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STUDIES ON AFRICAN SAURIAN MALARIAS: *PLASMODIUM* PARASITES OF CORDYLID LIZARDS

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SUMMARY. The saurian malarial parasite *Plasmodium zonuriae* Pienaar, 1962 is redescribed from the type host *Cordylus vittifer* (Reichenow), collected near the type locality. It is characterized by polymorphic schizonts, larger than host cell nuclei, which produce 12-28 merozoites, usually arranged around the schizont margin. Gametocytes, most often elongate, are larger than host cell nuclei, which are often partially encircled by the parasite. Infected erythrocytes are enlarged and may show nuclear hypertrophy. In a second host, *Pseudocordylus microlepidotus melanotus* (Smith), schizonts and gametocytes of *P. zonuriae* are smaller but closely resemble those from the type host. Merozoite range and mean numbers are virtually identical. Another malarial parasite of cordylid lizards, *Plasmodium cordyli* n. sp., is described from Tanzanian *Cordylus cordylus tropidosternum* (Cope). Gametocytes are typically round or oval and average smaller than host cell nuclei. The variably shaped schizonts are also smaller than host cell nuclei and produce 5-11 merozoites in the type host. Asexual parasites and young gametocytes are commonly nucleophilic. Cells host to gametocytes, and their nuclei, may be enlarged; this effect was not observed when erythrocytes were parasitized by schizonts. A small *Plasmodium* parasite found in *Cordylus vittifer* from Transvaal is probably conspecific with *P. cordyli*. Gametocytes are virtually identical morphometrically and in appearance, but schizonts are larger, about the size of host cell nuclei, and produce 8-14 merozoites.

Key-words: Systematic. *Plasmodium zonuriae*. *P. cordyli*. Lizards.

Études sur le paludisme des sauriens africains : les plasmodiums parasites de lézards Cordylidés.

RÉSUMÉ. Le parasite de Saurien *Plasmodium zonuriae* est redécrit chez l'hôte type *Cordylus vittifer* collecté près de la localité type et chez un second hôte, *Pseudocordylus microlepidotus melanotus*.

Un deuxième parasite de Cordylidés, *Plasmodium cordyli* n. sp. est décrit chez *Cordylus cordylus tropidosternum* de Tanzanie.

Un plasmodium trouvé chez *Cordylus vittifer* du Transvaal est rattaché à *P. cordyli*.

Mots-clés : Systématique. *Plasmodium zonuriae*. *P. cordyli*. Lézards.

The endemic African saurian family, Cordylidae, is distributed primarily in the southern third of the continent, with a few species occurring in East Africa. One haemosporidian parasite, *Plasmodium zonuriae* Pienaar, 1962 is known from the

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family, described from *Cordylus vittifer* (Reichenow) at Elandsfontein near Fochville, between Johannesburg and Potschefstroom in the Transvaal. In 1972 I obtained two cordylids identified as *Pseudocordylus subviridis*, purportedly from Southwest Africa, which were infected by a *Plasmodium* species apparently conspecific with *P. zonuriae*. Examination of the host specimens shows them to be *Pseudocordylus microlepidotus melanotus* (Smith), 1838, known only from the Transvaal, Swaziland and Natal, i. e., southeastern not Southwest Africa. The type host, *Cordylus vittifer*, is sympatric with *P. microlepidotus melanotus*, which supports identification of this parasite as *P. zonuriae*.

In 1984, I collected several *Cordylus cordylus tropidosternum* in Tanzania which had infections of a very different, smaller *Plasmodium*. Professor M. B. Markus and Dr. E. McClain, Department of Zoology, University of the Witwatersrand, Johannesburg, kindly obtained smears from a series of *Cordylus vittifer*, collected from four localities in the southwestern Transvaal, including near the type locality, and sent them to me for study. *Plasmodium zonuriae* was present on most slides, as was a second, smaller parasite which I believe to be conspecific with that found in Tanzania. With the availability of this new material, I redescribe *P. zonuriae* below, and describe the second species found in Tanzania and South Africa.

Materials and methods

The Tanzanian lizards examined were collected from tree trunks, a wooden fence, and within a hollow, dead limb by hand or noose. Thin smears were made from clipped toes, fixed in absolute methanol, and stained by Giemsa at dilution of 1 : 10 and pH 7.0 for one hour. The South African smears were fixed in absolute methanol there; upon receipt, I stained them with May-Grünwald-Giemsa. Slides were screened at 400×, and parasites studied, measured by calibrated ocular micrometer, and photographed at 1,000× under oil immersion. Statistical analysis was done using the Microstat package (Ecosoft, Inc.) for I. B. M. personal com-

FIGS. 1-14. — *Plasmodium zonuriae* from *Cordylus vittifer*.
1, 2: trophozoites.
3-13: schizonts.
14: segmenter.
Host cells 3, 4, 12 are proerythrocytes, remainder erythrocytes.

