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A New Saurian Malarial Parasite *Plasmodium balli* from Panama

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SYNOPSIS: *Plasmodium balli* sp. nov. is described from *Anolis lionotus* and *A. poecilopus* of central Panama.

Large, elongate gametocytes and segmenters containing up to 100 merozoites are produced by *P. balli*. Proerythrocytes and normoblasts are more commonly parasitized than erythrocytes. Pigment is uncommon, but when present consists of a minute dot. Hypertrophy, distortion and lysis of host cell nuclei may result from parasitization of immature blood cells by gametocytes, while enucleated host cells are common.

DURING the course of a survey of Panamanian lizards for leptomonad parasites, several undescribed species of *Plasmodium* were encountered. A species of the subgenus *Sauramoeba* is described herein. The subgenus *Sauramoeba*, as defined by Garnham (3), contains those saurian malarial parasites with large segmenters, while the species producing small segmenters (12 or fewer merozoites) are placed in *Carinamoeba*.

The Panamanian herpetofauna is rich in species of iguanid lizards, and especially of the genus *Anolis*. While the taxonomy of this group is yet unsettled, I would estimate that the number of species occurring in Panama exceeds 20. One group of anoles has become structurally and behaviorally specialized for a semiaquatic existence, and at least 3 species, *Anolis lionotus*, *A. poecilopus*, and *A. aquaticus* are found in Panama. The first 2 species are probably closely related; *aquaticus* is derived from a different stock. Both *lionotus* and *poecilopus* are sympatric in some areas, but presumably occupy different drainage systems. *A. aquaticus* enters Panama only in the mountains near the Costa Rican border.

Two species of *Plasmodium* are found commonly in *Anolis lionotus* and *A. poecilopus* in every locality so far collected. A species of the subgenus *Carinamoeba* is most abundant and will be described in the future, while a less common *Sauramoeba* is considered here.

MATERIALS AND METHODS

Between January and December, 1968, a total of 230 semiaquatic anoles was examined for blood parasites. Malarial parasites of the subgenus *Sauramoeba* were present in 29 of 128 *Anolis lionotus* (22.6%) and 15 of 120 *A. poecilopus* (12.5%). Lizards were collected in the jungle by hand or noose and brought alive to the laboratory. Blood smears were prepared immediately from specimens which died en route, and from the remainder usually on the next day. In some cases, lizards were kept alive in the laboratory for up to 4 months and blood smears were made at weekly intervals or less. Blood was obtained by toe clipping of live lizards, and from the heart of dead specimens. Slides were fixed in absolute methanol and stained with a 1:10 dilution of Giemsa at pH 6.8 for 1.0-1.5 hours.

Blood smears were examined under oil immersion at 1000×, and parasites were measured at that magnification. The nomenclature proposed by Pienaar (6) has been followed in the description of saurian blood cells. The level of statistical significance chosen was 0.05.

