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PARASITES IN A BIODIVERSITY HOTSPOT: A SURVEY OF HEMATOZOA AND A MOLECULAR PHYLOGENETIC ANALYSIS OF *PLASMODIUM* IN NEW GUINEA SKINKS

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ABSTRACT: A sample of 204 skinks (Squamata: Scincidae) from 10 genera representing 24 species were collected from 10 different localities in New Guinea and examined for blood parasites. Hemogregarines, trypanosomes, microfilarial worms, and 8 infections showing 2 distinct morphological types of malaria parasites (*Plasmodium* sp.) were observed. Molecular sequence data, in the form of mitochondrial cytochrome b sequences from the *Plasmodium* infections, showed 2 distinct clades of parasites, 1 in *Sphenomorphus jobiense* hosts and 1 in *Emoia* spp., which correspond to the 2 morphotypes. There was substantial genetic variation between the 2 clades, as well as within the clade of *Emoia* parasites. Nearly half of the skinks sampled had green blood pigmentation, resulting from the presence of biliverdin in the plasma; however, only 1 of these lizards was infected with *Plasmodium* sp. and only 2 had any blood parasites. These preliminary results suggest a high degree of phylogenetic diversity but a very low prevalence of *Plasmodium* spp. infections in the skinks of this globally important biodiversity hot spot.

The island of New Guinea is on the border of one of the most distinct biogeographic demarcations in the world. Wallace's line, which coincides with the boundary between the ancient supercontinents of Laurasia and Gondwana, separates the dramatically different Oriental and Australian faunas. Although the island represents less than 0.6% of Earth's landmass, it is estimated that 5–7% of the world's biodiversity is found on New Guinea, and thus it has been identified as a megadiverse region (Mittermeier and Mittermeier, 1997). Although poorly understood and inadequately described, the vast diversity of biological life on New Guinea, much of which is endemic, is a result of the island's diverse topography, extensive range of habitat types, and complex geological history.

Despite its importance as a biodiversity hotspot (Mittermeier et al., 1998), the parasites of New Guinea wildlife, particularly hematoparasites, are poorly studied. Malaria parasites (species of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*), as well as trypanosomes and microfilarial worms, have been observed in both captive and wild birds in Papua New Guinea (Ewers, 1967, 1973; Jones, 1985; Varghese, 1987; Beadell et al., 2004); species of *Hepatozoon* and trypanosomes have been reported from marsupials and rodents from the island (Anderson, 1990), but few have been identified past the generic level. Likewise, only 3 species of malaria parasites have been described from the diverse herpetofauna of New Guinea (Thompson and Hart, 1946; Telford and Wellehan, 2005), and the systematic relationships, biogeography, and in some cases even the taxonomic status of these species are not well understood.

Scincid lizards are the most visible and widely dispersed component of the New Guinea reptile fauna and represent 70% of the overall lizard species from the island (Allison, 1996). Here, we report blood parasites that were observed from 204 specimens of scincid lizards representing 24 species from 10 genera that were collected from Papua New Guinea over a 10-yr period between 1991 and 2001, from 10 localities in New Guinea, including the main island and associated archipelagos (Fig. 1). Of the 4 subfamilies of skinks, Acontinae, Feyliniinae, Lygosominae, and Scincinae (Greer, 1970), Lygosomine skinks, the only subfamily occurring in New Guinea, are globally the most diverse and widely distributed. Within the Lygosominae, the Australasian species have been separated into 3 presumed monophyletic groups, the Egernia, Eugongylus, and Sphenomorphus groups, named for the prominent genus in each group (Greer, 1979, 1989; Hutchinson, 1993). Our survey includes all 3 groups (although the majority of the Egernia group diversity is found in Australia).

Nearly half (47%) of the skinks sampled belonged to one of the most unusual genera of lizards from New Guinea, *Prasinohaema*. These lizards are the only amniotes with green blood (Greer and Raizes, 1969; Austin and Jessing, 1994). The green coloration of the blood is caused by the bile pigment, biliverdin, which is a toxic degradation product of hemoglobin (Austin and Jessing, 1994). In all other vertebrates, the accumulation of biliverdin, or bilirubin, or both, in the circulatory system and tissues causes the pathological condition known as jaundice (Gray, 1953; Gray et al., 1961). The divergent physiology of these lizards results in a striking lime-green coloration of the blood, muscles, bones, and mucosal lining. *Prasinohaema* includes 5 described species from New Guinea and associated archipelagos, and we surveyed 4 of the 5 described species.

Molecular data, in the form of mitochondrial cytochrome b DNA sequences, have recently been used to identify species of saurian malaria (Perkins, 2000), track their biogeographic patterns (Perkins, 2001), and infer the systematic relationships of saurian, avian, and mammalian malaria parasites (Perkins and Schall, 2002). In an effort to investigate the genetic diversity and evolutionary relationships of the malaria parasites observed from New Guinea, we also generated cytochrome b data from the *Plasmodium* spp. infections and analyzed these in the context of other species of lizard malaria parasites from around the globe.

