

ΑΝΙΧΝΕΥΣΗ ΠΥΡΗΝΟ-ΚΥΤΤΑΡΟΠΛΑΣΜΑΤΙΚΩΝ ΑΝΩΜΑΛΙΩΝ ΚΑΙ ΠΑΡΑΣΙΤΩΝ ΣΕ ΕΡΥΘΡΟΚΥΤΤΑΡΑ ΔΥΟ ΜΕΤΑΝΑΣΤΕΥΤΙΚΩΝ ΠΤΗΝΩΝ ΣΕ ΠΕΡΙΟΧΗ ΣΤΑΘΜΕΥΣΗΣ ΣΤΟ ΝΗΣΙ ΤΩΝ ΑΝΤΙΚΥΘΗΡΩΝ

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Κύριος στόχος της παρούσας μελέτης ήταν η ανίχνευση πυρηνο-κυτταροπλασματικών ανωμαλιών στα ερυθροκύτταρα δύο μεταναστευτικών ειδών πτηνών, του τρυγονιού *Streptopelia turtur* και του συκοφάγου *Oriolus oriolus*, καθώς επίσης και η παρασιτολογική μελέτη στα ερυθροκύτταρα του είδους *Streptopelia turtur*. Συγκεκριμένα, μετά από σύλληψη και δακτυλίωση ατόμων του κάθε είδους (N>25) στο νησί των Αντικυθήρων (περιοχή στάθμευσης κατά την μετανάστευση), κατά τους μήνες Απρίλιο-Μάιο 2017, ακολούθησε καταγραφή των μορφομετρικών τους στοιχείων και συλλογή μικρής ποσότητας αίματος από τη βραγχίονια αρτηρία κάθε ατόμου. Ακολούθησε επίστρωση του αίματος σε αντικειμενοφόρο πλάκα και κατάλληλη προετοιμασία των δειγμάτων για προσδιορισμό πυρηνο-κυτταροπλασματικών ανωμαλιών και παρασίτων. Τα αποτελέσματα έδειξαν την ύπαρξη πυρηνο-κυτταροπλασματικών ανωμαλιών στα ερυθροκύτταρα και των δύο ειδών, γεγονός που υποδηλώνει την περιβαλλοντική καταπόνησή τους. Παρά την απουσία στατιστικών διαφορών μεταξύ της συχνότητας εμφάνισης κυτταρικών αλλοιώσεων και της ηλικίας ή/και του φύλου των ατόμων, παρατηρήθηκαν σημαντικές διαειδικές διαφορές, με μεγαλύτερες συχνότητες εμφάνισης αλλοιώσεων να παρατηρούνται στα τρυγόνια. Η παρασιτολογική ανάλυση έδειξε την προσβολή ενός μεγάλου ποσοστού ατόμων τρυγονιών (36%) από πρωτόζωα του γένους *Haemoproteus* και *Leucocytozoon*. Η εμφάνιση γενετοξικών και μολυσματικών φαινομένων στα ερυθροκύτταρα των δύο μεταναστευτικών πτηνών υποδεικνύει για πρώτη φορά την ύπαρξη περιβαλλοντικών παραγόντων καταπόνησης στα εν λόγω είδη, που θα μπορούσαν να επηρεάσουν την φυσιολογική τους κατάσταση και κατ' επέκταση διαφορετικά στάδια του κύκλου ζωής τους.

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DETECTION OF CYTONUCLEAR ABNORMALITIES AND PARASITES IN THE RED BLOOD CELLS OF TWO TRANS-EQUATORIAL MIGRATORY BIRDS IN A STAGING AREA ON THE ANTIKYTHIRA ISLAND

