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ORIGINAL PAPER

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Skull morphometry of *Pygoscelis* (Sphenisciformes): inter and intraspecific variations

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Abstract Morphological diversity of 47 pygoscelid skulls was tested empirically through geometric morphometrics approach. Using 14 landmarks, shape is analyzed independently of other aspects of the body form. The shape disparity within and between the three living species of *Pygoscelis* is explored as well as how the structure of the skull is related to food preferences. Comparison of the three mean configurations of the species suggests that differences among groups are small relative to the variability within each group. However, some differences at the posterior portion of the braincase are indicated. Sexual dimorphism within each species is not noticeable. Comparison with a piscivorous species (*Spheniscus humboldti*) shows two cranial patterns: pygoscelid type with wide nasal gland depression limited by well-marked edges, shallow temporal fossae, and a poorly developed temporal nuchal crest; the second type represented by *Spheniscus humboldti* with laterally open nasal gland depression, deep temporal fossae, and a well-developed temporal nuchal crest.

Introduction

Penguins are one of the oldest, most aquatic, and possibly most mystifying group of birds. Within marine birds, they show the best performance in the water, being able to swim at high speeds and great depths. This is because their wings become into flippers and the body is fusiform, allowing them to "fly" under the water. On the other hand, their legs are short and located just below the body, so their locomotion on land is very particular (Pinshow et al. 1976; Griffin and Kram 2000).

Today, 17 species and six genera occur along the coasts and oceans of the Southern Hemisphere. The majority of the species live between 45° and 60°S, and display the greatest diversity on the mainland and islands of southern New Zealand and Islas Malvinas (Williams 1995). They breed in very big colonies constituted by one or more species, no more than four (Woehler 1993). *Pygoscelis*, Wagler (1832), one of the best-known penguins, includes three living species: Chinstrap penguin, Adelie penguin, and Gentoo penguin distributed around Antarctica and sub-Antarctic islands. Any combination of all these species is displayed in the breeding colonies (Diego Montalti, personal communications), but there is always one dominant species in the colony.

Every species can be externally identified on the basis of characters of the head, particularly on the bill, plumage color pattern and color distribution (Martínez 1992). The Adelie penguin *Pygoscelis adeliae* (Hombron and Jacquinot 1841) is a medium sized penguin of around 71 cm in height with a short bill feathered to half way along its length, while the Chinstrap penguin *Pygoscelis antarctica* (Forster 1781) is between 68 and 71 cm in height (Martínez 1992), its bill is completely black with a distinctive white face (Williams 1995). The largest of the genus is the Gentoo penguin *Pygoscelis papua*, Forster (1781), that reaches 76–81 cm in height (Martínez 1992), being easily recognizable because of its bright bill, which is orange-red on the sides (Williams 1995).

Sometimes small differences in the external anatomy are attributed to sex, so *P. adeliae* and *P. papua* females are smaller than males; while both sexes are similar in *P. antarctica*. But it is still unknown if there are any other anatomical differences within a given population which cannot be attributed to sex. What is more, few studies have investigated whether anatomical differences in a population (sexual or nonsexual variation) are observable in skull morphology. An attempt to determine sex in Adelie penguins using morphometric parameters, more indeed measures of the bill and head, found small

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differences (Knowies et al. 1992). An essential basis for any investigation that tries to understand the morphology and function of the skull of penguins is the excellent work that Richard Zusi (1975) had written three decades ago and of course, the historical paper of Pycraft (1898) that gives an appropriate idea of the shape disparity on the different regions of the skull. We try here to determine if morphology (excluding the size effect) can be used to distinguish different species or males and females belonging to the same species.

To evaluate characters useful in systematics it is obviously necessary to study the degree of variation within and between species. The present work is part of an ambitious project that attempts to find patterns in cranial and postcranial skeletal morphology as a basis for penguin classification. Theoretically, species in homogeneous habitats are precluded from partitioning food resources by using different microhabitats (Barbosa and Moreno 1999). In this context, it can be assumed that this partition implies differences in foraging strategies, and such differences would be reflected by disparities in morphology. This information can be very useful to understand the role of penguins in the marine trophic chains and their relationships with other organisms of the same environment. Additionally, this kind of data could be applied to paleoecological interpretations of past ecosystems, where extinct species are under study.

