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Reprinted from THE JOURNAL OF PROTOZOOLOGY,
J. PROTOZOOL., 17(3), 400–405 (1970).
Made in United States of America

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Plasmodium chiricahuae sp. nov. from Arizona Lizards

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SYNOPSIS. *Plasmodium chiricahuae* sp. nov. from the Arizona lizards *Sceloporus jarrovi* and *S. clarki* is similar to *P. mexicanum* Thompson and Huff, but differs by having larger gametocytes and smaller erythrocytic schizonts (4-8 merozoites). It produces low-level parasitemia with early appearance of gametocytes. Gametocytes may persist over one year following cessation of erythrocytic schizogonic activity. *P. chiricahuae* varies in prevalence at different altitudes, being most common between 6000 and 8000 feet. Infected hosts were collected at a maximum of 9200 feet.

SAURIAN malaria parasites have been reported twice from lizards of western United States. Wood and Wood (10) mentioned the presence of plasmodia in *Sceloporus occidentalis* and *S. graciosus* in the Berkeley, California area, but did not describe the parasites in detail. Jordan (2) identified malaria parasites in *S. occidentalis* from several California counties as *P. mexicanum*. These constitute the only published records of *Plasmodium* from lizards in western North America north of San Luis Potosí, Mexico.

In 1966, Richard C. Goris of Ikuei Technical College, Tokyo, gave me 4 *Sceloporus jarrovi* purportedly from southern Arizona. One lizard had a few *Plasmodium* gametocytes and some asexual stages. The parasitemia remained very low for 2 months. In view of the inexact locality data and low parasitemia, I was reluctant to report this finding until more material became available. The gametocytes, however, were similar to those described for *P. mexicanum* by Pelaez, Perez-Reyes and Barrera (5), but seemed larger. In September 1968, Professor C. H. Lowe of the Univ. of Arizona generously gave me 9 *Sceloporus jarrovi* from the Chiricahua Mountains of southern Arizona; all were infected with apparently the same parasite. Five of the lizards had active infections in various stages and 4 had only light gametocytemias. Two of the infections were sufficiently heavy to permit thoro study of the parasite.

In early 1969, Dr. T. A. Burns of Northwestern State College of Louisiana sent me a large series of slides from *S. jarrovi* collected at different altitudes in the Pinaleño Mountains of Arizona; many of these contained the same parasite. Recently, Mr. Stephen C. Ayala of the Univ. of California, Berkeley sent me slides of *Sceloporus clarki* and *S. jarrovi* collected in Carr Canyon, Cochise County, Arizona which contained this *Plasmodium*.

MATERIALS AND METHODS

Infected lizards were maintained in the laboratory on a diet of *Tenebrio* larvae, mosquitoes and occasional grasshoppers. Room temperature was maintained at approximately 70 F. A 150-watt light bulb was placed in the cage as a heat source, and was operated usually from 0730 to 1630 hours daily.

Blood smears were made usually at 5-day intervals from clipped toes. Smears were fixed in absolute methanol and stained with Giemsa. The slides from the Pinaleño Mountains were prepared with Wright's stain. Slides were examined at 600 ×; measurements were made with an ocular micrometer at 1000 ×. All measurements are expressed in micra. Photomicrographs were taken at 1000 ×.

RESULTS

This *Plasmodium* is possibly related to *P. mexicanum*, but differs from it in gametocyte size, merozoite number and, apparently, in the course of infection. Altho study of more comparative material in the future may justify use of the trinomial to express its relationship to *P. mexicanum*, I think it is appropriate at present to consider this parasite as a distinct species:

Plasmodium chiricahuae sp. nov.

DIAGNOSIS: A saurian malaria parasite characterized by large oval to round gametocytes, and small schizonts which produce 4-8 merozoites in erythrocytes. It gives rise to a low-level parasitemia in its natural host, with early production and long persistence of gametocytes. Gametocytes are usually lateral and cause great distortion and hypertrophy of the host cell and its nucleus, which is always displaced. Asexual stages have little effect upon host cells. Infections are predominantly in mature erythrocytes. Exoerythrocytic stages are found usually in thrombocytes, but may occur in eosinophils and other leucocytes.

DESCRIPTION: *Trophozoites*. The smallest stages observed were 2 by 1.5 and consisted only of a red chromatin mass with a minute edge of pale cytoplasm. As the young parasite grows the cytoplasm enlarges to about twice the size of the nucleus (Fig. 1). Large trophozoites 2.5 by 2, occasionally form vacuoles. A small black pigment granule appears in large trophozoites.

