

**STATISTICAL MEASURES OF ASSOCIATION BETWEEN
AMBLYOMMA SABANERAE STOLL (ACARI: IXODIDA: IXODIDAE) AND
THE FURROWED WOOD TURTLE, *RHINOCLEMMYS AREOLATA*
(DUMÉRIL AND BIBRON) (TESTUDINES: EMYDIDAE),
IN NORTHERN BELIZE**

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Abstract.—Between April 1997 and June 1998, 159 tick collections, comprising 411 specimens, were made from 261 adults and juveniles of the furrowed wood turtle, *Rhinoclemmys areolata* (Duméril and Bibron), on a tract of pine forest and savanna in northern Belize. All ticks were determined to be *Amblyomma sabanerae* Stoll, a common parasite of testudines in the lowland tropics of Central America. Male ticks were collected from more turtles (94) than were females (48) or immatures (82). However, despite the likelihood that male turtles have greater home ranges, significantly more ticks parasitized female turtles than males. It is thought that overdispersion of both ticks and turtles declines in the equable climate of the Neotropics, in which case female turtles, being larger than males, would be expected to acquire more ticks. Collections of immature *A. sabanerae* were more frequently made on fleshy areas of *R. areolata*, but the mean number of nymphs and larvae collected from anterior areas was not significantly different from that for posterior areas.

Key Words: Acari, Ixodidae, *Amblyomma sabanerae*, host relationships, Belize

Neotropical turtles and tortoises (Testudines) are the principal hosts of only 8 tick species: the argasid *Ornithodoros transversus* (Banks), and the ixodids *Amblyomma crassum* Robinson, *A. humerale* Koch, *A. macfarlandi* Keirans, Hoogstraal and Clifford, *A. pilosum* Neumann, *A. sabanerae* Stoll, *A. testudinis* (Conil), and *A. usingeri* Keirans, Hoogstraal and Clifford (Hoogstraal and Aeschlimann 1982). Yet, the ecology, population dynamics and disease relationships of these acarines have seldom been addressed, the most comprehensive

studies to date being those of Schneider et al. (1971) and Ernst and Ernst (1977). This is lamentable because, in recent years, testudine populations have been declining precipitously worldwide (Ernst and Barbour 1989), with the result that of 247 nominal species listed by King and Burke (1989), 70 (28%) appear in Appendix I (species threatened with extinction that are or may be affected by trade) or Appendix II (species not necessarily threatened with extinction but that may become so unless trade is strictly regulated) of the Convention on In-

ternational Trade in Endangered Species of Wild Fauna and Flora. Between April 1997 and June 1998, we were able to methodically collect ticks from a Belizean population of the furrowed wood turtle, *Rhinoclemmys areolata* (Duméril and Bibron), a semiterrestrial species that is still common over most of its range: south-central Veracruz, Tabasco and eastern Chiapas to the Yucatán Peninsula and Cozumel Island in Mexico, south through Belize, eastern Guatemala, and perhaps eastern Honduras (Ernst and Barbour 1989). Our results contrast markedly with those from a similar study of tick populations on tortoises in the arid subtropics of southwestern Russia (Robbins et al. 1998).

MATERIALS AND METHODS

Turtles were collected on a privately owned 405-ha tract (17.22N, 88.34W) of pine forest and savanna adjacent to the Western Highway in northern Belize, approximately 48 km west of Belize City. The flora of this area is characterized by an overstory of Caribbean pine, *Pinus caribaea* Morelet, and oak, *Quercus oleoides* Schlechtendal and Chamisso, with an understory of craboo, *Byrsonima crassifolia* (L.) Kunth, sandpaper tree, *Curatella americana* L., wax myrtle, *Myrica* spp., miconia, *Miconia* spp., and eugenia, *Eugenia* spp. Savannas are dominated by grasses (Gramineae) and sedges, *Rhynchospora* spp. and *Scleria* spp., with scattered pines, craboo, and clusters of paurotis, *Acoelorrhaphe wrightii* (Grisebach and Wendland) Wendland ex Beccari (Furley 1989). The climate of northern Belize is considered tropical because the average temperature each month exceeds 18°C. Warmest temperatures are recorded during the dry season in April and May, with average maxima of 32.8 and 33.1°C, respectively. At this season, natural and anthropogenic fires occur in both pine forest and savanna, as they did in April and May 1997, when our study site was swept by a series of catastrophic blazes. Precipitation at our site averages 150 cm/yr, with

a pronounced wet season from June to November, though this may vary annually (Hartshorn et al. 1984).