Specifically, the goals of the present contribution are (1) to determine the skull intraspecific variation in *P. adeliae*, *P. papua*, and *P. antarctica*, (2) to determine if there is any skull variation attributed to sexual dimorphism in each species of *Pygoscelis*, (3) to determine the skull interspecific variation in *Pygoscelis*, and (4) to analyze the morphometric differences and their potential relationships with the feeding requirements of the taxa.

Materials and methods

According to the main objectives, a series of hypotheses were formulated to be tested during this work: (1) adult penguin skulls of the same population display the same morphometric pattern, (2) there are morphological differences between males and females of the same species, (3) it is possible to distinguish the three species of *Pygoscelis* by analyzing the skull features, and (4) braincase morphology and feeding preferences are correlated.

To test each hypothesis, a sample constituted by the skulls housed at the collections of the División Paleontología Vertebrados at Museo de La Plata (MLP) and Instituto Antártico Argentino (IAA), Argentina was studied.

Each skull was photographed in dorsal view with the same focal distance. A digital camera was used to take images of 48 adult specimens (Appendix). The *x*, *y* coordinates of 14 landmarks were digitized from these images using TpsDig 2.04 (Rohlf 2005). Dorsal view was preferred because the configuration of the nuchal crests and the shape of the rostrum are clearly seen.

Landmark selection

Fourteen points were chosen to analyze in the skull, including landmarks of type I, II, and III (Rabello Monteiro and Furtado dos Reis 1999), which are shown in Fig. 1.

Landmarks 2, 3, 4, 12, and 14 are located on joints (type I) and indicate the extension of the nasal foramen (2) and the nasal processes (3), the position of the lacrimal bone (4), the development of the temporal fossa (12), and configuration of the nuchal crest (14).

Landmarks 7, 9, 10, and 11 belong to type II. The first three shows the expansion and width of the salt gland depression. Landmark 11 is located on the temporal nuchal crest, showing its degree of development.

Finally, landmarks 1, 8, and 13 corresponding to type III indicate the end of the bill, the postorbital process, and the most posterior end of the braincase, represented by the cerebellar prominence. These points show the relative extension and width of the skull.

Morphometric and comparative methods

The nuisance parameters from the dataset, such as differences in size, orientation, and position, were removed through procrustean reorientation, so only the variation in shape of the landmarks configurations was compared (Small 1996). Procrustes superimposition was used (Rohlf and Slice 1990) to accomplish this task. This technique scales all configurations so as to have centroid size (square root of the summed squared distances from all landmarks to the configuration centroid) equal to 1, translates all configurations so that all centroids are located at the origin, and rotates all configurations by an optimum angle. These superimposition steps are taken to minimize the sum of squared distances between corresponding landmarks. The generalized Procrustes superimposition (Rohlf and Slice 1990) allows the calculation of mean shapes from samples. The computer graphics used here are the thin plate splines, whose function minimizes the energy required during the transformation and the ones made by the Morpheus software (Slice 1994–1998). This energy is a function not only of the amount of transformation in shape, but also

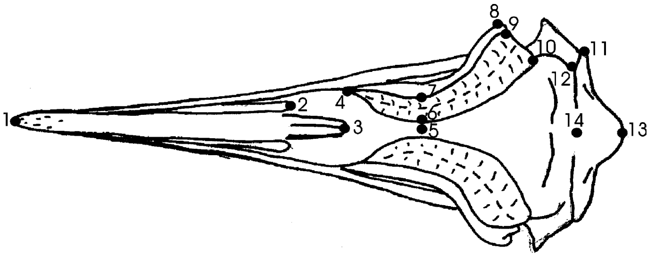

Fig. 1 Landmarks selection on penguin skull in right side of *dorsal view*

of the degree of closeness among the configuration points.

Once these values were obtained, a relative warp analysis, a modification of principal component analysis for shape coordinate data was made using the software tpsRelw (Rohlf 2005).

Skulls of the sexed specimens were compared to detect the intraspecific differences between females and males. Once that variation was established, skulls of the same population belonging to the same species were analyzed. Three independent analyses were performed, comparing 22 individuals of *P. adeliae*, 16 of *P. antarctica*, and nine of *P. papua* in each case.

The consensus configuration was determined for each species and these new configurations were compared between them. It allowed visualizing the interspecific variation and testing our third hypothesis.

