Biometry and sex discrimination of first calendar year Cetti's Warbler (*Cettia cetti*) by discriminant analysis

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Abstract - A method is proposed here for distinguishing the sexes of Cetti's warblers in their first calendar year (Euring age code=3) based on the use of discriminant analysis. In the initial phase of the study, 118 subjects of known sex (62 males and 56 females), captured and marked in the Palude Brusà, were subjected to analysis as a calibration sample. The analysis yielded a discriminant function based on the two most significant variables (third primary length, tarsus length), enabling the sexes to be classified with a 99.2%. The function was subsequently tested on the validation sample (n=106), composed of 54 males and 52 females of known sex ringed in Punte Alberete (north-eastern Italy) and the Trasimeno Emissary (central Italy), obtaining an accuracy classification equal to 99.1%. A global discriminant analysis was subsequently conducted, integrating the data from the three locations into a unified sample (n=224). This analysis yielded a function that accurately classified 99.1% of the females and 100% of the males. The resulting equation represents a rapid and precise method for distinguishing the sexes of first-calendar year Cetti's warblers. Additionally, it can assist in the appropriate selection of ring size, which differs between the sexes.

Keyword: Cetti's Warbler, sex identification, bird ringing, discriminant analysis, biometry.

Riassunto - Biometria e determinazione del sesso mediante analisi discriminante in giovani del primo anno di Usignolo di fiume (*Cettia cetti*).

Viene qui proposto un metodo per distinguere i sessi dei giovani di Usignolo di fiume (codice età Euring =3), basato sull'utilizzo dell'analisi discriminante. Nella fase iniziale dello studio, 118 individui catturati ed inanellati nella Palude Brusà, (62 maschi e 56 femmine) di sesso conosciuto, sono stati sottoposti ad analisi fungendo da campione di calibrazione. L'analisi ha prodotto una funzione discriminante basata sulle due variabili più significative (lunghezza della terza remigante e lunghezza del tarso), che ha consentito di classificare i sessi con un'accuratezza globale pari a 99.2%. La funzione è stata successivamente testata sul campione di validazione (n =106), composto da 54 maschi e 52 femmine di sesso conosciuto, inanellati a Punte Alberete (Italia nord-orientale) e ad Emissario Trasimeno (Italia centrale), rivelando un'accuratezza di classificazione pari a 99.1%. È stata infine condotta un'analisi discriminante globale, integrando i dati delle tre località in un unico campione (n =224). Questa analisi ha prodotto una funzione che ha classificato accuratamente il 99.1% delle femmine e il 100% dei maschi. L'equazione risultante rappresenta un metodo rapido e preciso per distinguere i sessi nei giovani Usignoli di fiume, e può aiutare nella scelta appropriata della misura dell'anello da applicare, che in questa specie differisce a seconda dei sessi.

Parole chiave: Usignolo di fiume, determinazione del sesso, inanellamento, analisi discriminante, biometria.

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INTRODUCTION

The Cetti's Warbler (*Cettia cetti*) is a mainly non-migratory passerine species whose range extends from western Europe to west-central Asia (Sharrock & Grant, 1982; Geister & Ivanov, 1997). In Italy, the species is sedentary and reproduces in the wet habitats and hygrophilous thickets of the peninsula, as well as in Sicily, Sardinia, Corsica, and some smaller islands. The sex of an organism is a significant factor in ecological studies, including those dealing with demographic, behavioural, or movement patterns. The determination of sex in birds during their first calendar year is of great importance in ringing operations, as males, which are on average larger than females, require a ring of a larger diameter (Fig. 1). As the species is sexually monochromatic, ringers employ biometric differences between males and females to determine the sex of juvenile birds (Bibby *et al*., 1984; Cramp, 1992; Svensson, 1992; Villarán, 2000; Licheri & Spina, 2002; Guallar *et al*., 2010; Demongin, 2015). Nevertheless, the overlap of certain measurements, precludes the accurate determination of the sex of some subjects. The present study aims to propose a method of sexing birds in their first calendar year (Euring age code=3) through the use of discriminant analysis. This methodology has previously been employed to determine the sex of Cetti's Warblers (Guallar *et al*., 2010) and other avian species (Green & Theobald, 1989; Desrochers, 1990; Scott, 1993; Sweeney & Tatner, 1996; Walton & Walton, 1999; Balbontin *et al*., 2001; Bertellotti *et al*., 2002; Gordo *et al*., 2016; Zenatello & Kiss, 2005; Lequitte-Charransol *et al*., 2021). The output of the dis-

criminant analysis is a function that is a linear combination of multiple biometric variables, weighted to maximize the statistical differences between birds whose sex has been ascertained. The sex of birds, including those of a monomorphic species, can be determined through the use of several DNA-based techniques for ascertaining sex in monomorphic species (Griffiths *et al*., 1998; Sacchi *et al*., 2004; Morinha *et al*., 2012; Çakmak *et al*., 2017); however, these techniques are not applicable during ringing operations in the field. In contrast, the use of a straightforward formula represents a rapid, noninvasive and practical technique for the determination of sex in subjects of uncertain determination.

