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Short Communication

Microscopical analyses on characterization of peripheral blood cells of Western pond turtle (*Actinemys marmorata*) (Baird and Girard, 1852)

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Abstract

The aim of the present study is to investigate the microscopical characterization of peripheral blood cells namely erythrocytes, and leucocytes of Western pond turtle. However, no previous report on microscopical characterization of blood cells of Western pond turtle has been reported. One turtle was taken for this study. Blood sample was collected and blood smears were immediately prepared without any anticoagulant. Then after drying in air, fixed with methanol and stained with Giemsa stain. The erythrocytes observed were mainly elliptical in shape with ellipsoid or irregularly round nuclei. Besides that different shapes of erythrocytes were also observed. Five types of leucocytes including lymphocytes, monocytes, heterophils, eosinophils and basophils are seen in the blood smears of peripheral blood. The aim of the study is to provide baseline data on the description and morphological classification of blood cells in Western pond turtles by which their physiological states can also be known and they can be better treated. The data serve as a baseline for future hematologic studies in this species.

Keywords: Basophils, eosinophils, erythrocytes, heterophils, lymphocytes, monocytes, western pond turtle.

Introduction

The *Actinemys marmorata* (Western pond turtle) is a medium sized turtle having a smooth, keel-less low domed carapace that is olive, dark brown, or black in coloration[1]. The carapace generally has spots having network, flecks or extension of lines from the growth centre of each scute of carapace although unmarked carapace have also been seen occasionally[1]. In this study unmarked carapace was observed in turtle. Six pairs of scutes are present in plastron, and is cream or yellowish with or without dark blothes[2]. The Western pond turtle adopts extensively both aquatic and terrestrial habitats to complete its life cycle. In aquatic habitat it may be found in rivers, creeks, small ponds and lakes, marshes, and slow-moving sloughs, in both permanent and ephemeral water bodies up to 2000m in elevation[3]. Aquatic habitats are primarily used by Western pond turtle for foraging, thermoregulation, and avoidance of predators. Dispersal along aquatic corridors has also been noticed[4]. As regards to Rathbun et al. in them timing with the region the timing of egg laying varies[5] and in case of scanty resources it may skip a breeding year[4]. They are associated with those water bodies which are adjacent to open terrestrial habitats with good solar exposure and cover with low canopy like oak savannah, prairie or pasture[6]. Nests of them are generally seen within 200m of the edge of water[4]. The morphologies of peripheral blood cells of Western pond turtle are not documented in literature. Therefore the present study is investigated to describe the microscopical analyses of peripheral blood cells of Western pond turtle (*Actinemys marmorata*).

Materials and methods

One Western pond turtle was used in this study. From the dorsal coccygeal vein of the turtle 0.5ml of blood was collected[7], by the help of a veterinary expert. Blood sample was collected from them minimizing the discomfort and pain to them and then preparation of blood smears were done on clean microscopical slides. Then they were air dried and for fixation methanol was used. Then staining of slides were done by the use of Giemsa stain using the standard protocol as described by manufactures instruction (Himedia Laboratories Pvt. Ltd.) and then air-dried. The stained smears were observed under microscope (Carl Zeiss Axio imager Z2) under 40X objectives and photographs of blood cells were taken.

Results and discussion

From the observation of stained smear it was found that mature erythrocytes were typically ellipsoidal or ovoid in shape, with elliptical or round nuclei of irregular shapes in which dense and dark chromatins, as like amphibians, other reptiles and aves. The cytoplasm of erythrocytes were light or pale pink in the smears of blood (Figure-1).

