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**Hematology, Morphology, and Ultrastructure of Blood Cells of Juvenile Olive Ridley Sea Turtles (*Lepidochelys olivacea*)**

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**ABSTRACT.** – Hematologic data, morphology, and ultrastructure of peripheral blood cells of 5 juvenile olive ridley turtles (*Lepidochelys olivacea*) were evaluated. The following blood cells were identified: mature and immature erythrocytes, heterophils, large and small eosinophils, basophils, lymphocytes, monocytes, and thrombocytes. Unique blood cell features of olive ridley turtles that are useful for health evaluations within sea turtle care and conservation were identified in hematologic, morphological, and ultrastructural studies.

There are 7 species of sea turtles worldwide, and all are listed on the IUCN Red List (Seminoff and Shanker 2008). Five species are found in the waters surrounding mainland China and the associated islands, including leatherbacks (*Dermochelys coriacea*), loggerheads (*Caretta caretta*), green turtles (*Chelonia mydas*), hawksbills (*Eretmochelys imbricata*), and olive ridleys (*Lepidochelys olivacea*) (Chan et al. 2007). Research and data on the hematology and morphology of blood cells are useful for the veterinary surgeons involved in sea turtle conservation throughout the world and in China as well (Casal and Orós 2007). Data on these topics have been collected for most sea turtle species: green turtles (Samour et al. 1998; Work et al. 1998), loggerheads (Bradley et al. 1998; Keller et al. 2004; Stamper et al. 2005; Casal and Orós 2007; Casal et al. 2007; Deem et al. 2009), leatherbacks (Deem et al. 2006), hawksbills (Zhang et al. 2009), as well as for Kemp’s ridley turtles (*Lepidochelys kempii*; Cannon 1992; Innis et al. 2009). However, data on hematology and blood cell morphology are lacking for olive ridley turtles and flatback turtles (*Natator depressus*). Herein we describe hematologic parameters, morphological classification, and ultrastructure of blood cells for olive ridley turtles.

**Materials and Methods.** — Five olive ridley turtles caught as local bycatch were raised for 1 to 2 years at Huidong Gangkou Sea Turtle Nature Reserve of China and used in this study, which was permitted by the Oceanic and Fisheries Administrator of Guangdong Provincial of China. The turtles were raised in indoor enclosures exposed to natural sunlight with temperature ranging from 16°C to 28°C. Turtles were fed an assortment of marine fish, squid, and crab. All turtles were juveniles, clinically normal, and in good physical condition. Average individual straight carapace length was 50.9 cm (range: 49.4–52.9 cm) while average body weight was 17.2 kg (range: 13–23 kg).

