

Genotoxicity in Free-living Birds: Detecting Nuclear and Cytoplasmic Abnormalities in the Red Blood Cells of two Trans-equatorial Migrants that have just Arrived to Europe to Breed

Papadimitraki Maria¹, Barboutis Christos², Kassara Christina¹, Tsarpali Vasiliki¹, Giokas Sinos¹, Dailianis Stefanos¹

¹Department of Biology, University of Patras, GR-26500 Rio Patras, Greece

²Antikythira Bird Observatory, Hellenic Ornithological Society/BirdLife Greece, GR-10681 Athens, Greece

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, 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, 1000x magnification) to detect the presence of cytoplasmic and/or nuclear abnormalities (i.e., micronuclei formation; **Fig. 3**). Intra- and interspecies differences were assessed via Man-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. 4**), 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. 4**).

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. 4**). Considering age and sex-based differences, higher abnormalities were observed in Turtle Dove individuals compared to Golden Oriole as follows (**Fig. 4 & Table 1**): females – micronuclei (**MN**) & eight-shaped cells (**EightCyt**), adults (age class 6) – micronuclei (**MN**) & vacuolated cells (**VN**), subadults (age class 5) – binucleated cells (**BN**) & acanthocytes (**AcanCyt**).

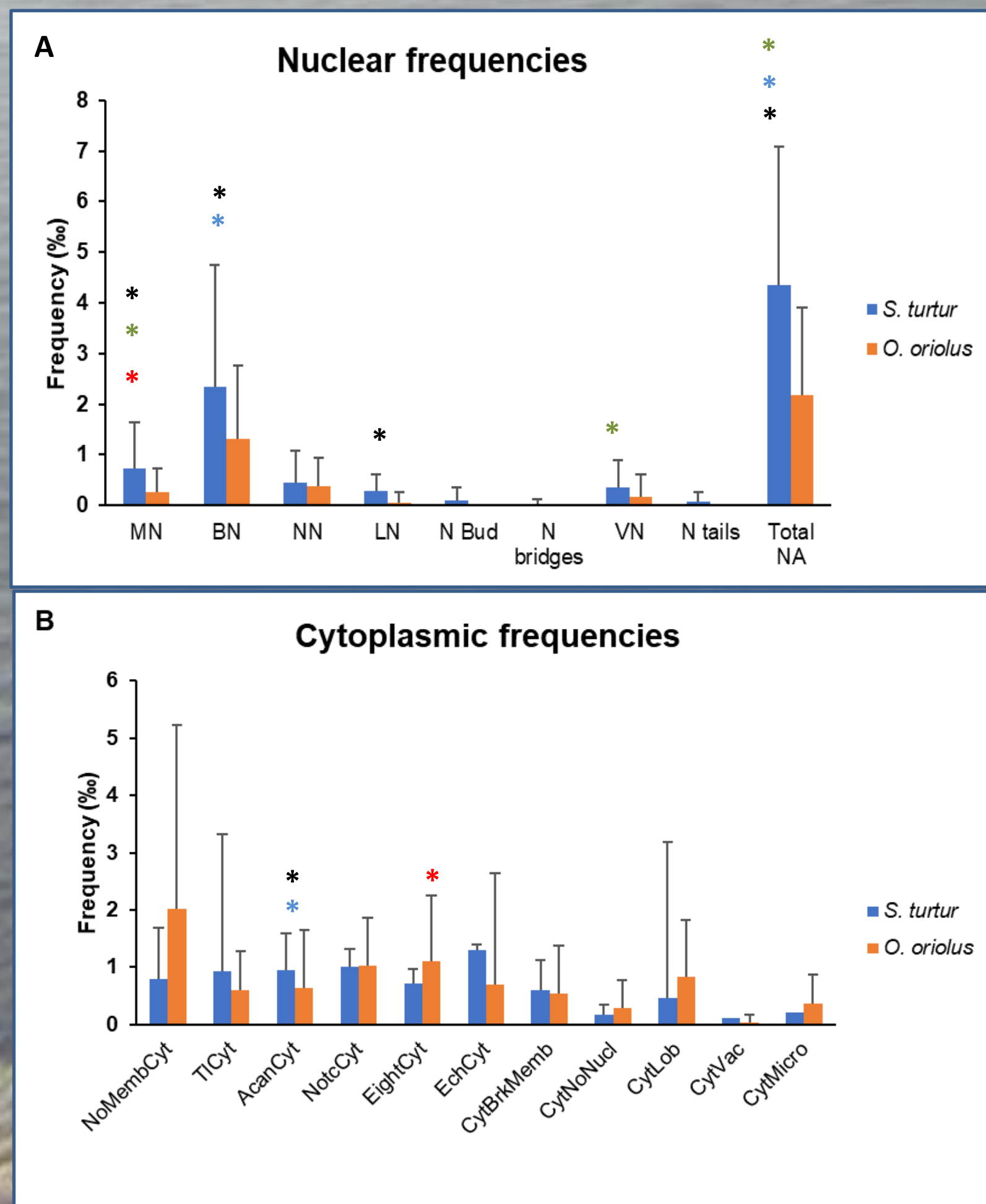


Fig 4. Mean frequencies and standard errors of nuclear (A) and cytoplasmic (B) abnormalities for Turtle Dove and Golden Oriole. Interspecies significant differences (p<0.05): * among all individuals, * among individuals of age class 5 (sub-adults), * among females, * among individuals of age class 6 (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
	CytLob	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