Normal erythrocytes (Figure-1a), oval shaped erythrocyte (Figure-1b), elliptical shaped erythrocytes (Figure-1c), erythrocyte with irregular shaped nucleus (Figure-1d), circular erythrocyte (Figure-1e), circular erythrocyte having eccentrically placed nucleus (Figure-1f), circular erythrocyte

lacking nucleus (Figure-g), erythrocyte lacking distinct cell membrane (Figure-1h), comma shaped erythrocytes (Figure-1i and j) were seen. Erythrocyte peculiarities were also observed (Figure-2). Comma shaped peculiar erythrocyte (Figure-2a), tear drop shaped erythrocyte having peripheral nucleus (Figure-2b), tear drop shaped erythrocyte having centrally placed nucleus (Figure-2c), tear drop shaped erythrocyte having eccentrically placed nucleus (Figure-2d), tear drop shaped erythrocyte lacking nucleus (Figure-2e), spindle shaped erythrocyte lacking nucleus (Figure-2f), different shapes of erythrocytes [Figure-2(g), (h), (i), (j), (k), (l), (m), (n), (o), (p)], erythrocyte with one bulging end (Figure-2q), Erythrocyte having separated bulging tip (Figure-2r), dividing erythrocyte (Figure-2s), degenerating erythrocyte (Figure-2t), fragmented erythrocyte (Figure-2u), rhombic shaped erythrocyte (Figure-2v), partially folded erythrocytes (Figure-2w and x), tear drop shaped erythrocytes with early crenulations (Figure-2y and z), rouleax formation (Figure-2z') were noticed.