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The main goal of the present study was to investigate cellular and nuclear abnormalities in red blood cells of two trans-migratory avian species, the turtle dove *Streptopelia turtur* and the Eurasian Golden Oriole *Oriolus oriolus*, as well as the presence of parasites in turtle dove individuals. Specifically, 25 individuals of *Streptopelia turtur* and 28 individuals of *Oriolus oriolus* were captured during April-May 2017, on Antikythira island (staging area). After ringing and collection of morphometric measurements for each individual (i.e. body mass, wing and tarsus length, sex and age class), a small volume of blood was collected from the brachial vein, blood smears were prepared in all cases and finally analyzed for cytoplasmic and nuclear abnormalities (i.e. micronuclei formation), as well as for the presence of blood parasites. According to per species results of the present study, there was no age- and/or sex-related differences in the frequency of the examined stress indices in almost all cases. On the other hand, inter-species variations were recorded, with higher frequencies of specific abnormalities to be observed in *Streptopelia turtur* compared to *Oriolus oriolus* (e.g. higher micronuclei frequency in females and adults). Moreover, the parasitological analysis revealed the presence of protozoans of the genus *Haemoproteus* and *Leucocytozoon* in the blood of 9 individuals (36%) of *Streptopelia turtur*. The results of the present study revealed for the first time the induction of genotoxic and contagious effects in blood cells of these avian species, which in turn could affect their health status and, subsequently, different stages of their life cycle.

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1. Introduction

Cellular abnormalities in erythrocytes have been extensively used as biomarkers to assess the environmental quality and specifically the presence of genotoxic factors in the habitat of free-living animals. In particular, birds are considered ideal model organisms in such assays. We focused on two trans-equatorial migratory birds, the Turtle Dove (*Streptopelia turtur*) and the Golden Oriole (*Oriolus oriolus*) (**Fig. 1**), to detect the presence of a battery of stress indices, namely micronuclei, nuclear and cytoplasmic abnormalities, as well as the presence of parasites in Turtle Dove individuals, during the return journey to their breeding grounds.

2. Materials & Methods

During April-May 2017, 25 individuals of Turtle Dove and 28 individuals of Golden Oriole were trapped in mist nets on Antikythira island, S Greece (**Fig. 2**), which is a well-known staging area of both species. Birds encountered on the island during spring have just crossed the Mediterranean Sea. All captured individuals were ringed and aged by plumage. Standard morphological measurements were also taken. In continuation, by puncturing the brachial vein a drop of blood was transferred to a slide and smeared. The blood smears were air-dried, fixed in methanol and stained with Giemsa 5% v/v. Two blood smear samples from each individual were microscopically scanned (1,000 erythrocytes per blood smear, 100x magnification) to detect the presence of cytoplasmic, nuclear abnormalities (i.e., micronuclei formation; **Fig. 4**) and parasites. Intra- and interspecies differences were assessed via Mann-Whitney U tests.

3. Results

According to **per species results** of the present study, **there was no significant age- and/or sex-related differences** in the frequency of the examined stress indices in almost all cases.

- Among nuclear abnormalities, the most common abnormality in the erythrocytes of both species was the formation of binucleated cells (**BN**; **Fig. 5**), whose mean value was higher than those reported in literature.
- Regarding cytoplasmic abnormalities, high frequency of echinocytes (**EchCyt**) was observed in Turtle Dove individuals, whereas an increased number of cells without membranes (**NoMembCyt**) was found in Golden Oriole individuals (**Fig. 5**).

The **interspecies results** indicated significantly **higher frequencies in specific nuclear** (Micronucleus-MN, binucleated cell-BN, lobed nucleus-LN, total nuclear-TotalNucl) **and cytoplasmic abnormalities** (acanthocyte-AcanCyt) in Turtle Dove individuals compared to Golden Oriole individuals (**Fig. 5**). Considering age and sex-based differences, higher abnormalities were observed in Turtle Dove individuals compared to Golden Oriole as follows (**Fig. 5** & **Table 1**): females- micronuclei (**MN**) & eight-shaped cell (**EightCyt**), adults (age class 6) – micronuclei (**MN**) & vacuolated cells (**VN**), subadults (age class 5) – binucleated cell (**BN**) & acanthocyte (**AcanCyt**).

