UDC 575.
https://doi.org/10.2298/GENSR1901103A
Original scientific paper

CHROMOSOMAL EVOLUTION AND MOLECULAR GENETIC ANALYSIS OF FOUR SPECIES OF GENUS ANAS (AVES: ANATIDAE)

Ali Hussein ABU-ALMAATY[1*], Mohamed K. HASSAN[1], Neveen E. R. EL BAKARY[2], Sarah H. AHMED[1]

[1]Zoology Department, Faculty of Science, Port Said University, Egypt
[2]Zoology Department, Faculty of Science, Damietta University, Egypt

Abu-Almaaty A. H., M. K. Hassan, N. E. R. El Bakary, S. H. Ahmed (2019): *Chromosomal evolution and molecular genetic analysis of four species of genus Anas (Aves: Anatidae).*- Genetika, Vol 51, No.1, 103-119.

Birds are considered one of the least karyotypically examined animal groups due to their karyotype specificity, i.e. small chromosomes, a large diploid chromosome number and the separation of chromosomes into macro- and microchromosomes. The present work was aimed to investigate the number of chromosomes and their karyological and molecular genetic relationships of four species of genus *Anas* (*Anas crecca, Anas penelope, Anas acuta and Anas clypeata* (Family: Anatidae). All four species have the same diploid chromosome number of 2n=80. The four investigated species have shown five pairs of macrochromosomes and the remaining 35 pairs were of microchromosomes. Ten RAPD primers were used for molecular discrimination by polymerase chain reaction (PCR). The (PCR) showed polymorphic bands, which were used for the construction of the dendrogram and a similarity matrix. A total of 133 bands were obtained; 37 of them were polymorphic and 27 unique bands. Similarity values among the species under study ranged from 79% to 85%. The highest similarity was between *A. Penelope* and *A. acuta* (85%) while the lowest similarity was between *A. acuta* and *A. clypeata* (79 %). RAPD analysis confirmed that the four *Anas* species under study are genetically different from each other and a genetic variation was found between and within the three species tested

Corresponding author: Ali Hussein Abu-Almaaty, PhD., Biotechnology Program, Department of Zoology, Faculty of Science, Port Said University, Port Said, 42521, Egypt.
E-mail: ali_zoology_2010@yahoo.com; Tel: 00201093041699

in this study. The karyotypic features are also suitable as cytotaxonomic markers of Anatidae.

Keywords: Micro/Macro chromosomes, karyotype, RAPD, DNA, *Anas*, Anatidae.

INTRODUCTION

Karyotyping is the process in which chromosomes are paired and rearranged, thus providing a genome-wide image of an individual's chromosomes. Karyotypes are made by using standardized staining steps that detect structural characteristics for each chromosome (O'CONNOR, 2008).

The recent interest in comparative bird cytogenetic allowed us to estimate such factors as the comprehensive variations of karyotype in avian populations and to consider the role that chromosomal variability plays in bird speciation (SHIELDS, 1982). Birds are one of the least deliberated animal groups owing to karyotype specificity, i.e. small chromosomes, a large diploidal chromosome number or the splitting of the chromosomes into macro- and microchromosomes (WÓJCIK and SMALEC, 2007; ABU-ALMAATY, 2017). The macro-chromosomes comprise only few pairs which their size ranging from four to eight microns the remaining ones termed microchromosomes are usually smaller than two microns and in many cases are seen as points (WÓJCIK and SMALEC, 2007). The diploid chromosome number of order Anseriformes ranges from 72 to 80 and the macro-chromosomes ranges from 5 to 10 pairs while micro-chromosomes ranges from 28 to 35 pairs (TEGELSTRÖM and RYTTMAN, 1981; DE OLIVEIRA *et al.,* 2001; SHAHIN *et al.,* 2014). Other authors reported that the diploid chromosome number of order Anseriformes ranges from 72 to 84 (HAMMAR, 1966, 1970; TAKAGI and SASAKI, 1974; SHIELDS, 1982).

The improvement of molecular techniques has generated new chances for genetic evolution of animals. DNA markers such as RAPD (Randomly Amplified Polymorphic DNA), it allows the polymorphism of DNA fragments or entire genomes to be studied (BAWEJ *et al.,* 2012). RAPD is a useful technique dealing with the phylogenetic relationships between closely related populations and determine the genetic relationships among different populations in relation to their degree of connectivity (LI *et al.,* 2006). RAPD is an extremely diverse nuclear marker that supplies dependable information on genetic divergence, polymorphisms and the relationships within populations of variable origins (WILLIAMS *et al.,* 1990). RAPD effectively exposes polymorphisms depend on comparisons throughout the genome (WELSH and MCCLELLAND, 1990). The major advantages of the RAPD technique lie in its rapidity and the fact that it can be used to any organism without previous knowledge of a nucleotide sequence (WILLIAMS *et al.,* 1990).

The aim of this study was to provide information about the chromosome numbers and karyotypes of the four species of genus *Anas*, moreover to determine the molecular genetic variations and phylogenetic relationships among the four species that might have occurred during the differentiation of these species by different cytogenetic techniques to characterize the molecular nature and patterns of distribution.

MATERIALS AND METHODS

Samples collection

All the investigated animals were mature and healthy. Four species of ducks *(Anas crecca, Anas penelope, Anas acuta and Anas clypeata*) were obtained from the local markets in

Port Said and Damietta (Egypt). This study followed the guidelines for the care and use of laboratory animals and the animal welfare and Ethics Committee of the Faculty of Science, Port Said University according to the Egyptian's laws, approved it, in which satisfactory measures were taken to minimize pain or discomfort.

Karyotype preparation

Chromosomal preparations have been made from usual air drying technique according to BLOOM (1969); DEGOKE and EJERE (1991). 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 centrifuged and decanted, then the residual mass was fixed with ethyl alcohol: acetic acid solution at a ratio of 3:1. The samples were taken through three changes of fixative, each change for twenty minutes at least and centrifuged through the three changes the few drops of the cell suspension were placed on the slides which were gently dried on hot plate. The dried slides were soaked for 5-7 minutes in 5% buffered Giemsa stain, the stained slides were examined by light microscope using 10x or eyepieces, together with 100x objective for chromosomal analysis and the selected chromosomes spreads were photographed, chromosomes spread were printed. Karyotypes were made from good spreads of chromosome. Classification of chromosomes in karyotype studies relating to centromeric index was done according to LEVAN *et al*., 1964; IMAI, 1991).

Genomic DNA extraction and PCR

DNA was extracted from the four samples by DNeasy Mini Kit (Qiagen Santa Clarita, CA). Ten primers were used in RAPD – PCR analysis to study the difference between four specimens of ducks, the code and sequences of these primers are shown in Table 1.

