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# Is the skull of the black-headed gull dimorphic? Analysis of shape variation

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**Abstract**
The black-headed gull (*Chroicocephalus ridibundus*) is a small bird belonging to the Laridae family. Their distribution and breeding broad area primarily cover the northern part of the Eurasian continent. Gulls are considered monomorphic birds, and distinguishing the sexes based on external morphological features is extremely difficult. In this study, a 3D shape analysis of the skull of black-headed gulls from central-northern Poland was performed to reveal the variation in shape between individuals and whether male skulls differ from females. For these purposes, 82 skulls were used (32 females and 50 males). 3D surface models of the skull were extracted using computed tomography. Twenty-one landmarks were designed on each skull. The principal components method was used to assess the variability of skull shapes. The first two principal components accounted for 48.28% of the cumulative shape variation. The most significant shape variation relative to PC1 was observed in the braincase. Positive PC2 values reflected the neurocranium expanding more rapidly dorso-ventrally with a wider orbit, and the nasofrontal hinge moved dorsally. According to the Procrustes ANOVA, the centroid size was statistically different between the sexes ($p < 0.0001$). Male skulls were longer than females. Neurocranium was wider in male gulls. The nasofrontal hinge was more caudal in females. The results of this study are consistent with the observations of other authors applying linear morphometry.

**Keywords:** *Avian anatomy*, *shape analysis*, *sex determination*

## Introduction

The black-headed gull *(Chroicocephalus ridibundus*) is a small gull species that belongs to the family *Laridae*. It is widely distributed across Europe and Asia and is a familiar bird in urban and suburban areas where it commonly nests (BirdLife International 2023). Most of the population winters further south, but some birds reside in the milder westernmost areas of Europe. Within this breeding area, northern populations are migratory, while populations further south are partially migratory. As with many gulls, it was previously placed in the genus Larus (Pons et al. 2005). The genus name Chroicocephalus comes from the ancient Greek khroizo, "to colour", and kephale, "head". The specific ridibundus is Latin for "laughing", from ridere "to laugh" (Jobling 2010).

Gulls (Laridae) are monomorphic birds in plumage and appearance (Del Hoyo et al. 1996); hence, determining the sex of wild-caught specimens poses considerable difficulties.

Sexual dimorphism in the skeleton is studied in all tetrapod groups and often varies between species (Ralls 1977). It is most clearly marked in the pelvic girdle but also affects the skull (Schutz et al. 2009).

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Many avian species do not show such apparent sexual dimorphism as mammals. This applies both to the external appearance and to the skeletal dimensions and shape. However, researchers have been trying to find features that can distinguish males from females also in monomorphic birds for a long time (Hutt 1929; Livezey & Humphrey 1984; Verdiglione & Rizzi 2018; Gündemir et al. 2020a; Szara et al. 2022).

Males tend to be larger than females in most bird species, but "inverted size dimorphism" is observed in many species, with females being the larger sex. This applies chiefly to raptors, owls, shorebirds, and seabirds (Fairbairn & Shine 1993). Rensch's rule states that sexual size dimorphism increases with increasing body size when the male is the larger sex and decreases with increasing average body size when the female is the larger sex (Rensch 1950). It seems evident that the differences in the size of the skulls in males and females must entail variability in their shape, if only because of the more extensive attachments that stronger muscles require (Dillon 2014).

Black-headed gulls show little sexual dimorphism, and courtship behaviors were rarely observed during the chick-raising period when most re-sightings occurred (Péron et al. 2010). Attempts to determine the sex of gulls, including the black-headed gull, have been made by several authors (Ingolfsson 1969; Schnell et al. 1985; Mawhinney & Diamond 1999; Chochi et al. 2002; Lindenfors et al. 2003; Galarza et al. 2008; Herring et al. 2010; Meissner et al. 2017; Indykiewicz et al. 2019; Pazvant et al. 2022). Székely et al. (2000) noted that Charadria (coastal birds, seagulls, and alcids) exhibit diverse plumage and size dimorphism, ranging from monomorphism to male-biased or female-biased dimorphism.

The ability to determine sex in birds is necessary, among others, in breeding work and population research.

This paper aims to answer whether there are differences in the shape of the skeleton of the head and its parts between males and females of the black-headed gull.