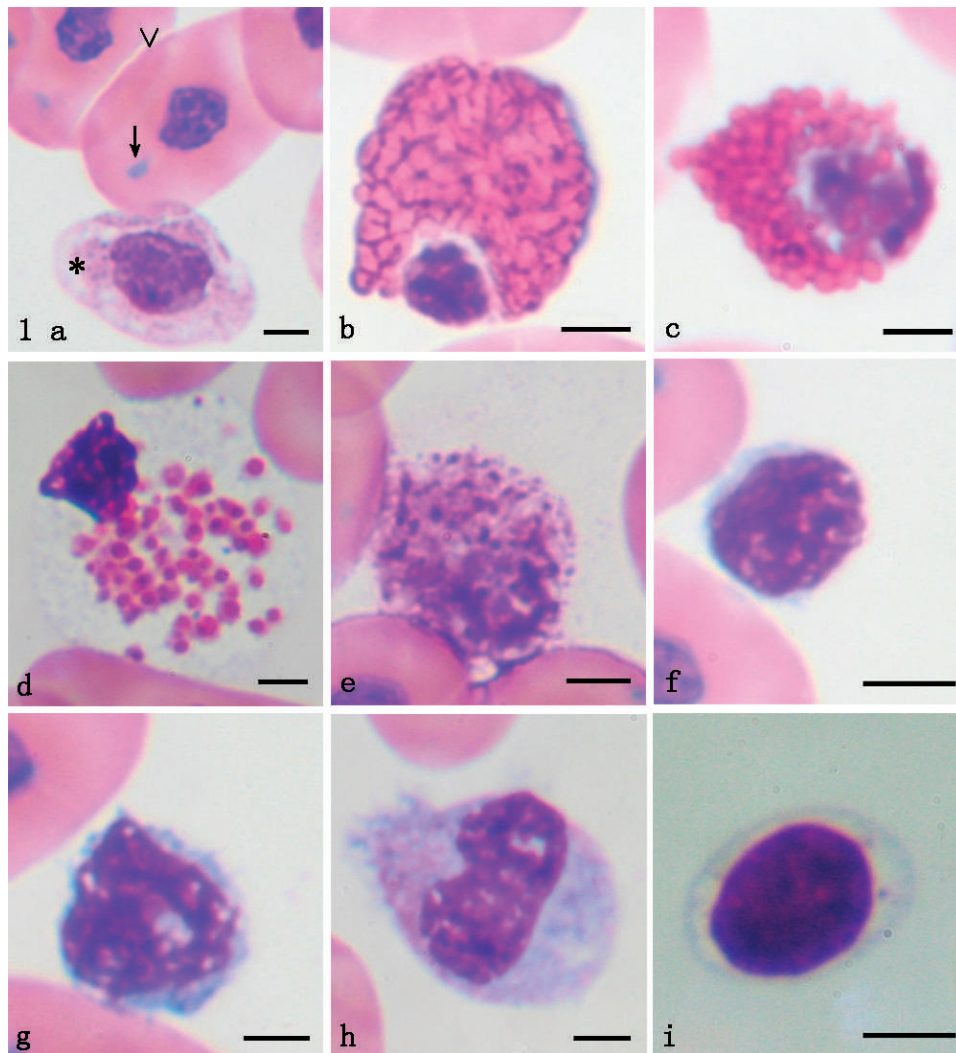
A small amount of peripheral blood (~2 mL) was collected from the dorsal cervical sinus (Owens and Ruiz 1980) using 21-gauge needles and 5-mL syringes. A 0.2-mL aliquot from each sample was used immediately for hematologic analysis and light microscopy, while the remaining blood was saved in tubes with heparin anticoagulant for transmission electron microscopy (TEM).

For light microscopy, 3 blood smears for each turtle were prepared and stained with Wright–Giemsa stain. The blood smears were examined and photographed under a Motic microscope using the immersion ×100 objective. The maximum and minimum diameter of blood cells (20 erythrocytes and 15 leucocytes) and their nuclei were measured for each smear with a calibrated ocular micrometer. Blood cell and nuclear areas were calculated according to the following formula:  $(\text{length} \times \text{width} \times \pi) / 4$  (Uğurtş et al. 2003).

For TEM, blood samples with heparin anticoagulant were centrifuged to discard the plasma. The upper layer of white blood cells and thrombocytes plus a small part of the red blood cells layer were removed and fixed in phosphate-buffered glutaraldehyde 2.5%. These samples were refrigerated for 4 hours, and the fixed cell layer was removed and stored in fresh glutaraldehyde at 4°C until being processed for TEM. Samples were postfixed in 1% osmium tetroxide and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate. Observation and microphotography was carried out using a Philips TECNAI-10 transmission electron microscope and Morada soft imaging system.

The total erythrocyte, leukocyte, and thrombocyte counts were determined manually using a hemocytometer after blood was diluted with Natt and Herrick’s solution (Natt and Herrick 1952). Differential leukocyte counts were made by counting 200 leukocytes from the Wright–Giemsa stained blood smears, and the percentage of each cell type was calculated. For immature erythrocyte counts, the percentage of immature erythrocytes was determined per 1000 red blood cells.

The Shapiro–Wilk statistic, kurtosis, and skewness were used to evaluate the distribution of the data. The mean ± SD is reported for normally distributed data, and



**Figure 1.** Photomicrographs of blood cells from olive ridley turtles (*Lepidochelys olivacea*) stained with Wright-Giemsa. a) A mature erythrocyte (arrowhead) with basophilic intracytoplasmic inclusions (arrow), and a immature erythrocyte (\*); b) a heterophil; c) a small eosinophil; d) a large eosinophil; e) a basophil; f) a small lymphocyte; g) a medium-size lymphocyte; h) a monocyte; i) a thrombocyte. Scale bar in each photograph = 4  $\mu$ m.

the median is reported for nonnormally distributed data (Deem et al. 2009).