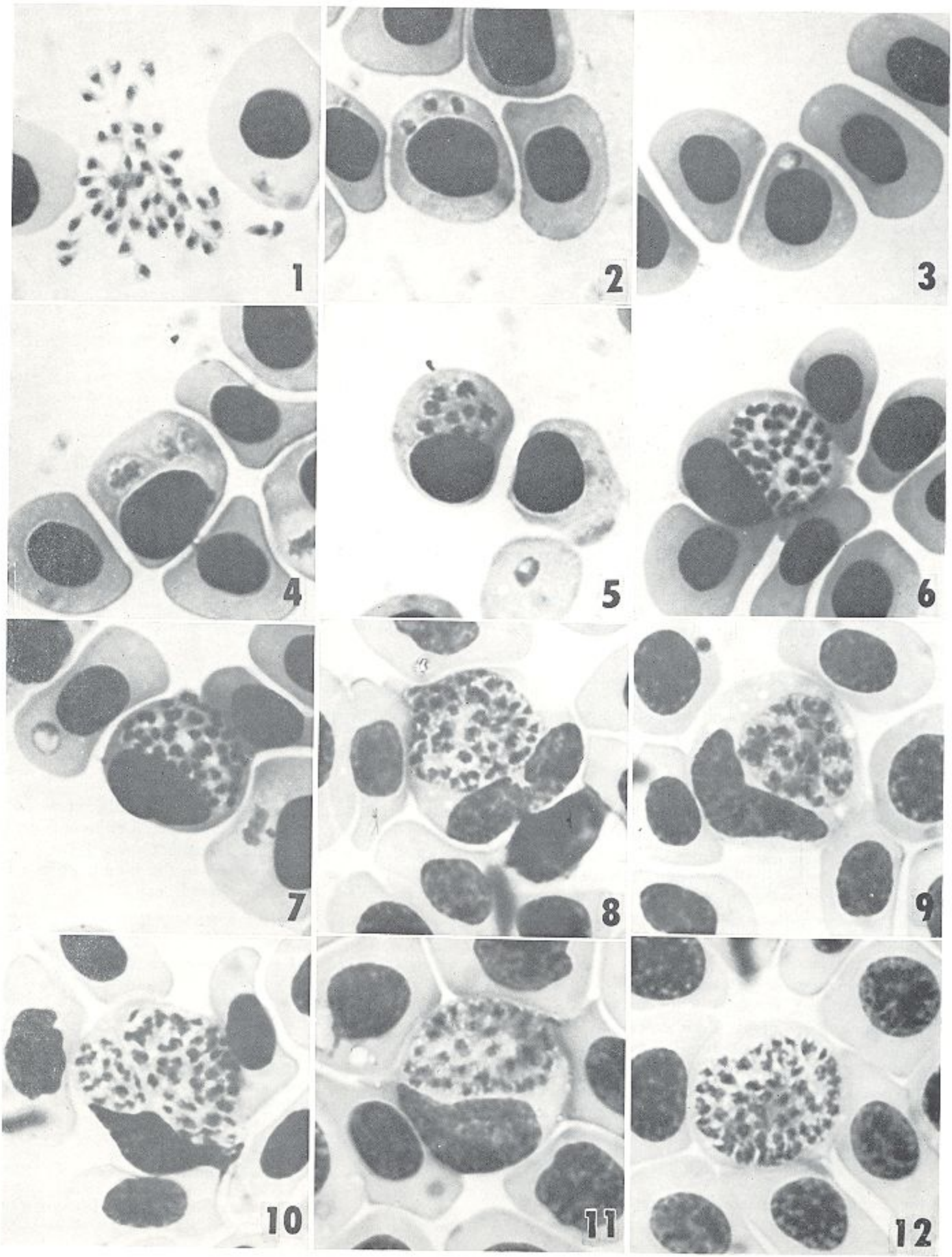
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TABLE 1. *Comparison of segmenters from different hosts and localities.*

Host species and localities	N	parasite length μ	parasite width μ	number merozoites	% with pigment	% in erythrocytes	% in distorted cell	% in cell w/distorted nucleus	% in cell w/displaced nucleus	% in enucleated cell
Anolis lionotus										
Achiote										
RR5-7II	21	9-18	7-12	18-44	0	29	95	81	91	10
mean		13.8	10.0	29.0						
LF4LR4	23	12-19	8-13	30-60	0	0	90	85	85	15
mean		15.1	11.5	44.5						
Gaspar Sabana										
RF3LR3	40	10-16	8-13	15-40	0	25	100	100	100	0
mean		13.1	10.7	26.5						
El Aguacate										
RF3RR5	22	8-12	6-9	26-45	0	0	100	95	95	5
mean		9.6	7.4	34.9						
LF3LR2	22	9-17	8-13	29-52	0	9	100	95	95	5
mean		13.0	11.0	41.9						
Species total	128	8-19	6-13	15-60						
mean		12.9	10.1	35.4						
Anolis poecilopus										
Q. Juan Grande										
2205	21	9-15	7-12	22-64	0	25	100	95	100	0
mean		11.8	9.6	37.7						
Frijolito Creek										
RF5LR2	20	12-17	8-15	33-100	0	0	100	100	100	20
mean		14.4	11.1	55.8						
Species total	41	9-17	7-15	22-100						
mean		13.1	10.4	46.8						
TOTAL	169	8-19	6-15	15-100						
Overall mean		13.0	10.3	41.1						

RESULTS

The structural characters of this parasite resemble those of no other New World *Plasmodium* yet described, and I therefore propose that it be named in honor of Professor Gordon H. Ball, whose contributions to the study of the haemosporozoa need no enumeration:

Plasmodium balli sp. nov.

