A TAXONOMIC STUDY OF THE HAEMOPROTEIDAE (APICOMPLEXA: HAEMOSPORINA) OF THE AVIAN ORDER STRIGIFORMES

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by

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ABSTRACT

The avian order Strigiformes consists of two families, the Strigidae (136 species) and the Tytonidae (12 species). Thirteen species of haemoproteids (Haemosporina: Haemoproteidae) have previously been described from the Strigidae while none have been described from the Tytonidae. A review of these species based on the morphometric parameters of the gametocyte indicates that *Haemoproteus asio* and *H. otus* are *nomina nuda*; *H.bubonis*, *H. aluci* and *H. glaucidiumi* are *nomina dubia*; *H. glaucidii, in partim., H. bramae, H. cellii, in partim.* and *H. nebraskensis* are synonyms of *H. noctuae* and *H. glaucidii, in partim., H. cellii, in partim., H. multiparasitans* and *H. aegyptius* are synonyms of *H. syrnii*. *Haemoproteus noctuae* and *H. syrnii* are herein redescribed and neohapantotypes and paraneohapantotypes are designated. *Haemoproteus phodili* n. sp. is described from the Tytonidae.

Other blood parasites which were recorded from the Strigiformes included: *Leucocytozoon ziemanni*, *Plasmodium relictum, P. vaughani, P. polare, P. fallax, P. circumflexum, P. elongatum, Trypanosoma avium, T. calmettei, T. everetti*, *Atoxoplasma* sp., *Babesia* sp., microfilaria and unidentified parasites.

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INTRODUCTION

Members of the order Strigiformes (owls) are distinguished by their large forward facing eyes, pronounced facial discs, large heads and large, unequal-sized ears. They can be found worldwide except on a few oceanic islands and Antarctica and occupy a wide range of ecological niches from barren deserts and tundra to tropical rain forests. There are 2 families in this order, the Strigidae (136 species) and the Tytonidae (12 species); the former are the "owls" proper while the latter are the "barn owls". These two families are separated by differences in skeletal structure and shape of the facial disc (Burton, 1973).

Avian blood parasites were first observed by Danilewskyi in 1885 when he recorded *Trypanosoma avium* from the tawny owl, *Strix aluco*, and the first haemoproteids were described in 1890 by Kruse. Many studies on haemoproteids have been carried out since that time, the majority being summarized by Bennett *et al.* (1982). There is more information concerning the taxonomy of haemoproteids for such groups as the Passeriformes, Anseriformes and Galliformes than any other avian orders. Little work has been done in the past 30 years on the taxonomy of the haemoproteids which infect the Strigiformes, probably due to the fact that owls are relatively few in number and difficult to capture (Sacchi and Prigioni, 1984).

Blood parasites commonly recorded from strigids include species of the families Haemoproteidae (genus *Haemoproteus* Kruse), Leucocytozoidae (genus *Leucocytozoon* Sambon), Plasmodiidae (genus *Plasmodium* Marchiafava and Celli),

Trypanosomatidae (*Trypanosoma* Gruby), and members of the superfamily Filarioidea. Parasites belonging to the genera *Atoxoplasma* Garnham, *Babesia* Starcovici, *Haemogregarina* Danilewskyi, *Hepatozoon* Miller, *Lankesterella* Labbé, *Nuttalia* França and *Toxoplasma* Nicolle and Manceaux are infrequently encountered in strigid blood smears.

Species of the family Haemoproteidae (suborder Haemosporina; phylum Apicomplexa) are the most commonly occurring parasites found in avian blood (Bennett *et al.*, 1982). The genus *Haemoproteus* erected by Kruse in 1890 contains the avian-infecting species of this family. In 1965 Bennett *et al.* designated a second avian-inhabiting genus, *Parahaemoproteus*, based on differences in schizogony, sporogony and invertebrate host species (Ceratopogonidae, genus *Culicoides* Latreille; not Hippoboscidae). Since these criteria were known for only a minority of species and the gametocytes of the two genera could not be separated, the genus *Parahaemoproteus* was reduced to subgeneric level by Levine and Campbell (1971) until such a time when more information concerning the life cycles is known. This action has been accepted by Bennett and his colleagues (Bennett and Campbell, 1972 *et seq.*) in their reviews of the haemoproteids of various avian families.

Haemoproteid parasites are two-host parasites in that the asexual cycle occurs in the avian host and the sexual cycle occurs in the invertebrate host. For most of the species only the gametogonic stages in the blood of the vertebrate host are known. The gametocytes of these parasites are found in red blood cells and are studied by examination of thin blood smears. The gametocytes of *Haemoproteus* species occur in five morphological forms: microsomal, halteridial (macrosomal),

circumnuclear, rhabdosomal and discosomal (Bennett and Peirce, 1988). Species of haemoproteids are distinguished by the morphology of the gametocyte and the host family. Several species of haemoproteids have been shown experimentally to be host family specific (Atkinson, 1986; Bennett and Peirce, 1988), that is a haemoproteid species only infects species of a single avian family. The vectors are known for only 11 of 113 haemoproteid species (eight are biting midges of the genus *Culicoides* (Ceratopogonidae) and three are louse flies (Hippoboscidae) (Bennett and Peirce, 1988).

The descriptions of many haemoproteids are inadequate according to present day standards as they lack illustrations and virtually none include measurements. Many were described from smears taken from dead birds where *'post mortem'* changes had resulted in alteration of parasite morphology (White and Bennett, 1979). Others were described on the basis of the "one host-one parasite" concept, popular in the early 1900's (Bennett and Campbell, 1973). Frequently, authors were unaware that species had previously been described and proceeded to describe them as new species. In many cases hapantotype and parahapantotype material were not designated. Hapantotype refers to the name-bearing type preparation (blood smear in this case) designated for species of Protozoa where a number of directly related individuals occur in a preparation (Article 72, item c(iv), International Code of Zoological Nomenclature (ICZN), 1985) (formerly called types and syntypes). The parahapantotypes are preparations of the type series other than the hapantotype (formerly called paratypes). In most cases neohapantotypes and paraneohapantotypes, (new name-bearing types and type series for species which

were previously described, but for which no hapantotype, lectohapantotype or parahapantotype are believed to exist) must be designated.

Taxonomic problems in the Haemoproteidae stem from the fact that there were no guidelines to follow when defining a species. Helmy Mohammed (1958) defined what he felt were the valid taxonomic characteristics in determining haemoproteid species (gametocyte form; gametocyte length, width, area; gametocyte nucleus length, width, area; pigment granule number; % hypertrophy/atrophy and NDR). Modified versions of these characteristics have subsequently been used in taxonomic studies by Bennett and his colleagues (Bennett and Campbell, 1972 *et seq.*).

Thirteen species of haemoproteids have been described from the order Strigiformes, all from the family Strigidae:

Haemoproteus noctuae Celli & Sanfelice, **1891**
Host: *Athene noctua* Locality: Rome, Italy

Haemoproteus aluci Celli and Sanfelice, **1891**
Host: *Strix aluco* Locality: Rome, Italy

Haemoproteus bubonis Celli & Sanfelice, **1891**
Host: *Asio flammeus* Locality: Rome, Italy

Haemoproteus syrnii (Mayer, **1910**) Coatney, 1936

Host: *Strix aluco* Locality: Lübeck, West Germany or Vienna, Austria

Originally described as *Halteridium syrnii* by Mayer, 1910

Haemoproteus glaucidiumi houssayi Jörg, **1931**

Host: *Glaucidium nanum* Locality: Chaco District, Argentina

Haemoproteus bramae de Mello, **1936**

Host: *Athene brama* Locality: Nagoa (*sic* Nova-Goa), Portuguese India

Haemoproteus glaucidii de Mello, **1936**

Host: *Glaucidium radiatum* Locality: Canacona, Portuguese India

Haemoproteus cellii (Coatney & Roudabush, **1937**) Helmy Mohammed, 1958

Host: *Otus asio* Locality: Nebraska, U.S.A.

Originally described as a variety of *H. noctuae* by Coatney & Roudabush in 1937.

Haemoproteus nebraskensis (Coatney & Roudabush, **1937**) Levine & Campbell, 1971

Host: *Bubo virginianus* Locality: Nebraska, U.S.A.

Originally described as a variety of *H. noctuae* by Coatney & Roudabush, 1937.

Haemoproteus multiparasitans Covaleda Ortega and Gállego Berenguer, **1950**

Host: *Athene noctua* Locality: Granada, Spain

Haemoproteus aegyptius (Helmy Mohammed, **1958**) Levine and Campbell, 1971

Host: *Bubo bubo* Locality: Cairo Zoological Gardens, Guiza, Egypt

Originally described as a variety of *H. cellii* by Helmy Mohammed in 1958.

Haemoproteus asio Zeiniev, **1975**

Host: *Asio otus* Locality: Azerbaidzhan, U.S.S.R.

Haemoproteus otus Musaev & Zeiniev, **1977**

Host: *Otus scops* Locality: U.S.S.R.

Review, description, redescription and synonymy of the 13 species of haemoproteids described from the Strigiformes is required to clarify this chaotic situation; this is the thrust of this study.

MATERIALS AND METHODS

A total of 519 blood smears containing blood parasites from 30 species of Strigiformes, (27 of the family Strigidae and three of the family Tytonidae) (Appendix A) were available for study at the International Reference Centre for Avian Haematozoa (IRCAH). This material, collected by collaborators worldwide, provided the sample for this taxonomic study. This data set is extensive; however, there is a preponderance of material from Southeast Asia. Additionally, many of the Strigiformes are fairly limited in their distribution thus biasing the sample by host species. It must be emphasized that no negative material was available and hence no prevalence of infection could be determined. Therefore a valid zoogeographic study was not possible.

Preparation of blood smears

A large proportion of the blood smears for this study were collected for the Migratory Animal Pathological Survey (MAPS) under the supervision of H. Elliott McClure from 1963 through 1971 in Southeast Asia. The majority of blood films were taken from netted live birds by clipping a toenail and making a smear of the issuing drops of peripheral blood. The blood films were fixed with methanol and were shipped to MAPS headquarters in Bangkok, Thailand for staining and preliminary screening (McClure *et al.*, 1978). Of the remaining blood films, most were stained in their country of origin; a few were stained at the IRCAH with Giemsa's stain (buffered to pH 7.2) for 45 minutes and washed in slightly acidic (pH

6.5) tap water. Additionally, smears which had been stained with any of the "quick stains" such as Wright's or Leishman's (Russell *et al.*, 1963) and had become faded were restained at the IRCAH using 1 ml Giemsa's : 9 ml distilled water solution until an adequate stain was obtained.

Examination of blood smears

The blood specimens used in this study were previously examined by the contributors or by the staff at the IRCAH and identifications had generally been made to the genus level. For this study they were re-examined under 40x and 100x oil immersion objectives on a Zeiss II Photomicroscope. Each blood film was examined a minimum of two times for a period of 10 to 30 minutes each time and the parasites present were identified.

Taxonomic analysis of haemoproteids

Gametocytes of the haemoproteids of the Strigidae were studied according to the protocols described by Bennett and Campbell (1972) and Forrester *et al.* (1977). Gametocytes and infected and non-infected erythrocytes were drawn with the aid of a camera lucida. Parasite length, width and area, parasite nucleus length, width and area, erythrocyte length, width and area, and erythrocyte nucleus length, width and area were measured using a Zeiss MOP-3 Digital Analyzer, which provides mechanical reproducibility of results to within 0.1%.

A percentage ratio of the parasite area to the infected erythrocyte area determined the percent area of the host-parasite complex that the parasite occupied,

indicating the size of the parasite relative to the erythrocyte. The percent hypertrophy or atrophy (the percentage ratios of the increase or decrease, respectively, in the measured morphological parameters of the erythrocyte and its nucleus due to invasion by the haemoproteid) was determined. The nuclear displacement ratio which indicates the amount of lateral displacement of the erythrocyte nucleus caused by the parasite is defined by the equation:

$$NDR = 2X/X+Y$$

For halteridial forms (Figure 1):

Y = the distance between the erythrocyte nucleus periphery and the erythrocyte cell membrane on the side of the cell containing the gametocyte

X = the distance between the same points on the opposite side

For circumnuclear forms (Figure 2):

Y = the distance between the erythrocyte nucleus periphery and the erythrocyte cell membrane on the side of the cell containing the gametocyte nucleus.

X = the distance between the same points on the opposite side

In an uninfected erythrocyte, the NDR = 1; if the nucleus is displaced laterally towards the side of the erythrocyte away from the parasite the NDR approaches zero, and if the nucleus is displaced towards the parasite the NDR > 1. Since the parasite's influence on the host cell is consistent within haemoproteid species, these ratios can be important in distinguishing among species of *Haemoproteus*.

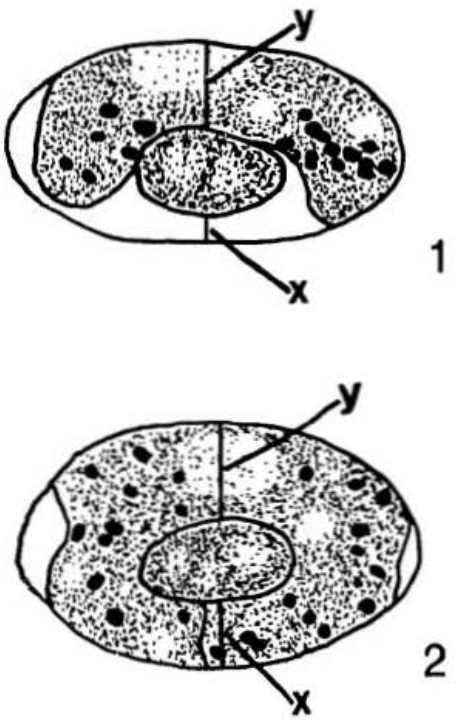
Haematin (also referred to as haemazoin) and a protein or polypeptide, both

Figure 1

Nuclear displacement ratio of a halteridial haemoproteid (NDR = 2X / X+Y)

Figure 2

Nuclear displacement ratio of a circumnuclear haemoproteid (NDR = 2X / X+Y)


y
x
1
y
x
2
10µm

waste products of haemoglobin digestion, are stored in vacuoles within the parasite (von Brand, 1966; Garnham, 1966); these vacuoles and their contents appear as yellow-brown granules and are referred to as "pigment granules". The number, arrangement and shape of these granules are characteristic of species of haemoproteids (White and Bennett, 1978) and were, therefore, recorded. Volutin granules or metachromatic inclusion bodies, which probably contain a high proportion of RNA (von Brand, 1966), sometimes occur in haemoproteids; little is known about their properties but the presence (or absence) and arrangement of these granules may be of taxonomic importance and hence were recorded. The gametocyte shape which may be microsomal, halteridial (macrosomal), circumnuclear, rhabdosomal or discosomal (Bennett and Peirce, in press), the shape of the gametocyte margins (amoeboid or entire), and the position and shape of the gametocyte nucleus were recorded.

