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ORIGINAL ARTICLE

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New records of *Haemoproteus* and *Plasmodium* (Sporozoa: Haemosporida) of rock pigeon (*Columba livia*) in India

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Abstract The prevalence, intensity and morpho-variants of new species of haemosporida (*Haemoproteus* and *Plasmodium*) from the rock pigeon, *Columba livia* are described and illustrated for the first time from Uttar Pradesh state of India. Thin blood smears from 266 *C. livia* indicated 55.63% (*Haemoproteus*) and 6.76% (*Plasmodium*) prevalence and 1–6 pars/100 RBC's (*Haemoproteus*) and 1–2 pars/100 RBC's (*Plasmodium*) intensity of infection. The fully grown intracellular gametocytes of *Haemoproteus* were differentiated into microgametocyte (14.0 × 4.3 μm) and macrogametocyte (13.9 × 4.7 μm). Extracorpuscular gametocyte (15.0–17.8 μm in length, 3.9–7.3 μm in width) were occasionally visible. Nuclear displacement ratio was 0.2. *Plasmodium* species was characterized by rounded schizonts and elongated microgametocyte (7.8 × 7.6 μm) and macrogametocyte (7.8 × 7.7 μm) with irregular margins. Cells containing schizonts are often rounded and enlarged and those parasitized by gametocytes may be somewhat distorted in shape by lateral hypertrophy. Host cell nuclei are also displaced. Double gametocyte infection of *Haemoproteus* occasionally present but that of *Plasmodium* lacking.

Keywords Avian haematozoa · Erythrocytic gamogony · Uttar Pradesh

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Introduction

Haemosporida of the genera *Haemoproteus* and *Plasmodium* are relatively well known (De Mello 1936; Chakravarty and Kar 1945; Zargar 1945; Ray and Bhatnagar 1953; Mc Clure et al. 1978, Ishtiaq et al. 2007; Leppert et al. 2008). A check list and index catalogue of avian haematozoa from India was published by Nandi (1984). Vectors of *Plasmodium* are culicid mosquitoes and those of *Haemoproteus* are haematophagus dipteran flies (Valkiunas 1997).

The prevalence and intensity of haemoparasites in columbids have been reported by Klei and DeGiusti (1975) and Mandal (1990). *Haemoproteus columbae* Kruse, 1890 was the first haemoproteid species to be described. The presence of haemoproteids in the blood of free living pigeons and doves have been reported by Levine and Kantor (1959), Levine (1961), Stabler and Halt (1962), Kinsley and Herman (1967), Greiner (1970, 1975), Klei and DeGiusti (1975), Shamis and Forrester (1977), Dias et al. (1984), Bennett and Peirce (1990) and Mandal (1990). Mio et al. (2005) detected *Haemoproteus* sp. and microfilaria from *Columbia janthina*. Valkiunas et al. (2008) observed new species of haemosporidian parasite from African rainforest birds.

Differences in the prevalence, geographic distribution and host of haemoproteids are associated with the habitat preference of the bird hosts, the abundance and feeding habits within the habitats of suitable insect vector and innate physiological differences make some avian hosts more susceptible than others.

The rock pigeon, *Columba livia* Gmelin belonging the avian family, Columbidae has a restricted natural resident range in western and southern Europe, North Africa and into south Asia. Helminth parasitic fauna of columbids

have been extensively studied but reports on their blood parasites are not so frequent. The haemosporida of *C. livia* from Rohilkhand region of Uttar Pradesh, India are virtually unreported. The aim of this article is to report the prevalence and intensity of haemosporidian species from the avian host mentioned above. Two new haemosporida (*Haemoproteus* and *Plasmodium*) were encountered from this bird and description and taxonomic designation of these species are provided.

Materials and methods

Study birds

Columba livia Gmelin ($n = 266$) weighing 400–500 g were collected from different sources of Bareilly including bird market, college campus, hostel's garden, and old buildings and kept in cages. They were maintained in the laboratory at 38–40°C, fed on grains (30 g per day) and provided water (1 l per 20 pigeons).

Blood sampling and slide preparation

Blood was collected directly from the clipped nail by a fingernail clipper or from the brachial vein, a drop placed on a clean microscopic slide and blood smears were prepared according to Gupta (1986), stained in Giemsa's with phosphate buffer (pH 7.4) in the ratio of 1:7 for 3 h. The slides were washed, dried and examined for blood parasites at ×1,000. Parasitaemia were calculated from counts of 100 red blood cells at ×1,000 except in the case of chronic cases where infection could be detected in up to 50 fields at ×400 to provide crude estimates. The positive slides were mounted in DPX and observed under oil immersion objective.

The parasites and blood cells were drawn to scale and measured in micron. Photographs were taken under LEICA DMLB photoautomat at a magnification of ×1,000 under standardized conditions.

Holotypes and paratypes of the two haemosporida are deposited in the Zoological Survey of India, Northern Regional Station, Dehradun, India.

Calculation of nuclear displacement ratio (NDR)

NDR was calculated according to the ratio.

$$\text{NDR} = 2x/x + y$$

where x is the distance between the periphery of the cell and the periphery of the nucleus and Y is the distance between the cell and periphery of the nucleus on the other side.

Results

Parasite: Haemoproteus

Taxonomic summary

Phylum	Apicomplexa
Class	Aconoidasida
Order	Haemosporida
Suborder	Haemosporina
Family	Haemoproteidae
Genus	*Haemoproteus*
Species	*nasimii* sp. nov

Parasite profile

Type host	*Columba livia*
Type locality	Bareilly (28°10′ N, 78°23′ E)
Additional localities	Badaun (28°02′ N, 79°10′ E), Shajahanpur (27°53′ N, 79°55′ E)
Site of infection	Blood
Holotype	ZSI/NRS/IV. 399
Paratype	ZSI/NRS/IV. 400 (Zoological Survey of India, Northern Regional Station, Dehradun, India)
Etymology	The parasite is named after the name of the author
Prevalence	55.63%
Intensity	1–6 pars/100 RBC's

General organization and generic diagnosis

Haemoproteus Kruse 1890 (*Haima*—blood and *Proteus*—sea god having the power of assuming different shapes) is a genus of Apicomplexa that are parasitic in birds, reptiles and amphibians. Three other genera, *Halteridium*, *Haemocystidium* and *Simondia* are now considered to be synonyms of *Haemoproteus.* Within the genus, there are 133 species, 5 varieties and 1 subspecies, maximum occurring in birds (114). They are transmitted by blood sucking insects including mosquitoes, louse flies (*Hippoboscidae*) and biting midges (*Culicoides*). Infection with this genus is sometimes known as pseudomalaria because of the parasites' similarities with *Plasmodium* species. Diagnosis of *Haemoproteus* infection is generally accomplished by microscopic examination of a Giemsa-stained peripheral blood smear. Gametocytes are only present within erythrocytes. Organisms may appear similar to *Plasmodium*, but the pigment within the intraerythrocytic gametocytes is more dispersed and schizonts are not seen in the peripheral blood smears. These pigment granules (haemozoin) are derived from the digestion of haemoglobin found within the host's erythrocytes and appear as refractile, yellow to brown granules within the host's erythrocyte (Friend and Franson 1999). The gametocytes partially encircle the

erythrocyte nucleus forming a halter-shaped appearance with little displacement of the host cell nucleus. *Haemoproteus* gametocytes often occupy over one half of the erythrocyte cytoplasm. Parasite may cause slight enlargement of infected host cells and displacement of the red blood cell nucleus to one side.

