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Sex identification of Jack Snipe *Lymnocryptes minimus* by discriminant analysis of morphometric measurements

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Jack Snipe *Lymnocryptes minimus* is a small wader showing sexual monomorphism in plumage and some dimorphism in size. To study whether parameters of body size may be used to reliably sex individuals of this species, a discriminant function analysis was applied to a set of morphometric traits measured in birds caught during migration and the wintering period in northern Poland. Birds were sexed molecularly based on size differences in CHD-linked sequences from W- and Z-chromosomes. Males were significantly larger than females in wing length, head length, skull length, bill depth, bill width and the length of middle toe with claw, while females had longer bills. All traits except for bill width were highly repeatable. A stepwise discriminant function analysis selected wing length, skull length, bill length and bill depth as the best discriminators of sex in Jack Snipe. The discriminant function based on these four traits enabled reliable sexing of 99% of individuals. The alternative function, excluding wing length, which may not always be available for measurement, showed 96.7% classification success. Such very high success in sex identification by a discriminant analysis of morphometric traits may promote this approach as an alternative to molecular sexing techniques in Jack Snipe, when non-invasive sampling is required.

Key words: DNA-based sexing, discriminant function, sexual size dimorphism, Jack Snipe

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INTRODUCTION

A number of techniques have been developed to enable sexing in bird species that exhibit identical plumage between the sexes. However, some of these techniques may be used only during the breeding season (sex-specific breeding behaviour, the existence of a brood patch) or are invasive or time-consuming (laparotomy, karyotyping) (Griffiths 2000). An increasing number of studies rely

on molecular techniques for sex identification, which are based on differences in DNA sequences between males and females (Griffiths *et al.* 1998, Fridolfsson & Ellegren 1999). DNA-based sexing is relatively fast, but requires expensive equipment and collection of a DNA source, such as blood or feathers, which may be stressful for birds. Therefore, the development of non-molecularly based procedures allowing for reliable sex identification is still necessary. Given that sexually mono-

chromatic species show some sexual dimorphism in size, it may be possible to select the characters that may be successfully used in sex identification.

In this study we investigated whether morphometric measurements were useful in sexing Jack Snipe *Limnocryptes minimus* caught during migration and the wintering period in northern Poland. Jack Snipe is a small wader that breeds in boreal and subarctic parts of Eurasia and winters in western and southern Europe, and northern and central Africa (Glutz *et al.* 1977, Cramp & Simmons 1983, Smiddy 2002). Similar to other Charadrii waders, Jack Snipe shows sexual monomorphism in plumage and some sexual dimorphism in body size (Prater *et al.* 1977, Cramp & Simmons 1983). However, contrary to the majority of Charadrii of the Western Palearctic, Jack Snipe males are larger than females (Prater *et al.* 1977, Cramp & Simmons 1983). The measurements of different traits of body size overlap to a great extent, which does not allow for accurate identification of sex in this species based on univariate techniques. Therefore, we applied a discriminant function to a set of morphometric traits to determine which continuous variables discriminate between sexes (Quinn & Keough 2006). The sex of individuals used to develop a discriminant function was assessed molecularly.

METHODS

Field studies were conducted in Gdańsk Pomerania and Warmia region (N Poland) during autumn migration and the wintering period between September 2004 and March 2005. The wintering grounds of Jack Snipe discovered in the regions of Gdańsk Pomerania and Warmia in 1998 are located on the northeastern boundary of its wintering range (Sikora & Maniakowski 2000). The number of wintering individuals is estimated to be as high as 100–200. Recoveries indicate that birds using Gdańsk Pomerania and Warmia region as a stopover site during autumn migration move to wintering grounds in W and S Europe (unpubl. data). Jack Snipes were caught with dip-nets at 33

