Comparative haematological investigations in two bird species from the order *Psittaciformes*: *Psittacula krameri* and *Melopsittacus undulatus*

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Abstract
The knowledge of the hematological parameters it's one of the most important indicators of the health evaluation. The aim of these studies was to investigate the main hematological values, including the morphology and the morphometry of the blood cells, in a comparative way of two bird species belonging to the order Psittaciformes: Psittacula krameri and Melopsittacus undulatus. Five adult birds from each species were analyzed, the blood samples being taken from the jugular vein on EDTA containers. In Psittacula krameri the values obtained were: RBC = 2.98x10^6/µl (±0.16); Hb = 14.46 g/dl (±0.76); HCT = 43.4% (±3.21); MCV = 145.62 fl (±2.95); MCH = 48.58 pg (±1.23); MCHC = 33.36 g/dl (±1.33); thrombocytes = 116x10^3/µl (±25,84); leucocytes: WBC = 14.46x10^3/µl (±1.21), heterophils = 52.74% (±4.04), eosinophils = 0.48% (±0.44), basophils = 1.28% (±1.7), monocytes = 6.18% (±1.47) and lymphocytes = 39.32% (±3.79). In Melopsittacus undulatus the following values were obtained: RBC = 4.04x10^6/µl (±0.15); Hb = 15.26 g/dl (±0.86); HCT = 54.2% (±5.12); MCV = 134.08 fl (±7.98); MCH = 37.81 pg (±1.14); MCHC = 28.26 g/dl (±1.39); thrombocytes = 86.2x10^3/µl (±6.98); leucocytes: WBC = 21.42x10^3/µl (±1.85), heterophils = 39.4% (±3.93), eosinophils = 0.28% (±0.33), basophils = 4.22% (±1.36), monocytes = 8.82% (±1.32l) and lymphocytes = 47.28% (±3.89). H/L ratio was higher in parrots. Average morphometric values of the blood cells in showed significant differences for monocytes which are larger in Psittacula krameri (p≤0.001), less differences for the heterophils, lymphocytes and platelets which are also larger in Psittacula krameri (p≤0.01) and very reduced differences for erythrocytes (except the nucleus width), eosinophils and basophils (p≤0.01). The researches concluded that there are differences regarding the most of the parameters, even if they are related species, including the morphometric values.
Key words: *comparative hematology, Psittaciformes, blood cells, morphology, morphometry*

Introduction

Clinical hematology is the examination of the cellular and fluid components of the blood and a study of the tissue that form, store and circulate the blood cells (5). The interpretation of avian blood cell provides many challenges; a veterinarian uses the result of hematology test to help determine the health of avian. These results are used in conjunction with history, physical examination and other finding (6). Clinical signs in birds are very non-specific, and physical exams provide limited information. Blood exams are an indispensable tool in bird medicine (3, 8).

Comprehensive health assessments on wild bird populations, as hematology, can be used to assess the effects of many health related problems, such as contaminant intoxication, malnutrition, and exposure to infection (10). With hematological exams, it is possible to qualitatively and quantitatively measure changes in the red and white blood cell fractions as well as changes in cell morphology that can assist in the diagnosis of several diseases and pathologies (2).

Although there are similarities of the hematological profile between the species within a class of vertebrates, however, some parameters may vary widely from one species to another, thus requiring a detailed knowledge of the values of each species. In birds, although the hematological profile is well-known in the main species of economic interest, at present, more attention is paid to these examinations to other species, such as exotic or cage birds, grown as "pets", in this way being

necessary to know the normal cytological and biochemical parameters. From the order *Pssitaciformes* there are many species grown as cage birds, but the species *Psittacula krameri* and *Melopsittacus undulatus* are two of the most common, which is why we found it's useful to start some hematological investigations of these species.

