supported by two genera of lice that already shows that the Turacos are far apart from cuckoos. The feather lice found on South American seedsnipes (Thinocoridae) firmly show that this bird belongs to the Charadriiformes and indicates closer relationship to the Scolopacidae which is also suggested by DNA evidence. The Mallophaga fauna of the Crotophaginae is quite peculiar and distinctly separates their hosts from the cuckoos, though classically these birds have been counterfeit as a subfamily of the cuckoos. DNA evidence agrees with this separation (Mauersberger & Mey 1993).

The third category contains all lice data that show contradictory results to morpho-taxonomy but no DNA studies have been carried out to either confirm or invalidate the findings. Elbel & Emerson (1958) successfully distinguished between the Asiatic scops owl of Thailand and the North American screech owl based on the differences in species of lice on these birds. *Otus asio* of America is host to *Kurodaia painei* and *Strigiphilus otus*, whereas Thai *Otus* is host to two related but different species of Mallophaga, *Kurodaia sp.* and *Strigiphilus heterogenitalis*. Ignoring the possibility of straggler or host switching, two different hosts may share a common ancestor if they are parasitised by similar species of lice. Ostriches and Rheas harbour the same Ischnoceran parasites although these birds are clearly separated, inhabiting different continents. This finding suggests that these birds originated from the same

region (Harrison 1916). Harrison (1914) noted that *Goniodes* and *Goniocotes* are commonly present in Tinamous and Gallinaceous birds. These lice are also present on pigeons, and one species of *Goniocotes* has been found on *Opisthocomus*. All these four birds are closely related in classical taxonomy. These lice, however, have also been recorded on Penguins (order Sphenisciformes), and this suggests that all these five groups of birds may have a common origin. Straggling between Penguins and the other birds is virtually impossible since they live in different habitats and are geographically separated. Moreover, these lice are absent from other seabirds such as petrels or gulls, so these seabirds are unlikely to be a vector for lice transportation through straggling. These facts suggest that Sphenisciformes must have a common ancestral stock with the Tinamiformes, Galliformes, and Columbiformes. Penguins therefore, may have undergone a comparatively recent and rapid specialisation to an aquatic life, and may not be such an ancient group as has generally been considered (Harrison 1914).

Feather lice data may also show discordance with the arrangements of either one or both other approaches, and all these data has been classified into a fourth section. The systematic position of Flamingos is controversial. Some ornithologists place them in the Ciconiiformes (Storks) and others in the Anseriformes (Ducks and Geese), in both arrangements as a separate suborder. The communities of Mallophaga present on flamingos, storks, and ducks, clearly lump flamingos together with ducks rather than storks (Hopkins 1942). In the case of the hoatzin (*Opisthocomus hoatzin*), Mallophaga show that this bird is not related either to Cracidae (Galliformes) as indicated by classical techniques or to cuckoo (subfamily Crotophaginae) as indicated by DNA data, but suggest that it is more closely related to gruiforms. Lice from sandgrouse (Pteroclididae) suggest that these birds do not belong to either Columbiformes or Charadriiformes. The Family Otidae (Bustards) was classified together with Gruiformes although they have no clear relationship to the Gruidae (Cranes). Mallophaga collected from Bustards all belong to Ischnocera which indicate that this host is primitive in contrast to Gruidae which normally harbour Amblycera. In addition, circumfasciate Ischnocera also predominate on Bustards. All this evidence indicates that Otidae do not

belong to the Gruiformes, and strongly suggests that they are an exceedingly ancient and primitive group.

In addition to these four categories, some feather lice also assist in elucidating systematic relationships by giving some indications about taxa *incertae sedis* when DNA evidence is unavailable (Mauersberger & Mey 1993). The New Zealand wattlebirds (Callacatidae) can be used as a good example because even though they are very dissimilar externally compared to Corvidae, most of them have been placed together based on the similarity in their feather lice. Whereas on Huia, *Huiacola extinctus*, lice are totally different to those on Passeriformes so that the affiliation with the Callacatidae appears highly questionable. In a study on madagassic mesites (Mesitornithidae), Mallophaga suggests that this bird is not related to gallinaccous or gruiform groups but rather is closer to the Cuculiformes or piciform-passeriform complex (Mauersberger & Mey 1993).

4.1.3. Aims of This Chapter

This chapter explores the relationships between skuas and their feather lice, especially ecological and behavioural associations between both taxa, and postulate the interpretations of this association. Various lice extraction methods, including from various forms of host such as live, frozen, and museum specimens is also discussed. In particular, the distribution of lice on their hosts, their diversities, and infestation rates is examined. A brief explanation about useful lice characters and how they can play an important role as an alternative model for providing supplementary evidence to other taxonomic methods (molecular taxonomy or classical techniques) is presented. This contribution will be very useful when molecular or classical approaches are unable to provide adequate data to resolve taxonomic problems.

4.2. Materials and Methods

It would be ideal to conduct this study on live birds, but widespread distributions of skuas make this intention very difficult to implement. Most Mallophaga studies, including this one, used museum specimens, either skin or frozen form as the major source of material. Whenever data from live birds are available (e.g. Furness & Palma 1992), it is included into the study. Hopefully by combining these data (current and previous studies), it will form a satisfactory picture about relationships between skuas and feather lice.

Using museum specimens has its own disadvantages. These drawbacks, mostly related to the problem of losing some ectoparasites during preparation of bird skins. Fortunately, results from previous studies on skin specimens showed that these still manage to present a general pattern about lice diversity and their distribution on hosts. Ward (1957) stated that adequate quantities of lice remain on birds after the preservation process and this amount is enough to produce reliable results. Initial comparisons between data from live birds and that from museum specimens shows that the former harbour more lice but that museum specimens produce the same species and relative abundances of lice as found on live birds.

4.2.1. Visual Examination.

For dead birds, the most satisfactory method of searching for the ectoparasites is raising the host feathers with forceps, and removing lice intact, or snipping off with a fine pair of scissors (Ash 1960). Each region of host body was examined carefully and ectoparasites were removed and placed in 70% alcohol, their location on the bird being recorded. Later, the feather lice were separated on the basis of species, sex, and stage of development, and were counted. The number of bird specimens of each taxon used in this study was arbitrarily determined because in many cases only a limited number of good, undistorted specimens were available for study, so all available skins were used.

4.2.2. Feather Fumigation

This is the most suitable method for extracting lice from live birds. This method was never applied in this study but, since some of the data were obtained by this method, it is worth discussing. This method has a successful record as shown by Fowler & Cohen (1983), who demonstrated that (by adopting a standardised procedure) representative samples of ectoparasites could be removed quickly and safely from large numbers of birds for comparative analyses. This method, originally proposed by Dunn (1932), involved exposing the plumage of a bird (excluding its head) to a delousing agent such as chloroform or ethyl acetate. It was modified by Williamson (1954) through construction of the 'Fair Isle Apparatus' and perfected later by Ontario Bird Banding Association (1960) and successfully used by Fowler & Cohen (1983).

Live birds were placed individually in clean linen bags, measured and weighed in the normal way, and deloused individually for 20 minutes in suitable size glass vessels containing chloroform vapour. Ectoparasites which had collected in the bottom of the vessels were transferred to vials of 70% ethanol in which they were preserved until identification and mounted on slides in Canada balsam (Fowler & William 1985). After delousing, the host was removed from the fumigation chamber and suspended over a sheet of white paper. The feathers of the host were agitated vigorously for a period of 1 minute, with attention directed to all regions of the body (Clayton *et al.* 1992). This precaution is to make sure that all ectoparasites were remove from host feathers.

Freshly dead birds were placed in individual paper bags which were rolled shut to prevent ectoparasites from transferring between hosts. Each bird was later fumigated for at least 10 minutes in a glass vessel containing cotton soaked in a delousing agent, which kills ectoparasites rapidly. Following fumigation, birds were 'quantitatively' or qualitatively sampled for lice, as described above (Clayton *et al.* 1992). There are two limitations to this method as far as Phthiraptera are concerned; many lice, especially some Ischnocera, attach themselves firmly to a feather when they die and are most difficult to remove, and since the bird's head is kept clear from the chloroform,

parasites in that region will remain unaffected (Ash 1960). These problems were overcome by visually examining each bird for the presence of ectoparasites before release.

4.2.3. Identification of Lice

All the parasites were separated and identified based on morphological criteria using a binocular microscope. In some cases parasites were sent to New Zealand Natural History Museum for confirmation by Dr. Ricardo L. Palma.

4.2.4. Statistical Analysis

Statistical analysis in this study is based on Marshall (1981) and Kim (1985). Two parameters, incidence rate (the percentage of host infested; infestation rate of Kim) and infestation rate (the mean number of ectoparasite per host examined; population rate of Kim) have been used. A new parameter proposed by Choe & Kim (1987), called infestation density (the mean number of ectoparasites per infested host) has also been used as an addition to the above parameters. Other indices, H' (Shannon & Weaver 1963) and J' (Pielou 1966) were also used to calculate the diversity and evenness of the ectoparasite communities respectively. These indices are represented by formulae written below;

$$H' = - \sum_{i=1}^{s} Pi \log Pi \quad \text{and} \quad J' = H'/\log S$$

where S = the total number of ectoparasites genera

Pi = relative frequency of the ith genera

Degree of structural similarity between the ectoparasite communities is calculated by using Sorensen's similarity index (Sorensen 1948), as shown below;

$$Cs = 2S_{AB} / (S_A + S_B)$$

where S_A = number of ectoparasite genera on bird species A

S_B = number of ectoparasite genera on bird species B

S_{AB} = number of ectoparasite genera in common between two bird species

4.3. Results

4.3.1. Lice on Skuas

A total of 956 lice were extracted from 364 skuas (334 museum skins, 26 frozen specimens, and 4 live samples from Furness & Palma (1992)). The majority of lice present (all except one species) are non-haematophagous insects, feeding principally on sloughed off skin and feather debris. These lice comprise four genera and can be separated into two groups; suborder Ischnocera (Family Philopteridae), consists of three genera (*Haffneria grandis, Saemundssonia stresemanni*, *Saemundssonia insexpectata*, *Saemundssonia cephalus* and *Quadraceps normifer normifer*), and suborder Amblycera (Family Menoponidae) which only has a single species, *Austromenopon fuscofasciatum*.

All nine taxa of skua bear more than one genus of Mallophaga, but these do not occur with equal frequency on their particular hosts. Chilean skua (*Catharacta chilensis*) and Pomarine skua (*Stercorarius pomarinus*) harbour the largest number of genera of lice, both being parasitised by four genera of Mallophaga, the maximum number of genera discovered in this study. On the other hand, Long-tailed skua (*Stercorarius longicaudus*) is the only host being infested by just two genera of lice (*Saemundssonia sp.* and *Quadraceps normifer normifer*). Other skuas have been parasitised by three genera of lice.

Sorensen's index shows that Pomarine and Chilean skuas possess the most similar communities of lice (Table 4.1). The lowest similarities exist between Brown and Long-tailed skuas, and between Tristan and Long-tailed skuas where both taxa pairs have an index value of 0.40%.

Table 4.1. Values of Sorensen's index of similarity between the ectoparasite communities (lower diagonal) and number of genera in common (upper diagonal) among study skuas.

	Great	Falkland	S. Polar	L-tailed	Arctic	Brown	Tristan	Chilean	Pomarine	n
Great skua	-	3	3	2	2	2	2	3	3	53
Falkland skua	0	-	3	2	2	2	2	3	3	67
South Polar skua	0	0	-	2	2	2	2	3	3	42
Long-tailed skua	0.80	0.80	0.80	-	2	1	1	2	2	23
Arctic skua	0.80	0.67	0.80	0.80	-	2	2	3	3	83
Brown skua	0.80	0.67	0.67	0.40	0.67	-	3	3	3	22
Tristan skua	0.67	0.67	0.67	0.40	0.67	0	-	3	3	30
Chilean skua	0.86	0.86	0.86	0.67	0.86	0.86	0.86	-	4	19
Pomarine skua	0.86	0.86	0.86	0.67	0.86	0.86	0.86	0	-	25

Great skua has a very diverse louse community with the highest diversity index, H' of 1.18, in contrast to the lowest value, possessed by the Pomarine skua which has a value of 0.15. The majority of H' values from lice communities of skuas are in the range of 0.24 to 0.42 except the South polar skua (H'=1.05). In addition to being very diverse the lice community on Great skua is also more even compared to other lice communities. This lice community shows an evenness value, J' of 2.47. Other high evenness values are obtained from lice communities infesting South polar skua, with a value of 2.22. Other lice communities on skuas possessed an evenness values in a range of 0.68 to 0.93 except the lowest value, possessed by Pomarine skua lice with a value of 0.31 (Table 4.2).

There is a varying degree of Mallophaga infestation on different species of birds. Some lice heavily infest certain species of host whereas other lice concentrate on another. Taking taxa from which reasonably large samples have been obtained, of 41 skins of South Polar skua examined, only 12 (29.26%) were parasitised, whilst at the other extreme 13 (72.22%) Chilean skuas were infested out of 18 examined (Table 4.2). All lice present on skuas are oligoxenous; that is infesting two or more congeneric host species. *Haffneria grandis* (Plate 10) for example is present on all large skuas. This is the biggest louse found on skuas and contributed the largest number of lice present overall. Examination of museum specimens reveals that this louse heavily infests Falkland skua (30.59%), South polar skua (18.83%), and Chilean skua (18.83%) compared to other skuas. The percentage presence of *H. grandis* is less than 11% (Table 4.3) except in Brown skua where it is 16.47%. Examination of museum and frozen specimens, indicates that *Haffneria grandis* failed to colonise small skuas, although some of them (9 individuals) were present on museum specimens of Pomarine skua; possibly due to straggling. More lice can be recovered from frozen and live specimens than from museum specimens (87 lice on 22 frozen Great skuas and 160 lice on 4 live Tristan skuas; Table 4.4). Although a larger number of *H. grandis* were found on the latter specimens, all hosts present a similar pattern of lice-age distribution; nymphal stages outnumber adult lice (Table 4.3 and 4.4).

Table 4.2. Diversities and evenness of lice communities of adult skuas inferred from museum specimens.

Species	Number of bird examined (infected)	Number of lice genera (amount)	Number of Genera		Diversity (H')	Evenness (J')
			Mean	S.D.		
Great skua	25 (6)	3 (9)	1.80	1.09	1.18	2.47
Falkland skua	57 (19)	3 (44)	2.32	2.43	0.39	0.82
Brown skua	22 (9)	3 (23)	2.56	2.06	0.41	0.85
Tristan skua	23 (3)	2 (4)	1.34	0.58	0.24	0.81
South polar skua	41 (12)	3 (23)	1.92	1.31	1.06	2.22
Chilean skua	18 (13)	3 (38)	2.92	2.63	0.42	0.88
Pomarine skua	19 (11)	3 (172)	15.64	41.65	0.15	0.31
Arctic skua	60 (39)	3 (182)	4.67	5.10	0.33	0.68
Long-tailed skua	19 (6)	2 (10)	1.67	1.03	0.28	0.93

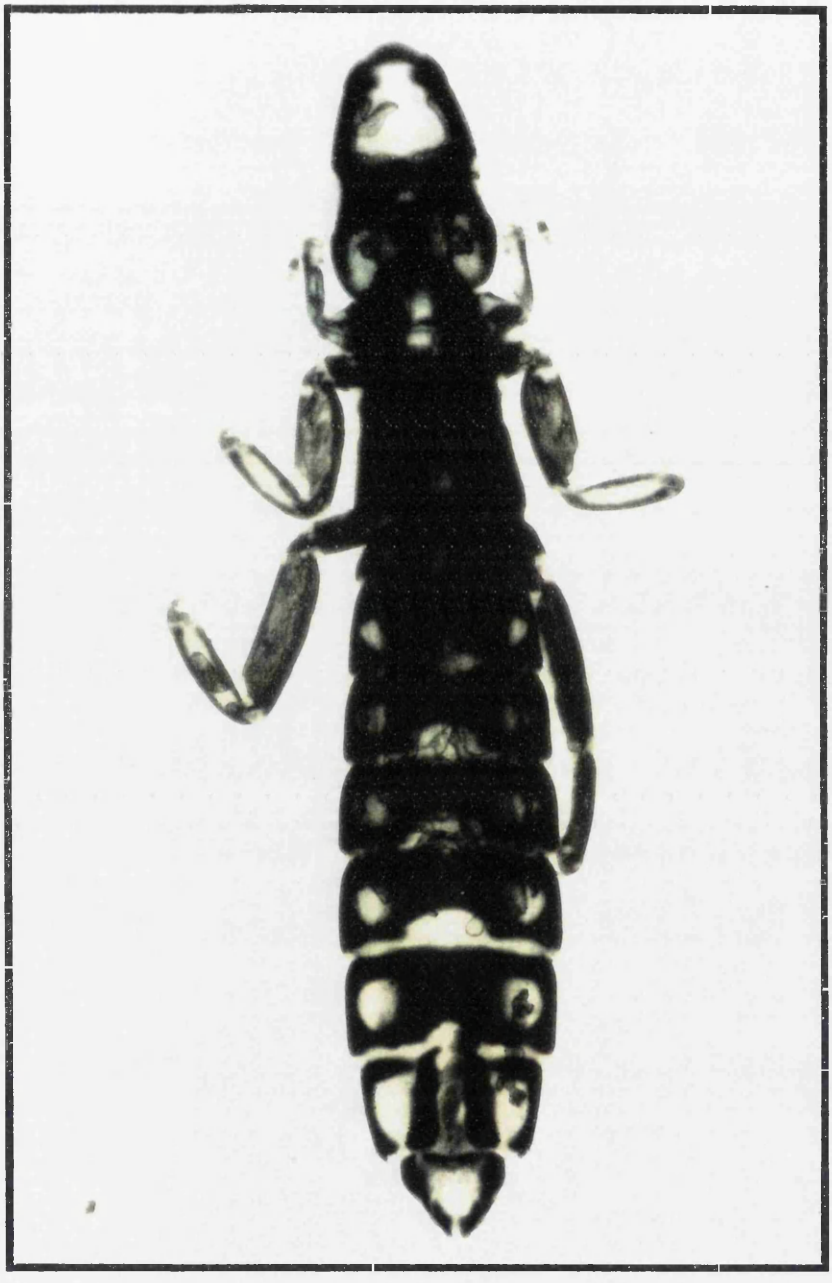


Plate 10: *Haffneria grandis*

Table 4.3. Number of lice on each taxon of skuas skins. Various stages of lice development is represented by F = adult female, M = adult male and N = nymphs.

Taxon	*Haffneria grandis*	*Saemundssonia sp.*	*Quadraceps n. normifer*	*Austromenopon fuscofasciatum*	Total
Great skua	3 (3.52%)	4 (1.89%)	2 (0.94%)	-	9 (1.29%)
	1F; 0M; 2N	3F; 0M; 1N	0F; 0M; 2N		4F; 0M; 5N
Falkland skua	26 (30.59%)	15 (7.11%)	4 (1.89%)	-	45 (6.45%)
	11F; 3M; 12N	8F; 3M; 4N	1F; 3M; 0N		20F; 9M; 16N
Brown skua	14 (16.47%)	5 (2.37%)	-	4 (2.11%)	23 (3.29%)
	5F; 4M; 5N	4F; 1M; 0N		1F; 1M; 2N	10F; 6M; 7N
Tristan skua	1 (1.17%)	2 (0.95%)	-	1 (0.53%)	4 (0.57%)
	0F; 0M; 1N	1F; 1M; 0N		1F; 0M; 0N	2F; 1M; 1N
South polar skua	16 (18.83%)	6 (2.84%)	1 (0.47%)	-	23 (3.29%)
	6F; 3M; 7N	4F; 1M; 1N	0F;1M; 0N		10F; 5M; 8N
Chilean skua	16 (18.83%)	18 (8.53%)	4 (1.89%)	4 (2.11%)	42 (6.02%)
	5F; 0N; 11N	13F; 1M; 4N	3F; 1M; 0N	1F; 1M; 2N	22F; 3M; 17N
Pomarine skua	9 (10.59%)	40 (18.96%)	9 (4.25%)	157 (82.63%)	215 (30.80%)
	1F; 2M; 6N	11F; 7M; 22N	2F; 1M; 6N	35F; 11M; 111N	49F; 21M; 145N
Arctic skua	-	101 (47.87%)	165 (77.83%)	24 (12.62%)	290 (41.55%)
		31F; 25M; 45N	69F; 45M; 51N	10F; 4M; 100N	110F; 74M; 106N
Long-tailed skua	-	20 (9.48%)	27 (12.73%)	-	47 (6.74%)
		6F; 5M; 9N	8F; 5M; 14N		14F; 10M; 23N
Total	85 (100%)	211 (100%)	212 (100%)	190 (100%)	698 (100%)
	29F; 12M; 44N	81F; 44M; 86N	83F; 56M; 73N	48F; 17M; 215N	241F; 129M; 328N

Table 4.4. Number of lice present on frozen and live specimens of skuas. Various stages of lice development is represented by F = adult female, M = adult male and N = nymphs.

Frozen specimens

Taxon	*Haffneria grandis*	*Saemundssonia sp.*	*Quadraceps n. normifer*	*Austromenopon fuscofasciatum*	Total
Great skua	87 (100.00%) 14F; 12M; 61N	4 (80.00%) 3F; 0M; 1N	-	-	91 (93.81%) 17F; 12M; 62N
Arctic skua	-	-	2 (100.00%) 1F; 1M; 0N	-	2 (2.06%) 1F; 1M; 0N
Pomarine skua	-	1 (20.00%) 1F; 0M; 0N	-	3 (100.00%) 1F; 1M; 1N	4 (4.12%) 2F; 1M; 1N
	87 (100.00%) 14F; 12M; 61N	5 (100.00%) 4F; 0M; 1N	2 (100.00%) 1F; 1M; 0N	3 (100.00%) 1F; 1M; 1N	97 (100.00%) 20F; 14M; 63N

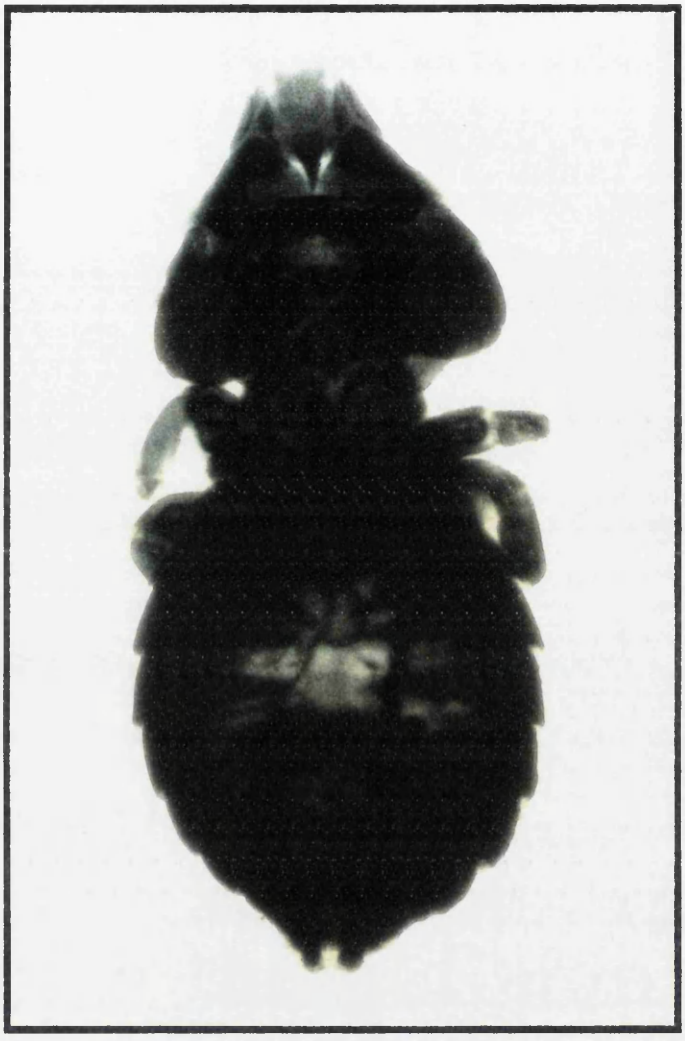
Live specimens

Tristan skua	160 (99.37%) 18F; 21M; 121N	1 (0.63%) 1F; 0M; 0N	-	-	161 (100.00%) 19F; 21M; 121N

The most widely distributed louse found on skuas are from genus *Saemundssonia*. This genus consist of three species which are very difficult to differentiate visually. Male lice of these species however, differ in genitalia morphology (Plate 11). *Saemundssonia sp.* present on all skuas skins, but heavily concentrated on Arctic skua (101 lice individuals 47.87%). This is well understood because many Arctic skua skins have been examined (81 specimens or 24.25%; Table 4.5a) and from this amount, most of them are infested by this insect (50 hosts or 61.72%). Apart from Arctic skua, Pomarine and Long-tailed skuas also show a heavy infestation. Other skuas, however, show a very light infestation with average rate below 20 lice. The lowest infestation density occurred in the Tristan skua skins, only harbouring 2 lice (0.95%). From 211 *Saemundssonia sp.* specimens found on skuas skins, 81 individuals were adult females, 44 were adults males, and 86 were nymphs (Table 4.3). Except in the small skuas group, the majority of this louse were adults (> 73.33%). This insect is predominantly in nymph stages on small skuas (> 44.55%), showing the same age-fraction pattern as *H. grandis,* contrary to the pattern discovered from frozen and live specimens (more than 75.00% were adults; Table 4.4).

