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FULL PAPER

# Quantitative and qualitative morphologic, cytochemical and ultrastructural characteristics of blood cells in the Crested Serpent eagle and Shikra

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## Abstract

**The Crested Serpent eagle (*Spilornis cheela*) is a bird of prey found in the tropical rain forest in Thailand. The Shikra (*Accipiter badius*) is a sparrow hawk and common resident in Thailand. Blood samples from 9 Crested Serpent eagles and 12 Shikras were obtained from September 2010 to November 2014. They were clinically healthy and negative for blood parasites detectable by light microscopy and molecular techniques (partial cytochrome *b* gene for avian malaria and partial 18S rRNA gene for trypanosome). Cytochemical staining (Sudan black B, peroxidase, α-naphthyl acetate esterase, and β-glucuronidase) and transmission electron microscopy were performed. Hematological results were reported as the mean ± standard deviation and median. Heterophils were the most prevalent leukocytes in the Crested Serpent eagle, but in the Shikra, lymphocytes were the most prevalent leukocytes. In the Shikra, some vacuoles were observed in the cytoplasm of the eosinophils. All blood cells in both types of raptors stained positively for β-glucuronidase but negatively for peroxidase. The ultrastructure of heterophils showed more clearly differentiate long rod granules in Crested Serpent eagle and spindle-shaped granules in Shikra. The ultrastructure of the eosinophils in the Crested Serpent eagle revealed varied electron-dense, round-shaped granules with round, different electron-dense areas in the centers of some granules, which differed from the structure reported for other raptors. These quantitative results may be useful for clinical evaluations of Crested Serpent eagles and Shikras that are undergoing rehabilitation for release.**

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## Introduction

The Crested Serpent eagle (*Spilornis cheela*) is a medium-sized bird of prey found in the tropical rain forest and forest fringe in Thailand. This species has a wide range of habitats across its range, which spans the Indian subcontinent and southern Asia[9]. The Shikra (*Accipiter badius*) is a small-sized hawk, 25–35 cm in length, which is found widely distributed in Africa and Asia. In Africa, it is also called the little banded goshawk. Both raptors are in the family *Accipitridae*. In Thailand, the Shikra is a breeding resident and lives in forest edges, woodlands, parks and gardens[9]. Although the Crested Serpent eagle and the Shikra are classified as being of the Least Concern[2,3], in Thailand, they have been protected under the Wildlife Protection and Preservation Law since 1997. The Kasetsart University Raptor Rehabilitation Unit (KURRU) has admitted various species of raptors for health rehabilitation since 2007. Shikras are the third most common species to be submitted to the KURRU, while Crested Serpent eagles are admitted more rarely. Hematologic analyses and interpretation of abnormal values may offer valuable insights into the nutrition and possible pathologies of individual raptors[5,7]. There are many factors influencing the standard values in each species, so a basic hematological study should be performed in endemic raptors for more accurate reference values for use in evaluations of avian health status. To be considered for release, raptors undergoing rehabilitation must have recovered from their initial injury and be clinically healthy. For that purpose, a good understanding of reference hematologic values is important in determining release criteria for raptors in a rehabilitation unit[4]. The literature regarding hematological data from raptorial birds is limited[5].

Cytochemical staining requires special stains for observing the macromolecular and enzymatic contents such as lipids, carbohydrates and enzymes[11]. A periodic acid Schiff (PAS) reaction reveals carbohydrate. Sudan black B (SBB) is used to observe variety in lipids, including phospholipids, sterols and neutral fat. The myeloperoxidase (PO) is present in the granules of the myeloid cells. Alpha-naphthyl acetate esterase (ANAE) is a nonspecific esterase enzyme found in large amounts in monocytes, but minor concentrations may found in myeloid or lymphoid cells[11]. β-glucuronidase (BG) is a hydrolytic enzyme found in lymphocytes, monocytes and granulocytes[11]. Although there have been some reports on the morphology, cytochemistry and ultrastructure of blood cells in birds from Thailand[13,14,15,19] or in hemograms from other hawks[4,10], variations in cell characteristics exist among species within the class Aves[5,7,8]. However, data regarding the morphology, cytochemistry and ultrastructure of blood cells in these two species of raptor have not been reported. The main purpose of the present study was to obtain information on the hematology, morphology, cytochemistry and ultrastructure of blood cells from the Crested Serpent eagle and the Shikras submitted to the KURRU.

## Materials and Methods

*Sample collection:* From September 2010 to November 2014, 9 Crested Serpent eagles (4 males and 5 females) and 12 Shikras (4 males and 8 females) were submitted to the KURRU for rehabilitation. They recovered from illness, became clinically healthy and were ready to be released. They were negative for blood parasites detectable by light microscopy and molecular techniques (partial cytochrome *b* gene for avian malaria[17] and partial 18S rRNA gene for trypanosome[18]). Blood samples were collected from

the wing vein or the jugular vein and put into a 3-mL tube containing ethylenediaminetetraacetic acid (EDTA). Complete blood cell counts were performed within 2 hours of collection.