Relative warps

The relative warps were computed with the uniform component included and no weighting by bending energy (Bookstein 1996). A relative warps analysis is reported by the joint distribution of weighted scores together with the diagrams of the grid deformations corresponding to the eigenvectors that generated those scores.

Food preferences

The information on foraging areas, ranges, and food preferences of penguins is limited and patchy. Studies are generally restricted to a few specific areas, usually for a single season and to the breeding season (Bost et al. 1997; Kooyman et al. 1992). To test the last hypotheses, we follow Williams (1995) who states that *P. papua* takes large amounts of Antarctic krill, other different crustaceans, and small quantities of cephalopods, polychaetes, and fishes. *P. adeliae* takes mainly krill with smaller quantities of fishes, amphipods, and cephalopods. *P. antarctica* almost exclusively feed on krill, but also takes a few fishes and other species of crustaceans. Comparison with a piscivorous species was made using *S. humboldti*. It is an opportunistic feeder, taking equal proportions of pelagic fish, squid, and some crustaceans.

Results

Our results will be described in relation to the four hypotheses previously proposed. When females and males were analyzed, definitively scarce morphological distances were found (Fig. 2). For this reason, all the specimens pertaining to the same species were examined altogether, either are female or males. Specimen comparison within each species suggests that the intraspecific variation is small (Fig. 3). However, a number of small differences are located on the base of the rostrum and the preorbital region.

The greatest variation in the skulls of *P. adeliae* is registered on landmarks 1, 4, 7, and 13 (Fig. 3a) that correspond to the extension of the skull, the preorbital region, and the width of the nasal gland depression, respectively. By comparison, landmarks 3, 8, 9, and 11, corresponding to the frontonasal hinge region, postorbital at the salt gland ridges, and temporal region, respectively, are conservative and show little variation.

When the intraspecific variation of *P. antarctica* is analyzed (Fig. 3b), the regions of greatest variability are marked by landmarks 4, 11, and 14 that correspond to the preorbital region, temporal and the nuchal crest of the skull, respectively. On the other hand, the regions that exhibit less change are marked by landmarks 5, 6, 7, 8, and 9, and correspond to the orbital and interorbital regions.

P. papua (Fig. 3c) shows similar skull morphology to *P. antarctica*, with regions of morphological change located in the interorbital, temporal, and nuchal zones (landmarks 5, 6, 11, and 14). The base of the rostrum and postorbital region and the roof of the braincase (cerebellar prominence) are the most stable regions (landmarks 1, 3, 4, 6, 9, and 10).

Comparison of the three mean shapes (Fig. 4a) suggests that also the differences among groups are small relative to the variability within each group. However, nasal foramen, preorbital and postorbital zones are relatively more variable between species. These results

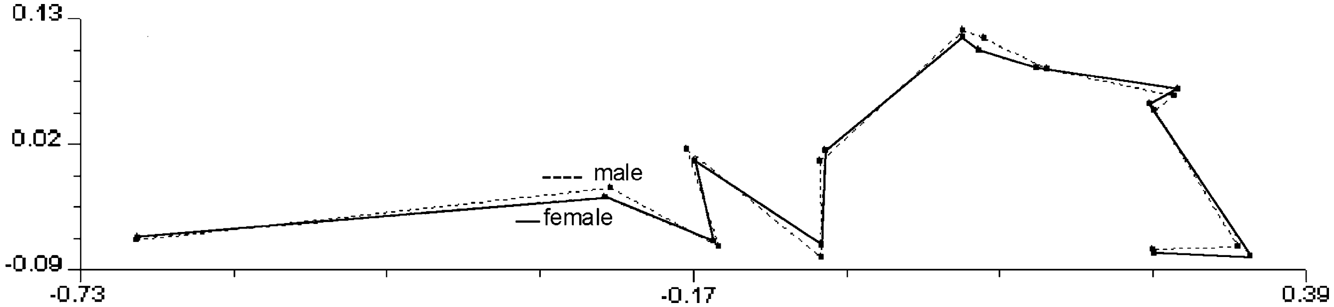

Fig. 2 Comparative graphic showing the skull configuration of males and females in *dorsal view*