Schizonts. Binucleate schizonts are from 2.5 by 2 to 5 by 3 and are usually oval or round. The cytoplasm may be light blue or paler. As nuclear division continues,

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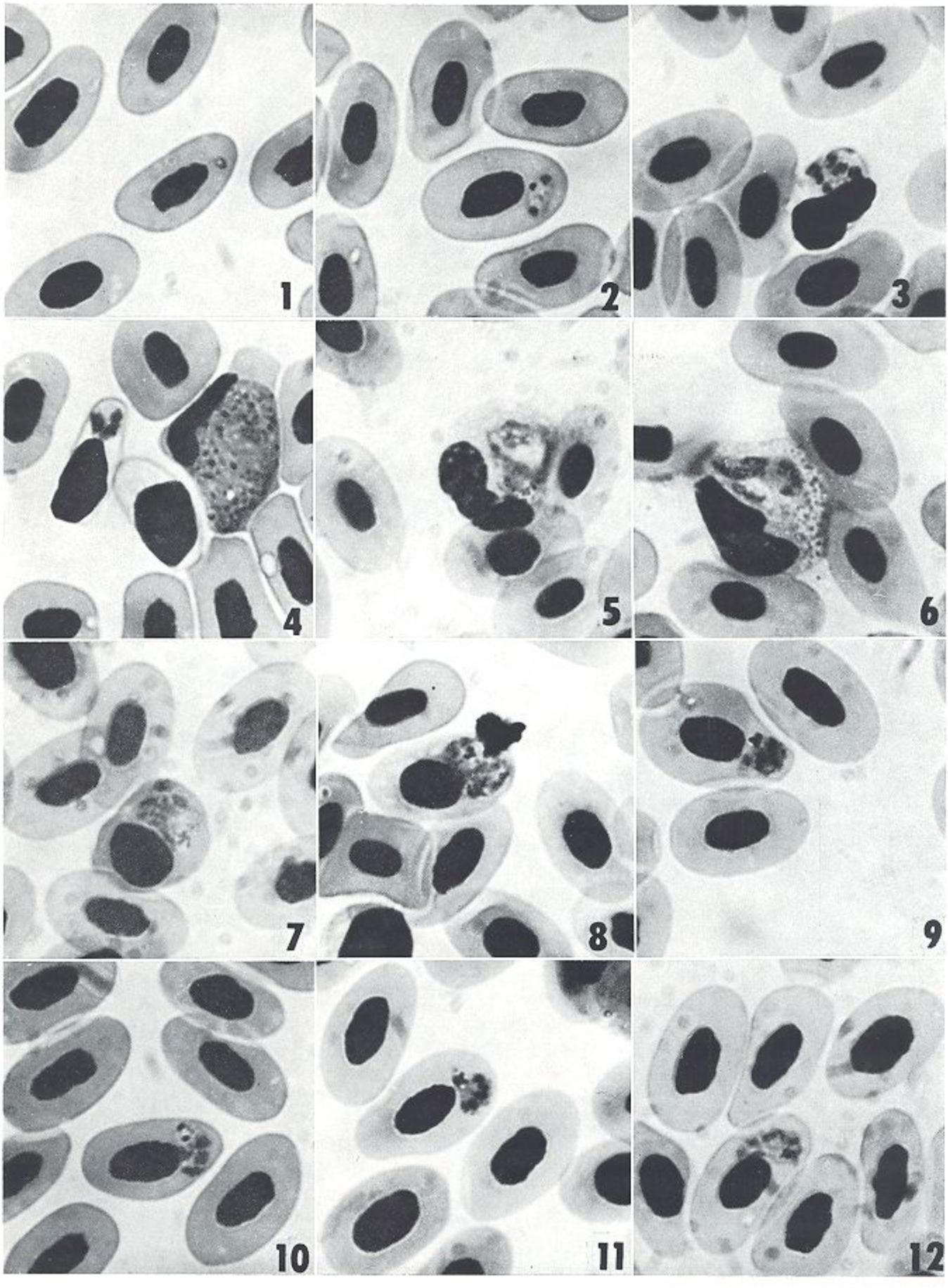
All photomicrographs taken at × 1000.