*Hematological study:* Blood smears were immediately prepared, air-dried and stained with Wright's stain; an in-house preparation using Wright eosin methylene blue and Giemsa azura eosin methylene blue (Merck KGaA, Darmstadt, Germany), to determine the differential white blood cell (WBC) count, grading of blood parasite infection and morphologic evaluation of all blood cells.

All blood samples were confirmed as negative for both avian malaria (*Plasmodium* sp. and *Haemoproteus* sp.) and *Trypanosoma* sp. by molecular study, as previous described[17,18]. Leukocyte differential counts were based on average count of 200 cells by 2 veterinary hematologists (C. Salakij and P. Suwannasaeng). Morphometric measurements, using an image analysis program (DP73 digital camera and CellSens standard®, Olympus, Tokyo, Japan), were performed to obtain the width, length, area, perimeter and diameter of 150–300 randomly selected blood cells from 6 birds in each species.

Packed cell volumes (PCV) were determined using a microhematocrit centrifuge at 10,000xg for 5 minutes. The total red blood cell (RBC) count and the WBC count were determined manually with the counting chamber after the blood was diluted 200 times with Natt and Herrick's solution, as described previously[13]. The hemoglobin (Hb) concentration was determined by the cyanmethemoglobin method in which free RBC nuclei were removed by centrifugation before reading the absorbance. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the PCV, Hb concentration, and RBC count. Reticulocyte counts (aggregate and punctate) were determined by staining with new methylene blue (NMB) using a wet preparation. Total protein and fibrinogen concentrations were determined using a refractometer (Atago, Tokyo, Japan) and the heat precipitation method for fibrinogen (56°C for 3 minutes).

*Cytochemical staining:* The characteristics of blood cells were evaluated using 5 air-dried blood smears from 4 birds of each species of raptors. Cells were stained with PAS, SBB, PO, ANAE and BG as previously described[13]. Normal dog blood smears were used as positive controls to ensure correct staining procedures.

*Ultrastructural study:* EDTA samples from 3 birds of each species of raptors were processed for transmission electron microscopy (TEM) as described previously[13]. Briefly, buffy coats from microcapillary tubes were fixed immediately after microcentrifugation in 2.5% glutaraldehyde in 0.1 M phosphate buffer at 4°C for 24 hours, post-fixed in 1% osmium tetroxide, and embedded in Spurr's epoxy resin. The fixation of buffy coats was performed within 30 minutes after blood collection. Ultrathin sections stained with uranyl acetate and lead citrate were examined with a JEM 1230 transmission electron microscope (JEOL, Tokyo, Japan). The light and ultrastructural characteristics of individual blood cells were evaluated.

*Statistical analysis:* Data were presented as the mean ± standard deviation. The Independent-Samples Mann-Whitney U test was used to identify the significant differences in hematological values of both species of raptor. Statistical comparisons based on morphometry of the cells were made using independent sample *t*-test (SPSS 17.0, SPSS Inc, Chicago, USA). A *p*-value less than 0.05 was considered significant.

## Results

The hematological results, cytochemical staining patterns of blood cells and morphometric

analysis are summarized in Tables 1, 2 and 3, respectively. Due to the low numbers of both raptors, data from both sexes were pooled together. The RBC count in the Crested Serpent eagle was lower than that of the Shikra. The some RBC indices (MCV and MCH) in the Crested Serpent eagle were higher (Table 1). However, while MCV and MCH are significantly different, MCHC values are equivalent between Crested Serpent eagle and Shikra The absolute number of lymphocytes and the percentage of aggregate reticulocytes in the Crested Serpent eagle were significantly less than those of the Shikra, whereas the plasma protein concentration was higher (Table 1).

*Morphological, cytochemical and ultrastructural characteristics of individual blood cells*

*Erythrocytes* (RBCs) were oval shaped with an elliptical outline. The nuclei were slightly heterogeneous in size (Figs. 1A and 1D). RBCs were nearly negative for all cytochemical stains except for a small dot of BG staining (Figs. 1B and 1E, Table 2). RBCs in the Crested Serpent eagle were significantly longer and had more area but less width than the RBCs of the Shikra (Table 3). Ultrastructurally, RBCs contained oval nuclei with clumped chromatin, homogeneous