Materials and methods

The research was carried out on 10 birds, 5 parrots *(Psittacula krameri)* – 3 males and 2 females (6-8 years old) and 5 budgerigars (*Melopsittacus undulatus)* – 3 males and 2 females (2-4 years old), the blood samples being taken from the right jugular vein, the preferred vein in the case of the small birds (1). After the local antisepsis, the blood was collected in EDTA containers which are recommended (11). The samples were labeled and stored in the refrigerator until processing.

The analyzed parameters were: RBC, WBC and differential WBC (no. of heterophils, basophils, eosinophils, lymphocytes and monocytes), H/L ratio, the number of thrombocytes, HCT, Hb, MCV, MCH, MCHC.

Total Red Blood Cells (RBC) counts was determined by hemocytometer method using the Neubauer Chamber and Natt-Herrick solution; hematocrit (HCT) and hemoglobin (Hb) values were measured by microhematocrit in capillary tubes and colorimetric Sahli methods respectively (9). The calculated erythrocyte indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the results determined from the red fraction (7) and the values were expressed in femtoliters (fl), picograms (pg) and grams per deciliter (g/dL), respectively.

Total White Blood Cell (WBC) counts were performed by a manual method using Natt-Herrick solution (1) and the results were expressed in units per microliter (μL). Thin smears on glass slides were made immediately after the blood collection to avoid any interference on cell structure. Differential WBC counts, using an average of 100-150 cells, were made from blood films stained with Fast Panoptic Diff Quick method and examined under an optical microscope with a 100X objective (immersion oil). Images were captured using a digital camera (Motic) with the aid of a computer and the Software ImagesPlus. Erythrocytes, leukocytes and thrombocytes were identified and morphologically characterized, and their length and width were measured (increase 1000x).

For statistical analysis we used Student's t-test to qualitatively and quantitatively analyze the components of the blood.

Results and discussions

Based on the morphological characters, the blood cell series were identified: erythrocytes, leukocytes (heterophils, basophils, eosinophils, lymphocytes, monocytes) and thrombocytes.

The average comparative values of the parameters obtained from the hematological determinations are given in the table 1.

Table 1. The average values of the main blood parameters in *Psittacula krameri* and *Melopsittacus undulatus*

Evaluated parameters	**Parrots** *(Psittacula krameri)*		**Budgerigars** *(Melopsittacus undulatus)*	
	N[l]	**Values obtained**	**N[l]**	**Values obtained**
RBC (x10^6/μl)	5	2.98±0.16	5	4.04±0.15***
Hb (g/dl)	5	14.46±0.76	5	15.26±0.86

HCT (%)		5	43.4±3.21	5	54.2±5.12**
MCV (fl)		5	145.62±2.95	5	134.08±7.98*
MCH (pg)		5	48.58±1.23	5	37.81±1.14***
MCHC (g/dl)		5	33.36±1.33	5	28.26±1.39**
WBC ($x10^3/\mu l$)		5	14.46±1.21	5	21.42±1.85***
Diff. WBC % ($x10^3/\mu l$)	**Heterophils**	5	52.74±4.04 (7.6±0.57)	5	39.4±3.93** (8.48±1.44)
	Eosinophils	5	0.48±0.44 (0.07±0.06)	5	0.28±0.33 (0.06±0.07)
	Basophils	5	1.28±1.7 (0.18±0.15)	5	4.22±1.36** (0.9±0.27**)
	Monocytes	5	6.18±1.47 (0.9±0.28)	5	8.82±1.32* (1.9±0.41**)
	Lymphocytes	5	39.32±3.79 (5.69±0.79)	5	47.28±3.89** (10.06±0.15***)
	H/L Ratio	5	1.36±0.24	5	0.85±0.14**
Thrombocytes ($x10^3/\mu l$)		5	116±25.84	5	86.2±6.98*

[1] Number of samples; *** p≤0.001; ** p≤0.01; * p≤0.05

Regarding the red series, the higher values of RBC (p≤0.001) and HCT (p≤0.01) can be found in budgerigars, while the Hb values and the erythrocyte constants were higher in parrots (MCV, MCH, MCHC). The values of the WBC were higher in the budgerigars (p≤0.001). Of these, lymphocytes predominated in the budgerigars (p≤0.01), while in parrots, the most numerous were heterophils (p≤0.01). In this way, the H/L ratio was significantly higher (p≤0.01) in parrots. The basophils of the budgerigars were more numerous (p≤0.01), the values of the eosinophils being similar. The platelets were slightly more numerous in budgerigars (p≤0.05).