The third Ischnoceran lice on skuas, *Quadraceps n. normifer* (Plate 12) normally occurs on the head and neck area of the host together with *Saemundssonia sp.* These lice firmly affix themselves by their mandibles to a barbule. In some cases the louse had to be removed together with a bird feather in order to avoid destruction of the louse in the extraction process. The examination of skua skins reveals that this louse did not affect Brown and Tristan skuas but heavily parasitised Arctic skua (77.83% or 165 individuals). Again, Long-tailed and Pomarine skuas were second and third highest affected birds with the lice population of 27 and 9 individuals respectively. Other skuas possessed less than 5 individuals of this louse (Table 4.3). *Quadraceps n. normifer* population was formed by 83 adult females (38.78%), 56 adult males (26.17%), and 75 nymphs (35.05%). Different developmental stages of lice were not uniformly distribute among hosts. In some hosts (for example Long-tailed, Pomarine,

Plate 11. *Saemundssonia sp.* Figures at the bottom represent male genitalia for each species. A = *S. cephalus* ; B = *S. stresemanni* (both figures were extracted from Timmermann 1936) and C = *S. inexspectata* (from Timmermann 1957).



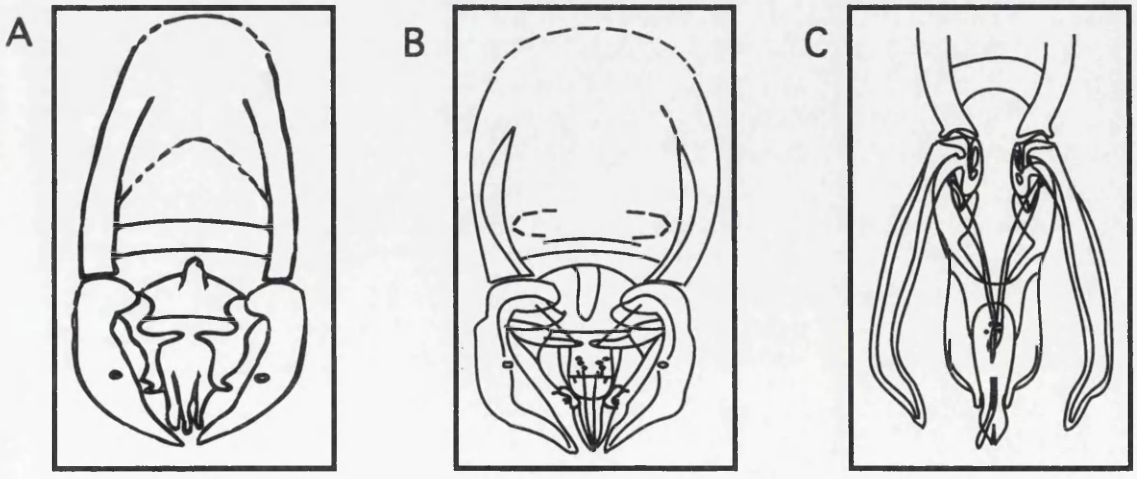


Table 4.5a. The distribution of feather lice found on museum specimens of various taxa of skua. The skuas have been divided into different age stages to facilitate comparison. The letter *n* represent total number of skua individuals harbouring lice.

	Number of bird studied				Number of bird infected by lice			
Taxon	adults	immatures	chicks	n	adults	immatures	chicks	n
Great skua	25	2	3	30	6	0	0	6
Falkland skua	57	8	2	67	19	1	0	20
Brown skua	22	0	0	22	9	0	0	9
Tristan skua	23	1	2	26	3	0	0	3
South polar skua	41	1	0	42	12	0	0	12
Chilean skua	18	1	0	19	13	1	0	14
Pomarine skua	19	5	0	24	11	5	0	16
Arctic skua	60	19	2	81	39	11	0	50
Long-tailed skua	19	4	0	23	6	3	0	9
Total	284	41	9	334	118	21	0	139

Table 4.5b. The distribution of feather lice on frozen specimens of various taxa of skua. The skuas have been divided into different age stages to facilitate comparison. The letter *n* represent total number of skuas individuals harbouring lice.

	Number of bird studied				Number of bird infected by lice			
Taxon	adults	immatures	chicks	n	adults	immatures	chicks	n
Great skua	22	1	0	23	21	0	0	21
Pomarine skua	1	0	0	1	1	0	0	1
Arctic skua	2	0	0	2	1	0	0	1
Total	25	1	0	26	23	0	0	23

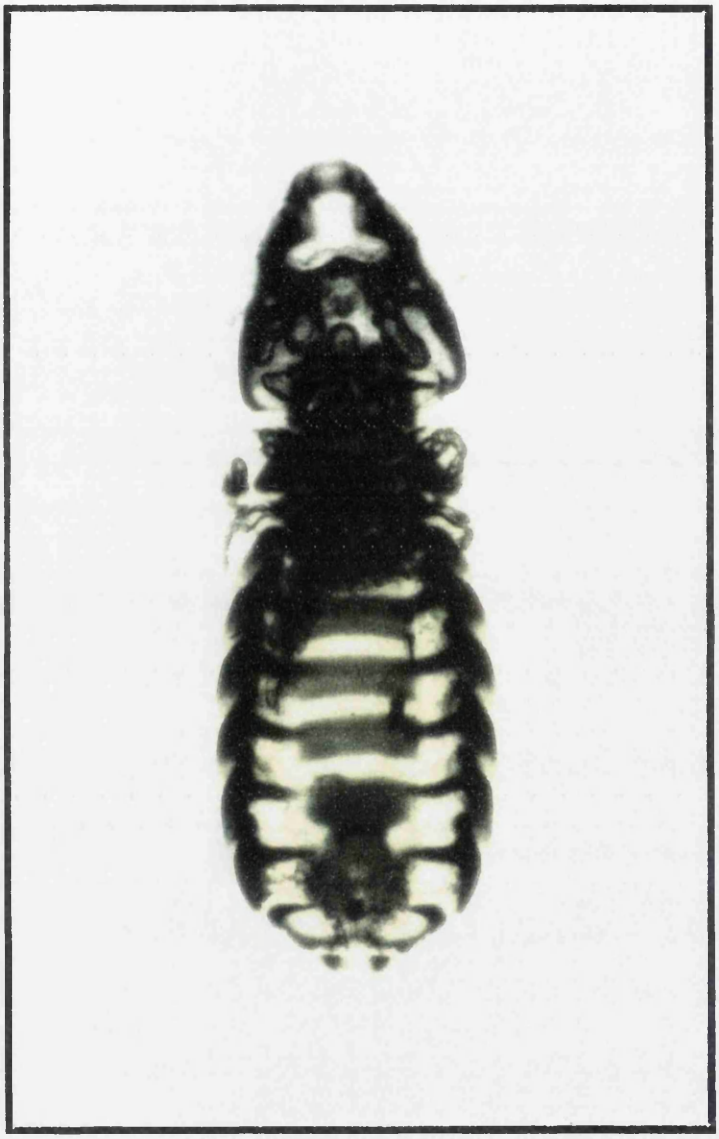


Plate 12: *Quadraceps normifer normifer*

and Great skuas) nymphs outnumber adults but on other hosts (such as Arctic, Chilean, South polar, and Falkland skuas), adult stages were present in greater number than nymphs. This pattern of age distribution also occurred in lice from frozen specimen of Arctic skua.

The single Amblyceran representative, *Austromenopon fuscofasciatum* (Plate 13) was only present in small numbers. Only five taxa of hosts were infected by them. Although it shows heavy infestation on Pomarine skuas (157 individuals or 82.63%), it was absent from Great, Falkland, South Polar, and Long-tailed skuas. Heavy infestation on Pomarine skua was not evenly distributed since one host harboured 138 lice (72.63%). This insect showed very light infestation on other hosts, having four individuals (2.11%) on Brown and Chilean skuas skins, and only a single louse (0.53%) on the Tristan skua skin. There were 24 lice on Arctic skuas skins (12.62%; Table 4.3). In total, 190 individual lice from this species were discovered on skuas. Fractionation of life stages for this insect was 48 adult females (25.26%), 17 adult males (8.95%), and 125 nymphs (65.79%). Generally, nymphs were present in larger numbers compared with adult stages (> 50.00% except in Tristan skuas).

4.3.2. Patterns of Parasitism

From 284 adult skua skins examined, only 118 (41.54%) were parasitised by Phthiraptera (Table 4.5a). Parasitism pattern is different from one taxon to another. Three out of nine taxa of skuas were heavily infected. These are Chilean, Pomarine, and Arctic skuas with more than 50% of the samples parasitised by lice. On the other hand, Tristan, South Polar, and Great skuas skins are less affected by lice. Less than 30% of the skins from the latter group of hosts are infected by lice. Frozen and live specimens of these species are heavily affected by lice. More than 95% of frozen samples of adult Great skuas for example are affected by lice (Table 4.5b), whereas all live Tristan skuas studied by Furness & Palma (1992) harbour *H. grandis*.

Plate 13: *Austromenopon fuscofasciatum*

Of the nine taxa of skuas, 312 adult specimens were studied and 46.15% (144 individuals) were infested by lice. In addition to adult samples, 43 immature and 9 chicks were examined. The majority of immatures available were Arctic skuas (19 individuals or 44.18%), and unfortunately, no Brown skua immatures were available for parasite examination. Limited chick samples were obtained from various hosts such as Great (3), Falkland (2), Tristan (2), and Arctic (2). Although all chicks were free from lice infection most immatures were affected by lice (22 individuals or 51.16%), and sometimes the incidence rate (the percentage of hosts infested) was higher such as in Pomarine and Chilean skuas where all immatures were affected by lice. Some immatures harboured a large number of lice. Two Arctic skua immatures, for example, possessed 29 and 28 lice each.

The numbers of lice genera present on each host vary. The majority of individual hosts only harboured one genus of parasite (112 hosts or 67.47%), while others were either infested by two genera (44 birds or 26.50%) or three species of lice (10 specimens or 6.03%). Only four taxa of hosts had been colonised by three genera of lice, four Arctic skuas, three Pomarine skuas, two Chilean and a single Falkland skua.

Lice also show different infestation rates and densities on each host taxon. These values are greatly affected by the condition of samples studied. Frozen and live specimens will normally present a higher value compared to skins specimens. All four samples of live Tristan skuas for instance were affected by a larger number of *H. grandis* (160 individuals), giving the incidence rate of 100.0 and the infestation density and infestation rate of 40.0 (Table 4.6). This louse also shows a higher parasitism value on frozen specimens of Great skua (95.45 for incidence rate, 3.96 for infestation rate, and 4.14 for infestation density).

Among skin specimens, the highest incidence rate was shown by *Q. n. normifer* on Arctic skua and *Saemundssonia sp.* on Pomarine skua, both with the values of 63.34 and 63.16 respectively. These lice however, failed to maintain the same position for infestation rate and infestation density on other skua taxa. The presence of the larger number of lice on small number of hosts gives a higher value of infestation rate (8.26)

Table 4.6. The degree of parasitisation (infestation rate and density of lice) on skuas

1) Museum specimens of Great skuas (*Catharacta skua*) (n = 30)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
H. grandis	3	2	8.0	0.12	1.50
Saemundssonia sp.	4	2	8.0	0.16	2.00
Q. n. normifer	2	2	8.0	0.08	1.00

2) Frozen specimens of Great skuas (*Catharacta skua*) (n = 23)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
H. grandis	87	21	95.45	3.96	4.14
Saemundssonia sp.	4	4	18.18	0.18	1.00

3) Museum specimens of Falkland skua (*Catharacta antarctica antarctica*) (n = 67)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
H. grandis	26	17	25.37	0.39	1.53
Saemundssonia sp.	14	7	10.45	0.21	2.00
Q. n. normifer	4	1	1.49	0.06	4.00

4) Museum specimens of Chilean skua (*Catharacta chilensis*) (n = 19)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
H. grandis	16	7	36.84	0.84	2.28
Saemundssonia sp.	18	8	42.11	0.95	2.25
Q. n. normifer	4	1	5.26	0.21	4.00
A. fuscofasciatum	4	3	15.79	0.21	1.33

5) Museum specimens of Long- tailed skua (*Stercorarius longicaudus*) (n = 23)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
Saemundssonia sp.	20	6	26.08	0.87	3.33
Q. n. normifer	21	5	21.74	0.91	4.20

6) Museum specimens of Arctic skua (*Stercorarius parasiticus*) (n = 81)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
Saemundssonia sp.	101	32	53.34	1.68	3.15
Q. n. normifer	165	38	63.34	2.75	4.34
A. fuscofasciatum	24	7	11.67	0.40	3.43

7) Frozen specimens of Arctic skua (*Stercorarius parasiticus*) (n = 2)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
Q. n. normifer	2	1	50.00	1.00	2.00

8) Museum specimens of Brown skua (*Catharacta antarctica lonnbergi*) (n = 22)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
H. grandis	14	6	27.27	0.64	2.33
Saemundssonia sp.	5	4	18.18	0.23	1.25
A. fuscofasciatum	4	1	4.54	0.18	4.00

9) Museum specimens of Pomarine skua (*Stercorarius pomarinus*) (n = 24)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
H. grandis	9	5	26.32	0.47	3.80
Saemundssonia sp.	40	12	63.16	2.11	3.34
Q. n. normifer	8	6	31.58	0.47	1.50
A. fuscofasciatum	157	7	36.84	8.26	22.43

10) Frozen specimens of Pomarine skua (*Stercorarius pomarinus*) (n = 1)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
Saemundssonia sp.	1	7	100.00	1.00	1.00

11) Museum specimens of South Polar skua (*Catharacta maccormicki*) (n = 42)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
H. grandis	16	8	19.04	0.38	2.00
Saemundssonia sp.	6	6	14.28	0.14	1.00
Q. n. normifer	1	1	2.38	0.02	1.00

12) Museum specimens of Tristan skua (*Catharacta antarctica hamiltoni*) (n = 26)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
H. grandis	1	1	4.35	0.04	1.00
Saemundssonia sp.	2	2	8.69	0.08	1.00
A. fuscofasciatum	1	1	4.35	0.04	1.00

13) Live specimens of Tristan skua (*Catharacta antarctica hamiltoni*) (n = 4)

	Total lice	No. of birds infested	Incidence rate	Infestation rate	Infestation density
H. grandis	160	4	100.00	40.00	40.00
Saemundssonia sp.	1	1	25.00	0.25	1.00

and infestation density (22.43) for *A. fuscofasciatum* infesting Pomarine skua. *Quadraceps n. normifer* on Falkland skua, has the minimum incidence rate (1.49), on South Polar skua, the lowest infestation rate (0.02), and lowest infestation density on Great and South Polar skuas (1.00). *Saemundssonia sp.* also shows minimum infestation density (1.00) on Tristan and South Polar skuas.

4.3.3. Distribution of lice on host

Certain species of Mallophaga favour certain areas on birds. To study this behaviour the bird's body was classified into eight regions (Figure 4.1). These regions were as follows; 1) head, 2) neck, 3) breast, 4) abdomen, 5) back or dorsal, 6) wings (right and left), 7) legs (right and left), and 8) tail. All parasites obtained from each region were separated, identified, and recorded.

Some species prefer to settle on the host head area and are modified to adapt to that area. Lice in this area, are normally small and round. *Saemundssonia sp.* is a good example of this type of louse. They generally occupy the area of head and neck of host, and are only present in small quantities on other areas (Table 4.7). Only three individuals of this louse were found outside their regular territories (head and neck). All of these individuals were infesting Pomarine skua and one of them was on the breast area, while two were on the dorsal region. *Saemundssonia sp.* normally lay their eggs in head or neck areas unless the population increases, when eggs will be found spread to breast or back (see Nelson & Murray 1971).

Other lice such as *Haffneria grandis* are also highly site-specific, being present largely on wing, abdomen, and back area and sometimes also on breast and tail regions (Table 4.8). These latter areas, however, are rarely colonised by this louse, only occupied twice in two different specimens; both Great skuas. *H. grandis*, normally referred to as wing lice, are attached to the rachis of body feathers in the silky zone and will lay their eggs in wing areas or surroundings (e.g. body sides). In cases of heavy infestation, wing lice will lay their eggs on axillary feathers (Clay 1949). This type of

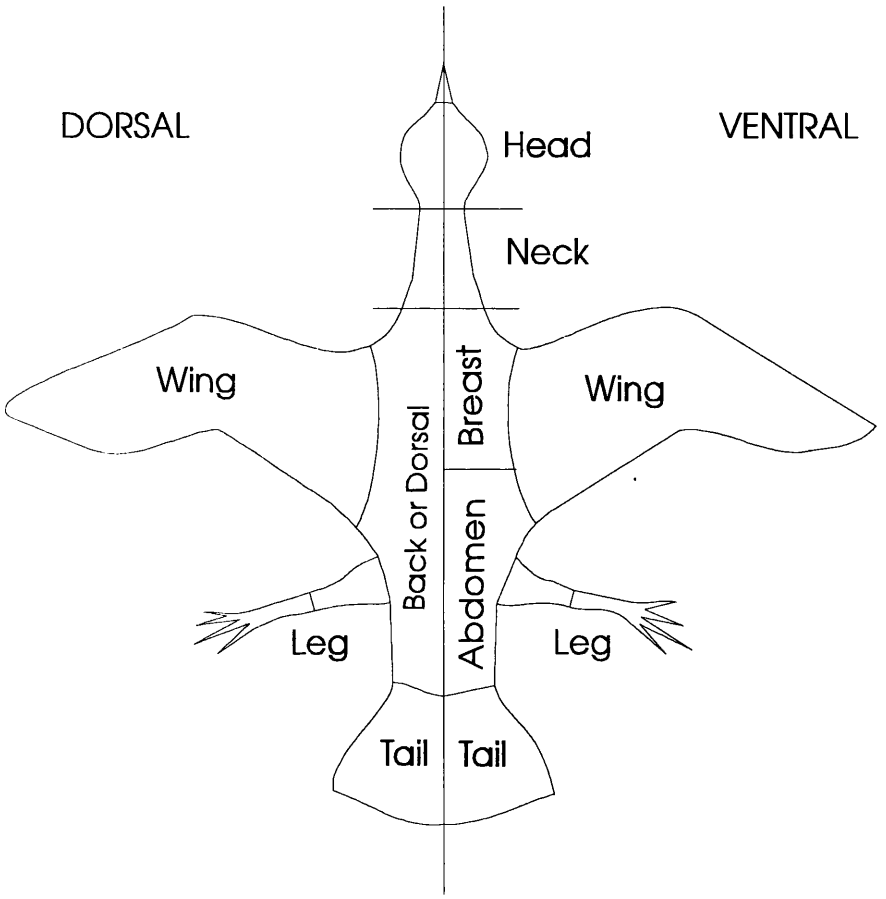


Figure 4.1. Schematic diagram of bird showing partition of body regions of hosts examined (excerpt from Eveleigh & Threllfal 1976).

Table 4.7. Distribution of *Saemundssonia sp.* on skuas skins and frozen specimens.

Region	Arctic	Brown	Chilean	Falkland	Great	Long-tailed	South Polar	Pomarine	Tristan
Head	67	5	16	13	4	14	4	27	1
Neck	34	0	2	2	4	6	2	11	1
Breast	0	0	0	0	0	0	0	1	0
Abdomen	0	0	0	0	0	0	0	0	0
Back	0	0	0	0	0	0	0	2	0
Wing	0	0	0	0	0	0	0	0	0
Leg	0	0	0	0	0	0	0	0	0
Tail	0	0	0	0	0	0	0	0	0
Total	101	5	18	15	8	20	6	41	2

Table 4.8. Distribution of *Haffneria grandis* on the skuas skins and frozen specimens.

Region	Brown	Chilean	Falkland	Great	South Polar	Pomarine	Tristan
Head	0	0	0	0	0	0	0
Neck	0	0	0	0	0	0	0
Breast	0	0	1	1	0	2	0
Abdomen	0	0	0	13	0	1	0
Back	7	4	11	47	5	1	1
Wing	7	12	14	25	11	5	0
Leg	0	0	0	0	0	0	0
Tail	0	0	0	4	0	0	0
Total	14	16	26	90	16	9	1

louse is larger than head lice and are not normally present on smaller birds (e.g. Passeriformes) due to a general correlation between size and shape of the Mallophaga and size of feathers (Clay 1949).

Contrary to the above two species, *Austromenopon fuscofasciatum* and *Quadraceps n. normifer* are not really site-specific. They normally settle down in several body regions depending on which host they parasitise. On Arctic skua, for instance, both lice have a wide distribution on head, neck, breast, and abdomen of the host (Table 4.9 and 4.10). *Austromenopon fuscofasciatum* living on Pomarine skua also shows a wide distribution pattern, being present on head, neck, abdomen, and back. As pointed out by Ash (1960), this Amblyceran lice may be found on any part of the body, although their oviposition, and probably their feeding, are confined to restricted areas.

The frequency distribution of lice on skuas is represented as the 'hollow curve' type as described by Williams (1964). In this type of distribution, most of the hosts have a few parasites, and only a few hosts have a large number of lice (Eveleigh & Threlfall 1976). In the study of museum skins, 53 infected hosts (38.13%) harbouring one louse compare to 19 (13.66%) and 20 (14.38%) hosts possessing two and three lice respectively (Figure 4.2). The majority of hosts (116 or 83.45%) harbour less than 6 lice whereas 16.5% of hosts (23 hosts) possess more than seven lice. The sample set contained only two cases of extreme infestation. These skuas (both are Pomarine), were heavily infested by 112 lice and 141 lice respectively. Lice on frozen specimens also possessed a similar pattern of distribution (Figure 4.3).

4.4. Discussion

Only six species of lice of four different genera (*Saemundssonia stresemanni, Saemundssonia inexspectata, Saemundssonia cephalus, Austromenopon fuscofasciatum, Haffneria grandis,* and *Quadraceps n. normifer*) were recovered from the skuas examined. These species are not equally distributed among hosts. Some lice exist on certain host taxa whereas others are present either on both groups or on large skuas

Table 4.9. Distribution of *Quadraceps n. normifer* on skuas skins and frozen specimens.

Region	Arctic	Chilean	Falkland	Great	Long-tailed	South polar	Pomarine
Head	71	0	1	0	13	0	3
Neck	41	4	3	0	11	1	4
Breast	14	0	0	0	0	0	0
Abdomen	8	0	0	0	0	0	1
Back	24	0	0	2	0	0	1
Wing	9	0	0	0	3	0	0
Leg	0	0	0	0	0	0	0
Tail	0	0	0	0	0	0	0
Total	167	4	4	2	27	1	9

Table 4.10. Distrbution of *Austromenopon fuscofasciatum* on skuas skins and frozen specimens.

Region	Arctic	Brown	Chilean	Pomarine	Tristan
Head	12	2	1	56	1
Neck	8	0	1	58	0
Breast	2	0	0	11	0
Abdomen	2	0	0	3	0
Back	0	1	1	0	0
Wing	0	1	1	32	0
Leg	0	0	0	0	0
Tail	0	0	0	0	0
Total	24	4	4	160	1

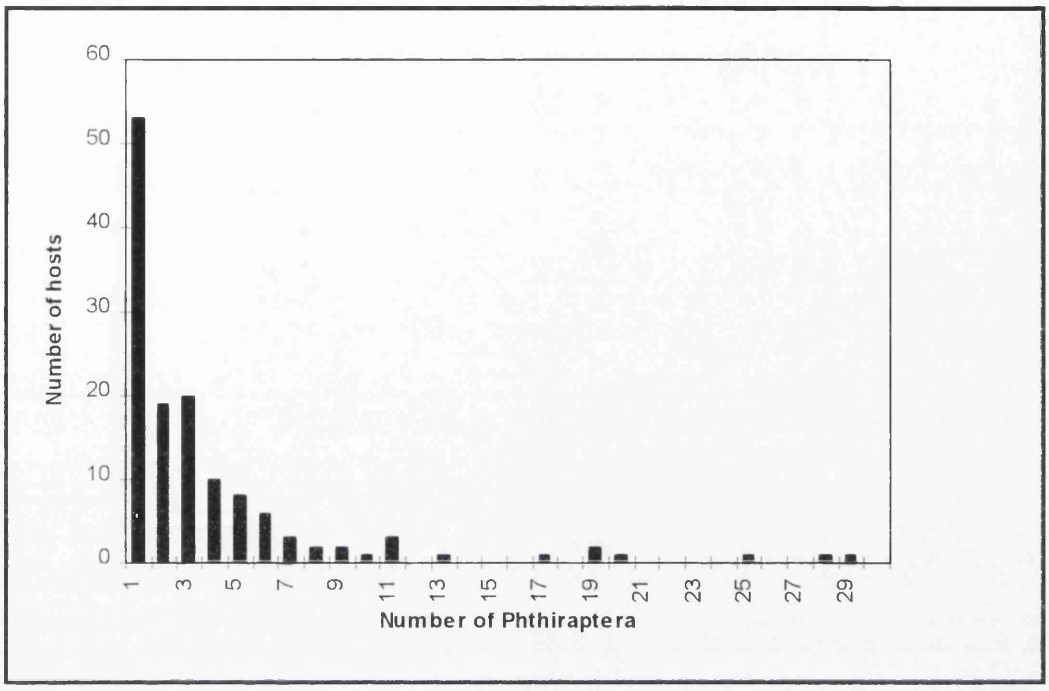


Figure 4.2. Frequency distribution of feather lice on museum specimens of skuas.

