

# GUANA REPORT

## 2006

by  
J. Lazell



Vagrant African Emperor  
photo by Andre Guenther

# *The Conservation Agency*

*Exploration, Education, and Research*

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7 July 2007

Dr. Henry Jarecki  
10 Timber Trail  
Rye, NY

Dear Henry:

Apologies for the lateness of this report. As you know, we were in China until 8 May and didn't get back to Rhode Island until late June. The two-plus year China project is completed and I have been able to refocus on Guana.

Our month in 2006 was early, as in 2005. Without that last week of October we get less good stuff -- especially insects and migrating birds. We are happy to be rescheduled for all October in 2007.

Here is a sketch of what follows:

**Cover Story** - The second New World record for this big dragonfly and a record year for all Odonata. More details and an annotated list of all the species appears below

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**Birds** - Another excellent season for the Boal team with rare residents like the Virgin Islands Yellow Warbler and rare migrants like the Black-throated Warbler .....

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**Turtle** - Just like the introduced "red-eared slider" of the universal pet trade, but now a Tortola resident.....

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**Stout Iguana** - Are Guana's bigger and faster growing? Looks that way. Can we ever prove the geographic origin of iguana bones? Dr. Sam Turvey has a plan .....

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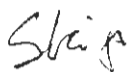
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That's it for now. See you in October.

All the best,



James Lazell, Ph.D.

## COVER STORY

### AFRICAN VAGRANT EMPEROR

No, not Haile Selassie reincarnate, but a magnificent big dragonfly (Hemianax ephippiger). Jill Silsby, in her book *Dragonflies of the World*, says: "This species occurs throughout Africa, breeding in any suitable water from the Cape to the Mediterranean coast; its movements appear to be complexly related to weather systems and seasons. It quite regularly crosses the Mediterranean into Europe and Asia...."

It is the only dragonfly known to have reached Iceland. However, there was only one lonely record of its ever reaching the New World: One was found on Guadeloupe, French West Indies, in January, 2006.

Fred Sibley got the second one on Guana this October. It has made the rounds of experts for verification, and it took this long to be absolutely certain just what it was.

When I wrote the book, there were ten species of dragonflies and damselflies known from Guana. Three of those were only caught by Fred in the weird El Nino year of 1997, and we suspected they had been blown eastward from their regular migratory route through the Greater Antilles. Fred got all three again this year - with no El Nino effect at all. Remind anyone of our migratory bird records?

**ORNITHOLOGICAL MONITORING AND RESEARCH ON  
GUANA ISLAND, BRITISH VIRGIN ISLANDS**

**PROJECT REPORT 2006**

**Clint W. Boal, Ph.D.**

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3 April 2007



**Yellow Warbler**

## ORNITHOLOGICAL RESEARCH AND MONITORING ON GUANA ISLAND, BRITISH VIRGIN ISLANDS: PROJECT REPORT 2006

Dr. Clint W. Boal, *USGS Texas Cooperative Fish and Wildlife Research Unit, Texas Tech University, Lubbock, TX 79409-2120*

### INTRODUCTION

In terms of ornithological reports, the Virgin Islands, especially the British Virgin Islands (BVI), have received very little attention compared to the rest of the West Indies (Wiley 2000). Thus, ornithological work on Guana Island makes substantive contributions toward a better understanding of the Virgin Islands as stop-over habitat for migrant birds and the basic ecology of Caribbean birds. A key component of the avian research on Guana Island is the annual mist-netting and banding of neotropical songbirds that migrate through the Caribbean region during the autumn migration. This activity alone has increased the scientific understanding of neotropical migrant use of the BVI and led to documentation of previously unknown migrants in the BVI (McNair et al. 2002, Boal et al. 2006, Boal and Estabrook 2007).

In the 2005 annual report (Boal 2006), objectives for avian research during the 2006 Science Month on Guana Island were identified as:

- Continuation of annual mist-netting of neotropical migrants.
- Initiation of annual surveys to develop an understanding of bird species distributions and relative abundances across Guana Island, and how these parameters change in relation to environmental events.
- Continuation of a color-banding program to facilitate an ongoing study of population ecology of Bananaquits.
- Continuation of a Mangrove Cuckoo study.

All of these objectives were satisfactorily met during the 2006 Science Month. Here I provide data and discussion of the Results of the 2006 field season, a review of research productivity stemming from avian research on Guana Island, and plans for the 2007 Science Month.

### RESULTS AND DISCUSSION

#### *Mist-Netting*

I operated mist-nets from 29 September through 20 October 2006 for a total of 400 net hours. I captured 209 new birds of 24 species in 2006. This included several hummingbirds ( $n = 9$ ) which were released at the point of capture and not banded, and a Scaly-naped Pigeon ( $n = 1$ ) for which I did not have the correct band size. I recaptured 9 birds from 2006, and 56 birds banded prior to 2006. I also captured and euthanized 10 Pearly-eyed Thrashers for stomach contents analysis. Therefore, I captured and removed 284 birds from nets during the 2006 netting operation (Table 1). This was considerable fewer birds, but 3 more species, than the banner year of 2005 during which I captured 428 birds. This difference is primarily due to the migrant fallout, which was huge in 2005 and only slightly better than average in 2006. However, I captured more birds in 2006 than in 2003 or 2004. My capture rate of 0.71 birds per net hour in 2006 was the lowest I have experienced since I began mist-netting operations on Guana Island in 2003 (Table 2), but not unexpected given the inconsistent pattern of migrant fallouts on the island.

Compared to 2005, when I banded 176 Blackpoll Warblers and 26 Swainson's Thrushes, 2006 was not nearly as productive in terms of neotropical migrants. However, I still captured a large number of Blackpoll Warblers ( $n = 63$ ), and some infrequent migrants such as Hooded Warblers, Black-and-white Warblers, Gray-cheeked Thrush, and Indigo Buntings. More unusual was the capture of an American Redstart and a Black-throated Blue Warbler, which are rare on Guana Island.

Of substantive interest from an ornithological conservation perspective is my capture of 3 Yellow Warblers. This species had apparently disappeared from Guana Island after 1955, was observed once in 1976, and then again but sporadically since 1993 (Lazell 2005) with an active nest found in June 2005 (Lazell, pers. comm.). In addition to the captures, several individuals were observed in the wooded areas around the flat and salt pond. However, it remained unknown as to the residency status of the species on Guana Island. Based on wing measurements, the Yellow Warblers captured were the Caribbean resident form (*Dendroica petechia cruziana*) and not the North American continental form and migrant (*D. p. petechia*). I suspect they are resident to Guana Island and are again present as a breeding bird.

An item for consideration is the timing of bird captures during mist-netting activities. Science month has been starting during the last 8-10 days of September and ending approximately 20-23 October. When examining 2005 and 2006, two of the biggest years in terms of number of migrants, it becomes apparent that migrants do not start arriving in any substantial numbers until after the 15<sup>th</sup> of October. In fact, over 80% of neotropical migrants captured were captured during the 16-20 of October (Fig. 1). This is not an artifact of when the nets are open; we are engaged in mist-netting throughout Science Month. Efforts toward capturing neotropical migrants would be facilitated by shifting Science Month into late October. Given the past schedules, I suspect the majority of migrants arrived after Science Month was not completed.

### Surveys

In 2006 I initiated an island wide bird survey that I anticipate conducting annually. This is important in documenting changes in species abundance and distribution over time. The methodological approach was a replication of that used by Arendt (1995) and Wunderle (2001) during previous surveys on Guana Island. The only difference is that there are no permanent records of their survey points, so neither survey can be exactly replicated. Instead, I developed 53 survey points assorted among dry forest, gnut forest, the hotel area and the flat. To facilitate time efficiency, I followed Wunderle's (2001) example and used the existing trail system to assign survey points. I conducted surveys along the Pyramid, Snake Transect, Lao Wei Ping, Long Man Point, Monkey Point, and Sugar Loaf trails, and in the vicinity of the Hotel and the Flat.

I found both similarities and differences among surveys of 1994 (Arendt 1995), 2001 (Wunderle 2001) and my surveys (Table 3). Whereas Arendt (1995) and Wunderle (2001) detected similar numbers of Scaly-naped Pigeons and Bridled Quail-doves, I found more of the former and none of the latter. It is important to note, however, that I believe Bridled Quail-doves were generally abundant in 2006, as I saw them in several locations. However, they were not calling or visually detected during surveys. In contrast, Arendt (1995) found more Common Ground-doves and Zenaida Doves than did I or Wunderle (2001). Arendt (1995) also detected more crested hummingbirds than Wunderle (2001) or myself, and I detected far fewer caribs. However, I detected several hummingbirds that I could not identify; when I pooled all hummingbirds, the detection



rate was similar to that of Arendt (1995) and Wunderle (2001) for caribs. I suspect there was not a real difference in detection of hummingbirds, only in my ability to discern their identity by sound.

An item of potential concern is the status of Caribbean Elaenias. Arendt (1995) detected 0.43 per 25m survey plot in 1994. Seven years later, Wunderle (2001) detected only 0.23 per 25m plot in 2001, and five years later in 2006, I detected only 0.08 per 25m plot. Detections may be influenced by activity such as breeding and territoriality, which may have varied among survey years. Still, this species is not difficult to detect, and I and my assistants observed very few elaenias during our other research activities. Due to the dramatic decrease in detections, I will place special emphasis on evaluating elaenias during the next field season to better assess any changes in population.

On a positive note, Guana Island had an incredible number of White-cheeked Pintails this year. Although numbers have been increasing each year, it was surprising to count 64 individuals one afternoon. Similarly, but less quantitatively, the Bridled Quail-doves appear to be doing well. Although they were not calling, we had numerous visual detections, especially in the vicinity of the workshop where I counted 7 one morning. I also encountered a flock of at least 4 near Sugar Loaf peak.

The two most common birds on Guana Island are probably the Pearly-eyed Thrasher and the Bananaquit. My thrasher detections were lower and Bananaquit detections were higher than previous surveys (Table 3). Even though detections of thrashers were low compared to previous surveys, more thrashers were mist-netted in 2006 than in the 3 previous years. I suspect Bananaquit detections are a reasonably accurate representation of reality; Bananaquits were quite abundant in general in 2006. These data, however, are indicative of the potential variability of conducting bird surveys in the tropics. During breeding periods, many species are more vocal and active and, hence, more easily detected. Most of these species breed year-round but breeding is related to rainfall patterns. Thus, variability in detections may be related to vagaries of local climate patterns rather than a real change in populations. I anticipate attempting to build in weather variables, and breeding information for future modeling of population trends based on survey data.

#### *Bananaquit Demography*

Similar to previous years, the most commonly captured resident bird species was the Bananaquit, both in terms of new captures ( $n = 63$ ) and recaptures of birds banded in previous years (Table 1). I am currently examining patterns of annual survival based on mark-recapture analysis, and annual variation in age and sex composition of the Bananaquit population on Guana Island. These parameters will be included in a model examining the influences of weather patterns on the Bananaquit population. To expand on this, I am also investigating behavioral ecology and dispersal of the species. To do this, I banded 69 Bananaquits in 2005 with color bands, and another 40 Bananaquits in 2006. This involves placing a silver metal band on one leg and a combination of two plastic colored bands on the other leg (Fig. 2). This allows the identification of individuals without having to recapture them, and allows identification of mated pairs of birds and breeding territories, pair and site fidelity over time, and dispersal across the island.

The Bananaquit can be viewed as a representative of other island resident bird species, and information I collect and resulting analysis may be extrapolated to those other



species and, possibly, other islands. These are important data in context of assessing the probable impacts of global climate change.

#### *Mangrove Cuckoo Ecology*

The lead field investigator for this project is Tracy S. Estabrook (M.S.). The Mangrove Cuckoo is one of the least-studied North American birds and baseline population estimates and habitat requirements have been identified as among the most important research needs for the species. In 2005 we initiated a standardized call-playback survey along trails on Guana to establish baseline information on minimum number of individual cuckoos, pairs, and/or family groups present. A call-playback survey consists of broadcasting the call of the species over a loudspeaker. If an individual of the species is present, it may interpret the broadcast as the vocalization of a territorial intruder, and respond by approaching the surveyor while calling in response.

We established survey points ~100 m apart along established trails on Guana Island. At each survey point we played a pre-recorded Mangrove Cuckoo vocalization 3 times, spaced 1 minute apart, and recorded number, estimated distance, and estimated compass direction of visual and audio responses from Mangrove Cuckoos in the area.

In 2006, we surveyed 110 points for Mangrove Cuckoos between 27 September and 20 October. We modified the survey points from 2005 by dropping 12 points and adding 18 new points on new routes to better survey the island. We obtained responses from cuckoos at 35 (32%) of survey points. We had 7 points in which we could not be certain the responding cuckoo was not the same bird from a previous point. We will attempt to resolve this detection issue in 2007. Additionally, at 13 of the 35 points (37%) in which cuckoos responded, multiple cuckoos were detected. At one point, there were at least 6 cuckoos detected. It remains unknown if these were multiple unrelated individuals, adjacent pairs, or adjacent family groups responding.

On 16 October we had the opportunity to conduct surveys on Necker Island. We conducted broadcast surveys at 12 points and obtained responses at 4 points (33%). However, at two points we could not be certain that the responding bird was a new detection and not the same bird from a previous point. Also, we detected no more than one cuckoo at any point.

In subsequent field seasons we hope to refine this technique to establish a more robust survey protocol and distribution data set, and repeat surveys to record potential changes in Mangrove Cuckoo numbers and distribution on Guana Island over time. We anticipate expanding these surveys to other islands in the BVI. The relevancy of this study, in addition to acquiring basic biological information for the species, is the utility of it as a tool for conservation of Mangrove Cuckoos. The species is suspected of being substantially impacted by habitat loss and degradation due to conversion of low lying vegetation areas on islands to urbanization, resorts, and agricultural production. Refinement of our survey protocol may enable detection, monitoring, and enhanced conservation of Mangrove Cuckoos not just in the Caribbean, but in coastal areas of North America.

#### *Project Productivity*

Avian research is of little value if not made available to both the scientific community and the general public. Since engaging in avian research on Guana Island in 2003, I and my colleagues have attempted to make our research on Guana Island available to the

broader scientific and lay audiences. To date, 3 manuscripts have been published or are submitted, and 5 presentations have been made. Other papers and presentations are anticipated as existing data are analyzed and new data are collected.

#### Publications

- Boal, C.W., F. Sibley, T.S. Estabrook, and J.D. Lazell. 2006. Insular migrant species, longevity records, and new species records on Guana Island, British Virgin Islands. *Wilson Journal of Ornithology* 118:218-224.
- Boal, C.W., and T.S. Estabrook. 2007. Occurrence and condition of migrant Swainson's Thrushes in the British Virgin Islands. *Wilson Journal of Ornithology: In Press*
- Boal, C.W., B. Dankwa-Wiredu, J. Goldfarb, K. MacDonald, J. Owen, L. Smythe, J. Tsai, and G. Perry. Pearly-eyed thrasher and Anolis lizard population densities and association with vegetation communities on Guana Island, BVI. *In revision.*
- Boal, C.W. Nest defense of Antillean Crested Hummingbird toward Anolis lizards. *Submitted - Caribbean Journal of Ornithology*
- Boal, C.W. Nest attendance by Antillean Crested Hummingbird. *In prep.*

#### Presentations:

- Boal, C.W. 2003. Birds of prey in the British Virgin Islands. H. Levity Stoutt Community College, Roadtown, Tortola, British Virgin Islands.
- Boal, C.W. 2005. Avian research on Guana Island: a decade in review. H. Levity Stoutt Community College, Roadtown, Tortola, British Virgin Islands.
- Boal, C.W. 2006. New bird species in the British Virgin Islands: evidence for migration pattern changes? H. Levity Stoutt Community College, Roadtown, Tortola, British Virgin Islands.
- Estabrook, T.S. 2005. Mangrove Cuckoos: where the heck are they and what the heck are they doing? H. Levity Stoutt Community College, Roadtown, Tortola, British Virgin Islands.

#### **FUTURE WORK**

Avian study during Science Month in 2007 will essentially be continuation of the current projects. These are:

1. Operation of the banding station to monitor species diversity and abundance of Neotropical Migrant land birds using Guana Island during fall migration.
2. Conduct point count surveys at established locations to develop an understanding of species distribution and abundances across Guana Island, and how these parameters change in relation to climate patterns.
3. Continuation of the color-banding mark-recapture program with Bananaquits.
4. Continuation, and possible expansion, of the Mangrove Cuckoo study.

#### **ACKNOWLEDGEMENTS**

First and foremost, I thank Dr. James Lazell and Dr. Gad Perry for continuing to facilitate avian research activities on Guana Island. I thank Gloria and Henry Jarecki for providing the opportunity for me and other researchers to conduct our studies on Guana Island. I thank the several people who assisted with ornithological studies on Guana Island during the 2006 season. Specifically, in 2006 these were Brandon Best, Tracy Estabrook, Susan Valentine, and Tom Willard. Funding for this research was provided by The Conservation Agency through a grant from the Falconwood Foundation and by the U.S. Geological Survey Texas Cooperative Fish and Wildlife Research Unit.

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- Boal, C.W., F. Sibley, T.S. Estabrook, and J.D. Lazell. 2006. Insular migrant species, longevity records, and new species records on Guana Island, British Virgin Islands. *Wilson Journal of Ornithology* 118:218-224.
- Boal, C.W., and T.S. Estabrook. 2007. Occurrence and condition of migrant Swainson's Thrushes in the British Virgin Islands. *Wilson Journal of Ornithology: In Press*
- McNair, D.B., F. Sibley, E.B. Massiah, and M.D. Frost. 2002. Ground-based nearctic-neotropic landbird migration during autumn in the eastern Caribbean. Pages 86-103 in *Studies in Trinidad and Tobago Ornithology Honouring Richard French* (F. E. Hays and S. A. Temple, Eds.). Department of Life Sciences, University of West Indies, St. Augustine, Occasional Paper 11.
- Wunderle, J.M., Jr. 2001. Avian surveys on Guana Island, B.V.I. (4-10 October 2001). Final Report to The Conservation Agency, 20 November 2001.

Table 1. Species captured, new captures, and recaptures of birds on Guana Island, British Virgin Islands, 29 September – 20 October 2006.

<u>pecies</u>	<u>New captures in 2006</u>	<u>Recaptures of 2006 birds</u>	<u>Recaptures of pre-2006 birds*</u>	<u>Total birds</u>
American Kestrel	1	0	0	1
Yellow-billed Cuckoo	3	1	0	4
Common Ground-dove	2	0	0	2
Scaley-naped Pigeon**	1	0	0	1
Green-throated Carib**	8	0	0	8
Antillean Crested Hummingbird**	1	0	0	1
Gray Kingbird	1	0	1	2
Caribbean Elaenia	2	0	0	2
Pearly-eyed Thrasher	37	2	6	45
Gray-cheeked Thrush	1	0	0	1
Red-eyed Vireo	1	0	0	1
Indigo Bunting	3	0	0	3
Ovenbird	1	0	0	1
Bananaquit	63	4	39	106
American Redstart	1	0	0	1
Blackpoll Warbler	63	0	0	63
Black-throated Blue Warbler	1	0	0	1
Black-and-white Warbler	3	0	0	3
Hooded Warbler	3	1	0	4
Prairie Warbler	1	0	0	1
Yellow Warbler	3	0	0	3
Black-faced Grassquit	17	1	10	28
Barn Swallow	1	0	0	1
Belted Kingfisher	1	0	0	1
Totals	219	9	56	284

\* May include multiple recaptures of the same individuals.

\*\* Not banded

Table 2. Comparison of mist-netting effort and capture rates at the Guana Island field site, British Virgin Islands, 2003-2006.

<u>Year</u>	<u>Net hrs.</u>	<u>Birds Captured</u>	<u>Birds /net hr.</u>	<u>Species Captured</u>
2003	184	185	1.00	25
2004	218	168	0.80	20
2005	403	428	1.10	21
2006	400	284	0.71	24

Table 3. Abundance of terrestrial bird species in 65 point counts in October 1994 (Arendt 1995) and 2001 (Wunderle 2001) and at 53 point counts in October 2006 (this study) on Guana Island, BVI. Shown are the mean detections per point within 25-m fixed-radius point counts [ $M(\leq 25)$ ]; proportion of 25-m fixed-radius point counts in which the species was detected [ $f(\leq 25)$ ]; proportion of unlimited radius point counts [ $f(u)$ ] in which the species was represented by at least one individual; and the species detection ratio [D-ratio] which is the number of point counts at which a species was recorded only beyond the 25-m radius.

Species	1994				2001				2006			
	$M(\leq 25)$	$f(\leq 25)$	$f(u)$	D-ratio	$M(\leq 25)$	$f(\leq 25)$	$f(u)$	D-ratio	$M(\leq 25)$	$f(\leq 25)$	$f(u)$	D-ratio
<i>Resident Species</i>												
Red-tailed Hawk	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	1.00
American Kestrel	0.00	0.00	0.02	1.00	0.00	0.00	0.00	0.00	0.02	0.02	0.08	0.75
Scaly-naped Pigeon	0.10	0.08	0.07	0.44	0.12	0.11	0.15	0.20	0.27	0.13	0.13	0.00
Bridled Quail-Dove	0.10	0.03	0.00	0.00	0.12	0.11	0.11	0.29	0.00	0.00	0.00	0.00
Common Ground-Dove	0.17	0.15	0.33	0.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zenaida Dove	0.55	0.43	0.67	0.61	0.23	0.17	0.18	0.00	0.23	0.15	0.17	0.11
Mangrove Cuckoo	0.13	0.12	0.17	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Smooth-billed Ani	0.03	0.02	0.05	0.75	0.00	0.00	0.02	1.00	0.04	0.02	0.02	0.00
Antillean Mango	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Antillean Crested Hummingbird	0.18	0.17	0.00	0.00	0.05	0.05	0.05	0.00	0.08	0.08	0.08	0.00
Green-throated Carib	0.22	0.22	0.00	0.00	0.25	0.23	0.28	0.00	0.06	0.06	0.06	0.00
All Hummingbirds	Na	na	na	na	na	na	na	na	0.23	0.23	0.23	0.00
Gray Kingbird	0.18	0.13	0.10	0.46	0.09	0.08	0.18	0.67	0.23	0.19	0.29	0.33
Caribbean Elaenia	0.43	0.30	0.25	0.44	0.23	0.18	0.26	0.18	0.08	0.06	0.06	0.00
Northern Mockingbird	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	1.00
Pearly-eyed Thrasher	2.12	0.87	0.78	0.47	1.54	0.85	0.86	0.59	0.81	0.52	0.61	0.16

Species	<u>M(≤25)</u>	<u>f(≤25)</u>	<u>f(u)</u>	<u>D-ratio</u>	<u>M(≤25)</u>	<u>f(≤25)</u>	<u>f(u)</u>	<u>D-ratio</u>	<u>M(≤25)</u>	<u>f(≤25)</u>	<u>f(u)</u>	<u>D-ratio</u>
Bananaquit	1.38	0.75	0.18	0.20	0.82	0.57	0.58	0.05	1.75	0.79	0.79	0.00
Black-faced Grassquit	0.13	0.12	0.05	0.30	0.17	0.15	0.15	0.00	0.96	0.15	0.15	0.00
<i>Migrant Species</i>												
Barn Swallow	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
Blackpoll Warbler	0.10	0.05	0.00	0.00	0.03	0.03	0.03	0.00	0.25	0.04	0.04	0.00
Black-and -White Warbler	0.02	0.02	0.00	0.00	0.02	0.02	0.02	0.00	0.00	0.00	0.00	0.00
Prairie Warbler	0.07	0.05	0.00	0.00	0.03	0.03	0.05	0.00	0.04	0.02	0.02	0.00
Cape May Warbler	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
American Redstart	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.02	0.02	0.00

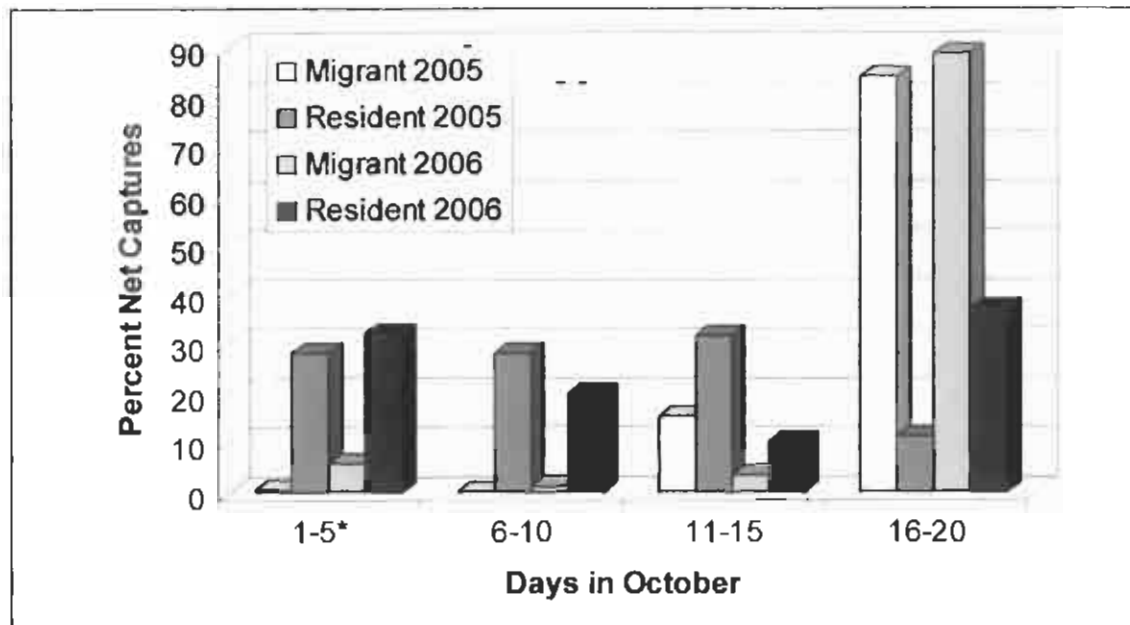


Figure 1. Proportion of Migrant and Resident birds captured by 5 day intervals in October of 2005 and 2006. Figure demonstrates most migrants do not start arriving until the middle of October. \*September 29 is included in days 1-5 for 2006.



Figure 2. Color-banded Bananaquit, identified as Silver - Red Yellow or, X-RY.





Female American Redstart



Prairie Warbler



2006 Banding Crew



Yellow-billed Cuckoo



Belted Kingfisher



Scaly-naped Pigeon

**DEVELOPMENT OF A NESTLING ANTILLEAN CRESTED HUMMINGBIRD**

In 2006, many people on Guana Island enjoyed watching an Antillean Crested Hummingbird raise a nestling in front of the White Bay Beach House. This is a selection of images of different growth stages of nestling. Top left is approximately 1 day old. Bottom right is at 20 days old and was taken the day the nestling fledged. For size perspective, the inside of the nest cup is 3cm (just over 1 inch) across.

## The red-eared slider, *Trachemys scripta elegans*, in the British Virgin Islands

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**Key data.** *Trachemys scripta elegans*; Emydidae; red-eared slider; population; British Virgin Islands. Tortola; Road Town; pond in J.R. O'Neal Botanic Gardens (18°25'38"N, 64°37'22"W); 21 October 2003; collected by Fred Sibley; MCZ R183688. Virgin Gorda; Spanish Town (18°26'41"N 64°26'23"W); 28 October 2004; collected by Jim Egelhoff; MCZ R184120. Both verified by Joe Martinez, Museum of Comparative Zoology, Harvard University.

These are the first records of this widely-distributed species from the British Virgin Islands (BVI). The pond at the J. R. O'Neal Botanic Gardens in Road Town, Tortola (<100 m<sup>2</sup>, <1 m depth), was constructed in the early 1980s. It is one of the few permanent bodies of fresh water in the BVI. Adult sliders were first noted there soon after construction was completed, and excavations and nests were first observed in the vegetation surrounding the pond in the mid-1990s. Hatchlings were first sighted and captured in 1998, and the population grew to 15-20 adults by the late 1990s (Arona DeWindt, pers. com.). Seining of the pond in 2003 produced 12 adults and approximately 20 juveniles. This indicates the presence of an established, reproducing population on Tortola. Additional capture efforts produced further adults and juveniles in July and October 2004, but were not sufficient to eradicate the population. A second species of freshwater turtle, *Pseudemys nelsoni*, has also been found in the same pond (Owen et al., 2006).

*Trachemys s. elegans* has been introduced around the world, including the Caribbean, as a result of its prevalence in the pet trade (Lever, 2003; Emer, 2004). Staff at the O'Neal Botanic Gardens observe visitors releasing turtles into the pond several times per year (A. DeWindt, pers. com.), and the chief source of these animals is presumably the pet trade (the two pet stores on Tortola report importing

baby sliders for at least the past 20 years). In addition to direct releases by owners, at least 10 turtles donated to the Humane Society on Tortola in recent years have been released at the Gardens (Tessa Gunter, pers. com.; it is also reported that sliders are occasionally "released" into the sea).

No additional breeding populations are known from the BVI, and few suitable habitats exist. The Virgin Gorda specimen does not appear to be part of a population, but rather an escaped pet. It might have originated on Tortola, either bought at a pet store or from the O'Neal Botanic Gardens, whose staff report that visitors occasionally capture and remove juveniles, presumably for pets. At least one of these is known to have escaped elsewhere on Tortola (A. DeWindt, pers. com.). Additional turtles originating in pet stores in the British and US Virgin Islands have been released on Jost Van Dyke, according to multiple residents interviewed in 2006. Unconfirmed reports of "pond turtles" at the concrete freshwater retention ponds near the airport at Beef Island probably reflect the presence of this species there as well. We expect further spread into agricultural ponds and other artificial water bodies to eventually take place. However, scarcity of suitable habitat will likely restrict range expansion of this species in the BVI. Ecological impacts remain unstudied, but are likely to be small because of the localized nature of the invasion and the artificial nature of the habitat.

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ERIE G. D. PERRY ET AL.

RRH: BODY SIZE AND REPRODUCTION STOUT IGUANA

**Body Size and Timing of Reproduction in the Highly Endangered Stout Iguana, *Cyclura pinguis*, in the British Virgin Islands**

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**Abstract.**--The stout iguana, *Cyclura pinguis* (also known as the Anegada iguana), survives only in the British Virgin Islands and the total population is believed to be about 200 individuals. The sole remaining natural population, on Anegada, is declining in both numbers and area inhabited. Several extra-limital populations have been established, and here we report on body size and timing of egg-laying in the Guana Island population, which is thriving. Egg laying occurs in June and July, and hatchlings emerge in September and October. For hatchlings, mean snout-vent length was 108 mm, mean undamaged tail length was 197 mm, and mean mass was 60 g. Growth of hatchlings is rapid. Overall, the relationship between log mass and log body length is statistically significant. Comparison to data published for Anegada animals shows that iguanas of similar length weigh more on Guana, suggesting possible food limitation in the Anegada population. Until progress is made towards establishing a national park on Anegada, reintroduced populations, such as the one on Guana, constitute a critically important precautionary measure for *Cyclura pinguis*. Moreover, we strongly recommend enhancing the Guana gene pool.

**Keywords.**--Conservation, British Virgin Islands, lizard, Iguanidae, Guana Island



All iguanas of the genus *Cyclura* are considered threatened or endangered under the World Conservation Union (IUCN) Red List criteria. Still, most remain poorly studied (Alberts, 2000). The stout iguana, *C. pinguis* (also known as the Anegada iguana), is thought to have originally inhabited the entire Greater Puerto-Rico Bank (Pregill, 1981; Lazell, 2002, 2005). Today, however, the species survives only in the British Virgin Islands (BVI; Binns, 2003). Until recently, the only surviving population existed on the island of Anegada (approximately 38 km<sup>2</sup>), where introduced herbivores, feral predators, and increasing development greatly degraded conditions for the species and caused a major population decline (Carey, 1975; Mitchell, 1999; Lazell, 2002). Because the species is so rare, our data are considerably less extensive than we would like. Unfortunately, similar problems plague many species of Caribbean iguanas (e.g., Mitchell et al., 2002, for *C. carinata* in the Turks and Caicos).

After reviewing the current situation in the wild, Lemm et al. (2005) concluded that conditions "justify an ex-situ conservation program as a safeguard against extinction" for this species. Over the last twenty years, *C. pinguis* has been translocated to a number of islands in the BVI (Lazell, 2002; J. Lazell, unpubl. data). Of the translocated populations, the one on Guana Island has been best studied (e.g., Goodyear and Lazell, 1994; Mitchell, 1999; Perry et al., 2003) and appears to be flourishing (Mitchell, 2000; Perry and Mitchell, 2003). The population on Necker Island is also expanding (Lazell, 2000; Binns, 2003). However, the other reintroductions are too recent for proper evaluation. Conservation efforts also include a headstarting facility on Anegada, which has lately released 24 individuals back into the wild (Binns, 2003; Bradley, 2004).

With only about 250 individuals thought to remain in all extant wild populations, *C. pinguis* is considered critically endangered (Alberts, 2000, 2004; Hudson and Alberts, 2004). The Anegada population appears to be shrinking both in numbers and in geographic scope on the island (Gerber, 2004). This severe threat to the species' survival demands that detailed information about its



biology be obtained to implement successful conservation and restoration efforts. However, the small number of remaining animals makes obtaining such information difficult, especially on Anegada where only a few free-ranging individuals remain. Probably the densest extant population, numbering about 100 individuals (Perry and Mitchell, 2003) and common enough such that it is both frequently observed and reintegrated into the food chain, is the one restored to Guana Island (LeVering and Perry, 2003; Perry et al., 2003). However, little is known about the life history of this critically endangered species anywhere, including Guana Island. We begin addressing this lack of data by reporting on timing of reproduction and body size, two important aspects of the biology of *C. pinguis* on Guana Island.

Data on timing of breeding activities were taken from a survey of the literature (see below) and interviews with BVI residents. Body size measurements were obtained from systematic collection efforts conducted in October of 2003 and 2004. We surveyed the island on foot and with a vehicle and, using nooses, attempted to capture every iguana sighted. Capture success was high for hatchlings (~75% of sighted animals) but much lower for adults (~25%). Captured animals were measured (snout vent length [SVL] and tail length [TL] to the nearest mm), weighed (for hatchlings, < 1 g, using a Pesola scale; for adults, > 100 g, using a Pesola scale or the change in weight reported by a bathroom scale when an individual picked up the animal), PIT tagged (AVID Identification Systems Inc., Norco, CA, USA), and released at the site of capture. Not all measurements were obtained for all individuals. Animal-related procedures were carried out under Texas Tech University ACUC permit 05006-01. Statistical analyses were conducted in SPSS using two-tailed probabilities. Whenever comparisons were conducted between measures representing different dimensionality (i.e., mass vs. length), a log transformation was used to linearize the data.

Published data on timing of reproduction in *C. pinguis* are sparse but consistent in two respects: oviposition occurs in June and July and eggs are deposited in sandy areas. On Guana,

Fazell (2005: 185) documents some nesting in late June and a gravid female in late July whereas Bruns (2003) reported that some females were heavily gravid in late July of 2002. As part of the headstarting efforts on Anegada, nests are located every year in June and July (Gerber, 2004). Incubation on Guana Island appears to take about three months, with hatchlings appearing in late September or October and rapidly dispersing into the higher elevations in the center of the island. E. Jurecki and F. Kraus (pers. com.) found iguana eggs that had been accidentally dug up on the Guana Island beach in late September or October 1991. These hatched three days later. Roger Miller also reports a large number of hatchlings being seen in late September of 2004. Our own observations indicate that the prevalence of recently-hatched hatchlings on Guana occurs in late September and October of most years. Hatching on Anegada also occurs at that time (Gerber, 2004) and there have been no sightings of hatchling on Guana at any other time of the year.

In 2003 we obtained body size data for 29 hatchlings and four adults. Data collected in 2004 included 18 hatchlings and two adults, one of which was previously captured in 2003. In 2005 we captured 13 new hatchlings on Guana and recaptured an adult previously measured in 2003 and 2004. Mean hatchling SVL was 108 mm (SD = 10.8 mm, N = 59), mean undamaged tail length was 197 mm (SD = 12.9 mm, N = 21), and mean mass was 60 g (SD = 28.3 g, N = 52). For animals with complete tail, SVL and tail length were directly related (linear regression: tail length =  $133.6 + 0.604 \cdot (\text{SVL})$ ,  $R^2 = 0.32$ , N = 20,  $p = 0.008$ ). Of the animals for which tail measurements exist, tail damage consisting of missing tips (Figure 1) was noted in three, a rate comparable to that seen on Anegada (Gerber 2000 in Iverson et al., 2004, Table 13.8).