In order to determine the morphometric values of the cells, 20 cells were measured in the case of red blood cells, 10 in the case of platelets, heterophils, lymphocytes and monocytes, 3 in the case of basophils and 1 for eosinophils. The average values obtained and the standard deviations are shown in the table 2.

Table 2. Morphometrical mean values (μm) of the blood cells in *Psittacula krameri* and *Melopsittacus undulatus*

Cells		**N/S**[1]	**Parrots** *(Psittacula krameri)*	**Budgerigars** *(Melopsittacus undulatus)*
Erythrocytes	Cell	20	9.72/4.87±0.45/0.25	10.2*/4.4*±0.47/0.23
	Nucleus	20	4.2/2.3±0.23/0.1	4.3*/1.9**±0.2/0.12
Heterophils		10	9.99±0.48	9.12**±0.44
Basophils		3	8.17±0.39	8.2±0.4
Eosinophils		1	9.5±0.38	8.9*±0.32
Monocytes		10	10.16±0.47	6.92***±0.27
Lymphocytes		10	6.96±0.28	5.89**±0.25
Thrombocytes		10	5.43±0.25	3.8**±0.2

[1]Number of cells/smear; *** p≤0.001; ** p≤0.01; * p≤0.05

It can be observed that if in the case of erythrocytes, basophils and eosinophils the differences are reduced or insignificant ($p \leq 0.05$; $p \geq 0.05$), except the nucleus width of the erythrocytes ($p \leq 0.01$), slightly higher values were observed regarding the heterophils, lymphocytes and platelets in parrots compared to the budgerigars ($p \leq 0.01$), significant differences being recorded in the case of the monocytes ($p \leq 0.001$), these cells having larger dimensions in the parrots.

The normal morphology of the blood cells corresponds generally with that described in the literature (11): the mature erythrocytes are flattened and elliptical cells, with an elliptical or oval nucleus, centrally positioned and smooth nuclear margins (11), dark-purple stained, while the cytoplasm appears orange-pink with a uniform texture; it can be noted that the nucleus is more elongated in budgerigars (Fig. 1, 2, 9, 10). As the data on literature says (4), variations from the typical erythrocyte are occasionally seen; the shape can vary (poikilocytosis) from irregular to round or elongated.

The heterophils appeared as round cells with bilobular or trilobular nucleus, located off-center or on the periphery, purple colored. The cytoplasm of normal mature heterophils appears colorless and contains granules that stain an eosinophilic color. In accordance with the description of Weiss and Wardrop (2010), the cytoplasmic granules of psittacine heterophils appear elongated (rod or spicular shaped) (Fig. 3, 11) and frequently contain a central refractile body. Vacuoles were seen in the cytoplasm of some heterophils.

The basophils appears as round cells, the cytoplasm containing basophilic, large or round granules that frequently obscure the nucleus (Fig. 4, 12).

Due to the reduced number of the eosinophils observed, we correlated their morphology with that of other descriptions (11) as being generally similar in size to the heterophils in the same blood film. The nucleus of the eosinophil is lobed and usually stains darker than a heterophil nucleus and the cytoplasm stains clear blue in contrast to the colorless cytoplasm of normal mature heterophils. The cytoplasmic granules of these cells are strongly eosinophilic and are typically round in shape and tend to stain more intensely compared to granules of heterophils.