Fig. 1 – A juvenile Cetti's Warbler captured in the Palude Brusà (VR) in northeastern Italy. In this species the ring size differs between males and females (Photo: Roberto Pollo). / Giovane Usignolo di fiume catturato nella Palude Brusà (VR), Italia nord-orientale. In questa specie la dimensione dell'anello differisce tra maschi e femmine (Foto: Roberto Pollo).

MATERIALS AND METHODS

Study area

Biometric data was collected at three ringing stations located in freshwater wetlands in north-eastern Italy [Palude Brusà (PB), Punte Alberete (PA)], and in central Italy [Emissario Trasimeno (ET)] (Fig. 2).

PB (45°10'17" N, 11°13'02" E) is a 117-hectare marsh located at an altitude of 13 m a.s.l. in the municipality of Cerea (province of Verona). The habitats are characterized by the extensive reedbeds dominated by the common reed (*Phragmites australis*), interpenetrated by sedges (*Carex* sp. pl.) and mixed shrubs (*Salix cinerea, Sambucus nigra,*

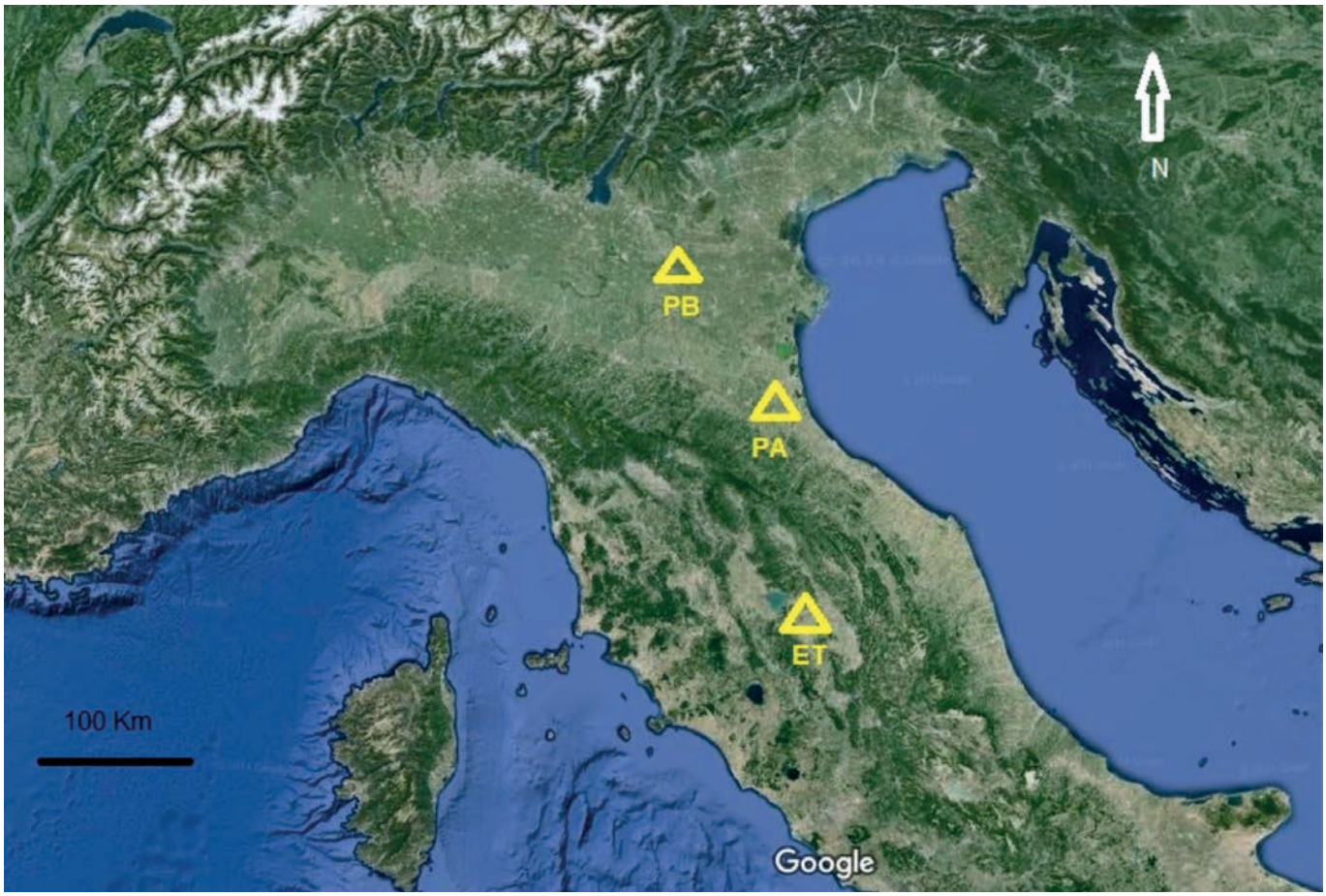

Fig. 2 – Geographical location of the three ringing stations. PB, Palude Brusà (VR); PA, Punte Alberete (RA); ET, Emissario Trasimeno (PG). (Source: Google Earth). / Localizzazione geografica delle tre stazioni di inanellamento. PB, Palude Brusà (VR); PA, Punte Alberete (RA); ET, Emissario Trasimeno (PG). (Fonte: Google Earth).

Cornus sanguinea) arranged around a pond that is about 1.5 hectares in size. PA (44°31' N, 12°13'15" E) is a 150-hectare coastal marsh situated in the southern Po Delta,10 km north of the town of Ravenna. The flooded ancient dune cordons are covered by shrubs (*Prunus spinosa*, *Crataegus monogyna*, *Sambucus nigra*) and tall trees (*Populus alba, Fraxinus angustifolia, Salix alba, Ulmus minor*) while the lower areas are covered by reedbeds dominated by *Phragmites australis* and small areas of open water in the deepest and permanently submerged areas. ET (43°06'23" N, 12°11'08" E) is located on the southeastern shore of Lake Trasimeno in the municipality of Magione (province of Perugia), at an altitude of 257 m above sea level. The shore area is characterized by the presence of semi-submerged helophytic communities and riparian scrublands, which dominated by *Salix alba, Salix purpurea, Populus nigra* and *Populus canescens* (Venanzoni & Gigante, 2000).

Methods

