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Source: Waterbirds, 37(2):183-190. 2014.
Published By: The Waterbird Society
DOI: http://dx.doi.org/10.1675/063.037.0207
URL: http://www.bioone.org/doi/full/10.1675/063.037.0207

Using Morphometry and Molecular Markers for Sexing South American, Cayenne and Royal Terns Breeding in Patagonia, Argentina

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Abstract.—The first information on the morphometry and sexual size dimorphism of the sympatric South American (*Sterna hirundinacea*), Cayenne (*Thalasseus sandvicensis eurygnathus*) and Royal (*T. maximus maximus*) terns from the Patagonia region in Argentina is provided, and a discriminant analysis to sex the three species is used. Morphological characters were obtained from South American Terns (n = 83), Cayenne Terns (n = 63) and Royal Terns (n = 20). All species were sexed using polymerase chain reaction-based molecular techniques. Sexes in the three tern species were only slightly dimorphic in size. Male Cayenne and South American terns were significantly larger than females in bill length, bill depth and head length. Royal Terns, in contrast, showed a high overlap in most morphological measurements, with head length being the only measurement that differed significantly between sexes. Head length correctly sexed 89% of South American Terns and 75% of Royal Terns, while a function including bill depth and head length correctly sexed 78% of Cayenne Terns. Our results provide a valuable tool for rapid sexing in the field of these three Patagonian terns, although reliable sexing in Cayenne and Royal terns should be preferentially achieved using a combination of morphometric and molecular sexing. *Received 18 September 2013, accepted 10 November 2013.*

Key words.—discriminant analysis, morphometric traits, **s**ex determination, sexual dimorphism, *Sterna hirundinacea*, *Thalasseus maximus maximus*, *Thalasseus sandvicensis eurygnathus*.

Waterbirds 37(2): 183-190, 2014

Seabirds are sexually dimorphic, with body size differences between males and females varying among different taxa (Fairbairn and Shine 1993; Gaston 2004; Serrano-Meneses and Székely 2006). As in other seabird families, species within the Sternidae—terns and allies—exhibit sexual differences in body dimensions with males being larger than females (Gochfeld and Burger 1996), although these differences are slight and difficult to determine visually. The ability to sex individuals is particularly important to answer many ecological and behavioral questions and to effectively develop conservation and management actions. For example, sexing of individuals has been fundamental for the study of tern foraging patterns (Bluso-Demmers *et al.* 2008), feeding behavior (Wagner and Safina 1989), mating systems (Nisbet *et al.* 2007), demography (Dittmann *et al.* 2005), and differential effects of pollutants (Herring *et al.* 2010b).

Despite the similarity in body size between sexes in most terns, individuals of several species have been successfully sexed using a combination of body measurements such as head length, bill length, bill depth, wing length and/or tarsus length. Sex identification using these external measurements and discriminant analyses has been shown in several tern species (Stern and Jarvis 1991; Fletcher and Hamer 2003; Reynolds *et al.* 2008; Ledwon 2011). Discriminant functions developed in these studies allowed the correct assignment of sex in 72% to 95% of individuals.

Three tern species are found breeding along the Patagonian coast of Argentina: the South American (*Sterna hirundinacea*), Cayenne (*Thalasseus sandvicensis eurygnathus*) and Royal (*T. maximus maximus*) terns. The South American Tern is endemic to South America, breeding from the coasts of southern Peru and central Brazil to Tierra del Fuego, Argentina, including the Malvinas Islands (Gochfeld and Burger 1996). In Patagonia, they have been recorded breeding in over 40 locations, with a yet unknown total breeding population (Yorio *et al.* 1999; Yorio 2005). Cayenne and Royal terns are widely distributed in the Americas (Gochfeld and Burger 1996; Shealer 1999; Buck-