Fig. 1. Trophozoite.
Fig. 2. Immature schizont.
Figs. 3, 4. Schizonts in thrombocytes.
Figs. 5, 6. Schizonts in leucocytes.
Figs. 7, 8. Schizonts in proerythrocytes.
Figs. 9-12. Mature schizonts.
Host cells erythrocytes except where otherwise noted.
Host—*Sceloporus jarrovi*.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nuclei become arranged peripherally (Fig. 2), with the pigment usually in one or 2 masses in the center of the parasite. Mature erythrocytic schizonts (Figs. 9-14) are 4-8 by 3-6, mean 5.3 by 3.6, and may vary in form from a crude rosette to fan-shaped (Fig. 11), with round to elongate merozoites. Merozoites in erythrocytes number 4-8 with a mean of 5.8 (N=56). Up to 14 merozoites may be present when proerythrocytes are parasitized (Figs. 7, 8). Schizonts tend to lie close to the host cell nucleus, often in contact with it when in a lateral position (15%) or polar-lateral position (62%) within the cell. Schizonts in polar position (23%) occasionally touch the host cell nucleus.

Exoerythrocytic Schizonts. Most of the exoerythrocytic schizonts found were in thrombocytes (Figs. 3, 4), in which there were 4-6 nuclei. One apparently mature schizont in a leucocyte contained 10 nuclei (Fig. 5), and another, 18 nuclei (Fig. 6). No parasites have been found in fixed cells of liver, lung, spleen, brain or bone marrow in several lizards which died during the acute phase of infection.

Gametocytes. The smallest parasites distinguishable as gametocytes are approximately 4-6 in diameter, and are usually round to oval. As they grow, they elongate (Figs. 17, 18) and may become spindle-shaped, with one or both ends drawn out into a point. The smallest spindle-shaped gametocyte seen was 6 by 3. Male and female gametocytes may be distinguished at this size, the latter staining deep blue. Newly mature gametocytes (Figs. 15, 16) are elongate with blunt or slightly pointed ends. The nucleus in female gametocytes often stains as a pink band at midbody, with a prominent round, red nucleolus (Fig. 18) which may be 2 in diameter. Newly mature male gametocytes stain pink or are unstained. Their nuclei, when visible as an entity, are rather irregular. Nucleoli are rarely seen. The pigment in immature gametocytes is golden brown, with the granules clumped into one or 2 masses which often lie at the ends of the parasite.

Mature macrogametocytes (Figs. 19-22) are 14-19 by 8-14, and average 16.6 by 10.5 (N=44). Mature microgametocytes (Figs. 23, 24) are 11-17 by 7-12, and average 14.9 by 9.0 (N=31). Macrogametocytes are round to broadly oval in shape, while microgametocytes are usually oval but occasionally round. The pigment is dark brown to black and characteristically forms 20-50 or more minute, bacilliform granules scattered over the gametocyte surface. Occasionally some of the granules clump together to form larger masses. Mature macrogametocytes stain deep blue with an irregularly round pink area where the nucleus lies. There are often one or 2 small vacuoles in the cytoplasm. Microgametocytes are unstained, sometimes with pale pinkish vermiculations on the surface. The nucleus is indicated by a broad pinkish area, more prominent in younger gametocytes. Round nucleoli are often prominent in female but are less commonly seen in male gametocytes.

Types of Host Cells Parasitized. In one active infection studied, 97% of the immature parasites were found in erythrocytes and 3% in proerythrocytes. In another lizard, immature parasites were distributed among several cell types: erythrocytes (57%), proerythrocytes (17%), thrombocytes (13%), leucocytes (7%), normoblasts (3%), and eosinophils (3%). Segmenters were found in erythrocytes (96%) and proerythrocytes (4%), while mature gametocytes in 5 samples usually parasitized erythrocytes.

Effects Upon Host Cells. Immature asexual parasites have no discernible effect upon host cells. Schizonts displaced host cell nuclei in 12% of those studied, but caused neither hypertrophy nor distortion of the host cell and its nucleus. Gametocytes caused distortion of the host cell (Figs. 15-23) and its nucleus, and displaced the latter. Host cells were hypertrophied laterally and their nuclei usually became elongated and flattened. Occasional host cell nuclei were rounded.

TYPE HOST: *Sceloporus jarrovi* Cope (Sauria, Iguanidae).

OTHER HOSTS: *Sceloporus clarki* Baird and Girard.

TYPE LOCALITY: Arizona, Cochise County, Chiricahua Mountains, 1.8 miles above Onion Saddle, elevation 7,700 feet.