**Table 1. Comparative hematology (Mean ± SD) of 9 Crested Serpent eagles and 13 Shikras**

| Parameter[a] | Units | Crested Serpent eagle (Median) | Shikra (Median) |
|---|---|---|---|
| Packed cell volume | L/L | 0.39 ± 0.06 (0.41) | 0.38 ± 0.03 (37) |
| Hemoglobin | g/L | 132.0 ± 20.1 (138.0) | 129.3 ± 11.8 (131) |
| RBC | $x10^{12}$/L | 1.87 ± 0.47 (1.91) | 2.86 ± 0.35 (2.93)* |
| MCV | fL | 215 ± 38 (202) | 133 ± 14 (131)* |
| MCH | pg | 73.3 ± 13.9 (70.4) | 45.4 ± 3.6 (44.7)* |
| MCHC | g/dL | 34.1 ± 1.0 (34.4) | 34.2 ± 1.6 (34.5) |
| WBC | $x10^9$/L | 13.95 ± 1.70 (13.60) | 17.34 ± 4.17 (15.84) |
| Absolute differential count | | | |
| Heterophils | $x10^9$/L | 6.71 ± 1.49 (7.41) | 6.16 ± 2.74 (5.65) |
| Lymphocytes | $x10^9$/L | 4.43 ± 1.86 (3.49) | 7.27 ± 3.52 (7.88)* |
| Eosinophils | $x10^9$/L | 1.47 ± 0.77 (1.62) | 2.48 ± 1.18 (1.92) |
| Basophils | $x10^9$/L | 0.33 ± 0.20 (0.25) | 0.55 ± 0.32 (0.47) |
| Monocytes | $x10^9$/L | 0.94 ± 0.49 (0.74) | 1.19 ± 0.62 (1.19) |
| Relative differential count | | | |
| Heterophils | % | 48.7 ± 12.0 (49.0) | 36.0 ± 15.3 (33.5) |
| Lymphocytes | % | 31.1 ± 10.3 (30.0) | 41.2 ± 16.3 (34.0) |
| Eosinophils | % | 10.5 ± 5.6 (10.0) | 14.2 ± 5.7 (13.5) |
| Basophils | % | 2.3 ± 1.1 (2.0) | 3.2 ± 1.8 (3.0) |
| Monocytes | % | 6.9 ± 4.3 (5.5) | 7.3 ± 4.1 (6.5) |
| Heterophil:lymphocyte ratio | | 1.57 | 0.87 |
| Plasma protein | g/L | 55.8 ± 7.0 (56.0) | 44.8 ± 4.8 (46.0)* |
| Fibrinogen | g/L | 2.6 ± 1.1 (2.0) | 1.9 ± 1.0 (2.0) |
| Thrombocytes | /100 WBC | 176 ± 83 (199) | 196 ± 44 (190) |
| Aggregate reticulocytes | % | 13.7 ± 7.4 (15.7) | 22.6 ± 7.5 (22.4)* |
| Punctate reticulocytes | % | 28.6 ± 14.4 (31.4) | 40.4 ± 20.4 (39.2) |

[a]*RBC* red blood cells, *WBC* white blood cells, *MCV* mean cell volume, *MCH* mean cell haemoglobin, *MCHC* mean cell haemoglobin concentration, *fl* femtoliters, *pg* picograms, *SD* standard deviation.
*Significantly different from the values of Crested Serpent eagles at $P < 0.05$.

electron-dense hemoglobin and tiny vacuoles (Figs. 1C and 1F).

*Leukocytes* (WBCs) were classified as granulocytes (heterophils, eosinophils and basophils) and agranulocytes (lymphocyte and monocyte).

Heterophils were the most prevalent leukocytes in the Crested Serpent eagle and the

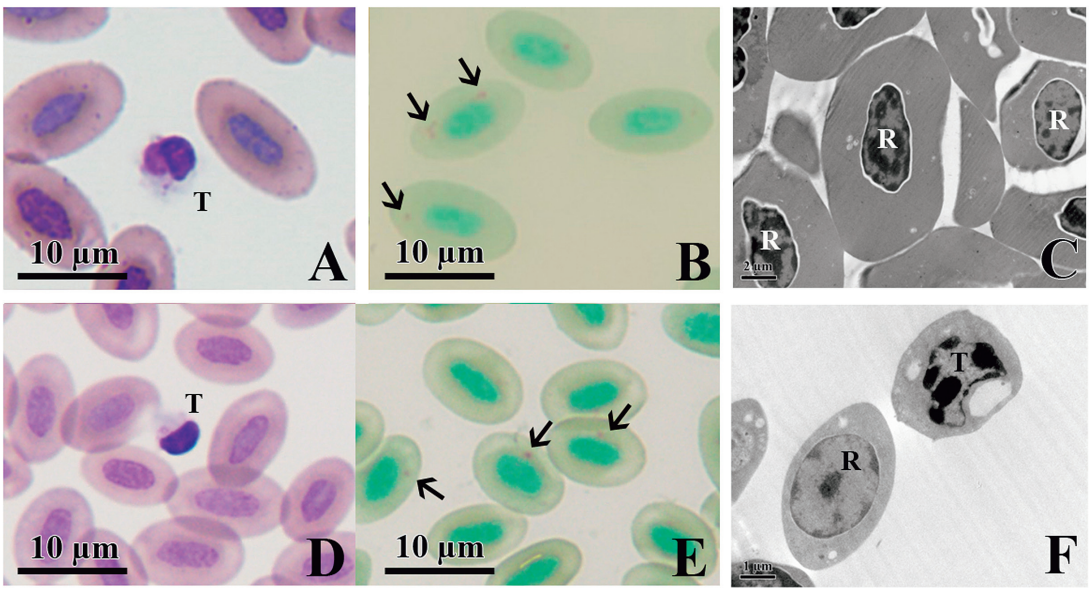


**Fig. 1. Light and electron micrographs of blood cells in the Crested Serpent eagle (A–C) and Shikra (D–F). (A and D)** Red blood cells (RBCs) and thrombocyte (T), Wright's stain. **(B and E)** β-glucuronidase dot-positive (arrows) in RBCs. **(C and F)** RBCs (R) and a thrombocyte (T). TEM, uranyl acetate and lead citrate stains.