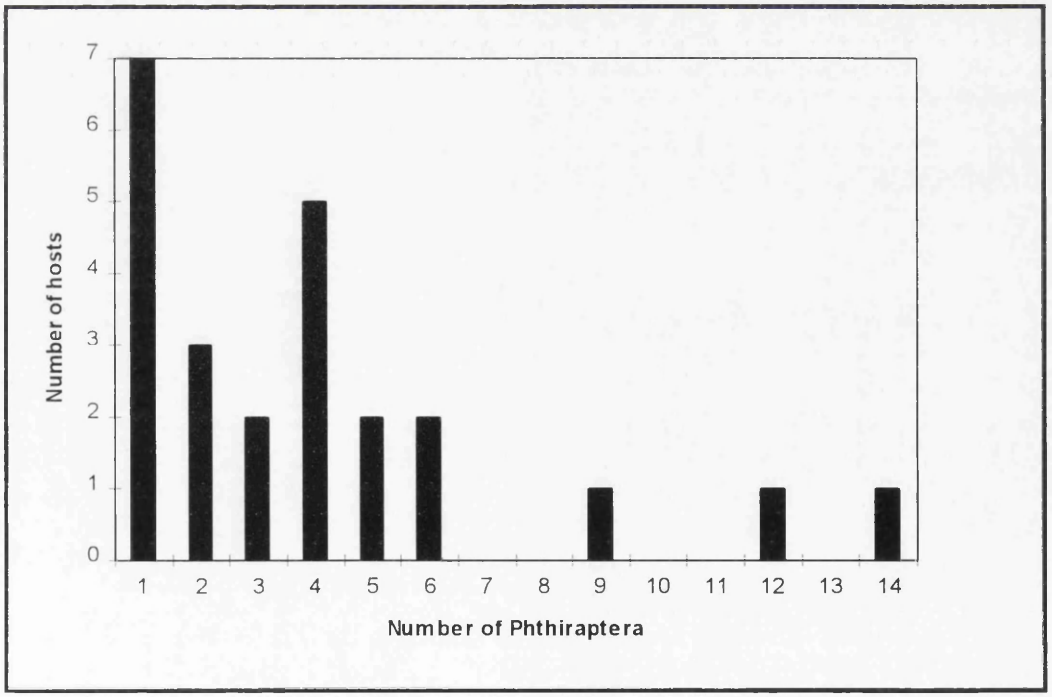


Figure 4.3. Frequency distribution of feather lice on frozen specimens of skuas.

only. This differs from an original hypothesis that nine taxa of skuas should possess nine different species of lice. This distribution of parasites raises two main possibilities; either lice are not highly host specific, or classifications of lice or of skuas are not accurate as they are presented.

The wide distribution of *Saemundssonia sp.* (*S. stresemanni*, *S. insexpectata*, *S. cephalus*) indicate that these Mallophaga are a general lice for skuas. Review of previous studies shows that these species have never been found on other groups of birds. The fact that they exist on all skuas but are absent from gulls (their sister taxon) shows that this louse can be used to differentiate between skuas and its closely related species.

Feather lice normally expand their population just prior to their hosts breeding season or before migration. This assures that they have ample opportunities for survival and dispersal by transferring to the host's partner and fledglings. During the breeding season much body contact occurs between potential hosts and this will increase the rate of transfer which will be more likely if there are large numbers of lice (Ash 1960). Oniki (1990) shows that the presence of *Brueelia sp.* nymphs on *Catharus fuscescens* shortly before hosts breed proved that parasites eggs were laid within the preceding month or two as the entire lice life cycle is very short. Foster (1969) also found that there is a correlation between the timing of breeding in *Ricinus picturatus* and *Menacanthus sp.* and the timing of breeding in their host, the orange-crowned warbler, *Vermivora celata.* By possessing this behaviour, feather lice safeguard themselves against migratory losses. *Colpocephalum* and *Ibidoecus* on the Glossy Ibis begin to produce many eggs just prior to the hosts migration, but contrary to swift's Mallophaga the number of active Mallophaga in their population decreases (Dubinin 1938). In his study, Ash (1960) also noted that the number of infested birds increased after the breeding season was over. This indicates that lice do transfer by direct contact during copulation. Lice have a good strategy for survival by laying eggs in severe conditions; e.g. they normally laid their eggs before death (e.g. *Campanulotes bidentatus compar*) when exposed to 40°C (Nelson & Murray 1971).

Results from this study also demonstrate that the patterns of louse distribution on the host's body are strongly affected by their site-specificity. Distributions of Amblyceran lice, however, are not affected by this factor. This finding is strongly supported by several ecological studies on infestation patterns by Phthiraptera (see Ash 1960; Foster 1969; Post & Enders 1970). Data from these studies show that no lice occupy the same habitat when they are parasitising closely related hosts. Later, Fitzpatrick & Threlfall (1977) and Ballard & Ring (1979) proved that feather lice exhibit regional specificity. They showed that *Saemundssonia lari* and *Saemundssonia calva* are usually closely associated with the head and neck regions, while *Quadraceps ornatus* prefer to live in the breast region. A similar conclusion also applies to lice discovered in this study such as *Saemundssonia sp.* (restricted to the head and neck region) and *Haffneria grandis* (present on wings and back). This behaviour can be explained by studying lice claws. Each species of louse has a very specific claw for gripping the feather of their host. Variations in claw size enable feather lice to attach to other hosts whose feathers are different diameters (Chinery 1993). Along with site specificity, infestation size also has a major influence in determining the distribution of Ischnoceran as shown in *L. lawrensis tropicalis* and *G. gallinae* (which show a wide range of distribution in heavy infestations) by Trivedi *et al.* (1991). Although some lice prefer a certain habitat, when population increase, they will spread widely to other regions of host body.

The distribution of feather lice is also influenced by host grooming behaviour (Waage 1979). Host grooming and preening is effective against lice and mites infesting rodents (Murray 1990), ticks infesting penguins (Brooke 1985), and lice infesting doves (Clayton 1991). This host defence behaviour will result in restriction of ectoparasite distribution on respective hosts. Clayton (1991) supports this fact by showing that birds with deformed or missing feet sometimes have elevated populations of ectoparasites that are restricted to the head and upper body, i.e. those regions that must be groomed with the feet.

Distribution of lice on their host body is also influenced by their feeding behaviour. Lice which feed on head or neck's feather will reside in these regions while larger lice which favour wing feathers will colonise this area. However, this rule does not apply for all lice. Some Ischnoceras live on one part of their host's body but feed on another. A good example for this violation is *Columbicola columbae* which lies between the barbs of the primary, secondary, and tail feathers, lays eggs on the under-wing coverts, but appears to feed entirely on the feathers of the upper breast (Nelson & Murray 1971). Several *Bruelia sp.* on Turdidae normally occur on the breast, lower back and rump feathers, where they lay their eggs, but occasionally rest on the inner surface of the secondary and tail feathers (Ash 1960).

This study reveals that more than a single species of lice can be occured on a single host. This is not an unfamiliar phenomenon. Earlier studies indicated that up to five or six species of lice or sometimes more can be encountered from a single bird. Twelve species of Mallophaga belonging to eight genera and three families have been recorded from one species of Tinamidae (Tinamous), *Crypturellus obsoletus punensis*, and fifteen species belonging to twelve genera and three families from another, *Tinamus major* (Ash 1960). During the preservation process, some parasites may be unintentionally washed away or fall off their hosts. Comparison of data obtained from this study using skins and frozen specimens with previous ones using live birds (Furness & Palma 1992) indicate that the effect of host conditions influences the results. Although data from live birds show the same lice communities and all species have been discovered in this study, live specimens produce more lice per bird compared to skins and frozen specimens.

The existence of some lice on hosts can be influenced by several factors. In his study, Ash (1960) discussed that changes in individual infestation by parasites happen regularly and are unpredictable since birds which were uninfected when trapped for the first time were infested when later retrapped. Either the bird has acquired some lice from some other individual of the same species, or else adult lice have been present and have been overlooked during earlier examinations, or unnoticed eggs have been hatched

during examination period. It is hardly likely, however, that these could have been overlooked when the bird has been retrapped and re-examined on several subsequent circumstances. On other occasions, birds which were infested when first trapped were later uninfected. In this case, the possibility of lice being overlooked on subsequent examinations cannot be entirely excluded. Nevertheless, there can be no doubt that birds do rid themselves of parasites, as is demonstrated by the fact that many fewer adults and nymphs than hatched and unhatched eggs are found on a bird. Hatched eggs are soon preened from the feathers, as are a number of unhatched ones (Ash 1960).

It is surprising that all skua chicks (downing stages) managed to escape lice infestation. Young hosts are often more affected by ectoparasites, perhaps because they possess a higher ratio of accessible surface to body volume, or because their grooming behaviour and other defence mechanism are less efficient (Lehmann 1993). The smaller number of samples may be the reason for the apparent lack of lice on skua chicks. The condition of feathers of skuas' chick may also prevent Mallophaga from affixing themselves, which may result in lice being easily washed away during the preservation process. An earlier study by Van Den Broek (1967) showed that adult Mallophaga could be found on Black-headed gull (*Larus ridibundus*) chicks several hours old. He also noted that the number of parasites generally decreased on the hosts more than one week old, but eggs were present in most cases, normally in the triangular area between bill and eyes. These findings agreed with Stenram (1956) on his observation on pigeon-louse, *Columbicola columbae*.

In terms of the degree of infestation, the majority of skuas seem very successful in controlling their ectoparasite load. Only a few hosts possess large numbers of parasites. All examined skins were normally infested by less than seven lice. These small populations probably have very little effect on the host and through a variety of defence mechanisms ranging from behavioural responses like grooming and dust-baths to immune reactions, the healthy bird is able to keep the parasite numbers in check. The presence of lice has no effect on body weight or mortality rate among hosts (Ash 1960). Due to coevolution, most individual birds will normally harbour only small,

relatively harmless loads of ectoparasites (Rothschild & Clay 1952; Marshall 1981; Clayton 1991).

Wild birds like skuas normally have smaller population of ectoparasite and heavy infestations are very rare (Meinertzhagen & Clay 1948). Ectoparasite populations are normally at low levels on healthy wild hosts, and larger populations of parasite indicate that the host is unhealthy (Marshall 1981). The number of parasites required to kill a host will vary in different circumstances and death does not result from a simple interaction between host and parasite (Crofton 1971). This is because the death of the host inevitably means the death of the parasites, so there is no advantage to the host-specific parasite (Hopkins 1942).

Harbouring large populations of lice will cause severe damage to skuas. The feeding habit of lice, for example, can cause considerable irritation to the host, which results in almost continuous scratching when infestation is heavy. Amblycera are responsible for skin damage, loss of blood, and entry of disease germs (Nelson *et al.* 1977; Clayton 1990; Chinery 1993) which are a major factor reducing egg production of poultry (DeVaney 1976). Transmission of viral diseases can cause fetal abortions and sometimes death to host if the infection is heavy (Ash 1960; Marshall 1981). Clayton (1990) shows that feather damage by Ischnocera not only impairs the thermoregulatory ability and winter survival of wild hosts but also reduces the ability of infected hosts to attract mates. As a result, the reproductive success in hosts (e.g. in house martin, Delope *et al.* 1993; and swallow, Moller 1990) will be reduced as some female birds recognise and avoid infested males (Borgia & Collis 1989; Delope & Moller 1993).

Skuas can maintain their ectoparasite population at low level by various ways. The most obvious defence is grooming (Nelson *et al.* 1977; Waage 1979). The major form of this behaviour is preening, which may be operationally defined as manipulation of the plumage with the bill. Another form of grooming is foot scratching, which is usually used to control ectoparasites on the head and other regions inaccessible to preening. The efficiency of this behaviour in controlling ectoparasite populations was proved in studies of birds with impaired beaks (the tip of the upper mandible has been

removed to prevent feather-picking) or feet. Studies on birds with deformed feet or beaks and single leg show that they often have large ectoparasite loads, apparently because of their inability to groom properly (Kartman 1949; Boyd 1951; Rothschild & Clay 1952; Ash 1960; Marshall 1981). Impaired preening experiments also showed increased ectoparasite populations (Kartman 1949; Nelson & Murray 1971; DeVaney 1976). Although Simmonds (1957) stated that there is no direct connection between anting behaviour (anoint the feathers with ant fluids) and deparasitisation there is no doubt that this behaviour can be used in removing ectoparasites from the bird body. Accidental deparasitisation also can occur sometimes, for example when waders overwinter in highly alkaline water (Meinertzhagen 1950).

The composition of food, microclimate, and other ecological conditions found near the skin of the hosts, and lice tolerances to these factors, are the greatest influences in determining host specificity of the Mallophaga. Feather lice really depend on continuous association with their host and live at most only a few days when deprived of their normal food (Emerson & Price 1985). This is because they are extremely food specific. Some of them only feed on the feathers of a particular part of the body, and if they are presented with the feathers from other parts of the body, they will eat them but fail to breed and soon die (Ash 1960). In *Columbicola columbae* and *Colpocephalum turbinatum* for example, body feathers are essential for them since they eat the fluff (Nelson & Murray 1971). Humidity and temperature of the microclimate next to the host skin also appear to affect Mallophaga survival (Emerson & Price 1985). Lice are very sensitive to temperature and have a narrow range of preference. Although they can tolerate a lower temperature most of them cannot survive temperatures higher than that of their normal host. In addition, various stages in their life cycle require slightly different conditions, as shown by large mortality rates amongst incubator-bred nymphs when only one situation has been presented to these lice (Ash 1960). Survival requires special modification of physical appearance, behaviour, and sometimes physiology to adapt well to the host in pursuing their need to avoid the beak and claws (Brooke & Birkhead 1991). These alterations produce host-specific Mallophaga, which often

parasitise only a single species or subspecies of host as they are unable to live on different hosts with distinct characters (Clay 1949). In addition, parasites will also change due to interactions between themselves.

Different microhabitats offered by various hosts result in variation in parasite diversity inhabiting a distinct host. Thus some species of parasite will be present in some host species but not in others. As a result, feather lice behave like a secondary aid in understanding the taxonomic relationship of their host (Pilgrim & Palma 1982). Some feather lice studies already proved that Mallophaga can be used to clarify host relationships which were poorly known or speculative before. This kind of study also shows that an analysis of the distribution of Mallophaga genera on their hosts indicates that they can be used to distinguish host categories of greater than generic rank. The Mallophaga species group define host genera and provide exact data on their relationships. In addition, feather lice evidence also give hints about possible affinities of taxa, the position of which could neither be elucidated by classical methods, nor by DNA material.

Feather lice transfer between hosts only occurs through body-to-body contact. Regular occurrence of such contacts can be imagined primarily between individuals of the same species, such as parent-offspring and sexual contacts (a clear exception is the predator-prey contact which normally happens in a short time). Even if this contact occurs between two different species of birds, and lice successfully transferred to a new host, the possibility that highly specific lice will survive is very low. A good example can be inferred from Cuckoo broods (*Cuculus canorus*) which made countless contact with their foster parents, but are still infested by typical cuckoo lice and not by those of their passerine foster parents (Mauersberger & Mey 1993).

Although feather lice already proved that they are able to provide a great contribution in systematic studies, owing to the possibility of secondary infestations, data obtained from parasites must be used cautiously (Imber 1985). Furthermore, parasite evidence is not a panacea to host systematics. This information has to be used very carefully to avoid misinterpretation about taxonomic conclusions. Unrelated bird

species may have similar lice through the process of straggling due to specific habits and breeding sympatrically (e.g. predatory birds may, at least temporarily, acquire lice from their prey, and birds breeding on the ground may become infested by lice from other species breeding nearby; Pilgrim & Palma 1982). However, if two groups of birds have two or three identical species of Phthiraptera their close affinity is established (Hopkins 1942, Mauersberger & Mey 1993). In the case where evidence from parasites is against the conventional position of a bird in classification, ornithologists should consult more evidence from various methods before any conclusions can be derived.

There are several possible objections to the use of parasites in inferring host phylogeny (Stresemann 1959; Downes 1990). These objections were made on the basis of several judgements and can be categorised into three groups. First, classification of parasites is not totally independent from their host. Affinities of the parasite therefore, will be obscured by convergent structures and host-induced phenomena. Secondly, existence of host switching (transmission of parasites from one host lineage to another), extinction, and incomplete sampling of some parasites will obscure phylogenetic evidence of cospeciation (Price 1980; Page 1993). Finally, not all host-parasite relationships result in cospeciation due to the lack of strict host specificity.

On the other hand, it has already been shown that every higher bird taxon (e.g. orders or isolated families) appears to be infested by particular species of Mallophaga, which means reciprocally that a number of Mallophaga genera colonise only avian genera that are doubtless related to each other. If free inter-host mobility of parasite exists (which has sometimes been claimed) one might expect, after an evolution lasting millions of years, bird predators to be infested by species (or close relatives of them) which parasitised their prey species and families. There is little evidence of transfers from bird prey species to bird predator species.

This study has been conducted purely based on museum and frozen specimens. Clearly feather louse is a highly host-specific ectoparasite as showed before. Feather louse therefore, can be used as an indicator for their hosts systematic relationships. Any further researches using live birds should be welcome and the results can be compared

with this study. Samples of hosts from different geographical regions should also be collected in presenting a more diverse and fully representative situation. It will be good if further study is also carried out to determine the degree of host specificity indicated by lice; if live lice are transferred to different species of host, do they still manage to survive?

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Chapter 5

Morphological Correlations Between Ectoparasites, Feather Lice (Insecta: Phthiraptera) and Their Hosts, Skuas (Aves: Stercorariidae)

5.1. Introduction

Similarities in morphology between organisms may arise from common ancestry, convergence, or by reversion to an apparently ancestral condition (Wake 1991). Convergence is a process where similar characters arise independently in distinct or unrelated taxa in response to some common pressure (Futuyma 1986; Pankhurst 1991). Convergence has sometimes been confused with parallelism, so that in many cases putative convergence has turned out to be predominantly parallel evolution (Zuckerkandl 1994). Parallelism can be distinguished from convergence in three ways; 1) it occurs more frequently in closely related taxa, 2) it normally refers to populations which have the same ancestral character state, and 3) it often occurs in organisms that have similar developmental pathways (Kocher *et al.* 1993).

Morphological variations occur in several ways. For example, by divergence, when two closely related taxa have evolved rapidly at some stage and become physically distinct (Pankhurst 1991). Differences in expressed phenotypes may also be produced by mutation of certain DNA sites.

Morphological diversity plays an important role in classical taxonomy. The degree of similarity or difference among taxa has been used by morpho-taxonomists to classify organisms. Therefore, any non-genetical (non-inherited) morphological change has the potential to cause error for morpho-taxonomists. The existence of similarities in some characters (especially for closely related taxa) constrains morpho-taxonomists from making an accurate assessment of an organism's relationships (for example similarities in cichlid fishes in east African lakes, Kocher *et al.* 1993). Identical

organisms are formed when particular developmental phenomena are triggered by different stimuli or when constraints shape external form or limit morphological expression to a few options (Wake 1991). This similarity can only be detected by obtaining further indications from other characters from related taxa (Futuyma 1986).

Convergent evolution, a good example of homoplasy, occurs in various living systems. This phenomenon provides valuable information for the study of evolutionary morphology and can serve as a substitute for laboratory controls. Convergent evolution, therefore, is the greatest hope for the formulation of operationally valid theories of (morphological) causation (Emberton 1994). Examples of this phenomenon can be seen in the study of the granivorous and the insectivorous songbirds (cardueline finches (Fringillidae: Carduelini) and turdid chats (Turdidae: Saxicolini)) living in alpine habitat in Central Himalayas and Nepal. These birds possess slightly different phenotypes due to living in different habitats i.e. altitude. Species that live at higher altitudes have heavier bodies, more pointed wings, and longer claws than their lower altitude counterparts. In addition, shrub-dwelling and ground-dwelling species tend to have longer tarsi and toes (Landman & Winding 1995).

Animals living sympatrically are more prone to convergent evolution since they are exposed to the same environmental pressure. Shells of sympatric land-snails (Triodopsinae *Neohelix major* (Binney) and Polygyrinae *Mesodon normalis* (Pilsbry), for example, are more convergent in size and shape compared to their allopatric counterparts (Emberton 1995). A study of three extant species of canid living sympatrically in east Africa showed that the sidestriped (*Canis adustus*), the golden (*C. aureus*), and the blackbacked (*C. mesomelas*) jackals have converged to a similar size and overall morphology (Vanvalkenburgh & Wayne 1994). Two ascid mites (*Mycolaelaps maxinae* and *Hoploseius tenuis* Lindquist) show morphological resemblance as an adaptation to a way of life inside the lumen of bracket fungus pores (Lindquist 1995). This is an excellent example of convergence in organisms living in the same niche.

In host-parasite systems, information about host phylogeny can be inferred from their parasite species composition (and population general structure) provided that the parasites are highly host-specific and no host-switching occurs. The structure of ectoparasite communities in this system is strongly influenced by the host (Choe & Kim 1988). The development of feathers by bird ancestors or hair by mammal ancestors for example, provided a new type of habitat for their ectoparasites, mainly feather lice and mites (Clay 1949). This empty ecological niche, with plentiful food and lack of competition, would attract new users to colonise it. However, if the new habitat is slightly different from the previous one, the parasites are then required to modify some of their features to ensure successful colonisation. The adaptation process will normally invoke alterations in behaviour, diet or morphology.

The environment of ectoparasites, such as feather lice and mites is formed by the chemical composition and physical structure of the feathers, the texture of the skin and certain physiological characters of the host such as temperature and body secretions. Thus, any changes in host morphology (or parasites' microhabitat) will induce parasites to adapt or evolve to this modification. After the initial period of rapid evolution, this change will tend to occur at a slower rate and is reflected in the general correlation found between the classifications of host and parasite (Clay 1949).

There are several studies on the effects of hosts on parasite morphology. Kellog (1913) pointed out that the majority of variation occurs within every Mallophaga species, caused by the separation, approximately complete and persisting, of its individuals into little groups and family strains each isolated on its host island or succession of self-reproducing islands. This study of small-scale conspicuous morphological variation indicates that those apparent conspecifics are different species (Johannesson *et al.* 1993). Based on simplified phylogenies of gophers and lice, Harvey & Keymer (1991) showed that evolution of louse body size is highly correlated with that of their host. Each species of chewing louse possesses a specific groove size which correlates with the width of the hair of the host (Page *et al.* 1996). Thus lice are coadapted with their host (Hafner & Page 1995).

This study is designed to examine any morphological correlation between skuas (host) and feather lice (ectoparasite) by studying the effects of different microhabitat provided by various taxa of host on the morphology of the lice. Ectoparasite achievement in partitioning available resources among sympatric species is also examined.

5.2. Materials and Methods

5.2.1. Collection and Measurement of Ectoparasites

Feather lice were extracted from their skua hosts. Skuas were obtained from various sources such as the Glasgow Museum and Art Gallery, the Royal Scottish Museum at Edinburgh, the British Museum of Natural History at London and a few specimens from the Hunterian Museum in the Division of Environmental and Evolutionary Biology Collection (DEEB, formerly known as the Zoology Department), University of Glasgow. The majority of hosts were preserved either as dried skins or as frozen birds. For museum skins, lice were extracted by thoroughly combing each feather. This process was done very carefully to minimise damage to preserved birds. In the majority of cases, every area of bird external morphology was examined. In a few cases, the inner side of the bird's wing was inaccessible because it was tightly fastened after preservation. Frozen birds did not present any problems of inaccessibility but they had to be thawed before any combing process was possible. More details about this method are given in Chapter 4.

Only well preserved lice (which possessed all of the characters required for measurement) were used in morphometric analysis. In addition to these, some lice donated by Dr. R.L. Palma (New Zealand Natural History Museum) were also used. All donated lice were preserved in ethanol (70% by volume). Measurements were made using a binocular microscope fitted with a special micrometer scale in the eye-piece. Measurement data obtained by this method were calibrated later with a standard

eye-piece calibration scale. All measurements were recorded in millimetres. The study has been divided into two sections; 1) examination of size differences, and 2) investigation of shape variations. Eleven characters were measured for the study of size differences, while an additional four derived characters (ratios of some characters to others) were selected to investigate shape differences. All characters are based on those used by Eveleigh & Amano (1977) and were chosen for ease and repeatability of measurement and overall morphological representation. These characters are explained in Table 5.1 and illustrated in Figure 5.1.

5.2.2. Analysis of Morphological Differences

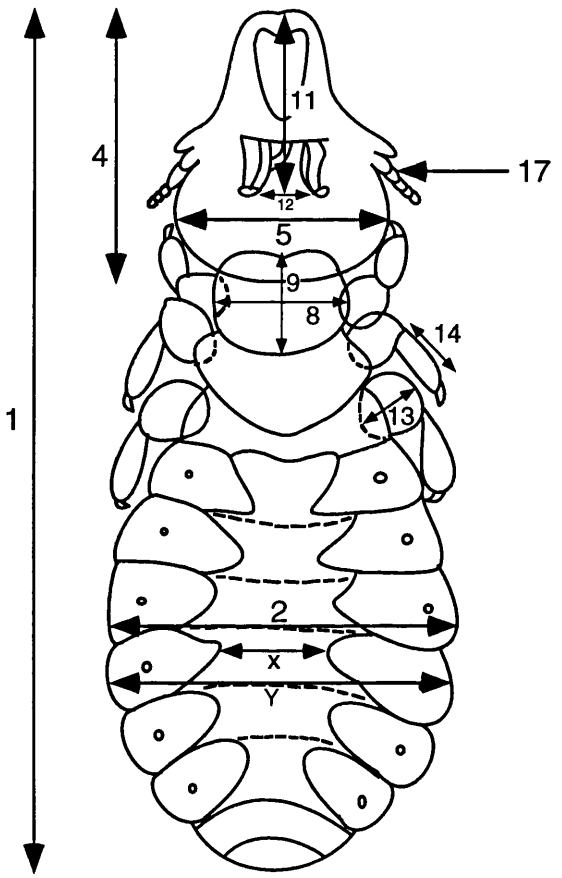
The analysis procedure begins with all measurements being standardised with the assumption that all variables should contribute equally to morphological variations. All four genera of lice (*Haffneria grandis* [HG], *Austromenopon fuscofasciatum* [AF], *Saemundssonia sp.* [SS] and *Quadraceps normifer normifer* [QN]) were separated into eight groups based on genera and sex. To facilitate analysis, all individuals of *Saemundssonia sp.* were lumped together and been considered as a single taxon. Separation between louse sexes has been done on the assumption that sexual dimorphism occurs. In addition to these eight groups, HG and SS have also been separated into several groups based on the taxon of infected host. This separation investigates the effects of variation in host species on lice morphology. The main assumption for this investigation is that similar taxa of lice may possess slightly different morphologies due to long term coevolution with their host.