sites. The habitats surveyed were mainly drained fish ponds and the shorelines of other water reservoirs in autumn and field irrigation ditches and fish ponds in winter. The relative proportion of each of the different habitat types surveyed in each season of the year corresponded to seasonal changes in habitat use by Jack Snipe (Sikora 2005). The following body size measurements were taken: 1) head plus bill length (HEAD-L; from the tip of the bill to the back of the skull), 2) wing length (WING-L; maximum flattened chord), 3) bill length (BILL-L; from the tip of the bill to the feathering), 4) bill depth (BILL-D; height at the base of feathers at upper mandible), 5) bill width (BILL-W; height at the base of feathers at upper mandible), 6) middle toe length (TOE-L; distance from the first scale of the middle toe to the base of the nail), 7) middle toe plus claw length (TOE-CL-L), 8) tarsus length (TARSUS-L; Svensson 1992: page 27, Fig. 18B) and 9) body mass (BMAS). Wing was measured to the nearest 1 mm with a zero-stop ruler and other linear measurements to the nearest 0.1 mm with callipers. Birds were weighed to the nearest 1 g with a Pesola spring balance. All measurements were taken by AS. The difference between head plus bill length and bill length was used as a measurement of the skull length (SKULL-L) (Åkesson *et al.* 2007).

Sex of Jack Snipe was identified molecularly based on size differences in CHD-linked (chromodomain helicase DNA binding protein gene) sequences from W- and Z-chromosomes (Griffiths *et al.* 1998). At the time of catching, a small amount of blood was obtained from the wing vein and then stored in ethanol at room temperature until analysed. DNA was extracted with Chelex (BioRad) (Walsh *et al.* 1991) and the W- and Z-linked sequences were amplified with P2 and P8 primers (Griffiths *et al.* 1998) with the following protocol: initial denaturation 94°C/2 min, 30 cycles: denaturation 94°C/30 s, annealing 52°C/45 s, template extension 72°C/45 s, and a final extension 72°C/5 min. Each 10 µl PCR sample contained: 2 µl DNA, 1.5 mM MgCl₂, 0.2 mM dNTP, 0.5 mM of primers P2 and P8, 0.5U/µl Taq polymerase and 1 µl buffer (Fermentas). Polymerase

chain reaction (PCR) products were visualized with a 3% agarose gel stained with ethidium bromide following 2-hours long electrophoresis at 5V/cm. Individuals with two bands were scored as females, and with one band as males.

The measurements of all morphometric traits, body mass and blood samples were obtained for 299 birds. Sixty-one individuals were captured more than once including 40 birds captured twice, 15 birds captured three times and six birds captured four times. If the bird was captured more than once, only one randomly selected capture event was included in the discriminant analysis. The time span between the subsequent captures ranged from one to 71 days (mean \pm SD: 20.4 \pm 15.3). The repeatability of measurements was calculated in the group of birds captured at least twice as the intraclass correlation coefficient based on variance components derived from a one-way analysis of variance (Lessells & Boag 1987). Variables with a coefficient between 0.7 and 0.9 may be considered as highly repeatable and a coefficient higher than 0.9 as very highly repeatable (Harper 1994). Only highly and very highly repeatable characters were used in the stepwise procedure as inconsistent measurements may be associated with increased variance and therefore a reduced discriminatory power of the character. Inter-sexual differences in morphological traits were tested with a two-sample *t*-test. The sexual dimorphism in size (%) in each trait was calculated following the formula $(\bar{x}_m - \bar{x}_f) / \bar{x}_m$, with \bar{x}_m and \bar{x}_f as the mean values of the traits in males and females, respectively (Genovart *et al.* 2003). The discriminant function was generated by a stepwise discriminant analysis in SPSS for Windows 12.0.1. At each step the variable that minimized the overall Wilk's Lambda ratio was entered into the model. The default minimum partial *F* to enter (3.84) and maximum partial *F* to remove (2.71) were chosen. The discriminant function equation is given with unstandardized canonical discriminant function coefficients. We also presented the standardized discriminant function coefficients, which reflect the contribution of one predictor in the context of the other predictors

