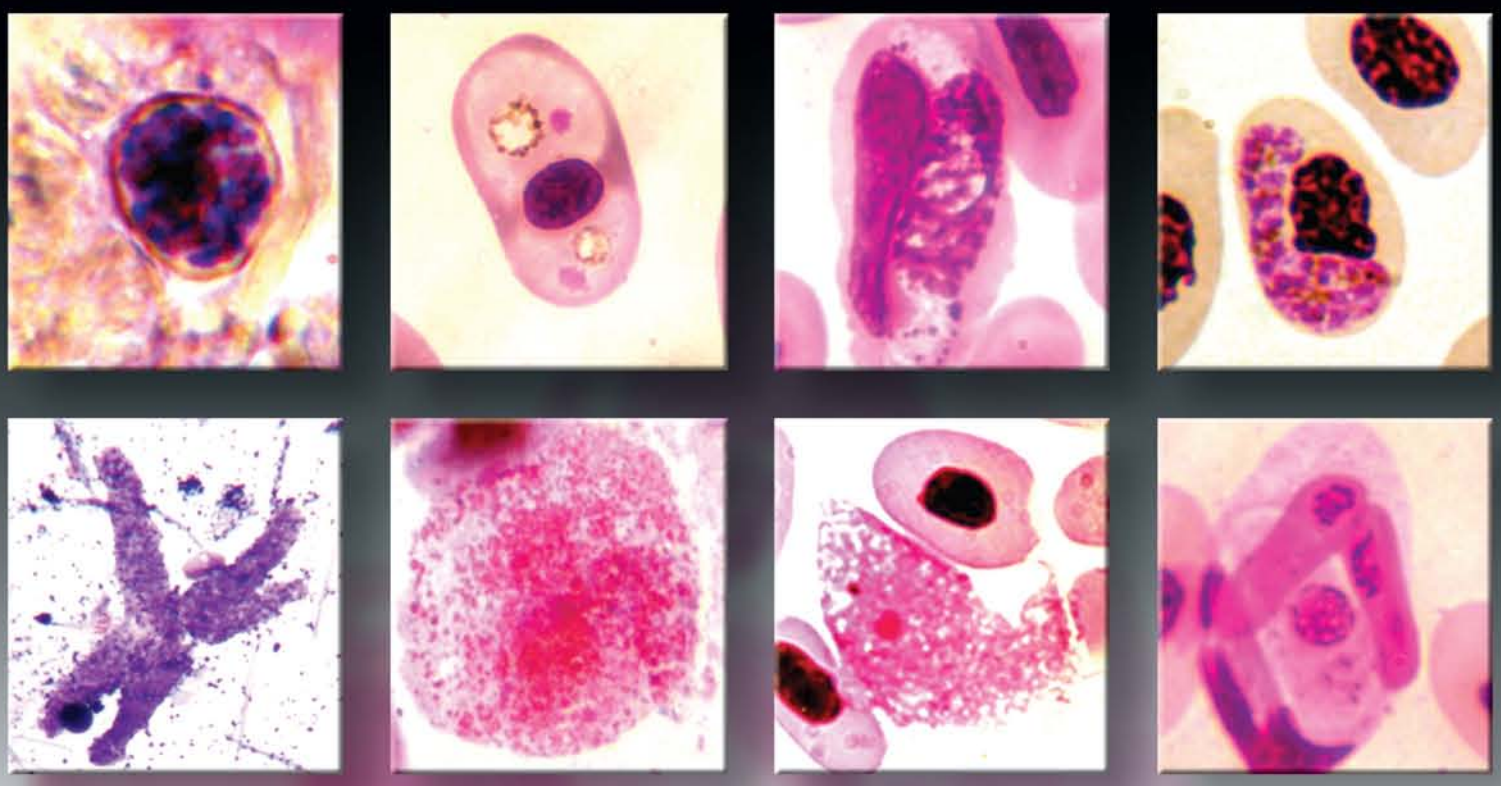
HEMOPARASITES OF THE REPTILIA

COLOR ATLAS AND TEXT



SAM R. TELFORD, JR.

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PREFACE

Morphological variability is a basic characteristic of all species from Protista through the vertebrates and is the critical factor allowing their evolution as their environment changes. Probably, most of the known species of hemoparasites have been poorly described in terms of their variable morphological characters visible under light microscopy. This is unlikely to change as variation among genomes replaces visible morphological characters as the basis for taxonomic distinction. Yet, the need for the ability to distinguish one taxon from another by visible characters will not completely disappear.

The blood parasites of reptiles are both diverse and morphologically variable to an extent not present, or at least not as well reported, among mammalian and avian hosts. Perhaps this is related to the larger size of reptilian erythrocytes and the presence of prominent nuclei within them that can strongly influence the appearance of the parasite within the cell. The student of the mammalian malarial parasites has a far simpler job of identifying what he or she is looking at than does one who samples populations of reptiles host to *Plasmodium* and its relatives. The diversity of reptilian hemoparasites is greater than that of mammals and birds in the numbers of genera and species, although all three of the tetrapod classes are host to the same important groups of unicellular parasites (i.e., plasmodiids, hemogregarines, and trypanosomatid flagellates). The lower vagility of terrestrial reptiles and their more restricted or isolated habitats are major factors in the increased taxonomic diversity of their parasites, probably influenced considerably by the greater phyletic age of reptiles.

The intent of this work is to compile in a single location all of the published data on the morphology of the unicellular parasites of reptilian blood. It is supplemented by the data acquired but unpublished during my 45 years of research, collected primarily from the field while resident in North, Middle, and South America; eastern, southeastern, and southern Asia; and East Africa, as well as from material sent to me for identification by numerous students, veterinarians, and colleagues. The species accounts also contain host and geographic distribution, with precise localities when possible; prevalence, life cycles, and vectors where known; effects on the host; and ecology of the host–parasite relationship. Not all of the published reports have been read because of inability to obtain the original papers (or in a few cases, to read them), but most of the literature on reptilian unicellular hemoparasites is cited. No attempt has been made to survey the veterinary literature, as this consists largely of

reports of individual "disease" cases from captive reptiles, with little demonstrated significance to the natural populations, or reviews based on previous reviews, many of which are now outdated.

The scope of the species considered varies according to the taxonomic group. All reptilian species of the Plasmodiidae are described. Only those hemogregarines, the most speciose group of reptilian blood parasites (over 300 spp.), for which at least partial development in a vector is known are included, which reduces the number of species accounts to less than 50. Trypanosome species for which the descriptions are sufficient in terms of dimensions and locations of structures to permit identification, and a few of the leishmanial species known from reptiles, for which morphology is available and useful, are described. Only a general account has been possible of the several species of uncertain classification, except if ultrastructural characters indicate their bacterial or viral natures. Several new species are described, mostly from slides collected decades ago for which additional material has not become available. The most recent classification published by the Society of Protozoologists (2000) has been followed except for my recognition of the genus *Haemocystidium* within the Plasmodiidae, containing those species parasitic in lizards that were previously considered to be *Haemoproteus*. Tissue meront morphology demonstrates generic affinity with *Plasmodium* rather than *Haemoproteus*, and this is supported by recent molecular phylogeny, as cited here. Subgenera of the reptilian *Plasmodium* species are those defined by Telford (1988a). The host taxonomic names used are those most familiar to herpetologists and have not been updated to reflect the genomic analyses of recent years, which have synonymized many names based on characters derived from visible morphological or ecological characters. Usually, the host name stated in original descriptions of hemoparasites has been used, except when the designation is long outdated, with little or no use in the last half of the past century. Many of the recent changes proposed from DNA analysis are not yet generally accepted, and some already have joined the taxonomic synonymy of the taxa studied.

Materials and Methods

Morphometric data from the slides of haemosporidiids, hemogregarines, hemococcidia, and trypanosomatids were originally obtained by measurement of adequate series of parasites from slides using a calibrated ocular micrometer with a Nikon compound microscope. Perhaps ten of the several hundred samples measured over the years were obtained with a Zeiss microscope; the remainder were made using the same Nikon microscope that survived my many international moves from 1965 to 1985. A minimum of 25 parasites of each stage needed for description (i.e., meronts, gametocytes, gamonts, sporozoites, trypomastigotes, and amastigotes) were measured with data recorded on a standard sheet, which also contained observations on immature forms, locations of important individual parasites on the particular slide, and, of considerable importance, the grid locations of the vertical paths searched on the individual slide. When these individual parasites were photographed, a notation to that effect was almost always made. If a single slide contained too few parasites to meet the sample desired, a second or more slides made on the same date, if available, was searched. At times, it was necessary to use slides from subsequent dates to meet the standard desired. Rarely, it was necessary to base a description on smaller samples, then usually because of age of infection, which often affects parasitemia and the stages present. When available, infections of the same parasite from at least three different individuals of the same host species were studied. When a parasite species infected additional host species, the measurements obtained were never combined into a single sample from all hosts but were analyzed separately. Total sample sizes comprised up to several hundred in some plasmodiid species.

Initially, because more than one sample often was used for description of a parasite, the mean values were reported as mean plus or minus standard error of the mean, but in later years the more usual mean plus or minus standard deviation was stated. The laborious calculations necessary before

the arrival of desktop computers involved rather primitive (by today's standards), glorified adding machines and handheld scientific calculators. With the advent of appropriate computer programs, all data from the large number of data sheets were recorded in Lotus 1-2-3 spreadsheets, then exported to a statistical program called Microstat© (1984, Ecosoft, Indianapolis, IN) for analysis. When samples did not require more complex techniques, they were simply summarized using the Lotus procedures. With the availability of Excel spreadsheets, all of the original Lotus sheets were copied into that system. Individual infections for each host species were combined by host species for the descriptions presented in this book, and the statistics given, recalculated, represent the entire sample for that parasite, again with separations by host species and, if logical, geographic origin. Significant differences between samples were based on one-way analysis of variance (ANOVA) comparisons, with significance taken at $P \leq .05$. All of the original data sheets as well as the computerized formats are deposited in the herpetology collection of the Florida Museum of Natural History, where most of the host specimens obtained by the author are preserved, and field notes, when recorded, are on file. Wherever in the text the author's name appears as (Telford) with no date citation, especially within Other Localities or Prevalence sections, this indicates unpublished data of the author. Prevalence of a parasite in a sample size of less than ten is not expressed as a percentage.

Except on rare occasions when material was prepared by others, all of the blood slides I collected were fixed in absolute methanol and stained by the Giemsa technique for at least 55 minutes or more at pH 6.8 during residence in Japan (1965–67) or 7.0 thereafter. Many slides stained over 40 years ago have retained the original results, but sadly, many more have partially or largely destained. A great error was committed when I mounted much of the type material in a supposedly neutral euparal mounting medium, which usually resulted in rapid, near total destaining. Results were mixed when Permount® (Fisher Scientific) was the mounting medium, but were generally much better than with euparal. Slides of some value with nearly or completely vanished stain were sometimes destained in slightly acidic ethanol and neutralized with basic ethanol, then restained using the Kimsey (1992) Giemsa staining technique. Again, results were mixed, with some slides successfully restained almost to the original colors, while often the results did not proceed beyond shades of basophilia. A slide of *Plasmodium minasense carini* gametocytes digitally photographed for **Plate 17B, e–l**, prepared by C. M. Wenyon in 1915 on Trinidad, has retained the original staining remarkably well, while slides I made within the last 10 years are already destaining. It is impossible to generalize what the duration and quality of stain on a given slide will be. Hapantotype slides of all species I described in the past will be deposited as time permits in the U.S. National Parasite Collection in Beltsville, Maryland. Although some type material of plasmodiids was deposited in the Garnham collection in London or in the Muséum National d'Histoire Naturelle, Paris, many were retained for "eventual deposition with the Telford collection" because of my opinion of the postal system in developing countries where I lived. The entire Telford collection is well under way in cataloguing and will be offered to an appropriate depository when completed. The preparation of tissue samples other than blood was reported in the various articles in which they were used, but standard techniques were always employed.

More than 10,500 digital images were obtained with a Nikon Coolpix© digital camera during about a year and one-half following the completion of most of the text. Most were unsuitable for publication, but those that best showed the details of the species involved were in sufficient numbers to provide some idea of the considerable variation normal to reptilian hemoparasite species.

Many of the infections from which hemoparasite species were described were followed for considerable intervals after capture, even exceeding 4 years. The host animals were maintained by appropriate measures on similar diets, in most cases, to those that were natural to the species. I almost always made host species identifications, utilizing my knowledge and experience in herpetology, which now exceeds 60 years. Voucher specimens of most host species, often in very large series (~2000 *Takydromus tachydromoides*, for example), are on deposit in the herpetology collection of the Florida Museum of Natural History.

ACKNOWLEDGMENTS

In an earlier work (Telford, 1988a), I acknowledged the help of numerous colleagues who contributed specimens, information, and advice toward the several studies that formed the basis of this work, and their assistance continues to be appreciated. M. B. Markus and E. McClain must be added to that group. Two colleagues in particular more recently sent me slides that facilitated the inclusion and preparation of species accounts that would otherwise have not been included. Juergen Stein, during his doctoral studies in Australia, referred well over 100 slides from the host he studied, *Egernia stokesii*, to me for identification of the several hemoparasite species present. David Modry provided slides of *Haemogregarina stepanowi* from the type host, *Emys orbicularis*, and of *Hemolivia mauritanica* in *Testudo marginata*. Their contributions are greatly appreciated.

My sons have contributed continuously to my studies since they dispersed. Sam collected material from Venezuelan Amazonas, Sabah, and Russia during projects in those areas. Randy's lifelong enthusiasm has obtained dozens of snakes in south Florida, from which at least 10 undescribed *Hepatozoon* species became available for sporogonic studies. Robert assisted in the acquisition of computer components and in the necessarily repetitive explanations of how to use them. And, all three sons contributed digital equipment and other logistical support. Jim Schram struggled with my incompetence in matters digital and eventually succeeded in enabling me to overcome some of my computer ignorance.

Finally, throughout the course of a 50-year marriage, Michiko Miyazawa Telford put up with a lot of strange activities by her husband, with the presence of hundreds of reptiles over time in and around her home, wherever we lived in seven countries abroad and in the United States, some of which required frequent admonitions on her part to avoid widowhood. A good example is the occasion in December 1981 on which she watched her eldest son and his father manhandling an 8-foot black mamba on the highway south of Tanga in Tanzania. No one was bitten, the blood slide (*Hepatozoon* positive) is in the Telford collection, and the snake is deposited in the Florida Museum of Natural History. Her competence in the handling of household affairs and financial matters provided the needed stability of the home environment for my research. None of my hemoparasite studies ever received institutional or grant support, apart from my various professional assignments, which happily

placed me into situations that permitted a fascinating utilization of my spare time. It has been a long and fun journey for a boy who once roamed barefoot the scrubs, flatwoods, hammocks, and swamps of central Florida, developing his herpetological expertise, and exercising his unlimited curiosity for the natural world. Finally, I must thank my deceased parents, Sam Rountree Telford and Ann Marion Frances Schiller, who never really understood, but did not forbid, when once they could.

ABOUT THE AUTHOR

Sam R. Telford, Jr., a fourth-generation Floridian, was born and raised in Winter Haven, Florida, and attended local schools. He was active in scouting for 7 years, camping throughout much of Florida before it became the urban/suburban mess that it is today, and achieved the rank of Eagle Scout. Fortunate to grow up on a large tract of land comprised of pine woods, swamp, and citrus that fronted on one of the area's many lakes, he began learning herpetology at the age of 11, and during high school maintained a large collection of snakes at his home on Lake Shipp. Sam's first article, reporting a litter of 101 green water snakes, was published in *Herpetologica* around the time of his 16th birthday.

His first 2 years of college were at the University of Florida, where he spent much of the time, when he should have been studying, in the field with zoology graduate students, collecting reptiles and amphibians. Realizing the need for a more intensive academic environment, he transferred to the University of Virginia, graduating with a B.A. in biology in 1955. After a semester in law school at the University of Florida, he entered the U.S. Army, was trained at the Army Intelligence Center, and served for 3 years with two military intelligence units near Tokyo, Japan. Here, he met and married his lifelong companion, Michiko Miyazawa, who also worked for one of his intelligence units. Her first realization of what kind of biologist she had married was when she discovered a couple of preserved Japanese rat snakes in bottles that had previously contained bourbon.

When they returned to the States in 1959, Sam entered graduate school at the University of Florida, intending to pursue herpetology. After taking a stimulating first course in parasitology from George W. Hunter III, he realized that virtually nothing was known of reptilian parasitology apart from scattered reports. Under the influence of Eugene C. Bovee, his master's thesis, "Studies on the Incidence of Intestinal Protozoan Inquilines in Snakes and Lizards of the Southeastern United States," was completed in 1961. Simultaneously with his graduate research, he reviewed the taxonomy of the southeastern crowned snakes, genus *Tantilla*. Sam accepted a National Institutes of Health (NIH) Predoctoral Fellowship at the University of California at Los Angeles (UCLA) and conducted his doctoral research under Gordon H. Ball, completing "A Comparative Study of Endoparasitism Among Some Southern California Lizard Populations" in 1964.