The parasitological analysis of blood smears revealed the presence of protozoans of the genus *Haemoproteus* and *Leucocytozoon* in the blood of 9 individuals (36%) of *Streptopelia turtur* (**Fig. 3**).

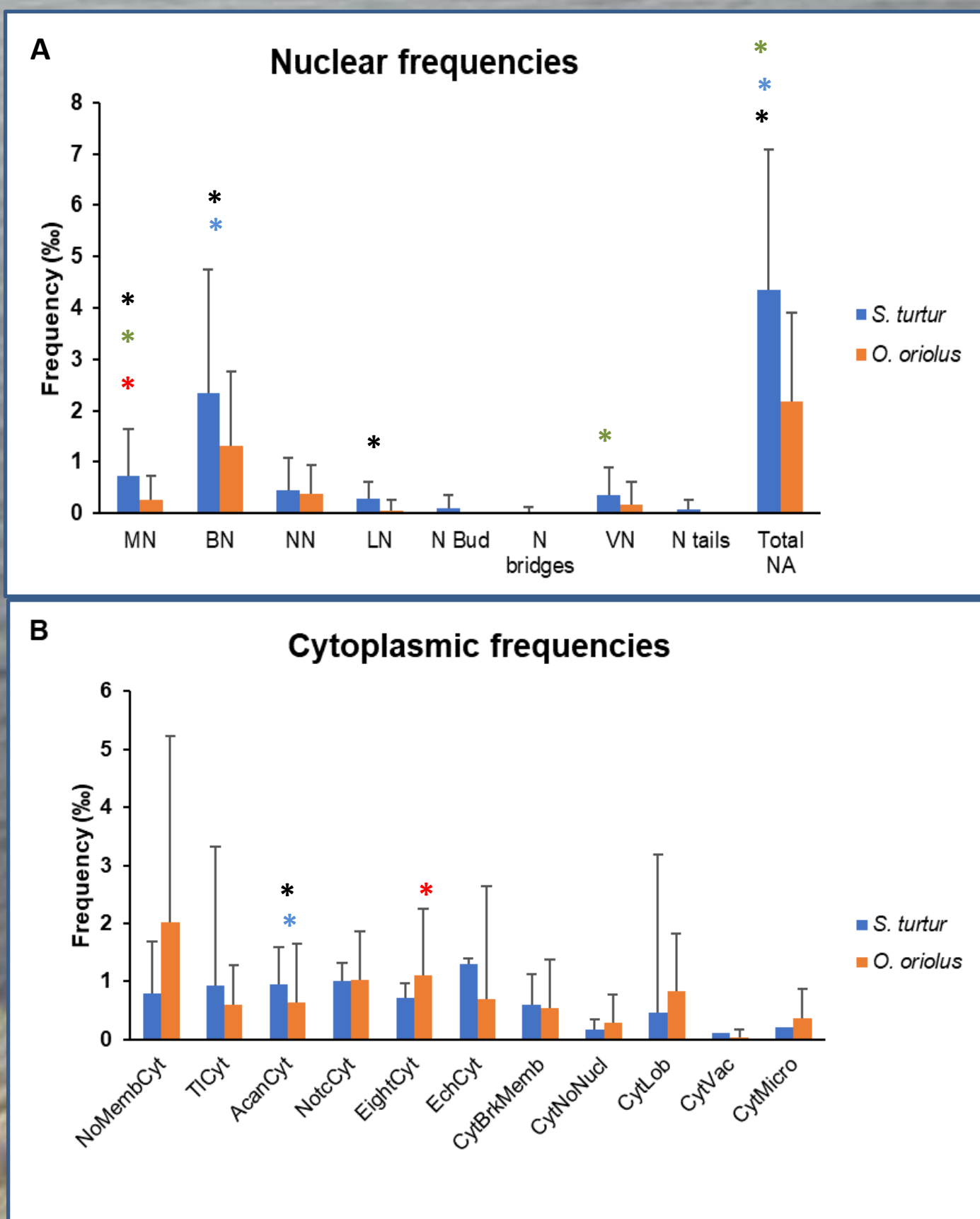


Fig. 5. Mean frequencies and standard errors of nuclear (A) and cytoplasmic (B) abnormalities for Turtle Dove and Golden Oriole. Interspecies significant differences ($p < 0.05$): * among all individuals, * among individuals of age class 5 (sub-adults), * among females, * among individuals of age class 6 (adults).

