

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 It is supplemented by the data acquired but unpublished during my 45 years of research, collected primarily from the field while resident in North, Middle, and South America; eastern, southeastern, and southern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–i**, 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.