While at UCLA, he necropsied countless snakes and lizards in return for identifying them for a dealer in exotic reptiles. After finding high prevalence of filarial worms in Mexican boa constrictors,

he obtained a grant from Sigma Xi and took his wife and two small boys to Manzanillo in Colima, Mexico, for 6 weeks in 1963, obtained blood from 100 snakes, and began his career with reptilian hemoparasites. After obtaining his Ph.D. in 1964, he received a 3-year NIH postdoctoral fellowship in the Institute for Infectious Diseases, University of Tokyo, Japan, in the laboratory of Professor Manabu Sasa. Sam studied the population biology of a Japanese lizard and its relationship to its 20 species of symbiotes, published 30 years later as *The Ecology of a Symbiotic Community* in two volumes, and began his studies of reptilian malarial parasites and other blood parasites that continue today.

After the birth of their third son in Tokyo, Sam and Michiko moved to Panama, where he worked at Gorgas Memorial Laboratory from 1967 to 1970 as a vertebrate ecologist on studies of leishmaniasis in forest mammals. In 1970, they returned to Florida, where Sam taught biology to undergraduates for 3 years until he was recruited by the World Health Organization (WHO) for a project on Chagas' disease in Venezuela. After nearly 2 years there, WHO sent him to Karachi, Pakistan, to study rodent-borne diseases (1975–77), then to WHO Headquarters in Geneva, Switzerland, for administrative work on leishmaniasis and Chagas (1977–78). Boring quickly of the desk job, he transferred to Rangoon, Burma, to pursue rodents responsible for plague. Sam resigned his WHO appointment in 1980 and returned briefly to Florida and then was selected by the Danish International Development Agency as project leader for their research and training program in rodent biology and control on the campus of Sokoine University in Morogoro, Tanzania.

After 4 years in Morogoro, Sam and Michiko tired of living abroad and returned to Gainesville, Florida, in 1985, where they presently live. The nearly 20 years abroad provided the material for authoring or coauthoring well over 200 research papers and several book chapters on reptilian, avian, and mammalian blood and intestinal parasites; Florida's endemic reptile fauna; cutaneous leishmaniasis; Chagas' disease; and, strangely, mesostigmatic mites. Sixty years after publication of his first paper, Sam continues his research and writing with no intention of quitting and taking up fishing or golf.

1
THE PLASMODIID PARASITES

In the first decade of the 20th century the first reptilian malarial parasites were recognized, joining those reported from humans and birds within the previous 20 years. Wenyon (1909a), during his tenure as traveling protozoologist for the Wellcome Research Laboratories, found *Plasmodium agamae* and *P. mabuiae* in agamid and scincid lizards of the Sudan. In the same year, and with priority to Wenyon's discovery, two species, *Plasmodium diploglossi* and *P. tropiduri*, were described from Brazil in anguid and tropidurid lizards (Aragão and Neiva, 1909). The pace of species discovery and description rose slowly until the 1960s, with only 29 species and subspecies recognized by Garnham from reptiles in his classic *Malarial Parasites and Other Haemosporidia*, which appeared in 1966. At the end of that decade, the recognition of *Plasmodium* species and species of related genera began to rise in seemingly geometric progression, with 87 taxa known by 1989 (Telford, 1994), then slowed in the 1990s, with 101 species and subspecies of *Plasmodium sensu stricto* described or under description by 2007, as well as 37 other related species of plasmodiids: *Garnia* (10), *Fallisia* (10), *Haemocystidium* (14), *Saurocytozoon* (2), and *Progarnia* (1). To a considerable extent, this proliferation resulted from long-term residence in endemic areas by parasitologists interested in these organisms, in contrast to brief visits with the limited collections possible by traveling scientists or physicians, often with other primary interests.

Most articles dealing with reptilian plasmodiids have been taxonomic until recently, when interest arose in using plasmodiids as examples supporting ecological theory. Of late, genomic analysis has begun, but until a broad spectrum of known species has been studied, the results will remain tantalizing but far from definitive in tracing systematic/phylogenetic relationships, which must remain based on morphological and life history traits.

Morphology and Life Cycles

Vectors

Until 1970 the complete sporogony of a saurian *Plasmodium* species remained unknown. There were occasional reports of oocysts present on the midguts of culicine mosquitoes from experimental feedings on infected lizards. Huff (1941) found an oocyst on the midgut of an *Aedes aegypti* that had ingested a saurian malaria parasite later described as *Plasmodium floridense* (Thompson and Huff, 1944b), but subsequent attempts to infect *A. aegypti* and *Culex pipiens* by *Plasmodium mexicanum* and *Plasmodium rhadinurum* were unsuccessful. In the case of *P. rhadinurum*, all fed mosquitoes died within a day of feeding on the infected lizards, but those fed on an uninfected lizard survived, leading the investigators to suggest that the parasite was lethal to the mosquitoes. In Liberia, Baker (1961) fed four *Aedes* species on *Agama agama* infected by *Plasmodium giganteum* or *Plasmodium agamae*. Male and female gametes and ookinetes of *P. giganteum* were seen in several *A. aegypti*, but no other sporogonic stages were found.

Greater success was obtained by Jordan (1964) when she fed eight mosquito species on *Anolis carolinensis* and *Sceloporus undulatus* infected by *P. floridense*. Four species could not be infected, although they took blood meals (*Aedes atlanticus-tormentor, A. triseriatus, Mansonia perturbans, Psorophora* sp.), and another ten mosquito species common in the area refused to feed on the lizards. All *Aedes aegypti* and *Culex quinquefasciatus*, nearly 500, that fed on *A. carolinensis* had a negative result. One of 80 *A. aegypti* fed on *S. undulatus* had an oocyst on its midgut when dissected, and on two occasions single *C. quinquefasciatus* from 150 dissected showed one and three oocysts present. Four of 75 *Culex territans* had 1, 2,

16, and 23 oocysts present on midguts. Three individuals of a small, dark, unidentified *Culex* species were fed on an infected lizard, and one had 70 oocysts present when dissected. Salivary gland dissections all were negative for sporozoites.

Speculation concerning the natural vectors of saurian malarial parasites took a different direction in 1970 when Stephen C. Ayala described complete sporogony of *Plasmodium mexicanum* in two species of phlebotomine sand flies, *Lutzomyia vexator* and *L. stewarti*, in California. Some parasitologists even assumed that all saurian malarias utilized sand flies for their invertebrate hosts, relying on the dictum that the first host found to support sexual reproduction of the parasite inevitably would prove to be the characteristic vector group for all related species. The oocysts on midguts, sporozoites in salivary glands of *L. vexator*, and transmission of *P. mexicanum* by inoculation of sporozoites into laboratory hatched or uninfected, wild-caught juvenile lizards were convincing evidence that the sand fly could indeed transmit the parasite (Ayala and Lee, 1970). Sand flies collected from ground squirrel burrows were fed on *Sceloporus occidentalis* infected with *P. mexicanum*, and within the next 7–10 days, oocysts in profusion formed on midguts, and sporozoites matured within oocysts, which then ruptured, releasing hundreds of sporozoites into the sand fly hemocoel. Some of them entered the salivary glands of the sand fly. Experimental infections in the young lizards became patent within 22 days postinoculation with sporozoites. Vector competence was confirmed by Klein (1985), who fed colony-raised *L. vexator* of North Florida origin on *S. occidentalis* from California, infected with *P. mexicanum*, and after sporogony was completed, infected North Florida *Sceloporus undulatus* with *P. mexicanum* by sand fly bite (Klein et al., 1987b). More recently, Fialho and Schall (1995) explored the effect of temperature on development of *P. mexicanum* in *L. vexator* and the relationship of temperature to the ecology of the vector, both within the ground squirrel burrows in which it shelters and in the laboratory. They concluded that *P. mexicanum* enhanced transmission success through its comparatively rapid rate of development at an optimum temperature range of 22.9–24.9°C, and suggested that the parasite adaptively manipulates the thermoregulatory behavior of the sand fly. Further confirmation of the vector role of *L. vexator* for *P. mexicanum* resulted from inoculation of a crushed midgut with mature oocysts into an uninfected lizard, producing an infection within 2 weeks.

Involvement of another dipteran family in possible transmission of saurian *Plasmodium* species was suggested when Petit et al. (1983) obtained mature oocysts containing sporozoites of the African *Plasmodium agamae* from the gut of the European ceratopogonid fly *Culicoides nubeculosus*. Perhaps because of the unnatural host, oocysts did not rupture and release sporozoites capable of entering the salivary glands. When Klein (1985) fed *Lutzomyia vexator* on lizards infected with *Plasmodium floridense*, sporozoites were produced by the oocysts on the sand fly midgut but were retained within the oocysts and did not enter the salivary glands within an observation period of 14 days.

As described, Jordan (1964) found 70 oocysts on the midgut of a small, dark *Culex* species that she fed on lizards infected with *P. floridense* in Georgia. Klein (1985) demonstrated that *P. floridense* in both species of lizard hosts in northern Florida, *Anolis carolinensis* and *Sceloporus undulatus*, readily underwent complete sporogony in *Culex erraticus*, an abundant local mosquito that could be described as small and dark, similar to the unidentified species in which Jordan found the largest number of oocysts of *P. floridense*. The sporogonic pattern of *P. floridense* was typical of the other, nonreptilian *Plasmodium* species that develop in mosquito hosts. Mosquitoes infected in the laboratory that took second blood meals 2 weeks or so following their infective meal transmitted *P. floridense* to uninfected local lizards, with patency of infection evident in 18–40 days following the second feeding (Klein et al., 1987a). *Culex erraticus* readily entered mosquito traps baited with *A. carolinensis* and fed on them. Additional implication as a vector of *P. floridense* came from the light trap collections that demonstrated that the peak of abundance of *C. erraticus* in north Florida occurs in late summer, just preceding the appearance of new infections in both young and mature anoles. *Culex territans*, however, in which Jordan found up to 23 oocysts, reaches its peak of abundance 2 months earlier than *C. erraticus*, and Klein was unable to infect the species in the laboratory. Occasional transmission of *P. floridense* by *C. territans* possibly occurs in the spring and early summer when *C. erraticus* is not present in abundance.

Another plasmodiid, *Saurocytozoon tupinambi* in Brazil, produced mature oocysts containing sporocysts in *Culex pipiens* (Landau et al., 1973), but sporozoites were retained within oocysts and did not infect salivary glands, in a similar manner to the sporogony of *P. floridense* in *Lutzomyia vexator*, and *P. agamae* in *Culicoides nubeculosus*. Retention of the sporozoites within the oocysts is probably due to the dipteran being an unnatural and not completely capable invertebrate host. There is another dipteran family proven to transmit a haemosporidian parasite of reptiles, *Chrysops callidus* (Tabanidae), the natural vector of *Haemoproteus "metchnikovi"* in the northern United States (DeGiusti et al., 1973). Transmission by acarinids of reptilian plasmodiids has not been clearly demonstrated, but Peláez and Perez-Reyes (1952) thought that *P. mexicanum* infections that appeared in newborn *Sceloporus torquatus* caged with infected adult lizards infested by

mites, a *Hirstiella* sp., might have been acquired from the mites. Similar transmissions occurred in two additional trials (D. Peláez, personal communication, 1986). Transmission of avian *Plasmodium* by ingestion of infected vectors is possible (Et. Sergent and Sergent, 1912; Et. Sergent, 1937; Young, 1941), and this may explain the observations of Peláez on *P. mexicanum*. Another possibility is congenital transmission from infected females of ovoviparous species, such as *S. torquatus*, to the developing young, as can happen in *Hepatozoon* species of snakes, but neither of these alternative methods of transmission, presumably mechanically at least in the latter method, has been critically studied.

Course of Infection in the Vertebrate Host

Preerythrocytic Phase No one has studied the development of sporozoites in the lizard host once experimental infection has occurred. Restained tissue sections from natural infections of *Plasmodium sasai* in four lizards were studied by Telford (1989), which provided information for an outline of this portion of the life history that might be pertinent to other saurian *Plasmodium* species. Uninucleate parasites were present in hepatic parenchymal cells of a lizard captured immediately after emergence from hibernation, which may have been infected late in the fall, immediately preceding hibernation. The lizard showed an acute parasitemia at capture. The uninucleate parasites were contained within parasitophorous vacuoles, as were binucleate and multinucleate meronts. It was suggested that the uninucleate stages were comparable to the hypnozoites of a primate malarial parasite, *Plasmodium cynomolgi*, reported by Krotoski et al. (1982a). These would enable sporozoites introduced just prior to cessation of lizard activity before winter to begin preerythrocytic merogony immediately following hibernation before the first generation of vectors could appear. The merogonic stages present in hepatic parenchymal cells probably represent the cryptozoic generation. Multiply infected macrophages present in hepatic sinuses apparently were host to the metacryptozoite generation, which then gave rise to phanerozoites in the endothelium and connective tissues of heart, lungs, femoral muscles, testes, and brain. Telford (1994) summarized the preerythrocytic phase of infection thus:

> Sporozoites inoculated by the vector appear to enter parenchymal cells of the liver. Some of them may remain inactive, as hypnozoites, whereas others may undergo at least one merogony as cryptozoites before becoming inactive. Some of the progeny may enter macrophages and form metacryptozoic meronts. Merozoites from the merogony or subsequent merogonies then are likely to parasitize the capillary endothelium and connective tissues of various organs to begin the phanerozoic merogonic cycles.

In some species of *Plasmodium* from lizards, macrophages containing developing meronts have been found when erythrocytic parasitemia has begun, suggesting some continuation of the metacryptozoic cycle after phanerozoites have appeared and erythrocytes were invaded. Alternatively, these could represent a later invasion of macrophages by the progeny of phanerozoites.

Phanerozoic meronts of *P. mexicanum* were termed "*gallinaceum* type" by Thompson and Huff (1944a). They have been observed in sections of endothelium and connective tissue in most organs during the course of active and chronic *Plasmodium* infections, and have been found in the following species, in addition to *P. mexicanum*: *P. sasai* (Telford, 1989, 1996b, 1998b); *P. agamae* (Telford, 1994); *P. michikoa-P. gologoloense* mixed infection (Telford, 1988b); *P. pitmani* (Garnham, 1950; Telford, 1994); *P. mackerrasae* (Telford and Stein, 2000); *P. floridense, P. aurulentum, P. loveridgei, P. holaspi, P. cordyli, P. lionatum,* and *P. fischeri-P. acuminatum* mixed infection (Telford, unpublished). In the host of *P. sasai* from mainland Japan, *Takydromus tachydromoides*, a lizard that must hibernate during winter, some phanerozoites occur with a cyst wall around them (Telford, 1989, 1996b), which may be an adaptation for survival of the infection through winters exceptionally prolonged or perhaps, although doubtful, as a defense against immune response. The encysted phanerozoites were termed "chronozoites" by Telford (1989) and are similar to the encysted "relapse schizonts" reported for *Leucocytozoon simondi* (Desser et al., 1968).

Erythrocytic Phase The course of infections has been studied in experimental infections of only four *Plasmodium* species of lizards: *P. mexicanum* (Thompson, 1944; Thompson and Huff, 1944a; Jordan, 1970a; Klein, 1985); *P. floridense* (Thompson, 1944; Goodwin, 1951; Goodwin and Stapleton, 1952; Jordan, 1975; Klein, 1985); *P. tropiduri* (Scorza, 1970a); and *P. sasai* (Telford, 1972a). The prepatent period, time required to reach peak of parasitemia, maximum parasitemia, and duration of infection are similar in each species. Although the prepatent or incubation periods have been reported to range from 2 to 45 days, infection in less than 5 to 7 days resulting from inoculation of infected blood probably represents the presence in circulating blood of infected erythrocytes from the inoculum. The appearance of tiny trophozoites, clearly resulting from merogony in the new host, is a better indicator of the onset of parasitemia than simply finding parasites of various stages in the blood. The use of intracardial or intravenous inoculation, sometimes utilized by Thompson and

Huff (1944a) and by Goodwin and Stapleton (1952), would be expected to result in circulating parasites more quickly than using intraperitoneal infection as they did in some cases and as used by Scorza (1970a) and Telford (1972a). Klein (1985) found that prepatent periods for sporozoite-induced infections by *P. mexicanum* and *P. floridense* were similar to those resulting from inoculation of infected blood, 23–40 days and 13–25 days, respectively. The length of time is determined by both the time required for preerythrocytic development and the need for invasion of an adequate number of infected erythrocytes for detection of infection by examination of slides.