Table 1. Sequence of the ten decamer arbitrary primers assayed in RAPD-PCR

No	Primer Code	Sequence (5′-3′)
1	OP-A11	CAATCGCCGT
2	OP-A18	AGGTGACCGT
3	OP-B03	CATCCCCCTG
4	OP-C02	GTGAGGCGTC
5	OP-C03	GGGGGTCTTT
6	OP-D11	AGCGCCATTG
7	OP-D16	AGGGCGTAAG
8	OP-E05	TCAGGGAGGT
9	OP-H05	AGTCGTCCCC
10	OP-H07	CTGCATCGTG

The amplification reaction was carried out in 25 µl reaction volumes containing 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 25pmol primer, I U *Taq* DNA polymerase and 30 ng templates DNA. PCR amplification was performed in Perkin-Elmer/GeneAmp PCR system 9700 (*PE* Applied Biosystems) programmed to fulfill 35 cycles after an initial denaturation cycle for 5 min at 94ºC for 50s, and an elongation step at 72 ºC for 1 min. The primer extension segment was extended to 7 min at 72 ºC in the final cycle. The amplification products were resolved by

electrophoresis in 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts.

RESULTS

Four species of ducks, belonging to family Anatidae (Order: Anseriformes), were cytogenetically and molecularly studied, using air drying technique and RAPD-PCR analysis, respectively.

The chromosomal numbers of all species under the study were the same, with 2n=80, but differ in the karyotype in some species. Ten single 10-mer primers (OP-A11 OP-A18, OP-B03, OP-C02, OP-C03, OP-D11, OP-D16, OP-E05, OP-H05, OP-H07) with G+C contents of 60% - were used in the present investigation to determine the genetic differences among four species. The DNA fragments generated by the ten primers from the genomic DNA of the four species were separated using agarose gel electrophoresis and illustrated in Figures (5-9). The banding patterns of these DNA fragments were analyzed by gene profiler computer software program and results were summarized in charts representing each primer in Table (6). Following are the karyotypes and amplification results of the four species obtained from this study.

1. *Anas crecca*

The photographs of cell spread and karyotypes of this species was found to have a diploid chromosome number of 2n=80 and FN=14, as shown in (Fig.1). These numbers of chromosomes are allocated into four groups group A: consists of one pair metacentric chromosome with relative length 26.25%, arm ratio of 1.4, centromeric index 41.27% and mean length 0.63 µm, group B: consists of one pair sub-metacentric chromosome with relative length 32.08%, arm ratio of 1.75, centromeric index 36.36% and mean length 0.77 µm group C: consists of three pairs of acrocentric chromosomes with relative length ranged from 11.25% to 18.33%, arm ratio ∞, centromeric indices equal zero and mean length ranged from 0.27 to 0.44 and group D: consists of 35 pairs of microchromosomes as shown in Table (2).

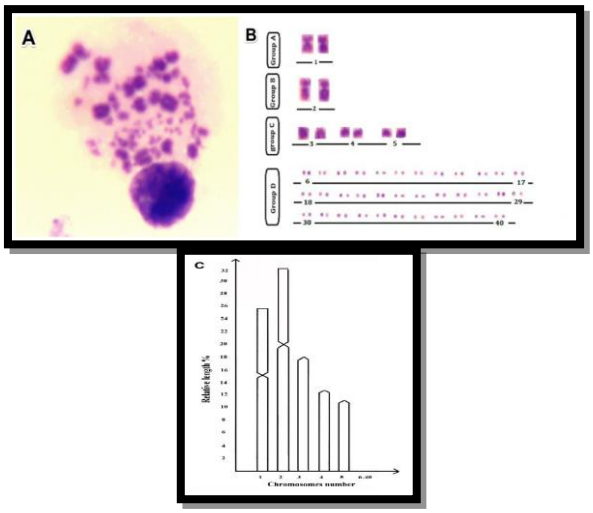

Fig. 1. Cell spread (A), Karyotype (B) and ideogram (C) of *A. crecca*

Table 2. Average of ten cells spreads of chromosomes measurements and classification of A. crecca

Chromosome number.	Chromosome length			Relative length			Arm ratio	Cintromeric index%	Classification
	Long arm	Short arm	Total	Long arm	Short arm	Total			
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	
1	0.37±0.05	0.26±0.03	0.63±0.04	15.42±0.05	10.83±0.03	26.25±0.06	1.4±0.06	41.27±0.04	M
2	0.49±0.03	0.28±0.03	0.77±0.05	20.42±0.05	11.66±0.03	32.08±0.05	1.75±0.06	36.36±0.05	SM
3	0.44±0.03	0.0	0.44±0.05	18.33±0.03	0.0	18.33±0.05	∞	0.0	A
4	0.29±0.03	0.0	0.29±0.03	12.08±0.03	0.0	12.08±0.04	∞	0.0	A
5	0.27±0.03	0.0	0.27±0.05	11.25±0.03	0.0	11.25±0.03	∞	0.0	A
6-40	-	-	-	-	-	-	-	-	Micro
Sum			2.4±0.05						

The RAPD PCR analysis indicated that all amplified primers produced fragments with this duck, 102 are all the bands varied from 7 by the primers OP-C03 to 13 by the primers OP-D16, the size of these bands varies approximately from 190 bp by the primer OP-E05 to 1000 bp by the primer OP-H07 as shown in figures (5-14) - lane (1).

2. *Anas penelope*

The photographs of cell spread and karyotypes of this species was found to have a diploid chromosome number of 2n=80 and FN=14, as shown in Figure 2. These numbers of chromosomes are allocated into three groups group A: consists of one pair of sub-metacentric chromosome with relative length 33.56 %, arm ratio of 1.74, centromeric index equal 36.56 and mean length 0.93, group B: consists of three pairs of acrocentric chromosomes with relative length ranged from 9.28% to 13.91%, arm ratio ∞, centromeric indices equal zero and mean length ranged from 0.32 to 0.48 and group C: consists of 35 pairs of microchromosomes. Z-chromosome is metacentric with relative length 20.58, arm ratio 1.63 and centromeric index equal 38.03%, W-chromosome is submetacentric with relative length 19.42, arm ratio 1.79 and centromeric index equal 35.82% as shown in Table 3.

