Original Article

Study of Morphological Characteristic and Prevalence of *Haemoproteus* Blood Parasite in Passerines in Bung Boraphet

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Abstract

Haemoproteus is one of the world's widespread avian blood parasite. However, it is rarely reported in Thailand; even at Bung Boraphet, which is an important wetland bird area located in the central part of Thailand, it still has never been reported. Therefore, information on species of haemoproteid and their epidemiology is important for the health of wildlife in Thailand. Samples of blood smear were collected from 633 passerines consisting of 6 orders, 15 families, 25 genera, and 35 species in total from Bung Boraphet. This is the first study to report 8 haemoproteid blood parasite species including *Haemoproteus herdiadis, H. fallisi, H. dicruri, H, payevski, H. otocompsae, H. sanguinis, H. paseris* and *H. orizivorae* infecting 9 avian host species. *Haemoproteus* species were identified and distinguished using morphological characters and physical measurement. Prevalence of haemoproteids infection was 12.01±0.46. This prevalence varied within avian species. The highest prevalence was found in *Lonchura punctulata* infected with *H. orizivorae* and the lowest prevalence was found in *Rhipidura javanica* infected with *H. fallisi*. Moreover, the study found relationship between *Haemoproteus* infection and feather's ectoparasite in all avian samples except in every avian host population that was infected with *Haemoproteus*.

Keywords: birds, Bung Boraphet, haemoproteid, *Haemoproteus*, identification, prevalence

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Introduction

Bung Boraphet, located in the central part of Thailand, is Thailand's largest freshwater wetland. On top of that, it has been classified as an Important Bird Area (IBA), where grassland and larval insect food sources are abundant, suitable for bird nestling or transition station for migratory birds all year round. Therefore, using this location for field investigation into natural birds is appropriate. The ecological appropriateness in this wetland provides suitable conditions for infectious pathogens to transmit, especially vector borne pathogens including Japanese encephalitis virus (JEV). Moreover, there are bird communities all year round, zoonotic diseases such as avian influenza (AI) virus can therefore be possible (Tiawsirisup and Nuchprayoon, 2010; Ueta and Ryabtsev, 2001; Newman et al., 2012; Chaichana and Choowaew, 2013). Several studies investigated the role of bird community in infectious disease which had impact on public health, however information on the epizootic disease in bird communities of Thailand has been rare. Consequently, the purpose of the present study was to describe any important information about infectious agent in bird communities.

Basic information on haematozoa in bird community such as species level and pattern of infection is necessary for haematozoa investigation. *Haemoproteus* is an interesting haematozoa according to its reported abundance. Results of this study can be used a baseline information on blood parasite and epizootic disease in bird community in Thailand. Consequently, this investigation aimed to identify *Haemoproteus* at species level and its prevalence in bird community, as well as any factor associated with other infections.

Materials and Methods

***Bird sample collection*:** Bird samples were collected throughout the year during February 1999 to January 2000 using mist-nets at Bung Boraphet (15°40′-15°45′N, 100°10′-100°23′E), Nakorn Sawan province. The mist-nets were set in the early morning around 4:00 a.m., before the birds came out of their nests, until 10:00 a.m. The mist-nets were monitored every 15 minutes and trapped birds were put in transparent wool bags. The trapped birds were identified by using morphological characters, following the bird guide of Thailand, and biological measuring information was used as supporting information. Bird species, sex and age were recorded for classification in species level (Bunsong, 1991).

***Haemoproteid blood parasite collection*:** Thin blood smears were obtained from brachial wing vein of each bird, and they were stopped from bleeding before release. The smears were air-dried before being fixed with absolute methanol, then stained with 3% Giemsa, prepared in phosphate buffered solution at pH 7.2, for 45-60 minutes.

***Haemoproteid blood parasite identification*:** Microscopic examination was performed with oil immersion objective for 20-30 minutes for each blood smear sample. *Haemoproteus* parasites were identified by gamete's morphological characters which differentiated by morphological forms of *Haemoproteus* (Bennett and Peirce, 1988), and physiological measurement as previously performed by Bennett and Campbell (1972), the traditional *Haemoproteus* identification using Zeiss compound microscope with MRC camera and measurement with Axio version 4.8.1 software. Nuclear displacement ration (NDR) was calculated by 2X/(X+Y), where X is the distance between nuclear membrane to cell membrane of the host cell and Y is the length of the opposite side that the parasite occupies. All information collected was used to identify the species. Short description of important morphology characters of each species is shown below. The morphological characters distinctly differentiated the closely related genera of blood parasite: *Haemoproteus, Plasmodium* and *Leucocytozoon*, by blood stages and pigment presentation. The pigments were present in both genera *Haemoproteus* and *Plasmodiun*, but absent in the genus *Leucocytozoon*. The erythrocytic merogony stages were present only in the genus *Plasmodium,* but absent in the genus *Haemoproteus.* Although recently new species have been reported and a fragment of sequencing mitochondrial (mt) DNA is the supporting information in species description, traditional morphometric characters and life story of bird host are still necessary in taxonomic study (Valkiunas et al., 2009).

***Epidemiological study of haemoproteid*:** Each blood smear slide was examined at 1000x magnification for 10 minutes or until 100 parasites were found (Riper III et al., 1986). Only one parasite found during the examination was considered as a positive slide. Percentage of positive samples was calculated with standard deviation and 95% confident intervals (CI), the percentage reflecting prevalence of infection was defined in parasitology's ecological terms (Margolis et al., 1982). Difference between each avian hosts' infection prevalence was determined by chi square test. P value of 0.05 or less was considered significant.

***Relationship study with feather ectoparasite infection*:** During sample collection, additional information on feather ectoparasites was also recorded with infection status, without identification to species level. Normally, lice and mite infect feathers of the host. The infections of both *Haemoproteus* and feather ectoparasites were examined by Pearson's chi square test for dependencies, and relative risk (prevalence study) and odd ratio (incidence rate) were calculated to estimate relationship between both parasitic infections.

