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SEX DETERMINATION OF ADULT HUMBOLDT PENGUINS USING MORPHOMETRIC CHARACTERS

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Abstract.—The Humboldt Penguin (*Spheniscus humboldti*) is one of the most endangered and least-studied species of penguins. Studies of its breeding ecology are needed to develop management plans for its conservation, and sex determination is essential to measure certain reproductive parameters. Humboldt Penguins, like other species of penguins, showed sexual size dimorphism, with males being heavier and larger than females. One discriminant function was derived from external measurements of 297 wild adult Humboldt Penguins of known sex (146 females and 151 males) at Punta San Juan, Peru. The sex of 95% of these birds could be correctly determined using the discriminant function $D_1 = -38.98 + 3.16(WH) + 3.69(BL)$ where WH is width of the head and BL is bill length. In addition, the sex of 91% of the birds were correctly classified by means of the bill length using the univariate function $D_2 = -6.31 + BL$. In both equations, if $D > 0$ the bird was classified as male, if $D < 0$ it was a female, and if $D = 0$, the sex could not be identified. The discriminant function D_1 was not accurate to sex a group of captive Humboldt Penguins (71% successfully classified) because there were differences in size of the head between wild and captive birds. However, we obtained 83% of cases successfully classified using the function D_2 as bill length was similar between wild and captive birds. The discriminant function may not be applicable to other wild and captive populations of Humboldt Penguins.

DETERMINACIÓN DEL SEXO EN ADULTOS DE *SPHENISCUS HUMBOLDTI* UTILIZANDO CARACTERÍSTICAS MORFOMÉTRICAS

Sinopsis.—Dentro de las 17 especies de pingüinos, el de Humboldt (*Spheniscus humboldti*) es uno de los más amenazados y menos estudiados. Para llevar a cabo planes de manejo dirigidos a su conservación, son necesarios estudios sobre su ecología reproductiva, siendo la determinación del sexo esencial para medir algunos de sus parámetros reproductivos. Los pingüinos de Humboldt son sexualmente dimórficos en sus medidas corporales, siendo los machos más pesados y grandes que las hembras. Una función discriminante fue obtenida a partir de las medidas de 297 adultos de sexo conocido (146 hembras y 151 machos) en Punta San Juan, Perú. El sexo de estas aves pudo ser identificado correctamente en un 95% de los casos utilizando la función discriminante $D_1 = -38.98 + 3.16\ (WH) + 3.69\ (BL)$, donde WH es el ancho de la cabeza y BL la longitud del pico. De igual manera se identificó el sexo del 91% de los pingüinos por medio de la ecuación univariada $D_2 = -6.31 + BL$. Para ambas ecuaciones, si $D > 0$ el ave fue clasificada como macho, si $D < 0$ como hembra y si $D = 0$, el ave no era clasificada. La función discriminante D_1 no fue tan exitosa en la determinación del sexo de pingüinos cautivos (sólo el 71% de los casos fue correctamente clasificado) debido a que estas aves exhibieron cabezas más grandes que aquellas medidas en el campo. Sin embargo, el sexo del 83% de los pinguinos pudo ser identificado utilizando la ecuacion D_2 debido a que la longitud del pico fue similar entre los pingüinos silvestres y los cautivos. La función discriminante desarrollada no necesariamente pudiera aplicarse a otras poblaciones de pingüinos de Humboldt ya sea silvestres o cautivos.

The Humboldt Penguin (*Spheniscus humboldti*) is one of the most threatened and least-studied species of penguins (Boersma 1991, Hays

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1984). Because of a sharp decline in the wild population (Hays 1984), studies of their ecology and behavior are needed for developing effective conservation plans. To achieve most of these studies, the ability to determine the sex of individuals is essential for investigating sex-specific behavior (Davis 1988) or measuring certain reproductive parameters (CCAMLR 1994). Determining sex of captive Humboldt Penguins in zoos is also important in order to maximize successful reproduction in captive birds (Cheney 1990, McGill and Perkins 1993, Steveson 1993).