Table 3. Average of ten cells spreads of chromosomes measurements and classification of *A. Penelope*

Chromosome number	Chromosome length			Relative length			Arm ratio	Centromeric index %	Classification
	Long arm	Short arm	Total	Long arm	Short arm	Total			
	Mean ± S.D	Mean± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	
1	0.59±0.03	0.34±0.03	0.93±0.03	17.1±0.05	9.86±0.03	33.56±0.06	1.74±0.04	36.56±0.05	SM
2	0.48±0.03	0.0	0.48±0.06	13.91±0.05	0.0	13.91±0.08	∞	0.0	A
3	0.34±0.05	0.0	0.34±0.05	9.86±0.05	0.0	9.86±0.05	∞	0.0	A
4	0.32±0.03	0.0	0.32±0.03	9.28±0.04	0.0	9.28±0.06	∞	0.0	A
5-39	-	-	-	-	-	-	-	-	Micro
Z	0.44±0.03	0.27±0.03	0.71±0.03	12.75±0.05	7.83±0.05	20.58±0.05	1.63±0.03	38.03±0.03	M
W	0.43±0.03	0.24±0.04	0.67±0.03	12.46±0.06	6.96±0.05	19.42±0.06	1.79±0.08	35.82±0.04	SM
Sum.			3.45±0.05						

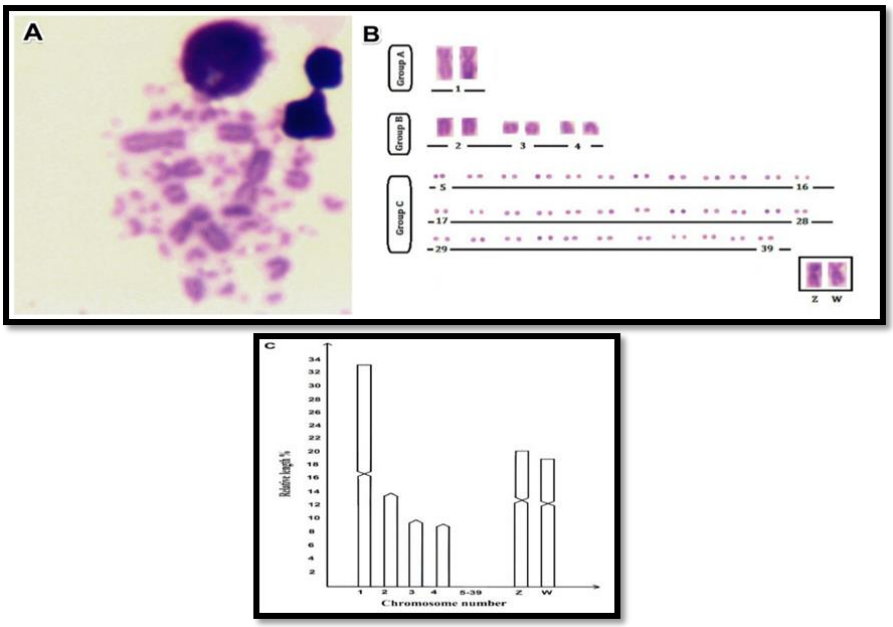

Fig. 2. Cell spread (A), karyotype (B) and ideogram (C) of *A. Penelope*

The RAPD PCR analysis indicated that all amplified primers produced fragments with this duck, 106 are all the bands varied from 7 by the primers OP-C03 to 14 by the primers OP-A11 and the size of these bands varies approximately from 190 bp by the primer OP-E05 to 1120 bp by the primer OP-B03 as shown in figures (5-14) - lane (2).

3. *Anas acuta*

The photographs of cell spread and karyotypes of this species was found to have a diploid chromosome number of 2n=80 and FN=14, as shown in Figure 3. These numbers of chromosomes are allocated into three groups group A: consists of one pair of metacentric chromosome with relative length 17.32%, arm ratio of 1.2, centromeric index equal 45.9 % and mean length 0.61µm, group B: consists of three pairs of acrocentric chromosomes with relative length ranged from 8.19% to 14.69%, arm ratio ∞, centromeric indices equal zero and mean length ranged from 0.29 µm to 0.52 µm and group C: consists of 35 pairs of microchromosomes. Z-chromosome is metacentric with relative length 26.83%, arm ratio 1.32 and centromeric index equal 43.16%, W-chromosome is submetacentric with relative length 25.16, arm ratio 1.93 and centromeric index equal 34.15% as shown in Table 4.

The RAPD PCR analysis indicated that all amplified primers produced fragments with this duck, 101 are all the bands varied from 7 by the primers OP-H07 to 13 by the primers OP-A11and the size of these bands varies approximately from 190 bp by the primer OP-E05 to 1000 bp by the primer OP-H07 as shown in figures (5-14) - lane (3).

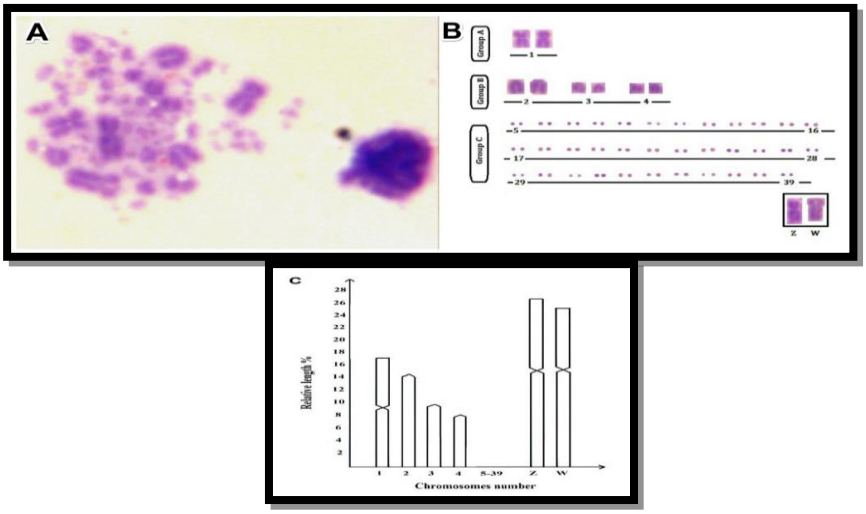

Fig. 3. Cell spread (A), karyotype (B) and ideogram (C) of *A. acuta*

Table 4. Average of ten cell spreads of chromosomes measurements and classification of A. acuta

Chromosome number	Chromosome length			Relative length			Arm ratio	Centromeric index %	Classification
	Long arm	Short arm	Total	Long arm	Short arm	Total			
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	
1	0.33±0.03	0.28±0.03	0.61±0.03	9.32±0.05	7.91±0.03	17.32±0.06	1.2±0.04	45.9±0.05	M
2	0.52±0.03	0.0	0.52±0.06	14.69±0.05	0.0	14.69±0.08	∞	0.0	A
3	0.35±0.05	0.0	0.35±0.05	9.89±0.05	0.0	9.89±0.05	∞	0.0	A
4	0.29±0.03	0.0	0.29±0.03	8.19±0.04	0.0	8.19±0.06	∞	0.0	A
5-39	-	-	-	-	-	-	-	-	Micro
Z	0.54±0.03	0.41±0.03	0.95±0.03	15.25±0.05	11.58±0.05	26.83±0.05	1.32±0.03	43.16±0.03	M
W	0.54±0.03	0.28±0.04	0.82±0.03	15.25±0.06	7.91±0.05	25.16±0.06	1.93±0.08	34.15±0.04	SM
Sum.			3.54±0.05						

4. *Anas clypeata*

The photographs of cell spread and karyotypes of this species was found to have a diploid chromosome number of 2n=80 and FN=14, as shown in Fig.4. These numbers of chromosomes are allocated into three groups, group A: consists of one short metacentric chromosome with relative length 22.01 %, arm ratio of 1.06 and centromeric index equal 48.6 and mean length 0.37, group B: consists of three pairs of Acrocentric chromosomes with relative length ranged from 10.1% to 14.9 %, arm ratio ∞, centromeric indices equal zero and mean length ranged from 0.17 to 0.25 and group C: consists of 35 pairs of microchromosomes. Z-chromosome is submetacentric with relative length 23.83, arm ratio 1.85 and centromeric index equal 35, W-

chromosome is metacentric with relative length 17.83, arm ratio 1.14 and centromeric index equal 47% as shown in Table 5.