Lymphocytes found had a round shape and different sizes. The nucleus was non-lobed, with rounded shape, occasionally slightly indented, located slightly to one side or at the center of the cell, purple in color with areas of condensed chromatin. Homogeneous cytoplasm, stained in blue, ranged in number of low to moderate, being scarce especially in small lymphocytes and increasing in volume in larger lymphocytes. Small mature lymphocytes are the most frequent form of lymphocyte in blood films; medium and large lymphocytes with more abundant and lighter staining cytoplasm may occur as well (Fig. 5, 6, 13). Occasionally, lymphocytes in the blood film of normal psittacine birds may contain distinct azurophilic granules or irregular cytoplasmic projections.

The observed monocytes were large cells, especially in parrots, with a round or irregular shape. These had nuclei without divisions or were bilobular, and were pleomorphic, localized off center or in the center and had loose chromatin and small areas of condensed chromatin. The cytoplasm was blue-grey and had small discrete vacuoles that were also found in the nucleus (Fig. 7, 14).

Thrombocytes appeared as small, mononucleated cells that were round or oval. The shape of the nucleus corresponded to the shape of the cell. It was located in the center or on the periphery and was dark purple in color with clumped chromatin (Fig. 8, 15). In some platelets the nucleus presented a polar granule (Fig. 16). The cytoplasm was small and clear. We also occasionally found clusters of thrombocytes.

The studies were conducted to evaluate the blood components in these two Psittacines species with the goal of determining the hematologic parameters in captive birds. We can affirm it's a limited study due to the reduced number of birds and the difficulty of the blood sample collection.

The values obtained are similar to the data in the literature regarding the hematological constants of the birds, ranging between the known minimum and maximum values (11) except the values of HCT in budgerigars which were higher than the maximum of the species studied.

The blood cells of *Psittacula krameri* and *Melopsittacus undulatus* had similar morphological and morphometric characteristics described for the birds, and particularly for *Psittacines* (11, 4), the interspecific differences recorded being more significant for the morphometric aspects.

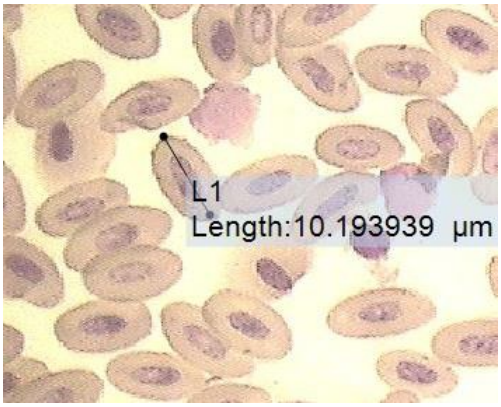

Fig. 1. Morphological and morphometric aspects of the parrot *(Psittacula krameri)* erythrocytes (x1000) – cell length

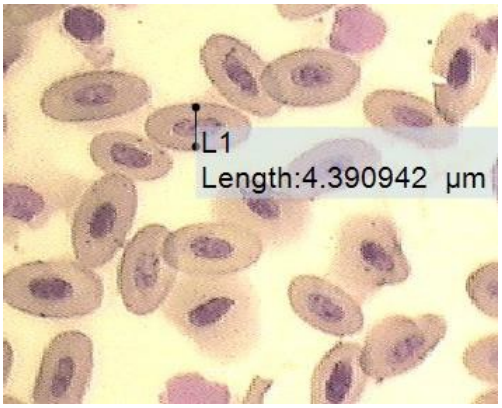

Fig. 2. Morphological and morphometric aspects of the parrot *(Psittacula krameri)* erythrocytes (x1000) – cell width