ley and Buckley 2002; Yorio and Efe 2008). Although the Cayenne Tern has long been treated as one of two subspecies of Sandwich Tern in the Americas (Shealer 1999), recent molecular evidence (Efe *et al.* 2009) does not support subspecific status for Cayenne Tern. Rather, mtDNA analysis indicates two species: the nominate Sandwich Tern (*T. sandvicensis*), which breeds in Eurasia and winters in Africa, and Cabot's Tern (*T. acuflavida*) which breeds along eastern coastal North and South America and in the Caribbean (Efe *et al.* 2009). The British Ornithologists' Union has accepted this split (British Ornithologists' Union 2013), but the American Ornithologists' Union has not (Remsen *et al.* 2013). We therefore retain the original nomenclature for Cayenne Tern (*T. sandvicensis eurygnatha*) in this paper, recognizing that it is likely conspecific with what is now referred to as Cabot's Tern (*T. acuflavida*), as proposed by Efe *et al.* (2009). In Argentina, Cayenne Terns have been recorded in at least 23 locations with an estimated population of less than 10,000 pairs, while Royal Terns have been recorded in at least 14 locations with an estimated breeding population of less than 5,000 pairs (Yorio and Efe 2008). Very little is known about their breeding ecology and behavior (Yorio 2005), and the development of a method for rapid sexing in the field will greatly aid in future theoretical and applied research on these species. Our goals were to: 1) provide the first information on the morphometry and sexual size dimorphism of South American, Cayenne and Royal terns from Argentine Patagonia; and 2) develop a discriminant function based on morphometric data and molecular markers to reliably sex these three tern species.

METHODS

Study Sites and Species

The study was conducted in the Punta Loma (42° 49' S, 64° 53' W) and Punta León (43° 04' S, 64° 29' W) Provincial Protected Areas, Chubut, Argentina, between 2004 and 2006. South American Terns were studied at the Punta Loma colony. Their colony was located on the edge of the cliffs and comprised about 3,200 pairs (A. Gatto and P. Yorio, unpubl. data). South American Terns arrive at Punta Loma during mid-October and settle at the colony site during the first and second week of November. First chicks hatch during the first and second week of December and start to fledge at about 4 weeks of age (Villanueva Gomila *et al.* 2009). Cayenne and Royal terns were studied at the Punta León colony. At this location, a silt platform of ~5 ha lying to the seaward side of the cliffs and covered by vegetation is used for nesting by several seabird species. Colony sizes of Cayenne and Royal terns were estimated at 1,050 and 450, respectively (Gatto 2009). Cayenne and Royal terns arrive at Punta León during mid-September and settle in a single colony during the second and third weeks of October (Yorio *et al.* 1994). First chicks hatch during the first and second weeks of November and stay at their nest until they are 20 days old, when they start to congregate in crèches (Quintana and Yorio 1997).

Field Methods

Morphometric measurements were obtained from South American Terns ($n = 83$), Cayenne Terns ($n = 63$) and Royal Terns ($n = 20$). Most of the birds were captured at night using mist nets at the colony periphery and some using nest traps during the egg-laying, chick hatching and late chick periods. Eight Cayenne Terns and one Royal Tern were found freshly dead near the colony. Six morphological characters were measured, including body mass, head length (from the back of the head to the tip of the bill), bill length (from the feathering at the base of the bill to the tip of the bill), bill depth (from the bottom of the mandible to the top of the maxilla above the nostril), tarsus length (from the midtarsal joint to the distal end of the tarsometatarsus), and wing length (from the carpal joint to the tip of the longest primary feather). Body mass was recorded only on live individuals using spring scales (to the nearest 2 g for South American and Cayenne terns and to the nearest 5 g for Royal Terns). Head length, bill length and depth, and tarsus length were measured to the nearest 0.1 mm using calipers, while wing length was measured to the nearest mm using a metal ruler. For some individuals, gape width, tarsus diameter, and tail length were also measured. Measurements were taken by three trained researchers following an established protocol. Whole blood samples were extracted from the brachial vein of a sample of individuals, and a portion was placed on a commercial filter paper, air-dried, and stored in a sealed plastic bag.