It is evident now that the rather long periods of prepatency in infections induced by inoculation of infected blood may be due, at least in part, to the development of phanerozoites, possibly several generations of them, within the endothelial and connective tissue cells of the recipient lizard. Following intraperitoneal inoculation of blood from lizards infected with *P. sasai*, phanerozoites were found in capillary endothelium and connective tissue of most organs examined of juvenile *Takydromus tachydromoides* killed at 48, 72, and 96 hours postinoculation (Telford, 1998b). Erythrocytic infections in the series of lizards, at 0.04% parasitemia, were not detected until the 6th day postinoculation. The inocula were massive, 450,000 parasites per 10^4 erythrocytes. Other *T. tachydromoides*, both juveniles and adults, infected by inocula at levels of less than 15,000 to 108,000 parasites per 10^4 erythrocytes, that died 21–296 days postinoculation were examined histologically, and all had phanerozoites present in their tissues. The length of the prepatent period is also affected by host species and the level of inoculum (Thompson, 1944; Thompson and Huff, 1944a; Telford, 1972). In the *P. sasai* study (Telford, 1972a), the mean prepatent periods were 34.7, 27.2, and 11.3 days when inocula were less than 4,000, 40,000–46,000, and 57,000–68,000 parasites per 10^4 erythrocytes, respectively. The prepatent period was no shorter when the inoculum varied from 108,000 to 167,000 than when 57,000–68,000 parasites per 10^4 erythrocytes were used. Given the prepatent period of 6 days in the series of juveniles given 450,000 parasites per 10^4 erythrocytes, this is perhaps the minimum time for one generation of phanerozoic merogony in *Plasmodium sasai*, in its type host, that is adequate to provide erythrocytic infection detectable by microscopy.

Erythrocytic Merogony Meronts of the reptilian plasmodiid species undergo from two to seven nuclear divisions, resulting in 4–130 merozoites. In two subgenera, *Sauramoeba* and *Garnia*, the largest meronts occur in mature erythrocytes, but in many species of other subgenera, meronts are larger and contain more merozoites in immature erythroid cells, in particular proerythrocytes, than in mature cells. There may be no difference in size of the meronts in mature and immature cells, but they contain fewer merozoites when mature. During the course of infection, as parasitemia increases, there is a tendency by some species, such as *P. floridense* (Jordan, 1975) and *P. tropiduri* (Scorza, 1970a), to produce more merozoites during the period of acute rise in the infection. This may result from greater utilization of immature host cells as erythropoiesis increases. When peak parasitemia is attained, crisis forms may appear in these two species, and as the infection enters chronic phase, meronts again largely parasitize mature erythrocytes. In natural, active-phase infections of *P. colombiense*, larger meronts with more merozoites occurred in immature cells than in chronic-phase meronts (Ayala and Spain, 1976). Telford (1994) suggested that mature erythrocytes perhaps possess less of some resource essential for nuclear division than do immature cells, despite the presence of less hemoglobin in the latter group.

In some but not all saurian malaria species, there is a distinct peak of infection following acute rise in parasitemia, and this can vary by host species. Jordan (1975) inoculated a pathogenic strain of *P. floridense* from *Sceloporus undulatus* into *Anolis carolinensis*. In *S. undulatus,* the period of acute rise in parasitemia required about 65 days; peak occurred at 13,400 parasites per 10^4 erythrocytes; mean merozoite number per meront during acute rise was 10.7; but following peak, as parasitemia declined, meronts averaged 8.8 merozoites. The hosts died 4 days after peak. In *A. carolinensis*, however, the period of acute rise took 41 days, peak parasitemia reached only 2,400 parasites per 10^4 erythrocytes, and patent parasitemia lasted for 81 days, with all hosts surviving the infection. The mean numbers of merozoites before and after peak were higher than in *S. undulatus*, 13.7 and 11.2, respectively.

Geographically different strains of *P. sasai* were found to produce different patterns of infection in their natural hosts (Telford, 1972a). In the host of *P. sasai* in central Honshu, *Takydromus tachydromoides*, patency required 7–45 days, with peak parasitemia occurring 25–96 days postinoculation, usually between 2,500 and 6,000 parasites per 10^4 erythrocytes, and varying from 730 to 12,700 parasites per 10^4 erythrocytes. The acute phase of infection was usually about 80 days but ranged from 15 to 183 days. Infections became chronic following the peak parasitemia, with some lizards surviving up to 291 days postinoculation, during which one or two relapses occurred in which the level of parasitemia was far lower than the previous peaks of those infections. The course of infection by the strain of *P. sasai* in *Takydromus smaragdinus* from Amami Oshima in the Ryukyu Islands had a more benign effect on the hosts. Infections were patent in 11–50 days at low parasitemia, about 200 parasites per 10^4 erythrocytes, and

about half of the infections showed no peak parasitemia. Acute infections averaged about 16 days, ranging from 5 to 24 days, and developed between days 30 and 85 postinoculation. Peak parasitemias occurred between 35 and 90 days and averaged 879 parasites per 10^4 erythrocytes, varying from 332 to 2,278 parasites per 10^4 erythrocytes. Infections persisted up to 150 days, with no host mortality. The Ryukyu strain of *P. sasai* retained its characteristics when inoculated into *T. tachydromoides* from Honshu, producing a low-level parasitemia without clear peaks of infection. One infection only of the Honshu strain resulted from inoculation into *T. smaragdinus*, lasted only 1 month, and produced a peak of only 680 parasites per 10^4 erythrocytes. The mild infections produced by the Ryukyuan strain of *P. sasai* in *T. tachydromoides* suggests that the intrinsic characteristics of the strain limited the infection, producing mild parasitemias and short duration of infection, regardless of development in a host where parasitemias by the Honshu strain achieved high levels and lasted for many months.

In nature, the course of infection by saurian *Plasmodium* species may not show the characteristics of experimental infections in the laboratory. Bromwich and Schall (1986) did not observe an acute rise and a distinct peak followed by a sharp decline in a field study of *P. mexicanum* in California lizards. The course of infection as determined from recaptures of infected lizards remained relatively stable during the summer. Although these observations could have been a sampling artifact (samples were taken only during the summer), other *Plasmodium* species characteristically show stable infections over time. Certainly in laboratory studies of *P. tropiduri* (Scorza, 1971b), *P. floridense* (Jordan, 1975; Thompson, 1944; Goodwin and Stapleton, 1952; Klein et al., 1987a), *P. mexicanum* (Thompson, 1944; Jordan, 1970; Klein et al., 1987b), and *P. sasai* (Telford, 1972a), the classical characteristics of infection (i.e., acute rise, distinct peak, and decline [often sharp]) into chronicity were observed. The inoculation of sporozoites that begins natural infections may well produce different patterns of infection than that resulting from the large numbers of parasites inoculated to establish infections in the laboratory.

Synchronicity of Merogony Synchronous division of meronts was suggested by Jordan (1975) for *P. floridense*, and Scorza (1970a) found a merogonic cycle of 48 hours in a single *Tropidurus hispidus* infected with *P. tropiduri* and followed for 9 days. Telford and Ball (1969) found clear evidence of synchronous division in three *Takydromus tachydromoides* infected with *P. sasai*, followed for a period of 114 hours. Merozoites were released usually between midnight and 0600 hours, with peaks occurring at 24-hour intervals under normal day-night cycle, and at ambient temperatures of 25–28°C. Thompson and Huff (1944a, 1944b), however, found no evidence of synchronicity in the division of *P. mexicanum* in an unnatural host species, *Crotaphytus collaris*, and in one *Sceloporus undulatus*, a natural host of *P. floridense*.

Paraerythrocytic Merogony Meronts utilize both fixed cells of various organs and circulating leukocytes and thrombocytes in addition to erythrocytes. Nonerythrocytic cells may provide some protection against immune responses directed at parasites of erythroid cells, but this has not been established. The most commonly infected cells are lymphocytes, monocytes, and thrombocytes, but a variety of granulocytes can also be infected. *Plasmodium mexicanum* produces exoerythrocytic (EE) meronts in both circulating nonerythroid cells and fixed cells of organs, a pattern described by Thompson and Huff (1944a) as "both *elongatum* and *gallinaceum* types," respectively, of exoerythrocytic merogony from the patterns described originally in two species of avian *Plasmodium*. This combination was called the "*mexicanum*" type by Garnham (1966), who described it as distinct from both avian and mammalian patterns. Merogony in endothelium suggested a close relationship between avian *Plasmodium* and *P. mexicanum*. The evidence available from 12 of the saurian *Plasmodium* species listed above as host to "*gallinaceum*" type EE merogony suggests that the "*mexicanum*" type may be characteristic of most reptilian *Plasmodium* species inasmuch as all but the mixed infection of *P. acuminatum/fischeri* also had *elongatum*-type meronts in circulating cells. Exoerythrocytic meronts were produced by *P. mexicanum* in all species of hosts infected by blood inoculation (Thompson and Huff, 1944a). *Plasmodium sasai* infections induced by inoculation of infected blood produced phanerozoic meronts that persisted throughout the course of infection and for the remaining lifetime of the lizard hosts (Telford, 1998b). It is probable that both the initial erythrocytic infection and subsequent relapses are derived from phanerozoic meronts in the endothelium and connective tissues of the hosts.

In addition to the evidence that phanerozoic and erythrocytic infection by *P. sasai* persisted for the remaining life of the experimental lizards, there are several examples cited by Telford (1994) of infections remaining for some years following capture of naturally infected lizards. Ayala (1977) reported asexual stages of a *Plasmodium* species persisting for 3 years. In the absence of asexual stages of *P. chiricahuae*, gametocytes remained present continuously for 495 and 369 days in *Sceloporus jarrovii* (Telford, 1970b), and it was suggested that exoerythrocytic meronts may have produced them directly, as reported for a rodent parasite, *Plasmodium berghei yoeli* (Killick-Kendrick and Warren, 1968). Two *Cordylus cordylus tropidosternum* were

positive at capture for asexual stages of *P. cordyli* (Telford, 1989), and the lizards remained positive for 49 months in one case and for 36 months in the other. In each lizard, there was a rise in parasitemia during May and June each year, and gametocytes appeared briefly during this period. In the longest surviving lizard, phanerozoites were abundant in the heart and other organs at death. In the very early spring, *Sceloporus occidentalis* with barely detectable gametocytemias and no asexual parasites present had many phanerozoic meronts of *P. mexicanum* present in tissues. The phanerozoites are probably the direct source for the "spring relapse" of gametocytes in *P. mexicanum* (Ayala, 1970b; Bromwich and Schall, 1986).

Gametocytes In many, if not most, saurian *Plasmodium* species, gametocytes appear early in the course of infection, simultaneously with or soon after the appearance of asexual stages, and usually all of the stages are present throughout the course of active infection and in some species during the chronic phase as well. There may be changes in the morphology of gametocytes correlated with the infection phase. Chronic-phase gametocytes may be either smaller or larger than those in active phase and are often rounder (see individual species accounts). In some species, gametocytes are the dominant stage present in established infections, and in others their appearance seems transitory. Gametocytes are seldom seen in infections of *P. rhadinurum* and *P. minasense carinii* of *Iguana iguana* (Thompson and Huff, 1944b; Ayala, 1977) and in *P. cordyli*, as described above. At least in the latter species gametocyte appearance appears to be seasonal, and it is reasonable to suggest that their appearance is related to the seasonal abundance of whatever vector is responsible for the transmission of *P. cordyli*. In two species with marked dominance of gametocytes following the initial acute infection, *P. mexicanum* and *P. chiricahuae* (Jordan, 1970a; Ayala, 1970b; Telford, 1970b), chronic-phase gametocytes are much larger than those in active infection. In its natural host in California, *Sceloporus occidentalis*, *P. mexicanum* gametocytes appeared late in the active phase, 40 and 58 days postcapture, but in experimental sporozoite-induced infections of the unnatural host *Sceloporus undulatus* (Klein, 1985), asexual stages were found on average on day 31, ranging from 26 to 40 days postinfection, while the appearance of gametocytes averaged 39 days, ranging from 32 to 44 days. *Plasmodium mexicanum* infections induced in *S. undulatus* of north Florida produce very high parasitemia and inevitably kill the host. In the natural Mexican host of *P. mexicanum*, *Sceloporus torquatus*, young gametocytes appeared 8 days following asexual stages, but they grew slowly, attaining two-thirds the size of mature gametocytes in 23 days (Peláez et al., 1948). In *P. chiricahuae* of *S. jarrovii*, gametocytes appeared at the same time as asexual stages and quickly attained the length of mature gametocytes but remained more slender for some time (Telford, 1970b). Klein (1985) found asexual stages of *P. floridense* in sporozoite-induced infections of its natural host, *Anolis carolinensis*, present on day 24 postinfection, with gametocytes appearing on days 32 and 36. In blood-induced infections of *P. sasai* in *Takydromus tachydromoides*, asexual parasites appeared on average on day 25 postinoculation, with a range of 7–45 days, while gametocytes were present on average on day 33, ranging from 7 to 51 days and often (19%) present on the day asexual stages appeared (Telford, unpublished).

Few studies have given data on sex ratio of gametocytes. Ayala and Spain (1976) found a ratio of 1.57 in favor of macrogametocytes in a sample of over 900 *P. colombiense* gametocytes. Schall (1989) also found macrogametocytes to be more common in three species of *Plasmodium*: The proportions of microgametocytes in 54 *P. mexicanum* infections of *S. occidentalis* averaged 0.474 (0.25–0.73), in 30 infections of *P. agamae* in *Agama agama* at a proportion of 0.401 (0.29–0.63), and in 30 infections of *P. giganteum* in *A. agama* at a proportion of 0.371 (0.22–0.51), with no evidence of a characteristic proportion of microgametocytes for any species. The sex ratio, however, could remain constant within individual infected lizards over time regardless of changes in gametocytemia.

Ultrastructure of Reptilian Plasmodiids

Sporogonic Stages

Plasmodium In *Plasmodium agamae* (Boulard et al., 1983) developing in the laboratory vector *Culicoides nubeculosus* (Ceratopogonidae), oocysts form between intestinal epithelial cells into which they are invaginated and the basal membrane. A trilaminate wall is present: The outer layer has a fibrous structure, and the innermost layer, the plasma membrane, is thin, and surrounds the cytoplasm of the oocyst. The round or oval nuclei are situated near the periphery of the oocyst. Numerous mitochondria and about ten vesicles containing a granular, electron-dense material surround a clear central zone. Several well-defined pigment grains are visible in oocysts. With maturation, the inner plasma membrane moves away from the outer fibrous wall, and the cytoplasm contained within condenses. Cryptomitosis is centered on a centriolar plate, while sporozoites differentiate in several sites within the oocyst. They have a trilaminar pellicle and two large, elongate rhoptries and lack a conoid in the apical complex. There are 26 subpellicular microtubules distributed along the two halves of the circumference in two groups, one-half containing 18 microtubules, and the other half 8. The

large number of subpellicular microtubules resembles the numbers described for *Leucocytozoon* species, 29–35, and *Haemoproteus* species, 22–23, in contrast to 11–18 found in the mammalian and avian *Plasmodium* species that have been studied (Boulard et al., 1983).

In *Plasmodium mexicanum* (Klein et al., 1988b), oocysts formed extracellularly between epithelial midgut cells and in contact with their basal membrane. As in *P. agamae*, large nuclei are scattered within the cytoplasm but are not especially peripheral in position. Vacuolation of the oocyst indicates the beginning of its differentiation, and sporoblastoids form early. As vacuoles form in the sporoblastoid body, its plasma membrane contracts away from the oocyst capsule. Large vacuoles within the sporoblastoid form and coalesce, producing clefts as they extend to the surface of the sporoblastoid. The clefts divide the cytoplasm of the oocyst into sporoblasts. Sporozoites then bud off the sporoblastoid and become elongate, stout, and crescent-shaped bodies enclosed within a pellicle composed of outer and inner plasma membranes and bilaminate, in contrast to the trilaminate pellicle described by Boulard et al. (1983) for sporozoites of *P. agamae*. Structures present in sporozoites of *P. mexicanum* include polar rings, a cytostome, 2 rhoptries, micronemes, and 14 subpellicular microtubules. As in *P. agamae*, the distribution of the microtubules is asymmetrical, with 9 of 14 contained in one-half of the circumference of the sporozoite, and the remaining 5 in the other half. Although this unequal distribution is similar in both species, there are nearly twice as many (26) subpellicular microtubules present in the sporozoites of *P. agamae* as in *P. mexicanum* (14), with the latter species more consistent with the lower number of microtubules recorded from avian and mammalian *Plasmodium* species.

Oocysts of *P. floridense*, as described by Klein et al. (1988c), have a thick, trilaminate wall, similar to that of *P. agamae*. Oocyst position with regard to the midgut is variable; most oocysts protrude slightly into the hemocoel, but some are "tightly packed between midgut epithelial cells or protruded into the midgut lumen" (Klein et al., 1988c). The structure of undifferentiated oocysts is similar to that described for other *Plasmodium* species. Differentiation of the sporoblastoid and formation of sporozoites resembles that of *P. mexicanum* (Klein et al., 1988b), but daughter sporoblastoids do not form from the sporoblastoid until somewhat later than in *P. mexicanum*. Some sporoblastoids remain after sporozoites complete their differentiation, and the mature oocysts rupture. Sporozoites, elongate and thin as in other *Plasmodium* species transmitted by mosquitoes, have comparable structure to other species but differ in number and arrangement of subpellicular microtubules from those present in the short, stout sporozoites of *P. mexicanum* and *P. agamae*. While within oocysts, there are up to 10 or 11 microtubules arranged asymmetrically in the anterior third of the sporozoite. Two microtubules are located along one surface of the sporozoite, and the remainder are along the opposite surface. Subpellicular microtubules of sporozoites within the salivary glands are infrequently visible in the anterior third of the sporozoite and are clumped together in small groups. Sporozoites of *P. floridense* are intracellular in the salivary gland, similar to those of other mosquito-transmitted *Plasmodium* species, in contrast to the extracellular position of *P. mexicanum* sporozoites, which occupy the lumen of the gland. The extracellular position of *P. mexicanum* sporozoites "may be related to the morphological structure of the salivary gland because the gland of *L. vexator* is only a fluid-filled sac" (Klein et al., 1988b).

