

PLASMODIUM SPP. IN RAPTORS ON THE EURASIAN-AFRICAN MIGRATION ROUTE

PAPERNA I.*, YOSEF R.** & LANDAU I.***

Summary:

Examination of blood smears obtained from raptors trapped while on migration at Eilat, Israel, demonstrated *Plasmodium* infection in *Accipiter brevipes* and *Buteo buteo*. The following species are described, from *A. brevipes*: *Plasmodium alloelongatum* n. sp., *P. accipiteris* n. sp. and from *B. buteo*: *P. buteonis* n. sp. and *Plasmodium* sp. for which we lack sufficient data for adequate species description. Overall prevalence of infection with *Plasmodium* spp. was very low: among 38 examined *A. brevipes* 5 % and among 56 *B. buteo* 3.6 %.

KEY WORDS : *Plasmodium* spp., *Accipiter brevipes*, *Buteo buteo*, Israel, migration.

Résumé :

PLASMODIUM SPP. DE RAPACES EN MIGRATION ENTRE L'ÉURASIE ET L'AFRIQUE
L'examen de prélèvements sanguins effectués chez des rapaces capturés et bagués à Eilat (Israël), au cours de leur migration, a révélé la présence de *Plasmodium* spp chez *Accipiter brevipes* et *Buteo buteo*. Les espèces suivantes sont décrites: *Plasmodium alloelongatum* n. sp. et *P. accipiteris* n. sp. chez *A. brevipes*, *P. buteonis* n. sp. et *Plasmodium* sp. chez *B. buteo*.

MOTS CLÉS : *Plasmodium* spp., *Accipiter brevipes*, *Buteo buteo*, Israël, migration.

INTRODUCTION

Raptors crossing Israel en route between Africa and their breeding locations in the north across Europe and Asia are caught each migration season (predominantly during spring) in Helgoland traps for ringing at the International Birding & Research Center at Eilat at the northern end of the Gulf of Aqaba. This provided an opportunity to study the blood parasites of these birds. *Plasmodium* infections among avian raptor populations are considerably less abundant than the other haemosporidians, *Haemoproteus* sp. and *Leucocytozoon* sp. In *Accipiter brevipes* and *Buteo buteo* examined during 2004-2005, among 38 *A. brevipes*: 22 (58 %) were infected with *Haemoproteus* spp., five (13 %) with *L. toddi* and only two (5 %) with *Plasmodium* spp. Among 56 *B. buteo*: 50 (89 %) were infected with *Haemoproteus* spp., 47 (84 %) with

L. toddi and only two (3.6 %) with *Plasmodium* spp.; all birds but one, *A. brevipes*, were caught during spring (Paperna & Yosef, unpublished). Available records on *Plasmodium* infections among Palearctic raptors (Bennett *et al.*, 1982; Valkiunas, 1997/2005) lists about 10 species, all of which are also reported from a wider range of non-raptor (passerine) hosts. In this communication we describe three new species of *Plasmodium* infections from *A. brevipes* and *B. buteo* with a further species from *B. buteo* with as yet insufficient data to be assigned as a new species.

MATERIALS AND METHODS

Birds were trapped in Helgoland traps at the International Birding & Research Center at Eilat, at the northern end of the Gulf of Aqaba (29° 33' N, 34° 57' E). The site supports native species of *Acacia* spp., *Zizyphus spina christi* and a variety of arid native and exotic shrubs and trees typical of arid areas. Numbered metal rings (of Tel Aviv University) were attached onto the tarsus of each bird, and morphometric data was recorded. The brachial vein of the bird was pierced by a sterile fine needle and a blood sample withdrawn into a heparinized hematocrit capillary and smeared onto a clean glass slide (two per bird). The bird was then released. Air dried smears were flooded by absolute methanol and before drying were stained for 1 h to 1 h and 15 min. with 12 %

* Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Sciences, of the Hebrew University of Jerusalem, Rehovot 76100, Israel.

** International Birding & Research Center, P.O.B. 774, Eilat, Israel.

*** Parasitologie Comparée et Modèles Expérimentaux (USM 307), Muséum National d'Histoire Naturelle, 61, rue Buffon, 75231 Paris Cedex 05.

Correspondence: I. Paperna, Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Sciences, of the Hebrew University of Jerusalem, Rehovot 76100, Israel.

Tel.: 972-8-9489945 – Fax: 972-8-9465763.

E-mail: ipaperna@agri.huji.ac.il

Giemsa (Merck), in pH 7.4 buffer phosphate. The entire stained smear was screened with microscope at $\times 1,000$ magnification, to include at least 50,000 erythrocytes. Holotypes: for each species, one schizont considered as typical was marked by a ring with a diamond marker and the slides are deposited in the collection of the Muséum National d'Histoire Naturelle, Paris.

RESULTS

PLASMODIUM ALLOELONGATUM N. SP.

Host: *Accipiter brevipes* – ring no. EE17661 (male, adult, weight: 187 gr).

Date of capture: 24.4.04 (Eilat).

Etymology: reminiscent of *P. elongatum*

Holotype: slide 441LV; PXIII67

Description (Figs 1-18, 65-68 – photographs)

Schizonts occur in immature erythrocytes (erythroblasts and reticulocytes), usually in polar position (Figs 1-9). Gametocytes occur in premature (reticulocytes) and mature erythrocytes, alongside their nucleus (Figs 10-18). Neither the schizonts, nor the gametocytes cause noticeable deformation of the erythrocyte or displacement of its nucleus. Schizonts ready for, and after first division, are as large as the proceeding stages, rectangular, sometimes with tapering lateral tips. They contain several large vacuoles and small aggregate of black pigment (Figs 1-5). After subsequent divisions, schizonts rounds up and attain the same size as the erythrocyte nucleus (Figs 5-7). The number of observed merozoites never exceeded six. At final stage of differentiation merozoites become elongated and clamp together along their longitudinal axis, the pigment granule remains single (Figs 8, 9).