Molecular Sexing

Molecular sexing was performed using Fridolfsson and Ellegren (1999). We used a pair of highly conserved primers (2550F_5'-GTTACTGATTCGTCTACGAGA-3' and 2718R_5'-ATTGAAATGATCCAGTGCTTG-3') that amplified different sizes of an intron within the CHD1 gene in each of the sex chromosomes. CHD1-Z fragments vary between 600 and 650 bp in size, and CHD1-W fragments vary between 400 and 450 bp. DNA extraction was performed using a modification of the salting-out method proposed by Aljanabi and Martinez (1997). Dried blood samples preserved in filter paper were cut into small pieces and placed into 1.5 ml Eppendorf

tubes. The lysis buffer consisted of 1 M Tris-HCl, 0.5 M EDTA, 20% SDS, 5 M NaCl at pH 8 and Proteinase K (1 mg/ml). After incubation for 3 hr at 56 °C, the extraction was performed with 5 M NaCl and DNA was precipitated using Isopropanol 100%, washed with 70% ethanol, dried and re-suspended in TE buffer. Purified DNA was preserved at 4 °C. Fragments of the CHD1 gene were polymerase chain reaction (PCR) amplified in a 25 µl-volume reaction containing 0.25 µL Taq (Invitrogen), 5 µL of PCR Buffer 10X, 4 µL $MgCl_2$ 50 mM, 1 µL dNTPs 10 mM, 1.5 µL of each primer (10 pmol), and 1 µL of template DNA. PCR amplifications were performed in a Veriti Thermal Cycler (Applied Biosystems) and the cycling conditions were as follows: initial denaturing step of 2 min at 95 °C, then 40 cycles of 95 °C for 30 sec, 48 °C for 45 sec and 72 °C for 45 sec, and a final extension of 6 min at 72 °C. The PCR products were separated by electrophoresis at 1% agarose gels in TAE 1X buffer, stained in ethidium bromide solution at 1% and visualized on a UV transilluminator, revealing the presence of one or two bands, being males or females respectively.

Analysis

To characterize sexual size dimorphism and variability in morphometric traits of the three tern species studied here, six variables were selected: body mass, bill length, bill depth, tarsus length, head length, and wing length. Sex differences in the selected traits were tested by one-way ANOVA using R software (R Development Core Team 2013). Normal probability plots were used to test if measured traits were normally distributed. Coefficients of variation in all selected measurements were calculated for each sex, and the average within a species was reported to indicate the degree of variability (Fletcher and Hamer 2003). Also a sexual size dimorphism index (SSD) was calculated for all selected variables: SSD = 100 x [(mean male size/mean female size) - 1] (Fletcher and Hamer 2003).

Discriminant analyses were performed to obtain reliable functions to determine the sex of the three tern species studied here based on external measurements. Univariate discriminant analyses were performed for each of the selected morphometric measurements. Then forward discriminant analyses were performed to obtain a combination of measurements that best classified the sexes. Two variables were excluded from the discriminant analyses: bill length because it is included in the head length measurement, and body mass because it may show annual and seasonal variation. To avoid reducing the sample size in the data sets, the discriminant analyses were performed with only those variables that had no missing values. The criterion used for variable selection was the statistic Wilks' Lambda obtained using R software (R Development Core Team 2013). The effectiveness of the classification was first assessed in terms of the proportion of birds of known sex that were correctly classified and, finally, by a jackknife procedure (cross-validation) in which each individual was classified using a function derived from the total sample excluding the individual being classified (Tabachnick and Fidell 1996).

RESULTS

The three species showed differences in all body measurements, with the Royal Tern the largest species and the South American Tern the smallest (Table 1).