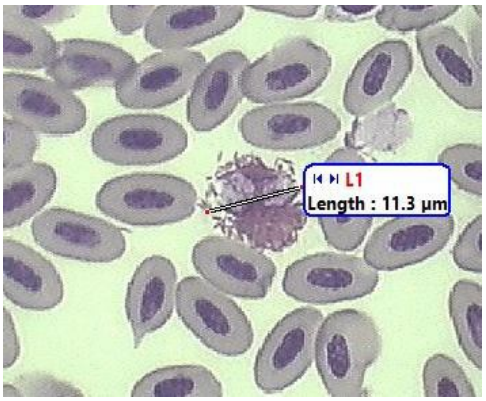

Fig. 3. Morphological and morphometric aspects of the parrot *(Psittacula krameri)* heterophil (x1000)

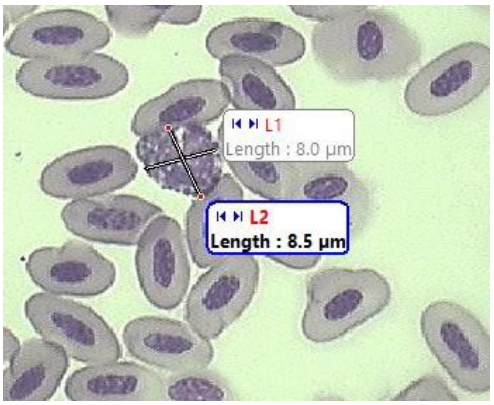

Fig. 4. Morphological and morphometric aspects of the parrot *(Psittacula krameri)* basophil (x1000)

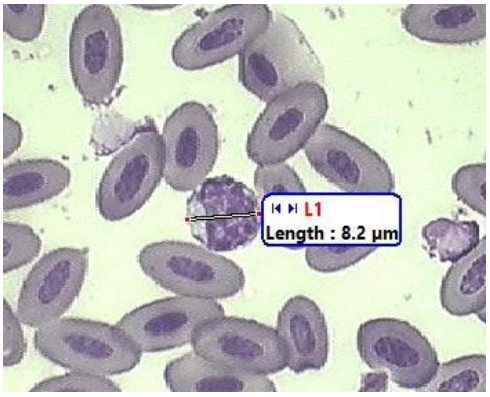

Fig. 5. Morphological and morphometric aspects of the parrot *(Psittacula krameri)* lymphocyte (x1000)

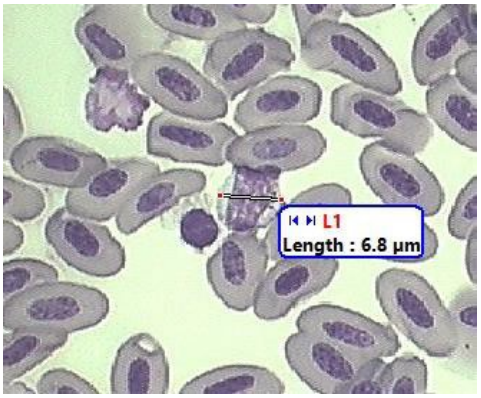

Fig. 6. Morphological and morphometric aspects of the parrot *(Psittacula krameri)* lymphocyte (x1000)

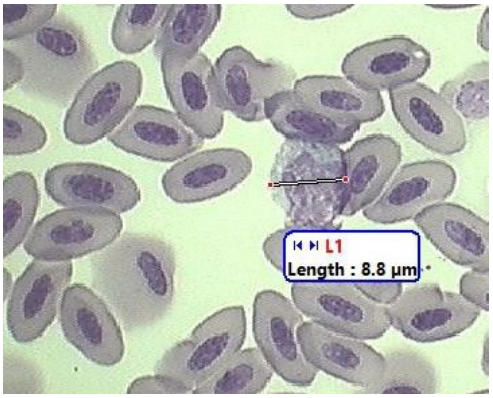

Fig. 7. Morphological and morphometric aspects of the parrot *(Psittacula krameri)* monocyte (x1000)

