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APPLICATION OF BIRD EGG MORPHOMETRICS IN PHYLOGENY

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The aim of this report was to to build phylogenies using bird egg data, because egg data are available for a larger range of species than molecular data, it is cheaper to collect egg data than molecular data, moreover, building trees using egg data provide verification of molecular phylogenies.

We used egg morphological data from the collections of zoological museums in Ukraine and Russia. Two models of egg geometry were used: composite ovoid and polynomial. For the first model we used seven indices of description of eggs, including the traditional elongation index as well as six original indices: index of infundibular (blunt) area, index of lateral area, index of local area, index of asymmetry, Equatorial index, and index of complimentary. All the necessary parameters were obtained from the digital pictures of eggs, processed by original computer software in accordance with suggested schemes. Using Falconiformes species as a case study, we found that measures of egg geometry gave important phylogenetic information: egg parameters clearly separated Falconiformes species from Gaviiformes, Pelicanidae, and Podicepediformes. We proposed usage of the morphological parameters of eggs as additional information in bird systematics.

Key words: birds, eggs, systematic, geometrical parameters.

INTRODUCTION

A variety of methods have been used to study the systematics, evolution, and phylogenetic relationships. Before the advent of molecular technology, scientists built phylogenetic trees based on morphological and anatomical features, using methods such as the Wetmore system. The accumulation of paleontological and comparative anatomical data contributed to understanding of the age and origin of bird groups, the direction of evolution, and relationships both within and between clades.

Near the end of the 20th century, C. Sibley and J. Ahlquist used data from DNA hybridization to inform a fundamental revision of bird systematics (Sibley & Ahlquist, 1990). These results sparked critical publications and inspired further systematics research using molecular techniques (Andersen et al., 2014; Clarke et al., 2005; Gamauf & Haring, 2004; Harshman, 1994; Slack, 2012; Amaral & Jorge, 2003), as well as morphological (Zelenitsky et al., 2012; Davis & Page, 2014; Deeming & Ruta, 2014a) and comparatively-anatomic (Kurochkin, 2000; Barta & Székely, 1997; Demming & Ruta, 2014b; Livezey & Zusi, 2007; Mayr, 2005; Welch et al., 2014) research to examine their data in a new way .

Another approach to bird systematics, while not well-known, deserves consideration. We conditionally named this the oological method, because it is based on the morphological parameters of bird eggs. In former USSR its founder was A.P. Kuziakin. He compared the nests and clutches of species from different genera, families, and orders to determine whether this information could improve the modern classification of birds (Kuziakin, 1954).

The oological method confirms the generally accepted interpretations of phylogenetic connections in most, but not all, cases. If the oological method suggested different phylogenetic relationships than molecular methods, that we should question the accuracy of molecular methods. We used the oological method to study the classification of modern ostriches and the extinct moa, using both information about the destruction of the shell and mitochondrial DNA (Huynen et al., 2010; Lerner et al., 2008; Lerner & Mindell, 2005). These works prompted the current broader investigation of the utility of eggs geometry data in bird systematics.

An egg is an autonomous system that grows within another system, the female organism. Both systems are realized on the basis of a single genetic code. Thus, the tree diagrams built using the morphological characters of eggs are expected to be similar to diagrams built using other morphological or molecular characters of adult birds. The problem with using morphological data was that morphological characters can appear similar when they are in fact convergent – molecular data would indicate that the taxa are unrelated, even though they share certain morphological characters due to convergent evolution. The identity of data depends on the amount of the analyzed criteria and their quality, i.e. they must be integrative (Troscianko, 2014).

The aim of this report was to determine the extent to which morphological features of eggs provide additional information useful for understanding the phylogenetic relationships of birds.

MATERIAL AND METHODS

We collected measurement data and photos of eggs from the collections of zoological museums of Ukraine and Russia (National Science and Natural History Museum National Academy of Sciences of Ukraine, the Zoological Museum of Kiev, National Taras Shevchenko Museum of Zoology, Lviv National Ivan Franko University, State Museum of Natural History National Academy of Sciences of Ukraine (Lviv), the Nature Museum of Kharkiv National University, Cherkassy Regional History Museum, the Zoological Museum of Moscow State University).