MATERIALS AND METHODS

Lizards were captured alive by hand or blowpipe. Blood was collected with a heparinized capillary tube from the postorbital sinus. The blood sample was used to prepare thin blood smears, and, for lizards collected after 1995, blood blots onto filter paper or FTA paper or unstained thin blood smears were also made for subsequent isolation of parasite DNA.

Thin blood smears were stained with Giemsa and examined under a microscope at ×400 for approximately 1 min to look for larger parasites, e.g., trypanosomes and microfilariae, and then examined for an additional 3 min at ×1,000, which allows for careful inspection of approximately 5,000 lizard red blood cells (Schall, 1996). When infections with *Plasmodium* spp. were detected, smears were examined for extended periods of time to assess variation in parasite morphology.

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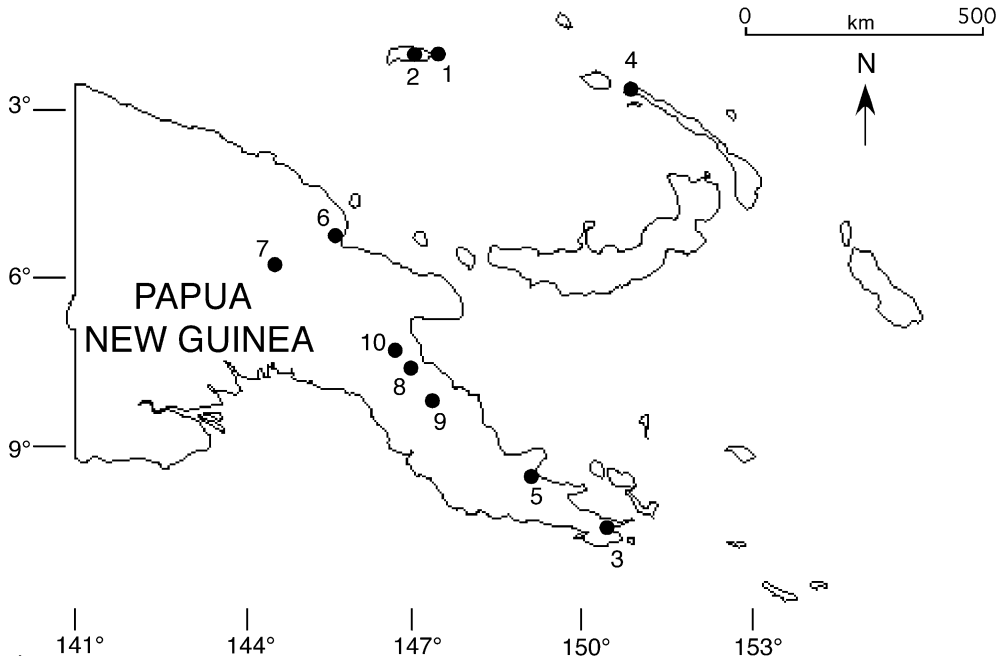

FIGURE 1. Map of Papua New Guinea showing collection localities for lizards surveyed for hemoparasite infection. Numbers correspond to province (locality) data presented in Table I.

DNA samples were also available in the form of either unstained slides or filter/FTA paper. Blood was scraped from the unstained slides using a new, sterile razor blade and placed into a 1.5-ml tube. Or, if blood had been blotted onto paper, a small section, approximately 25 mm^2, was cut from the stain and placed into a 1.5-ml tube. The DNeasy extraction kit (Qiagen, Valencia, California) was used to isolate DNA, following manufacturer's protocols, except eluting the DNA in the final step with just 50 μl of buffer to enhance concentration of DNA.

A 1132-bp portion of the mitochondrial cytochrome b gene was amplified via nested polymerase chain reaction using DNA from lizards found to be infected with *Plasmodium* spp. Reactions were all performed with Ready-to-Go PCR beads (Amersham Pharmacia, Piscataway, New Jersey). The outer reaction used 2 μl of extracted genomic DNA as template and primers DW2 (5′-TAA TGC CTA GAC GTA TTC CTG ATT ATC CAG-3′; Perkins and Schall, 2002) and DW4 (5′-TGT TTG CTT GGG AGC TGT AAT CAT AAT GTG-3′ [=AL1356; Escalante et al., 1998]). Two nested reactions used primers DW1 (5′-TCA ACA ATG ACT TTA TTT GG-3′; Creasey et al., 1993) and DW3 (5′-TGC TGT ATC ATA CCC TAA AG-3′; Creasey et al., 1993) in 1 amplification and primers DW8 (5′-GCA CAA ATC CTT TAG GGT ATG ATA C-3′; Perkins and Schall, 2002) and DW6 (5′-GGG AGC TGT AAT CAT AAT GTG-3′ [=AL1413; Escalante et al., 1998]) in the other, along with 0.5 μl of the outer reaction product as template. All products were visualized in 1% agarose gels with ethidium bromide over UV light.