in the model. The cut-off point, which sets ranges of the discriminant scores for classifying cases, was calculated as the weighted average of the values at group centroids (mean discriminant scores for males and females) (Garson 2006). If the discriminant score was above the cut-off point the case was classified as male and if below as female. The data were tested to meet the assumptions of the discriminant function analysis (Garson 2006). Because head plus bill length is the function (the sum) of the two other predictors, which results in ill-conditioned matrix and there was a very high within-group correlation between this measurement and bill length, which violates the assumption of low multicollinearity of the independents, this measurement was excluded from the discriminant function analysis (Garson 2006). Prior probabilities were set equal in both groups. The classification success rate was cross-validated with a jack-knife, a procedure in which each case is classified using a discriminant function based on all cases except the given case (Sokal & Rohlf 1981). Moreover, we tried to assess the general applicability of discriminant functions derived from our study population. To achieve this goal, two groups of birds were selected: caught only during the autumn migration period, and therefore classified as migratory birds (group 1) and caught both during migration and the wintering period or only during the wintering period, and hence classified as wintering birds (group 2). The wintering period, 22 December – 21 March, was selected based on the mean number of birds observed per survey during successive 10-day periods in years 1996–2005 (Sikora 2005). If different strategies, e.g. using study region only as a stopover site or as wintering grounds, are related to different population origin, testing discriminant functions derived from the group 1 to assign the sex of birds in the group 2, and vice versa, may be a crude estimation of the applicability of discriminant functions derived from our population in other European Jack Snipe populations. Furthermore, by fitting a non-linear curve to the points describing the relationship between the discriminant scores and a posterior probability of a group membership, we

generated the equation to calculate the probability of classifying the sex (following Phillips and Furness 1997). Body mass was excluded from the analyses, because it may considerably vary during migration and wintering period (unpubl. data).

RESULTS

In a set of 299 molecularly sexed birds, there were 193 males and 106 females. Males and females differed in seven of nine morphometric traits. Males had longer wing, larger head, larger skull, deeper and wider bill and longer middle toe with claw, while females had longer bill. However, in all these traits, there was overlap between the sexes (Table 1). Males and females did not differ in tarsus length and middle toe length. Sexual dimorphism was most pronounced in bill depth, bill width, wing length and skull length (Table 1). All measurements except for bill width were highly or very highly repeatable (Table 2).

Only five measurements were used in a stepwise discriminant function analysis: wing length, skull length, bill length, bill depth, middle toe plus claw length. Tarsus length and middle toe length were excluded from the analysis because males and females did not show significant differences in

these measurements. Bill width, despite being the second most sexually dimorphic trait, was not used as it was only moderately repeatable and head length was not used because its inclusion violated the assumptions of the discriminant function analysis (see Methods for details).

Four characters were selected by a stepwise analysis (Wilks' Lambda = 0.177, $\chi^2_2 = 511.7$, $P < 0.001$) producing the following equation:

$$D = -64.798 + 0.259 \text{ WING-L} + 1.105$$

Table 2. Repeatability (intraclass correlation coefficient derived from ANOVA with $F_{60,88}$) of morphometric measurements in Jack Snipe.

Measurement (mm)	Repeatability	F	P
Wing length	0.98	122.77	<0.001
Head length	0.98	114.98	<0.001
Skull length	0.92	31.21	<0.001
Bill length	0.95	48.70	<0.001
Bill depth	0.80	10.98	<0.001
Bill width	0.55	3.98	<0.001
Tarsus length	0.91	25.05	<0.001
Middle toe length	0.94	41.58	<0.001
Middle toe length with claw	0.92	30.42	<0.001

Table 1. Morphometrics of male and female Jack Snipes caught during the migration and wintering period in northern Poland. Mean and SD are presented. The difference in the measurements between the sexes was tested with a *t*-test. See Methods for calculation of dimorphism.