Statistical analysis

Means and standard deviations for each of the parameters measured were obtained using the Statistics procedure of SPSS^x (Statistical Package for the Social Sciences, 1983). Morphometric parameters of haemoproteids were compared using multivariate analyses of variance (SPSS^x procedure MANOVA). Means of certain morphological parameters obtained from previous species descriptions were compared with samples from this study using special case T-tests for comparison of a single specimen with a sample (Sokal & Rohlf, 1981). In both cases a significance level of $= 0.01$ was chosen to minimize effects of compounding Type I error rates. All of the above procedures were run on VAX 8800 - VMS v.4.7.

RESULTS AND DISCUSSION

Blood Parasites of the Strigiformes

Samples were available from Strigiformes throughout their range (with the exception of Australia), from North and South America through Europe and Africa to Asia. A total of 519 blood smears from 30 species of birds positive for blood parasites were examined. Three identified species of *Haemoproteus* were recorded (Table 1). A single species of *Leucocytozoon*: *L. ziemanni*; six species of *Plasmodium*: *P. relictum, P. vaughani, P. polare, P. fallax, P. circumflexum* and *P. elongatum*; three species of *Trypanosoma*: *T. avium* (by definition) and two species similar to descriptions of *T. calmettei* and *T. everetti*; microfilaria; *Atoxoplasma* sp.; *Babesia* sp. and unidentified parasites were also recorded (Appendix B). The frequencies of occurrence of the avian haematozoa in this study were given in Table 2. Species of the genus *Haemoproteus* were the most commonly occurring (79.4%) which corresponds with haemoproteid frequencies reported for the Strigiformes by McClure *et al.*(1978), White *et al.* (1978) and Peirce (1981).

Haemoproteidae of the Strigiformes

Both *Haemoproteus bubonis* from *Asio flammeus* and *Haemoproteus aluci* from *Strix aluco* described by Celli and Sanfelice (1891) in Rome, Italy pose problems for haemoproteid taxonomy. Each is presented as a two line description with table listing and two figures. The descriptions are "meagre and decidedly confusing" (Coatney and Roudabush, 1937; Helmy Mohammed, 1958). Celli and Sanfelice

TABLE 1. Haemoproteids in the Strigiformes (based on the files of the IRCAH).

	Total Examined	*Haemoproteus*				
		syrnii	*noctuae*	*phodili*	*H.*sp.	*noctuae /syrnii*
STRIGIDAE						
Aegolius acadicus	26					
Asio flammeus	10					
Asio otus	3	1			1	
Athene brama	20	3	7			9
Athene noctua	3					
Bubo africanus	13	1	1			9
Bubo bubo	1	1				
Bubo coromandus	3	1				2
Bubo virginianus	17	2	4		1	5
Ciccaba woodfordii	2		1			1
Glaucidium brodiei	12	2	3			7
Glaucidium cuculoides	14	3	3		2	6
Glaucidium radiatum	1					1
Ninox connivans	1		1			
Ninox philippensis	45	3	21		3	13
Ninox scutulata	30	4	11			11
Nyctea scandiaca	10		1			1
Otus asio	10	1	1			2
Otus bakkamoena	131	43	10		5	65
Otus choliba	7	4				2
Otus rufescens	4	2	1			
Otus scops	70	15	23		3	27
Otus spilocephalus	14	5	4		1	3
Otus trichopsis	1	1				
Strix aluco	30	3	1			10
Strix occidentalis	1	1				
Strix varia	11	5	2		1	3
Unknown	6					
TOTAL	496	101	95		17	177
TYTONIDAE						
Phodilus badius	11			11		
Tyto alba	10				9	
Tyto longimembris	2				2	
TOTAL	23			11	11	
GRAND TOTAL	519	101	95	11	28	177

TABLE 2. The frequency of the occurrence of blood parasites of the Strigiformes.

	Total		Strigidae		Tytonidae	
	N	%	N	%	N	%
Haemoproteus	412	79.4	390	78.6	22	95.6
Leucocytozoon	197	38.0	192	38.7	5	21.7
Plasmodium	60	11.6	60	12.1		
Trypanosoma	36	6.9	36	7.2		
Microfilaria	22	4.2	21	4.2	1	4.4
Atoxoplasma	1	0.2	1	0.2		
Babesia	1	0.2	1	0.2		
Haemoproteus or *Plasmodium*	6	1.2	6	1.2		
Unknown	67	12.9	66	13.3	1	4.4

N - number positive for a given taxon
Total number of Strigiformes examined - 519
Total number of Strigids examined - 496
Total number of Tytonids examined - 23

based their description on fresh blood which was neither fixed nor stained. Under these circumstances morphological characters may have been distorted (Helmy Mohammed, 1958). For *H. bubonis* Celli and Sanfelice described a large endoglobular form which had left the red blood corpuscle and from which smaller masses had budded off. They mentioned that *H. aluci* had fewer refractive (pigment) granules than *H. bubonis*, but no numbers were given. *Haemoproteus bubonis* was illustrated by Celli and Sanfelice in two figures, one of an immature gametocyte in which not much can be determined of the eventual form of the mature gametocyte and the other of a parasite free from the erythrocyte which is distorted and therefore of no use when determining species. *Haemoproteus aluci* was also represented by two figures, one a halteridial haemoproteid which may be either an adult halteridial or an immature circumnuclear form and the second, which may be an exflagellating microgamete, is an elongated free form which has a flagella-like projection at one end. By modern standards (Article 17, item 1, ICZN, 1985) these two descriptions are not adequate to determine haemoproteid species. Indeed there seem to be no distinctive characteristics which distinguish either of them as being separate species. Celli and Sanfelice probably based these two new species on the "one host-one parasite" theory, popular at the time (Bennett and Campbell, 1973). These two species are therefore here designated as *nomina dubia* and invalid and they are removed from the list of valid species of *Haemoproteus*.

In 1931 Jörg described *Haemoproteus glaucidiumi* from *Glaucidium nanum* in Argentina (Appendix C). He described this parasite as one which totally invades the host cell without destruction or displacement of the host cell nucleus (a

circumnuclear parasite). The parasites in the appropriate figures appear to be halteridial parasites, none of which encircle the host cell nucleus and do not represent what is described in the text. They may be immature forms of what he described but this is not stated. Additionally, in his description the title of the appropriate section refers to *H. glaucidiumi* but the title of the figures uses a trinomial, *H. glaucidiumi houssayi* therefore adding to the confusion. The description and the figures may indeed be of different parasites. These problems have been previously referred to by Bennett *et al.* (1972) in a review of the haemoproteid species of the Alcedinidae; they designated *H. glaucidiumi houssayi (H. glaucidiumi?)* and *H. podicepsi houssayi (H. houssayi?)* as *nomina dubia* (in accordance with Article 17, item 2, of the ICZN, 1963 now Article 17, item 1, of the ICZN, 1985) and these have been removed from the list of valid species of *Haemoproteus*.

Haemoproteus asio Zeiniev, 1975 from *Asio otus* and *Haemoproteus otus* Musaev & Zeiniev, 1977 from *Otus scops* were listed as new species in what are essentially survey tables. There are no descriptions, measurements or drawings. This is not in agreement with Article 13, item a(i) of the ICZN (1985) as neither of these species can be separated from other taxa.. These two species were designated by Peirce and Bennett, (1979) as *nomina nuda* and were removed from the list of valid species of haemoproteids.

Taxonomic Review

Strigidae

Haemoproteus noctuae Celli and Sanfelice, 1891

Type Host: *Athene noctua* (Scopoli)

Type Locality: Rome, Italy

Synonyms: *Haemoproteus bramae* de Mello, 1936

Haemoproteus glaucidii de Mello, 1936, *in partim.*

Haemoproteus nebraskensis (Coatney and Roudabush, 1937) Levine and Campbell, 1971

Haemoproteus cellii (Coatney and Roudabush, 1937) Helmy Mohammed, 1958, *in partim.*

Immature gametocyte: (Figures 3, 4 and 5). Youngest forms seen initiate growth in a central or subpolar position, in both cases older immature forms approach a halteridial shape. Volutin granules if present, are usually randomly distributed but sometimes may be clumped at the poles.

Macrogametocyte: (Figures 6 and 7; Table 3). Circumnuclear parasite of medium to large size; occupying approximately 75% of the erythrocyte-parasite complex causing host cell hypertrophy; cytoplasm finely granular, staining deep blue with Giemsa's stain; margins of the parasite may be amoeboid, entire or a combination of both, with neither the inner or outer margin appressing to the erythrocyte nucleus

Figure 3

Haemoproteus noctuae

immature gametocyte

Figure 4

Haemoproteus noctuae

immature gametocyte

Figure 5

Haemoproteus noctuae

immature gametocyte

Figure 6

Haemoproteus noctuae

mature macrogametocyte

Figure 7

Haemoproteus noctuae

mature macrogametocyte

Figure 8

Haemoproteus noctuae

mature microgametocyte

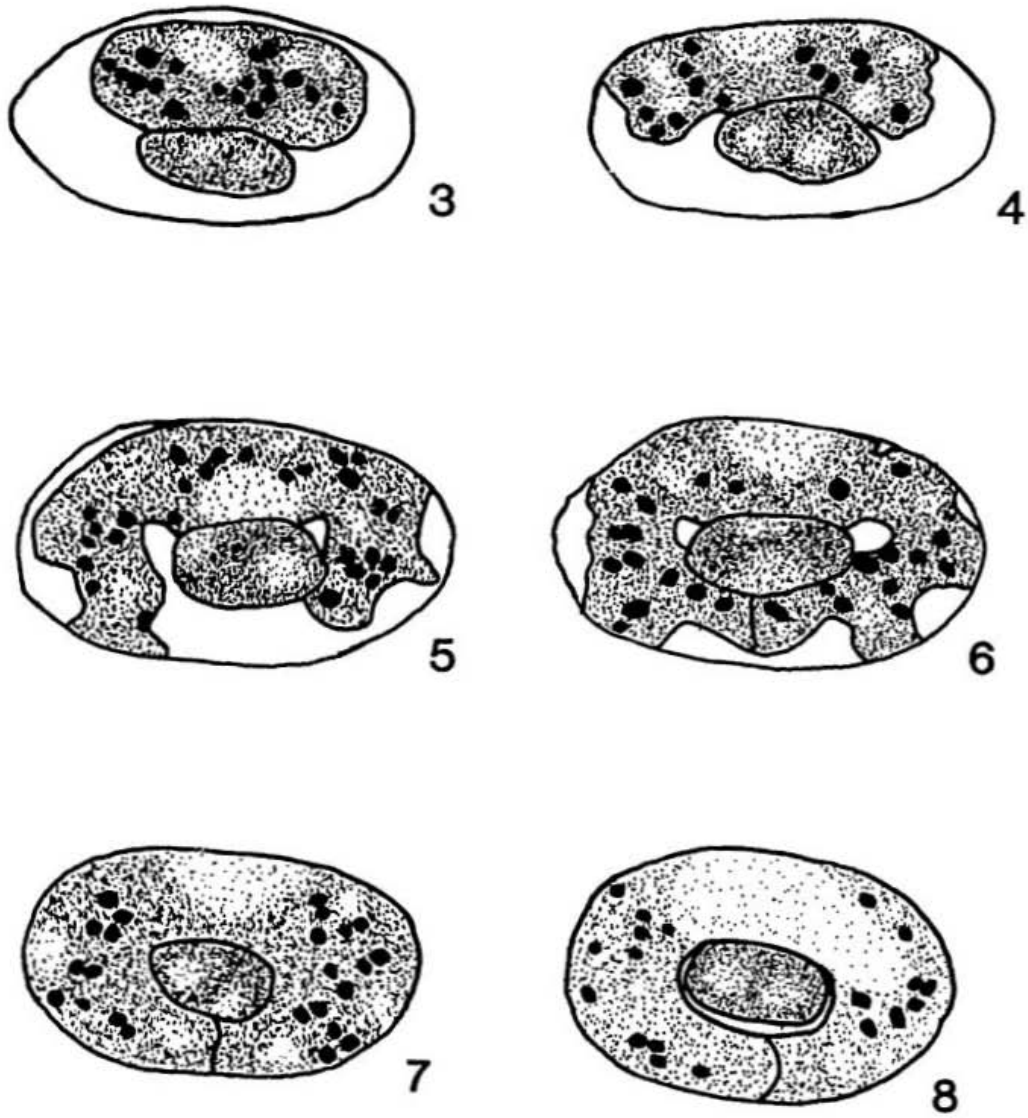

3
4
5
6
7
8
10μm

TABLE 3. Morphometric parameters of the haemoproteids of the Strigiformes.

	noctuae	*syrnii*	*phodili* n.sp.	*H.*sp.
Uninfected erythrocyte	N=50	N=50	N=40	N=5
length	13.0	13.0	12.7	14.0
	0.7*	0.7	0.6	0.3
width	7.5	7.7	7.6	8.1
	0.6	0.3	0.4	0.4
area	79.2	79.5	76.4	90.4
	9.8	5.4	5.8	4.6
Uninfected erythrocyte nucleus	N=50	N=50	N=40	N=5
length	6.1	5.6	5.5	5.6
	0.4	0.4	0.4	0.5
width	2.9	2.8	2.6	2.8
	0.3	0.2	0.3	0.3
area	14.9	12.5	11.7	12.3
	1.7	1.4	1.3	1.5
% Area of erythrocyte	18.8	15.7	15.3	13.6
Erythrocyte infected by macrogametocyte	N=70	N=65	N=60	N=15
length	14.4	14.2	14.2	15.7
	0.9	1.0	0.7	0.9
% Hypertrophy/Atrophy	10.8**	9.2	11.8	12.1
width	8.5	7.7	7.3	8.3
	0.6	0.5	0.5	0.5
% Hypertrophy/Atrophy	13.3	no change	-3.9	2.5
area	98.5	87.8	83.2	103.2
	9.2	8.3	6.3	9.9
% Hypertrophy/Atrophy	24.4	10.4	8.9	14.2

TABLE 3 (cont'd). Morphometric parameters of the haemoproteids of the Strigiformes.

	noctuae	*syrnii*	*phodili* n.sp.	*H.*sp.
Infected erythrocyte nucleus	N=70	N=65	N=60	N=15
length	5.4	5.0	5.5	4.8
	1.2	0.5	0.6	0.5
% Hypertrophy/Atrophy	-11.5	-10.7	no change	-14.3
width	2.8	2.8	2.4	2.4
	0.4	0.3	0.3	0.3
% Hypertrophy/Atrophy	-3.4	no change	-7.7	-14.3
area	12.1	11.6	11.1	9.5
	1.7	1.5	2.0	1.3
% Hypertrophy/Atrophy	-18.8	-7.2	-5.1	-22.8
% Area of erythrocyte-parasite complex	12.3	13.2	13.3	9.2
Macrogametocyte	N=70	N=65	N=60	N=15
length	25.3	15.7	14.3	22.9
	1.9	1.1	0.9	1.8
width	3.0	3.1	3.3	2.7
	0.4	0.5	0.4	0.6
area	74.5	53.8	48.0	60.6
	8.3	4.8	5.2	14.1
% Area of erythrocyte-parasite complex	75.6	61.3	57.7	58.7
Macrogametocyte nucleus	N=70	N=65	N=60	N=1
length	3.8	3.3	2.6	3.2
	1.2	0.8	0.4	
width	2.5	2.2	2.2	2.8
	0.5	0.4	0.3	
area	8.7	5.7	4.5	8.0
	3.4	1.0	0.9	