South American Tern

Males were significantly larger than females in bill length, bill depth and head length ($P < 0.05$; Table 2). The morphometric measure with the highest sexual size dimorphism was bill length, while body mass and tarsus length presented the lowest (Table 2). Tarsus length showed the highest within sex variation, and head length the lowest (Table 2). The single measured characters that significantly predicted the sex of South American Terns were head length and bill depth ($P < 0.05$; Table 3). Head length was the most accurate single variable indicating sex (89% of South American Terns

Table 1. Morphological measurements (mean ± SD, Range (sample size)) of South American, Cayenne and Royal terns. All measurements are given in mm, except body mass in g.

Variable	Royal Tern	Cayenne Tern	South American Tern
Body mass	465 ± 30.6, 405-510 (19)	246 ± 21.0, 210-320 (56)	177 ± 15.6, 150-246 (83)
Head length	124.5 ± 2.96, 118.0-129.7 (20)	108.1 ± 4.3, 97.9-116.3 (63)	86.4 ± 3.7, 78.4-102.6 (83)
Bill length	63.8 ± 3.09, 56.8-69.8 (20)	59.2 ± 2.9, 52.6-66.1 (63)	41.2 ± 2.4, 34.8-46.6 (83)
Bill depth	16.1 ± 0.49, 14.8-16.6 (12)	12.1 ± 1.1, 8.1-15.6 (55)	9.3 ± 0.6, 8.0-11.0 (73)
Gape width	22.9 ± 2.40, 16.9-25.8 (11)	18.6 ± 2.1, 14.6-23.6 (54)	16.1 ± 2.28, 11.5-22.4 (72)
Tarsus length	36.0 ± 2.82, 32.2-42.5 (20)	29.5 ± 3.0, 24.3-36.4 (63)	23.3 ± 4.2, 18.4-54.5 (83)
Tarsus width	5.6 ± 0.23, 5.4-5.8 (3)	3.9 ± 0.7, 2.6-5.0 (22)	3.5 ± 0.5, 3.0-4.2 (4)
Wing length	375.6 ± 10.8, 356-392 (18)	310.9 ± 12.9, 246-332 (59)	306.8 ± 13.4, 280-393 (79)
Tail length	—	138.5 ± 9.1, 126-159 (24)	—

Table 2. Body dimensions and masses (mean ± SD, Range (sample size)), sexual size dimorphism (SSD) and coefficients of variation (CV) of female and male South American, Cayenne and Royal terns. All measurements are given in mm, except body mass in g. F-values and df are given for the results of ANOVAs comparing the two sexes.