Humboldt Penguins, like most seabird species, lack plumage characters by which sexes may be recognized. However, small differences in morphometric parameters reveal sufficient dimorphism to distinguish sexes (Murphy 1936:452, Scholten 1987). Characters used to determine sex in penguins include vent measurements (Boersma and Davies 1987), sexual behavior (Davis 1988, Edgington 1989, Scholten 1992), morphometric differences in mated pairs (Edgington 1989) and temporal attendance during incubation (Kerry et al. 1993). Some of these methods have been used successfully in the wild and in captivity, but only on reproductive individuals during early parts of the breeding season. Cloacal examination has been used in wild Adélie Penguins (*Pygoscelis adeliae*; Lishman 1985, Sladen 1978), Chinstrap Penguins, (*Pygoscelis antarctica*; Lishman 1985) and captive Humboldt Penguins (Yamazaki et al. 1994). However, this technique requires trained researchers and specialized equipment to complete with minimal stress to the animal. Other methods, such as chromosome analysis, hormone analysis, and laparoscopy are effective but time-consuming and expensive (Edgington 1989).

Sex determination by discriminant analysis of external measurements has been used successfully in the wild in several penguin species such as Magellanic Penguin (*Spheniscus magellanicus*, Scolaro et al. 1983), Yellow-eyed Penguin (*Megadyptes antipodes*, Darby and Seddon 1990), all pygoscelid penguins (Amat et al. 1993, Kerry et al. 1992, Williams 1990), Little Blue Penguin (*Eudyptula minor novaehollandiae*, Gales 1988), and others. Discriminant analysis uses morphometric differences between known male and female birds to calculate a function that predicts the sex of unknown individuals. This technique is reliable, practical, fast, inexpensive, non-invasive, and can be used outside of the breeding season.

The objectives of this study were (1) to determine discriminant function(s) using external measurements with which to determine the sex of wild adult Humboldt Penguins at Punta San Juan, Peru and (2) to test the applicability of these functions on captive Humboldt Penguins.

MATERIALS AND METHODS

Measurements of wild penguins.—Between September 1992 and June 1993, the bodies of 223 dead adult Humboldt Penguins were recovered from the port of San Juan de Marcona, 3 km north of Punta San Juan (15°22′S, 75°12′W), a 54-ha guano bird reserve on the southern coast of Peru. This reserve holds one of the largest populations of Humboldt Penguins in the country (Hays 1984). The birds were accidentally caught and drowned in the nets of small boats in the course of normal fishing operations based at Punta San Juan along 50–60 km of the coast (within 5 km offshore). Thus, the penguins caught may originate from Punta San Juan as well as from neighboring colonies such as Sombrerillo, San Nicolás, and San Fernando.

Immediately after collection, carcasses were weighed to the nearest 100 g with a 10-kg Pesola spring balance. Head and bill were measured with vernier calipers to the nearest 0.1 mm and the flipper was measured with a ruler to the nearest 0.1 cm. The measurements included: total length of the head (LH) from supraoccipital to the tip of the bill; width of the head (WH) in a crevice just posterior to the bulge behind the eyes; bill length (BL) from the edge of implantation of feathers to the tip of the culmen; bill depth (BD) measured dorso-ventrally at the nostrils and flipper length (FL), maximum flattened chord, from the humero-radial joint to the tip of the flipper (Fig. 1). The sex of each individual was determined by gonad examination after dissection.

Discriminant functions were derived from the measurements taken from the 223 carcasses (analysis sample). These data were analyzed by stepwise discriminant function analysis with the SPSS/PC + 4.0.1 statistical package (Norusis 1990). The Wilks' lambda ratio was used as a criterion for variable selection. All other statistical analysis were carried out using the SYSTAT 5.0 statistical package (Wilkinson 1991).

To test the accuracy of our results, we measured 74 breeding wild penguins of known sex (validation sample). These animals were banded and measured between 1992 and 1994 at Punta San Juan as part of a long-term study of the breeding ecology of this species. The sex of these birds was identified from their position during copulation.

Body mass was excluded from the discriminant analysis because there was a significant amount of seasonal variability (CV = 13.6%). Also, the majority of dead penguins were wet and their lungs were full of water (due to drowning) at the time of weighing, which would bias this measurement (Table 1). These penguins were 4.8% heavier than a sample of 288 living penguins of known sex (the sex of these individuals was determined from the discriminant function reported below) weighed at the same time at Punta San Juan (males: $\bar{x}$ = 4711.06 g, SE = 31.55, range = 3450–6000, n = 165; females: $\bar{x}$ = 4047.39 g, SE = 35.15, range = 2950–5400, n = 123; pooled: $\bar{x}$ = 4427.62 g, SE = 30.42, range = 2950–6000, n = 288). These differences were significant (z-test, z = 98, $P <$ 0.05).

