Antarctic Science 27(4), 327–332 (2015) © Antarctic Science Ltd 2015 doi:10.1017/S0954102014000820

Morphometric variation in chinstrap penguins: molecular sexing and discriminant functions in the South Shetland Islands, Antarctica

JAQUELINE BRUMMELHAUS[1,2], VICTOR HUGO VALIATI[2] and MARIA VIRGINIA PETRY[1]

[1]*Universidade do Vale do Rio dos Sinos, Graduate Program in Biology, Laboratory of Ornithology and Marine Animals, Center for Health Sciences. Av. Unisinos, 950, 93022-000, PO Box 275, São Leopoldo, Rio Grande do Sul, Brazil*
[2]*Universidade do Vale do Rio dos Sinos, Graduate Program in Biology, Laboratory of Molecular Biology, Center for Health Sciences. Av. Unisinos, 950, 93022-000, PO Box 275, São Leopoldo, Rio Grande do Sul, Brazil*
jaquebrummelhaus@gmail.com

Abstract: Chinstrap penguins (*Pygoscelis antarcticus*) show little sexual dimorphism and sexing by direct observation can be difficult. Through molecular techniques, male and female adults were identified at Stinker Point, Elephant Island, South Shetland Islands, in the 2011–12 and 2012–13 breeding seasons. In the assessment of sexual dimorphism using morphological characteristics, males were 6.0–9.4% larger than females. From the most significant morphological measurements, a discriminant function was formulated that classified 80.6% of the birds correctly. In addition, our data on bill length and depth were compared with those in the literature to evaluate sexual dimorphism between different breeding locations and to test the performance of the discriminant function. There were no differences in sexual dimorphism between breeding locations. However, the discriminant function should be used with caution because some penguins may be misclassified. Therefore, when there is doubt about the accuracy of morphometric approaches, application of molecular sexing techniques is recommended.

Received 4 November 2013, accepted 1 October 2014, first published online 9 January 2015

Key words: CHD gene, genetic sexing, morphological sexing, *Pygoscelis antarcticus*, sexual dimorphism

Introduction

Chinstrap penguins (*Pygoscelis antarcticus* (Forster)) are an Antarctic species that breeds during the summer in colonies on ice-free areas of the coast, mainly on sub-Antarctic islands and the Antarctic Peninsula. In the South Shetland Islands, the total breeding population was estimated to be 1 248 350 pairs (Harris 2006). As in other penguin species, they show little sex-linked size and plumage dimorphism, although males are usually bigger than females (Davis & Speirs 1990, Zavalaga & Paredes 1997, Bertellotti *et al*. 2002, Valenzuela-Guerra *et al*. 2013).

Knowing the sex of individuals is essential for measuring several parameters of interest to the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) and the Ecosystem Monitoring Program (CEMP). For chinstrap penguins, weight on arrival at the breeding colony and age-specific annual survival and recruitment are important population parameters that involve a knowledge of the penguin's sex (CCAMLR 2004). Moreover, sex classification is important in studies of life history, ecology, behaviour, demography, conservation and evolutionary change.

Previous studies have determined the sex of penguins using morphometric measurements and discriminant functions (Amat *et al*. 1993, Zavalaga & Paredes 1997, Bertellotti *et al*. 2002, Hart *et al*. 2009, Poisbleau *et al*. 2010, Polito *et al*. 2012, Valenzuela-Guerra *et al*. 2013). However, as is common for philopatric seabirds, many penguin species show considerable geographic variation in body size (Williams 1995), which makes it difficult to apply discriminant functions in locations other than where they were developed. Moreover, morphometric tests are potentially prone to a bias because males that are unusually small or late to develop may be scored as female (Hart *et al*. 2009). Immature penguins have smaller measurements than adults (Minguez *et al*. 1998), thus immature males may be misclassified as adult females.

