

A better sex discriminator? Validity of sex-discriminating formulas in water rails *Rallus aquaticus* L., 1758 (Rallidae)

Alexandru Nicolae STERMIN¹, Alin DAVID^{1,*}, Alexander EILERS², Martin HAASE² and Angela Schmitz ORNÉS²

1. Department of Taxonomy and Ecology, Babeş-Bolyai University, Clinicilor Str., no. 5-7, Cluj-Napoca, Romania.

2. Vogelwarte, Zoological Institute and Museum, Ernst Moritz Arndt University, Soldmann Str., no. 23, Greifswald, Germany.

*Corresponding author, A. David, E-mail: adavid.ubb@gmail.com

Received: 20. November 2016 / Accepted: 13. August 2017 / Available online: 13. August 2017 / Printed: December 2018

Abstract. The water rail, *Rallus aquaticus*, has no visible sexual dimorphism. In previous studies, where morphometric measurements and molecular data had been used, a sex-discriminating formula was described from a German water rail population. We tested this formula in 21 water rails from a Romanian population and found that 90% of them were correctly sexed. A new formula was calculated specifically for the new population, and this one had a 95 % correct discriminating score in our population and 67.7% in the German population. The data analysis had revealed that these two populations were significantly different in terms of body measurements and the reasons for this difference were discussed. We concluded that the formula calculated in the German population had a higher resolution, being established from a larger number of individuals with a lower degree of variance, and therefore it may have a high validity in sex discrimination in other populations. The formula developed for the Romanian population has a high discriminant level only in a local scale and a low resolution when used on a population with high variability.

Key words: regional differences, sexing, morphometry, molecular sexing.

Introduction

To determine the sex of individuals in species which do not exhibit observable sex-specific morphological characteristics, usually we have to rely on molecular analyses. However, for the sex determination, while conducting field ornithological monitoring of populations, these methods are not easily applicable. An alternative of using a discriminant formula based on different morphological traits measured on a sample of some individuals of the population has been proposed (Kenward et al. 2004, Jakubas & Wojczulanis 2007, Herring et al. 2008, Liordos & Goutner 2008). Discriminant Function Analysis (DFA) is a multivariate method that, in these cases, provides possible equations based on morphological measurements in order to predict the sex of individuals. Here, molecular data regarding the sex of individuals are used primarily to calibrate the equation, and every discriminant equation has its estimated proportion of correctly sexed individuals (Dechaume-Moncharmont et al. 2011).

Discriminant analysis has been used as an statistical approach to sex bird species from morphometric measurements in many bird orders, such as: Anseriformes (Calabuig et al. 2011), Sphenisciformes (Zavalaga & Paredes 1997), Podicipediformes (Kloskowski et al. 2006), Procellariiformes (Weidinger & van Franeker 1998), Pelecaniformes (Glahn & McCoy 1995, Liordos & Goutner 2008); Falconiformes (Sarasola & Negro 2004, Pitzer et al. 2008), Gruiformes (Martin et al. 2000, Eilers et al. 2012), Charadriiformes (Grecian et al. 2003, Reynolds et al. 2008, Yannic et al. 2016), Strigiformes (Delgado & Penteriani 2004) and Passeriformes (Sweeney & Tatner 1996, Sandoval & Mennill 2013, Henry et al. 2015).

Studies in biometry suggest that there is a large temporal and spatial variation in morphometric traits that may be used to discriminate the sexes (Wiedenfeld 1991, Bull 2006, Van de Pol et al. 2009). This variation can be a result of selection and phenotypic plasticity (Kingsolver et al. 2001, Van de Pol et al. 2009), in which the biometry of a population often depends on the environment, generally changing not only in time but also in space.

When sexing birds using DFA, the most used morphological traits are bill, tarsus and/or wing (Croxall 1995, Bosch 1996, Eilers et al. 2012, Kulaszewicz et al. 2013). It is well known that there is a strong correlation between food availability and bill morphology, which indicates that environmental changes might produce bill morphological changes (e.g. Grant 1999, Badyaev et al. 2000, Marquiss & Rae 2002, Van de Pol et al. 2009). Further, length of the tarsus may be related with body weight and also with the structure of substrates (Grant 1971, Martin 1991). Wing shape may also be dependent on habitat use, foraging habits, predator evasion behaviour and on the demands of annual migration (Savile, 1957, Videler, 2005, Fernández et al. 2007, Kralj et al. 2010). Intraspecific studies in songbirds revealed that northern populations have often longer and more pointed wings and those from southern populations are more rounded (Pérez-Tris & Tellería 2001, Peiró 2003, Fiedler 2005, Arizaga et al. 2006).