The relationship between SVL and mass was positive and significant for Guana animals (Figure 2; squares and regression line: linear regression:  $\log(\text{mass}) = -3.213 + 2.440 \cdot \log(\text{SVL})$ ,  $R^2 = 0.65$ , N = 51,  $p = 0.0011$ ). Comparing current Guana size data to those collected by Carey (1975) and Mitchell (1999) on Anegada (Figure 2) shows that the difference between the three studies in

the size-mass relationship was statistically significant (ANCOVA of log-transformed adult mass as a function of sampling effort [present study for Guana; Carey, 1975 and Mitchell, 1999 from Anegada], with log[SVL] as the covariate;  $F_{1,22} = 4.343$ ,  $p = 0.026$ ). Guana animals reach similar masses at shorter SVLs, and Anegada animals did not fare as well in the late 1980s as they did two decades earlier. This supports the view of Carey (1975) and Mitchell (1999) that Anegada iguanas suffered from food limitation for a long period and that the condition of Anegada animals deteriorated between the 1960s (Carey, 1975) and the late 1980s (Mitchell, 1999).

Little is known about growth rates in the genus *Cyclura* (Iverson et al., 2004). On Guana, hatchling SVL was significantly and positively correlated with capture date. The difference between years (Figure 3) was not statistically significant (ANCOVA of SVL with year as random factor and day since work began that year as covariate; day:  $F_{1,50} = 5.396$ ,  $p = 0.024$ ; year:  $F_{1,50} = 2.010$ ,  $p = 0.144$ ). These data suggest that growth occurred even within the relatively brief sampling period available to us. However, the data do not make it possible to estimate growth rate for individuals as each animal was only measured once. A linear regression of SVL on day measured produced a highly significant but weakly predictive relationship ( $R^2 = 0.12$ ,  $N = 59$ ,  $p = 0.007$ ). Both this and the regression equation ( $SVL = 102.6 + 0.438 \cdot \text{day}$ ) suggest that hatchlings grow by almost 0.5 mm each day. Young *C. pinguis* are known to face predation from feral cats (Mitchell, 2000), snakes, and birds (LeVering and Perry, 2003) and may therefore have low survivorship (Mitchell 1999). Rapid growth has thus clear benefits and is to be expected at this early stage. Data for captive animals held in a headstarting facility on Little Hatch Island provide some additional insight. Between October 2003 and 2004, one animal grew from 122 mm SVL and 70 g to 156 mm and 240 g. By October 2005 it measured 206 mm SVL and 385 g. From 2004 to 2005, one female (hatched in 2004 on Guana) grew from 102 mm and 48 g to 150 mm and 169 g; another (hatched in 2003 on Necker) grew from 132 mm and 129 g to 154 mm and 190 g, and one

male (hatched in 2004 on Guana) grew from 101 mm and 50 g to 160 mm and 201 g. The only adult for which we have repeated measurements is a free-ranging adult female, measured at 370 mm SVL in 2003 (unfortunately with no data on weight), 380 mm and 3000 g in 2004, and 422 mm and 3300 g in 2005. This suggests that females of that size range are approaching the growth asymptote (Stamps et al., 1994), perhaps as a result of most energy being invested in egg production.

Ex-situ captive husbandry of *C. pinguis* is finally enjoying modest success (R. Hudson, pers. com.; Lemm et al., 2005) and the headstarting program on Anegada has become successful in raising releasable animals (Gerber, 2004) with high survival rates (K. Bradley, pers. com.). At the same time, there has been little progress in establishing protected habitat for *C. pinguis* on Anegada (Mitchell et al., 2002) and iguana habitat appears to be shrinking from ongoing degradation by feral livestock (Gerber, 2004). Until progress is made in establishing a national park on Anegada, reintroduced populations, such as the one on Guana, function as critically important safety nets for this species. However, all Guana Island iguanas are descended from only eight individuals (Lazell, 2002) and other reintroduced populations form a subset of that already-limited genetic stock. Despite the findings of Knapp and Malone (2003), who found that a translocated population of *C. cyclura mornoti* is doing well despite having originated from eight founders, we strongly recommend enhancing the gene pool.

*Acknowledgments.*—We thank the staff of Guana Island, especially Lynford Cooper and Roger Miller, for technical assistance. Henry and Gloria Jarecki kindly allowed us access to Guana Island, Richard Branson to Necker Island, and John and Jill Maynard to Little Thatch. A number of people, especially Clint Boxl and Jacob Goldfarb, were helpful in capturing iguanas. Allison

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Ms. Received 28 May 2005; accepted 17 September 2006

## Figure legends

Figure 1. A young *Cyclura pinguis* showing tail regeneration after unusually extensive damage of unknown origin. The arrow indicates where the original tail ends and the regenerated tail begins.

Figure 2. The relationship between snout-vent length and mass in *Cyclura pinguis*. Guana data are from the present study, those for Anegada are from Carey (1975) for the 1968 values (full circles) and Mitchell (1999) for 1988-89 values (empty circles). The line represents the relationship for Guana animals. Previously measured Anegada animals had a lower mass at a given body size than seen on Guana today.

Figure 3. Snout-vent length of juvenile *Cyclura pinguis* upon first capture. The increase over time is statistically significant, whereas the difference between years is not. The line represents the linear regression of the combined data.

**Date Sent:** Friday, February 02, 2007 8:44 AM

**From:** "Samuel Turvey" <Samuel.Turvey@ioz.ac.uk>

**To:** wenhua

**Subject:** FWD: Virgin Island iguanas

Hi Skip,

Sorry for not replying to your e-mails sooner – hope that things are going well with you in China (and that you're witnessing fewer large-scale extinction events than I was forced to on my recent trip). Below is a message which I've just sent to Wolfgang Mueller, a stable isotope researcher based at Royal Holloway, University of London, who does a lot of research on using stable isotopes to ascertain geographical distributions and former migrations (he worked on Otzi the Iceman, for example). Fingers crossed that we could do a really interesting project about pre-human iguana distribution...

Cheers,  
Sam

Dr Samuel Turvey  
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Regent's Park,  
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-----Original Message-----

**From:** Samuel Turvey  
**Sent:** 02 February 2007 13:42  
**To:** 'w.mueller@gl.rhul.ac.uk'  
**Subject:** Virgin Island iguanas

Dear Wolfgang,

Ian Barnes tells me that he's talked to you about my suggestions for a research project looking at pre-human iguana distribution in the Virgin Islands. The story is that today, Critically Endangered stout iguanas (*Cyclura pinguis*) are only found on Anegada Island, a low-lying limestone island situated at the easternmost end of the Virgin Islands. However, iguana remains referable to this species have also been reported in quite large numbers from archaeological sites on other, geologically different islands further west in the Virgin Islands (e.g. St Thomas, St. John, Guana). Over the past few years, conservationists have carried out a successful series of translocations of iguanas from Anegada to various people suggesting that these translocations don't represent re-introductions to areas of their former range, but instead introductions to ecologically unsuitable regions where the animals won't be able to persist. These critics suggest that the zooarchaeological iguana remains present on many of these islands might represent animals which were brought over from Anegada for Amerindian feasts, meaning that these islands don't in fact represent parts of their former range. My idea is, presumably the stable isotopic composition of iguana material from different islands with different geologies would reflect whether they had lived on these islands all their lives, or whether they'd been transferred from one island to another for food?

I was wondering whether you would be interested in exploring these questions as a stable isotope research project? I should be able to access iguana samples from archaeological sites fairly easily, and should also be able to get hold of a few specimens from Anegada Island which are in US museum collections. Please let me know what your thoughts are on this – I would be extremely interested in using the stable isotope approach to investigate the prehistoric occurrence of these endangered animals to inform their appropriate conservation management.

Many thanks in advance,  
Sam Turvey

**ANOLIS PULCHELLUS** (Grass Anole). **NECTIVORY.** Nectar feeding has previously been reported in anoles (e.g., Liner 1996, Herpetol. Rev. 27:78). Perry and Lazell (1997, Herpetol. Rev. 28:150–151) argued that, though episodic, it is more common than usually thought, and recent reports (Campbell 2000, Herpetol. Rev. 31:239; Echternacht et al. 2000, Herpetol. Rev. 31:173; Rios-López 2004, Herpetol. Rev. 35:386; Townsend 2004, Herpetol. Rev. 35:141–142) support that view. Here we report on another such case.

On the afternoon of 24 September 2005, we observed an adult (ca. 4.5 cm SVL) male *Anolis pulchellus* licking at a button-like structure near the base of the leaf of a small *Leucaena leucocephala* tree (Leguminosae). The lizard was about 1 m above the ground and the behavior continued for about a minute. Although nectar was not apparent to the naked eye, these structures are known to be extrafloral nectaries (Minu 1991, Acta Botanica Indica 19:49–54).

This is but the second report of lizards using extrafloral nectaries; the first (Rios-López 2004, Herpetol. Rev. 35:386) occurred in a closely related lizard species of similar size that also occurs on the Puerto Rico bank. Anoles also occasionally engage in outright herbivory (e.g., Lazell and Mitchell 1998, Herpetol. Rev. 29:237), and episodic nectivory also occurs in other lizard clades (e.g., Perry and Ritter 1999, Herpetol. Rev. 30:166–167).

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Submitted by **GAD PERRY**, Department of Range, Wildlife, and Fisheries Management, Texas Tech University, Lubbock, Texas 79409, USA (e-mail: gadperry@ttu.edu); and **JAMES LAZELL**, The Conservation Agency, 6 Swinburne St., Jamestown, Rhode Island 02835, USA.

## Are anoles on Little St. James different than on Guana?

Prepared by Gad Perry, Texas Tech University

June 2007

The Virgin Islands offer a unique laboratory in which to study the evolution of animals because they were united in a single landmass until a few thousand years ago, when rising sea level separated the current islands from one another. Different islands became separated at different times, and experienced different selection regimes following this isolation. I have long been interested in looking at the relatedness of island populations at the genetic level, and how this genetic relatedness translates into ecological, physiological, and behavioral differences.

Past visits to Little St. James (LSJ) have identified a number of questions within this broad area of inquiry. For example, the ground lizards of LSJ appear different in size and coloration from those commonly encountered in the BVI, even though they supposedly belong to the same species. In 2006, we had an opportunity to begin addressing some of these questions. A team of four visited LSJ and conducted work that tied in with ongoing work in the BVI and elsewhere in the USVI. The team consisted of Dr. Jonathan Losos (Professor and Curator, Museum of Comparative Zoology, Harvard University), Dr. Kevin De Queiroz (Curator/Research Zoologist, National Museum of Natural History, Smithsonian Institution), Dr. Molly Morris (Associate Professor, Biological Sciences, Ohio University), and myself. During work on other islands we were joined by Dr. Renata Platenberg of the USVI Division of Fish and Wildlife. The specific questions being addressed all had to do with the *Anolis* lizards found throughout the VIs. A general goal was to collect genetic material for future analysis which would allow us to correlate genetic differentiation at the molecular level with geographic separation, as determined by the timing of island split events during the sea-level rise that created the BVI starting some 10,000 years ago. We collected specimens on LSJ, as well as St. Thomas and St. John in the USVI, and Guana, Norman Island, Carrot Rock, Peter Island, and other BVI locations. This material is currently awaiting analysis in the Smithsonian.

Another ongoing project of which LSJ data are only a small part involves examination of the spread of the agave weevil (*Scyphophorus acupunctatus*) in the VIs, its impacts on the native century plant (*Agave missionum*), and the hoped-for recovery of the plant over time. These data will also not be presented here. The trip was also used to document the arrival of a number of invasive reptile and amphibian species on LSJ, a report on which is to appear in Applied Herpetology within the next few months.

The other questions being addressed are ecological in nature and have to do with how the unique species composition on LSJ (the saddled anole, *Anolis stratulus*, common in the VIs, is missing for reasons we do not understand) affects the species found there. Specifically, we set out to measure four parameters: perch height (how high above ground lizards are found) and diameter (how big around is the branch it is on), degree of insolation (how sunny was the perch site), and body size (which in other species varies as a function of the presence or absence of other species). We focused our work on the grass anole, *Anolis pulchellus*, which in a preliminary site visit appeared to be larger than those found in the BVI. We combined our data with ones collected during a previous visit, in which Dr. Razi Dmi'el participated and in which water loss rates were compared between Guana and LSJ. Thus, we have data on five parameters, compared among those two islands: perch height and diameter, insolation, body size, and water loss rate.

*Perch height, diameter, and insolation*

The data collected are summarized in Table 1. Statistical comparisons using a Mann-Whitney U-test (a non-parametric test which avoids making assumptions about data distributions) show no significant difference in perch height or insolation ( $p > 0.25$  in both cases; a  $p$  of 0.05 or below is needed to identify significant differences). However, perch diameter on Guana was significantly larger than on LSJ (the test parameter  $Z = -1.056$ ,  $p = 0.005$ ).

Table 1. Summaries of perch characteristics of *Anolis pulchellus* on Guana Island in the BVI and Little St. James (LSJ) in the USVI. N is the sample size. SD is the standard deviation, a measure of the variation in the data. Persun is the percent of the perch in direct sunlight.

Parameter	Island	N	Mean	SD
Height	Guana	36	57.2	37.41
	LSJ	37	45.5	24.02
Diameter	Guana	36	17.5	27.67
	LSJ	36	5.9	12.83
Persun	Guana	36	26.4	36.81
	LSJ	34	30.9	32.60

*Body size and water loss rates*

The data collected are summarized in Table 2. Statistical comparisons using a Mann-Whitney U-test show no significant difference in any characteristic ( $p > 0.25$  in all cases). The lack of difference in water loss rates is further illustrated in Figure 1, which shows that raw water loss rates on the two islands are very similar for lizards of similar body size.

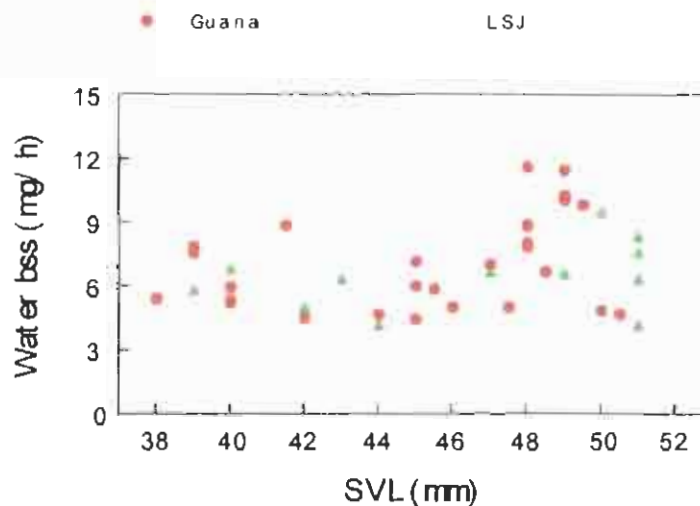
Table 2. Summaries of body size and water loss rates of *Anolis pulchellus* on Guana Island in the BVI and Little St. James (LSJ) in the USVI. N is the sample size. SD is the standard deviation, a measure of the variation in the data. SVL is the snout-to-vent length, or body size (in mm). Mass is given in grams. R is the skin resistance to water loss, measured in sec/cm.

	Island	N	Mean	SD
SVL	Guana	30	45.0	4.73
	LSJ	12	46.5	4.64
Mass	Guana	30	1.7	0.47
	LSJ	12	1.9	0.53
R	Guana	30	113.3	37.71
	LSJ	12	124.	30.63

### Conclusions

Our findings differed considerably from our expected outcomes in several respects. Despite the lack of similar-sized anoles on LSJ, the grass anole did not change the height of perch it used, nor the degree of insolation it exposed itself to, nor its body size. This was the opposite of what had been seen in other species (e.g., Losos, 2004). Despite apparent differences in water availability – LSJ appears more arid than Guana, although rainfall data do not exist for the former – the water loss rates we measured at the two islands were virtually identical. This is unlike what we have previously documented with other anoles in the BVI, which vary their water loss rates as a function of both short-term and long-term water availability (Dmi'el et al., 1997; Perry et al., 1999, 2000). The only difference between the two islands, in perch diameter, is likely to be a function of plant availability, rather than lizard behavior. The hotel area on Guana has a number of century plants, which the grass anoles use on occasion. On LSJ, those broad-leaved plants are only found on the dry side of the island, where we encountered few grass anoles. Thus, it seems unlikely that the difference reflects anything more than opportunistic use of available perches.

Figure 1. Total water loss rates of *Anolis pulchellus* on Guana (red circles) and Little St. James (green triangles). When corrected for body size (SVL), the rates do not statistically significantly differ between islands.



The lack of differences between the islands is hard to explain, given our current knowledge of the biology of the lizard and conditions on the islands. It is possible that weather conditions on the two islands are not as different as we perceive, but this will be impossible to test until rainfall data are collected on LSJ that can be compared to the Guana data. It is possible that the missing anole on LSJ was there until recently, and the lack of changes in perch or size indicates the lack of time for those changes to occur. We are not aware of any data suggesting



the saddled anole was ever on LSJ, but many of the smaller islands and cays were never carefully surveyed. Finally, it is also possible that the crested anole (*Anolis cristatellus*) on LSJ has shifted its ecology or behavior in a way that precludes the grass anole from changing, although we did not see any qualitative indications that this is the case. In the absence of a chance to spend considerably more time on LSJ, which appears unlikely, we are unlikely to be able to explain our findings or further examine the processes responsible for them.

One item of future work we would definitely like to conduct on LSJ, if the chance for another brief visit occurs, would involve further studying the ground lizard on the island. As stated above, they appear both more numerous and different in size and coloration than in the BVI, and we would love to have a chance to further study them over the course of a day or two.

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**ALSOPHIS PORTORICENSIS ANEGADAE** (NCN). **AQUATIC ACTIVITY.** West Indian racers in the genus *Alsophis* are generally characterized as diurnal, primarily terrestrial, actively foraging snakes. Many of the islands on which they live do not have permanent bodies of fresh water and few references address aquatic activity.

In the course of a multi-year study of population structure of *A. portoricensis anegadae* on Guana Island (British Virgin Islands), we recorded four instances of aquatic activity. In October 2003, an adult snake escaped capture by entering and swimming across an intermittent pool of water several meters wide and about 20 cm deep. Three additional observations were made during an unusually pluvial year (2005). On 6 October, an individual (579 mm SVL) attempted to escape capture by diving into a temporary pool near the base of Quail Dove Ghut. Once submerged, it remained motionless with its anterior body adpressed against accumulated debris about 30 cm deep until disturbed by the collector. On 8 October, a female (653 mm SVL) was found near North Beach swimming in a large pool covering a flooded dirt track and adjacent forest. On 9 October, another female (570 mm SVL) was found in the southeastern lowlands resting at the edge of a large puddle with over half of its body in the water, suggesting that it had just crossed the water. Members of the staff residing on the island also reported sporadic observations of snakes swimming in the sea (T. Peliwan, pers. comm.). All but one sighting occurred in shallow water within 20 m of shore, but one snake was reportedly seen swimming toward a nearby island.

We have found no previous records of aquatic behavior in this species, but some exist for congeners (Schwartz and Henderson, 1991, *Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History*, Univ. Florida Press, Gainesville, xvi + 720 pp.). Although anecdotal, our observations suggest that *A. p. anegadae* is at least opportunistically aquatic, presumably in

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much the same fashion as it is facultatively arboreal. Moreover, ocean swimming might explain how some snakes disperse from one Caribbean island to another.

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## *Alsophis portoricensis anegadae* (Squamata: Colubridae): Morphometric Characteristics, Activity Patterns, and Habitat Use

ARIJANA BARIĆ, GAD PERRY, ROBERT W. HENDERSON, AND ROBERT POWELL

From 2001–2005, we collected and individually marked 219 *Alsophis portoricensis anegadae* from Guana Island, British Virgin Islands, during the months September–October to determine morphometric characters, evaluate incidence of scarring and tail damage, and assess habitat use and activity. Males were longer than females and significantly heavier and heavier per unit length. Sex ratio was almost exactly 1:1 (102 M:104 F). Undamaged tails of males were significantly longer than those of females, but rates of tail damage did not differ by sex. Scarring and tail damage were more abundant posteriorly on the body and tail, and were cumulative, occurring with significantly greater frequency in larger individuals. Most damage was probably attributable to unsuccessful predation attempts by Soldier Crabs (*Coenobita clypeatus*). We found snakes in all habitats except an open grassy plain. Activity appeared to be bimodal, with a depression during the heat of the day. Although usually diurnal, three snakes were observed active at night. Most snakes were on the ground, but a small number were in water or climbing on vegetation or human-made structures. Most were in full shade and very few in full sun when first observed. Snakes most frequently were sprawled, rarely coiled, and many were first seen while moving.

**S**NAKES of the genus *Alsophis* (frequently referred to as “racers”) are mostly terrestrial and range from the Bahamas to Dominica (Schwartz and Henderson, 1991; Powell et al., 1996). They were historically common on many islands, but introduced invasive species such as the Indian Mongoose (*Herpestes javanicus*) and Black Rat (*Rattus rattus*) prey on snakes and other native species (Sajdak and Henderson, 1991; Duhv et al., 2001; Henderson, 2004; Powell and Henderson, 2005). Consequently, they have suffered more extirpations and extinctions than any other reptilian or amphibian genus in the region. Other important factors include habitat degradation and human persecution, both common throughout the region (Duhv et al., 2001; Powell and Henderson, 2005). Despite the wide distribution, conservation concern, and large populations on mongoose-free islands, we know surprisingly little about the ecology of most members of the genus beyond basic habitat associations (Schwartz and Henderson, 1991) and diets (Henderson and Sajdak, 1996; for exceptions see Duhv et al., 2001; Heinz et al., 2004, 2005; Savit et al., 2005). The lack of even basic biological information often hampers our ability to protect remaining populations by identifying locations that are of particular biological importance.

*Alsophis portoricensis* inhabits the Puerto Rican Bank, including Puerto Rico proper and the Virgin Islands. MacLean (1982) reported the species to be rare or extirpated on the larger islands but still moderately common on Puerto Rico and many smaller islands. Especially in light of these

declines, studies on the biology of the species are both desirable (Perry and Gerber, 2006) and difficult to implement on most remaining populations. Our goal was to document the basic biology of the species in a relatively intact system in order to provide crucial information for attempts to protect and restore populations elsewhere. We focused on two broad questions: What are the morphometric characteristics of the species and what do they tell us about its biology? Where and when are these animals active, and what ambient conditions affect activity levels?

### MATERIALS AND METHODS

**Study site.**—Guana Island (18°38'N, 65°25'W) is situated in the West Indies and is part of the British Virgin Islands (BVI). Like most islands in the region, it is largely igneous in origin and is characterized by many steep slopes. Human activity on Guana, a private wildlife sanctuary, has been largely restricted to small portions of the island associated with an upscale resort hotel. Guana is primarily covered in dry tropical forest, with smaller contributions from forested ravines (gullies), sun-baked rock faces, mangrove stands, and sand flats (Lazell, 2005). Biological research on the island has been ongoing for over 20 years, leading to an exceptional understanding of its biology (Lazell, 2005). The island is mongoose-free and has few, if any, feral cats because of frequent bait trapping. Previous observations (Lazell, 2005) have shown that *Alsophis* is abundant on Guana and included some anec-

digital observations (e.g., Perry and Lazell, 2000; Powell et al., unpubl. data), but no detailed study has been conducted.

**Morphometrics.**—From 2001–2005, as part of an ongoing mark-recapture study, we collected and individually marked snakes from throughout the island during September–October. Snakes were visually located, manually captured, measured, uniquely marked with PIT tags, and released at the site of capture. For each animal, we recorded date, time of day, location, sex, snout-vent length (SVL), tail length, weight, tail damage, scarring, habitat, activity of the snake (moving, sprawled, coiled), and degree of insolation (full sun, sun-shade mosaic, or full shade). We determined weight to the nearest gram using Pesola spring scales, and SVL and tail length to the nearest mm using a metric measuring tape.

**Habitat and activity.**—We conducted systematic surveys during October 2001, using a 1-km trail through dry forest as our main transect. Dry weather resulted in a paucity of low vegetation and unusually good visibility. A single researcher (AB) walked the transect at 2-h increments, starting at 0600 and ending at 2130 h. During each survey, which lasted 1.5 h and encompassed the entire transect, she visually scanned a 2-m belt on each side of the trail for presence of snakes. To avoid disturbing the snakes and ensure that normal behavior was observed, the transect was visited once per day on all but two occasions, on which one visit occurred in the morning and the other during late afternoon or at night. To reduce systematic biases and ensure replication, each survey began at a different end of the transect and each sampling time was repeated twice. She avoided sampling on days when ambient conditions were not conducive to snake activity (cool, rainy days). Whenever a snake was encountered, she recorded time of day, activity (moving, sprawled, coiled), degree of insolation (full sun, sun-shade mosaic, or shade), and body, air, and substrate temperatures, measured using a Miller & Weber quick-reading cloacal thermometer. Transect data were augmented by additional observations conducted by us from 2001–2005 and by the activities of multiple researchers studying a variety of organisms on Guana at different times of day and night, revealing no snake activity at other times of day.

**Statistical methods.**—Statistical analyses were carried out using SPSS for Windows (SPSS Inc., Chicago, Illinois). Whenever possible, we used non-parametric tests to assure that our data met the assumptions of each test. When non-parametric

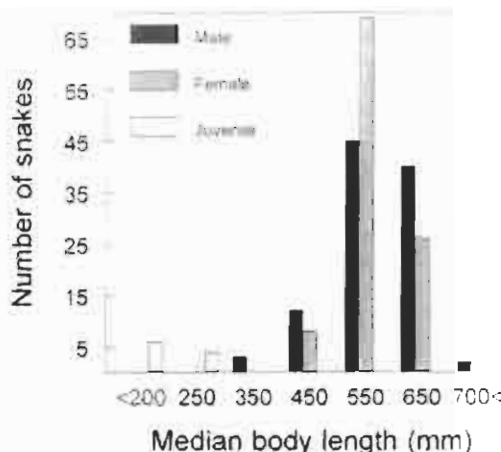


Fig. 1. Sizes (SVL) of *Allophis portoricensis anguina* on Guana Island ( $n = 219$ ).

metric tests were inappropriate, we used parametric tests after transforming the data. All  $P$  values reported are two-tailed.

## RESULTS

**Morphometrics.**—Snake SVL ranged from 185–855 mm ( $n = 219$ ,  $\bar{x} \pm \text{SD} = 555.4 \pm 93.7$  mm) and mass ranged from 2–308 g ( $n = 219$ ,  $\bar{x} = 62.1 \pm 31.7$  g). Nearly all of our adult animals were of moderate size (450–650 mm SVL; Fig. 1). Only two males (SVL = 767 and 855 mm) exceeded that range.

Sex ratio was almost exactly 1:1, with 102 males and 104 females in our sample. Despite the two large individuals, males were not significantly longer than females (male SVL:  $\bar{x} = 574.6 \pm 75.0$  mm,  $n = 102$ ; female SVL:  $\bar{x} = 564.0 \pm 57.1$  mm,  $n = 104$ ; Mann-Whitney  $U$ -test:  $u = 206$ ,  $U = 4563.5$ ,  $Z = -1.73$ ,  $P = 0.08$ ). Males were, however, significantly heavier than females (male mass,  $\bar{x} = 71.2 \pm 35.5$  g,  $n = 101$ ; female mass,  $\bar{x} = 56.8 \pm 17.0$  g,  $n = 104$ ;  $u = 205$ ,  $U = 3450.5$ ,  $Z = -4.24$ ,  $P < 0.001$ ). We used an ANCOVA of log-transformed values to ask whether the relationship between body size and mass (Fig. 2) was significantly different between the sexes. Not surprisingly, mass and body length were significantly associated ( $F_{1,202} = 513$ ,  $P < 0.001$ ). We also found a highly significant sexual difference, whereby males of a given length were heavier than similarly-sized females ( $F_{1,202} = 23.3$ ,  $P < 0.001$ ).

Tail length ranged from 25–354 mm ( $\bar{x} = 232.8 \pm 58.5$  mm,  $n = 219$ ). However, most tails (71% of measured tails) were stubbed to some degree (Fig. 3A). Undamaged tails ranged in length from 72–330 mm ( $\bar{x} = 228.1 \pm 61.3$  mm,  $n = 56$ ). Undamaged male tails ( $\bar{x} = 258.6 \pm$

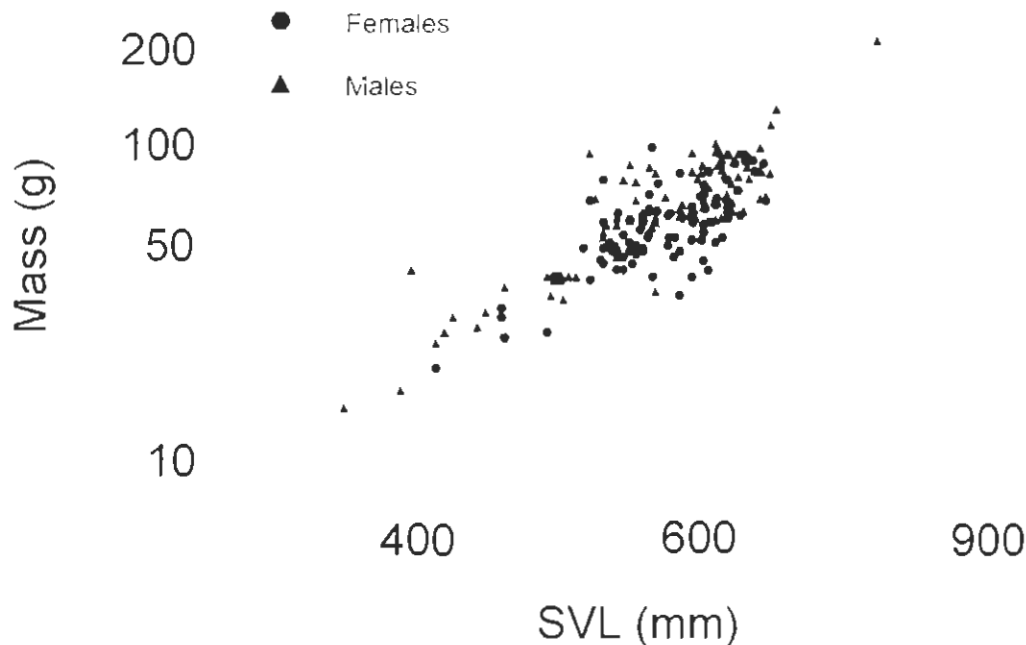


Fig. 2. The relationship between body length and mass in females (dots, full lines) and males (triangles, dotted lines) is significantly different.

37.2 mm,  $\bar{x} = 50 \pm 5.5\%$  of SVL,  $n = 25$ ) were longer than those of females ( $\bar{x} = 236.6 \pm 45.5$  mm,  $\bar{x} = 44 \pm 8\%$  of SVL,  $n = 31$ ). The difference between the sexes in undamaged tail length was statistically significant (ANCOVA using SVL as covariate;  $F_{1,50} = 2.96$ ,  $P < 0.027$ ).

Frequency of tail damage was essentially the same for the two sexes (males 69%, females 74%;  $df = 1$ ,  $\chi^2 = 0.18$ ,  $P = 0.68$ ). We did not observe tail damage in the smallest animals, but the incidence of damage rapidly and significantly increased once SVL exceeded 450 mm (Fig. 4A;  $n = 9$  size categories,  $\rho = 0.94$ ,  $P < 0.001$ ). The relationship between SVL and tail length in undamaged animals was positive and statistically significant (log tail length =  $-0.009 + 0.009 \cdot \log$  SVL; linear regression,  $r^2 = -0.73$ ,  $P < 0.001$ ). Using this equation, we calculated the predicted tail length for animals that had sustained tail damage and correlation analysis to ask whether larger animals had more extensive damage than did smaller ones. Indeed, the percentage of the tail damage, compared to predicted values, increased significantly with body size ( $n = 143$ ,  $\rho = 0.19$ ,  $P = 0.02$ ), suggesting that additional damage occurs over time.

Scarring of the body showed a pattern similar to that for tail damage (Fig. 3B, C). Scarring was more likely to be seen posteriorly than near the head (Table 1) and increased in frequency as

snakes grew larger (Fig. 4B;  $n = 9$  size categories,  $\rho = 0.96$ ,  $P < 0.001$ ). Frequency of scarring was almost identical for the two sexes (percent scarred: males 52.7% of 93 animals; females 53.3% of 90 animals,  $df = 1$ ,  $\chi^2 = 0.003$ ,  $P = 0.95$ ). Animals with damaged tails were also more likely to have scarring than those with no tail damage ( $\chi^2 = 7.21$ ,  $df = 1$ ,  $P = 0.007$ ).

**Habitat and activity.**—Snakes were abundant ( $n = 105$ ) in and around the substantially altered areas associated with the hotel, donkey corrals (mostly decrepit and unoccupied), the “orchard” (a cultivated area with numerous fruit trees), and the section of beach devoted to tourism ( $n = 7$ ). Snakes were similarly abundant in dry forest ( $n = 72$ ), ghut forest ( $n = 31$ ), and beach strand ( $n = 9$ ). Only in the open grassy flat did we fail to encounter animals, although we found individuals in “islands” of vegetation or debris within the plain.

Most snakes we encountered were on the ground, but we observed four animals in water (Powell et al., 2006) and at least 11 animals (three juveniles) that had climbed on trees or human-made structures. As indicated by our transect study, snakes ( $n = 20$ ) were active throughout the day, with activity peaks in the morning and afternoon and lower activity during the heat of the day (Fig. 5A). Activity was significantly negatively correlated with ambient



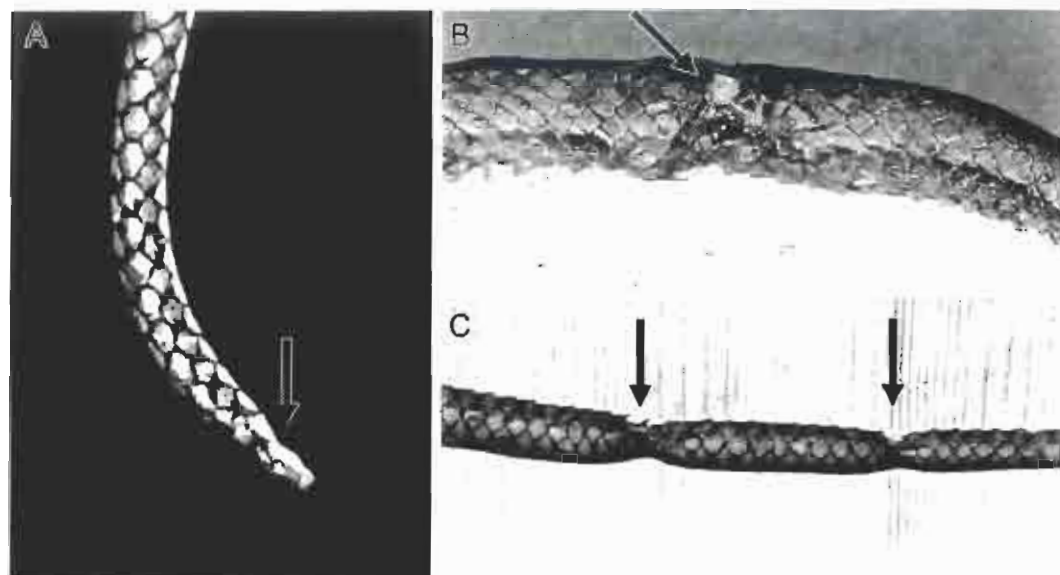


Fig. 3. Damage presumably inflicted by Soldier Crabs (*Coenobita clypeatus*) on *Aliophis portoricensis* from Guana Island, British Virgin Islands. (A) Damaged tail tip. The arrow indicates scarring just above the stump. (B) Recent damage to the posterior part of the body. The arrow indicates exposed bone. (C) Healed damage to tail. The arrows indicate two of three such scars on this individual.

temperature (Spearman correlation:  $\rho = -0.71$ ,  $n = 8$ ,  $P = 0.05$ ). Snake body temperature was significantly correlated with both air ( $\rho = 0.86$ ,  $n = 41$ ,  $P < 0.001$ ) and substrate temperatures ( $\rho = 0.88$ ,  $n = 37$ ,  $P < 0.001$ ). However, multiple regression showed that substrate temperature had no immediate effect on body temperature ( $t = 0.88$ ,  $P = 0.39$ ) once the statistically significant effects of air temperature ( $t = 3.50$ ,  $P = 0.001$ ) were removed. Total snake encounters over the five-year study period ( $n = 205$  for which time of day was recorded) show a similar pattern (Fig. 5B), although encounter rates were not corrected for search effort. We did observe three snakes active at night, one of them at a night-light (Perry and Lazell, 2000).