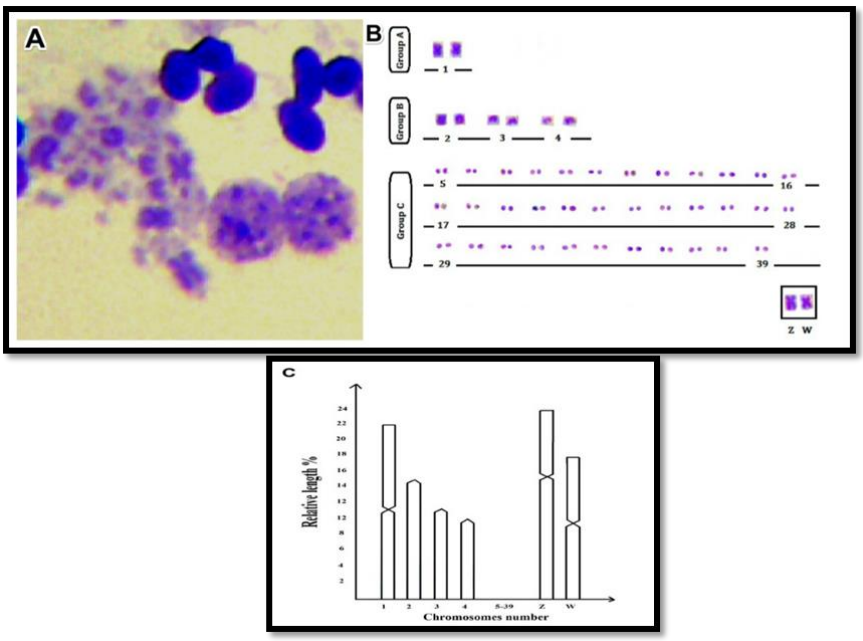

Fig. 4. Cell spread (A), karyotype (B) and ideogram (C) of *A. clypeata*

Table 5.Average of ten cells spreads of chromosomes measurements and classification of A. clypeata

Chromosome number	Chromosome length			Relative length			Arm ratio	Centromeric index %	Classification
	Long arm	Short arm	Total	Long arm	Short arm	Total			
	Mean ±S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	
1	0.19±0.05	0.18±0.03	0.37±0.05	11.3±0.05	10.7±0.06	22.01±0.05	1.06±0.05	48.6±0.04	M
2	0.25±0.05	0.0	0.25±0.04	14.9±0.05	0.0	14.9±0.07	∞	0.0	A
3	0.19±0.03	0.0	0.19±0.03	11.31±0.05	0.0	11.31±0.05	∞	0.0	A
4	0.17±0.05	0.0	0.17±0.05	10.12±0.05	0.0	10.12±0.05	∞	0.0	A
5-39	-	-	-	-	-	-	-	-	Micro
Z	0.26±0.03	0.14±0.03	0.40±0.03	15.5±0.05	8.33±0.06	23.83±0.05	1.85±0.05	35±0.05	SM
W	0.16±0.04	0.14±0.03	0.30±0.04	9.52±0.06	8.33±0.05	17.85±0.06	1.14±0.05	47±0.06	M
Sum.			1.68±0.05						

The RAPD PCR analysis indicated that all amplified primers produced fragments with this duck, 103 are all the bands varied from 5 by the primers Op-C03 to 14 by the primers OP-

A11and the size of these bands varies approximately from 190 bp by the primer OP-E05 to 1090 bp by the primer OP-H07.

A total of 133 DNA bands were generated by ten primers for all studied duck species, out of these DNA bands out of these DNA bands 69 (51.9%) were conserved among all specimens while 37 bands were polymorphic with percentage (27.81%) and 27 bands were unique (20.30%) of all the 10 tested primers with polymorphism 48.12% in all specimens (Table 7).

Table 6. Survey of RAPD Markers using ten primers (1- Anas crecca, 2- Anas penelope 3- Anas acuta and 4- Anas clypeata

primer OP-A11						primer OP-C02					
No.	MW	(1)	(2)	(3)	(4)	No.	MW	(1)	(2)	(3)	(4)
1	820	1	1	1	1	1	860	1	1	1	1
2	770	1	1	1	1	2	730	0	0	1	1
3	710	1	1	1	1	3	680	0	1	0	0
4	630	1	1	1	1	4	670	0	0	1	0
5	580	1	1	1	1	5	660	0	0	0	1
6	530	1	1	1	1	6	630	1	0	0	0
7	480	0	1	0	1	7	600	0	1	1	1
8	440	1	1	1	1	8	530	1	1	1	1
9	400	1	1	1	1	9	490	0	1	1	1
10	380	0	1	1	1	10	460	1	1	1	1
11	350	1	1	1	1	11	420	1	1	1	1
12	330	0	0	0	1	12	380	1	1	1	1
13	300	1	1	1	1	13	350	1	0	0	0
14	260	0	1	1	1	14	320	1	1	1	1
15	230	1	1	1	0	15	290	1	1	1	0
primer OP-A18						16	270	1	1	0	1
No.	MW	(1)	(2)	(3)	(4)	17	220	1	0	0	1
1	900	1	1	1	1	primer OP-C03					
2	830	0	0	0	1	No.	MW	(1)	(2)	(3)	(4)
3	790	1	0	0	0	1	990	0	0	1	0
4	720	1	1	1	1	2	920	1	0	0	0
5	660	1	1	1	1	3	790	0	0	1	0
6	580	1	1	1	1	4	780	0	0	0	1
7	550	0	1	0	0	5	710	0	0	1	0
8	490	1	1	1	1	6	700	1	1	0	0
9	400	1	1	1	1	7	610	0	1	1	0
10	340	1	0	1	1	8	480	1	1	1	0
11	310	1	1	1	1	9	430	0	0	1	0
12	270	1	1	1	1	10	400	1	1	0	1
13	240	0	0	1	1	11	360	1	1	1	1
primer OP-B03						12	310	1	1	1	1
No.	MW	(1)	(2)	(3)	(4)	13	270	1	1	1	1
1	1120	0	1	0	0	primer OP-D11					
2	940	1	1	0	1	No.	MW	(1)	(2)	(3)	(4)
3	840	1	1	1	1	1	900	1	1	1	1
4	720	0	1	1	0	2	780	1	1	1	1
5	650	1	1	1	1	3	670	1	1	1	1
6	580	1	1	0	1	4	610	0	1	1	1
7	530	1	1	1	1	5	560	1	1	1	1
8	460	1	1	1	1	6	520	1	1	1	1
9	400	1	1	1	1	7	470	1	1	1	1
10	370	1	1	1	1	8	420	1	0	0	0
11	340	0	1	1	1	9	390	1	1	1	1
12	310	1	1	1	1	10	360	1	1	0	1
13	270	1	1	0	1	11	330	1	1	1	1
						12	290	1	1	1	1
						13	270	1	1	1	0