The elongated macro and microgametocytes are as long as the erythrocyte longitudinal axis and have undulating or even rugged rims with tapering filaments at one or both ends (Figs 11-18). Their cytoplasm contains variable amounts of vacuoles. Pigment material forms one or two large aggregates, accompanied with 1-12 (mean $6.6 \pm SD 4.0$, $n = 7$), pigment material sometimes adjoins a vacuole. Pigment granules also enter into the filaments. Parasitaemia: 0.15 %

Remarks

P. elongatum Huff, 1930 has been described first from *Serinus canaria* and subsequently from *Passer domesticus* in USA (Huff & Bloom, 1935). Raffaele (1934) reported this species from *Carduelis carduelis* in Italy. It has been affiliated with the subgenus *Huffia*, Corradetti, Garnham & Laird, 1963. The presently described species and *P. elongatum* share common characters: blood stages (schizonts as well as gametocytes)

develop in immature erythrocytes and the merozoites elongate towards final differentiation. The two species, however, differ in two distinct details: The schizont's progeny is limited to 6 (6-12 in *P. elongatum*) and unlike the smoothed outlines of *P. elongatum* gametocytes, the presently described have undulating or rugged outlines and tapering ends, extending usually into a distal spine or filaments.

PLASMODIUM ACCIPITERIS N. SP.

Host: *Accipiter brevipes* ring no. EE17662 (male, adult, weight: 173 gr).

Date of capture: 24.4.04 (Eilat).

Etymology: named by its host generic name.

Holotype: slide 442LV; PXIII68

Description (Figs 19-28, 69, 70 – photographs)

Schizonts and gametocytes occur only in mature erythrocytes, neither of the stages causes displacement of the host-cell nucleus. Schizonts at all stages of maturity do not vary much in size and remain smaller than the erythrocyte nucleus. Schizonts are rounded or oblong and demonstrate at all stages conspicuous one, and sometimes two, faint-blue staining (in Giemsa) globules (Figs 21-26). The limited volume of cytoplasm contains variable number of vacuoles and 1-7 (2.5 ± 2.5 ; $n = 8$) scattered pigment granules. Schizonts formed four to six merozoites, and exceptionally 7-8 (Figs 25, 26). The macrogametocyte, prolonged or jagged (Fig. 27), the microgametocytes long and slender (Fig. 28), both extend alongside the erythrocyte nucleus. Their cytoplasm contains many vacuoles and sometimes up to four blue staining globules. Pigment forms either a single aggregate or up to seven granules. Parasitaemia: 0.14 %.

Remarks

The small schizonts with the conspicuous blue globules and the progeny of six nuclei are reminiscent of *P. tenuis* Laveran & Marullaz, 1914, parasite of *Leiothrix lutea* (Scopoli, 1786) and *P. merulae* Corradetti & Scanga 1973, parasite of *Turdus merula* L., 1758. Both are *P. vaughani*-like species (Manwell, 1935), affiliated with the subgenus *Novyella* Corradetti, Garnham & Laird, 1963, with a same progeny of merozoites and with a conspicuous blue staining refractile globule. However, unlike the latter species, the cytoplasm in the presently described species is more abundant and blue globules form also in the gametocytes.