Haemoproteus The only ultrastructural studies of a reptilian *Haemoproteus* species are those on *H. "metchnikovi"* by Sterling (1972) and Sterling and DeGiusti (1972, 1974), in which the fine structures of sporogonic stages, merogony, and gametocytes were described.

Sterling and DeGiusti (1974) obtained oocysts of *H. "metchnikovi"* from the midgut wall, and sporozoites from the salivary glands of the natural vector *Chrysops callidus* (Tabanidae). Oocysts form beneath the basement membrane of the midgut epithelium, "deep within the tortuous folds of the fly midgut." The oocyst capsule appears to form from the basement membrane. Young oocysts, 7–9 µm in diameter, have a deeply infolded marginal cell membrane, and their cytoplasm contains abundant ribosomes and some pigment granules along with aggregates of crystalloid material. As oocysts mature, they reach a maximum diameter of 18–20 µm, contain many nuclei, and bulge outward from the midgut epithelium. When differentiation into sporozoites begins, cytoplasm contracts away from the capsule, and regions of thickened membrane appear beneath the cytoplasmic-limiting membrane. Cellular components and nuclei become arranged beneath the thickenings. Sporozoites form from a single sporoblastoid body. The membrane thickenings form part of the double inner pellicular membrane of the sporozoite. A nucleus, mitochondrion, and other cytoplasmic elements move into the sporozoite buds as they form, after which the buds pinch off from the sporoblastoid cytoplasm. This becomes the residual body that contains the persistent remains of the anterior-end complex of the ookinete.

The sporozoite pellicle is comprised of the outer limiting membrane and a double inner membrane complex. A cytostome is present midway between the anterior end of the sporozoite and the subcentrally located nucleus. There are three dense concentric polar rings at the anterior end of the sporozoite, followed posteriorly by a pellicular cavity. The subpellicular microtubules, 23 in number and apparently originating near the cavity, are arranged at

regular intervals around the sporozoite circumference and extend its full length. Two and sometimes three or four rhoptries are present in the anterior portion of the sporozoite, along with small micronemes, near which "crystalloid" material is present in the sporozoite cytoplasm. A mitochondrion and a spherical body 0.3 µm in diameter are present in the cytoplasm near the cytostome. Tubular cristae are contained in the mitochondrion. A double-membrane envelope surrounds the sporozoite nucleus, the outer membrane of which is continuous with Golgi and endoplasmic reticulum located anterior to the nucleus. No nucleolus was observed.

The oocysts and sporozoites of *H. "metchnikovi"* are similar in their morphology to both *Plasmodium* and *Leucocytozoon*, but many of the shared features differ between the last two genera. As in *Plasmodium*, the mature oocysts of *H. "metchnikovi"* lie external to the basement membrane of the midterm epithelium, although their early site lies beneath the membrane, as in *Leucocytozoon* and probably *Plasmodium* as well (Sterling and DeGiusti, 1974). In comparison to the much larger, expanding oocysts of *Plasmodium* species, both *Leucocytozoon* and *Haemoproteus* species of birds form mature oocysts of relatively small size, and *H. "metchnikovi"* is similar to these last genera in this character. The formation of sporozoites around a single sporoblastoid body, as in *Leucocytozoon*, contrasts with the multiple sporoblastoids present in *Plasmodium* oocysts. Although avian *Haemoproteus* species vary in the number of sporozoites formed within oocysts, the 100–200 sporozoites per oocyst of *H. "metchnikovi"* far exceed the numbers produced by oocysts of *Leucocytozoon* and most *Haemoproteus* species. *Plasmodium* sporozoites arise from an initial subcapsular vacuolation, followed by cytoplasmic cleft formation, processes not described for *Leucocytozoon* (Sterling and DeGiusti, 1974), but present in *H. "metchnikovi."* Crystalloid material, although present initially, is not found in differentiated oocysts and sporozoites of *Plasmodium* but is present in these stages of *Leucocytozoon* and *H. "metchnikovi."* The subpellicular microtubules are distributed regularly around the entire circumference of *H. "metchnikovi"* sporozoites and those of *H. columbae* and *L. simondi* (Sterling and DeGiusti, 1974), in contrast to the arrangement found in sporozoites of reptilian, avian, and mammalian *Plasmodium* species, in which most of the microtubules occur in one-half to two-thirds of the circumference, with fewer than half present in the remaining circumference of the sporozoite. In most other structural features, the sporozoites of *Plasmodium, Haemoproteus*, and *Leucocytozoon* species are similar. Although a cytostome is present in sporozoites of *H. "metchnikovi,"* its presence is inconsistent in avian *Haemoproteus* species and in *Plasmodium*, and it is absent in *L. simondi* (Sterling and DeGiusti, 1974). One character apparently unique to the sporozoites of *H. "metchnikovi"* is the presence of a spherical body associated with the mitochondrion.

Intrinsic Stages

The fine structure of reptilian plasmodiid stages within blood cells has been described for four pigmented *Plasmodium* species, two unpigmented *Plasmodium* (*Garnia*) species, one *Fallisia*, *Haemoproteus "metchnikovi,"* and five species of *Haemocystidium*. All later studies support the conclusion indicated by Aikawa and Jordan (1968) that reptilian plasmodiid stages do not differ essentially in either structure or sequence of development from the avian and mammalian *Plasmodium* species. There are some differences, as would be expected among the various species studied, but the relevance of these differences to the systematics of the plasmodiids cannot be given much importance until many more species have been examined, a situation comparable to the use of genome analysis, by which fewer than 10% of the known reptilian *Plasmodium* species have been studied.

Plasmodium In *P. floridense*, the dedifferentiation of merozoites following entry into erythrocytes, ingestion of host cell cytoplasm within food vacuoles, nuclear division, and migration of newly formed organelles into the budding areas of meronts to form merozoites occur as in avian and mammalian *Plasmodium* species; gametocytes have trilaminate membranes as do avian parasites; and in macrogametocytes the greater abundance of ribosomes and rounded osmiophilic bodies distinguish them from microgametocytes (Aikawa and Jordan, 1968). Subsequently, Aikawa et al. (1969) distinguished avian and reptilian *Plasmodium* species by the trilaminate pellicle of gametocytes from those of mammals, in which it is apparently bilaminate; by the distinct mitochondria with cristae of tubular form and rounded osmiophilic bodies in contrast to the mammalian condition of elongated osmiophilic bodies and for which a double membrane-bound structure possibly represents the mitochondria; and by the presence of nucleoli in trophozoites and gametocytes of the avian and reptilian species, which do not occur in mammalian *Plasmodium* species.

The ultrastructure of *Plasmodium tropiduri* was described in detail by Scorza (1971a). Only two important differences were found between *P. tropiduri* and *P. floridense*, as described by Aikawa and Jordan (1968). Merozoites in the latter species formed a stellate structure, with their anterior ends projecting toward the cytoplasm of the host erythrocyte, which was not typical of *P. tropiduri*, and the rounded structures present in merozoites of *P. floridense* were not present in *P. tropiduri*. In gametocytes of *P. tropiduri*, the clear vacuole, commonly seen in other saurian *Plasmodium* species, was associated with

the endoplasmic reticulum, thereby possibly connecting to the cell surface, and Scorza suggested that its function might be osmoregulatory or as a reservoir of water. Merozoites containing similar vacuoles might become gametocytes following completion of merogony. Gametocytes of *P. tropiduri* contained an apparent exflagellatory apparatus, similar to coccidian and avian *Plasmodium* species. The *P. floridense* material examined by Aikawa and Jordan (1968) contained few gametocytes, which might account for their failure to report the exflagellatory apparatus. Scorza considered *P. tropiduri* to be closely related to *P. floridense* and to avian *Plasmodium* species. Both asexual and sexual stages, apparently of *P. tropiduri*, occur in thrombocytes of *Tropidurus hispidus*, in addition to the far more common erythrocytic infections (Scorza, 1970b). Except for the absence of pigment, the thrombocytic parasites were similar in ultrastructure to those occupying erythrocytes, and the structure of the large, uninucleate thrombocytic forms clearly indicated their identity as gametocytes.

Moore and Sinden (1973) studied the ultrastructure of *P. mexicanum* and found close similarity to both *P. floridense* and *P. tropiduri*. The rounded, moderately electron transparent bodies in merozoites of *P. floridense* were not present in *P. mexicanum* and *P. tropiduri*, and the merozoites of the last two species did not project beyond the original area occupied by the meront into the host cell cytoplasm, which was reported for *P. floridense*. There was greater similarity of the micropores in the trophozoites of *P. mexicanum* to those of *P. floridense* than to those present in *P. tropiduri*, but their size was about one-half the internal diameter in *P. mexicanum* than in *P. floridense*. *Plasmodium mexicanum*, and apparently *P. floridense* as well, lacked the umbrella-shaped structure associated with gametocyte micropores in *P. tropiduri*. In all three species, there was greater density of ribosomes in macrogametocytes than in microgametocytes.

In their study of a *P. tropiduri*-like species from *Kentropyx calcarata* in Brazil, Paperna and Lainson (2002) clarified the origin of the outermost membrane surrounding all stages of the parasite as the boundary of the parasitophorous vacuole "which becomes detached in processing" (Aikawa, 1971). In gametocytes, the two inner membranes were of equal size, in comparison to other species in which the middle membrane may be very thin. Although the "general ultrastructure" is in conformity with the reptilian and avian species of *Plasmodium* so far studied, Paperna and Lainson found that their transmission electron microscopic (TEM) images did not "clearly resemble" the ultrastructural description of *P. tropiduri* as described by Scorza (1971a), suggesting that the *P. tropiduri*-like parasite differs taxonomically. Cytoplasm of the trophozoites contained a mitochrondrion and cytoplasmic clefts. Of possible importance to the studies cited below, a large food vacuole remained in the meront residuum after merozoites were formed, as in *P. mexicanum* (Moore and Sinden, 1974), and the emerging merozoites contained a "large, conspicuous cytostome as described for *P. tropiduri* (Scorza, 1971) and *P. floridense*" (Aikawa and Jordan, 1968). Gametocytes, however, apparently lack cytostomes, although these usually are visible in that stage (Aikawa et al., 1969). Pigment granules "were conspicuous only in the meront residuum and in its food vacuole," their scarcity possibly due to age of the infection or processing of the material.

Garnia and Fallisia Ultrastructural evidence from their study of *Garnia gonatodi, G. uranoscodoni*, and *Fallisia effusa* was considered by Boulard et al. (1987) to support the familial distinction of those genera, as Garniidae, from the Plasmodiidae, Haemoproteidae, and Leucocytozoidae. Their basic structure demonstrates their haemosporidian identity. Absence of pigment from the erythrocytic *Garnia* species, and of course from the thrombocytic *Fallisia effusa*, as well as absence of a vacuolar digestive system showing phagocytic or pinocytic activity in both *Garnia* and *Fallisia* contrast to their presence in plasmodiids. Instead, a transfer of nutrients across the pellicular complex is suggested. In comparison to the location of centrosomes within the cytoplasm of the *Plasmodium, Haemoproteus, Parahaemoproteus,* and *Leucocytozoon* species that have been studied, caryokinesis arises from a centriolar plaque situated within a nuclear pore in *F. effusa* gametocytes and *G. gonatodi* schizonts (Boulard et al., 1987). Primary emphasis was placed by these authors on the absence of pigment and apparent absence of a vacuolar digestive system to justify the distinction of *Garnia* and *Fallisia* at the familial level. A subsequent study of the ultrastructure of *Garnia gonatodi* (Diniz-Jose et al., 2000) again confirmed the absence of hemozoin and the presence of ultrastructural features common to other genera of apicomplexans. Besides the presence of subpellicular microtubules, other microtubules were associated with the mitochondrion. A structure resembling the acidocalcisome of trypanosomatid flagellates was present, and the endoplasmic reticulum of the host erythrocyte, particularly the cisternae, was strongly associated with the parasitophorous vacuole. Although the previously described absence of a vacuolar digestive system in *G. gonatodi* (Boulard et al., 1987) was not mentioned in this study, it is interesting that a cytostome was present in trophozoites, and electron-dense material "similar to that found in the host erythrocyte cytoplasm" was present within it.

Silva et al. (2005) further studied the ultrastructure of *Fallisia effusa*, demonstrating that thrombocytes parasitized by gametocytes showed a distinctive "circumferential coil of microtubules," in comparison to uninfected thrombocytes in which microtubules were arranged as "bundles."

Macrogametocytes were bordered by "a four-layered pellicle composed of a plasma membrane and a three-layered inner complex, formed by three closely-apposed unit membranes." Microgametocytes, in contrast, were less electron dense than macrogametocytes and had a two-layer pellicle formed by "the plasma membrane and underlying membrane complex." Macrogametocytes also displayed invaginations of the pellicle that were sometimes deep, crossing large areas of the gametocyte. No cytostome was observed. The ultrastructure of meronts was similar to that of other plasmodiid species studied by electron microscopy. The authors believed that the ultrastructure of *F. effusa* had "characteristic features distinguishing it from other members of the Haemosporidian families," but the systematic significance of the described ultrastructural differences must await confirmation by study of other *Fallisia* species.

Haemocystidium The fine structure of gametocytes from five species of *Haemocystidium*, considered by the authors to be *Haemoproteus*, was described by Paperna and Boulard (2000). The species studied represented much of the geographic distribution of this saurian plasmodiid genus: Europe (*H. tarentolae*), the area of Cisjordan in western Asia (*H. edomensis, H. ptyodactyli*), and Australia (*H. oedurae, H. mackerrasi*). Except for *H. edomensis*, an agamid parasite, all are from gekkonid hosts. The ultrastructure of the gametocytes from these *Haemocystidium* species was consistent with that shown for the other reptilian plasmodiids studied, *Plasmodium floridense* (Aikawa and Jordan, 1968), *P. mexicanum* (Moore and Sinden, 1974), *P. tropiduri* (Scorza, 1971a), *P. gonatodi* and *Fallisia effusa* (Boulard et al., 1987), *F. copemani* (Paperna and Boulard, 1990), and *Haemoproteus "metchnikovi"* (Sterling, 1972).

All plasmodiid species of reptilian and avian hosts thus far studied have a gametocyte pellicle comprised of three layers. Much of the intraspecific variation appears to result from differences in gametocyte sex and age (juvenile, differentiated, "waiting", senile). The two species from Cisjordan, *H. edomensis* of agamids and *H. ptyodactyli* from gekkonids, were the most similar in structure. *Haemocystidium edomensis* was distinguished from the other species by presence of "An electron-dense, trapezoid body with a granular medium-electron-dense halo bordered by electron-dense droplets" (Paperna and Boulard, 2000). Paperna and Boulard suggested that it may represent a food vacuole, and is perhaps homologous to the "lipid-like" vesicle in *H. "metchnikovi"* (Sterling, 1972). TEM images of *H. tarentolae* gametocytes contained a "large electron-lucent central space," which may represent a "poorly stained zone" commonly seen in Giemsa-stained gametocytes of both sexes, reported as a "cisterna" by Paperna and Landau (1991).

Haemoproteus "metchnikovi" The ultrastructure of gametocytes and gametogenesis of *H. "metchnikovi"* were described by Sterling (1972) apparently from naturally infected turtles.

Gametocytes The structure of gametocytes is essentially similar to that of other species of reptilian and avian haemosporinids. The pellicle is trilaminate, with the inner membrane formed by two "closely apposed" membranes. Macrogametocytes have a more granular cytoplasm, resulting from the presence of numerous ribosomes; more numerous osmiophilic bodies in the cytoplasm; a more extensive, granular endoplasmic reticulum, often appearing as vesicular bundles that apparently store lipid material; more numerous mitochondria, which possess tubular cristae; and a more compact nucleus that contains a nucleolus. In microgametocytes, the cytoplasm is less granular; there are fewer osmiophilic cytoplasmic bodies and mitochondria; less-prominent vesicular bundles associated with endoplasmic reticulum; and a more poorly defined, although membrane-bound, nucleus that lacks a nucleolus. Gametocytes of both sexes contain food vacuoles surrounded by a single membrane, within which the digestion of hemoglobin leaves behind hemozoin granules. A single cytostome is found in gametocytes, surrounded by two electron-dense rings.