All measurements were normally distributed (Kolmogorov-Smirnov one sample test, $P >$ 0.05 in all tests) with some overlap between sexes. In order to determine a discriminant function, we established the same cri-

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FIGURE 1. Variables measured to sex adult Humboldt Penguins. BL = bill length; BD = bill depth; LH = length of the head; WH = width of the head; FL = flipper length.

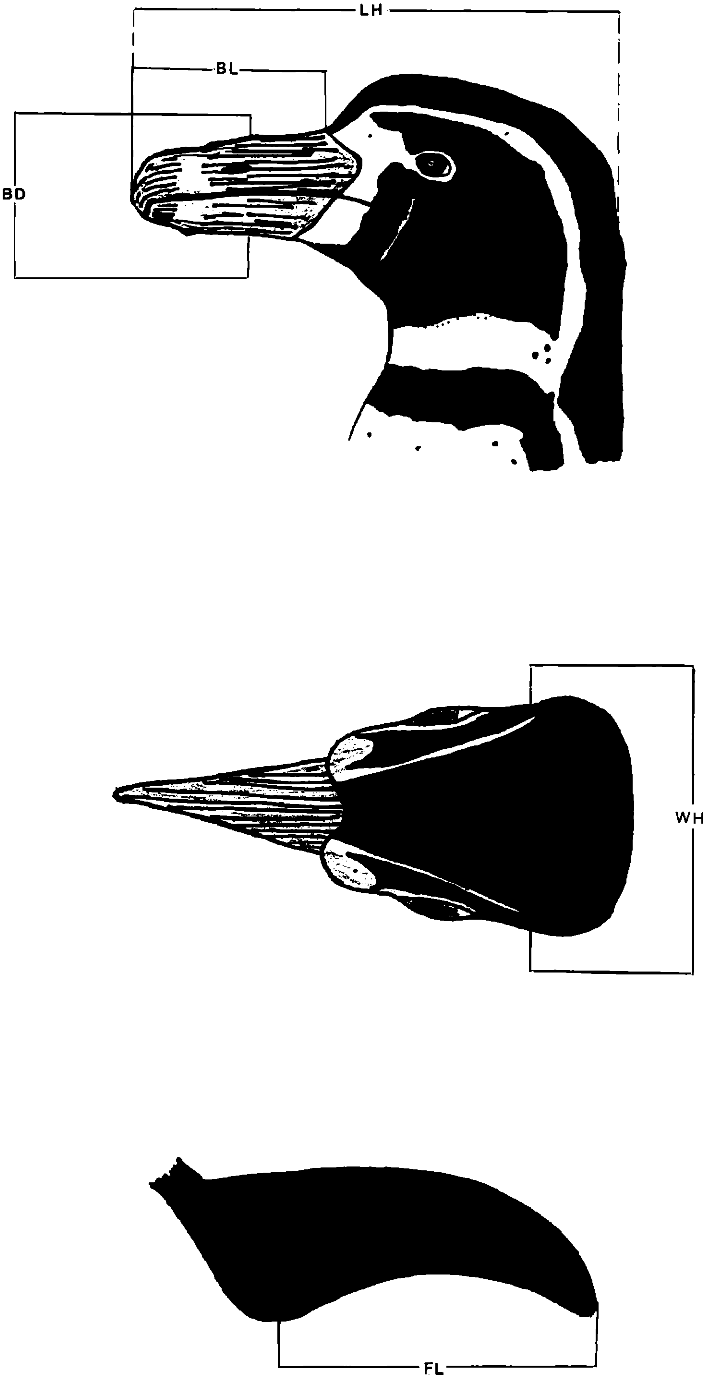
LH
BL
BD
WH
FL

TABLE 1. Morphometric data of drowned Humboldt Penguins (*Spheniscus humboldti*). Range is given in parentheses. Body mass is in grams, other measurements are in cm.