PCR products were sequenced in both directions with either ABI BigDye (Foster City, California) or Amersham Dyenamic (Piscataway, New Jersey) sequencing premixes using the nested primers to generate overlapping double-stranded segments. Reactions were run on an MJ Research BaseStation 51 or an ABI 3730 (Applied Biosystems, Foster City, California) automated sequencer. Sequences were edited using the software Sequencher (Gene Codes Corporation, Ann Arbor, Michigan).

Newly recovered sequences from the malaria parasites of the New Guinea skinks; published sequences of other *Plasmodium* spp. sequences from North American, South American, African, and Australian lizards (Perkins and Schall, 2002); and 3 *Plasmodium* species that infect mammals (the human malaria parasites *Plasmodium falciparum*, *Plasmodium vivax*, and the rodent malaria parasite *Plasmodium chabaudi*), which were used as outgroups, were aligned in clustalw (Thompson et al., 1994). Phylogenetic analyses were run on PAUP* v. 4.0 using unweighted parsimony and 10 random addition sequences and excluding the characters that represented the DW3/DW8 primer region. Nodal support was determined via jackknife analysis with 37% deletion of characters each round and the full heuristic search, again with 10 random addition sequences.

RESULTS

Our survey of 10 localities showed similar diversity of blood parasites, i.e., haemogregarines, trypanosomes, microfilariae, and malaria parasites, as described by Thompson and Hart (1946) in their survey of skinks collected on Goodenough Island, located approximately 25 km east of mainland New Guinea. Table I summarizes these findings according to host species and collection localities. The blood parasites other than *Plasmodium* spp. are shown in Figure 2. The hemogregarines in all infections (n = 10), observed in 9 species and 4 of the localities, showed the same morphology, with the host nucleus greatly distorted (Fig. 2A). Trypanosomes (4 infections from 3 host species) were large (>50 μm) and leaflike, with undulating membranes and visible flagella (Fig. 2B). One *Sphenomorphus jobiense* individual had microfilariae in the blood. These were unsheathed (Fig. 2C), and in the 1 infection where they were observed, they were very abundant, with microfilariae present in nearly every field of view in the thin smear. Further identification of these parasites was not performed, but each is consistent with the morphological description of these parasites in Thompson and Hart (1946).

Of the 204 New Guinea skinks examined, 8 individuals belonging to 6 species, i.e., 3.9% of the lizards, were infected with *Plasmodium* spp. Two basic types of malarial parasite infections, based on morphology of various blood stages, were observed (Fig. 3). One type, observed in 1 *Prasinohaema prehensicauda* specimen (Fig. 3A–C) and 2 *S. jobiense* individuals (Fig. 3D–F), had trophozoites with a large vacuole and often

TABLE I. Survey of hematoparasite infection in 204 New Guinea skinks representing 24 species from 10 genera across 10 localities. Locality numbers in parentheses refer to Figure 1.

Genus	Species	N	Province (locality)	Number (%) of individuals infected			
				Plasmodium	*Hepatozoon*	Trypano-somes	Microfilarial worms
Carlia	*ailanpalai*	5	Manus (1)	—	—	—	—
		1	Manus (2)	—	—	—	—
Carlia	*eothen*	3	Milne Bay (3)	—	—	—	—
Carlia	*mysi*	5	New Ireland (4)	—	—	—	—
		1	Oro (5)	—	1 (100)	—	—
Emoia	*atrocostata*	2	Madang (6)	—	—	1 (50)	—
		1	New Ireland (4)	—	—	—	—
Emoia	*caeruleocauda*	1	Manus (1)	—	—	—	—
		2	Manus (2)	—	—	—	—
		1	Oro (5)	1 (100)	—	—	—
Emoia	*cyanura*	4	New Ireland (4)	—	—	—	—
Emoia	*jakati*	3	Madang (6)	1 (33)	1 (33)	—	—
Emoia	*kordoana*	3	Madang (6)	—	—	—	—
		1	Manus (2)	1 (100)	—	—	—
		2	Oro (5)	—	—	—	—
Emoia	*longicauda*	2	Milne Bay (3)	2 (100)	2 (100)	—	—
		1	Oro (5)	—	—	—	—
Emoia	*mivarti*	6	Manus (1)	—	—	—	—
		2	Manus (2)	—	—	—	—
Emoia	sp.	1	Oro (5)	—	—	—	—
		1	Milne Bay (3)	—	—	—	—
Eugongylus	*albofasciolatus*	1	Oro (5)	—	1 (100)	—	—
Lamprolepis	*smaragdina*	2	Milne Bay (3)	—	—	—	—
		1	Oro (5)	—	—	—	—
		4	Madang (6)	—	—	—	—
		1	Madang (7)	—	—	—	—
		2	Manus (2)	—	—	—	—
		2	New Ireland (4)	—	—	—	—
Lipinia	*noctua*	3	Madang (6)	—	—	—	—
		4	New Ireland (4)	—	—	—	—
Lobulia	*elegans*	3	Morobe (8)	—	—	—	—
Papuascincus	*stanleyanus*	7	Madang (7)	—	1 (14)	1 (14)	—
		1	Morobe (8)	—	—	—	—
Prasinohaema	*flavipes*	10	Madang (7)	—	1 (10)	—	—
Prasinohaema	*prehensicauda*	23	Madang (7)	1 (4)	1 (4)	—	—
Prasinohaema	*semoni*	1	Milne Bay (3)	—	—	—	—
		1	Morobe (9)	—	—	—	—
Prasinohaema	*virens*	52	Madang (6)	—	—	—	—
		8	Milne Bay (3)	—	—	—	—
		1	Oro (5)	—	—	—	—
Sphenomorphus	*jobiense*	2	Madang (6)	—	—	—	—
		1	Manus (2)	1 (100)	—	1 (100)	1 (100)
		2	Milne Bay (3)	—	1 (50)	—	—
		7	Morobe (9)	—	—	—	—
		2	Oro (5)	1 (50)	1 (50)	1 (50)	—
Sphenomorphus	*leptofasciatus*	4	Madang (7)	—	—	—	—
Sphenomorphus	sp.	3	Madang (6)	—	—	—	—
		1	Morobe (10)	—	—	—	—
		6	Morobe (9)	—	—	—	—
Tribolonotus	*brongersamai*	1	Manus (2)	—	—	—	—