## Materials and methods

### *Samples*

In the study, 82 skulls of black-headed gulls were used (32 females, 50 males). All skulls were retrieved for study from jays found dead in four breeding colonies between 2014 and 2015. These colonies were located in central-northern Poland on islands of different sizes of water reservoirs. Pathological or broken specimens were not used in the study.

### *Sex determination*

The sex of the birds was determined based on an autopsy, i.e., the presence of testes or ovaries (66 individuals). However, when it was impossible due to the advanced decomposition of soft parts (internal organs), a genetic method of sexing was applied (16 individuals). We used a molecular approach developed by Fridolfsson and Ellegren (1999).

### *DNA extraction and molecular sexing*

Nuclear DNA was extracted from the feather using Bio-Trace DNA Purification Kit (EURx, Gdańsk, Poland), following the protocol recommended by the manufacturer. After extraction, amplifications of the chromo-helicase-DNA-binding (CHD) region were performed with the primer pairs P2 (5'TCTGCATCGCTAAATCCTTT-3") and P8 (5"-TCCCAAGGATGAGRAAYTG-3') (Griffiths et al. 1998) (samples from 2014) or 2550F and 2718R (Fridolfsson & Ellegren 1999) (samples from 2015). The PCR mix contained 1× PCR buffer, 1.5 mm MgCl2, 200 jiM dNTP, 1 U Tag polymerase (Promega), 30 pmoles each of primer (Oligo), 1 µl of genomic DNA and distilled water to 50 µl. The PCR cycling conditions consisted of an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 48°C (P2/P8 primer pair) or 50°C (2550F/2718 R primer pair) for 45 s and an extension of 72°C for 45 s. Finally, one cycle of annealing at 48°C for 1 min and an extension at 72°C for 3 min was performed. PCR fragments were separated by horizontal electrophoresis through 2.5% agarose gels. Gels were viewed under UV light and photographed. A female produced two bands (ca. 650 bp and 450 bp), and the male was indicated by one band (ca. 650 bp).

### *CT scanning and 3D reconstruction*

Computed tomography scans were taken using a 16-row scanner (Philips & Neusoft Medical Systems). Scanning parameters for all the samples were 0.75 mm slice thickness, 120 kV, and 28 mA, total scanning time of approximately 14 s. After the scanning process, the images were transferred to the computer, and the segmentation process was performed. 3D models of skulls were extracted using the 3D Slicer (5.1.0 version)

program. The Stratovan Checkpoint program was used for landmark operations. A total of 12 landmarks were designed: 1 – the bill tip, 2 – the midpoint of the nasofrontal hinge, 3 – most rostral point of the antorbital fenestra, 4 – the orbital process of the lacrimal bone, 5 – edge of the orbit on the level of the frontoprefrontal junction, edge of the orbit, 6 – edge of the orbit in middle between the orbital and postorbital processes, 7 – postorbital process, 8 – otic process, 9 – mandibular process, 10 – dorsolateral point of the foramen magnum, and 11 – ventrolateral point of the foramen magnum 12 – the most ventral point of the occipital condyle

The landmarks used are shown in Figure 1.

### *Statistical analysis*

MorphoJ (version 1.07a, Klingenberg 2011) was used to perform statistical analyses of 3D landmark data and evaluate skull shape variation. Procrustes superimposition was applied to the landmarks. This process removes position, orientation, and size differences, focusing solely on shape information. The within-individual variation and measurement error were reduced by averaging Procrustes coordinates of the skull. Principal component analysis was performed to reveal the shape variation. Effect sizes for females and males were obtained, and p-values were obtained using a t-test using Past software. Multivariate regression of shape onto size for the symmetric component was performed to analyze the shape allometric effect. This analysis examined the regression between Procrustes coordinates and centroid size. The regression analysis can help quantify and understand the specific shape changes associated with size, highlighting the patterns of shape variation that are directly related to size changes and distinguishing them from other sources of variation.

## Results

### *Size*

Mean values of centroid size were obtained for males ($7.59 \pm 0.04$) and females ($7.25 \pm 0.04$). The minimum centroid size value for males was 7.07, and the maximum was 8.28. These values were 6.69 and 7.67 for females, respectively. The difference between males and females was statistically significant ($p < 0.01$).