Variable	Female	Male	*F*-value	*P*-value	SSD (%)	CV (%)
South American Tern						
Body mass	178 ± 14.7, 150-198 (20)	176 ± 13.2, 160-200 (18)	$F_{1,36}$ = 0.1	0.740	-0.86	7.890
Bill length	39.4 ± 1.78, 34.8-41.9 (20)	42.8 ± 2.19, 38.2-46.6 (18)	$F_{1,36}$ = 27.5	0.001	8.59	4.825
Bill depth	9.0 ± 0.38, 8.6-9.7 (15)	9.5 ± 0.63, 8.7-11.0 (15)	$F_{1,28}$ = 7.5	0.011	5.77	5.420
Tarsus length	22.8 ± 2.90, 19.9-28.3 (20)	22.6 ± 2.43, 19.0-29.2 (18)	$F_{1,36}$ = 0.1	0.749	-1.23	11.725
Head length	84.7 ± 2.42, 78.7-87.9 (20)	89.7 ± 3.77, 84.9-102.6 (18)	$F_{1,36}$ = 24.5	0.001	5.95	3.530
Wing length	303.3 ± 11.07, 280-324 (18)	309.6 ± 22.32, 285-393 (18)	$F_{1,34}$ = 1.1	0.293	2.07	5.430
Cayenne Tern						
Body mass	241 ± 26.46, 212-310 (11)	245 ± 15.49, 214-270 (25)	$F_{1,35}$ = 0.4	0.529	1.83	8.630
Bill length	57.3 ± 2.69, 52.6-62.95 (17)	60.7 ± 2.58, 56.7-66.1 (28)	$F_{1,43}$ = 17.0	0.001	5.81	4.475
Bill depth	11.7 ± 0.65, 10.61-12.9 (17)	12.6 ± 0.84, 11.6-15.6 (28)	$F_{1,43}$ = 13.2	0.001	7.40	6.125
Tarsus length	28.1 ± 2.54, 24.3-35.9 (17)	28.7 ± 2.09, 24.5-35.3 (28)	$F_{1,43}$ = 0.8	0.379	2.21	8.170
Head length	105.5 ± 3.28, 97.9-113.2 (17)	109.2 ± 4.49, 98.9-116.3 (28)	$F_{1,43}$ = 8.8	0.005	3.53	3.610
Wing length	307.5 ± 17.58, 246-324 (16)	308.4 ± 10.22, 280-330 (25)	$F_{1,39}$ = 0.0	0.829	0.31	4.515
Royal Tern						
Body mass	454 ± 34.03, 405-510 (10)	477 ± 22.24, 445-510 (9)	$F_{1,17}$ = 2.9	0.107	5.00	6.075
Bill length	63.5 ± 3.52, 56.8-69.8 (11)	64.10 ± 2.64, 59.6-68.2 (9)	$F_{1,18}$ = 0.2	0.663	0.99	4.835
Bill depth	15.9 ± 0.61, 14.8-16.6 (7)	16.3 ± 0.23, 16.1-16.6 (5)	$F_{1,10}$ = 1.1	0.311	1.92	2.600
Tarsus length	35.7 ± 3.04, 32.2-42.5 (11)	36.3 ± 2.67, 32.7-39.9 (9)	$F_{1,18}$ = 0.2	0.683	1.51	7.940
Head length	123.3 ± 2.44, 118.0-127.6 (11)	125.9 ± 3.03, 121.7-129.7 (9)	$F_{1,18}$ = 4.5	0.048	2.10	2.190
Wing length	371.9 ± 12.37, 356-392 (10)	380.1 ± 6.40, 370-387 (8)	$F_{1,16}$ = 2.9	0.108	2.21	2.505

correctly classified). South American Terns with head length values larger than 87.2 mm (cut-off value) were classified as males while those with lower values were classified as females (Table 3). Bill depth was also a good predictor of sex (60% of South American Terns correctly classified). South American Terns with bill depth values larger than 9.2 mm (cut-off value) were classified as males while those with lower values were classified as females (Table 3). Forward discriminant analysis applied to the two selected variables that maximized sample size (head length and tarsus length) produced one significant discriminant function ($P < 0.001$). The discriminant function obtained included head length and tarsus length as discriminatory variables (Table 3). The discriminant function was:

$$D_1 = (\text{head length} \times 0.34) - (\text{tarsus length} \times 0.16) - 25.97$$

This discriminant function correctly sexed 81% of the South American Terns (Table 3), less than the head length as a

Table 3. Percentage of correctly classified South American, Cayenne, and Royal terns by single measurements and discriminant functions (D_1).

Variable	Wilk's Lambda	*F*-value	*P*-value	Males	Females	Total
South American Tern (n = 38)						
Head length	0.595	$F_{1,36}$ = 24.5	$P < 0.001$	83	95	89
D_1	0.553	$F_{2,35}$ = 14.1	$P < 0.001$	78	85	81
Cayenne Tern (n = 45)						
Bill depth	0.765	$F_{1,43}$ = 13.2	$P < 0.007$	61	71	64
Head length	0.829	$F_{1,43}$ = 13.2	$P < 0.049$	71	71	71
D_1	0.694	$F_{2,42}$ = 9.2	$P < 0.001$	71	88	78
Royal Tern (n = 20)						
Head length	0.800	$F_{1,18}$ = 4.5	$P < 0.048$	67	82	75

single variable. The discriminant function misclassified four males and three females; however, head length misclassified only one male and three females. The jackknife validation provided the same classification as that produced by the discriminant analyses in both cases.