with spindled ends. The schizonts of this type were either fan or rosette shaped (Fig. 3B–E), and the gametocytes were rounded to slightly reniform or ovoid, always polar, and commonly had a large vacuole with small pigment granules around the periphery of this vacuole (Fig. 3C–F). The second type of infection, observed in 4 *Emoia* species, i.e., *E. jakati*, *E. caeruleocauda*, *E. kordoana*, and 2 individuals of *E. longicauda*, consisted of small, ringlike trophozoites (Fig. 3G); large schizonts (approximately 10 μm in average diameter) with no specific shape, often in hypertrophied cells (Fig. 3H); and very large (~15 μm in length) elongate to reniform gametocytes that sometimes filled approximately three-quarters of the host eryth-

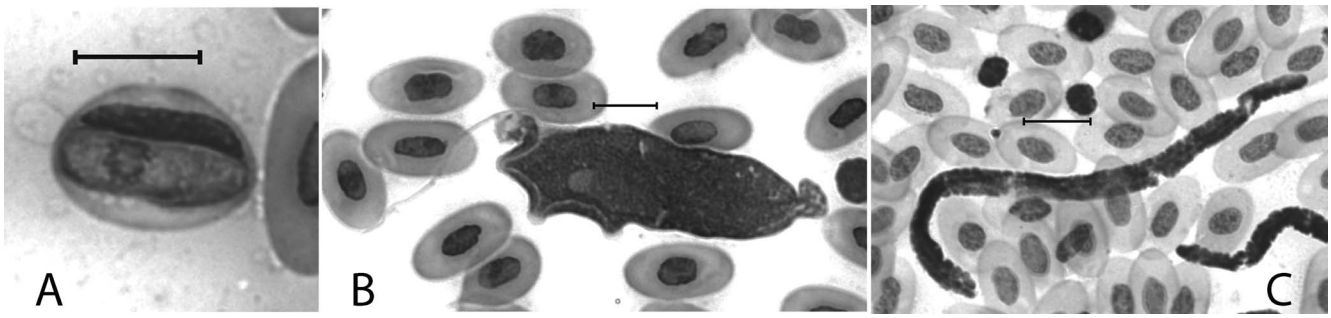

FIGURE 2. Non-*Plasmodium* blood parasites observed in New Guinea skinks. (**A**) *Hepatozoon* sp. (**B**) Trypanosome. (**C**) Microfilariae. Scale bar equals 10 μm.

rocyte (Fig. 3I). Some smaller gametocytes, again elongate and adjacent to the nucleus, were sometimes observed; these may represent immature gametocytes. These suites of life history stage morphotypes were consistent in all infections, e.g., the first type of trophozoite was never found in combination with the second type of gametocyte, and mixtures of the 2 morphological types were never found within the same individual host.

Although 96 of the 204 lizards surveyed (47%) had green blood pigmentation, only 2 of these individuals were infected with any hematozoa. Thus, the green-blooded lizards accounted for only 1 of the 8 (12.5%) *Plasmodium* spp. infections ($\chi^2 = 2.56$; $P = 0.11$) and 2 of the 9 ($\chi^2 = 1.34$; $P = 0.24$) hemogregarine infections (1 *P. prehensicauda* individual had both *Plasmodium* spp. and hemogregarines).