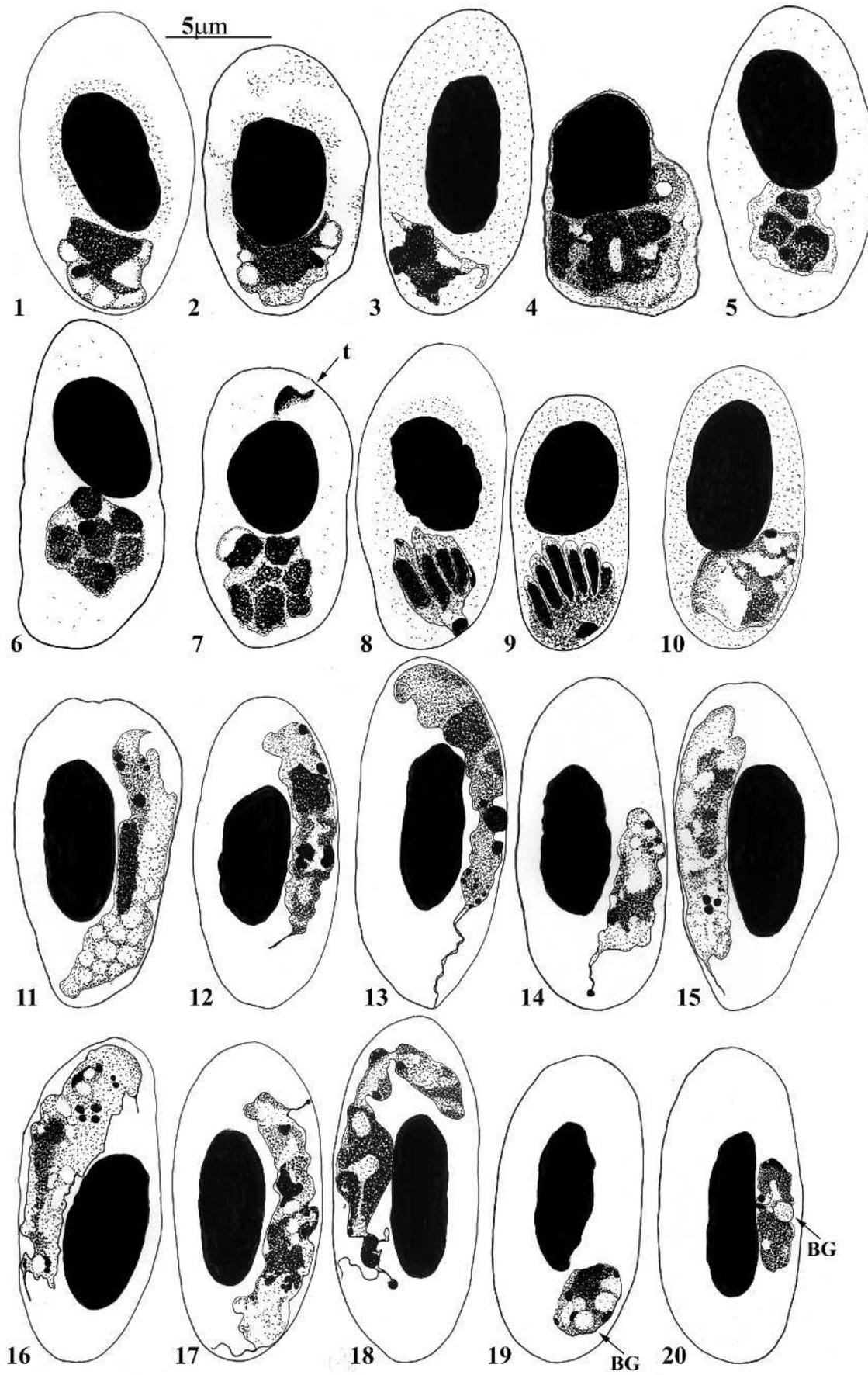
PLASMODIUM BUTEONIS N. SP.

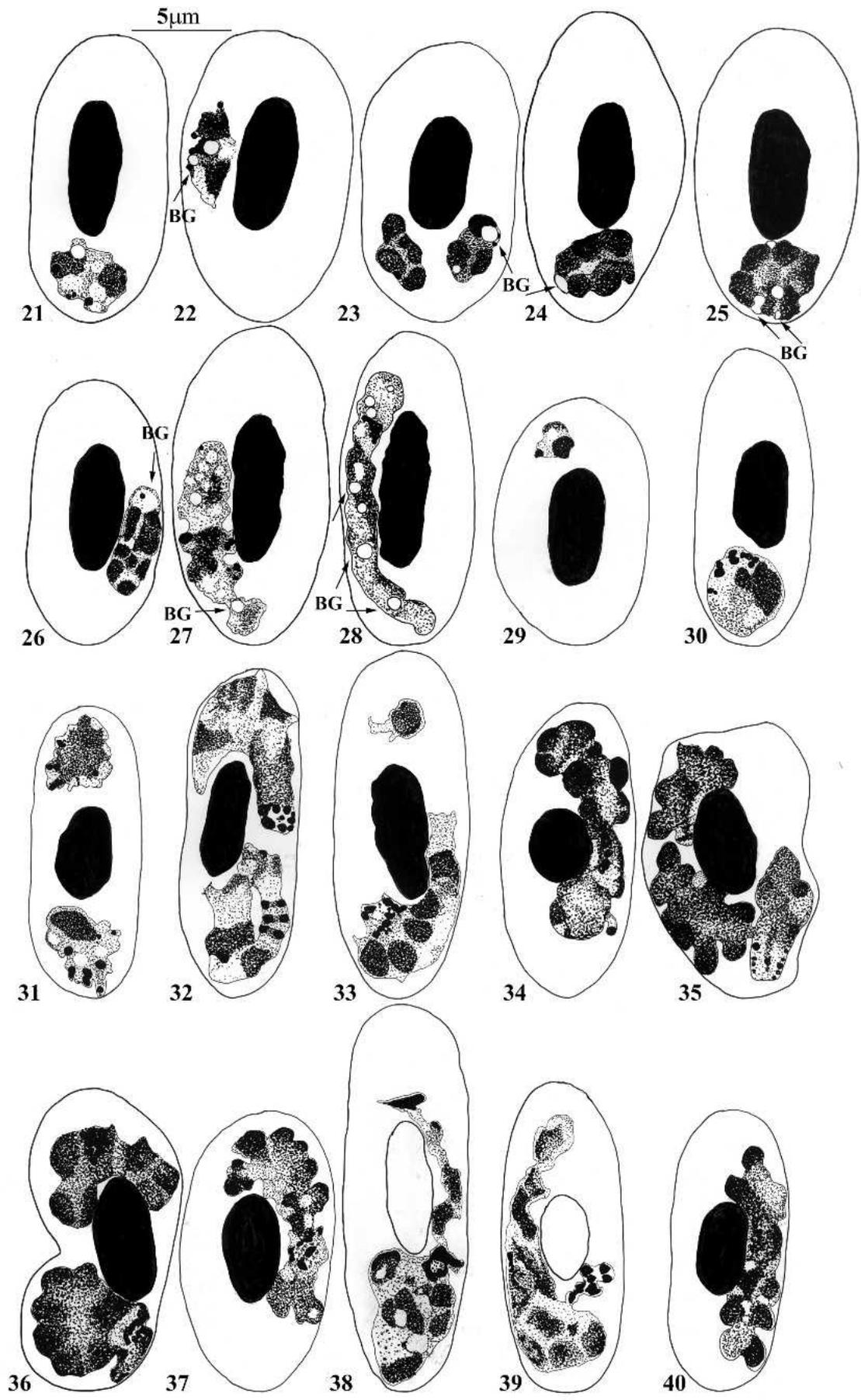
Host: *Buteo buteo*, ring no. GG4725 (Juv. 2d year, weight: 491 gr).