Gametogenesis Atypical centrioles are present in both young and mature gametocytes of both sexes and are comprised of nine peripheral tubules "with electron-dense fibers which merge at a single central tubule." The aggregation of electron-dense nuclear material near an atypical centriole signals the beginning of microgametogenesis. This aggregate is associated with microtubules within the nucleus that "end at the nuclear membrane at a dense plaque which may correspond to the atypical centriole." Basal bodies adjacent to atypical centrioles are attached to variably developed axonemes. Axonemes variably assembled are found in the cytoplasm before the gametocyte becomes extracellular. Their structure is comprised of "9 peripheral doublets of microtubules arranged around two central tubules" and appears the same in the microgametes. Development of many axonemes can be completed simultaneously near the nucleus periphery in the extracellular microgametocyte. At the periphery of the irregularly shaped nucleus, electron-dense nuclear material in aggregates apparently "leave the nucleus and assemble around the developing axonemes." After passing through the inner membranes of the microgametocyte,

the nuclear buds become surrounded by the outer membrane, which possibly contributes to the membrane surrounding the individual microgamete. Microgametocytes contain "a single axoneme … surrounded by a centrally located nucleus" and apparently lack mitochondria when mature. Overall, microgametogenesis of *H. "metchnikovi"* resembles that described for *Leucocytozoon simondi* and *Haemoproteus columbae* (Sterling, 1972), although timing of some events may differ in *H. "metchnikovi."* The direct budding of microgametes from the microgametocyte of *H. "metchnikovi"* is similar to that of *H. columbae* but differs from the microgametogenesis of *L. simondi*. There is some disparity in reports of axoneme numbers in microgametes, one or two in *L. simondi*, two in *H. columbae*, and one in *H. "metchnikovi,"* although a few microgametes of *H. "metchnikovi"* did contain more than one axoneme (Sterling, 1972).

Merogony Tissues from naturally infected turtles were examined by Sterling and DeGiusti (1972) to describe the merogony of *H. "metchnikovi."* Both merozoites and meronts were found only in tissues of the spleen. The pellicle of histotropic merozoites is comprised of three layers, beneath which lies a layer of subpellicular microtubules. These extend into a clear pellicular cavity in the anterior portion of the merozoite where the innermost membrane layer terminates. The nucleus, oval or round in shape, is located in the posterior region, and a large food vacuole containing membrane-bound "boluses" is present anterior to the nucleus. Within the boluses are particles that resemble ribosomes of the host cell cytoplasm. A cytostome may be present on the merozoite surface, near the food vacuole. Dedifferentiation of tissue merozoites into trophozoites is marked by disappearance of the thickened inner membrane and anterior-end complex, and the appearance of microvillus-like projections around the outer membrane. Cytoplasm of the trophozoite is surrounded by the middle membrane after disappearance of the inner membrane. Mitochondria, food vacuoles, and a "chromatinlike area" without a clear nuclear envelope are present in the trophozoite, and apparently as merogony approaches, microtubules radiate toward the chromatin-like area from a centriolar plaque.

As young meronts grow, nuclear division occurs, and the cytoplasm divides to form "discrete islands." Numerous ribosomes form a granular matrix within the islands, accompanied by less-granular areas at their periphery that contain chromatin-like material. Nuclear division is suggested by the presence of centriolar plaques and spindle fibers in the less-granular areas. Merozoite formation within mature megalomeronts results from "increased nuclear multiplication, cytoplasmic vacuolation, and differentiation within the islands" of the meront. Beneath the outer limiting membrane of the vacuolated islands, membrane thickenings occur in proximity to nuclei within the islands. Nuclear division and merozoite budding proceed at the same time, as indicated by centriolar plaques and spindle microtubules in association within nuclei. The conoids form from differentiation of the limiting membrane and thickened membranes at the free end of the merozoite, and "subpellicular microtubules extend posteriorly from the region of the polar rings." The limiting membranes of the multinucleated island give rise to the cytostome, which is incorporated into the merozoite pellicle. As budding merozoites grow outward, a thickened membrane complex develops and terminates where merozoites attach to the islands of the meront. As merozoite budding is completed, a mitochondrion and other elements within the cytoplasm become included within the body of the merozoite, and the inner membrane complex extends around the posterior portion of the merozoite bud. The completed merozoites are 2.5 × 1 μm in average size. Their structure is similar to that of the tissue merozoites at the beginning of dedifferentiation into trophozoites: an outer limiting membrane, two closely opposed membranes forming the inner membrane complex, and three polar rings above the clear pellicular cavity, on the top of which and to the exterior the inner membrane complex terminates. Microtubules, along one side only of the merozoite, originate near the pellicular cavity and extend at least one-half of the merozoite length. Elongate, tear-shaped paired organelles are present at the anterior end, associated with micronemes. At midbody of the merozoite, a mitochondrion with tubular cristae is usually bent around the associated spherical body. There is also a cytostome in the midregion of the merozoite. The oval or round nucleus is located in the posterior third of the merozoite, with chromatin material concentrated around its periphery. There are numerous ribosomes within the cytoplasm of the merozoite. When merozoites enter erythrocytes, they undergo a dedifferentiation similar to that of the histotropic merozoite when it becomes a trophozoite. The megalomeronts may contain both vacuolated islands and fully formed merozoites. Megalomeront margins often appear with irregular folds and have many uniformly spaced "microvilluslike projections" extending outward from the perimeter. These projections contain a central core. Megalomeronts are enveloped by extensive giant cell formation, and the perimeter of the granuloma within which the meront lies is comprised of giant cell fibers and fibroblast-like cells.

The pattern of exoerythrocytic merogony of *H. "metchnikovi"* resembles that demonstrated for *Plasmodium fallax* in vitro (Sterling and DeGiusti, 1972), that is, dedifferentiation, growth, and redifferentiation phases.

> Merozoite formation and structure … is similar in most respects to in vivo … descriptions of exoerythrocytic schizogony and merozoites in other malaria and malarialike parasites. Merozoite formation and structure in *H. "metchnikovi"* also show similarities with descriptions of erythrocytic schizogony in the Plasmodiidae. … The characteristic structures within merozoites including the polar rings, subpellicular microtubules, paired organelles, micronemes, mitochondrion, spherical body, cytostome, and nucleus, are observed in *H. "metchnikovi"* merozoites. In addition, there is a clear pellicular cavity which extends around the anterior end of the merozoite and appears to be similar to the pellicular cavity described in *H. columbae* sporozoites. (Sterling and DeGiusti, 1972)

Taxonomic Characters

The descriptions of most reptilian haemosporinid species prior to the late 1960s were inadequate, primarily verbal, and contained little, if any, quantitative data that would permit comparisons with other species. In areas where the diversity of saurian *Plasmodium* species is great, notably the neotropics and East Africa, only morphometric comparisons could have effectively separated samples into species with distinctive and characteristic morphology. In a supplementary role, the effects of the parasite on host cells can be useful, but not primary. And, with the methods of genomic identification available today, species can be readily separated from each other, although the common presence of mixed infections can cause complications. Nevertheless, morphology visible under light microscopy is essential in the definition and description of species, whatever relevance it may possess to genetic relationships among a fauna. A species description must be based on the measurement of adequate series of mature sexual and asexual stages, that is, gametocytes and meronts. Telford (1974) listed both direct and indirect characters useful in making taxonomic decisions. The direct characters include those derived from measurement or obtained from calculations of those measurements: the length and maximum width of gametocytes and meronts in micrometers, the size or LW value (Length × Maximum width) stated as square micrometers, the shape or L/W ratio, and the merozoite numbers present in mature or nearly mature meronts. These quantitative data should be expressed as the mean ± 1 SD (standard deviation) and range. Shape can be verbally described as round, oval, elongate, lentiform, or bulky, and the arrangement of merozoites in meronts as a fan, rosette, morulum, cruciform, or amorphous. Describe the presence and relative quantity of pigment, its distribution as scattered granules or clumped as masses, and pigment color. Relative characters are host cell type; position of the parasite within the host cell usefully described as lateral, lateropolar, polar, halteridial, or filling most of the host cell; and the relative size of the gametocyte and meront to the host cell nucleus (LW/HNLW) and to the nuclei of uninfected erythrocytes (LW/NNLW). Indirect characters are hypertrophy or hypotrophy of the host cell, distortion of the host cell and its nucleus, and displacement of the nucleus.

Species Accounts

HAEMOSPORIDIA, Plasmodiidae

PLASMODIUM SPECIES OF AFRICAN LIZARDS

AFRICAN *SAURAMOEBA* SPECIES

Plasmodium michikoa Telford 1988 (**Plate 1**)

Diagnosis A *Plasmodium* (*Sauramoeba*) species with variably shaped meronts 6–15 × 4–8 µm, and LW 28–78 µm² that produce 12–32 merozoites. Meronts are usually erythrocytic, but when proerythrocytes are parasitized, there is no difference in meront dimensions or number of merozoites produced. Meront size relative to host cell nucleus averages 1.91, and to normal erythrocyte nuclei is 1.56. Gametocytes are usually elongate, 6–14 × 4–8 µm, with LW 36–80 µm² and L/W 1.0–3.5. Gametocyte size relative to host cell nucleus is 1.81, and to normal erythrocyte nuclei is 1.75. The golden pigment granules are usually dispersed within mature meronts and macrogametocytes and tend to be marginal in microgametocytes. Both meronts and gametocytes cause hypotrophy of host erythrocytes and their nuclei. Gametocytes are not sexually dimorphic in size, but macrogametocytes are more elongate than microgametocytes.

Type Host *Bradypodion oxyrhinum* Klaver and Böhme (Sauria: Chamaeleonidae) (syn. *Chamaeleo tenuis* of Telford, 1988b).

Type Locality Eastern Udzungwa Mountains above Sanje, Kilombero District, Morogoro Region, Tanzania.

Other Hosts None known.

Other Localities None known.

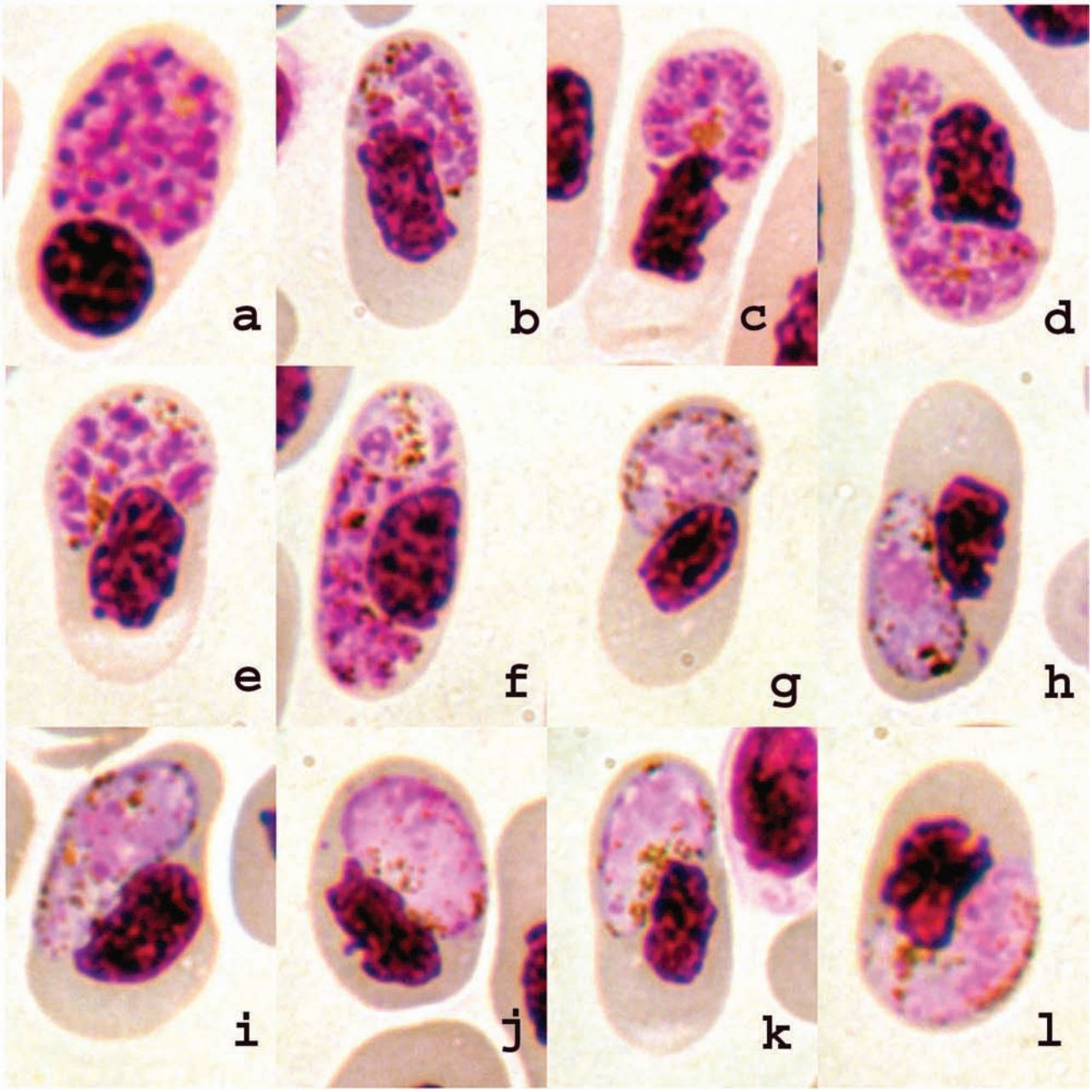


Plate 1 *Plasmodium michikoa* from *Bradypodion oxyrhinum* of Tanzania. Meronts, **a–f**; macrogametocytes, **g–i**; microgametocytes, **j–l**.

Prevalence The only *B. oxyrhinum* examined by Telford (1988b) was host to *P. michikoa*.

Morphological Variation Meronts are variably shaped, 8.4 ± 1.9 × 6.2 ± 1.0 µm (6–15 × 4–8, N = 75), with LW 51.2 ± 10.8 µm² (28–78), and contain 19.6 ± 3.8 (12–32) merozoites. Meront size relative to host cell nucleus is 1.91 ± 0.66 (0.8–4.0, N = 61), and to normal erythrocyte nuclei is 1.56 ± 0.40 (0.70–2.6). Meronts usually parasitize erythrocytes, but when proerythrocytic, neither meront dimensions nor number of merozoites produced differs. Golden pigment granules or small clumps are usually dispersed among merozoites in the meronts, sometimes clustered. Gametocytes are 9.9 ± 1.6 × 5.7 ± 1.0 µm (6–14 × 4–8, N = 105), with LW 56.1 ± 9.2 µm² (36–80) and L/W 1.82 ± 0.56 (1.0–3.5). Gametocyte size relative to host cell nucleus averages 1.88 ± 0.47 (1.0–3.2, N = 77), and to normal erythrocyte nuclei is 1.75 ± 0.38 (0.88–2.57, N = 105). Gametocytes do not differ sexually in size, but macrogametocytes are more elongate than microgametocytes: 10.3 ± 1.7 × 5.6 ± 1.0 µm (6–14 × 4–8, N = 57), LW 56.4 ± 9.8 µm² (36–80), and L/W 1.93 ± 0.59 (1.0–3.5) versus 9.5 ± 1.4 × 5.9 ± 1.0 µm

(7–12 × 4–8, N = 48), LW 55.6 ± 8.6 µm² (40–77), and L/W 1.68 ± 0.49 (1.0–3.0), respectively. Small, dark golden pigment granules are dispersed in macrogametocytes and usually lie along microgametocyte margins.

Exoerythrocytic Merogony Large phanerozoites were present in a fulminating mixed infection of *P. michikoa* and *P. gologoloense* but cannot be assigned to either species with confidence. Similarly, meronts present in thrombocytes might belong to either species. Free EE meronts appeared in circulating blood 5 days before the host died and infected polymorphonuclear leukocytes 2 days prior to death, while cardiac blood at death contained many heavily infected, large macrophages (Telford, 1988b), but examination of liver, lung, and spleen sections and smears showed no parasites in fixed cells.

Sporogony Unknown.

Effects on Host Erythrocytes host to both mature meronts and gametocytes, and their nuclei, are hypotrophic in size. Host cells of meronts and their nuclei are often distorted and nuclei displaced. Erythrocytes infected by gametocytes are seldom distorted, and their nuclei rarely so, but the latter were usually displaced (Telford, 1988b).

Remarks The rarely encountered host species has a disjunct range in the Uluguru and Udzungwa mountains of Tanzania, at a height of 1400–1900 m (Spawls et al., 2002). The type host specimen was collected in montane forest at 2100 m.

Plasmodium giganteum Theiler 1930 (**Plate 2**)

Diagnosis A *Plasmodium* (*Sauramoeba*) species characterized by rounded or ovoid meronts 9–18 × 4–11 µm, with LW 52–165 µm², that produce 28–74 merozoites, occasionally nearly 100. Meront size relative to host cell nucleus size is 2.0–5.9 and to normal erythrocyte nucleus size is 1.9–8.0. Pigment usually forms from small yellowish-brown granules into a large, blackish-brown mass. Gametocytes are nearly round to elongate or bulky, 9–22 × 4–10 µm, with LW 45–145 µm² and L/W 1.1–5.0. Gametocyte size relative to host cell nucleus size is 1.2–5.3, and to normal erythrocyte nuclei is 1.6–6.3. Pigment is dispersed in both gametocyte sexes, and forms as yellowish-brown granules. In older active and in chronic infections, gametocytes are dimorphic in dimensions, with macrogametocytes averaging larger in width and LW, sometimes in length, than microgametocytes.

Type Host *Agama agama* (Linnaeus) (syn. *A. colonorum*) (Sauria: Agamidae).