Of 154 snakes for which insolation data were collected, seven (4.5%) were found in full sun, 89 (57.8%) were in full shade, and 58 (37.7%) were first seen in a sun-shade mosaic. Snakes were seen in the full sun only early and late in the day (Fig. 6), although shade and sun are available in nearly all habitats on most days. Of 188 snakes for which posture data are available, 76 (40.4%) were moving when first seen, 90 (47.9%) were sprawled, and 22 (11.7%) were coiled.

#### DISCUSSION

*Size and tail damage.*—Individuals of *A. portoricensis* on Puerto Rico, St. Thomas, and Burk Island (off St. Thomas) routinely reach large size

(Schwartz, 1966; Schwartz and Henderson, 1991), but those on Guana Island rarely exceed 650 mm SVL. This is consistent with the characterization of *A. p. anegadæ* by Schwartz (1966) as a subspecies of "moderate size." We lack the data to determine whether the paucity of large individuals on Guana is genetically or energetically constrained. However, these snakes presumably subsist almost entirely on the ubiquitous anoles (*Anolis* spp.), the largest of which (*A. cristatellus*) attain SVLs of only about 75 mm. Larger prey (e.g., *Ameiva*, *Cyclura*, or rodents) appear to be taken only rarely. Furthermore, *Ameiva*, although abundant, are not nearly as ubiquitous as anoles (Nicholson et al., 2005), and, at least during our September–October sampling periods, most individuals were small (juveniles or subadults with SVL  $\leq 55$ –60 mm; pers. obs.). Rock lizards (*C. pinguis*) have only recently been introduced to the island (Lazell, 2002). Their consumption by snakes (LeVering and Perry, 2003) is probably adventitious and limited to larger snakes, as hatchling iguanas are stocky and have an average SVL of about 108 mm and mass of 60 g (Perry et al., in press). We have no records of snakes on the island consuming rodents (*Mus* or *Rattus*).

Many snake studies report SVL (e.g., Fitch, 1981), but fewer report mass or the relationship between SVL and mass. In contrast, many studies of lizards report such data (e.g., Pough, 1973; Perry, 1989), and such relationships provide important

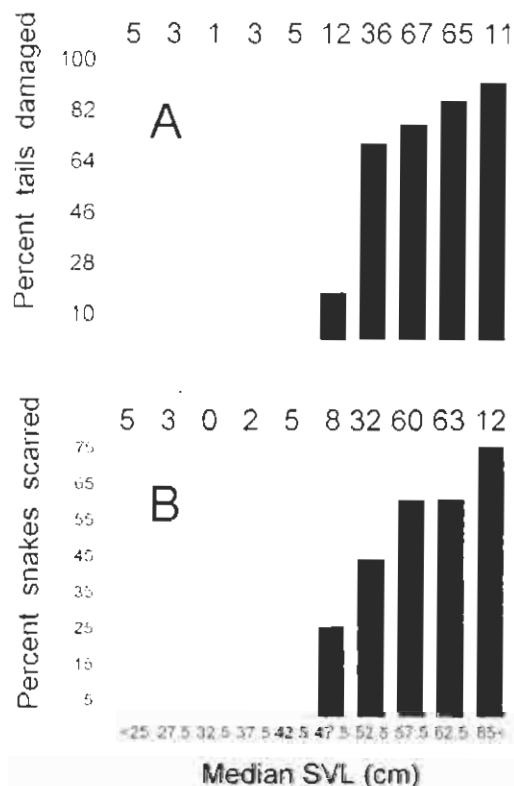


Fig. 4. Frequency of injuries as a function of size in *Alsophis portoricensis*. The number above each column represents sample size. (A) Tails are significantly more likely to be damaged as snakes grow larger. (B) The frequency of overall scarring increases with body size.

information on the biology of a species (Gayer and Donnelly, 1990). For example, we know that the Guana population is flourishing, suggesting our snakes are not unduly stressed. Consequently, demonstrations that animals in other populations have lower mass for a given body length could indicate compromised condition that might be attributable to pressures imposed by introduced predators or poor habitat quality.

Reptiles typically show female-biased sexual size dimorphism (SSD; Fitch, 1981), and this also is true for snakes (Shine, 1994). Our data indicated that *A. portoricensis* is an exception to that rule, as males were longer and significantly heavier and heavier per unit length than were females. The opposite pattern was found in *A. antiguae* (Daltro et al., 2001), but we have not been able to locate additional studies reporting such information for *Alsophis*. The relatively low mass of females compared to males of similar size may indicate a difference in the cost of reproduction between the sexes (e.g., Shine, 1980), and the longer tails of males likely reflect the need to hold the reproductive organs, as in other species (Shine et al., 1999).

The tail is important to at least juvenile *A. portoricensis*, which exhibit caudal luring as a foraging strategy (Leal and Thomas, 1994). The level of damage we found—nearly 70%—is high compared to other records we have been able to locate: about 10% in *Thamnophis sirtalis* (Jayne and Bennett, 1989), 26% in *Atrypa lateralis* (Burns and Heatwole, 2000) and *Pseustes phillipsii* (Akani et al., 2002), 42% in *A. antiguae* (Daltro et al., 2001), and 55% in *Scaphiodontophis annulatus* (Henderson, 1984). However, our findings are consistent with other reports for *A. portoricensis* (Schwartz, 1966). What might be causing damage at these levels? Potential avian and mammalian predators, with the exception of the Pearly-eyed Thrasher (*Margarops fuscatus*), are uncommon on Guana (Lazell, 2005). Given that damage appears in large snakes rather than small ones, thrashers are probably not responsible. Avian and mammalian species also are likely to be fairly effective as predators and thus unlikely to leave many scarred survivors (Jaksic and Greene, 1984). In contrast, crabs, and especially Soldier Crabs (*Cenobita clypeatus*), are common on the island (Harrison et al., unpubl. data) and are unlikely to frequently kill snakes. This species is known to prey on eggs of ducks (Meier et al., 1989) and doves (Rivera-Milan and Schaffner, 2002) in Puerto Rico and

TABLE 1. THE FREQUENCY OF SCARS IN *Alsophis portoricensis*. Numbers represent the number of individuals with at least one scar on the head, body (divided into three equal parts), or tail. Note that the frequency of scars increases from the head toward the tail.

	Head	Scar location			Tail	Confirmed
		Body	Body	Body		
		Anterior 1/3	Middle 1/3	Posterior 1/3		
Scarred	3	18	30	36	79	99
Unscarred	188	174	162	136	112	93
Percent unscarred	98.4	90.6	84.4	70.1	58.6	48.4
n	191	192	192	192	191	192

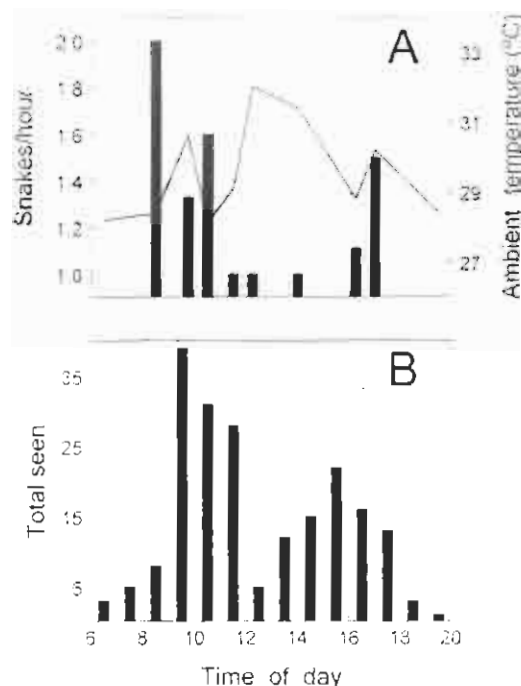


Fig. 5. Activity time of *Alsophis portoricensis* on Guana Island, British Virgin Islands. Snakes are significantly more active during the morning and afternoon hours, when ambient temperatures are slightly cooler. (A) Effort controlled transect study ( $n = 20$ ). The line shows ambient temperatures measured during the study. (B) Total observations over the five-year study period ( $n = 205$ ).

parrots in the Cayman Islands (Wiley et al., 2004). Other species of crabs also are known to prey on terrestrial snakes (Hecht et al., 1955; Maitland, 2003), marine snakes (Voris and Jeffries, 1995), sea turtles (Wood, 1986), frogs (Ryan et al., 1981), and other species (Greene, 1988; Schwartz and Henderson, 1991:560). Crabs, and especially Soldier Crabs, appear to be a plausible culprit for the tail loss and scarring we observed (Fig. 3C).

**Habitat and activity.**—Although not corrected for effort, our data emphasize the fact that these snakes occur virtually everywhere on the island and that proximity to humans does not deter their presence. Because Guana is a “snake-friendly” island, we believe that our results indicate that, under the right conditions, snakes and humans can peacefully co-exist.

Our data indicate a bimodal activity period, with a relative lull during the warmer times of the day (approximately 1200–1400 h). A bimodal pattern of activity in snakes is not unusual (Gibbons and Semlitsch, 1987) and has been

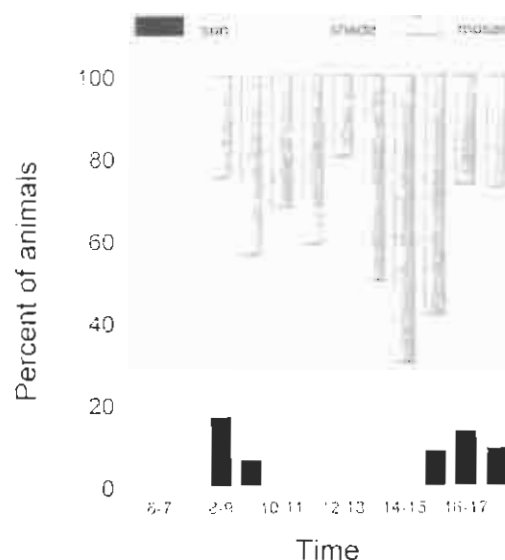


Fig. 6. Insolation conditions at locations where *Alsophis portoricensis* were observed on Guana Island, British Virgin Islands, as a function of activity time. Despite ongoing availability, snakes avoid direct sunlight during the warmest parts of the day.

seen in the closely related *A. rufiventris* on St. Eustatius (Savitt et al., 2005) and ecologically similar *Liophis poecilogyrus* in Brazil (Maclei et al., 2003). Bimodal activity often is related to avoidance of high daytime temperatures (Gibbons and Semlitsch, 1987; Peterson et al., 1993; Macleai et al., 2003; Savitt et al., 2005). Our data show that the body temperatures of *A. portoricensis* tracked those of an animal's immediate environment, and especially air temperature, which changes more rapidly than ground temperature. In the absence of data on availability of thermal resources in the environment, we do not know whether this represents thermoconformism, active thermoregulation, or some intermediate degree of thermal control. However, the apparent avoidance of full sun during the warmer parts of the day suggests that at least some degree of active choice is involved.

Members of the genus *Alsophis* are generally considered to be diurnally active (e.g., Schwartz and Henderson, 1991). *Alsophis portoricensis* was not known to be active at night until a recent observation of an individual exploiting the “night-light” niche (Perry and Lazell, 2000), something few snakes are known to do (Perry and Fisher, 2006). With extensive sampling effort by multiple searchers extending throughout the day, we observed a few more instances of snakes active at night away from lights. These indicated two things. First, *A. portoricensis* is active, albeit rarely, after dark, and this doubtlessly facilitated



the exploration of the night-light niche. Second, with similar effort, additional species may be found occasionally active at atypical times. Similarly, although species of *Alosophis* are usually terrestrial, previous reports indicated that some individuals swim (Powell et al., 2006) and climb (Pérez-Rivera and Lahov-Rivera, 1996), even as high as 18–20 m in rainforest habitat (Thomas and Kessler, 1996).

Racers such as *A. portoricensis* are important predators in intact Caribbean ecosystems. However, accelerated rates of development and invasive species dispersal in the Caribbean mean that few intact communities remain, and snakes in the genus *Alosophis* are among the species disappearing most rapidly. Despite this, our knowledge of their biology, a prerequisite for any conservation work, remains limited. The present study begins to address this knowledge gap for *A. portoricensis* and reiterates the need for similar work on related species.

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*ALSOPHIS PORTORICENSIS ANEGADAE* (Racer). **CANNIBALISM.** Feeding on conspecifics is an uncommon but broadly observed phenomenon in snakes (e.g., Loveridge 1944, Copeia 1944:254; Lourdaïs et al. 2005, Biol. J. Linn. Soc. 84:767–774). However, it has not been previously reported in *A. portoricensis* (Schwartz and Henderson 1991, Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History. University of Florida Press, Gainesville. 720 pp.). Here we report on several such cases observed on Guana Island, British Virgin Islands, in 2006.

The most definitive evidence for cannibalism occurring in nature was obtained on 12 October 2006, when staff alerted us to the presence of a dead snake at the dump. We found an adult male (snout-vent length [SVL] = 596 mm, tail length [TL] = 229, MCZ field tag Z39456) with the posterior end of a smaller female (SVL = 545, TL = 177, MCZ 39457) emerging from its mouth (Figure 1). Because the two snakes may have tried to swallow a single prey item, we dissected the specimen but found no prey item. The larger snake had abundant fat deposits and appeared in good condition. A second case occurred in captivity on 7 October, when two snakes were placed in a single cloth bag. Two days later, only the larger animal (female, SVL = 440, TL = 247, MCZ 39455) could be found. Upon dissection it was found to contain the other individual, a hatchling (SVL = 241, TL = 116, also MCZ 39455). Hotel staff brought a third instance to our attention. They had observed two snakes “fighting” and photographed the event (Figure 2). Unfortunately, they did not allow the interaction to continue, but the photograph is inconsistent with known snake fighting behavior, which has never been observed in this species. Instead, the larger individual had grasped the smaller snake behind the head, presumably in an

attempt to subdue it for ingestion. The smaller snake was bleeding profusely, according to the report.

We are surprised that a behavior not noted in the past, despite extensive work on the species (e.g., Barun et al. 2007. *Copeia*: in press), appeared to be common this year. The past year had been unusually wet on Guana Island, vegetation is abundant, and anoles, the principal prey of *A. portoricensis*, are common. Consequently, the prevalence of cannibalism is difficult to explain. However, if cannibalism occurs frequently, that could explain why smaller animals were relatively uncommon in the sample examined by Barun et al. (Op. Cit.).

We thank April Bates, Val Jardine, James Lazell, and Kate LeVering for bringing some of these instances to our attention. The work was conducted under ACUC permit 05006-01 from Texas Tech University. Support for this project was provided by The Conservation Agency and Texas Tech University. This is manuscript T-9-1119 of the College of Agricultural Sciences and Natural Resource Management, Texas Tech University.

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Figure 1. Dead adult *Alsophis portoricensis* with a smaller individual protruding from its mouth. Photograph by Gad Perry.

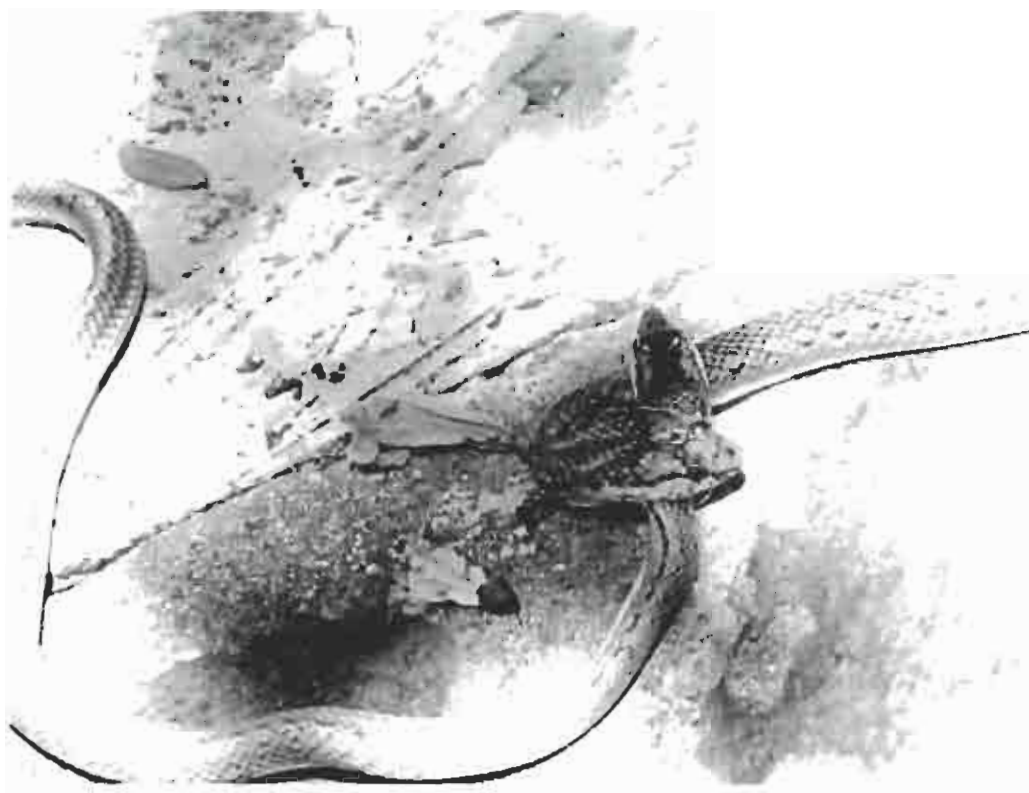


Figure 2. Adult *Alsophis portoricensis* apparently trying to subdue a smaller individual. Photograph by April Bates.

## Conservation of amphibians and reptiles in the British Virgin Islands: Status and patterns

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**Abstract.** The British Virgin Islands (BVI) forms part of the Puerto Rican Bank and its fauna is closely related to that of the US Virgin Islands and Puerto Rico. Humans have inhabited the region for several thousand years and their impacts have continuously included habitat modification, hunting, and introduction of invasive species. Those are the three main causes of concern for the present-day herpetofauna of the BVI, which contains 34 amphibian and reptile species. Of these, five (15%) are common, three (9%) are at risk, eight (23%) are endangered, ten (29%) are data deficient, two (6%) are extinct, and six (18%) are introduced or cryptogenic. Several endemic species are only found on tiny islets where stochastic events can easily lead to their extinction. The native iguana, *Cyclura pinguis*, and the sea turtles found in the region are in an especially sensitive condition. Habitat destruction and fragmentation are ongoing and likely to accelerate. Invasive species are still arriving, establishing, and spreading. Thus, although the short-term conservation status of the BVI herpetofauna appears unlikely to change, we are more concerned about the mid-term outlook. We recommend some specific remedies intended to offset these pressures and provide long-term protection to the biota of the BVI.

**Key words:** Amphibians; British Virgin Islands; conservation; *Cyclura pinguis*; invasive species, reptiles; sea turtles.

### Introduction

Human impacts on species and ecosystems are still expanding, despite often already being more pervasive than can be sustainably supported. Global amphibian declines have drawn considerable attention and debate. They appear primarily correlated with a complex and often site-specific combination of the loss of appropriate habitat, pollution, and the introduction of invasive predators and diseases (e.g., Adams, 1999; Stallard, 2001; Blaustein and Kiesecker, 2002; Muths et al., 2003; Lannoo, 2005). Less attention has been paid to the rapid disappearance of reptile species

(Gibbons et al., 2000). The IUCN (1996) classified vertebrates into four categories based on conservation status and found similar rates of endangerment in both groups (Critically endangered: 9% in amphibians, 10% in reptiles; Endangered: 16% and 14%, respectively; Vulnerable: 39% and 38%). In a recent update (IUCN, 2004) they identified 61% of evaluated reptile species as endangered, compared to 32% of evaluated amphibians. Similar patterns and concerns are apparent in the Caribbean. For example, Powell and Henderson (2005) estimated that almost half of Lesser Antillean terrestrial reptiles have been substantially negatively affected by human activities. Unfortunately, the herpetofauna of the British Virgin Islands (BVI, about 150 km<sup>2</sup> total land area) has not been systematically evaluated since MacLean (1982), the exception being a recent book (Lazell, 2005) that focuses on a single island. Here we review the current conservation status of all reptiles and amphibians known from the BVI, drawing upon both published and unpublished work. We begin with a brief description of the historical and biogeographical setting, then provide species accounts. Taxa of special concern are then discussed in additional detail, and we close with a review of broad patterns and likely future trends.

## **The Setting**

### *History*

Humans first arrived in the BVI some 4000-6000 years ago, primarily inhabiting the larger islands (Wilson, 2001) but utilizing some of the smaller ones for protracted periods. Their presence has clearly had extensive impacts on the local fauna (Lazell, 2005), which suffered both additions and extinctions. The current name was given to the islands by Columbus, whose 1493 visit ultimately resulted in the extirpation of Amerindians from the area and in additional plant and animal introductions and extinctions. European settlement began in 1648, when the Dutch claimed Tortola (Jenkins, 1923), and the islands changed hands multiple times before the British finally acquired them in 1672 (Rogozinski, 1992). Slaves were brought in from Africa to support the agricultural effort which converted much of the land area into plantations. England abolished slavery in 1808 and enacted the Emancipation Proclamation in 1834, and the islands have enjoyed various levels of self-rule ever since. The population in 1844 was 6,689 (Fergus, 2003), declined to 4,639 by 1891, grew to 10,000 in the late 1970s, and is currently estimated at 22,643 people, more than three times what it was 150 years ago (BVI Government, 2005). The current annual population growth rate is 2.06%, and population pressure in the BVI is growing, especially on Tortola (which has over 80% of the total population on less than 40% of the BVI land area). The second largest island, Virgin Gorda, doubled its population between 1960 and 1980 and saw an increase of almost 75% from 1980 to 1991. Despite a relatively high emigration rate, the population of the BVI is expected to double in about 35 years (BVI Government, 2005). The current economy is primarily based on tourism and offshore banking. Growth in population



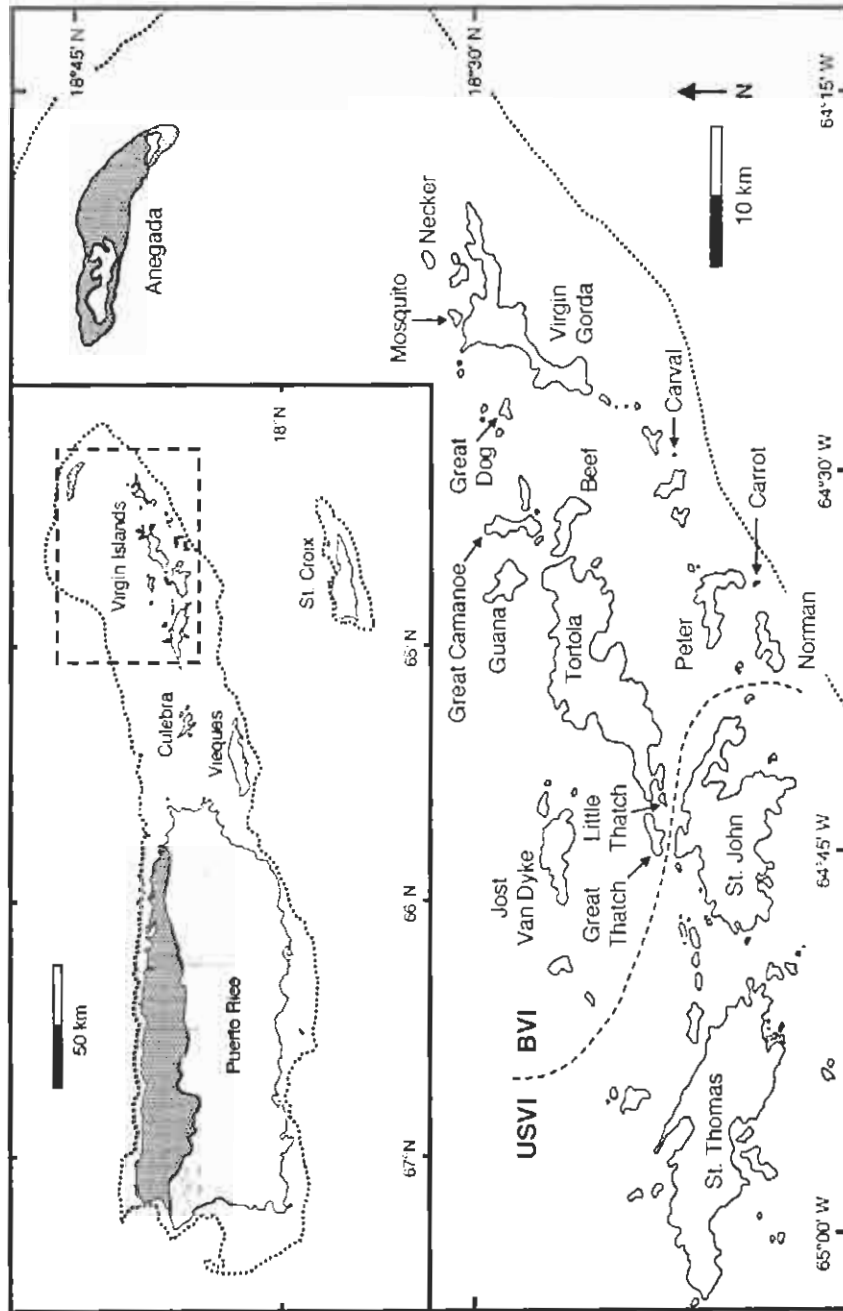
and tourism has resulted in ongoing construction throughout the islands, with roads and buildings being added on a regular basis, natural habitats increasingly being lost and fragmented, and increasing problems with trash disposal.

### *Biogeography*

In the late Pleistocene, a single land mass stood where Puerto Rico, its associated islands, the US Virgin Islands (USVI, except St. Croix) and the BVI now lie (fig. 1). Today called the Puerto Rican Bank, it was roughly twice the area of Puerto Rico today, and became increasingly fragmented as sea-levels rose in the Holocene (Heatwole and MacKenzie, 1967). As a result, the biotas of these politically disparate units are highly similar (see for example, Platenberg and Boulon, 2006). However, rapid evolution has produced a number of unique species on some of the islands, and dispersal and extinction related to island biogeographic processes resulted in a mosaic of distribution patterns. Some of the species, such as *Anolis cristatellus* and *Sphaerodactylus macrolepis*, are present on nearly every piece of dry land in the region (Lazell, 1983), whereas others, such as *A. ernestwilliamsi*, are only found on a single small rock. The genera, however, are all wide-spread throughout the region: there are no endemic genera on the Puerto Rican Bank, and the taxa found there have been shown to be related to species found both to the east and west (Thomas, 1999). Much additional information about the biogeography of the region can be found in Woods and Sergile (2001).

### **Species Accounts**

Information in this section is heavily based on MacLean (1982), with updates drawn from our own observations and from additional sources, which are the only ones cited in the text. Scientific names follow the most accepted current usage. Common names given are those normally in general or regional use. These are often abbreviated for local use, and 'Puerto Rican racer,' for example, is normally locally simply referred to as 'racer'. Distribution information primarily follows MacLean (1982) and Mayer and Lazell (1988). The 'major islands' referred to are Anegada, Tortola, and Virgin Gorda (fig. 1). Few studies have assessed the population sizes of any of these species, and our assignment of status is based on what literature exists and our personal experience. Species are identified as Common (present on many islands in large or stable numbers); At risk (distribution is limited and numbers are declining); Endangered (the species has a very limited distribution and small numbers and is thus in immediate risk); Data deficient (studies identifying population trends are lacking); Extinct (in the BVI — the species may still exist elsewhere); Introduced (non-native taxa which arrived in the BVI relatively recently and with human assistance); or Cryptogenic (impossible to reliably classify as native — see Carlton, 1996). Most species are not locally protected or considered to be of concern, the exception being the iguana and sea turtles.



**Figure 1.** Map of the Puerto Rico Bank (inset) and the British Virgin Islands. Islands named in the text are indicated, but not all islets are named on the map. Dotted lines represent the 100 m depth contour and the approximate areas above sea level during the last glacial maximum. Stippling shows the distribution of karst habitats on present day islands.

### Amphibia

**Leptodactylidae.** *Leptodactylus albilabris* (white-lipped frog). All major and some smaller islands: At risk. This species requires fresh water for reproduction. Although it can breed in ditches, populations appear to be declining. Its tadpoles may be vulnerable to competition from *Osteopilus septentrionalis* tadpoles (see Smith, 2005, for related work). A study of population trends is desirable. *Eleutherodactylus antillensis* (bo-peep, Antillean or churi coqui). Tortola, Virgin Gorda, and many small cays: Common. MacLean (1982) reported it as being found on 'all major islands' but we are not aware of any populations on Anegada. This species appears to be declining wherever *Osteopilus septentrionalis* spreads, but numbers are still high. Populations appear to positively respond to increased rainfall (Ovaska, in Lazell, 2005). *Eleutherodactylus cochranae* (bo-peep, whistling coqui). Tortola, Virgin Gorda: Data deficient. This species appears to be declining wherever *Osteopilus septentrionalis* spreads. *Eleutherodactylus schwartzi* (bo-peep, Virgin Islands coqui). Tortola, Virgin Gorda, Great Dog; (re?)introduced: Little Thatch (Lazell, 2002): At risk. This species appears to be declining wherever *Osteopilus septentrionalis* spreads, has disappeared in the USVI (Philibosian and Yntema, 1976; Platenberg and Boulon, 2006), and has been identified by the IUCN as Endangered (IUCN, 2004). The Great Dog population is somewhat distinct (Ovaska et al., 2000), extremely limited in range, and very susceptible to development there. Additional study, and perhaps protection, of that population is urgently needed, and protection of other remaining BVI populations may well be warranted.

**Bufonidae.** *Bufo (Peltophryne) lemur* (Puerto Rican crested or lowland Caribbean toad). Virgin Gorda: Extinct. No specimens have been collected from the BVI in many decades. Captive husbandry and reintroduction are ongoing in Puerto Rico (Miller, 2005). Whether such a program can succeed in the BVI will depend on the ability to provide and protect appropriate low-elevation habitat, much of which has been destroyed by development.

**Hylidae.** *Osteopilus septentrionalis* (Cuban treefrog). Tortola, Virgin Gorda, Beef Island, Peter Island (Owen et al., 2005a, 2006): Introduced. Individuals have also been collected on Necker and Guana Island (Owen et al., 2005a, 2006), but neither island appears to have a breeding population at the moment. This species is spreading rapidly, with frequent new island records. The diet suggests the potential to severely impact native species (Owen, 2005) and the tadpoles are known to impact those of some native anurans (Smith, 2005).

### Testudines

**Sea turtles.** See additional information under Species of Special Concern, below. Dermochelyidae. *Dermochelys coriacea* (leatherback or trunk turtle). Nesting only, primarily on Tortola and adjacent Beef Island: Endangered. Cheloniidae. *Chelonia*

*mydas* (green turtle). Common in coastal waters, nesting widespread in low numbers: Endangered. *Eretmochelys imbricata* (hawksbill turtle). Common in coastal waters, nesting widespread in low numbers: Endangered. *Caretta caretta* (loggerhead turtle). Infrequent in coastal waters, nesting extremely rare, if at all: Endangered.

**Testudinidae.** *Geochelone carbonaria* (red-legged or red-footed tortoise). Tortola, Virgin Gorda; (re?)introduced: Guana, Necker (Lazell, 2002); At risk (Introduced?). These turtles may not be native to the BVI, as populations have been widely transported throughout the Caribbean starting in Amerindian times (Censky, 1988). Both the Guana and Necker populations appear to be breeding well in their new habitats (Lazell, 2002), but Tortola and Virgin Gorda animals are uncommon in the wild, presumably as a result of habitat loss.

**Emydidae.** *Pseudemys nelsoni* (Florida red-bellied turtle). Tortola (Owen et al., 2005b): Introduced. A few individuals have been collected at the botanical garden ponds, but absence of suitable habitat and fresh water bodies makes them of relatively little concern. *Trachemys scripta elegans* (red-eared slider). Tortola, Virgin Gorda (Owen et al., 2005b): Introduced. Several individuals of this damaging invasive, including juveniles, have been collected, suggesting reproduction is occurring. However, absence of suitable habitat and ecologically similar native species makes them of relatively little concern.

### Sauria

**Polychrotidae.** *Anolis cristatellus* (Puerto Rican crested anole; man-lizard [mostly used for the males]). All major islands and most small rocks and cays: Common. This species is ubiquitous, highly tolerant of human presence and development, and well-studied (Perry, 2005). It has been introduced in other locations (e.g., Brach, 1977). Diet occasionally includes other lizards (Owen and Perry, 2005). *Anolis ernestwilliamsi* (Carrot Rock or Ernest's anole). Carrot Rock, Peter Island: Endangered (possibly extinct on Peter Island; Perry, 1995). This large anole is only found on Carrot Rock, a tiny and highly exposed location. Population size is doubtlessly small and susceptible to stochastic events such as hurricanes. *Anolis stratulus* (Puerto Rican saddled anole). All major islands and many smaller cays: Common. BVI populations (Rodda et al., 2001) are much less dense than those seen in Puerto Rico (Reagan, 1992), and the species is rarely seen on Anegada, for example. However, there is no indication of population declines. Contrary to the view of MacLean (1982), these animals will occasionally take vegetable material (Perry and Lazell, 1997; Rios-López, 2004). *Anolis pulchellus* (Puerto Rican grass or bush anole). All major islands and some smaller ones: Common. This species is restricted to open or edge habitats where grass or narrow stems are available, and can tolerate human-impacted areas. They are often hard to see, both because of their excellent camouflage and tendency to be active during cooler times of day (G. Perry and

K.R. LeVering, unpublished). Like *A. stratulus*, these animals will occasionally take vegetable material (Perry and Lazell, submitted). *Anolis roosevelti* (Culebra Island giant anole). Tortola: Extinct (in the BVI). MacLean (1982) was not sure whether Tortola sightings were genuine but Gaa (1987) unearthed museum specimens. Some animals may remain on Culebra off the Puerto Rico shore and a recovery plan exists (Campbell and Dodd, 1982), but the species may be globally extinct.

**Iguanidae.** *Cyclura pinguis* (Anegada or stout iguana). Anegada; (re?)introduced: Guana, Necker (Lazell, 2002), Norman, Little Thatch (Perry et al., 2006): Endangered. See expanded discussion in Species of Special Concern, below. *Iguana iguana* (green or common iguana). Peter Island (Carey, 1972), Tortola: Introduced. Green iguanas are certainly native to part of the Lesser Antilles (e.g., Saba, Netherlands Antilles; Powell et al., 2005), and MacLean (1982) considered Virgin Islands populations to also be native. However, BVI animals appear more similar in appearance to animals found in the pet trade than to native Caribbean populations. Genetic studies to identify their origin are warranted. The main concern is their ability to take over habitat that might otherwise be appropriate for the native *Cyclura pinguis*.

**Gekkonidae.** *Hemidactylus mabouia* (common house gecko; wood slave). All major islands and some smaller ones: Cryptogenic. This species is common around human habitation, but generally uncommon or absent in native habitats (Rodda et al., 2001). There is no evidence of broad impacts on native species, but little effort has been devoted to identifying such patterns. The house gecko was likely introduced throughout the region in slave ships arriving from Africa. *Thecadactylus rapicaudus* (turnip-tailed gecko). Necker Island: Cryptogenic. This species is widespread in the Lesser Antilles but is only recorded from one location in the BVI. The population on Necker is often associated with human habitation, but can also be found in native habitats (Lazell, 1995). It may be anthropogenic in origin. Despite being small the population appears stable.

*Sphaerodactylus macrolepis* (Puerto Rican dwarf gecko or sphaero). All major islands and most small cays and rocks: Common. This species reaches incredibly high densities in areas with deep leaf-litter (Rodda et al., 2001). It shows some signs of physiological variation even within islands (MacLean, 1985), suggesting that future speciation may occur on a small scale. *Sphaerodactylus parthenopion* (BVI dwarf gecko or sphaero). Tortola (?), Virgin Gorda: Data deficient. Carey (1972) and MacLean (1982) only reported this species from Virgin Gorda, but Procter and Fleming (1999) also report it from Tortola and Heatwole et al. (1981) from Mosquito Island. One of the world's smallest lizards, the biology of this species is poorly understood and population trends are unknown. Distribution within the islands it resides on appears fairly limited and likely to be further curtailed by development. *Sphaerodactylus* sp. (proposed: Carvel Rock dwarf gecko or sphaero). Carvel Rock: Endangered. This population appears morphologically distinct but

is yet to be described. Given the extremely limited range – a small part of a single small rock – it appears especially susceptible to stochastic events such as hurricanes.

**Scincidae.** *Mabuya sloanii* (Antillean slipperyback or skink). All major islands and some smaller ones: Data deficient. This species appears widespread but is never common. Its biology is poorly understood and population trends are unknown. Carey (1972) reports that their numbers in the BVI were much reduced because of the introduced mongoose (*Herpestes javanicus*). Given the broad range of the mongoose in the BVI, this is a source of concern. *Mabuya macleani* (Carrot Rock slipperyback or skink). Carrot Rock: Endangered. Only recently described (Mayer and Lazell, 2000), this species has an extremely limited range and is thus highly vulnerable. However, the Carrot Rock population is relatively dense. Almost nothing is known about its biology or population trends.