**Table 2. Cytochemical staining patterns[a] of blood cells in 4 Crested Serpent eagles and 4 Shikras**

| Cell types | SBB | PO | PAS | ANAE | BG |
|---|---|---|---|---|---|
| **Crested Serpent eagle** | | | | | |
| Heterophils | ++ | − | − | − | + (inter-granular) |
| Eosinophils | ++ | − | − | ++ | ++ |
| Basophils | − | − | − | − | ++ (inter-granular) |
| Lymphocytes | − | − | − | −, + (fine granular) | −, + (dot) |
| Monocytes | − | − | − | ++ | ++ |
| Thrombocytes | − | − | − | + | + |
| Erythrocytes | − | − | − | − | Dot + |
| **Shikra** | | | | | |
| Heterophils | −* | − | +* | − | + |
| Eosinophils | −* | − | − | + | ++ |
| Basophils | − | − | − | − | + |
| Lymphocytes | − | − | − | − / fine granular | − / fine granular |
| Monocytes | − | − | ±* | −* | +++ |
| Thrombocytes | − | − | +* | −* | + |
| Erythrocytes | − | − | − | − | Dot + |

[a]SBB, Sudan black B; PO, peroxidase; ANAE, α-naphthyl acetate esterase; BG, beta-glucuronidase; −, negative; ±, positive or negative; +, faintly positive; ++ strongly positive.
*Different cytochemical staining patterns from those of Crested Serpent eagles.

**Table 3. Comparative morphometry (Mean ± SD) of blood cells between 6 Crested Serpent eagles and 6 Shikras**

| Parameter | Number | **Crested Serpent Eagle** | **Shikra** |
|---|---|---|---|
| Red blood cell width (μm) | 300 | 7.1 ± 0.7 | 7.3 ± 0.4* |
| Red blood cell length (μm) | 300 | 12.7 ± 0.9 | 11.9 ± 0.8* |
| Area of Red blood cell ($\mu m^2$) | 300 | 72.7 ± 11.1 | 68.5 ± 6.5* |
| Heterophils (μm) | 150 | 9.3 ± 1.7 | 10.4 ± 1.0* |
| Eosinophils (μm) | 150 | 8.8 ± 1.6 | 13.3 ± 1.2* |
| Basophils (μm) | 150 | 7.4 ± 1.2 | 7.2 ± 0.9 |
| Lymphocytes (μm) | 150 | 6.7 ± 1.0 | 6.8 ± 1.6 |
| Monocytes (μm) | 150 | 11.0 ± 2.1 | 12.3 ± 1.4* |

*Significantly different from the values of Crested Serpent eagles at $P < 0.05$.

second most frequent leukocytes in the Shikra (Table 1). Heterophils in the Crested Serpent eagle were the largest granulocytes (Table 3). Heterophils contained lobed nuclei and numerous long rod (Fig. 2A, Crested Serpent eagle) to spindle-shaped (Fig. 2D, Shikra) eosinophilic granules. Heterophils in the Crested Serpent eagle were positive for SBB (Fig. 2B) and BG, whereas in the Shikra, they were positive for PAS and BG (Table 2, Fig. 2E). Heterophils in the Shikra were significantly larger than those of the Crested Serpent eagle (Table 3). Ultrastructurally, they contained numerous membrane-bound granules, mitochondria, rough endoplasmic reticulum and ribosomes (Figs. 2C and 2F). They were more clearly differentiated long rod granules in Crested Serpent eagle (Fig. 2C) and spindle-shaped granules in Shikra (Fig. 2F).

Lymphocytes were the most prevalent leukocytes in the Shikra and the second most frequent leukocytes in the Crested Serpent eagle (Table 1). They were the smallest leukocytes (Table 3), were well-differentiated, and each contained a round eccentric nucleus (Figs. 3A and 3D). Lymphocytes were negative with SBB, PO and PAS but showed 2 patterns of cytochemical staining with ANAE and BG: negative and finely granular (Figs. 3B and 3E, Table 2). Ultrastructurally, lymphocytes contained round nuclei with dense heterochromatin, scant cytoplasm and a few mitochondria. A few lymphocytes (less than 5% WBC) contained azurophilic granules (Figs. 3A, 3C and 3F, arrows).

Eosinophils in the Shikra were the largest granulocytes (Table 2). Eosinophils in both raptors contained lobed nuclei and numerous spherical acidophilic granules within light blue cytoplasm (Figs. 4A and 4D). In the Shikra, some vacuoles were observed in the cytoplasm of the eosinophils (Figs. 4D and 4E). Cytochemically, eosinophils in the Crested Serpent eagle were positive for SBB, ANAE (Fig. 4B) and BG, whereas eosinophils in the Shikra were positive only for ANAE (Fig. 4E) and BG (Table 2). Ultrastructurally, eosinophils in the Shikra contained oval to lobed nuclei, variable sizes of spherical granules, few mitochondria (Fig. 4C, white arrows) and rough endoplasmic reticulum (Fig. 4F, triple arrows). Granules of eosinophils in the Crested Serpent eagle were heterogeneously electron-dense, with a different round electron-dense area in the center of some granules (Fig. 4C), while those of the Shikra were homogeneously electron-dense (Fig. 4F).