Results

Haemoproteid identification: Bird samples and blood smears were collected from a total of 633 birds comprising 35 species within 15 families and 6 orders. Only 5 families from 2 orders were infected. In the first order, Ciconiformes, only one species (*Ixobrychus sinensis*) of the Aedeidae family was infected with *Haemoproteus herodiadis* and in the second order, Passeriformes, 4 families out of 8 were infected with 7 other haemoproteids. As for the order Passeriformes,

firstly there were 2 species, *Rhipidura javanica* and *Dicrurus macrocercus* of the Corvidae family, which were infected with *H. fallisi* and *H. dicruri*, respectively. Secondly, *Acrocephalus bistrigiceps* and *A. orientalis* of the family Sylviidae were infected with *H. payevski*. Thirdly, *Pycnonotus goiavier* and *P. blanfordi* of the family Pycnonotidae were infected with *H. otocompsae* and *H. sanguinis*, respectively. Lastly, *Ploceus philippinus* and *Lonchura punctulata* of the family Passeridae were infected with *H. paseris* and *H. orizivorae*, respectively.

The measurements of the morphological character and RBCs infected with 8 *Haemoproteus* species are shown in Table 1, and distinct characters of all 8 haemoproteid parasitic species are described below.

Table 1 Morphological characters of *Haemoproteus* in avian host families (genus) with morphological parasite observation and dominated character of *Haemoproteus* species and morphological impact on host's red blood cells (RBCs). Information are shown on both microgametocyte and microgametocyte of parasitic protozoa.

Family of host (Genus)	Species of parasitic protozoa	Morphological character				Efficacy of parasitical infection		
		Shape	Pigment	Outline	Highlight	NDR	%Hypertrophy	%P*HPC^{-1}
Aedeidae (*Ixobrychus*)	*Haemoproteus herodiadis*	M	5-7	E	MD	0.61/0.65	6.85/10.04	45.01/38.78
Corvidae (*Rhipidura*)	*Haemoproteus fallisi*	M	5-10	PA	MD	0.64/0.67	10.15/13.06	45.97/39.74
Corvidae (*Dicrurus*)	*Haemoproteus dicruri*	H	10-11	PA/ME	MD	0.46/0.62	16.89/8.09	55.41/52.00
Sylviidae (*Acrocephalus*)	*Haemoproteus payevski*	H	11-13	PA/E	MD	0.74/0.77	15.61/18.31	56.41/52.01
Pycnonotidae (*Pycnonotus*)	*Haemoproteus otocompsae*	H	11-13	E/PA	MD	0.74/0.75	16.79/15.47	55.93/54.48
Pycnonotidae (*Pycnonotus*)	*Haemoproteus sanguinis*	H	8-12	E	MD	0.74/0.85	14.35/17.87	56.93/36.15
Estrildidae (*Ploceus*)	*Haemoproteus paseris*	H	8-13	IA/ME	MD	0.54/0.68	19.20/20.23	50.76/51.21
Estrildidae (*Lonchura*)	*Haemoproteus orizivorae*	H	13-15	E	MD	0.62/0.72	25.61/20.49	62.46/62.06

Morphological characters belong to five basic shapes of gametocytes defined as circumnuclear form (C), discoid form (D), halteridial form (H), microhalteridial form (M) and rhabdosomal form (R) (Bennett and Peirce, 1988). Pigment is the number of pigment granules found in microgametocyte in mature gametocyte. Outline of growing gametocytes wavy: entire margin (E), amoeboid (A), poles amoeboid (PA), immature amoeboid (IA) and mature entire (ME). Highlight is the erythrocyte nucleus feature that the *Haemoproteus* caused marked displacement (MD) and appearing bilobed (B). Nucleus displacement ratio (NDR) was used to indicate the degree of lateral displacement of the erythrocyte nucleus caused by the gametocyte. Percentage of hypertrophy (%hypertrophy) is the ratios between the increase erythrocytes and its nucleus due to invasion by the *Haemoproteus*. Percentage of host-parasite complex (%P*HPC^{-1}) is the ratio between the parasite area and the infected erythrocyte area.

Table 2 Prevalence of *Haemoproteus* spp. infection in different bird species with standard deviation and 95% confident intervals (CI) of each avian host species

Host Species	*Haemoproteus*	N(n)*	%	95% CI	
All	*Haemoproteus* spp.	633(76)	12.01±0.46	12.90	11.11
Ixobrychus sinensis	*Haemoproteus herodiadis*	28(1)	3.57±0.12	3.81	3.34
Rhipidura javanica	*Haemoproteus fallisi*	38(1)	2.63±0.08	2.79	2.47
Dicrurus macrocercus	*Haemoproteus dicruri*	28(3)	10.71±0.41	11.51	9.92
Pycnonotus goiavier	*Haemoproteus otocompsae*	5(1)	20.00±0.77	21.52	18.48
Pycnonotus blanfordi	*Haemoproteus sanguinis*	41(11)	26.83±1.05	28.88	24.78
Acrocephalus bistrigiceps	*Haemoproteus payevski*	6(1)	16.67±0.64	17.93	15.41
Acrocephalus orientalis	*Haemoproteus payevski*	87(12)	13.79±0.53	14.83	12.76
Ploceus philippinus	*Haemoproteus paseris*	51(5)	9.80±0.37	10.53	9.08
Lonchura punctulata	*Haemoproteus orizivorae*	62(41)	66.13±2.61	71.24	61.02

*N(n) is number of birds examined and number of infected birds within the paraphrase

Haemoproteus herodiadis (1 to 5 in Fig 1) had one of the two microhalteridial haemoproteid forms. The outline of this parasite could entirely be seen, with 5-7 malarial pigments. There was marked displacement of the host's RBC nucleus (NDR: 0.61/0.65), while the host cell was slightly hypertrophied. The percentage of hypertrophy was 6.85/10.04% (caused by macrogametocyte and microgametocyte, respectively), and the parasite invaded 45.01/38.78% (macrogametocyte and microgametocyte, respectively) of the infected host cell.

Haemoproteus fallisi was another microhalteridial haemoproteid (6 to10 in Fig 1) with polar amoeboid outline, with 5-10 pigments (NDR: 0.64/0.67), causing the host cell to be slightly hypertrophied (10.15/13.06%), although the percentage of parasite area was 45.97/39.74% of the host-parasite complex.

Haemoproteus dicruri, found in the blood of *Dicrurus macrocercus*, was a halteridial haemoproteid (11 to 17 in Fig 1). The polar outline showed amoeboid form, except in mature gametocytes in which the outline was entirely distinct, with 10-11 malarial

pigments distributed around the nucleus in the cytoplasm. The nucleus was markedly displaced (NDR: 0.46/0.62), particularly with macrogametocyte infection. Hypertrophy of the host cell in macrogametocyte infection was greater than in microgametocyte infection, even if the percentage of the parasite in the host cell was not much different (55.42/52.00%).