**Results.** — In the peripheral blood of olive ridleys, the following blood cells were identified: mature and immature erythrocytes, heterophils, large and small eosinophils, basophils, lymphocytes, monocytes, and thrombocytes (Fig. 1). Dimensions of the blood cells are shown in Table 1. Since small eosinophils and basophils were scarce, measurements were not made. Differential counts of each cell type were as follows: heterophils (62.6%), lymphocytes (20.1%), monocytes (12.8%), eosinophils (4.4%), and basophils (0.2%). More detailed results can be found in Table 2.

Mature erythrocytes (Fig. 1a) were nucleated oval cells with centrally positioned nuclei containing dense blue chromatin clumps. Cytoplasm was uniform orange-pink under Wright-Giemsa stain. Some mature erythrocytes had oval basophilic intracytoplasmic inclusions. These cytoplasmic inclusions were stained blue and measured from

0.8 to 2  $\mu$ m in diameter (Fig. 1a). Ultrastructurally, erythrocytes were oval and contained an elliptical nucleus with dense heterochromatin (Fig. 2a). Some erythrocytes had small electron-dense intracytoplasmic inclusions without recognizable organelles that are considered to be degenerating organelles.

Immature erythrocytes (Fig. 1a) occasionally were seen in the peripheral blood. Immature erythrocytes were ellipsoidal and were smaller than mature cells (Table 1). Immature erythrocytes had larger, rounder nuclei and less dense chromatin clumping than mature erythrocytes. The cytoplasm was pale red and filled with numerous small basophilic granules. These cells accounted for 1% of all erythrocytes. Ultrastructurally, some immature erythrocytes were seen with an oval nucleus containing moderate amounts of heterochromatin with 2 nucleoli.

Heterophils (Fig. 1b) were the predominant leukocyte in the peripheral blood. These cells had a round shape, and contained a dense, blue, generally eccentric

**Table 1.** Blood cells morphological parameters of juvenile olive ridley turtles (*Lepidochelys olivacea*).

Cell type	Maximum length diameter ( $\mu\text{m}$ )	Minimum length diameter ( $\mu\text{m}$ )	Cell area ( $\mu\text{m}^2$ )	Nuclear area ( $\mu\text{m}^2$ )
Erythrocyte				
Median	20.35	12.55	201.65	19.79
Range	14.50–24.00	7.00–15.00	102.49–263.89	14.14–29.45
Immature erythrocyte				
Mean $\pm$ SD or median	16.00	10.59 $\pm$ 1.86	127.63	32.99
Range	11.00–20.00	7.00–15.00	86.39–235.62	23.56–66.37
Heterophil				
Mean $\pm$ SD or median	16.00	15.00	193.01	22.28 $\pm$ 4.54
Range	11.40–19.50	10.00–19.00	89.54–283.53	12.57–32.99
Large eosinophil				
Mean $\pm$ SD or median	18.00	16.70 $\pm$ 1.90	245.83	33.68 $\pm$ 6.85
Range	13.00–20.00	11.00–20.00	112.31–314.16	25.13–46.73
Lymphocyte				
Mean $\pm$ SD or median	8.90 $\pm$ 1.68	8.32 $\pm$ 1.43	56.54	49.48
Range	6.00–13.00	6.00–12.00	28.27–122.52	23.56–86.39
Monocyte				
Mean $\pm$ SD or median	13.49 $\pm$ 1.78	11.57 $\pm$ 1.24	115.45	56.65 $\pm$ 7.51
Range	11.00–18.00	9.00–15.00	86.39–188.50	39.27–69.12
Thrombocyte				
Mean $\pm$ SD or median	8.65 $\pm$ 1.94	5.23 $\pm$ 0.86	34.16	27.49
Range	5.50–13.00	3.60–7.00	21.99–71.47	17.67–56.55

nucleus. The abundant cytoplasm was filled with eosinophilic granules, generally spindle, rod, or oval shaped. Ultrastructurally, heterophils had oval nuclei with moderate amounts of heterochromatin (Fig. 2a, b). The cytoplasm contained numerous, moderately electron-dense, elongated to round granules and a small number of very electron-dense round granules. Some mitochondria and endoplasmic reticula were also observed (Fig. 2b).