Cont. Table 6.

primer OP-D16						primer OP-H05					
No.	**MW**	**(1)**	**(2)**	**(3)**	**(4)**	**No.**	**MW**	**(1)**	**(2)**	**(3)**	**(4)**
1	**860**	**1**	**1**	**1**	**1**	**1**	**860**	**0**	**1**	**1**	**0**
2	**800**	**1**	**0**	**0**	**1**	**2**	**720**	**0**	**1**	**1**	**0**
3	**710**	**1**	**1**	**0**	**1**	**3**	**640**	**0**	**1**	**1**	**0**
4	**600**	**1**	**0**	**0**	**1**	**4**	**580**	**1**	**1**	**0**	**1**
5	**560**	**1**	**0**	**0**	**1**	**5**	**530**	**0**	**1**	**1**	**0**
6	**510**	**1**	**1**	**1**	**1**	**6**	**490**	**1**	**1**	**1**	**1**
7	**460**	**1**	**1**	**1**	**0**	**7**	**420**	**1**	**1**	**1**	**1**
8	**410**	**1**	**1**	**1**	**1**	**8**	**380**	**1**	**1**	**1**	**1**
9	**350**	**1**	**0**	**1**	**1**	**9**	**340**	**0**	**1**	**1**	**0**
10	**320**	**1**	**1**	**1**	**1**	**10**	**310**	**1**	**1**	**1**	**1**
11	**300**	**1**	**1**	**1**	**1**	**11**	**300**	**1**	**0**	**1**	**1**
12	**260**	**1**	**0**	**0**	**1**	**12**	**260**	**1**	**1**	**1**	**1**
13	**220**	**1**	**1**	**1**	**1**	**13**	**240**	**0**	**1**	**0**	**0**
14	**210**	**0**	**1**	**0**	**1**	**14**	**210**	**1**	**0**	**1**	**1**
primer OP-E05						**primer OP-H07**					
No.	**MW**	**(1)**	**(2)**	**(3)**	**(4)**	**No.**	**MW**	**(1)**	**(2)**	**(3)**	**(4)**
1	**830**	**1**	**1**	**1**	**1**	**1**	**1090**	**0**	**1**	**0**	**1**
2	**680**	**1**	**1**	**0**	**1**	**2**	**1000**	**1**	**1**	**1**	**1**
3	**600**	**1**	**1**	**1**	**1**	**3**	**910**	**1**	**1**	**1**	**1**
4	**530**	**1**	**1**	**1**	**1**	**4**	**820**	**1**	**1**	**1**	**0**
5	**480**	**1**	**1**	**1**	**1**	**5**	**750**	**1**	**0**	**0**	**0**
6	**440**	**1**	**1**	**1**	**1**	**6**	**680**	**1**	**1**	**1**	**1**
7	**400**	**1**	**1**	**1**	**1**	**7**	**590**	**1**	**1**	**1**	**1**
8	**330**	**1**	**1**	**1**	**1**	**8**	**490**	**1**	**1**	**1**	**1**
9	**300**	**1**	**1**	**1**	**1**	**9**	**420**	**1**	**1**	**1**	**1**
10	**260**	**1**	**0**	**1**	**1**						
11	**230**	**1**	**1**	**1**	**0**						
12	**190**	**1**	**1**	**1**	**1**						

Table 7. The number of the amplified bands and polymorphic DNA- fragments occurred in the studied species

Primer	Primer code	No. of Amplified bands				Total amplified bands	Monomorphic bands	Polymorph. bands	Unique bands	Polymorphism
		A. crecca	*A. penelope*	*A. acuta*	*A. clypeata*					
1	A-11	11	14	13	14	15	10	2	3	33.33%
2	A-18	10	9	10	11	13	8	4	1	38.46%
3	B-03	10	13	9	11	13	7	2	4	46.15%
4	C-02	11	11	11	12	17	6	7	4	64.7%
5	C-03	7	7	9	5	13	3	8	2	76.92%
6	D-11	12	12	11	11	13	9	1	3	30.77%
7	D-16	13	9	8	13	14	6	5	3	57.14%
8	E-05	12	11	11	11	12	9	0	3	25%
9	H-05	8	12	12	8	14	5	6	3	64.29%
10	H-07	8	8	7	7	9	6	2	1	33.33%
Total		102	106	101	103	133	69	37	27	48.12%

The number of fragments amplified per primer varied between 9 (OP-H07) and 17 (OP-C02) and had a size range from 190 bp (OP-E05) to 1120 bp (OP-B03). Data of the presence/ absence of DNA fragments the four investigated species, were used to calculate the genetic similarity, based

on the calculated genetic similarity presented in Table 8 and Dendrogram as in figure 10, an estimation of the relationship between the above species was concluded where highest similarity was between *A. Penelope* and *A. acuta* (85%) while the lowest similarity was between *A. acuta* and *A. clypeata*. (79 %)

Table 8. Similarity matrix UPGMA Dice coefficient

	A. crecca	*A. penelope*	*A. acuta*	*A. clypeata*
A. crecca	100			
A. penelope	81	100		
A. acuta	80	85	100	
A. clypeata	84	82	79	100

The results of cytogenetic analysis (karyotyping) and of RAPD-PCR analysis were compared with those obtained from the classical methods in taxonomy using morphological and anatomical characters. This research is an initial study reporting the chromosome numbers, karyotypic characters and RAPD analysis of four species, *Anas crecca*, *Anas Penelope*, *Anas acuta* and *Anas clypeata* in Egypt.