Cetti's warblers were captured during standardised ringing sessions carried out as part of two national monitoring schemes: PRISCO (Volponi *et al*., 2002) and MoniTRing. The aim of these programmes was to monitor the birds during the breeding and migration periods, respectively. Birds were captured using standard mist nests (four bags, mesh of 16 mm) and marked with metal rings provided by the Italian Ringing Centre of ISPRA before release. Juveniles (Euring age code=3) were distinguished from adults by the presence of dark spots on the tongue (Cramp, 1992; Svensson, 1992), by growth bars on the rectrices (Mead, 1965) (Fig. 3), by differences in the degree of wear of the outer primary feathers and in the extent and pattern of post-juvenile moult (Gargallo, 1997; Demongin, 2015). Only birds with complete feather growth were measured, sexed and included in the study. As the species is predominantly sedentary in the study area (Pollo *et al*., 2011), we consider that our sample include only individuals with biometric measurements that are typical of the local population. The following measurements were taken from each bird: weight (to the nearest 0.1 g) and wing length (measured as the maximum chord), length of the third primary feather (counted in ascending order), tarsus length, bill length (from the base of the skull to the tip of the bill), and tail length using the standard method (to the nearest 0.1 mm) (Demongin, 2015). In the case of birds that were captured on more than one occasion, a mean average of each measure was calculated, and the individual was only included once in the dataset. During the first capture, sex was assigned principally based on wing length and weight (Svensson, 1992; Licheri & Spina, 2002; Demongin, 2015). However, sex was subsequently confirmed by at least one recapture of these subjects, which occurred in the years following that of birth. Individuals exhibiting the presence of incubation patches, were confirmed as female, as they are the only ones that incubate (Bibby, 1982; Cramp, 1992). Conversely, those displaying a cloacal protuberance and long, sharp claws were confirmed as male. Discriminant analysis was conducted with the measurements of the third primary length, tarsus length, and weight as variables. The tail length was excluded from the analysis as the heavy wear present in the rectrices (Fig. 3) could influence the measurements taken at different periods of the year. Additionally, the length of the bill was excluded, as its measurements appeared less homogeneous in the three stations compared to the others, and it was not present in a statistically adequate number in the data sets of the three stations.