Based on the above generic characters, the genus is identified as *Haemoproteus* Kruse, 1890.

Haemoproteus nasimii sp. nov.

General morphology (Fig. 1a–z)

The blood of *C. livia* revealed gamogonic stages of *Haemoproteus* (Fig. 1a–z). The male (microgametocyte) is distinguishable from the female (macrogametocyte) by its larger and more diffuse nucleus. Usually the concentration of the parasite was sparse (1–6 pars/100 RBC) but occasionally a high degree of erythrocytes parasitization was visible (10–20 pars/100 RBC). Occasionally, the parasite infected two adjacent cells (Fig. 2a), at times there was close approximation of cells parasitized with micro and macrogametocyte (Fig. 2c). Immature and mature gametocytes were visible in blood films.

Immature gametocyte (Fig. 2b) ($n = 10$)

The young and immature forms (8.4 × 3.7 μm) develop lateral to the host cell nucleus and have no contact with the host cell membrane or the host cell nucleus.

Mature form

Mature forms could be differentiated into macrogametocytes (randomly scattered granules, nucleus with clear margins) and microgametocytes (granules polar, nucleus diffused with cytoplasm) (Fig. 2c).

Macrogametocyte (Figs. 2c–f, 3a–f) ($n = 27$)

Macrogametocytes are broadly sausage shaped, slightly halteridial and usually laterally situated to the erythrocytic nucleus. The fully grown parasite reached the poles of the infected erythrocyte but never encircled its nucleus (Fig. 2d). The margins of the gametocyte were mostly smooth and rarely amoeboid. Variations in the shape of the macrogametocyte were quite evident. Sometimes, a large space between the gametocyte and the host cell membrane

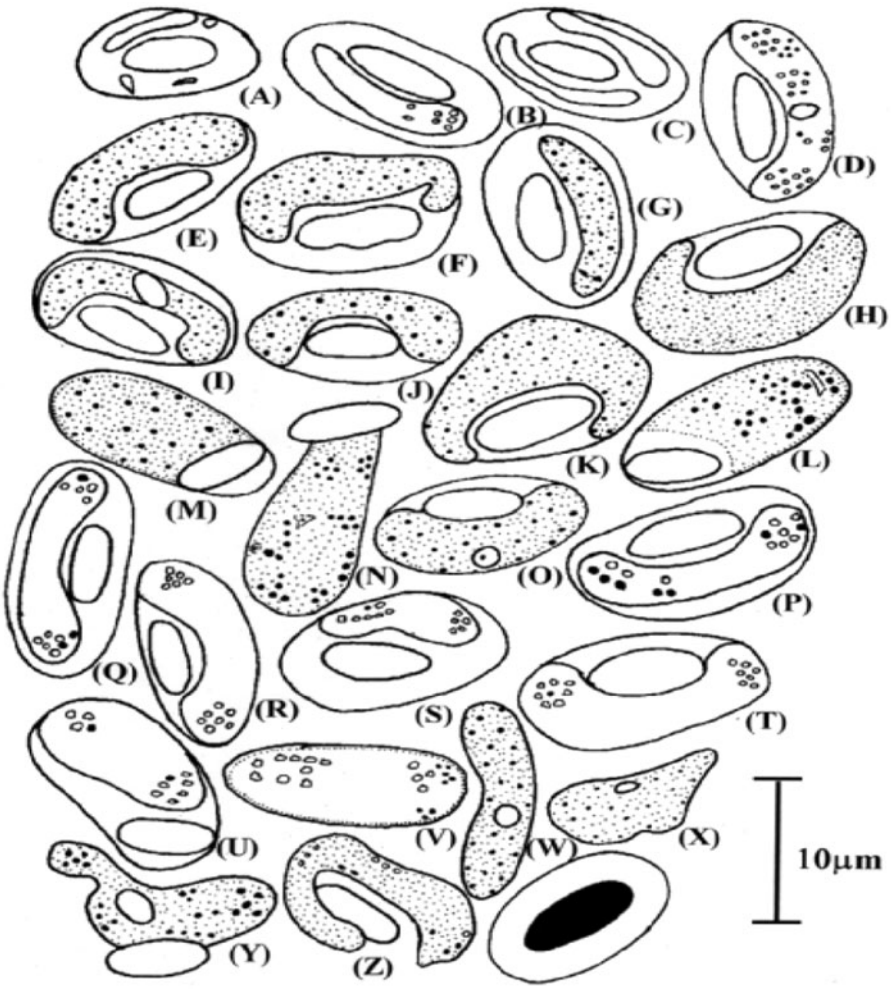

Fig. 1 Camera lucida diagrams of *H. nasimii* sp. nov. **a–c** Immature forms. **d–o** Macrogametocytes. **p–v** Microgametocytes. **w–z** Extracellular forms

