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# DETERMINATION OF SOMATIC AND SEX CHROMOSOMES OF THREE EGYPTIAN BIRDS SPECIES USING CYTOGENETIC ANALYSIS

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Study of karyotypes has been revealing important information on the taxonomic relationships and evolutionary patterns in various groups of birds. Karyotypes analysis and morphometric measurement of the chromosomes of three birds species of ***Geopelia cuneata*** (Columbiformes), ***Oriolus oriolus*** and ***Corvus ruficollis*** (Passeriformes) and their karyological have been studied. The diploid chromosome numbers of three species were, 2n=72, 2n=80 and 2n =80 respectively. The karyotypes of three species were different. The somatic and sex chromosomes and karyotypes of three species discussed and compared with other related species in the present study.

*Key words:* birds, cytogenetics, karyotype, macrochromosomes, microchromosomes, ZW sex chromosome.

## INTRODUCTION

The number of birds species and sub-species that occur in Egypt are 515 (123 species and 392 sub-species), 186 of them are resident birds, 12 are extinct and 17 are endemic. The rest of birds' species and sub-species are migratory birds which visit Egypt in the summer from the south or in the winter from the north. Some are passers that visit Egypt twice a year, in the autumn and in the spring in their way to Affrica or back to Europe. All the species and sub-species belong to 71 families and 23 orders THARWAT (1997).

Cytotaxonomy is a branch of cytogenetics, devoted to the comparative study of karyological features for systematic and evolutionary purposes (SILJAK and PERUZZI, 2012). Cytogenetic data are important for characterization of each species and can help to establish their karyotype evolution.The comparative cytogenetic studies now allow us to assess such factor as

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the overall karyotypic variability to consider the role that chromosomal change plays in speciation (CASTRO *et al*. 2002).

In the framework of the available cytogenetic techniques, avian karyological studies have always been arrears of those mammals, this is mainly because of inherent difficulties in handling avian chromosomes. In the class Aves which comprise more than 8500 species, only about 6-7% of them have been karyologically investigated as compared to 30% of mammalia (SULTANA and BHUNYA, 1987; SASAKI *et al*., 1994).

Cytogenetic technique for sex determination of monomorphic birds is now well established. Sexing chromosomal determination is an invasive procedure, which has been accurately performed. Most avian Karyotypes comprise a small number of macrochromosomes and a large number of small chromosomes is microchromosomes. Female is the heterogametic sex (ZW) and male is the homogametic sex (ZZ). Sex identification by sex chromosomes is easily accomplished because large- sized z chromosome can be discriminated from the smaller w chromosome **(**MITTAL and SHARMA, 1993; SOLARI, 1994; FILLON and SEQUELA, 1995; ELLEGREN, 2001; ARCHAWARNON, 2004).

Cytogenetic studies of birds are relatively rare as compared with similar studies in other vertebrates (DE LUCCA and ROCHA, 1992). Most of the chromosomes of nearly all avian species are microchromosomes, which are difficult to count accurately. Consequently, it's difficult to determine whether differences in the total microchromosomes number reported by authors are real or are artifacts of preparation and analysis. This problem can be resolved only when large numbers of karyotypes per individual and meiotic analysis are included in a research plane. Such detailed studies are uncommon in current research on avian chromosomes, and most comparative studies emphasize variation in macrochromosome (SHIELDS, 1982).

The karyotype studies of bird species not yet analyzed can offer important information towards a better knowledge of mechanisms of evolution and phylogenetic connection with the group. Conventional staining of chromosomes allows for the identification of number, structure and sub-structure of the chromosome (GOLDSCHMIDT, *et al*., 2000).