**Teiidae.** *Ameiva exsul* (Puerto Rican ground lizard). All major islands and some smaller ones: Data deficient. Members of the genus *Ameiva* appear especially susceptible to predation by the introduced mongoose (*Herpestes javanicus*; Henderson, 1992). Given the broad range of the mongoose in the BVI, this is a source of concern for many populations. This species is common on the mongoose-free islands of Guana (Nicholson et al., 2005) and Anegada (G. Gerber, unpublished) but infrequently seen on most other islands, despite being very tolerant of development and fragmentation.

**Amphisbaenidae.** *Amphisbaena fenestrata* (Virgin Islands worm lizard). Great Camanoe, Great Thatch, Guana, Tortola, Virgin Gorda: Data deficient. This secretive species spends most of its time underground as it cannot avoid dehydration on the surface (Perry et al., 2004). Very little is known about its biology, but there is no indication of population decline and it is likely to exist on additional islands.

### *Serpentes*

**Typhlopidae.** *Typhlops richardi* (Virgin Islands blind snake). Guana, Necker (?), Tortola: Data deficient. These subterranean animals are not often seen above-ground and little is known about their biology. However, data collected by Rodda et al. (2001) suggest they are found in large numbers. The Necker population may belong to *T. catapontus* (J. Lazell, pers. com.) and needs to be reexamined. Populations are known from additional islands but which of the two species they belong to has not been studied. *Typhlops catapontus* (BVI blind snake). Anegada, Virgin Gorda: Data deficient. These subterranean animals are not often seen above-ground and little is known about their biology and population trends. Blind snakes are known from additional islands, but whether they belong to this species or to the one that precedes remains unstudied.

**Boidae.** *Epicrates monensis* (Virgin Islands boa). Tortola: Data deficient. This species is considered endangered in the USVI (US Fish and Wildlife Service, 1980), where a reintroduction program is ongoing (Tolson, 2005). In contrast, Lazell (2005) believes it 'is the most common snake on Tortola'. Proposed studies of population status in the USVI (R. Platenberg, pers. com.) may help shed light on this issue, but a study of the BVI population is highly desirable.

**Colubridae.** *Arrhyton exiguum* (Puerto Rican garden or ground snake). All major islands and some smaller ones: Data deficient. This small, nocturnal snake is only found at low densities and is very poorly studied. Sightings on Guana appear fairly constant, but broad population trends are unknown. *Alsophis portoricensis* (Puerto Rican racer). All major islands and many smaller ones: Data deficient. Members of the genus *Alsophis* appear especially susceptible to predation by the introduced mongoose (*Herpestes javanicus*; Henderson, 1992). Given the broad range of the mongoose in the BVI, this is a source of concern. Populations on the mongoose-free islands of Guana (Barun et al., submitted) and Anegada (G. Gerber, unpublished) appear healthy, but numbers on other islands are generally much lower. Recent information suggests these animals swim well, perhaps enhancing their inter-island dispersal abilities (Powell et al., in press).

### Species of Special Concern

Two types of organisms native to the BVI are of particular concern, and we provide more detailed information about both in this section. The first is the endemic iguana, *Cyclura pinguis*, which is one of the most endangered lizards on Earth and the most endangered animal species in the BVI. *Cyclura pinguis* is listed as Critically Endangered in the IUCN Red List of Threatened Species (IUCN, 2004) and Endangered in the U.S. Fish and Wildlife Service List of Endangered and Threatened Wildlife and Plants (USFWS, 1999). It is protected from international trade by inclusion in Appendix I of the Convention on the International Trade of Endangered Species of Wild Fauna and Flora (CITES, 2005). The other is a group of species, marine turtles, which are at risk world-wide and which are likewise protected by a number of international agreements, including CITES. Their situation will likely require regional cooperation to resolve.

#### *The endemic iguana*

*Cyclura pinguis* was described approximately 90 years ago (Barbour, 1917), at which time it was restricted to Anegada, a 39 km<sup>2</sup> island located on the northeastern edge of the Puerto Rican Bank in the BVI (fig. 1). However, fossils of *C. pinguis* are common in late Pleistocene deposits (15,000–20,000 years old) from limestone caves in northern Puerto Rico (Pregill, 1981). There is considerable debate over when population declines occurred. Pregill (1981) and Pregill and Olson (1981)

provide evidence of the disappearance of *C. pinguis* and other xerophylic reptile species in Puerto Rico at the end of the Pleistocene, well before human colonization of the region. They hypothesize that the extinctions resulted from a significant reduction in xeric habitats, brought about by climatic changes associated with the end of the last ice age and rising ocean levels throughout the West Indies. Pregill (1981) and Pregill and Olson (1981) view Anegada as exemplifying prototypical *Cyclura* habitat and serving as a climatic refugium for *C. pinguis*. Anegada is similar to Caribbean islands with other *Cyclura* (Alberts, 2000) and different in substrate, topography, and vegetation from other large islands on the Puerto Rican Bank. Anegada is composed entirely of sedimentary substrates (limestone, coral, and sand), is flat and low-lying (8.5 m max.), and supports only xerophylic vegetation. In contrast, other islands are characterized by volcanic and metamorphic substrates, mountainous terrain (up to 523 m in the BVI), and semi-mesic vegetation. However, remains of *C. pinguis* have been recovered from Native American middens on St. Thomas, USVI (Barbour, 1919; Pregill, 1981), and some authors (e.g., Wing, 2001; Lazell, 2005; S.T. Turvey, pers. com.) believe the iguana survived much later and was extirpated from most of its range by humans. Ultimately, this issue must take a back seat to the urgent problem of guaranteeing the survival of the species beyond the next few years.

Iguanas were reported to be plentiful and sometimes hunted in parts of Anegada in the early 1800's (Schomburgk, 1832), but were described as rare by the early 1900's (Barbour, 1917). In 1968, Carey (1975) observed that adult iguanas outnumbered juveniles and concluded that the population was in decline. He attributed this to negative interactions with introduced mammals, which included rats, cats, dogs, goats, sheep, donkeys, and cattle. Mitchell (1999), who conducted her studies between 1988 and 1994, corroborated Carey's conclusions and described further declines in range, abundance, and individual condition. She estimated the total population at 164 animals. Consistent with this information, Perry et al. (2006) showed that body condition declined from Carey's (1975) work to that of Mitchell (1999). The most recent studies on Anegada, ongoing since 1998, indicate the population is largely restricted to a core area of 3 km<sup>2</sup> on the north coast and likely numbers less than 300 individuals, although a recent population estimate is needed. Most of the current population is comprised of adults, and although successful reproduction is still noted, almost no recruitment occurs because of predation of juveniles by feral cats (Gerber, 2004).

Current conservation efforts consist of two largely disparate programs; one aimed at preserving the species and its habitat on Anegada, another that seeks to establish the species on other islands as a precaution against extinction. In 1997, the BVI National Parks Trust and the IUCN Iguana Specialist Group (ISG) established an in situ headstart and release program for *C. pinguis* on Anegada to bolster recruitment until feral cats and other introduced mammals could be controlled (Bradley and Gerber, 2005). Nests are located each summer and fenced off for protection. When hatchlings emerge in the fall they are collected for captive rearing until large enough



to survive with cats. Since 2003, 12 male and 12 female headstarted iguanas have been fitted with radiotransmitters and released each October. Survival of released iguanas has averaged 85%, providing the population with a substantial increase in young adults (Bradley and Gerber, 2006).

The other conservation program has focused on translocating *C. pinguis* to privately owned islands with limited development and few introduced mammals. Between 1984 and 1986, eight *C. pinguis* (three males and five females, 224–509 mm SVL) were moved from Anegada to Guana Island, north of Tortola (Goodyear and Lazell, 1994). The translocation was successful and in 2002 the Guana population of *C. pinguis* was estimated at 100 individuals (Perry and Mitchell, 2003). Four hatchling *C. pinguis* from Guana were released on Necker Island, north of Virgin Gorda, in 1995 (Lazell, 1995). This translocation has also been successful, with reproduction reported on site (Lazell, 2002), but no population estimate is available. Two adults (a male and a female) and 10 juveniles from the Guana population have recently been released on Norman Island, south of Tortola, and five hatchlings have been placed in a headstart facility on Little Thatch Island, between Tortola and St. John (J. Lazell, pers. com.), but it is too early to evaluate the success of either translocation.

Despite these efforts, full recovery of *C. pinguis* will require considerable additional effort. The Anegada population, potentially the largest and certainly the best documented historically, requires habitat protection and eradication of feral mammals. Anegada's western salt ponds and surrounding habitat were declared a Ramsar site in 2000, providing limited protection for approximately half of the core iguana area on the island. However, establishment of a proposed National Park, encompassing the entire core iguana habitat, is needed to adequately protect this area from ongoing development and other direct human impacts. A detailed feral mammal eradication plan for Anegada is currently being developed, but funding for implementation has not been obtained yet. Other populations likely remain extremely genetically limited (Perry et al., 2006), raising concerns about their long-term viability without an influx of additional animals. A recovery plan for the Anegada population (in press) does not take into consideration the translocated populations, as lack of coordination between the two programs has hampered cooperative efforts.

### *Sea turtles*

Four species of marine turtle, belonging to two families, are known from the BVI. Adult leatherbacks are largely pelagic, feeding on jellyfish and other soft-bodied invertebrates. They can be found in the BVI between March and July, when females visit coastal waters and come onto sandy north-shore beaches to nest. Juvenile leatherbacks have been reported from a variety of coastal waters worldwide (Eckert, 1999), but not from the BVI. In contrast, both adult and juvenile green and hawksbill turtles are commonly found in BVI coastal waters year-round and predominantly feed on marine vegetation and sponges, respectively. Both species nest, in low

numbers, on beaches throughout the BVI. Loggerheads, which feed primarily on crustaceans and mollusks, are the least common turtle in the BVI.

Sea turtles have historically been harvested in the BVI, and are consequently much less abundant today than during past times (Eckert et al., 1992; Godley et al., 2004). Eggs of all marine turtles have been exploited for food in the BVI, as elsewhere. Leatherbacks (locally known as trunks) were traditionally slaughtered for their meat and to render oil, thought to have medicinal value, when they came ashore to nest (Lettsome, 1989). In the 1920s and 1930s, 'trunkers' recall as many as six females coming ashore per night on several Tortola beaches (Lettsome, 1989; Eckert et al., 1992). In the 1960s, two or more females were still coming ashore each night on some beaches, but by the 1980s leatherbacks had all but disappeared from the BVI, with fewer than 10 nesting attempts per year (Chambers and Lima, 1990; Eckert et al., 1992; Hasting, 2003). Other marine turtle species were also taken on nesting beaches in the BVI but levels of exploitation and numbers of females nesting are not well documented. In 1981, Fletemeyer (1984) estimated the number of females in the BVI nesting population at  $75 \pm 25$  greens,  $50 \pm 25$  hawksbills, and a 'few' loggerheads. In contrast, surveys conducted in 1990 and 1991 (Hasting, 1992) reported only 6 and 17 nests for the three species combined, respectively. Unlike leatherbacks, other sea turtles, primarily greens and hawksbills, have traditionally also been harvested from the sea. Fletemeyer (1984) reported a direct catch of 600 greens and 300 hawksbills, and an incidental catch of 200 unspecified turtles by other fisheries, in 1981.

All of the marine turtle species reported from the BVI are listed in the IUCN Red List of Threatened Species (IUCN, 2004) as either Critically Endangered (leatherback, hawksbill) or Endangered (green, loggerhead). All marine turtles are also protected by inclusion in Appendix I of two Multilateral Environmental Agreements to which the BVI is a party; CITES and the Convention on Migratory Species (CMS). BVI legislation pertaining to marine turtles includes the Turtles Ordinance 1959, the Turtles Protection Amendment Notice 1986, the Fisheries Act 1997, and the Fisheries Regulation 2003. In compliance with CITES, the BVI prohibits international trade of marine turtles and products. It is currently illegal to (1) take leatherback and loggerhead turtles, (2) disturb or harm sea turtle eggs or nesting females, (3) use any turtle capture device within 100 m of shore, (4) fish for turtles from 1 April-30 November, or (5) take green and hawksbill turtles with carapace lengths less than 61 cm (24 inches) and 38 cm (15 inches), respectively. Additional recommendations to amend legislation, including maximum size limits to protect reproductive stocks, establishment of a limited turtle fishing licensing scheme with strict regulations, catch quotas, compulsory biometric reporting prior to slaughter, and increased fines for infringements were proposed by Godley et al. (2004). Although all nesting marine turtles and their eggs have been protected in the BVI since 1986, commercial trade of marine turtles and products is permitted within the BVI. Government-regulated turtle harvest appears to go beyond accommodating 'the needs of subsistence users', as provided by the CMS. Godley et al. (2004)

estimated that over 150 green and 50 hawksbill turtles are taken annually, and illegal take is still ongoing as well.

Sea-turtle soup and products can still be readily obtained in the BVI, but increased law enforcement, public awareness, and monitoring and conservation activities have allowed sea turtle populations to begin recovering. The Wider Caribbean Sea Turtle Network (WIDECAST) has provided training in marine turtle biology and management and assisted in development of a national sea turtle recovery action plan (Eckert et al., 1992). Leatherback nesting activities have been monitored annually by the Conservation and Fisheries Department since the 1980s (Hasting, 2003; Godley et al., 2004). Most recently, the BVI participated in two UK sponsored projects aimed in whole or in part at marine turtle conservation: Turtles in the Caribbean Overseas Territories (TCOT), from 2001-2004, and the Darwin Initiative Assessment of Coastal Biodiversity in Anegada, from 2003-2006. TCOT-initiated activities in the BVI included training and capacity building, extensive sociological surveys to document trends in the harvest of marine turtles and attitudes toward conservation efforts, aerial and ground surveys of beaches to assess marine turtle nesting activities, and an overall assessment of the status and exploitation of marine turtles in the BVI (Godley et al., 2004). The ongoing biodiversity assessment of Anegada ([www.seaturtle.org/mtrg/projects/anegada](http://www.seaturtle.org/mtrg/projects/anegada)) initiated an extensive in-water tagging, monitoring, and sampling program for marine turtles. Several hundred green and hawksbill turtles have been marked to date, and an action plan for preserving Anegada's biodiversity is being produced. As a result of this effort, the outlook for marine turtles in the BVI has improved dramatically in recent years. As many as 65 leatherback nesting attempts per year have been reported for the north-shore beaches of Tortola and adjacent Beef Island (Hasting, 2003; Godley et al., 2004). Populations of other species also appear to be on the rise, but have not rebounded to the extent that leatherbacks have (Godley et al., 2004).

The take of marine turtles and their eggs by humans remains the greatest threat to BVI turtle populations. Other threats reported by Godley et al. (2004) include accidental boat strikes; loss of beach nesting habitat from erosion, sand mining, and development; indirect impacts from increased development, including artificial lighting; pollution, including litter at sea and on beaches; and general environmental degradation. In addition, fibropapilloma disease has been observed in green turtles in the BVI (Overing, 1996) and, although undocumented, introduced predators, such as mongoose and dogs, likely prey on turtle eggs in the BVI, as they do in the US Virgin Islands (Platenberg and Boulon, 2006). Livestock, which on some islands (e.g., Anegada) frequent beach areas in large numbers at night, probably also damage nests. To adequately protect marine turtle stocks in the BVI and aid in their recovery, we strongly urge authorities to (1) increase protection of critical nesting and foraging habitats, (2) implement country-wide beach and in-water monitoring programs to better document turtle population trends, and (3) increase oversight of the turtle fishery, including strict enforcement of regulations, detailed documentation of the annual take and evaluation of its impacts, and establishment of

size limits that encourage maximum reproduction. Experience has shown that taking primarily large individuals in fish populations results in a reduction in reproductive output, and we urge the government to consider setting both upper and lower limits on size of turtles which can be killed.

## Discussion

Of the 34 amphibian and reptile species documented in the BVI, five (15%) are common, three (9%) are at risk, eight (23%) are endangered, ten (29%) are data deficient, two (6%) are extinct, and six (18%) are introduced or cryptogenic. There are several causes for alarm in this breakdown. One is that we have little or no information about the population trends of almost a third of the species, a lack that urgently needs to be rectified. A second concern is that another third of the species are already at immediate or low-level risk and an additional number are already extinct. Given the increasing population and development pressure, this is not a good sign for the future. Of especially great concern are the sea turtles, which have traditionally been harvested in the area (Eckert et al., 1992) and remain an exploited resource; the native iguana; and species found on single, tiny, and highly vulnerable islets. However, leatherback nesting activities appear to be on the rise (Hasting, 2003), and both in-situ and ex-situ efforts to protect the iguana are having some success (Lazell, 2002; Bradley and Gerber, 2006; Perry et al., 2006). Finally, over a tenth of the herpetofauna of the BVI is already comprised of introduced species, some of which have documented negative impacts on native species. Additional species may have been introduced long enough ago and their origin is hard to ascertain.

Primary causes of endangerment and extinction in the BVI include habitat loss and introduction of invasive species such as mongooses, cats, goats, and sheep. Development is an especially large concern for sea turtles, both because of the loss of nesting beaches and the additional night-lights that are detrimental to juvenile post-hatching dispersal (Salmon, 2005). Some work has taken place or is planned for control of invasives. For example, goats were successfully eradicated from Norman Island. However, most invasive species remain common and prospects for eradicating species such as the mongoose in the foreseeable future are minimal. Worse, additional species keep arriving in the BVI, and some taxa that are already in the region, such as *Bufo marinus* and *Eleutherodactylus coqui* have great potential for harm should they invade the BVI. Luckily, we have not yet seen signs of chytridiomycosis, a disease implicated in the severe declines of many amphibian populations (Berger et al., 1998), in BVI amphibian samples sent to the United States Geological Survey National Wildlife Health Center in Madison, Wisconsin for analysis (D.E. Green, Pers. Com.). Moreover, most BVI amphibians do not match the profile of especially susceptible taxa identified by Lips et al. (2003). Nonetheless, monitoring of BVI populations must be a high priority, given the broad declines of amphibians identified in Puerto Rico (Burrowes et al., 2004).

For species with single-island distributions, the risk of extinction as a result of stochastic events such as storms remains a major concern. On Puerto Rico, hurricanes are known to affect birds (Wauer and Wunderle, 1992) and boas (Wunderle et al., 2004). Similar effects are likely in the BVI, especially given the prediction that global climate change will result in more frequent and more severe storms (Goldenberg et al., 2001; Emanuel, 2005). Climate change is expected to affect forests in additional ways as well (Dale et al., 2001), and changes in factors such as the frequency of drought are sources for concern. Although extinctions of small isolated populations are a natural event, the increased likelihood caused by human activities may merit efforts to protect such taxa. For example, Carrot Rock may not be facing development but the unique species it contains are nonetheless affected by developments on nearby Peter Island (where the Carrot Rock anole is probably already extinct) and by global climate change, thought to be responsible for increased storm severity and higher sea levels.

In the short-term, the conservation status of the BVI herpetofauna appears unlikely to change. However, we are more concerned about the mid-term outlook, which includes increasing impacts from habitat loss and invasive species. To offset these pressures, we recommend the following strategies be adopted:

1. Set aside additional areas for conservation. This is especially true for marine turtle nesting beaches; the proposed National Park on Anegada, which is essential for the survival of *C. pinguis*; and for expanding the size of the two peak reserves already recognized, Gorda Peak and Sage Mountain, both of which are very small. Islets containing unique taxa, such as Carrot Rock, Carvel Rock, and Great Dog, should also be a high priority for protection.
2. Minimize the impacts of development via better enforcement of existing regulations and improved planning. For example, impacts to sea turtles from beach-side night-lights can be reduced by use of improved lighting technology (Bertolotti and Salmon, 2005). Improved international collaboration on conservation of sea turtles would also benefit the conservation of these animals, which migrate among political units and are therefore subject to variable protection.
3. Establish a baseline for population size of data-deficient species and a long-term program for monitoring all amphibian and reptile species to ensure declines are not occurring. Studies of the molecular systematics of local species should be a high priority, as such work has repeatedly identified the presence of cryptic species.
4. Support the eradication of existing harmful invasives, a process which has become increasingly feasible in recent years (Veitch and Clout, 2001; Krajick, 2005), and enhance prevention of additional invasive species arrivals. New Zealand is a leader in this, and much information is available on their governmental web site (<http://www.biosecurity.govt.nz/>) and on the IUCN Invasive Species Specialist Group web site (<http://www.issg.org/>).

5. Improve public education about the value of conservation in general and herpetofauna in particular.

The motto of the BVI is 'nature's little secrets', but its ability to sell itself as an ecotourism destination is rapidly being eroded by the same processes that are endangering the herpetofauna of the nation. The five strategies we identified above are not unique to the herpetofauna, but will rather provide protection to many species and habitats. We hope that the government of the BVI will continue to show foresight in protecting its natural assets.

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## Keeping Invasive Species Off Guana Island, British Virgin Islands

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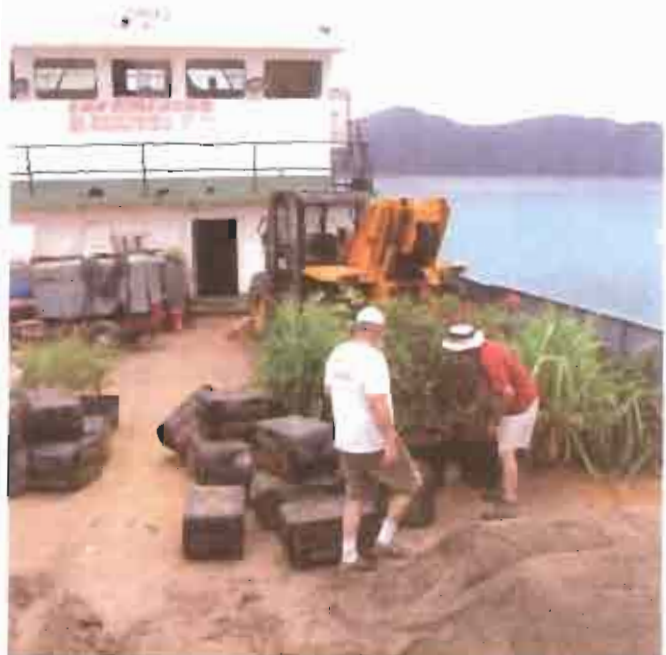
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Volume 37

Only habitat loss is a bigger threat to native species than invasive species, which are found in increasing numbers in both terrestrial and aquatic habitats and cause over \$100 billion in estimated economic damage every year in the United States alone. In recent years, one of us has documented the ongoing expansion of the Cuban Treefrog in the British Virgin Islands. This frog invades the water cisterns that are the main source of drinking water for many residents, causes the decline of native frogs, and also feeds on lizards, snakes, birds, and invertebrates. The owners and management of Guana have been committed to keeping these frogs off the island and have fumigated plants and construction materials with vehicle exhaust, a practice which has proved ineffectual and has been stopped.

In October 2006, we inspected a barge delivering over 220 potted plants to Guana Island and originating in Florida via Beef Island. The search was conducted by a team of eight and involved individually searching each plant and removing invasive organisms. During the search we removed eight lizards belonging to three species, a spider, several snails, and two nests of the imported Red Fire Ant which is also plaguing Texas.

Both the number of organisms discovered on this barge and their diversity is alarming because they indicate that a broad range of organisms can and is transported in such shipments, which are common. BVI law offers little guidance about inspecting and sanitizing shipments arriving from other locations, such as Florida, and additional measures are needed to prevent the arrival of invasive species and eradicate those already in the BVI.



*Plants and other landscaping supplies being inspected on the barge.*

# Keeping Invasive Species Off Guana Island, British Virgin Islands

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Only habitat loss is a bigger threat to native species than invasive species, which are found in increasing numbers in both terrestrial and aquatic habitats and cause over \$100 billion in estimated economic damage every year in the United States alone (Pimentel et al. 2000). Such species often arrive via the transportation network (reviewed in Ruiz and Carlton 2003), whether voluntarily (intentional introductions) or involuntarily (accidental immigrants). That has been the case of the Cuban Treefrog (CTF), *Osteopilus septentrionalis*, which has been spreading in the Caribbean, primarily as a stowaway in ornamental plants and construction materials (e.g., Townsend et al. 2000, Powell et al. 2005, Powell 2006). In recent years, one of us has documented its ongoing expansion in the British Virgin Islands (BVI), where it is now known from breeding populations on Tortola, Virgin Gorda, Beef Island, and Peter Island (Owen et al. 2005, 2006). Single individuals have been documented on Necker Island and Guana Island, but searches of both in 2006 revealed no breeding populations, adults, or tadpoles. This frog is of concern for several reasons: It appears to cause the decline of native frogs, at least partially by direct predation; it also feeds on lizards, snakes, birds, and — primarily — invertebrates. In addition, it invades the water cisterns that are the main source of drinking water for many BVI residents, raising human health concerns.

Even before the collection of a CTF on Guana Island, a private wildlife preserve, the owners and management have been committed to keeping these frogs off the island. Over the years, they have fumigated plants and construction materials with vehicle exhaust, but the frogs appear remarkably resistant to long exposures to the toxic fumes, and this practice has been stopped. Nursery staff is supposed to inspect all plants for frogs before loading. In addition, all arriving nursery and construction materials are individually inspected before they are allowed onto the island. So far, these searches have resulted in the capture and destruction of three CTFs, two in a single shipment in 2005 (S. Western, pers. comm.) and one in 2004 (H. Watson, unpublished). Here, we report on one such search.

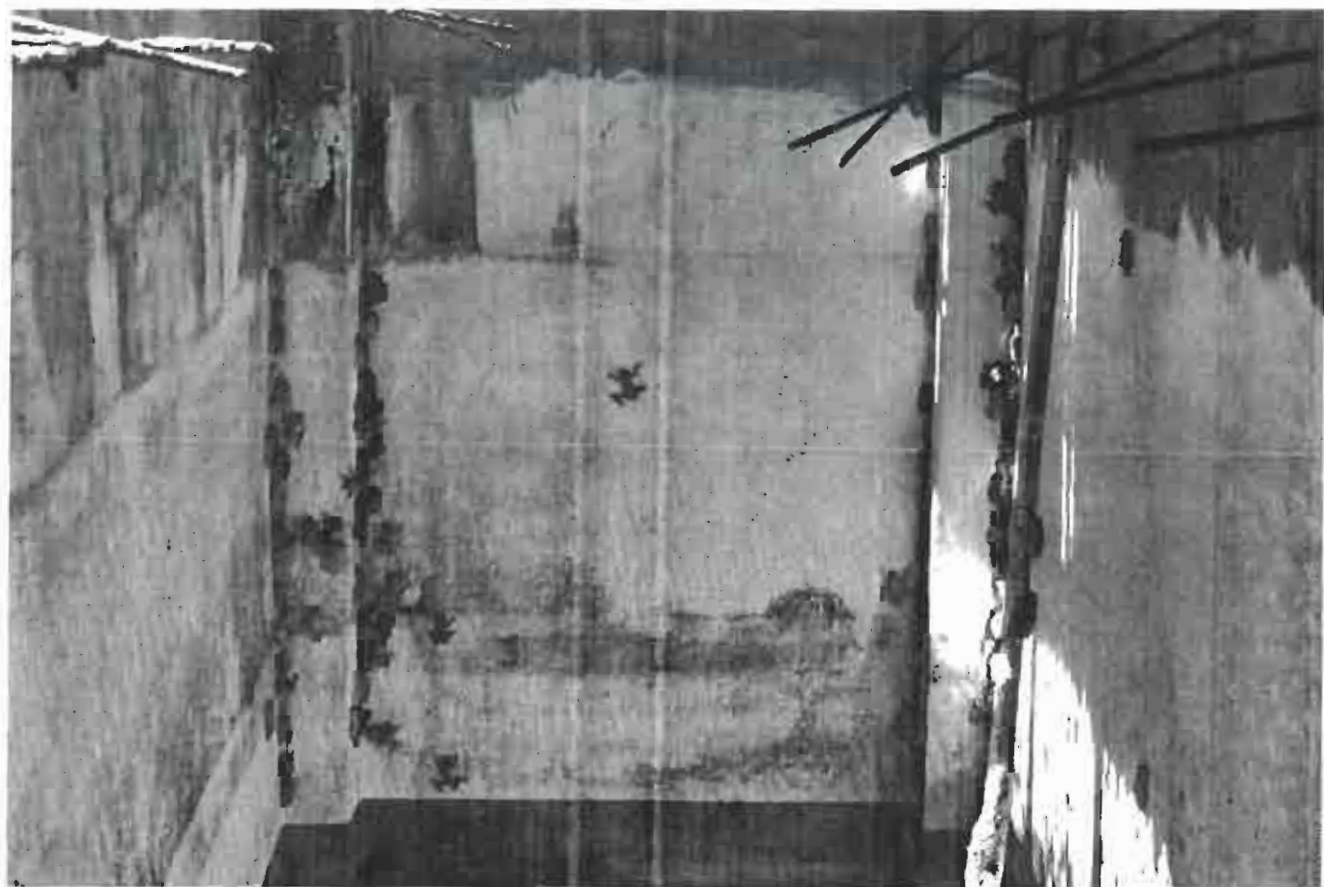
On 12 October 2006, the barge *Deriece-W*, normally based on Beef Island, delivered over 220 potted plants to Guana Island: *Ficus microcarpa*, *Clusia guttifera*, *Bougainvillea* "Helen Johnson," *Pandanus utilis*, *Jatropha integerrima*, *Suriana maritima* (Bay Cedar), and *Coccoloba uvifera* (Seagrape). As with many ornamental plants used throughout the Caribbean, they originated in Florida, USA, which is inundated with invasive reptiles and amphibians (e.g., Meshaka et al. 2004). Prior to being loaded on the barge, plants were housed at Minnie's Plants, a nursery on Beef Island that is heavily infested with CTFs (Owen, 2005). Some of the material (*Clusia* and *Jatropha*) had arrived from Florida 10 days previously, while other plants had been on Beef Island for up to 18 months. In addition to the plants, the barge carried soil and several vehicles, including a truck loaded with additional plants. Thus, we were concerned about both frogs (either arriving directly from Florida or acquired at the nursery) and other organisms that might have arrived from either location. One species of particular concern was the Brown Anole, *Anolis sagrei*, which also has invaded Florida and is spreading in the Caribbean (e.g., Greene et al. 2002, Henderson and Powell 2005), often to the detriment of native species (Kolbe et al. 2004). So far, it has not been found in the BVI. The search was conducted by a team of eight, including the authors, and lasted approximately one hour. Each plant was individually searched, with both foliage and soil being sifted for invasive organisms. Those found were recorded and removed.

We removed from the plants inspected five juvenile anoles, later identified as the native Crested Anole (*Anolis cristatellus*),



A Cuban Treefrog (*Osteopilus septentrionalis*) on an ornamental plant at the Beef Island nursery.





A cistern on Beef Island, the source of water for a household, is teeming with dozens of Cuban Treefrogs (*Osteopilus septentrionalis*).



Guana Island, like the many other islands of various sizes that comprise the British Virgin Islands, is separated from other islands only by short distances across intervening channels. As part of the Greater Puerto Rico Bank, these islands were once connected when sea levels were much lower than today. At least those populations of plants and animals with limited abilities to disperse across water have evolved separately on individual islands, and efforts should be made to avoid diluting potentially unique gene pools by transferring even common species from island to island.

and sighted at least one other that was not recovered. In the soil of the planters, we collected one dwarf gecko, later identified as a native *Sphaerodactylus macrolepis*. In addition, we recovered one immature spider (an unidentified member of the genus *Selenops*; catalog number TTU-Z 31,098) and three individual snails, *Zachrysia provisoria* (MCZ 356974). This species originates in Cuba, but has been reported in Florida and elsewhere in the Caribbean, including the U.S. Virgin Islands (A. Baldinger, pers. comm.; Kraus 2005). The soil in two pots contained nests of the imported Red Fire Ant (*Solenopsis invicta*), and had to be fumigated. This species is not listed among those



The barge, with its gate down, is ready to discharge its load of plants and soil on Guana Island.



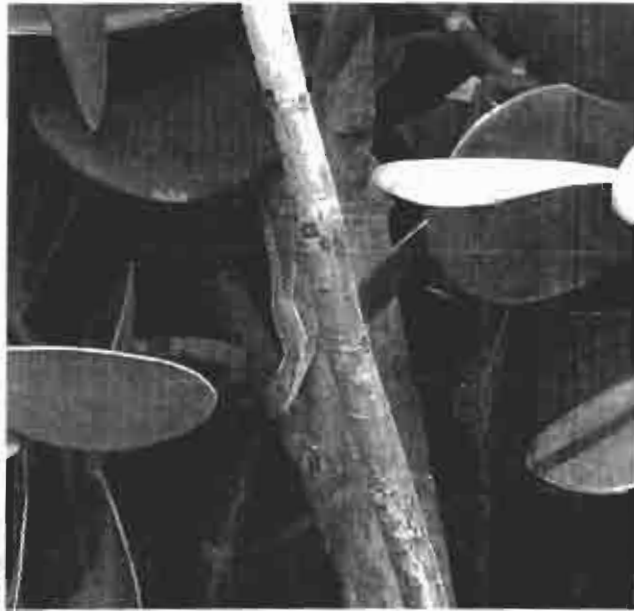
The Cuban Brown Anole (*Anolis sagrei*) is an aggressive invader originating in Cuba. These lizards thrive in habitats altered by human activity.



James Lazell and Howard Watson inspecting the leaf axils of *Pandanus utilis* for stowaways.

located by Miller (1994), who searched a similar barge at the same location, but looked for invertebrates.

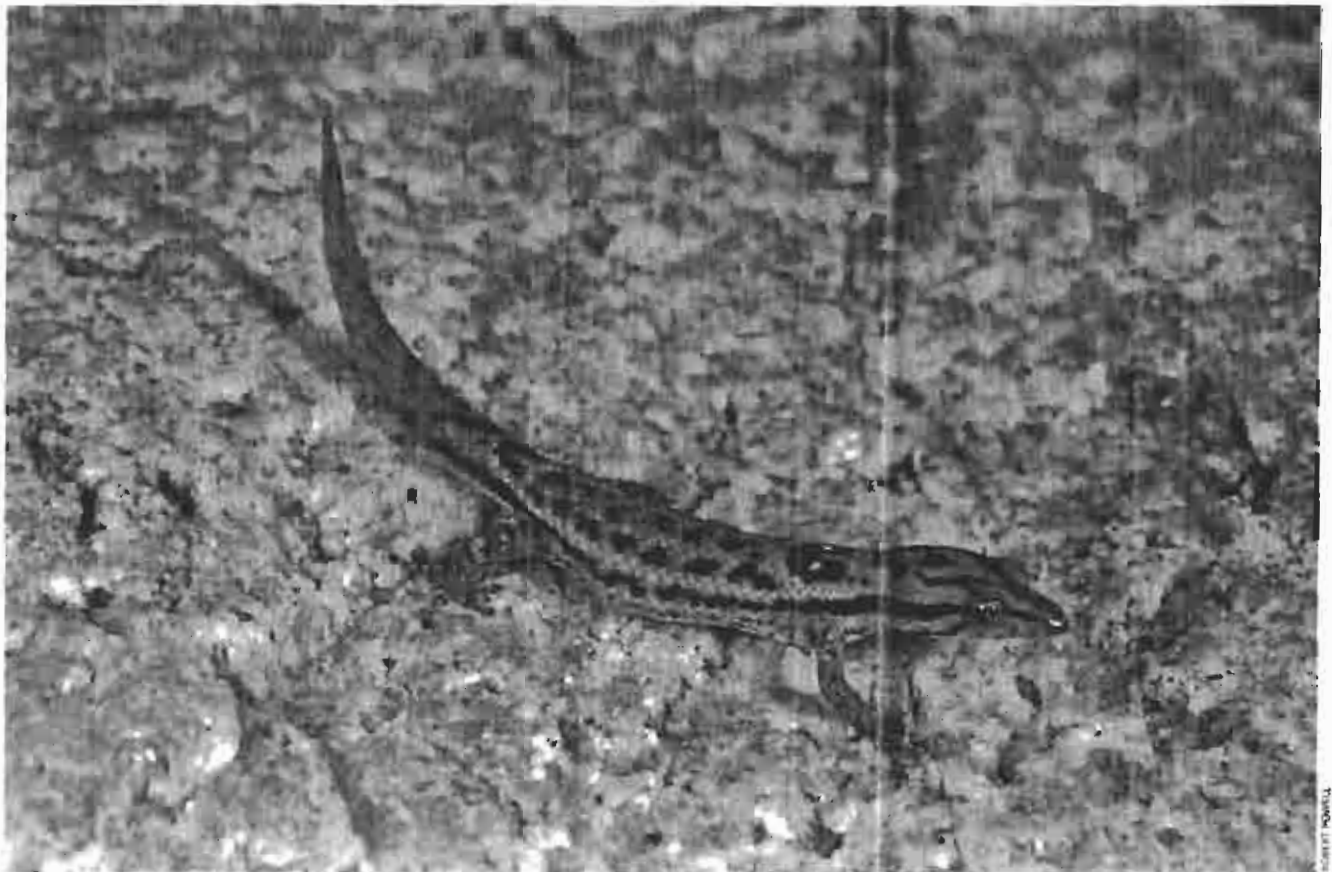
In addition to these cargo-related species, we recovered two juvenile ground lizards, later identified as the native *Ameiva*



A juvenile Crested Anole (*Anolis cristatellus*) on a nursery plant on top of a truck loaded on the barge. The animal escaped before it could be captured.