In the first step of the data analysis, 118 juvenile Cetti's warblers (62 males and 56 females) captured in PB between 2011 and 2022 were employed. These data represent 53% of the overall sample and were used, as recommended by Frank *et al*. (1965), as a calibration sample to generate the first discriminant function. The remaining 47% of the dataset, comprising 54 males and 52 females ringed in PA and ET, was used to form the validation sample. The percentages of correct classification were then estimated from this sample. As the results derived from the validation sample were as good as those from the calibration sample, the entire dataset was used to derive a global discriminant function that could be applied to all three populations under study. Discriminant analysis was performed using

Fig. 3 – A juvenile Cetti's Warbler (Euring age code=3) showing conspicuous and typical growth bars on their rectrices (Photo: Roberto Pollo). / Giovane Usignolo di fiume (codice età Euring=3) con le tipiche ed evidenti barrature di crescita sulle timoniere (Foto: Roberto Pollo).

the non-standardised canonical discriminant procedure of SPSS 13.0 for Windows, with an alpha level of 0.01.

RESULTS

Statistics for the biometric variables of the calibration sample, disaggregated by sex, are presented in Tab. 1. The sex ratio of the sample was not found to differ significantly from a ratio of 1:1 (Single proportion test, Z=-0.37; p=0.71). The results of the t-tests indicate a clear sexual dimorphism in Cetti's Warbler.

Statistics for the biometric variables of data recorded at the three ringing stations PB, PA, and ET disaggregated by sex, are presented in Tab. 2. The analysis of variance test indicates that the biometric measurements are globally homogeneous between the three stations, with the exception of the third primary length in females and the weight in males.

Discriminant analysis

A preliminary analysis utilising the "stepwise" procedure was conducted using the data from the calibration sample (N=118) on third primary length, tarsus length and weight. This revealed that the analysis could also be conducted using only the two best-performing variables: third primary length and tarsus length. The classification accuracy obtained with two variables is, in fact, slightly higher (99.2%) than that obtained with three variables (99.1%). The following equation was subsequently derived: D1=0.949 third primary length+0.687 tarsus length–56.675

The above equation can be used directly for the classification of individual birds, as positive D scores indicate males, and negative scores indicate females. This function was found to be highly significant, with an Eigenvalue of 10.577; Wilk's Lambda of 0.086, and a Chi-squared value of 281.639 with two degrees of freedom and a p-value of less than <0.001 (Fig. 4). The confusion matrix is provided in Tab. 3.

The equation was then applied to the remaining 47% of the sample (PA+ET; 54 males and 52 females), resulting in 98.2% of males and 100% of females being correctly classified. The global accuracy was 99.1%. A subsequent discriminant analysis was then carried out using the overall sample (N=224), comprising 116 males and 108 females. This yielded the following equation: D2= 0.748 third primary length+0.920 tarsus length–52.623

This function was also found to be highly significant. The eigenvalue was 8.873, Wilk's Lambda was 0.101, and the chi-squared value was 506.053 with two degrees of freedom. The resulting p-value was less than 0.001 (Fig. 5). The global accuracy was 99.6 % (100% for the males and 99.1 % for the females). The confusion matrix is provided in Tab. 4.

In accordance with the calculations described by Green

Tab. 1 – Biometric measurements of juvenile Cetti's Warblers (Euring age code=3) ringed in Palude Brusà which composed the calibration sample. The presented statistics comprise the mean and standard deviation, measurement range, sample size, and t-test with probability value. The asterisk indicates high statistical significance. / Misure biometriche di giovani Usignoli di fiume (codice età Euring=3) catturati presso Palude Brusà e utilizzati per il campione di calibrazione. Le statistiche presentate comprendono media, deviazione standard, intervallo di misurazione, dimensione del campione e il t-test con il valore di probabilità.

	Third primary length (mm)	Tarsus length (mm)	Weight (g)
Males	46.9±0.8; (45.5-48.5); N=62	22.3±0.6; (20.1-23.6); N=62	14.1±1.0; (12-16.8); N=60
Females	41.5±1.1; 39-46.5; N=56	20.3±0.5; (19.2-22); N=56	11.4±0.8; (9.9-13.4); N=53
Comparison of males and females	t_{116}=32.32, p<0.001	t_{116}=18.56, p<0.001	t_{111}=15.74, p<0.001

N, sample size / dimensione del campione.