Variable	Males (n = 112) $\bar{x} \pm$ SE	Females (n = 111) $\bar{x} \pm$ SE	Pooled (n = 223) $\bar{x} \pm$ SE	z-test z	z-test P
Body mass[a]	4931.08 ± 56.05 (3500–7000)	4317.85 ± 52.15 (3200–6000)	4643.54 ± 43.90	8.00	<0.001
Length of the head	13.52 ± 0.03 (12.82–14.95)	12.68 ± 0.03 (11.92–13.72)	13.10 ± 0.04	19.04	<0.001
Width of the head	5.13 ± 0.02 (4.73–5.84)	4.75 ± 0.02 (4.33–5.22)	4.92 ± 0.02	16.04	<0.001
Bill length	6.54 ± 0.02 (5.98–7.02)	6.08 ± 0.02 (5.55–6.58)	6.31 ± 0.02	16.80	<0.001
Bill depth	2.56 ± 0.01 (2.16–2.90)	2.27 ± 0.01 (1.90–2.73)	2.42 ± 0.01	15.71	<0.001
Flipper length[b]	15.63 ± 0.06 (14.4–17.3)	14.93 ± 0.05 (13.5–16.5)	15.29 ± 0.05	8.47	<0.001

[a] Body mass was recorded for 111 males and 98 females only. These birds were wet at the time of collection and were 4.8% heavier than a sample of 288 wild living penguins (see text).

[b] Flipper length was recorded for 98 males and 103 females only.

teria used by Kerry et al. (1992) for discriminant analysis: (1) characters were not linear combinations of each other; (2) correlation coefficients between characteristics used for the final discriminant function were less than 0.60; and (3) the variance-covariance matrices were not significantly different (Box's M-statistic = 6.02; $F = 1.98$; $P = 0.1140$).

Measurements of captive penguins.—to test the efficiency of the discriminant functions for determining the sex of captive Humboldt Penguins, we evaluated measurements of 35 birds of known sex. These birds were measured during May 1995 by the bird keepers at the Metro Washington Park Zoo, Portland, Oregon, USA. The sex of these individuals was determined by genetic karyotyping.

RESULTS

Determination of sex of wild penguins.—The measurements of 223 dead adult Humboldt penguins (112 males and 111 females) are presented in Table 1. Males were significantly larger than females (*z*-test, $P < 0.05$) for all morphometric characters (Table 1). Given some overlapping male and female distributions of each univariate character, the interception point between both curves was determined from the analysis sample, obtaining the following values for sex separation: LH = 13.09, WH = 4.94, BL = 6.31, BD = 2.42, FL = 15.29 (all measurements in cm). If the measurement of some of these variables was higher than its respective cutoff value the penguin was classified as a male, if lower as a female, and if equal the sex could not be determined. The percentage of cases correctly classified for the validation sample were 91%, 89%, 88%, 78% and 70% for BL, BD, LH, WH and FL, respectively.

Following the criteria of Kerry et al. (1992), only WH and BL were significantly selected by the stepwise discriminant analysis (Wilks' Lambda = 0.321, $\chi^2 = 250.03$, df = 2, $P < 0.001$). On the basis of Wilks' Lambda values and the number of cases correctly classified, the inclusion of the other variables was not justified. By using WH and BL simultaneously, we obtained the following unstandardized discriminant function:

$$D = -38.60 + 3.36(WH) + 3.48(BL), \qquad (1)$$

If $D > 0$ the penguin was classified as male and if $D < 0$ the penguin was classified as female. By using this function, we correctly sexed 94% of the wild penguins in the analysis sample: 103 of 111 females (93%) and 106 of 112 males (95%) were correctly identified.

When we applied the discriminant function to the validation sample (39 males, 35 females), we correctly classified 97% of the birds, with two males and no females being misclassified.

The classification accuracy of the analysis sample ($n = 223$, 94%) and the validation sample ($n = 74$, 97%) were similar, suggesting minimal sampling bias. The samples were combined and a new function based on the larger sample size was derived. This second unstandardized function also accurately discriminated between the sexes (Wilks' Lambda = 0.3132, $\chi^2 = 341.31$, df = 2, $P < 0.001$):