Two types of eosinophils could be observed: small and large. Small eosinophils (Fig. 1c) had dense purple-blue nuclei. The cytoplasm was filled with well-defined round eosinophilic granules. Large eosinophils (Fig. 1d) were round with an eccentrically positioned nucleus. The cytoplasm was abundant and stained weakly basophilic, appeared pale blue with numerous coalescing small vacuoles, and contained well-defined round eosinophilic granules. These granules had a bright orange core and were surrounded by a blue-orange slightly refractile halo. Ultrastructurally, large eosinophils had an oval nucleus

with moderate amounts of heterochromatin. Few large, well-defined round, homogeneous electron-dense granules were found on one side of the cell (Fig. 2c). Ultrastructural characteristics of small eosinophils were not observed since this cell was scarce.

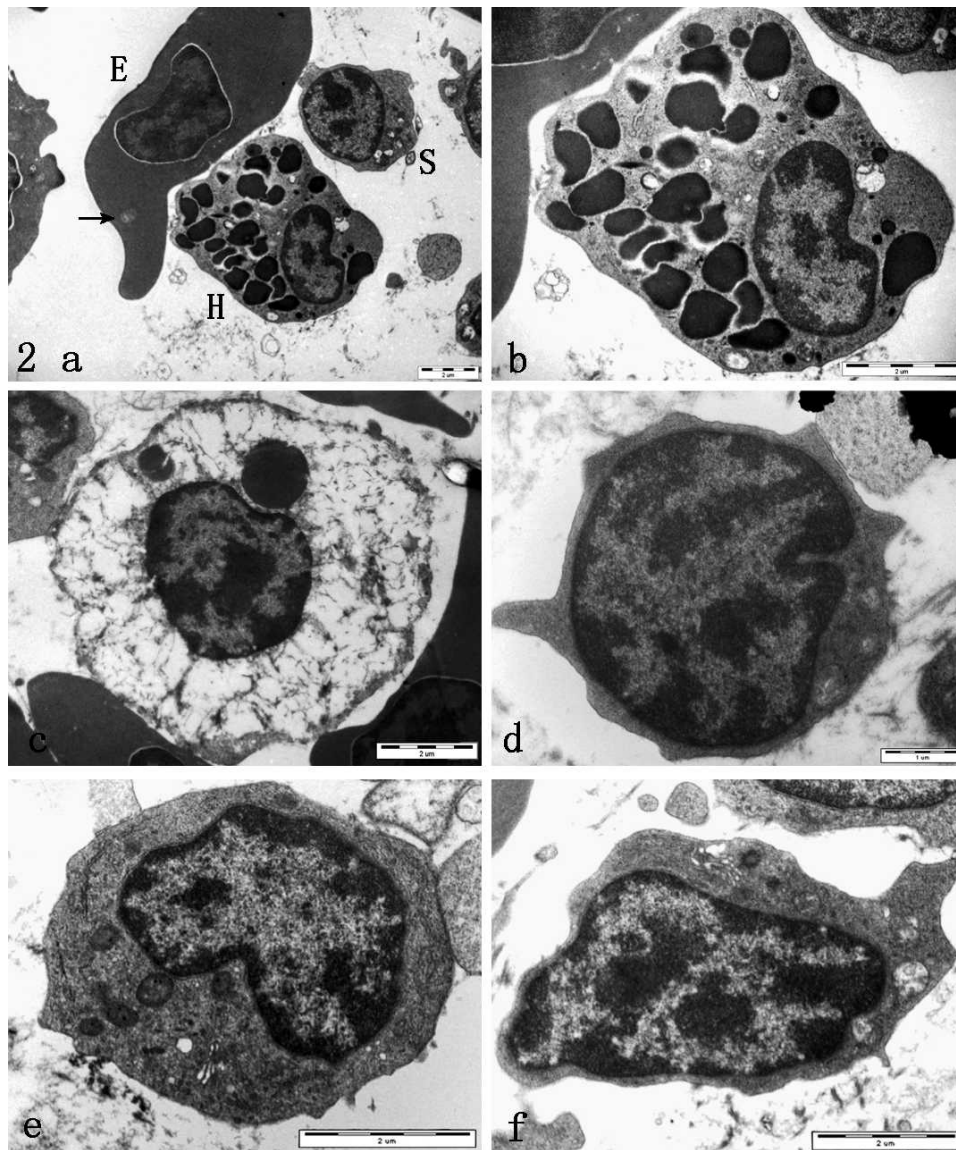
Basophils (Fig. 1e) were the most rarely observed leukocyte. These cells were round with an oval and eccentric nucleus. The cytoplasm was filled with round or oval granules with dark blue color under Wright–Giemsa stain. The basophilic granules often obscured the nucleus. Ultrastructural characteristics of basophils were not determined because of their low numbers.