TABLE 3 (cont'd). Morphometric parameters of the haemoproteids of the Strigiformes.

	noctuae	*symii*	*phodili* n.sp.	*H.*sp.
% Area of gametocyte	11.7	10.6	9.4	13.2
Pigment granules	20.3	20.6	14.1	23.3
	3.3	5.1	1.7	2.0
Nuclear displacement ratio	0.9	0.6	0.6	0.9
	0.1	0.2	0.2	0.1
Erythrocyte infected by microgametocyte	N=20	N=20	N=23	N=10
length	14.2	14.0	14.3	15.6
	1.0	1.0	1.0	0.7
% Hypertrophy/Atrophy	9.2	7.7	12.6	11.4
width	8.5	7.9	7.3	8.4
	0.5	0.5	0.5	0.4
% Hypertrophy/Atrophy	13.3	2.6	-3.9	3.7
area	96.2	89.3	84.8	104.4
	10.4	7.9	7.3	5.2
% Hypertrophy/Atrophy	21.5	12.3	11.0	15.5
Infected erythrocyte nucleus	N=20	N=20	N=23	N=10
length	5.4	5.2	5.4	4.9
	0.4	0.5	0.5	0.5
% Hypertrophy/Atrophy	-11.5	-7.1	-1.8	-12.5
width	2.8	2.9	2.3	2.5
	0.2	0.3	0.4	0.2
% Hypertrophy/Atrophy	-3.4	3.6	-11.5	-10.7
area	12.3	12.1	9.9	9.7
	1.1	1.3	2.5	1.2
% Hypertrophy/Atrophy	-17.4	-3.2	-15.4	-21.1
% Area of erythrocyte-parasite complex	12.8	13.5	11.7	9.3

TABLE 3 (cont'd). Morphometric parameters of the haemoproteids of the Strigiformes.

	noctuae	*symii*	*phodili* n.sp.	*H.*sp.
Microgametocyte	N=20	N=20	N=23	N=10
length	25.9 1.6	15.3 1.7	13.8 1.1	23.2 1.9
width	3.0 0.4	3.5 0.6	3.3 0.4	3.0 0.6
area	76.1 10.7	53.9 5.2	46.1 6.9	64.3 13.9
% Area of erythrocyte-parasite complex	79.1	60.4	54.4	61.6
Microgametocyte nucleus	N=20	N=10	N=23	N=2
length	10.5 2.2	5.6 1.1	3.9 0.5	11.7 0.4
width	3.2 0.5	3.2 0.4	2.1 0.4	3.4 1.1
area	31.6 7.1	14.9 2.9	6.7 1.2	32.1 10.5
% Area of gametocyte	41.5	27.6	14.5	49.9
Pigment granules	18.7 4.3	15.2 6.0	13.4 2.5	22.1 2.7
Nuclear displacement ratio	0.9 0.1	0.6 0.2	0.6 0.2	0.9 0.2

Note: N is the number measured. Linear measurements in m; area measurements in m^2

* Standard deviations are given below means

** Hypertrophy is indicated by +, atrophy by -

or host cell membrane respectively; ends may be pointed or round; pigment granules, round to oval, staining yellow-brown, scattered throughout the parasite cytoplasm; volutin granules sometimes present, staining dark blue to violet, randomly distributed or clumped at both ends, may block pigment granules from view causing the pigment granule count to be artificially low; parasite nucleus, median, compact, oval to elongate, staining bright pink; host-cell nucleus usually atrophied but rarely displaced.

Microgametocyte: (Figure 8; Table 3). Circumnuclear form; cytoplasm staining pale blue to colourless with Giemsa's stain; occupying approximately 79% of the erythrocyte-parasite complex causing host cell hypertrophy; inner and outer margins may be amoeboid or entire, or a combination of both; the inner and the outer margin do not always appress to the erythrocyte nucleus or cell membrane respectively; ends may be pointed or round; pigment granules slightly fewer than in macrogametocyte, staining yellow-brown, scattered throughout the cytoplasm; volutin granules sometimes present, staining dark blue to violet; may be clumped at the poles or scattered throughout the parasite cytoplasm and may confuse the pigment granule count (see above); parasite nucleus diffuse, irregularly shaped, staining pale pink to colourless making it hard to distinguish, usually lying lateral to the host-cell nucleus; host-cell nucleus atrophied but rarely displaced.

Schizogony: The schizogonic stages of this parasite are unknown. The lungs, kidneys, spleen and liver of the vertebrate host have been shown (Aragão, 1908;

Wenyon, 1926; Fallis and Desser, 1977; Bennett, 1987) to be sites for schizogony of other *Haemoproteus* species and this may be also true for this species.

Sporogony: The invertebrate hosts which transmit *Haemoproteus noctuae* are not known. For the 11 species whose sporogonic cycles are known eight are transmitted by ceratopogonids (genus *Culicoides*) and three by hippoboscids (Bennett *et al.*, 1985). The vectors of this species are most probably species of the Ceratopogonidae or possibly the Hippoboscidae. McClure *et al.* (1978) showed that the prevalence of both *Haemoproteus noctuae* and *H. symii* infections for several species of owls did not change throughout the year. This suggests that the vector or vectors of *H. noctuae* are present year round.

Geographic Range: North and South America, Europe, Asia, Africa and potentially throughout the range of the Strigidae.

Neohapantotype: Celli and Sanfelice (1891) failed to designate a type specimen and it is assumed that their personal collections have been lost. The hapantotype slide for *H. noctuae* var. *nebraskensis* was studied for this review. This slide however, is of an immature infection. Additionally volutin granules are present in many of the parasites. This may result in pigment granules being obscured and counts therefore being artificially low. This slide is not acceptable as a neohapantotype slide. No suitable material was available from either the same host or locality from which the species was described originally. Blood film #80420 of the collection at

the IRCAH was of the best quality and the closest to the original type host and type locality available, in keeping with article 75 of the ICZN and is designated as the neohapantotype slide for *Haemoproteus noctuae*. This blood sample was taken from *Strix aluco,* the Eurasian tawny owl by C. J. Mead at Woburn, Bedfordshire, England on May 30, 1978.

Paraneohapantotype: Blood film #11080 at the IRCAH collected by H. Elliott McClure from Siaton, Negros Oriental, Philippine Islands on February 3, 1965 from *Ninox philippensis*, the Philippine boobook-owl is designated as the paraneohapantotype.

Additional Host Records: *Asio flammeus, A. otus, Athene brama, A. noctua, Bubo africanus, B. coromandus, B. virginianus, Ciccaba woodfordii, Glaucidium brodiei, G. cuculoides, G. radiatum, Ninox connivans, N. novaeseelandiae, N. scutulata, N. strenua, Nyctea scandiaca, Otus asio, O. bakkamoena, O. brucei, O. rufescens, O. scops, O. spilocephalus, Strix varia.*

Comments: Celli and Sanfelice (1891) described *Haemoproteus noctuae* varieties A and C from several young *Athene noctua* from Rome, Italy. Variety C was designated *Plasmodium oti* by Wolfson in 1936 while variety A is accepted as *H. noctuae*.

Celli and Sanfelice (1891) described *Haemoproteus noctuae* in comparison with *H. columbae* (Appendix C). This species was described from live material and

some characters were distorted. It can be determined from the description that this is a circumnuclear parasite with a smooth or indented (amoeboid) periphery and thick ends. It may or may not fill the entire erythrocyte (excluding the nuclear region). Celli and Sanfelice (1891) illustrated these characteristics in immature and mature forms with and without indentations. They also illustrated forms free from the host cell. This description is adequate for identifying parasites of the same form (circumnuclear) but lacks many important points by today's standards.

Schaudinn (1904) who studied the life cycle of this species extensively (but incorrectly believed *H. noctuae* to be a stage in the life cycle of a *Trypanosoma* species), failed to give satisfactory descriptions of mature gametocytes. He did, however, illustrate an immature gametocyte with distinctive indentations.

Sergent and Sergent recorded this species from an owl in 1907. They illustrated adult and immature circumnuclear forms with indentations and also free forms but did not redescribe the species.

In 1958 Helmy Mohammed adequately redescribed this species in a lengthy description including morphometric parameters and illustrations but did not designate a neohapantotype or paraneohapantotypes. One major problem with Helmy Mohammed's description is that he described this species from *Tyto alba* of the Tytonidae, not the Strigidae, and if one accepts the hypothesis of familial specificity (Bennett *et al.*, 1972), this parasite cannot be *H. noctuae.*

De Mello described *Haemoproteus glaucidii* from a single bird, *Glaucidium radiatum* shot at Canacona, Portuguese India in 1936 and he redescribed this same species at greater length in his 1936-1937 publication (Appendix C). The

macrogametocytes are described as almost completely embracing the host cell nucleus (circumnuclear) with the nucleus usually central while the microgametocytes displace it and do not tend to surround it. The pigment granules may be scattered or clumped at the poles. De Mello's figures illustrate several parasites at different stages of rounding up and popping out of the erythrocytes while others were completely free and had taken on a round - ovoid configuration which suggested 'post mortem' conditions (White and Bennett, 1979). This indicates that neither the description nor the illustrations for this parasite are accurate. It is assumed from the figures and the description that the above smear contained a mixed infection of a halteridial haemoproteid and a circumnuclear form.

Haemoproteus bramae was also described and redescribed by de Mello (1936, 1936-1937) (Appendix C) from a single *Athene brama* shot at Nagoa (*sic* Nova-Goa) (Salcete), Portuguese India. De Mello described this parasite as typically halteridial but sometimes totally surrounding the host cell nucleus, pigment granules scattered or in clusters and, sometimes, with the host cell nucleus displaced to the periphery. De Mello's illustrations indicate the presence of volutin granules. No counts of either pigment granules or volutin granules were given. The fact that samples had been taken from a dead bird and 'post mortem' changes had occurred, which caused the parasites to round up and leave the host cell, explains why some parasites displaced the host cell nucleus (immature circumnuclear) and others did not. From the description and figures this parasite is probably a circumnuclear parasite like *H. noctuae*.

Haemoproteus noctuae var. *nebraskensis* was described by Coatney and

Roudabush in 1937 (Appendix C), from a single specimen, *Bubo virginianus* from Peru, Nebraska, U.S.A. This parasite was described as almost completely surrounding the host cell nucleus but not in close contact with either the nucleus or the host cell membrane. The parasite contained many small vacuoles and it displaced the host cell nucleus in 50% of the cases but did not hypertrophy the host cell. Coatney and Roudabush's illustrations show halteridial parasites with pointed extending ends indicating that these are immature gametocytes of a circumnuclear form. Coatney and Roudabush stated that this parasite was similar to *H. noctuae* but unlike *H. noctuae* did not completely enclose the host cell nucleus. The hapantotype slide was examined in this study and showed an infection mainly of immature circumnuclear parasites with volutin granules similar to *H. noctuae*. Several mature forms which completely surround the host cell nucleus were also seen. In their 1971 checklist of the species of the genus *Haemoproteus* Levine and Campbell emended this variety to species level. This change was not based on criteria from the species description but on the premise that different host genera are infected with different haemoproteid species. Since *H. noctuae* was described from *Athene noctua* and *H. noctuae* var. *nebraskensis* was described from *Bubo virginianus* the latter was designated as a distinct species.

Coatney and Roudabush (1937) described *H. noctuae* var. *cellii* (Appendix C) from a single *Otus asio* from Peru, Nebraska, U.S.A. It was described as a halteridial parasite but examination of the hapantotype for this study showed that this blood film contains a mixed haemoproteid infection and there are not only halteridial but also circumnuclear forms present.

All of these haemoproteid species are circumnuclear forms with similar morphology. None have unique characters. It is proposed that these are, in fact, the same species. The valid name for this species is *Haemoproteus noctuae*, as it was the first to be described. The margins of this parasite may be amoeboid or entire. Gametocytes may or may not have volutin granules in the cytoplasm. The occurrence of volutin granules is not consistent as some birds from the current study which were sampled several times contained volutin granules each time while others contained volutin granules intermittently (Table 4). Volutin granules are not restricted by locality or host species but are common in the avian species *Otus scops* and *Otus bakkamoena* in Southeast Asia. Indeed in some blood films, volutin granules were also present in specimens of *Leucocytozoon ziemanni*. These granules are thought to be chromatin or a forerunner of chromatin (Wenyon, 1926) because they stain deeply with chromatin stains. The particular physiological state of the host may cause these granules to be produced.

Haemoproteus noctuae is herein redescribed with neohapantotype and paraneohapantotype slides designated.

TABLE 4. Occurrence of volutin granules in strigid haemoproteids sampled over time (all records from the files of IRCAH).

Species	Capture	Parasite	Volutin
Otus bakkamoena Bird # 1	62.IX.12	*H. syrnii*	+
		H. noctuae	+
	62.XI.30	*H. noctuae*	+
Otus bakkamoena Bird # 2	61.VI.21	*H. syrnii*	+
		H. noctuae	-
		P. vaughani	+
	61.VIII.11	*H. syrnii*	some
		P. vaughani	+
	61.IX.13	*H. syrnii*	+
		P. vaughani	+
	61.XII.20	*H. syrnii*	+
		H. noctuae	-
		P. vaughani	some
Otus bakkamoena Bird # 3	60.VI.16	*H. syrnii*	+
	60.IX.07	*H. syrnii*	-
	60.X.26	*H. syrnii*	-
		H. noctuae	-
	61.XII.22	*H. syrnii*	+
	62.IV.02	*H. syrnii*	+
		H. noctuae	+
Otus bakkamoena Bird # 4	60.II.11	*H. syrnii*	+
	61.I.02	*H. syrnii*	-
		H. noctuae	-
	62.II.03	*H. syrnii*	-
		H. noctuae	-
Otus bakkamoena Bird # 5	62.XI.02	*H. syrnii*	+
		H. noctuae	+
	62.XI.28	*H. syrnii*	+
	62.XII.07	*H. syrnii*	+
Otus bakkamoena Bird # 6	62.I.19	*H. syrnii*	+
		P. vaughani	+
	62.I.24	*H. syrnii*	some
		P. vaughani	+

TABLE 4 (cont'd). Occurrence of volutin granules in strigid haemoproteids sampled over time (all records from the files of IRCAH).

Species	Capture	Parasite	Volutin
Otus scops Bird # 7	62.I.24	*H. syrnii*	-
		H. noctuae	-
	62.XI.14	*H. syrnii*	+
		H. noctuae	+
	62.XII.13	*H. syrnii*	+
		H. noctuae	+

+ - volutin granules present
some - volutin present in some gametocytes
- - volutin granules absent
P. - *Plasmodium*
H. - *Haemoproteus*

Haemoproteus syrnii (Mayer, 1910)

Type Host: *Strix aluco* L.