We described eggs using methods presented previously (Mityay, 2003; 2008). We used two models of egg geometry: composite ovoid and polynomial. For the first model, we used seven indices of description of eggs: the traditional elongation index ($I_{el} = L/D$), and six indices that we originated. These six new indices are: index of infundibular (blunt) area, $I_{iz} = r_i/D$; index of lateral area, $I_{lz} = r_l/D$; index of local area, $I_{cz} = r_c/D$; index of asymmetry, $I_{as} = r_c/r$; equatorial index, $I_{eq} = b = L-(r_c+r_i)$; and index of complimentary, $I_{com} = (r_c+b)(r_i+b)BL$. Note that L is the egg length, D is the egg diameter, r_c, r_l, and r_i are radiuses of cloacal, lateral, and infundibular areas, and $b = L - (r_c+r_i)$. We obtained all the necessary parameters from digital pictures of the eggs, processed by originally designed computer software in accordance with proposed schemes (Fig. 1).

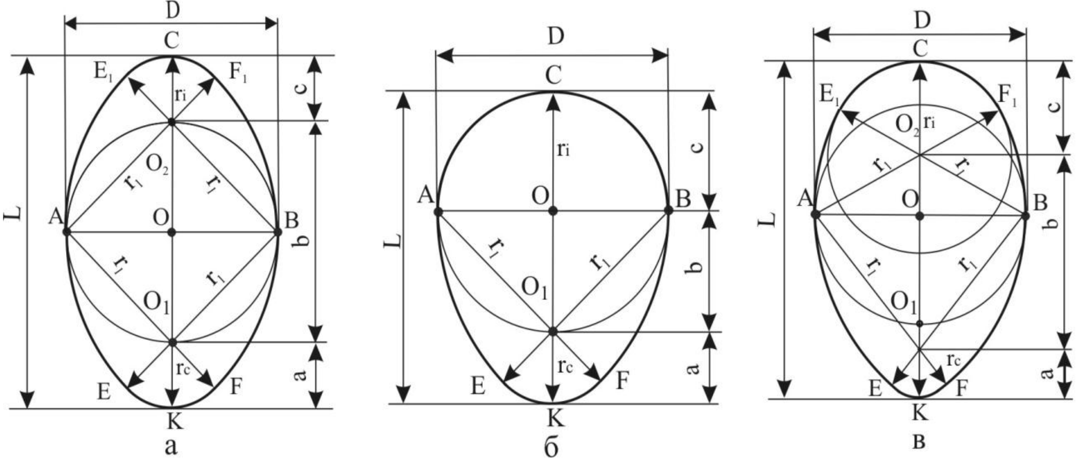

Fig. 1. The schemes of measurements of different egg types. A and B - symmetric and asymmetric pseudoovoids, C - ovoids.

The second model presented egg description by means of polynomial coefficients k^0, k^1, k^2, and k^3, as suggested by Frantsevich (Frantsevich, 2015).

Thus we need to use the closely-related parameters for our analysis. These ovoid parameters could be the arcs, united by the laws of interface (model of component ovoid). Their radiuses correspond to the mentioned arcs, and the length of ovoids can be considered as the complete unit. In the second model the polynomial coefficients are the composites, united by a polynomial equation (physical model), which reflects the aggregation of transformations of the sphere into ovoid.

All the research related to the phylogeny and systematic of Falconiformes could be summarized in these: a) position of order in class Aves; b) subdividing of order into families; c) interrelation of representatives of families and genera inter se and with the representatives of other orders. In regard to the position of Falconiformes among other bird orders there is a great number of contradictory literature data. The basic reason of such variation is one-tailed approach, when the whole system uses one or two criteria.

Morphological evidence suggests that birds of prey are closely related to the families Ciconiformes and Pelicaniformes (Mayr & Clarke, 2003), although other authors have suggested that weak similarity instead exists between small predators (hawks, harriers) and Little Bittern and White Stork (Dementyev, 1951; Kuziakin, 1954).