Basophils were the smallest granulocytes (Table 3). Basophils contained numerous dark blue cytoplasmic granules, which obscured the central round nuclei (Figs. 5A and 5D). With the NMB stain, basophil granules were more clearly identified by numerous spherical, intensely dark blue, cytoplasmic granules surrounding a central, round nucleus. Cytochemically, some granules of the basophils were only positive for BG (Figs. 5B and 5E). Ultrastructurally, they contained large

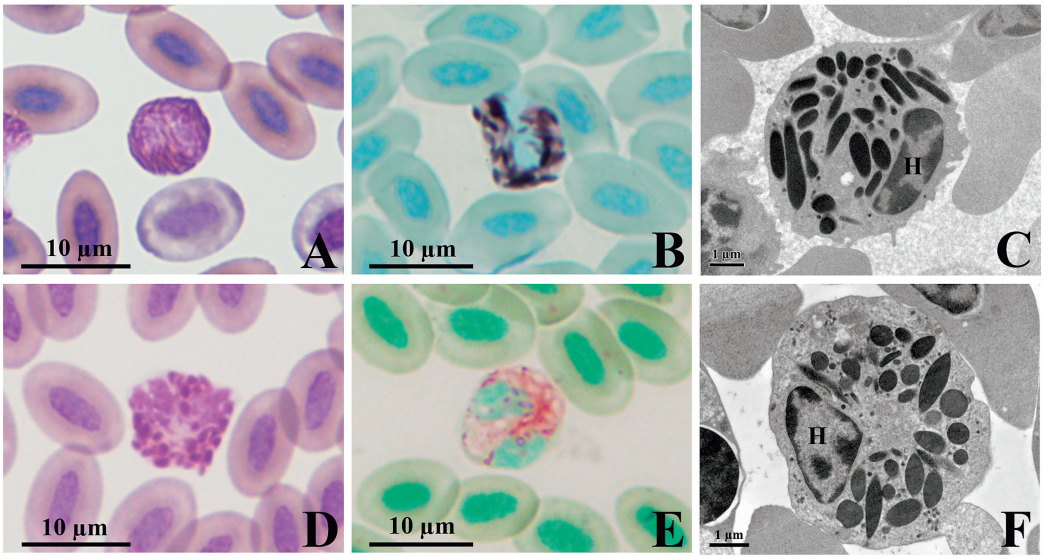


**Fig. 2. Light and electron micrographs of heterophils in the Crested Serpent eagle (A–C) and Shikra (D–F). (A and D)** Heterophils, Wright's stain. **(B)** Sudan Black B-positive heterophil in Crested Serpent eagle. **(E)** β-glucuronidase positive heterophil in Shikra. **(C and F)** Ultrastructure of heterophils (H). It was clearly differentiated long rod granules in Crested Serpent eagle (C) and spindle-shaped granules in Shikra (F). TEM, uranyl acetate and lead citrate stains.