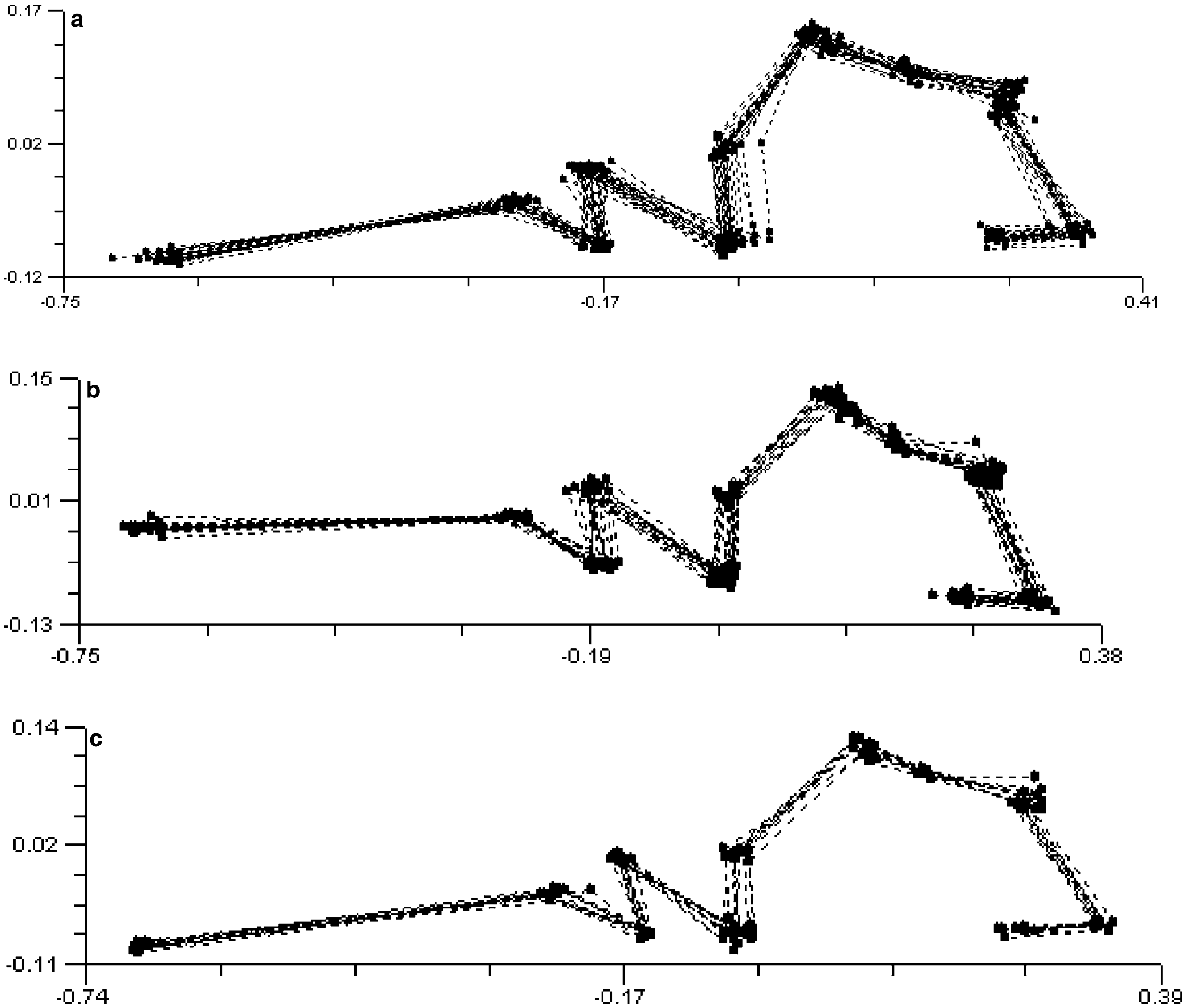

Fig. 3 Intraspecific skull variation in *dorsal view*: **a** *Pygoscelis adeliae*, **b** *Pygoscelis antarctica*, **c** *Pygoscelis papua*

show variations at the rostrum and the interorbital region, landmarks 1, 2, 3, 5, and 6, whereas the preorbital, temporal, and nuchal regions (landmarks 4, 10, 11, and 14) seem to be the most constant in the genus.