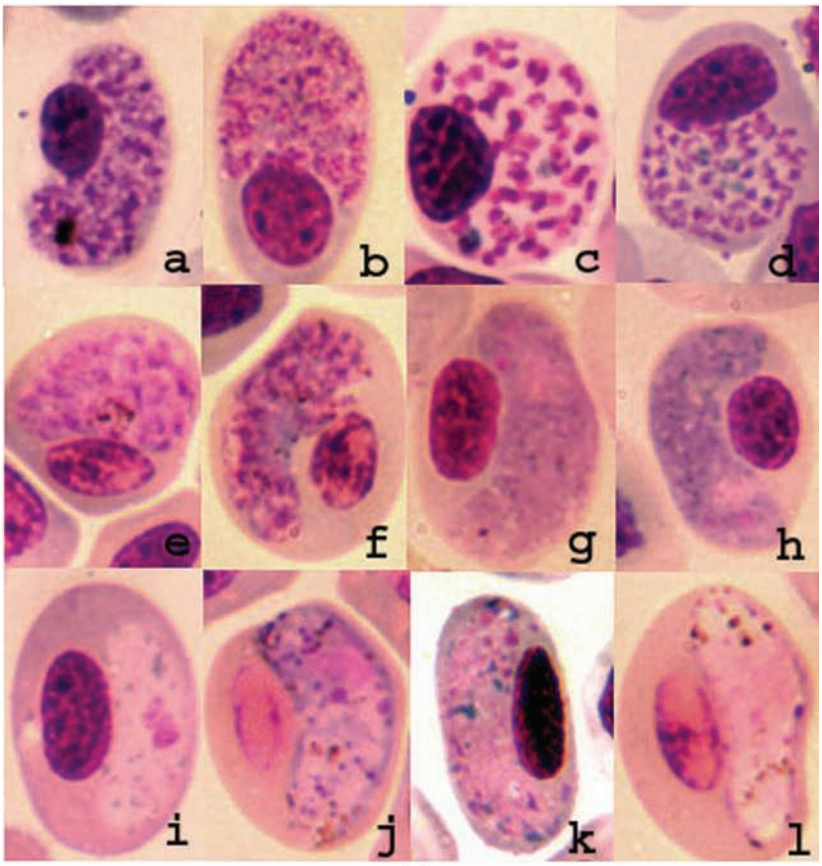
Type Locality Gbanga, Liberia, West Africa.

Other Hosts *Agama mossambica* (Telford), *A. cyanogaster* (Southgate, 1970).

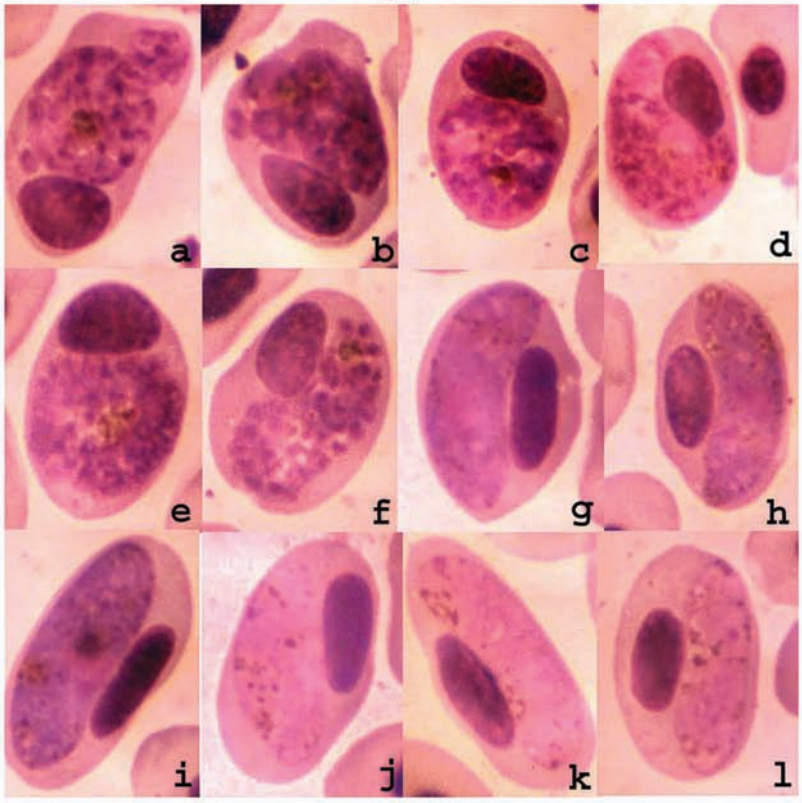
Other Localities Nigeria (Macfie, 1914); Sierra Leone (Adler, 1924, as *P. agamae*; Garnham, 1966), Freetown (Garnham, 1966) and 22 other localities of Sierra Leone (Schall and Bromwich, 1994); Mali (Rousselot, 1953, as *P. agamae*); Harbel, Liberia (Bray, 1959); 12 km west southwest of Kinshasa, Kinsuka, on Congo River, Republic of the Congo (Telford); Marimonte (Ball, 1967a) and Pole, Kacheliba (Mutinga [Telford, 1994]), Bondo, Siakago, Kiambere, and Kiboku, Kenya (Southgate, 1970); Gonja, Pare Mountains (Pringle, vide Ball, 1967a), and Morogoro, Tanzania (Telford).

Prevalence In *A. agama*, the prevalence is as follows: Liberia, 2 of 30 (6.7%; Theiler, 1930), and 6 of 21 (28.6%; Bray, 1959); Charlesville, 36 of 139 (36%); Harbel, 2 of 28 (7%); Uoinjama, 1 of 12 (8%; Baker, 1961); Congo, 3 of 21 (14.3%; Telford); and Kenya, 3 of 48 (6.3%; Ball), and 2 of 77 (2.6%; Mutinga Collection [Telford, 1994]). In *A. cyanogaster*, the prevalence in Kenya, 6 of 125 overall, 6 of 57 (10.5%) in four positive localities (Southgate, 1970). In *A. mossambica*, prevalence is 25 of 69 (36.2%; Telford) in Tanzania.

Morphological Variation No dimensional data were provided by Theiler (1930), Bray (1959), or Ball (1967a). Garnham (1966) described macrogametocytes of *P. giganteum* as 16 to 18 µm in length and 6 or 7 µm in width, and microgametocytes as 12 × 8 µm. Meronts were said to reach 12 µm in diameter and produce 24–96 merozoites; the latter number was reported by Bray (1959). In active infection of *A. agama* from Sierra Leone, gametocytes were 15.8 ± 1.5 × 7.0 ± 0.9 µm (12–19 × 6–10, N = 50), with LW 109.3 ± 15.2 µm² (84–144), and L/W 2.31 ± 0.39 (1.3–3.2). Gametocytes in active infection of *A. agama* in Congo were 14.6 ± 2.0 × 5.7 ± 1.2 µm (9–20 × 4–9, N = 50), with LW 81.7 ± 17.4 µm² (45–104) and L/W 2.69 ± 0.71 (1.1–5.0). In a chronic-phase *A. agama* from Kenya, gametocytes were 17.9 ± 1.9 × 7.0 ± 1.0 µm (13–21 × 5–10, N = 25), with LW 125.1 ± 21.0 µm² (90–184.5) and L/W 2.62 ± 0.49 (1.4–3.7). Active-phase gametocytes in *A. mossambica* of Tanzania were 14.9 ± 1.9 × 5.8 ± 0.8 µm (10–22 × 4–8, N = 75), with LW 85.4 ± 14.5 µm² (52–132) and L/W 2.65 ± 0.58 (1.4–4.5). Macrogametocyte width and LW were greater than in



(A)



(B)

Plate 2 (**A**) *Plasmodium giganteum* from *Agama agama* and *A. mossambica*. Meronts, **a–f**; macrogametocytes, **g**, **h**, **j**; microgametocytes, **i**, **k**, **l**. Origin: *A. agama* from **a**, **b**, **g**, **h** Nigeria, and **c**, **d**, **i**, **k** Congo; *A. mossambica* from Tanzania **e**, **f**, **j**, **l**. (**B**) *Plasmodium* sp. *cf. giganteum* from *Mabuya striata* of Kenya. Meronts, **a–f**; macrogametocytes, **g–i**; microgametocytes, **j–l**.

microgametocytes in the samples from Sierra Leone (16.2 ± 1.8 µm length, 7.4 ± 1.0 µm width, and 118.0 ± 14.8 µm² LW vs. 15.4 ± 1.1 µm, 6.6 ± 0.5 µm, 100.7 ± 10.0 µm², respectively) and in Kenya were greater in length and LW but not width (18.7 ± 1.1 µm, 7.1 ± 1.0 µm, 133.2 ± 22.0 µm² vs. 16.9 ± 2.3 µm, 6.9 ± 1.1 µm, 114.8 ± 14.9 µm², respectively). Macrogametocytes from *A. mossambica* had greater average values in length, width, and LW (15.5 ± 2.0 µm, 6.0 ± 0.8 µm, 97.5 ± 13.6 µm²) than did microgametocytes (14.3 ± 1.6 µm, 5.5 ± 0.8 µm, 78.2 ± 11.7 µm², respectively). In two apparently younger active infections of *A. agamae* from Congo, there were no dimensional differences in any characters: Macrogametocytes were 14.6 ± 1.7 µm, 5.7 ± 0.9 µm, and 83.0 ± 11.9 µm² versus 14.5 ± 2.4 µm, 5.6 ± 1.4 µm, and 80.4 ± 21.7 µm² in microgametocytes. Regardless of infection phase, host, or locality, gametocyte shape as indicated by L/W ratio did not differ by sex. Schall (1989) also reported macrogametocytes of *P. giganteum* as larger than microgametocytes in size (presumably LW).

Meronts averaged 11.2 ± 0.9 µm × 9.4 ± 0.7 µm (10–14 × 8–10, N = 25), with LW 105.5 ± 9.1 in Sierra Leone, and 10.8 ± 1.4 × 8.5 ± 1.3 µm² (9–16 × 4–11, N = 47), LW 90.6 ± 15.3 µm² in Congo and produced 48.3 ± 5.6 (39–61) and 42.2 ± 7.5 (28–66) merozoites, respectively. In Tanzanian *A. mossambica*, meronts were 13.1 ± 2.1 × 7.6 ± 1.4 µm² (9–18 × 5–11, N = 50), with LW 98.9 ± 21.2 µm² (72–165), and contained 41.0 ± 8.2 (28–74) merozoites. Relative to host cell nuclei, meront averages are 3.0–3.4, and to normal erythrocyte nuclei are 3.2–4.7, while gametocyte averages are 2.6–3.7 and 3.3–4.7, respectively.

Exoerythrocytic Merogony Bray (1959) reported two EE meronts from *A. agama* in Liberia. One, vermicular in shape, occupied a endothelium cell in brain capillary and contained 48 nuclei. The other, in a fixed macrophage cell of the liver, had 16 nuclei. Tissue smears of liver from *Agama mossambica* had both large and small meronts in abundance (**Plate 11B**), but these could not be identified as *P. giganteum* because of the presence of *P. "agamae."*

Sporogony Baker (1961), in Liberia, fed four *Aedes* species (*aegypti, simpsoni, apicoargenteus, africanus*) on *A. agama* infected with either *P. giganteum* or *P. agamae*. In a single *A. aegypti*, male and female gametes were seen in a smear of stomach contents about 15 minutes after ingesting *P. giganteum*. The macrogamete was oval, 10.3 × 7.4 µm, and an adjacent microgamete was about 15 µm in length. Ookinetes were found in six *A. aegypti* examined 1 day postfeeding (PF) and in four at 2 days. Ookinetes were 16.3 × 3.4 µm² (14.3–20.3 × 2.3–4.6, N = 10) and contained "a relatively large amount of pigment."

Effects on Host Schall (1990b) compared hematological parameters and oxygen consumption for *A. agama* from Sierra Leone infected with *P. giganteum* alone, mixed infections with *P. agamae*, and uninfected lizards. Uninfected lizards had 0.574% immature erythrocytes in their blood, on average, but in lizards infected with *P. giganteum* alone, the percentage of immature blood cells was 4.85%; in mixed infections, it was 5.91%. Normal hematocrit values were little different in these comparisons, as were hemoglobin concentrations. There were slight but significant increases in oxygen consumption for both categories of infected lizards. Running stamina was not affected by *P. giganteum* alone, but in mixed infection there was a significant reduction in capacity. Infection in either category did not affect ovary or testis mass or the prevalence of broken or regenerated tails, as an indication of predator evasion. In all samples of *P. giganteum*, gametocytes distorted 96–100% of host cells and displaced 93–100% of their nuclei. Erythrocytes of *A. agama* host to gametocytes were 2–13% larger than uninfected cells, but hypertrophy was greater in *A. mossambica* infected by *P. giganteum*, with 25–28% enlargement. Erythrocyte nuclei were distorted in 92–100% of cells in samples from *A. agama* but in only 16% of cells from *A. mossambica*. In both host species, the nuclei of infected erythrocytes were hypertrophied, by 30–36% in *A.* agama and by 26–52% in *A. mossambica*. Meronts in all samples of *P. giganteum* distorted 93–100% of host cells and displaced their nuclei, distorting nuclei in 96% and 98% in Sierra Leone and Congo, respectively, but only in 80% int *A. mossambica*. Erythrocytes of *A. agama* in one infection from Congo, parasitized by meronts, were enlarged by 11% and their nuclei by 28%. Erythrocytes host to *P. giganteum* meronts in *A. mossambica* were hypertophied by 23–26% and their nuclei by 35–44% over normal size. In *A. agama* from Sierra Leone and Congo, 32% and 69% of meronts, respectively, parasitized proerythrocytes, but only 4% of cells containing meronts in *A. mossambica* were immature. In the Congo samples, 35% of gametocytes occupied immature host cells, but all cells host to gametocytes in the Sierra Leone sample were erythrocytes only. Only 5% of gametocytes were proerythrocytic in the chronic infection from Kenya, and all gametocytes occupied erythrocytes in *A. mossambica*.

Remarks Schall and Bromwich (1994) examined 4772 blood smears from 2870 *A. agama* in Sierra Leone and apparently found an overall prevalence of 12.1% of *P. giganteum* (577 infections, their Table 2). Although not clearly stated, the authors presumably examined multiple slides of some lizards until they were satisfied about species present. In an analysis of ten single-species infections each of *P. giganteum* and *P. agamae*, they concluded that their data showed that *P. giganteum* primarily used immature

host cells, and *P. agamae* occupied mature erythrocytes, leading to the conclusion that the presence of *P. agamae* infection in a lizard facilitated establishment of infection by *P. giganteum*, thus demonstrating that "*Plasmodium* species form interactive assemblages" (Schall and Bromwich, 1994). This conclusion is not strongly supported by the infections of *A. agama* from Sierra Leone and Congo reported above and not at all by infections of *P. giganteum* in *Agama mossambica*, where *P. giganteum* seldom to rarely infected proerythrocytes: 36 of 69 *A. mossambica* were infected by *Plasmodium* species, with single infections by *P. giganteum* present in 11, only *P. "agamae"* infections were in 13, and 12 lizards had mixed infections of the two species.

Plasmodium heischi Garnham and Telford 1984 (**Plate 3A**)

Diagnosis A *Plasmodium* (*Sauramoeba*) species with large, spindle-shaped gametocytes in which the nuclei usually occupy subterminal positions. Gametocytes are 8–12 × 4–9 µm, with LW 60–120 µm² and L/W 1.5–4.8. Their size relative to host cell nucleus size is 2.1–6.3, and to normal erythrocyte nuclei is 3.1–6.9. Meronts are elongate, often nearly halteridial, 8–18 × 6–11 µm, with LW 48–144 µm², and produce 20–65 merozoites. Meront size relative to host cell nucleus size is 1.8–5.3, and to normal erythrocyte nucleus size is 2.8–8.3. Although width does not differ, gametocytes are sexually dimorphic in length and LW, with macrogametocytes larger than microgametocytes, with the latter less elongated in shape. Pigment is dispersed as dark granules in gametocytes but tends to form small clumps in meronts.

Type Host *Mabuya striata* (Peters) (Sauria: Scincidae).

Type Locality Nairobi, Kenya.

Other Hosts None known.

Other Localities None known.

Prevalence *P. heischi* was identified in 11 of 60 *M. striata*.