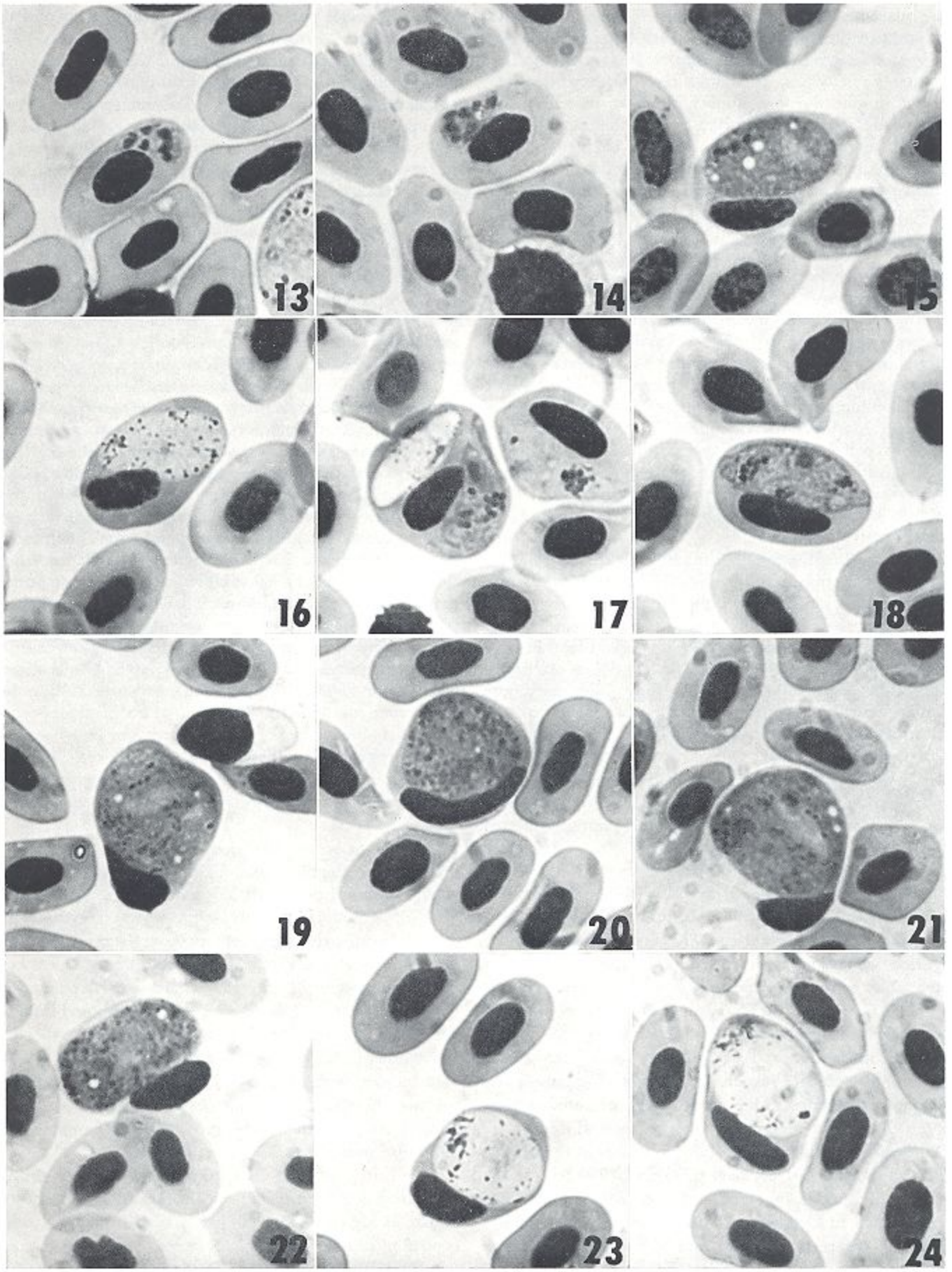
LOCATION OF TYPE MATERIAL: Type slides retained at present in author's collection. Paratype slides are deposited in the Dept. of Zoology, Univ. of California, Los Angeles, and with Professor P. C. C. Garnham, Imperial College Field Station, Silwood Park, England.

GEOGRAPHIC RANGE: At present, Cochise, Graham and Pima counties, Arizona.