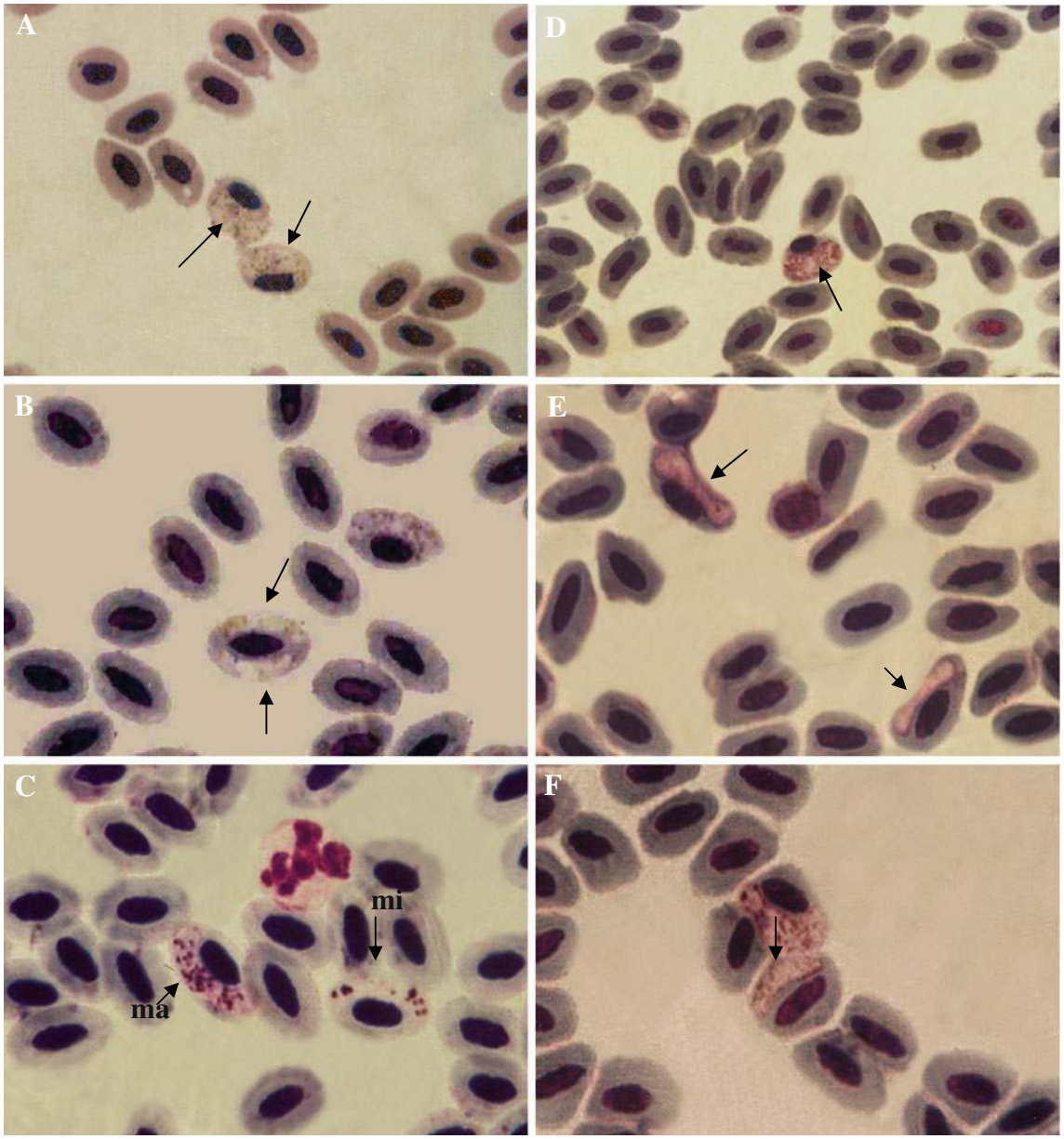

Fig. 2 Microphotographs of *H. nasimii* sp. nov. (×1,000): **a** Macrogametocyte infecting two adjacent cells. **b** Two young gametocytes laterally along the long axis of the cell. **c**. Macrogametocyte (ma) and microgametocyte (mi). **d** Fully grown macrogametocyte reaching the poles of the infected erythrocyte. **e** Macrogametocytes slender centrally and broad at the ends. **f** Macrogametocyte broad in the central area and thin at the poles

could be seen in the central zone and in such cases, the gametocyte was thin in the central zone and broad at the ends (Fig. 2e). On the other hand, some gametocytes adhered to the host cell membrane in the central area thereby broadening it (Fig. 2f). Some gametocytes were broad at one end and narrow at the other (Fig. 3a). Occasionally, the ends of the parasite curved around the erythrocytic nucleus (Fig. 3b). Almost mature forms displaced the host nucleus towards one pole (Fig. 3c) and sometimes, the parasite twists the host cell nucleus (Fig. 3d). A nearly mature form with host cell nearing enucleation could be seen in typical polar position (Fig. 3e).

Cytoplasm of the parasite was moderately coarse and stained pale blue with Giemsa's stain. The granules were median or small sized and dispersed randomly in all parts of the cytoplasm averaging 20 per parasite. When the granules were small, their number was higher and were black to yellow- brown in colour. The parasite nucleus was median and stained pink with Giemsa's stain, averaging 1.8 μm in length and 1.6 μm in width. The measurements

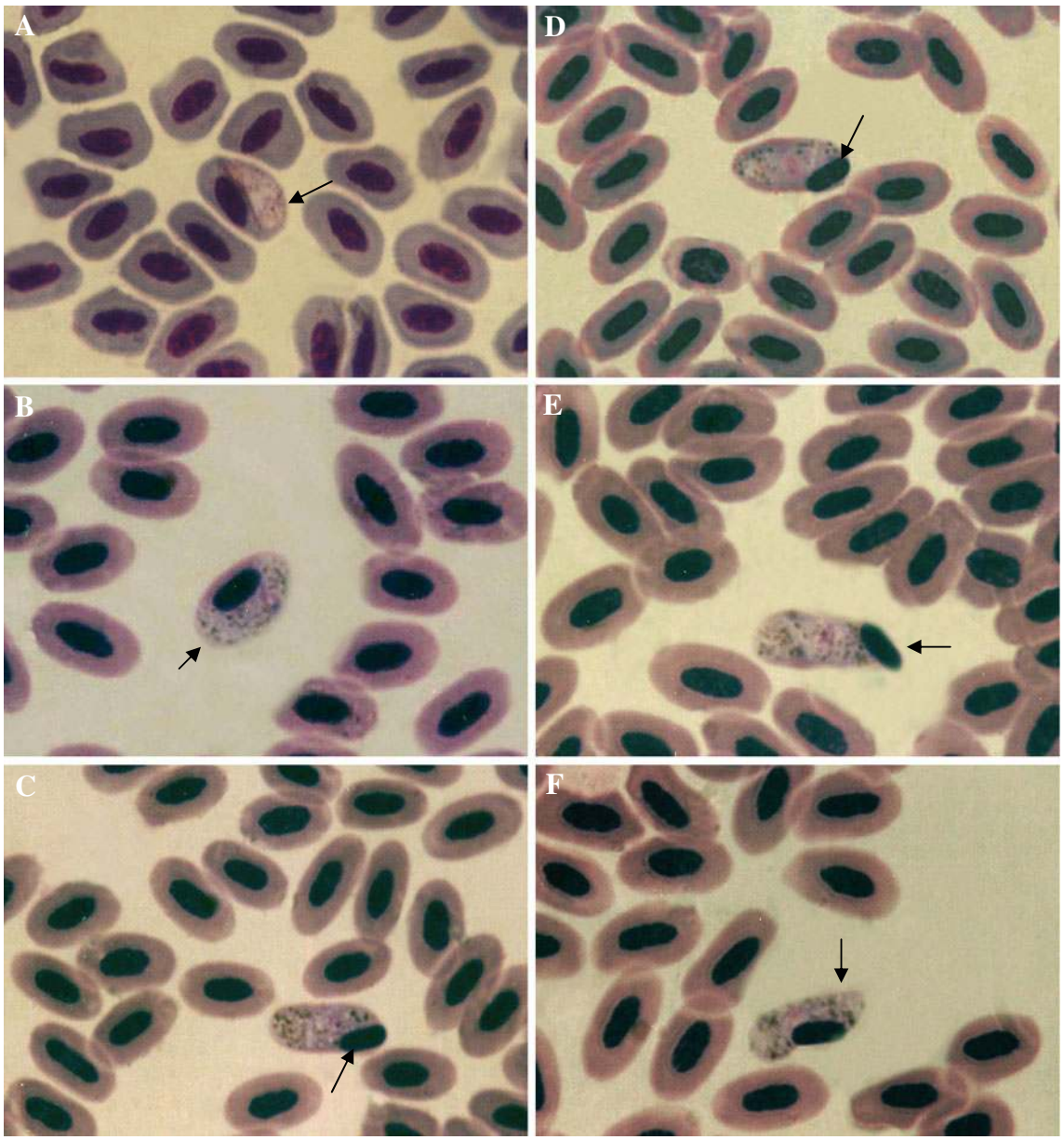

Fig. 3 Microphotographs of macrogametocytes of *H. nasimii* sp. nov. (×1,000): **a** Broad at one end and narrow at the other. **b** Curved around the host cell nucleus. **c** The host cell nucleus displaced towards the pole. **d** Host cell nucleus twisted. **e** Host cell nearing enucleation. **f** Host cell cytoplasm completely filled with macrogametocyte

of mature form varied from 13.0 to 16.0 μm in length and 4.0–6.9 μm in width (average length 13.9 μm and width 4.7 μm in). The parasite occupied approximately three-quarters of the host cell and sometimes completely filled the host cell cytoplasm (Fig. 3f).