Tab. 2 – Mean values and range of aggregate biometric data recorded at the three ringing stations Palude Brusà, Punte Alberete, Emissario Trasimeno. It also shows the results of homogeneity tests between the data from the three ringing stations, using one-way analysis of variance. The α level was set at 0.05, with the asterisk indicating a high level of statistical significance. / Valore medio e intervallo di variazione delle biometrie aggregate, rilevate presso le tre stazioni di inanellamento Palude Brusà, Punte Alberete, Emissario Trasimeno. Risultati dei test di omogeneità tra i dati delle tre stazioni calcolato mediante analisi della varianza ad una via. Il livello α è stato fissato a 0.05; l'asterisco indica elevata significatività statistica.

	Third primary length (mm)	Tarsus length (mm)	Weight (g)
Males	46.84±0.98; (43-49); N=116; *F*=2.347; p=0.100	22.22±0.59; (20.1-23.6); N=116 *F*=0.700; p=0.486	14.42±1.01; (11.5-17); N=114 *F*=5.871; p=0.004*
Females	41.36±1.14; (38-46.5); N=108; *F*=6.748; p=0.002*	20.22±0.48; (19.2-22); N=108 *F*=2.911; p=0.06	11.35±0.69; (9.9-13.4); N=105 *F*=0.064; p=0.938

N, sample size / dimensione del campione.

& Theobald (1989), a scatter plot was constructed with measures of third primary and tarsus lengths, with contours lines corresponding to specified probabilities that a bird is male (Fig. 6). This graphical representation allows for a more rapid and straightforward evaluation of the probability that a capture young Cetti's Warbler may be sexed.

Tab. 3 – Confusion matrix of the discriminant function obtained from the analysis carried out on 53% of subjects of known sex (62 males and 56 females) captured in Palude Brusà. / Matrice di dissimilarità della funzione discriminante ottenuta mediante analisi del 53% dei soggetti di sesso conosciuto (62 maschi e 56 femmine) catturati in Palude Brusà.

	Predicted Group Membership	
	Males	Females
Males	62	1
Females	0	55
Accuracy	100%	98.2%
Global Accuracy	99.2%	

DISCUSSION AND CONCLUSIONS

The analysis conducted on the calibration sample enabled the derivation of a preliminary discriminant function with the capacity to differentiate between the sexes of first-year Cetti's Warblers with a 99.2% accuracy rate. Subsequently, the function was tested on the validation sample, with a classification accuracy of 99.1%. This outcome prompted us to undertake a comprehensive analysis, integrating the data from the three sites into a unified sample. The level of accuracy achieved in this final analysis was similarly high, with the function correctly identifying 99.1% of females and 100% of males, resulting in a global accuracy of 99.6%.

A critical factor in this study is the fact that the three populations under investigation had their biometric data collected by three different ringers. Consequently, it was not possible to verify the repeatability of field measurements (Hernandez *et al*., 2011). It should be noted, ho-