DISCUSSION

Plasmodium chiricahuae is most similar to *P. mexicanum* as described by Pelaez et al. (5) from the Mexican lizards *Sceloporus ferrariperezi* and *S. microlepidotus*. It can be distinguished from all other saurian malaria parasites, however, by the combination of small schizonts and large gametocytes. The gametocytes, in fact, average larger than those reported for any saurian *Plasmodium*, with the possible exception of *P. egerniae* described by Mackerras (4) from an Australian skink. *P. egerniae*, however, has more slender gametocytes and causes greater distortion of the host cell nuclei. If *P. chiricahuae* were not apparently related to *P. mexicanum*, it would have

Figs. 13, 14. Mature schizonts.
Figs. 15, 16. Gametocytes from *Sceloporus clarki*:
Fig. 15. ♀ Gametocyte.
Fig. 16. ♂ Gametocyte.
Fig. 17. Double infection of haemogregarine and immature gametocyte.
Fig. 18. ♀ Gametocyte nearing maturity.
Figs. 19-22. ♀ Gametocytes.
Figs. 23, 24. ♂ Gametocytes.
Host cells erythrocytes.
Host *S. jarrovi* except 15 and 16.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to be placed in Garnham's subgenus *Carinamoeba* which contains parasites producing less than 12 merozoites (1). In view of its possible relationship to *P. mexicanum,* and its huge gametocytes, it should be considered a *Sauramoeba,* despite the low number of nuclei in schizonts.

Merozoite number in saurian malaria parasites can be unreliable as sole criterion for specific distinction, since Thompson and Huff (9) found merozoites to differ in number among different species of experimental hosts of *P. mexicanum.* Saurian malaria parasites within single host species are also more highly variable in this taxonomic character than are avian and mammalian parasites (1, 8). However, since there is no overlap in range of merozoite numbers between *P. chiricahuae* (4-8) and *P. mexicanum* (10-20), and since the mean number of merozoites in the latter exceeds the mean in *P. chiricahuae* by a factor of 2-4, I think it is realistic to consider the Arizona parasite a distinct species. The fact that it parasitizes a host which can be considered a relict (*S. jarrovi* is a component of the Sierra Madrean herpetofauna, 7), may further support its specific identity. There is little probability of mixed infections accounting for the odd combination of large gametocytes and low number of merozoites. Only one type of gametocyte has been observed thruout the course of active infections.

Altitudinal Distribution. Examination of slides from 149 *S. jarrovi* collected at different altitudes in the Pinaleño and Chiricahua Mountains indicates that *P. chiricahuae* varies altitudinally in prevalence: it was present in 54% of 57 lizards collected between 6000 and 7900 feet, in 26% of 35 lizards from 8000-8900 feet, and in 6% of 36 lizards collected between 9000 and 9900 feet. The maximum elevation at which an infected lizard was found was 9200 feet. Samples of 22 lizards from 9400 feet and 21 from 10,700 feet were negative.

An interesting contrast is provided by the analysis of hemogregarine infection rates at the same altitudes. Hemogregarines were present in 42% of the sample collected at 6000-7900 feet, 17% from 8000-8900 feet, 28% from 9000-9900 feet, and 48% in lizards from 10,700 feet. It appears that *P. chiricahuae* is transmitted by a vector with an altitudinally zoned distribution, presumably with maximum density between 6000 and 7900 feet, while the hemogregarine which parasitizes *S. jarrovi* utilizes a different vector which is not restricted altitudinally, or has multiple vectors. *S. jarrovi* is heavily parasitized by blood-ingesting red mites of the genus *Geckobia.* Examination of several smears of mites removed at intervals from lizards with high gametocytemias revealed no evidence of exflagellation or zygote formation, altho gametocytes persisted apparently unchanged in the guts of mites for several days.

Seasonality. Comparison of infection rates in the sample of 32 lizards collected between 6000 and 7900 feet in the Pinaleño Mountains in June, and the 16 from that altitude collected in October and November, revealed no significant difference in infection rate between June and late fall. There was also no significant difference in the prevalence of asexual parasites in the June infections and those from the fall.

Relationship of Infection to Host Size. Sceloporus jarrovi is ovoviviparous, giving birth in May and June to young which average about 24 mm snout-vent length (7). The smallest specimens examined were a 35 mm lizard collected 29 October and a 43 mm specimen in June. Size distribution data of the Pinaleño Mountains sample imply that lizards one year old in June are usually between 43 and 70 mm snout-vent length. The smallest lizard found infected was the 43 mm June specimen, which had a one-plus infection, apparently solely of gametocytes, thus suggesting that the infection was acquired within its first year. All active infections (*i.e.,* those showing asexual stages) were in lizards 61-78 mm long (mean 68 mm), while old infections (*i.e.,* gametocytes only) were, with one exception, in lizards of 62-96 mm long (mean 78 mm) snout-vent. These data suggest that *Plasmodium* infections can be found in lizards less than one year old, but that infection usually occurs in the 2nd year.