Microgametocyte (Fig. 4b–f) ($n = 20$)

Microgametocyte was slightly larger than the macrogametocyte (Fig. 4b), slightly halteridial and usually lateral to the host cell nucleus similar to the macrogametocyte. The ends of the parasites are usually rounded and the margin entire. The gametocytes almost adhere to the host cell membrane at the polar zone but sometimes, in the central zone as well. A fully-grown microgametocyte fills the poles of the affected erythrocyte (Fig. 4c) and may displace its nucleus towards the pole (Fig. 4d). An enucleated erythrocyte also contained microgametocyte (Fig. 4e).

Microgametocytes are also capable of twisting the host cell nucleus by approximately 90° similar to the macrogametocyte (Fig. 4f). Cytoplasm of the mature form was fairly granular and stained only lightly with Giemsa'stain or occasionally it was colourless. The granules were localized only at the poles of the parasite averaging 11 per parasite and are yellow–brown or black in colour. Parasite nucleus was

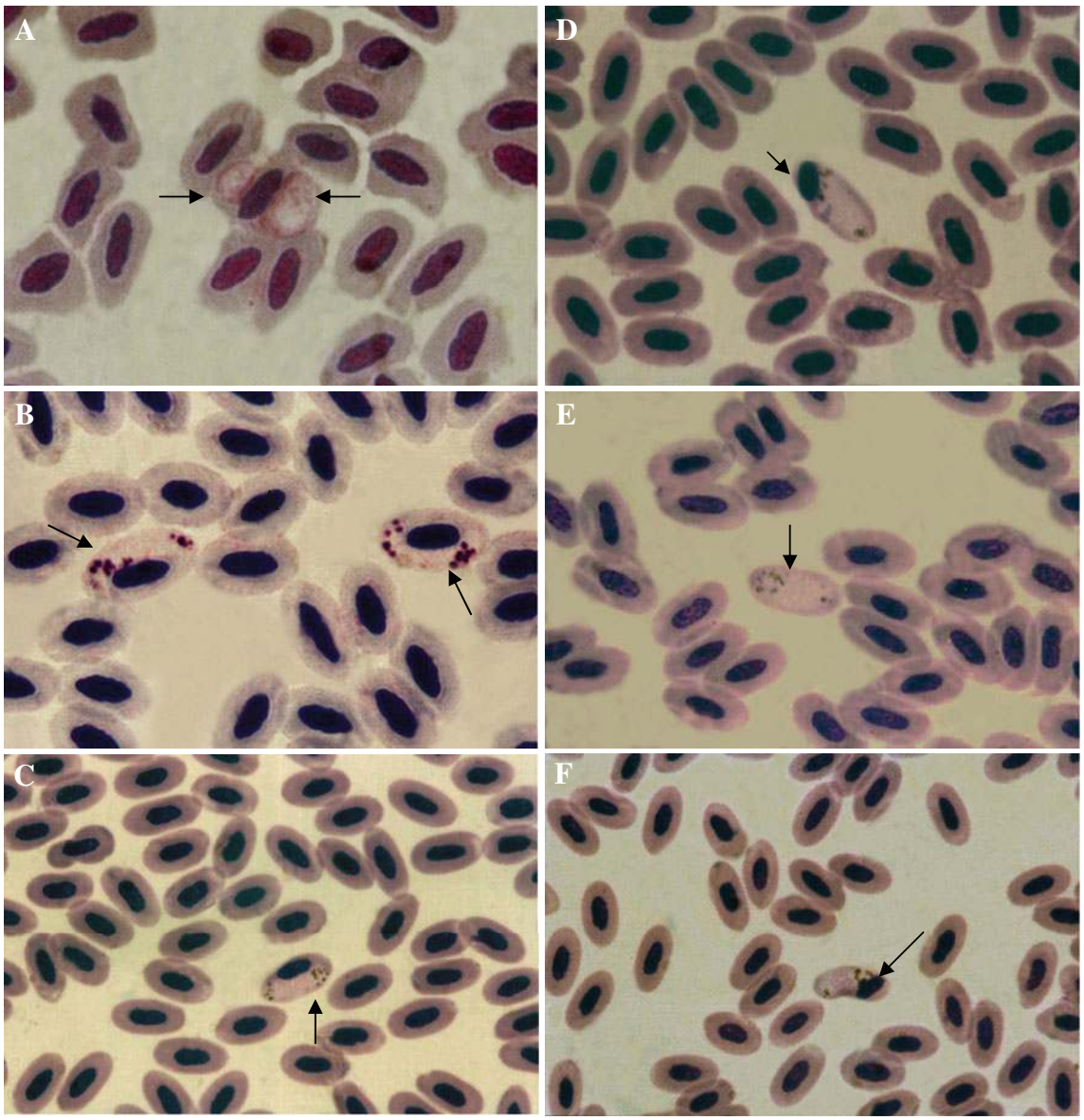

Fig. 4 Microphotographs of gametocytes of *H. nasimii* sp. nov. (×1,000): **a** DGI. **b** Two microgametocytes. **c** A fully-grown microgametocyte filling the poles of the host cell. **d** Host cell nucleus shifted towards the pole. **e** An enucleated erythrocyte containing microgametocyte **f** Host cell nucleus twisted

diffused and not easily distinguishable from the cytoplasm of the parasite. Microgametocytes varied in size from 13.0 to 15.0 µm in length and 4.0 to 6.0 µm in width (average 14.0 µm in length and 4.3 µm in width). Mature form occupied the major part of the infected erythrocytes.

Host nucleus

Erythrocytic *Haemoproteus* displaced the host cell nucleus and NDR was 0.2 in the parasitized erythrocytes. In some cases the nucleus shifted to one corner of the cell.

Extra corpuscular form (Fig. 5a–d) ($n = 10$)

Macrogametocytes could be seen escaping from the red blood cells (Fig. 5a) or lying free in the plasma. The extra corpuscular forms lying in the plasma were halteridial (Fig. 5b), elongated (Fig. 5c) or spindle-shaped (Fig. 5d) in shape. Cytoplasm was fairly granular, granules being dispersed throughout the parasite (Fig. 5b). The extra corpuscular forms varied in size from 15.0 to 17.8 µm in length and 3.9 to 7.3 µm in width while the average measurement was 16.7 µm in length and 5.8 µm in width. The nucleus took a pink stain with Giemsa's stain and is situated at the center of the parasite averaging 2.0 µm in length and 1.8 µm in width.

Double gametocyte infection (DGI) (Figs. 2b, 4a)

DGI and trigametocyte infection (TGI) are rarely reported in vertebrate erythrocytes. This phenomena has