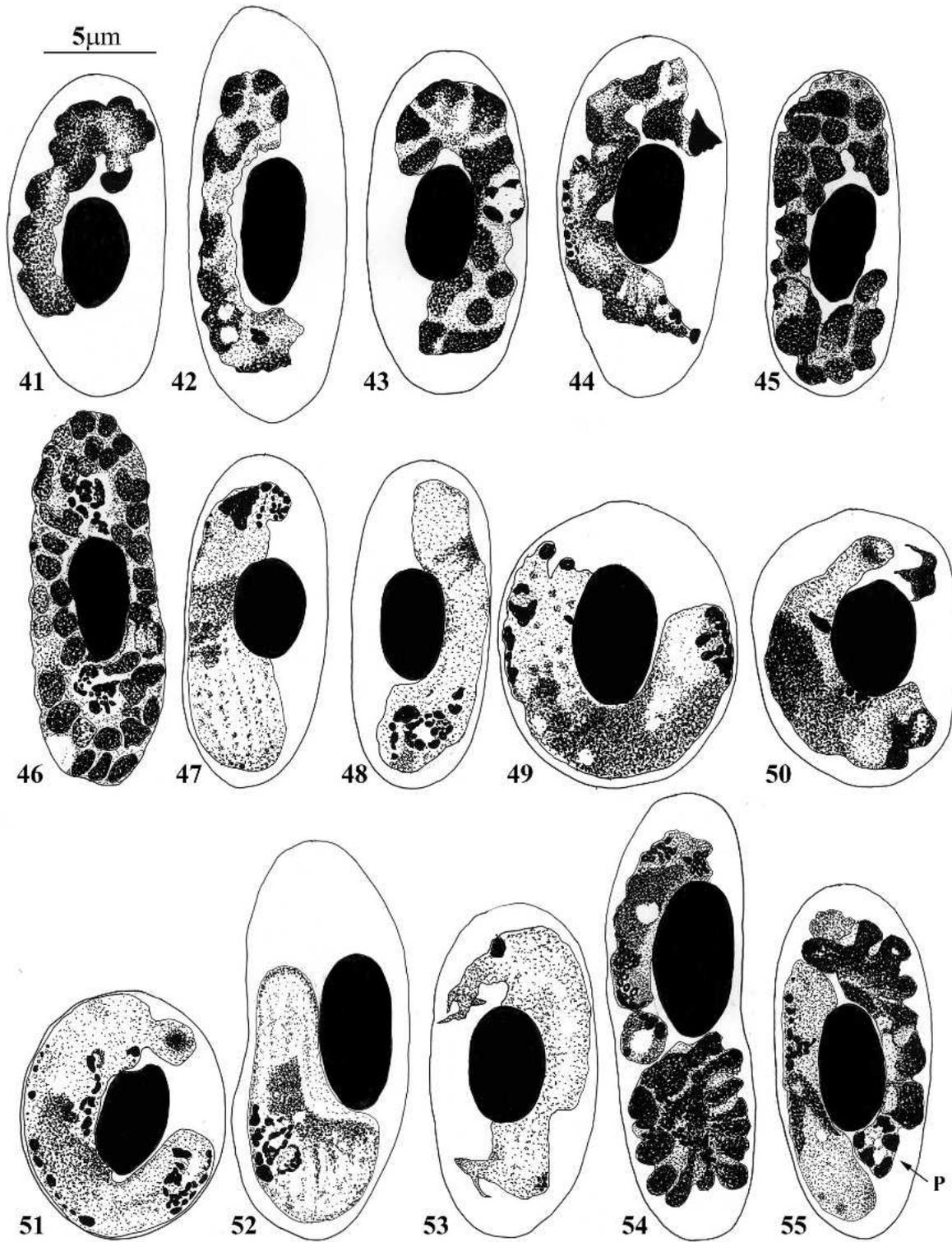
Date of capture: 21.5.05 (Eilat).

