Description of a New Haemogregarine, *Haemogregarina sundarbanensis* n. sp. (Apicomplexa: Haemogregarinidae) from Mud Turtle of Sundarban Regions, West Bengal, India

Hindistan (Batı Bengal)'daki Kanat Kabuklu Çamur Kaplumbağası'ndan Yeni Bir *Haemogregarin Haemogregarina sundarbanensis* n. sp. (Apicomplexa: Haemogregarinidae)'in Tavsifi

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ABSTRACT

Objective: The aim of this study was to provide a description of a new haemogregarine, *Haemogregarina sundarbanensis* n. sp., from mud turtles collected from Sundarban regions, West Bengal, India.
Methods: The turtles were collected from fish markets near Canning, South 24-Parganas District, West Bengal. Thin blood films were prepared using the blood drawn from the head and feet. The blood films were then air-dried, fixed in absolute methanol, and stained with Giemsa.
Results: The erythrocytic stages could be differentiated into small and large forms. The young gamonts measured 7.896×3.469 µm, microgamonts measured 8.876×4.425 µm, and macrogamonts measured 8.919×4.272 µm. The cytoplasm of the macrogamonts was stained deep blue with Giemsa, and many metachromatic granules are found uniformly distributed. The oval or rounded nucleus was central in position and stained deep red with Giemsa stain, whereas in microgamonts, the nucleus was not at all compact but with a few dispersed chromatin granules.
Conclusion: The prevalence of infection was found to be 33.3% (5/15). The infected erythrocytes reveal shape alteration, marginal and atrophic nucleus, and were larger than non-parasitized erythrocytes. The cytoplasm of the infected erythrocytes stained darker than that of the uninfected erythrocytes. *(Turkiye Parazitol Derg 2015; 39: 131-4)*

Keywords: Haemogregarine, *Haemogregarina sundarbanensis* n. sp., erythrocyte, India

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ÖZET

Amaç: Bu çalışmanın amacı Hindistan'ın Batı Bengal bölgesindeki çamur kaplumbağasından yeni bir hemogregarin türünün, *Haemogregarina sundarbanensis* n. sp. (Apicomplexa: Haemogregarinidae)'in tavsifidir.
Yöntemler: Kaplumbağalar Batı Bengal bölgesindeki balıkhanelerden elde edilmiştir. İnce kan filmler baş ve ayaktaki kandan yapılmıştır. Kan filmleri hava yardımıyla kurutulmuş, absolü metal alkolde tespit edilmiş, Giemsa boyalarıyla boyanmıştır.
Bulgular: Eritrositik safhalar küçük ve büyük formlara farklılaşabilirler. Genç gamontlar 7,896x3,469 µm, mikrogamontlar 8,876x4,425 µm, makrogamontlar 8,919x4,272 µm'dir. Makrogamontların sitoplazması Giemsa'yla koyu mavi boyanmıştır ve çok sayıdaki metakromatik granüller düzgünce dağılmıştır. Orta pozisyondaki oval veya yuvarlak nukleus Giemsa'yla koyu kırmızı boyanmıştır, fakat mikrogamontlarda nukleus tamamiyle kompakt değildir ve bir kaç dağılmış kromatin granülleri içerir.
Sonuç: Enfeksiyon yaygınlığı %33,3 (5/15)'tür. Enfekte eritrositler şekil değişikliği, marjinal ve atrofik nukleus gösterirler ve parazitlenmemiş eritrositlerden daha büyüktürler. Enfekte eritrositlerin sitoplazması enfekte olmamış eritrositlerden daha koyudur.
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Anahtar Sözcükler: Hemogregarin, *Haemogregarina sundarbanensis* n. sp., eritrosit, Hindistan

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INTRODUCTION

Intracellular haemogregarine parasites are often found in fresh water turtles, appearing as banana-shaped structures in the cytoplasm of the host erythrocytes. *Haemogregarina stepanowi* was first described from the European tortoise *Emys orbicularis* (Linn.) (1). Detailed account of the structure and life cycle of *H. stepanowi* in the same tortoise was established (2). After that, a number of studies have described different species from different parts of the world (3-8).