Table 1. Significant age- and/or sex-related differences in the frequency of nuclear and cytoplasmic abnormalities between the two species, based on Mann-Whitney U tests. Mean \pm SD values are given. P-values smaller than 0.05 are highlighted in bold.

Age class 5				
Abnormalities	Type	<i>Streptopelia turtur</i> mean \pm SD	<i>Oriolus oriolus</i> mean \pm SD	Mann Whitney test
Nuclear	BN	2.16 \pm 1.80	0.57 \pm 0.73	U=25.00 p=0.015
	TotalNucl	3.92 \pm 2.33	1.07 \pm 0.67	U=12.50 p=0.001
Cytoplasmic	AcanCyt	0.97 \pm 0.98	0.21 \pm 0.27	U=30.50 p=0.035
Age class 6				
Abnormalities	Type	<i>Streptopelia turtur</i> mean \pm SD	<i>Oriolus oriolus</i> mean \pm SD	Mann Whitney test
Nuclear	MN	0.75 \pm 0.52	0.29 \pm 0.49	U=30.00 p=0.057
	VN	0.67 \pm 0.52	0.21 \pm 0.49	U=26.00 p=0.031
	TotalNucl	5.83 \pm 3.49	2.57 \pm 1.80	U=18.00 p=0.007
Females				
Abnormalities	Type	<i>Streptopelia turtur</i> mean \pm SD	<i>Oriolus oriolus</i> mean \pm SD	Mann Whitney test
Nuclear	MN	0.61 \pm 0.49	0.18 \pm 0.46	U=48.00 p=0.021
	TotalNucl	4.50 \pm 2.70	2.50 \pm 2.18	U=57.50 p=0.062
Cytoplasmic	NoMembCyt	0.86 \pm 1.98	2.00 \pm 3.41	U=57.50 p=0.062
	EightCyt	0.54 \pm 0.84	1.43 \pm 1.24	U=54.50 p=0.044
	CytlLob	0.46 \pm 0.60	1.18 \pm 1.15	U=57.00 p=0.062
Males				
Abnormalities	Type	<i>Streptopelia turtur</i> mean \pm SD	<i>Oriolus oriolus</i> mean \pm SD	Mann Whitney test
Nuclear	TotalNucl	4.23 \pm 2.83	1.89 \pm 1.08	U=40.00 p=0.044



Fig. 1. (A) *Streptopelia turtur* and (B) *Oriolus oriolus* captured on Antikythira island. © C. Barboutis/Hellenic Ornithological Society's Archive.