In general, all these morphological measurements are not independent from each other or from the environment. In this context we expect morphological differences among intraspecific bird populations, as a consequence of different physiological, and behavioural adaptations along their geographical ranges, moreover when birds show wide distributions with the potential of occurring in different habitats (Grant 1971, Herrera 1978, 1981; James 1983, Leisler & Winkler 1985, Kralj et al. 2010).

The water rail *Rallus aquaticus* is occurring within a large geographical range of three subspecies: *R. a. aquaticus* in Europe including the British Isles and also Northern Africa to North-Western Asia; *R. a. korejewi* in Western to Middle Asia, and *R. a. hibernans*, in Iceland, which became extinct around 1965 (De Kroon 1991, Taylor 1998, Tavares et al. 2010).

In contrast with Central Europe, populations which are mainly migratory, birds from Northern and Central Europe move to the south and southwest to winter in the Mediterranean basin, North Africa, and east to the south Caspian Sea area (Taylor 1998, Tavares et al. 2010, Stermin et al. 2014b).

In the context of different movements' status and different gene flow levels between water rail populations a formula for the sex discrimination, as the one proposed by Eilers et al. (2012), based on individuals from one population may be not valid for sexing other populations on different areas across the species range.

The main goal of our study was (i) to reveal the validity of the sex discrimination formula calculated based on a North-Western European water rail population (Eilers et al. 2012) but applied to the Eastern European population (ii) to calculate another formula specific for the new population and (iii) to compare both formulas and analyse the results in terms of general validity and population differences.

Material and methods

Study sites

The studied South-Eastern Europe population of water rails occurs in the Fizeş Basin in central part of the Transylvanian Plain (24°10'E; 46° 50'N) in Romania. The wetlands of the Fizeş Basin represent 1/3 of all Transylvanian wetlands and the area is one of Romanian Important Bird Areas, and was recently designated as a "Natura 2000" site, part of the "Natura 2000" European network of protected areas (Stermin et al. 2011). Birds were captured from two natural wetlands, located 5 km away from each other: a) Sic Reed Beds - the largest reed bed area [250 ha] in Transylvania, where the vegetation is dominated by reed *Phragmites australis*, and water level does not exceed 1 m, and b) Pike Lake - wetland with a maximum depth of 7 m, but with a large marsh vegetation area around, where the water level does not exceed 1.5 m (Stermin et al. 2011).

Collecting data and measurements

A total of 41 individuals were collected, 23 males and 18 females. However, 20 individuals were juveniles and 21 adults (13 males and eight females). Birds were captured using Potter's traps and automatic fall traps (Bub 1991, Eilers et al. 2012, Stermin et al. 2014a), during the breeding seasons (April - August) in 2010 and 2011. We used five morphological traits: the length of the bill along the culmen, maximum wing chord length, tarsus length, body mass and maximum tail length (Svensson 1992). All birds were measured by the same person (ANS). Samples of breast feathers and blood (approx. 50 µl) by brachial venepuncture into capillaries were collected during the ringing process and preserved frozen or in ethanol 96% for DNA extraction.

Molecular methods and data analysis

In order to determine the exact sex of the collected birds we conducted DNA analyses following Eilers et al. (2012). DNA was either isolated from feathers or blood samples preserved using QIAGEN's DNeasy® Blood & Tissue Kit. In order to amplify sex-specific DNA fragments, according to Shizuaka & Lyon (2008) we used a set of three primers 1237L (5'-GAGAACTGTGCAAAACAG-3'), 1272H (5'-TCCAGAAATATCTTCTGCTCC-3') and GWR2 (5'-CCTGTAAAAACCAACC-3').