DIAGNOSIS: A species of *Plasmodium* (*Sauramoeba*) which produces segmenters containing up to 100 merozoites; macro- and microgametocytes usually elongate and at least twice the size of nuclei in uninfected erythrocytes, with compact, centrally located nuclei. Trophozoites tend to parasitize normoblasts or proerythrocytes. Pigment often absent, but when present seldom more than a minute blackish brown dot. Parasitized cells sometimes enucleated, with hypertrophy and distortion of cell and nucleus, and host nuclear displacement usually present after the 1st parasite nuclear division; a lytic effect on the host cell nucleus often evident, most pronounced in cells parasitized by gametocytes.

DESCRIPTION: *Trophozoites.* The smallest stages (Fig. 2) have oblong to squarish nuclei 1.5-2 μ in diameter, with light blue cytoplasm ½ or less the size of the nucleus. No vacuole or pigment is present. The host cells are usually proerythrocytes but may be erythrocytes, normoblasts or stem cells. When the trophozoite reaches a length of 3.5 μ (Fig. 3), the cytoplasm is at least twice the size of the nucleus. Trophozoites vary in shape from triangular or drop-shaped to round or almost square. Uninucleate forms as large as 7 by 2.5 μ may be developing gametocytes, since the smallest binucleate schizonts observed were 5 by 3 μ. Uninucleate parasites 5 μ in length have deep blue cytoplasm. The largest trophozoites produce no apparent effect on the host cell.

Schizonts. Binucleate schizonts (Fig. 4) are 5-8 by 3-5 μ, with nuclei 1-2.5 μ in diameter. No clearly defined vacuole is present, but the cytoplasm is often paler around the nuclei. As nuclear division proceeds, schizonts broaden more rapidly than they increase in length: parasites with 5 or 6 nuclei may be only 8 μ long, but 5-7 μ wide. At the same time the host cell nucleus is displaced laterally and the cell itself may become broader than long. A single small dot of black pigment may occasionally be present anywhere in the parasite as early as the tetranucleate stage. All host cells containing parasites past the 1st nuclear division show some tendency towards enlargement, rounding, nuclear hypertrophy and displacement.

Segmenters. Segmenters are 8-19 by 6-15 μ, with a mean size of 12.6 by 9.9 μ, and may vary in shape from round to almost rectangular, but usually are oval (Figs. 6-12).

Figs. 1-12: *Plasmodium balli* asexual stages in *Anolis lionotus*. 1, brood of free merozoites; 2, triple infection of early trophozoites; 3, older trophozoites; 4, double infection of binucleate schizonts; 5, immature schizont; 6-12, segmenters; 10, double segmenter infection. Host cells 2-7, 10 are proerythrocytes; cells 8, 9, 11 probable stem cells; 12, enucleated host cell.