Haemoproteus payevski was found in two species in the Sylviide families, *Acrocephalus orientalis* and *A. britrigiceps*, and had halteridial haemoproteid form (16 to 20 in Fig 1) with 11-13 pigments spread all over the cytoplasm around the parasite's nucleus. Immature Trophozoites showed a polar amoeboid form, but the gametocyte showed an entire outline. The nucleus of the host cell was obviously displaced with NDR 0.74/0.77. Host cell hypertrophy occurred less in macrogametocyte than in microgametocyte, although the percentage of parasite area in the host-parasite complex was not different.

Table 3 Relationship between genera and species of blood parasitic protozoa (*Haemoproteus*) and ectoparasite of each avian host

Avian host species	Parasite	Infection status		Ectoparasite infection status uninfected % (n)	infected % (n)
All host species	*Haemoproteus* spp.	uninfected	% (n)	49.25 (165)	28.66 (96)
		infected	% (n)	12.54 (42)	9.55 (32)
Ixobrychus sinensis	*Haemoproteus herodiadis*	uninfected	% (n)	89.29 (25)	7.14 (2)
		infected	% (n)	3.57 (1)	0.00 (0)
Rhipidura javanica	*Haemoproteus fallisi*	uninfected	% (n)	26.32 (10)	71.05 (27)
		infected	% (n)	2.63 (1)	0.00 (0)
Dicrurus macrocercus	*Haemoproteus dicruri*	uninfected	% (n)	71.43 (20)	17.86 (5)
		infected	% (n)	10.71 (3)	0.00 (0)
Pycnonotus blanfordi	*Haemoproteus sanguinis*	uninfected	% (n)	73.17 (30)	0.00 (0)
		infected	% (n)	24.39 (10)	2.44 (1)
Acrocephalus orientalis	*Haemoproteus payevski*	uninfected	% (n)	36.78 (32)	49.43 (43)
		infected	% (n)	5.75 (5)	8.05 (7)
Ploceus philippinus	*Haemoproteus paseris*	uninfected	% (n)	72.55 (37)	17.65 (9)
		infected	% (n)	9.80 (5)	0.00 (0)
Lonchura punctulata	*Haemoproteus orizivorae*	uninfected	% (n)	17.74 (11)	16.13 (10)
		infected	% (n)	27.42 (17)	38.71 (24)

Table 4 Prevalence study and incidence rate of *Haemoproteus* infection and ectoparasite infection

Avian host species	Parasite	Prevalence study %	95% CI		Incidence rate %	95% CI	
All avian host species		1.18±0.16	1.48	0.87	1.31±0.27	1.83	0.79
Infected with *Haemoproteus*							
All 8 avian species	*Haemoproteus* spp.	1.18±0.16	1.34	1.02	1.31±0.27	1.58	1.04
Acrocephalus orientalis	*Haemoproteus payevski*	1.02±0.26	1.53	0.50	1.04±0.63	2.28	-0.19
Lonchura punctulata	*Haemoproteus orizivorae*	1.23±0.26	1.75	0.71	1.55±0.54	2.61	0.49

Haemoproteus otocompsae was found in one out of the two species of *Pycnonotus*, which is *Pycnonotus goiavier*. It was a halteridial parasite (1 to 5 in Fig 2) with both amoeboid and entire polar outline seen. It had 11-13 pigments of the same size distributed within the cytoplasm around the parasite's nucleus. Host nucleus displacement could be noticed in both macrogametocyte and microgametocyte (NDR: 0.74/0.75). Furthermore, the hypertrophy of the infected cell was at intermediate level (16.76/15.47%) and more than half of the infected cell was occupied by the parasite (55.93/54.48%).

Haemoproteus sanguinis infected the other bird in *Pycnonotus*, *P. blanfordi*. The parasite had slightly halteridial form, with obvious outline. In the host cell with microgametocyte infection, the nucleus was normal with noticeable displacement (0.74/0.85). There were 8-12 pigments appearing in the parasitic cytoplasm. The effects on the host cell with *H. sanguinis* infection, both hypertrophy (14.35/17.87%) and percentage of parasites in the host-parasite complex (56.93/36.15%), were somewhat different between macrogametocyte and microgametocyte (6 to 8 and 9 to 10 in Fig 2, respectively).

Haemoproteus paseris was found in one species of Estrildidae, *Ploceus philippinus*. The parasite had halteridial form (11 to 15 in Fig 2), and immature amoeboid or mature amoeboid haemoproteid, with 8-13 pigments thoroughly distributed in the cytoplasm around the parasite's nucleus. The parasite slightly displaced the host's nucleus (0.54/0.68), however, causing major hypertrophy (19.20/20.23%) of the host cell, with 50.76/51.21% of parasites within the host-parasite complex.

Haemoproteus orizivorae infected *Lonchura punctulata*, which is another species in the Estrildidae family, and had halteridial form (16 to 20 in Fig 2) and an abundance of pigments (13-15), with entirely distinct outline and causing intermediate displacement of the host nucleus (0.62/0.72). Although there was no difference in the percentage of macrogametocyte and microgametocyte hypertrophy within the infected cell (62.46/62.06%), the hypertrophy of host cells infected with macrogametocytes tended to be more than in the

one that was infected with microgametocytes (25.61/20.49%).

***Prevalence of Haemoproteus*:** The prevalence of *Haemoproteus* infection was 12.01±0.46 (95% CI: 12.90-11.11) from 76 infected birds out of 633 (Table 2), calculated from 9 species of bird that were infected with 8 *Haemoproteus* species. The infection was found in 2 species of *Pycnonotus* infected with different *Haemoproteus* species, whereas 2 species of *Acrocephalus* birds were infected with a single *Haemoproteus* species. The prevalence of infection varied with each *Haemoproteus* species (n =9, df =8, P-value <0.005), where *Lonchura punctulata*, an avian host that was infected with *H. orizivorae* (41 out of 62 infected birds), had the highest prevalence (66.13%). Moreover, the second and third highest prevalence were *H. sanguinis* (infected *P. blanfordi*) and *H. otocompsae* (infected *P. goiavier*), respectively. On the other hand, The lowest prevalence was found in *Rhipidura javanicus* infected with *H. fallisi*, 2.63% (95% CI: 2.79-2.47), with only one bird out of 38 birds infected, while the second and third to the lowest prevalence was 3.57 and 9.80% found in *Ixobrychus sinensis* and *Ploceus philippinus* infected with *H. herodiadis* and *H. paseris*, respectively.