HAKAN *et al.* (1983) reported that 65 % of all avian species investigated have diploid chromosome number ranging from 78 to 82 with 7 to 9 pairs of macro-chromosomes and 30 to 34 pairs of micro-chromosomes and karyotype can be divided into four groups characterized by number of macro and micro-chromosomes. While most remaining species have a karyotype with a lower diploid number of chromosome ranging from 66 to 74 showing 12 pairs of macro-chromosomes and 21 to 25 pairs of micro-chromosomes (GOLDSCHMIDT *et al*., 1997; PIGOZZI and SOLARI, 1998; DERJUSHEVA, *et al*., 2001; RIBEIRO, *et al*., 2003).

On other hand some authors concluded that the diploid chromosomes number in birds, except order falconiformes ranging from 40 to 126 with a great tendency for an increase or decrease in the number of micro and macro-chromosomes (GUNSKI and GIANNONI, 1998; ARCHAWARNON and MEVATEE, 2002; HASSAN, 2003a; BED'HOM, *et al*., 2003; LUNRADI *et al.*, 2003; HASSAN and EBIED, 2004; EBIED *et al.*, 2005a; EBIED *et al.*, 2005b; DE OLIVEIRA *et al*., 2005; HASSAN *et al*., 2006; LEONARDO *et al.*, 2012; ADEL *et al.*, 2014; RAFAEL *et al*., 2015a, RAFAEL *et al*., 2015b).

Karyological studies on Egyptian birds are generally few, so the main aim of this study is to provide information about the somatic and sex chromosomes and karyotypes of the three species under this study. To the best of author knowledge these results are reported for the first time in Egypt.

## MATERIALS AND METHODS

Specimens of *Geopelia cuneata* (Columbiformes), *Oriolus oriolus* and *Corvus ruficollis* (Passeriformes) used in the present study were collected from different localities in Egypt. Specimens were regularly collected in an adequate numbers during the period of study. The classification and identification of the specimens was carried out by the help of the description and key provided by (JANSSON, 1992; ALDERTON 1993; PORTER, *et al*., 1996; THARWAT, 1997).

Chromosomal preparations have been made from usual air drying technique according to ADEGOKE and NADESON, (1986), with some modifications made by ADEGOKE and EJERE, (1991) and SALAMA *et al.* (1995) as follow. Intraperitoneal injection of 0.05 % colchicine to specimen for one to two hours, bone marrow of humerus and tibia flushed out with hypotonic solution 0.56 or 0.75 % potassium chloride, the extract centeifuged and decanted and, the residual mass was fixed with ethanol – acetic acid solution by ratio three volumes ethanol to one volume glacial acetic acid, the samples were taken through three changes of fixative, each change for twenty minute at least and centrifuged through three changes, few drops of cell suspension were placed on the slides which were gently dried on hot plate.

The dried slides were soaked for five minutes in 5 % buffered giemsa stain, the stained slides were examined by light microscope for chromosomal analysis and the selected chromosomes spread were photographed, chromosomes spread were printed and developed by poly – contrast rapid paper.

Karyotypes were made from good spreads of chromosomes. Classification of chromosomes in karyotype studies and nomenclature of chromosomes morphological types relating to centromeric index was done according to (LEVAN *et al*., 1964; ABRAHAN and NAGENDRA, 1983; IMAI, 1991).

Chromosomes were cut from photography, assorted and stuck in pairs to a suitable white paper on the bases of centromeric position, size, length, and arm ratio. For each chromosome, the length of short arm, the length of long arm and the absolute length of a given pair of chromosomes were obtained. Relative length (RL), arm ratio (AR), and centromeric index (CI) were calculated.

## RESULTS

The cytogenetic analysis of three species of birds; *Geopelia cuneata* (Columbiformes), *Oriolus oriolus* and *Corvus ruficollis* (Passeriformes), including the chromosome number, sex chromosomes and karyotypes were investigated in the present study. The diploid chromosome numbers of three species were, 2n=72, 2n=80 and 2n =80 respectively. The karyotypes of three species were different. Following were the results of chromosomal analysis of the three species in this study.