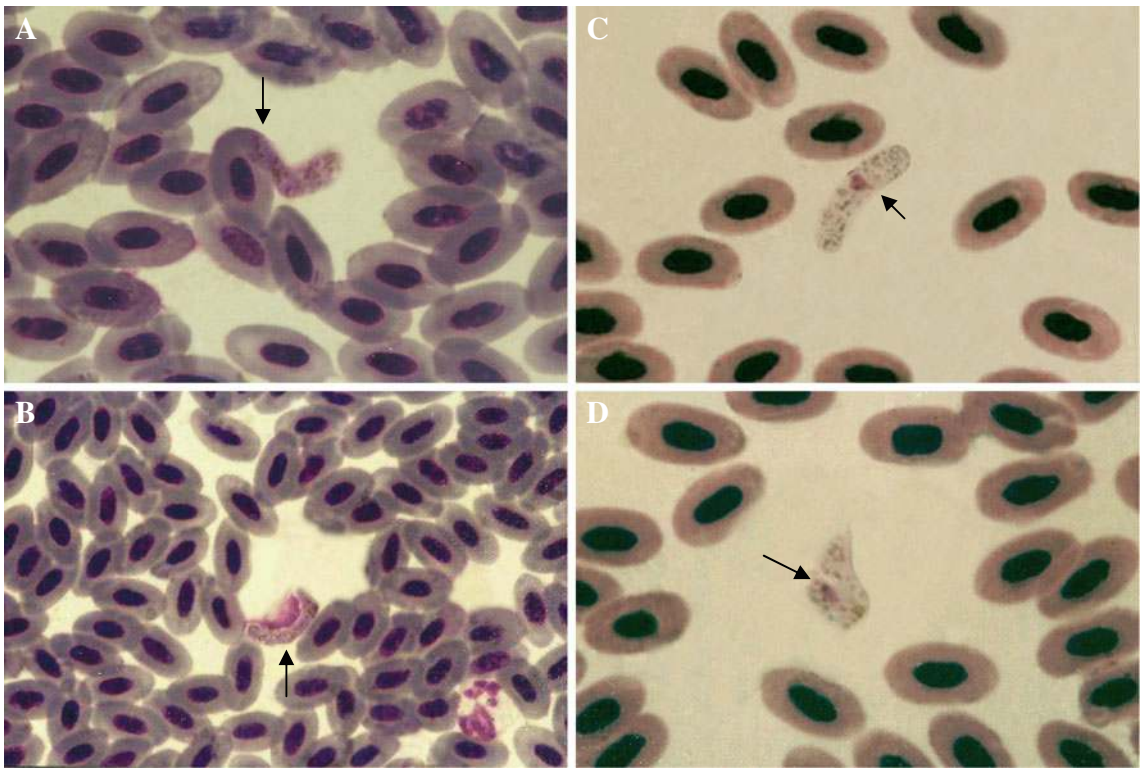

Fig. 5 Microphotographs of extracorpuscular form (macrogametocyte) of *H. nasimii* sp. nov. (×1,000): **a** Escaping from the RBC. **b** Halteridial form in the plasma. **c** Elongated form. **d** Spindle-shaped form

been suggested to enhance apicomplexon transmission (Jovani et al. 2004). During the present investigations, infrequently cases of DGI (Figs. 2b, 4a) were encountered. No cases of TGI or multiple gametocyte infection (MGI) were recorded.

Discussion

Haemoproteus species have been described based on statistical measurements of parasite's size and the changes incurred in the RBC's size (Campbell 1995). *H. columbae* Kruse 1890 was the earliest species of *Haemoproteus* described from *C. livia* being 14.7 µm in length and 3.9 µm in width. *H. passeris* from *Paser hispaniolensis* was also described by the same author (Kruse 1890). *H. fringillae* from *Fringlla coelebs* (Labbe 1894); *H. sacharovi* and *H. maccalumi* from *Zenaidura macroura* (Novy and Mac Neal 1905); *H. melopeliae* from *Melopedia leucoptera* (Laveran and Petit 1909); *H. dicruri* and *H. lanii* from *Lanius callurio* (De Mello 1936); *H. meropis* from *Merops orientalis* (Zargar 1945); *H. sanguinis* from *Pychnontus jocosus* (Chakravarty and Kar 1945); *H. turtur* from *Streptopelia turtur* (Ortega and Berenguer 1950); *H. piresi* from *C. livia* (Son 1960); *H. palumbis* from *Columba p. palumbus* (Baker 1966a); *H. fallisi* from American robins (Bennett and Campbell 1972) and *H. bennetti* from *Picus flainura* (Greiner et al. 1977) have been described from avian hosts. From Columbidae avians, *H. columbae*, *H. sacharovi, H. maccalumi, H. melopeliae, H. turtur, H. piresi* and *H. palumbis* have been described. Levine (1961) regarded only *H. columbae* and *H. sacharovi* as distinct, the remaining being synonyms of *H. columbae*. However, both *H. sacharovi* and *H. melopeliae* can be distinguished morphologically from *H. columbae* by the large size of their gametocytes. Baker (1966b) also questioned the validity of some of these species. Bennett and Peirce (1990) proposed that only *H. columbae* and *H. sacharovi* are valid species in their taxonomic review of the haemoproteid parasites of columbids, the other five species can be separated morphologically from *H. columbae* and are considered to be synonyms of this species. Adriano and Cordeiro (2001) described *H. columbae* from three species of wild doves.

A skilful examination of the present form with the above mentioned species indicated that *H. sacharovi, H. fringillae, H. lanii* and *H. palumbis* are larger than the present species therefore uncomparable. The gametocyte of present

species is comparable to *H. columbae, H. passeris, H. meropis, H. sanguinis, H. pallidus* and *H. payevskyi* in the parasite length. However, the parasite width of the present parasite species is greater than *H. columbae, H. passeris, H. pallidus, H. payevskyi* and *H. fallisi.* On the other hand, *H. bennetti* is smaller in length than the present species while its width is comparable to the present form. The present species also enclosed a nucleus 1.8 μm in length and 1.6 μm in width. The measurements suggest that gametocytes of the present species are comparable to *H. columbae* in intracorpuscular gametocyte cell length but differ considerably in gametocyte width (*H. columbae* 3.9 μm, present species 4.7 μm) and in the presence of nucleus.

Moreover, the extracorpuscular free form of the present species is also longer in all dimensions as compared to *H. columbae.* NDR of the two species are also different (*H. columbae* 0.5, present species 0.2). It is also interesting to note that the nucleus in the present species was observed not only in the intracorpuscular form but in the extracorpuscular form as well whereas it was not reported in the compared species (*H. columbae*).

The above critical comparison with the earlier reported species and discussion indicates that the *Haemoproteus* species encountered from the blood of *C. livia* is distinct and is thus designated as a new species, *H. nasimii* sp. nov. with the specific characters as mentioned in this account.

Parasite: Plasmodium

Taxonomic summary

Phylum	Apicomplexa
Class	Aconoidasida
Order	Haemosporida
Suborder	Haemosporina
Family	Plasmodiidae
Genus	*Plasmodium*
Species	*guptii* sp. nov

Parasite profile

Type host	*Columba livia*
Type locality	Bareilly (28°10′ N, 78°23′ E)
Additional localities	Badaun (28°02′ N, 79°10′ E), Shajahanpur (27°53′ N, 79°55′ E)
Site of infection	Blood
Holotype	ZSI/NRS/IV. 399
Paratype	ZSI/NRS/IV. 400 (Zoological Survey of India, Northern Regional Station, Dehradun, India)
Etymology	The parasite is named after the name of the author
Prevalence	6.76%
Intensity	1–2 pars/100 RBC's

General organization and generic diagnosis

The genus *Plasmodium* belongs to the family Plasmodiidae given their own order Haemosporidia. There are currently 450 recognized species in this order. The genus *Plasmodium* includes 13 subgenera in which 5 subgenera *Giovannola, Haemamoeba, Huffia*, *Novyella* and *Bennettinia* were created for the known avian malaria species (Corradetti et al 1963; Valkiunas 1997).

According to Corradetti et al. (1963) and Garnham (1966), the subgenera can be identified as follows:

Keys for the identification of the species in the subgenus *Bennettinia*

- Schizonts contain scant cytoplasm and are often round.
- Schizonts do not exceed the size of the host nucleus and stick to it.
- Gametocytes while varying in shape tend to be round or oval, do not exceed the size of the nucleus and stick to it.

Keys for the identification of the species in the subgenus *Giovannola*

- Schizont contain plentiful cytoplasm, are larger than the host cell nucleus and frequently displace it.
- They are found only in mature erythrocytes.
- Gametocytes are elongated.
- Exoerythrocytic schizogony occurs in the mononuclear phagocyte system.