Type Locality: Lübeck, West Germany or Vienna, Austria

Synonyms: *Haemoproteus glaucidii* de Mello, 1936, *in partim.*

Haemoproteus cellii (Coatney and Roudabush, 1937) Helmy Mohammed, 1958, *in partim.*

Haemoproteus multiparasitans Covaleda Ortega and Gállego Berenguer, 1950

Haemoproteus aegyptius (Helmy Mohammed, 1958) Levine and Campbell, 1971

Immature gametocyte: (Figures 9 and 10). Youngest forms usually initiate growth lateral to the erythrocyte nucleus but some may initiate growth in a polar position (Figure 10); parasite margins entire; volutin granules may be present.

Macrogametocyte: (Figure 11; Table 3). Halteridial parasite of medium size occupying approximately 60% of the erythrocyte-parasite complex causing host cell hypertrophy; cytoplasm finely granular, staining deep blue with Giemsa's stain; margin of the parasite entire with inner margin not usually appressing to the host cell nucleus; ends of the parasite usually round but may be pointed; pigment granules round to oval, yellow-brown, and scattered throughout the parasite's cytoplasm; volutin granules sometimes present, staining dark blue to violet, usually clumped at both poles of the parasite but sometimes scattered, may block pigment granules from view causing the pigment granule count to be artificially low; parasite nucleus compact, oval to elongate usually median, staining dark pink; host cell

Figure 9

Haemoproteus syrnii

immature gametocyte

Figure 10

Haemoproteus syrnii

immature gametocyte

Figure 11

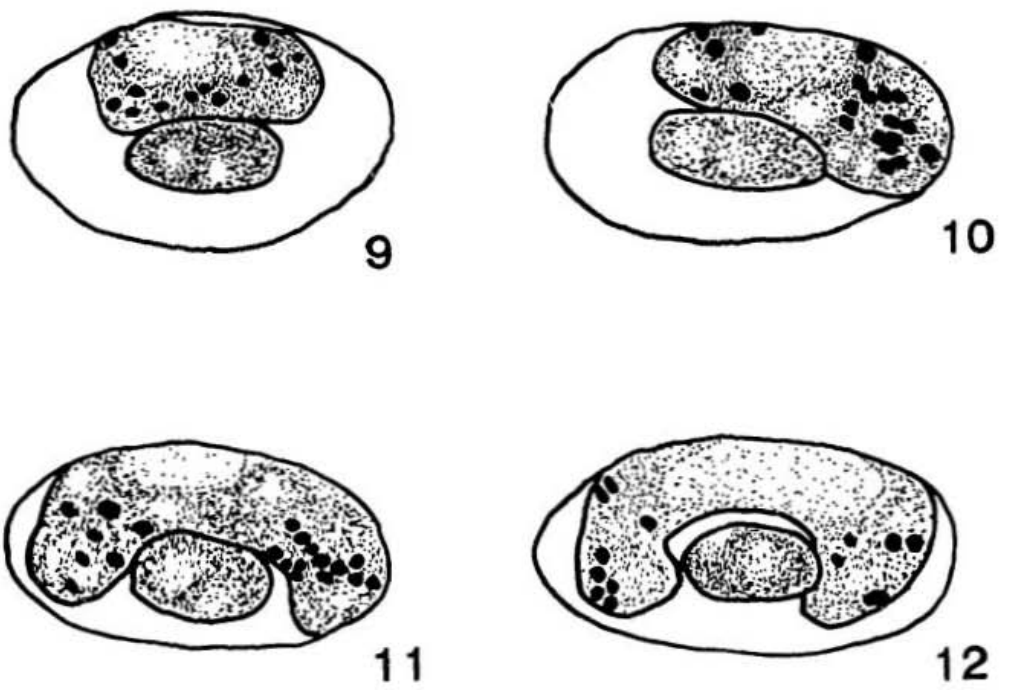
Haemoproteus syrnii

mature macrogametocyte

Figure 12

Haemoproteus syrnii

mature microgametocyte


9
10
11
12
10µm

nucleus is usually atrophied and displaced slightly.

Microgametocyte: (Figure 12; Table 3). Medium size halteridial parasite occupying approximately 60% of the erythrocyte-parasite complex causing host cell hypertrophy; cytoplasm is finely granular; staining pale blue to colourless with Giemsa's stain; margins entire with inner margin usually not appressing to the host cell nucleus; ends usually round but may be pointed; pigment granules, fewer than macrogametocyte, round to oval, staining yellow-brown, scattered throughout the cytoplasm of the parasite; volutin granules sometimes present, staining dark blue to violet, usually clumped at both poles of the parasite but sometimes scattered throughout, often confusing pigment granule counts (see above); parasite nucleus large, elongate, diffuse, median, staining pale pink with Giemsa's; host-cell nucleus usually atrophied and displaced slightly.

Schizogony: The stages involved in this part of the life cycle are unknown. The lungs, kidneys, spleen and liver of the vertebrate host have been shown to be the sites of schizogony for some haemoproteids (Aragão, 1908; Wenyon, 1926; Fallis and Desser, 1977; Bennett, 1987) and this is likely to be so for this species as well.

Sporogony: The vectors for *Haemoproteus syrnii* are not known but are probably species of the family, Ceratopogonidae or possibly the family, Hippoboscidae. Of the 11 species for which this part of the life cycle is known, eight are transmitted by ceratopogonids (genus *Culicoides*) and three by hippoboscids (Bennett *et al.*, 1985).

McClure *et al.* (1978) in their Southeast Asian study (1978) showed that the prevalence of *Haemoproteus syrnii* and *H. noctuae* infections of several species of owls did not change year round. This suggests that the vector or vectors of *H. syrnii* are present throughout the year.

Geographic Range: North and South America, Asia, Europe, Africa and potentially throughout the range of the Strigidae.

Neohapantotype: Mayer (1910) did not designate a type specimen for *H. syrnii.* The location of his personal collection is unknown and it is presumed lost. The hapantotype slide of *H. noctuae* var. *cellii* has been examined in this study. It contains a mixed infection of *H. cellii (H. syrnii)* and *H. noctuae.* There are many multiple infections of two and three parasites per erythrocyte which often results in distortion of the parasites. Large numbers of volutin granules are present and may block the pigment granules from view, resulting in pigment granule counts being low. This slide is therefore unacceptable as a neohapantotype. The parahapantotype slide for *H. cellii* var. *aegyptius* was also examined but it was taken from neither the type host nor type locality. The neohapantotype should be from the same host and locality as the original but if no suitable material is available from either of these, a specimen from the nearest locality and the closest related host species can be designated as the neohapantotype. Blood smears from the type species were available but those infected with *Haemoproteus syrnii* were either of poor quality or contained mixed haemoproteid infections and were inadequate for neohapantotype

designation. In the absence of the original material the blood film #44851 which has been deposited in the collection at the IRCAH is designated as the neohapantotype for *Haemoproteus syrnii* in keeping with article 75 of the ICZN. This smear was taken from *Strix varia*, the barred owl, collected in Oklahoma City, Oklahoma, U.S.A. by Alan Kocan on March 3, 1975.

Paraneohapantotype: Blood film #10688 from *Otus scops*, the Eurasian scops-owl collected by H. Elliott McClure, November 12, 1967 at Chi Tou, Taiwan; blood film # 102692, of *H. aegyptius* from *Bubo bubo*, the northern eagle-owl, collected by A. H. Helmy Mohammed, November 30, 1949 in Cairo, Egypt.

Additional Host Records: *Asio otus, Athene brama, A. noctua, Bubo africanus, B. coromandus, B. lacteus, B. poensis, B. virginianus, Ciccaba woodfordii, Glaucidium brodiei, G. cuculoides, Ninox philippensis, N. scutulata, O. asio, O. bakkamoena, O. choliba, O. rufescens, O. spilocephalus, O. trichopsis, Strix aluco, S. occidentalis.*

Comments: In 1910 Mayer named and briefly described *Halteridium syrnii* from *Syrnium aluco (Strix aluco)*. In 1911 he redescribed the same material in more detail (Appendix C). In both cases Mayer stated that a portion of the smears were taken in Lübeck, West Germany and a portion in Vienna, Austria but did not note the collection localities of the individual smears. This species was listed as *Haemoproteus syrnii* by Coatney (1936) because *Halteridium* is considered to be a synonym of *Haemoproteus*. There is however some confusion as to when these two

genera were synonymized. As early as 1904 Schaudinn recognized *Haemoproteus* as the valid genus, "*Halteridium* (former name) now called *Haemoproteus*" (translated) but the two names were used interchangeably for a number of years.

Mayer found two characteristics which he felt distinguished this parasite as a separate species. One was the presence of double nuclei in immature (round and elongate) gametocytes. The second nucleus stained darker than the main ("true") nucleus, and was found either lying next to the true nucleus (in round, immature gametocytes) or in one pole of the parasite (elongate, immature gametocytes). From his descriptions and figures the second nucleus appears to have been clumped pigment granules, or more likely, volutin. Mayer believed that the second nucleus disappeared when the parasite matured. This may occur as the clumped pigment (volutin) tends to separate into discrete distinct granules as the parasite matures.

The second distinguishing characteristic of this species according to Mayer (1911) was the presence of "Alkaliphile" granules. When smears were lightly stained many small vacuoles were present but when smears were stained heavily, dark violet granules were present. These dark violet 'alkaliphile' granules are also probably volutin. Mayer's figures show that *H. symii* is a halteridial parasite of medium size which may or may not contain purple volutin granules depending on the intensity of the stain.

Schwetz (1935) also described this species from *Syrnium nuchale* from Stanleyville, 'Belgian Congo' (now Zaire). He wrote of a halteridial parasite with "gros grain rond" in small immature forms and "beaucoup plus petits" granules in the mature forms. He described the so-called second nucleus as a large round grain which

occurred in immature gametocytes and noted that when the gametocytes matured many small granules were present. It appears that Schwetz believed the dark violet - black mass to be a type of pigment.

Schwetz's figures of mature gametocytes were similar to those of Mayer's. Each shows typically halteridial parasites in which the number of "granules" is not constant. These granules correspond to the dark purple volutin granules recorded from the material used in this study.

Coatney and Roudabush (1937) believed that *H. syrnii* was the same as *H. noctuae* var. *A* except that it had basophilic granules in specimens which were heavily stained. However, *Haemoproteus noctuae* was described by Celli and Sanfelice as a circumnuclear parasite and *H. syrnii* by Mayer as a halteridial form. This major morphological difference separates these two species.

Coatney and Roudabush in 1937 described a new variety of haemoproteid, *Haemoproteus noctuae* var. *cellii* (Appendix C) from a single *Otus asio* from Peru, Nebraska, U.S.A. They described a halteridial parasite in close contact with the host cell membrane but not with the host cell nucleus. Linear hypertrophy was evident and the erythrocyte nucleus was often displaced. The parasite nucleus was found towards the periphery and indistinct vacuoles were present. Two figures of this typical halteridial parasite, one each of a macrogametocyte and a microgametocyte, are given. The ends of these parasites do not appear to be as pointed or extending as the description suggests. Coatney and Roudabush distinguished this parasite from *H. noctuae* on the basis of (1) all growth stages had smooth contours, (2) macrogametocyte nuclei lay near the periphery of the parasite next to the host cell

membrane, and (3) a greater number of pigment granules. From Coatney and Roudabush's description and figures, Helmy Mohammed (1958) decided that *H. noctuae* var. *cellii* differed from *H. noctuae* in several major characteristics including gametocyte form. As a result he decided that this variety should be raised to the rank of a species. It appears from Coatney and Roudabush's figures and Helmy Mohammed's comments that *H. cellii* is a typical halteridial parasite similar to *H. syrnii* but lacking volutin granules.

The hapantotype slide of *H. cellii* designated by Coatney and Roudabush was examined in this study. The slide contains a mixed haemoproteid infection of a halteridial form and a circumnuclear form. There are many multiple infections of two or three parasites per erythrocyte which often causes the parasites to be distorted. Also, the presence of volutin granules leads to confusion in that they may be misidentified as pigment granules, thereby giving an artificially high pigment granule count which may account for the greater numbers reported for the species by Coatney and Roudabush (1937). These factors cast doubt on the validity of this slide as a hapantotype and on *H. cellii* as a distinct species.

Covaleda Ortega and Gállego Berenguer (1950) described a new parasite, *H. multiparasitans* (Appendix C) from a single specimen, *Athene noctua* from Granada, Spain. Based on their description and figures it is assumed that this species was described from either blood of a dead bird or from smears which had dried slowly as many parasites had rounded up and popped out of the erythrocytes, an artifact of the conditions under which the smear was made (Bennett *et al.*, 1975). These parasites were described as almost globular, initiating growth lateral to the host cell

nucleus or in a pole in which case one end appeared to grow faster resulting in a halteridial haemoproteid form. They also reported that no circumnuclear forms were seen and that there were many multiple infected erythrocytes. From the figures it appears that the borders of this parasite are irregular but not amoeboid. The phenomenon of multiple infected erythrocytes occurs when the intensity of a haemoproteid infection is high and is not a valid character on which to base a new species. Additionally the number of granules varied between the sexes (> 30 - female; < 20 - male) which is unusual for pigment granules. Indeed the *Haemoproteus columbae* complex is the only known haemoproteid in which the pigment granule numbers differ between sexes (Female=2N, Male=N; Bennett and Peirce, in press). It appears likely, therefore, that there was some degree of confusion between pigment granules and volutin granules. Under better preparatory conditions this parasite would likely appear as a medium-sized halteridial parasite very similar to *H. syrnii*.

Haemoproteus cellii var. *aegyptius* was described by Helmy Mohammed, 1958 from a lone *Bubo bubo* in the Cairo Zoological Gardens at Guiza, Egypt. His description of this parasite is clear and concise (Appendix C). It includes measurements of morphometric parameters as well as several figures. He described a typical halteridial parasite in which the parasites do not have much contact with the host cell nucleus or the host cell membrane, they have dissimilar ends and the nucleus is central but closer to the host cell periphery.

Helmy Mohammed separated this parasite from *H. cellii* on the basis of shorter gametocytes, wider macrogametocytes, and longer microgametocyte nuclei. One of

Helmy Mohammed's parahapantotype slides of this parasite was examined in this study and Helmy Mohammed's description was confirmed. Levine and Campbell (1971) raised *H. cellii* var. *aegyptius* to species level, *H. aegyptius*, on the same premise as for *H. noctuae var. nebraskensis*. This parasite is a typical halteridial parasite similar to *H. syrnii*.

All of the above species are halteridial haemoproteids. The descriptions are similar with none having any distinguishing features. It is proposed that these species are synonyms. The name *Haemoproteus syrnii* takes priority as the valid name since it was the first described.