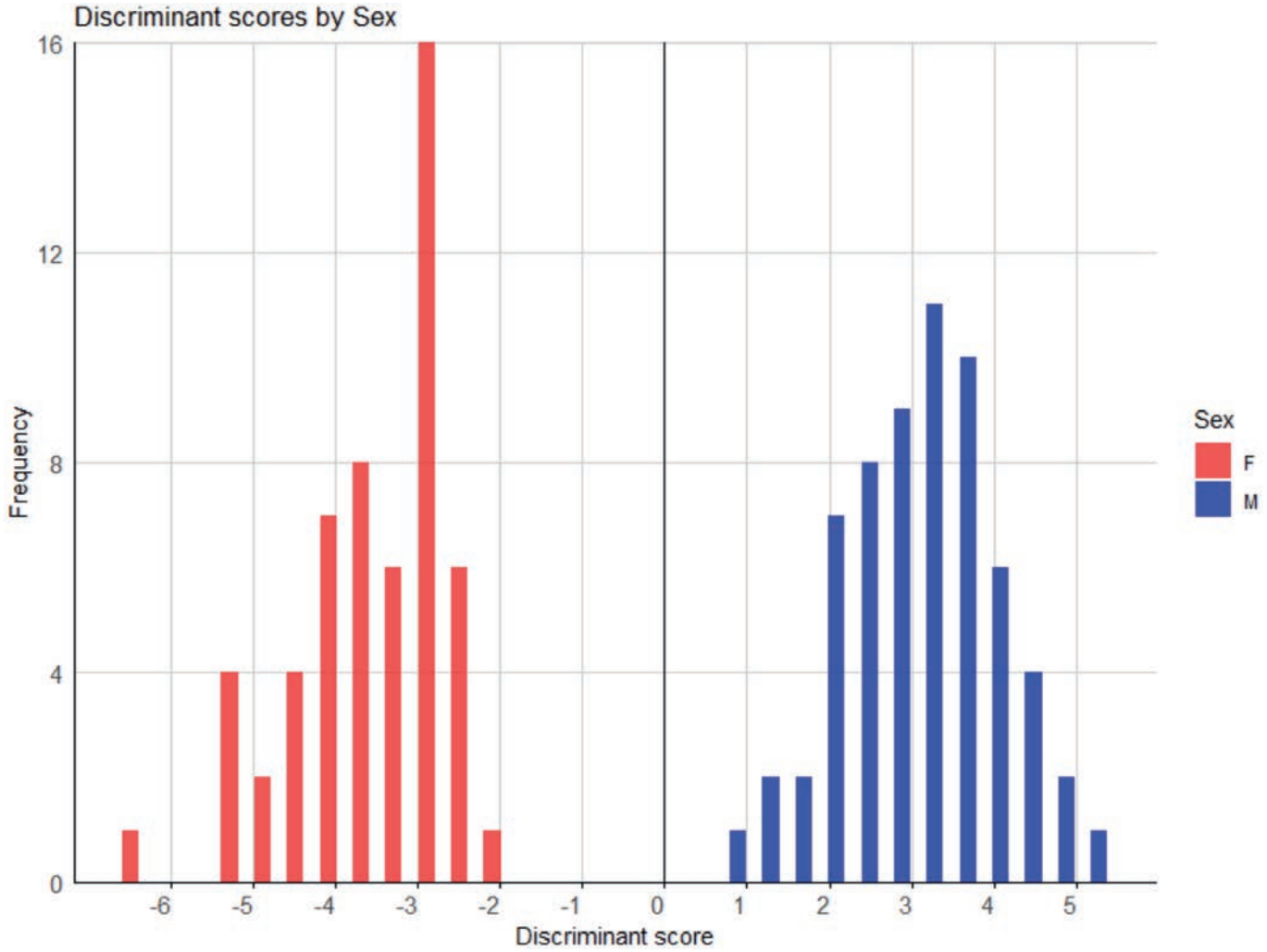

Fig. 4 – The plot shows the results of a discriminant analysis conducted on the data of the third primary length and tarsus length of juvenile Cetti's Warblers captured at Palude Brusà ringing station. / Grafico con i risultati dell'analisi discriminante ottenuta con i dati della lunghezza della terza remigante primaria e del tarso di giovani Usignoli di fiume catturati nella stazione di inanellamento di Palude Brusà.

wever, that the biometric data used in the analysis are standard measurements commonly taken during ringing activities and readily collected by experienced ringers. Tab. 2 illustrates that, except the measurement of the third primary length of the females, the measurements taken in the three stations and used in the discriminant analysis are homogeneous. Moreover, in the case of wing length, the third primary length was selected over the maximum chord due to its superior standardisation in the measurement method. Furthermore, the reliability of the procedure is reinforced by the fact that the first discriminant function obtained with the PB data, when applied to the validation sample, demonstrated the same high classification accuracy. We are, therefore, confident that these potential sources of error are unlikely to compromise the reliability of the global discriminant function. With regard to its applicability, the equation can be used in north-east and central Italy on subjects in their first calendar year, but great caution must be exercised on subjects captured in other European areas. For instance, the mean weight derived from the combined data from the three Italian stations is, for both sexes, higher than that observed in juvenile subjects captured near the Atlantic coast of Portugal and is slightly

Tab. 4 – Confusion matrix of the final discriminant function obtained from the analysis carried out on the sample obtained from the three ringing stations of Palude Brusà, Punte Alberete and Emissario Trasimeno (N=224). / Matrice di dissimilarità della funzione discriminante finale elaborata con il campione globale di dati raccolti nelle tre stazioni di inanellamento di Palude Brusà, Punte Alberete ed Emissario Trasimeno (N=224).