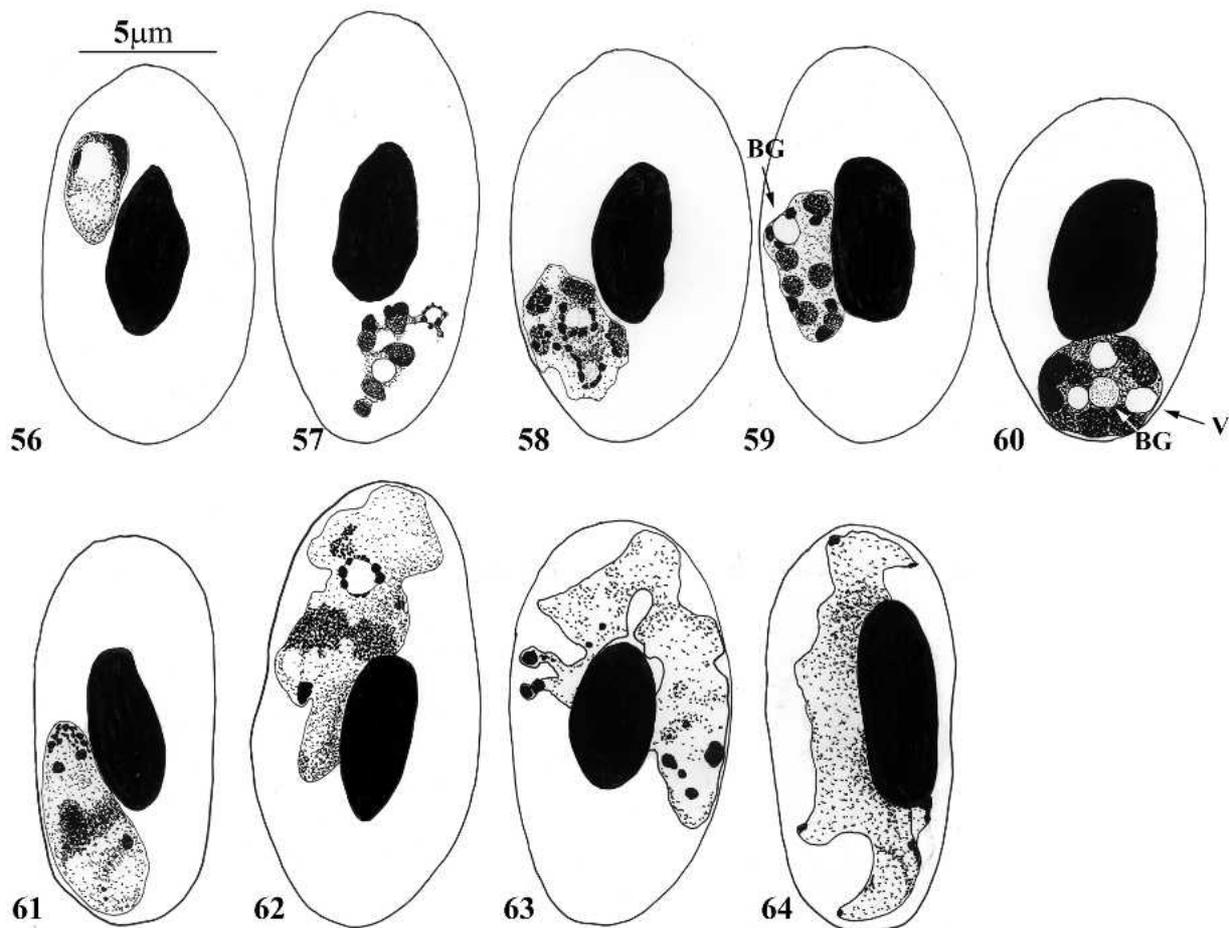
Etymology: named by its host generic name.

Holotype: slide 443LV; PXIII69









Figs 1-18. – *Plasmodium alloelongatum* n. sp. from *Accipiter brevipes*. All schizogony stages (Figs 1-9) in premature erythrocytes. Figs 1-3: undivided schizonts. Fig. 4: dividing schizont. Fig. 5: schizonts of four to six nuclei. Figs 8, 9: mature schizont with elongated merozoites. Fig. 10: young macrogametocyte. Figs 11-13: macrogametocytes. Figs 14-18: microgametocytes. Same scale for all figures.

Figs 19-28. – *Plasmodium accipiteris* n. sp. from *Accipiter brevipes*. Fig. 19: young schizont; y. Figs 20-22: schizonts after 1st division. Figs 23-26: schizonts of four to six nuclei. Fig. 27: macrogametocyte. Fig. 28: microgametocytes. BG = blue staining globule. Same scale for all figures.

Figs 29-55. – *Plasmodium. buteonis* n. sp. from *Buteo buteo*. Fig. 29: trophozoite. Figs 30, 31: early schizonts. Fig. 32: young schizonts, already elongated. Fig. 33: trophozoite (top) and five-nucleate schizont. Fig. 34: schizont with nine nuclei. Figs 35, 36: multiple infections of schizonts and gametocytes. Figs 37-44: further stages in schizont's differentiation – elongation and nuclei multiplication. Fig. 45: schizonts of ~20 nuclei. Fig. 46: schizont of ~ 36 nuclei. Figs 47-50: macrogametocytes. Figs 51-53: microgametocytes. Fig. 54: triple infection: from top-macrogametocyte, young schizont and 16-nucleated schizont. Fig. 55: double infection of eight-nucleate schizont (right) and macrogametocyte. P = pigment; t = trophozoite. Same scale for all figures.