*Shape variation.* As a result of the Principal Component Analysis, the total number of dimensions was reduced, and 29 principal components (PCs) were generated. PC1 explained 28.88% of the total shape variation (eigenvalue: 0.00129768). PC2 explained 19.40% of the total shape variation (eigenvalue: 0.00087197). PC3 explained 12.66% of the total shape variation (eigenvalue: 0.00056904). The first two principal components accounted for 48.28% of cumulative shape variation. The scatter plot for PC1 and PC2 showed distributions for male and female skulls. The females and males highly overlapped in the morphospace described by the first two PCs (Figure 2).

The most significant shape variation relative to PC1 was observed in the braincase (Figure 3). The facial part of the skull was lower, and the base of the nasal bone moved slightly upward with increasing PC1. The relative cranial height was slightly lower than the mean with increasing PC1 value. Individuals with the more ventral position of the foramen magnum have higher PC1 scores. The increased PC2 value represented a narrower cranium. Positive PC2 values reflected the neurocranium expanding more

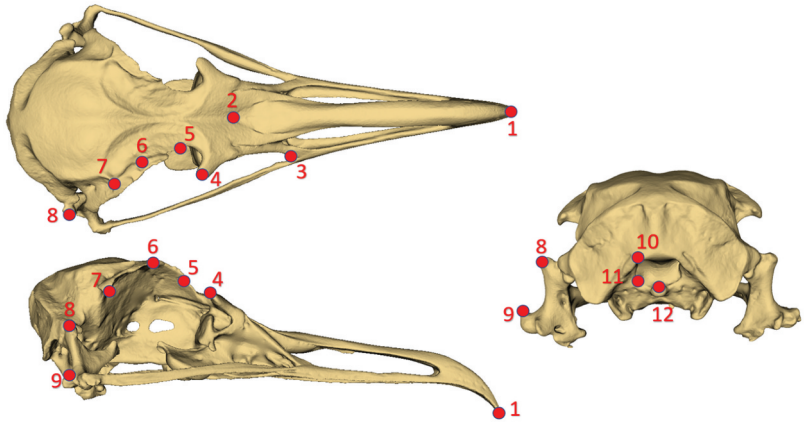


Figure 1. Landmarks.

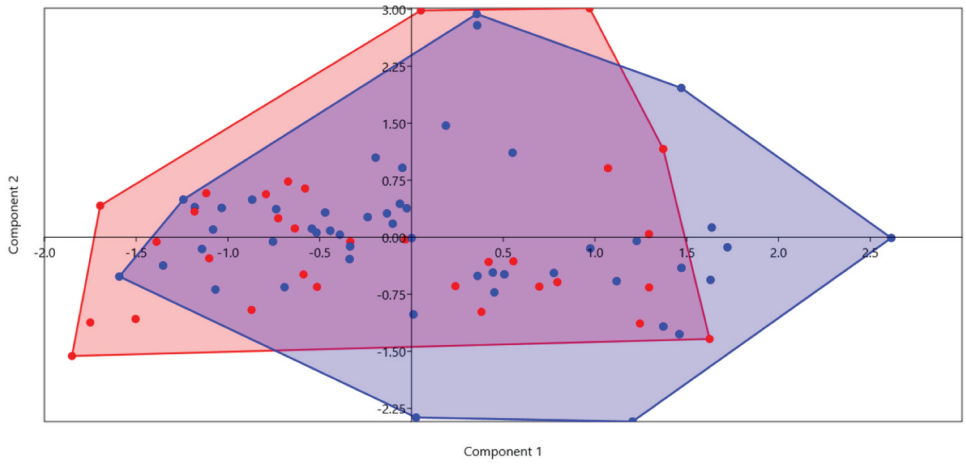


Figure 2. Principal component analysis scatter plot comparing skull morphology of black-headed gulls with convex hulls (female: red; male: blue).