Multivariate analysis was then used on standardised data starting with principal component analysis (PCA). This analysis examines morphological variations among feather lice and provides an overview of the patterns of relationships among observations in the data-set. A major advantage of PCA over other methods is the ability to analyse a range of variables simultaneously and to represent these in fewer axes without significant reduction in the information content of the data-set. This was

Table 5.1. Characters used in this study and brief explanation about each of them (modified from Eveleigh & Amano 1977). Characters 3, 6, 7 and 10 were derived characters.

Abbreviation	Characters explanation (number corresponds to Figure 5.1)
1. bodylen	Total body length (1)
2. bodywid	Body width, taken at widest point (2)
3. bltobw	Ratio of body length to body width (3)
4. headlen	Head length (4)
5. headwid	Head width, taken at widest point (5)
6. hltohw	Ratio of head length to head width (6)
7. bltohl	Ratio of body length to head length (7)
8. prolen	Prothorax length (8)
9. prowid	Prothorax width (9)
10. pwtopl	Ratio of prothorax width to prothorax length (10)
11. httomb	Distance between headtip to mouthpart bases (11)
12. mouthbas	Distance between mouthpart bases (12)
13. femur	Length of femur of third leg (13)
14. tibia	Length of tibia of second leg (14)
15. sclero	Degree of sclerotization (ratio of width of nonsclerotized zone of fourth spiracle-bearing segment (x) to total width of that segment of abdomen (y) (15)

Figure 5.1. Schematic diagram of lice showing all characters used in this study (modified from Eveleigh & Amano 1977)



achieved by transforming correlated variables into uncorrelated ones and then representing these relatively large percentages of the total variability as linear combinations (Airoldi & Flurry 1988). Transformed values or scores produced by this analysis can be used to explain relationships between characters. Extreme eigen-values normally are providing more information than others.

Another multivariate analysis method used in this study was canonical discriminant analysis. This analysis is often capable of better discrimination between observations when PCA gives poor results. The data are treated with one classification variable (with each value denoting a different group) to several quantitative variables. This technique will also calculate the distance index within and between groups in a data-set. The likelihood ratios produced by this analysis can be used as a preliminary judgement to determine how many axes can be used in differentiating observations in a data-set. This will lead to group allocation when canonical discriminant values derived from analysis are being plotted.

5.3. Results

5.3.1. Variation in Lice Composition

A total of 445 adult lice were extracted from 360 hosts. However, not all specimens were suitable for morphometric analysis due to imperfect preservation. Only 325 lice (73.03%) possessed all of the characters required for measurement and were therefore suitable for morphometric analysis. The composition of lice samples varied among hosts. Some species of lice exist in larger numbers on certain hosts but are present in smaller quantities or absent on others. QN, for example, was the most abundant, with 114 occurrences or 35.07% of lice occurrences, followed by *Saemundssonia sp.* (100 lice or 30.77%), HG (56 specimens or 17.23%), and finally AF with only 45 individuals or 16.93% (Table 5.2). More abundant lice, such as QN could be examined more thoroughly but unfortunately no QN specimens occurred on Brown, Chilean, Great, or

Table 5.2. The lice on preserved skuas. Figures show the numbers of lice discovered in this study while the number in parentheses shows the number of lice examined for morphometric analysis. Some lice were not available for examination and these are represented as (-).

	H. grandis		*Saemundssonia sp.*		*Q. n. normifer*		*A. fuscofasciatum*	
	male	female	male	female	male	female	male	female
Arctic skua	0 (-)	0 (-)	25 (20)	31 (16)	46 (39)	70 (57)	4 (3)	10 (6)
Brown skua	4 (2)	5 (-)	2 (5*)	4 (3)	1 (-)	1 (-)	0 (1*)	0 (1*)
Chilean skua	0 (-)	5 (2)	1 (3*)	13 (9)	1 (-)	3 (-)	1 (-)	1 (-)
Falkland skua	3 (2)	11 (6)	3 (2)	8 (9*)	3 (3)	1 (1)	0 (-)	0 (-)
Great skua	12 (12)	15 (15)	0 (-)	6 (4)	0 (-)	0 (-)	0 (-)	0 (-)
Long-tailed skua	0 (-)	0 (-)	5 (3)	6 (4)	5 (5)	8 (5)	0 (-)	0 (-)
Pomarine skua	2 (-)	1 (-)	7 (1)	12 (9)	1 (2*)	2 (1)	12 (10)	36 (24)
South polar skua	3 (3)	6 (7*)	1 (4*)	4 (4)	1 (1)	0 (-)	0 (-)	0 (-)
Tristan skua	21+ (5)	18+ (2)	1 (2*)	2 (2)	0 (-)	1 (-)	0 (-)	0 (-)
Total	45 (24)	61 (32)	45 (40)	86 (60)	58 (50)	86 (64)	17 (14)	47 (31)

Notes:

* = Includes some donated by R.L. Palma, New Zealand Natural History Museum

+ = Includes data from Furness & Palma (1992)

Tristan skuas. The majority of QN specimens (96 individuals or 84.21%) occur on Arctic skua. Further study revealed that this pattern of distribution was not only shown by QN, but also by other lice. Measurements of SS for example were mainly done on samples obtained from Arctic skua (36 lice or 36.00%) whereas HG and AF were primarily from lice inhabiting Great (27 individuals or 48.21%) and Pomarine skuas (34 specimens or 75.56%) respectively (Table 5.2).

Some hosts are parasitised by a small quantity of feather lice and, therefore, only a limited number of lice were successfully extracted from these hosts providing only a small number of lice for morphometric analysis. In extreme cases, only a single louse was available for measurement. This occured in SS from male Pomarine skua, in QN on female Falkland and Pomarine skuas and male South polar skua, and in AF on the Brown skua (Table 5.2). Small sample sizes always present major analytical difficulties. Single observations reduce the level of reliability and may result in an inaccurate picture of relationships between lice and their hosts.

5.3.2. Differences in Louse Morphology

As anticipated, results from PCA clearly indicate that all lice used in this study could be allocated into four clusters (Figure 5.2 to 5.5). This separation, based on size and shape differences, occurs in both sexes. Female HG possess longer body, femur and tibia in comparison with other lice in this study. These characters distinguished female HG from other lice by PCA. Female SS differ from other lice by possessing a large head which also influenced measurements of the distance between head tip to mouthpart base (Figure 5.2; Table 5.3). On the basis of shape variations among female lice, HG possess a smaller prothorax, as compared to AF. The other two female lice (SS and QN) have an intermediate prothorax shape but they have a larger body proportion compared to their head (Figure 5.3; Table 5.4).

PCA of size differences among male lice shows a concordance with results from females. Results from male lice also revealed that for measurement of body length,

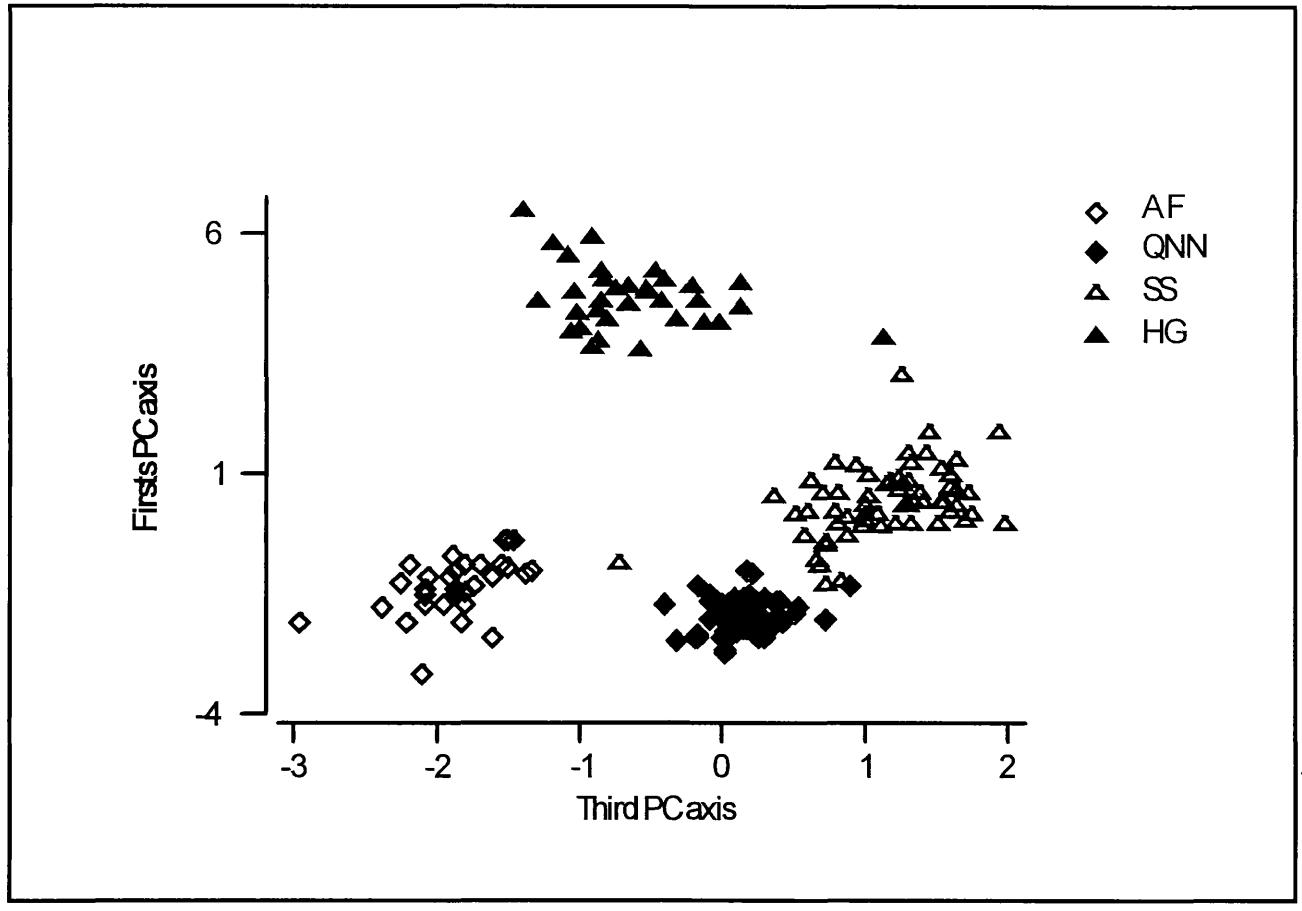


Figure 5.2. Scatter plot of PC values (PC1 against PC3) for size differences of 191 individuals of female lice inhabiting skuas.

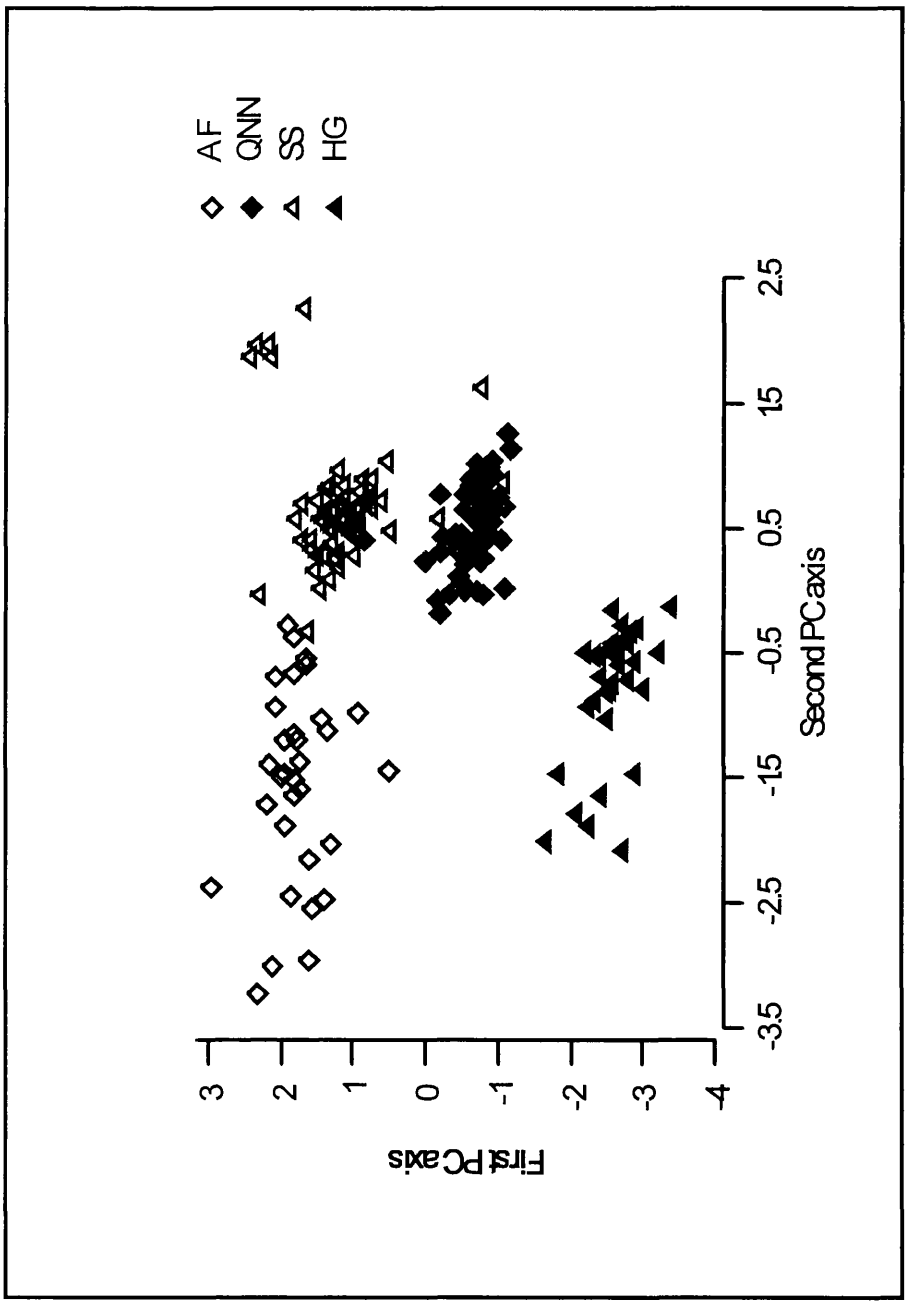


Figure 5.3. Scatter plot of PC values (PC1 against PC2) for shape variations of 191 individuals of female lice inhabiting skuas.

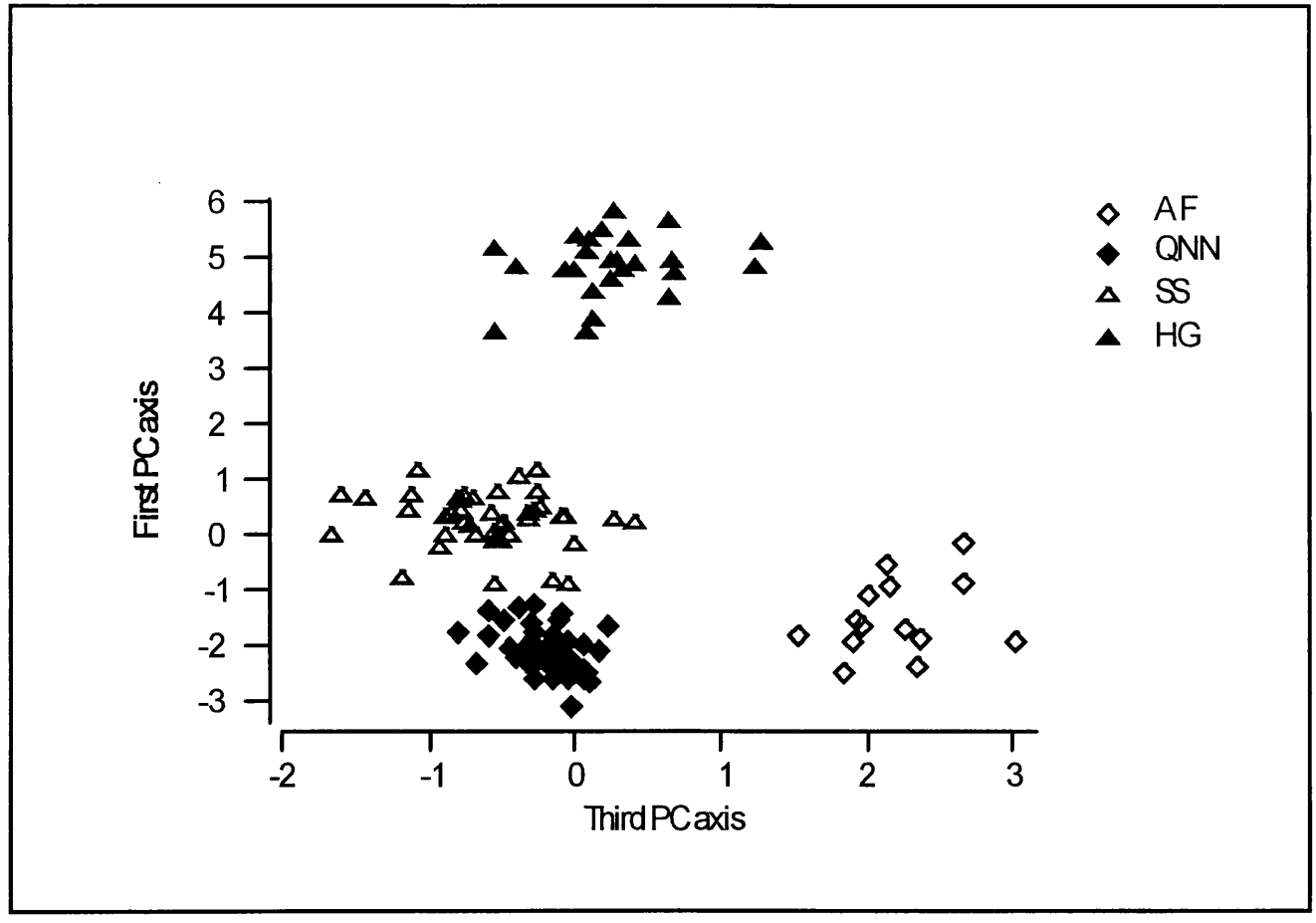


Figure 5.4. Scatter plot of PC values (PC1 against PC3) for size differences of 134 individuals of male lice inhabiting skuas

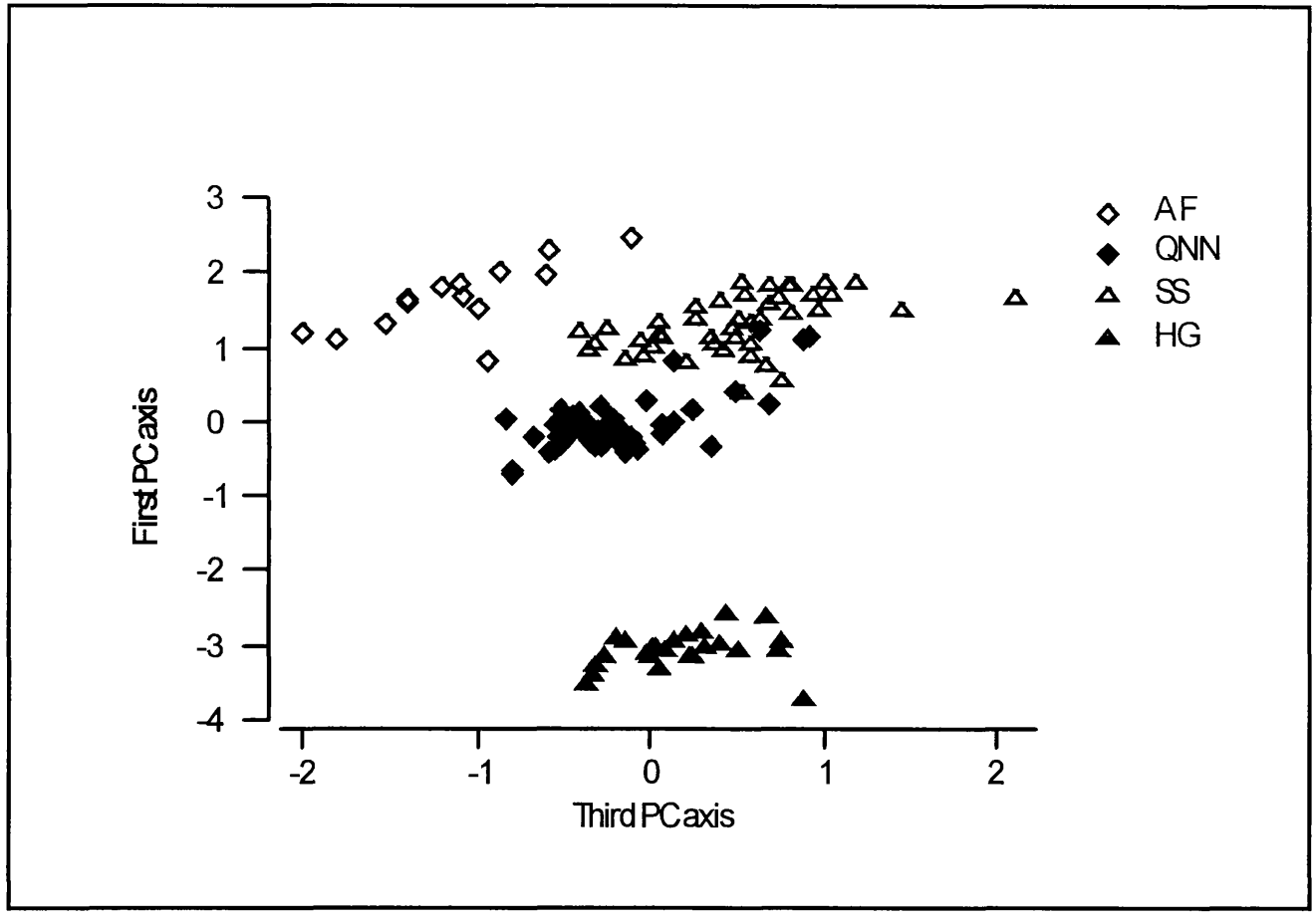


Figure 5.5. Scatter plot of PC values (PC1 against PC3) for shape differences of 134 individuals of male lice inhabiting skuas

Table 5.3. Eigenvalues and component loading for the first three principal components analysed based on size differences characters of 191 female lice extracted from skuas.

Components	1	2	3
Eigenvalue	5.808	2.653	1.231
Cumulative (%)	52.8	76.9	88.1
Characters[1]	Component loadings		
bodylen	0.373	-0.232	-0.091
bodywid	0.255	0.372	0.077
headlen	0.339	-0.168	0.392
headwid	0.194	0.460	0.355
prolen	0.287	-0.261	-0.401
prowid	0.200	0.411	-0.401
httomb	0.352	-0.077	0.382
mouthbas	0.343	0.129	0.201
femur	0.372	-0.083	-0.253
tibia	0.374	-0.114	-0.251
sclero	-0.015	-0.536	0.232

Note:

[1]Explanation for character names can be obtained from Table 5.1.

Table 5.4. Eigenvalues and component loading for the first three principal components analysed based on shape variations characters on 191 female lice infesting skuas.

Components	1	2	3
Eigenvalue	2.521	1.037	0.383
Cumulative (%)	63.0	89.0	98.6
Characters[1]	Component loadings		
bltobw	-0.595	-0.132	0.400
hltohw	-0.211	-0.921	-0.071
bltohl	-0.566	0.327	0.382
pwtopl	0.530	-0.165	0.830

Note:

[1]Explanation for character names can be obtained from Table 5.1.

third femur and second tibia length, male HG possess maximum values. Male AF also have a wider prothorax and body than other lice (Figure 5.4; Table 5.5). Shape variations among male lice (body and head formations) distinguish HG from the other lice (Figure 5.5; Table 5.6).

In addition to component loading for each louse character, Table 5.3, 5.4, 5.5 and 5.6 also present the first three eigenvalues obtained from all lice. These first three scores alone are adequate in presenting an overall pattern of morphological variations among the lice. A cumulative proportion of eigenvalues of female lice for instance represents 88.1% (for size differences) and 98.6% (for shape variations) of total morphological variations. Among male lice, 89.6% of size differences and 98.4% of shape variations were represented by the first three principal components.

The classical approach of comparing measurements of morphological characters is also very useful in differentiating unknown organisms (e.g. feather lice in this case). This straight-forward technique has suggested that HG is the largest louse discovered in this study (by possessing a total body length that ranges from 3.847 to 4.503 cm for females and 3.564 to 4.657 cm for males), followed by SS, QN and AF (Table 5.7 to 5.10). Therefore, all maximum morphometric values of examined characters will be dominated by HG. This was true for most characters except for features such as body width and relative size of head to body. In body width measurements for example, although a majority of HG possess a wider body compared to other lice (range from 0.648 to 0.851 cm for females and 0.567 to 0.729 cm for males) other lice such as female SS and AF show a higher measurement value (with a mean value of 0.795 cm and 0.732 cm respectively, Table 5.8 and 5.10). HG also seems to have a slender head (long but narrow) compared to SS which possesses the widest head (ranging from 0.546 to 0.735 cm for male lice and 0.588 to 0.850 cm for female lice, as compared with 0.256 to 0.607 cm and 0.526 to 0.810 for male and female HG respectively). However, HG has a relatively large prothorax both in terms of length (ranging from 0.283 to 0.405 cm for female and from 0.273 to 0.405 cm for male) and width (ranging from 0.231 to 0.486 cm for female and from 0.364 to 0.486 cm for male). However, some

Table 5.5. Eigenvalues and component loading for the first three principal components analysed based on size differences characters on 134 male lice infesting skuas.

Components	1	2	3
Eigenvalue	6.615	2.455	0.791
Cumulative (%)	60.1	82.5	89.6
Characters[1]	Component loadings		
bodylen	0.368	-0.184	-0.010
bodywid	0.280	0.295	0.316
headlen	0.348	-0.078	-0.407
headwid	0.158	0.519	-0.362
prolen	0.303	-0.316	0.289
prowid	0.289	0.299	0.414
httomb	0.323	0.006	-0.444
mouthbas	0.309	0.103	-0.249
femur	0.358	-0.165	0.163
tibia	0.368	-0.145	0.172
sclero	-0.031	-0.596	-0.182

Note:

[1]Explanation for character names can be obtained from Table 5.1.