In the comparison of the pygoscelid skulls with that of *Spheniscus*, several differences are noticeable. The configuration of the base of the bill, lacrimals and nuchal crests (landmarks 4, 11, and 12) show morphological diversity of these avian skulls (Fig. 4b). These morphological differences are characteristics of the two extreme morphological types represented by *Spheniscus* and *Pygoscelis* (Fig. 5). Comparing the areas corresponding to the landmarks 4, 11, and 12, the skull is proportionally wider in *Spheniscus* than in *Pygoscelis*. The nuchal region displays significant differences in the degree of development of the temporal crests, which are expanded like wings in *Spheniscus* and short in *Pygoscelis*.

Discussion and conclusions

We started our analyses expecting to find morphological differences in *Pygoscelis* that can be used in systematic analysis, and among them with *Spheniscus*, as the representative of two different ecological types.

Morphometric measurements of the head were used to sex yellow-eyed penguins (Setiawan et al. 2004). But this more traditional approach showed, indeed males are longer than females, that head lengths between sexes overlap for most of the measurement range (see also Scolaro 1987; Eason et al. 2001). Although we did not find any study of this type done in *Pygoscelis*, the difficulties to determine sexes with other penguin species, made us prefer the use of other techniques, independent of the size. However, in regard to our goals, we did not find important interspecific morphological differences

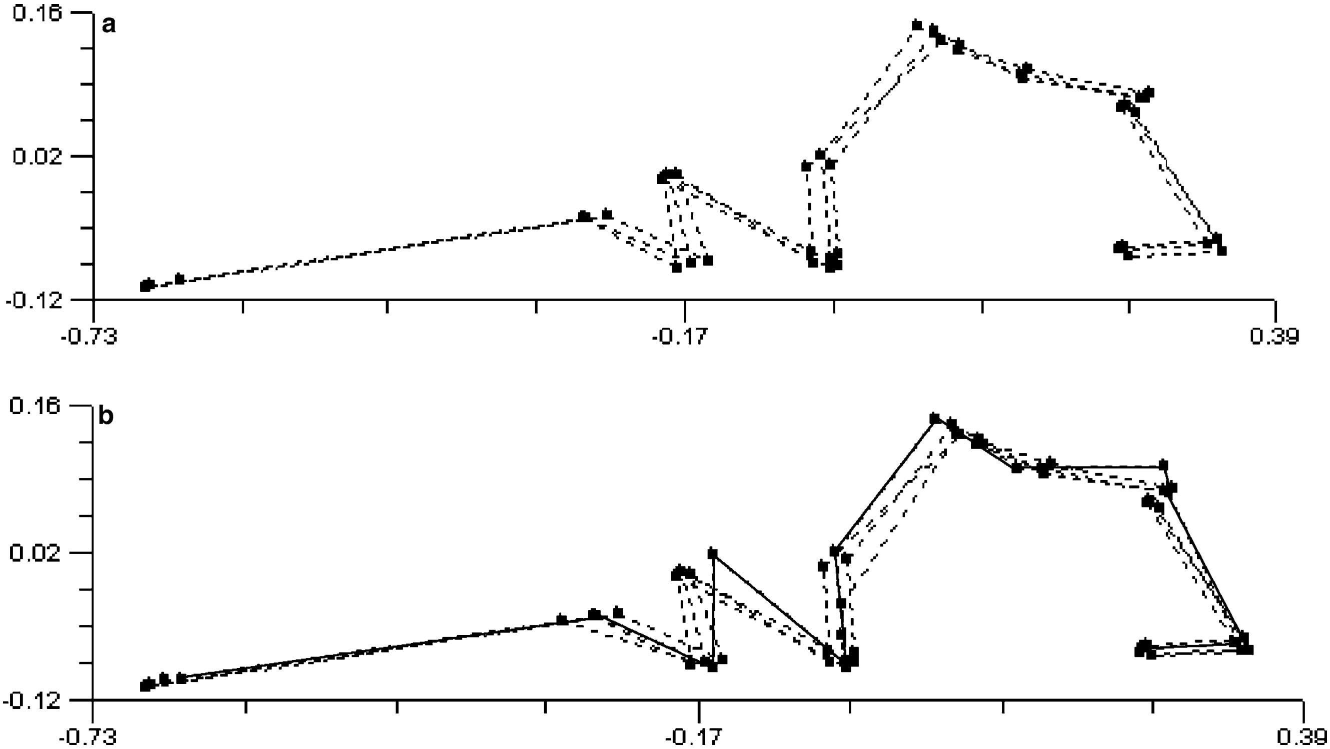

Fig. 4 Interspecific skull variation in *dorsal view*: **a** comparing the consensus configuration of the three species of *Pygoscelis*, **b** comparing the species of *Pygoscelis* with *Spheniscus humboldti* (*full line*)

and the small differences we found apparently do not correspond to sex. So, adult members of a single species also may show the same morphological pattern. This is not surprising because all *Pygoscelis* species belong to the same ecomorphological type.