Wood turtles were sought during the morning (0700–1000 hr) and evening (1530–1800 hr), when they are most active (Vogt and Platt, in press). Although common on the study site, turtles proved difficult to locate in the thick ground vegetation, and most (>75%) were found with the aid of a trained dog. Captured turtles were taken to a field laboratory, where a variety of morphometric data were collected. Sex was determined on the basis of tail morphology: males have long, thickened tails with the vent well beyond the carapace margin, whereas females have short tails with the vent beneath the carapace margin (Ernst and Barbour 1989). Sex could not be determined for juveniles (carapace length < 11 cm). Each turtle was permanently marked by notching a unique series of marginal scutes (Cagle 1939) and was released at its point of capture within 24 hours. Of 261 male, female, or unsexed juvenile *R. areolata* taken in this manner, 159 (61%) were parasitized by ticks: 52 (32.7%) male turtles, 75 (47.2%) females, and 32 (20.1%) juveniles. Infested turtles were assigned accession numbers (RA1-RA159) and MEDARKS (Medical Archives) numbers (1997-0546 through 1997-0703) by the Wildlife Conservation Society (formerly New York Zoological Society), Bronx, New York. Tick collections corresponding to these numbers were shipped to RGR in vials of Formalin, later changed to 70% isopropyl alcohol, for identification and analysis. All tick collections have been deposited at the American Museum of Natural History, New York, New York.

RESULTS

A total of 411 tick specimens were removed from our 159 infested *R. areolata* during this study. All were *A. sabanerae*, whose range overlaps that of *R. areolata* and extends southward as a parasite of various turtles, e.g., *R. annulata* (Gray), *R. fu-*

Table 1. Descriptive statistics for all stages of *Amblyomma sabanerae* from 261 *Rhinoclemmys areolata*; Belize District, northern Belize, April 1997–June 1998 (collectors S. G. Platt and T. R. Rainwater).

Tick Sex/Stage	Number (%)	Range*	Mean with Standard Error	Standard Deviation	Coefficient of Variation
Males	126 (31)	0–4	0.5 ± 0.05	0.8	160.0
Females	55 (13)	0–3	0.2 ± 0.03	0.5	250.0
Nymphs	172 (42)	0–13	0.6 ± 0.09	1.5	250.0
Larvae	58 (14)	0–24	0.2 ± 0.10	1.7	850.0

* Ticks/turtle.

nerea (Cope), *R. pulcherrima* (Gray) and *Trachemys scripta* (Schoepf), into the low-land tropics of Panama (Fairchild et al. 1966) and perhaps northern South America (records of *A. sabanerae* from Colombia (Schulze 1937, Osorno-Mesa 1940) and Suriname (Oudemans 1902) require confirmation). Adults of *A. sabanerae* were separated from other turtle-infesting amblyomines using the key of Jones et al. (1972). The preimaginal stages (larva, nymph) of *A. sabanerae* have not been described and were “identified” by association with adults. Occasional nymphs of the reptile and amphibian parasite *A. dissimile* Koch were found on *R. areolata* at other locations in northern Belize, but the nymphal dentition of *A. dissimile* is 3/3 (Keirans and Durden 1998), whereas in nymphs that we are calling *A. sabanerae* it is 2/2.

Descriptive statistics for *A. sabanerae* on *R. areolata* appear in Table 1. Males of *A. sabanerae* were recovered from 94 turtles, nymphs from 77 turtles, but females and larvae from just 48 and 12 turtles, respectively. Because male ticks were least overdispersed (clumped) on the turtle population, they were employed to test whether observed differences in parasitization between male and female turtles were statistically significant (juvenile turtles were excluded from this analysis because too few were parasitized). The mean number of male *A. sabanerae* on male *R. areolata* was 0.6 ± 0.1 (mean \pm standard error), while that for female turtles was 1.0 ± 0.1 . This difference is significant at $t = 2.765$, $df = 126$, $P < 0.01$. The higher mean infestation