	Predicted Group Membership	
	Males	Females
Males	116	1
Females	0	107
Accuracy	100%	99.1%
Global accuracy	99.6%	

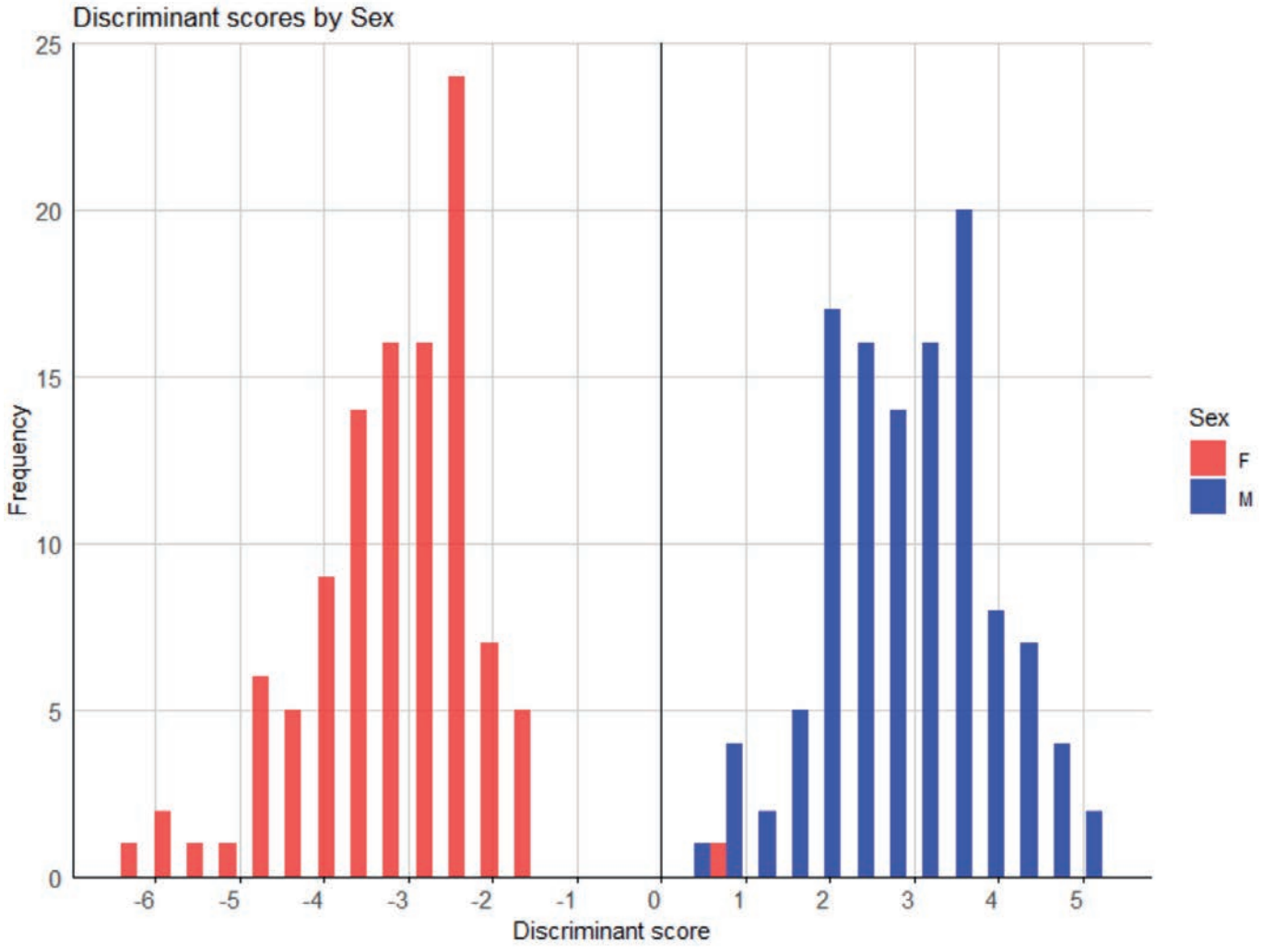

Fig. 5 – The plot shows the results of the final discriminant analysis conducted on the data of the third primary length and tarsus length of juvenile Cetti's Warblers captured in Palude Brusà, Punte Alberete and Emissario Trasimeno (N=224). / Grafico con i risultati dell'analisi discriminante finale ottenuti con i dati di lunghezza della terza remigante e del tarso dei giovani Usignoli di fiume catturati nelle stazioni di Palude Brusà, Punte Alberete ed Emissario Trasimeno (N=224).