Cayenne Tern

Males were significantly larger than females in bill length, bill depth and head length ($P < 0.05$; Table 2). The morphometric measure with the highest sexual size dimorphism was bill depth (Table 2). Body mass was the variable with the highest within sex variation while head length presented the lowest (Table 2). The single measured characters that significantly predicted the sex of Cayenne Terns were bill depth and head length ($P < 0.05$; Table 3). Head length was the most accurate single variable indicating sex (71% of Cayenne Terns correctly classified). Cayenne Terns with head length values larger than 107.4 mm (cut-off value) were classified as males while those with lower values were classified as females (Table 3). Bill depth was also a good predictor of sex (64% of Cayenne Terns correctly classified). Cayenne Terns with bill depth values larger than 12.2 mm (cut-off value) were classified as males while those with lower values were classified as females (Table 3). Forward discriminant analysis produced one significant discriminant function ($P < 0.001$). The discriminant function obtained included bill depth and head length as discriminatory variables (Table 3). The discriminant function was:

$$D_1 = (\text{bill depth} \times 0.95) + (\text{head length} \times 0.14) - 26.44$$

The discriminant function correctly sexed 78% of the Cayenne Terns (Table 3). The discriminant function misclassified eight males and two females. The jackknife validation provided the same classifications as those produced by the discriminant function.

Royal Tern

Males were significantly larger than females only in the head length ($P < 0.05$; Table 2). The morphometric measure with highest sexual size dimorphism was body mass. Tarsus length was the variable with the highest within sex variation while head length presented the lowest (Table 2). Forward discriminant analysis applied to the two selected variables (head length and tarsus length) resulted in head length being the only variable that significantly discriminated sex ($P < 0.05$). Royal Terns with head length values larger than 124.6 mm (cut-off value) were classified as males while those with lower values were classified as females (Table 3). Head length correctly sexed 75% of the Royal Terns (Table 3). This variable misclassified three males and two females. The jackknife validation provided the same classification as that produced by the discriminant analysis.

DISCUSSION

Our results constitute the first morphological characterization of South American, Cayenne and Royal terns breeding in Argentina, although data is available from Brazil for Cayenne Terns (Efe *et al.* 2004). No information on body dimensions and mass is available for South American Terns, and published information on body-size measurements of Cayenne and Royal terns is scant, based on a small sample of individuals, and mostly museum specimens (Voous 1968; Escalante 1970, 1985). Unfortunately, the characteristics of published data sets, including small sample sizes, do not allow adequate comparisons with our data. The three tern species studied here showed differences in body measurements, with Royal Tern the largest species and South American Tern the smallest. Body-size differences have been shown to be important in the ecological segregation of several sympatric terns, contributing to prey-size partitioning (Ashmole 1968; Lemmetyinen 1976; Hulsman 1987). Royal and Cayenne terns nest in mixed-species colonies throughout their distributional range in South America (Yorio and Efe 2008), and there are several sites along the Patagonian coast where these two species also nest syntopically with the South American Tern (Yorio 2005).

As shown in other Sternidae species, South American, Cayenne and Royal terns are only slightly dimorphic. Although males were on average larger than females in both the South American and Cayenne terns, significant differences were only found in bill length, bill depth and head length. Royal Terns showed a high overlap in most morphological measurements with significant sex differences only in the head length. Body mass in this species exhibited the highest sexual size dimorphism, although this variable did not differ significantly between males and females. Within Sternidae, the degree of sexual differences in body dimensions and body mass depends on the species considered. In some species, such as the Brown Noddy (*Anous stolidus*) (Chardine and Morris 1989), Roseate Tern (*S. dougallii*) (Palestis *et al.* 2012) and Whiskered Tern (*Chlidonias hybrida*) (Lewdon 2011), significant differences between sexes were found in all of the analyzed variables. In the Caspian Tern (*Hydroprogne caspia*), in contrast, most morphological measurements analyzed were similar between sexes (Ackerman *et al.* 2008), as observed in the Royal Tern in the present study.