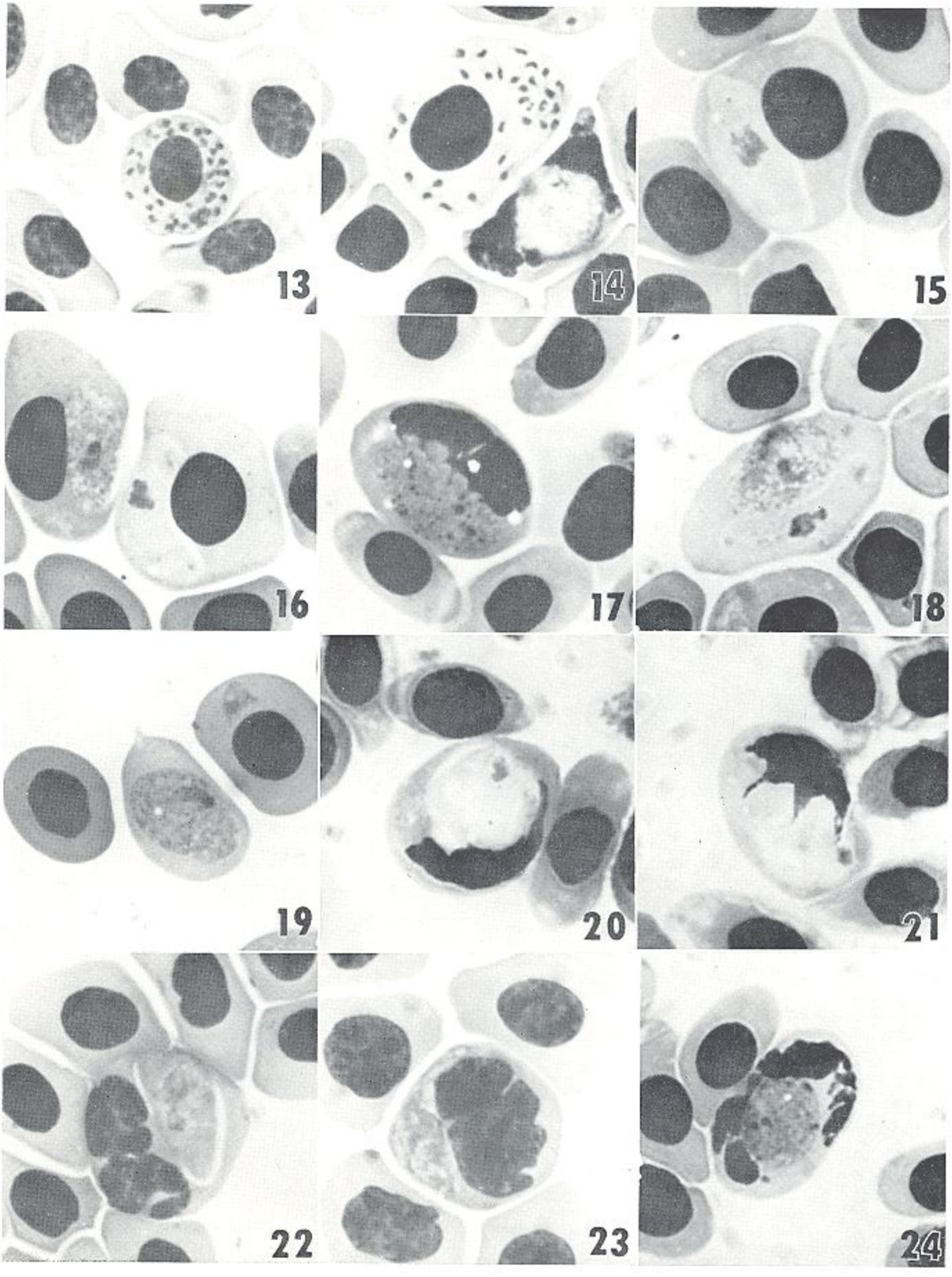
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TABLE 2. *Comparison of gametocytes from different hosts and localities.*

Host species and localities	N	parasite length μ	parasite width μ	mean width /length ratio	% with pigment	% in erythro-cytes	% in distorted cell	% in cell w/distorted nucleus	% in cell w/displaced nucleus	% in enucleated cell
Anolis lionotus										
Achiote										
RR5-7II	20	10-17	5-10	0.50	0	20	100	95	95	5
mean		14.7	7.3							
LF4LR4	21	10-18	4-11	0.54	0	0	85	95	100	5
mean		12.9	7.0							
Gaspar Sabana										
RF3LR3	20	10-17	5-9	0.54	0	0	100	100	100	0
mean		13.6	7.3							
El Aguacate										
LF2RR4	30	9-23	5-7	0.34	30	67	100	90	43	0
mean		17.4	5.9							
Species Total	91	9-23	4-11	0.34-0.54						
mean		14.7	6.9	0.48						
Anolis poecilopus										
Q. Juan Grande										
2205	100	8-18	6-14	0.75	23	98	95	95	100	0
mean		12.8	9.7							
Frijolito Creek										
RF5LR2	58	11-19	4-12	0.44	17	22	100	100	100	12
mean		16.1	7.1							
Species Total	158	8-19	4-14	0.44-0.75						
mean		14.5	8.4	0.58						
TOTAL	249	8-23	4-14	0.34-0.75						
Overall mean		14.6	7.7	0.53						

Merozoites number 13-100, with an overall mean of 41.1, and are usually uniformly distributed thruout the cell. When present, the blackish brown pigment may be a minute dot or a mass larger than a single merozoite. Merozoite nuclei are 1-1.5 μ in diameter, and merozoites may sometimes remain within the intact host cell after rupture of the segmenter (Figs. 13, 14). Free merozoites are usually drop-shaped (Fig. 1).