Keys for the identification of the species in the subgenus *Haemamoeba*

- Mature schizonts are larger than the host cell nucleus and commonly displace it.
- Gametocytes are larger, round, oval or irregular in shape.
- Gametocytes are substantially larger than the host cell nucleus.

Keys for the identification of the species in the subgenus *Huffia*

- Mature schizonts, while varying in shape and size, contain plentiful cytoplasm.
- Schizonts are commonly found in immature erythrocytes.
- Gametocytes are elongated.

Keys for the identification of the species in the subgenus *Novyella*

- Mature schizonts are either smaller than or only slightly larger than host nucleus.
- Schizonts contain scanty cytoplasm.
- Gametocytes are elongated.

- Sexual stages in this subgenus resemble those of *Haemoproteus.*
- Exoerythrocytic schizogony occurs in the mononuclear phagocyte systems.

Definitive diagnosis of a *Plasmodium* is dependent on detecting the presence of asexually reproducing stages of its life cycle (schizonts) in the red blood cells of the infected host (Figs. 6, 7). The U-shaped forms, resembling the elongate ones except in position, are unlike the usual type of *Haemoproteus*. Mature schizonts are larger than the host cell nucleus and commonly displace it. Microgametocytes and macrogametocytes are also formed within erythrocytes in *Plasmodium* infections but are observed infrequently. Gametocytes are larger, round, oval or irregular in shape and substantially larger than the host cell nucleus (Corradetti et al. 1963). *Plasmodium* produce an insoluble golden brown or black deposits of haemozoin pigments in the parasite cells (Friend and Franson 1999).

The morphological characteristics of *Plasmodium* species discovered from *C. livia* clearly place it in the subgenus *Haemameoba* with round or oval gametocytes, schizonts in mature erythrocytes and with erythrocytic gametocytes lacking noticeable cytoplasm and being larger than the host cell nucleus and is identified as such.

Plasmodium (*Haemamoeba*) *guptii* sp. nov.

General morphology (Fig. 6a–r)

Erythrocytic and exoerythrocytic forms of this parasite were observed in the blood smears.

Description of erythrocytic stages

Trophozoites (Fig. 7a–e) ($n = 10$)

The smallest parasites (1.5 × 1.5 μm) have no visible cytoplasm, vacuole or pigments (Fig. 7a). A thin gray cytoplasm visible in 2.7 × 2.7 μm sized trophozoites which lacked pigment (Fig. 7b). Uninucleate parasites

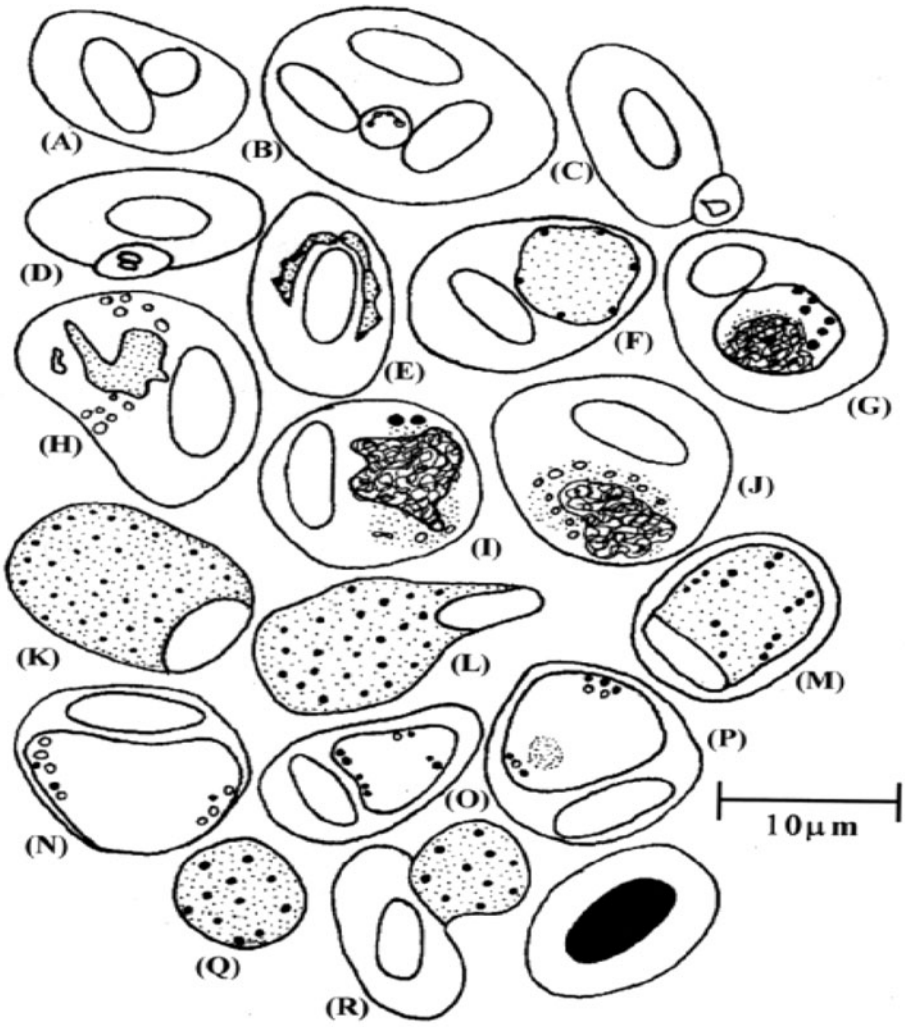

Fig. 6 Camera lucida diagrams of *Plasmodium guptii* sp. nov. **a–e** Trophozoites. **f–j** Schizonts. **k–m** Macrogametocyte**. n–p** Microgametocyte. **q–r** Exoerythrocytic forms