Parasite cytochrome b sequences were obtained from all *Plasmodium* spp. infections (GenBank Accession numbers DQ337362–DQ337366), with the exception of those in the *E. jakati* and *P. prehensicauda* specimens, for which only stained blood smears with cover slips were available. In all other infections, a single sequence was recovered, with no ambiguous peaks in the electropherogram data, suggesting single infections. The phylogenetic analyses of these sequences along with additional ingroup (other lizard malaria parasites) and outgroup (mammalian malaria parasites) produced 11 equally parsimonious trees of 658 steps; the strict consensus is shown in Figure 4. The 6 Papua New Guinea skink *Plasmodium* infections cluster into 2 well-supported monophyletic groups, 1 in *S. jobiense* and 1 in *Emoia* spp. These New Guinea parasites, then, are part of a larger group of lizard malaria parasites that includes those from Central America (*Plasmodium fairchildi*) and the Caribbean (*Plasmodium azurophilum* ''red'' and *P. azurophilum* ''white''; Perkins, 2000). Elsewhere in the phylogeny is a clade containing the Old World taxa, *Plasmodium agamae* from Africa, *Plasmodium mackerrasae*, from Australia, and 2 parasites in *Haemocystidium*, i.e., *Haemocystidium ptyodactyli* Paperna and Landau, 1991, from Israel, and *Haemocystidium kopki*, Wenyon, 1926, from Pakistan. The western North American lizard parasites, *Plasmodium mexicanum* Thompson and Huff, 1944, and *Plasmodium chiricahuae* Telford, 1970, are basal to all others in a clade with 100% jackknife support.

The average sequence divergence in the cytochrome b fragment between the 2 groups of New Guinea skink *Plasmodium* isolates is 4.4%. The 2 *Plasmodium* spp. isolates from *S. jobiense* had identical cytochrome b sequences, even though the individuals possessing the infections came from locales approximately 800 km apart (Fig. 1, locations 2 and 3). Within the *Emoia* parasite clade, there was substantial diversity, with pairwise divergences ranging from 0.48% between the infection from the 2 *E. longicauda* collected from Milne Bay Province (Fig. 1, location 3) and the infection from *E. caeruleocauda* collected from Oro Province (Fig. 1, location 5), to 1.88% divergence between the 2 *E. longicauda* samples, both collected in Milne Bay (Fig. 1, location 3).

DISCUSSION

This is the first survey of lizard hematozoa in mainland New Guinea and the largest sampling effort for blood parasites in this region, with collections from 10 of 19 (52%) skink genera and 24 of 128 species (9.3%) from Papua New Guinea. Our survey, however, generally only sampled a small number of individuals for each skink species. Nonetheless, our results already suggest that a diverse hematoparasite community is present on the island. This initial survey, however, needs to be expanded to understand the full diversity of *Plasmodium* species and other blood parasite species richness and the congruence, if any, between lizard host phylogeny. Hampering efforts in this regard is the poorly understood phylogenetic relationships for hosts within both the Eugongylus and Sphenomorphus groups.

Prior to this study, just 3 species of *Plasmodium* in lizards had been described from the island of New Guinea and only 8 had been described from all of Oceania. The first report of lizard blood parasites from New Guinea was by Thompson and Hart (1946), who examined 1 species of scincid lizard (*Leiopisma fusca*, now *Carlia mysi* Zug, 2004) sampled from Goodenough Island. These blood parasites included haemogregarines, trypanosomes, and microfilariae worms, as well as at least 1 species of *Plasmodium*. Thompson and Hart (1946) named this species, *Plasmodium lacertiliae*, and noted that it resembled *Plasmodium tropiduri* Aragão and Neiva, 1909, a malaria parasite described from Brazilian iguanid lizards, i.e., *Tropidurus* sp. Subsequent ultrastructural work on the intraerythrocytic stages (Scorza, 1970) concluded that the 2 species were, for all intents and purposes, identical. Artificial infections demonstrated that *P. tropiduri* was capable of establishing infections in skinks from the Neotropics (Lainson and Shaw, 1969); thus, Scorza (1970) hinted that *P. lacertiliae* and *P. tropiduri* should be synonymized. However, the prospect of parasites maintaining gene flow and remaining a single species over the enormous