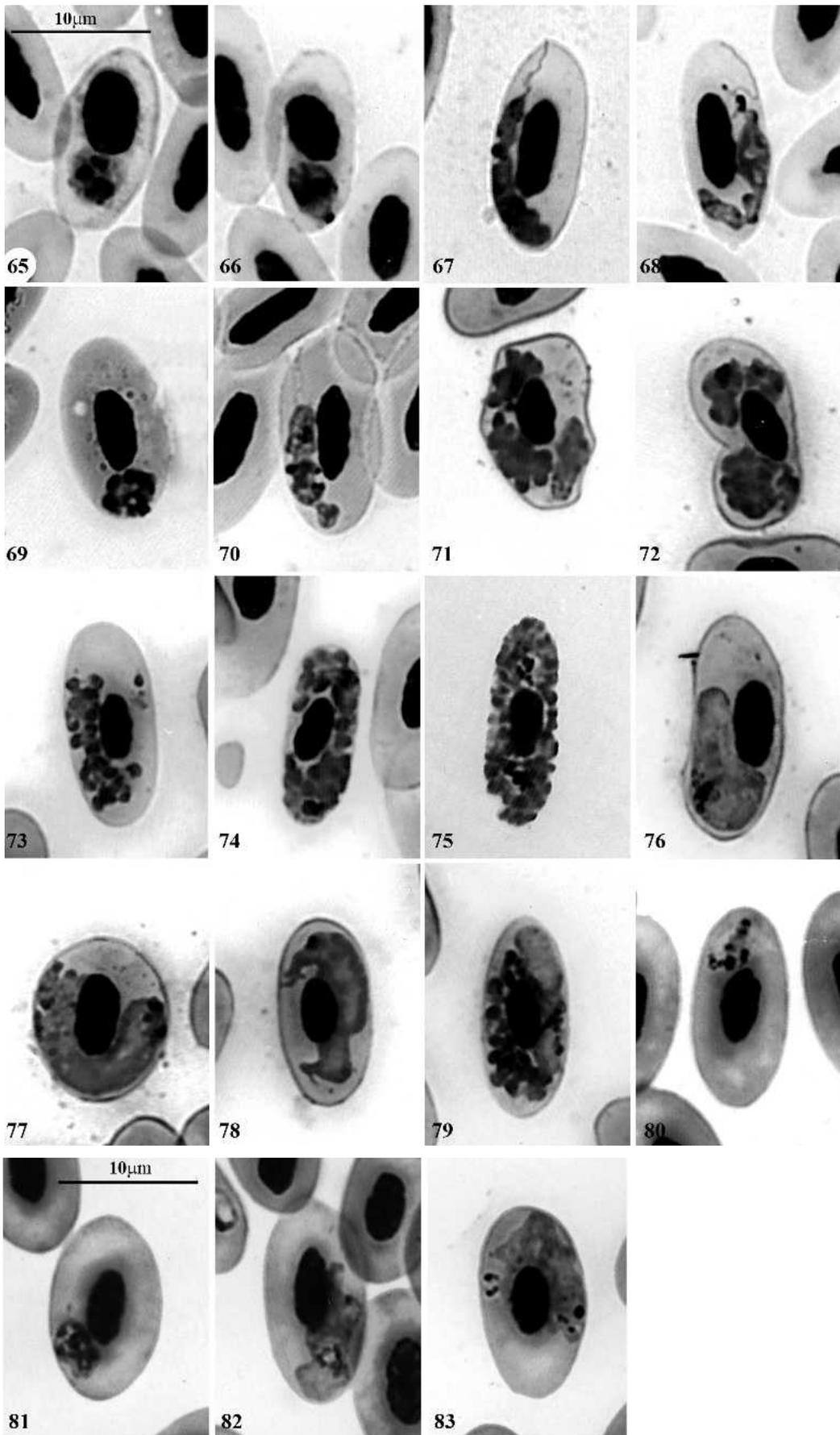
Figs 56-64. – *Plasmodium* sp. from *Buteo buteo*. Fig. 56: trophozoite. Fig. 57: reticulated schizont. Figs. 58-60: clumped, rounded schizonts. Fig. 61: young macrogametocyte. Fig. 62: mature macrogametocyte. Figs 63, 64: microgametocytes. BG = blue staining globule; V = vacuole. Same scale for all figures.

Figs 65-68. – *Plasmodium alloelongatum* n. sp. from *Accipiter brevipes*. Figs 65, 66: schizonts. Fig. 67: macrogametocyte. Fig. 68: microgametocyte.

Figs 69, 70. – *Plasmodium accipiteris* n. sp. from *Accipiter brevipes*. Fig. 69: schizont. Fig. 70: macrogametocyte.

Figs 71-79. – *Plasmodium. buteonis* n. sp. from *Buteo buteo*. Figs 71, 72: multiple infections with young schizonts. Figs 73, 74: on-growing schizont. Fig. 75: mature schizont. Fig. 76: macrogametocyte. Figs 77, 78: microgametocytes. Fig. 79: double infection of macrogametocyte and schizont.

Figs 80-83. – *Plasmodium* sp. from *Buteo buteo*. Fig. 80: reticulated schizont. Fig. 81: clumped, rounded schizont. Fig. 82: macrogametocyte. Fig. 83: microgametocyte.



Description (Figs 29-55, 71-79 – photographs)

Schizonts and gametocytes occur only in mature erythrocytes. Parasitized erythrocytes may become enlarged or prolonged, particularly in multiple infections, or round up when infected with gametocytes. Only exceptionally the erythrocyte nucleus becomes displaced from its central position. Infection was very heavy (with relapse of over 50 % trophozoites), with numerous double or multiple infections of trophozoites, schizonts, or of schizonts with gametocytes.

The youngest parasite had round nucleus and an extended cytoplasm, which may already contain pigment granules (Fig. 29). The differentiating schizont initially occupied a polar position, particularly in multiple infections (Figs 30-33, 35, 36), but with subsequent nuclear division it elongates and aligns alongside the erythrocyte nucleus (Figs 34, 37-44). The dark pigment granules may number up to 33 (11.7 ± 7.2 , $n = 16$) when few, occur in several (usually 4-6) aggregates of variable sizes, often accompanied by vacuoles. Further differentiated schizonts (with > 8 nuclei) usually occur as solitary infections.

With further nuclear divisions the schizont grows around the erythrocyte nucleus (Fig. 45) until it becomes completely encircled (Fig. 46). Such stages contained about 36 nuclei, but were rare.