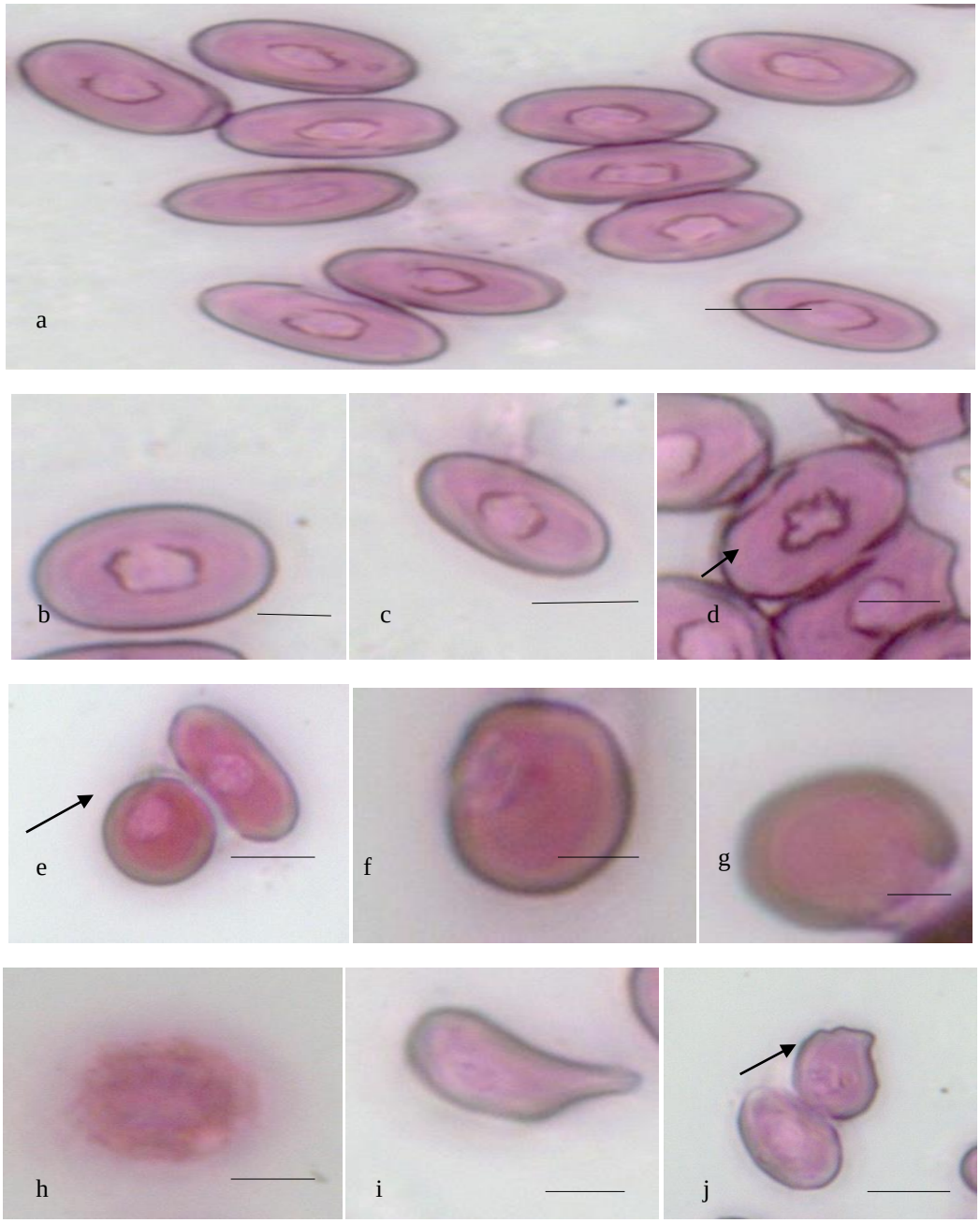
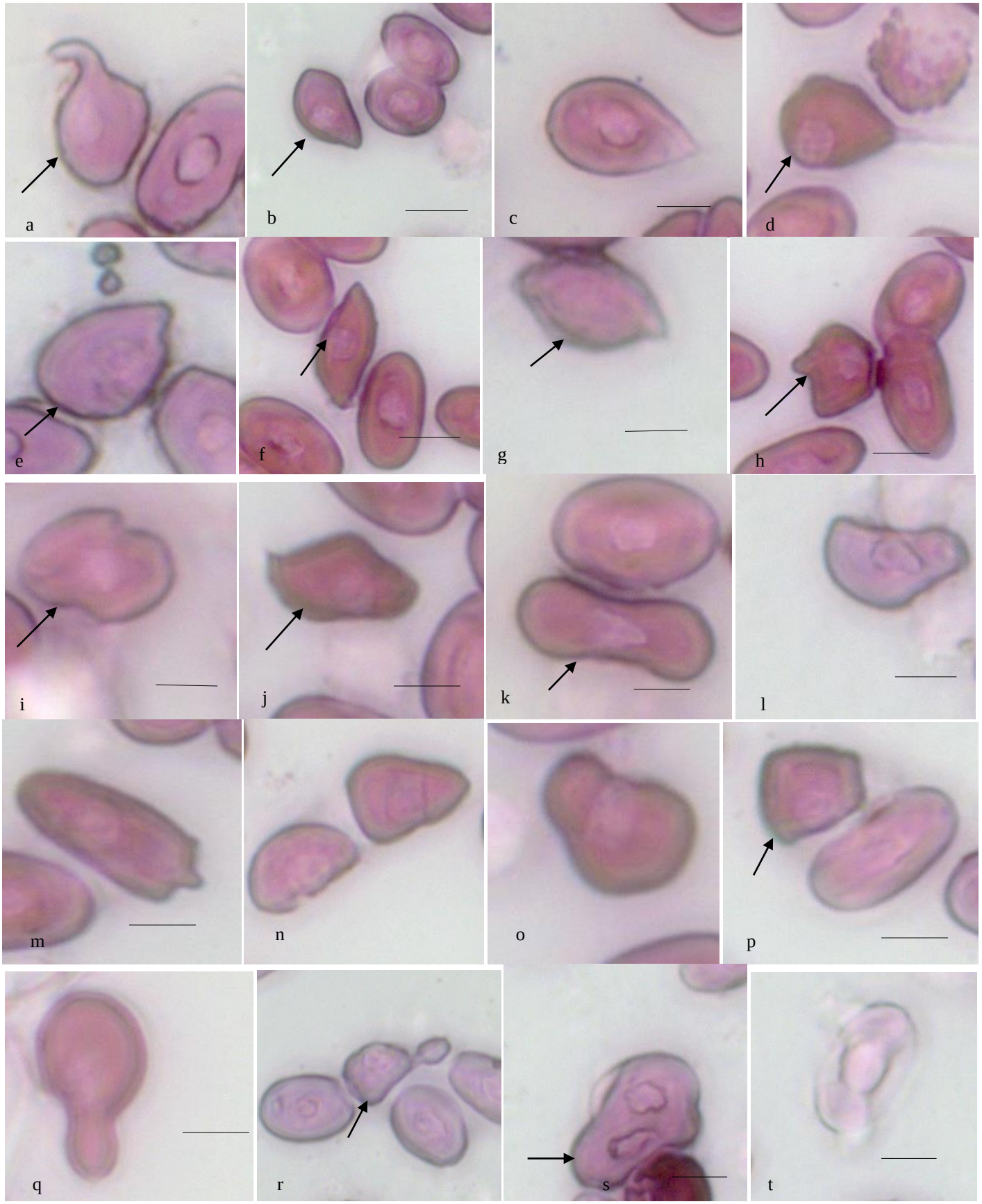