Gametocytes. Macro- and microgametocytes may vary from oval (Fig. 17) to elongate banana-shape (Fig. 16), and are usually somewhat flattened on the side adjacent to the host cell nucleus. Macrogametocytes stain deep blue and have a granular cytoplasm, while microgametocytes have a pale, non-granular cytoplasm. The nucleus is compact in both types, located centrally, and stains red. In macrogametocytes it may be rounded to elongate, while in microgametocytes it often appears bilobate (Fig. 15). In the latter, the nucleus is often situated within a clear area in the cytoplasm. Gametocytes are 9-23 by 4-14 μ, with mean dimensions of 14.5 by 7.3 μ. Macro- and microgametocytes are similar in size and proportions. Gametocytes may occur in either proerythrocytes or erythrocytes, with presence of pigment apparently correlated with parasitization of erythrocytes. When present, pigment consists of one to several minute black dots. The position within the host cell is usually lateral, with a tendency to curve slightly around the host cell nucleus (Fig. 16).

TYPE HOST: *Anolis lionotus* Cope (Sauria, Iguanidae).

OTHER HOSTS: *Anolis poecilopus* Cope.

TYPE LOCALITY: Three miles southeast of Achiote (Colon Province), Canal Zone, Panama.

GEOGRAPHIC RANGE: Colon and Panama provinces, Republic of Panama, and the Canal Zone.

LOCATION OF TYPES: The series of type slides is retained at present in the collection of the author. A duplicate series is on deposit in the Dept. of Zoology, Univ. of California, Los Angeles.

INTERPOPULATION VARIATION

Variation in segmenter structure. Table 1 gives data on dimensions of segmenters, numbers of merozoites, and effects upon host cells.

Dimensions of segmenters from all populations are generally similar. Mean merozoite numbers from the infections listed in Table 1 were compared statistically; significant differences were present both within and among all populations, which suggests that little taxonomic significance should be attributed to differences in mean merozoite numbers in this species. In all other respects, the parasites were similar.

Effects of segmenters upon host cells. In all infections, similar effects upon host cells were observed. Distortion of

Figs. 13-24: *Plasmodium balli* asexual and sexual stages in *Anolis lionotus* and *A. poecilopus*. 13, 14 free merozoites within host cells; 15, 20, 21 male gametocytes; 14, 17, 19, 24, female gametocytes; 16, male and female gametocytes in adjacent cells; 18, double infection of male and female gametocytes in enucleated cell; 22, 23, immature gametocytes. Host cells 13, 14 (with merozoites), 15, 16, erythrocytes; cells 14 (with gametocyte), 17, 20, 21, 24, presumably proerythrocytes; cells 22, 23, probable normoblasts.

TABLE 3. *Effect of gametocytes upon host cell nuclei.*

Host species and population	no apparent effect %	enlarged & rounded %	lobulated %	lysing %
Anolis lionotus				
Achiote				
RR5 - 7 II	5	0	75	20
LF4LR4	8	12	60	20
Gaspar Sabana				
RF3LR3	0	0	92	8
El Aguacate				
LF2RR4	20	64	16	0
Anolis poecilopus				
Quebrada Juan Grande				
2205	5	0	25	70
Frijolito Creek				
RF5LR2	0	40	53	7

the cell and displacement of the nucleus almost always occurred, whatever the type of cell parasitized.

Types of host cells parasitized by segmenters. In most *Anolis lionotus* and *A. poecilopus*, segmenters occasionally parasitized erythrocytes but were usually in proerythrocytes or normoblasts. In *A. lionotus* LF4LR4, however, 70 percent of the segmenters were in proerythrocytes, and 30 percent in probable stem cells (Figs. 8, 9, 11).

Variation in gametocyte structure. Table 2 presents data on gametocyte structure and effects on host cells.

Gametocytes from all populations were similar in proportions except in *A. poecilopus* 2205 (Figs. 20, 21), in which the width/length ratio of gametocytes was somewhat larger. This is not considered significant, however, since slides from this host were prepared a couple of hours postmortem, and some alteration of gametocyte shape could have occurred. Staining reactions were similar in all infections, with macrogametocytes appearing blue and microgametocytes pale to pinkish.

Effects of gametocytes upon host cells. In all infections host cells were distorted. Host cell nuclei were displaced and distorted in all. Host cell nuclei were lobulated and enlarged (Figs. 17, 20, 22, 23), or apparently lysing (Figs. 14, 24), often with streams of nuclear material extending over the gametocyte (Fig. 21). Enucleation of host cells was common in heavy infections of *P. balli*, (Figs. 12, 18, 19), and was not limited to cells parasitized by gametocytes. Table 3 presents the effects of gametocytes upon host cell nuclei.