FIGS. 15-36. — *Plasmodium zonuriae* from *Pseudocordylus microlepidotus*.
15, 16, 18, 19, 22-25: host cells containing trophozoites and schizonts.
17, 20, 21, 26-28: schizonts.
29, 30: segmenters.
22, 31-36: immature gametocytes.
Host cells are erythrocytes. Vertical bars represent 10 μm.


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FIGS. 1-36.

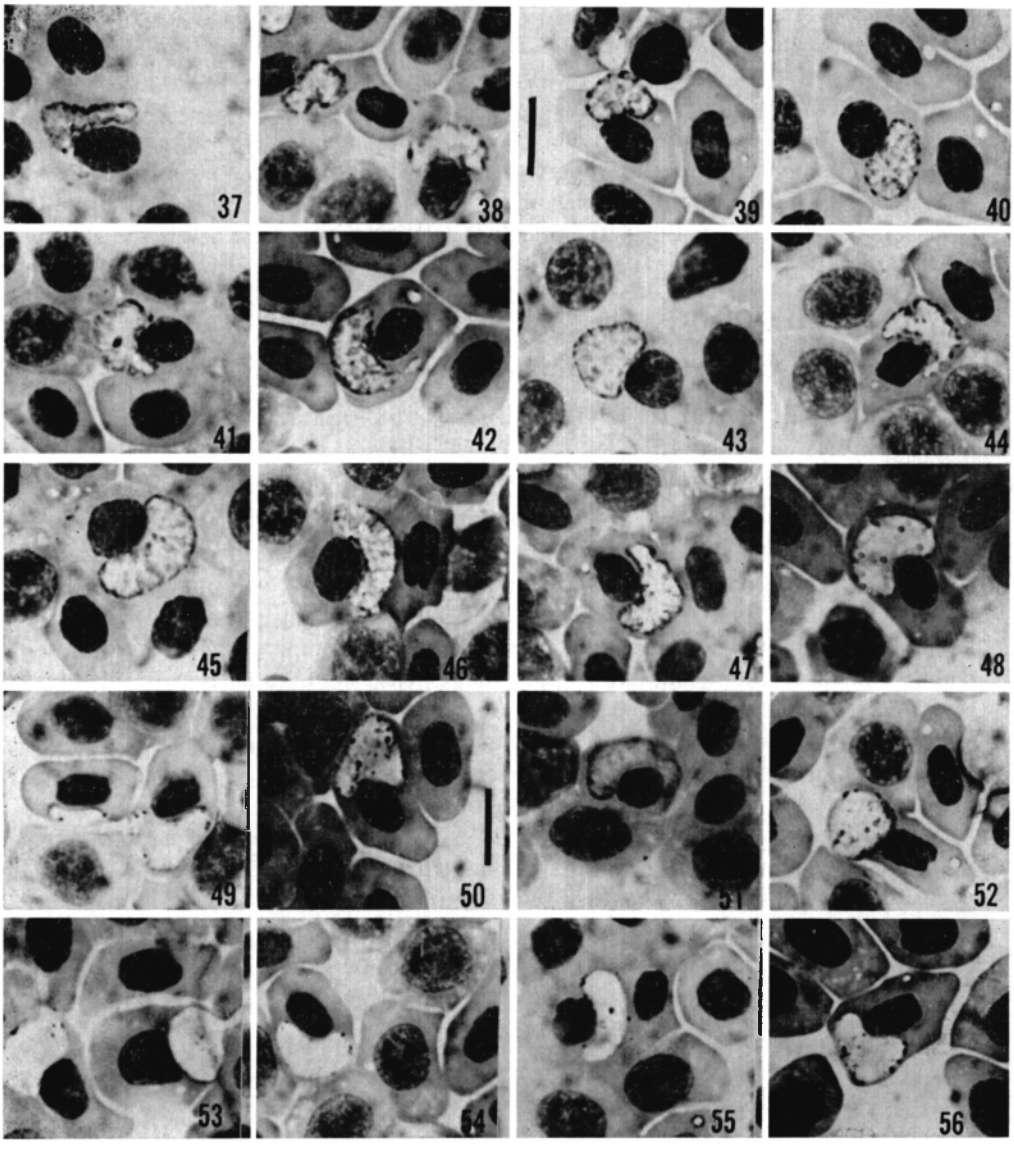
puters. Host lizards were maintained on diets of orthopterans, coleopteran or lepidopteran larvae, and examined at 3-7 day intervals when active infections were present, and at greater intervals during chronic phase. One *Pseudocordylus microlepidotus* was followed from March 1972-May 1973, while two infections in *C. cordylus tropidosternum*, collected in June and August 1984 are still being observed (March 1987).