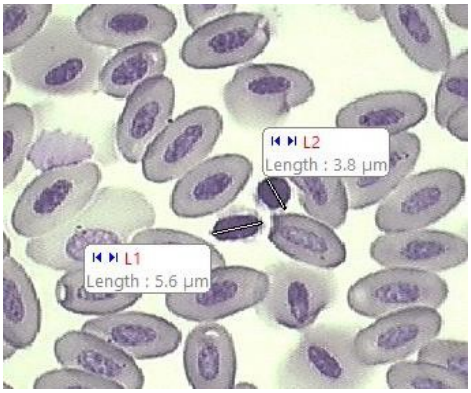

Fig. 8. Morphological and morphometric aspects of the parrot *(Psittacula krameri)* thrombocyte (x1000)

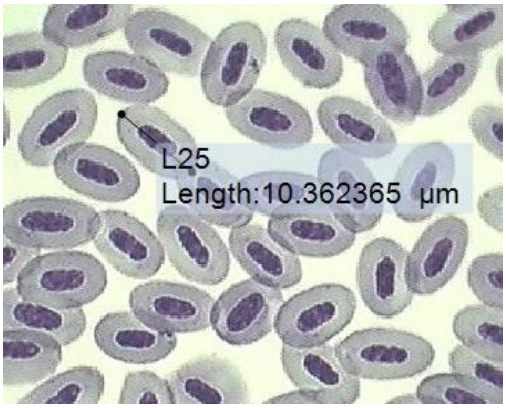

Fig. 9. Morphological and morphometric aspects of the budgerigar *(Melopsittacus undulatus)* erythrocytes (x1000) – cell length

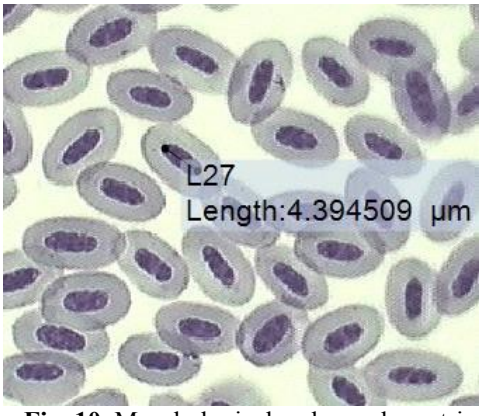

Fig. 10. Morphological and morphometric aspects of the budgerigar *(Melopsittacus undulatus)* erythrocytes (x1000) – nucleus length

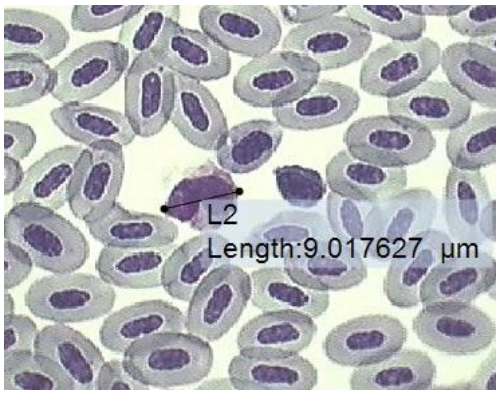

Fig. 11. Morphological and morphometric aspects of the budgerigar *(Melopsittacus undulatus)*heterophil (x1000)

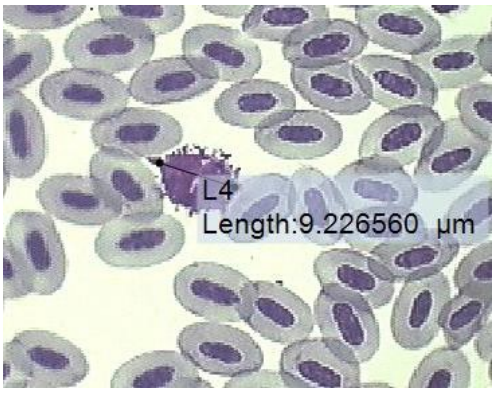

Fig. 12. Morphological and morphometric aspects of the budgerigar *(Melopsittacus undulatus)*basophil (x1000)