This parasite is distinguished from *H. noctuae* by its morphological form; *H. syrnii* - halteridial, *H. noctuae* - circumnuclear. Also the margins of *H. syrnii* are always entire, never amoeboid while those of *H. noctuae* may be either. *Haemoproteus syrnii* is similar to *H. noctuae* in that it may or may not contain volutin granules in the cytoplasm and it occurs throughout the studied range of the family.

Haemoproteus syrnii is herein redescribed with neohapantotype and paraneohapantotype slides designated.

Tytonidae

Haemoproteus phodili n. sp.

Type Host: *Phodilus badius* (Horsfield)

Type Locality: Malaya

Immature gametocyte: (Figure 13). Youngest forms seen initiate growth lateral to the erythrocyte nucleus; margins entire.

Macrogametocyte: (Figure 14; Table 3). Medium sized parasite occupying approximately 55% of the erythrocyte-parasite complex causing slight hypertrophy of the erythrocyte; slightly halteridial (microsomal); ends round; cytoplasm finely granular staining deep blue with Giemsa's stain; margins entire; pigment granules yellow-brown, small, round and scattered throughout the parasite cytoplasm; volutin granules not present; parasite nucleus distinct, compact, round to oval, centrally positioned staining dark pink; host cell nucleus is slightly atrophied and displaced.

Microgametocyte: (Figure 15; Table 3). Medium, slightly halteridial (microsomal) parasite occupying approximately 53% of the erythrocyte-parasite complex causing slight hypertrophy of the erythrocyte; ends round, cytoplasm staining light blue to colourless; margins entire; pigment granules yellow-brown, small, round, randomly distributed throughout the cytoplasm; volutin granules not present; parasite nucleus diffuse, colourless, slightly larger than that of the macrogametocyte; host cell nucleus is atrophied and slightly displaced.

Figure 13

Haemoproteus phodili n. sp.

immature gametocyte

Figure 14

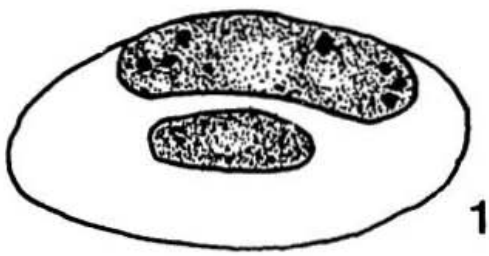
Haemoproteus phodili n. sp.

mature macrogametocyte

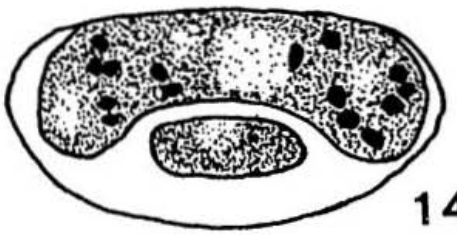
Figure 15

Haemoproteus phodili n. sp.

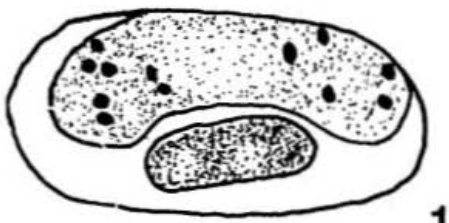
mature microgametocyte



13



14



15

10µm

Schizogony: The schizogonic stages of this parasite are unknown. For other *Haemoproteus* species the lungs, kidneys, spleen and liver of the vertebrate host have been shown to be sites for schizogony (Aragão, 1908; Wenyon, 1926; Fallis and Desser, 1977; Bennett, 1987). This may also be true for this species.

Sporogony: The vector or vectors of this parasite are unknown. They may be members of the families Hippoboscidae or Ceratopogonidae as is the case for other species for which this part of the life cycle is known (eight transmitted by ceratopogonids (genus *Culicoides*) and three by hippoboscids) (Bennett *et al.* 1985).

Geographic Range: Asia. The actual range of this parasite potentially extends further than the Asian continent throughout the whole range of the Tytonidae.

Hapantotype: Blood film #36958 deposited at the IRCAH from *Phodilus badius*, the Oriental bay-owl, collected by H. Elliott McClure on February 22, 1970 in Malaya.

Parahapantotypes: Blood film numbers 2631 and 2633 collected by H. Elliott McClure in Rantau Panjang, Malaysia each from *Phodilus badius*, the Oriental bay-owl on August 25, 1960 and February 3, 1961 respectively.

Comments: *Haemoproteus phodili* n. sp. is a microsomal to halteridial parasite occupying approximately 55% of the erythrocyte-parasite complex. The microgametocyte nucleus is only slightly larger than the macrogametocyte nucleus.

This is unusual for haemoproteid species and has only been recorded for one other species, *H. lophortyx* (Bennett and Peirce, in press) from the Phasianidae. *Haemoproteus phodili* n. sp. and *H. syrnii* are similar in appearance but differ for some of the parameters measured (see Appendix D). Only future transmission studies will reveal if these are indeed distinct or one species. If the latter is the case, *H. phodili* n. sp. will fall as a synonym of *H. syrnii*. Until such time the premise of host family specificity (Bennett and Campbell, 1972; Bennett *et al.*, 1975; Williams *et al.*, 1975) will be followed and *Haemoproteus phodili* n. sp. is considered to be a distinct and separate species. This species is hereby named *Haemoproteus phodili* n. sp. after the genus in which it most commonly occurs.

Haemoproteus sp.

Haemoproteus sp. is recorded from both *Tyto longimembris* and *Tyto alba* in this study. The immature gametocytes (Figure 16) initiate growth in a lateral or subpolar position and their margins may be entire or amoeboid.

Mature gametocytes (Figure 17, Table 1) are circumnuclear; medium size, occupying approximately 59% of the erythrocyte-parasite complex causing host cell hypertrophy; cytoplasm granular staining blue with Giemsa's; margins amoeboid or entire or a combination of the two not always appressing to either the erythrocyte nucleus or cell membrane; ends may be pointed or round; pigment granules small to medium staining yellow-brown, scattered throughout the cytoplasm; volutin granules not present ; parasite nucleus not distinguishable in the majority of parasites in the available smears, so macro- and microgametocytes are not distinguished; host-cell nucleus is atrophied but rarely displaced.

Haemoproteus sp. is quite distinct from *Haemoproteus phodili* n. sp. The latter is a halteridial parasite with entire margins and the former is a circumnuclear parasite with margins entire or amoeboid. This species is in many respects quite similar to *Haemoproteus noctuae* of the Strigidae. Both are circumnuclear parasites which may have both the inner and outer margins entire or amoeboid or a combination of both. The pigment granule number is also quite similar. Haemoproteids have been shown experimentally to be family specific (Bennett and Campbell, 1972; Bennett *et al.*, 1975; Williams *et al.*, 1975) and by this premise the species which infects the tytonids is distinct from *Haemoproteus noctuae* of the strigids. At the present time however no adequate material is available to be

Figure 16

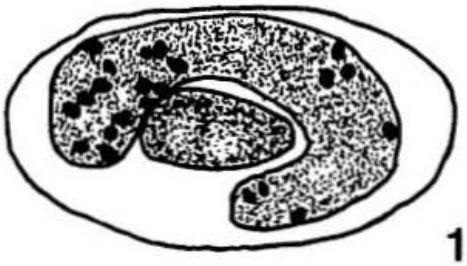
Haemoproteus sp.

immature gametocyte

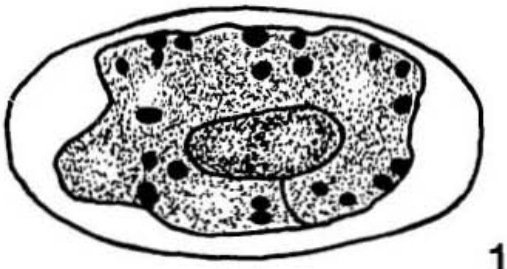
Figure 17

Haemoproteus sp.

mature gametocyte



16



17

10µm

designated hapantotype or parahapantotypes. When material becomes available this haemoproteid will be described as a new species until such a time when experimental studies may show that it is indeed *Haemoproteus noctuae*.

Conclusions

The material examined in the present study contains two morphological types of haemoproteids, a circumnuclear type and a halteridial type in the Strigidae and in the Tytonidae.

In the Strigidae these two morphological forms can be distinguished quite easily upon examination of a blood smear. The halteridial form (1) always has smooth margins, (2) the macrogametocyte does not affect the erythrocyte width or the erythrocyte nucleus width, (3) the microgametocyte slightly hypertrophies erythrocyte width and nucleus width and (4) noticeably displaces the erythrocyte nucleus. On the other hand the circumnuclear form (1) may have amoeboid margins, (2) does not noticeably displace the erythrocyte nucleus and (3) both the macro- and microgametocytes greatly hypertrophy the erythrocyte width and noticeably atrophy the erythrocyte nucleus width.

The halteridial form in this study could not be separated from *H. syrnii* nor could the circumnuclear form be separated from *H. noctuae*. Comparisons of the measurements of morphometric parameters of the two forms in this study with those given in previous descriptions are given in Appendix D. Differences were not considered relevant because morphometric parameters are variable even for a single haemoproteid species within the same blood smear (Bennett *et al.*, 1985).

Additionally Atkinson (1986) showed experimentally that determination of haemoproteid species should be based on morphological types as suggested by Greiner *et al.* (1977) and Bennett and Peirce (in press) and not on minor morphological differences between hosts.

No haemoproteid species have been previously described from the Tytonidae. The material used in this study contains two distinct haemoproteids, one a circumnuclear form and the other a halteridial form. The halteridial form is herein described as *Haemoproteus phodili* n. sp. The circumnuclear form is a distinctive parasite but because no adequate material was available for designation as hapantotype and parahapantotypes it is not herein described as a new species.

Comparisons of the two halteridial parasites, *H. syrnii* of the Strigidae and *H. phodili* of the Tytonidae (Appendix D) indicated differences for many parameters. These differences may reflect variability between species, families and geographic locations (Bennett *et al.*, 1986) or may be actual parasite species differences. In either case these two will remain distinct species on the basis of family specificity until such a time when this premise is disproven by cross transmission studies.

Comparison of the two circumnuclear parasites, *H. noctuae* of the Strigidae and *Haemoproteus* sp. of the Tytonidae showed differences between the uninfected and infected erythrocytes and microgametocytes but none between the macrogametocytes. It appears that these two may in fact be the same species because the macrogametocyte parameters are not significantly different and indeed the two are not distinguishable by observation in a blood smear. However, unless cross-infectivity studies prove differently the two shall remain distinct species on the basis

of family specificity.

As a result of this study a dichotomous key to the haemoproteids of the Strigiformes was constructed as follows:

1. Mature gametocyte parasitizing species of the avian family Strigidae 2

   Mature gametocyte parasitizing species of the avian family Tytonidae 3

2. Mature gametocyte encircling the erythrocyte nucleus (circumnuclear); margins amoeboid or entire ..... *Haemoproteus noctuae*

   Mature gametocyte not encircling the erythrocyte nucleus (halteridial); margins always entire ..... *Haemoproteus syrnii*

3. Mature gametocyte encircling the erythrocyte nucleus (circumnuclear); margins amoeboid or entire ..... *Haemoproteus* sp.

   Mature gametocyte not encircling the erythrocyte nucleus (microsomal to halteridial); margins always entire ..... *Haemoproteus phodili* n. sp.

These species are all morphologically similar to other species of haemoproteids; *H. syrnii* and *H. phodili* to other halteridial parasites of the same type (eg. *H. nettionis, H. beckeri* and *H. formicarius*) and *H. noctuae* to other circumnuclear parasites (eg. *H. velans, H. greineri, H. circumnuclearis* and *H.*

archilochus). Until such a time that family specificity is disproven the three species described in this study will be considered distinct (Bennett *et al.*, 1986).

As Helmy Mohammed stated in 1958 "our ignorance of the complete life cycles of development should not be taken forever as an excuse to refrain from identifying parasites".

SUMMARY

1. *Haemoproteus* was the most commonly occurring genus of blood parasites found in 79.4% of the Strigiformes examined, followed by *Leucocytozoon* in 38.0%, *Plasmodium* in 11.6%, *Trypanosoma* in 6.9%, Microfilaria in 4.2% and Others in 14.5%.
2. One species of *Leucocytozoon* - *L. ziemanni*; six species of *Plasmodium* - *P. circumflexum, P. elongatum, P. fallax, P. polare, P. relictum* and *P. vaughani*; three species of *Trypanosoma* - *T. avium, T. calmettei* and *T. everetti* were identified from the Strigiformes. The microfilaria were not identified to species.
3. *Haemoproteus noctuae* and *H. syrnii* were recorded from the Strigidae. *Haemoproteus glaucidii, in partim., H. bramae, H. nebraskensis* and *H. cellii, in partim.* were synonymized with *H. noctuae*. *Haemoproteus glaucidii, in partim., H. cellii, in partim., H. multiparasitans* and *H. aegyptius* were synonymized with *H. syrnii*.
4. *Haemoproteus noctuae* and *H. syrnii* were redescribed.
5. *Haemoproteus phodili* n. sp. and *Haemoproteus* sp. were recorded from the Tytonidae.
6. *Haemoproteus phodili* n. sp. was described.
7. A dichotomous key to the haemoproteid species of the Strigiformes was constructed.

LITERATURE CITED

American Ornithologists' Union. 1983. Check-list of North American birds. Sixth edition. Allen Press, Inc., Lawrence, Kansas. 877pp.

Aragão, H. de B. 1908. Über den entwicklungsgang und die übertragung von *Haemoproteus columbae*. Arch. Protistenkd. 12: 154-167.

Atkinson, C. T. 1986. Host specificity and morphometric variation of *Haemoproteus meleagridis* Levine, 1961 (Protozoa: Haemosporina) in gallinaceous birds. Can. J. Zool. 64: 2634-2638.

Bennett, G. F. 1987. Chapter 20. Hematozoa. In: Companion Bird Medicine. E. Burr editor. Iowa State University Press. Ames, Iowa, U.S.A. 247 pp.

Bennett, G. F., J. R. Caines and M. A. Whiteway. 1986. Avian haemoproteidae. 22. The haemoproteids of the New World flycatchers, the Tyrannidae. Can. J. Zool. 64: 774-777.

Bennett, G. F. and A. G. Campbell. 1972. Avian Haemoproteidae. I. Description of *Haemoproteus fallisi* n. sp. and a review of the haemoproteids of the family Turdidae. Can. J. Zool. 50(10): 1269-1275.

Bennett, G. F. and A. G. Campbell. 1973. Avian haemoproteidae. III. Description of *Haemoproteus fusca* sp. n. and redescription of *Haemoproteus halcyonis* de Mello, 1935, parasites of the kingfisher family Alcedinidae. J. Parasitol. 59(2): 337-343.

Bennett, G. F., D. J. Forrester, E. C. Greiner and A. G. Campbell. 1975. Avian haemoproteidae. 4. Description of *Haemoproteus telfordi* sp. nov., and a review of the haemoproteids of the families Gruidae and Otidae. Can. J. Zool. 53(1): 72-81.