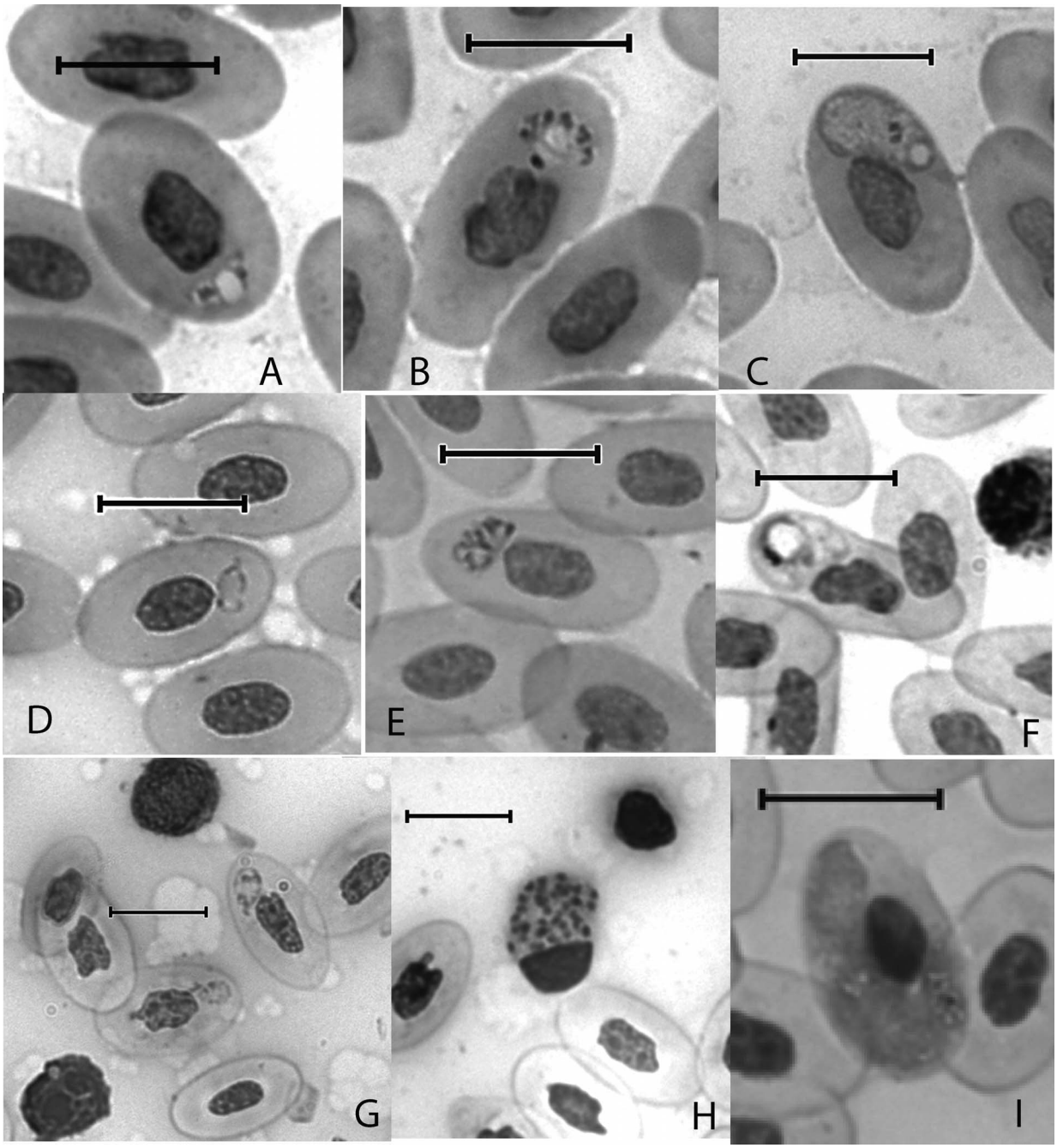

FIGURE 3. Morphological types of *Plasmodium* observed in the 3 skink genera: (**A**) trophozoite in *Prasinohaema prehensicauda*; (**B**) schizont in *P. prehensicauda*; (**C**) gametocyte in *P. prehensicauda*; (**D**) trophozoite in *Sphenomorphus jobiense*; (**E**) schizont in *S. jobiense*; (**F**) gametocyte in *S. jobiense*; (**G**) trophozoite in *Emoia longicauda*; (**H**) schizont in *E. longicauda*; and (**I**) gametocyte in *E. longicauda*. Scale bar in each photo equals 10 μm.

geographic distance separating Brazil and New Guinea seems highly implausible, and they have remained separate species in the literature.

Recently, Telford and Wellehan (2005) described 2 species, *Plasmodium tribolonoti* and *Plasmodium gracilis*, both from a single crocodile skink (*Tribolonotus gracilis*) obtained from the pet trade, which they presumed to have come from the Indonesian province of Papua, at the western half of the island of New Guinea (formerly called Irian Jaya). Unfortunately, the geographic provenance of the single host animal is entirely unknown. That is, neither a type locality nor any interim localities where the lizard may have acquired the infection were known to Telford and Wellehan (2005). Furthermore, their belief that the host lizard had come from Indonesia was based on an assumption that exportation from Papua New Guinea would have been less likely. Telford and Wellehan (2005) differentiated the 2 species primarily using schizont shape, with *P. tribolonoti* having ''no particular arrangement of nuclei'' and *P. gracilis*