Figure-1: Different shapes of erythrocytes. (a) Normal RBCs, (b) Ovoid shaped RBCs, (c) Ellipsoid erythrocyte, (d) RBC with irregular shaped nucleus, (e) Circular RBC, (f) Circular RBC having eccentrically placed nucleus, (g) Circular erythrocyte lacking nucleus, (h) RBC having no distinct cell membrane, (i) and (j) Comma shaped erythrocytes.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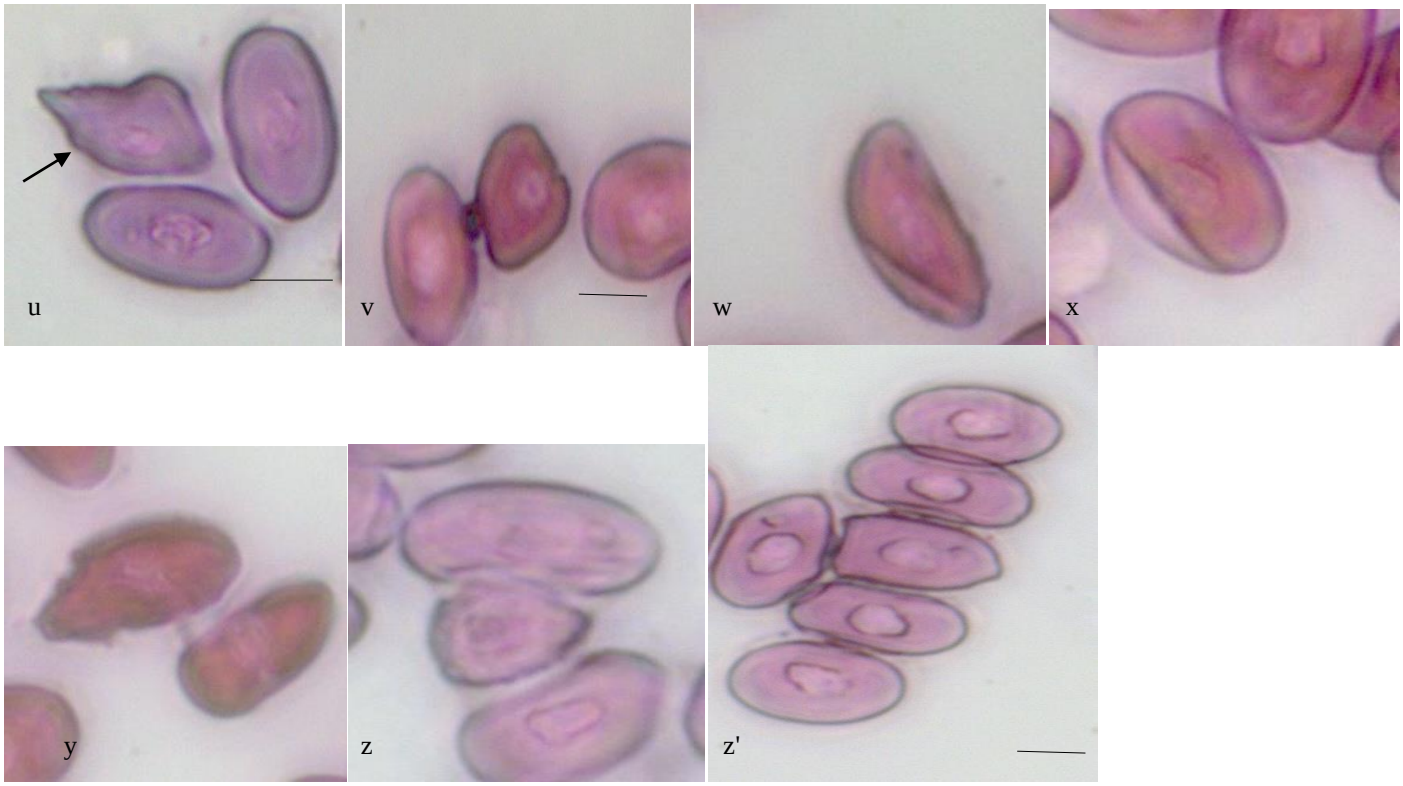