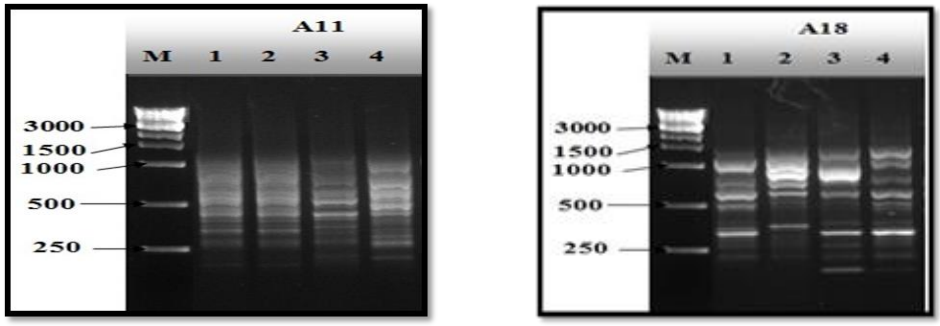

Fig. 5. Agarose-gel electrophoresis of RAPD products generated with primer OPA-11 and OPA-18 with the four samples 1- *Anas crecca*, 2- *Anas penelope*, 3-*Anas acuta* and 4- *Anas clypeata*

Fig. 6. Agarose-gel electrophoresis of RAPD products generated with primer OPB-03 and OPC-02 with the four samples 1- *Anas crecca* , 2- *Anas penelope*, 3-*Anas acuta* and 4- *Anas clypeata*

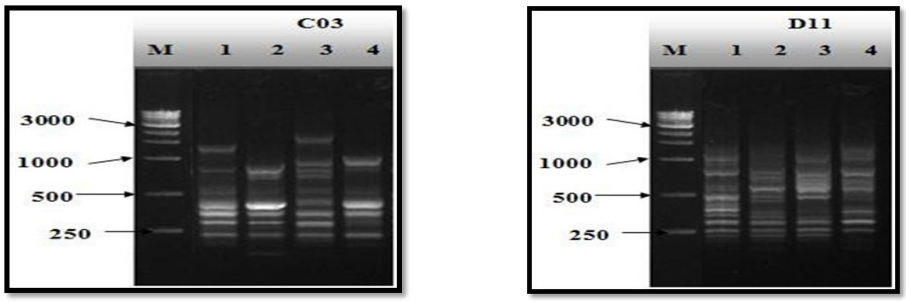

Fig. 7. Agarose-gel electrophoresis of RAPD products generated with primer OPC 03 and OPD-11 with the four samples 1- *Anas crecca*, 2- *Anas penelope*, 3-*Anas acuta* and 4- *Anas clypeata*

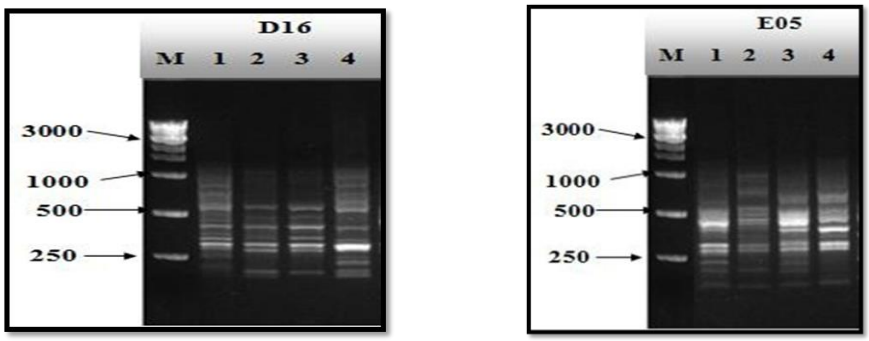

Fig. 8. Agarose-gel electrophoresis of RAPD products generated with primer OPD-16 and OPE-05 with the four samples 1- *Anas crecca* , 2- *Anas penelope*, 3-*Anas acuta* and 4- *Anas clypeata*

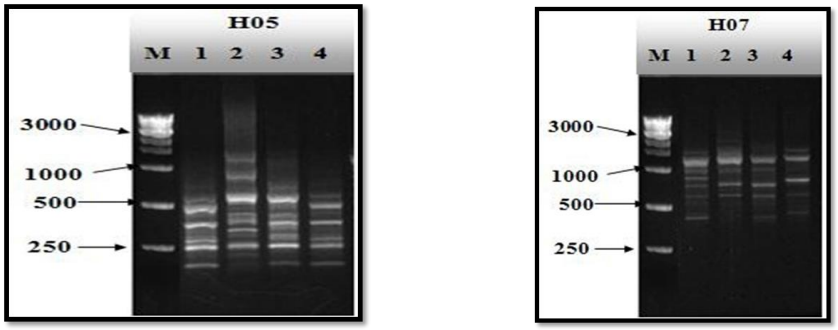

Fig. 9. Agarose-gel electrophoresis of RAPD products generated with primer OPH-05 and OPH-07 with the four samples 1- *Anas crecca*, 2- *Anas penelope*, 3-*Anas acuta* and 4- *Anas clypeata*

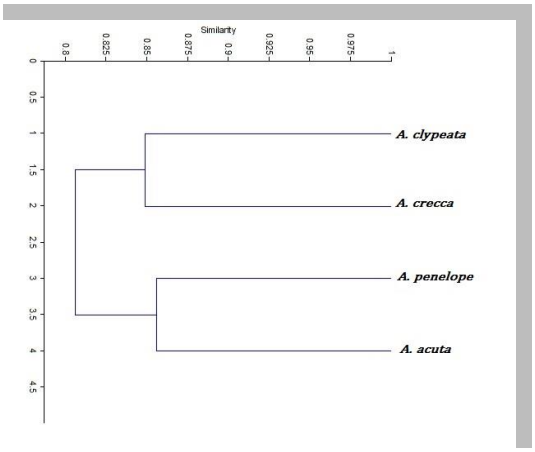

Fig. 10. Dendrogram for four species constructed from the RAPD data

DISCUSSION

The structure of most avian karyotypes consists of a few pairs of macrochromosome and many microchromosomes. The standard karyotype study for chicken that have eight macrochromosomes plus the Z and W sex chromosomes has been previously concluded by LADJALI-MOHAMMEDI *et al.* (1999). In the analysis of the karyotypes of birds, it is important to take into consideration not only the larger chromosomes but also the complete karyotype including macro- and microchromosomes (HAMMAR, 1966; DEGRANDI *et al*., 2018). The present study is a contribution to the karyological data on four species of ducks (*A. crecca, A. penelope, A. acuta and A. clypeata*) belonging to family Anatidae, Order Anseriformes. The four investigated species have identical chromosome number 2n = 80 and a very few differences in the karyotype.