Bennett, G. F., P. C. C. Garnham and A. M. Fallis. 1965. On the status of the genera *Leucocytozoon* Ziemann, 1898 and *Haemoproteus* Kruse, 1890 (Haemosporidiida: Leucocytozoidae and Haemoproteidae). Can. J. Zool. 43: 927-932.

Bennett, G. F., E. C. Greiner and A. G. Campbell. 1975. Avian haemoproteidae. 5. The haemoproteids of the family Threskiornithidae. Can. J. Zool. 53: 634-638.

Bennett, G. F., N. O. Okia, R. G. Ashford and A. G. Campbell. 1972. Avian haemoproteidae. II. *Haemoproteus enucleator* sp. n. form the kingfisher, *Ispidina picta* (Boddaert). J. Parasitol. 58(6): 1143-1147.

Bennett, G. F. and M. A. Peirce. (in press) The haemoproteids of the avian family Phasianidae. Can. J. Zool.

Bennett, G. F. and M. A. Peirce. 1988. Morphological form in the avian Haemoproteidae and an annotated checklist of the genus *Haemoproteus* Kruse, 1890. J. Nat. Hist. 22: 1683-1696.

Bennett, G. F., M. A. Peirce and J. R. Caines. 1985. Avian haemoproteidae. 19. The haemoproteids of the sunbirds (family Nectariniidae) and morphometric variation in *Haemoproteus sequeirae*. Can. J. Zool. 63: 1371-1376.

Bennett, G. F., M. Whiteway and C. Woodworth-Lynas. 1982. A host-parasite catalogue of the avian haematozoa. Memorial Univ. Nfld. Occas. Pap. Biol. No. 5. 243pp.

von Brand, T. 1966. Biochemistry of Parasites. Academic Press, New York. 429pp.

Burton, J. A. 1973. Owls of the World. Their evolution, structure and ecology. E. P. Dutton and Co., Inc. New York. 216pp.

Celli, A. and F. Sanfelice. 1891. Sui parasiti del globulo rosso. Nell'uomo e negli animali. Instituto d'Igiene sperimentale della R. Università di Roma 1(1): 33-63.

Coatney, G. R. 1936. A check-list and host-index of the genus *Haemoproteus*. J. Parasitol. 22 (1): 88-105.

Coatney, G. R. and R. L. Roudabush. 1937. Some blood parasites from Nebraska birds. Am. Midl. Nat. 18: 1005-1030.

Covaleda Ortega, J. and J. Gállego Berenguer. 1950. Hemoproteus aviares. Rev. Iber. Parasitol. 10(2): 141-185.

Danilewskyi, V. Ya. 1885. Zur parasitologie des blutes. Biol. Zentralbl. 5: 529-537.

Edwards, E. P. 1982. A Coded Workbook of Birds of the World. Volume 1: Non-passerines. Second edition. Ernest P. Edwards, Sweet Briar, VA. 134pp.

Fallis, A. M. and S. S. Desser. 1977. Chapter 5. On species of *Leucocytozoon, Haemoproteus and Hepatocyctis. In:* Parasitic Protozoa. Volume III. Gregarines, Haemogregarines, Coccidia, Plasmodia and Haemoproteids. J. P. Kreier editor. Academic Press. New York, U.S.A. 563pp.

Forrester, D. J., E. C. Greiner, G. F. Bennett and M. K. Kigaye. 1977. Avian Haemoproteidae. 7. A review of the haemoproteids of the family Ciconiidae (storks) and descriptions of *Haemoproteus brodkorbi* sp. nov. and *H. peircei* sp. nov. Can. J. Zool. 55(8): 1268-1274.

Garnham, P. C. C. 1966. Malaria parasites and other Haemosporidia. Blackwell Scientific Publications, Oxford. 1114pp.

Greiner, E. C., A. K. Mandal and N. C. Nandi. 1977. *Haemoproteus bennetti* sp. n. and a review of the haemoproteids from the Picidae (woodpeckers). J. Parasitol. 63(4): 651-656.

Helmy Mohammed, A. H. 1958. Systematic and experimental studies on protozoal blood parasites of Egyptian birds. Vol. II. Cairo University Press. 298pp.

International Code of Zoological Nomenclature. 1985. Third edition. American Association for Zoological Nomenclature, Washington D.C. 338pp.

Jörg, M. E. 1931. Nuevos protozoos parasitos de la republica Argentina. Arch. Soc. Biol. Montev. 5(suppl.): 1141-1165.

Kruse, W. 1890. Ueber blutparasiten. Archiv fuer Pathologische Anatomie und Physiologie und fuer Klinische Medicin 120: 541-559.

Laveran, M. A. 1903. Contribution a l'étude de *Haemamoeba ziemanni*. C. R. Seances Soc. Biol. Fil. 55: 620-623.

Levine, N. D. and G. R. Campbell, 1971. A check-list of the species of the genus *Haemoproteus* (Apicomplexa, Plasmodiidae). J. Protozool. 18(3): 475-484.

Mayer, M. 1910. Über die entwicklung von *Halteridium* (Vorläufige Mitteilung). Archiv für Schiffs-und Tropen-Hygiene 14(7): 197-202.

Mayer, M. 1911. Über ein *Halteridium* und *Leucocytozoon* des waldkauzes und deren weiterentwicklung in Stechmücken. Arch. Protistenkd. 21: 232-254.

McClure, H. E., P. Poonswad, E. C. Greiner and M. Laird. 1978. Haematozoa in the birds of Eastern and Southern Asia. Memorial University of Newfoundland, St. John's, Newfoundland, Canada. 296pp.

de Mello. I. F. 1936. New haemoproteids of some Indian birds. Proc. Indian Acad. Sci. Sect. B. 2: 469-475.

de Mello, I. F. 1936-1937. On Haemoprotozoa of Indian birds. Comptes Rendus XII. Congrès International de Zoologie 2: 1391-1445.

Musaev, M. A. and N. R. Zeiniev. 1977. Nekotorye voprosy sistematiki kroveparazitov ptits roda *Haemoproteus* na primere analiza materialov vtorogo vsesoyuznogo s"ezda. (Questions of the systematics of blood parasites of the genus *Haemoproteus* during the analysis of material of the Second All Union Conference on Protozoology. (In Russian). Izv. Akad. Nauk Az. SSR Ser. Biol. Nauk 1: 51-59.

Peirce, M. A. 1981. Distribution and host-parasite check-list of the haematozoa of birds in Western Europe. J. Nat. Hist. 15: 419-458.

Peirce, M. A. and G. F. Bennett. 1979. Avian haematozoa: some taxonomic and nomenclatural problems arising from the Russian literature. J. Protozool. 26(3): 357-359.

Peters, J. L. 1940. Check-list of Birds of the World. Volume IV. Harvard University Press, Cambridge, Mass. 291pp.

Russell, P. F., L. S. West, R. D. Manwell and G. Macdonald. 1963. Practical Malariology. Second Edition. Oxford University Press, London. 750pp.

Sacchi, L. and C. Prigioni. 1984. Occurrence of *Leucocytozoon* and *Haemoproteus* (Apicomplexa, Haemosporina) in Falconiformes and Strigiformes of Italy. Annales de Parasitologie Hum. Comp. 59(3): 219-226.

Schaudinn, F. 1904. Generations-und wirtswechsel bei *Trypanosoma* und *Spirochaete*. Arbeiten aus dem kaiserlichen Gesundheitsamte 20: 387-439.

Schwetz, J. 1935. Recherches morphologiques sur un *Haemoproteus* spécial et deux *Plasmodiums* du hibou *Syrnium nuchale* et sur deux *Plasmodiums* du petit tisserin *Brachycope anomala* (Fam. Ploceidae). Rivista di Malariologia 14(3): 214-247.

Sergent, Ed. and Ét. Sergent. 1907. Études sur les hématozoaires d'oiseaux. *Plasmodium relictum, Leucocytozoon ziemanni* et *Haemoproteus noctuae, Haemoproteus columbae*, trypanosome de l'hirondelle. Ann. Inst. Pasteur (Paris) 21: 251-280.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. Second edition. W. H. Freeman and Company. 859pp.

Statistical Package for Social Sciences. 1983. SPSS* Users Guide. McGraw - Hill Book Company. New York. 806pp.

Wenyon, C. M. 1926. Protozoology. A manual for medical men, veterinarians and zoologists. Volumes 1 and 2. Bailliére, Tindall and Cassel Ltd., London. 1563pp.

White, E. M. and G. F. Bennett. 1978. Avian haemoproteidae. 9. Description of *Haemoproteus stellaris* n. sp. and a review of the haemoproteids of the swallow family Hirundinidae. Can. J. Zool. 56(10): 2110-2116.

White, E. M. and G. F. Bennett. 1979. Avian haemoproteidae. 12. The haemoproteids of the grouse family Tetraonidae. Can. J. Zool. 57(7): 1465-1472.

White, E. M., E. C. Greiner, G. F. Bennett and C. M. Herman. 1978. Distribution of the hematozoa of Neotropical birds. Rev. Biol. Trop. 26: 43-102.

Williams, N. A., G. F. Bennett and J. L. Mahrt. 1975. Avian haemoproteidae. 6. Description of *Haemoproteus caprimulgi* sp. nov., and a review of the haemoproteids of the family Caprimulgidae. Can. J. Zool. 916-919.

Wolfson, F. 1936. *Plasmodium oti* n. sp. a *Plasmodium* from the eastern screech owl (*Otus asio naevius*), infective to canaries. Am. J. Hyg. 24: 94-101.

Zeiniev, N. R. 1975. Paraziticheskie prosteishie krovi ptits severo-vostochnogo Azerbaidzhana. (Parasitic protozoa in the blood of birds from North-Eastern Azerbaidzhan). (In Russian). Izv. Akad. Nauk Az. SSR Ser. Biol. Nauk 4: 86-89.

APPENDIX A

SPECIES OF STRIGIFORMES EXAMINED IN THIS STUDY

STRIGIDAE

Aegolius acadicus (Gmelin)	Northern saw-whet owl*
Asio flammeus (Pontoppidan)	Short-eared owl*
Asio otus (Linnaeus)	Long-eared owl*
Athene brama (Temminck)	Spotted owlet/little-owl**
Athene noctua (Scopoli)	Little owl**
Bubo africanus (Temminck)	Spotted/African eagle-owl**
Bubo bubo (Linnaeus)	Northern eagle-owl**
Bubo coromandus (Latham)	Dusky eagle-owl**
Bubo virginianus (Gmelin)	Great horned owl*
Ciccaba woodfordii (A. Smith)	African wood-owl**
Glaucidium brodiei (Burton)	Collared owlet/pygmy owl**
Glaucidium cuculoides (Vigors)	Asian barred owlet**
Glaucidium radiatum (Tickell)	Jungle owlet**
Ninox connivens (Latham)	Barking owl**
Ninox philippensis Bonaparte	Philippine boobook-owl**
Ninox scutulata (Raffles)	Brown hawk-owl**
Nyctea scandiaca (Linnaeus)	Snowy owl*
Otus asio (Linnaeus)	Eastern screech-owl*
Otus bakkamoena Pennant	Collared scops-owl**
Otus choliba (Vieillot)	Tropical screech-owl*

APPENDIX A cont'd

Otus rufescens (Horsfield)	Reddish scops-owl**
Otus scops (Linnaeus)	Eurasian scops-owl**
Otus spilocephalus (Blyth)	Mountain scops-owl**
Otus trichopsis (Wagler)	Whiskered screech-owl*
Strix aluco Linnaeus	Eurasian tawny owl**
Strix occidentalis (Xantus de Vesey)	Spotted owl*
Strix varia Barton	Barred owl*

TYTONIDAE

Phodilus badius (Horsfield)	Oriental bay-owl**
Tyto alba (Scopoli)	Common barn-owl*
Tyto longimembris (Jerdon)	Eastern grass-owl**

* A. O. U., 1983

** Edwards, 1982

Note: Authorities from Peters, 1940

APPENDIX B

Leucocytozoa in the Strigiformes (based on the files of the IRCAH).

	Total Examined	*Leucocytozoon*	
		ziemanni	*L.*sp.
STRIGIDAE			
Aegolius acadicus	26	25	
Asio flammeus	10	10	
Asio otus	3	3	
Athene brama	20	1	
Athene noctua	3	3	
Bubo africanus	13	4	
Bubo bubo	1		
Bubo coromandus	3		
Bubo virginianus	17	12	
Ciccaba woodfordii	2		
Glaucidium brodiei	12	4	2
Glaucidium cuculoides	14	10	1
Glaucidium radiatum	1		
Ninox connivans	1		
Ninox philippensis	45	24	1
Ninox scutulata	30	10	1
Nyctea scandiaca	10	7	
Otus asio	10	8	
Otus bakkamoena	131	8	
Otus choliba	7		
Otus rufescens	4		
Otus scops	70	19	1
Otus spilocephalus	14	5	
Otus trichopsis	1		
Strix aluco	30	21	
Strix occidentalis	1	1	
Strix varia	11	5	
Unknown	6	6	
TOTAL	496	186	6
TYTONIDAE			
Phodilus badius	11	1	
Tyto alba	10	4	
Tyto longimembris	2		
TOTAL	23	5	
GRAND TOTAL	519	191	6

APPENDIX B cont'd

Plasmodia in the Strigiformes (based on the files of the IRCAH).

	Total Examined	Plasmodium						
		circumflexum	elongatum	fallax	polare	relictum	vaughani	P.sp.
STRIGIDAE								
Aegolius acadicus	26							
Asio flammeus	10							
Asio otus	3							
Athene brama	20	1		1		4	1	
Athene noctua	3							
Bubo africanus	13							1
Bubo bubo	1							
Bubo coromandus	3							
Bubo virginianus	17							
Ciccaba woodfordii	2							
Glaucidium brodiei	12							
Glaucidium cuculoides	14		1			1		2
Glaucidium radiatum	1							
Ninox connivans	1							
Ninox philippensis	45			1		3		3
Ninox scutulata	30					1		1
Nyctea scandiaca	10				2	2		1
Otus asio	10						1	1
Otus bakkamoena	131			2	3	6	7	6
Otus choliba	7						2	
Otus rufescens	4							
Otus scops	70				3	1	2	1
Otus spilocephalus	14			1	1	1		1
Otus trichopsis	1							

APPENDIX B cont'd

Plasmodia in the Strigiformes (based on the files of the IRCAH).

	Total Examined	Plasmodium						
		circumflexum	elongatum	fallax	polare	relictum	vaughani	P.sp.
Strix aluco	30							
Strix occidentalis	1					1		
Strix varia	11							
Unknown	6							
TOTAL	496	1	1	5	9	20	13	17
TYTONIDAE								
Phodilus badius	11							
Tyto alba	10							
Tyto longimembris	2							
TOTAL	23							
GRAND TOTAL	519	1	1	5	9	20	13	17

Note: As a result of multiple infections in individual birds the total number of infections listed may exceed the total number of infected birds.

APPENDIX B cont'd

Trypanosomes in the Strigiformes (based on the files of the IRCAH).