Measurement (mm)	Males (n = 193)			Females (n = 106)			P	% dimorphism
	Mean	SD	Range	Mean	SD	Range		
Wing length	118	2	114–125	112	2	108–118	<0.001	5.1
Head length	67.4	1.1	64.3–70.4	66.3	1.0	63.6–69.7	<0.001	1.6
Skull length	26.4	0.4	25.2–27.8	25.1	0.4	24.1–26.0	<0.001	4.9
Bill length	41.0	0.9	38.5–43.6	41.2	0.9	38.5–43.8	0.041	0.5
Bill depth	9.2	0.2	8.5–10.0	8.5	0.3	7.7–9.0	<0.001	7.6
Bill width	5.5	0.2	5.0–6.1	5.2	0.2	4.8–5.7	<0.001	5.5
Tarsus length	24.5	0.6	22.6–26.2	24.4	0.6	22.9–25.6	0.355	0.4
Middle toe length	27.8	0.8	25.4–30.1	27.7	0.8	26.1–30.3	0.250	0.4
Middle toe length with claw	31.5	1.0	28.7–34.2	31.1	1.0	29.0–34.3	0.006	1.3

$$\text{SKULL-L} + 2.035 \text{ BILL-D} - 0.297 \text{ BILL-L} \quad (1)$$

The characters were introduced into the model in the following order: skull length, bill depth, wing length, bill length. The cut-off point was -0.655 and all birds with discriminant scores above this value were classified as males and below this value as females (Fig. 1A). The function correctly classified 99.0% of males and 99.1% of females, which gives the overall classification success of 99.0%. Cross-validation with a jackknife produced the same classification success rate. The standardized coefficients for skull length, bill depth, wing length and bill length were 0.477, 0.529, 0.534 and -0.274 , respectively, indicating that wing length and bill depth had the highest discriminatory power.

As wing length may not be measured reliably during moult and may be subject to wear over the season, we also tested the model without this trait in the analysis (Wilks' Lambda = 0.223, $\chi^2_2 = 442.9$, $P < 0.001$, Fig. 1B):

$$\begin{aligned} D &= -55.165 + 1.621 \text{ SKULL-L} + 2.446 \\ \text{BILL-D} - 0.215 \text{ BILL-L} & \quad (\text{cut-off point: } -0.565) \end{aligned} \quad (2)$$

Overall, the function correctly classified 96.7% of birds: 97.9% of males and 94.3% of females. Cross-validation with a jackknife produced the same classification success rate. The standardized coefficients were 0.700 for skull length, 0.636 for bill depth and -0.198 for bill length.

Additionally, we produced the functions with the exclusion of bill depth from the analyses because this character is not commonly measured and therefore may limit the application of equations 1 and 2:

$$\begin{aligned} D &= -63.179 + 1.461 \text{ SKULL-L} + 0.315 \\ \text{WING-L} - 0.274 \text{ BILL-L} & \quad (\text{cut-off point: } -0.559) \end{aligned} \quad (3)$$

$$\begin{aligned} D &= -52.938 + 2.315 \text{ SKULL-L} - 0.172 \\ \text{BILL-L} & \quad (\text{cut-off point: } -0.441) \end{aligned} \quad (4)$$

Equation 3 (Wilks' Lambda = 0.227, $\chi^2_2 = 438.1$, $P < 0.001$) gives the overall classification success rate of 97.3% in a self-test (males: 97.4%,