The order Anseriformes to which *A. crecca* belongs has diversity in the chromosome number as follows; the diploid chromosome number of *Anser anser* L., was about 80 as reported before by HAMMAR (1966), the diploid chromosome number of *Mergus cucullatus* (Hooded merganser) 2n = 82±, *Aix galericulata* (Mandarin duck) 2n = 90 as concluded by BENIRSCHKE, *et al*. (1975). The diploid chromosome numbers of *Cygnus melancoryphus* was 2n = 78 as concluded by DE OLIVERA *et al.* (2001). According to tour results, *A. crecca* is shown to have a diploid chromosome number of 2n=80 (First recorded) and the karyotype consists of one metacentric chromosome, one submetacentric chromosome, three pairs of Acrocentric chromosomes and 35 pairs of microchromosomes.

HAMMAR (1970) reported that the diploid chromosome number of *Tadorna tadorna* (Common shelduck) was 2n = 80, with karyotype build of 10 or more metacentrics, submetacentrics or subtelocentrics, the remainder chromosomes were presumed to be acrocentrics. Z-chromosome was Acrocentric, W-chromosome was acrocentric. According to

our results, *A. penelope* is shown to have a diploid chromosome number of 2n=80 (First recorded) and the karyotype consists of one pair of sub-metacentric chromosome, three pairs of Acrocentric chromosomes and 35 pairs of microchromosomes. Z chromosome is metacentric, W-chromosome is sub-metacentric.

BENIRSCHKE *et al*. (1975) studied the diploid chromosome number of *Oxyura jamaicensis jamaicensis* (Ruddy duck) 2n = 80 with 4 Submetacentrics. The remainder chromosomes were presumed to be acrocentric or telocentric. Z-chromosome was subtelocentric or submetacentric, W-chromosome was acrocentric or subtelocentric. Our results showed that, *A. acuta* has a diploid chromosome number of 2n=80 and the karyotype consists of one pair of metacentric chromosome, three pairs of Acrocentric chromosomes and 35 pairs of microchromosomes. Z-chromosome is metacentric, W-chromosome is submetacentric. Our results run in consistence with those by SHIELDS (1982) who reported the same number of chromosomes.

According to our results, *A. clypeata* has a diploid chromosome number of 2n=80 and the karyotype consists of one metacentric chromosome, one submetacentric chromosome, three pairs of Acrocentric chromosomes and 35 pairs of microchromosomes. Z-chromosome is submetacentric, W-chromosome is metacentric. The number of chromosomes agrees with that reported by SHIELDS (1982).

The results of the current study addressed two major features of the bird karyotypes. One is the origin of the many tiny microchromosomes in all the bird karyotypes, and the other is the evolution of the ZW sex chromosomes and their sex determination (SHETTY and GRIFFIN, 1999).

In this study, we further used RAPD markers for studying genetic similarity among the four investigated species because RAPD-PCR is a useful tool for estimating the genetic variability and degree of similarity among avian species (KULIKOVA *et al.,* 2002, 2003; SPIRIDONOVA *et al*., 2003; EL-GENDY *et al.,* 2005; MACIUSZONEK *et al.*, 2005; BARBANERA *et al.*, 2007; TUBELYTE *et al*., 2011; BAWEJ *et al*., 2012; VOLKOVSKY *et al.*, 2013; MUHAMMAD *et al.,* 2015).

RAPD bands in this experiment showed high variability (i.e. strong, faint, fuzzy and sharp bands) generated with each primer because one or more copies of DNA may occur per genome or may be referred to the varying of the annealing process between the primer and the DNA, this problem of mixed bands shows the sensitivity of PCRs (BIELAWSKI *et al*., 1995)

In the present study, the similarity relationship between *A. crecca* and *A. penelope* was 81%, between *A. crecca* and *A. acuta* was 80%, between *A. crecca* and *A. clypeata* was 84%, between *A. penelope* and *A. acuta* was 85%, between *A. penelope* and *A. clypeata* was 82% and between *A. acuta* and *A. clypeata* was 79%. According to our best knowledge, this is the first report on the relationship between the four species in the present study. The results are the first recorded ones.

The polymorphism produced between the studied species using primers A-11, A-18, B-03, C-02, C-03, D-11, D-16, E-05, H-05 and H-07 were 33.33%, 38.46% , 46,15% , 64.7% , 76.92%, 30.77% , 57.14% , 25% , 64.29% and 33.33% respectively.

CONCLUSION

The present study not only reflected for the first time the taxonomical relationship between the four species studied but also, confirmed that the combination of chromosome complement and RAPD-PCR are useful tools for estimating the genetic variability and degree of

similarity among avian species. Further studies are needed to investigate if some genes have specific sequence in each species.

Received, March 21th, 2018
Accepted November 18th, 2018

REFERENCES

ABU-ALMAATY, A.H. (2017): Determination of somatic and sex chromosomes of three Egyptian birds species using cytogenetic analysis. Genetika, *49*(1): 285-295.

ADEGOKE, J.A., V.C., EJERE (1991): Description of the chromosomes of three lizard species belonging to the genus Mabuya (Scincidae, Reptilia). Caryologia, *44*(3-4): 333-342.

BARBANERA, F., M., GUERRINI, P., HADJIGEROU, P., PANAYIDES, C., SOKOS, P., WILKINSON, F., DINI (2007): Genetic insight into Mediterranean chukar (Alectoris chukar, Galliformes) populations inferred from mitochondrial DNA and RAPD markers. Genetica, *131*(3): 287-298.

BAWEJ, M., D., KOKOSZYŃSKI, Z., BERNACKI (2012): Evaluation of genetic similarity between white and grey varieties of Guinea fowl (Numida meleagris). J. Central European Agric., *13*(4): 654-661.

BENIRSCHKE, K., T.C., HSU, M.L., BEÇAK, W., BEÇAK, T.R., CHEN, R.N., SHOFFNER (1975): Anseriformes. In: *Chromosome Atlas: Fish, Amphibians, Reptiles, and Birds* (pp. 157-192). Springer Berlin Heidelberg.

BIELAWSKI, J.P., K., NOACK, D.E., PUMO (1995): Reproducible amplification of RAPD markers from vertebrate DNA. Biotechniques, *18*(5): 856-860.

BLOOM, S.E. (1969): Mitotic chromosomes of Mallard ducks. J. Heredity, *60*(1): 35-38.

DEGRANDI, T.M., J.C.P., DE OLIVEIRA, A., DE ARAÚJO SOARES, M.A., LEDESMA, I., HASS, A., DEL VALLE GARNERO, R.J., GUNSKI (2018): Karyotype description and comparative analysis in Ringed Kingfisher and Green Kingfisher (Coraciiformes, Alcedinidae). Comparative Cytogenetics, *12*(2): 163-170.

DE OLIVEIRA, M.D.V., W., JORGE, C.P., BAREZANI (2001): Chromosome study in six Brazilian birds. Caryologia, *54*(3): 235-244.

EL-GENDY, E.A., M.A., HELAL, N.H., GOHER, A., MOSTAGEER (2005): Molecular characterization of genetic biodiversity in ducks, using RAPD-PCR analysis. Arab J. Biotech., *8*(2): 253-264.