	Total Examined	*Trypanosoma*			
		avium	*calmettei*	*everetti*	*T.*sp.
STRIGIDAE					
Aegolius acadicus	26	1			3
Asio flammeus	10				
Asio otus	3				
Athene brama	20				
Athene noctua	3				
Bubo africanus	13	1			
Bubo bubo	1				
Bubo coromandus	3				
Bubo virginianus	17				
Ciccaba woodfordii	2				
Glaucidium brodiei	12	2	3		
Glaucidium cuculoides	14	2			
Glaucidium radiatum	1				
Ninox connivans	1				
Ninox philippensis	45	3	1		
Ninox scutulata	30		1		
Nyctea scandiaca	10	1			
Otus asio	10	2			1
Otus bakkamoena	131		2		2
Otus choliba	7				
Otus rufescens	4			1	
Otus scops	70		1		
Otus spilocephalus	14				
Otus trichopsis	1				
Strix aluco	30	4	1		1
Strix occidentalis	1				1
Strix varia	11			1	1
Unknown	6				
TOTAL	496	16	9	2	9
TYTONIDAE					
Phodilus badius	11				
Tyto alba	10				
Tyto longimembris	2				
TOTAL	23				
GRAND TOTAL	519	16	9	2	9

APPENDIX B cont'd

Microfilaria in the Strigiformes (based on the files of the IRCAH).

	Total Examined	Microfilaria	
		described	undescribed
STRIGIDAE			
Aegolius acadicus	26		5
Asio flammeus	10		
Asio otus	3		
Athene brama	20		
Athene noctua	3		
Bubo africanus	13		
Bubo bubo	1		
Bubo coromandus	3		
Bubo virginianus	17		
Ciccaba woodfordii	2		
Glaucidium brodiei	12		
Glaucidium cuculoides	14	2	3
Glaucidium radiatum	1		
Ninox connivans	1		
Ninox philippensis	45		2
Ninox scutulata	30	1	1
Nyctea scandiaca	10		
Otus asio	10		1
Otus bakkamoena	131	1	1
Otus choliba	7		
Otus rufescens	4		
Otus scops	70		3
Otus spilocephalus	14		
Otus trichopsis	1		
Strix aluco	30		
Strix occidentalis	1		1
Strix varia	11		
Unknown	6		
TOTAL	496	4	17
TYTONIDAE			
Phodilus badius	11		1
Tyto alba	10		
Tyto longimembris	2		
TOTAL	23		1
GRAND TOTAL	519	4	18

APPENDIX B cont'd

Other haematozoa in the Strigiformes (based on the files of the IRCAH).

	Total Examined	Other			
		Atoxoplasma	*Babesia*	*H.*sp. /*P.*sp.	Unidentified
STRIGIDAE					
Aegolius acadicus	26	1			
Asio flammeus	10				
Asio otus	3			1	
Athene brama	20				
Athene noctua	3				
Bubo africanus	13				1
Bubo bubo	1				1
Bubo coromandus	3				
Bubo virginianus	17				1
Ciccaba woodfordii	2				
Glaucidium brodiei	12				
Glaucidium cuculoides	14				
Glaucidium radiatum	1				
Ninox connivans	1				
Ninox philippensis	45			1	
Ninox scutulata	30			1	
Nyctea scandiaca	10			1	
Otus asio	10				
Otus bakkamoena	131			2	37
Otus choliba	7		1		
Otus rufescens	4				1
Otus scops	70				24
Otus spilocephalus	14				2
Otus trichopsis	1				
Strix aluco	30				
Strix occidentalis	1				
Strix varia	11				
Unknown	6				
TOTAL	496	1	1	6	66
TYTONIDAE					
Phodilus badius	11				
Tyto alba	10				1
Tyto longimembris	2				
TOTAL	23				1
GRAND TOTAL	519	1	1	6	67

APPENDIX C

ORIGINAL DESCRIPTIONS OF THE HAEMOPROTEIDS OF THE STRIGIFORMES

Haemoproteus glaucidiumi Jörg, 1931

(Translation from Jörg, 1931)

Type Host: *Glaucidium nanum* Type Locality: Chaco District, Argentina

The *Haemoproteus* of the cabure is characterized by its constant adhesion to the nucleus, the concentrated pigment masses which are always in the medial part of the parasite and the tendency to grow so as to totally invade the erythrocytes without destruction of either the nucleus nor its position within the cell.

The younger forms have a uniformly basophilic cytoplasm, more stained around the borders than in the centre, we have not observed the nucleus because the chromatic masses are well pigmented since the specimens were prepared with the haematic pigments reacted with lignum vitae (Guaiacum) resin according to the particular technique which will be the subject of a later communication, however the technique is still not perfect.

The older forms of *Haemoproteus*, have various vacuoles which ultimately form up and result in the almost total destruction of the parasite.

APPENDIX C cont'd

Haemoproteus noctuae Celli and Sanfelice, 1891

(Translation from Celli and Sanfelice, 1891)

Type Host: *Athene noctua* Type Locality: Rome, Italy.

In some young owls (*Athene noctua*) one can find parasites which resemble those previously mentioned (Table II, fig. 22 - 38). The only difference is that the cells are larger, with thicker poles and with a more full bodied form. The protoplasmic extensions are shorter, thicker and less numerous. When parasites occupy a full section of a red blood cell, they often show regular indentations (fig. 29 - 33), which can be so distinguishable at the poles by a darkened area in the middle, that they seem separated from the actual blood cell. These darkenings are possibly due to spores, because the corpuscles look very much like young endoglobular forms. And, we have already observed indentations followed by young endoglobular forms. The developmental cycle to reach full maturity is complete in 4 to 5 days. At this point, all activity stops for several days or more. Since the endoglobular forms of parasites are larger than those found in the dove, not only can they encircle the nucleus, but even invade the entire red blood cell, with the exception of the nuclear zone (fig. 35). Accordingly, the free parasite forms are also larger. Endoglobular forms like the free parasite forms tend to become circular, and are then enclosed by a residue of faded blood cells. Once circular, these forms show a very active movement in the process of degeneration of the black granules, however, we did not have the opportunity to witness the formation of flagella.

APPENDIX C cont'd

Haemoproteus glaucidii de Mello, 1936

(from de Mello, 1936)

Type Host: *Glaucidium radiatum* Type Locality: Canacona, Portuguese India

Glaucidium radiatum Tickell. Shot at Canacona and identified by Mr. Cann. *Haemoproteus glaucidii* sp. n. ? var. nov.? [? = *H.* of *Glaucidium perlatum* recorded by A. and M. Leger (Niger 1914);? Var. of. *H.* noctuae Celli and S. Felice (1891)]. Sexual dimorphism. Female deep blue, vacuolated. Nucleus rose, spheric, central or elongated and situated on the convex border. Pigment scattered. Male almost colourless, nucleus large without definite borders, constituted by irregular chromatic masses, sub-central in haltheridial forms, central in the roundish ones. Red cell hypertrophied; nucleus keeps the normal position when the parasite is female, and is displaced by the male forms.

Haemoproteus glaucidii de Mello, 1936

(from de Mello 1936-37)

Female gametocyte with alveolar protoplasm, staining deep blue by Leishman, and having sometimes very small vacuoles, especially in the polar region. Nucleus generally spherical, pink, central or subcentral, seldom elongated and situated on the convex border of the parasite. Pigment granules, more or less abundant, scattered throughout the body. Form halteridial when intraglobular, halteridial or spherical when free. In this last case the protoplasm denser and the pigment granules more abundant arround (sic around) the nucleus.

APPENDIX C cont'd

Male gametocyte with almost colourless protoplasm; large, indistinctly outlined nucleus constituted by irregular chromatic masses generally scattered in the nuclear endosome, its position subcentral in the halteridial forms, more or less central in the roundish ones. Pigment granules generally more abundant at the poles, often in one pole only. Intragobular form halteridial, spherical, pyriform. Free parasites spherical, with more or less excentric nucleus, or halteridial.

Red cell hypertrophied. Its nucleus keeps the normal position when the parasite is female and is almost embraced by the protozoon; when the gametocyte is male, the nucleus is always displaced to the periphery. The nucleus often takes an oblique position, keeping however its normal place.

Some globules were infected by two parasites and in one case both sexes were present, their protoplasm being almost fused together so difficult was it to recognise the line of separation between the both protoplasms.

The infection was very severe.

We will name this species: *Haemoproteus glaucidii* n. sp.(?) or n. var. of *H. noctuae*(?). We must however note that morphologically it is entirely different from the *Haemoproteus* of the *Athene bramae*.

APPENDIX C cont'd

Haemoproteus bramae de Mello, 1936

(from de Mello, 1936)

Type Host: *Athene brama* Type Locality: Nagoa (sic Nova-Goa) (Salcete), Portuguese India

Athene brama Temm. Shot at Nagoa (sic Nova-Goa) (Salcete), identified by Mr. Cann. *Haemoproteus bramae* sp. n. (recorded already by Donovan, 1904). Sexual dimorphism. Female haltheridic, slender, more or less irregular showing some degree of constriction in the middle. Rare oval forms. Protoplasm homogeneous, blue, the staining being more pronounced in the poles. Nucleus oval, pale rose, central or sub-central. Pigment granules isolated or in clusters, irregularly scattered. Male haltheridic, comma-shaped, oval. Nucleus without definite outline, often scarcely visible. Pigment granules thinner than in female, generally having a polar location but often covering the whole body. Red cell unaltered or slightly hypertrophied; nucleus displaced.

Haemoproteus bramae de Mello, 1936

(from de Mello 1936-37)

Female gametocyte generally halteridial, often more or less irregular, slender or showing some degree of constriction in the middle. We have found also one perfectly oval form, embracing the nucleus of the red cell. Protoplasm sometimes homogeneous, others vacuolated; staining blue, the coloration being more pronounced at the poles and lighter in the centre. Nucleus oval, central or

APPENDIX C cont'd

subcentral, pale pink, often scarcely visible. Pigment granules irregularly scattered throughout the body, isolated or in clusters.

Male gametocyte with the same morphology as in females: halteridial, crescent or comma-shaped, oval. Nuclear mass without distinct outline, also often scarcely visible even stained by May-Grünwald-Giemsa. Protoplasm colourless. Pigment granules finer than in the female, generally having a polar situation but sometimes scattered throughout the whole body.

Red cell unaltered or slightly hypertrophied. Nucleus displaced to the periphery.

Our *Haemoproteus* is certainly the same as that seen by Donovan.

Haemoproteus noctuae var. *nebraskensis* Coatney and Roudabush, 1937 = *Haemoproteus nebraskensis* (Coatney and Roudabush, 1937) Levine and Campbell, 1971

(from Coatney and Roudabush, 1937)

Type Host: *Bubo virginianus* Type Locality: Peru, Nebraska, U.S.A.

The *Haemoproteus* reported here was found in the blood of a Great Horned Owl (*Bubo virginianus*). The bird also harbored *Leucocytozoon* and, during the twelve days this owl was under observation, one trypanosome was seen.

The full grown female gametocyte measured from 17.48 μm to 24.84 μm in total length and from 2.76 μm to 4.14 μm in width. The mean size was 20.18 μm by 3.05 μm. The parasites had rather pointed ends and, in many cases, they almost

APPENDIX C cont'd

completely enclosed the host cell nucleus. They were found not in close contact with the host cell membrane or its nucleus. A clear zone was particularly evident around the nucleus of the host cell. The cytoplasm stained a purplish-blue and showed many small indistinct vacuoles. The pigment granules were round to oval and numbered from 17 to 29 with a mean of 20. The nucleus was roughly spherical and stained a light red. This structure measured from 1.84 μm to 3.68 μm in length and from 1.38 μm to 3.20 μm in width with a mean size of 2.64 μm to 2.28 μm.

The adult male gametocytes measured 16.56 μm to 22.08 μm in length and from 1.84 μm to 3.68 μm in width with a mean size of 18.54 μm by 2.93 μm. The parasites had rather pointed ends and, as with the macrogametocytes, many of them almost enclosed the host cell nucleus. These parasites did not touch the host cell nucleus. They contacted the periphery of the host cell only along the side while at the ends, or poles of the cell, they pulled away leaving a clear area. The cytoplasm and nucleus of these parasites stained a dark pink, the latter structure was so diffuse that its boundaries could not be made out with certainty. The granules were rounded to oval and in many cases were clustered together at the ends of the parasites. Where counts of the individual granules could be made the number ran from 12 to 21 with a mean of 15.5. Nuclear displacement was seen in about half of the cells harboring adult parasites of both sexes. There was no host cell hypertrophy.

APPENDIX C cont'd

Haemoproteus syrnii Mayer, 1910

(Translation from Mayer, 1911).

Type Host: *Strix aluco* Type Locality: Lübeck, West Germany or Vienna, Austria

Unstained preparations of halteridia showed no peculiarities not even in the immediately occurring exflagellation of the microgametocytes. On the other hand there appeared in the smears stained with Giemsa, two characteristics which allow differentiation from those previously described owl halteridia.

I. Double Nuclei: all pigment free young forms show two nuclei. The youngest forms appear round; the main nucleus is round to elongate and appears a shining red colour. Adjacent to it lies a circular chromatin nucleus which is similar to the blepharoplasts. It is coloured somewhat darker often with a lighter centre (figs. 1 & 2). In the somewhat older already elongated specimens the main nucleus lies approximately in the middle of the blue protoplasm while the second nucleus lies at one of the ends (figs. 3 & 4).

In the older pigment containing parasite this second nucleus can no longer be demonstrated most of the time. Proof for an expulsion, or invasion into the main nucleus was not found.

A similar occurrence of a double nucleated halteridium was described by Woodcock (5) in the *Halteridium* of the chaffinch. He found dual nuclei in the female, indifferent, and young forms. Woodcock considers his findings as the first definitive proof to support at least one of the famous conclusions of Schaudinn. According to Berliner (6) *Haemoproteus noctuae* often has a small blepharoplast

APPENDIX C cont'd

lying near the nucleus.

II. "Alkaliphile" (OH group) Granules. In all forms of older and mature individuals the protoplasm when stained lightly appears like a cone-like structure so that light holes ("lacunae") occur fairly regularly in the blue plasmaton. When coloured more strongly there appears in some of the individuals shiny red dots in typical chromatin colouring in the lacunae. In very strongly stained preparations the granules take on a dark violet colour - in contrast to the nucleus - and appear circular and larger than before. Only through the addition of "alkali" (an alkali chemical) to the colouring solution (1 drop 1% soda solution to 10cc colour solution) one succeeds to make the granules appear in a consistent manner and they then appear depending on the degree of colouring, red-violet to black-violet and possibly entirely black. "Diese aviditat" for "alkali" has been demonstrated for those granules by Mr. Giemsa who supported me in the most friendly manner in the experiments of their consistent depiction . The granules always lie in the protoplasm to both sides of the nucleus, in mature forms in the approximate numbers of 20 - 30 in each half. They appear circular in the "Alkali" colouring also. Besides these, golden yellow pigment is always present often lying above these forms. The pigment nuclei are often of the same size and form and thus one cannot exclude that the granules stand in a relation to the pigment. It is beyond doubt that we are not dealing here with nuclear substance as appears at the first view. Cardamatis (7) has seen similar forms in the *Halteridium* of doves.