The gametocytes are tongue-like (Figs 47-52), sometimes with spiny projections (Fig. 53) and located alongside the erythrocyte nucleus. Some cause rounding of the erythrocyte while embracing the erythrocyte nucleus (Figs 49, 50). The black pigment material is numerous (22.3 ± 12.1 , $n = 11$), when fewer, assembles like in the schizonts in several variable size aggregates, sometimes accompanied by a vacuole. Multiple infections of gametocytes and differentiated schizonts are not uncommon (Figs 54, 55). Parasitaemia: 6.6 %

Remarks

The mature schizont bears close resemblance to *P. circumflexum* Kikuth, 1931 (subgenus *Giovannolaia* Corradetti, Garnham & Laird, 1963), isolated from *Turdus pilaris* and described from an experimentally infected canary (see Valkiunas 1997/2005). In contrast to ~ 24 reported in the latter, those presently described reach higher merozoite progeny, of about 36. Pigment material in the gametocytes forms aggregates or even clumps rather than scattered as shown in *P. circumflexum* and the gametocytes never seem to entirely enclose the erythrocyte nucleus. The infection in *B. buteo* is characterized by its high intensity that results in high rates of multiple infections of schizogony stages and of schizonts with gametocytes. *P. circumflexum* has been reported by Yakunin (1972) from *B. buteo* (among other avian hosts) from Kazakhstan, but no detailed description has been provided. *P. heroni* Basu, 1938, from *Ardeola grayii* in India, another species which

develops a “*circumflexum*” schizont that engulfs the erythrocyte nucleus, was synonymised by Valkiunas (1997/2005) with *P. circumflexum*. The fully differentiated schizont of Basu’s species yields 26 merozoites. Basu (1938) could not infect with *P. heroni* canaries, which are susceptible to *P. circumflexum* (Manwell & Goldstein 1938) and reports its development up to oocyst stages in *Culex fatigans* (= *C. quinquefasciatus*). The vectors of *P. circumflexum* are *Culiseta* spp. and *Mansonia crassipes* (Valkiunas, 1997/2005).

Type material from Basu was found in Emile Brumpt’s collection at the Museum of Paris and detailed comparisons with other “*circumflexum*” like species will be published later.

PLASMODIUM SP.

Host: *Buteo buteo* ring no. GG4724 (Juv. 2d year, weight: 570 gr.).

Date of capture: 21.5.05 (Eilat).

Description (Figs 56-64, 80-83 – photographs)

Infection was scanty, revealing only few trophozoites, schizont stages and gametocytes. Trophozoites, schizonts and gametocytes occur only in mature erythrocytes, neither of the stages causes displacement of the host-cell nucleus. Trophozoites are ring-shaped (Fig. 56). Schizonts at all stages are smaller than the erythrocyte nucleus. Schizonts with scanty reticulated cytoplasm connecting five nuclei, blue staining globule and a vacuole surrounded with dark pigment grains seems to represent early stage of differentiation (Fig. 57). Differentiated schizonts consolidate into oval cytoplasmic body with 4-6 nuclei and blue staining globule, with additional transparent vacuole (Figs 58-60). Numerous black pigment granules (6-28) aggregate around the globule and the vacuole. Both traced macrogametocyte (Fig. 62) and the microgametocytes (Figs 63, 64) are large, crooked-shaped, or weavy, and extend either alongside the erythrocyte long axis or wrap the nucleus at one pole, both contain dark 8-15 pigment granules of variable sizes (11 ± 2.9 , $n = 4$). One macrogametocyte shows a blue staining vacuole surrounded by pigment granules, none seen in the others.

Remarks

This species may be affiliated with the subgenus *Novyella* Corradetti, Garnham & Laird, 1963. With the limited number of stages available for study we refrain from naming this parasite. Cytoplasm limited to reticulated frame is characteristic to *P. rouxi* as well as to further undescribed species (Paperna *et al.* in prep.). *P. rouxi* yields only four merozoites, *Plasmodium* sp. yields 5-6 merozoites. The differentiated clustered schizonts with the blue staining globule is reminiscent of the species *P. tenuis*, and *P. merulae* and could also be conspecific with *P. accipiteris* n. sp. (this communication). It

is not exceptional to find prolonged crooked-shaped gametocytes with pointed tips among species included in the subgenus *Novyella*. Blue staining globules form also in gametocytes of several species (see *P. accipiteris*, this communication), the macrogametocyte shown in figure 62, however, contains an extended blue staining vacuole rather than a (refractile and thick walled) globule.

DISCUSSION

What is the extent of host specificity among avian species of *Plasmodium*? All species of *Plasmodium* thus far recorded from raptors such as *P. relictum*, *P. circumflexum*, *P. fallax*, *P. lophurae* and *P. polare*, were additionally (or predominantly) recorded from non-raptors, including passerine species (Bennett *et al.*, 1982).

Do raptors share *Plasmodium* species with passerine birds? Taxonomical records often list numerous hosts for a particular species of *Plasmodium* (Bennett *et al.*, 1982; Bishop & Bennett, 1992; Valkiunas, 1997/2005). In fact, much of the available taxonomic descriptions are from secondary, experimental hosts (see Valkiunas, 1997/2005): infections of many species of wild birds of diverse families were induced by blood inoculation to canaries (*P. relictum*, *P. circumflexum*, *P. polare*, a further example: *P. oti* Wolfson, 1936 of the owl *Megascops asio naevius*, or to pigeons, ducklings and chicks (*P. fallax* and *P. lophurae*). One may conclude that there is no host specificity among avian *Plasmodium* species, which spreads randomly among birds and birds contract infection if circumstances for transmission are appropriate. The alternative explanation could be that artificial inoculation exposes experimental hosts (in analogy to the case of white mice in mammalian malaria infections) – canaries, chicks or pigeons to multifold dose of infection which compromises the birds' natural resistance.