Results

Taxonomic redescription

Plasmodium zonuriae Pienaar, 1962 *(figs. 1-72).*

Redescription from 4 natural infections of *Cordylus vittifer (figs. 1-14, 37-56)*, 3 of which were topotypic. *Trophozoites:* Smallest 2 × 1 μm, nearly rectangular, with a minute cytoplasmic projection at one end which contained a tiny pigment dot. Larger trophozoites *(figs. 1, 2)* irregular or elongate, becoming oblong and nearly pear-shaped at 4-6 × 2-3 μm, with nucleus usually terminal and no distinct vacuoles. *Schizonts:* Binucleate *(figs. 3-6)* 6-8 × 3-5 μm, still oval, oblong or elongate, occasionally with one end pointed. As nuclear division proceeded *(figs. 7-13)*, shape remained variable. Schizonts commonly 10 × 7 μm without indication of segmentation *(figs. 12, 13)*. Nuclei usually peripheral, with pigment as dark dispersed dots, seldom a discrete mass. Mature schizonts *(figs. 14, 37-47)* 7-17 by 4-9 μm ($\bar{x}$ 10.3 ± 0.2 × 6.6 ± 0.1, N = 102), with LW 67.6 ± 1.6 μm² (36-120). Mean LW varied 56.8-85.5 μm². Merozoites 12-28, x 18.6 ± 0.3 (N = 105); means ranged 17.3-21.2. Schizonts polymorphic: oval, round oblong, elongate, fan-shaped, lentiform, rosette, irregular or curved around host cell nucleus (HCN). *Gametocytes:* Youngest had smooth outlines, early on assuming oval, elongate or lentiform shape, 3 × 2.5 μm, with a few dark pigment dots. Pigment rarely clumped in immature gametocytes, usually as distinct, individual dots, often peripheral but as commonly dispersed. Apparently mature gametocytes *(figs 48-56)* 7-20 × 4-10 μm, $\bar{x}$ 10.9 ± 0.2 × 6.7 ± 0.01 μm (N = 125), with LW 72.2 ± 1.2 μm² (42-114). Mean LW varied 61.6-79.6 μm². Gametocyte shape usually elongate, with L/W 1.0-5.0, $\bar{x}$ 1.70 ± 0.01. Gametocyte LW: HCN LW ratio 1.69 ± 0.05 (0.7-2.8). No sexual differences in morphometric parameters. In 3 of 4 infections, *P. zonuriae* schizonts caused host cell hypertrophy, and in 2 infections, of HCN. Erythrocytes usually distorted, with HCN displaced and distorted. Schizonts most often polar or lateropolar, rarely lateral to HCN. Gametocyte host cells enlarged in all samples, with HCN hypertrophy in one. Erythrocytes usually distorted, with HCN displaced and distorted *(figs. 53, 55)*. Elongate schizonts and gametocytes curved somewhat around HCN as halteridia *(figs. 41, 44, 48, 51)*. Gametocytes usually polar or lateropolar, seldom lateral to HCN. Small parasites variably placed, without obvious effect; later, HCN became displaced as cell hypertrophy increased. Both schizonts and gametocytes occasionally occupied proerythrocytes.



FIGS. 37-56. — *Plasmodium zonuriae* from *Cordylus vittifer*.

37-47: nearly mature schizonts and segmenters.

48-52: macrogametocytes.

53-56: microgametocytes.

Host cells are erythrocytes. Vertical bars represent 10 μm.

DIAGNOSIS: A *Plasmodium* parasite of African cordylid lizards characterized by tpolymorphic schizonts which produce 12-28 merozoites typically arranged around he margins of both immature and mature schizonts. Gametocytes are usually elongate. Both schizonts and gametocytes are larger than the host cell nuclei, which they often partially encircle. Erythrocytes infected by either stage are enlarged and may show nuclear hypertrophy. The dark pigment is usually dispersed as separate granules in both sexual and asexual stages. Host cells are normally erythrocytes, but proerythrocytes and erythroblasts may be parasitized during acute infection.

TYPE LOCALITY: Potchefstroom District, SW Transvaal, South Africa.