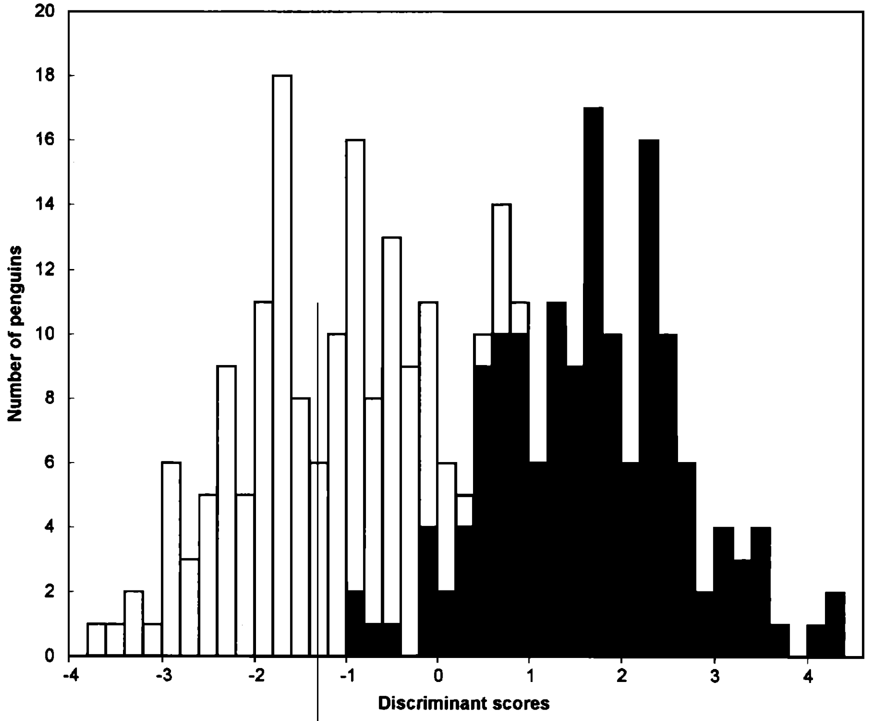

FIGURE 2. Distribution of discriminant scores of males (filled bars) and females (open bars) wild Humboldt penguins from Punta San Juan, Peru.

$$D = -38.98 + 3.16(WH) + 3.69(BL) \quad (2)$$

Again, if D was positive the bird was a male, if negative, a female. This function correctly classified 95% of the penguins. The slightly higher accuracy and the larger sample size used to derive function 2 suggest it would be the most useful function. The average of the discriminant scores (group centroid) for females was −1.50 and for males 1.45. No birds with scores higher than 0.69 (males) or with scores below −1.17 (females) were misclassified (Fig. 2). Thus, for greatest accuracy, we recommend that sex of individuals whose discriminant score falls within this range should not be identified.

Determination of sex of captive population.—Except for bill length, captive penguins were significantly larger than wild birds (t-test, $P < 0.05$) for all measurements. Bill depth showed the highest difference, captive birds having 18% thicker bills than wild penguins. On the other hand, except for the length of the head, there were significant differences between sexes in all measurements (t-test, $P < 0.05$), males being larger than females (Table 2).

We obtained poor sex discrimination of captive birds using equation

TABLE 2. Morphometric data of captive Humboldt Penguins (*Spheniscus humboldti*) held in the Metro Washington Park Zoo. Range is given in parentheses. Body mass is in grams, other measurements are in cm.

Variable	Males (n = 19) x̄ ± SE	Females (n = 16) x̄ ± SE	Pooled (n = 35) x̄ ± SE	t-test t	P
Body mass	4802.6 ± 100.5 (4100–5850)	4328.0 ± 92.6 (3500–5000)	4585.7 ± 79.2	3.42	<0.001
Length of the head	14.24 ± 0.126 (13.33–15.03)	14.04 ± 0.145 (13.17–15.08)	14.15 ± 0.094	1.08	0.28
Width of the head	5.49 ± 0.045 (5.19–5.99)	5.22 ± 0.055 (4.83–5.64)	5.36 ± 0.042	3.7	<0.001
Bill length	6.44 ± 0.075 (5.68–6.92)	6.03 ± 0.057 (5.66–6.40)	6.25 ± 0.059	4.16	<0.001
Bill depth	2.97 ± 0.045 (2.96–3.25)	2.71 ± 0.04 (2.51–3.04)	2.85 ± 0.037	4.04	<0.001

(2), derived from wild penguins. This function correctly classified 71% of penguins. Males were successfully classified in 84% of cases while females had 56% successful allocation. However, the sex of 83% of the birds could be correctly identified using only bill length as the variable for sex separation. We chose only this variable because the means (t-test, $t = 0.9$, $P = 0.36$) and variances (F-test, $F_{34,222} = 1.29$, $P = 0.14$) were not significantly different between captive and wild birds.

DISCUSSION

All penguins exhibit some sexual size dimorphism, with males generally being heavier and having larger flippers and bills than females (Farbain and Shine 1993, Williams 1995). We have shown that for Humboldt Penguins, males are also larger than females.