Table 5.6. Eigenvalues and component loading for the first three principal components analysed based on shape variations characters on 134 male lice infesting skuas.

Components	1	2	3
Eigenvalue	2.805	0.715	0.415
Cumulative (%)	70.1	88.0	98.4
Characters[1]	Component loadings		
bltobw	-0.557	-0.188	0.422
hltohw	-0.548	-0.244	0.457
bltohl	-0.435	-0.713	-0.492
pwtopl	0.447	-0.630	0.609

Note:

[1]Explanation for character names can be obtained from Table 5.1.

Table 5.7. Variation in morphometric data for *Haffneria grandis* parasitising skuas. Measurements in millimetres on 32 female and 24 male lice. Refer to Table 5.1 and Figure 5.1 for explanation of characters.

	Female			Male		
Characters	Mean	S.D.	Range	Mean	S.D.	Range
1. bodylen	4.2570	0.1650	3.847 - 4.503	4.1942	0.2667	3.564 - 4.657
2. bodywid	0.7918	0.0582	0.648 - 0.851	0.6835	0.0534	0.567 - 0.729
3. bltobw	5.3726	0.3082	4.926 - 6.187	6.1499	0.3014	5.651 - 6.756
4. headlen	0.8912	0.0792	0.688 - 0.972	0.9186	0.0384	0.850 - 0.972
5. headwid	0.5936	0.0456	0.526 - 0.810	0.5353	0.0693	0.256 - 0.607
6. hltohw	1.5086	0.1688	1.051 - 1.714	1.6950	0.1222	1.499 - 2.089
7. bltohl	4.7972	0.3645	4.389 - 5.834	4.5672	0.2713	4.000 - 5.112
8. prolen	0.3341	0.0369	0.283 - 0.405	0.3218	0.0309	0.273 - 0.405
9. prowid	0.4264	0.0417	0.231 - 0.486	0.4278	0.0265	0.364 - 0.486
10. pwtopl	1.2870	0.1663	0.814 - 1.576	1.3401	0.1514	1.101 - 1.711
11. httomb	0.6529	0.0489	0.567 - 0.769	0.6108	0.1079	0.284 - 0.729
12. mouthbas	0.1583	0.0257	0.122 - 0.203	0.1588	0.0335	0.122 - 0.243
13. femur	0.3328	0.0648	0.243 - 0.607	0.3206	0.0313	0.243 - 0.405
14. tibia	0.4664	0.0395	0.364 - 0.526	0.4928	0.0368	0.446 - 0.567
15. sclero	0.3201	0.0779	0.157 - 0.471	0.3542	0.1030	0.167 - 0.556

Table 5.8. Variation in morphometric data for *Saemundssonia sp.* infesting skuas. Measurements in millimetres on 60 female and 43 male lice. Refer to Table 5.1 and Figure 5.1 for explanation of characters.

	Female			Male		
	Mean	S.D.	Range	Mean	S.D.	Range
1. bodylen	1.8868	0.2775	1.092 - 2.308	1.7876	0.1343	1.491 - 2.100
2. bodywid	0.7950	0.1293	0.420 - 1.113	0.6235	0.0864	0.420 - 0.777
3. bltobw	2.4323	0.4840	1.200 - 3.750	2.9151	0.4629	2.389 - 4.182
4. headlen	0.6712	0.0607	0.504 - 0.777	0.6426	0.0606	0.462 - 0.756
5. headwid	0.7301	0.0566	0.588 - 0.850	0.6742	0.0417	0.546 - 0.735
6. hltohw	0.9261	0.0849	0.756 - 1.212	0.9533	0.0771	0.709 - 1.129
7. bltohl	2.8264	0.4240	1.542 - 3.571	2.7976	0.2481	2.419 - 3.500
8. prolen	0.1822	0.0438	0.126 - 0.420	0.1653	0.0185	0.147 - 0.210
9. prowid	0.3813	0.0463	0.168 - 0.446	0.3686	0.0392	0.315 - 0.588
10. pwtopl	2.1490	0.4786	0.182 - 3.167	2.2493	0.2923	1.700 - 3.112
11. httomb	0.5145	0.0637	0.284 - 0.651	0.4768	0.0483	0.357 - 0.567
12. mouthbas	0.1310	0.0251	0.084 - 0.231	0.1233	0.0256	0.084 - 0.189
13. femur	0.1426	0.0310	0.084 - 0.231	0.1228	0.0255	0.084 - 0.231
14. tibia	0.1728	0.0248	0.122 - 0.231	0.1672	0.0197	0.126 - 0.210
15. sclero	0.1577	0.0840	0.000 - 0.334	0.0370	0.0717	0.000 - 0.285

Table 5.9. Variation in morphometric data for *Quadraceps n. normifer* parasitising skuas. Measurements in millimetres on 64 female and 50 male lice. Refer to Table 5.1 and Figure 5.1 for explanation of characters.

	Female			Male		
	Mean	S.D.	Range	Mean	S.D.	Range
1. bodylen	1.8090	0.0916	1.6380 - 1.9950	1.4939	0.0996	1.3020 - 1.8480
2. bodywid	0.5440	0.0392	0.4200 - 0.6720	0.4746	0.0372	0.4200 - 0.6090
3. bltobw	3.3354	0.2026	2.7860 - 4.1500	3.1536	0.1549	2.7930 - 3.5200
4. headlen	0.5362	0.0389	0.4410 - 0.6300	0.5032	0.0323	0.4620 - 0.5880
5. headwid	0.4433	0.0246	0.3990 - 0.5250	0.4187	0.0410	0.3570 - 0.5460
6. hltohw	1.2110	0.0900	1.0450 - 1.4730	1.2082	0.0994	0.9230 - 1.4730
7. bltohl	3.3867	0.2381	2.8920 - 3.9090	2.9745	0.1870	2.5000 - 3.5200
8. prolen	0.2080	0.0211	0.1260 - 0.2310	0.1856	0.0252	0.1260 - 0.2310
9. prowid	0.2828	0.0212	0.2520 - 0.3990	0.2629	0.0196	0.2310 - 0.3150
10. pwtopl	1.3802	0.2259	1.1820 - 2.6670	1.4573	0.3330	1.1000 - 2.5000
11. httomb	0.3779	0.0424	0.2730 - 0.5040	0.3598	0.0347	0.2940 - 0.4410
12. mouthbas	0.0824	0.0114	0.0630 - 0.1050	0.0794	0.0115	0.0630 - 0.1050
13. femur	0.1066	0.0173	0.0630 - 0.1470	0.1012	0.0138	0.0630 - 0.1260
14. tibia	0.1338	0.0185	0.1050 - 0.1680	0.1209	0.0144	0.0840 - 0.1470
15. sclero	0.4207	0.0800	0.0000 - 0.6250	0.4523	0.0934	0.0000 - 0.5710

Table 5.10. Variation in morphometric data for *Austromenopon fuscofasciatum* infesting skuas. Measurements in millimetres for 31 female and 14 male. Refer to Table 5.1 and Figure 5.1 for explanation of characters.

	Female			Male		
	Mean	S.D.	Range	Mean	S.D.	Range
1. bodylen	1.4219	0.1193	1.1970 - 1.8690	1.1130	0.1629	0.9030 - 1.4490
2. bodywid	0.7327	0.1369	0.0540 - 0.8190	0.6315	0.0484	0.5670 - 0.7560
3. bltobw	1.9155	0.2067	1.6750 - 2.7500	1.7569	0.1613	1.5350 - 2.0310
4. headlen	0.3082	0.0445	0.2310 - 0.3780	0.2925	0.0291	0.2520 - 0.3570
5. headwid	0.5717	0.0608	0.3570 - 0.6510	0.4935	0.0668	0.2940 - 0.5670
6. hltohw	0.5432	0.0851	0.3790 - 0.7640	0.6042	0.1090	0.4620 - 0.9280
7. bltohl	4.6940	0.6840	3.5560 - 6.0910	3.8310	0.6120	3.0000 - 4.9170
8. prolen	0.2134	0.0217	0.1470 - 0.2520	0.1890	0.0218	0.1470 - 0.2100
9. prowid	0.4756	0.0487	0.2940 - 0.5670	0.4140	0.0363	0.3570 - 0.4830
10. pwtopl	2.2545	0.3504	1.4000 - 3.2860	2.2056	0.1930	1.9000 - 2.5710
11. httomb	0.2703	0.0523	0.1890 - 0.3990	0.2550	0.0533	0.1680 - 0.3150
12. mouthbas	0.0942	0.0202	0.0420 - 0.1260	0.0825	0.0153	0.0630 - 0.1050
13. femur	0.1470	0.0248	0.0840 - 0.1890	0.1200	0.0209	0.0840 - 0.1470
14. tibia	0.1836	0.0271	0.1470 - 0.2310	0.1650	0.0307	0.1260 - 0.2100
15. sclero	0.0093	0.0519	0.0000 - 0.2890	0.0000	0.0000	0.0000 - 0.0000

female SS show a closer value of prothorax width to HG (ranges from 0.168 to 0.446 cm; Table 5.8).

5.3.3. Separation of Hosts by Lice Morphology

Results from canonical discriminant analysis indicate that three out of the four genera of lice are morphologically distinct when parasitising different hosts. This is most strongly shown by HG (identifying members of large skuas group), followed by QN (distinguishing members of small skua group from large skuas group), and SS (differentiating Chilean skua from the others), whereas AF failed to shows any significant variation in morphology.

HG parasitised all taxa of the large skua group, but unfortunately female lice from Brown skua and male lice from Chilean skua were unavailable for analysis. Detailed study on female HG revealed that, although these lice belong to the same species, they are slightly different in morphology. These differences vary according to the taxa of host which they parasitise. Plots of canonical variables of female HG produced three clusters which represent three different groups of HG (Figure 5.6). Explanation about variations in HG morphology can be obtained by studying the first two canonical variates (from canonical discriminant analysis) which represent 93.17% of total variations (Table 5.11). The first canonical variable shows that female HG from Tristan, Great, and Falkland skuas were morphologically similar but slightly different from female HG living on South polar or Chilean skuas (ANOVA: $F=3.22$; $df=70.81,44$; $p<0.0001$). Lice from the former hosts differ from those on the latter in head length, body length and the distance between head tip to mouthpart base (Table 5.11). The lice of the South polar skua are significantly distinct from HG on other skuas (ANOVA: $F>3.24$; $df=18,11$; $p<0.013$) by having a shorter femur and a narrow head. Second canonical variables suggest that the lice of the Chilean skua possess a longer femur and prothorax and, therefore, are distinct from other HG on other large skuas (ANOVA: $F=1.82$; $df=56.44,30$; $p<0.025$).

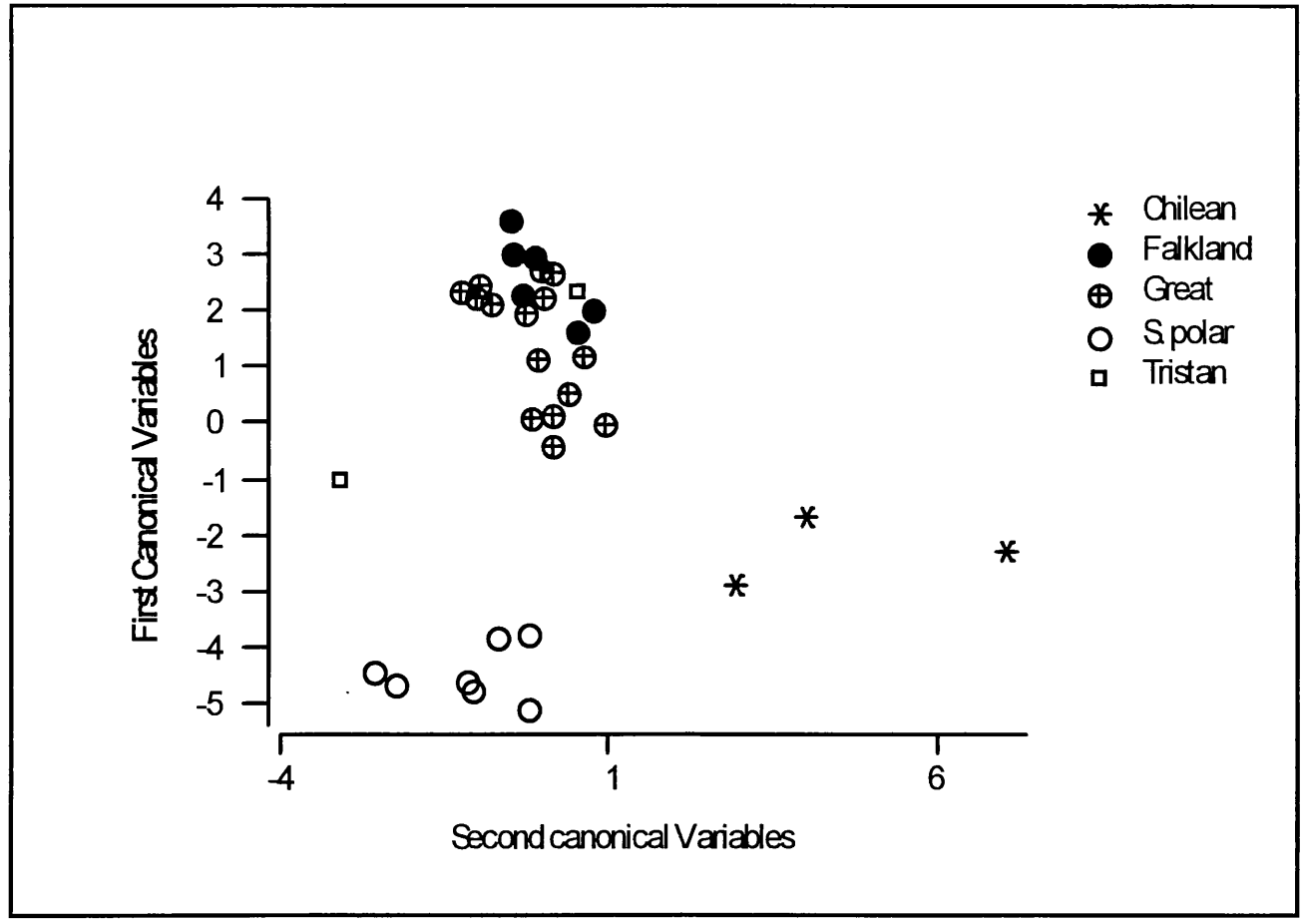


Figure 5.6. Canonical discriminant analysis of lice. Female *H. grandis* from South Polar and Chilean skuas are different in size from their cospecies in Falkland, Tristan and Great skuas.

Table 5.11. Total canonical structure score from canonical discriminant analysis on size differences among female *Haffneria grandis* infesting skuas.

Components	Canonical variate 1	Canonical variate 2	Canonical variate 3
Eigenvalue	8.195	2.833	0.655
Cumulative (%)	69.24	93.17	98.71
Characters[1]	component loadings		
bodylen	0.541	0.136	-0.494
bodywid	0.430	0.359	-0.699
headlen	0.777	0.143	-0.024
headwid	-0.336	0.024	-0.270
prolen	0.116	0.514	-0.047
prowid	0.412	0.323	-0.184
httomb	0.524	0.306	0.132
mouthbas	-0.642	0.397	0.266
femur	-0.017	0.869	-0.200
tibia	0.295	-0.595	-0.199
sclero	-0.331	-0.130	0.212

Note:

[1]Explanation for character names can be obtained from Table 5.1.

Female HG reveal a similar pattern but the details of differences among skuas are slightly different (Figure 5.7). This analysis revealed that HG from South polar skua possess a slightly different morphologies than HG from other large skuas (ANOVA: $F>5.17$, $df=25,4$; $p<0.003$) except one which parasitised Chilean skua. HG from South Polar skuas have a smaller body length relative to their body-width and head-length (Table 5.12). Apart from being similar to the lice of the South polar skua, HG from Chilean skua are also not significantly different from the lice of the Great skua. Chilean skua lice tend to have a smaller head, whereas HG living on Falkland and Tristan skuas generally have a large prothorax. All these variations have been presented by the first two canonical variates which already account for 96.84% of total variation (Table 5.12).

Size differences among male HG also produce three groups as has been revealed by their female counterparts (Figure 5.8). However, the allocation of skuas species by these data is slightly different from female HG. This may be caused by the absence of HG from Chilean skua and the presence of HG on Brown skua. Again, the first two canonical variates represent a higher cumulative value; i.e. 80.72% from total variations, and this value is assumed to be more than adequate in representing lice variation (Table 5.13). The second canonical variable successfully separates HG on Great, South polar and Falkland skuas from HG on Brown and Tristan skuas. HG from the former hosts have a narrow prothorax and a shorter femur and tibia. Data from the first canonical variables show that HG on Great, Brown and Tristan skuas are significantly different from HG on South polar and Falkland skuas (ANOVA: $F=2.53$; $df=40.21,44$; $p<0.0017$). HG from the former skuas differs from the latter in body length and body width characters. The lice of the former skuas are also larger in body size compared to lice from the latter hosts (Table 5.13).

Shape analysis of male HG shows that lice from Great skuas possess a wide range of body morphologies (Figure 5.9). Further investigation of the first canonical variate indicates that lice from Brown and Tristan skuas are slightly different from those from South polar and Falkland skuas (Table 5.14). Male HG from the former

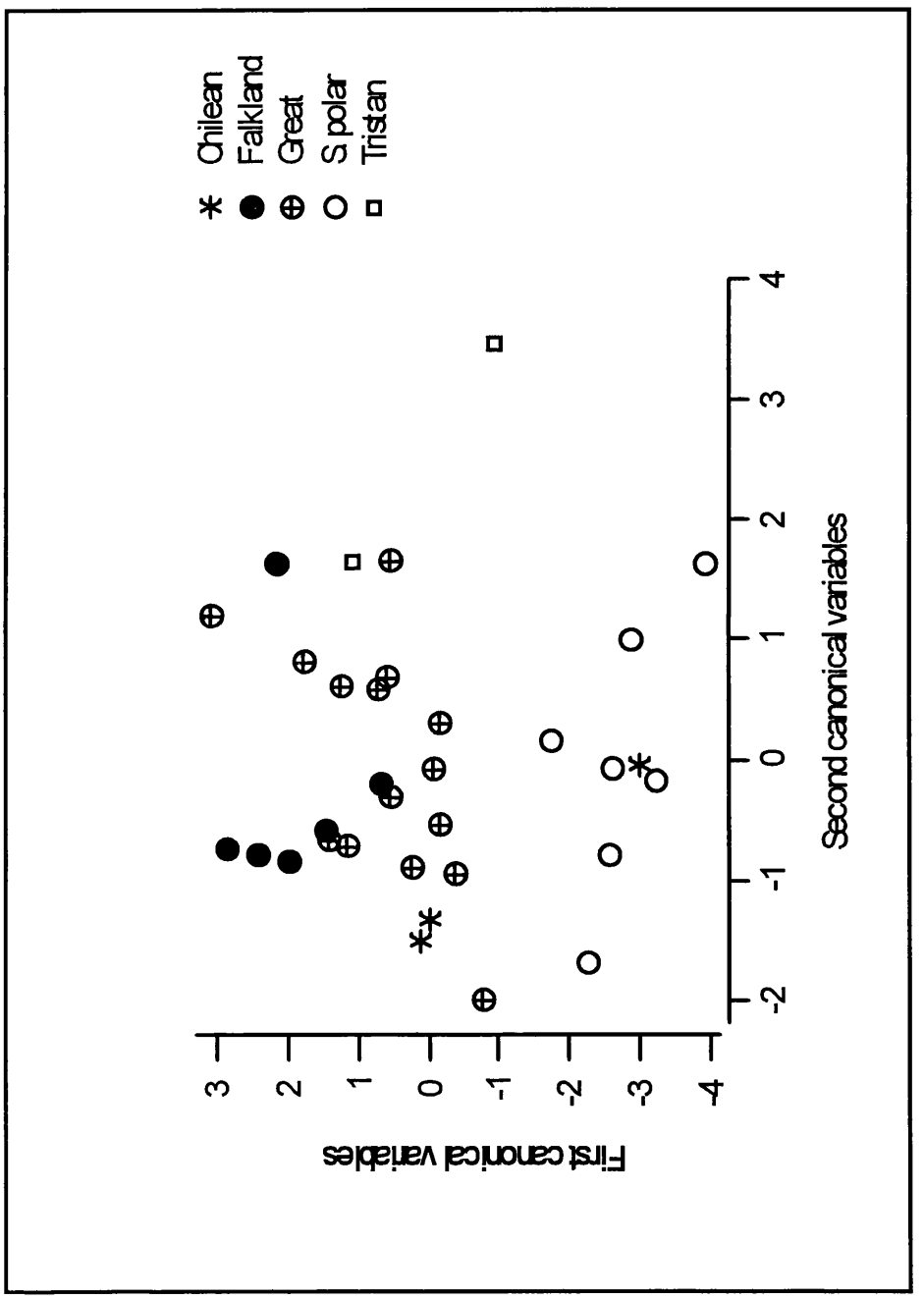


Figure 5.7. Plot of shape differences among female *H. grandis.*

Table 5.12. Total canonical structure score from canonical discriminant analysis on shape variations among female *Haffneria grandis* infesting skuas.

Components	canonical variate 1	canonical variate 2	canonical variate 3
Eigenvalue	3.069	0.587	0.108
Cumulative (%)	81.28	96.84	99.71
Characters[1]	component loadings		
bltobw	-0.524	0.843	0.094
hltohw	0.992	0.104	-0.040
bltohl	-0.955	-0.184	0.231
pwtopl	0.738	-0.022	0.609

Note:

[1]Explanation for character names can be obtained from Table 5.1.

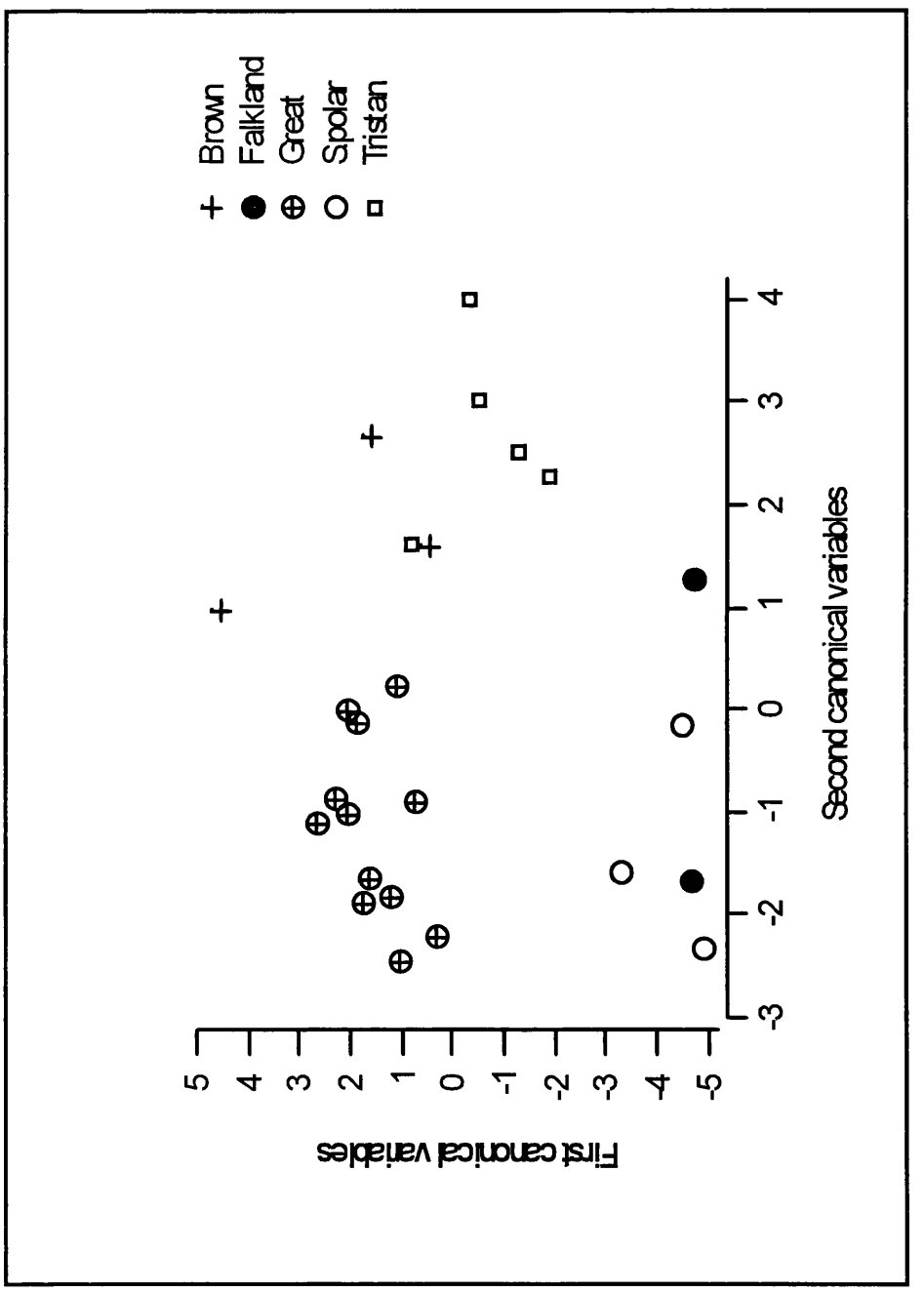


Figure 5.8. Plot first two canonical variables of differences in size of male *H. grandis* inhabiting large skuas.

Table 5.13. Total canonical structure scores from canonical discriminant analysis on size differences among male *Haffneria grandis* infesting skuas.