*Geopelia cuneata***:** The chromosomal analysis of this species showed that it has a diploid chromosome number 2n=72 and FN (Fundamental Number) = 26. The karyotype was divided into four groups as following; one pair metacentric; two pairs submetacentric and five pairs acrocentric chromosomes and twenty seven pairs of microchromosomes, with a pair of sex chromosomes Z and W, which were submetacentric and subtelocentric respectively (Fig. 1). The morphometric measurements of the chromosomes of this species were calculated. (Table 1)

*Oriolus oriolus*: The karyotype of this species was found to have a diploid chromosome number 2n=80 and FN=22. The chromosomes were arranged in four groups as following; one pair metacentric; one pair subtelocentric and five pairs acrocentric chromosomes and thirty two pairs of microchromosomes, with a pair of sex chromosomes Z and W, which were metacentric and

submetacentric respectively (Fig. 2). The morphometric measurements of the chromosomes of this species were calculated. (Table 2)

*Table (1). Averages of chromosomes measurements and classification, obtained from observations on ten cell spreads of Geopelia cuneata*

| Chromosome Number | Chromosome Length | | | Relative Length % | | | Arm Ratio Mean± S.D. | Centromeric Index Mean± S.D. | Classification |
|---|---|---|---|---|---|---|---|---|---|
| | Long Arm Mean± S.D. | Short Arm Mean± S.D. | Total Mean ± S.D. | Long Arm Mean ± S.D. | Short Arm Mean ± S.D. | Total Mean ± S.D. | | | |
| 1 | 0.69±0.05 | 0.42±0.02 | 1.11±0.05 | 13.19±0.04 | 8.03±0.06 | 21.22±0.06 | 1.64±0.03 | 37.84±1.14 | M. |
| 2 | 0.51±0.05 | 0.28±0.03 | 0.79±0.04 | 9.75±0.05 | 5.35±0.06 | 15.10±0.04 | 1.82±0.07 | 35.44±0.55 | S.M. |
| 3 | 0.30±0.04 | 0.12±0.05 | 0.42±0.03 | 5.74±0.06 | 2.29±0.03 | 8.03±0.04 | 2.50±0.04 | 28.57±1.43 | S.M. |
| 4 | 0.60±0.04 | 0.0 | 0.60±0.04 | 11.47±0.05 | 0.0 | 11.47±0.05 | ∞ | 0.0 | Acro. |
| 5 | 0.24±0.02 | 0.0 | 0.24±0.02 | 4.59±0.05 | 0.0 | 4.59±0.05 | ∞ | 0.0 | Acro. |
| 6 | 0.20±0.03 | 0.0 | 0.20±0.03 | 3.82±0.04 | 0.0 | 3.82±0.04 | ∞ | 0.0 | Acro. |
| 7 | 0.18±0.03 | 0.0 | 0.18±0.03 | 3.44±0.04 | 0.0 | 3.44±0.04 | ∞ | 0.0 | Acro. |
| 8 | 0.16±0.03 | 0.0 | 0.16±0.03 | 3.06±0.03 | 0.0 | 3.06±0.03 | ∞ | 0.0 | Acro. |
| 9 - 35 | - | - | - | - | - | - | - | - | Micro. |
| Z | 0.60±0.05 | 0.28±0.02 | 0.88±0.03 | 11.47±0.04 | 5.35±0.04 | 16.82±0.04 | 2.14±0.04 | 31.82±0.76 | S.M. |
| W | 0.51±0.05 | 0.14±0.02 | 0.65±0.02 | 9.75±0.05 | 2.68±0.05 | 12.43±0.03 | 3.64±0.05 | 21.54±1.04 | S.T. |
| Sum. | | | 5.23±0.06 | | | | | | |