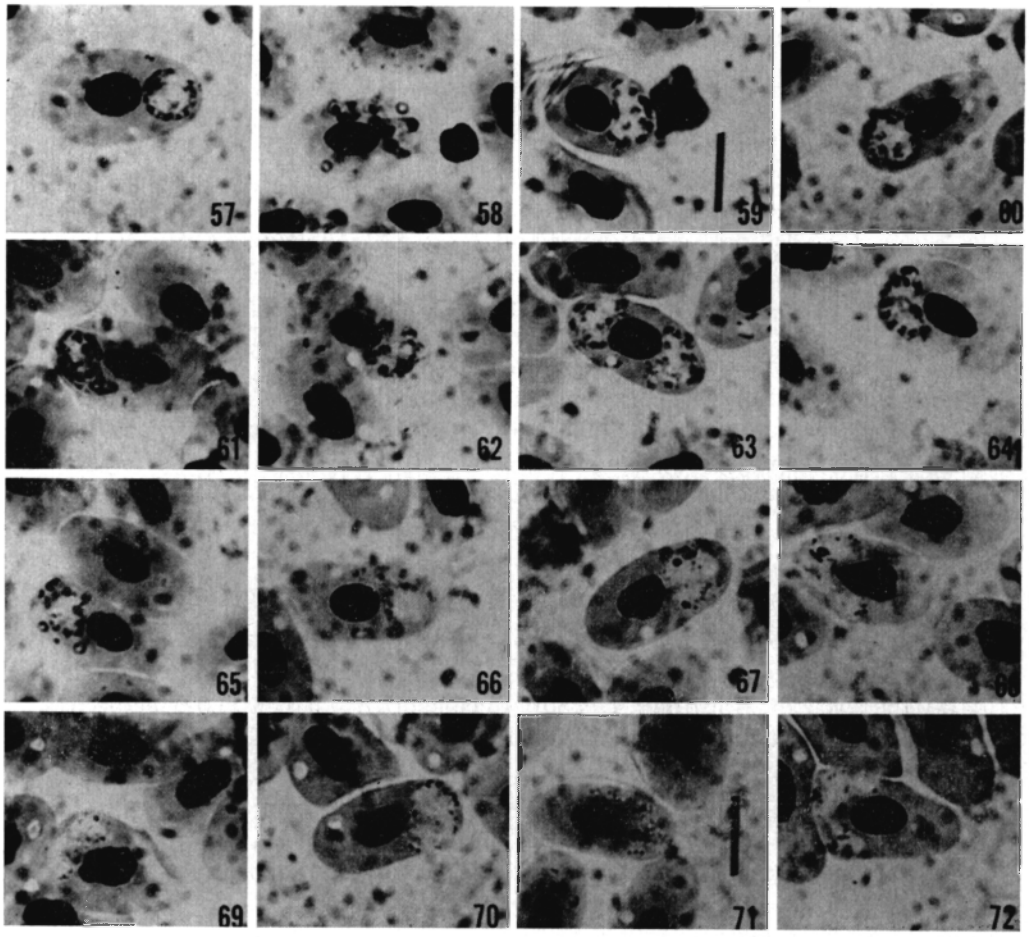
GEOGRAPHICAL DISTRIBUTION: Known only from southwestern Transvaal.

HOST SPECIES: *Cordylus vittifer* (Reichenow), 1887 (Sauria: Cordylidae).

Plasmodium zonuriae from *Pseudocordylus microlepidotus (figs. 15-36, 57-72).*

Trophozoites: Smallest 1 × 0.8-1 μm, largely chromatin, triangular, unpigmented *(figs. 19, 22, 24).* Minute pigment dots at 1.5 × 1 μm, always in larger parasites. At 2-2.5 × 1.5-2 μm, some had small vacuoles. Nuclei marginal, often as thin chromatin bands. Largest 3.5-5.5 × 3-4 μm, oblong or oval *(figs. 15, 16, 23, 25),* with peripheral nuclei, no vacuole, and prominent pigment granules usually clustered along one side opposite nucleus; no cytoplasmic projections. *Schizonts:* Binucleate *(figs. 15-19)* elongate, oblong, usually oval, 5-5.5 × 2-5 μm. Nuclei marginal, usually opposite, often as thin band *(fig. 16).* Pigment granules often clustered, peripheral. Schizonts grew little more *(fig. 20)* until after third nuclear division at 5-8 × 4-5 μm, usually oblong to round *(figs 21-25),* with nuclei marginal. After fourth division *(figs. 26-28, 57-63),* most nuclei remained peripheral, ringing schizonts. At segmentation, schizonts elongated *(figs 29, 30, 64),* often curving around HCN. Mature schizonts *(figs. 29, 30, 64-66)* 7-11 × 4-7 μm ($\bar{x}$ 8.1 ± 0.2 × 6.0 ± 0.2, N = 25), with LW 48.4 ± 1.8 μm² (32-63), and 12-23 merozoites (18.2 ± 0.5). Most formed rosettes, rarely fans.