Components	Canonical variate 1	Canonical variate 2	Canonical variate 3
Eigenvalue	7.261	3.373	2.109
Cumulative (%)	55.12	80.72	96.74
Characters[1]	component loadings		
bodylen	0.897	0.292	-0.149
bodywid	0.690	0.379	-0.380
headlen	0.327	0.031	-0.207
headwid	0.039	0.237	-0.062
prolen	0.435	0.451	-0.034
prowid	0.377	0.074	-0.018
httomb	0.402	0.194	0.720
mouthbas	-0.509	0.193	-0.426
femur	0.394	-0.033	0.395
tibia	0.333	0.055	0.402
sclero	-0.171	0.726	0.071

Note:

[1]Explanation for character names can be obtained from Table 5.1.

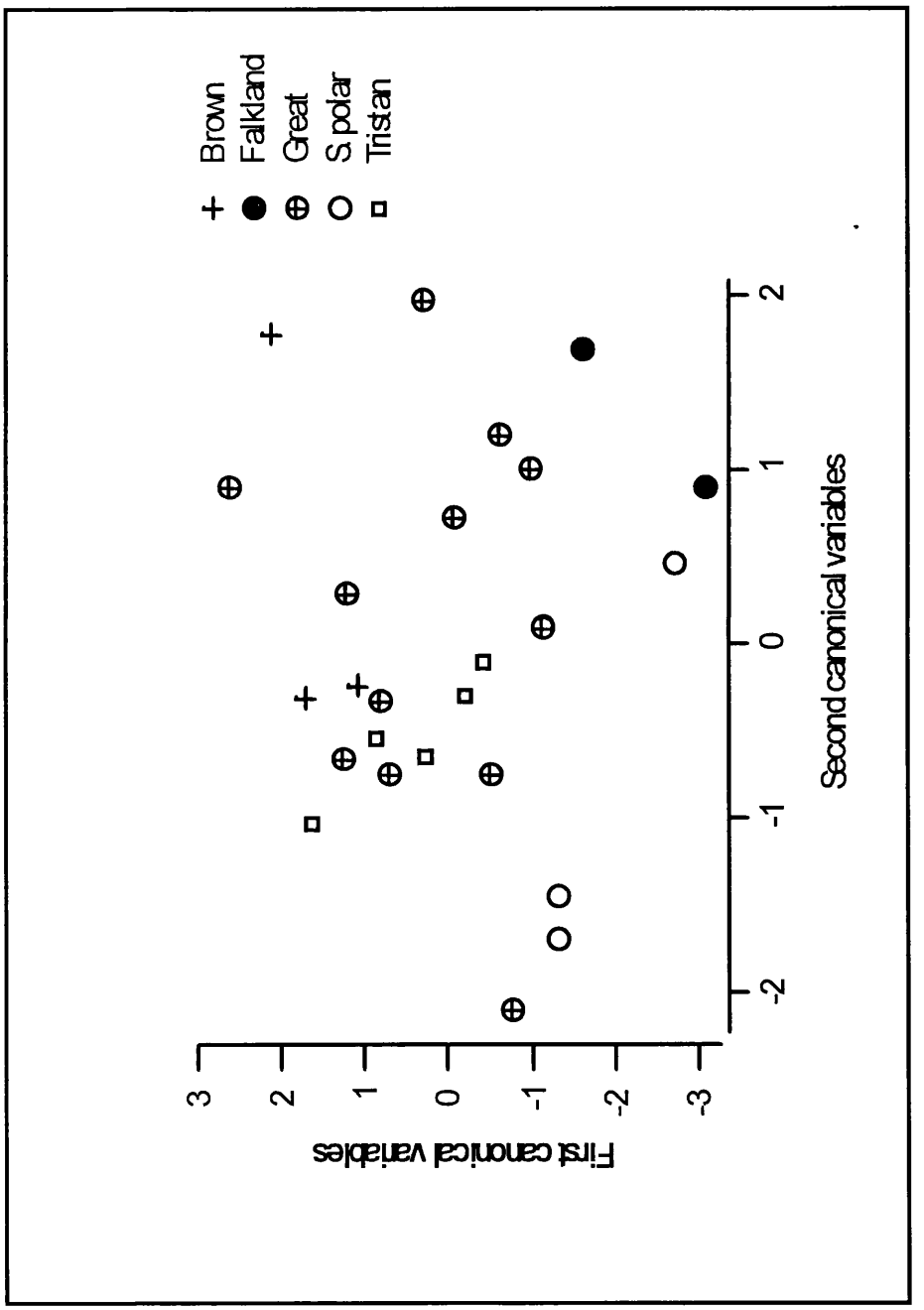


Figure 5.9. Plot of shape differences among male *H. grandis.*

Table 5.14. Total canonical structure score from canonical discriminant analysis on shape variations among male *Haffneria grandis* infesting skuas.

Components	canonical variate 1	canonical variate 2	canonical variate 3
Eigenvalue	1.519	0.393	0.177
Cumulative (%)	72.70	91.53	100.0
Characters[1]	component loadings		
bltobw	-0.151	0.857	0.302
hltohw	-0.143	-0.197	0.592
bltohl	0.933	0.231	0.126
pwtopl	-0.319	-0.206	0.721

Note:

[1]Explanation for character names can be obtained from Table 5.1.

hosts have a significantly higher value of body length to head length ratio compared to lice from Falkland or South polar skuas (ANOVA: $F>4.27$; $df=17,4$; $p<0.014$; Table 5.14). All first three canonical variates (which represent total variations in lice morphology) also show that HG on Falkland skuas have a larger body than lice from South polar or Tristan skuas (ANOVA: $F>3.16$; $df=17,4$; $p<0.04$).

Morphometric analysis of QN shows that they can be used to separate small skuas from large skuas. This louse only infected a few specimens of large skuas (five hosts or 2.18%). Female QN only parasitised Falkland skuas, whereas male lice also occured on South polar skuas. A study of size variation among female QN shows that QN of Falkland skua is clearly different from QN on other skuas (Figure 5.10). Morphological differences among this louse are clearly indicated by the value from their first three canonical variables which represent total morphological variations (Table 5.15). QN from Falkland skuas is separated from other QN by first canonical variables while second canonical variables successfully isolates QN of Pomarine skua. Female QN indicate that in parasitising skuas, physical modifications are necessary concordant with host morphology. Lice inhabiting Falkland skuas for instance have a wider body, head and prothorax (ANOVA: $F=3.51$; $df=150.95,33$; $p<0.0001$) as represented in the first canonical variates (Figure 5.10 and Table 5.15). However, lice extracted from small skuas have a shorter prothorax than other female QN (ANOVA: $F=2.03$; $df=104,20$; $p<0.01$). Study on shape variations among female QN also gives a similar result; QN from Falkland skua is different from other skuas (Figure 5.11). First canonical variables for QN indicate that the QN of the Falkland skua has a rounder prothorax (higher ratio of width to length) than QN from small skuas (Table 5.16).

Similar results were obtained from a study of the shape and size variations of male QN. From 93.52% of total variation (represented by first two canonical variates; Table 5.17), 81.91% of variation is present in the first canonical variables. These large variations successfully separated QN of Falkland and South polar skuas from those of Arctic, Long-tailed and Pomarine skuas. Again male QN yielded a similar result, with male QN from the former hosts differing from the latter hosts in body, prothorax, and

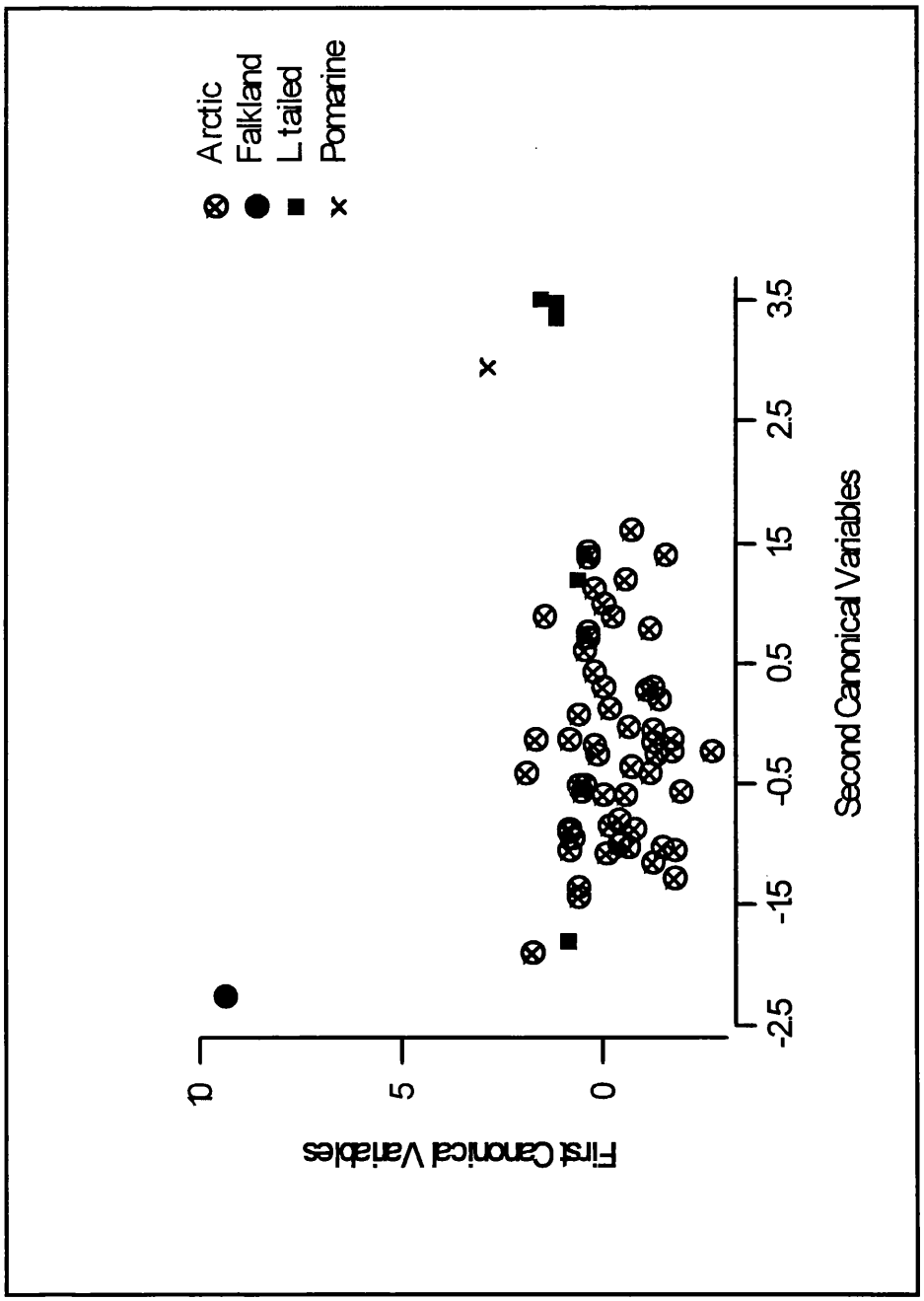


Figure 5.10. Plot of size differences among female *Q. n. normifer.*

Table 5.15. Total canonical structure score from canonical discriminant analysis on size differences among female *Quadraceps normifer normifer* infesting skuas.

Components	Canonical variate 1	Canonical variate 2	Canonical variate 3
Eigenvalue	1.758	0.572	0.232
Cumulative (%)	68.59	90.93	100.0
Characters[1]	component loadings		
bodylen	0.357	0.026	-0.012
bodywid	0.624	0.115	0.486
haedlen	0.374	-0.135	-0.021
headwid	0.687	0.313	0.191
prolen	-0.556	0.292	0.479
prowid	0.438	-0.041	0.310
httomb	0.115	0.162	-0.390
mouthbas	0.352	0.059	-0.216
femur	0.226	0.088	0.174
tibia	0.052	0.334	0.340
sclero	-0.401	-0.603	0.529

Note:

[1]Explanation for character names can be obtained from Table 5.1.

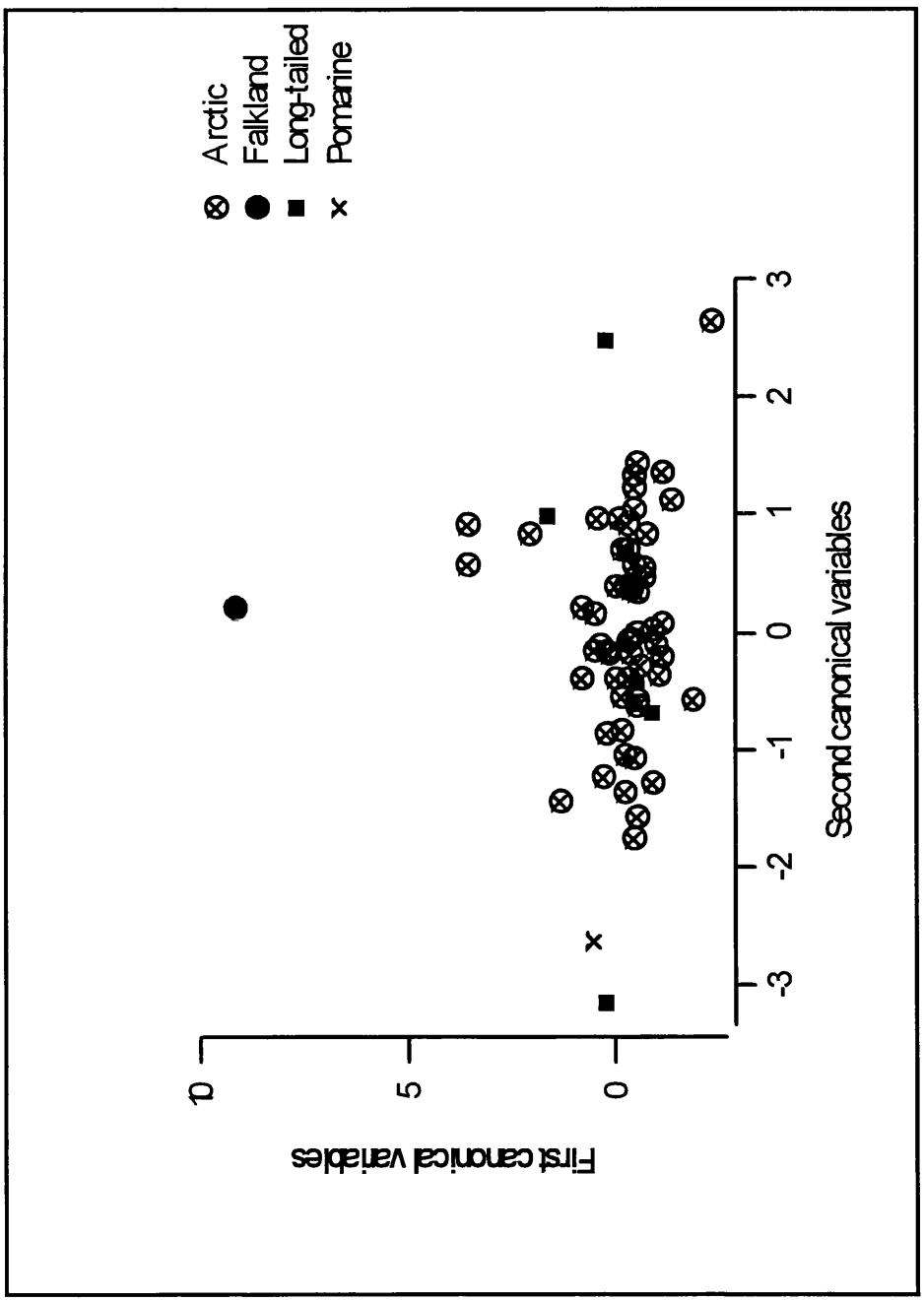


Figure 5.11. Plot of shape differences of female *Q. n. normifer*.

Table 5.16. Total canonical structure score from canonical discriminant analysis on shape variations among female *Quadraceps n. normifer* infesting skuas.

Components	canonical variate 1	canonical variate 2	canonical variate 3
Eigenvalue	1.430	0.118	0.028
Cumulative (%)	90.66	98.20	100.0
Characters[1]	component loadings		
bltobw	-0.323	0.825	-0.448
hltohw	-0.047	0.605	0.609
bltohl	-0.147	-0.101	-0.308
pwtopl	0.946	0.120	-0.289

Note:

[1]Explanation for character names can be obtained from Table 5.1.

Table 5.17. Total canonical structure score from canonical discriminant analysis on size differences among male *Quadraceps n. normifer* infesting skuas.

Components	Canonical variate 1	Canonical variate 2	Canonical variate 3
Eigenvalue	5.948	0.843	0.339
Cumulative (%)	81.91	93.52	98.21
Characters[1]	component loadings		
bodylen	0.563	-0.017	0.122
bodywid	0.773	-0.388	0.147
headlen	0.527	0.088	0.056
headwid	0.828	0.041	0.058
prolen	-0.705	0.068	0.225
prowid	0.864	0.151	-0.061
httomb	0.496	0.293	0.330
mouthbas	0.375	0.181	0.253
femur	-0.059	-0.286	-0.636
tibia	0.091	0.326	0.047
sclero	-0.534	-0.535	0.061

Note:

[1]Explanation for character names can be obtained from Table 5.1.

head width (ANOVA: F=3.72; df=139.68,44; p<0.0001; Figure 5.12, Table 5.17). Cross examination of shape variation data shows that QN from small skuas have a higher value in length to width ratio of head and body (Figure 5.13, Table 5.18). This variation is represented in the first canonical variate which represents 91.81% of total variation. Detailed examination of lice data from each of the small skuas, indicates that QN from the Arctic skua is morphologically similar to those from Long-tailed skua, but slightly distinct from lice of the Pomarine skua (ANOVA: F=3.44; df=36,11; p<0.01 for shape and F=3.64; df=43,4; p<0.001 for size differences). QN of large skuas does not show any significant differences among taxa either in shape or size.

Evidence from female SS suggests that the lice of Chilean skua are clearly separated from those of other skuas (Figure 5.14). SS from Chilean skua differ from SS of other skuas by possessing longer tibia (Table 5.19). This difference however, is not detected in shape variation among female or male SS.

5.4. Discussion

5.4.1. Suitability of Methods Used in This Study

Two main approaches have been used in this study. These methods, principal component analysis and canonical discriminant analysis, are commonly used in various fields of study. PCA for instance, already proved its capability in morphometric studies (e.g. in Reyment *et al.* 1984; Airoldi & Flury 1988) and in ecological studies (e.g. in Legendre & Legendre 1983; Pielou 1984). In this study, PCA has clustered lice into four groups. This method has successfully reduced the original values into a few transformed variables that explain much of the variation, because all measured morphological dimensions were highly correlated with each other. Therefore, by applying PCA to morphometric measurements alone an initial idea about identification of lice (or other organisms) can be obtained, and the clusters are absolutely in agreement with the classification of skua feather lice into four distinct genera.

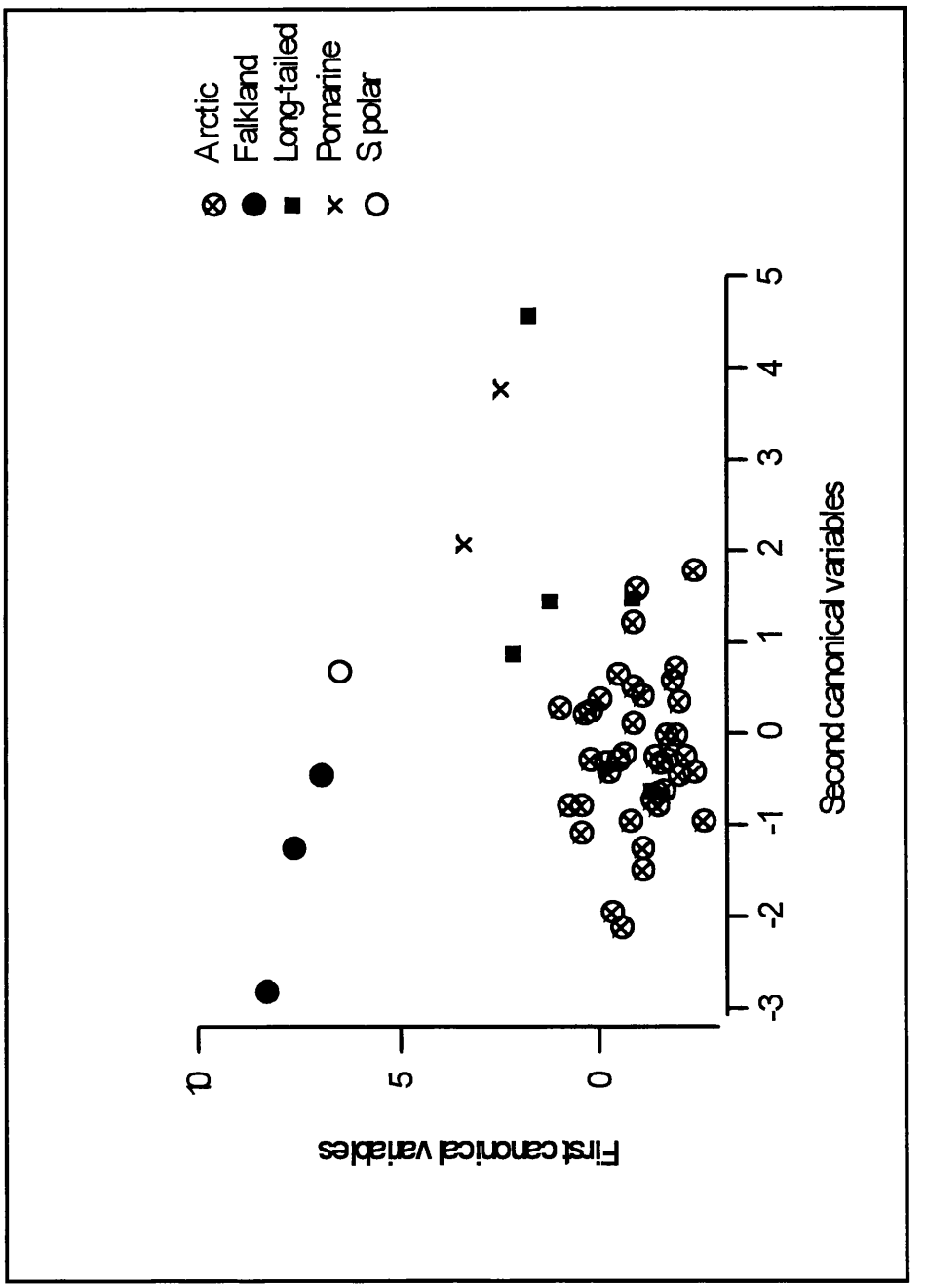


Figure 5.12. Plot of size differences among male *Q. n. normifer*.

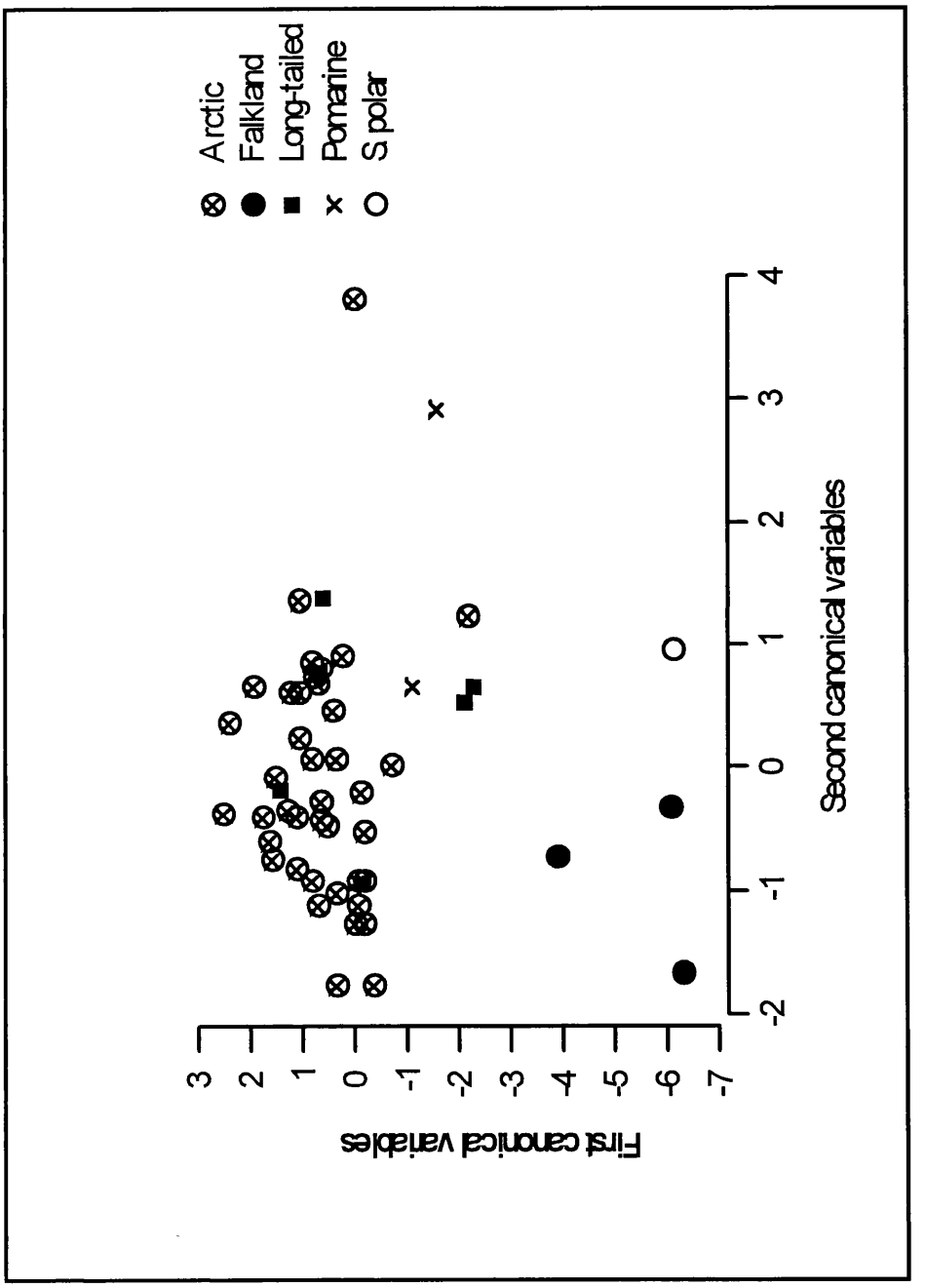


Figure 5.13. Plot of shape differences among male *Q. n. normifer.*

Table 5.18. Total canonical structure scores from canonical discriminant analysis on shape variations among male *Quadraceps normifer normifer* infesting skuas.