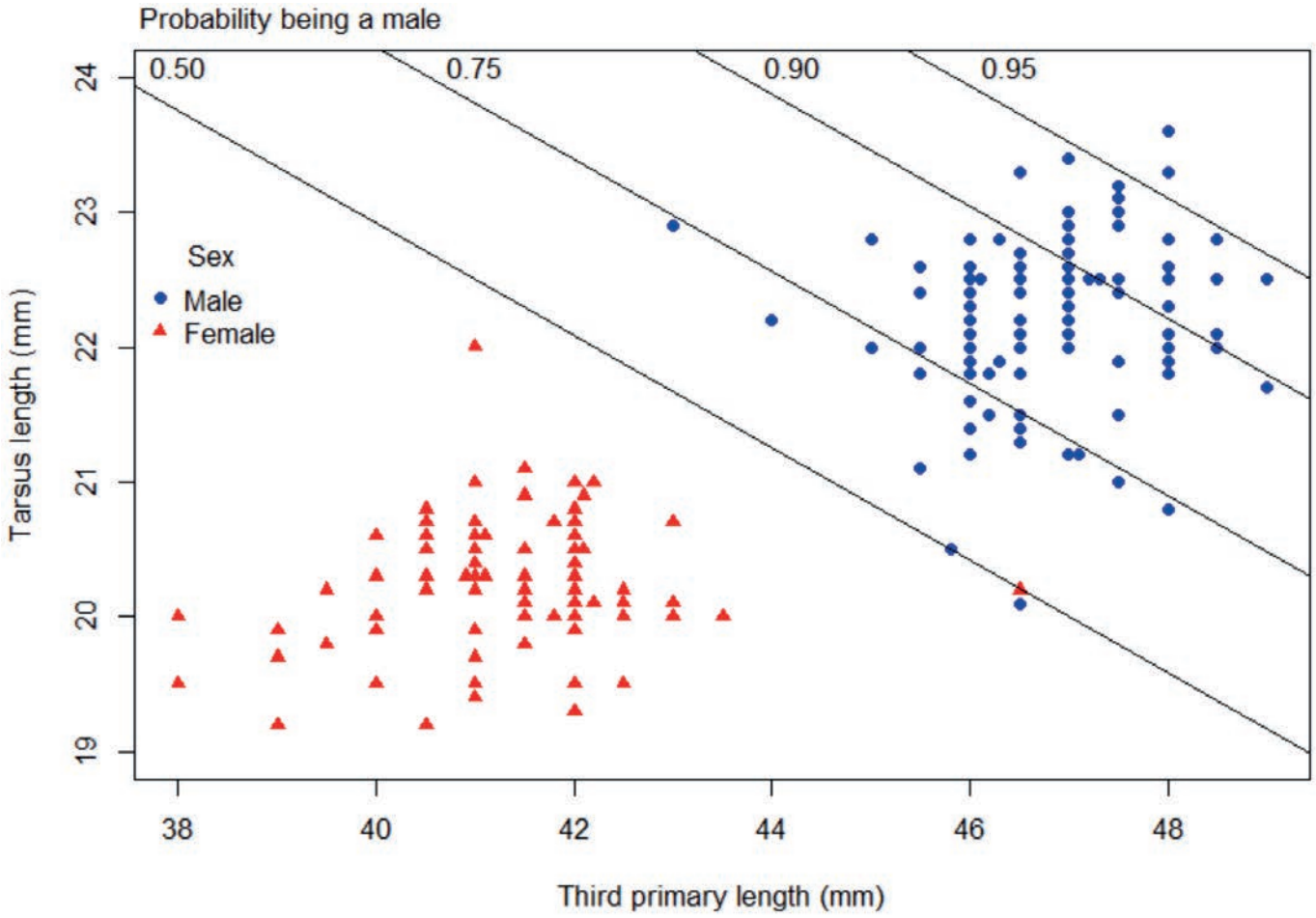

Fig. 6– Sex distribution of third primary and tarsus lengths of young Cetti's warblers. Lines represent the 0.50, 0.75, 0.90 and 0.95 probability contours of being male calculated as described in Green & Theobald (1989). Whole dataset (N=224). / Distribuzione delle misure di lunghezza del tarso e della terza remigante di giovani Usignoli di fiume. Le linee di contorno, calcolate secondo il metodo descritto in Green & Theobald (1989), rappresentano la probabilità dello 0.50, 0.75, 0.90 e 0.95 di un individuo di essere maschio (intero campione, N=224).

lower than that recorded in the north-west of France (Bibby *et al*., 1984).

In conclusion, the utilisation of this straightforward equation in the field offers the advantage of a rapid and effective method of selecting the most appropriate ring size with a high degree of accuracy for captured subjects. We hope that the function obtained in this study, despite being tested on a limited area of the Cetti's Warbler breeding range, can be used by other ringers to verify its validity in other Italian regions or to compare the biometry of subjects belonging to other European populations.

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