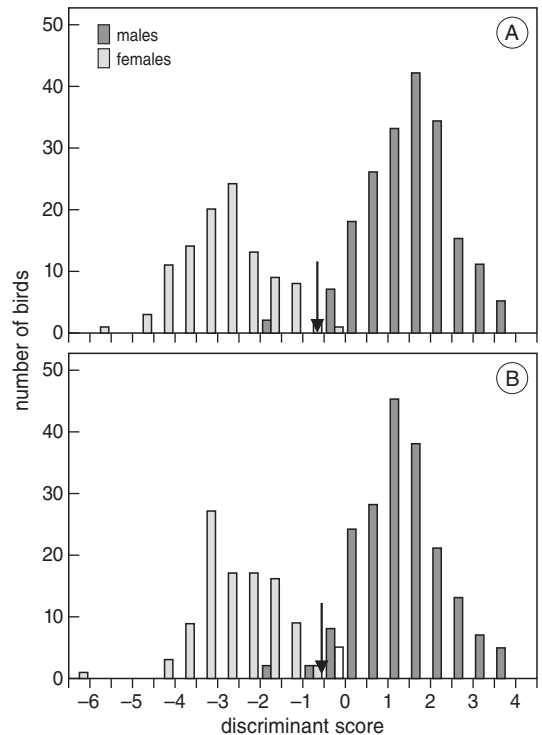


Figure 1. Frequency distributions of discriminant scores derived from the equation based on wing length, skull length, bill depth and bill length (A) and the equation based on skull length, bill depth and bill length (B). The arrow indicates the cut-off point.

females: 97.2%) and 97.0% in a jackknife test (males: 96.9%, females: 97.2%), while equation 4 (Wilks' Lambda = 0.321, $\chi^2_2 = 336.4$, $P < 0.001$) gives 94.6% in a self-test (males: 94.8%, females: 94.3%) and 94.0% in a jackknife test (males: 94.3%, females: 93.4%).

Discriminant functions (equations not shown) derived from birds, which were caught only during the autumn migration period assigned the sex in wintering birds with 98.1% success based on equation 1 and 96.1% success based on equation 2. When discriminant functions were derived from a group of wintering birds the success rate was 98.5% and 96.6% for equation 1 and 2, respectively.

Given that 1.0% and 3.3% of Jack Snipes may

be misclassified using the equation 1 and 2, respectively, the accuracy of sexing may be increased by limiting sex assignment only to birds with a high probability of a group membership. The relationship between posterior probabilities of a group membership and discriminant scores for equation 1 and 2 are given in Fig. 2. By fitting a non-linear curve to these points the following equations for the probability P of a group membership were developed (Phillips & Furness 1997):

$$P = 1/(1 + e^{4.5003 * (\text{discriminant score} + 0.65472)})$$

(for equation 1)

$$P = 1/(1 + e^{3.8842 * (\text{discriminant score} + 0.5651)})$$

(for equation 2)

If sexing of birds is limited only to individuals with a probability of a group membership $P > 0.95$, 98.0% of females and 100% of males could be classified correctly in case of the equation 1 and 97.9% of females and 100% of males in case of the equation 2, leaving 15 (5%) and 32 (10.7%) of birds unclassified, respectively.

DISCUSSION

Jack Snipes caught during migration and the wintering period in northern Poland showed sexual dimorphism in wing length, skull length, head length, bill depth, bill width, bill length and the length of middle toe with claw. The ranges of these morphometric traits overlapped substantially, thereby limiting the utility for reliable sexing based on the raw values. Application of a discriminant analysis to a set of five morphometric measurements produced a discriminant function that enabled correct identification of sex in 99% of individuals. Measurements that best discriminated between males and females in Jack Snipe caught in northern Poland included wing length, skull length, bill length and bill depth. Obtained accuracy is one of the highest ones reported for discriminant functions aiming at sex identification in birds (e.g. Sweeney & Tatner 1996, Genovart *et al.* 2003, Setiawan *et al.* 2004) and the highest in