Morphological Variation Gametocytes are 16.1 ± 1.9 × 5.7 ± 0.3 µm (12–20 × 4–9, N = 100), with LW 91.4 ± 12.9 µm² (60–126) and L/W 2.90 ± 0.60 (1.5–4.8). Gametocyte size relative to host cell nucleus size is 3.51 ± 0.76 (N = 100), and to normal erythrocyte nuclei is 4.48 ± 0.93. Gametocytes are sexually dimorphic in length, LW, and L/W: Macrogametocytes are 17.1 ± 1.6 × 5.7 ± 0.9 µm (13–20 × 4–9, N = 50), with LW 96.7 ± 11.7 µm² (68–126) and L/W 3.10 ± 0.65 (1.6–4.8); microgametocytes are 15.1 ± 1.7 × 5.7 ± 0.8 µm (12–18 × 5–8, N = 50), LW 86.1 ± 11.8 µm² (60–108), and L/W 2.70 ± 0.49 (1.5–3.6). Chronic-phase gametocytes differ sexually only in LW, with macrogametocytes averaging 97.4 ± 10.5 µm² (N = 25) and microgametocytes 90.4 ± 10.0 µm² (N = 25). Within the same sex, microgametocytes differ only in LW, 81.8 ± 12.0 µm² in active phase, versus 90.4 ± 10.0 µm² in chronic phase; while macrogametocytes differ by infection phase in length, width, and L/W, but not in LW: active 18.2 ± 1.0 × 5.3 ± 0.5 µm, LW 95.6 ± 12.5 µm², and L/W 3.18 ± 0.45 versus 16.1 ± 1.5 × 6.1 ± 1.0 µm, LW 97.4 ± 10.5 µm², and L/W 2.72 ± 0.60. Gametocytes have distinctly pointed ends in active infection and usually when chronic, although some in the latter phase of infection have more broadly rounded, nonpointed ends. Meronts are usually elongate and may curve incompletely around the host cell nucleus, but occasionally take an elongated ovoid shape, nearly filling the host cell. Meronts are 14.3 ± 2.8 × 7.7 ± 1.3 µm² (8–18 × 6–11, N = 25), with an LW of 109.4 ± 23.3 µm² (48–144). Size relative to host cell nucleus size is 4.11 ± 0.81, and to normal erythrocyte nuclei is 6.32 ± 1.34 (N = 25).

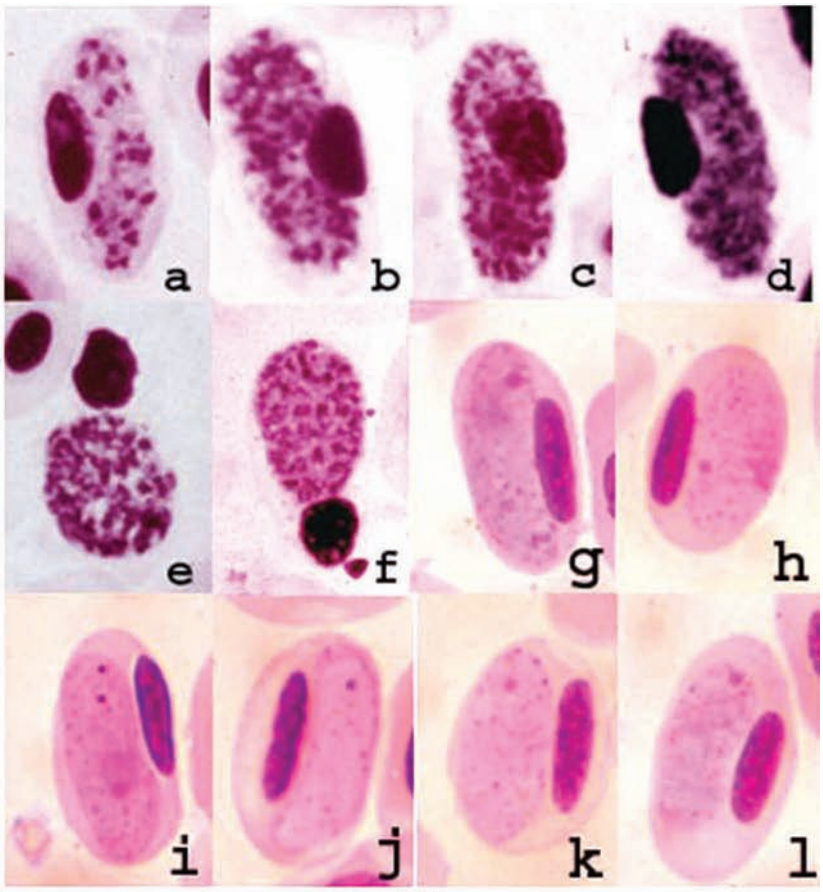
Exoerythrocytic Merogony Unknown.

Sporogony Unknown.

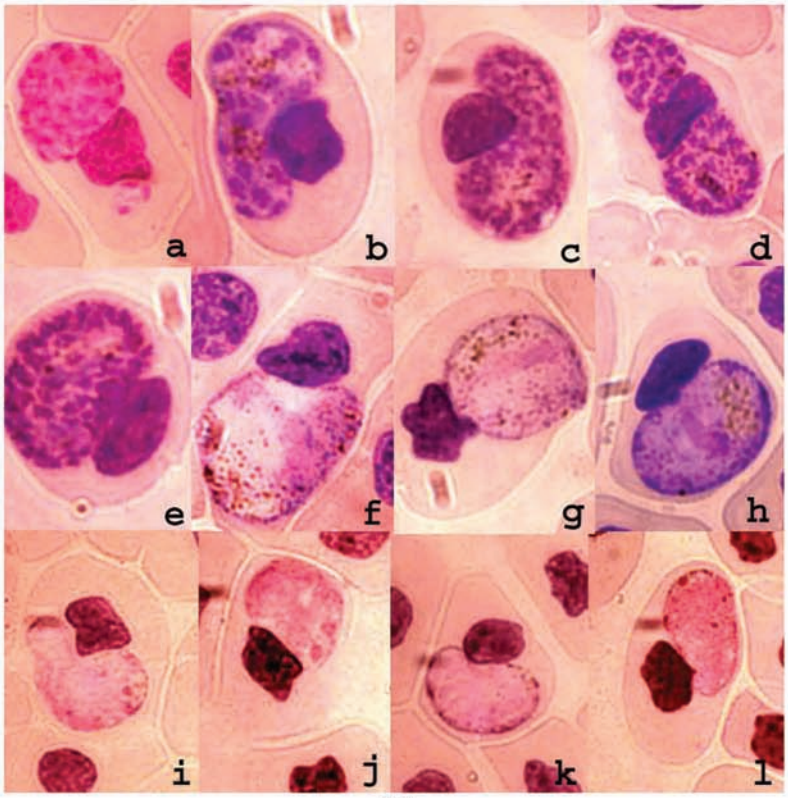
Effects on Host Immature parasites usually occur in proerythrocytes, which mature by the time the parasite matures. Both meronts and gametocytes produce significant hypertrophy of host erythrocytes, with host cells about 25% larger when parasitized by meronts and 40% larger when infected by gametocytes, and their nuclei are two-thirds larger than normal nuclei. Almost all host erythrocytes and their nuclei are distorted, and the latter are displaced, usually laterally, occasionally in a polar direction.

Remarks The infected series of *M. striata* were collected between 1949 and 1965 by R. B. Heisch in the vicinity of the Medical Research Laboratory in Nairobi, from rocky gullies on hillsides above the Athi Plains. In this habitat, *M. striata* and *Agama agama* coexist in boulder piles and termite nests. A single infection of *Plasmodium* in an *M. striata*, collected in 1949, appears to be *Plasmodium giganteum* instead of *P. heischi* and possibly represents one of the very few cross infections of a saurian *Plasmodium* species between host families (**Plate 2B**). Meronts are rounded or ovoid, usually filling the host cells, nearly half of which are proerythrocytes. Meronts average 11.2 ± 1.4 × 8.9 ± 0.8 µm (10–15 × 7–10, N = 25), with LW 99.2 ± 13.0 µm² (80–135), and contain 38.7 ± 10.5 (22–68) merozoites. Gametocytes are elongate with broad, rounded ends, not pointed as in *P. heischi*. Most gametocyte nuclei are large and central, never subterminal, the usual position of macrogametocyte

Plate 3 (**A)** *Plasmodium heischi* from *Mabuya striata* of Kenya. Meronts, **a–f**; macrogametocytes, **g–i**; microgametocytes, **j–l**. (Figures **a** and **b** from Garnham, P. C. C., and Telford, S. R., Jr., *J. Protozool*., 31, 518, 1984, with permission, Blackwell Publishing.) (**B**) *Plasmodium robinsoni* from *Chamaeleo parsoni* of Madagascar. Meronts, **a–e**; macrogametocytes, **f–h**; microgametocytes, **i–l**.



(A)



(B)

nuclei in *P. heischi*. Gametocytes are 15.4 ± 1.5 × 6.1 ± 0.9 µm (11–18 × 5–8, N = 50), with LW 92.8 ± 12.3 µm² and L/W 2.59 ± 0.48 (1.4–3.4). Their size relative to host cell nucleus averages 3.26 ± 0.64, and to normal erythrocyte nuclei is 4.20 ± 0.55. As in *P. giganteum* and *P. heischi*, macrogametocytes are longer, with greater LW, and more slender than microgametocytes but are similar in width. Macrogametocytes are 16.1 ± 0.5 × 6.1 ± 0.8 µm (13–18 × 5–8, N = 25), with LW 97.4 ± 9.5 µm² (75–112) and L/W 2.71 ± 0.47 (1.6–3.4); microgametocytes are 14.6 ± 1.4 × 6.1 ± 1.0 µm (11–17 × 5–8, N = 25), with LW 88.3 ± 13.2 µm² (65–128) and L/W 2.47 ± 0.47 (1.4–3.4). The slide of this infection is no. 998 in the Garnham Collection.

Plasmodium robinsoni (Brygoo) 1962 Telford and Landau 1987 (**Plate 3**)

Diagnosis A *Plasmodium* (*Sauramoeba*) species with large round, oval, or elongate meronts that approximate gametocytes in size. Meronts are 11–23 × 7–11 µm, with LW 90–184 µm², and contain 40–74 merozoites. Gametocytes are oval to elongate or bulky, 9–20 × 5–13 µm, with LW 72–221 µm² and L/W 1.0–3.4. Meront size relative to host cell nucleus size is 2.0–4.3, and to normal erythrocyte nuclei is 1.9–4.3. Gametocyte size relative to host cell nucleus size is 1.4–5.4, and to normal erythrocyte nuclei is 2.1–4.7. Pigment forms a light golden mass in meronts, often centered among nuclei, and is dispersed as several loose clumps of dark granules in gametocytes. Gametocytes are sexually dimorphic in dimensions, with microgametocytes longer and more slender than macrogametocytes.

Type Host *Chamaeleo brevicornis* Gunther.

Type Locality Fiherenana, Moramanga Subprefecture, Madagascar.

Other Hosts *Chamaeleo parsoni crucifer*.

Other Localities Périnet, Moramanga Subprefecture, Madagascar.

Prevalence Three *C. brevicornis* from the type locality and 2 of 47 (4.3%) collected at Périnet were infected by *P. robinsoni* (Brygoo, 1962).

Morphological Variation The type infection of *P. robinsoni* was in chronic phase at a parasitemia of less than 0.1% (Telford and Landau, 1987). Brygoo (1962) provided no dimensional data for either gametocytes or meronts but did comment that mature meronts contained 40–70 nuclei, and that gametocytes were variably shaped, elongate, ellipsoidal, sometimes with a projection that resembled a tennis racquet, sometimes curving around the erythrocyte nucleus in a halteridial form. Garnham (1966) described their form as a tennis racquet, which is not typical of the species. Examination of the type slide found a single mature meront, 10 × 9 µm, that contained 47 nuclei. In an active infection of *P. robinsoni* in *C. parsoni*, meronts are 16.1 ± 3.9 × 8.8 ± 1.2 µm (11–23 × 7–11, N = 17), with LW 138.8 ± 25.2 µm² (99–184) and 56.6 ± 12.0 (40–74) merozoites. Meront size relative to host cell nucleus is 3.13 ± 0.07 (2.0–4.3, N = 15), and to normal erythrocyte nuclei is 3.05 ± 0.70 (1.9–4.3, N = 16). Meronts are usually round or ovoid, but when elongate are halteridial around the erythrocyte nucleus or even occasionally divided into two portions by the latter, only one of which would contain the pigment mass. Pigment granules are clustered into a large, light golden mass. In *C. brevicornis*, gametocytes are 15.0 ± 1.5 × 7.4 ± 1.8 µm (12–18 × 5–12, N = 50), with LW 110.5 ± 24.5 µm² (75–170) and L/W 2.14 ± 0.57 (1.1–3.4). Their size relative to host cell nucleus is 3.10 ± 0.97 (1.4–5.4), and to normal erythrocyte nuclei is 3.04 ± 0.67 (2.1–4.7). Gametocytes in *C. parsoni* are 13.9 ± 2.4 × 9.4 ± 1.6 µm (9–20 × 6–13, N = 70), with LW 130.2 ± 32.6 µm² (72–221) and L/W 1.54 ± 0.42 (1.0–2.8). Gametocyte size relative to host cell nucleus size is 3.12 ± 0.79 (1.6–5.0, N = 25), and to normal erythrocyte nuclei is 3.35 ± 0.84 (1.9–5.7, N = 70). Gametocytes from *C. brevicornis* are longer and narrower than those from *C. parsoni*, with smaller LW, and are more elongate in shape, with a greater L/W ratio. Sexual dimorphism is present among gametocytes from both host species, varying between the sexes in exactly the same pattern: Macrogametocytes are shorter and wider, have greater LW values and lower L/W ratios (i.e., are more rounded) than in microgametocytes. In *C. brevicornis*, microgametocytes average 15.3 ± 2.2 × 6.4 ± 1.2 µm (12–18 × 5–9, N = 8), with LW 95.4 ± 9.1 µm² (84–108) and L/W 2.49 ± 0.67 (1.3–3.4) versus, respectively, in macrogametocytes, 14.9 ± 1.3 × 7.6 ± 1.8 µm (12–17 × 5–12, N = 42), 113.4 ± 25.5 µm² (75–170), and 2.07 ± 0.54 (1.1–3.2). Microgametocytes in *C. parsoni* are 14.7 ± 2.2 × 8.5 ± 1.4 µm (10–20 × 6–11, N = 25), with LW 124.8 ± 30.1 µm² (90–200) and L/W 1.78 ± 0.38 (1.0–2.5) versus, respectively, in macrogametocytes, 13.5 ± 2.4 × 9.9 ± 1.6 µm (9–20 × 6–13, N = 45), 133.2 ± 33.9 µm² (72–221), and 1.40 ± 0.38 (1.0–2.8). The dark pigment granules are distributed in several variably discrete groups in both gametocyte sexes.

Exoerythrocytic Merogony Unknown. Brygoo (1962) did not find EE meronts in sections of liver and spleen from two heavily parasitized chameleons.

Sporogony Brygoo (1962) fed *Culex fatigans* on a gametocyte-rich infection in *C. brevicornis*, but sporogonic development was not observed in 30 mosquitoes dissected between day 5 and 21 PF.

Effects on Host Infected erythrocytes were described as hypertrophied when host to meronts or mature gametocytes by Brygoo (1962). Host cells utilized by *P. robinsoni* are predominantly erythrocytes. Meronts and gametocytes are most commonly polar or lateropolar in *C. parsoni*; gametocytes are more commonly lateral or lateropolar in *C. brevicornis*. "In both hosts cells infected with meronts or gametocytes enlarged and distorted, with nuclei always displaced and often distorted; only meronts produced nuclear hypertrophy" (Telford and Landau, 1987).

Remarks Brygoo (1962) inoculated infected blood containing *P. robinsoni* subcutaneously into two *Chamaeleo lateralis* and one *C. verrucosus*, but neither species became infected. He also attempted transmission from a heavily infected *C. brevicornis* by subcutaneous inoculation of a broth prepared from liver and spleen into another *C. verrucosus* and two more *C. lateralis*, without success.

Plasmodium acuminatum Pringle 1960

Diagnosis A *Plasmodium* (*Sauramoeba*) species in which young asexual stages and gametocytes have prominent, pointed cytoplasmic projections at each end. Dark pigment granules tend to clump together at an extremity or along one side in gametocytes. Largest meronts observed contained six to nine nuclei, but mature meronts are undescribed. Immature meronts can equal host cell nuclei in size. Immature gametocytes are elongate and acuminate at both ends. Mature gametocytes, with irregularly rounded or bluntly tapered extremities, can occupy nearly the entire host erythrocyte, curving around the nucleus.

Type Host *Chamaeleo f. fischeri* (Reichenow) (Sauria: Chaemaeleonidae).

Type Locality Amani, Eastern Usambara Mountains, Tanga Region, Tanzania.

Other Hosts None known.

Other Localities None known.

Prevalence One of 42 *C. fischeri* collected at Amani was infected by *P. acuminatum* (Ball, 1967a).

Morphological Variation No dimensional data are available for this species.

Exoerythrocytic Merogony Unknown.

Sporogony Unknown.

Effects on Host Meronts "partially embraced the swollen and displaced red cell nucleus" while gametocytes (immature) "caused only a minor distortion of the host cell" (Pringle, 1960) and did not displace the nucleus.

Remarks This *Plasmodium* species has not been reported since its description by Pringle (1960). He did not describe mature meronts and gametocytes, but in an addendum to the description article, reported a third infection in which

> the blood contained scanty gametocytes apparently in a more advanced stage of development … the largest forms, which occupy almost the entire red cell, displace and partially embrace the red cell nucleus; they have a less characteristic shape, with irregularly rounded or bluntly tapered extremities … the macrogametocyte, usually a larger and more irregularly shaped parasite [than the microgametocyte]. … Rarely the shape of the microgametocyte approximates to the rounded bean shape. (Pringle, 1960)

This species is probably a *Sauramoeba* species based on having very large gametocytes that are sexually dimorphic in size and shape. The very large immature meronts figured by Pringle indicate that mature meronts would also be large, as with other *Sauramoeba* species. The host species was identified by Pringle (1960) as *Chamaeleo fischeri tavetanus*, which is incorrect.