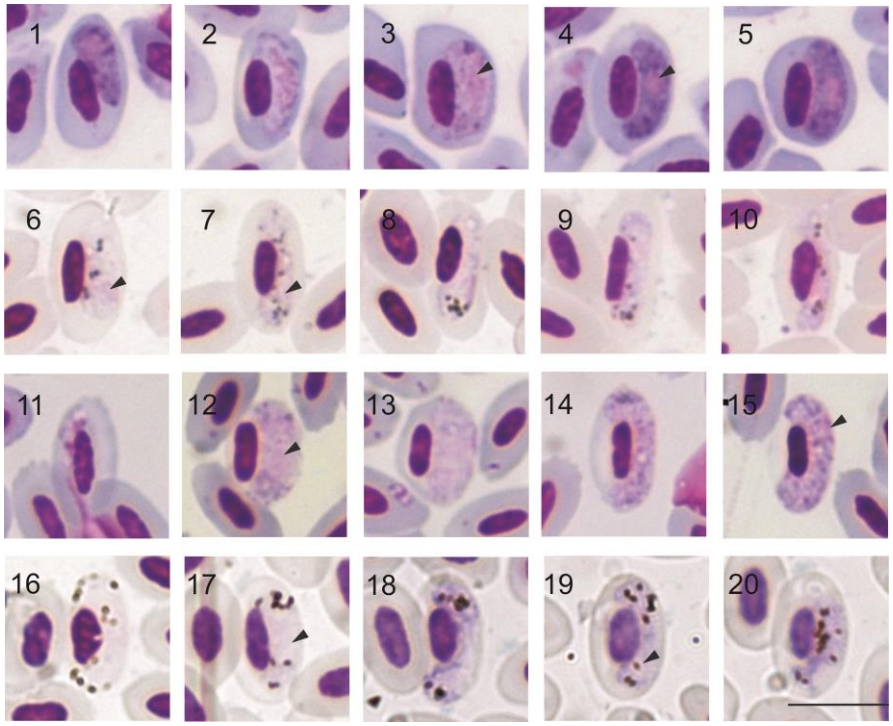

Figure 1 *Haemoproteus* spp. of Aedeidae (I), Corvidae (II and III) and Sylviidae (IV) in Bung Boraphet: (1-5) I. *Haemoproteus herodiadis*; entire outline of parasite, the host cell is slightly hypertrophy, (1) young gametocyte (2 and 3) microgametocyte, (4 and 5) microgametocyte; (6-10) II. *Haemoproteus fallisi*; polar amoeboid outline, host cell is slightly hypertrophy, (6) microgametocyte, (7-10) microgametocyte; (11-15) III. *Haemoproteus dicruri*; markedly displaces host nucleus and host cell hypertrophy in macrogametocyte infection is greater than in microgametocyte infection, (11) young gametocyte, (12 and 13) microgametocyte, (14 and 15) microgametocyte; (16-20) IV. *Haemoproteus payevski*; pigments spread all over cytoplasm around parasite's nucleus, host cell hypertrophy occurs lesser in macrogametocyte than in microgametocyte. Arrow heads, nuclei of *Haemoproteus* spp. Geimsa-stained thin blood film. Scale bar = 10 µm.

***Relationship between Haemoproteus and feather ectoparasite infection*:** There was a negative relationship between host and parasite in almost every host-parasite complex. Yet, there might be a positive commensal or a negative relationship between different parasite species. The infection relationship was verified by the amount of each parasite infection, co-infection and non-infection. All 633 birds were examined for infection relationship between each other. Two infections were dependent with p value <0.005 (n =633, df =1), whereas the examination between avian host with *Haemoproteus* infection, and feather ectoparasite infection was independent (n =335, df =1, 0.90< p value <0.10). Moreover, the same happened when examining each host species. Only two avian host species, *Acrocephalus orientalis* and *Lonchura punctulata* hosting *H. payevski* and *H. orizivorae*, respectively, and infected with feather ectoparasites, could be used for evaluation (Table 3).

All avian hosts infected with *Haemoproteus* were examined using the prevalence study and incidence rate, and it was found that the ratio of prevalence study was around 1 (0.87 to 1.48), which means that there was no relationship between *Haemoproteus* and feather ectoparasite infection. On top of that, the two avian hosts mentioned above were also not related either (Table 4). The other calculation was the incidence rate of all *Haemoproteus* infected avian hosts, for which the rate was 1.31 (95% CI: 0.79-1.83), meaning that every 1.31 birds infected with *Haemoproteus* will have feather ectoparasite infection. Hence, it seems to be related to similar incidence rate in *A. orientalis* and *L. punctulata* of all birds infected with *Haemoproteus* at 1.04 and 1.55, respectively.