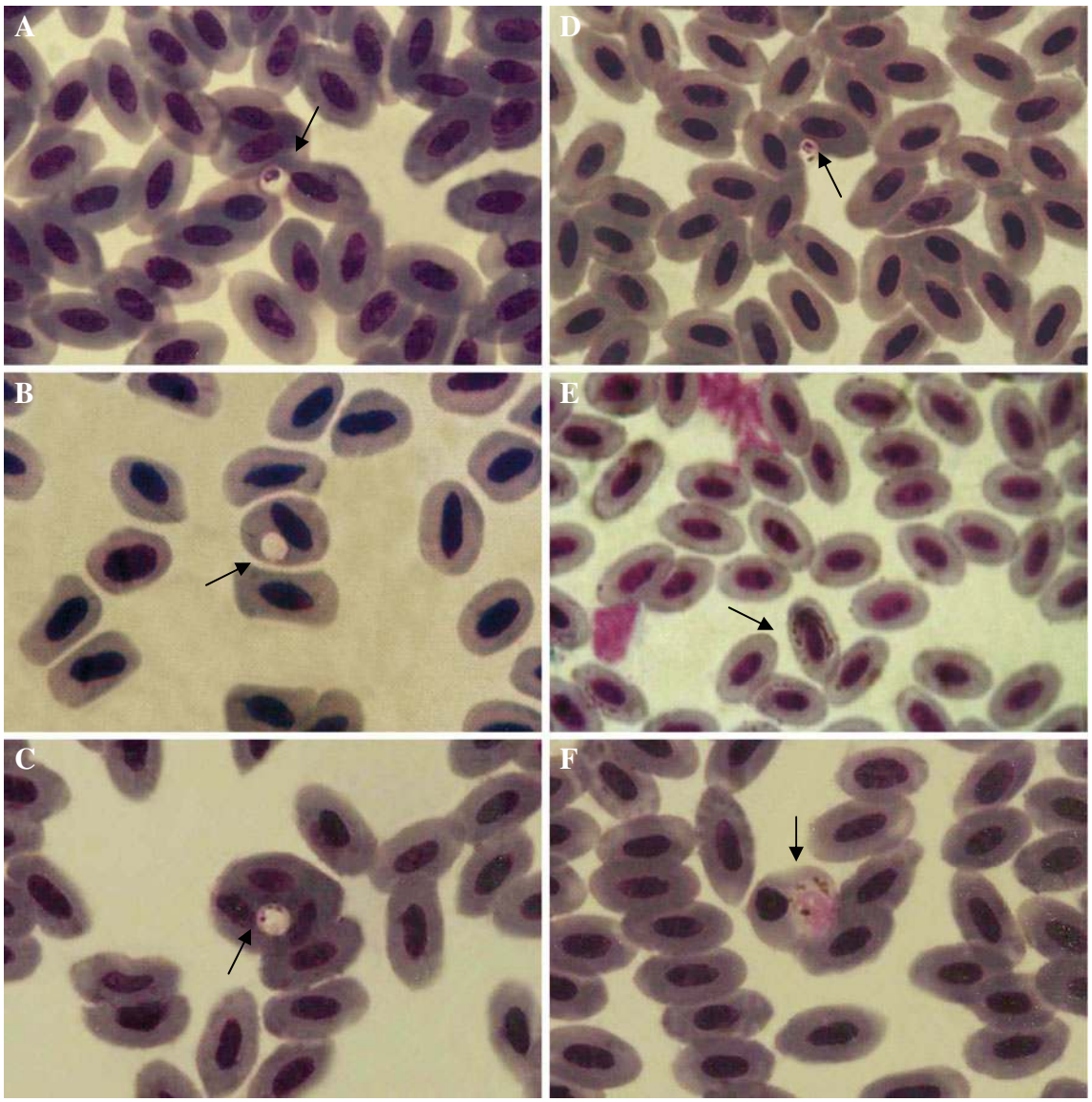

Fig. 7 Microphotographs of *Plasmodium guptii* sp. nov. (×1,000): **a** Trophozoite form. **b** Trophozoite with no pigments. **c** Round trophozoite. **d** Oval trophozoite. **e** U-shaped trophozoite. **f** Early schizont stage

usually elongate, but as they approach the first nuclear division, often become rounded (Fig. 7c) or oval (Fig. 7d). As they grow, some of them appear to migrate to the polar end of the host cell where they often assume characteristic U-shape, bending about the end of the erythrocyte nucleus (Fig. 7e). No nuclear displacement of the host cell due to trophozoites was evident.

Schizonts (Figs. 7f, 8a, b) (n = 16)

Schizonts usually lateral to the host cell nucleus, always marginal and visible in various stages of development: early schizont (Fig. 7f), rosette shaped schizont (Fig. 8a) and mature schizont (Fig. 8b). They change the shape of the infected erythrocyte and displace the host cell nucleus towards one side (Fig. 7f). Pigments usually found in clumps and are more conspicuous at the extremities of the parasite. Schizonts were 5.9 × 4.1 µm in size and their nuclei usually distributed in the form of a rosette (Fig. 8a).

Gametocytes (Figs. 8c–f, 9a, b) (n = 16)

Stained mature gametocytes showed characteristic sexual differences, macrogametocytes (Fig. 8c) staining blue and microgametocytes appearing pink or white in colour (Fig. 8d). Gametocytes usually appeared oval or round when occurring in a polar position in the cell (Fig. 8e) and sometimes the host cell nucleus was oblique in position (Fig. 8f). Mature gametocytes can fill the entire host cell cytoplasm (Fig. 9a). Pigment granules small, dispersed and vary greatly in number in macrogametocytes (Fig. 8c)

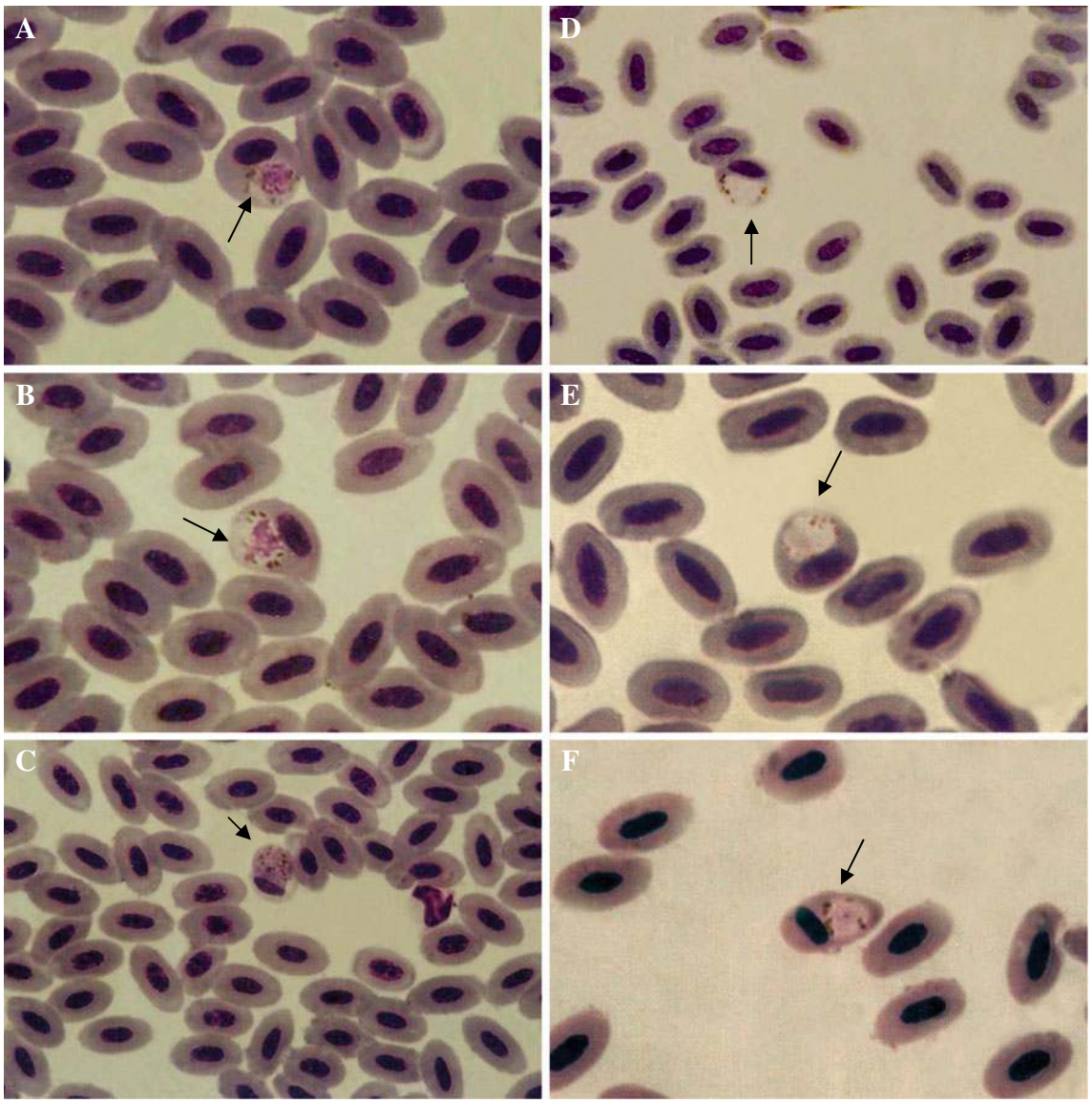

Fig. 8 Microphotographs of *Plasmodium guptii* sp. nov. (×1,000): **a** Schizont in the rosette form. **b** Mature schizont stage. **c** Mature macrogametocyte. **d** Mature microgametocyte. **e** Polar position of gametocyte and host cell nucleus. **f** Host nucleus twisted

whereas in microgametocytes, they cluster at one end of the parasite (Fig. 8d). Macrogametocytes averaged 7.8 × 7.7 μm and microgametocytes 7.8 × 7.6 μm in size. All gametocytes seen were pigmented.