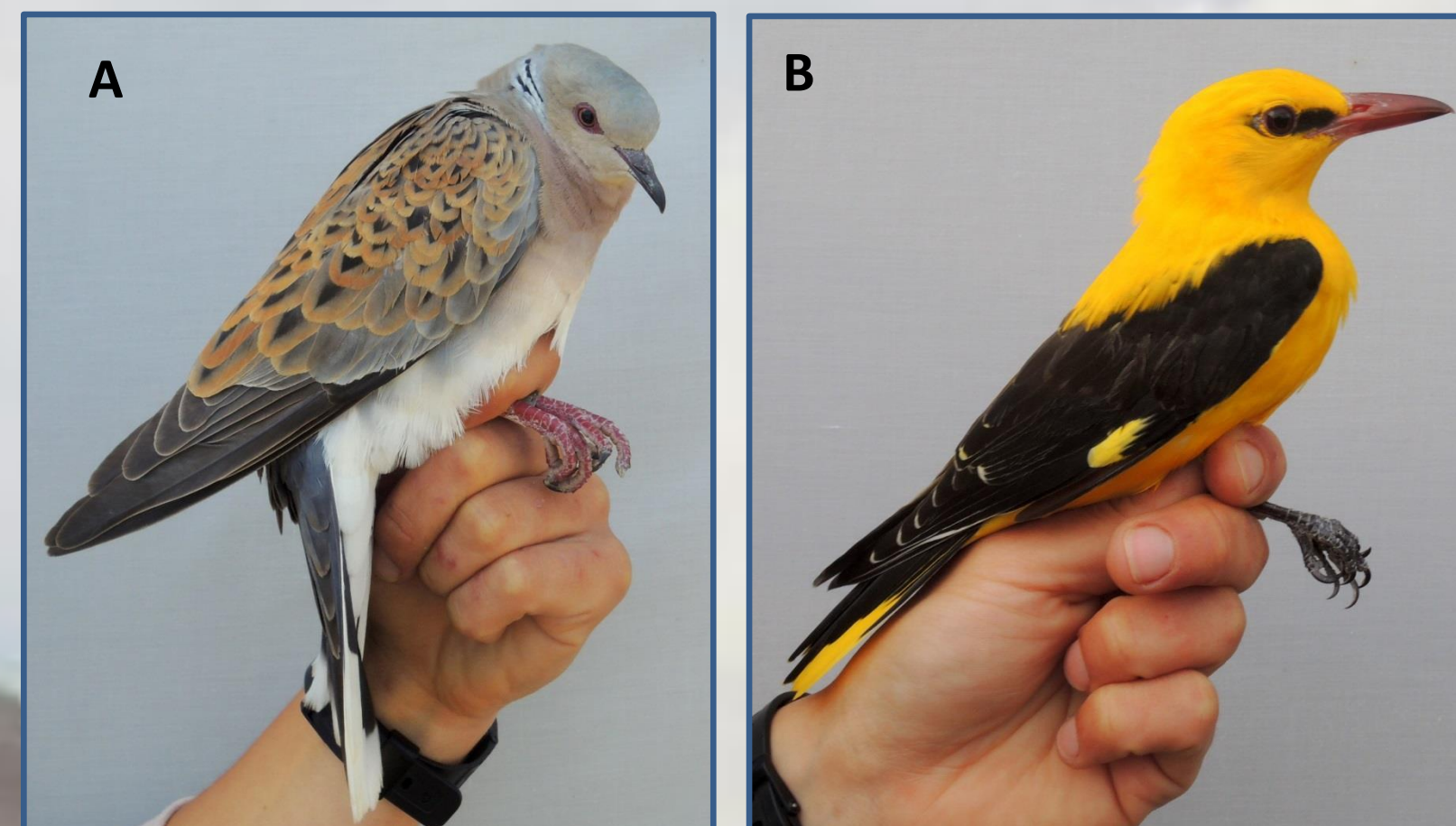


Fig. 1. (A) *Streptopelia turtur* and (B) *Oriolus oriolus* captured on Antikythira island. © C. Barboutis/Hellenic Ornithological Society's Archive.