Discriminant analysis has been used successfully to determine the sex of penguins and other seabirds such as gulls (Evans et al. 1995, Fitzpatrick et al. 1988), cormorants (Glahn and McCoy 1995), and petrels (Lorentsen and Røv 1994, van Franeker and ter Braak 1993). The accuracy of our discriminant function for sexing wild adult Humboldt Penguins is similar to those reported for other penguin species (Amat et al. 1993, CCAMLR 1994:Appendix 2.1, Gales 1988, Scolaro et al. 1983, Williams 1990). Agnew (1992) has shown that if the discriminant function is greater than 80% successful and the sample size less than 600 birds, the apparent mean (derived from discriminant functions) of a character for males and females is unlikely to be significantly different from the true mean (derived where the sex of birds is known). In this case, the sexes can be distinguished and sex determination by discriminant analysis will give acceptable indices.

Although head width and bill length yielded the most accurate discrimination of sex, a procedure requiring only one measurement could be useful in some cases. For example, when sex determination of a large number of birds is required in a short period of time. In addition, birds cannot be sexed reliably using head measurements during the molt due to the deposition and depletion of fat as ocurrs in other penguin species (Boersma 1976, Cooper 1978). In these cases we recommend to use the equation $D = -6.31 + BL$ as criterion of sex discrimination because the bill length was one of the simplest measurement to take on living birds and the number of cases correctly classified (91% of successful allocation) was higher than other univariate characters.

The discriminant function reported in this paper to sex wild adult Humboldt Penguins is an easy, fast, inexpensive, and minimally stressful technique. However, studies of other penguin species (Bost and Jouventin 1990, Duffy 1987, Gales 1988, Gandini et al. 1992, Kerry et al. 1992, Murie et al. 1991), gulls (Evans et al. 1995), and petrels (van Franeker and ter Braak 1993) have noted geographic variation in morphometry. Thus, geographic variation may invalidate the function's applicability to populations other than the one from which it was derived. Sex identification by external measurements of other wild populations of Humboldt Penguins must be interpreted with caution until a sample of known sex animals can be developed to act as a control.

It was not appropriate to apply the two-variable discriminant function derived from wild penguins at Punta San Juan to sex captive birds because there were differences in size of the head between captive and wild birds. The extent by which captive birds differ from wild ones may be significant. Also, Humboldt Penguins held in the Metro Washington Park Zoo exhibited overgrown bills, not so much in length as in depth. We observed the same pattern in 30 captive Humboldt Penguins held in the Parque de Las Leyendas Zoo in Lima, Peru. The causes of this extra deposition of material are unknown. It may be nutrition-related or due to the absence of bill abrasion from digging, fighting or swimming, which is normal in wild penguins. However, we obtained 83% successful allocation of captive birds using the univariate function $D = -6.31 + BL$ obtained from wild birds, as bill length was similar between wild and captive birds. Thus, if the means and variances of the bill length are similar between wild penguins at Punta San Juan and other captive populations of Humboldt Penguins, the use of this function may be accomplished.

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SEVENTY-FIFTH ANNIVERSARY MEETING ASSOCIATION OF FIELD ORNITHOLOGISTS

The Association of Field Ornithologists will hold its 75th anniversary meeting jointly with the American Birding Association in San José, Costa Rica, 21–28 Jul. 1997, hosted by the *Associación Ornitológica de Costa Rica.* Scientic paper sessions, symposia, workshops, and field trips are planned. There will be a focus on conservation and research in Costa Rica and post-meeting trips to major field stations for researchers and educators. Travel and per diem funds are being sought for Latin American participants. Likely symposium topics include Conservation of New World Psitticines, Bird Sound Recording, Raptor Migration between the Americas, Bird Observatories, and Birds in Environmental Education for Indigenous Peoples. Registration will begin 10 Feb. 1997. Call for papers: Scott K. Robinson, Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, IL 61820 (217-333-6857; scottr@mail.inhs.uiuc.edu), and Rafael Campos Ramierez (fax 506-494-3346). For information about symposia (suggestions for additional topics welcome): Charles D. Duncan, Institute for Field Ornithology, University of Maine at Machias, Machias, ME 04654 (207-255-1358); cduncan@aced.umm.maine.edu). Registrar: Carol Wallace, American Birding Association, P.O. Box 6599, Colorado Springs, CO 80934-6599 (800-850-2473 or 719-578-1614; fax 719-578-1480; member@aba.org).