AFRICAN *LACERTAMOEBA* AND *CARINAMOEBA* SPECIES

Plasmodium brygooi Telford and Landau 1987

Diagnosis A *Plasmodium* (*Lacertamoeba*) species with oval, oblong, or lentiform meronts, 6–9 × 5–8 µm, with LW 36–64 µm², that produce 10–16 merozoites. Meront size relative to host cell nucleus size is 0.5–1.2, and to normal erythrocyte nuclei is 0.5–1.1. Pigment in meronts is usually formed as three or four dark clusters, rarely coalesced into a single golden mass. Gametocytes are usually oval or elongate, 9–15 × 5–10 µm, with LW 66–126 µm² and L/W 1.1–3.0. Size of gametocytes relative to host cell nucleus is 1.0–3.4, and to normal erythrocyte nuclei is 1.2–2.3. Dark pigment granules in gametocytes are not widely dispersed but are somewhat localized.

Type Host *Chamaeleo brevicornis* Gunther (Sauria: Chamaeleonidae).

Type Locality Périnet, Madagascar.

Other Hosts None known.

Other Localities None known.

Prevalence Unknown.

Morphological Variation Meronts are variably shaped, oval, oblong, or lentiform, with merozoites arranged along the periphery as a rosette. They average 7.9 ± 0.9 × 6.3 ± 0.8 µm (6–9 × 5–8, N = 16), with LW 49.0 ± 6.7 µm² (36–64). Merozoites number 13.8 ± 2.1 (10–16). Meront size relative to host cell nucleus is 0.79 ± 0.17 (0.5–1.2), and to normal erythrocyte nuclei is 0.88 ± 0.12 (0.6–1.1). Pigment usually forms three or four prominent dark clusters, rarely coalescing as a golden mass. Gametocytes are usually oval or elongate, 11.5 ± 1.4 × 8.1 ± 1.3 µm (9–15 × 5–10, N = 48), with LW 92.5 ± 16.0 µm² (66–126) and L/W 1.47 ± 0.38 (1.1–3.0). Gametocyte size relative to host cell nucleus size is 1.74 ± 0.50 (1.0–3.4), and to normal erythrocyte nuclei is 1.65 ± 0.29 (1.2–2.3). There appears to be no sexual dimorphism in gametocyte dimensions.

Exoerythrocytic Merogony Unknown.

Sporogony Unknown.

Effects on Host Both meronts and gametocytes usually occupy polar or lateropolar positions in host erythrocytes, which are hypertrophied and distorted, with nuclei displaced and often distorted. Only meronts cause hypertrophy of erythrocyte nuclei.

Remarks Although Brygoo (1962) examined 47 *C. brevicornis* from the type locality, Périnet, between 1954 and 1962, he found only *Plasmodium robinsoni* present in their blood. A single *C. brevicornis* collected in 1972 was host to *P. brygooi*.

Plasmodium holaspi Telford 1986 (**Plate 4**)

Diagnosis A *Plasmodium* (*Lacertamoeba*) species with young asexual stages that occupy marginal positions in erythrocytes. Meronts are usually oblong or formed as rosettes, 5–13 × 4–7 µm, with LW 25–66 µm², and contain 8–18 merozoites. Meront size relative to host cell nucleus is 1.26, and to normal erythrocyte nuclei is 1.50. Pigment forms a single, dark irregular mass variably located within the meront. The usually elongate gametocytes are 6–18 × 3–8 µm, with LW 28–98 µm² and L/W 1.13–4.67. Their size relative to host cell nucleus size is 2.13, and to normal erythrocyte nuclei is 2.25. Dimensions of gametocytes are not sexually dimorphic. Large masses of apparent chromatin that stain intensely reddish occur in both sexes of maturing gametocytes, more dispersed in microgametocytes, becoming less prominent in the larger gametocytes. Irregular dark pigment granules are conspicuous and dispersed in both sexes.

Type Host *Holaspis guentheri* Gray (Sauria: Lacertidae).

Type Locality Kimboza Forest, 1 km north of the Ruvu River below Kibungo Village, south side of Uluguru Mountains, Morogoro Region, Tanzania.

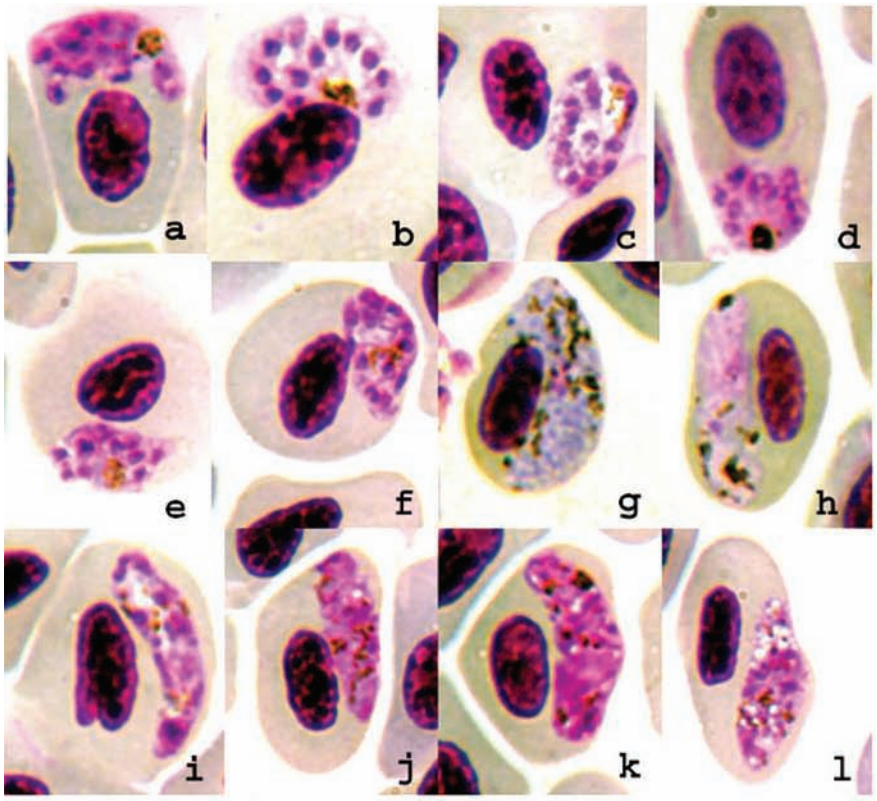
Other Hosts None known.

Other Localities None known.

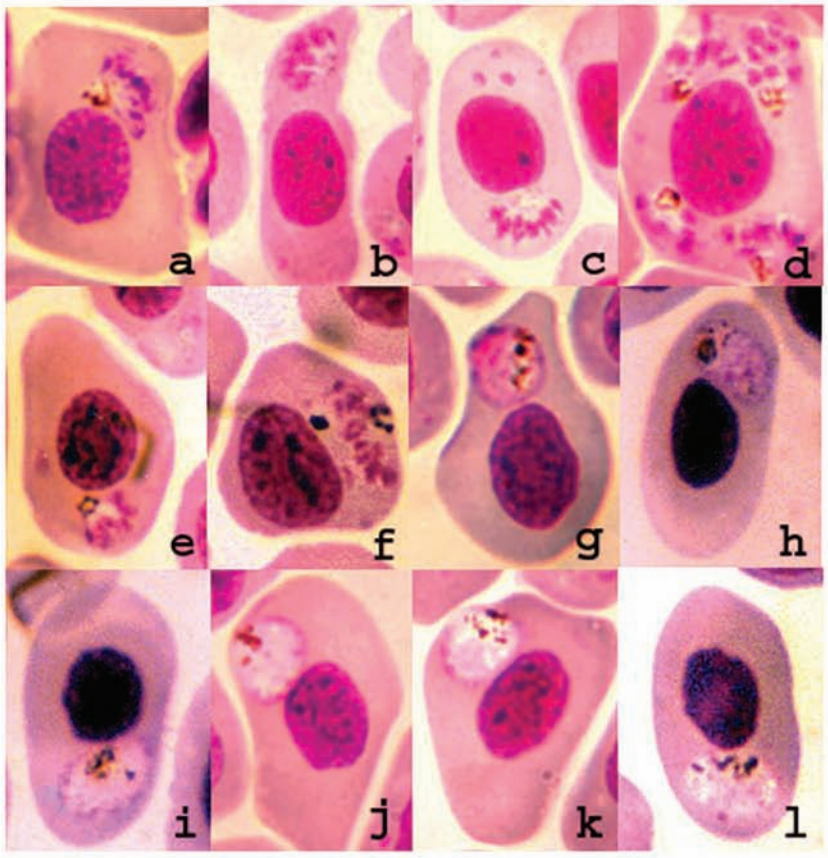
Prevalence Two of seven *H. guentheri* from the type locality were infected by *P. holaspi* (Telford, 1986a).

Morphological Variation Meronts are 7.8 ± 1.6 × 5.4 ± 0.8 µm (5–13 × 4–7, N = 66), with LW 42.1 ± 9.4 µm² (25–66), and produce 12.3 ± 2.3 (8–18) merozoites. Meront size relative to host cell nucleus is 1.26 ± 0.32 (0.73–2.29, N = 62), and to normal erythrocyte nuclei is 1.50 ± 0.52 (0.78–2.77). Meront shape is most often oblong or with merozoites arranged in a rosette, rarely as a fan or other forms, with a single dark irregular mass of pigment granules, variably located within the meront. Gametocytes, usually elongate, are 11.8 ± 2.4 × 5.0 ± 1.0 µm (6–18 × 3–8, N = 85), with LW 58.9 ± 15.2 µm² (28–98) and L/W 2.47 ± 0.75 (1.13–4.67). Gametocyte size relative to host cell nucleus size is 2.13 ± 0.77 (0.7–4.3, N = 60), and to normal erythrocyte nuclei is 2.25 ± 0.75 (0.9–4.1, N = 85). Gametocytes are not sexually dimorphic in dimensions: Macrogametocytes are 12.2 ± 2.1 × 4.9 ± 0.8 µm (7–18 × 3–7, N = 44), with LW 59.8 ± 14.1 µm² (35–90) and L/W 2.54 ± 0.66 (1.2–4.5); microgametocytes are 11.5 ± 2.6 × 5.1 ± 1.1 µm (6–15 × 3–8, N = 41), with LW 58.0 ± 16.4 µm² (28–98) and L/W 2.39 ± 0.83 (1.1–4.7). The prominent pigment granules are dispersed and irregular in shape. Blocks of apparent chromatin that stain deep reddish appear in the larger immature gametocytes and nearly mature gametocytes that mask the cell nucleus, more dispersed in micro- than in macrogametocytes. In the larger gametocytes, the blocks are somewhat reduced in size.

Exoerythrocytic Merogony EE meronts were common in the lungs of two *H. guentheri* (**Plate 11A**, **m–p**). Ovoid to elongate in shape, dimensions varied, 9–27 × 4–16 µm (14.6 ± 3.7 × 9.6 ± 2.4, N = 25). Most meronts were stained too intensely for an accurate count of nuclei in a single focal plane, but in six meronts less heavily stained, nuclei numbered approximately 66, 69, 78, 85, 99, and 210.



(A)



(B)

Plate 4 **(A)** *Plasmodium holaspi* from *Holaspis guentheri* of Tanzania. Meronts, **a–f**; macrogametocytes, **g**, **h**; prematuration gametocyte, **i**; microgametocytes, **j–l**. (Figures **a** and **b** modified from Telford, S. R., Jr., *J. Parasitol.,* 72, 271, 1986, Figs. 6 and 11, with permission.) (**B**) *Plasmodium uluguruense* from *Hemidactylus platycephalus* of Tanzania. Meronts, **a–f**; macrogametocytes, **g**, **h**; microgametocytes, **i–k**.

Sporogony Unknown.

Effects on Host Meronts often distort the host cell and displace its nucleus and can cause both the erythrocyte and its nucleus to become hypertrophied. Gametocytes commonly distort host cells and displace their nuclei, but both meronts and gametocytes only rarely distort the nuclei. In an infection in which hypertrophy of host cells and their nuclei appeared when meronts occupied erythrocytes; the same effect occurred with cells host to erythrocytes.

Remarks *Plasmodium holaspi* is distinguished from all other described malarial parasites of African lizards by the marginal position of young parasites and from all known *Plasmodium* species by the prominent blocks or masses of apparent chromatin present in maturing gametocytes (Telford, 1986a).

Plasmodium uluguruense Telford 1984 (**Plate 4**)

Diagnosis A *Plasmodium* (*Lacertamoeba*) species with asexual stages parasitic in both mature and immature erythrocytes. Meronts are 4–10 × 2–6 µm, with LW 12–54 µm², and contain 4–12 merozoites, usually arranged as a fan. Meront size relative to host cell nucleus averages 0.65, and to normal erythrocyte nuclei is 0.73. Proerythrocytic meronts produce more merozoites than erythrocytic meronts. Light golden pigment granules aggregate into a mass at the base of fans. The usually ovoid gametocytes are 5–10 × 4–7 µm, with LW 20–63 µm² and L/W 1.00–2.50. Gametocyte size relative to host cell nucleus averages 0.97, and to normal erythrocyte nuclei is 1.07. Dark greenish-yellow to black pigment granules are not dispersed in either sex of gametocyte but tend to aggregate in a single focus near the gametocyte margin. Microgametocytes exceed macrogametocytes in length and size but do not differ in shape.

Type Host *Hemidactylus platycephalus* Peters (Sauria: Gekkonidae).

Type Locality North slope of the Uluguru Mountains at Morogoro, Morogoro Region, Tanzania.

Other Hosts None known.

Other Localities Mindu Mountain about 5 km northwest of Morogoro; Kimboza Forest, 1 km north of the Ruvu River below Kibungo Village, south side of Uluguru Mountains, Morogoro Region; Mgeta, about 30 km southwest of Morogoro.

Prevalence *P. uluguruense* parasitized 46 of 71 (64.8%) *H. platycephalus* in the four localities in which it was found: 13 of 21 (61.9%) at the type locality, 26 of 41 (63.4%) in Kimboza Forest, 6 of 8 on Mindu Mountain, and the only gecko taken at Mgeta.

Morphological Variation Meronts, usually fan shaped, are 6.1 ± 1.0 × 4.2 ± 0.8 µm (4–10 × 2–6, N = 133), with LW 25.8 ± 6.4 µm² (12–54), and produce 7.2 ± 1.4 (4–12, N = 135) merozoites. Meront size relative to host cell nucleus is 0.65 ± 0.14 (0.63–1.00, N = 51), and to normal erythrocyte nuclei is 0.73 ± 0.16 (0.34–1.42, N = 133). Proerythrocytic meronts are similar in size to erythrocytic, 6.2 ± 1.1 × 4.1 ± 0.8 µm (4–10 × 2–6, N = 82), LW 25.7 ± 7.1 µm² (12–54), versus 5.9 ± 0.8 × 4.4 ± 0.7 µm (4–8 × 3–5, N = 51), LW 25.8 ± 5.2 µm², respectively, but produce more merozoites, 7.4 ± 1.5 (4–12, N = 84) versus 6.9 ± 1.2 (4–9, N = 51). Aggregations of the light golden pigment granules form a mass at the base of the fan or at one end of elongated meronts. Gametocytes are ovoid, 7.1 ± 1.1 × 5.2 ± 0.6 µm (5–10 × 4–7, N = 150), with LW 37.1 ± 7.2 µm² (20–63) and L/W 1.39 ± 0.28 (1.00–2.50). Gametocyte size relative to host cell nucleus is 0.97 ± 0.24 (0.58–2.29, N = 141), and to normal erythrocyte nuclei is 1.07 ± 0.19 (0.66–1.74, N = 150). Microgametocytes are longer and larger in size than macrogametocytes but do not differ in L/W ratio, 7.4 ± 1.2 × 5.4 ± 0.6 µm (6–10 × 4–7, N = 82), LW 39.5 ± 6.7 µm² (30–50), and L/W 1.40 ± 0.30 (1.00–2.50) versus 6.8 ± 0.9 × 5.0 ± 0.6 µm (5–10 × 4–7, N = 68), LW 34.2 ± 6.9 µm² (20–63), and L/W 1.38 ± 0.26 (1.00–2.50), respectively. Pigment is not dispersed in gametocytes but forms as an aggregate of dark greenish-yellow granules, usually situated near the cell margin in both sexes.

Exoerythrocytic Merogony Up to seven nuclei were present in thrombocytic meronts of one infection (Telford, 1984a).

Sporogony Unknown.

Effects on Host In one of three active infections, cells host to meronts were hypertrophied and occasionally distorted but were normal in size and less often distorted in chronic and relapse infections. Host cell nuclei were hypertrophied in two of three active infections, seldom distorted but usually displaced (Telford, 1984a). Nuclei were not distorted but were usually displaced in the chronic and relapse infections. In erythrocytes parasitized by gametocytes, hypertrophy of the cell was present in one active infection, and distortion of the cells was common in three of five infections. Erythrocyte nuclei were enlarged in all active infections of gametocytes but were normal in size