Components	canonical variate 1	canonical variate 2	canonical variate 3
Eigenvalue	3.217	0.269	0.012
Cumulative (%)	91.81	99.50	99.85
Characters[1]	component loadings		
bltobw	0.417	0.782	0.041
hltohw	0.500	-0.077	0.837
bltohl	-0.035	-0.123	-0.527
pwtopl	-0.976	-0.001	0.179

Note:

[1]Explanation for character names can be obtained from Table 5.1.

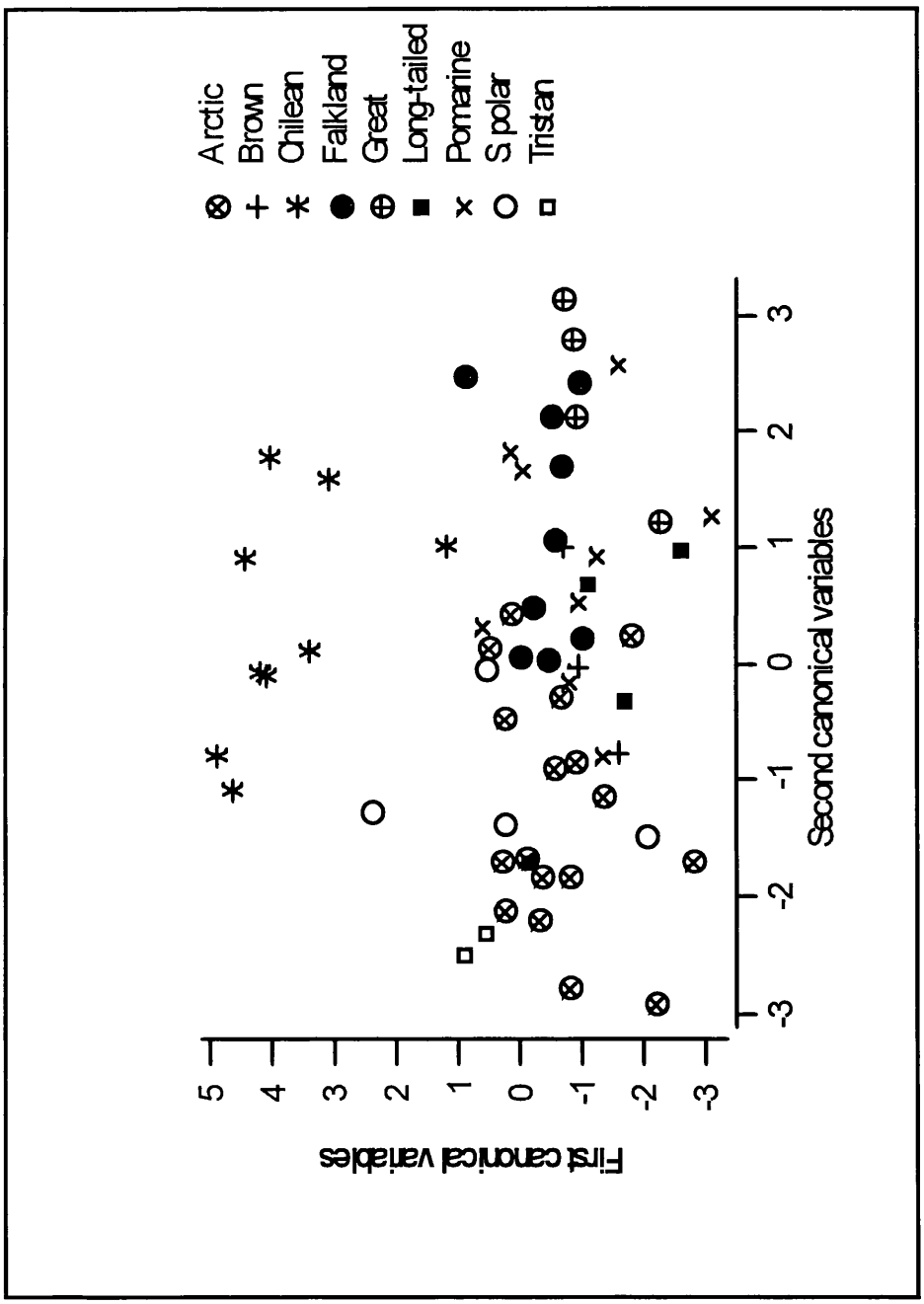


Figure 5.14. Plot of size differences among female *Saemundssonia sp.*

Table 5.19. Total canonical structure score from canonical discriminant analysis on size differences among female *Saemundssonia sp.* infesting skuas.

Components	Canonical variate 1	Canonical variate 2	Canonical variate 3
Eigenvalue	3.022	0.671	0.266
Cumulative (%)	71.35	87.19	93.48
Characters[1]	component loadings		
bodylen	0.201	0.430	-0.294
bodywid	0.287	0.476	0.611
headlen	-0.163	-0.042	-0.131
headwid	-0.205	-0.055	0.136
prolen	-0.063	0.114	0.147
prowid	0.003	0.109	-0.189
httomb	-0.172	-0.043	-0.246
mouthbas	0.318	0.620	-0.267
tibia	0.891	-0.118	0.103
sclero	-0.161	0.042	0.069

Note:

[1]Explanation for character names can be obtained from Table 5.1.

However, problems occur with PCA when trying to differentiate the same species of lice from various hosts. This problem, although not a complete failure, is mainly associated with the nature of PCA methodology. The analysis has been designed to formulate a new axis for a set of multivariate data without considering the degree of association among data. For presenting such information, discriminant analysis or its derivatives are more successful than PCA. Discriminant analysis is sensitive to any relations among data-sets, and already provides a useful assistance in several morphometric studies such as in kangaroo rats (Baumgardner & Kennedy 1994) and birds of prey (Hertel 1994).

5.4.2. Suitability of Characters Used in This Study

The amount of characters used in this study is assumed to be adequate. This conclusion was based on several publications which normally obtained their morphological data from various numbers of characters; ranging from twenty (Rogovin & Surov 1990), twenty-five (Sever 1991), thirty (Seidel & Palmer 1991; Winemiller 1991), forty (Mironov 1991), and up to sixty-two characters (Vanvalkenburgh & Wayne 1994) depending on the type of specimens involved. Pankhurst (1991) suggested that more reliable results can be deduced when more characters are applied, but this does not always guarantee better results. This study shows that from fifteen characters used (including derived characters), only some of them are useful either in recognising feather lice's hosts or distinguishing between them. Analysis of HG for example successfully separated their hosts (large skuas) by using data associated mainly with head length, femur length, prothorax length, and sometimes from the measurements of the distance between mouthpart base. For QN, variations in prothorax shape were enough to provide a clear separation of their hosts; small and large skuas.

Increasing the number of characters may also produce negative effects on overall results. Results from analysis of variance indicate that male SS possess four informative characters, but the total effect from all variables completely eliminates this

information and produces no evidence for host separation. This effect also occurs with AF which has a single informative character but failed to show host differences.

Several previous researches proved that, although many characters are available for analysis, only some of them are informative for displaying the differences between sets of morphological data. From twenty-one morphological and behavioural characters, Alexander (1991) successfully analysed the dance language of *Apis* (Insecta: Apidae) by using only data on genitalia. In analysis on cloacal anatomy of salamanders, Sever (1991) found from twenty-five characters used in his study, only twelve were useful for phylogenetical analysis. In another study, DeSalle & Grimaldi (1992) discovered ten out of 210 morphological characters were capable of presenting good information about phylogenetical relationships among *Drosophila* (Insecta: Drosophilidae). Brust & Munstermann (1992) supported this finding by correctly separating *Communis* complex (Insecta: Diptera) to each species based on pointed larval comb scale only.

5.4.3. Differences in Lice Morphology and Host Classification

Evidence from PCA and simple morphological comparisons leads to the conclusion that all lice examined in this study can be divided into four taxa which are clearly different from each other. Their morphologies are distinct either in size or in shape. The fact that concordant results were obtained from both sexes supported the conclusion that all these lice were truly different. In addition to a concordant result between sexes, canonical discriminant analysis also used similar characters in discriminating between HG and AF. This indicates that variations among the lice in this study really exist and that the possibility of separation among them is more than that expected by chance.

Generally, all lice in this study can be differentiated by using several criteria. HG for example is easily recognised due to its large size. AF differs in possessing a more rounded body (shorter but wider) and its prothorax is relatively large to total body size. In addition, this louse is also distinct in possessing shorter antennae which are concealed in the groove on the side of the head, a typical feature of Amblyceran lice.

SS and QN are somewhat similar, but the former taxon normally has a relatively large head and a higher value for the distance between head tip to mouthpart base. Generally, SS is slightly larger than QN. Both taxa can also be distinguished by using an additional character; the number of segments in their antenna. SS normally has up to six segments whereas QN only possess four or five segments.

The results from this study also show that variations in lice morphology can be used as an indicator in separating their hosts. Various degrees of morphological variation exhibited by the same taxon of lice when on different species of hosts indicate that morphological coevolution does exist in skua-lice systems. This modification may be due to special adaptation by lice to utilise available resources (such as feather size and shape) provided by their host. Morphological adaptations in convergent systems have already been shown in various systems. Emberton (1995) showed that land-snails (*Mesodon normalis* and *Neohelix major*) will modify their shell formation in response to environmental conditions. The differences in the shell shape and size of land-snails clearly indicates that both taxa have responsed to the same physical environment. The reverse phenomenon may be occurring in lice-skua system. In this system, similar species of feather lice were exposed to different environmental pressure provided by different species of hosts. However, the degree of these morphological variations is too small and would not be presented as a clear distinct morphology. These small scale variations can be detected only by differences in some morphological measurements.

Several factors may be responsible for variations in feather lice morphology. An interaction within and among species living in a similar environment can modify the overall size of an organism. In feather lice, this interaction may be due to inter and intra-species competition for limited resources provided by their host. Inter and intra-species competition can act as a major factor in morphological modification of feather lice anatomy. Increments in body-size may also evolve due to selection for increased fecundity and intraspecific niche partitioning, as in octopuses (Voight 1994). The fact that lice only differ slightly in overall body size on different hosts indicates that this variation is more greatly influenced by micro-environmental pressure than breeding

improvement. The differences among characters clearly shows adaptation of a louse to different hosts. Modifications in femur or tibia length will improve movement of lice on host. The ability to move faster is a key feature in enhancing lice efficiency in avoiding the beak and feet of the host during grooming. By increasing the efficiency of avoidance to host grooming, lice indirectly increase their survival rate.

Morphological evidence from QN morphology clearly distinguished small skuas from large skuas, while HG can be a useful species for allocating members within the large skuas group. Only female SS successfully separate Chilean skua from other skuas. Male SS failed to provide any significant variations among their hosts. Separation of hosts has distributed SS among skuas but this ectoparasite may possess a specific mechanism in reacting or adapting to host pressure which is not apparent in their morphology. The other investigated louse, AF, failed to provide any evidence about their hosts. This discovery is not unexpected as this louse belongs to the Amblyceran group, which is less specific than Ischnoceran lice (HG, SS and QN).

5.4.4. Limitation of This Study

These findings should be used with caution since the number of samples involved with this study is rather limited and sometimes not representing all skuas. In addition, lack of well preserved lice from certain hosts prohibits absolute comparison between hosts. Absence or lack of host samples also contributes a major problem to this study.

5.4.5. Suggestions for Future Work

Results from this study indicate that ectoparasites can provide useful information about their host's phylogenetic relationships. However, complete data covering all aspects of interaction for both taxa (host and ectoparasite) should be investigated before reliable results can be obtained. Future study in this area should be carried out on more feather lice, especially on fresh specimens, with the emphasis on increasing and diversifying

morphological characters. Ectoparasites should be obtained from various hosts to increase diversity, this can only be implemented if a wide ranges of hosts in different conditions (including live birds) are available for lice examination.

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Chapter 6

Inferring Systematic Relationships Among Cospeciated Species Using Mitochondrial DNA

6.1. Introduction

Various studies of host-parasite coevolution have indicated that birds and their feather lice (Insecta: Phthiraptera) are cospeciated (e.g. Paterson *et al.* 1993, Paterson 1994). This finding is in concordance with the fact that feather lice are obligate ectoparasites and highly host-specific, characters that prevent them from switching host species readily. As obligate ectoparasites, feather lice must complete whole life cycles on a single host and only transfer with physical contact between hosts (e.g. during courtship or from parental birds to their offspring). Long-term coevolution with a single host requires specific adaptation by the lice (i.e. a highly specific character) to ensure successful colonisation since different hosts possess distinctive morphologies which provide different microhabitats for feather lice.

Variations in bird morphology (especially feather structure) have led to the suggestion that different birds tend to harbour specific lice (Clay 1951; Ash 1960). However, closely related hosts do sometimes possess similar lice for example *Halipeurus sp.* parasitised on procelariid petrels (Paterson *et al.* 1993), and *Saemundssonia sp.* on the skuas (this study, Chapter 4). The presence of different lice on particular birds can be taken to indicate separation between hosts, whereas similarity in lice communities denotes close relationships among hosts. Some host classifications derived from parasite evidence have been supported by the concordance of results with molecular studies (see Sibley & Ahlquist 1990; Mauersberger & Mey 1993). Unfortunately, only very few molecular studies have been conducted on lice to investigate the reliability of their classification, or to deduce the degree of molecular cospeciation between host and ectoparasite; for instance cospeciation studies of pocket

gopher and their chewing lice by Nadler *et al.* (1990), Hafner *et al.* (1994) and Hafner & Page (1995).

The cospeciation process between host and parasite can be synchronous or not. Synchronous cospeciation will involve equivalent amounts of morphological or molecular change in associated host and parasite lineages (presented as similar patterns in their cladograms). In a strict cospeciation model, host and parasite phylogenies must agree not only in branching pattern, but also in branch lengths, which are proportional to the amount of morphological or molecular change (Hafner & Nadler 1988). To assess molecular change, comparison must be made between a similar gene of both systems. Hafner & Nadler (1990) suggested that two types of evidence are necessary (and sufficient) in documenting widespread cospeciation in the host-parasite assemblage; 1) evidence to demonstrate that host and parasite are derived independently, and 2) the topological similarity of host and parasite trees must exceed chance expectations.

Several indicators can be used in deducing systematic relationships among cospeciated species. The most widely used in molecular studies are mitochondrial DNA (mtDNA) and nuclear DNA. The use of mitochondrial DNA is more popular than nuclear DNA since mtDNA sequences are derived from molecules which have several advantages over nuclear DNA. These advantages include the fact that mtDNA is smaller than nuclear DNA, circular in form, simple in structure, lacks recombination, is maternally inherited, has a rapid evolutionary rate, a conserved genome size, and is easy to extract and analyse without necessarily having to kill animals (Lansman *et al.* 1981; Brown 1985, Wilson *et al.* 1985; Avise 1986; Moritz *et al.* 1987; Simon 1988). Thus, mtDNA analysis has been used extensively to study the genealogies of particular individuals (Avise 1986).

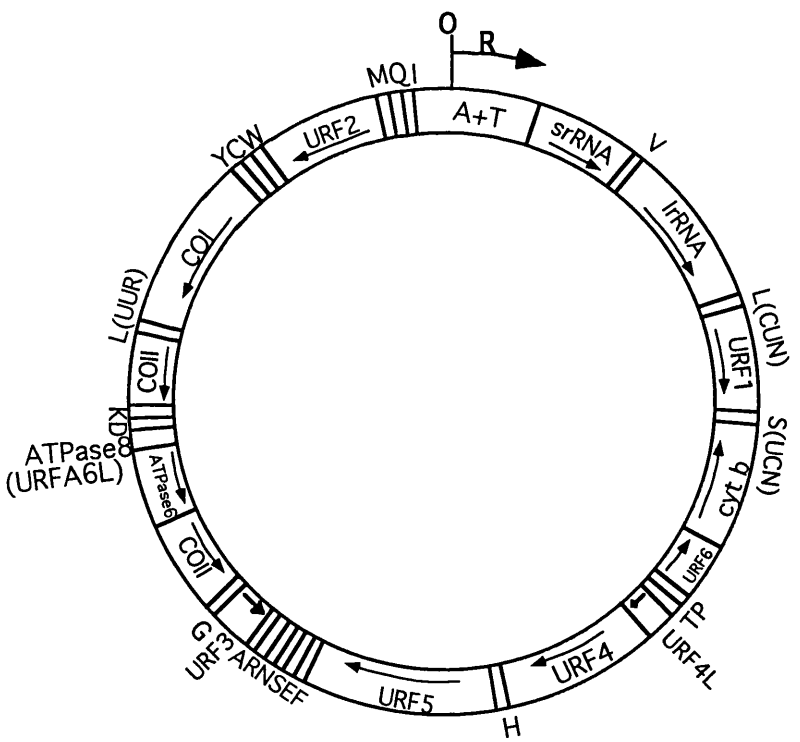
The mitochondrion is an energy-producing cellular organelle, which contains its own genome. There may be up to ten thousand mitochondria per cell i.e. up to ten thousand copies of the mitochondrial genome. This genome contains two ribosomal RNA genes (16S rRNA and 12S rRNA), 22 transfer RNA (tRNA) genes, 13 genes

encoding proteins involved in electron transport or ATP synthesis (i.e. genes for cytochrome oxidase subunits (COI, COII, and COIII)), ATPase subunit IV, and cytochrome b (Sederoff 1984; Brown 1985; Chomyn *et al.* 1985; Chomyn *et al.* 1986; Moritz *et al.* 1987). In addition, there are eight unidentified open reading frames (URF), which are presumed also to code for polypeptides (Wolstenholme *et al.* 1982). Each molecule also has a control region containing sequences that function in initiation of replication (which is normally unidirectional and highly asymmetric) and transcription (Clayton 1984; Harrison 1989). Available data indicate that all higher animals have a common mtDNA structure (Figure 6.1) of nearly constant size (ranges from 14,300 to 19,500 base pairs), which varies greatly in primary nucleotide sequence (Brown 1983; Avise 1986; Vawter & Brown 1986). Base composition in animal mtDNA also varies, for example guanine (G) and cytosine (C) differ considerably from as low as 21% in *Drosophila* species to a maximum of 46% in chicken (Brown 1983).

Mitochondrial DNA can be a useful indicator for showing different patterns in geographical and population structure because it exhibits considerable variation among individuals both within and between populations (Avise *et al.* 1987). This molecule is suitable for study at or near the species level, but less recognised for resolving deeper branches in genealogical trees (Hayasaka *et al.* 1988; Harrison 1989; Miyamoto & Boyle 1989; Meyer & Wilson 1990; Irwin *et al.* 1991). Information from mtDNA analysis may be presented as DNA sequences, restriction site maps, or restriction fragment mobility (Moritz *et al.* 1987).

Growing numbers of studies are using mtDNA as a tool for phylogenetic inference, especially among closely related taxa (Wilson *et al.* 1985; Avise 1986; Birley & Croft 1986). Large-coding sequence duplications normally have the potential to provide phylogenetic information within or between species (Moritz *et al.* 1987). Due to the differences in the divergence rate among particular segments of mtDNA, an appropriate gene can be selected to address specific systematic questions in phylogenetic studies. A rapidly evolving gene for instance is suitable for investigating

Figure 6.1. The arrangement of genes in insect mitochondrial DNA (in this case, *Drosophila yakuba*). The letters **O** and **R** denotes the origins of replication within the A+T rich region and the arrow pointing in the direction of replication. Each section was located to various genes and tRNA gene is identified by one-letter amino acid code (Extracted from Clary & Wolstenholme 1985).



recently diverged taxa, but may be too different to be useful in differentiating genera or species belonging to particularly ancient taxa (Simon 1991).

Amplification of segments of the mitochondrial genome with PCR (Polymerase Chain Reaction) facilitates the process of extracting information. However, this information is obtained from a specific section of mtDNA only and therefore, only generates phylogenies for a particular genetic section. This information may not reflect the true phylogeny of the organisms under investigation, but it can be used to provide guidelines in inferring evolutionary history for closely related individuals (Vogler & DeSalle 1993). Success in extraction of mtDNA from lice will allow the assessment of the degree of cospeciation between birds and their feather lice. This becomes more important in systems where several species of lice are present on different hosts. Lice can then be used to examine the relationship of these hosts. Therefore, the study of molecular cospeciation between birds and feather lice will also examine the reliability of coevolution data in deducing host phylogeny.

6.2. Materials and Methods

6.2.1. MtDNA Extraction

Several methods have been used in attempts to extract mtDNA from dried lice. The majority of extraction protocols share similar basic steps in their methodology (Simon *et al.* 1991) i.e. homogenisation of the samples, destruction of extraneous or harmful proteins (such as nucleases), lysis of membranes to release DNA, and the separation of DNA from cell debris, digested proteins and other extraneous materials. Some tissues contain more mtDNA than others. In insects, flight muscle and egg masses are two major sources of mtDNA (Simon *et al.* 1991). Unfortunately, this is not applicable to feather lice since they do not have wings and long-time preservation of museum skins used in this study made locating the egg masses (if they still exist) difficult.

First, the caesium-chloride gradient technique (Dowling *et al.* 1990) was used to extract mtDNA. Between one and ten lice were soaked in liquid nitrogen for a few seconds to facilitate grinding. Lice were crushed in a mortar in cold STES buffer (one part of 1.5M sucrose in TE; 5 parts of the combination of 10 mM Tris, 100 mM EDTA, and 10 mM NaCl pH7.5). This homogenate was centrifuged at 1200g for 5 minutes, and the supernatant transferred to another tube and re-centrifuged at 23,000g for 20 minutes. The subsequent pellet was resuspended in 1.0 ml TE at 22^{0}C. Sodium dodecyl sulphate (20% w/v) was added to lyse the membranes. The mixture was incubated at 22^{0}C for 10 minutes. Caesium-chloride-saturated water was added to precipitate nuclear DNA and the mixture was incubated on ice for 15 minutes. The solution was centrifuged at 17,000g for 10 minutes. The density of the decanted supernatant was adjusted to 1.4g/ml by the addition solid CsCl or milli Q™ water to facilitate the addition of propidium iodide. Later, the solution was mixed with different density solutions (1.40, 1.55, and 1.70 gm/ml CsCl stock solutions) to produced step gradient, overlain with mineral oil, and centrifuged at 140,000g for 24 hours. The mtDNA layer was detected using UV light (305 nm) in which the mtDNA appears light pink. This layer was transferred to a clean tube using a syringe. Propidium iodide was then removed using isopropyl alcohol followed by centrifugation to 12,000g for two minutes. The mtDNA was isolated by dialysis in 8mm dialysis tubing in 0.5X TE for 24 hours. The presence and purity of the mtDNA extracted was assessed using agarose electrophoresis (0.6% agarose, 30 minutes, 150 volts).

The second mtDNA extraction method involved in this study was modified from Boom *et al.* (1990). This method used guanidium thiocyanate and a silica resin to separate DNA from other components. Crushed samples were incubated at 60^{0}C with 1ml extraction buffer (10M GuSCN, 0.1M Tris-HCl pH 6.4, 0.02M EDTA pH 8.0, and 1.3% Triton X-100) for eight to twelve hours with occasional mixing. The solution was then centrifuged at 5,000g for 5 minutes. The supernatant (0.5ml) can then be either added to 40µl silica suspension and 0.5ml extraction buffer (GuSCN, Tris-HCl, EDTA, Triton X-100, and distilled water) or 1ml DNA-clean-up kit (Promega Corp.; Cat No.

A7390). When using the DNA clean-up kit, all protocols for subsequent steps were as directed by the manufacturer. Otherwise, sample solution was incubated at 22^{0}C for 10 minutes before the silica was pelleted with centrifugation (5000g for 5 minutes). The pellet was then washed using washing buffer (10M GuSCN, 0.1M Tris-HCl), followed by ethanol and acetone drying at 56^{0}C. The pellet was then resuspended in 65µl distilled water and heated at 56^{0}C to elute the DNA. Finally, the resin was washed several time with 2ml 80% isopropanol and DNA eluted in 50µl distilled water. Agarose gel with ethidium bromide were used to examine the mtDNA. The QIAquick-spin PCR purification kit (Diagen, GmbH, Hilden, Germany) also can be used to replace this conventional protocol since it employs the same procedures.

The third method used in this study was phenol-chloroform extraction (modified from Towner (1991)). Single lice were homogenised. Proteinase K (0.1% w/v final concentration) and RNAse (0.1% w/v final concentration) were added in Tris buffer (10mM pH 8) with EDTA (2mM), NaCl (10mM) and SDS (0.1% w/v final concentration). After 12 hours incubation at 55-65^{0}C, one volume of phenol-chloroform (phenol buffered with 0.1M Tris-HCl pH 8; one volume of chloroform; 0.1% alcohol quinoline) was added to the protease digest. The sample was mixed gently and one volume of chloroform added. One volume of isopropanol was then added and the solution centrifuged at 12,000g for 30 minutes. The supernatant was removed by aspiration, and the pellet recovered by redissolving the dry pellet in TE (10 mM Tris-HCl pH 8; 1 mM EDTA).