The reaction volume of 10 µl for the PCR consisted of 5.42 µl H₂O, 1 µl of BIOLINE™ 10x Reaction Buffer, 0.8 µl of 50 mM MgCl₂,

0.2 µl of 10 mM dNTPs, 0.3 µl of each 10 pM Primer, 0.6 µl of 15 ng/µl DNA, and 0.08 µl of 5 U/µl BIOLINE™ DNA polymerase. Amplification of the fragments was conducted on an ABI-cycler using "touchdown" program as follows: denaturation at 92°C for 2 min followed by 14 cycles at 92°C for 30 sec. Elongation temperature was 72°C for 45 sec followed by 21 denaturation cycles at 52°C for 45 sec, using the same temperature and time as to the first 14 cycles and a final extension at 72°C for 5 min. PCR products were separated on a 2% ethidium bromide-stained agarose gel.

We applied the sexing formula from Eilers et al. (2012) in our adult individuals to quantify its validity comparing these results with the molecular ones. To establish a new formula specific for our population we carried out the statistical analyses following Eilers et al. (2012) with SPSS17 (SPSS, Inc. 2008) at a significance level of $\alpha = 0.05$. Other statistical analyses conducted to compare the morphological measurements of both populations, including a Principal Component Analysis, were done using PASTv.3 (Hammer et al. 2001).

Results

The morphological measurements of the 21 adult individuals are presented in Table 1. The formula from Eilers et al. (2012) was able to sex correctly 90 % of the individuals collected by us (100 % of females and 84 % of males). In this case, two males were incorrectly considered to be females.

In order to develop a specific formula considering only the Romanian water rail population, the hypothesis of equal covariance matrices between males and females was tested (Box's M: 5.330, $F = 1.52$, $p > 0.05$). The stepwise discriminant analysis included: bill length along the culmen and wing length as predictive factors for the model (Wilk's- $\lambda = 0.319$, $\chi^2 = 18.257$, $p < 0.001$), and the resulting discriminant function was: $D = (0.064 * \text{wing} + 0.265 * \text{bill}) - 17.707$. The formula was able to discriminate the sexes correctly in 95 % of the individuals (100 % females and 92 % males) with a cut-off value of -0.371 (Fig. 1).

Applying our formula towards Eilers et al. (2012) data, 67.6 % of the individuals were correctly sexed (100 % of males and 34 % of females). From the females, 23 were incorrectly attributed to the male group.

We have combined both data sets and explored regional variations in the morphological measurements by using a PCA with culmen, wing and tarsus length as variables. These two data sets are separated in two groups, corresponding with the two regions. The individuals of Romanian population showed more variation in body measurements than the German ones (Fig. 2). The highest variance was revealed in the wing length (85.70 %), followed by the culmen length (12.38 %) and the lowest one was found in the tarsus (1.91 %) (Fig. 2).

By applying a MANOVA test we found statistically significant differences between Eilers et al. (2012) and our data

Table 1. Morphological measurements of 21 molecularly sexed water rails. M – male (13), F – female (8), BL – bill length along the culmen (mm), WL – maximum wing chord length (mm), TL – tarsus length (mm), BM – body mass (g), SD – standard deviation, CV – coefficient of variation

	F			M		
	Min-Max	Mean ± SD	CV	Min-Max	Mean ± SD	CV
BL	34.47- 41.98	37.36 ± 2.40	6.43	37.93 - 49.9	42.26 ± 3.05	7.21
WL	67.2 - 110	96.76 ± 13.52	13.98	112 - 146	119.66 ± 8.58	7.17
TL	38.86-50.02	42.97 ± 3.54	8.23	43.2- 52.24	46.30 ± 2.46	5.31
BM	85- 145.1	108.16 ± 21.6	19.97	104.4 -162.6	134.48 ± 19.58	14.56

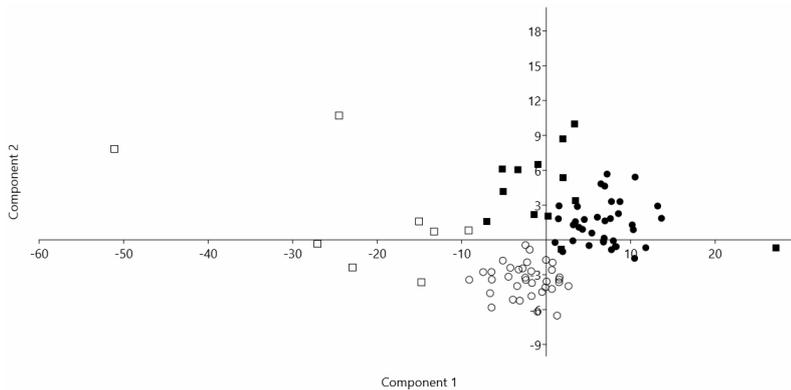


Figure 2. Principal component analysis scatter plot of water rail individuals' distribution according to standard morphological measurements (tarsus, culmen and wing length) of two different European populations. "■" males and "○" females from Romania and "●" males and "○" females from Germany according to Eilers et al. 2012 database.