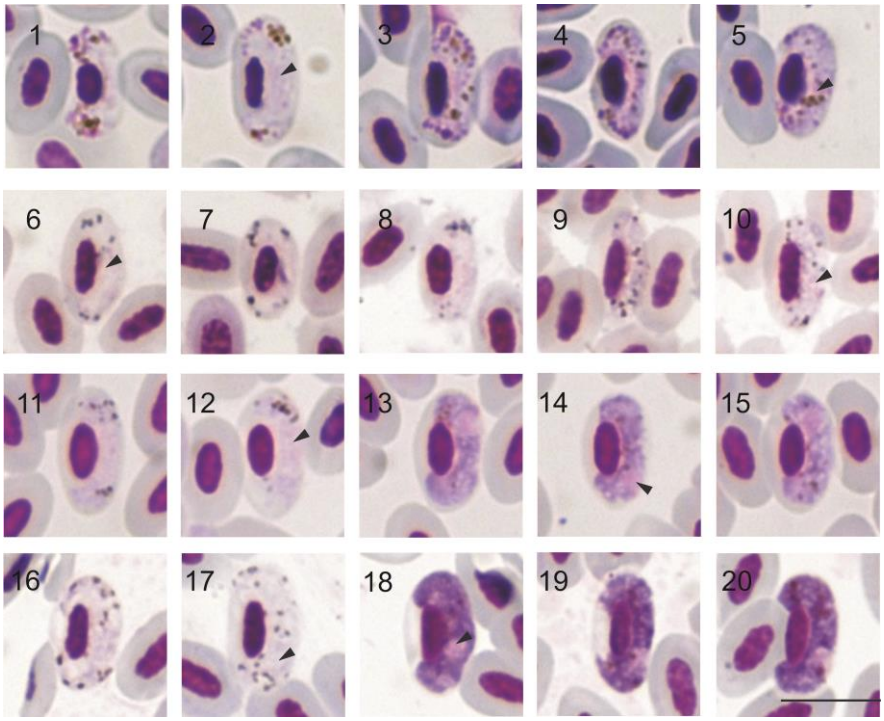

Figure 2 *Haemoproteus* spp. of Pycnonotidae (I and II) and Estrildae (III and IV) in Bung Boraphet: (1-5) I. *Haemoproteus otocompsae*; both amoeboid and entire polar outline, pigments distribute within cytoplasm around parasite's nucleus, noticed host nucleus displacement, (1 and 2) macrogametocyte, (3-5) microgametocyte; (6-10) II. *Haemoproteus sanguinis*; obvious outline, difference in both hypertrophy and percentage of parasite in host-parasite complex between macrogametocyte and microgametocyte, (6-8) macrogametocyte, (9 and 10) microgametocyte; (11-15) III. *Haemoproteus paseris*; slightly displace host nucleus, however, causing major hypertrophy, (11 and 12) macrogametocyte, (13-15) microgametocyte; (16-20) IV. *Haemoproteus orizivorae*; abundance of pigments, entirely distinct outline, (16 and 17) macrogametocyte, (18-20) microgametocyte. Arrow heads, nuclei of *Haemoproteus* spp. Geimsa-stained thin blood film. Scale bar = 10 µm.

Discussion

Since 1972, *Haemoproteus* has been described using morphological measurements to differentiate one species from other *Haemoproteus* species. The technique was first used to distinguish *H. fallisi*, which is found in the American robin (*Turdus migratorius* L.) and a member of the family Turdidae (Bennett and Campbell, 1972). Moreover, morphological parameters of RBCs and parasites, the statistical significance of different species, and historical exposés of vectors of avian hosts can also be used as supplemental information in identifying and describing haemoproteids at the species level (Bennett et al., 1975). Additionally, morphological forms of the parasite are fundamental characteristics to classify these parasite's forms, as well as outlines, marked displacement of RBC's nucleus and pathogenic effects of the host cell (Bennett and Peirce, 1988). However, bias of morphology and size may happen; therefore the information on morphological development of *Haemoproteus* in infected vectors (parasitic insects of the family Hippoboscidae) should also be provided in new species descriptions (Valkiunas et al., 2002). Thus, studies of morphology in each sexual development stage can only be performed in appropriate insect vectors, specifically in each *Haemoproteus* which is still unknown.

This investigation identified *Haemoproteus* based on morphological characteristics and the sizes of gametocyte of *Haemoproteus* (Bennett and Campbell, 1972; Bennett and Peirce, 1988), supporting the Haemosporidia and other avian malaria (Valkiunas, 2005). There are two different forms, including microhalteridial and halteridial haemoproteid, distinguished by size of the parasite. The microhalterial form appeared both in *H. herodiadis* and *H. fallisi*, although their pigment, outline and the percentage of RBC hypertrophy were different; the former had microhalteridial form with less hypertrophy and the latter had halteridial form with larger hypertrophy, differentiating the parasites from each other. In Corvidae, there were two haemoproteids found in two different avian hosts. *H. dicruri*, infecting *Dicrurus macrocercus*, gave a different hypertrophy effect between macro- and microgametocyte. On the other hand, *H. fallisi*, infecting *Rhipidura javanica*, which is smaller in size, with microhalteridial form, did not give a different hypertrophy effect between both gametocytes. The size and shape of individual parasite can vary with what it gets from the host it infects, also depending on the stage/age of the parasite, where fine and scattered pigments in its cytoplasm can be seen in young form, and these tend to accumulate when it turns to a mature gametocyte. *H. payevski* was found in Sylviidae, both *Acrocephalus orientalis* and *A. bistrigiceps*, and was considered to have medium size and distinctively displaced the host's nucleus. The displacement of host nucleus was compared with uninfected erythrocyte to determine the degree of displacement. However, this parameter can vary by various artifacts: 1) mechanical artifacts, during preparation of blood smear between marginal and

center of blood smear (Godfrey et al., 1987) and 2) physical factors. Appearance variations of the *Haemoproteus* itself when affecting the host cell and other artifacts of the avian host-parasite complex include age of the host, stage of infection or development of parasite cycle, and intensity of infection (Booth and Elliott, 2002; Holmstad et al., 2003). *H. otocompsae* and *H. sanguinis* were found in different hosts in Pycnonotidae, being *Pycnonotus goiavier* and *P. blanfordi*, respectively. Even if the sizes between these two parasites were not so different, the outlines and pigments were classified into two parasitic species of *Pycnonotus*. With reference to evidence of co-occurring evolutionary divergent parasite lineage, the parasite's shape varies in different geographical areas, especially in a Pycnonotidae host (Silva-Iturriza et al., 2012). Likewise, there were two haemoproteids found in Estrildidae, both *H. paseris* and *H. orizivorae* belonging to *Ploceus philippinus* and *Lonchura punctulata*, respectively. *H. orizivorae* had relatively larger hypertrophy and higher percentage of host-parasite complex than *H. paseris*. Similarly, pathogenic effects on erythrocyte, marked hypertrophy, width and changing shape, were used to distinguish *H. jenniae* from *H. lame,* which produce similar gametocytes and have closely related avian host species (Levin et al., 2012). Unfortunately, the result was an incomplete re-description because of lack of molecular information and fragment of mitochondrial cytochrome b gene supporting information, but morphological characters were able to classify *Haemoproteus*, following necessary traditional microscopic examination. In addition, the present study of *Haemoproteus* using its marked morphological character (Table 1 and Figs 1 to 2) indicates genetic divergence of more than 5%, to allow following investigations to be comprehensive in the re-description of all eight *Haemoproteus*.