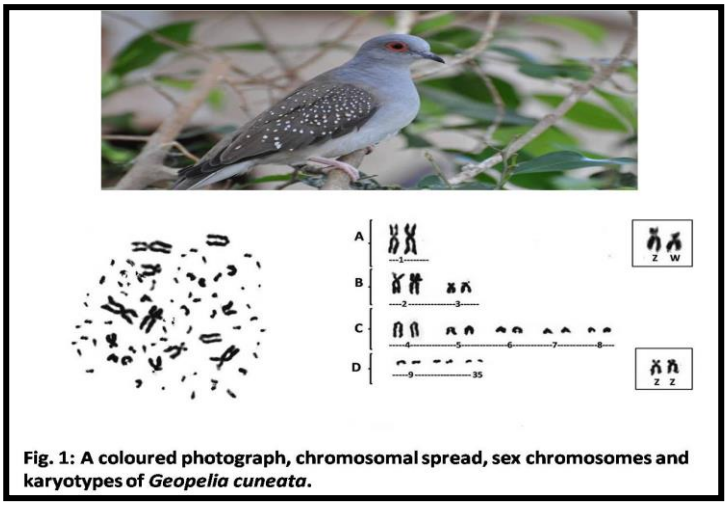


**Fig. 1: A coloured photograph, chromosomal spread, sex chromosomes and karyotypes of *Geopelia cuneata*.**

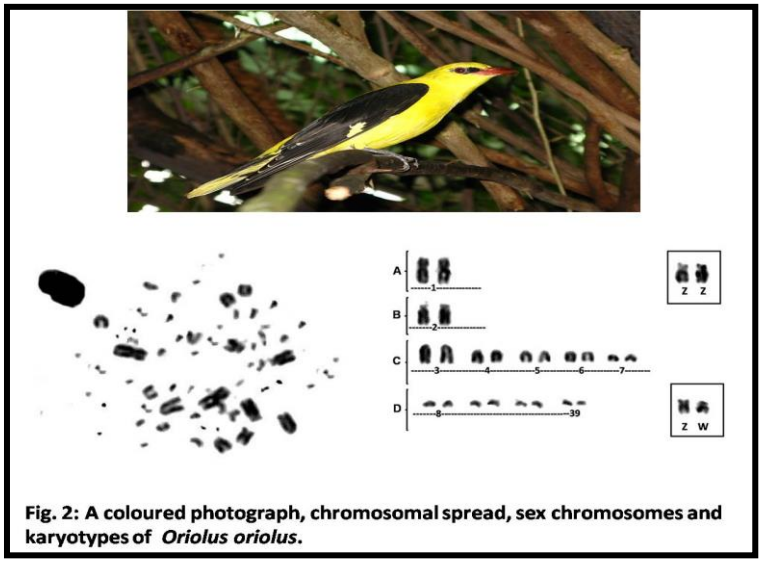


**Fig. 2: A coloured photograph, chromosomal spread, sex chromosomes and karyotypes of *Oriolus oriolus*.**

*Table 2. Averages of chromosomes measurements and classification, obtained from observations on ten cell spreads of Oriolus oriolus.*