Other methods, such as observing mating behaviours are also used. However, this only covers a small window of the breeding season (Bertellotti *et al*. 2002). A potential alternative for accurately sexing penguins is a DNA-based method that uses polymerase chain reaction (PCR) to target CHD1-Z and CHD1-W genes using the primers P2/P8 (Griffiths & Tiwari 1993, Griffiths 2000). This tool has been broadly applied to birds (except ratite species) (Griffiths & Tiwari 1993, Griffiths 2000). The P2/P8 primer pair gives high concordance with the results of morphometric sexing, improving the certainty of sexing birds (Hart *et al*. 2009). Although genetic procedures have

been applied to some species of penguins (Costantini *et al.* 2008, Hart *et al.* 2009, Poisbleau *et al.* 2010, Valenzuela-Guerra *et al.* 2013), these techniques have only been tested once in chinstrap penguins (Polito *et al.* 2012). An earlier study investigated sexual dimorphism in chinstrap penguins but only through the use of a morphometric discriminant function (Amat *et al.* 1993). Temporal attendance during incubation has also been used to determine sex in chinstrap and other penguin species (Williams 1995). Nevertheless, previous studies show that molecular sexing can complement methods based on discriminant function analysis in species with weak size dimorphism (Genovart *et al.* 2003, Jakubas & Wojczulanis 2007, Hart *et al.* 2009, Calabuig *et al.* 2011).

The goals of this study were, first, to identify male and female adult chinstrap penguins using a DNA-based molecular sexing technique at Stinker Point, Elephant Island. Second, sexual dimorphism between males and females was evaluated using morphological characteristics previously assessed in the literature. Third, a discriminant function, based on the characteristics that best identify the sex of chinstrap penguins, was obtained. In addition, the measures were compared with those described by Amat *et al.* (1993) and Polito *et al.* (2012) to evaluate sexual dimorphism between breeding locations and to test the performance of the discriminant function.

Methods

Chinstrap penguin breeding adults were captured by hand near their nests at Stinker Point (Elephant Island; 61°13'20"S, 55°21'36"W), South Shetland Islands, Antarctic, in the 2011–12 and 2012–13 breeding seasons. Four morphological measurements were taken using a calliper. Bill (culmen) length (BL) and commissure width (CW, taken at the base of the bill where the mandibles join) were measured to an accuracy of 0.1 mm. Bill depth (BD) was measured through the centre of the nostrils. Flipper length (FL) (average of right and left flippers) was measured with a graduated rule. All measurements were conducted by the same person. In total, 35 birds (for BD, $n = 31$) were measured (November 2011 and 2012, laying stage) and blood samples were taken from the foot.

Molecular sexing

Genomic DNA was isolated from blood samples through a standard phenol/chloroform technique with digestion by Proteinase K. Blood samples were refrigerated and stored in a sample bank at Laboratório de Biologia Molecular, Universidade do Vale do Rio dos Sinos. The CHD region of the sex chromosomes was amplified by PCR using the primers P2 and P8 (Griffiths & Tiwari 1993, Griffiths 2000). The PCR products were analysed by electrophoresis in 3% agarose gels stained with ethidium bromide and visualized under ultraviolet transillumination. Females are the heterogametic sex with both CHD-Z and CHD-W genes, which differ in length and appear as two bands on the gel. Males are homogametic and thus show a single band (Griffiths & Tiwari 1993, Griffiths 2000). The P2/P8 primers may, in some species, produce only one fragment in both males and females; therefore, females may be misidentified as males. Independent PCR amplification was made for all individuals who had only one fragment for sex identification.

Statistical analysis

Dimorphism between males and females was calculated for each measurement using the adapted Storer's index (SI):

$$\mathrm{SI} = [(\mathrm{Vm} - \mathrm{Vf})/((\mathrm{Vm} + \mathrm{Vf}) \times 0.5)] \times 100, \qquad (1)$$

where, *Vm* corresponds to the mean value for males and *Vf* to the mean value for females of the variable considered (Storer 1966, Blondel *et al.* 2002, González-Solís 2004, Mariano-Jelicich *et al.* 2007). Morphological measurements were compared between sexes and breeding locations (Stinker Point, Elephant Island and Admiralty Bay, King George Island), and interactions were assessed using two-way analyses of variance (ANOVAs). Pearson's correlations were used to examine the relationships among the morphometric characteristics.