6.2.2. mtDNA Amplification

Two common methods in amplifying DNA are cloning target DNA in a specific vector such as bacteria and Polymerase Chain Reaction (PCR). Cloning however, is not suitable for preserved materials due to severe modification done by post-mortem damage (Paabo *et al.* 1989). The experimenter faces two major problems if damaged DNA is subject to cloning. These problems are; 1) low cloning efficiency due to the

majority of the vector molecules becoming ligated to damaged molecules, and 2) production of wrong information because cloning tends to repair damaged molecules, often by processes that are error-prone. Therefore an alternative approach, PCR, has been used. The capability to amplify small numbers of intact DNA molecules existing in the tissue extraction becomes a major advantage of this method (Paabo 1989, Paabo *et al.* 1989, Hoss *et al.* 1994).

Three sections of mtDNA genes were chosen for amplification. The universal primers were used to amplify mtDNA sequences from the 12S rRNA gene (Kocher *et al.* 1989), the cytochrome b gene (Kocher *et al.* 1989; Edwards *et al.* 1991), and the noncoding control region or displacement-loop or A+T rich region gene (Kocher *et al.* 1989). The first two genes have been chosen for their highly conserved character and the last for its rapid evolution. In addition to these sequences, I also used several primers dedicated to specific genes such as cytochrome oxidase I (COI) and 18S rRNA for comparison purposes. These latter primers were all used successfully by me with fruit fly (*Drosophila sp.*) and brachiopod (*Terebtratulina sp.*) mtDNA, in an initial test of methodology.

For amplification purposes, between 1-5µl of extracted DNA were used in 100µl PCR mix (5.0 µl PCR buffer [100 mM Tris-HCl pH 8.3; 500mM KCl; 15 mM $MgCl_2$; and 0.01% w/v gelatine], 200 µM dNTPs, 1.0 µM primers, 2.5 units Taq DNA polymerase, and sterile milli Q™ water). Positive controls were included; *Terebratulina*, species of articulated brachiopod and DNA from a single individual of a *Drosophila* species. The amplification solution was overlain with mineral oil to prevent evaporation. DNA was denatured by heating at 92^0C for 2 minutes. Twenty five cycles of annealing (annealing at 65^0C for 1 minute), extension (extension at 72^0C for 1 minute) and denaturation (94^0C for 1 minute) were then applied. At the end of the last cycle, the heat denaturation step was omitted and the extension step was allowed to proceed for another five minutes. The purity of the amplified product was then assessed using agarose gel electrophoresis and the amount of DNA was quantified by spectrophotometer. The PCR process was optimised by diversifying the primers, and

modifying some steps including using different numbers of cycles, varying the time and temperature for each stage, and altering the concentration of dNTP and PCR mix.

6.3. Results

None of the DNA in any target section of mitochondrial DNA of feather lice was successfully amplified. This complete failure meant that no further steps could be taken. Plans to sequence amplified mtDNA or to analyse by Restriction Fragment Length Polymorphisms (RFLPs) analysis had to be abandoned. Analysis on positive controls (single *Drosophila* and brachiopod, *Terebratulina sp.*) however, produced an amplified DNA from 18S rRNA region. All four solutions of positive controls including two were mixed with lice DNA (lane 2 and 4 in Figure 6.2) successfully produced a positive result. As well as suggesting that the methodology used was the appropriate one, this result also indicate the absence of inhibitor in PCR reactions.

6.4. Discussion

6.4.1. Failure in Extracting Lice mtDNA

Although some of the previous studies have proved that DNA can be extracted from preserved samples (Higuchi *et al.* 1984; Thomas *et al.* 1989, Cano *et al.* 1993), this study failed to deliver a similar positive result for feather lice. There are several possible explanations for this failure, which may apply either during the extracting and purifying process or in the DNA amplification stage. There is a possibility that preserved lice do not have amplifiable amounts of DNA due to heavy degradation. Soltis & Soltis (1993) have shown that heavy degradation of DNA in some specimens will lead to absence of DNA in preserved materials. Consequently, the PCR is unable to execute because the original DNA template is totally degraded (Wirgin & Waldman 1994). The degradation and modification of DNA occurs primarily by oxidation, which may produce a baseless site, oxidised pyrimidines, and cross-links (Paabo 1989). The

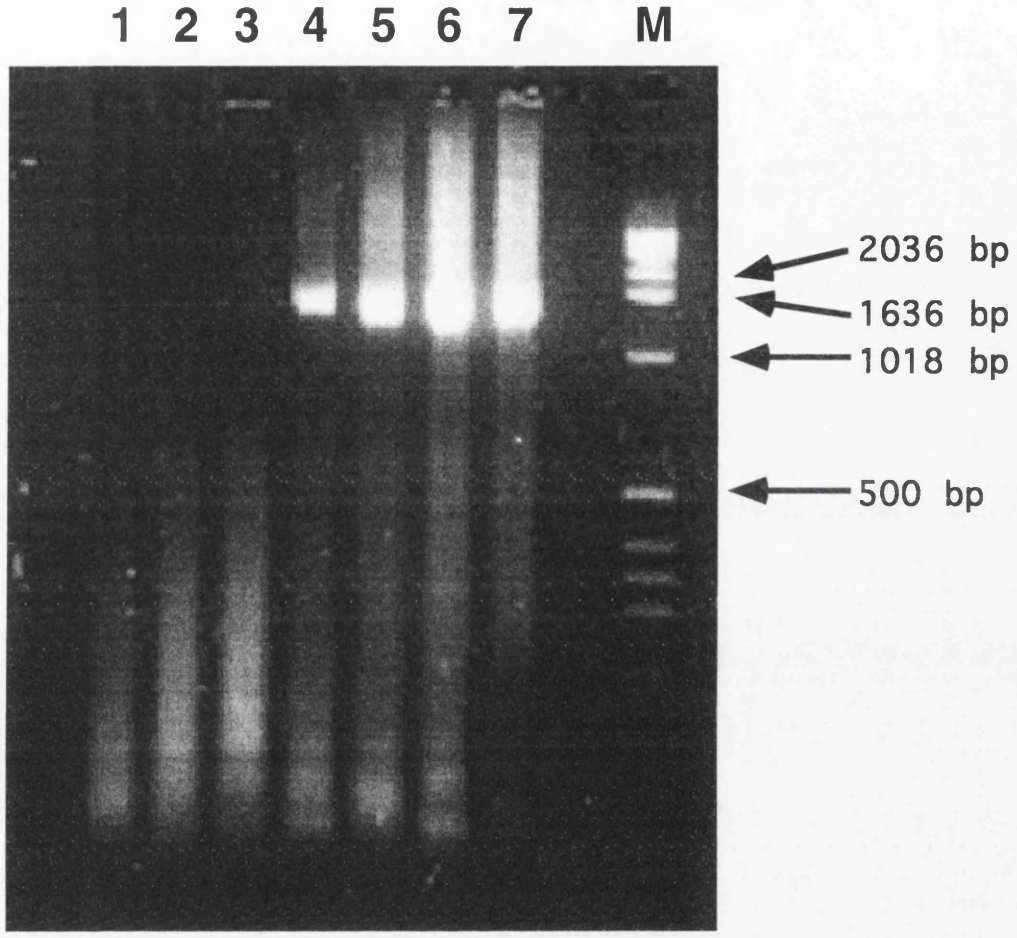


Figure 6.2. Photograph of the gel resulted from PCR amplification of 18S rRNA region. DNA was successfully amplified from both positive controls. Gel was stained for 30 minutes with ethidium bromide and was washed three times before photograph was taken. Lane 1 = negative control (no DNA was added); Lane 2 = 0.2 ul louse DNA; Lane 3 = 1 ul louse DNA; Lane 4 = 1 ul *Terebratulina* DNA + 1 ul louse DNA; Lane 5 = 1 ul *Terebratulina* DNA; Lane 6 = 1 ul *Drosophila* DNA + 1 ul louse DNA; Lane 7 = 1 ul *Drosophila* DNA.

processes of degradation and modification are greatly catalysed by the surrounding materials of the DNA template. Therefore, all chemicals used in the preservation of the specimen should be examined for their effects on DNA conservation. Formalin for example, is a main agent of DNA damage (Houde & Braun 1988).

Failure in amplification may also be caused by the presence of only a small amount of target sequence in the sample even when substantial quantities of DNA are available. Low quantity of target DNA will reduce the ultimate yield of PCR but increase the likelihood of amplifying contaminant DNA (Soltis & Soltis 1993). The absence of target sequence and the availability of dNTPs in reaction mixture will cause primers to flank to each other and amplify themselves, and as a result a primer-dimer is produced. This effect becomes obvious when the first round PCR products are subjected to another amplification.

The existence of low quality DNA may also be one of the reasons for amplification failure. Although DNA is present in all living animals, it begins to decay after animal death. The quality of DNA decreases with time following death, long intact sequences being degraded to short ones. Amplification of this damaged DNA template is slower and less efficient than with an intact template (Soltis & Soltis 1993). Furthermore, the universal primers would not normally be able to amplify the target sequence in this condition, and if they do, an erroneous result will be produced. This result will not present real genetic evidence of the study animal. This error happens through a process known as 'primer jumping' and it is a serious problem in the study of preserved DNA and should therefore, be treated cautiously. Jumping PCR is the recombination condition which can occur during PCR amplification using a damaged template. In this situation, the polymerase will stop at the site of damage on the template, but this partially extended primer can anneal to another template fragment in the next cycle and be extended to another damaged site. Thus, *in vitro* recombination can take place until the whole stretch encompassed by the two primers is synthesised and amplification enters the exponential part of the PCR (Paabo *et al.* 1990, Lawlor *et al.* 1991, DeSalle *et al.* 1993). Jumping PCR can be advantageous when working with a

homogenous system such as haploid organisms, since it helps to amplify longer fragments than are actually present in the extract (Handt *et al.* 1994).

Using an inappropriate combination of PCR reactions can also cause unsuccessful results. It has been shown by Douglas & Atchinson (1993) that PCR products can be degraded if incubated at 94^0C for longer than necessary. Suitable PCR conditions however, are controlled by several parameters which are very hard to configure. A lot of trial and error work has been carried out to achieve an optimum configuration. These processes are time consuming and increase experimental cost. As an alternative, previous works have been used as a guideline for the starting point for modification. Although after this approach was taken, the process of searching for optimum configuration was still unsuccessful due to limited time and tight budget which prevented more experiment to being carried out.

Failure of PCR in amplifying target DNA may be due to less suitable primers being used. Several sets of universal primers have been used in this study due to absence of specific primers for lice DNA. Although universal primers normally work with a wide-range of DNA, some animals do show slight incompatibility with them. Moreover, universal primers will tend to amplify a wide range of DNA (including human contamination) and may produce inaccurate results.

The presence of some contaminants can also produce some inhibitory effects and cause no DNA to be recovered. In this study I discovered that using water which was not fully sterilised totally inhibited the reactions. It is believed that the autoclave was not working properly and so the water was not sterilised completely. This effect was realised only after several changes had been made relating to the usage of chemicals and equipment. Human error was also tested by comparing the results of two similar experiments being conducted by different people.

Inhibitory effects can also prevent amplification of DNA. Some unknown substances which may be extracted together with the DNA can behave as an inhibitor to PCR reactions (Paabo *et al.* 1988, Golenberg 1991). Substances such as red blood cell component, sodium dodecyl sulphate, chelating agents, high salt concentration and

other unknown agents are good examples of PCR inhibitors (Shibata 1994). Fortunately this effect was reduced or may be absent from this study. This was proved by the presence of primer-dimer (Handt *et al.* 1994), successful in amplifying positive controls and the absence of any visible colour (usually light to dark yellow) in the extraction supernatant. The inhibitory effects are normally higher if the concentration of DNA in a preparation is high. Therefore, diluting the extraction products shall reduce the inhibitorary effects (Golenberg *et al.* 1990, Soltis *et al.* 1992).

The risk of contamination was reduced in this study by using sterile or disposable equipment. Additionally, an unexpected occurrence of contamination also has been checked by including negative controls in all reaction systems. The most likely sources of contamination may derive from micro-organisms (bacteria and fungi) since preserved materials were used in this study. To reduced these effects only specific animal genes were selected. This step can preclude some effects of DNA contamination associated with particular genes. The authenticity of final results can be tested by using two simple methods as suggested by Soltis & Soltis (1993). These were; 1) demonstrate that the results produced are unique and not because of contamination, and 2) comparison between experimental products and those from a closely related organism. As a rule of thumb, Paabo *et al.* (1989) and Handt *et al.* (1994) suggests that amplification efficiency will be reduced by DNA amplification size.

The possibility that unsuccessful amplification was due to experimenter error has also been tested and it has been proved that this was not the case. This was done by two different peoples using a similar method and extraction samples. This result, together with the fact that a positive result was recovered when the same protocols were applied to a positive control (a single *Drosophila*), clearly support the suggestion that the problems are related to specimens rather than the experimenter.

It has been proved that to amplify DNA from preserved specimens is more difficult than from fresh material (Soltis & Soltis 1993). This is because preserved DNA (e.g. from museum skin or ancient specimen) is exposed to post-mortem effects. The exact reasons for any failure in DNA extraction are very difficult to determine

since a lot of procedures and materials were involved. Hagelberg *et al.* (1991) in their analysis on ancient bone also failed to find a reasonable clue for unsuccessful amplification for one of their samples. In the majority of successful studies of preserved DNA, only a small amount of DNA is successfully extracted and amplified from preserved materials, fragments of around 100-150 bp for archaeological remains and up to 500 bp for museum specimens. Amplification of longer segments normally indicates contamination (Paabo *et al.* 1989).

6.4.2. Limitation of mtDNA Study

Based on its great features, it is not a foregone conclusion that mtDNA is able to present important evidence for phylogenetic reconstruction (Kessler & Avise 1984). However, due to several constraints and limitations especially related to the preservation processes, preserved mtDNA may experience severe degradation and, as a result, fail to present any clue about systematic relationships. It should be noted that mtDNA is maternally inherited and therefore, the information therein reflects female ancestry only, and need not be perfectly concordant with nuclear DNA genealogy. Moreover, mtDNA constitutes only a fraction of the total DNA in most animal cells. Therefore, the information they provide may reflect this set of genes only and not the overall study animals (Marjoram & Donnelly 1994).

Another important caveat about the genealogical reconstruction was that, with respect to a given microgeographical area, shared mtDNA genotypes were not necessarily synapomorphs. Therefore, individuals sharing a given genotype could represent descendants from separate immigrations from outside the area of study (Avise 1986). Barton & Jones (1983) conclude that mtDNA is able to penetrate the boundaries between species because it is not closely linked to genes responsible for maintaining reproductive isolation. In some cases where species are known to hybridise, differential introgression of mtDNA may explain the presence of similar or identical mtDNA genotypes in different species (Harrison 1989).

This study was tailored to overcome several constraints. The most common problem, limited budget, prohibited the analysis being carried out by a precise but expensive method such as DNA-bead analysis. Time constraints also prohibited analyses being carried out repeatedly. Lack of appropriate primers has proved to be a major obstacle in successful amplification. Problems with preserved lice and small sample numbers limited the experiments. Although mtDNA is not a panacea for phylogenetic reconstruction and should be used with considerable caution, the fact that this molecule had resolved many systematic problems where other approaches have failed cannot be denied (Wilson *et al.* 1985; Avise 1986; Birley & Croft 1986).

6.4.3. Suggestions for Future Study

Molecular study of feather lice and their coevolutionary hosts, birds, promises a very good opportunity in acquiring knowledge about systematic relationships for both taxa. However, it is a good idea to concentrate this study on live or fresh samples since study based on preserved lice seems to be very difficult (this study; Dr. R. Gray, pers. comm.). The success rate in amplifying preserved DNA can be improved by using several suggestions as put forward by Soltis & Soltis (1993). Using short primers (150 to 200 bp) may enhance results. This will increase the probability of faithful amplification and increase the pool of sequences available for priming. This method is useful when studying damaged DNA and has been used and proved successful by Lawlor *et al.* (1991). The damaged DNA also can be repaired before PCR is carried out. This can be done by treating nicked template DNA with Klenow and excess dNTPs. This will fill in all gaps in degraded template and create longer and intact DNA fragments. Any template DNA can also be linked together through subsequent treatment with T4 DNA ligase. However, both of these approaches have to be used cautiously since they could produce mosaic sequences derived by ligation of portions of different alleles (for nuclear genes) or genes (for a multicopy gene) or from ligation of template and contaminant DNA. The mosaics are like those produced by jumping PCR

(Paabo *et al.* 1988). The addition of bovine serum albumin and high quantities of heat-stable polymerase may circumvent the inhibitory effect of some co-precipitating substances (Paabo *et al.* 1988; Soltis & Soltis 1993). Finally, all findings or recovery related to negative or unsuccessful research should be reported to avoid similar mistakes being repeated by others. Similar to positive results, the negative finding also will contribute significantly to the field of molecular study as suggested by Handt *et al.* (1994).

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Chapter 7

General Discussion and Conclusion

7.1. Study Approaches

The use of various methods promised great advantages in revealing systematic relationships among skuas. This assumption is based on the common belief that each method normally possesses some limitation in presenting comprehensive information about systematic relationships. This restriction will normally restrain any one particular method from extracting some information from an available dataset. Therefore, diverse methods will collectively enhance the reliability of results by eliminating or at least reducing the limitations or disadvantages of any particular method. For this study, four methods have been used. These are morphometric and cladistic analysis of morphological data, a study of parasitological evidence and mitochondrial DNA.

Comparative studies can provide useful information on systematic relationships. Various studies on behaviour, population dynamics and morphometrics of feral Africanized honeybees (*Apis mellifera scutellata*), for example, have revealed that they are very similar to their African ancestor, *Apis mellifera* (Michener 1977; Winston 1992). Further taxonomic study of Black Korhann by Crowe *et al.* (1994) has suggested that the Black Korhann be considered as two species as proposed earlier by Clancey (1986, 1989). These earlier studies were based on aspects of congruency in geographical patterns and essentially qualitative divergence in plumage, morphometrics, behaviour, vocalizations, mitochondrial DNA structure and habitat.

In addition to diversification of methods, better results can also be obtained by increasing the numbers of characters applied in each method. Diverse results will be produced by analysing various characters. For this study of skuas, this has been done by using numerous characters of skins and skeletons specimen. However, all the

results that originate from various sources can only be useful to a systematic study if they produce parallel results.

7.2. Skua Classification

7.2.1. Morphological Studies of Skuas

Morphological evidence from skuas has been extracted by using two methods; morphometric and cladistic analyses. Results from both methods clearly indicate that all skuas should be separated into two different groups, small and large skuas. These results agree with previous phenetic studies by Wynne-Edwards (1935) and Howard & Moore (1980). It is clear, therefore, that skuas from the two groups are different based on physical appearances. Results from this study failed to resolve any clear relationship among the large skuas. Morphological evidence failed to present a clear evolutionary relationship among members of this group. This problem possibly arose because large skuas separated recently, i.e. about 50,000 years ago (Furness 1987). As a result, physical variations among them are too small to be used in phylogenetic analysis.

The failure of morphometric analyses to differentiate between large skuas may result from a failure to construct biologically meaningful links from morphology measurements or the results from the morphometric analyses may not have been translated into sensitive phylogenetic hypotheses. Both possible reasons have to be studied carefully before any conclusion can be made.

7.2.2. Parasitological Evidence

Earlier attempts to use comparisons of the systematics of feather lice (and some other parasites) with the current arrangement of birds for taxonomic decisions have been doubted or refuted by Stresemann (1959). This is because some of the parasitological evidence presented contradicts some of the results generated by classical taxonomy. However, later discoveries by molecular studies have provided some striking

coincidences with parasitological evidence and this has forced taxonomists to re-appraise this analytical method (Mauersberger & Mey 1993). Although it can be inaccurate to infer host similarity from shared lice, it is more conservative to infer host dissimilarity from unshared lice (Clayton 1990).

Feather lice are highly specific in choosing their host species. Each species of feather louse will normally only parasitise a specific host. This highly host specific character possessed by feather lice allows them to become an indirect taxonomic indicator for their host. Basically the distinctness of lice species raises the possibility of species variation among their hosts. Normally each species of feather louse will be restricted to either one species or subspecies of host. In this study, this highly specific character has been shown by two species of *Saemundssonia.* These are *Saemundssonia inexspectata* parasitised on Long-tailed skua and *Saemundssonia cephalus* infested on Arctic skua. This insect therefore, would suggest that these skuas are different species from each other. Whenever a similar species of feather louse has occured regularly on several individuals of the same species of host (e.g. *Menacanthus sinuatus* Burm. on the Great Tit), it has been presumed that all the lice of the same genus occuring on that host are of that species (Ash 1960).

Although most feather lice show a highly host specific character, some species may infect various hosts which share a similar descendant (Clay 1949). Therefore, there is a possibility that closely related hosts will have similar species of lice. This has been shown by *Halipeurus sp.* which parasitised procellariid petrels (Paterson *et al.* 1993) and *Saemundssonia stresemanni* on all skuas (this study, Chapter 4). *Haffneria grandis* also shows a similar phenomenon, occurring on large skuas and a rarely on Pomarine skuas, indicating a close relationship among large skuas members and between members of large skuas and Pomarine skuas. This result also supports the suggestion made by Furness *et al.* (1995) and Cohen *et al.* (in prep.) that Pomarine skua is more closely related to large skuas than to small skuas.

Parasitological evidence presents a challenging result when one or more similar species of lice are discovered on different hosts. However, Hopkins (1942) suggests that

whenever different birds share more than three species of feather lice these hosts should be classified as closely related species. A possible explanation may be that feather lice are an extant ectoparasite species which parasitised on a wide range of hosts due to its less specific requirements. Alternatively, host or feather lice or both systems may have been incorrectly classified. Both hypotheses should be treated delicately since they can lead to incorrect conclusions about the systematic relationships among the study objects.

A common parasite species may be passed down unchanged to the present distinct host species from their common ancestor. However, due to geographic wandering and isolation some hosts are split and start to diverge in plumage, colour and pattern, shape of bill or toes or wings, caused partly by adaptation and partly by the simple persistence of chance variations fostered by isolation and inbreeding (Kellog 1913). But the change in plumage markings, or of bill shape or even of food and flight habit, of the hosts splitting off from a common ancestor need not be of great significance for the parasite.

The life histories of different hosts from various geographical regions should be examined carefully if they share common parasites. There is a possibility that these hosts obtained similar lice because they are living sympatrically. Allopatric hosts therefore, are less prone to sharing similar lice (although by oligoxenous or pleioxenous lice) even though they are closely related, as shown by the owls (Strigiformes) which have been parasitised by *Strigiphilus sp.* (Clayton 1990).

Similar species of lice can also be found on different hosts due to the isolation of the same species of host. Host isolation begins with geographical separation or migration. If one of the isolated populations of bird species gives rise to a new species and even if later it becomes sympatric with its parent population, there would be no further interchange of lice owing to the discontinuance of interbreeding between the two bird populations, now distinct species (Clay 1949).

7.2.3. Coevolution Between Skuas and Feather Lice

Highly specific characters of feather lice force them to coevolve together with their host since this behaviour prevents feather lice from changing their host easily. They have to stay with the particular host and modify themselves to adapt with that bird species. When feather lice are removed from their original host they die almost immediately (Ash 1960). Micro-environmental factors such as humidity, body temperature and feather size provided by the specific host are believed to play an important role in parasite specificity. The combination of highly host specific characters and coevolution, provides an important regulation of feather lice and makes them a taxonomic indicator for their host. The existence of coevolution in host-feather lice systems allows specific hosts to harbour only particular species of feather lice.

Through coevolution, a particular skua not only possesses specific lice but these lice will also have special features which relate to that skua. This is because each skua has specific morphological characters which behave as a micro-environment to their feather lice. Study of feather lice morphology therefore, should reveal morphological evidence regarding the coevolutionary process between lice and their host. However, this particular study failed to present any obvious morphological variations among individuals of similar species of feather lice. One possible explanation for this finding may be that a particular louse may possess highly specific characters for a group of closely related birds (large or small skuas or skuas in general) and not be very highly specific to a single species of bird. Alternatively, the classification of skuas is less accurate. Some species of skuas therefore, should be lumped together rather than being separated as different species. This may apply particularly to the large skuas.

Coevolution can also be tested by looking at molecular evidence in both systems. Unfortunately, no feather lice DNA was available for comparison. Failure to extract any DNA from feather lice may be due to sample quality and quantity and the sensitivity of methods used. Samples of feather lice may be too dry to enable any DNA to be extracted. The quantity of DNA in this insect may be too low to allow it to be extracted by normal methods. Therefore, a more sensitive method may have to be

designed before any successful extraction can be done. Alternatively extraction from fresh or live specimens should be carried out. Previously no study on dried lice has been carried out and therefore there are no guidelines to follow.

Extraction of mtDNA from feather lice remains attractive since mtDNA sequences from skuas are already available for comparison (Cohen *et al.,* in prep). Furthermore, mtDNA is a popular means of making phylogenetical comparison. Each mtDNA molecule carries in its sequences the life history of its lineage (Wilson *et al.* 1985). The comparison of these sequences, assumed to be selectively neutral, can recover evolutionary relationships obscured by ecogenetic morphological variation (Wilson *et al.* 1985; DeSalle & Templeton 1988; Thorpe *et al.* 1993). Phylogeny constructed on the basis of DNA sequence normally gives more detailed resolution of relationships than the previously used protein electrophoresis study and tends to be in good agreement with traditional morphological and biological data (Seibold *et al.* 1993).

7.3. Conclusion

This study used various methods to obtain a wide range of data to build a systematic relationship among skuas. Unfortunately, some of the methods failed to present reliable results. Based on currently available data, it is obvious that skuas should be separated into two different groups; small and large skuas. Results from this study therefore, suggest that Pomarine skuas should be classified together with Arctic and Long-tailed skuas and not with Great skuas. Systematic relationships among large skuas also cannot be revealed due to insufficient data. More molecular studies have to be carried out on skuas and feather lice with more emphasis on fresh or live specimens.

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