*exul*, which appeared to have been resident on the barge. When chased, the lizards behaved in a manner we have never previously encountered or seen described. Both lizards used a drain hole on the edge of the deck to launch themselves into the sea, about 3 meters below. Once in the water, they swam as a crocodile or marine iguana would, tucking their limbs against their bodies and undulating the body and tail. Swimming occurred both under and on the surface of the water and lasted about 1 minute in each case. The lizards then swam back to the boat and climbed up its side. This ability may help explain why ground lizards are found on so many islands. We are happy to report that the owners of Guana Island, in keeping with their long-term policy of protecting the island, have now decided to suspend such shipments.

None of the reptiles collected were invasives, nor were CTFs found during our search, but some of the invertebrates collected were, similar to the findings of Miller (1994). Both the number of organisms discovered on this barge and their diversity is alarming for two reasons. First, they indicate that a broad range of organisms can and is transported in such shipments, which are not uncommon. In the last few years, Guana Island, which is not a major consumer of nursery plants, typically received 2–3 shipments of similar magnitude a year, as well as several smaller ones (H. Watson, unpublished). The potential for arrival of invasive species, such as the Fire Ants (already established on Guana) or CTF (which has not been noted this year, despite repeated surveys of all freshwater locations on the island) is alarmingly high. Second, transporting native species between

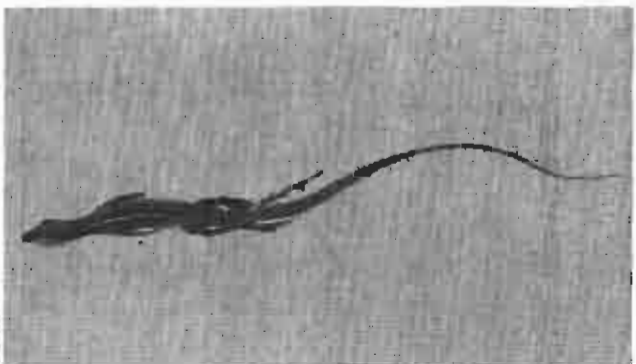


This dwarf gecko, *Sphaerodactylus macrolepis*, is common in the British Virgin Islands.





One of two juvenile ground lizards (*Ameiva exsul*) encountered on the barge.



*Ameiva exsul* swimming in the sea after leaping from the deck of the barge.

islands is not a benign activity. It swamps local genetic specializations that have evolved after the islands separated, leaving populations less well-adapted to local conditions. For example, water loss rates vary among islands in a manner related to their aridity (Dmi'el et al. 1997), and this variation has at least some genetic basis (Perry et al. 2000). Bringing in foreign genetic material could make an island population less capable of surviving a drought. In addition, such translocations make research into the biological history of the islands much more difficult. For example, an ongoing study of the genetic differences among Crested Anole populations and their relationship to other biogeographic patterns would be severely hampered if animals are regularly transported among islands.

BVI law offers little guidance about inspecting and sanitizing shipments arriving from other locations, such as Florida. We support the recommendation of Perry and Gerber (2006) that additional measures are needed to prevent the arrival of invasive species and eradicate those already in the BVI. In addition, however, we also recommend that internal biosecurity measures be established to reduce the risk of native species being accidentally moved among islands. Such measures are clearly needed to reduce the spread of invasives already found in the BVI, such as the Fire Ant or CTF, to new locations. We are not aware of such

regulations being in effect in any island-based nation. Attempts at quarantines of mainland invasives, such as the Fire Ant in the USA, often have failed because the volume of transport is high and inspection is lax, but the isolation provided by an island-based system offers some hope of success.

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Volume 37

## Variation in Soldier Crab Population Density and Size as a Function of Elevation and Vegetation Type

Carey Haralson, Jacob Goldfarb,  
AHM Ali Reza, Doni Schwalm,  
Bradley Strobel, Clint Boal, and Gad Perry

We used two sampling methods to describe the population density of soldier crabs across six cover types (dry forest, ghut forest, palm ghut forest, beach strand, rocky beach, human impacted) and three elevation bands on Guana Island, British



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Virgin Islands. Transect and quadrat surveys provided values for soldier crab numbers and relative sizes (based on claw length measurements). In the dry forest, both transects and quadrats showed a significant increase in crab densities as elevation increased. Both transect and quadrat methods yielded the highest number densities of crabs in the beach strand. The smallest crabs were located in the beach strand and rocky beach cover types, whereas the largest crabs were located in the dry forest and palm ghut forest. Overall, crabs found in transects were significantly larger than those found in quadrats. These values can be used to assess the future impacts of global climate change on the species.

# DRAGONFLIES

75

This is slightly modified summary I typed up for my notes. You will see that there are three new species for Island (two for BVI). One is an African species and only the 2nd record for New World - I was in Guadeloupe for first record but wasn't the one who caught it. Very exciting.

Mike Ivy has some neat stuff from Montserrat. His crew went well into the interior and were there in dry season - whine, whine.

Drop me a line if you need more information.

> Guana Island Dragonflies - 2006

>

>

> 13 species recorded from Guana in 2006 - the highest total ever.

> 5 appear every year (REG), 2 have appeared most years (IR), 3

> previously recorded only in 1997 ((&), 3 new this year (NEW).

> Everything more common than any year except 1997. Only *Brachymesia*

> *herbida*, of previously recorded species, not found. *Lestes*

> *forficula* and *Brachymesia furcata* the only BVI species not recorded

> on island.

>

> *Lestes forficula* (Rainpool Spreadwing) - 2m from Tortola, f from

> Beef Island. Never recorded from Guana - might appear in Garden of

> Eden. Regular at Community College and airport.

>

> REG *Ischnura ramburii* (Rambur's Forktail) - p Guana, p Tortola,

> 3p Beef. Recorded every year on Guana - more abundant this year

> with numerous pairs in wheel. All 2006 records from salt pond.

> Common and widespread on main islands.

> NEW *Hemianax ephippiger* (Vagrant Emperor) - f Guana. An African

> species and a notorious wanderer - only odonate recorded from

> Iceland. First record for the western Hemisphere in January during

> our trip to Guadeloupe (caught by the French biologist). The Guana

> island record is therefore 2nd record for New World. It was in

> mixed swarm of dragonflies over grassy field.

>

I'll quote a section from Philip Corbet's *Dragonflies - Behavior and ecology of odonata*.

pages 412 & 413 deal with *Hemianax ephippiger* and quote is from p. 412.

"Adults of *H. ephippiger* have been found at higher temperate latitudes than any other migrants that regularly travel outside the tropics: specimens, some still alive, have several times been reported from Iceland (Tuxen 1976)...Two of the arrivals in Iceland coincided with wind trajectories across the Atlantic from the eastern or western Mediterranean." Goes on to talk about flights of millions of individuals arriving in France and Italy behind mid-August and mid-October ahead of storm systems coming out of Africa.

They can breed in brackish water and develop in matter of a few months. Would be exciting if they are established somewhere in Lesser Antilles, but far more likely that these are just strays. They can't overwinter in Europe but do have summer hatches that then return to Africa in late Fall.

- >IR *Erythemis vesiculosa* (Great Pondhawk) - 2m Guana, m Tortola.
- >Recorded most years on Guana but never as common as this year. All
- >at salt pond.
- >
- >NEW *Erythrodiplax berenice* (Seaside Dragonlet) - 1m Guana. Always
- >common on Tortola and abundant on Anegada. The only odonate that
- >tolerates salt concentrations greater than sea water. Surprising
- >that it has not come to Guana before but possibly the old salt pond
- >was too salty. Females seen over flat and at south end of pond but
- >all males in the northern 2/3rds of pond. Presumably a reflection
- >of the salt concentration. Other species were concentrated in
- >southern (fresh?) 1/3rd although a few at north end.
- >
- >REG *Erythrodiplax umbrata* (Band-winged Dragonlet)- 2m, f Guana,
- >m Tortola. Always second commonest species on Guana. Common around
- >salt pond, but also at some of road puddles. Probably commoner than
- >in any other year. Egg laying in salt pond.
- >
- >REG *Orthemis ferruginea* (Roseate Skimmer - purple) - 8m Guana. It
- >is accepted that the purple and red *Orthemis* in the BVI are two
- >species, but still debate about what species. Correctly or not the
- >red species will be treated as the same red species found in the
- >Greater Antilles. The purple species on the Florida Keys would be
- >*ferruginea*. *Macrostigma* is being resurrected as a name for the
- >purple form on Guadeloupe so all Caribbean purple individuals will
- >be lumped in *macrostigma*. The red form may require a new name.
- >*Discolor* and *schmidtii* are Central and South American species that
- >have been poorly defined and these are being championed by some as
- >the equivalent of the red BVI form. A real rats nest.
- >
- >REG *Orthemis ferruginea* (Roseate Skimmer - red) - 2f, 9m, p Guana,
- >7m Tortola, 2m Beef. See above. Both forms recorded every year from
- >Guana and red form always more common. This year most of the
- >purples were over road puddles while reds were everywhere. The sea
- >level sites on Tortola and Beef have always produced 90+% red
- >individuals, the high stock pond 100% purple with pond at community
- >college being about 50-50.
- >
- >IR *Pantala flavescens* (Wandering Glider) - 1f Guana, 1m Tortola.
- >Common this year, but usually a few on Guana Island.
- >
- >97 *Pantala hymenaea* (Spot-winged Glider) - 1m Tortola. A number
- >seen on Guana with the more abundant *flavescens*. First record since

>the massive invasion of 1997.

>

>97 *Perithemis domitia* (Slough Amberwing) - 3m Guana. Several  
>others seen. At shaded pool near dock and shaded dump pit near  
>donkey pens. First record since 1997, but the two collected then  
>were close to death floating on water. This year the individuals  
>were holding territories at the respective pools.

>

>REG *Tamea abdominalis* (Vermilion Saddlebags) - 6m Guana, 1f  
>Tortola. Present most years on Guana and definitely common this  
>year. Most records at salt pond - others in mixed swarms over  
>grassy flat.

>

>97 *Tamea calverti* (Striped Saddlebags) - 3m Guana. Previously  
>record only in 1997 on Guana and one individual on Virgin Gorda in  
>2004. A migrant from Central America and Greater Antilles to  
>Venezuela and evidently easily blown off course. All three  
>collected same day at salt pond.

>

>NEW *Tamea onusta* (Red Saddlebags) - 6m, f Guana. Not previously  
>recorded from the Virgin Islands although Nick Donnelly (retired  
>professor who confirms identification on all may material - also  
>doing Mike Ivy's material from Montserrat so had a chance to see  
>that) remembers throwing his machete at one on St. Thomas (early  
>1960's) and cutting it in half, but he didn't save it. No reports  
>since. This is eastern most records - no records for Lesser Antilles.  
Surprising that it was as common as the regular *Tamea abdominalis*.

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## Evolutionary Dynamics of wAu-Like *Wolbachia* Variants in Neotropical *Drosophila* spp.

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*Wolbachia* bacteria are common intracellular symbionts of arthropods and have been extensively studied in *Drosophila*. Most research focuses on two Old World hosts, *Drosophila melanogaster* and *Drosophila simulans*, and does not take into account that some of the *Wolbachia* associations in these species may have evolved only after their fast global expansion and after the exposure to *Wolbachia* of previously isolated habitats. Here we looked at *Wolbachia* of Neotropical *Drosophila* species. Seventy-one lines of 16 Neotropical *Drosophila* species sampled in different regions and at different time points were analyzed. *Wolbachia* is absent in lines of *Drosophila willistoni* collected before the 1970s, but more recent samples are infected with a strain designated wWil. *Wolbachia* is absent in all other species of the willistoni group. Polymorphic wWil-related strains were detected in some saltans group species, with *D. septentrionalis* being coinfecting with at least four variants. Based on *wsp* and *ftsZ* sequence data, wWil of *D. willistoni* is identical to wAu, a strain isolated from *D. simulans*, but can be discriminated when using a polymorphic minisatellite marker. In contrast to wAu, which infects both germ line and somatic tissues of *D. simulans*, wWil is found exclusively in the primordial germ line cells of *D. willistoni* embryos. We report on a pool of closely related *Wolbachia* strains in Neotropical *Drosophila* species as a potential source for the wAu strain in *D. simulans*. Possible evolutionary scenarios reconstructing the infection history of wAu-like *Wolbachia* in Neotropical *Drosophila* species and the Old World species *D. simulans* are discussed.

*Wolbachia* strains are intracellular gram-negative, vertically transmitted *Alphaproteobacteria* that infect at least 20% of all insects (24, 47). In *Drosophila*, *Wolbachia* infections are capable of inducing cytoplasmic incompatibility (CI) or male killing (34). The CI phenotype increases the fitness of *Wolbachia*-infected females relative to uninfected females and drives *Wolbachia* through host populations. In recent years scientific interest has broadly focused on the evolutionary and functional interactions between *Wolbachia* and genetic model systems such as *D. melanogaster* and *D. simulans*, two well-studied Old World species belonging to the melanogaster group (42). In *D. melanogaster*, a single infection variant, wMel (50), had been described until not long ago (36). This infection is associated with variable levels of CI in its natural host. In *D. simulans*, five *Wolbachia* variants have been described: wRi, wHa, and wNo, which can induce CI, and wMa and wAu, which generally do not (29). Strains wMel of *D. melanogaster* and wAu of *D. simulans* are closely related in respect to the most sensitive molecular gene marker sets of *wsp* (50) and *ftsZ* (9, 35). There is a complete lack of *wsp* sequence polymorphism within wMel (36) and wAu (2, 23), which suggests either a strict clonality of the parasite or a recent acquisition by their host species. The phylogenetic relationship of these two *Wolbachia* strains has previously been analyzed (see, e.g., references 9, 21, and 50); however, the evolutionary origins of both the wAu and wMel associations remain unclear, including a possible recent acqui-

sition from other host species after the global expansion of both Old World *Drosophila* species.

In contrast to the well-studied *Wolbachia* associations in *D. melanogaster* and *D. simulans*, little is known about the occurrence of *Wolbachia* among American Neotropical *Drosophila* strains comprising two groups of species, the saltans group and the willistoni group (Fig. 1). There are presently two conflicting reports about the occurrence of *Wolbachia* in Neotropical *Drosophila*: Bourtzis et al. (4) screened a broad range of *Drosophila* species derived from various labs and from the *Drosophila* Species Stock Center (DSSC) in Bowling Green, Ohio (now held at the University of Arizona, Tucson). In their survey only two species out of the 41 stocks comprising 30 species were infected with *Wolbachia*. Interestingly, none of the analyzed DSSC fly lines was infected. The six Neotropical *Drosophila* species surveyed, including *D. willistoni*, *D. prosaltans*, and *D. sturtevantii*, were uninfected (4). The Neotropical samples surveyed originated from iso- or oligofemale lines kept at the DSSC since the 1950s. In contrast, Werren et al. (46) reported that a natural population of *D. willistoni* collected in the early 1990s in Panama was infected with *Wolbachia*. Its presence in *D. willistoni* was recently confirmed by discovering partial fragments of a *Wolbachia* genome in the Trace Archive of the *D. willistoni* genome sequencing project (37; J. Brownlie, personal communication). The genome sequence was derived from an isofemale line collected in the early 1990s in Guadeloupe (L. Ehrman, personal communication).

Here we reevaluated the *Wolbachia* infection status of Neotropical *Drosophila* species by conducting a large-scale survey. Seventy-one lines of 16 Neotropical *Drosophila* species belonging to the willistoni and saltans groups were searched for *Wolbachia*. We compared the occurrence of infection in old versus

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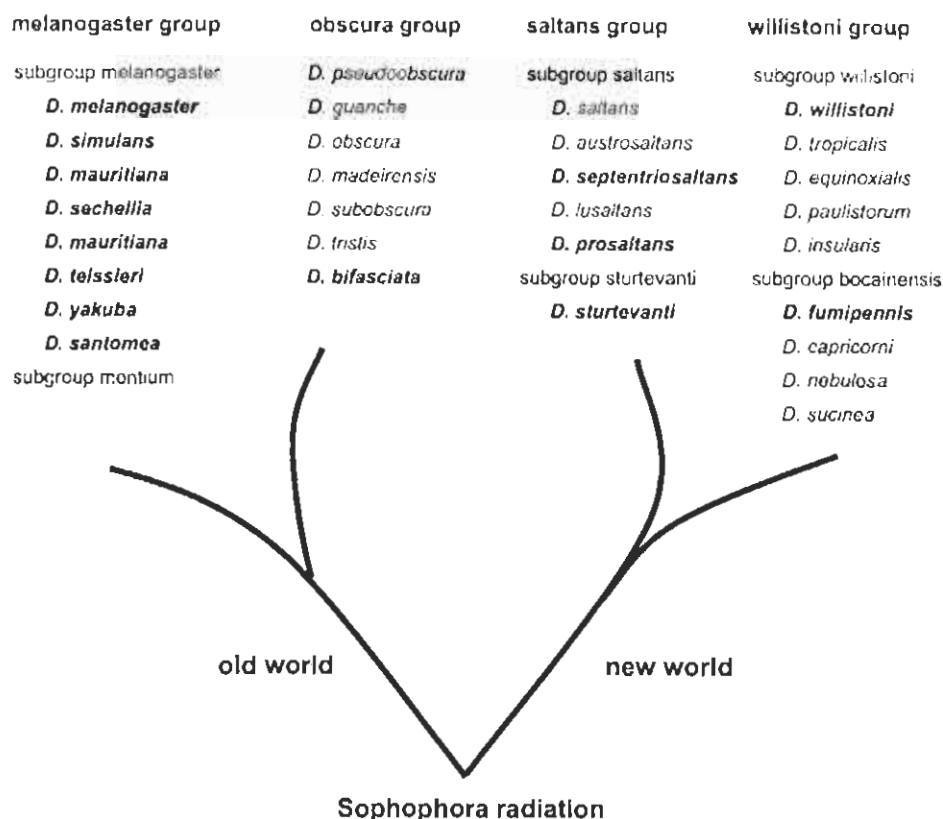


FIG. 1. Phylogenetic relationship of the *Sophophora* radiation (41). The *Wolbachia* infection statuses of the *Drosophila* species shown were deduced from data published previously (melanogaster and obscura groups) and from this study (saltans and willistoni groups). In boldface are species that have been found to be infected with various *Wolbachia* strains based on *wsp* sequences (see Materials and Methods).

recent population samples of different geographic origins and increased replicate numbers of analyzed lines per species, as analysis of only one or a few iso- or oligofemale lines would not detect low infection frequencies in species. Different diagnostic tools such as *Wolbachia*-specific *wsp* and *ftsZ* PCR, Southern blot hybridizations, and immunological diagnostic methods were applied for this purpose.

#### MATERIALS AND METHODS

***Drosophila* strains.** Fly samples were kindly provided by colleagues Margaret Kidwell, Egon Bartel, Kim van der Linde, Francisco Ayala, Jeff Powell, and Peter Chabona and by the DSSC, Tucson, Ariz. (For details about geographic origin, collector's name, and date of collection, see Tables 1 to 3.) All strains were kept on standard fly food in vials at a constant temperature of 21°C.

**PCR diagnostics, cloning, sequencing, and strain typing.** Genomic DNAs derived from single adult female flies were extracted according to the single-fly PCR protocol (14), and the quality of fly genomic DNAs was tested by control PCR experiments carried out with primers binding to conserved segments in exon 2 and exon 3 of the *Adh* gene (15). *Wolbachia*-specific PCRs were performed as previously described (24). In brief, 2 µl of the 50-µl single-fly sample was added to 20 µl of PCR mix (0.75 U *Taq* DNA polymerase [Promega] in 1× reaction buffer, 0.10 µM of each primer, and 75 µM of each deoxynucleoside triphosphate). PCR primer sets were used as described previously (24). The *Wolbachia* infection of *D. willistoni* was discriminated from *wAu* infection of *D. simulans* by the hypervariable VNTR-141 locus in *wMel* (primer set VNTR-141F-R), isolated by Riegler et al. (36). At least two independent PCRs were analyzed per sample. PCR fragments of the expected size were gel eluted, cloned into the pGEM-T Easy vector, and transformed into JM109 (Promega). Both strands of each clone were sequenced by GENEenterprise GmbH, Mainz, Germany.

*Wolbachia* strain names were assigned to *wsp* sequence variants deriving from different hosts according to current standards (34, 50). This is important in order to keep the ecological origin of the *Wolbachia* symbiosis transparent. The highly polymorphic *wsp* gene undergoes homologous recombination among strains, which is problematic for an evolutionary analysis of the symbiosis (1). Therefore, we used a multilocus approach, including *wsp* and *ftsZ* genes as well as the VNTR-141 locus.

**Phylogenetic analysis.** Multiple *wsp* sequence alignments, including the hyper-variable regions (bases 217 to 252 and 520 to 582), were generated using the Clustal X program (40). Alignments were based on amino acid translations followed by manual modifications. A base substitution was included in the analysis if it occurred in two or more plasmid clones obtained from independent PCRs. Other substitutions were eliminated. The final alignment is available at <http://ftp.ebi.ac.uk/pub/databases/emb/align/under> accession number ALIGN\_000917. Phylogenetic trees were constructed by applying PAUP\* (39) in the absence of an available outgroup. Neighbor-joining analyses after midpoint rooting and unweighted-pair group method with arithmetic mean analyses yielded similar phylogenies, supporting the close relationship of *wAu*-like *Wolbachia* variants.

**Single-fly Southern hybridization.** DNA extraction from individual 10-day-old female flies, restriction digestion with HindIII, vertical agarose gel separation, and membrane blotting were performed according to the protocol described by Jonakovic (26). Nylon membranes were probed with the eluted *wsp* PCR fragment of *wMel* derived from the *D. willistoni* strain Pan 02 (Table 1) cloned into the pGEM-T Easy vector.

**Semiquantitative genomic *wsp* PCRs.** The density of *Wolbachia* in *D. willistoni* was determined by semiquantitative *wsp* PCRs on 10 individual adult females of staged ages. After gel separation and SYBR Green I staining (Roche), the emission intensities of the obtained *wsp* fragments were determined and compared to *wsp* signal intensities derived from individual *D. simulans* (from a Cofts Harbor line) infected with *wAu* and *D. simulans* (from a Riverside line) infected with *wRi*.

TABLE 1. Distribution of *Wolbachia* in natural populations and stocks of *D. willistoni*

Region and fly line	Location, source <sup>a</sup>	Collection yr	PCR <sup>b</sup>	Southern blotting <sup>c</sup>
<b>American continental</b>				
Pan 02	Panama City, Panama; KL	2002	—	—
Lag	Laguna Negra, Rocha, Uruguay; LB	2000	—	—
Apa 5.1	Veraacruz, Mexico; JS	1998	+	—
Apa 8.2	Veraacruz, Mexico; JS	1998	—	—
Pan 98	Panama; EB	1998	+	+
JS 6.3	Jaton Sacha near Tena, Ecuador; PO	1997	+	+
JS 1	Jaton Sacha near Tena, Ecuador; PO	1997	+	+
Para 3	Belem, Pará, Brazil; MM	1997	+	+
Para 4	Belem, Pará, Brazil; MM	1997	+	—
RIP	Ribeirão Preto, São Paulo, Brazil; CR	1995	—	ND <sup>d</sup>
Pan 92	BCI, Panama; EB	1992	+	ND
Manaus	Manaus, Brazil; MM	1986	—	—
wilB6	Belize; FA	1974	+	+
wilC	Costa Rica; FA	1971	—	—
SP	Sao Pedro, Rio Grande do Sul, Brazil	1965	—	ND
WIP4	Ipitanga, Bahia, Brazil; HW and AC	1961	—	—
14030-0811.6	Fairchild Gardens, FL; WH	1959	—	—
14030-0811.1	San Salvador, El Salvador; WH	1955	—	—
14030-0811.0	San Maria d'Ostuma, Nicaragua; WH	1954	—	—
14030-0811.3	Atlixco, Veraacruz, Mexico; WH	1947	—	—
14030-0811.2	Royal Palm Park, FL; WH	1941	—	—
<b>Caribbean</b>				
wilG1-FW1	Basse Terre, Guadeloupe; PC	2000	+	+
L'Antilles 6	St. Vincent and Grenadines; HH	1997	+	+
L'Antilles 3	Grand Etang, Grenada; HH	1997	—	—
L'Antilles 4	St. Vincent and Grenadines; HH	1997	—	—
L'Antilles 1	Toro Negro, Puerto Rico; HH	1994	+	+
wilG2	Guana Island, Virgin Islands; PC	1991	+	+
wilG1	Basse Terre, Guadeloupe; PC	1991	+	+
wilH	Grande-Terre, Guadeloupe; PC	1991	—	—

<sup>a</sup> Collectors: CR, C. Rohde; EB, E. Bartel; FA, F. Ayala; HH, H. Hollocher; HW, H. Winger; KL, K. van der Linde; LB, L. Basso da Silva; MM, M. Martins; PC, P. Chaboras; PO, P. O'Grady; WH, W. Heed.

<sup>b</sup> Results obtained per line on individual flies from independent genomic PCRs with *ftsZ* and *wsp* primer sets ( $n = 6$  adult females per line).

<sup>c</sup> Results derived from genomic single-fly Southern blot hybridizations probed with the *wsp* fragment ( $n = 5$  adult females per line).

<sup>d</sup> ND, not determined.

**Immunological studies.** *Wolbachia* density and tissue tropism of *w-wil* in *D. willistoni* were determined using the polyclonal *Wolbachia* surface protein (WSP) antibody (11). WSP protein expression was analyzed via Western blotting of protein extracts derived from individual adult flies in independent replicates as well as whole-mount immunostainings on adult tissues and staged embryos (44). Rabbit anti-*wsp* antibody was used at a 1:500 dilution overnight at 4°C and detected after incubation with a 1:500 dilution of Alexa Fluor 488 goat anti-rabbit immunoglobulin G-labeled secondary antibody (Molecular Probes) at room temperature for 1 h. The total number of primordial germ line cells (PGCs) in stage 10 and later embryos of *D. willistoni* was determined using the pole cell-specific polyclonal rabbit anti-VASA antibody at a dilution of 1:1,000. Slides were stained for 3 min with 1 µg/ml DAPI (4',6'-diamidino-2-phenylindole) (Molecular Probes), rinsed, stained with 5 µg/ml propidium iodide (Molecular Probes) for 20 min, rinsed again, and mounted with ProLong antifade medium (Molecular Probes).

**Fluorescence microscopy.** Immunostainings of embryos and ovaries were examined by using a Zeiss Axiovert 2 Epifluorescence microscope. Images were processed using Photoshop 6.0 (Adobe).

**Nucleotide sequence accession numbers.** The *wsp* sequence data derived from Neotropical *Wolbachia* strains were deposited in GenBank under accession numbers AY620207 to AY620229 and DQ118779, as well as AY858801 for the respective sequence from *D. ananassae* collected in 2002 in São Tomé. Sequences of the diagnostic VNTR-141 loci of *D. samakani* (Coffs Harbor) and *D. willistoni* were deposited in GenBank under accession numbers DQ118777 and DQ118778, respectively.

## RESULTS

**Isolation of *Wolbachia* from *D. willistoni*.** Twenty-one continental American and eight Caribbean lines of *D. willistoni* were

screened for *Wolbachia* by using *wsp* PCR and single-fly Southern hybridization. Based on both molecular methods, 12 continental and 5 Caribbean lines tested positive. All five lines originating from the DSSC as well as most lines derived from collections before the 1980s were devoid of *Wolbachia* (Table 1). The five DSSC-derived fly lines collected in Central America and Florida in the 1940s and 1950s and the Brazilian and Costa Rican lines collected in the 1960s and 1970s lack *Wolbachia* (Table 1 and Fig. 2A). The oldest sample of *D. willistoni* infected with *Wolbachia* originates from a line of flies collected in Belize in 1974 (sample wilB6). The second-oldest infected line was collected in Panama in 1992. While the Brazilian line "Manaus" originating from a collection in 1986 is uninfected, all continental lines, ranging from Mexico to Uruguay, collected in the 1990s and later harbor *Wolbachia* infections (Table 1). Whereas older continental lines are devoid of *Wolbachia*, more recent samples are universally infected. Caribbean samples of *D. willistoni* show a more heterogeneous infection pattern. For example, recent collections from Grenada and St. Vincent (line L'Antilles 4) in 1997 are not infected. A line collected from Grand Terre, Guadeloupe (wilH), in 1991 is uninfected, whereas another one collected on the neighboring island Basse Terre (wilG1) in the same year is infected with *Wolbachia* (Table 1).



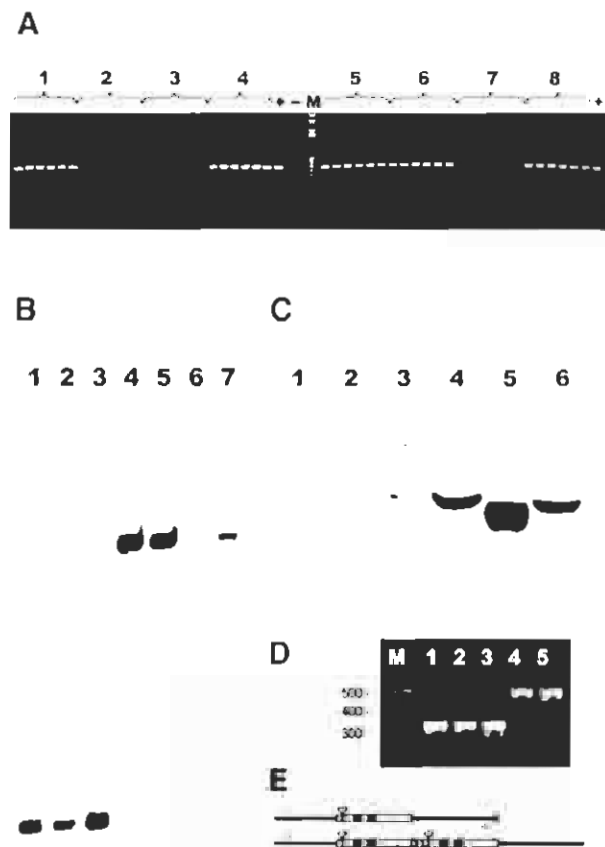


FIG. 2. Intra- and interspecific distributions of *Wolbachia* in Neotropical *Drosophila* species. (A) Single-fly *wsp* PCR on eight strains of *D. willistoni* collected at different American locations in different years (see Table 1). For each *D. willistoni* strain tested, PCRs were performed separately on six individual 2-day-old female flies. Lines are as follows: 1, Pan 02; 2, wilC; 3, wilH; 4, wilB6; 5, Apa 5.1; 6, Para 4; 7, WIP4; 8, wilG1. (B) Genomic single-fly Southern blot hybridization probed with the *wsp* plasmid of wWil on individual 10-day-old females of *D. melanogaster*/wMel CS (lane 1), *D. melanogaster*/wMel yw<sup>67</sup> (lane 2), *D. simulans*/wRi (lane 3), *D. simulans*/wAu (lanes 4 and 5), *D. willistoni*/wWil treated with tetracycline (lane 6), and *D. willistoni*/wWil strain Pan 02 (lane 7). (C) Western immunoblotting using the anti-WSP antibody (1:1,000) on single-fly protein extracts derived from *D. willistoni*-T, the tetracycline-treated control line of JS 6.3 (lane 1), *D. willistoni*/wWil (lane 2), *D. septentrionalis*/wSpt (lane 3), *D. simulans*/wAu (lane 4), *D. simulans*/wRi (lane 5), and *D. melanogaster*/wMel (lane 6). (D) VNTR-141 specific PCR on *D. willistoni*/wWil Pan 02 (lane 1), *D. willistoni*/wWil JS6.3 (lane 2), *D. willistoni*/wWil Para 4 (lane 3), *D. simulans*/wAu Coffs Harbor (lane 4), and *D. simulans*/wAu Yaounde 6 (lane 5). (E) Schematic comparison between the VNTR-141 loci (34) of wWil (top) and wAu (bottom). The basic unit is composed of a 15-bp repeat (stippled), a 23-bp hairpin (loop), an 18-bp insertion (hatched), and a 15-bp repeat (black). The size difference is caused by a 141-bp duplication in VNTR-141 of wAu.

Multiple *wsp* PCRs on individual flies from lines of *D. willistoni* confirmed the complete absence of *Wolbachia* in uninfected lines. Within infected fly lines, each individual tested was positive for *Wolbachia* (Fig. 2A and data not shown). These 100% infection frequencies suggest a close-to-complete vertical transmission efficiency of *Wolbachia* in *D. willistoni* hosts. This is corroborated by our observations that flies from naturally *Wolbachia*-infected populations of *D. willistoni* kept

in our lab maintained a stable 100% infection frequency in the 3 years since collection.

**Molecular characterization of the *D. willistoni*-specific *Wolbachia* strain wWil.** We sequenced fragments of two genes, *wsp* and *ftsZ*, from 12 *Wolbachia*-infected lines covering continental and Caribbean populations of *D. willistoni* in order to characterize the molecular structure and phylogenetic relationship of this *Wolbachia* association with other *Wolbachia* variants. Until recently these two diagnostic marker genes were regarded as the most informative for molecular *Wolbachia* variant classification (34). All isolated *Wolbachia* clones of *D. willistoni* were identical in their sequence. Below we refer to the strain as wWil. With respect to the *wsp* sequence of wWil obtained from the 12 infected lines (accession numbers AY620218 to AY620229, no sequence polymorphism could be detected. Moreover, all *wsp* and *ftsZ* sequences of *D. willistoni* were 100% identical to the respective *wsp* and *ftsZ* genes (accession numbers AF020067 and AY227739) of the *Wolbachia* variant wAu. As deduced from comparative Southern blots (Fig. 2B), the close relationship between wAu and wWil is corroborated by the conservation of the two HindIII restriction sites flanking the *wsp* locus.

In contrast to the identity of wWil and wAu at the *wsp* and *ftsZ* sequence level, comparative genomic single-fly Southern blots (Fig. 2B) and semiquantitative PCRs (data not shown) of infected individuals of *D. willistoni* and *D. simulans* showed clear quantitative differences. Strong signals comparable to those of wRi were obtained from wAu-infected *D. simulans* adults, and the intensity of wWil in similar-sized *D. willistoni* clearly showed a 70% reduction compared to that of wAu (Fig. 2B, lanes 4, 5, and 7). This quantitative effect was also detected at the WSP protein expression level by Western blots derived from single-fly protein extracts with the polyclonal anti-*wsp* antibody (Fig. 2C, lanes 2 and 4). The WSP proteins of wAu and wWil have the same molecular weight, whereas, for example, the homologues of two other *Wolbachia* variants that infect *D. melanogaster* and *D. simulans* (wMel and wRi, respectively) differ significantly (Fig. 2C, lanes 5 and 6).

In contrast to the *wsp* and *ftsZ* sequence identity between wWil and wAu, we were able to discriminate both strains at the genomic level by applying the recently isolated polymorphic marker VNTR-141 (36). This diagnostic marker covers the noncoding polymorphic VNTR-141 locus in wMel (positions 89003 to 90332 in the wMel chromosome). By performing VNTR-141-specific PCRs (Fig. 2D), we have obtained a 528-bp fragment from wAu (accession number DQ118777) and a 387-bp fragment from wWil (accession number DQ118778). The length difference is caused by a 141-bp duplication in wAu that is not present in wWil (Fig. 2E). Hence, wWil is closely related but not identical to wAu of *D. simulans*.