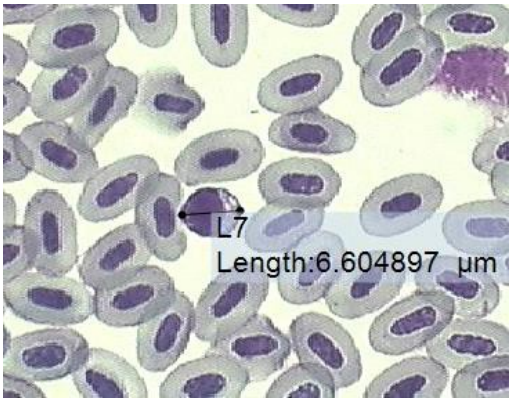

Fig. 13. Morphological and morphometric aspects of the budgerigar *(Melopsittacus undulatus)* lymphocyte (x1000)

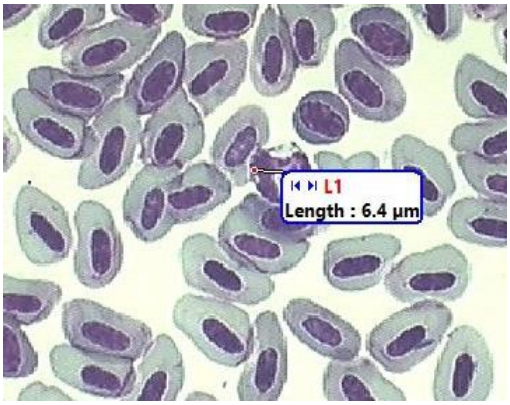

Fig. 14. Morphological and morphometric aspects of the budgerigar *(Melopsittacus undulatus)* monocyte (x1000)

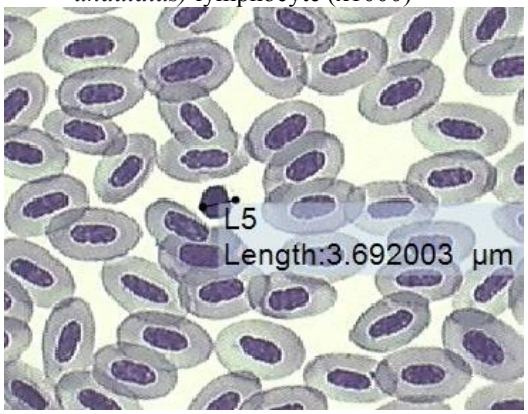

Fig. 15. Morphological and morphometric aspects of the budgerigar *(Melopsittacus undulatus)* thrombocyte (x1000)

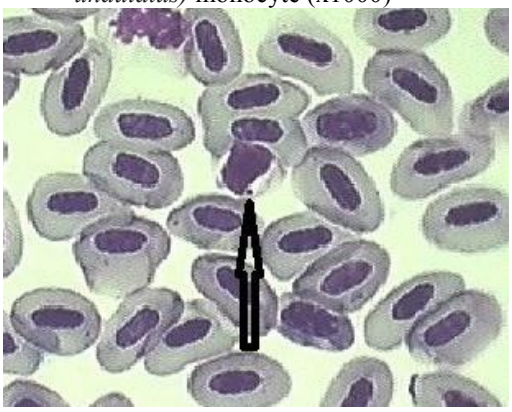

Fig. 16. Morphological and morphometric aspects of the budgerigar *(Melopsittacus undulatus)* thrombocyte (x1000) – polar granule (the arrow)

Conclusions

- The research concluded that there are differences regarding the most of the parameters, even if we talk about related species, including the morphometric values.
- The most significant differences regarding the hematological parameters were observed for HCT, RBC, MCH, total WBC and the number of lymphocytes.
- The morphological characteristics of the blood cells are similar for the species, but generally the blood cells are larger in parrots.

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