Gametocytes: Smallest *(fig. 22)* 3.5-4 × 2-3 μm, oval or slightly elongate, with a few scattered dots of black pigment. Nuclei, usually peripheral, visible at 5-6 × 2.5-4.5 μm *(figs. 31-35)* pigment granules clustered in larger gametocytes *(figs. 34-36).* At 7 × 6 μm gametocytes often broadly lentiform, with clumped pigment and slightly off-center nuclei, without differential staining reactions. Mature gametocytes *(figs 67-72)* round, oval, usually elongate, often curving around HCN *(figs. 63, 71, 72),* with dark pigment granules dispersed. Gametocytes 7-18 × 4-8 μm ($\bar{x}$ 8.7 ± 0.4 × 5.9 ± 0.2, N = 50), with LW 55.7 ± 1.8 μm² (35-88) and L/W ratios 1.76 ± 0.12 (1.0-4.5). Gametocyte LW: HCN 1.89 ± 0.01 (1.2-4.4). Microgametocytes more rounded (L/W $\bar{x}$ 1.47 ± 0.11) than macrogametocytes (2.09 ± 0.21, N = 24); no other morphometric differences. Cells with schizonts not enlarged, with little distortion of cell or HCN and only occasional nuclear



FIGS. 57-72. — *Plasmodium zonuriae* from *Pseudocordylus microlepidotus*.

57-63: schizonts.

64-66: segmenters.

67-69: macrogametocytes.

70-72: microgametocytes.

Host cells are erythrocytes. Vertical bars represent 10 μm.

displacement. Most schizonts polar to HCN, occasionally lateropolar or lateral. One of 2 gametocyte samples with significant host cell hypertrophy; HCN not enlarged. Host cells sometimes distorted; HCN displaced but rarely distorted. Gametocytes polar or lateropolar, seldom lateral.

TAXONOMIC DESCRIPTION

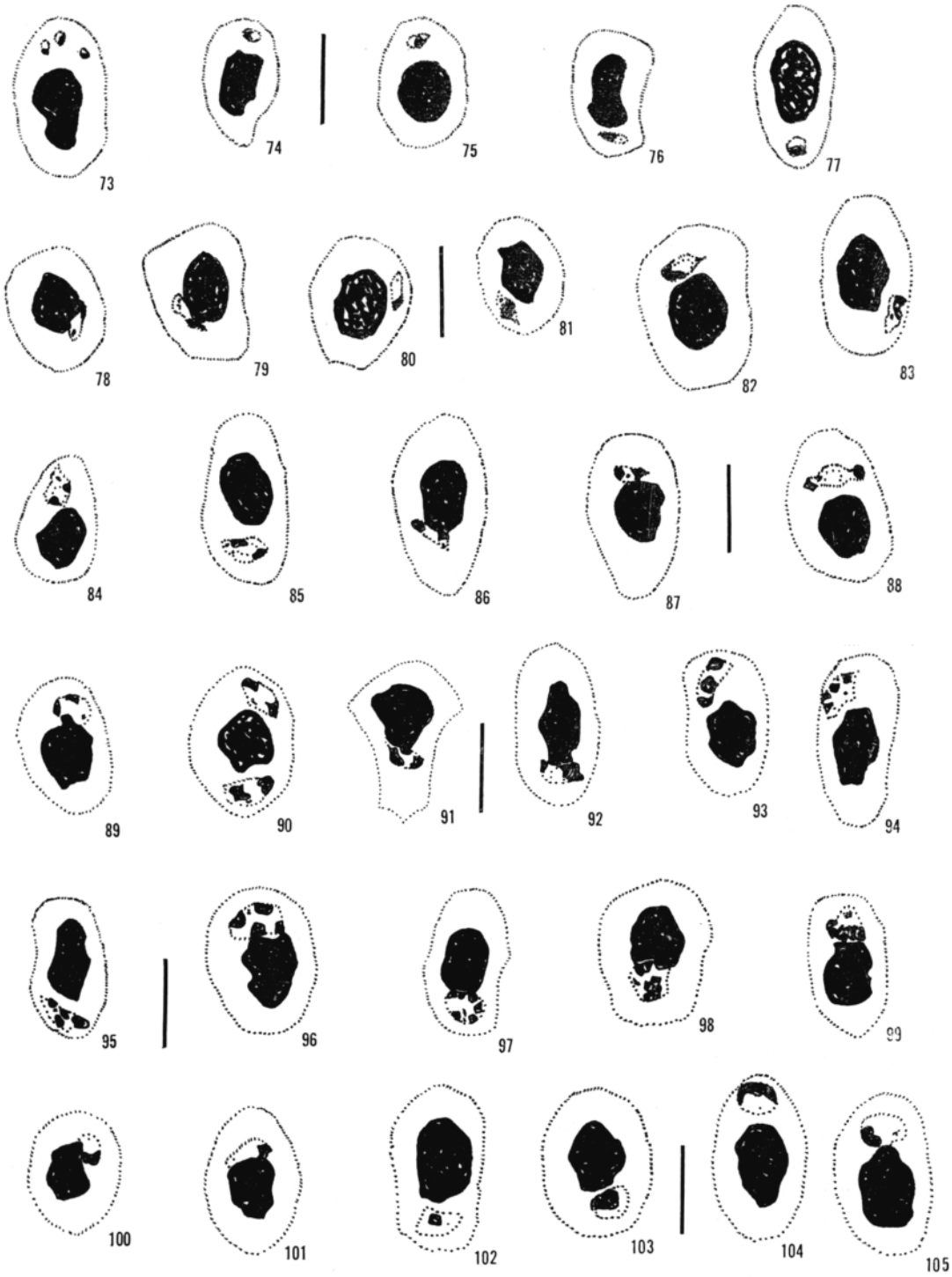
The malarial parasite of *Cordylus cordylus tropidosternum* is designated:

Plasmodium cordyli n. sp. *(figs. 73-117).*

Trophozoites: Smallest *(figs. 73-75)* 1 × 1-1.5 × 1 μm, triangular or tearshaped to elongate; unpigmented. Pigment appeared early on, at 2 × 1.5 μm *(fig. 76)*, but seldom prominent later, as 1-2 minute, grayish to black dots. Trophozoites *(figs. 73-82)* variable, usually elongated or oblong, occasionally tear-shaped. One or both ends sometimes formed a short, pointed pseudopod *(figs. 81, 82)*; occasional, usually central vacuoles *(fig. 82)*. Largest trophozoites 4-5 × 2-2.5 μm. *Schizonts:* Binucleate *(figs. 83-91)* 3.5-7 × 1.5-3 μm, elongate or oblong, with nuclei commonly opposite, and occasional vacuoles *(figs. 84, 85, 88)* or a short, pointed cytoplasmic projection. Tetranucleate *(figs. 91-96)* 5-6 × 2-3 μm, elongate or oblong, some rounded, nearly lentiform *(fig. 96)*. Size increased little after second nuclear division: schizonts with 6-7 nuclei 4-4.5 × 3.5-4 μm, most round *(figs. 97, 98)* before segmentation. Mature schizonts *(figs. 99, 106-111)* 4-7 × 3-6 μm ($\bar{x}$ 5.0 ± 0.1 × 4.1 ± 0.1, N = 88), with LW 20.9 ± 0.6 μm² (12-36). Merozoites 4-11 ($\bar{x}$ 6.6 ± 0.2, N = 90), with means 6.4-6.9. Erythrocytic schizonts smaller ($\bar{x}$ 4.8 ± 0.1 × 3.9 ± 0.1 μm, LW 18.8 ± 0.5 μm², N = 58), with fewer merozoites ($\bar{x}$ 6.1 ± 0.2) than those in immature cells ($\bar{x}$ 5.4 ± 0.8 × 4.6 ± 0.7 μm, LW 25.1 ± 1.0 μm², N = 30), which had 7.7 ± 0.3 (N = 32) merozoites. Schizonts most commonly fans, or round, oval, or oblong, with a few cruciform, rosette, or stellate.

Gametocytes: Apparent young gametocytes *(fig. 100)* were 3 × 2 μm, and clearly distinct at 4-6 × 2-3 μm *(figs. 101-105)*, usually oblong, oval or lentiform, some elongate or more variably shaped. Pigment not prominent, as 1-2 small, dark dots. Mature gametocytes *(figs. 112-117)* 5-8 × 4-7 μm ($\bar{x}$ 6.3 ± 0.1 × 5.5 ± 0.1, N = 50), with LW 35.1 ± 1.1 μm² (20-49), round or oval, with L/W ratio 1.15 ± 0.02 (1.0-1.6). Ratio LW : HCN 0.74 ± 0.02 (0.4-1.2, N = 49). Pigment dispersed in macrogametocytes, usually a single cluster in microgametocytes *(figs. 113, 115-117)*. Gametocytes had no sexual differences in dimensions. Schizonts did not cause enlargement of erythrocytes or HCN, distortion rare, but HCN sometimes displaced. Most schizonts and gametocytes were polar, rarely lateropolar or lateral. Gametocytes caused hypertrophy and distortion of host

FIGS. 73-105. — *Plasmodium cordyli* n. sp. from *Cordylus cordylus tropidosternum.*
73-82: trophozoites.
83-96: schizonts.
97-99: nearly mature schizonts and segmenters.
100-105: immature gametocytes.
Host cells 77, 80, 90 are proerythrocytes, remainder erythrocytes. Vertical bars represent 10 μm.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FIGS. 73-105.

cells and nuclei; HCN commonly displaced. Schizonts often proerythrocytic *(figs. 77, 80, 90, 108-111)*, but gametocytes rarely in immature host cells. Young asexual stages *(figs. 78, 79, 86, 87, 89, 91, 92, 96-98)*, mature schizonts *(figs. 99, 109)* and immature gametocytes *(figs. 100, 101)* showed a strong nucleophilic tendency (29 %).

DIAGNOSIS: A *Plasmodium* parasite of cordylid lizards characterized by round or oval gametocytes which average smaller than host cell nuclei, and by even smaller, variably shaped schizonts which produce 5-11 merozoites. The sparse pigment granules are dispersed in the cytoplasm of macrogametocytes but tend to be clumped in a single focus in microgametocytes. Both asexual parasites and young gametocytes are commonly nucleophilic.