**Extreme pole cell tropism of wWil in *D. willistoni* embryos.** Whole-mount immunostainings were performed on early embryos and ovaries of both fly species, using the anti-WSP antibody. In early embryos of *D. simulans*, wAu bacteria were detected in somatic and germ line tissues during all stages of embryonic development (Fig. 3A). Nuclei of earlier blastodermal stages were infected with *Wolbachia*, with some significant enrichment in the posterior pole cell region in both *D. simulans* and *D. willistoni*. Such posterior accumulations of wAu in *D. simulans* blastodermal embryos were reported recently (44). In

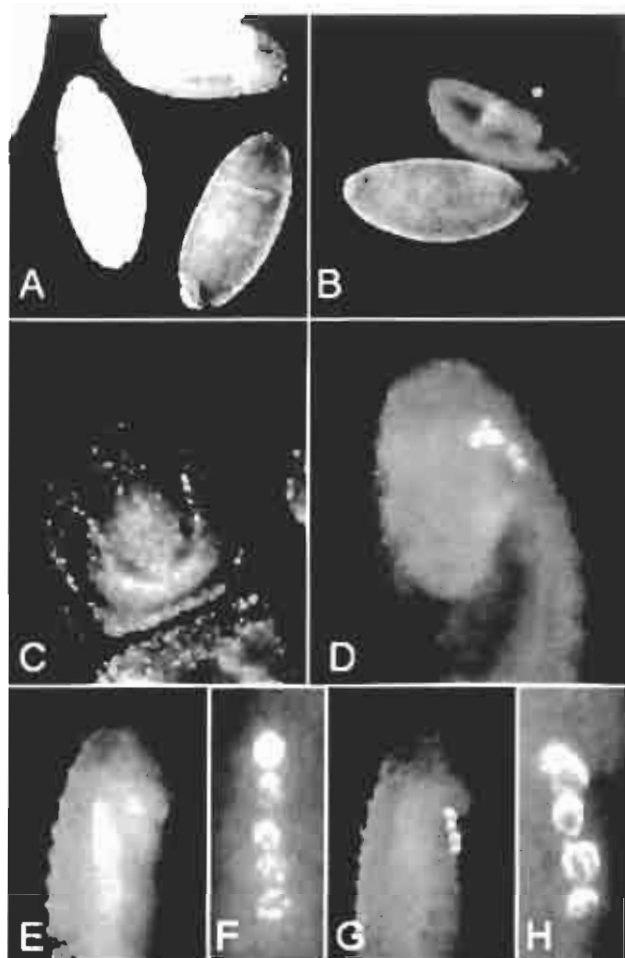


FIG. 3. Distribution of *Wolbachia* in *Drosophila* embryos. (A and B) Whole-mount immunostainings with rabbit anti-WSP antibody (green) on early-stage embryos of *D. simulans* infected with wAu (A) and *D. willistoni* JS 6.3 infected with wWil (B). (C and D) Stage 9 to 10 embryos of *D. simulans* (C) and *D. willistoni* (D) are shown in detail. Whereas wAu in *D. simulans* uniformly infects both somatic and germ line cells, wWil selectively targets a very limited number of primordial germ cells. In *D. willistoni*, wWil is not detectable at the immunological level in somatic cells at embryonic stage 9 or later. (E to H) Lateral views of a stage 12 embryo of *D. willistoni* infected with wWil (E and G) and their enhanced magnifications show a row of five heavily infected primordial germ cell nuclei on both lateral sides of the embryo (F and H).

contrast to this, during stages 9 and 10 of embryonic development of *D. willistoni*, wWil specifically targets the germ line (Fig. 3B). In early gastrulating embryos, shortly after pole cell invagination, somatic tissues of *D. simulans* were heavily infected by wAu (Fig. 3C). At this developmental stage, wWil bacteria in *D. willistoni* are selectively targeting a small number of primordial germ line cells, whereas somatic tissues are devoid of bacteria (Fig. 3D). Later, during stages 12 to 14, in the course of germ band retraction, only one lateral pair of five or six PGC nuclei was infected by wWil (Fig. 3E to H). Control immunostainings with the *Drosophila* germ line-specific VASA antibody (28) showed that, in contrast to *D. melanogaster*, the Neotropical species *D. willistoni* harbors a reduced number of

PGCs which perfectly colocalize with wWil (data not shown). Based on the tight temporal and spatial association between the host-encoded VASA protein and WSP-expressing wWil, we assume that this intracellular parasite possesses a molecular association with the host-expressed, pole cell-specific *vasa* RNA or with its encoded protein.

**Natural polymorphism of wAu-like *Wolbachia* in other Neotropical *Drosophila* species.** We have expanded our survey into species of the *willistoni* group in order to search for a potential origin of the wWil detected in recent collections of *D. willistoni*. Besides *D. willistoni*, 21 fly lines derived from eight species of this group, covering both the *willistoni* and *bocainensis* subgroups (Fig. 1), were screened for the presence of *Wolbachia* by using the *wsp* primer set. With the exception of *D. fumipennis*, a strain kept at the DSSC since 1958, all *willistoni* group species sampled were negative for *wsp* and *ftsZ* PCR (Table 2). On the basis of its *wsp* sequence, the infection in *D. fumipennis* (wFum; accession number AY620207) shows only a distant relationship to wWil (Fig. 4), similar to the A subgroup *Wolbachia* infection of *Pegoscopus longiceps* (accession number AF521161).

In contrast to the absence of wWil infections in the *willistoni* group, three out of the seven tested species belonging to the saltans group harbor *Wolbachia* (Table 3). The two saltans subgroup members *D. septentriosaltans* and *D. prosaltans* are infected with wWil-related *Wolbachia* strains, designated wSpt and wPro, respectively. The *wsp* sequence of the *Wolbachia* strain wPro SG1 (accession number AY620208) isolated from *D. prosaltans* shows 97.9% homology to wWil of *D. willistoni* and is almost identical (99.0%) to the wSpt PNM2 strain (AY622214) of *D. septentriosaltans*. Below we refer to these Neotropical strains wWil, wSpt, and wPro (Fig. 4) as wAu-like *Wolbachia* because of their close phylogenetic relationship with wAu of *D. simulans*.

Six wSpt *wsp* sequences were isolated from three different *D. septentriosaltans* lines collected in Panama between 1998 and 2002 (Table 3). At least four different wSPT subtypes can be distinguished according to their *wsp* sequences (Fig. 4): wSPT BC11 (accession number AY620209) is identical to wCer2 (accession number AF418557) of the cherry fruit fly *Rhagoletis cerasi* (33) and to wTei (accession number AY291347) and wYak (accession number AY291348) of *D. teissieri* and *D. yakuba*, respectively (8). The variant wSpt PLR1 (accession number AY620211) clusters with wSpt PLR2 (accession number AY620212), BC12 (accession number AY620210), and PNM1 (accession number AY620213). The latter three *wsp* clones are identical at the sequence level but stem from three different Panamanian *D. septentriosaltans* populations (Table 3). The fourth subtype, wSpt PNM2 (accession number AY620214), is the most divergent variant positioned between wMel (accession number AF020072) of *D. melanogaster* and the wAu-like *Wolbachia* clade (Table 4). All lines of *D. septentriosaltans* tested are multiply infected with *wsp* variants of wSpt. For example, individual flies from the PNM strain from Panama City harbor at least two different types of *wsp* sequences. Each *wsp* variant sequenced seems to be part of an intact open reading frame encoding a 196-amino-acid (aa) section of the WSP protein. The observed *wsp* sequence polymorphism of wSpt variants within *D. septentriosaltans* is manifested even at the protein level (Table 4). With respect to the

TABLE 2. Distribution of *Wolbachia* in the *willistoni* group

Species	Fly line, location, source*	Collection yr	PCR <sup>b</sup>
<i>willistoni</i> subgroup			
<i>D. tropicalis</i>	PNM; Panama City, Panama; KL	2002	—
	Panama; JS	1998	—
	BCL; Panama City, Panama; EB	1997	—
<i>D. insularis</i>	St. Kitts, St. Lucia; HH	ND <sup>c</sup>	—
<i>D. equinoxialis</i>	Apazapan, Veracruz, Mexico; JS	1998	—
	Gigante, Panama; EB	1997	—
	PLR; Gamboa, Panama; KL	2002	—
	ES; Colon, Panama; KL	2002	—
<i>D. paulistorum</i>	JS 5.2; Jatun Sacha, Tena, Ecuador; PO	1997	—
	Interior; LE	1970	—
	Central americas; LE	1959	—
	14030-0771.6; San Salvador, El Salvador	1955	—
	14030-0771.2; Mesitas, Mexico; LE	ND	—
	A28; LE	ND	—
<i>bocainensis</i> subgroup			
<i>D. cupricorni</i>	14030-0721.1; Canal Zone, Panama	1961	—
<i>D. sucinea</i>	Xalapa Botanical Gardens; Mexico; JS	1998	—
	14030-791.0; Medellin, Colombia	1958	—
<i>D. nebulosa</i>	Apazapan, Veracruz, Mexico; JS	1998	—
	14030-0761.0; Palmira, Columbia	ND	—
	14030-0761.1; San Jose, Costa Rica	ND	—
<i>D. fumipennis</i>	14030-0751.1; Arima Valley, Trinidad	1958	+

\* Collectors: EB, E. Bartel; HH, H. Hollocher; JS, J. Silva; KL, K. van der Linde; LE, L. Ehrman; PO, P. O'Grady.

<sup>b</sup> Results obtained per line on individual flies from independent genomic PCRs with *ftsZ* and *wsp* primer sets ( $n = 6$  adult females per line).

<sup>c</sup> ND, not determined.

WSP consensus sequence wBCL1 (accession number AY620209), two amino acid substitutions are found, i.e., in the sequence of wSpt PLR1, PLR2, BCL2, PNMI, and PNM2 at consensus position aa 24 (Tyr to His) and in the variant PLR1 (accession number AY620211) at position 126 (Asp to Gly).

At least two *wsp* variants of wPro were isolated from the *D. prosaltans* SG line from Panama. Both wPro variants share a host species diagnostic substitution at aa 23 (Thr to Ser), and wProSG1 has a substitution at aa 88 (Table 4).

**wStv *Wolbachia* in *D. sturtevantii*.** Our survey yielded another new *Wolbachia* variant, wStv, which was isolated from *D. sturtevantii*, a member of the *sturtevantii* subgroup (Fig. 1). The distribution pattern of the wStv infection within its host species is patchy; e.g., wStv is present in the isofemale line Pan 6 (accession number AY620216) but is absent from Pan 12 (Table 3). As deduced from *wsp* sequence data wStv belongs to A-group *Wolbachia* but is distantly related to the wAu-like variants (Fig. 4). Three closely related but distinctive variants of wStv were isolated as singly occurring infections from three Panamanian populations (accession numbers AY620215, AY620216, and AY620217) (Fig. 4). Interestingly, the *wsp* sequence of wStv MI (accession number AY620215) collected in Maria Eugenia, Panama, is identical to that of wWhi (accession number AF237886) isolated from the phlebotomine sand fly *Lutzomyia shannoni* in Colombia (31). Those authors proposed, based on an extensive data set showing that other non-American populations of *L. shannoni* are free of *Wolba-*

*chia*, that *L. shannoni* probably acquired wWhi recently from another host in America.

## DISCUSSION

**wWil infection of *D. willistoni*.** Our survey shows that Neotropical *Drosophila* species belonging to the *willistoni* and *saltans* groups are infected with various A-group *Wolbachia* strains. In *wsp* and *ftsZ* sequence analysis, wWil of *D. willistoni* is identical to wAu of *D. simulans*. However, wWil can be discriminated from wAu by the VNTR-141 polymorphism and the strict pole cell tropism in its natural host. Hence, wWil is closely related but not identical to wAu of *D. simulans*. Our biogeographic analysis suggests that the infection is absent in *D. willistoni* stocks collected before the 1970s. Two alternative hypotheses may explain this result, i.e., a stochastic loss in the stocks or a recent invasion in the field. All five DSSC-derived *D. willistoni* samples tested negative for wWil (Table 1) and were kept under artificial lab conditions since the 1940s and 1960s. The DSSC collection was moved first from Texas to Ohio and then to Arizona. We cannot exclude the possibility that the *Wolbachia* infection was present in all lines but was then stochastically lost in independent lines in the course of their long-term stock maintenance due to stress factors, starvation, dramatic reduction of population size, or application of antibiotics. This hypothesis cannot completely be dismissed; however, we have three arguments against it: (i) wWil infec-

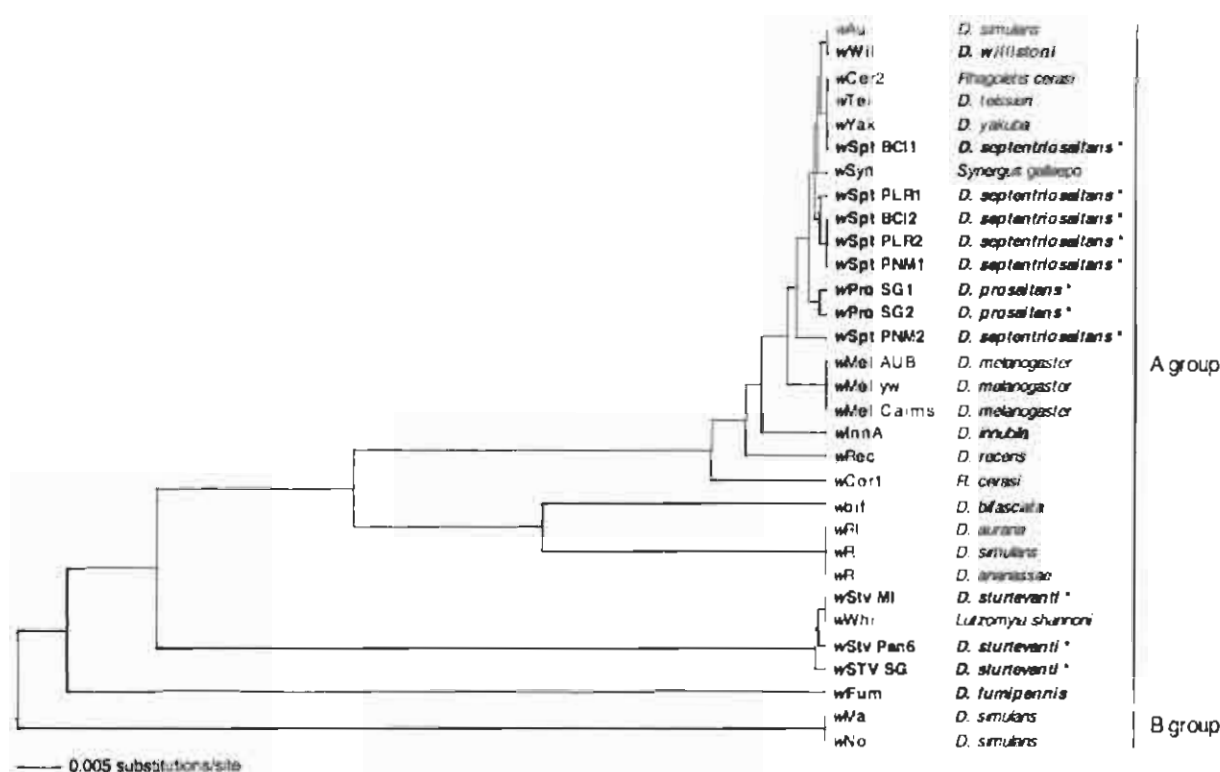


FIG. 4. Unweighted-pair group method with arithmetic mean cladogram based on *wsp* sequence alignment, including the hypervariable region of the *Wolbachia* strains (50) derived from Neotropical *Drosophila* species (boldface) and from earlier reported host species (lightface). Host species found to harbor polymorphic *Wolbachia* variants are indicated by asterisks.

tions in *D. willistoni* lines were completely stable under our lab conditions for more than 3 years, (ii) the DSSC contains infected *Drosophila* lines originating from equally old collections (e.g., *D. fumipennis*), and (iii) overall ratios of infected versus uninfected *D. melanogaster* fly lines in several other stock centers stayed constant over the last 80 years (36). Hence, we are in favor of the hypothesis of recent spreading, for which we can add three supporting observations: (i) the lack of sequence variation of all available wWil markers obtained from our samples suggests clonality of the infection and recent acquisition by horizontal transfer from an external source; (ii) individual adult flies of two alcohol samples of *D. willistoni* (DSSC stock numbers 14030-0811.4 and 14030-0811.5; kindly provided by S. J. Castrezana, *Drosophila* Species Stock Center, Tucson, Ariz.) collected in Mexico in the 1950s were uninfected, whereas control PCRs with *Adh*-specific primer sets were successful; and (iii) the two old strains wILC and wILB6, collected by F. Ayala in Central America in the 1970s, are uninfected and infected, respectively (Table 1). Additional analysis of *D. willistoni* populations collected between the 1970s and 1990s would doubtlessly improve our data set, although these strains would be difficult or impossible to obtain.

The complete absence of wAu-like *Wolbachia* in the related willistoni group species tested (Table 2) supports the idea that *D. willistoni* was infected after its speciation. Since all recently collected continental samples of *D. willistoni* are infected with wWil, we assume that this *Wolbachia* strain reached fixation in continental populations of *D. willistoni*. wWil's pole cell tropism and its 100% transmission rate, seen in lab lines, were

probably crucial factors. A recent *Wolbachia*-driven process should also be detected in the biogeographic distribution of mitochondrial variation, but this has not yet been looked at in the context of *Wolbachia* infections. A departure from an expected ratio of mitochondrial versus nuclear DNA polymorphism has been reported when comparing different populations of *D. willistoni*, and a selective mitochondrial sweep has been suggested as one plausible reason (38; J. Silva and M. Kidwell, personal communication).

**wAu-like *Wolbachia* originated in saltans group species.** We found polymorphic but closely related *wsp* sequences of wPro and wSpt in the host species *D. prosaltans* and *D. septentriosaltans*, respectively. This implies that these *Wolbachia* variants are an outcome of old associations with Neotropical *Drosophila* species. Independent multiple horizontal transfers with closely related *Wolbachia* strains are less likely. The progenitor of wPro and wSpt presumably infected the common ancestor of both host species before speciation and subsequently diverged at the *wsp* sequence level in the course of long-term vertical transmission. Host-specific diagnostic sites within *wsp* correspond with our hypothesis (Table 4). Therefore, we suggest that wAu-like variants evolved in the American Neotropical saltans group species and are potential donors for the horizontal transmission to *D. willistoni*. A similar event has been suggested for *Wolbachia* associations among the Old World sibling species *D. simulans* and *D. sechellia*, where original *Wolbachia* infections in an original species have not yet yielded a sequence divergence in *wsp* in the sibling species (6).

TABLE 3. Distribution of *Wolbachia* in the saltans group

Species	Fly line, location/source*	Collection yr	PCR*
<i>saltans</i> subgroup			
<i>D. saltans</i>	PNM; Panama City, Panama; KL	2002	—
	PLR; Gamboa, Panama; KL	2002	—
	FS; Colon, Panama; KL	2002	—
	BCI; Panama; KL	1998	—
<i>D. austrosaltans</i>	14030-0771.0; Pirassununga, Brazil	1959	—
<i>D. lusaltans</i>	14045-0891.0; Petionville, Haiti	ND*	—
<i>D. septentriosaltans</i>	PLR; Gamboa, Panama; KL	2002	+
	PNM; Panama City, Panama; KL	2002	+
	FS; Colon, Panama; KL	2002	+
	BCI; Panama; EB	1998	+
<i>D. subsaltans</i>	14044-0872.0; Balem, Brazil	1959	—
<i>D. prosaltans</i>	SG; Summit Gardens, Panama; EB	1998	+
	14045-0901.3; Balboa, Panama	1958	—
<i>sturtevantii</i> subgroup			
<i>D. sturtevantii</i>	PNM; Panama City, Panama; KL	2002	—
	PLR; Gamboa, Panama; KL	2002	—
	Barb 1; Turner's Hall, Barbados; H11	1999	+
	Barb 2; Turner's Hall, Barbados; H11	1999	—
	Pan 6; Panama; TM	1999	+
	Pan 12; Panama; TM	1999	—
	MI; Maria Eugenia, Panama; EB	1998	+
	SG; Summit Gardens, Panama; EB	1998	+

\* Collectors: EB, E. Bartel; HH, H. Hollocher; KL, K. van der Linde; TM, T. Markow.

<sup>b</sup> Results obtained per line on individual flies from independent genomic PCRs with *ftsZ* and *nsp* primer sets ( $n = 6$  adult females per line).

\* ND, not determined.

**Recent horizontal transfer into *D. similans*: origin of the wAu infection.** Non-CI-inducing wAu of *D. similans* (17) is found worldwide, including in Australia, Madagascar, Cameroon, parts of Europe and Japan, Ecuador, Jamaica, and the southern United States (2, 3, 7, 23). The overlapping geographic distribution of populations of *D. similans*, *D. willistoni*, and other Neotropical *Drosophila* species in Central America, together with *wsp* and *fstZ* sequence identity of the two *Wolbachia* variants wAu and wWil, strongly suggests a recent hor-

izontal transfer of *Wolbachia* from an original native Neotropical *Drosophila*-*Wolbachia* guild to the immigrating Old World species *D. simulans*. To date *D. willistoni* can be regarded as the most likely donor species of this transfer. Recent transfers of transposable elements between *D. willistoni* and another immigrating Old World *Drosophila* species, *D. melanogaster*, have been shown for the canonical *P* transposon (10, 18), and for the retrotransposon  *copia* (13, 25). Furthermore, the male-killing bacterium *Spiroplasma poulsonii* of the *D. wil-*

TABLE 4. Variable nucleotide and amino acid sites in the *wsp* sequence of the closely related wAu-like *Wolbachia* strains of *Drosophila*

Strain	Nucleotide at variable position in <i>wsp</i> DNA consensus <sup>a</sup>														Strain	Amino acid at variable position in <i>wsp</i> amino acid sequence									
	68	70	258	263	333	340	363	377	426	520	529	536	538	23		24	88	114	126	174	177	179	180		
Consensus	C	T	T	G	T	G	A	A	T	G	A	T	A	Consensus	T	Y	G	A	D	D	R	V	T		
wAu						A								wAu				T							
wWil						A								wWil				T							
wPro SG1	G	C		A										wPro SG1	S	H	E								
wPro SG2	G	C												wPro SG2	S	H									
wSpt PLR1		C							G					wSpt PLR1		H			G						
wSpt PLR2		C												wSpt PLR2		H									
wSpt BC12		C												wSpt BC12		H									
wSpt PNM1		C												wSpt PNM1		H									
wSpt PNM2		C	C		C			G						wSpt PNM2		H									
wSpt BC11														wSpt BC11											
wTei														wTei											
wYak														wYak											
wCer2														wCer2											
wMel										A	G	C	G	wMel					N	G	A	A			

\* Position 1 of the consensus sequence corresponds to position 164 in the wsp sequence of wAu of *D. undecimnotata* (accession number AF020067).

*willistoni* group species *D. nebulosa* has recently infected immigrating *D. melanogaster* populations in Brazil (30). Extensive phylogenetic studies of hosts and their parasites suggest horizontal transmission of *Wolbachia* variants between distantly related insect species (5, 16, 43, 46). Furthermore, it has been experimentally demonstrated that *Wolbachia* can be shuffled horizontally within and between *Trichogramma* parasitoid species (19, 20).

In agreement with the hypothesis of an American origin of wAu and opposed to an African origin (7) is the extensive analysis of mitochondrial variation in *D. simulans*. wAu is globally associated with the mitochondrial-sII haplotype of *D. simulans* (23). However, some African populations of *D. simulans* harboring the sII haplotype are uninfected. Ballard proposed recently that uninfected flies migrated to Ecuador and acquired wAu in a horizontal transmission event from an unknown host source (2). Subsequently, wAu spread throughout natural populations of *D. simulans* worldwide. The infection model outlined by Ballard, based on mitochondrial haplotypes and geographic distribution of wAu-infected *D. simulans*, is in line with our hypothesis that a Neotropical species such as *D. willistoni* could be the donor species of wAu.

In summary, we suggest a potential evolutionary scenario: wAu-like variants evolved in the guild of the Neotropical saltans group, being vertically transmitted and/or horizontally shuffled between related host species over a long period of time. More recently, a proto-wAu-like strain, the ancestor of wWil, infected horizontally a locally isolated population of *D. willistoni*, most likely in Central America. In this population, wWil evolved perfect maternal transmission through an extreme tissue tropism towards the germ line of *D. willistoni*. Within the last 300 years, immigrating *D. simulans* flies from Africa may have become infected by wWil or by another wAu-like strain from infected Neotropical *Drosophila* species through vectors such as parasitoid wasps (20, 43). wAu-infected *D. simulans* has then spread worldwide (2). An alternative source for wAu is an acquisition from outside the closely related *Wolbachia* pool of Neotropical *Drosophila* species, but if so, the fact that Neotropical *Drosophila* species are infected with closely related *Wolbachia* strains will need to be explained. It is unclear how wWil and wAu drove themselves through host populations. Presently, neither wAu in *D. simulans* nor wWil in *D. willistoni* is able to induce measurable levels of CI (17; W. J. Miller, unpublished data). The possibility that they did so in the past cannot be excluded. As reported by Ballard and coworkers, wAu seems to induce weak levels of CI in some infected populations of *D. simulans* from Florida (3, 23, 29). Alternatively, the driving force for the spreading of wAu-like strains could be a positive fitness contribution to their hosts that remains to be elucidated. The phenotypes of the Neotropical *Wolbachia* strains still need to be elucidated. The wAu-like strains are nested within the *Mel* cluster (50) of closely related *Wolbachia* strains that have a variety of phenotypic effects in other host species. Based on the *wsp* sequence, the variant wSpt BCII of *D. septentrionalis* is identical to the infection of the African species *D. yakuba* and *D. teissieri* (27) and to wCer2 of *R. cerasi* (33). Whereas wCer2 causes strong CI in *R. cerasi* and in transinfected *Ceratitis capitata* (49) and intermediate CI in transinfected *D. simulans* (35), wTei and wYak do not induce CI but are able to fully rescue the wRi

*mod* function in their original host (48). *Wolbachia* infections of *D. melanogaster* (32, 50) and the quinaria group member *D. recens* induce CI (45). The strain wInnA causes male killing in the related *D. imubila* (22), where it is regarded as an ancestral infection (12).

The present paper shows the complexity of evolutionary dynamics of *Wolbachia* in Neotropical *Drosophila* species and its success in colonizing the Old World species *D. simulans*. Both wWil and wAu successfully colonized natural populations of *D. willistoni* in America and of *D. simulans* globally. The detailed understanding of the evolutionary "jump-and-go" dynamics of *Wolbachia* will have important implications for practical applications of this symbiont as a vector system and in biological pest control management.

#### ACKNOWLEDGMENTS

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November 16, 1996.....

## Undesirable Sex Partners

*Bacteria manipulate reproduction of insects and other species*

By JOHN TRAITS

It took a while before the medical community paid attention. The first known cases of what the tabloids gleefully called virgin births appeared, amusingly enough, in Las Vegas. Then physicians across the United States began documenting similar events. In each case, an unfertilized egg in a woman had spontaneously begun to develop, ultimately producing a healthy female baby.

One young researcher, who had analyzed the timing and locales of the virgin births, suggested a spreading infection might be causing the incidents. The Federal Centers for Disease Control and Prevention in Atlanta quickly dismissed the idea, calling it "ridiculous."

Several months later came a well-publicized report in the *Journal of the American Medical Association* concluding that the number of infertile couples was rising rapidly worldwide. The international uproar intensified when physicians began to observe another reproductive curiosity: Some newborns that were genetically male appeared to be female. One week, the *New England Journal of Medicine* and the *National Enquirer* ran articles with the headline, "Is this the end of mankind, or just men?"

Science fiction? Definitely. For many insect species and other arthropods, however, the truth can be as strange as fiction when bacteria known as *Wolbachia* are around.

These microorganisms populate cells in the testes and ovaries of arthropods, often profoundly altering the reproduction of their hosts. In some species, infected males can generate offspring only if they mate with infected females. In others, infected females give birth without the need for the opposite sex. In one arthropod species, *Wolbachia* even transform embryos that would normally be males into females.

"These traits have all evolved because they increase the transmission of the microorganisms," says John H. Werren of the University of Rochester (N.Y.), who has documented the diversity of animals infected by *Wolbachia*.

There's no evidence that *Wolbachia* infects mammals, let alone humans, but that hasn't dulled biologists' fascination with them. "It's a very special group of bacteria," says



Werren.

Scientists first identified the bacteria in the reproductive tissues of a mosquito species in 1924.

Yet it took a mystery and several decades before *Wolbachia* truly entered the limelight. The mystery emerged in the 1950s, when insect geneticists encountered problems while trying to cross different strains of mosquitoes.

"They started to find all these crossing abnormalities," says Scott L. O'Neill of Yale University Medical School. The most obvious one, dubbed cytoplasmic incompatibility, centered on the failure of certain strains to produce offspring when mating with other strains of the same mosquito species.

Scientists argued for 20 years over what caused cytoplasmic incompatibility, says O'Neill. Then, in 1971, Janice Yen and Ralph Barr, biologists at the University of California, Los Angeles, tabbed *Wolbachia* as the culprit.

Cytoplasmic incompatibility, the researchers found, occurs when males infected with *Wolbachia* mate with uninfected females. In such unions, no offspring, or just a few in some host species, result. This reproductive barrier can be eliminated with antibiotics that rid the mosquitoes of the bacteria.

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**W**hy does *Wolbachia* generate cytoplasmic incompatibility? To favor reproduction by infected females, says O'Neill. That helps the bacteria, which dwell in the cytoplasm of egg cells, pass on to future generations.

In species affected by cytoplasmic incompatibility, infected females have no trouble reproducing with infected males. Infected females also breed easily with uninfected males. Both kinds of unions transfer *Wolbachia* to offspring. Consequently, cytoplasmic incompatibility can spread *Wolbachia* rapidly through an uninfected population, says O'Neill, who organized a session on *Wolbachia* at the recent Symbiosis 96! Meeting in Bar Harbor, Maine.

Researchers are finding that *Wolbachia* infects a surprisingly large variety of species. Werren and Donald Windsor of the Smithsonian Tropical Research Institute in Panama reported last year that 16 percent of Panamanian insect species, including some in all of the major insect orders, harbor *Wolbachia*. Since the estimated number of insect species ranges from 10 million to 30 million, that means roughly 2 million to 5 million insect species play host to the bacteria.

"That's very much an underestimate," adds Werren, noting that researchers have time to test only a limited number of insects from each species.

Scientists have also found that *Wolbachia* infects a variety of isopods (a subgroup of crustaceans that includes wood lice), at least one species of mites, and perhaps even a worm species.

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Wasps are among the favored hosts of *Wolbachia*. Take the jewel wasp, Werren's favorite research subject. *Wolbachia* infections in these insects produce an odd variation on cytoplasmic incompatibility: Uninfected female wasps mating with infected males can produce offspring, but their progeny are all male.

An explanation rests in the fact that wasps, like bees and ants, have an unusual mechanism for determining sex. In wasps, eggs fertilized by sperm contain a maternal and a paternal set of chromosomes and develop into females. Unfertilized eggs, with only a maternal chromosome set, develop into males.

When an infected male jewel wasp mates with an uninfected female, the paternal chromosomes from the sperm seem to fragment and fail to join the maternal set, says Werren. Consequently, only males result from such a mating. This indirectly aids the spread of *Wolbachia* by reducing the number of uninfected daughters produced by uninfected females, explains Werren.

*Wolbachia* sometimes takes a more feminist approach. In many parasitic wasps, which lay their eggs in developing insects that they have killed, *Wolbachia* infections eliminate the need for males. An infected female reproduces via an asexual process known as parthenogenesis. The unfertilized eggs simply duplicate their one set of chromosomes and develop into females.

These parthenogenetic wasps had long been a biological curiosity until a few years ago, when Richard Stouthamer, working with Werren, showed that the phenomenon stemmed from *Wolbachia* infection. With antibiotics, "you can cure a line of its parthenogenesis and make it sexual," says Stouthamer, now at the Wageningen Agricultural University in the Netherlands.

The clear preference for females isn't limited to *Wolbachia* strains that infect wasps. At the Bar Harbor meeting, Thierry Rigaud of the University of Poitiers in France, reported finding the bacteria in the wood louse *Armadillidium vulgare*.

In these lice, *Wolbachia* frequently overrides genetic inheritance. The bacteria, says Rigaud, "feminize" an embryonic wood louse that is genetically male by disrupting the

production or effects of masculinizing hormones during its development. The increased number of daughters allows *Wolbachia* to spread quickly.

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While scientists continue to tally the animals that *Wolbachia* infects, as well as the outcomes of those infections, they are also trying to unravel the mechanisms by which *Wolbachia* distorts its host's reproduction. Are cytoplasmic incompatibility, parthenogenesis, and feminization distinct strategies pursued by *Wolbachia*, or are they merely reflections of different ways in which host species react to the bacteria?

As the best-known phenomenon, cytoplasmic incompatibility has received the most attention. An initial hypothesis to explain it was that proteins made by *Wolbachia* in the testes bind to the chromosomes inside sperm and disrupt their later union with maternal chromosomes.

That simple idea has a major flaw, however. Researchers haven't found any

*Wolbachia* molecule incorporated into sperm. Nor are whole bacteria present in the sperm. As sperm mature, they lose most of their cytoplasm and squeeze out any *Wolbachia*.

The latest theory about cytoplasmic incompatibility comes from Timothy L. Karr of the University of Chicago. He argues that *Wolbachia*'s influence during the maturation of sperm somehow disturbs the carefully choreographed maneuvering that later brings together the sperm's chromosomes with the egg's. "It looks like the paternal and maternal chromosomes are out of sync," says Karr.

While comparing infected and uninfected eggs of a fruit fly species, Karr's group discovered that *Wolbachia* binds to specific egg proteins. One of those proteins normally resides in the nucleus of the fruit fly's egg cell. In an infected egg cell, however, the protein concentrates in sites throughout the cytoplasm.

The same phenomenon appears to occur in the testes, but it is complicated by the eventual eviction of *Wolbachia* from mature sperm, says Karr. "During spermatogenesis, *Wolbachia* binds proteins that should be in that sperm when it finally matures." But because *Wolbachia* gets kicked out of sperm, it takes that protein away, he says.

Though not necessary for early stages of fertilization, this sperm protein is crucial to coordinating the union of the two chromosome sets, proposes Karr. That would explain why infected males, whose sperm are stripped of the protein by *Wolbachia*, have trouble generating progeny in uninfected females, says Karr.

As for the pairings of infected females and infected males, which do produce offspring, Karr contends that the proteins stripped from sperm by *Wolbachia* are the same ones concentrated by the bacteria in the cytoplasm of the egg cell. The *Wolbachia* in the egg cells, along with the host proteins they are bound to, thus "rescues" the defective sperm from infected males, he says.

O'Neill has discovered a *Wolbachia* protein that may be relevant to Karr's theory. This protein varies in size among *Wolbachia* strains, and O'Neill suggests it may explain why some strains induce cytoplasmic incompatibility and others do not. Furthermore, the protein, seemingly from *Wolbachia*'s outer surface, binds to the same host cellular proteins identified by Karr. "It looks like our stories are coming together," says O'Neill.

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There is a practical side to all this *Wolbachia* research. Both Werren and Stouthamer, for example, are leading efforts to transfer parthenogenesis-inducing *Wolbachia* into commercially useful parasitic wasps.

"A lot of parasitic wasps are reared for the control of pest insects," notes Werren. "It would be highly desirable for these mass-rearing programs to use [parthenogenesis-inducing *Wolbachia*] because they could generate all females, and it's the females that go out and kill the insects."

O'Neill's ambitions center on eliminating insect-borne diseases, such as malaria and Lyme disease, by making use of *Wolbachia*'s ability to disperse through a population.

In one scenario, researchers would genetically engineer *Wolbachia* to make antibodies or other compounds that kill the malaria-causing parasite carried by mosquitoes in the wild. They would then infect mosquitoes with these *Wolbachia* and seed the countryside with the insects. Since the bacteria induce cytoplasmic incompatibility, the natural mosquito population would quickly give way to a population almost entirely infected with *Wolbachia* and presumably free of the malaria parasite.

The main obstacle to this idea, notes O'Neill, is that *Wolbachia* normally lives in the mosquito's reproductive tissues, whereas the malaria parasite inhabits its gut and salivary glands. Consequently, any compounds made by *Wolbachia* might not reach and kill the parasites.

O'Neill is searching for the *Wolbachia* gene that brings about cytoplasmic incompatibility. He intends to link that gene to a gene engineered to produce an antiparasitic compound in the tissues where the malaria parasite dwells. Once added to the mosquito's complement of genes, the two genes would be inherited by future mosquito generations and, with the aid of cytoplasmic incompatibility, would spread

quickly within a mosquito population, says O'Neill.

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The most provocative question surrounding *Wolbachia* may be whether the bacteria have played a role in the development of new species.