The pygoscelid penguins prey almost exclusively on Antarctic krill, making dives at 20–54 m depth during foraging (Clausen et al. 2005) around Antarctica and sub-Antarctic Islands where they live. As we mentioned before, the three species can be sympatric, nesting in the same colony. This leads us to believe that niche partitioning must surely be operating (e.g. inshore or offshore foraging areas, feeding time of day) that allow them to coexist in the same area while consuming similar types and sizes of prey. Even so, such partitioning is not displayed in the anatomy of the skull or, perhaps more specifically, patterns of morphological diversity are not directly related to the mechanism or strategies of feeding.

The techniques of geometric morphometry, particularly the thin plate splines, allowed a deep insight into skull shape variation and showed that there are some shape differences of small scale in the interorbital region, nasal processes, and nuchal crests regions (mostly muscle attachments areas) that do not seem to be a priori influenced by functional needs.

Our analysis, in addition, tried to find morphological differences between both ecological types—icthyophagous versus plankton eaters—that could be applied to infer habits of the extinct forms. In fact, the fossilization of bird skulls is exceptional and, in the few cases that it happens, the posterior portion of the skull presents greater preservation potential than the rostrum. For this reason, we tried to focus on this sector to get characters applicable to the extinct species.

It is widely thought that the feeding behavior can be inferred from skull morphology. Comparing both extreme ecological types (represented by *Spheniscus* and *Pygoscelis*), it is possible to find some differences at the base of the bill, lacrimal area and, particularly, at the nuchal crests. According to previous studies, *Spheniscus* is the most piscivorous of all the living penguins, feeding on fishes and squids, while *Pygoscelis* feeds mostly on krill and small crustaceans. These two cranial patterns were recognized in our results. The pygoscelid type, characterized by a wide nasal gland depression limited by well-marked edges, a shallow temporal fossa, and poorly developed temporal nuchal crests. The type represented by *Spheniscus* is characterized by a laterally open nasal gland depression (without edges), deep temporal fossa, and well-developed temporal nuchal crests.

In the powerful mechanism of the skull for depressing the mandible and protracting the upper jaw, is involved the temporal portion of the adductor mandibulae externus muscle (Zusi 1993) that originates in the temporal fossae and runs between the zygomatic and postorbital processes to be inserted finally in the jaw. An extensive muscle area on the braincase, as *Spheniscus* shows, could be indicated by greater forces acting in the closing of the jaws. Another feature that *Spheniscus* has, in relation-