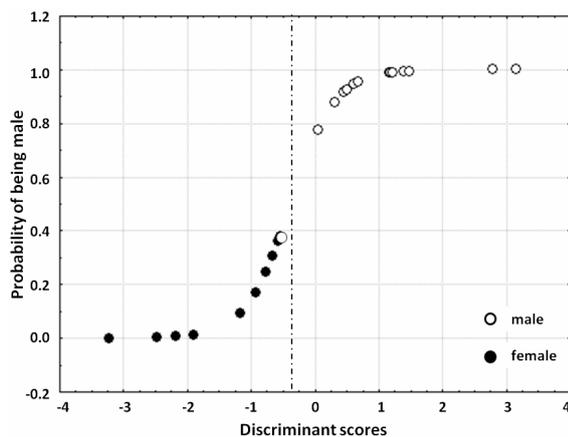


Figure 1. Posterior probabilities of being a male individual. Dotted line indicates the cut-off value.

(Wilks' lambda = 0.475; $F = 32.31$, $p < 0.001$). There were also significant differences regarding males (Wilks' lambda = 0.563; $F = 11.64$, $p < 0.001$) and females (Wilks' lambda = 0.302; $F = 30.05$, $p < 0.001$) between both data sets.

Discussion

The statistical comparison (PCA and MANOVA) of water rail populations coming from Northern Germany and Romania reveals regional differences in morphological measurements. These differences can be caused by regional differences or by the fact that the measurements were taken by different ringers with different instruments.

On the first hand, regional differences can be expected because the German population is supposed to be more sedentary due to the less extreme temperature differences as a consequence of the Gulf Stream in Middle Europe as compared to the high migratory behaviour showed by the Romanian population (Taylor 1998, Tavares et al. 2010, Stermin et al. 2014b). Sic Reed Beds is an important stop-over point in species migration (Stermin et al. 2012) within the main migratory water birds route through Transylvania. This attribute makes the water rails population more mixed and more variable and accordingly involve the measurements distribution in a larger cloud of the PCA scatter plot (Fig. 2). In terms of population genetics, based on the studies on mitochondrial DNA, North-Western European populations are not so different from the Romanian ones, but are very differ-

ent from the Siberian populations. At the same time, Romanian populations have the same genetic differences compared to the North-Western European and Siberian ones, which means that the Romanian population is a buffer population between those two parts of the species range (see Stermin et al. 2014b). Intraspecific geographical differences in bird species populations are a common characteristic which was also described and well documented in many other species (James 1982, Martin 1991, Kralj et al. 2010).

The measurements in wing length are larger with around 3.5 mm in German population compared with the Romanian one. Those differences may be an effect of the "Bergmann's rule" (Fiedler 2005, Ellrich et al. 2010, Meiri & Dayan 2003). The German population is a more northern population than the Romanian one and, according to the Bergmann's rule, we would expect longer wing lengths in the northern population (Flegg & Glue 1973, Von Blotzheim et al. 1973, De Kroon 1991, Fuertes et al. 2010). Assuming that in the German population there were already migratory individuals of more northern regions (e.g. Scandinavia) (Eilers et al. 2012), these individuals would increase the mean length additionally.

In this context, when we applied our formula based on more southern population with smaller wings to Eilers et al. (2012) data, the result of 23 incorrectly attributed females is easily explainable, because the wings of females in the German population are much longer than male wings of the Romanian population. For the same reason, applying Eilers et al. (2012) formula to our data, two males were incorrectly considered to be females.

On the other hand, the fact that the measurements were performed by different ringers with different instruments can be a cause for the revealed differences - measurement errors or biases. Francis & Mattlin (1986) found that there was a degree of uncertainty in many morphometric dimensions and it was proved that the discriminant power could fall from 89% to less than 50% when a small amount of bias exists in morphometric measurements. The effect and the importance of measurement biases has been investigated and has revealed as having a relevant effect on the results depending on the data set and the observers (Bailey & Byrnes 1990, Loughheed et al. 1991, Mallory & Forbes 2005, Dechaume-Moncharmont et al. 2011).