| Chromosome Number | Chromosome Length | | | Relative Length % | | | Arm Ratio | Centromeric Index | |
|---|---|---|---|---|---|---|---|---|---|
| | Long Arm Mean± S.D. | Short Arm Mean± S.D. | Total Mean ± S.D. | Long Arm Mean ± S.D. | Short Arm Mean ± S.D. | Total Mean ± S.D. | Mean± S.D. | Mean± S.D. | Classification |
| 1 | 0.47±0.04 | 0.38±0.05 | 0.85±0.05 | 11.38±0.03 | 9.20±0.05 | 20.58±0.03 | 1.24±0.08 | 44.71±1.85 | M. |
| 2 | 0.54±0.03 | 0.16±0.04 | 0.70±0.03 | 13.08±0.04 | 3.87±0.03 | 16.95±0.04 | 3.38±0.04 | 22.86±1.03 | S.T. |
| 3 | 0.60±0.03 | 0.0 | 0.60±0.03 | 14.53±0.06 | 0.0 | 14.53±0.06 | ∞ | 0.0 | Acro. |
| 4 | 0.40±0.06 | 0.0 | 0.40±0.06 | 9.69±0.05 | 0.0 | 9.69±0.05 | ∞ | 0.0 | Acro. |
| 5 | 0.36±0.05 | 0.0 | 0.36±0.05 | 8.72±0.03 | 0.0 | 8.72±0.03 | ∞ | 0.0 | Acro. |
| 6 | 0.32±0.04 | 0.0 | 0.32±0.04 | 7.75±0.02 | 0.0 | 7.75±0.02 | ∞ | 0.0 | Acro. |
| 7 | 0.18±0.03 | 0.0 | 0.18±0.03 | 4.36±0.03 | 0.0 | 4.36±0.03 | ∞ | 0.0 | Acro. |
| 8 - 39 | - | - | - | - | - | - | - | - | Micro. |
| Z | 0.24±0.03 | 0.16±0.03 | 0.40±0.03 | 5.81±0.04 | 3.87±0.04 | 9.69±0.04 | 1.50±0.07 | 40.00±0.85 | M. |
| W | 0.21±0.04 | 0.11±0.02 | 0.32±0.04 | 5.08±0.05 | 2.66±0.03 | 7.75±0.03 | 1.90±0.06 | 34.38±0.74 | S.M. |
| Sum. | | | 4.13±0.05 | | | | | | |

*Corvus ruficollis***:** The counted metaphase spread of this species showed that a diploid chromosome number 2n=80 and FN=22. The chromosomes were arranged in four groups as following; one pair submetacentric; two pairs subtelocentric and four pairs acrocentric chromosomes and thirty two pairs of microchromosomes, with a pair of sex chromosomes Z

acrocentric and W acrocentic but different in size hence Z more than W (Fig. 3). The morphometric measurements of the chromosomes of this species were calculated. (Table 3)
Ideograms of chromosomes of *Geopelia cuneata*, *Oriolus oriolus* and *Corvus ruficollis* which were constructed in respect to the relative length were illustrated in figures 4, 5 and 6.

To the best of author knowledge these results were reported for the first time in Egypt. This investigation confirmed that each species have a particular chromosome complement.

*Table 3. Averages of chromosomes measurements and classification, obtained from observations on ten cell spreads of Corvus ruficollis.*

| Chromosome Number | Chromosome Length | | | Relative Length % | | | Arm Ratio | Centromeric Index | Classification |
|---|---|---|---|---|---|---|---|---|---|
| | Long Arm Mean± S.D. | Short Arm Mean± S.D. | Total Mean ± S.D. | Long Arm Mean ± S.D. | Short Arm Mean ± S.D. | Total Mean ± S.D. | Mean± S.D. | Mean± S.D. | |
| 1 | 0.54±0.06 | 0.26±0.02 | 0.80±0.03 | 15.00±0.04 | 7.22±0.05 | 22.22±0.06 | 2.10±0.07 | 32.50±0.96 | S.M. |
| 2 | 0.46±0.03 | 0.12±0.03 | 0.58±0.04 | 12.78±0.05 | 3.33±0.06 | 16.11±0.05 | 3.83±0.04 | 20.69±1.45 | S.T. |
| 3 | 0.42±0.04 | 0.12±0.05 | 0.54±0.03 | 11.67±0.02 | 3.33±0.03 | 15.00±0.04 | 3.50±0.08 | 22.22±0.73 | S.T. |
| 4 | 0.40±0.03 | 0.0 | 0.40±0.03 | 11.11±0.03 | 0.0 | 11.11±0.03 | ∞ | 0.0 | Acro. |
| 5 | 0.32±0.06 | 0.0 | 0.32±0.06 | 8.89±0.05 | 0.0 | 8.89±0.05 | ∞ | 0.0 | Acro. |
| 6 | 0.24±0.03 | 0.0 | 0.24±0.03 | 6.67±0.03 | 0.0 | 6.67±0.03 | ∞ | 0.0 | Acro. |
| 7 | 0.18±0.04 | 0.0 | 0.18±0.04 | 5.00±0.02 | 0.0 | 5.00±0.02 | ∞ | 0.0 | Acro. |
| 8 - 39 | - | - | - | - | - | - | - | - | Micro. |
| Z | 0.36±0.03 | 0.0 | 0.36±0.03 | 10.00±0.06 | 0.0 | 10.00±0.06 | ∞ | 0.0 | Acro. |
| W | 0.18±0.02 | 0.0 | 0.18±0.02 | 5.00±0.03 | 0.0 | 5.00±0.03 | ∞ | 0.0 | Acro. |
| Sum. | | | 3.60±0.04 | | | | | | |