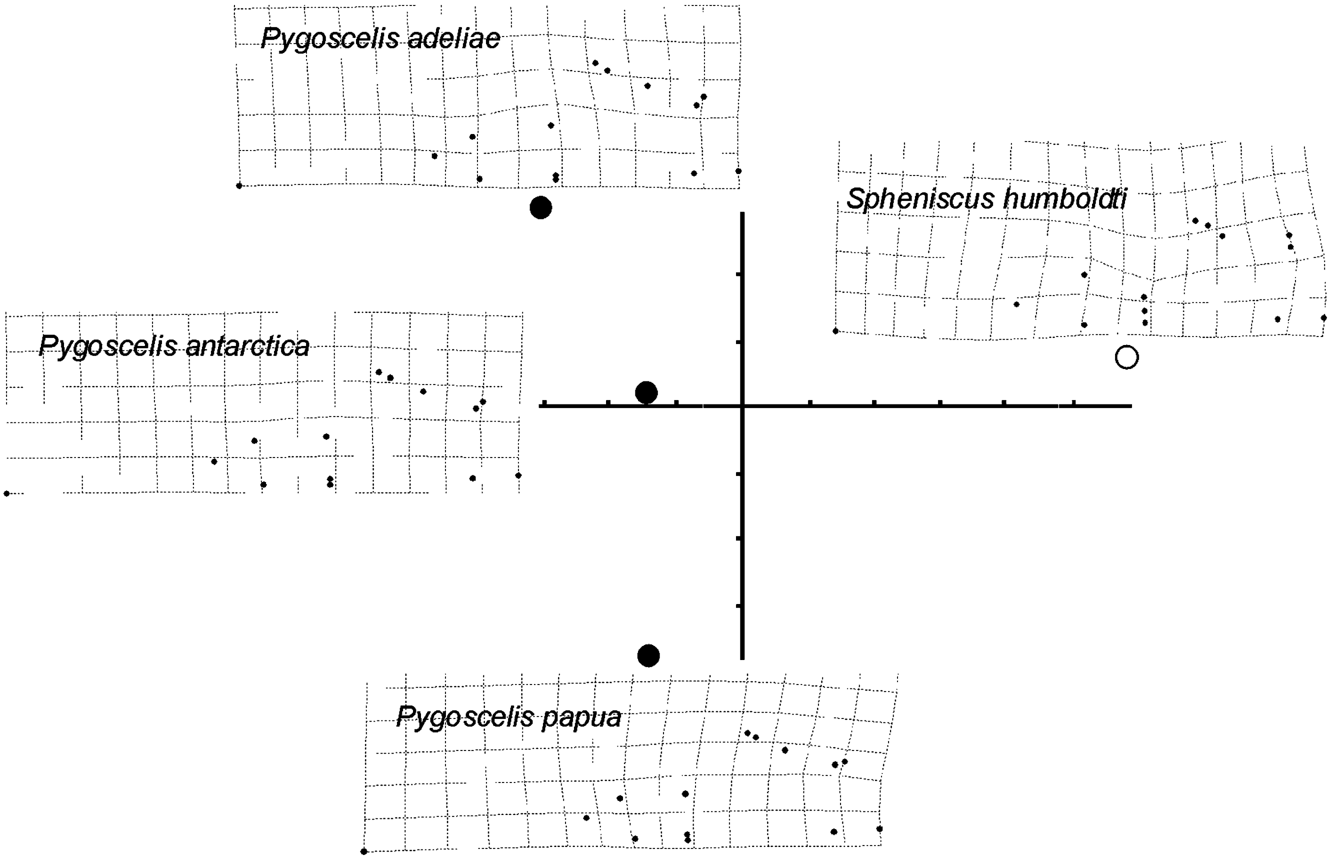

Fig. 5 Relative warp analysis of pygoscelids (*black dots*) and *Spheniscus* (*white dot*). *Grids* show the visualization of shape changes in each species

ship with the area of insertion of this muscle, is an enlarged temporal crest. However, in this interplay of forces and muscles, more than one muscle is working (e.g. protractor pterygoidei et quadrati muscle) and interpretations or inferences on feeding style are very complicated.

Recent studies of phylogeny on living penguins (Giannini and Bertelli 2004; Bertelli and Giannini 2005) provide a new opportunity for the analysis of skull structure in a phylogenetic context. They accepted that *Eudyptula* + *Spheniscus* constitute a different clade than that of *Aptenodytes* + *Pygoscelis*. The present state of our knowledge and our work on *S. humboldti* and the three living species of *Pygoscelis* allows us to suspect that each pattern that we distinguish in the skull appears in different clades. Comparison of morphometric studies of the skull in sister taxa, such as the present one, can further our understanding of the evolution and adaptation of these birds. Advances depend ultimately on the availability of anatomical specimens and on the surveys on the other penguins.

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Appendix: materials used in the morphometric analyses performed

P. adeliae: MLP 32, MLP 33, MLP 415, MLP 416, MLP 417, MLP 418, MLP 419, MLP 420, MLP 421, MLP 422, MLP 423, MLP 424, MLP 425, MLP 426, MLP 427, MLP 428, MLP 429, MLP 430, MLP 431, MLP 432, MLP 433, MLP 434. *Pygoscelis antarctica*: IAA 54, IAA 65, IAA 76, IAA 157, IAA 158, MLP 435, MLP 436, MLP 437, MLP 438, MLP 439, MLP 440, MLP 441, MLP 442, MLP 443, MLP 444, MLP 447. *Pygoscelis papua*: IAA 24, IAA 81, IAA 82, IAA 88, IAA 160, MLP 449, MLP 451, MLP 463, MLP 468. *Spheniscus humboldti*: MLP 686.

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