A stepwise discriminant function analysis was performed to assign individuals to a sex based on morphological measurements (BD, BL, CW and FL) that had significant influence on the classification of males and females (F-test of Wilk's Lambda, test of equality of group means). A cross-validation technique (leave-one-out test) was used to verify the accuracy of the discriminant function. This algorithm chooses the function that has the lowest percentage of misclassification. All data satisfied the Bartlett test of normality. The cut-off point was calculated as the weighted average of values of discriminant scores (i.e. average value of the mean of each sex; means were weighted by the number of males and females). Adults with discriminant scores greater than the cut-off point were classified as male and those with lower scores were classified as female. The discriminant functions from Amat *et al.* (1993) and Polito *et al.* (2012) were applied to the penguins from Stinker Point. With the raw measurement data that were available, the discriminant function from Amat *et al.* (1993) was also applied to the data of Polito *et al.* (2012) to assess the efficiency of the discriminant function. This allowed us to quantify how transferable the discriminant equation was to other breeding sites.

All statistical analyses were performed using SPSS 18.0 (SPSS 2009). All tests were two-tailed and differences were considered significant at $P \leq 0.05$ level.

https://doi.org/10.1017/S0954102014000820 Published online by Cambridge University Press

Table I. Morphological measurements for adult chinstrap penguins at Stinker Point, Elephant Island. Sexes and breeding locations were compared between Admiralty Bay, King George Island (Polito *et al.* 2012) and Stinker Point, Elephant Island using a two-way analysis of variance (ANOVA).

Measurements (mm)	Elephant Island						Two-way ANOVA		
	Female		Male			F-test of Wilk's lambda	Breeding location	Sex	Interaction
	n	Mean ± SD (range)	*n*	Mean ± SD (range)	SI (%)				
Bill depth	14	20.1 ± 1.4 (17.0–22.5)	17	21.8 ± 2.1 (19.3–27.2)	8.46	$\lambda = 0.80$ $F_{1,29} = 7.080$ $P = 0.013$	$F_{1,57} = 29.431$ $P = 0.000$	$F_{1,57} = 42.264$ $P = 0.000$	$F_{1,57} = 0.158$ $P = 0.775$
Bill length	16	46.9 ± 3.0 (42.0–50.5)	19	49.9 ± 2.3 (47.0–54.0)	6.20	$\lambda = 0.74$ $F_{1,33} = 11.723$ $P = 0.002$	$F_{1,61} = 0.926$ $P = 0.340$	$F_{1,61} = 43.436$ $P = 0.000$	$F_{1,61} = 3.081$ $P = 0.084$
Commissure width	16	29.8 ± 2.9 (26.3–36.0)	19	32.9 ± 3.3 (27.7–40.4)	9.89	$\lambda = 0.80$ $F_{1,33} = 8.249$ $P = 0.007$			
Flipper length	16	191.1 ± 6.3 (182.0–204.0)	19	194.1 ± 5.2 (185.0–202.5)	1.56	$\lambda = 0.93$ $F_{1,33} = 2.330$ $P = 0.136$			

SI = Storer's index, SD = standard deviation.

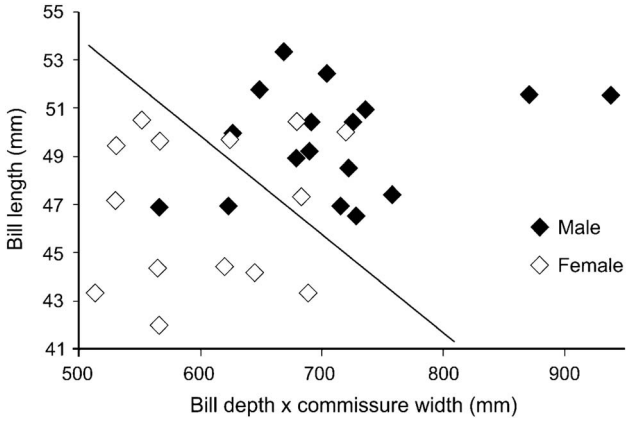

Fig. 1. Discriminant function separating male ($n = 17$) and female ($n = 14$) chinstrap penguins using bill length, bill depth and commissure width at Stinker Point, Elephant Island. The solid line represents the statistical boundary between males and females, derived from the discriminant function. True sexes were determined by a molecular technique: male $n = 17$ and female $n = 14$.