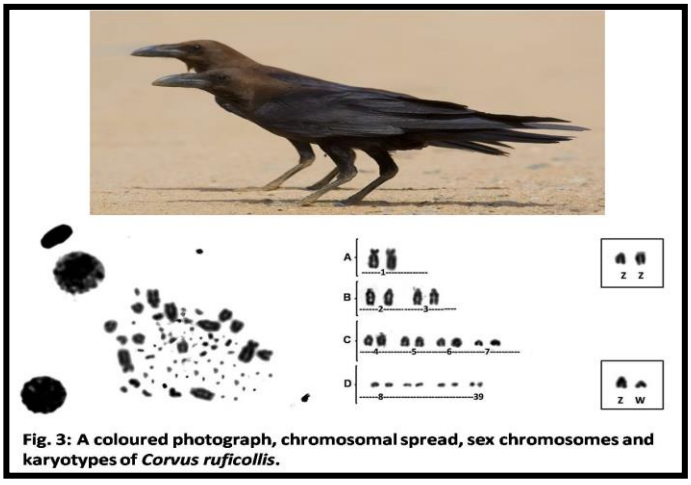


**Fig. 3: A coloured photograph, chromosomal spread, sex chromosomes and karyotypes of *Corvus ruficollis*.**

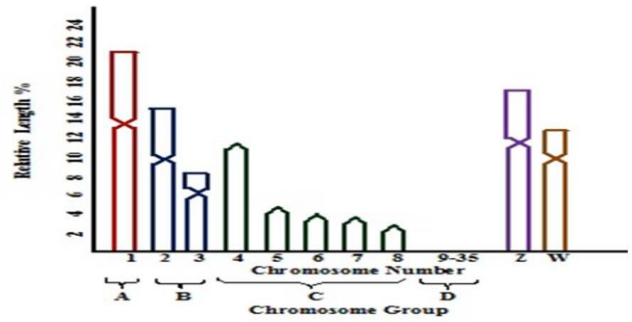


**Fig. 4: Ideogram of chromosomes of *Geopelia cuneata* which was constructed in respect to the relative length.**

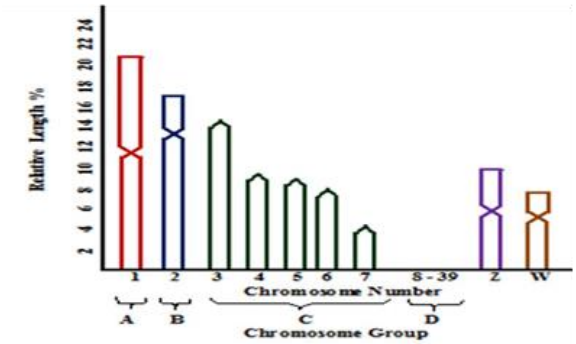


**Fig. 5: Ideogram of chromosomes of *Oriolus oriolus* which was constructed in respect to the relative length.**

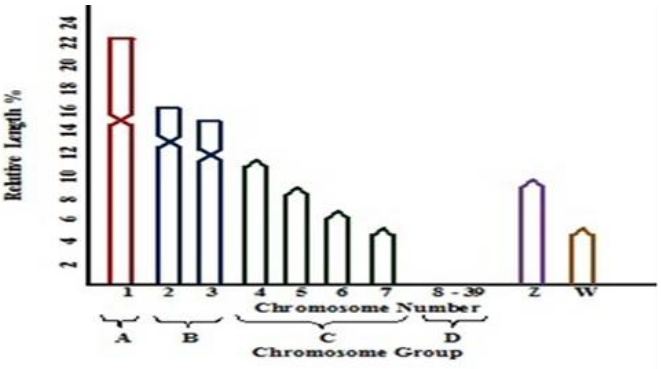


**Fig. 6: Ideogram of chromosomes of *Corvus ruficollis* which was constructed in respect to the relative length.**

## DISCUSSION

The diploid chromosome number of order Passeriformes ranging from 58 to 90, the macro-chromosomes ranges from 6 to 12 pairs. While micro-chromosomes ranges from 23 to 39 pairs (HASSAN, 2003a; GOLDSCHMIDT *et al*., 2000; ROSLIK and KRIUKOV, 2001; DERJUSHEVA *et al*., 2001; ARCHAWARANON and MEVATEE, 2002; ARCHAWARANON, 2004; RAFAEL *et al*., 2015a; RAFAEL *et al*., 2015b).

ROSLIK and KRIUKOV (2001) reported that the diploid chromosome numbers of *Corvus cornix, C. corone and C. macrorhynchos* (Family: Corvidae) were 2n=80, 80 and 82 respectively. YASEEN, (1998), reported that the diploid chromosome number of *Corvus splendens* 2n= 78 , its karyotype divided into three group group A has one pair of metacentric chromosomes, group B has six pairs of acrocentric chromosomes and group c has 31 pairs of microchromosome. Z chromosome was acrocentic, while W was microchromosome.

EBIED *et al.* (2005b) found that the diploid chromosome number of *Corvus corax* was 2n = 80, its karyotype divided into three group group A has one pair of sub-metacentric chromosomes, group B has eight pairs of acrocentric chromosomes and group c has 30 pairs of microchromosome. Z chromosome was acrocentic, while W was acrocentic but W smaller than Z in size.