of female wood turtles in the humid tropics of Belize stands in contradistinction to the much lower mean recorded for female spur-thighed tortoises, *Testudo graeca nikolskii* Chkhikvadze and Tuniev, relative to male tortoises infested with the tick *Hyalomma aegyptium* (L.) along the xeric Black Sea coast of southern Russia (Robbins et al. 1998). There are numerous possible explanations for this striking difference in mean infestation, among them sex- or species-specific differences in host behavior, the development in testudines of a degree of age-related immunity to tick parasitism (Wikel 1996, Tembo and Kiwanuka 1997), or the release of pheromones by female ticks that could affect the numbers of males (Sonenshine 1985, Gothe 1987). Unfortunately, virtually nothing is known about the physiological ecology of *A. sabanerae*, *H. aegyptium*, or the hosts of these tick species. Considering only habitat differences, it is possible to speculate that overdispersion of both ticks and testudines declines in the more equable climate of the Neotropics, where reduced saturation deficiency favors tick survival and luxuriant vegetation provides unbroken cover for turtles. By contrast, environmental extremes in the arid scrublands of southern Russia often confine ticks to “islands” of favorable habitat and restrict tortoises to their burrows. Male spur-thighed tortoises are thought to acquire more ticks chiefly because they have larger home ranges. Where environmental variables do not play a major limiting role, host attributes more likely account for observed differences in tick infestation. In the case at

hand, the mean carapace length (cm) of female turtles was 14.6 ± 0.2 , while that for males was only 12.6 ± 0.2 . This difference is highly significant at $t = 6.666$, $df = 126$, $P \ll 0.001$. Larger female turtles might acquire more ticks than males when moving through habitat in which ticks are less contagiously dispersed—even if male turtles have larger ranges.

Another attribute affecting tick loads is the nature of turtle integument. In Mesoamerican populations of four species of *Rhinoclemmys*, Ernst and Ernst (1977) noted that 78.5% of adult *A. sabanerae* were found attached to the shell; conversely, 95.5% of immature ticks were attached to exposed turtle skin, mostly at limb sockets or on the gular fold. Schmidt (1946) provided a photograph of the carapace of a Panamanian specimen of *R. annulata* on which numerous adult ticks, all clearly *A. sabanerae*, had attached at the seams between carapacial scutes. By a test of association (Sokal and Rohlf 1973), Ernst and Ernst (1977) were able to statistically demonstrate this segregation of adult and immature ticks on the basis of attachment site, reasoning that the shorter mouthparts of larvae and nymphs may restrict these stages to feeding at locations where the skin is thinner and that this situation may reduce competition between developmental stages. In our study, immature *A. sabanerae* were found attached to fleshy areas (head, neck, legs, tail) on *R. areolata* 113 times but to the carapace or plastron only 8 times. Collections of adult ticks were made from the carapace and plastron 116 times but from fleshy areas just 32 times. Again, a test of association reveals that these frequencies are highly dependent ($G = 157.818$, $P \ll 0.005$) upon the stage of tick development. However, despite our anticipation that preimaginal ticks would more often be encountered on anterior rather than posterior fleshy areas, as is generally the case with small mammals (Thompson 1974, Barré et al. 1991) and with the reptile tick *A. marmoreum* Koch on the leopard tortoise, *Geo-*

chelone pardalis (Bell), in South Africa (Fielden and Rechav 1994), the mean number of nymphs and larvae collected from anterior areas of *R. areolata* (3.7 ± 1.1) was not significantly different from that for posterior areas (2.8 ± 0.8 ; $t = 0.651$, $df = 111$, $P \gg 0.05$).

During the 15 months of this study, no seasonal trend in the percentage of parasitized turtles could be discerned. On a month-to-month basis, figures ranged from 0.0% (February 1998) to 85.7% (May 1997), but months are arbitrary time units that, coupled with wide variations in the number of turtles examined (99 in July 1997, none in November of that year), can easily give a false impression of the prevalence of parasitization. On our population of *R. areolata*, the overall prevalences, sensu Margolis et al. (1982), of adults, nymphs, and larvae of *A. sabanerae* were 45.2%, 29.5%, and 4.6%, respectively. Nothing is known of the life cycle of *A. sabanerae* or, indeed, of most Neotropical amblyommines. Until these baseline data are established through rearing studies, the temporal dynamics of tick populations in the field will remain unresolved.

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