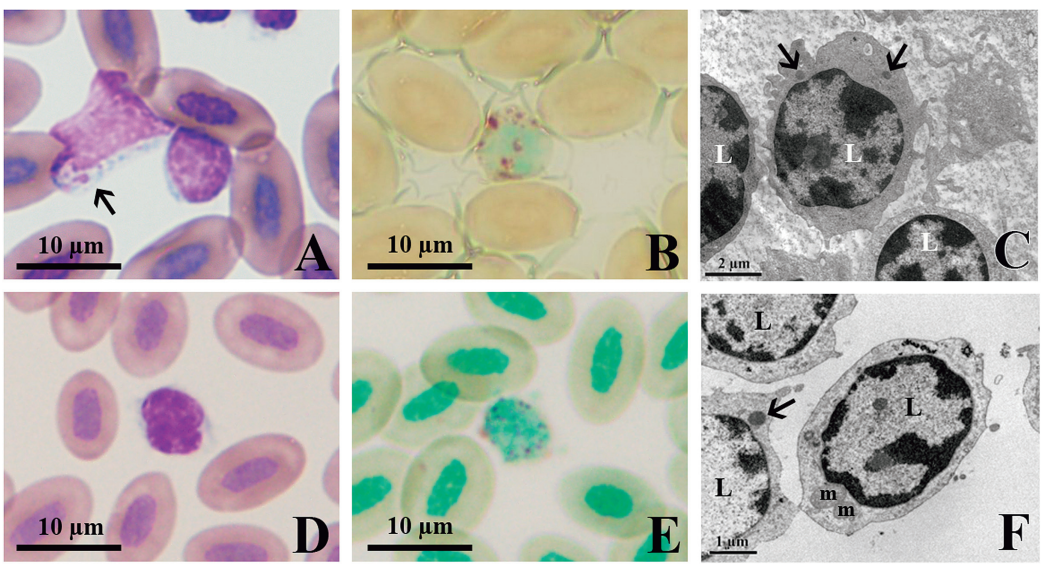


**Fig. 3. Light and electron micrographs of lymphocytes in the Crested Serpent eagle (A–C) and Shikra (D–F). (A and D)** Lymphocytes, Wright's stain. One lymphocyte contained azurophilic granules (arrow). **(B)** α-naphthyl acetate esterase-positive lymphocyte. **(E)** β-glucuronidase fine granular positive lymphocyte. **(C and F)** Ultrastructure of lymphocytes (L), showed round nuclei with heterochromatin and scanty cytoplasm. Some lymphocytes contained azurophilic granules (arrows). TEM, uranyl acetate and lead citrate stains.

spherical nuclei and numerous membrane-bound pleomorphic granules. Some granules were less electron-dense and finely reticulated (Figs. 5C and 5F). The other organelles, mitochondria, ribosomes and rough endoplasmic reticulum, were intermingled among the granules of the basophils.

Monocytes were the largest leukocytes (Table 3). They contained amoeboid nuclei and blue-gray cytoplasm (Figs. 6A and 6D). Cytochemically,

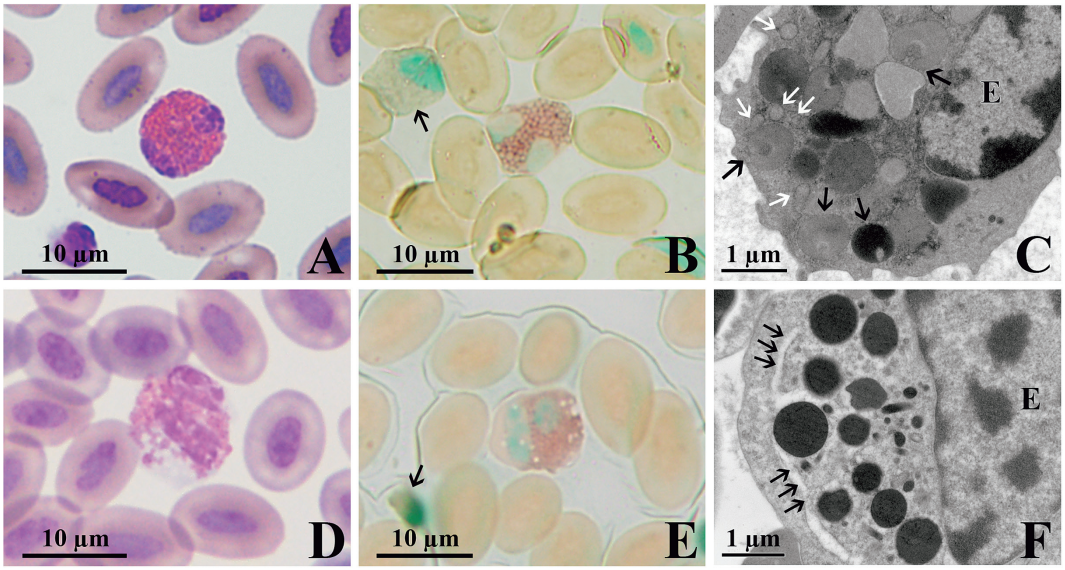


**Fig. 4. Light and electron micrographs of eosinophils in the Crested Serpent eagle (A–C) and Shikra (D–F). (A and D)** Eosinophils, Wright's stain. **(B)** α-naphthyl acetate esterase (ANAE)-positive eosinophil. ANAE-negative heterophil (arrow). **(C)** Ultrastructure of eosinophils (E) in Crested Serpent eagle, showing round, heterogeneous electron-density with round, different electron dense in the center of some granules (arrows). **(E)** ANAE-positive eosinophil in shikra and ANAE-negative thrombocyte (arrow). **(F)** Eosinophils (E) in Shikra showing round, homogeneous electron-dense granules. TEM, uranyl acetate and lead citrate stains.

