



International Journal of
**Zoological
Research**

ISSN 1811-9778



Academic
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**Morphological Observations on the Thrombocyte of
Eastern Sarus Cranes (*Grus antigone sharpii*)
in Northeastern Thailand***

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Abstract: Blood samples from eastern sarus cranes housed at Nakhonratchasima Zoo, Nakhon Ratchasima, northeastern, Thailand were collected in January, 2007. A morphological observation of the thrombocytes was examined using scanning electron microscopy. The results revealed the following information: The thrombocytes of the eastern sarus cranes are tiny cells and they display a smooth, irregular, spherical and pseudopodic membrane surface with long microfilament protrusions from their membrane. The thrombocytes of the eastern sarus cranes use these microfilaments to attach to other blood cells for movement and blood clumping. The long microfilaments were not found in fish, reptiles and other domestic birds. The morphological structure of the thrombocyte, the microfilaments and the activities of the eastern sarus crane has not been reported previously.

Key words: Eastern sarus cranes (*Grus antigone sharpii*), thrombocyte, microfilament, SEM

INTRODUCTION

The eastern sarus crane (*Grus antigone sharpii*) is the world's tallest flying bird (Jeltes, 2006). As one of three *Grus antigone* subspecies, it formerly inhabited Indochina; however, its population there has been greatly reduced in distribution and number. Flocks have been recently located in Myanmar, Cambodia and Vietnam, but it appears as if the population may now be fragmented into two separate sub-populations. Total numbers are estimated to be between 1,000 and 1,600 birds (Zoo, 1997). Jeltes (2006) reported that in this decade the total population of the eastern sarus crane has been reduced to between 500-1,500 birds. At present, sarus cranes are currently listed as vulnerable A1cde + 2cde on the IUCN red list. Nakhonratchasima Zoo obtained their eastern sarus cranes from the forests in Cambodia and keeps them in cages in the breeding section and public viewing section of the Zoo. At present, Nakhonratchasima Zoo has more than 40 eastern sarus cranes.

Thrombocytes have their origins in bone marrow and play a primary role in hemostasis (Sturkie, 1965) clot promotion (Shepro *et al.*, 1969) and tendency to clump in peripheral blood films

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*Originally Published in International Journal of Zoological Research, 2008

and may also have a phagocytic function to aid in the removal of foreign material from the blood (Campbell, 1995). Whereas, documents about the thrombocyte's characteristics in the eastern sarus crane are limited, the objective of this preliminary study was to examine the morphological aspect of the thrombocytes in eastern sarus crane by using SEM. Basic knowledge from this examination is important to any future in depth study of the hematology of this bird.

MATERIALS AND METHODS

Birds

Two eastern sarus cranes (male and female) were reared in the breeding section of Nakhonratchasima Zoo, Nakhon Ratchasima Province northeastern Thailand. Their blood samples were collected in January, 2007. Cranes were restrained manually and five milliliters of blood sample were collected from the wing vein (Rithchie *et al.*, 1994) using a 10 mL syringe, 21 gauge needle and 1.5 inch of length then placed in plastic tube with EDTA. The samples were cooled to approximately 4°C (Campbell, 1995) using icepacks and then taken to the Central Instrumentation Units, Faculty of Science, Mahasarakham University within 12 h of blood collection.

SEM Technique

Blood samples were dropped in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 overnight at 4°C then washed in the same buffer. Samples were postfixated with 1% Osmium tetroxide for 2 h then rinsed with distilled water, dehydrated in 20, 40, 60, 80 and 100% acetone and left to air dry. Gold coated blood films were examined with a SEM (JSM 6460LV).