A central concept in many theories of speciation is reproductive isolation. This idea holds that if two populations of a species cannot breed together, then the genes of each population will evolve independently and diverge (SN: 11/2/96, p. 284). "Reproductive isolation is a key component of speciation because without it, genomes would mix and you can't get divergence," says Werren.

Eventually, he explains, the genes of two populations would diverge so much that they become genetically incompatible for reproduction. At that point, most evolutionary biologists would argue, the single original species has given way to two species.

*Wolbachia* may serve as an excellent mechanism to engender reproductive isolation, argues Werren. He and other researchers have found that such isolation can arise in an insect species infected by different *Wolbachia* strains. Members of the species infected by one strain cannot reproduce with members infected by the other strain.

While theories about evolution are notoriously difficult to prove, Werren suggests that mapping the diversity of insect species infected and not infected by *Wolbachia* may bolster his theory. Species infected with *Wolbachia* should have many more closely related species than uninfected species do.

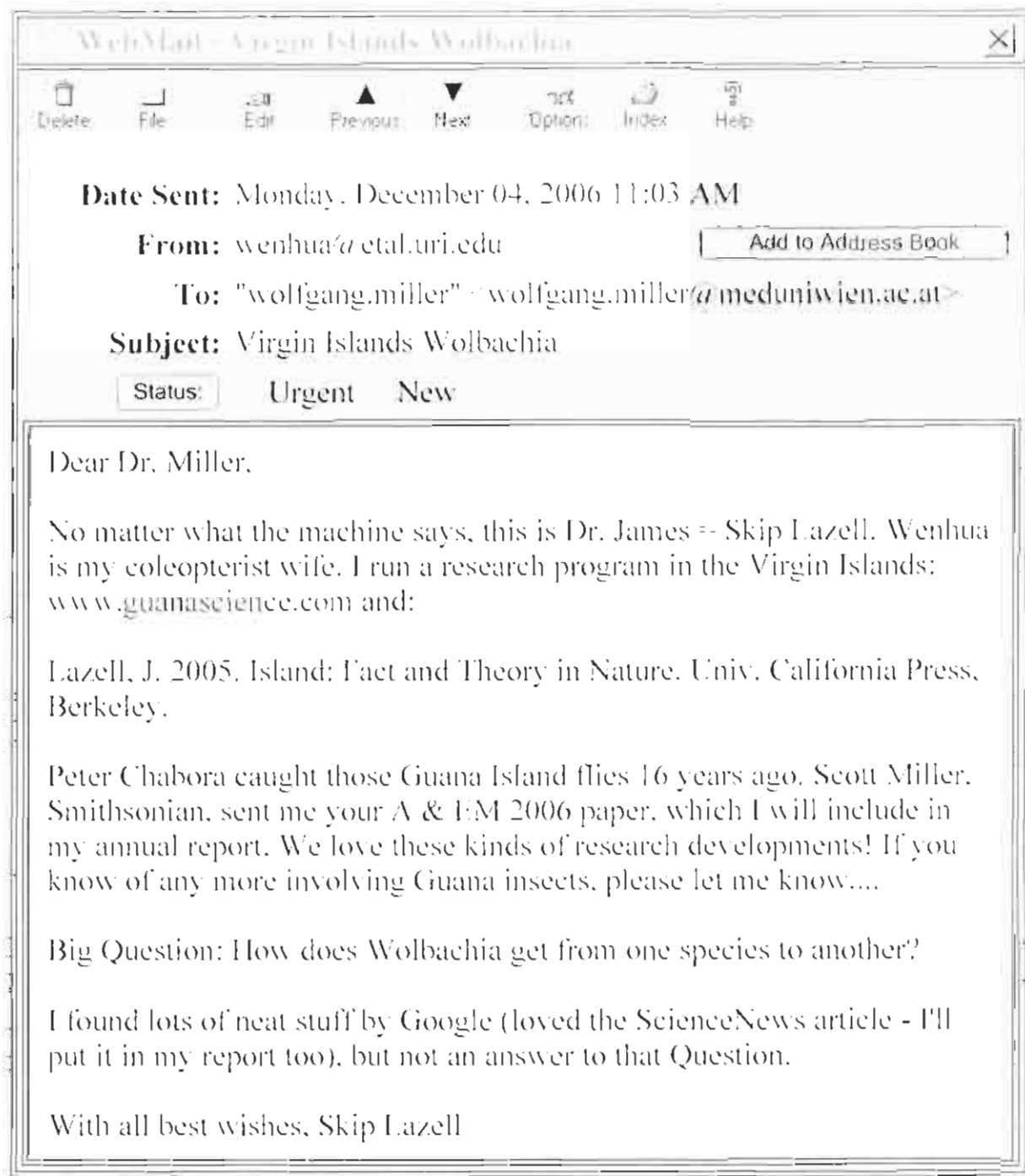
Could some *Wolbachia* species infect vertebrates, or even humans, and play a role in their speciation? Decades ago, researchers tried unsuccessfully to infect mice with strains of the bacteria. Moreover, O'Neill notes that all the *Wolbachia* found so far are temperature-sensitive and could not survive inside warm-blooded animals.

Still, Werren says it's too early to dismiss the possibility completely. "We don't have any idea whether these bacteria occur in vertebrates, either cold- or warm-blooded. We haven't really looked," he says.

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#### ● Sources

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WebMail - RE: Virgin Islands Wolbachia

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Dear Skip,

Thanks a lot for your interest, however, I am not aware of any other literature related to my field dealing with Guana insects.

The "big question" is perhaps the most important one but still an enigma: Primary suspects are parasitoid wasps that might be able to transmit Wolbachia from one organism to the other. In addition mites have been postulated for transferring transposable elements between Neotropical *Drosophila* species into *D. melanogaster* or transfer modes like introgression.

Best greetings from Vienna,

Wolfgang  
– Wolfgang J. Miller, Ph.D  
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Date Sent: Saturday, January 20, 2007 1:37 PM  
From: Peter.Chabora@qc.cuny.edu  
To: wenhua  
Subject: D. malerkotliana plus

Hi Skip,

I sent specimens only to U. Leiden, Ecology labs for identification because one of the researchers there found them in Panama to be rather abundant and not recorded earlier (that was the work of Svenster in his dissertation work). Most of the work that I know of on *D. malerkotliana* was done by various researchers at the CNRS labs at Gif-sur-Yvette on populations from Africa and various islands such as Maruitius and Seychelles. Offhand, I do not have those references available, but will check them out in my lab. Most had to do with *malerkotliana*'s ability to develop on a rather toxic fruit, locally called the "dog cucumber," or more formally, *Morinda citrifolia*. We found this to also be true in Guadeloupe. However, *D. malerkotliana* develops on a wide variety of resources - with a quick life cycle and skipping larvae.

The *D. willistoni* (and its sister variants such as *tropicalis*) along with many other insects seem to be hosts to *Wolbachia*, including many of the wasp parasitoids. Since *Wolbachia* inhabits the reproductive systems, it would be interesting to follow the virus from the parasitoid into hosts which may become infected during the egg laying attack, but survive the parasite by encapsulation or just the death of the egg or parasitoid larvae. Then the host becomes infected and subsequently passes the virus to its offspring. If they had the ability to survive parasitism because of resistance to the parasitoid, the trait would be selected for and be prevalent in the population.

As I mentioned, the *D. willistoni* population from Guadeloupe that I understand was selected for the genome analysis was infected with *Wolbachia*.

On another point, another aspect of interesting co-evolutionary adaptations that I was working on was antimicrobial peptides with Bruno Lemaitre at CNRS, Gif (PNAS, USA, 9 Aug. 2005, 11414-9). What I did was collect individual flies, as sterile as possible, along with samples of the substrate they were feeding upon, and drop them into vials of culture medium and send them to France ASAP. Neat stuff.

We'll keep in touch,

Peter

Peter C. Chabora  
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Flushing, NY 11367



## Clusiid Flies

Marshall, S.A. 2006. **Insects**

# Their Natural History and Diversity

With a photographic guide to insects of eastern North America

Firefly Books, Buffalo, NY

The Clusiidae (page 98) are small and rarely noticed, but these pretty little flies are easy to find once you know where to look, and their interesting behavior makes them worth looking for. Males of many clusiid species stake out a territory on large, pale fallen trees, and use their territories as mating arenas, or leks. Mated females usually lay their eggs in fallen trees as well, but usually in softer, wetter places than the lek sites chosen by, and defended by, males.

You should be able to find male clusiids patrolling and defending their little lengths of log on any spring day. The genus *Clusiodes*, for example, has about a dozen described North American species that regularly face off head-to-head as they hold their preferred bits of log surface. Some Australian species of *Clusiodes* do the same thing, but make the interactions more interesting by developing disproportionately wide heads with vibrissae (bristles that stick off the lower front corners of the head) that spiral like bedsprings. When two males meet, they flip their unusually long antennae out to the sides of their expanded heads and face each other. If one of the males cannot make the same width as his opponent, he abruptly leaves. If the flies are similar in width, they lock spiral vibrissae and fight until the vanquished male is forced to leave. In the meantime, small males without enlarged heads or spiral vibrissae lurk around the edges of the lek, presumably awaiting the chance to mate while the macho broad-heads are fighting it out.

# Redefinition of the Clusiinae and Clusiodinae, description of the new subfamily Sobarocephalinae, revision of the genus *Chaetoclusia* and a description of *Procerosoma* gen. n. (Diptera: Clusiidae)

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**Key words:** Clusiidae, Clusiinae, Clusiinae, Sobarocephalinae, *Chaetoclusia*, *Tetania fusca*, *Chaetoclusia*, *Procerosoma*, *Sobarocephalus*, *Sobarocephala*, revision

**Abstract.** The higher classification of the Clusiidae is reviewed, and the family is divided into the subfamilies Clusiinae, Clusiinae and the new subfamily Sobarocephalinae, all of which are redefined. The newly defined Clusiinae includes the genera *Phyllosclusia* Hendel, 1917, *Tetania* Coquillett, 1904, *Amecia* Zetter, 1987, *Clusia* Haliday, 1838, *Parachusia* Cerny, 1903, *Allochusia* Hendel, 1917 and *Apsichusia* Cerny, 1903. The Sobarocephalinae includes the genera *Sobarocephalus* Cerny, 1903, *Procerosoma* gen. n., and *Chaetoclusia* Coquillett, 1904. The new genus *Procerosoma* is described for two species, *Procerosoma alba* (Shatalkin) comb. n. from Brazil and *P. primum* sp. n. from Mexico. *Sobarocephalus* Cerny, 1902 is treated as a junior synonym of *Sobarocephala* and *Chaetoclusia* is redefined to include the monotypic genera *Chaetoclusia* Cerny, 1902, syn. n. and *Chaetoclusia* Cerny, 1902, syn. n. Six new species of *Chaetoclusia* are described (*C. ventricornis* sp. n., *C. amplipennis* sp. n., *C. tricolor* sp. n., *C. rubicollis* sp. n., *C. fusca* sp. n. and *C. hirsuta* sp. n.). *Chaetoclusia bakeri* permansu Henning, 1938 is included as a junior synonym of *C. bakeri* Coquillett, 1904. The phylogeny of *Chaetoclusia* is discussed and a key is provided for its 13 species.

## INTRODUCTION

Frey (1960) divided the Clusiidae into two subfamilies, Clusiinae (with one or more inclinate fronto-orbital bristles) and Clusiodinae (all fronto-orbitals reclinate). Later authors narrowed the Clusiinae to include only those genera with the anterior fronto-orbital bristle inclinate (Sasakawa, 1977; Pukin & Evenhuis, 1989; Figs 7, 11–13). Male and female genital characters, however, as well as previously unrecognized external morphological characters, suggest alternative subfamilial divisions of the Clusiidae. We here redefine the existing subfamilies, erect the new subfamily Sobarocephalinae, and revise *Chaetoclusia* Coquillett, 1904 and *Procerosoma* gen. n., which are the sobarocephaline genera other than *Sobarocephala* Cerny, 1903.

Most previous treatments of the New World tropical Clusiidae have focused on the immense sobarocephaline genus *Sobarocephala*, a frequently encountered genus of well over 200 Neotropical species. *Chaetoclusia*, in contrast, has received very little attention, because of the apparent rarity of species other than *C. bakeri* Coquillett. Six of the thirteen species of *Chaetoclusia* are described as new below, but the group's biology or immature stages remain unknown. *Procerosoma* is the least frequently encountered New World genus of Clusiidae, with its two species known from only three specimens collected in Mexico and Brazil.

## MATERIALS AND METHODS

Approximately 180 specimens were examined from the following institutions: University of Guelph Insect Collection (UGIC), British Museum of Natural History, London (BMNH),

Canadian National Collection, Ottawa (CNC), Carnegie Museum of Natural History, Pittsburgh (CMNH), Entomological Museum of Utah State, Logan (EMUS), Biological Collection, University of Bayreuth (BRUH), Instituto Nacional de Biodiversidad, Santo Domingo de Heredia (INB), Staatliches Museum für Tierkunde, Dresden (SMTD), United States National Museum, Washington, DC (USNM), Zoological Museum, University of Moscow (ZMUM).

Most specimens were pinned, either air-dried or prepared in a critical-point drier. BRUH specimens were stored in alcohol. Male and female genitalia were prepared by macerating abdomens in hot potassium hydroxide (10% solutions for 12–13 min, followed by washing in glacial acetic acid and deionized water). Terminology follows that in Calomen & Marshall (1998), with the exception of the pregonite and postgonite, which are illustrated in Figs 4 and 5. The  $M_1$  ratio is defined as the length of the ultimate section of vein  $M_1$  divided by the length of the penultimate section.

Representatives of all clusid genera were examined (excluding the fossil genus *Elctra fusca* Henning, 1965), as were representatives of all recognized sobarocephaline species (defined below) and a range of exemplars from possible out-group families, including all families in the superfamily Opiomyzoidea (in sense of McAlpine, 1989) except Xerostomatidae (cf. Papp, 1998). A phylogenetic hypothesis for the species of *Chaetoclusia* (Fig. 58) was generated through analysis of a morphological character matrix (Table 2) using PAUP version 4.0b10 [Phylogenetic Analysis Using Parsimony (Swofford, 2003)], using a simple heuristic search with all characters unweighted. To polarize characters, the lineage made up of *Sobarocephala* and *Procerosoma* was treated as the sister group to *Chaetoclusia*; this allowed for the construction of a hypothetical ancestor characterized by plesiomorphic states. Trees were drawn with the aid of Winclada (Nixon, 2002).

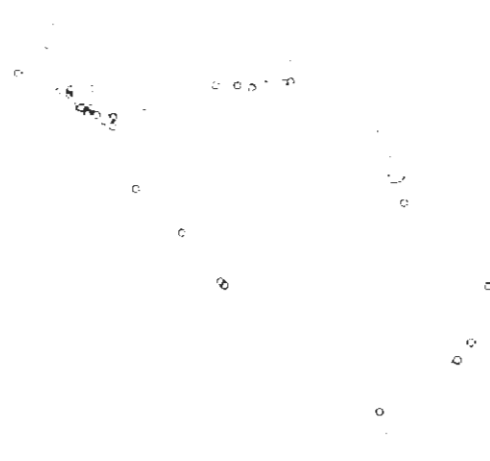
1st. Biol. L. Silva, 40, 150 ft, 10°26'N, 82°03'W, 311 1992 (1.7° INHC). Pinar del Rio, 25. Mami/24. Almondo, 80 m, Quépos, 100 1990, 40, 300 ft (1.3° INHC). Los Sirios, 90–100 m, P.S. Concentrado, 21 m, 25 ft, 1993. Z. Puentes, 11 ft (1.4° INHC). 1st. Catana Res. Biol. Canara, 200 m, 6 1990. Malaise (2.7° INHC). 6 km S San Vito, 10–21 ft 1967, 8–42 N, 83°00' W, 11 ft. Venus (1.5° FMUS). Rincón de la Osa, 26 ft, 1966. D. Venus (1.5° FMUS). Trinidad. Buchlingue, in 1938. M.R. Wheeler (2.5° USNM). Mexico. Chiapas. Tl. Tighondo (49 km S. Jaltenango), 13–18 ft, 1983. 1000–2000 m, W. N. Matius (1.5° USNM). Panama. Zona del Canal. Barro Colorado Island, 10 ft, 1980. R. Silberglied. A. Aethia. Barro Colorado (1.5° USNM). Barro Colorado Island, C. Z. Xu 1967. Infratrap W.W. Wirth (1.5° USNM). Colombia. C. Z. Papilme Rtl. in 1967. Malaise trap, W.W. Wirth (1.5° USNM). P. 4 m, 40 ft, 1980. H. J. Hurlan (1.5° USNM). Barro Colorado Nat. Monnt., 20 ft, 13 m, 1983. H. A. Grimaldi (1.5° USNM). C. Z. Ft. Kofke, 18 ft, 1958. W. J. Hanson (1.5° FMUS). Peru. Madre de Dios. Mami, Rio Mami, 240 m, Pakiza, 12–17 S, 70–88°W, 9–23 ft 1988. A. Freidberg (1.6° 3.5° USNM). Madre de Dios. Mami, Rio Mami, Cacha Salvado, 240 m, 14 ft, 1988. A. Freidberg (2.5° USNM). Madre de Dios. Mami, Rio Mami, Pakiza (5 km), 11. Aguiña, 40 ft, 1988. A. Freidberg (1.5° USNM). Madre de Dios. Mami, Pakiza (in Salvadour), 580 m, 5–6 ft, 1988. A. Freidberg (1.5° USNM). Depto. Loreto, 1.5 km N. Temmie Lopez, 18 ft, 1993. 240–405 m, FH, R. Leshon (1.5° USNM). Monzon Valley, Tingo Maria, J. J. Schlinger & J. S. Ross, 21 ft, 1954 (1.5° CASO). 26 ft, 1954 (1.5° CASO). Venezuela. San Vicente, Zula, 1970. J. Maldonado (1.5° USNM). Aragua. Rancho Grande Biol. Sta., H. Pitter N. Pl., 1250 m, 8 ft, 1993, and S. A. Marshall (1.5° DEBW). Provincia Pakon, Sierra de San Luis, 25 km 88°–400 m, forest in front of Cueva (Cave) Acenitz. Leaf litter on ground, along calcareous rocks and topsoil, swept, aspirated, 11°10–42°N, 69°17–76°W, 2650', 23 ft, 1998. M. C. Schimhan (1.5° FBH).

**Comments.** *Chaetoclusia bakeri* is characterized by a densely plumose arista and a reduction of the postvertical and ocellar bristles (Fig. 12). *Chaetoclusia bakeri* is also the most widespread and common species of *Chaetoclusia* on the continental mainland, with records not extending further from the coast than Trinidad or Barro Colorado Island.

The slight difference in thoracic coloration between the type specimen of the nominate subspecies (Cognilett, 1964) and the type specimen of the subspecies *Chaetoclusia bakeri peruviana* Hennig, 1938 was exaggerated by the more extensive pigmentation on the anepisternum of Hennig's specimen, which revealed the unpigmented median longitudinal stripe on the sclerite. This median portion of the anepisternum is always unpigmented, but was not apparent on the specimen examined by Cognilett since the background of this sclerite was also yellow. The extensive new material available to us has shown that the thoracic pigmentation of *C. bakeri* is quite varied, with different phenotypes often occurring in the same localities. There is a similar amount of variation in wing pigmentation, which ranges from light to dark. *Chaetoclusia bakeri peruviana* is therefore included here as a junior synonym of *C. bakeri*.

#### → *Chaetoclusia flava* sp. n.

(Figs 26, 50, 54, Map 1)



Map 3. Distribution of *Chaetoclusia bakeri* (Cognilett, 1964).

#### Description (Fig. 26)

**Male.** Body length 3.0–3.5 mm. Bristles yellow. One small bristle in front of anterior dorsocentral. Arista sparsely plumose. Ocellar bristle minute. Anterior lateral scutellar bristle minute or absent. Thorax yellow except as follows: antepisternum with dark ventral spot; one posterior pair of wide stripes on scutum (sometimes atrophied); proepisternum, katopisternum and meron white. Head predominantly yellow with ocellar tubercle light brown; first flagellomere light yellow to white, and face, gena and parafacial white. Legs yellow with coxae, basal half of femora and tarsi white. Abdomen yellow except as follows: tergite 2 brown posteriorly; tergite 3 to annulus brown with central yellow spot sometimes present on tergite 3; one pair of spots present laterally on epandrium.

**Female.** Externally as described for male except tergite 7 light brown and tergite 8 and terminalia yellow.

**Male terminalia** (Figs 50–54). Epandrium as wide as high, length 0.67 times height. Surstylus 0.67 times height of epandrium and elongate triangular (broadly rounded apically), distal third of inner-posterior margin with stout, pointed tubercles; inner-basal face with short pointed spur (ere) triangular with apical emargination, setose with one pair of longer central bristles. Hypandrium with ventral lobe and arm subequal in length; arm narrow medially and ventral lobe of hypandrium with one minute distal and two short medial bristles. Pregonite large and ovate with numerous central setulae. Postgonite and epiphallus well developed. Basiphallus saddle-shaped with slight dorsal arch. Base of distiphallus produced ventrally with ventral section 0.67 times length of dorsal section (from intersection with basiphallus); ventral section strongly curved, one pair of lateral spinulose swellings present medially, distally bifid and truncate, lateral lobe absent.

**Female terminalia.** As described for *C. bakeri* except spermathecal duct 2.5 times length of spermatheca.

**Etymology.** The specific name refers to the yellow coloration of this species relative to a similar species, *C. torva*.

**Distribution.** British Virgin Islands, Grand Cayman, U.S. Virgin Islands (Map 1).

**Holotype.** British Virgin Islands, St. John, Reef Bay, Hall (200) (Grimbold and Stark, insect stream bed) (3.ii.1992) (USNM).

**Paratypes.** British Virgin Islands, St. John, Reef Bay, Hall (0.10.1992) (Grimbold and Stark) (2♂, USNM); Grand Cayman, Georgetown (5–6.iii.1968) (R. McLachlan) (2♂, USNM); U.S. Virgin Islands, St. John, Reef Bay, Hall (200) (Grimbold and Stark, insect stream bed) (4.ii.1992) (♂, 4♀, USNM); 1♂ (J. H. Hall) (St. Thomas, Crown Mt., 1500) (3.ii.1992) (Grimbold and Stark, seeping forest floor) (1♂, USNM).

**Comments.** See comments for *Chaetoclusia livata* below.

***Chaetoclusia xanthops* (Wilkinson, 1896)**

(Figs 24, 48, 49, Map 1)

*Chaetoclusia xanthops* Wilkinson, 1896: 386 (in part), t. zern. (1903) 100.

*Chaetoclusia livata xanthops* Melander & Argo, 1924: 9–10.

**Redescription** (Fig. 24)

**Male.** Body length 3–4 mm. Bristles light brown. Two dorsocentral bristles. Arista sparsely plumose. Ocellar bristle minute. Anterior lateral scutellar bristle minute. Scutum brown with central stripe and anterior margin of postpronotum yellow. Scutellum yellow with lateral corners brown. Metatergites yellow with one pair of brown stripes lateral to scutellum. Pleuron and legs white. Head yellow with ocellar tubercle light brown and with first flagellomere; face, gena and parafacial white. Abdomen brown with epandrium orange and surstyli and cerci white. Wing dusky on distal half in first and second radial cells.

**Female.** Externally as described for male except abdomen entirely brown excluding yellow terminalia. Holotype with tergite 1 yellow.

**Male terminalia** (Figs 48, 49). As described for *Chaetoclusia livata* except as follows. Distiphallus deeply emarginate with tips rounded (not square), basiphallus more linear than saddle-shaped.

**Female terminalia.** As described for *C. livata* except spermatheca lightly pigmented.

**Distribution.** St. Vincent (Map 1).

**Holotype.** St. Vincent, W.I. Leeward side, H.H. Smith (USNM) (head missing).

**Additional material examined.** St. Vincent, W.I., 800 m (1957) (W.R. Heed) (1♀, USNM); Mingara, 28.iii.1989, A. Friedberg (1♀, USNM); Maricao, 1500', vii–viii.1972, A.D. Harrison (1♀, USNM); Malaise trap (1♀, USNM).

**Comments.** *Chaetoclusia xanthops* is the only known species of Sabarocephalinae with pigmented spermathecae.

***Chaetoclusia furva* sp. n.**

(Figs 25, 28, Map 1)

**Description** (Fig. 25)

**Male.** Body length 4.0–5.1 mm. Bristles light brown to dark brown. One small bristle in front of anterior dorsocentral. Acrostichal bristle absent. Presutural intra-alar bristle weak. Ocellar bristle minute. Anterior lateral scutellar bristle minute or absent. Arista sparsely plumose. Scutum yellow with notopleuron and one pair of wide

postsubmarginal stripes brown, sometimes sequum-right (narrow) basally in front of notopleuron. Scutellum yellow, sometimes with one pair of lateral postsubmarginal stripes. Metatergites yellow, sometimes with one pair of faded stripes lateral to scutellum. Pleuron and legs light yellow to wine with fore tarsi light brown to brown, sometimes with fore tibia or all tibiae brown. Head yellow with ocellar tubercle light brown and head white below antenna, sometimes with first flagellomere narrowly infuscated around base of arista. Abdomen brown with tergite 1 and surstyli yellow, sometimes tergite 2 only brown posteriorly and laterally. Wing dusky on distal half, darkest around R+1.

**Female.** Externally as described for male except as follows: colour often darker, sometimes anterior half of tergite 5 yellow; tergite 8 and terminalia yellow.

**Male terminalia.** As described for *C. livata*.

**Female terminalia** (Fig. 28). Spermatheca poorly sclerotized and with minute transverse wrinkles on basal third. Spermathecal duct up to half as wide as spermatheca and divided into two sections: basal section no more than 0.3 times width of spermatheca and approximately three times length of spermatheca; distal section wide, membranous and 1.2 times longer than spermatheca. Ventral receptacle approximately 1.5 times length of spermatheca; wide distally, sac-like and recurved ventrally.

**Etymology.** The specific name refers to the dark coloration of this species (i.e. "dark") relative to a similar species, *C. livata*.

**Distribution.** Barbados, Dominica, Dominican Republic, Puerto Rico (Map 1).

**Holotype.** Dominica, Port Casse, 22.xi.1964, P.J. Spangler (1♂, USNM).

**Paratypes.** Barbados, W.I. Sandicate, 6.ii.1964, H. Robinson (1♀, USNM); Dominica, W.I., Clarke Hall, Est. 15.ii.1966, R.J. Gagne (1♀, USNM); Dominican Republic, San Rafael, 18 km W. Hanfina, roadside ditch, 28.i.1989, S.A. Marshall (1♀, USNM); Pedernales, 23.5 km N Cabo Rojo, 18–06 N, 71–38 W, 540 m, 13–19.xii.1990, J. Masner, J. Rawlins, C. Young, deciduous forest, intercept trap (1♀, USNM); Pedernales, 26 km N Cabo Rojo, 18–06 N, 71–38 W, 730 m, 13–25.xii.1990, J. Masner, J. Rawlins, C. Young, wet deciduous forest, intercept trap (1♀, USNM); Hato Mayor, Parque Los Baños, 3 km W. Nuevo de Arena, 19–04 N, 69–29 W, 20 m, 7–9.xii.1992, R. Davidson, J. Rawlins, S. Thompson, C. Young, mesic lowland forest (2♂, USNM); Puerto Rico, Mayaguez, M. Wasserman, x.1957 (1♂, USNM).

**Comments.** *Chaetoclusia furva* is similar in appearance to *C. livata*, but it is distinguished by the derived female genitalia (Fig. 28) and differences in cephalic and axial coloration (see key) (Figs 25, 26). These two species appear to be most closely related to *C. xanthops*, which is also Caribbean in distribution, on the basis of chaetotaxy, coloration, and numerous derived characters of the male genitalia (see Discussion).

**PHYLOGENETIC DISCUSSION**

Cladistic analysis of the character matrix in Table 2 using PAUP produced three equally parsimonious trees (22 steps in length, CI = 0.82, RI = 0.89), one of which

## *Bat Flies and a Bat Bug*

**Date Sent:** Tuesday, November 07, 2006 8:06 PM

**From:** Michelle Theberge  
<mich\_theberge@yahoo.com>

**To:** wenhua

**Subject:** RE: Streblid

Hey Skip,

Hope you had a successful field trip this year! That's interesting about the streblids.

I've been corresponding with Carl Dick from the Chicago Field Museum. I haven't sent him the specimens yet, but he's provided insight into their ID. He has been willing to look at the specimens and has asked that some be kept at the Field Museum. Below is his contact, as well as what he said about the weird bat ectoparasite from 2005.

"The parasites from *Molossus* are not bat flies at all....not even Diptera. They are in the order Hemiptera, family polyctenidae, genus *Hesperoctenes*. These are commonly called "bat bugs" and are related to the family Cimicidae (which are sometimes mistakenly called "bat bugs" but are more precisely referred to as "bed bugs"). In the New World bugs of this family are exclusively associated with species of *Molossidae*."

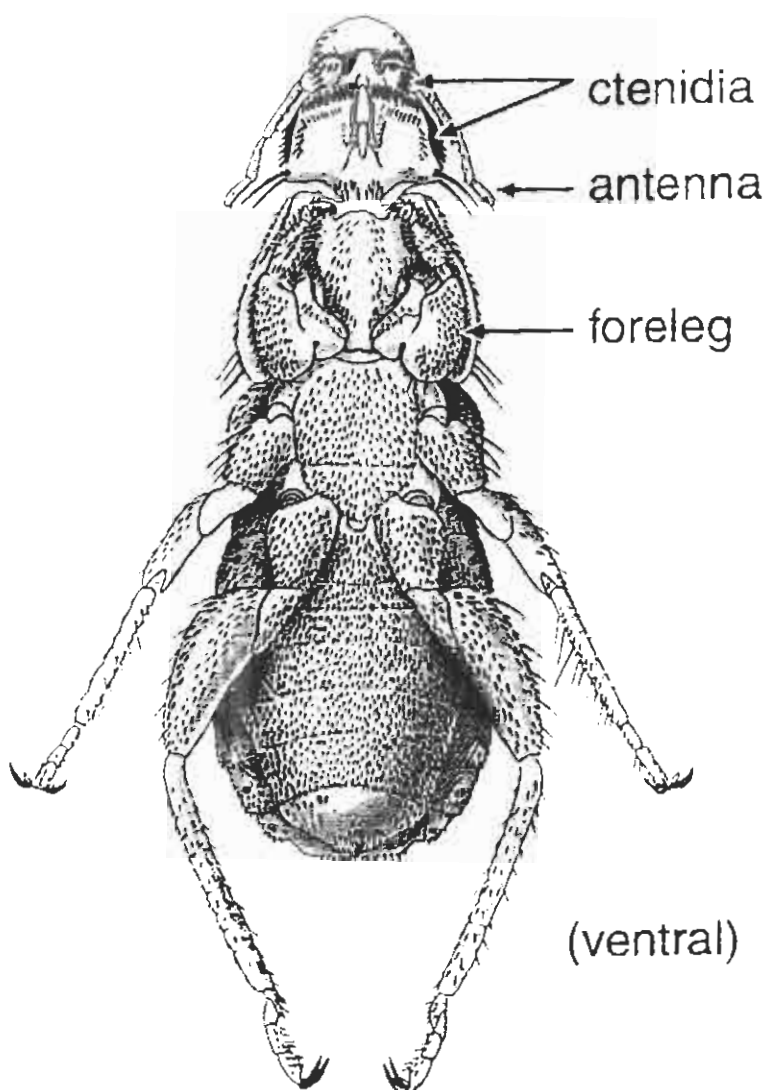
Carl W. Dick  
Brown Postdoctoral Fellow  
Department of Zoology  
Field Museum of Natural History  
1400 South Lake Shore Drive  
Chicago, IL 60605-2496 USA

312.665.7014 (t)  
312.665.7754 (f)  
cdick@fieldmuseum.org (e)

Hope that will help. I'd be interested in finding out what your streblids are if you get them identified! I'm going to send Carl the ectoparasites from Guana soon, so hopefully he'll be able to figure out the bat bug!

Missed being there this year... It's been a whole year since I've had my hands on a bat!

Michelle



*Polyctenes molossus*  
HETEROPTERA

Hi Michelle:

The ectoparasites arrived just fine. They were pretty straightforward to identify. I have placed them into better vials for storage, but will not print out labels until I (hopefully) can receive a few more details.

Please see the attached Excel file for more information on these specimens. The data fields that we commonly use for bat flies are included, but I have highlighted some missing fields in yellow. As you can, might you fill in any information that you can? I presume that you were not collecting bats along with the bat flies. Some ectoparasites here are from bats that were released and others are from ones that were collected for further study. Hence the "host number, host museum, etc." fields.

OK, all for now. Hope you are doing well.


Carl

Carl W. Dick

Collector	Field Number	Fly Collection	Host genus	Host species	Fly taxon	# Males	# Females
Theberge, M.	ARJA 8	FMNH	Artibeus	jamaicensis	Megistopoda aranea (Coquillett, 1899)	1	0
Theberge, M.	ARJA 3	FMNH	Artibeus	jamaicensis	Trichobius intermedius Peterson & Hurka, 1974	0	1
Theberge, M.	ARJA 6	FMNH	Artibeus	jamaicensis	Megistopoda aranea (Coquillett, 1899)	0	1
Theberge, M.	MOMO 05	FMNH	Molossus	molossus	Hesperoctenes fumarius (Westwood, 1874)	1	2



## New Longhorn Beetle

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1028

## Two new species of *Plectromerus* Haldeman (Coleoptera: Cerambycidae) from the West Indies

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### Abstract

Two new species of Curiini (Coleoptera: Cerambycidae: Cerambycinae), *Plectromerus lingafelteri* from the Dominican Republic and *Plectromerus ramosi* from Puerto Rico and the Virgin Islands are described. Features distinguishing the new species from their congeners are presented. *Plectromerus distinctus* (Cameron), previously synonymized with *P. serratus* (Cameron), is restored as a valid species.

### Resumen

Dos nuevas especies de Curiini (Coleoptera: Cerambycidae: Cerambycinae), *Plectromerus lingafelteri* de la República Dominicana y *Plectromerus ramosi* de Puerto Rico y las Islas Virgenes se describen. Se incluyen características para diferenciar estas especies de otros miembros del género. *Plectromerus distinctus* (Cameron), previamente sinonimizada con *P. serratus* (Cameron), se reconoce como especie válida.

**Key words:** Caribbean, West Indies, Dominican Republic, Puerto Rico, Virgin Islands, Cerambycidae, Cerambycinae, Curiini, *Plectromerus*, new species

### Introduction

Increased interest in the rich diversity of the Caribbean region has generated in-depth studies of its cerambycid fauna (Lingafelter & Micheli 2004, Micheli 2003, Micheli & Micheli 2004, Vitali & Rezbanyai-Reser 2003, Zayas 1975). Recent extensive collecting in the Dominican Republic, Puerto Rico, and the Virgin Islands has resulted in the discovery of new species, with estimated faunal counts of 131, 71, and 45 longhorned beetle species,

respectively, for the three areas. Continued surveys of varied habitats within the region are necessary for any future analyses of biodiversity and biogeography of West Indian Cerambycidae.

The genus *Plectromerus* Haldeman (1847) was first treated by LeConte (1873), LeConte & Horn (1883), and Leng (1885). There has been some confusion about the generic attributes of this genus and *Pentomacrus* White (Linsley 1963, Micheli 1983), but no thorough revisionary work has been done. Cameron (1910) described two species in *Pentomacrus* and provided a key for species of this genus only. Cazier & Lacey (1952) commented on the taxonomic problem clouding these two genera and included the species assigned to both within a single key. Subsequently, Giesbert (1985) stated that the supposed differences were not sufficient to justify two genera and synonymized *Pentomacrus* with *Plectromerus*. Vitali & Rezbanyai-Reser (2003) provided a key for all species of *Plectromerus*, which later was modified by Vitali (2004) to include a new fossil species and to subdivide the genus into two groups, *Plectromerus* and *Pentomacrus*. The resolution of generic issues within the Curiini is beyond the scope of this paper, and therefore we retain the generic concept of *Plectromerus* of Giesbert (1985) and include within this genus 15 species distributed in the Caribbean, southeastern USA, and southeastern Mexico (Monné & Hovore 2003, Monné 2005). A key to the species of *Plectromerus* will be provided at a later time by the junior author in his on-going revision and phylogenetic analysis of the tribe Curiini.