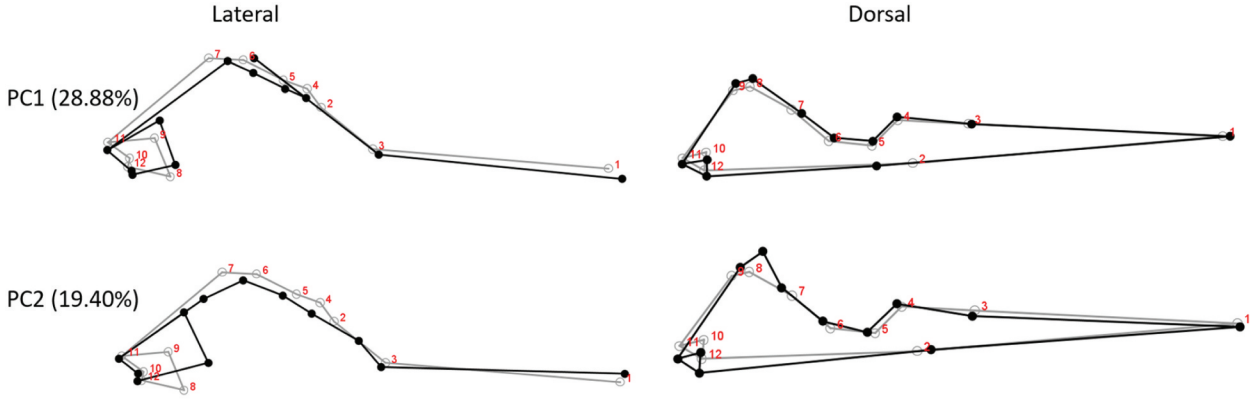


Figure 3. Wire-frame warp plots of changes in skull shape of PC1 and PC2. While the black plots indicate the positive limit of the principal component's scores, the gray plots indicate the mean shape.

dorso-ventrally with a wider orbit. The nasofrontal hinge also moved dorsally

Wire-frame warp plots comparing the average skull shape of females and males are shown in Figure 4. There is a noticeable lack of apparent differences between the sexes.

Multiple regression results are shown graphically in Figure 5. Regression of shape onto size for black-headed gull skull was significant ($R^2$: 02998, $p < 0.001$). About 4.39% of shape variation is explained by size variation.

Figure 6 shows the allometric effect on changes in the skull shape. The length and direction of the lines illustrate deviations from the average shape with increasing skull size.

With increasing size, the facial part of the skull lengthens. The neurocranium is relatively reduced in horizontal and vertical directions with simultaneous widening of the jugal arches.

## Discussion

Sexual dimorphism, although weakly expressed in gulls, appears in the embryonic period (Ležalová et al. 2005). Previous attempts to differentiate males from females in gulls have relied on behavioral characteristics and general appearance, including plumage (Ingolfsson 1969; Chochi et al. 2002; García-Tarrasón et al. 2015). However, the search for dimorphic morphometric, allometry-independent features has not yielded satisfactory results.

In this study, we compared the skull shape of the black-headed gull in search of sex differences using geometric morphometrics (Boz et al. 2023). The results presented above prove that the shape variability of black-headed gull skulls is not only related to sex differences. According to Shatkovska and Ghazali (2021), much of the variation in skull shape in birds remains unexplained.

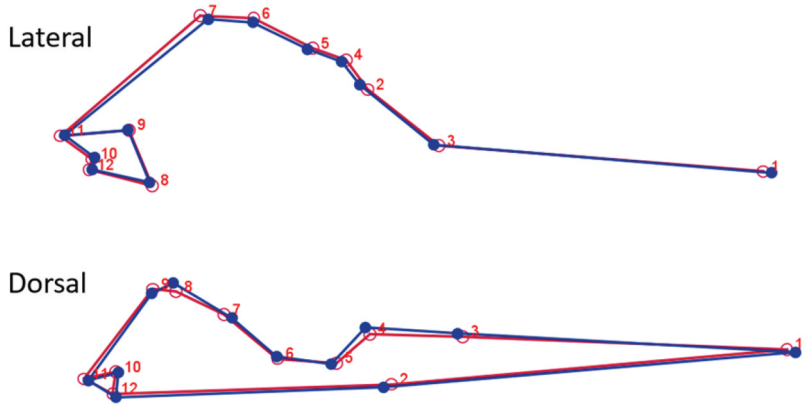


Figure 4. Mean shapes for females and males (female: red, male: blue).