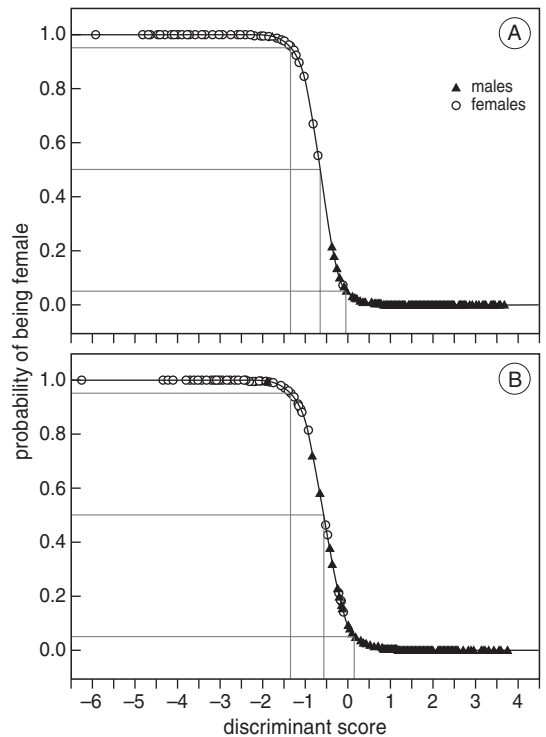


Figure 2. The relationship between *a posteriori* probability of being female and discriminant scores generated by the discriminant function based on wing length, skull length, bill depth, bill length (A) and the discriminant function based on skull length, bill depth and bill length (B). Dotted lines indicate 5%, 50% and 95% probability of being female and corresponding discriminant scores.

waders. In Dunlin *Calidris alpina* juveniles, Common Snipe *Gallinago gallinago delicata* and Sanderlings *Calidris alba* the accuracy of the discriminant function reached 90%, 88% and 92%, respectively (Maron & Myers 1984, McCloskey & Thompson 2000, Meissner 2005). Such high classification success promotes the application of a discriminant analysis as an alternative to molecular techniques in this species, when non-invasive sampling is required (Griffiths *et al.* 1998, Fridolfsson & Ellegren 1999).

Given the availability of large sample sizes, the accuracy of sex classification may be increased by limiting sex assignment to individuals with a very

high probability of a group membership (Phillips & Furness 1997). Such an approach should be especially applied when misclassification of sex would influence the interpretation of important biological processes. Alternatively, individuals with a low probability of a group membership could be sampled for blood and sexed molecularly to avoid the bias towards individuals with special morphometrics.

The four traits selected by a discriminant analysis were highly or very highly repeatable, which indicates their high potential for discrimination. Bill width is the second most dimorphic trait, however, its inclusion in the model was not justified as it may not be measured consistently. This inconsistency is most probably associated with the fact that this measurement is taken at the softest part of the bill. Bill depth had high discriminatory power and was considered highly repeatable (Harper 1994). However, small measurements are generally associated with a higher measurement error and the consistency might further decrease between observers taking the measurements.

Mallory & Forbes (2005) found that the variation in measurements of 10 morphometric traits in Northern Fulmars *Fulmarus glacialis* among six observers ranged from 20% to 90%. Because of this and the fact that bill depth is not commonly measured, we also derived the functions excluding this variable from the analyses. This resulted in 1.7% decrease in the classification success rate in case of the function including wing length and 4.4% decrease when only skull length and bill length were included.

The discriminant functions generated to identify sex in Jack Snipe caught during autumn migration and the wintering period in northern Poland should be applied with caution in other, geographically distinct, populations as geographical variation in body size is known to occur in many bird species (Zink & Remsen 1986, Weicker & Winker 2002). In case of Jack Snipe, the information on geographic variation of morphometric measurements is very scarce and based mainly on museum specimens (Table 3). As museum speci-

Table 3. Measurements of wing length, bill length, tarsus length and middle toe length in male and female Jack Snipes from different European populations. Mean, range of values and sample size (*n*) are presented.