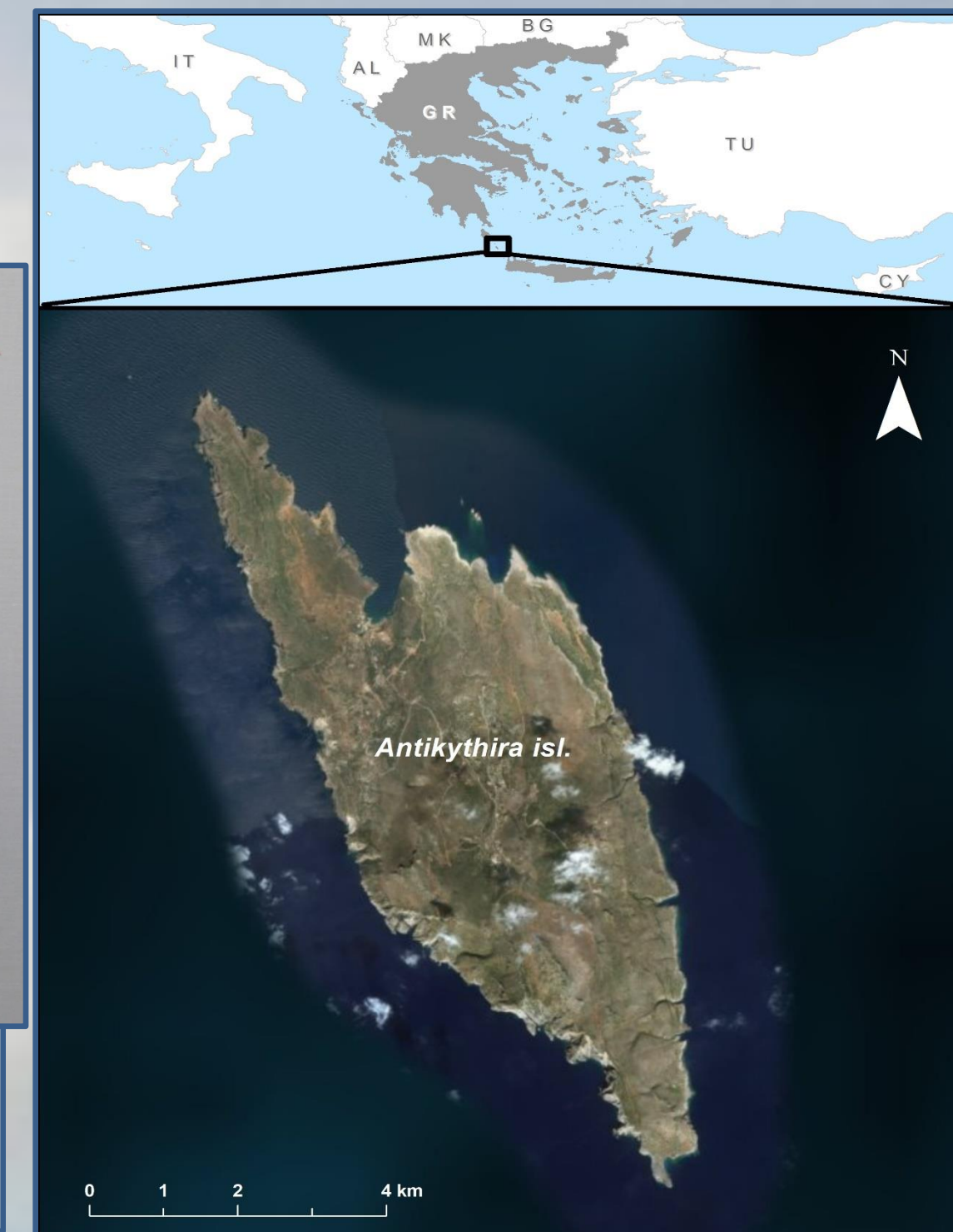


Fig. 2. Location of the study area

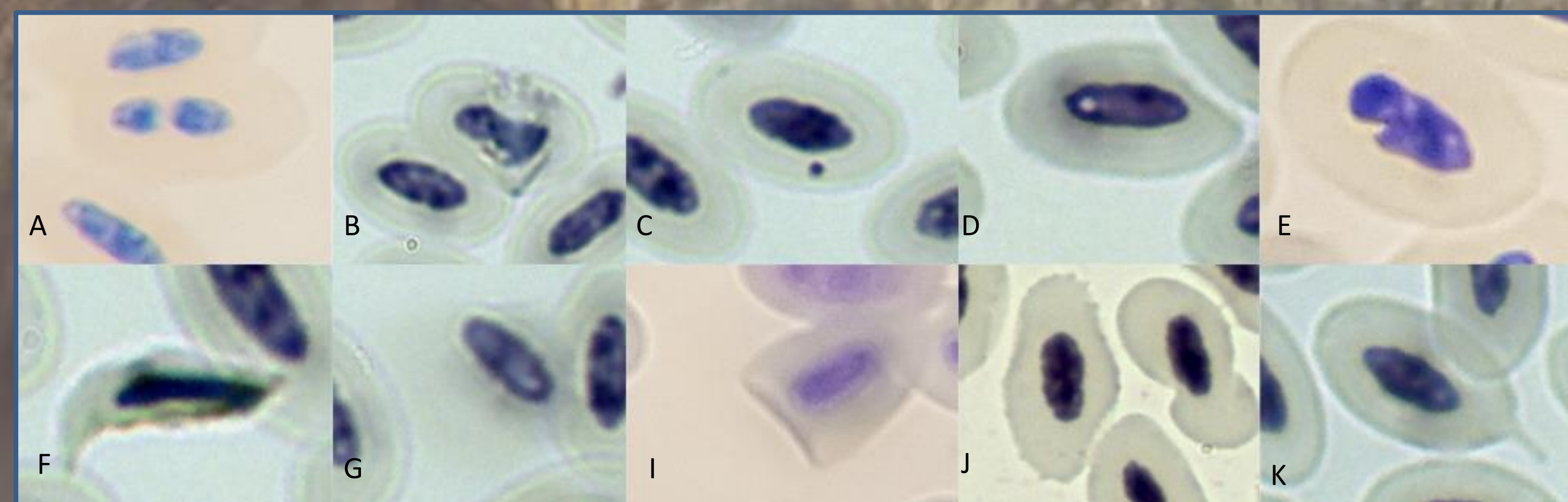


Fig. 3. Main nuclear and cytoplasmic abnormalities: (A) Binucleated cell-BN (B) Lobed Nucleus-LN (C) Micronucleus-MN (D) Vacuolated Nucleus-VN (E) Notched Nucleus-NN (F) Acanthocyte-AcanCyt (G) Cell without membrane-NoMembCyt (I) Notched Cytoplasm-NotcCyt (J) Echinocyte-EchCyt and eight-shaped cell-EightCyt (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.

5. References & Acknowledgements

- Baesae et al. 2015. Ecotoxicology and Environmental Safety, 115:223-228.
Birdlife International. 2015. Species factsheet: *Streptopelia turtur*.
Ekman & Hake 1990. Behavioural Ecology 1: 62–67.
Hussain et al. 2012. Pesticide Biochemistry and Physiology, 103: 38-42.
IUCN 2017. The IUCN Red List of Threatened Species. Version 2017-3.
Quero, et al. 2016. Environmental Science and Pollution Research, 23: 25224-25231.
Svensson et al. 2015. The Birds of Greece, Cyprus and Europe. Hellenic Ornithological Society, Athens.

The study was carried out in the framework of the project LIFE EIClima (LIFE13 NAT/GR/000909), which is implemented by the University of Patras, in collaboration with the Hellenic Ornithological Society (BirdLife Greece) and the NCC Ltd with the financial support of the European Union LIFE Instrument and the Hellenic Green Fund.