Results

Molecular sexing

Of 35 penguins at Stinker Point, Elephant Island, 19 were male and 16 were female. PCR produced a single band of about 375 base pairs (bp) for males and two bands of about 375 and 390 bp for females.

Morphometric differences between sexes and breeding locations

The BD, BL, and CW differed significantly between sexes, with males being 6.0–9.4% larger than females, even though there was overlap in some morphological measurements (Table I, Fig. 1; for all the measurements

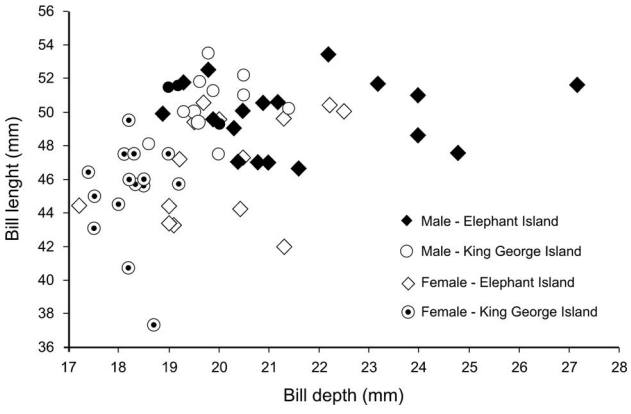

Fig. 2. Bill length and bill depth for breeding adult chinstrap penguins at Admiralty Bay, King George Island (Polito *et al.* 2012) and Stinker Point, Elephant Island.

https://doi.org/10.1017/S0954102014000820 Published online by Cambridge University Press

Table II. Pearson's correlation coefficient (r) among morphometric measurements for chinstrap penguins ($n = 31$) from Stinker Point, Elephant Island.

	Bill depth	Bill length	Commissure width
Bill length	0.397		
Commissure width	0.066*	0.238	
Flipper length	0.187	0.430*	-0.101

*$P < 0.05$.

see a supplemental table at http://dx.doi.org/10.1017/S0954102014000820). The SI for CW, BD and BL were 9.9%, 8.5% and 6.2%, respectively (Table I). Males also had a greater FL (1.5%), but this difference was not significant (Table I). There were significant effects of breeding location and sex on BD; even if the interaction between location and sex was not significant (Table I, Fig. 2). For BL, there were significant differences between males and females, but not between breeding locations (Table I, Fig. 2). Pearson's correlations among morphometric measurements are shown in Table II. Furthermore, the variance inflation factors (VIF) test showed that there is no multicollinearity between the morphometric measurements (BD = 1.188, CW = 1.205, BL = 1.728 and FL = 1.513).

Discriminant function analysis

For the discriminant function analysis, BD, BL and CW had the highest canonical correlations (0.629) and best classified breeding male and female adults at Stinker Point (Fig. 1). The accuracy of the classification of chinstrap penguins was 80.6% after cross-validation. The discriminant function was:

$$0.301\text{BD} + 0.207\text{BL} + 0.191\text{CW} - 22.349, \qquad (2)$$

where all measurements are in mm. The resulting cut-off point for the discriminant function was $D = -0.076$.