APPENDIX C cont'd

I have looked in the *Halteridium* of the rice bird and also in two year old - *Halteridium* smears of *Athene noctua* but I could not demonstrate it. In Figures 4 - 9 the different degrees of colouring and the alkalophile granules are shown.

Haemoproteus noctuae var. *cellii* Coatney and Roudabush, 1937 = *Haemoproteus cellii* (Coatney and Roudabush, 1937) Helmy Mohammed, 1958

(from Coatney and Roudabush, 1937)

Type Host: *Otus asio* Type Locality: Peru, Nebraska, U.S.A.

Halteridium parasites have been found in one Screech owl (*Otus asio*). The adult female gametocytes measured 13.78 μm to 22.44 μm in length and from 2.24 μm to 3.53 μm in width. The mean size was 17.96 μm by 2.89 μm. The ends of the parasites were pointed rather than rounded. The parasites were generally in close contact with the host cell membrane but they did not contact the nucleus of the host cell. The cytoplasm stained a dark blue and had a few indistinct vacuoles. The pigment granules varied in number from 19 to 26 with an average number of 22.5. The nucleus stained dark pink, was irregularly oval and was found toward the periphery of the host cell. The nucleus measured 1.92 μm to 3.85 μm in length and from 0.80 μm to 2.24 μm in width with mean size of 3.11 μm by 1.57 μm. Many host cells had the nucleus displaced. Linear hypertrophy of the host cell was evident.

The male gametocytes measured 11.54 μm to 20.82 μm in length by 2.24 μm to 3.21 μm in width with a mean size of 15.56 μm by 2.79 μm. The parasites were

APPENDIX C cont'd

close to the periphery of the host cell, except at the ends, but they did not contact the nucleus of the host cell. The granules were large, rod-like to irregular, and numbered form 8 to 24 with a mean of 17. In the younger forms the nucleus was distinct and stained a dark pink. In the older forms this body stained a light pink and was indistinct, sub-rectangular in outline and measured 3.85 μm to 7.69 μm in length and 2.20 u to 3.15 μm in width. The mean size was 6.18 μm by 2.74 μm. Linear hypertrophy of the host cell was evident and about half had the nucleus displaced.

The halteridia from the screech owl resemble *H. noctuae* but differ from this form in that the growth stages all had smooth contours and the nuclei of the macrogametocytes were located near the periphery of the parasite next to the host cell membrane. The screech owl halteridium also had a greater number of granules than *H. noctuae.*

Haemoproteus multiparasitans n. sp. Covaleda Ortega and Gállego Berenguer, 1950 (Translation from Covaleda Ortega and Gállego Berenguer, 1950)

Type Host: *Athene noctua* Type Locality: Granada, Spain

Frequently observed were erythrocytes infected with several, 4 - 5, gametocytes in young stages, but in those which had attained full development we verified at most two gametocytes within one erythrocyte - this being quite frequent.

When young, the gametocytes present a globular appearance and are located variably in the hematocyte. In forms in late stages of development, there is more

APPENDIX C cont'd

or less constant position in the erythrocyte. Some are situated laterally, others occupy the pole of the erythrocyte in a curved, horseshoe shaped form, but are not in contact with the nucleus. In fully developed forms the parasite is laterally situated but generally more displaced towards one of the poles of the hematocyte.

Its morphology is not halteridial, it is reminiscent (crudely) of a "L" with its borders very irregular, at full development it appears globulous and hypertrophies the hematocyte on its minor axis, finally it assumes a "spheroidal" form when free outside of the hematocyte (this being frequently observed). The nucleus of the erythrocyte is displaced towards a more oblique angle in relation to the major axis of the hematocyte.

It is characteristic of this parasite that it is not in contact with the nucleus of the red blood cell, inclusive of fully developed, globular forms.

Macrogametocytes: The protoplasm stains intense blue around the periphery, but the stain is lighter in the centre where vacuolization is more accentuated. The nucleus is round and of irregular contours, presenting a granular structure; measures 2.5 - 3 μm in diameter and is situated generally in the central part of the parasite. The pigment is granular, of violet-black coloration and is abundant with the number of granules usually surpassing 30, and generally disposed in all the cytoplasm, surrounding the vacuoles.

Microgametocytes: Protoplasm is very light blue, almost colorless and the color is only evident at the periphery of the parasite. The nucleus, very large, occupies a major part of the cytoplasm, it stains pale pink, and in it are perceptible numerous

APPENDIX C cont'd

granulations slightly more intense in color. The pigment is also granular and with same coloration as the female gametocytes but is less numerous. These rarely being more than 20 granules per gametocyte. The difference from this to the microgametocytes typical of the genus *Haemoproteus*, is that the pigment is not found solely at the poles, but appears also in the entire parasite, at the periphery in the gametocyte.

Haemoproteus cellii var. *aegyptius* Helmy Mohammed, 1958

= *Haemoproteus aegyptius* (Helmy Mohammed, 1958) Levine and Campbell, 1971 (from Helmy Mohammed, 1958)

Type Host: *Bubo bubo ascaphalus* Type Locality: Cairo Zoological Gardens, Guiza, Egypt

Macrogametocytes: Most of the macrogametocytes (about 95%) have the ordinary side-position in the cell, otherwise they grow asymmetrically having one end growing much further beyond the pole of the host-cell nucleus (fig. 11), or, again, the gametocytes may grow only around one of the poles of the nucleus assuming a cap-like position (fig. 12).

The gametocytes do not show a clear affinity towards the host-cell nucleus, and when they do not contact it, this usually takes place along its side (figs. 4 - 7). In many cases there is a narrow irregular clear zone between the parasite and the nucleus (figs. 8 and 9). Similarly, the gametocytes contact the membrane only along the side of the cell (figs. 4 - 8), but there are exceptional cases where the

APPENDIX C cont'd

gametocytes do not contact the membrane at all (fig. 9), or they contact it along their whole length (fig. 10). Thus, in most cases the ends of the parasite are free from both the host-cell nucleus and membrane (figs. 4 - 9)

Most of the gametocytes have dissimilar ends; one of them is larger than the other (figs. 5, 7, 8 and 9), and this feature seems to be characteristic to the parasite. In the majority of cases the two ends, even the larger one, are pointed. The gametocytes range between 12 and 19 μm in length, and between 2 and 5 μm in width, with a mean size of 15.3 x 2.94 μm.

The cytoplasm takes a vivid blue colour with a light purplish tint. It shows a fine mesh-work with dark islets and fine vacuolation. Some larger vacuoles may be seen scattered in the cytoplasm, but in about 7 % of the macrogametocytes a prominent vacuole appears at the broader end (fig. 7). The dark brown pigment granules are usually rounded and scattered all over the cytoplasm. They range in number between 12 and 32 with an average of 20.

The nucleus is usually central, and in many individuals, especially in those with a greater width, it is situated at the outer side of the parasite near to the host-cell membrane (figs. 7 - 10). In shape, it is rounded (figs. 9 and 11), oval (fig. 5), angular (figs. 4, 7 and 10) or irregular (figs. 8 and 12). The nucleus stains a dark pink and sometimes a darker granule is seen near the periphery. It ranges between 2 and 4 μm in length, and between 1 and 2 μm in width, with a mean size of 2.8 x 1.4 μm.

APPENDIX C cont'd

Microgametocytes: Microgametocytes assuming asymmetric positions in the host-cell are more numerous (about 20%) than the corresponding macrogametocytes. In their relations with the host-cell nucleus and membrane, the microgametocytes are more-or-less similar to the macrogametocytes. Here again the two ends are usually free form both the host-cell nucleus and membrane (figs. 14 - 17), and in the majority of cases they are dissimilar, but they are more irregular than in the macrogametocytes. The microgametocytes range in length between 11 and 17.5 μm, and in width between 2 and 3.5 μm, with a mean size of 13.8 x 2.68 μm.

When differentiation between the cytoplasmic and nuclear regions is clear, the nucleus ranges between 8.5 and 12.5 μm in length and between 2.0 and 3.5 μm in width, with a mean size of 10 x 2.5 μm. It extends along most the gametocyte pushing the cytoplasm to the poles, usually dividing the latter into two unequal parts, so unequal in some cases that one of them may just be a thin rim containing a number of the pigment granules (figs. 16 and 17). The nucleus stains a faint red, but darker than that of many haemoproteids. A very clear karyosome is present, usually terminal in position (figs. 16 and 17) and another similar but less distinct granule may occasionally be seen (figs. 14 and 15).

The cytoplasm stains in a very faint greyish blue colour, and contains the blackish brown pigment granules. The roundish granules ere (sic) coarser than those of the macrogametocytes. They range in number between 11 and 22 with an average of 17.

APPENDIX C cont'd

Effect on the host-cell: The average measurements for a normal uninfected erythrocyte are:

Long axis of cell 13.4 μm Long axis of nucleus 6.5 μm

Short axis of cell 6.2 μm Short axis of nucleus 3.0 μm

For cells infected by macrogametocytes the long and short axes measure, on the average, 14.0 and 6.6 μm respectively. Thus average hypertrophy along the long axis is 0.6 μm or 4.47 %, and along the short axis it is 0.4 μm or 6.4 %. Extreme cases of hypertrophy in length (fig. 4), and in width (fig 10) occur. In the latter case the infected cell is shortened and assumes a spheroidal form. Hypertrophy caused by the younger forms is naturally along the short axis (figs. 2 and 3).

For cells infected by microgametocytes the long and short axes are, on the average, 14.2 and 6.5 μm respectively. Thus average hypertrophy is 0.8 μm or 5.9 % along the long axis, and 0.3 μm or 4.8 % along the short axis.

Nucleus displacement is the rule, and it is only in the case of the gametocytes assuming the cap-like position in relation with the host-cell nucleus that the latter is not displaced (figs. 12 and 18). The ratio of displacement is 2.31 in cells infected by macrogametocytes and 2.2 in cells infected by microgametocytes. Rarely the host-cell nucleus is rotated during displacement (figs. 11 and 19), but figure 13 seems to depict a gametocyte that had been rounded prior to gamete formation.

APPENDIX D

Probability values of test statistics for comparison of morphometric parameters

[1] [2]	*H.aeg.*,Moh. *H.aeg.*Cur. t-test	*H.aeg.*,Cur. *H.syr.*,Cur. MANOVA	*H.cel.*,C&R *H.syr.*,Cur. t-test	*H.neb.*,C&R *H.noc.*,Cur. t-test	*H.noc.*,Moh. *H.*sp.,Cur. t-test	*H.noc.*,Moh. *H.noc.*,Cur. t-test	*H.syr.*,Cur. *H.pho.*,Cur. MANOVA	*H.noc.*,Cur. *H.*sp.,Cur. MANOVA
Pillais trace		<0.000*					<0.000*	>0.203
[3] UEL	>0.01	<0.000*			>0.01	>0.01	>0.964	<0.003*
UEW	>0.01	<0.000*			>0.01	>0.01	>0.659	>0.084
UEA		<0.008*					>0.662	>0.015
UNEL	>0.01	<0.000*			>0.01	>0.01	>0.427	<0.007*
UNEW	>0.01	>0.568			>0.01	>0.01	>0.517	>0.172
UNEA		<0.000*					>0.812	<0.002*
Macro								
IEL	>0.01	<0.000*			>0.01	>0.01	>0.394	<0.000*
IEW	>0.01	>0.012			>0.01	>0.01	>0.019	>0.310
IEA		>0.088					>0.017	>0.076
INEL		<0.000*					<0.000*	>0.090
INEW		<0.004*					<0.000*	<0.000*
INEA		<0.000*					<0.895	<0.000*
GL	<0.01*	<0.000*	>0.01	<0.01*	>0.01	<0.01*	>0.000*	>0.466
GW	>0.01	>0.725	>0.01	>0.01	>0.01	>0.01	>0.081	>0.803
GA		<0.000*					<0.000*	>0.365
GNL	>0.01	>0.144	>0.01	>0.01	-	>0.01	<0.000*	>0.581
GNW	<0.01*	<0.000*	>0.01	>0.01	-	<0.01*	>0.591	>0.547
GNA		<0.000*					<0.000*	>0.843
NDR	<0.01*	>0.850					>0.793	>0.580
PG	>0.01	>0.394	>0.01	>0.01	<0.01*	>0.01	<0.000*	>0.276

APPENDIX D cont'd

Probability values of test statistics for comparison of morphometric parameters.

	H.aeg.,Moh. *H.aeg.*Cur. t-test	*H.aeg.*,Cur. *H.syr.*,Cur. MANOVA	*H.cel.*,C&R *H.syr.*,Cur. t-test	*H.neb.*,C&R *H.noc.*,Cur. t-test	*H.noc.*,Moh. *H.*sp.,Cur. t-test	*H.noc.*,Moh. *H.noc.*,Cur. t-test	*H.syr.*,Cur. *H.pho.*,Cur. MANOVA	*H.noc.*,Cur. *H.*sp.,Cur. MANOVA
Pillais trace		>0.017					<0.000*	<0.006*
Micro								
IEL	>0.01	<0.006*			>0.01	>0.01	>0.374	<0.000*
IEW	>0.01	>0.017			<0.01*	<0.01*	>0.012	>0.514
IEA		>0.426					>0.181	>0.027
INEL		<0.000*					>0.044	<0.009*
INEW		<0.003*					<0.000*	<0.000*
INEA		<0.000*					>0.011	<0.000*
GL	>0.01	<0.000*	>0.01	<0.01*	>0.01	<0.01*	>0.045	>0.021
GW	<0.01*	>0.185	>0.01	>0.01	>0.01	>0.01	<0.001*	>0.397
GA		<0.002*					<0.001*	>0.086
GNL	<0.01*	<0.001*	>0.01		>0.01	>0.01	<0.000*	>0.462
GNW	>0.01	>0.143	>0.01		>0.01	<0.01*	<0.000*	>0.676
GNA		<0.008*					<0.000*	>0.929
NDR	<0.01*	>0.670					>0.018	>0.673
PG	>0.01	>0.727	>0.01	>0.01	<0.01*	>0.01	<0.000*	>0.681

1. *H.aeg.* = *Haemoproteus aegyptius*; *H.syr.* = *Haemoproteus syrnii*; *H.cel.* = *Haemoproteus cellii*; *H.neb.* = *Haemoproteus nebraskensis*; *H.noc.* = *Haemoproteus noctuae*; *H.*sp. = *Haemoproteus* species; *H.pho.* = *Haemoproteus phodili*; Moh. = Helmy Mohammed, 1958; Cur. = this study; C&R = Coatney and Roudabush, 1937

2. * significant at $\alpha = 0.01$

3. U = uninfected; I = infected; E = erythrocyte; Macro = Macrogametocyte; Micro = Microgametocyte; G = gametocyte; N = nucleus; L = length; W = width; A = area; PG = pigment granules; NDR = nuclear displacement ratio