Independent of the cause of the difference between the two populations considered for this study, the formula created by Eilers et al. (2012) for sex discrimination has a high efficiency in the Romanian population (90%). On the other

hand, our formula has a high efficiency only in the Romanian population (95 %) and a lower one in the German population (67.6%), especially when detecting the sex of females.

One of the causes for low efficiency of our formula in the German population is the fact that the individual measurements in the Romanian population have a higher variance in the measured traits compared with those from Germany (Fig. 2). This might have led to the lower resolution of the formula created for Romania.

In our population the female measurements have a higher level of variance, being distributed in a large cloud in the PCA, than values pertaining to the males (Fig. 2). In this case, the formula does not have a good power of discrimination between sexes in a population with low variance, as the German one, where the level of correct discrimination in females was 34 %. This high level of variance was caused first by the high variance of the wing length.

This variation in wing length may be a result of the collecting data period, as the individuals were captured during April-August period which overlaps with the moulting period. In water rail the post-breeding moult is complete, remiger and rectrices are moulted simultaneously between early June to early September, time when the birds are flightless for three weeks (Ginn & Melville 1983, Taylor 1998). The largest amount of individuals was trapped in the period between April and July, compared with Eilers et al. (2012), where the largest numbers of birds were trapped at the end of the breeding season (July - August), having more birds with a finished moult. In this context the timing of collecting data can be a reason for a lower variation of wing length in Eilers et al. (2012) data.

Some studies exclude wing length from discriminant analyses, because the differential wing tip wear and moult schedules affect its utility as a discriminant factor (Jodice et al. 2000, Mallory & Forbes 2005). We still use it because, as in other studies (Liordos & Goutner 2008, Sandoval & Mennill 2013), the wing length was selected in the stepwise discriminant analysis as the most powerful parameter in separating the sexes, followed by culmen and tarsus. In this context, we can agree that a high level of variance in the individual morphological traits is having a high effect on the efficiency of the discriminate formula revealed by the data.

Our formula takes into account two morphological traits (wing and culmen), which may decrease its discrimination power in populations with a low degree of variation, as is the case in Germany. Eilers et al. (2012) formula takes into account three morphological traits (wing, culmen and tarsus) which may increase its discrimination power. The discrimination power of the Eilers et al. (2012) formula can be related to a larger sample size ($n=71$), which can improve the efficiency of sex discrimination in some cases (Dechaume-Moncharmont et al. 2011). Even having a small sample size ($n=21$), our formula is still valid, showing a high rate of discrimination (95%). In other studies the sample size varied greatly, from 8 birds (4 males and 4 females) to 1891 (918 males and 973 females) and the discriminant rates ranged from 63% to 100% (Dechaume-Moncharmont et al. 2011).

Different levels of sex discrimination in different populations were also revealed in other studies. Walton & Walton (1999), developed a formula for reed buntings *Emberiza schoeniclus* from Southern-Eastern Scotland, which correctly

sexed 95.4% of the individuals. Ellrich et al. (2010) applied this formula to a reed buntings population from southwestern Germany and, as a result, 81.8% of all birds were sexed correctly (70.8% of males and 95.0% of females).

In the case where the molecular analysis is not possible we suggest to use the Eilers et al. (2012) formula, because it has a high level of discrimination that can be of help also for non-German populations. The Eilers et al. (2012) formula has a high resolution, being established from a larger number of individuals with a lower degree of variance in the measurements traits, and has a high validity in sex discrimination in other populations, like the Romanian one.

Acknowledgements. ANS was supported through an "Investing in people!" PhD scholarship and co-financed by the European Social Fund, Sectorial Operational Program Human Resources Development 2007- 2013 at Babeş-Bolyai University, Cluj-Napoca, Romania. Financial support was also provided by the Brehm Fonds für Internationalen Vogelschutz (to ASO).