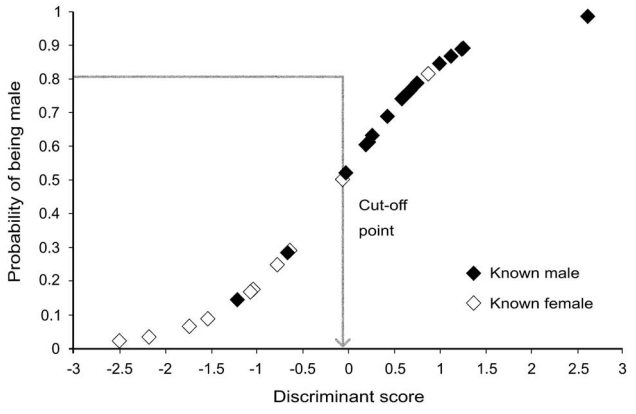

Fig. 3. Probability of a chinstrap penguin being male in relation to discriminant score at Stinker Point, Elephant Island.

Chinstrap penguins with a discriminant score greater than this were classified as male and those with a lower score were classified as female (Figs 1 & 3). In general, misclassifications occurred when females were larger.

Discussion

For chinstrap penguins, the results showed that males were significantly larger than females for three morphological measurements (BD, BL and CW) (Table I, Fig. 1). This shows that there is sexual dimorphism in this species, and it is possible to obtain information about sex in the field. Other authors have found similar results for seabirds (Amat *et al.* 1993, Serrano-Meneses & Székely 2006, Polito *et al.* 2012). In socially monogamous seabirds, sexual size dimorphism is correlated with sexually selected male displays, such that males are typically larger than females in those species in which the males display on the ground rather than in the air (Serrano-Meneses & Székely 2006). Other functional explanations that may account for male penguins being larger are intrasexual competition (males compete for access to females or for control of a resource) and intersexual competition (competition for food is reduced by males and females exploiting prey of different sizes) (Davis & Speirs 1990). Male chinstrap penguins are more aggressive in nest defence towards potential stone thieves than females; males also collect more and larger stones for nest maintenance (Moreno *et al.* 1995). Nevertheless, when it comes to feeding, there is no difference in meal size between male and female chinstrap penguins (Leon *et al.* 1998).

At Stinker Point, Elephant Island, BD, BL and CW were the most reliable morphological measurements for assessing sex, and enabled us to sex birds with an accuracy of 80.6% (Figs 1 & 3). Additionally, BD and BL are the most useful measurements for distinguishing between males and females, and are often highly dimorphic in a variety of penguin species (Amat *et al.* 1993, Zavalaga & Paredes 1997, Poisbleau *et al.* 2010, Polito *et al.* 2012). Morphometric sexing is useful when there is limited time to manipulate birds, and can provide instant information in field studies (Zavalaga & Paredes 1997, Poisbleau *et al.* 2010).

Previous studies claim that the chinstrap penguin does not display geographic variation (Marchant & Higgins 1990, Amat *et al.* 1993). In our study, this species exhibited morphological variation among breeding locations for one of our variables, BD, which differed significantly between Admiralty Bay and Stinker Point (Table I). However, the interaction between location and sex was not significant for CW, and there was only a significant difference for BL between males and females, but not between locations (Table I). Despite this, chinstrap penguins at Stinker Point appeared to have a

tendency to have larger morphometric measurements (Fig. 2). However, this trend should be considered with caution, as there is a need for a longer period of sampling. As highlighted by Zavalaga & Paredes (1997), the results of a discriminant function based on one breeding location are not necessarily widely applicable.

Several authors have found discriminant functions that classified adult penguins more accurately. Amat *et al.* (1993) correctly classified 94.6% of chinstrap penguins on Deception Island and Polito *et al.* (2012) correctly classified 96.7% of chinstrap penguins on King George Island. Similarly, Poisbleau *et al.* (2010) correctly classified 96.2% of rockhopper penguins, and Bertellotti *et al.* (2002) correctly classified 97.0% of magellanic penguins. Our discriminant function classified 80.6% of birds correctly; however, applying the discriminant functions of Polito *et al.* (2012) and Amat *et al.* (1993) to our data classified even fewer individuals correctly (67.7% and 71.0% of birds, respectively). Moreover, both discriminant functions also gave a bias towards identifying true females as males. Therefore, the reliability of sexing adults through biometry may be questioned, especially when applying a discriminant function to a region other than where it was developed. Large females, or males that are unusually small or late to develop, will be miscategorized. Despite these limitations, our discriminant function may be useful in the field. It is a fast technique, practical and cost-effective, especially if there is the need to sex a large number of adult penguins at any stage of the breeding season. For individuals in which the value of discriminant score is very close to the cut-off point of $D = -0.076$ or where the researcher has doubts regarding the sex, it is important to use another reliable technique such as molecular sexing. As has been reported previously (Bertellotti *et al.* 2002, Costantini *et al.* 2008, Hart *et al.* 2009, Polito *et al.* 2012), molecular analysis, which is now becoming a relatively easy and inexpensive technique (Costantini *et al.* 2008, Quintana *et al.* 2008), was found to be quite an effective and reliable method for sexing chinstrap penguins.