Host nucleus

The gametocyte displaced the host cell nucleus. Sometime nucleus shifted to the one pole of the cell. NDR was 0.3 with a range of 0.1–0.5 in the host cell.

Exo-erythrocytic stages (Fig. 9a–c) ($n = 10$)

The occurrence of exoerythrocytic forms in the blood is highly variable, sometimes being frequent or usually quite sparse. They are usually round in shape and may be seen escaping from the RBC (Fig. 9a, b) or lying free in the plasma (Fig. 9c).

DGI

No cases of DGI, TGI and MGI were recorded in this species.

Discussion

Haematozoa of the genus *Plasmodium* are common among wild birds. Their form and morphology is relatively well known in Western and Northern Europe and North America (Peirce and Mead 1977, 1978; Kucera 1981; Bennett 1982; Valkiunas 1997; Krone et al. 2001) but reports from India are few (Nandi 1984). Erythrocytic stages of *Plasmodium* from the blood of wild populations

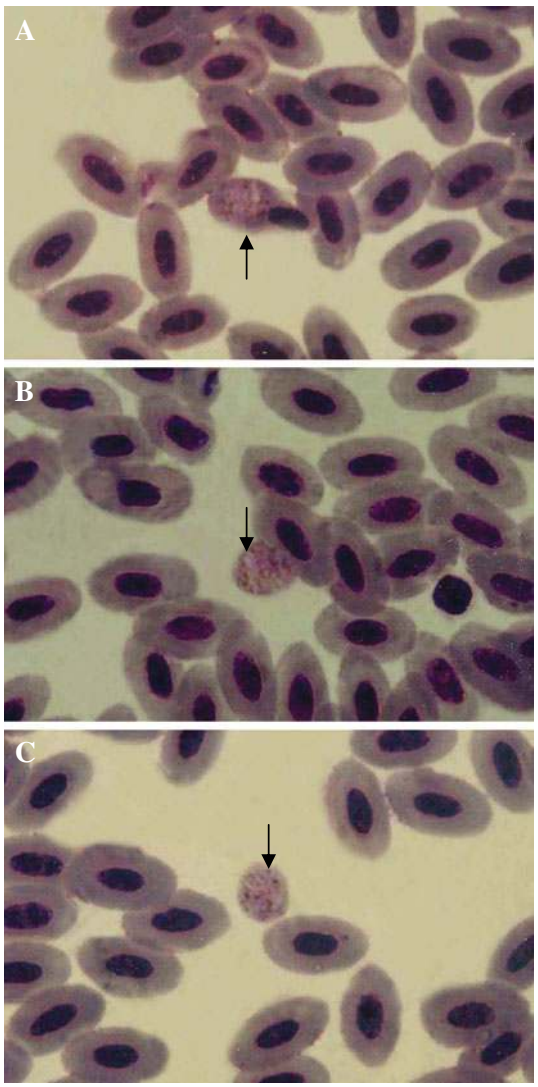

Fig. 9 Microphotographs of *Plasmodium guptii* sp. nov. (×1,000): **a** Macrogametocyte escaping from host cell. **b** Exoerythrocytic form escaping from the RBC. **c** Free exoerythrocytic form in the plasma

have been reported by various workers. Ray and Bhatnagar (1953) reported *P. rouxi* in the blood of a partridge from Delhi, India, Ray et al. (1953) recorded *P. polare* from the same host and locality. Amongst the early discovered species of avian *Plasmodium, P. vaughani* (Novy and Mac Neal 1904), *P. cathemerium* (Hartman 1927), *P. rouxi* (Sergent and Catanei 1928) and *P. circumflexum* (Kikuth 1931) are noteworthy.

P. vaughani and *P. circumflexum* were described by Laird and Lari (1956) and Laird (1962). A new species of *Plasmodium, P. formosanum* from *Arboriphila crudigularis* was described by Manwell (1962) and Manwell and Kuntz (1965) described *P. anasum* from *Anas clypeata*. *P. nucleophium* from *Ramphastos toca* and *P. paranucleophilum* from tangers were described by Manwell and Sessler (1971a, b).

The parasite described here in from wild pigeons bears certain morphologic similarities to *P. relictum* (subgenus *Haemameoba*). Within this subgenus, the present form resembles *P. relictum* and *P. cathemerium* most closely. The shape of gametocyte (round or oval shaped) indicates a closer morphological similarity of the present species to *P. relictum* and *P. cathemerium*. Parasites change the shape of infected erythrocytes and displace its nucleus, thereby the gametocyte of the present species is comparable to *P. relictum* and *P. cathemerium*. However, the parasite length and width of the present species is greater than *P. relictum* and *P. cathemarium* (*P. relictum* 6.7 × 6.1 µm, present species 7.8 × 7.6 µm) (Shurulinkov and Golemansky 2003). There are subtle morphological differences between these parasites, e.g. trophozoites do not displace the host cell nucleus in the present species whereas in *P. relictum*, trophozoites obviously displace the host cell nucleus (Shurulinkov and Golemansky 2003). The larger trophozoites and schizonts are especially characteristic of the present species just described because of the U-shape it so often exhibits. This peculiarity has not been reported from compared species. The most remarkable characteristic of this species is the frequent occurrence of extra-erythrocytic stages in the circulating blood not frequently reported by earlier authors.

Biological data, however, indicates the differences between the present species and *P. relictum* which are generally considered to be parasites of Passeriformes birds (Coulston and Huff 1947). Levi (1941) stated that *Plasmodium relictum* must be very rare in nature (among pigeons), since it has been found (in America) in only one free common pigeon and the vector is unknown. A single case was of a young pigeon observed at Peru, Nebraska, by Coatney (1938) from which stemmed the so-called 1-p strain of the parasite (Huff et al. 1942). Redmond (1944), Huff and Coulston (1946) and Coulston and Huff (1947) have adduced arguments for the better adaption of Coatney's strain to the canary than to the pigeon, leading to the suggestion it may have come originally from passerine birds.

It is possible that the Indian wild pigeon and its malaria represent a host-parasite relationship of longer duration than that of Passeriformes and *P. relictum*. Another reason, although of course not conclusive, for believing the parasite under discussion to be a new species is the fact that its host is a wild pigeon and is not previously known to be infected with malaria: indeed very few cases of naturally acquired malaria have been reported for any species of pigeons. Based on these facts, the parasite obtained from the blood of *C. livia* is considered to be new to science and a new name, *Plasmodium guptii* sp. nov. is proposed to account for the species with its specific characters as given in the account.

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