HAMMAR, B. (1966): The karyotypes of nine birds. Hereditas, *55*(2-3): 367-385.

HAMMAR, B. (1970): The karyotypes of thirty-one birds. Hereditas, *65*(1): 29-58.

IMAI, H.T. (1991): Mutability of constitutive heterochromatin (Cbands) during eukaryotic chromosomal evolution and their cytological meaning. Jpn. J. Genet., *66*:653-661.

KULIKOVA, I.V., G.N., CHELOMINA, Y.N., ZHURAVLEV (2002): RAPD–PCR Analysis of Genetic Diversity in the Manchurian Pheasant. Russian J. Genetics, *38*(6): 699-703.

KULIKOVA, I.V., G.N., CHELOMINA, Y.N. ZHURAVLEV (2003): Low genetic differentiation of and close evolutionary relationships between Anas platyrhynchos and Anas poecilorhyncha: RAPD–PCR evidence. Russian J. Genetics, *39*(10): 1143-1151.

LADJALI-MOHAMMEDI, K., J.J., BITGOOD, M., TIXIER-BOICHARD, F.A., PONCE DE LEON (1999): International system for standardized avian karyotypes (ISSAK): standardized banded karyotypes of the domestic fowl (*Gallus domesticus*). Cytogenetic and Genome Research, *86*(3-4): 271-276.

LEVAN, A., K., FREDGA, A.A., SANDBERG (1964): Nomenclature for centromeric position on chromosomes. Hereditas, *52*(2): 201-220.

LI, H., N., YANG, K., CHEN, G., CHEN, Q., TANG, Y., TU, Y., MA (2006): Study on molecular genetic diversity of native duck breeds in China. World's Poultry Science Journal, *62*(04): 603-611.

MACIUSZONEK, A., B., GRAJEWSKI, M., BEDNARCZYK (2005): RAPD-PCR analysis of various goose populations. Folia biologica, *53*(1-2): 83-85.

MUHAMMAD, S., A.A., KHAN, M., BABAR, M., RIAZ, N., AKHTAR, I., KHALIQ (2015): Population genetic structure of Rufous-Vented Prinia (*Prinia burnesii*) in Pakistan. African J. Biotech., *9*(53): 9077-9081.

O'CONNOR, C. (2008): Karyotyping for chromosomal abnormalities. Nature Education, *1*(1): 27.

SHAHIN A.A.B., A.T.M., ATA, A.S.M., ABU SHNAF (2014): Karyotype and C-banding pattern of the domestic geese Anser anser populations (Aves: Anatidae) in Egypt. Folia Biologica (Krakow) 62: 49-58.

SHETTY, S., D.K., GRIFFIN, J.A.M., GRAVES (1999): Comparative painting reveals strong chromosome homology over 80 million years of bird evolution. Chromosome Research, *7*(4): 289-295.

SHIELDS, G. F. (1982): Comparative avian cytogenetics: a review. Condor, *84*: 45-58.

SPIRIDONOVA, L.N., G.N., CHELOMINA, A.P., KRYUKOV (2003): Genetic diversity of the carrion and jungle crows as evidenced by RAPD–PCR analysis. Russian J. Genetics, *39*(11): 1281-1291.

TAKAGI, N., M., SASAKI (1974): A phylogenetic study of bird karyotypes. Chromosoma, *46*(1): 91-120.

TEGELSTRÖM, H., H., RYTTMAN (1981): Chromosomes in birds (Aves): evolutionary implications of macro-and microchromosome numbers and lengths. Hereditas, *94*(2): 225-233.

TUBELYTE, V., S., ŠVAŽAS, A., SRUOGA, D., BUTKAUSKAS, A., PAULAUSKAS, V., BAUBLYS, A., KOZULIN (2011): Genetic diversity of tufted ducks (*Aythya fuligula*, Anatidae) in Eastern Europe. Central European J. Biology, *6*(6): 1044-1053.

VOLKOVSKY, D.V., I.V., KULIKOVA, Y.N., GERASIMOV, Y.N., ZHURAVLEV (2013): Genetic diversity of Anser albifrons Scopoli, 1769 and Anser fabalis Latham, 1787 in the Russian Far East. Russian J. Genetics, *49*(4): 428-440.

WELSH, J., M., MCCLELLAND (1990): Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., *18*(24): 7213-7218.

WILLIAMS, J.G., A.R., KUBELIK, K.J., LIVAK, J.A., RAFALSKI, S.V., TINGEY (1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., *18*(22): 6531-6535.

WÓJCIK, E., E., SMALEC (2007): Description of the *Anser anser* goose karyotype. Folia biologica, *55*(1-2): 35-40.

HROMOZOMSKA EVOLUCIJA I MOLEKULARNA GENETIČKA ANALIZA ČETIRI VRSTA RODA *ANAS* (AVES: ANATIDAE)

Ali Hussein ABU-ALMAATY[1*], Mohamed K. HASSAN[1], Neveen E. R. EL BAKARY[2], Sarah H. AHMED[1]

[1]Departman za zoologiju, Prirodno-matematički fakultet, Port Said Univerzitet, Egipat
[2]Departman za zoologiju, Prirodno-matematički fakultet, Damietta Univerzitet, Egipat

Izvod

Ptice se smatraju jednom od najmanje kariotipski ispitivanih grupa životinja zbog njihove kariotipne specifičnosti, tj. malih hromozoma, velikog broja diploidnih hromozoma i odvajanja hromozoma u makro- i mikrokrohromozome. Cilj ovog rada bio je da se ispita broj hromozoma i njihovi kariološki i molekularno genetski odnosi četiri vrste roda Anas (*Anas crecca, Anas penelope, Anas acuta* i *Anas clipeata* (Porodica: Anatidae). Sve četiri vrste imaju isti broj diploidnih hromozoma od 2n = 80. Četiri ispitivane vrste su pokazale pet pari makrohromozoma, a preostalih 35 pari su bili mikrohromozomi. Deset RAPD prajmera je korišćeno za molekularnu diskriminaciju PCR reakcijom. PCR je dao polimorfne trake, koje su korišćene za konstrukciju dendrograma i matrice sličnosti. Ukupno je dobijeno 133 trake; 37 od njih su bile polimorfne, a 27 je bilo specifično. Vriednosti sličnosti između ispitivanih vrsta kretale su se od 79% do 85%. Najveća sličnost je bila između *A. Penelope* i *A. acuta* (85%), dok je najmanja sličnost bila između *A. acuta* i *A. clipeata* (79%). RAPD analiza je potvrdila da su četiri *Anas* vrste koje su proučavane genetski različite i da je pronađena genetska varijacija unutar I između ove tri vrste. Kariotipske osobine su takođe pogodne kao citotaksonomski markeri za *Anatidae*.

Primljeno 21.III.2018.
Odobreno 18. XI. 2018.