As a result of the molecular techniques used to determine the sex of chinstrap penguins it was possible to confirm the usefulness of a discriminant function constructed from morphological measurements at Stinker Point, Elephant Island. It is important to remember that discriminant function analyses should be used in the field with caution; where there is doubt, researchers should employ molecular sexing.

Acknowledgements

The Brazilian data were made possible through financial projects like INCT-APA (Instituto Nacional de Ciência e Tecnologia Antártico de Pesquisas Ambientais), (CNPq Process No. 574018/2008-5, FAPERJ E-26/170.023/2008) and were supported by Ministério do Meio Ambiente, Ministério da Ciência, Tecnologia e Inovação and Secretaria da Comissão Interministerial para os Recursos do Mar (SeCIRM) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors are grateful to Roberta da Cruz Piuco (UNISINOS) who conducted all morphological measures in the field activities, and laboratory operators Igor Radamés de Oliveira and Guilherme Pinto Cauduro (UNISINOS) for the technical support provided. The authors are also grateful to Dr Juliano Morales de Oliveira (UNISINOS) for his helpful suggestions and discussions. The authors also extend thanks to the reviewers for their valuable comments.

Author contribution

All authors authorize the publication of this paper and have reviewed the final version.

INCT-APA Project: MVP. Research design: JB, MVP and VHV. Data analysis: JB and VHV. Writing and intellectual contribution: JB and VHV.

Supplemental material

A supplemental table will be found at http://dx.doi.org/10.1017/S0954102014000820.

References

Amat, J.A., Viñuela, J. & Ferrer, M. 1993. Sexing chinstrap penguins (*Pygoscelis antarctica*) by morphological measurements. *Colonial Waterbirds*, **16**, 213–215.

Bertellotti, M., Tella, J.L., Godoy, J.A., Blanco, G., Forero, M.G., Donázar, J.A. & Ceballos, O. 2002. Determining sex of magellanic penguins using molecular procedures and discriminant functions. *Waterbirds*, **25**, 479–484.

Blondel, J., Perret, P., Anstett, M.C. & Thebaud, C. 2002. Evolution of sexual size dimorphism in birds: test of hypotheses using blue tits in contrasted Mediterranean habitats. *Journal of Evolution Biology*, **15**, 440–450.

Calabuig, C.P., Green, A.J., Ferrer, M., Muriel, R. & Moreira, H. 2011. Sexual size dimorphism and sex determination by morphometric measurements in the coscoroba swan. *Studies on Neotropical Fauna and Environment*, **46**, 177–184.

CCAMLR. 2004. *CCAMLR ecosystem, monitoring program, standard methods*. Hobart, TAS: Commission for the Conservation of Antarctic Marine Living Resources. Available at: http://www.ccamlr.org/ru/system/files/std-meth04_2.pdf.

Costantini, V., Guaricci, A.C., Laricchiuta, P., Rausa, F. & Lacalandra, G.M. 2008. DNA sexing in Humboldt penguins (*Spheniscus humboldti*) from feather samples. *Animal Reproduction Science*, **106**, 162–167.

Davis, L.S. & Speirs, E.A. 1990. Mate choice in penguins. *In* Davis, L.S. & Darby, J.T., *eds*. *Penguin biology*. San Diego, CA: Academic Press, 377–397.