However, recent molecular techniques are used as supplemental information to compare the close relationship between haemoproteids, combined with morphological diversity of *Haemoproteus* from sequencing of cytochrome b (cyt b) gene (Valkiunas et al., 2007; Krizanauskiene et al., 2010). Furthermore, the molecular approach recently seems to become useful in classifying *Haemoproteus* or in other haemosporidian investigation, involving distribution, evolution, host parasite interaction, and in describing characteristic keys about new species, especially for co-infection with other haemoproteids. However, morphologies of gametocyte, size measurements and life history of avian hosts are necessary for identifying haemoproteid parasites. Additionally, sexual reproductive stages might be considered during development within blood sucking insects to complete the *Haemoproteus* description.

During the investigation of blood parasitic protozoa in some birds in Bung Boraphet, there were 76 birds in 9 avian species infected with *Haemoproteus* spp., from all 633 birds. The total prevalence of *Haemoproteus* in the years 1999-2000 was 12.01. The prevalence of infection should be monitored together with other factors implicated to this infection, parasite and insect vector interactions or transmission pathways. This data may be able to provide applicable information for public health in the future. Hence, investigation into abundance needs to be considered in conjunction with other supporting factors, because the higher the host abundance, the greater the prevalence, without correlation to movement of host species. In other words, the prevalence will increase depending on specificity of host species, and the highest prevalence occurs in association with seasonal patterns (Ricklefs et al., 2011; Schultz et al., 2011).

From this investigation, there were 9 bird species out of all 35 which were infected with *Haemoproteus*. Thus, there were 26 (74.29%) bird species which were free from *Haemoproteus* infection. The negative results may be because of low susceptibility to local transmission in migratory birds compared with residential avian hosts. Infection in newly introduced species may occur if the parasite is specific to the host, or there is a phylogenetic connection with previously habituated avian species. Alternatively, high prevalence can be seen in some haemoproteid species that are able to infect more than one host species, even if there is no phylogenetic connection between the hosts. On the contrary, infection comparison between two shorebird species with different habitats, coastal marine and inland area, found different infection; the coastal marine birds were parasite free. The difference in prevalence of infection mainly occurs between tropical wetland, with freshwater inland habitat and marine coastal habitat. Migration through Europe and tropical Africa is not one of the factors associated with infection prevalence. However, the exposure of insect vectors is a factor that explains the habitat-related difference in prevalence of this hematozoa. Furthermore, another factor that affects the prevalence is spatial variation. This is supported by evidence of translocation of pigeons from a vector-free area to an area abundant in both parasites and vectors, and these unparasitized pigeons suffered from patent infection (Jovani et al., 2000; Mendes et al., 2005; Yohannes et al., 2009; Sol et al., 2012).

The prevalence of *Haemoproteus* infection varied with avian host species. *Lonchura punctulata* infected with *H. orizivorae* had the highest prevalence of 66.13%, with 41 infected birds out of all 62 birds, similar to an examination of haematozoa in Ghana, where Ploceidae and Estrildidae (Passeridae) were the most heavily infected. However, *Rhipidura javanica* infected with *H. fallisi* had the lowest prevalence of 2.63%, with only 1 out of 38 birds in total infected. *Haemoproteus* is the most common haematozoa in birds, although the prevalence of infection varies markedly by time and location (Bennett et al., 1975; Wink and Bennett, 1976; Bennett et al., 1978). Moreover, avian host species are the major factor for prevalence variance of *Haemoproteus* infection, matching the results of this investigation. Sample size may be the cause of the difference in prevalence of infection. Therefore, in this investigation, when only the infected population was used as sample size ($n = 135$, $df = 3$, p value > 0.90), the prevalence was still significantly different ($n = 16$, $df = 3$, p value < 0.005), which indicates that sample size is independent of the prevalence of *Haemoproteus* infection. Some theories suggest that insect vector density is an important cause of this parasite infection, but those theories lack detection of

the difference in exposure possibility to the parasite and specificity of transmitting vectors of each haematozoan. Haematozoan infection is significantly higher in tropical wetlands than in marine coastal habitat. The cumulative exposures to vectors increase probabilities of infection, namely the habitat-related prevalence differences (Sol et al., 2000; Mendes et al., 2005). Additionally, variations in the prevalence are dependent on environmental conditions, involving transmission dynamics, including seasonal or temporal variations, where bird communities may positively support haematozoan infection (Cosgrove et al., 2008; Fecchio et al., 2011).

During bird sample collection, other parasitism status was also observed. Feather ectoparasites may be seen on their hosts in any stages over their lifetime, given host-parasite fitness. Thus, the host as well has its ectoparasite defense mechanism for all its life stages (Vas et al., 2008; Hamstra and Badyaev, 2009; Koop et al., 2012). In this investigation, the status of ectoparasite infection was used, in relationship with *Haemoproteus* infection status, to answer whether these two infections have a negative or positive relationship to each other. The results from the prevalence study and the incidence rate showed that there was no relationship between both infection statuses. Despite the fact that *Haemoproteus* infections had a fair relationship to feather ectoparasite infections when considering all 633 avian samples (1.79 times greater in the prevalence study, ranging from 2.09 to 2.49, and the incidence rate of 2.37, which was greater than 1, meaning that the probability of shared infection in one bird was 2.37 times higher compared with non-infections). This clarifies that feather ectoparasites are one of the risk factors of *Haemoproteus* infection. In addition, this shared infection may be because of a reduction in the host's energy available for self-defense, and also feather ectoparasite infection may affect both biological systems and behavior of the avian hosts, consequently enhancing the transmission rate (Barbosa et al., 2002; Holmstad et al., 2008; O'Brien and Dawson, 2008).

Surprisingly, when considering only the avian host species with *Haemoproteus* infection, 335 out of 633 birds, with both *Haemoproteus* and ectoparasite infections, did not have statistically significant association ($n = 335$, $df = 1$, $0.90 < p \text{ value} < 0.10$) with each other. Moreover, the prevalence and incidence rate, 1.176 and 1.310 respectively, decreased compared with when investigating all birds. From this investigation, there were only two species of birds, *Acrocephalus orientalis* and *Lonchura punctulata*, infected with both parasites. However, there was no correlation of infections, as well as prevalence and incidence rate between these two species. Conditionally, transmission of *Haemoproteus* by other ectoparasites as insect vectors means that *Haemoproteus* infection does not depend on this feather ectoparasite. In reality, parasitism is rather common in societies and individuals living in larger groups. In particular, groups with species richness will significantly decrease ectoparasite transmission, then more social levels may also reduce ectoparasite diversity, resulting in loss of parasite transmission and exchange. In other words, feather ectoparasite transmission is concerned with the population size and diversity of the avian host, whereas *Haemoproteus* infection involves several conditions and appropriate transmission patterns, causing specificity in each avian host family and initiating patterns of infection. In terms of patterns of infection, host-parasite interaction may be involved. To build fitness effects, adjustment of both parasite and host is needed to balance their life cycle. The host's mechanical defense controls the intensity of infection, while the parasites require only adequate nutritional resources from their host. Consequently, parasites infecting a mutual host may have either positive or negative effects towards each other in order to maintain host-parasite fitness (Sol et al., 2003; Bize et al., 2008; Bordes et al., 2007; Owen et al., 2009).