Lymphocytes were round and had the highest nucleus:cytoplasm (N:C) ratio of all leukocytes. Lymphocytes varied in size from small to large. The small lymphocytes (Fig. 1f) had densely clumped chromatin in a round or notched nucleus that stained dark blue. The cytoplasm was scant and appeared as a thin rim of basophilic cytoplasm around the nucleus or in the notched side of the nucleus. The large lymphocytes (Fig. 1g) had more cytoplasmic volume than small lymphocytes and stained blue. These cells had round or oval nuclei. Nuclear chromatin was moderately condensed and stained blue. Ultrastructurally, the lymphocytes were round (Fig. 2a, d). The nucleus was round, often indented, with peripheral and central clumps of heterochromatin. The scant cytoplasm contained some mitochondria, endoplasmic reticula, and polyribosomes.

Monocytes (Fig. 1h) cells were round or occasionally amoeboid. The nucleus varied from oval or kidney shaped to rod shaped and also varied in position from eccentric to central. The nuclear chromatin was slightly less clumped

**Table 2.** Blood cells count of juvenile olive ridley turtles (*Lepidochelys olivacea*).

Cell type	No. of blood cells ( $\times 10^3/\mu\text{L}$ ), mean $\pm$ SD or median	Range ( $\times 10^3/\mu\text{L}$ )
Erythrocyte	230.00	120.00–405.00
Leukocyte	9.25 $\pm$ 3.64	3.75–13.00
Thrombocyte	10.50	6.75–19.75
Heterophil	5.35 $\pm$ 2.39	2.17–8.84
Eosinophil	0.30 $\pm$ 0.21	0.09–0.65
Basophil	0	0–0.07
Lymphocyte	1.53	0.92–2.54
Monocyte	1.30 $\pm$ 0.83	0.40–2.31



**Figure 2.** Transmission electron micrographs of blood cells from olive ridley turtles (*Lepidochelys olivacea*): a) an erythrocyte (E) with a degenerating organelle (arrows), a heterophil (H), and a lymphocyte (S), bar = 2  $\mu\text{m}$ ; b) a heterophil, bar = 2  $\mu\text{m}$ ; c) a large eosinophil, bar = 2  $\mu\text{m}$ ; d) a lymphocyte, bar = 1  $\mu\text{m}$ ; e) a monocyte, bar = 2  $\mu\text{m}$ ; f) a thrombocyte, bar = 2  $\mu\text{m}$ .

than that of the lymphocytes, and stained purple. The abundant cytoplasm was weak to moderately basophilic and sometimes contained extremely fine, light-purple granules and variably sized cytoplasmic vacuoles. Ultrastructurally, the cell was normally round and sometimes had pseudopods (Fig. 2e). The nucleus contained very little heterochromatin. The cytoplasmic organelles were easily observed, including mitochondria, endoplasmic reticula, and small dense granules.

Thrombocytes (Fig. 1i) were oval to round shaped. The nucleus was generally oval, contained clumped chromatin, and appeared blue. The cytoplasm was scant with a slim cytoplasmic halo around the nucleus and showed a very pale, almost transparent coloration. Ultrastructurally, the cell edges presented some finger-like projections (Fig. 2f). The nucleus was oval or round and contained peripherally located heterochromatin. The scant cytoplasm had canalicular

systems that provides direct access to the outside environment for the internal substances of thrombocytes. Mitochondria, microtubules, rough endoplasmic reticula, and Golgi complexes were often observed.