RESULTS AND DISCUSSION

Thrombocytes of the eastern sarus crane were examined by using SEM. The results revealed the following information: The thrombocytes are tiny cells (diameter = 2-3 μm); with a smooth, irregular and pseudopodic membrane surface with long microfilaments protruding from the membrane (Fig. 1A-H). The cell size of the thrombocytes in the eastern sarus crane were smaller than that of the chickens by about 2 times. Daimon and Uchida (1978) found that mature thrombocytes of the white leghorn chickens measured approximately 10 μm in length and 5 μm in width. Salakij *et al.* (2002) reported that the thrombocytes of the king cobra had an elongated shape with a smooth membrane. Moreover, Santos *et al.* (2003) and Sano-Martins *et al.* (1994) found that the thrombocytes of snakes and the roadside hawk, normally elliptical in shape, become spheroid with cytoplasmic protrusions and adhere to one another. This was accordant with the shape of the thrombocytes of the eastern sarus cranes in this study.

The membrane surface of the thrombocyte of the eastern sarus crane was similar to that reported by Shepro *et al.* (1969) and Maxwell (1974). They found that under ultra-structural observation, the membrane surface in dogfish and domestic birds had many forms: smooth, irregular, pseudopodia, lobopodia and villi like; whereas, the long microfilaments, which were found in the eastern sarus cranes, were not found in fish, reptiles and other domestic birds. Figure 1 showed that the thrombocytes of the eastern sarus cranes used their microfilaments to attach to other blood cells. Moreover, the thrombocytes used these structures for blood clumping.

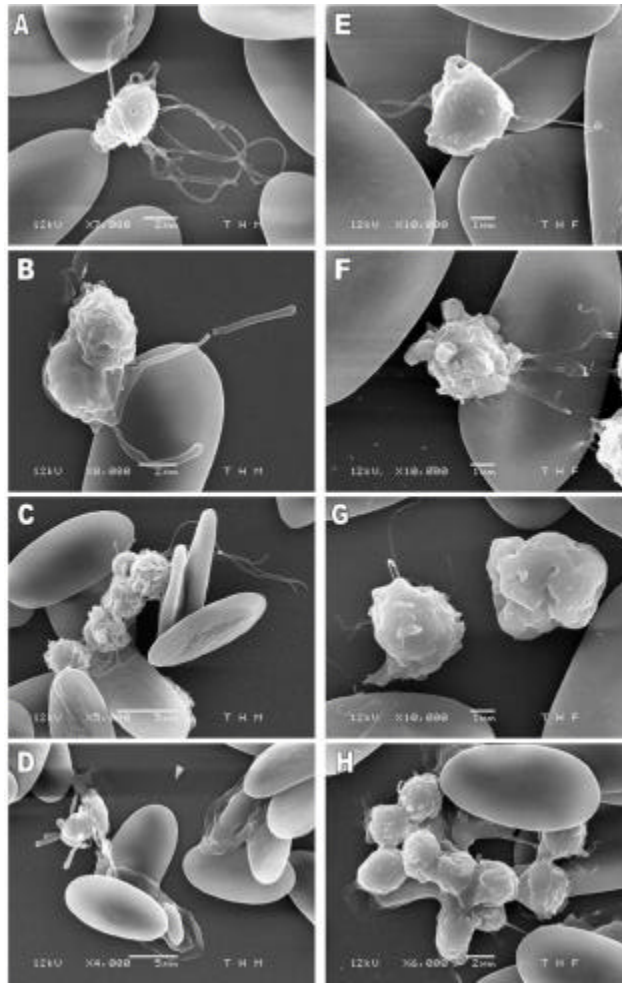


Fig. 1: SEM micrograph of thrombocyte in eastern sarus cranes (*Grus antigone sharpie*) (A) normal thrombocyte with long microfilament, (B) thrombocyte with microfilament attached to leukocyte and red blood cell, (C) clumping of thrombocyte, (D) thrombocyte with microfilament attached to mature and young red blood cell, (E) thrombocyte with microfilament attached to red blood cell, (F) thrombocyte with microfilament attached to red blood cell, (G) thrombocyte with microfilament and (H) clumping of thrombocyte (E, F and G = 1 µm; A, B and H = 2 µm; C and D = 5 µm)

CONCLUSIONS

The thrombocytes of the eastern sarus cranes are small and their surface membrane had several forms with long microfilament protrusions from their membrane. They used the microfilaments for

blood clumping and to attach to other blood cells. No previous reports on the morphological structure of the thrombocytes, the microfilaments or the activities of the eastern sarus cranes have been published.

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