This finding is the first report on *Haemoproteus* infection status in Bung Boraphet, Thailand. Future investigation should focus on *Haemoproteus* transmission and suitable assessment for *Haemoproteus* infection, which involve host specificity, cross transmission and morphogenetics of these *Haemoproteus*. Moreover, verifying relationships between *Haemoproteus* species or within one species between different avian host species, as well as between different *Haemoproteus* species within a single host species, may answer the questions of the haematozoa infection mechanism.

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References

Barbosa A, Merino S, de Lope F and Moller AP 2002. Effects of feather lice on flight behavior of male barn swallows (*Hirundo rustica*). Auk 119: 213-216.

Bennett GF, Blancou J, White EM and Williams NA 1978. Blood parasites of some birds from Senegal. Journal of wildlife diseases 14: 67-73.

Bennett GF and Campbell AG 1972. Avian haemoproteidae. I. Description of *Haemoproteus fallisi* n. sp. and a review of the haemoproteids of the family Turdidae. Canadian journal of zoology 50: 1269-1275.

Bennett GF, Forrester DJ, Greiner EC and Campbell AG 1975. Avian Haemoproteidae. 4. Description of *Haemoproteus telfordi* sp. nov., and a review of the haemoproteids of the families Gruidae and Otidae. Canadian journal of zoology 53: 72-81.

Bennett GF and Peirce MA 1988. Morphological form in the avian Haemoproteidae and an annotated checklist of the genus *Haemoproteus* Kruse, 1890. Journal of Natural History 22: 1683-1696.

Bennett GF, Smith AD, Whitman W and Cameron M 1975. Hematozoa of the Anatidae of the Atlantic Flyway. II. The Maritime Provinces of Canada. Journal of wildlife diseases 11: 280-289.

Bize P, Jeanneret C, Klopfenstein A and Roulin A 2008. What makes a host profitable? Parasites balance host nutritive resources against immunity. The American naturalist 171: 107-118.

Booth CE and Elliott PF 2002. Hematological responses to hematozoa in North American and neotropical songbirds. Comparative biochemistry and physiology. Part A, Molecular & integrative physiology 133: 451-467.

Bordes F, Blumstein DT and Morand S 2007. Rodent sociality and parasite diversity. Biology Lett 3: 692-694.

Bunsong L 1991. A Guide to the birds of Thailand / Boonsong Lekagul, Philip D. Round ; foreword by Ben King; illustrated by Mongkol Wongkalasin, Kamol Komolphalin. Bangkok: Saha Karn Bhaet Co.

Chaichana R and Choowaew S 2013. Ecological importance and biological resource conservation of Boraphet marsh, Thailand. Scienceasia 39: 105-110.

Cosgrove CL, Wood MJ, Day KP and Sheldon BC 2008. Seasonal variation in *Plasmodium* prevalence in a population of blue tits Cyanistes caeruleus. J Anim Ecol 77: 540-548.

Fecchio A, Lima MR, Silveira P, Braga EM and Marini MA 2011. High prevalence of blood parasites in social birds from a neotropical savanna in Brazil. Emu 111: 132-138.

Godfrey RD, Jr, Fedynich AM and Pence DB 1987. Quantification of hematozoa in blood smears. Journal of wildlife diseases 23: 558-565.

Hamstra TL and Badyaev AV 2009. Comprehensive investigation of ectoparasite community and abundance across life history stages of avian host. J Zool 278: 91-99.

Holmstad PR, Anwar A, Iezhova T and Skorping A 2003. Standard sampling techniques underestimate prevalence of avian hematozoa in willow ptarmigan (*Lagopus lagopus*). Journal of wildlife diseases 39: 354-358.

Holmstad PR, Jensen KH and Skorping A 2008. Ectoparasite intensities are correlated with endoparasite infection loads in willow ptarmigan. Oikos 117: 515-520.

Koop JAH, Huber SK and Clayton DH 2012. Does Sunlight Enhance the Effectiveness of Avian Preening for Ectoparasite Control. J Parasitol 98: 46-48.

Krizanauskiene A, Perez-Tris J, Palinauskas V, Hellgren O, Bensch S and Valkiunas G 2010. Molecular phylogenetic and morphological analysis of haemosporidian parasites (Haemosporida) in a naturally infected European songbird, the blackcap *Sylvia atricapilla*, with description of *Haemoproteus pallidulus* sp nov. Parasitology 137: 217-227.

Levin II, Valkiunas G, Iezhova TA, O'Brien SL and Parker PG 2012. Novel *Haemoproteus* Species (Haemosporida: Haemoproteidae) from the Swallow-Tailed Gull (Lariidae), with Remarks on the Host Range of Hippoboscid-Transmitted Avian Hemoproteids. J Parasitol 98: 847-854.

Margolis L, Esch GW, Holmes JC, Kuris AM and Schad GA 1982. The Use of Ecological Terms in Parasitology (Report of an Ad Hoc Committee of the American Society of Parasitologists). The Journal of Parasitology 68: 131-133.

Mendes L, Piersma T, Lecoq M, Spaans B and Ricklefs RE 2005. Disease-limited distributions? Contrasts in the prevalence of avian malaria in shorebird species using marine and freshwater habitats. Oikos 109: 396-404.

Newman SH, Hill NJ, Spragens KA, Janies D, Voronkin IO, Prosser DJ 2012. Eco-Virological Approach for Assessing the Role of Wild Birds in the Spread of Avian Influenza H5N1 along the Central Asian Flyway. Plos One 7.

O'Brien EL and Dawson RD 2008. Parasite-mediated growth patterns and nutritional constraints in a cavity-nesting bird. J Anim Ecol 77: 127-134.

Owen JP, Delany ME, Cardona CJ, Bickford A.A. and Mullens B.A. 2009. Host inflammatory response governs fitness in an avian ectoparasite, the northern fowl mite (Ornithonyssus sylviarum). Int J Parasitol 39: 789-799.