*Discussion.* — The morphological and ultrastructural characteristics of erythrocytes from juvenile olive ridleys found in this study were similar to those reported for green turtles (Work et al. 1998; Zhang et al. 2009), young loggerheads (Casal and Orós 2007; Casal et al. 2007), and hawksbills (Zhang et al. 2009) as well as other chelonians (Alleman et al. 1992; Uğurtuş et al. 2003). Basophilic inclusions are common in the erythrocytes of healthy reptiles (Strik et al. 2007) and commonly represent degenerating organelles (Alleman et al. 1992; Work et al. 1998; Casal et al. 2007).

We observed immature erythrocytes at a frequency of 1% in the peripheral blood of juvenile olive ridleys.

Similar morphological characteristics of immature erythrocytes were also seen in hatchling loggerheads (Bradley et al. 1998) and juvenile green turtles and hawksbills (Zhang et al. 2009), in addition to other reptilian species (Carvalho et al. 2006). A small number of immature erythrocytes are usually seen in the peripheral blood of reptiles, especially in young individuals (Campbell 2004). However, there are no descriptions of immature erythrocytes in the peripheral blood of juvenile Kemp's ridleys (Cannon 1992), green turtles (Work et al. 1998), and loggerheads (Casal and Orós 2007). This may be attributable in part to differences in staining methods used. Immature erythrocyte counts can be used to evaluate the erythrocytic regenerative response (Campbell 2004). Additional studies of factors that influence the number of immature erythrocytes in peripheral blood of sea turtles are warranted.

Heterophils of reptiles and birds are phagocytic and are analogous to neutrophils in mammals (Canfield 1998). Heterophils from olive ridleys were similar to those found in other sea turtles (Work et al. 1998; Casal and Orós 2007; Casal et al. 2007; Zhang et al. 2009), but heterophils were not described in Kemp's ridleys (Cannon 1992). Both heterophils and eosinophils have eosinophilic intracytoplasmic granules, and these 2 types of leukocytes are sometimes hard to distinguish. Heterophils and eosinophils are best differentiated by the shape of intracytoplasmic granules: eosinophils have round granules, while heterophils have elongated granules (Alexandre and Laurelúcia 2003).

Eosinophils from olive ridleys, Kemp's ridleys, and green turtles can be categorized into small and large types (Cannon 1992; Work et al. 1998; Zhang et al. 2009), unlike eosinophils from hawksbills and loggerheads that are similar in size (Casal and Orós 2007; Zhang et al. 2009). Small eosinophils from olive ridleys examined in this study had similar morphological and ultrastructural characteristics to the small eosinophils found in green turtles (Work et al. 1998), whereas large eosinophils observed in our study animals were similar to the large eosinophils found in green turtles and the eosinophils in loggerheads and hawksbills (Work et al. 1998; Casal and Orós 2007; Zhang et al. 2009). Eosinophils from olive ridleys and green turtles have round and eosinophilic cytoplasmic granules, similar to cytoplasmic granules that have been described in Kemp's ridley eosinophils; however, the 'large' eosinophils in Kemp's ridleys are generally smaller and have more cytoplasmic granules than those in both olive ridleys and green turtles (Cannon 1992; Work et al. 1998; Zhang et al. 2009).

Basophils were scarce in olive ridleys studied here, consistent with previous findings for green turtles (Work et al. 1998), loggerheads (Casal and Orós 2007), and hawksbills (Zhang et al. 2009). No basophils were identified in Kemp's ridleys (Cannon 1992) or leatherbacks (Deem et al. 2006). We did not observe basophils by TEM.

Monocytes from olive ridleys were similar to those from other sea turtles (Work et al. 1998; Casal and Orós 2007; Casal et al. 2007; Zhang et al. 2009). Work et al. (1998) reported monocytes were more difficult to differentiate from lymphocytes when smears were made from blood that had been chilled. In our study, monocytes were similar to large lymphocytes in size but could be easily differentiated by the shape of their nuclei and cytoplasm staining characteristics. Monocytes had oval or kidney shaped to rod shaped nuclei and pale purple-blue cytoplasm, while lymphocytes had round nuclei and blue cytoplasm.