Parasitemia and Course of Infection. Altho studies on the course of infection are not completed, it appears from naturally infected lizards that *P. chiricahuae* gives rise to a low-grade parasitemia in *S. jarrovi,* with early production and long persistence of gametocytes. The maximum parasitemia observed so far in 43 infections studied, was 219 parasites per 10,000 erythrocytes; this peak occurred in a naturally infected lizard followed for 75 days in the laboratory. When observation began, the parasitemia was 1%, and mature gametocytes comprised 4% of the parasite population. Immature gametocytes, however, accounted for 75% of the parasites. At peak, 20 days later, 57% were mature and 20% immature gametocytes, and 10 days following peak, these percentages had become 84 and 9, respectively. Three days before death (day 75), parasitemia was 169 per 10,000 erythrocytes, and mature and immature gametocytes comprised 98% and 2%, respectively.

In another active infection, followed for 75 days, the level of parasitemia remained much lower, never rising above 36 parasites per 10,000 erythrocytes, nor showing a peak of infection. Mature gametocytes comprised 17% of the parasitemia when observations began, and immature gametocytes were 22%. Ten days later, the respective percentages were 72 and 15. There was a resurgence of trophozoites after 10 more days, and mature and immature gametocyte percentages became 42 and 25, respectively. In another 5 days, 80% of the parasites were mature gametocytes, with no immatures present. During the succeeding 50 days, the gametocytemia remained between 75 and 100%, with no evidence of further production of immature gametocytes.

The course of infection appears to be very different from that described for *P. mexicanum* in natural hosts by Pelaez et al. (5). They found a high initial parasitemia consisting entirely of asexual stages. Young gametocytes appeared after several days, but these matured very slowly,

so that 23 days after first seeing young gametocytes, they had reached only ⅔ the size of mature gametocytes. *P. chiricahuae,* however, seems to produce gametocytes almost simultaneously with the appearance of asexual stages. These quickly reach the length of mature gametocytes, but remain more slender for awhile. A significant increase in the width dimension was also noted in samples measured 2-3 months apart. The level of parasitemia attained also differs from that of *P. mexicanum*: a maximum 219 parasites per 10,000 erythrocytes in contrast to the common occurrence of parasitemias exceeding 1,000 per 10,000 blood cells in *P. mexicanum* in its natural hosts.

Gametocytes apparently persist for long periods following cessation of erythrocytic schozogonic activity. One lizard, in which parasitemia was never observed to exceed 12 per 10,000 erythrocytes, has been followed for 495 days. Most of the parasites seen on the initial slide were asexual stages, but thereafter all other parasites observed were mature gametocytes except on days 15 (when one immature gametocyte was present among 5,000 erythrocytes) and 60 (when 2 young schizonts and one segmenter were seen among 5,000 erythrocytes). A 2nd lizard which also never showed a parasitemia greater than 12 per 10,000 erythrocytes, was followed for 369 days. On the initial slide it had only gametocytes; on day 30, a single trophozoite was seen among 5,000 erythrocytes. Only mature gametocytes were observed thereafter, until the final slide on day 369. Either parasitized host cells are very long-lived, or, more likely, some exoerythrocytic schizogony had continued during this long period of observation. Killick-Kendrick and Warren (3) reported gametogony directly from primary exoerythrocytic schizonts of *Plasmodium berghei yoeli,* and this is possibly the mechanism responsible for long persistence of gametocytes following disappearance of asexual stages in this species of saurian malaria parasite.

I wish to express my thanks to Richard C. Goris, C. H. Lowe, R. L. Bezy, T. A. Burns, M. J. Fouquette, Jr., J. A. Delahoussye, and Stephen C. Ayala for their generous contributions of specimens or slides which made this study possible. Professors Gordon H. Ball and P. C. C. Garnham reviewed the manuscript and offered useful suggestions. Partial support was furnished by NIH Grant AI-01251 to Gorgas Memorial Laboratory. My wife, Michiko M. Telford, has been most patient during my preoccupation with this study, and I give her my thanks.

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