Fig. 2. Location of the study area

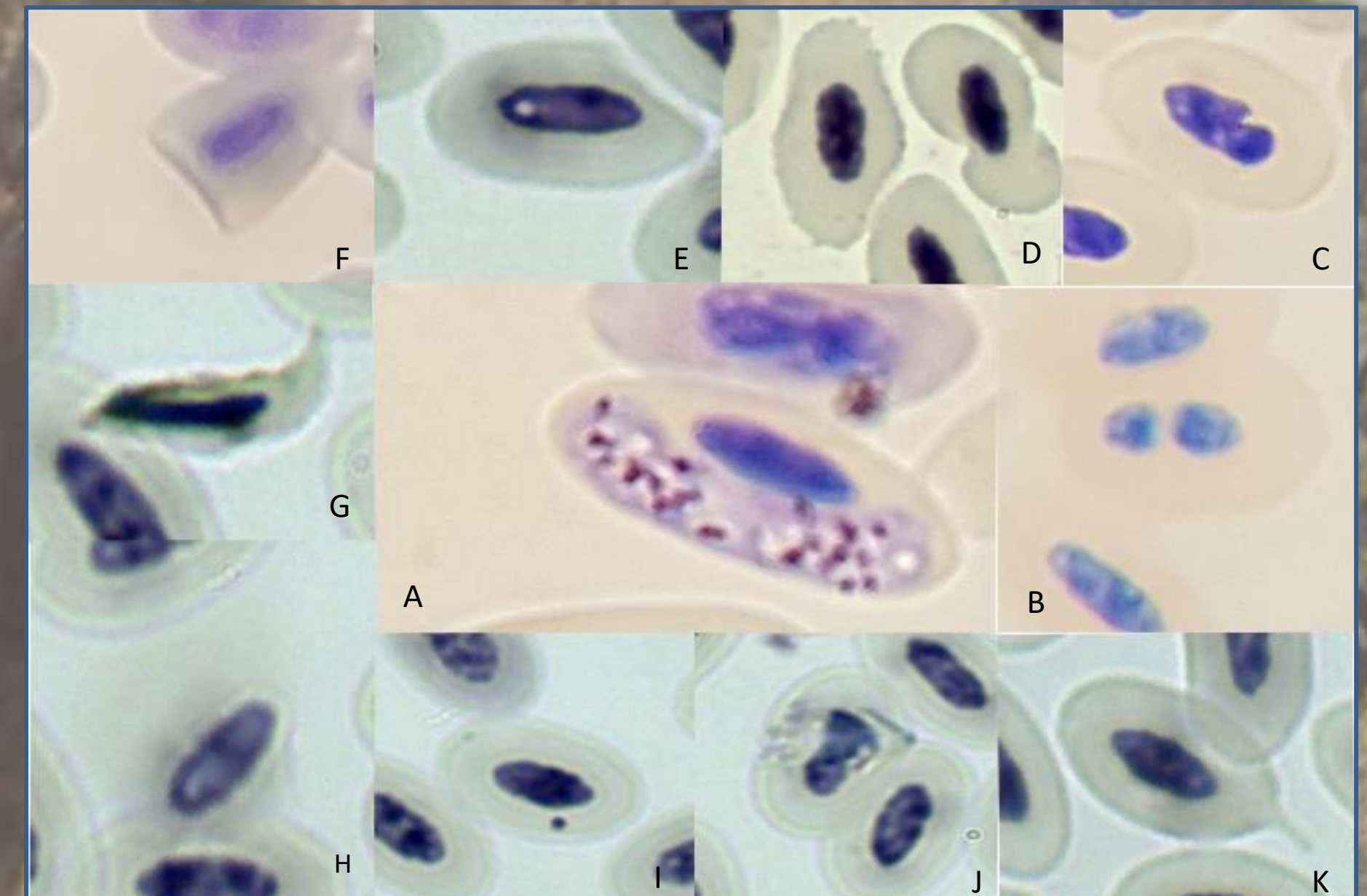


Fig. 4. Main nuclear and cytoplasmic abnormalities: (A) *Haemoproteus* sp. (B) Binucleated cell-BN (C) Notched Nucleus-NN (D) Echinocyte-EchCyt and Eight-shaped cell-EightCyt (E) Vacuolated Nucleus-VN (F) Notched Cytoplasm-NotCyt (G) Acanthocyte-AcanCyt (H) Cell without membrane-NoMembCyt (I) Micronucleus-MN (J) Lobed Nucleus-LN (K) Cytoplasmic tail-TICyt

4. Discussion

The results of the present study revealed for the first time the **induction of genotoxic effects** in red blood cells of these avian species, which in turn could affect their health status as well as their life cycle (i.e. migration, wintering, and breeding). This is the first experimental study in Greece, which profiles different nuclear and cytoplasmic abnormalities in the erythrocytes of these avian species during their migratory period. In the future, we will examine red blood samples of more species to elucidate the underlying factors influencing the susceptibility of spring migrants to genotoxic factors in the Palearctic migratory system.

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