De Leon, A., Fargallo, J.A. & Moreno, J. 1998. Parental body size affects meal size in the chinstrap penguin (*Pygoscelis antarctica*). *Polar Biology*, **19**, 358–360.

Genovart, M., McMinn, M. & Bowler, D. 2003. A discriminant function for predicting sex in the Balearic shearwater. *Waterbirds*, **26**, 72–76.

González-Solís, J. 2004. Sexual size dimorphism in northern giant petrels: ecological correlates and scaling. *Oikos*, **105**, 247–254.

Griffiths, R. 2000. Sex identification in birds. *Seminars in Avian and Exotic Pet Medicine*, **9**, 14–26.

Griffiths, R. & Tiwari, B. 1993. The isolation of molecular genetic markers for the identification of sex. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 8324–8326.

Harris, C.M. 2006. *Wildlife awareness manual: Antarctic Peninsula, South Shetland Islands, South Orkney Island*. Wildlife information publication No. 1. Prepared for the UK Foreign and Commonwealth Office. Cambridge: Environmental Research and Assessment, 139 pp.

Hart, T., Fitzcharles, E., Trathan, P.N., Coulson, T. & Rogers, A.D. 2009. Testing and improving the accuracy of discriminant function tests: a comparison between morphometric and molecular sexing in macaroni penguins. *Waterbirds*, **32**, 437–443.

Jakubas, D. & Wojczulanis, K. 2007. Predicting the sex of Dovekies by discriminant analysis. *Waterbirds*, **30**, 92–96.

Marchant, S. & Higgins, P.J. 1990. *Handbook of Australian, New Zealand and Antarctic birds*, vol. 1, Part A. Oxford: Oxford University Press, 1408 pp.

Mariano-Jelicich, R., Madrid, E. & Favero, M. 2007. Sexual dimorphism and diet segregation in the black skimmer *Rynchops niger*. *Ardea*, **95**, 115–124.

Minguez, E., Fargallo, J.A., de Leon, A., Moreno, J. & Moreno, E. 1998. Age-related variations in bill size in chinstrap penguins. *Colonial Waterbirds*, **21**, 66–68.

Moreno, J., Bustamante, J. & Viñuela, J. 1995. Nest maintenance and stone theft in the chinstrap penguin (*Pygoscelis antarctica*) 1. Sex roles and effects on fitness. *Polar Biology*, **15**, 533–540.

Poisbleau, M., Demongin, L., van Noordwijk, H.J., Strange, I.J. & Quillfeldt, P. 2010. Sexual dimorphism and use of morphological measurements to sex adults, immature and chicks of rockhopper penguins. *Ardea*, **98**, 217–224.

Polito, M.J., Clucas, G.V., Hart, T. & Trivelpiece, W.Z. 2012. A simplified method of determining the sex of *Pygoscelis* penguins using bill measurements. *Marine Ornithology*, **40**, 89–94.

Quintana, F., López, G.C. & Somoza, G. 2008. A cheap and quick method for DNA-based sexing of birds. *Waterbirds*, **31**, 485–488.

Serrano-Meneses, M.A. & Székely, T. 2006. Sexual size dimorphism in seabirds: sexual selection, fecundity selection and differential niche-utilisation. *Oikos*, **113**, 385–394.

SPSS. 2009. *SPSS base 10.0 user's guide*. Chicago, IL: SPSS, 548 pp.

Storer, R.W. 1966. Sexual dimorphism and food habits in three North American accipiters. *Auk*, **83**, 423–436.

Valenzuela-Guerra, P., Morales-Moraga, D., González-Acuña, D. & Vianna, J.A. 2013. Geographical variation of gentoo penguin (*Pygoscelis papua*) and sex identification: using morphometric characters and molecular markers. *Polar Biology*, **36**, 10.1007/s00300-013-1389-2.

Williams, T.D. 1995. *The penguins*. Oxford: Oxford University Press, 309 pp.

Zavalaga, C.B. & Paredes, R. 1997. Sex determination of adult Humboldt penguins using morphometric characters. *Journal Field Ornithology*, **68**, 102–112.