Source		Wing length		Bill length		Tarsus length		Middle toe length	
		Males	Females	Males	Females	Males	Females	Males	Females
Netherlands, September–February, skins (Cramp & Simmons 1983)	mean	114	110	40.3	40.1	23.8	24.0	28.7	28.4
	range	105–119	105–115	36.5–41.6	37.3–42.2	22.5–25	23–25.5	26.5–30.5	27.3–30.3
	<i>n</i>	36	21	31	19	27	16	24	14
Denmark, September–November, skins (Glutz <i>et al.</i> 1977; Coll. Zool. Mus. Copenhagen)	mean	114.0	109.9	39.7	40.4	24.4	24.6	-	-
	range	109–119	106–116.5	37–42	38.5–42	23–26	23–26		
	<i>n</i>	40	31	41	30	41	30		
Prater <i>et al.</i> 1977	mean	116.0	111.2	40.3	40.7	23.7	23.8	-	-
	range	110–121	107–119	38–42	39–43	23–25	23–25		
	<i>n</i>	29	22	26	19	26	19		
This paper	mean	118	112	41.0	41.2	24.5	24.4	27.8	27.7
	range	114–125	108–118	38.5–43.6	38.5–43.8	22.6–26.2	22.9–25.6	25.4–30.1	26.1–30.3
	<i>n</i>	193	106	193	106	193	106	193	106

mens tend to shrink, their measurements may be associated with a large error amounting even to 4% of the length of living body components (Herremans 1985, Winker 1993). Moreover, the comparisons between population from northern Poland and populations from other localities may be further complicated because of 1) the lack of information on the measurement methods, 2) small sample size and 3) different measurement accuracies (Cramp & Simmons 1983, Glutz *et al.* 1977, Prater *et al.* 1977). Therefore, based on the available data we were unable to assess whether there are differences in morphometrics among different European Jack Snipe populations. If body size indeed varies over the geographical range it is recommended to apply the function only to a local population (Evans & Cavanagh 1995).

We tried to address the issue of a more general applicability of functions derived from our population by developing functions separately for migratory and wintering birds and applying them to assign the sex of birds from the other group. Such approach could be used as a crude estimation of a general applicability given that the differences in the migration-wintering strategies are associated with different population origin. Functions developed in the group of migrating birds were highly successful in sex identification in wintering birds and vice versa. This may either suggest the lack of difference in body size between the two populations or that both groups of birds, the ones using the study region only as a stopover or as wintering grounds, originate from the same population.

To conclude, we showed that morphometric traits may be successfully used to sex Jack Snipes from migratory and wintering population in northern Poland. Based on only four characters it was possible to reliably sex 99% of individuals. However, we were unable to reliably verify whether the functions derived from the study population could be successfully applied to individuals in other European populations.

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SAMENVATTING

Het Bokje *Limnocyptes minimus* is een kleine steltlopersoort waarvan mannetjes en vrouwtjes niet op grond van het verenkleed te onderscheiden zijn, maar wel op basis van lichaamsgrootte. Dit artikel gaat over een onderzoek naar de betrouwbaarheid van structurele lichaamsmaten om individuen van deze soort te sexen. De auteurs gebruiken een discriminantanalyse om een serie morfologische gegevens te analyseren van Bokjes gevangen tijdens de trek en in de winter in het noorden van Polen. Van alle individuen is het geslacht bepaald met moleculaire technieken. Mannetjes hadden een grotere vleugellengte, hoofd lengte, schedellengte, snaveldiepte, snavelbreedte, en lengte van de middelste teen, terwijl vrouwtjes een langere snavel hadden. Al deze eigenschappen met uitzondering van snavelbreedte waren erg consistent. De discriminantanalyse selecteerde de combinatie van vleugellengte, schedellengte, snavellengte en snaveldiepte als beste maat om het geslacht van Bokjes te bepalen. De combinatie van deze vier eigenschappen bepaalt het geslacht van een individu met 99% betrouwbaarheid. Een alternatieve discriminantfunctie, zonder vleugellengte omdat vleugels soms niet gemeten worden en kunnen slijten, bepaalt het geslacht met een zekerheid van 96,7%. Dankzij de hoge betrouwbaarheid waarmee het geslacht van Bokjes bepaald kan worden op grond van morfologische kenmerken is moleculair sexen bij deze soort niet echt nodig. (BIT)

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