Figure-2: Erythrocyte peculiarities. (a) Comma shaped peculiar erythrocyte, (b) Tear drop shaped erythrocyte (dacryocyte) having peripheral nucleus, (c) Tear drop shaped erythrocyte having centrally placed nucleus, (d) Tear drop shaped erythrocyte having eccentrically placed nucleus, (e) Tear drop shaped erythrocyte lacking nucleus, (f) Spindle shaped erythrocyte lacking nucleus, (g), (h), (i), (j), (k), (l), (m), (n), (o), (p) different shapes of erythrocytes, (q) Erythrocyte with one bulging end, (r) Erythrocyte having separated bulging tip, (s) Dividing erythrocyte, (t) Degenerating erythrocyte, (u) Fragmented erythrocyte (schistocyte), (v) Rhombic shaped erythrocyte, (w) and (x) Partially folded erythrocytes, (y) and (z) Tear drop shaped erythrocytes with early crenulation, (z') Rouleax formation. (Scale bar= 20 micron in 40X objective)

The present study provides the leucocytes of five types namely lymphocytes, monocytes, heterophils, eosinophils and basophils in the peripheral blood smears of Western pond turtle (*Actinemys marmorata*). In the smears of blood both small (Figure-3a) and large lymphocytes (Figure-3b) were seen. The small lymphocyte was a small and round cell with a nucleus having clumped chromatin and a thin rim of cytoplasm was surrounded to it (Figure-3a). Large lymphocytes areas like as small lymphocytes only having a large round cytoplasm (Figure-3b). Monocyte was also large cell having a slightly indented nucleus (Figure-3c) but in comparison to large lymphocyte slightly. Less clumped chromatin present in it. Dense cytoplasmic granules are present in heterophils. Their nuclei were small and eccentric and placed close to the periphery of cell (Figure-3d).

Eosinophils were round in shape having eccentrically bulging nuclei. The nuclei were generally oval or round in shape and cytoplasm were filled by large, bright red color cytoplasmic granules (Figure-3e and 3f). Basophils present were round in shape and contains purple color nucleus. The cytoplasm contains purplish granules having butterfly shaped nucleus (Figure-3g) and irregular nucleus (Figure-3f).

Discussion: In this present study elliptical shaped nucleated erythrocytes having abundant pale pink cytoplasm were observed like those of other turtle species[8,9]. Turtles have several primitive cells in the circulating blood, namely red blood cells with nuclei help in transportation of oxygen and for clotting process thrombocytes were. There. For defense mechanism neutrophils, lymphocytes, basophils, monocytes, eosinophils, heterophils and azurophils present in the blood of turtle[10-13].

In reptiles, blood cell parameters may be influenced by several factors, such as age, sex, seasonality, reproduction, nutritional status and environmental parameters such as temperature, salinity, oxygen and light[14-23] and through the annual cycle and throughout the life of the individuals these parameters can vary. Different authors described circulating blood cells of different amphibian and reptilian species; moreover there are many chelonian species for those blood cell morphology and reference values are imprecise or still unknown.