In India, chelonian haemogregarines were studied (9). The author described two species, *H. laverani* and *H. mesnilli* from *Emydagranosa* and *Emys tectum*, respectively. In 1983, Bhatia in his monumental works on Fauna of British India mentioned a number of haemogregarine species described by different researchers from India (10). Two new haemogregarines were also reported from West Bengal, India (11, 12). In 1932, de Mello established a new species of haemogregarine in India (13).

The present paper deals with a new haemogregarine that infects the erythrocytes of a common mud turtle, *Lissemys punctata punctata* (Bonnaterre), from Canning, Sundarbans region, West Bengal, India.

METHODS

The turtles were collected from fish markets near Canning, South 24-Parganas District, West Bengal. These turtles were reported to be collected from the ponds near Port Canning of the Sundarbans region, West Bengal, India in the months of March–April, 2012. Thin blood films were prepared using the blood drawn from the head and feet by hypodermic syringes. The turtles were kept alive in a small reservoir along with small fishes and water weeds, such as *Hydrila*, and were observed at regular intervals.

The blood films were then air-dried, fixed in absolute methanol, and stained with Giemsa. Photomicrographs were taken with the help of a Carl Zeiss high-resolution microscope using a Digital Olympus Camera. The measurement and volume of the different stages of the parasite was taken using the LAS software, version 4.1.0 (Leica, Watzlar, Germany). To visualize the periphery of the parasites, the outline drawings were done using the related software.

The Type slides bearing no. HG/PARA/12 has been deposited in the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani, West Bengal, India.

RESULTS

Haemogregarina sundarbanensis n. sp.
Type-host: *Lissemys punctata punctata* (Bonnaterre)
Type-locality: Champahati, Canning, South 24 Parganas, West Bengal
Site of infection: Erythrocytes
Prevalence: Out of the 15 turtles, five (33.3%) were found to be infected.

Vector: Unknown

Description

Young Gamonts

They are elongated with one end narrower than the other which is blunt. They measure 7.896×3.469 µm with an average area of 20.794 µm^2. The cytoplasm is densely granular and stains light blue with Giemsa stain. The oval nucleus is central in position measuring 2.51×1.80 µm. It was stained pink in color. The sexes cannot be separated in this stage (Figure 1 a, b, Figure 2 a).