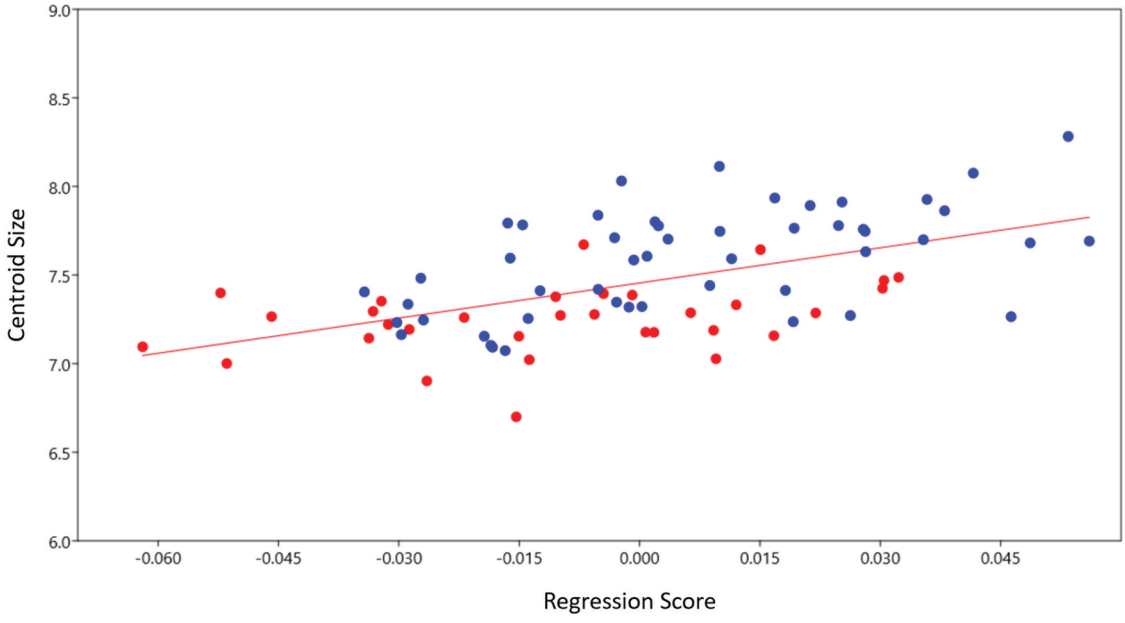


Figure 5. Multivariate regression plot for both sexes of black-headed gull by relating the centroid size vs. The shape scores (the regression score obtained from the procrustes coordinates; female: red, male: blue).

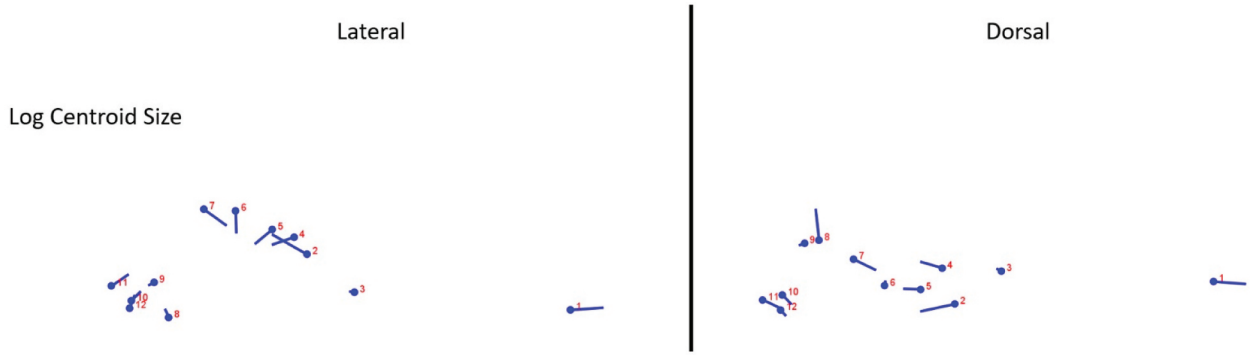


Figure 6. Lollipop plot of the shape changes in dorsal and lateral view (scaling factor = 10).

Sex differences in the skull were investigated using geometric morphometrics in poultry by Gündemir et al. (2020b). In the quail, the neurocranium of male birds was reported to be wider than in females (Szara et al. 2022). The authors noted that females and males showed close variation in Principal Component Analysis. The foramen magnum was narrower in female skulls. In our study conducted in black-headed gulls, the shape variation of male and female skulls was also similar, as shown by the Principal Component Analysis. However, similarly to quails, female gulls had a wider neurocranium.

There are also studies in which sex discrimination was performed using external head measurements in gulls (Chochi et al. 2002; Dubiec et al. 2015; Meissner et al. 2017; Meissner & Fischer 2017; Indykiewicz et al. 2019) and other birds (Gündemir et al. 2020b; Pazvant et al. 2022). In these studies, it was shown that the skull was linearly determinant in males and females. Male skulls were reported to be longer than females. The main measurements discriminating between sexes were the head length and the beak depth at gonys.