DISPOSITION OF TYPES: Hepantotype slide retained for deposition with the Telford collection. Parahepantotypes slides deposited at the Wellcome Museum of Medical Science, London, the Museum d'Histoire Naturelle, Paris, and the United States National Parasite Collection, Beltsville (No. 79519).

TYPE LOCALITY: Magrotto Mountain, Eastern Usambara Mountains, Tanga Region, Tanzania (5°07'S, 38°46'E).

GEOGRAPHICAL DISTRIBUTION: Known from Tanga and Lindi Regions, Tanzania, and apparently occurs in *Cordylus vittifer* in the Transvaal near Pretoria, South Africa.

Plasmodium cordyli from *Cordylus vittifer (figs 118-129).*

Due to mixed infections with *P. zonuriae* in *C. vittifer*, young parasites could not be identified with certainty as *P. cordyli*. Mature schizonts *(figs 118-123)* 6-9 × 5-7 μm ($\bar{x}$ 7.0 ± 0.1 × 5.6 ± 0.1, N = 25), with LW 38.2 ± 0.9 μm (30-49), had 8-14 merozoites ($\bar{x}$ 11.9 ± 0.3). Schizonts usually oblong, sometimes oval, round, rosette, or fan-shaped. Gametocytes *(figs 124-129)* 5-9 × 4-7 μm ($\bar{x}$ 6.5 ± 0.1 × 5.3 ± 0.1, N = 50), with LW 34.6 ± 1.0 μm² (20-49), round to elongate,

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FIGS. 106-117. — *Plasmodium cordyli* n. sp. from *Cordylus cordylus tropidosternum.*

106-111 : segmenters.

112 : macrogametocyte.

113-117 : microgametocytes.

Host cells 108, 110, 111 are proerythrocytes, remainder erythrocytes.

FIGS. 118-129. — *Plasmodium cordyli* n. sp. from *Cordylus vittifer.*

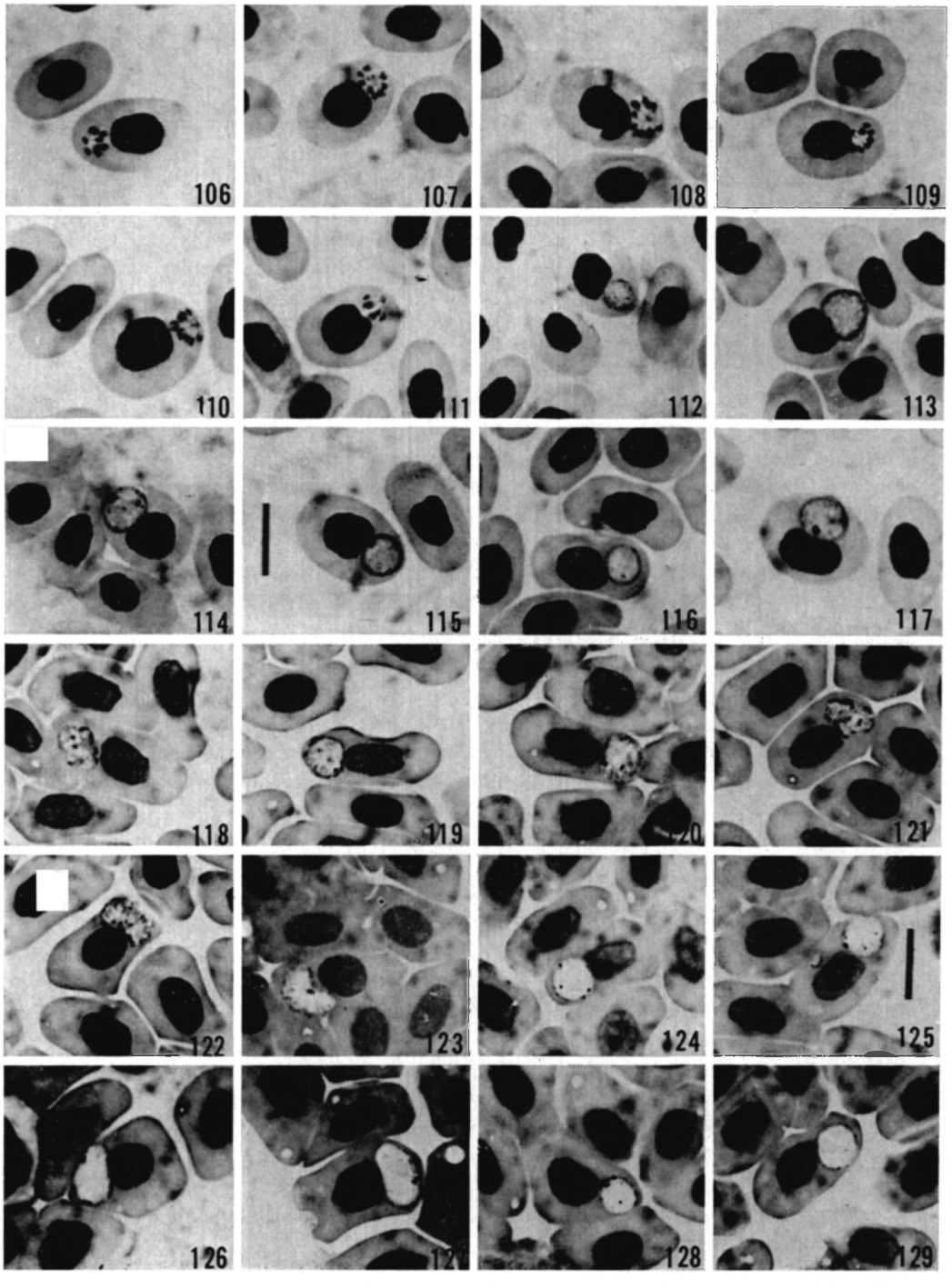
118-120 : nearly mature schizonts.