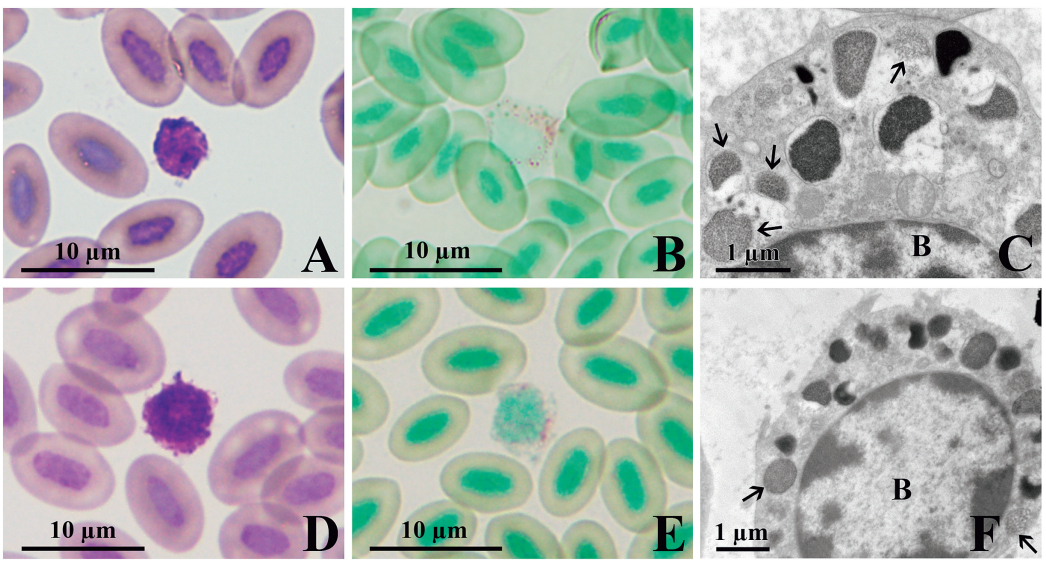


**Fig. 5. Light and electron micrographs of basophils in the Crested Serpent eagle (A–C) and Shikra (D–F). (A and D)** Basophils contained numerous round metachromatic granules, Wright's stain. **(B and E)** Some granules of basophils were positive for β-glucuronidase. **(C and F)** Ultrastructure of basophils (B), showed various electron-density granules. Some granules were finely reticulated (arrows). TEM, uranyl acetate and lead citrate stains.

monocytes in the Shikra were weakly positive with PAS and BG (Fig. 6B) but were negative with ANAE (Table 2). Monocytes in the Crested Serpent eagle were positive for BG (Fig. 6E) and ANAE (Table 2). By TEM, monocytes had variably shaped nuclei, several mitochondria, rough endoplasmic reticulum, numerous pseudopodia and a few azurophilic granules (Figs. 6C and 6F).

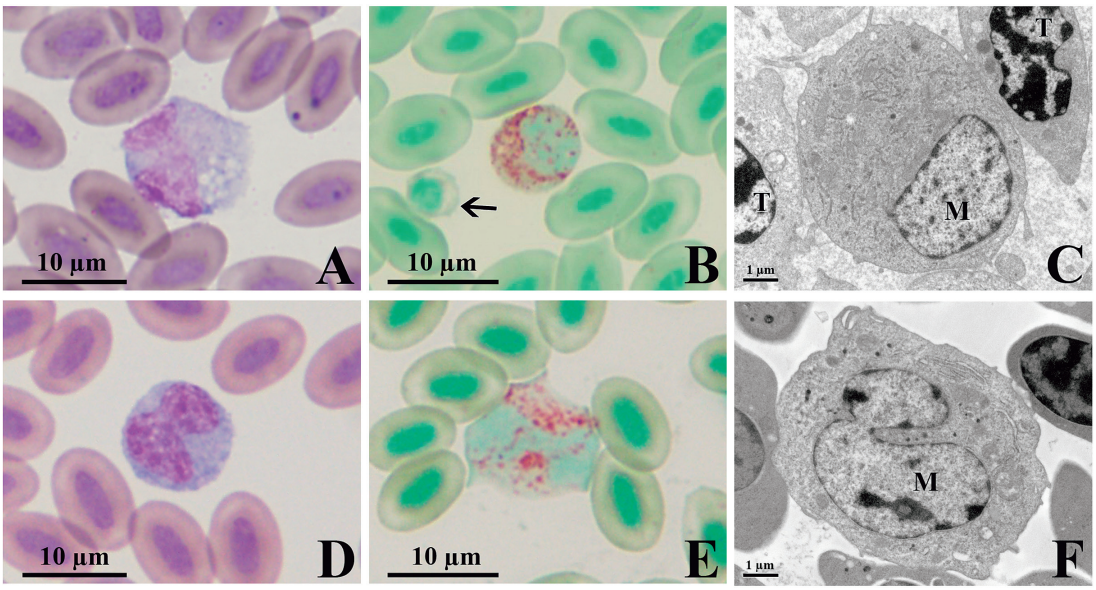


**Fig. 6. Light and electron micrographs of monocytes in the Crested Serpent eagle (A–C) and Shikra (D–F). (A and D)** Monocytes , Wright's stain. **(B and E)** β-glucuronidase (BG) positive monocytes. BG-negative thrombocyte (arrow). **(C and F)** Ultrastructure of monocytes (M), showed numerous mitochondria and rough endoplasmic reticulum. TEM, uranyl acetate and lead citrate stains.

*Thrombocytes* were oval to round cells, slightly smaller than lymphocytes. Nuclei were spherical and irregular with dense chromatin, while the cytoplasm was light blue and contained small azurophilic granules (Figs. 1A and 1D). Thrombocytes in the Crested Serpent eagle were positive for BG and ANAE (Table 2), whereas those of the Shikra were negative with nearly all cytochemical stains (Figs. 4E and 6B), except for some thrombocytes that showed a reaction with BG (Table 2). Ultrastructurally, thrombocytes contained an irregular nucleus with dense chromatin, mitochondria, dense granules and a large vacuole in the cytoplasm (Fig. 1F).