Head length was the best predictor of the gender of South American Terns, and the accuracy of sex determination achieved in this species (89%) was the highest among the three Patagonian terns. In the Cayenne Tern, head length also showed the highest discrimination power, but a greater accuracy was reached when it was used in combination with bill depth. In Royal Terns, head length accurately predicted only 75% of cases, although sample size used for the discriminant analysis was relatively small and further studies using a larger sample of individuals may be needed to adequately assess sexual size differences and develop sexing tools for this species. The percentages of successfully sexed individuals in the three Patagonian species are within the range recorded for other tern species in previous studies, which varied from 73% in Arctic Terns (*S. paradisaea*) (Fletcher and Hamer 2003) to 95% in Whiskered Tern (Lewdon 2011), but was generally under 90%.

Among the morphological measurements assessed in this study, head length proved to be an important variable in discriminant functions for the three Patagonian terns. This is a reliable variable easy to measure in the field, and in the three tern species it showed the lower coefficient of variation among measured body variables. Head length is the most important variable used to discriminate males from females in terns, gulls and skimmers (e.g., Coulson *et al.* 1983; Quinn 1990; Nisbet *et al.* 2007), and has been also used in species belonging to other seabird groups (Quintana *et al.* 2003; Guicking *et al.* 2004; Setiawan *et al.* 2004). Head length has been also used in combination with body mass to sex Black Terns (*C. niger*) (Shealer and Cleary 2007), or in combination with other body measures such as wing length in Sooty Terns (*Onychoprion fuscata*) (Reynolds *et al.* 2008) and Brown Noddies (Chardine and Morris 1989). As found for the Cayenne Tern in this study, discriminant functions combining head length and bill depth have been generated to sex Forster's Terns (*S. forsteri*) (Bluso *et al.* 2006), Arctic Terns (Devlin *et al.* 2004), and Caspian Terns (Ackerman *et al.* 2008).

Our results provide a valuable tool for rapid sexing in the field of three Patagonian terns. Reliable sexing in Cayenne and Royal terns, however, should be preferentially achieved using a combination of morphometric and molecular sexing. In addition, several studies in Larids and other seabirds have shown that individuals from different populations of the same species may differ in size (e.g., Granadeiro 1993; Shealer and Cleary 2007; Herring *et al.* 2010a). Although quantitative information on the regional variability of Patagonian terns is lacking, information presented in Efe *et al.* (2004) for Cayenne Terns in Brazil, for example, suggests individuals are smaller than in our study area. Thus, future studies should assess the validity of the functions generated in this study in other South American, Cayenne and Royal tern populations in the region.

ACKNOWLEDGMENTS

Research was supported by the Wildlife Conservation Society, The Waterbird Society 2005 Nisbet Re-

search Grant, and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). We thank Germán García, Luján Villanueva Gomila, Karina Cabral, Alexa Sapoznikow, Pim Edelaar, Javier Lenzi, Sebastián Jiménez, Fabián Pérez, María Cruz Sueiro, Laura Silva, and Verónica Borrell for field assistance; Bernabé Urrutia and people from Estancias 'El Pedral' and 'Bahía Cracker' for logistical support; and Marcio Efe and Kees Hulsman for helpful advice during field work. We thank Organismo Provincial de Turismo of Chubut for the permits to work at the Punta León and Punta Loma Protected Areas and the Dirección de Fauna y Flora Silvestre of Chubut for the permits to capture individuals.

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