The data thus collected were statistically analyzed by Non-Parametric Test (Kruskal-Wallis, H criterion), the data were also assessed with One-Way ANOVA (Homogeneity of variance test and Brown-Forsythe test) using IBM SPSS Statictics 18.0. Comparison of means was made through Post Hoc Multiple Comparisons. Hierarchical cluster analysis by the Ward algorithm was performed to build tree diagrams. We used four combinations of parameters: a) I_{cz}, I_{lz}, I_{iz}, I_{el}; b) I_{as}, I_{eq}, I_{com}; c) k^0, k^1, k^2, k^3; d) all transferred parameters.

We measured 16490 eggs from 8663 clutches, representing 21 orders, 88 families, 300 genera, and 681 species. The data on the taxonomic coverage of our measurements are presented in Table 1.

Table 1. **Raw data on the taxonomic coverage**

Order	Species	Clutches	Eggs
Anseriformes	48	1024	1415
Apodiformes	4	14	39
Caprimulgiformes	14	46	61
Charadriiformes	111	1201	2121
Ciconiiformes	14	492	821
Columbiformes	21	111	176
Coraciiformes	5	59	250
Cuculiformes	10	84	84
Falconiformes	47	1584	2315
Galliformes	26	114	446
Gaviiformes	5	169	233
Gruiformes	24	215	601
Passeriformes	290	2675	5600
Pelecaniformes	10	122	256
Piciformes	11	180	949
Podicipediformes	7	265	510
Procellariiformes	7	117	117
Sphenisciformes	5	29	39
Strigiformes	17	92	271
Struthioniformes	4	39	41
Upupiformes	1	31	145
Total	681	8663	16490

RESULTS

The modern variants of DNA-analysis have system character as the nucleotides form a double spiral on complimentary principle (Slack, 2012; Wang et al., 2013; Wink et al., 1996; Wink & Sauer-Gürth, 2000). Consequently, tree diagrams built by means of cluster analysis on system features must be similar (Wink et al., 1998; Zelenitsky et al., 2012). Our data presented on the tree diagram (Fig. 2).