Types of host cells parasitized by immature parasites. The distribution of immature parasites among various types of host cells is presented in Table 4.

Exoerythrocytic stages. No parasites clearly distinct from erythrocytic stages were observed in sections of liver, lung, brain and spleen.

TABLE 4. *Distribution of immature parasites among host cell types.*

Host species and population	Type of host cell parasitized (%) erythrocytes	proerythrocytes	normoblasts
Anolis lionotus			
Achiote			
RR5 - 7 II	2	96	2
LF4LR4	9	55	36
Gaspar Sabana			
RF3LR4	0	100	0
El Aguacate			
LF3LR2	2	66	32
RF3RR5	17	71	12
Anolis poecilopus			
Quebrada Juan Grande			
2205	0	97	3
Frijolito Creek			
RF5LR2	0	100	0

DISCUSSION

Seven species of the subgenus *Sauramoeba* have been described so far from the New World: *Plasmodium diploglossi, P. cnemidophori,* and *P. tropiduri* from Brazilian lizards; *P. floridense* from the southeastern United States and Panama; *P. mexicanum, P. beltrani,* and *P. brumpti* from Mexico. The last 2 species, described by Pelaez and Perez-Reyes (5) were overlooked by Garnham (3) in his monograph on malarial parasites.

P. balli resembles *P. diploglossi, P. cnemidophori,* and *P. beltrani* in having elongate mature gametocytes. *P. brumpti* gametocytes may vary in shape from round to reniform, while gametocytes of *P. tropiduri* and *P. mexicanum* are round. Those of *P. floridense* are round or elongate. Segmenters of the 4 species with elongate gametocytes are also similar in producing larger numbers of merozoites: 48 or more in *P. diploglossi;* over 100 in *P. cnemidophori*: 30-40 or more in *P. beltrani*; and up to 100 in *P. balli.* The other *Sauramoeba* species produce far fewer merozoites: a maximum of 12 in *P. tropiduri*; 10-20 in *P. mexicanum;* 6-21 or more in *P. floridense*; and 15-20 in *P. brumpti.*

P. diploglossi, P. cnemidophori and *P. beltrani* all parasitize erythrocytes, and as a consequence pigment is present and prominent, tho differing somewhat among them in degree of coarseness. *P. balli* differs from them all in usually parasitizing proerythrocytes or normoblasts. When erythrocytes are parasitized, pigment granules are minute and uncommon. All 4 of these species cause hypertrophy of the host cell, and all except *P. diploglossi* cause distortion of the host cell and displacement of its nucleus. *P. diploglossi* differs from the others by encircling the host cell nucleus. *P. beltrani* tends to curve around the nucleus somewhat, while an occasional gametocyte of *P. balli* may curve slightly. Distortion of the host cell nucleus does not occur with *P. cnemidophori* and *P. beltrani,* and only slightly with *P. diploglossi* in which the nuclei may become rounded. *P. balli* distorts considerably and even lyses nuclei of immature blood cells, and causes at least a rounding of erythrocyte nuclei. *P. balli* is most similar, perhaps, to *P. cnemidophori* but is easily distinguished by its virtual absence of pigment, parasitization of immature blood cells, distortion of the host cell nucleus even to the extreme of destroying it, and lack of the "fine, digitiform pseudopodia" characteristic of the young asexual stages of *P. cnemidophori,* according to Garnham (3). Only 3 of the Old World species of *Sauramoeba* produce numbers of merozoites comparable to *P. balli*: *P. giganteum* of Africa,

24-96 merozoites (3), and Australia, 22-49 (4); *P. robinsoni* of Madagascar, 50-70 (2); and *P. egerniae* of Australia, 40-50 or more (4). These also have elongate gametocytes. All have prominent pigment granules, in contrast to *P. balli*, and all produce some hypertrophy and distortion of the host cell and displacement of its nucleus. Only *P. egerniae* produces definite distortion of the host cell nucleus, and this is far less in degree than that characteristic of *P. balli* in immature red cells. *P. egerniae* and *P. robinsoni* parasitize erythrocytes; *P. giganteum*, according to Bray (1) never parasitizes mature erythrocytes, but produces prominent pigment granules. The African *P. giganteum* is similar to *P. balli* in having a wide range of merozoite numbers.

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