## Discussion

This paper presents the first record of the morphology of the blood cells of captive Crested Serpent eagle and Shikra and emphasizes the morphometry, cytochemistry and ultrastructure of the blood cells. The low value of the mean PCV (38%), compared with the other raptors[5,7], may be due to the tropical climate, which is correlated with the raptor's water intake. The RBCs in the Shikra were smaller than those of the Crested Serpent eagle and other reported birds such as the Painted Stork (*Mycteria leucocephala*)[13], the Lesser Adjutant (*Leptoptilos javanicus*) and the Greater Adjutant (*Leptoptilos dubius*)[14]. Among raptors, the RBCs of the Shikra had less area, but were wider, than those of the Crested Serpent eagle, and the Crested Goshawk (*Accipiter trivergatus*, $71 \pm 1\ \mu m^2$)[19].

The high number of eosinophils in the Crested Serpent eagles and the Shikras without parasite infection was characteristic of raptor species[5,7,8]. Lymphocytes were the most prevalent circulating leukocyte in the Shikra, similar to the Painted Stork[13] and other raptors (Red-tailed Hawk and Gyrfalcon)[12]. However, in the Crested Serpent eagle, the Lesser Adjutant, the Greater Adjutant[14], the Great-horn Owl, the Bald Eagle and the Peregrine Falcon[12], heterophil numbers exceeded lymphocyte number. Basophils were scarce in the Painted Stork[13], but we usually found a lower number of basophils (2–5%) than eosinophils in these two species of raptor. In many avian species, basophils are usually more

numerous than eosinophils[8].

The Crested Serpent eagles and Shikra had light morphologic features in its heterophils, eosinophils and basophils, similar to those of other raptors[19]. The light morphology and ultrastructure of the lymphocytes and monocytes were similar to those of other avians[8,13-15,19] and felids[16].

SBB reactions were negative in all blood cell types in the Shikra, similar to those of the Crested Goshawk[19]. However, in the Crested Serpent eagle and the Painted Stork[13], heterophils showed SBB positivity. All blood cells in the Crested Serpent eagle and the Shikra were negative for PO, but eosinophils in the Crested Goshawk[19] and the chicken[1] showed peroxidase activities. Heterophils in both raptors lack PO enzymes, but contained BG enzymes. These were cytochemical characteristics of avian heterophils, which were responsible for bactericidal activity[8]. Some granules of basophils in the Crested Serpent eagle and the Shikra stained only with BG, similar to those in the Painted Stork[13] and the Crested Goshawk[19]. The NMB-stained Shikra basophil granules showed metachromatic color, similar to that of the basophils of other avians[13,14] and domestic animals[11]. In the Shikra, only eosinophils showed ANAE reactions, whereas heterophils and monocytes in the Crested Serpent eagle, the Painted Stork[13], turkeys[6] and the Crested Goshawk[19] were also positive with ANAE. In the Crested Serpent eagle and the Shikra, all WBCs showed BG reactions similar to those of the Crested Goshawk[19] and the Painted Stork[13]. Basophils were negative for SBB and PO reactivity, similar to the other animals[11]. Basophils were positive only for BG, similar to that described in the Painted Stork[13], the Crested Goshawk[19] and felids[16]. All cytochemical results showed heterogeneity in the cytochemical reactions in blood cells among avian species.

Using electron microscopy, the granules of heterophils and eosinophils in the Shikra were similar in the homogeneous electron densities but different in shape and size of the granules. The ultrastructure of all granulocytes in the Shikra was similar to that described in the Painted Stork[13]. The Crested Serpent eagle had an ultrastructure of eosinophilic granules that differed from the other reported birds[13], whereas their round eosinophilic granules were similar under a light microscope. These data confirmed the heterogeneity of the granulocytes among avian species. Basophils in both raptors had different granule contents, and some granules were electron lucent. The heterogeneity of basophil granules was similar to those of the Painted Stork[13]. Because only some granules of basophils were positive for BG, this supports the existence of different contents in the granules.

In conclusion, the majority of light microscopic blood cell morphologies of the Crested Serpent eagle and the Shikra were similar to those reported in avian species, especially the round-shaped granules of the eosinophils. The vacuoles found in eosinophils of Shikra was characteristics of this species. Heterophils and eosinophils in the Crested Serpent eagle were stained with SBB, ANAE and BG. Heterophils, monocytes and thrombocytes in the Shikra were stained with PAS. These results showed different cytoplasmic components in blood cells in these two species of raptors. Ultrastructure revealed cytoplasmic granules and organelles in the blood cells and also showed a different electron density in the eosinophilic granules of the Crested Serpent eagle. The ultrastructure of heterophils showed more clearly differentiate long rod granules in Crested Serpent eagle and spindle-shaped granules in Shikra. Our results confirm the heterogeneity of blood cell morphology in birds. Although the number of raptors in this study was low for a determination of reference intervals, these results may be useful for evaluating the condition of raptors in a rehabilitation unit. In this study, we gathered the main characteristics of morphology, morphometry, cytochemistry and ultrastructure of all blood cell types in the Crested Serpent eagle and the Shikra as a baseline for future studies.

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