References

- Arizaga, J., Campos, F., Alonso, D. (2006): Variations in wing morphology among subspecies might reflect different migration distance in Bluethroat. *Ornis Fennica* 83: 162-169.
- Badyaev, A.V., Hill, G.E., Stoehr, A.M., Nolan, P.M., McGraw, K.J. (2000): The evolution of sexual size dimorphism in the House Finch. II. Population divergence in relation to local selection. *Evolution* 54: 2134-2144.
- Bailey, R.C., Byrnes, J. (1990): A new, old method for assessing measurement error in both univariate and multivariate morphometric studies. *Systematic Zoology* 39: 124-130.
- Bosch, M. (1996): Sexual size dimorphism and determination of sex in Yellow-legged Gulls. *Journal of Field Ornithology* 67: 534-541.
- Bub, H. (1991): Bird trapping and bird banding- a handbook for Trapping Methods all over the World. Cornell University Press. Ithaca, New York.
- Bull, L.S. (2006): Geographical variation in the morphology of the wedge-tailed shearwater (*Puffinus pacificus*). *Emu* 106: 233-243.
- 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.
- Croxall, J.P. (1995): Sexual size dimorphism in seabirds. *Oikos* 73: 399-403.
- De Kroon, G.H.J. (1991): A comparative study of the subspecies of *Rallus aquaticus*. *Beiträge zur Vogelkunde* 39(3): 155-158.
- Dechaume-Moncharmont, F.X., Monceau, K., Cezilly, F. (2011): Sexing Birds Using Discriminant Function Analysis: A Critical Appraisal. *The Auk* 128: 78-86.
- Delgado, M.D., Penteriani, V. (2004): Gender determination of Eurasian Eagle-Owls (*Bubo bubo*) by morphology. *Journal of Raptor Research* 38: 375-377.
- Eilers, A., Schmitz Ornés, A., Haase, M. (2012): Sex at second sight. Pitfalls of sexing Water Rails *Rallus aquaticus* and Spotted Crakes *Porzana porzana* using morphology and molecular techniques. *Acta Ornithologica* 47: 1-9.
- Ellrich, H., Salewski, V., Fiedler, W. (2010): Morphological sexing of passerines: not valid over larger geographical scales. *Journal of Ornithology* 151: 449-458.
- Fernández, G., Lank, D.B., Sandercock, B. (2007): Variation in the wing morphology of western sandpipers (*Calidris mauri*) in relation to sex, age class, and annual cycle. *The Auk* 124: 1037-1046.
- Fiedler, W. (2005): Ecomorphology of the external flight apparatus of blackcaps (*Sylvia atricapilla*) with different migration behaviour. *Annals of the New York Academy of Sciences* 1046: 253-263.
- Flegg, J.J.M., Glue, D.E. (1973): A Water Rail study. *Bird Study* 20: 69-80.
- Francis, R.I.C.C., Mattlin, R.H. (1986): A possible pitfall in the morphometric application of discriminant analysis: Measurement bias. *Marine Biology* 93: 311-313.
- Fuertes, B., Garcia, J., Fernandez, J., Suarez-Seoane, S., Arranz, J.J. (2010): Can Iberian Water Rail *Rallus aquaticus* be sexed reliably using simple morphometrics? *Ringing & Migration* 25:42-46.
- Ginn, H.B., Melville, D.S. (1983): Molt in birds. British Trust for Ornithology.