121-123 : segmenters.

124-127 : macrogametocytes.

128-129 : microgametocytes.

Host cells are erythrocytes. Vertical bars represent 10 μm.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FIGS. 106-129.

with L/W ratio 1.25 ± 0.03 (1.0-2.0). Ratio LW : HCN 0.85 ± 0.04 (0.4-1.4). Pigment dispersed as dark dots in macrogametocytes *(figs. 124-127)*, but tended to clump in microgametocytes *(fig. 128)*. There were no morphometric differences between sexes. Schizonts, always erythrocytic, did not enlarge either cell or nucleus, but HCN were distorted occasionally, and commonly displaced. Erythrocytes with gametocytes enlarged; one infection had enlarged HCN; a few gametocytes were proerythrocytic. Gametocytes occasionally distorted host cells and nuclei, often displacing HCN. Schizonts and gametocytes were normally polar or lateropolar.

Discussion

Two *Carinamoeba* species are known from Africa, *Plasmodium mabuiae* Wenyon, 1909 and *P. cordyli*. They differ by gametocyte shape: *P. mabuiae* has elongate gametocytes equal to or slightly larger than erythrocyte nuclei (Telford, 1983); those of *P. cordyli* are round or oval, smaller than host cell nuclei. Average gametocyte size of *P. mabuiae* is smaller than (LW 18-40 μm^2, means 23.1-32.4) *P. cordyli* (20-49 μm^2, means 33.9-36.3). Schizont size of *P. mabuiae* is similar (LW 10-30 μm^2, means 17.1-21.6) to *P. cordyli* (12-36 μm^2, means 18.8-25.1). The schizont LW:HCN ratio of *P. mabuiae* ($\bar{x}$ 0.77, 0.5-1.1) is greater than for *P. cordyli* (0.52, 0.3-0.8) but only shows that cordylid lizards have larger erythrocyte nuclei than do scincids, a conclusion pertinent also for relative gametocyte size. Schizonts of *P. mabuiae* are typically fan-shaped (80-83 %); those of *P. cordyli* are more variable, fewer (35 %) fan-shaped. Asexual stages and young gametocytes of both species are commonly nucleophilic. Merozoite range of *P. mabuiae* is 4-8; *P. cordyli* has 4-11 merozoites.

The *Carinamoeba* parasite of *Cordylus vittifer* is virtually identical in morphometric parameters of gametocytes to *P. cordyli*. Schizonts are larger (LW 30-49 μm^2) than in *P. cordyli* (12-36 μm^2) and produce more merozoites (8-14, 11.9 *vs.* 4-11, 6.1-7.7). The *C. vittifer* sample also showed no tendency to nucleophily, in contrast to those from the type host. I do not think there are adequate grounds for distinguishing them taxonomically, although it may be desirable with further study to use the trinomial.

Plasmodium zonuriae in *Pseudocordylus microlepidotus* has smaller schizonts and gametocytes than in *Cordylus vittifer*. However, merozoite ranges and even means are virtually identical, and gametocyte shape differs very little. With respect to the other described African saurian malarial parasites, *P. zonuriae* resembles none of them closely enough to merit comparison. Elsewhere it is perhaps most similar, as suggested by Pienaar (1962) to *Plasmodium floridense*, but there is little likelihood of close relationship, given the phylogenetic distance between host families and their intercontinental separation.

ACKNOWLEDGMENTS. The redescription of *P. zonuriae* would have been impossible without the splendid assistance of Professor M. B. Markus and Dr. E. McClain, and I am indebted to them. I thank Robert M. Telford for assistance in collecting and maintaining the Tanzanian lizards, and W. R. Smythe who collected the Lindi specimen. Dr. Jerry F. Butler provided administrative support toward publication of this study.

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