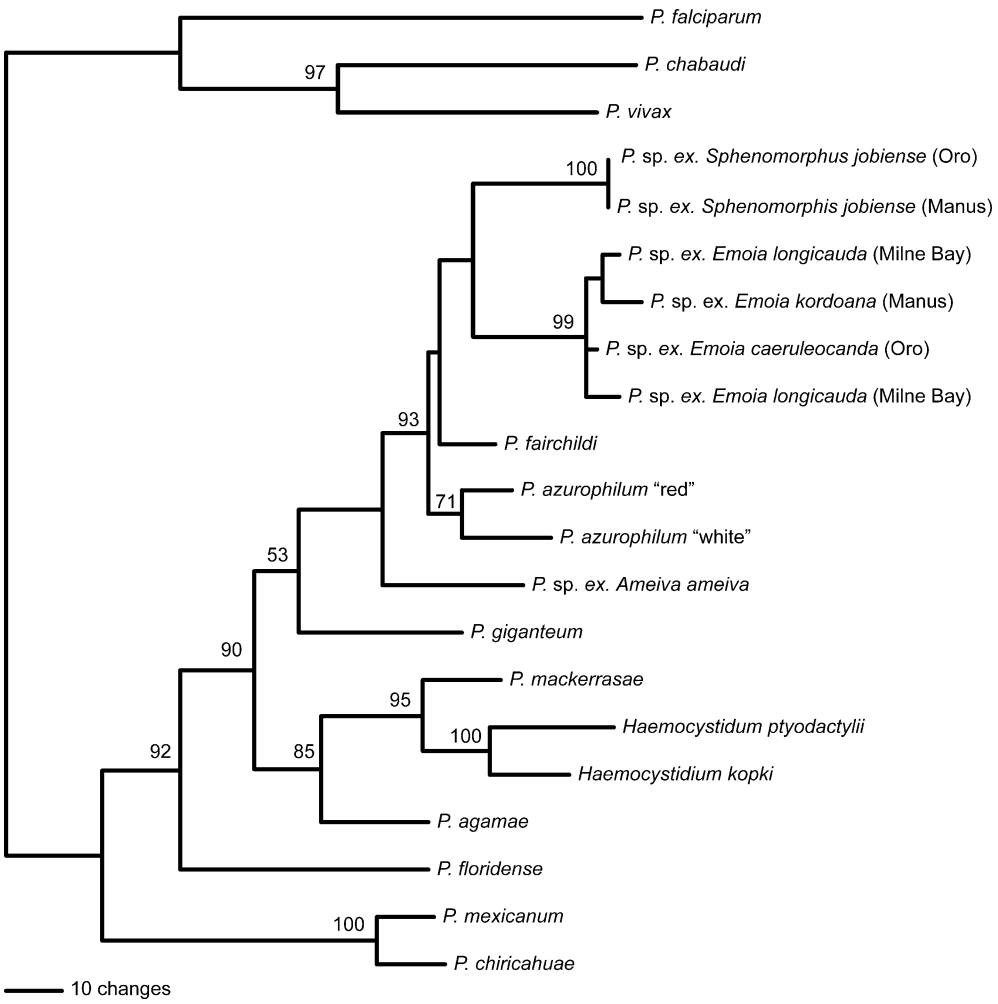

FIGURE 4. Phylogeny of mitochondrial cytochrome b sequence data from New Guinea lizard malaria and other saurian malaria parasites, rooted with mammalian *Plasmodium* species. The strict consensus of 11 equally parsimonious trees with jackknife support values >50% is shown.

having fan-shaped schizonts and larger gametocytes. However, instead of providing a statistical determination that gametocyte diameter failed to conform to a unimodal distribution, the authors separated the 2 putative species a priori on the basis of size and then differentiated the 2 species in their descriptions with these same size differences. On the whole, then, the findings of Telford and Wellehan (2005) should probably be interpreted with a great deal of caution.

Five other lizard malaria parasites have also been described from the Pacific region. Laird (1951) described *Plasmodium lygosomae* from a New Zealand skink, *Lygosoma moco* (now *Oligosoma moco*; Duméril and Bibron, 1839). Of particular note for this species were its unusual D-shaped trophozoites and extraerythrocytic schizogony, a phenomenon that, up until that time, had only been observed in *P. mexicanum* from western North America. The remaining 4 species of saurian malaria from the Pacific, i.e., *Plasmodium australis*, *Plasmodium egerniae*, *P. mackerrasae*, and *Plasmodium circularis*, have been described from Australia (Mackerras, 1961; Garnham, 1966; Telford, 1979; Telford and Stein, 2000). Based on morphology, 1 species, *P. australis*, was originally identified as *Plasmodium giganteum* (Mackerras, 1961), the type of which is a parasite of African agamid lizards; however, as with *P. lacertiliae*/*P. tropiduri*, *P. australis* was separated as a distinct species (Telford, 1988) because of the substantial current-day geographic separation.