Ricklefs RE, Gray JD, Latta SC and Svensson-Coelho M 2011. Distribution anomalies in avian haemosporidian parasites in the southern Lesser Antilles. J Avian Biol 42: 570-584.

Schultz A, Underhill LG, Earle R and Underhill G 2011. Seasonality, distribution and taxonomic status of avian haemosporidian parasites within the Greater Cape Town area, South Africa. Ostrich 82: 141-153.

Silva-Iturriza A, Ketmaier V and Tiedemann R 2012. Profound population structure in the Philippine Bulbul *Hypsipetes philippinus* (Pycnonotidae, Aves) is not reflected in its *Haemoproteus* haemosporidian parasite. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases 12: 127-136.

Sol D, Jovani R and Torres J 2000. Geographical variation in blood parasites in feral pigeons: the role of vectors. Ecography 23: 307-314.

Sol D, Jovani R and Torres J 2003. Parasite mediated mortality and host immune response explain age-related differences in blood parasitism in birds. Oecologia 135: 542-547.

Tiawsirisup S and Nuchprayoon S 2010. Mosquito distribution and Japanese encephalitis virus infection in the immigration bird (Asian open-billed stork) nested area in Pathum Thani province, central Thailand. Parasitol Res 106: 907-910.

Ueta M and Ryabtsev VV 2001. Migration routes of four juvenile Imperial Eagles *Aquila heliaca* from the Baikal region of eastern Russia. Bird Conserv Int 11: 93-99.

Valkiunas G 2005. Avian Malaria Parasites and Other Haemosporidia. Taylor & Francis.

Valkiunas G, Iezhova, TA, Loiseau, C, and Sehgal, RNM 2009. Nested Cytochrome B Polymerase Chain Reaction Diagnostics Detect Sporozoites of Hemosporidian Parasites in Peripheral Blood of

Naturally Infected Birds. Journal of Parasitology, 95(6), 1512-1515.

Valkiunas G, Krizanauskiene A, Iezhova TA, Hellgren O and Bensch S 2007. Molecular phylogenetic analysis of circumnuclear hemoproteids (Haemosporida : haemoproteidae) of sylviid birds, with a description of *Haemoproteus parabelopolskyi* sp nov. J Parasitol 93: 680-687.

Valkiunas G, Liutkevicius G and Iezhova TA 2002. Complete development of three species of *Haemoproteus* (Haemosporida, Haemoproteidae) in the biting midge *Culicoides impunctatus* (Diptera, Ceratopogonidae). J Parasitol 88: 864-868.

van RiperIII C, van Riper SG, Goff ML and Laird M 1986. The Epizootiology and Ecological Significance of Malaria in Hawaiian Land Birds. Ecological Monographs 56: 327-344. Vas Z, Csorgo T, Moller AP and Rozsa L 2008. The Feather Holes on the Barn Swallow *Hirundo rustica* and Other Small Passerines are Probably Caused by *Brueelia* Spp. Lice. J Parasitol 94: 1438-1440.

Ventim R, Mendes L, Ramos JA, Cardoso H and Perez-Tris J 2012. Local haemoparasites in introduced wetland passerines. J Ornithol 153: 1253-1259.

Wink M and Bennett GF 1976. Blood parasites of some birds from Ghana. Journal of wildlife diseases 12: 587-590.

Yohannes E, Krizanauskiene A, Valcu M, Bensch S and Kempenaers B 2009. Prevalence of malaria and related haemosporidian parasites in two shorebird species with different winter habitat distribution. J Ornithol 150: 287-291.

บทคัดย่อ

การศึกษาลักษณะทางกายภาพและความชุกของปรสิตในเลือดสกุลฮีโมโปรเตียสจากนกเกาะคอนในบึงบอระเพ็ด

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ปรสิตในเลือดนกสกุล *Haemoproteus* สามารถแพร่กระจายได้ทั่วโลก แต่ข้อมูลในเมืองไทยมีน้อยมาก แม้แต่พื้นที่แหล่งน้ำจืดที่มีความสำคัญต่อนกอย่างบึงบอระเพ็ดยังคงขาดข้อมูลของปรสิตสกุลนี้ ดังนั้นการศึกษาชนิดและการแพร่กระจายของปรสิตสกุล *Haemoproteus* นี้จึงมีความสำคัญต่องานด้านสุขภาพสัตว์ป่าของประเทศไทย ทำการเก็บตัวอย่างฟิล์มเลือดจำนวน 633 ตัวอย่างจากนก 35 ชนิด 25 สกุล 15 วงศ์ 6 อันดับในบึงบอระเพ็ด การศึกษานี้ได้รายงานการติดเชื้อปรสิตสกุล *Haemoproteus* เป็นครั้งแรก จำนวน 8 ชนิด ได้แก่ *Haemoproteus herdiadis, H. fallisi, H. dicruri, H, payevski, H. otocompsae, H. sanguinis, H. paseris* และ *H. orizivorae* ในนก 9 ชนิด ปรสิตสกุล *Haemoproteus* spp. จำแนกโดยใช้ลักษณะและการตรวจวัดทางกายภาพ ค่าความชุกของการติดเชื้อปรสิตสกุลนี้เท่ากับ 12.01±0.46 และนกผู้ให้อาศัยแต่ละชนิดมีค่าความชุกของการติดเชื้อแตกต่างกัน โดยนกผู้ให้อาศัยที่มีค่าความชุกสูงสุด คือ นก *Lonchura punctulata* และนกผู้ให้อาศัยที่มีค่าความชุกต่ำสุด คือ นก *Rhipidura javanica* ของการติดเชื้อปรสิต *H. orizivorae* และ *H. fallisi* ตามลำดับ การศึกษาความสัมพันธ์ระหว่างการติดเชื้อปรสิตสกุล *Haemoproteus* กับการติดเชื้อปรสิตภายนอกที่พบบริเวณขนนกพบความสัมพันธ์ของการติดเชื้อในระดับกลุ่มสังคมนกทั้งหมด แต่ไม่พบความสัมพันธ์ในประชากรนกในกลุ่มที่พบการติดเชื้อปรสิตสกุล *Haemoproteus*

คำสำคัญ: นก บึงบอระเพ็ด ฮีโมโปรตีอิด ฮีโมโปรเตียส การจำแนกชนิด ความชุกของการติดเชื้อ

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