- Glahn, J.F., McCoy, R.B. (1995): Measurements of wintering double-crested cormorants and discriminant models of sex. *Journal of Field Ornithology* 66: 299-304.
- Grant, P.J. (1999): *Ecology and Evolution of Darwin Finches*. Princeton: Princeton University Press.
- Grant, P.R. (1971): Variation in the tarsus length of birds in island and mainland regions. *Evolution* 25: 599-614.
- Grecian, V.D., Diamond, A.W., Chardine, J.W. (2003): Sexing razorbills *Alca torda* breeding at Machias Seal Island, New Brunswick, Canada, using discriminant function analysis. *Atlantic Seabirds* 5: 73-80.
- Hammer, Ø., Harper, D.A.T., Ryan, P.D. (2001): PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* 4: 9.
- Henry, L., Biquand, V., Craig, A.J.F.K., Hausberger, M. (2015): Sexing Adult Pale-Winged Starlings Using Morphometric and Discriminant Function Analysis. *PLoS ONE* 10(9): e0135628.
- Herrerca, G. (1978): Niche-shift in the genus *Parus* in southern Spain. *Ibis* 120: 236-240.
- Herrerca, G. (1981): Combination rules among western European *Parus* species. *Ornis Scandinavica* 12: 140-147.
- Herring, G., Gawlik, D.E., Beerens, J.M. (2008): Sex determination for the Great Egret and White Ibis. *Waterbirds* 31: 298-303.
- Jakubas, D., Wojczulanis, K. (2007): Predicting the sex of Dovekies by discriminant analysis. *Waterbirds* 30: 92-96.
- James, F.C. (1983): Environmental component of morphological differentiation in birds. *Science* 221:184-186.
- James, F.C. (1982): The ecological morphology of birds: a review. *Ann. Zool. Fennici* 19: 265-275.
- Jodice, P.G.R., Lanctot, R.B., Gill, V.A., Roby, D.D., Hatch, S.A. (2000): Sexing adult Black-legged Kittiwakes by DNA, behavior, and morphology. *Waterbirds* 23: 405-415
- Kenward, B., Rutz, C., Weir, A.A.S., Chapell, J., Kacelnik, A. (2004): Morphology and sexual dimorphism of the New Caledonian Crow *Corvus moneduloides*, with notes on its behaviour and ecology. *Ibis* 146:652-660.
- Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hill, C.E., Hoang, A., Gilbert, P., Beerli, P. (2001): The strength of phenotypic selection in natural populations. *American Naturalist* 157: 245-261.
- Kloskowski, J., Grela, P., Krogulec, J., Gaska, M., Tchorzewski, M. (2006): Sexing Red-necked Grebes *Podiceps griseogenus* by molecular techniques and morphology. *Acta Ornithologica* 41: 176-180.
- Kralj, J., Procházka, P., Fainová, D., Patzenhauerová, H., Tutiš, V. (2010): Intraspecific Variation in the Wing Shape and Genetic Differentiation of Reed Warblers *Acrocephalus scirpaceus* in Croatia. *Acta Ornithologica* 45: 51-58.
- Kulazewicz, I., Jakubas, D., Wojczulanis-Jakubas, K. (2013): Sex discrimination in the Savi's Warbler (*Locustella luscinioides*) using morphometric traits. *Ornis Fennica* 90: 203-210.
- Leisler, B., Winkler, H. (1985): Ecomorphology. *Current Ornithology* 2: 155-186.
- Liorios, V., Goutner, V. (2008): Sex determination of great cormorants (*Phalacrocorax carbo sinensis*) using morphometric measurements. *Waterbirds* 31: 203-210.
- Lougheed, S.C., Arnold, T.W., Bailey, R.C. (1991): Measurement error of external and skeletal variables in birds and its effect on principal components. *Auk* 108: 432-436.
- Mallory, M.L., Forbes, M.R. (2005): Sex discrimination and measurement bias in Northern Fulmars *Fulmarus glacialis* from the Canadian Arctic. *Ardea* 92: 25-36.
- Marquiss, M., Rae, R. (2002): Ecological differentiation in relation to bill size amongst sympatric, genetically undifferentiated Crossbills *Loxia* spp. *Ibis* 144: 494-508.
- Martin, C.A., Alonso, J.C., Alonso, J.A., Morales, M.B., Pitra, C. (2000): An approach to sexing young Great Bustards *Otis tarda* using discriminant analysis and molecular techniques. *Bird Study* 47: 147-153.
- Martin, J.L. (1991): Patterns and significance of geographical variation in the blue tit (*Parus caeruleus*). *Auk* 108: 820-832.
- Meiri, S., Dayan, T. (2003): On the validity of Bergmann's rule. *Journal of Biogeography* 30: 331-351.
- Peiró, I.G. (2003): Intraspecific variation in the wing shape of the long-distance migrant Reed Warbler *Acrocephalus scirpaceus*: effects of age and distance of migration. *Ardeola* 50: 31-37.
- Pérez-Tris, J., Tellería, J.L. (2001): Age-related variation in wing shape of migratory and sedentary Blackcaps *Sylvia atricapilla*. *Journal of Avian Biology* 32: 207-213.