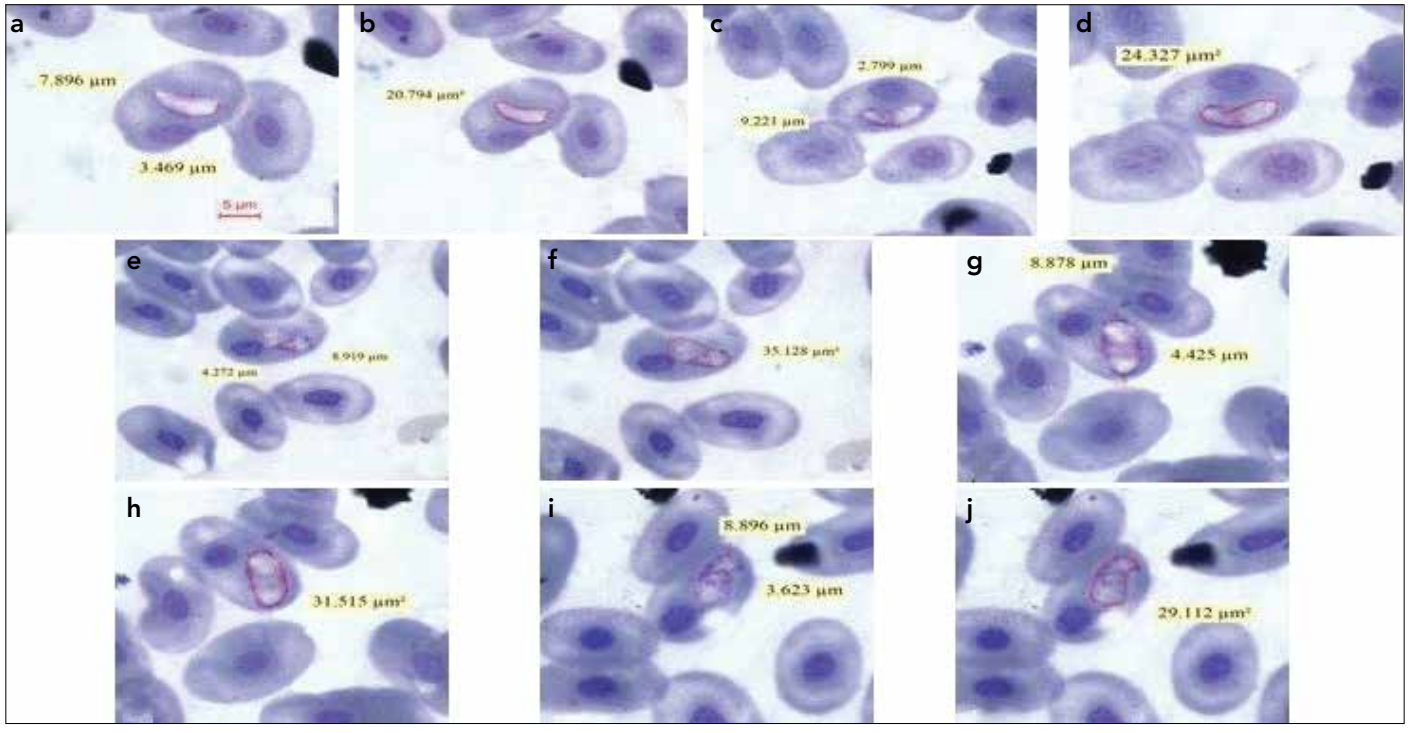

Figure 1. a-j. Multiple stages of intraerythrocytic haemogregarine gametocytes (a, b), young gamonts; (c, d), mature gamonts; (e, f), macrogamonts; (g, h), microgamonts; and erythrocytic schizogony (i, j)
The blood films were stained with Giemsa. The measurement and volume of the different stages of the parasite has been taken using the LAS software, version 4.1.0 (Leica, Watzlar, Germany).

Table 1. Comparative morphometric measurements of different *Haemogregarina* sp. of turtle recorded from India (All measurements in micrometers)

	H. choudhuryi **Ray et al. (12), 1984**	***H. gangetica*** **Misra (11), 1976**	***H. xaveri*** **de Mello (13), 1932**	***H. sundarbanensis*** **n. sp.**
Macrogametocytes	8.5×3.5 µm	9.6×4.8 µm	7.5–13.5 µm×2.5–6.2 µm	8.919×4.272 µm
Microgametocytes	8.5×2.0 µm	9.16×3.20 µm	9–10 µm×4.0 µm	8.876×4.425 µm
Schizonts	In erythrocytes and the lung tissue	In the lung tissue	In the lung, liver, and spleen tissue	In erythrocytes
Hosts	Lissemys punctate punctata	Trionyx gangeticus	Lissemys punctata	Lissemys punctate punctata