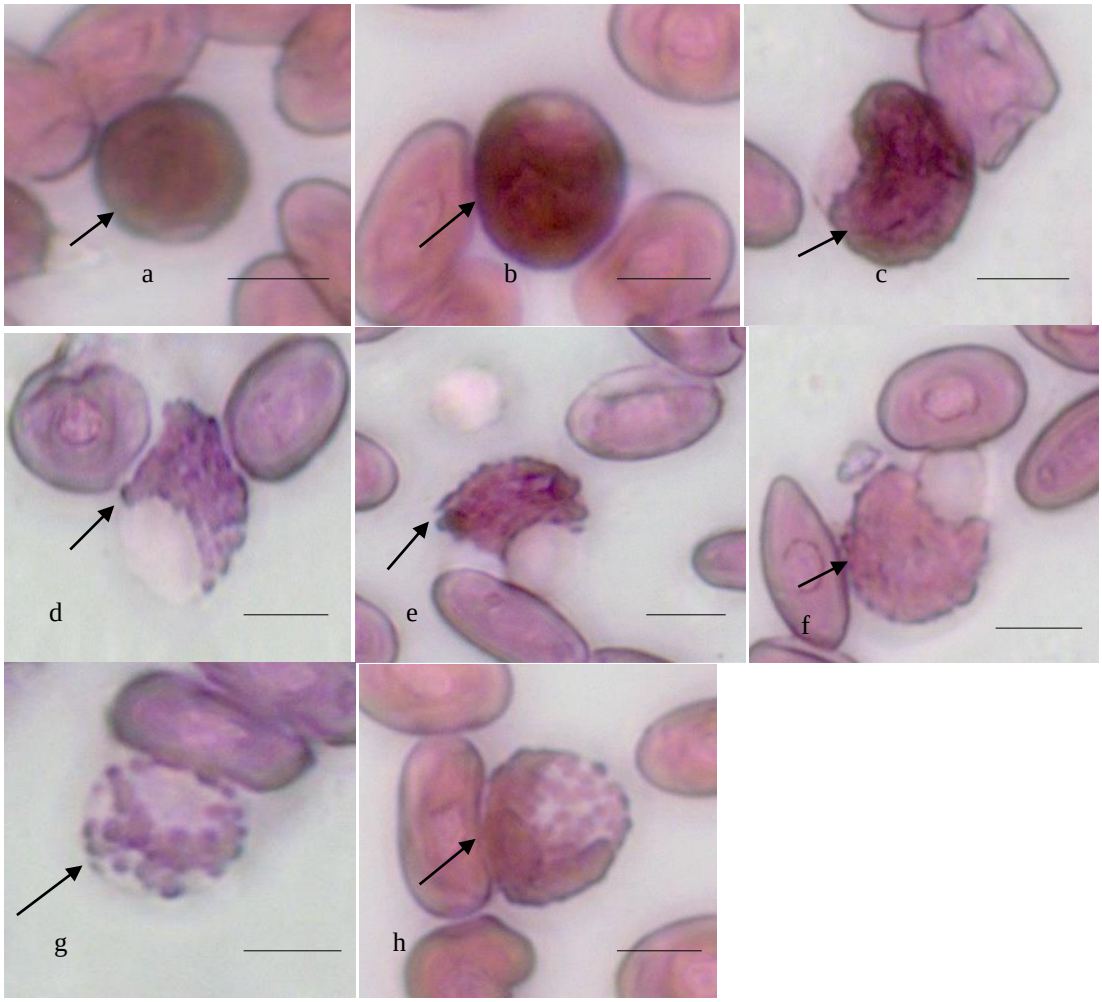

Figure-3: Different types of leucocytes. (a) Small lymphocyte, (b) Large lymphocyte, (c) Monocyte, (d) Heterophil, (e) and (f) Eosinophils, (g) Basophil having butterfly shaped nucleus, (h) Basophil (Scale bar= 20 micron in 40X objective).

Conclusion

By the help of this study baseline information regarding description and mirphological classification of blood cells of Western pond turtle is known by which their physiological states can also be known and they can be better treated. The data serve as a baseline for future hematologic studies in this species.

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