- Pitzer, S., Hull, J., Ernest, H.B., Hull, A.C. (2008): Sex determination of three raptor species using morphology and molecular techniques. *Journal of Field Ornithology* 79: 71-79.
- Reynolds, S.J., Martin, G.R., Wallace, L.L., Wearn, C.P., Hughes, B.J. (2008): Sexing sooty terns on Ascension Island from morphometric measurements. *Journal of Zoology* 274: 2-8.
- Sandoval, L., Mennill, J.D. (2013): Morphometric measurements permit accurate sexing of three species of Mesoamerican ground-sparrow (Genus: *Melospiza*). *The Wilson Journal of Ornithology* 125: 471-478.
- Sarasola, J.H., Negro, J.J. (2004): Gender determination in the Swainson's hawk (*Buteo swainsoni*) using molecular procedures and discriminant function analysis. *Journal of Raptor Research* 38: 357-361.
- Savile, D. (1957): Adaptive evolution in the avian wing. *Evolution* 11: 212-224.
- Shizuka, D., Lyon, B.E. (2008): Improving the reliability of molecular sexing of birds using a W-specific marker. *Molecular Ecology Resources* 8: 1249-1253.
- SPSS, Inc. (2008): *Advanced statistics v. 17.0*. SPSS, Inc., Chicago, Illinois
- Stermin, A.N., David, A., Pripon, L.R., Seviaru, E., Seifert, N., Eilers, A., Fregin, S., Haase, M., Schmitz Ornes, A. (2014b): Limited genetic structure and diversity in the water rail *Rallus aquaticus* L., 1758 (Aves: Gruiformes: Rallidae) revealed by mitochondrial DNA analysis. *Italian Journal of Zoology*: 1-5.
- Stermin, A.N., Pripon, L.R., David, A. (2012): The importance of homogenous vs. heterogeneous wetlands in rallid (Rallidae) phenological seasons. *Bruckenthal Acta Musei* 8: 549-554.
- Stermin, A.N., Pripon, L.R., David, A., Coroiu, I. (2011): Wetlands management for Little Crake (*Porzana parva*) conservation in a "Natura 2000" site. *International Conference for Environmental Sciences and Development IPCBEE* 4: 91-94.
- Stermin, A.N., Pripon, L.R., David, A., Seviaru, E., Coroiu, I. (2014a): Efficient methods in trapping Water Rails (*Rallus aquaticus*) and Little Crake (*Porzana parva*) for biological studies. *Studia Biologica* 59(1): 91-96.
- Svensson, L. (1992): *Identification guide to European passerines*. British Trust for Ornithology, Stockholm.
- Sweeney, J.J., Tatner, P. (1996): Sexing wrens *Troglodytes troglodytes* indigenus using morphological measurements and discriminant analysis. *Bird Study* 4: 342-350.
- Tavares, E., De Kroon, G.H.J., Baker, A.J. (2010): Phylogenetic and coalescent analyses of three loci suggest that the Water Rail is divisible into two species, *Rallus aquaticus* and *R. indicus*. *BMC Evolutionary Biology* 10: art.226.
- Taylor, B. (1998): *Rails - A guide to the rails, crakes, gallinules and coots of the world*. New Haven and London: Yale University Press.
- Van de Pol, M., Oosterbeek, K., Rutten, A.L., Ens, B.J., Tinbergen, J.M., Verhulst, S. (2009): Biometric sex discrimination is unreliable when sexual dimorphism varies within and between years: an example in Eurasian Oystercatchers *Haematopus ostralegus*. *Ibis* 151: 171-180.
- Videler, J.J. (2005): *Avian flight*. Oxford, UK: Oxford University Press.
- Von Blotzheim, G.U.N., Bauer, K.M., Bezzel, E. (1973): *Handbuch der Vogel Mitteleuropas*. Band 5. Akademische Verlagsgesellschaft, pp.376-395.
- Walton, C., Walton, P. (1999): Sexing first year reed buntings *Emberiza schoeniclus* using biometrics. *Ring and Migration* 19: 327-331.
- Weidinger, K., van Franeker, J.A. (1998): Applicability of external measurements to sexing of the cape petrel *Daption capense* at within-pair, within-population and between-population scales. *Journal of Zoology (London)* 245: 473-482.
- Wiedenfeld, D.A. (1991): Geographical morphology of male yellow warblers. *Condor* 93: 712-723.
- Yannic, G., Broquet, T., Strøm, H., Aebischer, A., Dufresnes, C., Gavrilo, M. V., Gilchrist, H. G., Mallory, M. L., Morrison, R. I. G., Sabard, B., Sermier, R., Gilg, O. (2016): Genetic and morphological sex identification methods reveal a male-biased sex ratio in the Ivory Gull *Pagophila eburnea*. *Journal of Ornithology* 157(3): 861-873.
- Zavalaga, C.B., Paredes R. (1997): Sex determination of adult Humboldt penguins using morphometric characters. *Journal of Field Ornithology* 68: 102-112.