Schnell et al. (985) evaluated in detail the differences in skeletal dimensions between the sexes of the California Gulls. The greater depth difference in the head was restricted to the facial part; the difference between males and females in skull depth was only 3.55%. The relatively greater bill depth in males is probably related to the importance and prominence of the bill in courtship displays and territorial defense. In the case of the black-headed gull, the more massive beak bones in the males are also associated with adaptations to fighting and territorial behavior (Bukacińska & Bukaciński 1993).

Many authors have noted head size dimorphism in different gull species (Ingolfsson 1969; Schnell et al. 1985; Fairbairn & Shine 1993; Székely et al. 2000; Galarza et al. 2008; Herring et al. 2010; Hallgrimsson et al. 2016; Indykiewicz et al. 2019). As our results show, the allometric effect is expressed mainly in the elongation of the facial part of the skull and the reduction of the neurocranium with a simultaneous clear expansion of its caudal part. This can be explained by strengthening the bill in larger individuals, which is necessary for foraging and fighting. This, in turn, forces the development of stronger muscle attachments that move the mandible (Zusi 1967).

Allometric analysis showed elongation of the bill, reduction in neurocranium dimensions, and widening of the jugal arches as the size of the entire skull increased. A similar phenomenon has been described by Bright et al. (2016), analyzing the allometric effect on head shape in raptor birds. The authors reported the allometric trend of posterior rotation of the occipital and dorsoventral compression of the braincase with increased bill length. Similar observations were made by Pecsics et al. (2023), examining the diversity of skull shapes in European woodpecker species. They noticed that larger species have longer bills compared to the neurocranium. Allometric changes can also be attributed to age and diet. Natale and Slater (2022) suggested that allometry contributes to avian morphological diversity. They found that foraging ecology explains more than two-thirds of the variation in skull shape in Charadriiformes. However, samples used in our study were obtained from dead birds. For this reason, we did not have enough information about their migrations and feeding, and the analysis on this topic could not be more detailed.

The test describing the allometric formation was found to be statistically significant in the study. However, the variation was very low. Size variation in mammals affects the shape of the skull. A larger skull requires more extensive muscle attachments (Cardini & Polly 2013). The relatively low shape-to-size regression coefficient in the black-headed gull, as in other birds, can be explained by poorly developed head muscles (Koenig et al. 2016).

Although we have captured differences in skull shape between the sexes, they are not clear enough to apply to any population. The high percentage of skulls falsely assigned to sex in the cross-validation proves that the shape dimorphism of the black-headed gull is very weakly expressed. Size dimorphism in these birds is more significant, as reported by other authors (Müller et al. 2007)

Székely et al. (2000) strongly suggest that sexual selection has had a significant influence on sexual size dimorphism in Charadrii. Black-headed gull males are about 15% heavier and skeletally larger than females (Glutz von Blotzheim & Bauer 1982)

Some authors point to the biased mortality of species with size dimorphism. Hasegawa and Arai (2018) showed that pronounced dimorphic features in swallows are associated with an increased risk of extinction. This phenomenon also applies to the black-headed gull (Müller et al. 2007).

The beak of birds is a classic example of dietary adaptation. The shape of the beak is strongly integrated with the rest of the skull and is influenced by allometric factors (Bright et al. 2016).

In our study, we have shown, among other things, that sexual dimorphism in the black-headed gull is not very clearly marked within the beak. These differences were noticed by Greenberg et al. (2013) in sparrows. However, the authors drew attention to

the seasonality of dimorphism in sparrows concerning the beak, particularly in the rhamphotheca. Based on the available material, we have not been able to analyze this phenomenon. It would certainly be worth undertaking in future studies.

We based our research on material from central-northern Poland only (Indykiewicz et al. 2019) pointed out that the discriminant functions developed for Western European populations of black-headed gulls showed relatively low efficiency in identifying sex with the inland Central European population, leading to incorrect estimation of the sex ratio. Also, Evans et al. (1993) reported that the highly effective discriminant function they developed for the Florida laughing gull misclassifies 40% of the New York black-headed gulls. Therefore, due to the pervasive nesting range of the black-headed gull, it seems reasonable to conduct similar studies in different habitats, enabling their subsequent comparison and perhaps generalization of the results.

In conclusion, we can state that the skulls of black-headed gulls show size dimorphism, and the variability of their shape is determined largely by factors other than sex.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request

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