Based on the observed morphological characteristics and the molecular date generated by the present study, it is likely that the 2 forms observed in the New Guinea skinks represent 2 new species of lizard malaria parasites. The first *Plasmodium* species infection type that we observed, in *P. prehensicauda* and *S. jobiense*, resembles *P. lygosomae*, reported from New Zealand skinks, in having small trophozoites with large vacuoles, hence producing the characteristic D shape noted by Laird (1951). The schizonts of this first type also resembled the intraerythrocytic forms described from *P. lygosomae* as described by Laird (1951); however, unlike the New Zealand infections, no exoerythrocytic schizonts were observed in the infections of the New Guinea lizards. The gametocytes in this first infection type also did not follow the pattern for *P. lygosomae*, which is said to have gametocytes that are ''commonly reniform in shape,'' that ''usually lie alongside the host nucleus,'' and with macrogametocytes that stained very darkly (Laird, 1951). As noted above, our New Guinea samples always had polar ga-

metocytes with prominent vacuoles and none stained darkly. The second infection type that was observed, always in *Emoia* skinks, shares characteristics with both *P. egerniae*, from skinks in Queensland, Australia (Mackerras, 1961), and with *P. lacertiliae*, the parasites described from Goodenough Island, off the coast of New Guinea, but does not conform exactly to either species description.

These 2 *Plasmodium* infection morphotypes correspond exactly to the 2 monophyletic groups of parasites obtained with the cytochrome b sequence data. The 4.4% divergence between these 2 groups is more than twice as much as the divergence between the erythrocytic and leucocytic forms of *P. azurophilum* (2.1%) or the divergence between *P. mexicanum* and *P. chiricahuae* (1.9%). Thus, it would seem that there are at least 2 distinct species present in our samples of mainland New Guinea skinks and the substantial genetic diversity within the *Emoia* parasite clade hints that there may be even more. Unfortunately, except for *P. mackerrasae*, DNA isolates were not available for any of the other Pacific saurian malarias, so we cannot, at present, be confident in claiming that the 2 groups from New Guinea are *P. lygosomae*, *P. lacertiliae*, *P. egerniae*, or perhaps 2 or more new species altogether without these data. The lack of a DNA sample for the infection from *P. prehensicauda* also precludes at this point any ability to discern whether this is a separate species or the same as the one observed in *Sphenomorphus*. The corroboration of the 2 morphotypes of parasites with 2 distinct clades in the phylogenetic tree suggest that there is an affinity of these 2 types of New Guinea lizard *Plasmodium* to the 2 different genera of skink hosts. The genetic, historical, phylogenetic, and/or biogeographic reasons for such host specificity are unclear, since in New Guinea there are no major ecological or biogeographic differences between the species-rich *Eugongylus* and *Sphenomorphus* group skinks. Both groups occupy a diversity of habitat types ranging from lowland tropical forest to montane habitats, and both groups have fossorial, terrestrial, and arboreal species and are broadly as well as locally sympatric across much of the island (Allison, 1996).

It is currently unknown whether the high concentration of biliverdin in the blood of lizards in species of *Prasinohaema*, resulting in a green color, is a physiological or ecological adaptation of any sort. One possibility is that the biliverdin accumulation might deter infection by malaria parasites and offer protection from any detriments to fitness caused by the parasites. The apparent low level of *Plasmodium* spp. infections in these skinks suggests that this could be the case. Although the proportion of infected green-blooded lizards was not significantly smaller statistically, this could be an effect of the small sample sizes of the red-blooded skink species. For each species of red-blooded skink where malaria was found, the prevalence was very high, i.e., 33% to 100%. If these values reflect true prevalence of *Plasmodium* spp. in these hosts, then there would be a highly statistically significant effect of blood pigmentation. For example, if an equivalent number of *E. jakati* as *P. prehensicauda* individuals had been sampled (23), and the prevalence of *Plasmodium* remained 33%, this would have meant 6 additional infections in this species, resulting in a χ^2 value of 7.4 ($P = 0.0065$).

The phylogenetic placement of the New Guinea parasites with several New World tropics species is intriguing. At present it is difficult to discern whether there are true biogeographic connections between these 2 regions for *Plasmodium* species and, if so, whether they are the result of ancient vicariance events associated with continental breakups or long-range dispersal events. No specimens were available to obtain DNA from *P. tropiduri*, so the hypothesis of Scorza (1970) that this species and *P. lacertiliae* are synonymous could not be tested. The New Guinea saurian malaria parasites do not appear to be closely related to the Old World saurian malarias; however, these results are based on a single-gene analysis of a gene that likely evolves very slowly (Perkins and Schall, 2002). Certainly there is a pressing need to sample malaria parasites from throughout the Australasian and Pacific regions and to continue efforts toward the integration of both morphological and molecular approaches for understanding the mechanisms responsible for the production and maintenance of parasite biodiversity, their evolutionary relationships, and the biogeographic patterns in this region.

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