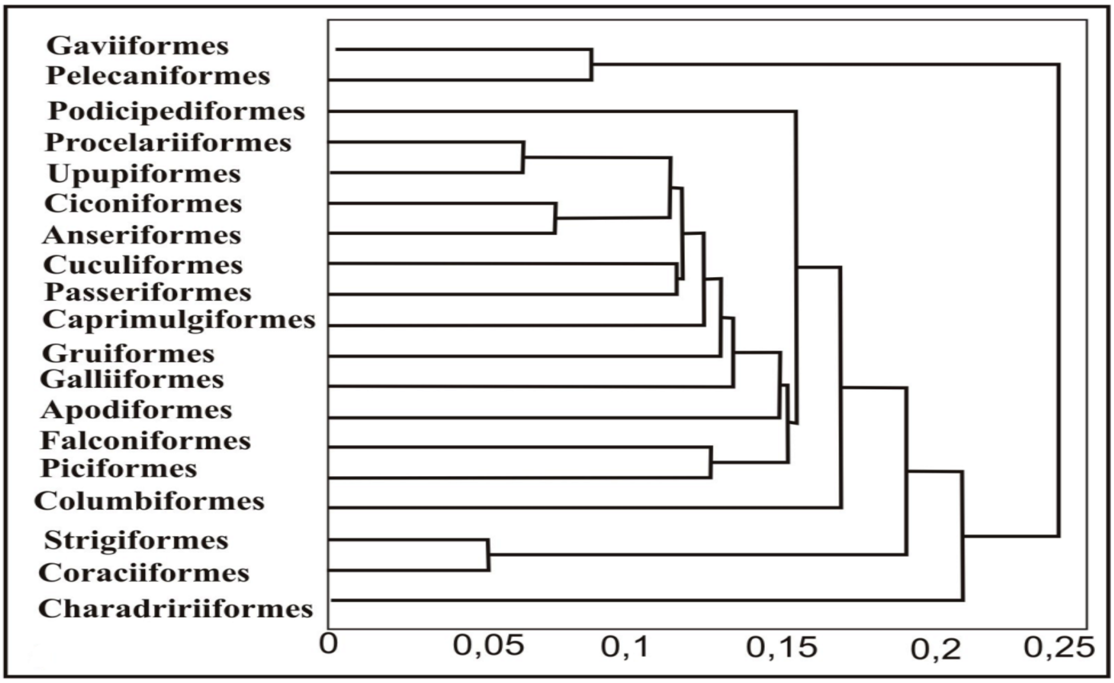

Fig. 2. Phylogenetic tree built on the data from this study

There is a clear differentiation of egg forms between bird orders. The similarity is only observed for third-order polynomial coefficients. The last is related to insignificant deformation of the lateral characteristics of eggs. At the same time this coefficient has strong and asymmetric influence on the form of circumpolar egg areas. In fact the radiuses of infundibular and cloack area of eggs differentiate considerably. For the predatory birds (n=1322) I_{iz} = 0.64±0.0001 and I_{cz} = 0.331±0.0003; for Ciconiformes (n=477) these indices were: I_{iz} = 0.409±0.0004 and I_{cz} = 0.312±0.0006. Moreover, the eggs of Falconiformes are shorter, I_{el} = 1.265±0.0004 than eggs of Ciconiformes, I_{el} = 1.397±0.0005 ($p \leq 0.05$; F = 48.8).

It should be noted that the eggs of Falconiformes differ from the eggs of representatives of other orders by major parameters (Chi-square = 1.775, Asymp. Sig. = 0,412, Kruskal-Wallis test). Therefore, we need to consider the relative character of similarity, i.e. more similar eggs are from taxonomically closely-related orders.

Indices of elongation for eggs from Falconiformes, Strigiformes, are Coraciiformes are similar: they are 1.265, 1.224, and 1.224 respectively (F = 10.941, Sig. = 0.148, Test of Homogeneity of Variances). This can be explained by different reasons. Previously it was suggested that spherical eggs are optimal in clutches of one to two or more than five eggs.

The maximum similarity of Ciconiformes eggs is proved with Strigiformes and Coraciiformes, and a bit less than - with Galliformes and Piciformes (F = 10.123, p = 0.001).

Maximal differences of Falconiformes egg parameters were registered with Gaviiformes, Pelicanidae, and Podicepediformes. The representatives of these orders have the most lengthened eggs among all considered birds. Their elongated indices are, accordingly, 1.602; 1.592; 1.482 (F = 11.345, p = 0.004). Significant differences are characteristic for the index of complementary: this was 1.078; 1.098; 1.082 respectively (F = 10.823, p = 0.005). The elongated index of Falconiformes was 1.285 and thus testified that the curvature of their local area is considerably lesser.

It is obvious that the Falconiformes and Strigiformes have strong similarity of egg shapes (F = 0.008, Sig. = 0.931, Independent Samples Test).

DISCUSSION

The method we suggested would assist the understanding of bird phylogeny by means of egg parameters analysis. In spite that nowadays the high technological methods seemed to be more attractive (Andersen et al., 2014; Slack, 2012; Torres & van Tuinen, 2013; Welch et al., 2014), egg morphometric measures still could be used in bird phylogeny (Deeming & Ruta, 2014a; 2014 b). On one hand, many aspects of the biology of bird eggs can be predicted from their weight and these predicted values can be used when empirical data are lacking (Hoyt, 1978), on other hand, we need to understand how many eggs is an adequate sample. According to Preston (1968) this depends on how much confidence we wish to feel the means or secondary, and this description is in terms of average standard error. He suggested getting 20 clutches and measuring one egg from each of these clutches. This reduces the standard error of the medium to about 0.7 percent for a great many species. As mentioned by Troscianko (2014), the geometrical properties of eggs – such as volume and surface area – have uses ranging from ecological, physiological and morphological studies in birds, to predictions of chick condition in the poultry industry.

We use more data in our research comparing to the above mentioned papers and extended egg parameters to bird systematic combining direct egg measures and computer images processing. Previous studies that have presented techniques for modeling egg shape from photographs have failed to test their models against real egg volumes in order to establish accuracy, instead pitting them against length and breadth-based models (Troscianko, 2014).

Our analyses suggest that, across the bird order we examined, the geometric parameters of bird eggs are species-specific. Tree diagrams and diagrams built using the quantitative values of oological features show considerable similarity with tree diagrams built using the morphological, comparatively-anatomical, paleontological, and molecular characters of adult birds. Therefore, we strongly recommend usage of the morphological parameters of eggs as additional information for understanding the systematics and phylogeny of birds.

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