Light and electron microscopy revealed that lymphocytes of olive ridleys were similar to those reported in other sea turtles (Cannon 1992; Work et al. 1998; Casal and Orós 2007; Casal et al. 2007; Zhang et al. 2009). In our study, the size of lymphocytes was within ranges in other sea turtles (Work et al. 1998; Casal and Orós 2007; Zhang et al. 2009).

Thrombocytes from olive ridleys were similar to those found in other chelonian species (Work et al. 1998; Casal and Orós 2007; Casal et al. 2007; Zhang et al. 2009). Thrombocytes can be difficult to distinguish from small lymphocytes by light microscopy (Alleman et al. 1992; Canfield 1998; Work et al. 1998). In our study, we found if the pH of phosphate-buffered saline for staining the smears is not appropriate, cytoplasm of thrombocytes will not stain, and the round forms of thrombocytes can be confused for small lymphocytes. However, thrombocytes usually clump together in the blood smear, which is helpful in differentiating thrombocytes from small lymphocytes.

In our study, azurophils were not identified in the peripheral blood of olive ridleys. Rare azurophils were observed in the blood of sea turtles (Samour et al. 1998; Keller et al. 2004; Deem et al. 2009). However, most other authors did not identify azurophils in sea turtles (Cannon 1992; Bradley et al. 1998; Work et al. 1998; Casal and Orós 2007; Zhang et al. 2009; Innis et al. 2009).

In the leukocyte differential counts, heterophils were the predominant leukocyte, followed by lymphocytes, monocytes, eosinophils, and basophils. Similar results were reported in leatherbacks (Deem et al. 2006) and captive green turtles and hawksbills (Zhang et al. 2009). In a study on rehabilitated juvenile loggerheads, heterophils were found to comprise 75.8% of the total leukocyte count, followed by lymphocytes, eosinophils, monocytes, and basophils (Casal and Orós 2007), and similar order of differential leukocyte found in foraging and nesting loggerheads (Deem et al. 2009). However in another survey on wild-caught juvenile loggerheads from inshore waters of North Carolina, lymphocytes were the most numerous circulating leukocytes, followed by heterophils, eosinophils, azurophils, and basophils (Keller et al. 2004). Lymphocytes were the predominant leukocytes in wild Hawaiian green turtle followed by eosinophils, heterophils, monocytes, and basophils (Work et al. 1998), while

in a study on free-living green turtles from the United Arab Emirates, heterophils were most abundant, followed by lymphocytes, eosinophils, monocytes, azurophils, and basophils (Samour et al. 1998). Age, geographic location, and differences in criteria used to identify blood cells may explain the difference in the results (Casal and Orós 2007). The stress effect was also documented, migratory loggerheads had higher percentage of heterophils and lower lymphocytes and eosinophils count than resident loggerheads (Stamper et al. 2005).

Red blood cell counts of juvenile olive ridleys were within ranges of green turtles (Samour et al. 1998; Zhang et al. 2009), nesting leatherbacks (Deem et al. 2006), captive juvenile hawksbills (Zhang et al. 2009), and loggerheads (Keller et al. 2004; Deem et al. 2009). The white blood cell count of juvenile olive ridleys was higher than those in nesting leatherbacks (Deem et al. 2006), wild-caught green turtles from United Arab Emirates (Samour et al. 1998), and captive juvenile green turtles and hawksbills (Zhang et al. 2009) but within the range of wild-caught juvenile green turtles from Hawaii (Work et al. 1998) and loggerheads (Keller et al. 2004; Deem et al. 2009). Thrombocyte counts of juvenile olive ridleys were higher than those of green turtles and hawksbills (Samour et al. 1998; Zhang et al. 2009). Differences in age, geography, diet, sex, season, or methods used may explain these differing results. Assessment of only 5 animals in this study provided a very limited sample size, and limitations of such data. Another reason may be that some animals were kept in captivity, and others were wild-caught. The factors including stress, nutrition, water conditions, and health could affect hematologic parameter of turtle maintained in captivity.

The results of this study provide information on the hematologic data and morphological and ultrastructural characteristics of blood cells from healthy juvenile olive ridleys. This information will be useful for future health evaluations of individuals of this species. Future hematologic study of olive ridley turtles should increase the sample size and compare individuals of different age classes and from different geographic locations.

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