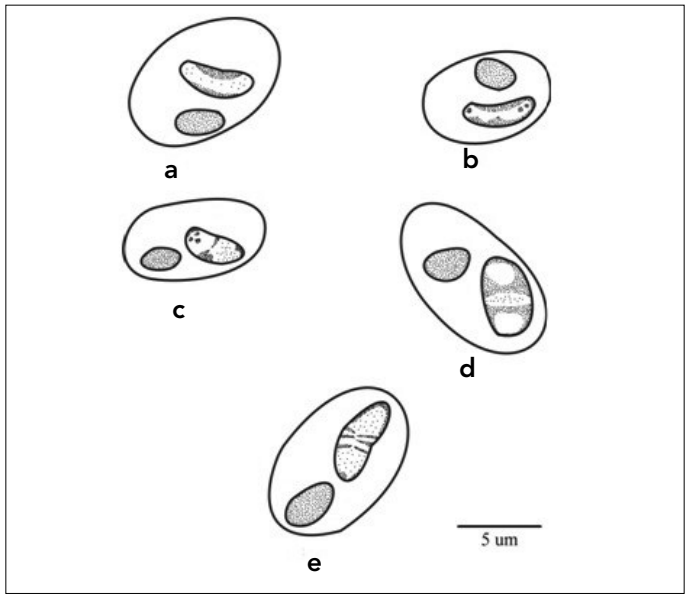

Figure 2. a-e. Drawings of multiple stages of intraerythrocytic haemogregarine gametocytes (a), young gamonts; (b), mature gamonts; (c), macrogamonts; (d), microgamonts; and (e), erythrocytic schizogony

Mature Gamonts

They are crescent shaped with both the ends rounded, measuring 9.221×2.799 µm with an average area of 24.327 µm^2. The cytoplasm is densely granular and stains blue with Giemsa stain. The sexes of the mature gamonts are quite apparent. The macrogamonts are elongated or "Banana-shaped" with both the ends equally rounded. The cytoplasm stains deep blue with Giemsa and many metachromatic granules are found uniformly distributed. The oval or rounded nucleus is central in position and stains deep red with Giemsa stain. They measure 8.919×4.272 µm with an average area of 35.128 µm^2. The microgamonts are smaller in size than the macrogamonts. They are also "kidney or bean shaped" with a few metachromatic granules and stains light blue with Giemsa. The nucleus is not at all compact but has a few dispersed chromatin granules. They measure 8.876×4.425 µm with an average area of 31.515 µm^2 (Figure 1 c-h, Figure 2 b-e).

Infected Erythrocytes

The infected erythrocytes demonstrate shape alteration, marginal and atrophic nucleus, and are larger than non-parasitized erythrocytes. The shape alterations consisted in lengthening of the red blood cells and presence of various abnormal shapes. Sometimes, the nucleus of the infected erythrocytes is displaced at one end. The cytoplasm of the infected erythrocytes became darker than the uninfected erythrocytes.

Erythrocytic Schizogony

As the parasites enlarge, the cytoplasm becomes vacuolated and division of the nucleus is initiated. Many erythrocytic schizonts were observed in the red blood cells of the circulating blood. Binucleate and tetranucleate schizonts commonly occurr. Successive divisions result in the formation of eight merozoites. The mature schizonts measure 8.896×3.623 µm with an average area of 29.112 µm^2. The merozoites are elongated with both the ends tapering. They are measure 5.0×1.0 µm with a central oval nucleus (Figure 1 i, j, Figure 2 e).

DISCUSSION

After reviewing earlier research work, it can be reported that there are many species of chelonian haemogregarines reported both from India and abroad. Moreover, there is confusion regarding the taxonomic status of those species described.

The present species has some resemblances with *Haemogregarina choudhuryi, H. balli*, and *H.stepanowi* by having similar type of erythrocytic schizonts with 6–10 merozoites. However, it differs from them in the type of localization of schizonts. Reichnow (1910) observed 12–24 merozoites in the erythrocytic schizonts and bone marrow. Paterson et al. (6) (1976) recorded the presence of the schizont of *H. balli* in a variety of cells of the liver, lungs, and spleen. In contrast, *H. sundarbanensis* shows only erythrocytic schizonts. It has some similarities with *H. pseudemydis* by not having tissue schizonts, and it differs from the same species in having 35–140 merozoites in a schizont in the erythrocyte and leucocytes. In *H. pseudemydis*, the sporogonic development is also unknown (Table 1).

CONCLUSION

The parasite under report differs from all the haemogregarines described so far from turtles and tortoises in morphometric parameters and other characteristics in detail. Because of it novelty, it is designated as *Haemogregarina sundarbanensis* sp. n. as it is named after the locality from which it was obtained, i.e., the Sundarbans region of West Bengal, India.

Ethics Committee Approval: The approval of the ethics committee has not been obtained because the host animal has not been killed.

Informed Consent: N/A.

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