In contrast to the loose host specificity reported in many avian *Plasmodium* infections, some communications report of restricted host specificity. Corradetti and Scanga (1973) demonstrated strict host specificity in *P. merulae* and *P. tenue*, both infections could not be transmitted to canaries. Iezhova *et al.* (2005) demonstrated high degree of host specificity in *P. nucleophilum*, from *Parus major* and *P. vaughani* from *Eritbacus rubecula*, species earlier reported from 40 and > 150 hosts respectively (Bennett *et al.*, 1982). Are all these listed hosts a result of a taxonomical misconception? Or evidence of intraspecific divergence? – Such as the division to subspecies as attributed to *P. nucleophilum* and *P. vaughani* (Manwell, 1935; Manwell & Sessler, 1971; Corradetti & Scanga, 1972).

Molecular studies provide evidences for relatively high fidelity between avian hosts and Haemosporidian species (Bensch *et al.*, 2000), but also host sharing and host shifts not only between host species of the same genus but between species of diverse families (Bensch *et al.*, 2004; Waldenström *et al.*, 2002). Unfortunately, presently, much of the published molecular data remains divorced from the microscopically determined taxonomic classification (see Valkiunas *et al.*, 2006), and as yet there is no way to link most molecular results with taxonomic data.

REFERENCES

- BENSCH S., STJERNMAN M., HASSELQUIST D., ÖSTMAN Ö., HANSSON B., WESTERDAHL H. & PINHERO R.T. Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. *Proceedings of the Royal Society of London*, B, 2000, 267, 1583-1589.
- BENSCH S., PÉREZ-TRIS J., WALDENSTRÖM J. & HELLEGREN O. Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic speciation? *Evolution*, 2004, 58, 1617-1621.
- BENNETT G.F., WHITEWAY M. & WOODWORTH-LYNAS C.B. Host-parasite-catalogue of the avian haematozoa. Memorial University of Newfoundland occasional papers in biology, 1982, 5, 243 p.
- BISHOP M.A. & BENNETT G.F. Host-parasite-catalogue of the avian haematozoa: Supplement 1 and Bibliography of the avian blood inhabiting haematozoa: Supplement 2. Memorial University of Newfoundland occasional papers in biology, 1992, 15, 244 p.
- CORRADETTI A. & SCANGA M. *Plasmodium (Novyella) vaughani* subsp. *merulae*, n. subsp., parasita di *Turdus merula*, con descrizione del ciclo pre-erythrocytico. *Parassitologia*, 1972, 14, 85-93.
- CORRADETTI A. & SCANGA M. The *Plasmodium vaughani*-complex. *Experimental Parasitology*, 1973, 34, 344-349.
- HUFF C.G. & BLOOM W. A malarial parasite infecting all blood and blood-forming cells in birds. *Journal of Infectious Diseases*. 1935, 57, 315-336.
- IEZHOVA T.A., VALKIUNAS G. & BAIRLEIN F. Vertebrate host specificity of two avian malaria parasites of the subgenus *Novyella*: *Plasmodium nucleophilum* and *Plasmodium vaughani*. *Journal of Parasitology*, 2005, 91, 472-474.
- MANWELL R.D. *Plasmodium vaughani* (Novy & MacNeal). *American Journal of Hygiene*, 1935, 21, 180-187.
- MANWELL R.D. & GOLDSTEIN F. Life history and immunity studies of the avian malaria parasite, *Plasmodium circumflexum*. *Proceedings of the Society for Experimental Biology and Medicine*. 1938, 39, 426-428.
- MANWELL R.D. & SESSLER G.J. Malaria parasites of toucans. *Journal of Protozoology*, 1971, 18, 570-574.
- RAFFAELE G. Un ceppo italiano di *Plasmodium elongatum*. *Rivista Di Malariologia* 1934, 13, 332-337.

- VALKIUNAS G. Bird Haemosporidia. *Acta Zoologica Lithuanica* 1997, 3-5, 607 p. (English translation: Avian malaria parasites and other haemosporidia). CRC Press Boca Raton, Florida, 2005, 946 p.
- VALKIUNAS G., BENSCH S., IEZHOVA T., KRIZANAUSKIENE A., HELLGREN O. & BOLSHAKOV C.V. Nested cytochrom B polymerase chain reaction diagnostics underestimate mixed infections of avian blood haemospridian parasites: microscopy is still essential. *Journal of Parasitology*, 2006, 92,418-423.
- WALDENSTRÖM J., BENSCH S., KIBO S., HASSELQUIST D. & OTTOSON U. Cross-species infection of blood parasites between resident and migratory songbirds in Africa. *Molecular Ecology*, 2002, 11, 1545-1554.
- WOLFSON F. *Plasmodium oti* n. sp., a *Plasmodium* from the eastern screech owl (*Otus asio naevius*) infective to canaries. *American Journal of Hygiene*, 1936, 24, 94-101.
- YAKUNIN M.P. Haemoparasites of wild birds of south-east Kazakhstan. *Trudi Instituta Zoologii Akamedia-Nauk Kazakhkoi SSR*, 1972, 33, 69-79.

Reçu le 21 février 2007

Accepté le 15 octobre 2007