ANSARI and KAUL, (1979) concluded that that the diploid chromosome number of *Oriolus xanthornus* (Family: Oriolidae) was 2n=80. There are 7 pairs of macrochromosomes in the karyotype, besides the sex chromosomes.

DE LUCCA (1984) reported that the diploid chromosome numbers of order Columbiformes ranges from 2n=68 to 2n=86, while ITOH, *et al.* (1969) concluded that diploid chromosome numbers of order Columbiformes ranges from 72 to 80 and the diploid chromosome numbers of *Geopelia cuneata* was 2n=72.

## CONCLUSION

The results obtained from the present investigation have revealed that the karyological study is a definite and constant feature for each species. These findings strengthen the opinion regarding the importance of using chromosomal analysis in addition to the classical taxonomy based on anatomical and morphological characters in recent taxonomy. The findings of this study can be further applied in research areas of bird's cytotaxonomy and evolutionary relationships.

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# ODREĐIVANJE SOMATSKIH I POLNIH HROMOZOMA KOD TRI VRSTE EGIPATSKIH PTICA KORIŠĆENJEM CITOGENETSKIH ANALIZA

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Izvod

Proučavanje kariotipa dalo je značajne informacije o taksnomskim vezama i evolucionim putevima kod različitih grupa ptica. Rađena je kariotipska analiza i morfmetrijska merenja hromozoma tri vrste ptica ***Geopelia cuneata*** (Columbiformes), ***Oriolus oriolus*** i ***Corvus ruficollis*** (Passeriformes). Diploidni brj hromozoma bio je 2n=72, 2n=80 i 2n =80. Kariotipovi tri vrste bili su različiti. U radu su proučavani somatski i polni hromozomi i kariotipovi su upoređivani sa drugim srodnim vrstama.

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