## Materials

Specimens from various collections were examined. The following acronyms are used throughout the paper:

BMNH—The Natural History Museum, London, United Kingdom

CMNH—Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, USA

DHPC—Daniel Heffern Private Collection, Houston, Texas, USA

ENPC—Eugenio Nearns Private Collection, Gainesville, Florida, USA

FSCA—Florida State Collection of Arthropods, Gainesville, Florida, USA

JAMC—Julio and Charyn Micheli Private Collection, Ponce, Puerto Rico, USA

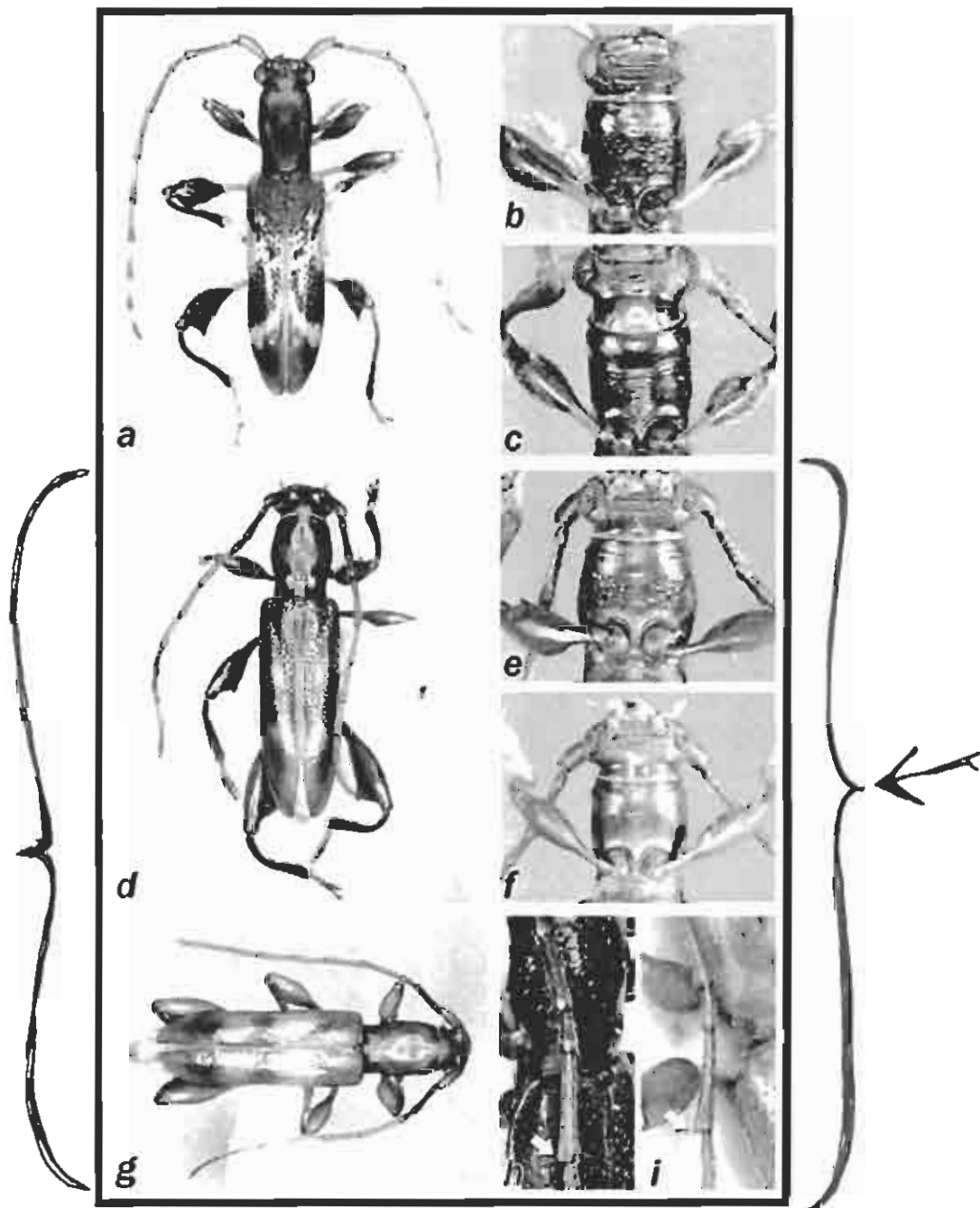
JEWC—James E. Wappes Private Collection, Bulverde, Texas, USA

RFMC—Roy F. Morris Private Collection, Lakeland, Florida, USA

RTPC—Robert H. Turnbow, Jr. Private Collection, Ft. Rucker, Alabama, USA

USNM—National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

WIBF—West Indian Beetle Fauna Project, Michael A. Ivic, Bozeman, Montana, USA



**FIGURE 2.** a–c, *Plectromerus lingafelteri* Micheli & Nearn, new species: a, holotype; b, closeup of prosternum, male; c, closeup of prosternum, female; d–h *Plectromerus ramosi* Micheli & Nearn, new species: d, holotype; e, closeup of prosternum, male; f, closeup of prosternum, female; g, lighter phenotype; h, closeup of fifth antennomere; i, *Plectromerus serratus* (Cameron), closeup of fifth antennomere of holotype.

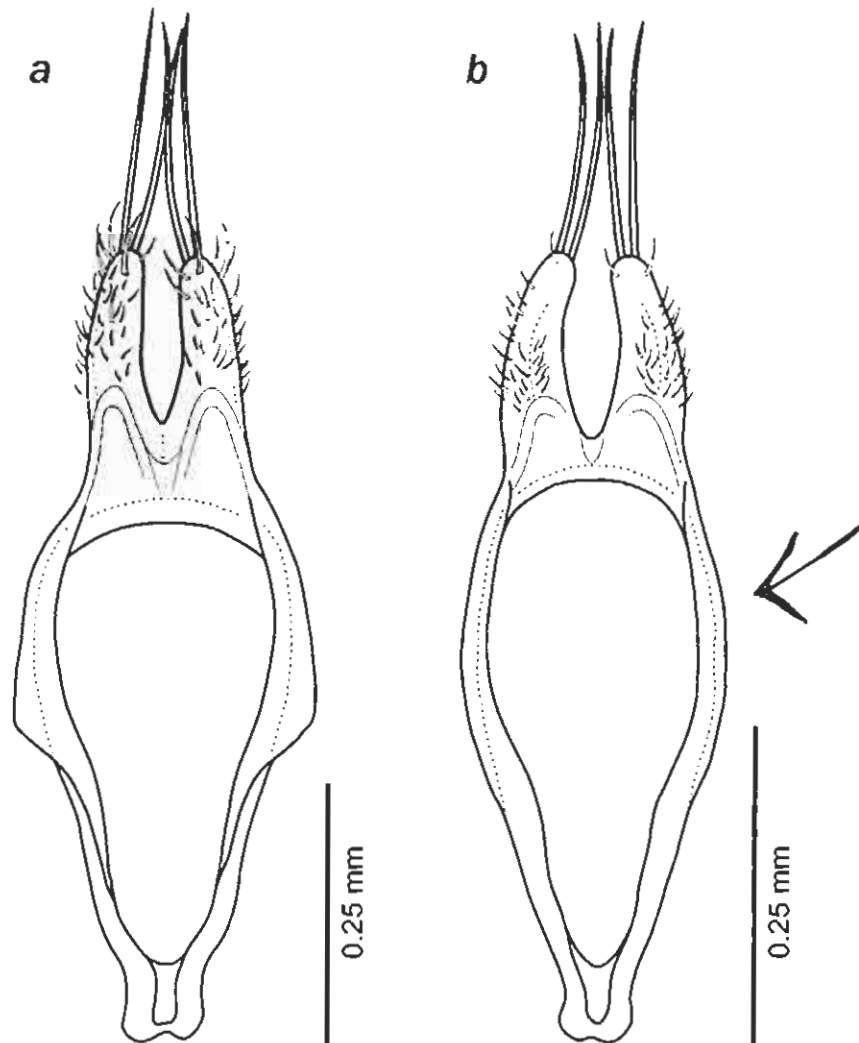


FIGURE 3. Tegmen and parameres, ventral view: a, *Plectromerus lingafelteri* Micheli & Nearn, new species; b, *Plectromerus ramosi* Micheli & Nearn, new species.

**Etymology.** This species is dedicated to Steven Lingafelter, who collected most of the type material and made the trip to the Dominican Republic by the senior author possible, and whose friendship, guidance, and companionship are truly appreciated.

**Types.** Holotype, male, DOMINICAN REPUBLIC, Pico Duarte Trail, 3300 ft., Los Tablones, beating, 19°08.222'N, 70°27.736'W, 29 June 2004, S. Lingafelter (USNM). Allotype, female, DOMINICAN REPUBLIC, Pedernales Prov., PN Sierra Baoruco, Las Abejas, 18°09.011'N, 71°37.342'W, 1150 meters, 11 July 2004, blacklight, C. J. Micheli,

coll. (USNM). Paratypes, 14 (all from the Dominican Republic): 1 male, same data as holotype, except day coll. (USNM); 2 males, Pico Duarte Trail, Ciénaga to Los Tablones, beating, 19°08.222'N, 70°27.736'W, 29 June 2004, C. J. Micheli (JAMC); 1 male and 1 female, Pedernales Prov., PN Sierra Baoruco, Las Abejas, 1150 m, 18°09.011'N, 71°37.342'W, ex. dead log w/ white fungus, 11 July 2004, S. Lingafelter (USNM); 2 males and 1 female, Pico Duarte Trail, 3300 ft., Los Tablones, blacklighting, 19°08.222'N, 70°27.736'W, 17 July 2004, S. W. Lingafelter (USNM); 1 male, Pedernales Prov., 25.5 km N. Cabo Rojo, 12-21-V-1992, coll. M. C. Thomas (FSCA); 1 female, Azua, East side of crest, Sierra Martín García, 7 km WNW Barrero, 18-21 N, 70-58W, 860m, 25-26 July 1992, C. Young, R. Davidson, S. Thompson, J. Rawlins, cloud forest adjacent to disturbed forest (CMNH); 2 males, Prov. Hato Mayor, Par. Nac. Los Haitises, 01-02 Apr 1992, bosque humido, W. Sabana dl Mar, M. Ivie, D. Sikes, Lanier (WIBF); 1 male, Barahona, 4.5 km. S Barahona, 22 May 1992, R. Turnbow (RTPC); 1 male, Pedernales, 25.5 km. N Cabo Rojo, 21 May 1992, R. Turnbow (RTPC).

**Discussion.** The intensity and breadth of maculations seem to be variable among specimens. Some specimens are mostly ferrugineous without any very dark areas but with the described light clytral pattern.

This species can be distinguished from the presently known congeners by the combination of the following characters: the opaque, microsculptured, finely punctate pronotum, the smooth metafemoral tooth, and the clytral maculation. At first glance, *P. lingafelteri* resembles *Plectromerus dentipes* (Olivier) (Fig. 1e) but this species has a shiny pronotum, the metafemoral tooth is serrate, and the clytral apex is moderately subtruncate (rounded in *P. lingafelteri*). Another species with a rather intricate clytral pattern is *P. exis* Zayas (Fig. 1f–g), but *P. lingafelteri* can be easily recognized by the shape and length of the pronotum, the length of the third antennomere, and the clytral punctation. In *P. exis*, the pronotum has a distinct tubercle in the center and the length is about 1.8 times the width (1.5 to 1.6 in *P. lingafelteri*), the third antennomere is distinctly longer than the scape (subequal in *P. lingafelteri*), and the clytral dark areas are opaque and microsculptured (not so in *P. lingafelteri*).



***Plectromerus ramosi* Micheli & Nearn, new species**  
Figs. 2d–h, 3b

**Description.** MALE. Length 4.3–6.5 mm, width 1.0–1.6 mm (measured across humeri). Small, narrow, subcylindrical. Integument ferrugineous, varying from light to dark, with two testaceous maculae (sometimes transverse fasciae) on each elytron, one at basal third, small, and oblong, and another just behind middle, this one oblique. *Head* with front nearly flat, transverse, with a median, shallow line from between eyes and antennal tubercles, slightly concave between antennal tubercles, which are slightly raised and widely separated. Surface moderately shining, with fine wrinkles, coarsely, rugosely, densely,

confluently punctate, punctures shallower beyond vertex. Head with a fine, short pale seta in each puncture and a few scattered long, pale, suberect hairs. Eyes prominent, transverse, subreniform. Antennae 11-segmented, slightly longer than body, third antennomere subequal to scape, about 1.3 to 1.8 longer than fourth, fifth antennomere about 1.4 longer than third, varying from slightly less than twice to three times the length of fourth, sixth subequal to seventh, eighth to tenth becoming progressively shorter, eleventh slightly longer than tenth; basal antennomeres subcylindrical, from antennomere 5 slightly flattened, with apices of antennomeres 5–10 produced externally (fifth only very slightly), more pronounced on antennomeres 7–10. Antennomeres feebly shining, scape moderately coarsely, moderately densely, shallowly punctate; clothed with fine, short, recumbent, pale pubescence with slightly longer, suberect hairs intermixed, sparser on basal segments, becoming denser on distal ones, antennomeres 2–6 ciliate beneath with coarse, moderately long, suberect, pale hairs. *Pronotum* about 1.3 longer than broad, widest at middle, slightly broader at base than apex, sides arcuately inflated, with a broad constriction at basal fifth, and a slight inflation just before apex; basal and apical margins slightly arcuate; disk slightly flattened medially, sometimes with three broad, rounded raised areas, one medial and two anterior to middle, one on each side. Surface moderately shining, often with fine wrinkles, sparse to moderately densely, shallowly, moderately coarse punctation on disk, laterally alutaceous with deeper punctures. Pronotum mostly glabrous except each side with two long, suberect setae, one anterolateral, the other one discal at basal third. *Scutellum* small, rounded, shining, impunctate. *Elytra* about 2.7 to 3 times as long as width at humeri, about 2.6 to 3 times as long as pronotal length, about 1.2 to 1.4 times broader basally than pronotum at widest (at middle); sides slightly sinuate, evenly rounded to apex which is rounded; epipleural margin sinuate. Disk slightly concave medially, subsuturally, creating a faint costa on each elytron. Surface shining; punctation moderately dense, coarse, punctures becoming finer towards apex and sides, almost obsolete at apical third; glabrous except for a few very fine, inconspicuous short hairs in punctures near apex. *Underside* with prosternum shining, rugose; apical fourth impunctate and one irregular patch of coarse punctures in front of each coxa (Fig. 2e); with very sparse, short, inconspicuous, pale hairs; narrowest area of prosternal process between coxae about 0.25 to 0.3 as wide as coxal cavity, and about 0.25 to 0.5 the width of apex of process which is subtriangular with rounded corners. Mesosternum shining; moderately finely to moderately coarsely punctate; with few short, inconspicuous pale hairs. Mesepisternum sparsely punctate; sparsely clothed with fine, short, pale hairs. Mesepimeron with denser pubescence. Metasternum shining; moderately finely to moderately coarsely, sparsely punctate; punctures with a fine, short, pale hair. Metepisternum moderately densely clothed with short, recumbent, pale pubescence, which is denser posteriorly. Abdomen shining; finely, shallowly punctate; abdomen with a few long, suberect pale hairs and punctures with a short, fine, pale hair; fifth sternite broadly rounded, slightly longer than preceding sternite. *Legs* with femora pedunculate-clavate, meso- and metafemora arcuate, shining, finely,

shallowly punctate, clothed with sparsely to moderately densely, recumbent, short, pale pubescence; underside of each femoral club with a broad triangular tooth with posterior edge strongly serrate; tibiae slightly arcuate, sinuate; clothed with sparse to moderately dense, fine, recumbent, pale pubescence, becoming longer and coarser apically. *Genitalia* see Fig. 3b.

**FEMALE.** Length 5.0–7.2 mm; width 1.2–1.7 mm (measured across humeri). Very similar to male. Antennae about as long as body. Lateral punctures on pronotum not distinctly deep and prosternum only finely punctate, lacking patches of coarse punctation (Fig. 2f). Narrowest area of prosternal process between coxae about 0.25 to 0.4 as wide as coxal cavity, and about 0.3 to 0.6 the width of apex of process.

**Etymology.** This species is named in memory of Dr. José A. Ramos for his invaluable contributions and his lifelong dedication to the study of insects in Puerto Rico, and for unconditional access to specimens from his remarkable personal collection, one of the largest on the Island.

**Types.** Holotype, male, PUERTO RICO, Maricao, Rd. 120, Km. 13.8, 26-IV-1980, J. & N. Micheli, coll., beating foliage (USNM). Allotype, female, PUERTO RICO, Maricao, Rd. 120, Km. 15.9, ex twigs *Eugenia* nr. *ligustrina*, coll. 17-X-1981, emerged XII-81, J. Micheli, coll. (USNM). Paratypes, 56: 1 female, same data as holotype (JAMC); 1 male, PUERTO RICO, Maricao, Rd. 120, Km. 13.8, 3-V-1980, J. Micheli, coll., beating dead foliage (JAMC); 1 male, same data as previous except, 10-V-1980 (JAMC); 3 males, PUERTO RICO, Maricao, Rd. 120, Km. 15.9, ex twigs *Eugenia* nr. *ligustrina*, coll. 17-X-1981, emerged XI-81, J. Micheli, coll. (JAMC, ENPC); 14 males and 2 females, same data as previous except, emerged XII-81 (JAMC, USNM, ENPC; 2 dissected); 1 male and 1 female, same data as previous except, emerged II-82 (JAMC); 4 males and 4 females, same data as previous except, emerged III-82 (JAMC, ENPC; 1 dissected); 1 male, PUERTO RICO, Maricao, Rd. 120, Km. 15.9, 18-X-1981, beating foliage, J. Micheli, coll. (JAMC); 1 male, PUERTO RICO, Maricao For., Water Filtration Plant, 18°09'N, 66°59'W, 17 June 2002, *Turpenia paniculata*, Steven W. Lingafelter (USNM); 1 female, PUERTO RICO, Maricao, Bosque Estatal de Maricao, 3.3 km SW Maricao, 18-09-39N, 67-00-05W, forest, 550 m, 10-11 June 1996, J. Rawlins, C. Young, R. Davidson, W. Zanol, S. Thompson, M. Klingler (CMNH); 1 female, PUERTO RICO, Hwy 120, km. 16.2, Hdqts. Maricao St. For. 8-8-1999, C. W. O'Brien (DHPC); 1 female, PUERTO RICO, Hwy. 120, K10H2, Maricao For. Res., July 26, 1979, L.B. O'Brien (JEWG); 1 male, PUERTO RICO, Guánica Forest, 6-IV-2001, ex dead log, Charyn J. Micheli, coll. (JAMC); 1 female, PUERTO RICO, Guánica Forest, Ballena trail, beating, 17°58'49"N, 66°51'74"W, 16 June 2002, Steven W. Lingafelter (USNM); 1 male, PUERTO RICO, Guánica Forest, Ballena trail, UV light, Spec ID:4228, Nearns & Lingafelter, 27-VII-2004 (ENPC); 1 male, PUERTO RICO, Ponce, Rd. 132, Km. 20, 26-VI-1972, J. Micheli, coll., at lights (JAMC); 2 males, PUERTO RICO, Ponce dry forest at Holiday Inn, 17°58'N, 66°38'W, 20 June 2002, beating, Steven W. Lingafelter (USNM, ENPC; 1 dissected); 1



male, PUERTO RICO, Ponce dry forest behind Holiday Inn, 17°58'N, 66°38'W, 1 July 2002, *Thotunia portoricensis*, Steven W. Lingafelter (USNM); 2 males and 2 females, PUERTO RICO, Guanica, Bosque Estatal de Guanica, 3.6 km E Guanica, 17-58-11N, 66-52-28W, thornscrub, 100 m, 12 June 1996, J. Rawlins, R. Davidson, C. Young, M. Klingler, W. Zanol, S. Thompson (CMNH); 1 female, 17°56'50"N, 066°51'48"W, PUERTO RICO, Guanica, Bosque Estatal de Guanica, just W. Punta Ballena on Rt. 333, 9.VIII.1999, P. W. Kovarik, collector, beating (WIBF); 1 female, PUERTO RICO, Humacao Dist., Casa Cabuy, Hwy.191 nr. Florida, 31-VII-2-VIII-1999, J. E. Eger, MV & UV lights (RFMC); 1 female, VIRGIN ISLANDS, St. John, Lameshur Bay – VIERS, 09 March 1984, at UV light, W. B. Muchmore colr. (WIBF); 1 female, VIRGIN ISLANDS, St. John, Est. Caneel Bay, Lind Point, December 1992, J. Comisky colr. (WIBF); 1 male and 1 female, VIRGIN ISLANDS, St. John, Lameshur Bay, VIERS, 21-28 July 1994, M. S. Becker colr, ultraviolet light (WIBF); 2 males, BRITISH VIRGIN ISLANDS, Guana Island, Sugarloaf trail, 100-800 ft., 09 OCT 1994, M. A. & L. L. Ivie (WIBF).

**Discussion.** Throughout the series there is some variation in color and slight variation in the shape of pronotal margins, pronotal texture, punctation on pronotum and mesosternum, and proportion and shape of the prosternal process. Specimens collected in the wet forest of Maricao are quite dark and the pale maculae on the elytra tend to be rather compact (Fig. 2d). Those from the drier areas of Guánica and Ponce (in Puerto Rico) and the Virgin Islands are lighter colored with the pale areas on the elytra more like fasciae (Fig. 2g). Except for color, other variation is slight and there is much overlap. To further investigate the possibility of two distinct species, dissections of male genitalia of several specimens from each phenotype were made by the junior author. Detailed study of the tegmen including the parameres (lateral lobes) and phallobase (basal piece) revealed no consistent morphological characters (Fig. 3b). Since we can find no significant differences between specimens from “wet” and “dry” areas, only a single species will be proposed.

This species can be confused with *Plectromerus serratus* (Cameron) but can be distinguished by the punctation of the pronotum: in *P. serratus*, the pronotum is impunctate and dull, whereas *P. ramosi* has a shiny pronotum and distinct punctation. Also, the fifth antennomere in *P. serratus* (Fig. 2i) is distinctly pronounced externally at apex whereas in *P. ramosi* (Fig. 2h) it is only slightly expanded. Some small, light specimens of *P. ramosi* are similar to *P. distinctus* (Cameron) but the latter species has long, suberect hairs on the elytra and granulate punctures on the pronotum, both lacking in *P. ramosi*. From other congeners, *P. ramosi* can be distinguished by the following combination of characters: the shape and punctation of pronotum (widest at middle, shallow, moderately coarse punctures), the punctation and macular pattern of elytra, the glabrous pronotum and elytra, and the serrate metafemoral tooth.

# INSECTS

Date Sent: Wednesday, May 30, 2007 2:38 AM

From: "B & B Valentine" - by [bnwes.com](mailto:bnwes.com) -

To: Wenhua Lu - [wenhua@etal.uri.edu](mailto:wenhua@etal.uri.edu) -

Here's an update on Wenhua's projects, and then general Guana news and progress:

Mordellids: The enclosed data sheet lists every Quail Dove Malaise specimen presently in my hands.

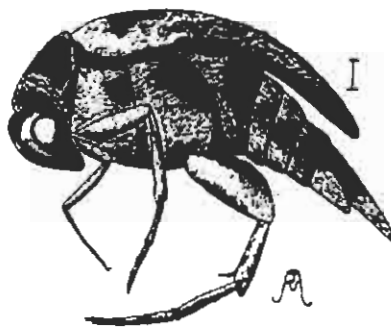
Wenhua can add those she has (should include the early years - for which I have no record). So, in the enclosed tabulation, missing months mean that Wenhua has the specimens, OR that none were

present. I've checked every pin and vial, so this is complete for BV material in Ohio and Florida.

Roaches: The early Quail Dove counts went to Wenhua - I have no record of these. I now have data for the last three years: November, 2003, through 15 October, 2006. These are enclosed. Let me know if you want me to continue this year.

General Insecta: All the larger insect orders have increased taxon counts, with the species total now in excess of 1,420. The largest increases have been in the groups studied by specialists, indicating my estimates have been too conservative. Significant new data are now available for Orthoptera, Homoptera,

Hemiptera, Neuroptera, Diptera, etc., all or parts of which are being or have been studied by specialists (see below). Other orders have also increased, but these are less diverse so the progress appears less exciting.



Mordellidae

COLEOPTERA: The largest and best-studied order has been increased by another family and about 55 species. To this must be added the many species from other islands which,

although not on Guana, add immeasurably to our reference set for the V.I. We expect many of these

will show up on Guana as we

try new habitats and techniques. Also, 50 species not listed in the V collection in your book are now present in our Guana set. There is also much information about individual

taxa...the first three families

will illustrate:

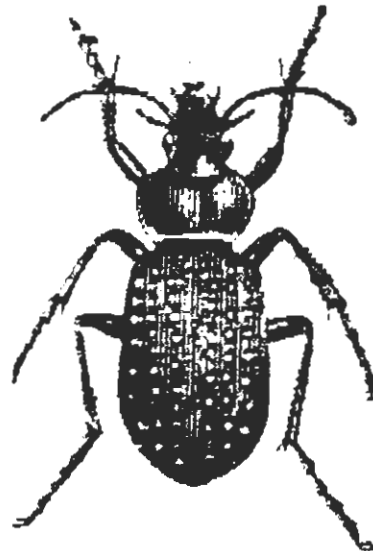
Carabidae: two genera and species have been added. Seven more species now have

representatives. Two of Ivie's species names have been changed. The NMNH specimen listed as *Pseud-*

*aptinus dorsalis* Brulle was checked for me by Dr. Terry Erwin and is not *dorsalis*, but either of two

Puerto Rican species (*insularis* Mutchler or *thoracicus* Wolcott) for which adequate information is not

available. Four non-Guana BVI carabid species are now on hand (Anagada, Tortola, Little St James), and these will help as new Guana taxa turn up.



Carabidae

Dytiscidae: a series of 6 *Eretes* from Guana is now in the V collection; I think the asterisk is incorrect.

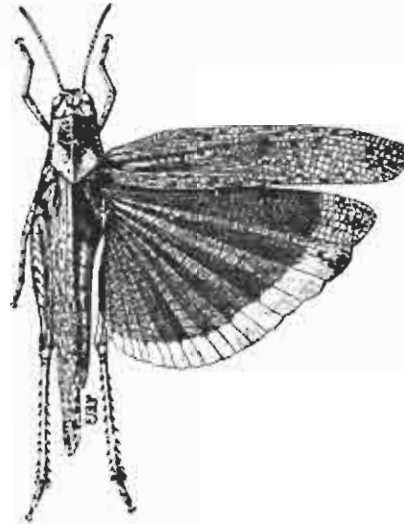
Also we have added another genus and species: *Thermoneetes basillaris* (Harris).

Hydrophilidae: we have two more taxa - a second species of *Berosus* (rare and still unidentified), and *Tropisternus lateralis* which is widespread in eastern U.S., Caribbean, etc.

Also, the large *Hydrophilus* is now in series and should probably lose the asterisk, etc. In addition, there were astonishing differences in abundance compared to previous years. For example,

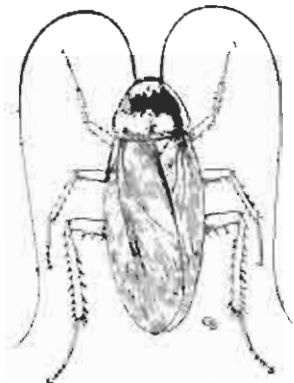
carabids and aphodiine scarabs were unusually abundant, and other scarabs, mordellids, cerambycids, cantharids, coccinellids, oedemerids, and tenebrionids were rare or greatly reduced.

ORTHOPTERA: real progress here! The many long-horned grasshoppers and katydids are identified, and the crickets are under study by the world authority who is revising the West Indian species. The count has jumped from 17 to about 30. Remaining problems are the short-horned grasshoppers and the two species of weird wingless camel-cricket-like beasts we looked at together. I suspect a 35+ species total.

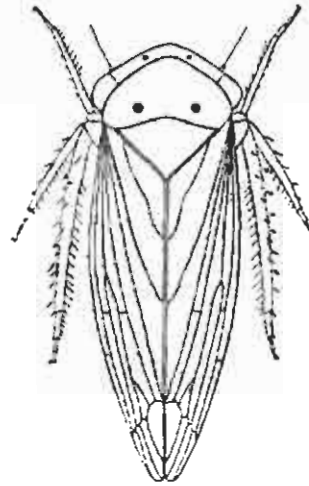


Acrididae

BLATTARIA: roach abundance has always fluctuated from year to year, however this year the ubiquitous green Panchlora was almost absent at our lights (from hundreds down to perhaps a dozen), and the previously very rare forest dweller Plectoptera was present by the dozens in several collections near the hotel (but not at Quail Dove Ghut).

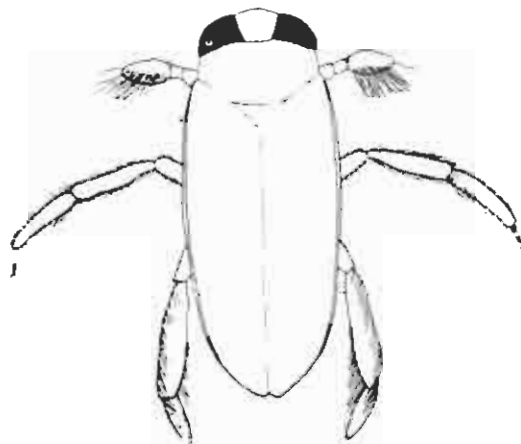


HOMOPTERA: these are a mixture of progress and frustration. We have increased Bartlett's (2000) count of 27 species of Fulgoroidea to at least 35. I suspect several of ours are undescribed, but Bartlett has problems accessing scattered types in obscure European museums, so the work remains unfinished. The family Cicadellidae (leafhoppers) is even more numerous, but the main expert has not answered my e-mails, and I do not have the extensive literature, nor knowledge, to do them myself. Both groups were in very unusual numbers - both abundance and diversity. The other families have not been studied by experts, except the Membracidae (treehoppers) where we have doubled the count - which sounds impressive until you hear we have gone from one species to two! The rest of this order: aphids, scales, whiteflies, etc. have been mounted and sorted but not yet identified.



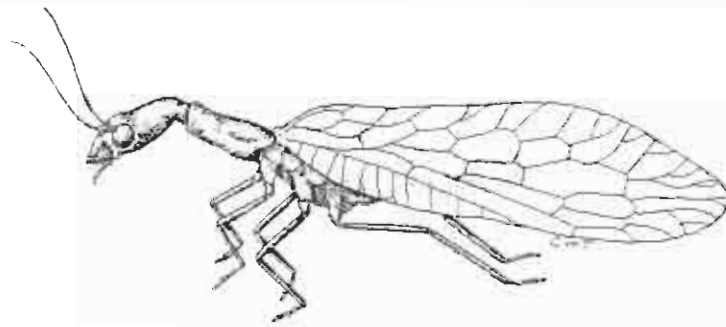
Cicadellidae

HEMIPTERA: Dr. Thomas Henry (Smithsonian Institution) did a great job on several families of our bugs - we hope he makes it to Guana this year. Many other families are still not studied, but Henry has the contacts to get the job done. His enthusiasm has spurred me to pay more attention to bugs. This past session we got 99 of his special pets, plus many others, and have a new (for Guana) species plus others which I think are new.

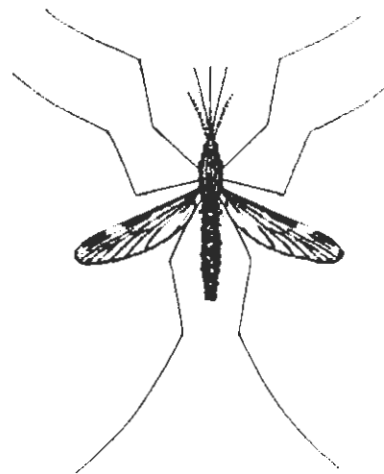


A water boatman

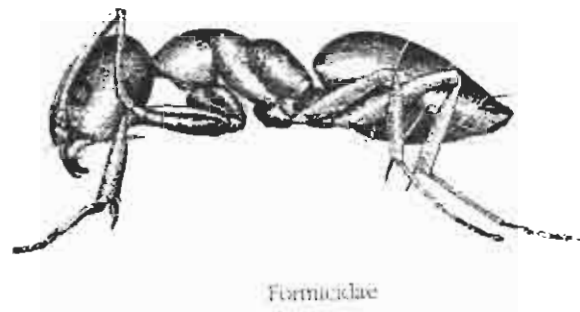
NEUROPTERA Also at the Smithsonian, an old college friend, Dr. Oliver Flint, has identified our 17 BVI species (15 from Guyana) through the 2005 season, and the 2006 material is now ready for study. I suspect some additional species...especially coniopterygids because I now have the search image for these minute, obscure, delicate beasts and am finding them by the dozen. It obviously helps to know what you are doing!  
 Note: I've now checked the older samples, and they were not present. There are still no Hemerobiidae. The ant lions are surprising. We have taken small series every year, but in 2006 we saw only one specimen on our last night.



DIPTERA: Flies are a black hole of ignorance (mine) but progress is slowly emerging. Mosquito identification is under way, several other families have been sorted out or are under study, and this past October we made special efforts to bolster and diversify the collection. We have an unending variety of flies, and are starting to farm them out to specialists. But don't hold your breath, it will take a while.



HYMENOPTERA: The myriads of tiny parasitoids are still a problem; however the larger aculeates (those with a female sting) are being prepared for shipment to Roy Snelling. We have I think a few taxa not in his reports. At least one ant is definitely new, and others may be. Odd bees were conspicuously present, and wasps were much less abundant. As in flies...hopfully more information next year.



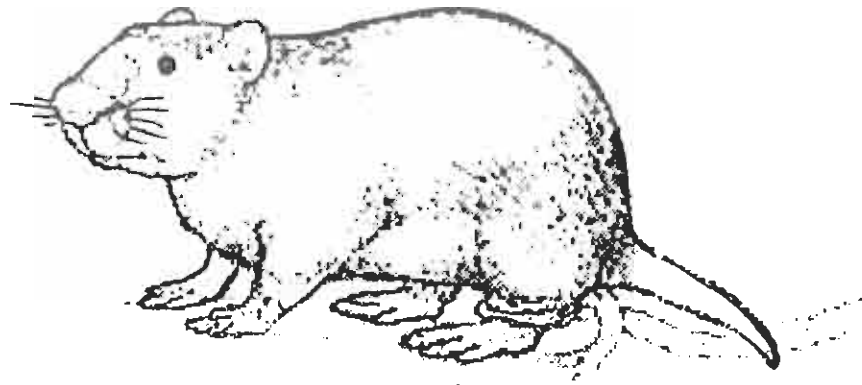
NON-INSECT ARTHROPODS: We have a surprising amount of material, most of which will be sent to James Cokendolpher. Although I am not familiar with all of his taxonomic work, he seems to have broad knowledge, so I will give him a try. He is certainly a dedicated and excellent collector, and he has been very generous with the insect components of his field work. A rare and very exotic fulgoroid comes to mind first, but there are others which he has donated. We really enjoyed his company and assistance, and I am certain he can help us with arachnids. As you know, every pair of hands on the island collects differently, and turns up new things, so we welcome the prospect of people like Thomas Henry and Cokendolpher. They have already made a difference.



We have developed excellent data for year-to-year fluctuations in October, but have nothing about distribution and abundance during the rest of the year when rainfall is less predictable. Also, we have little or nothing about life history patterns: breeding, climatic correlations, immatures, foods, predation, fecundity, etc. These are all heuristic projects and should be considered.

We send our very best wishes, and look forward to October.  
Barry (and Buena & Susan)





*Puerto Rican Hutia*  
(*Isolobodon portoricensis*)

REPORT OF 2006 INVESTIGATIONS AT A PRE-COLUMBIAN SITE ON  
THE FLAT AT GUANA ISLAND, BVI: PROGRESS TO DATE.

By Elizabeth Righter

Principal Investigator: Elizabeth Righter  
Crew: Robert Pederson  
Joshua Kehrburg  
Special Assistant: Sam Turvey  
Other Assistants: Tom Willard  
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During 2006, between September 23 and October 22, we continued our investigation of a pre-Columbian archaeological site on the flat at Guana Island, BVI. During the previous year, torrential rains, that continued for more than a week, severely hampered the investigation. Nevertheless, we were able to conduct a number of shovel tests that both delimited the midden area in the new donkey pen (a former garden) and allowed development of a plan for 2006.

During 2005, I presented a paper entitled: "Post-Saladoid Cultural Changes at Guana Island, BVI" to the XX1 Congress of the International Association for Caribbean Archaeology (IACA) held in Trinidad. During 2006, the paper (limited to a 10-minute presentation) was edited and presented for publication in the Proceedings of the Congress. The Proceedings will be distributed to all of those persons interested in Caribbean island archaeology and available for purchase.

When we arrived to conduct the 2006 investigations, we found that the grass on the flat had not been cut and the vegetative cover in the midden area in the new donkey pen was waist high. The fibrous nature of this cover and the well-established fire ant population prevented further archaeological investigation of this area. The long grass in the flat area of Guana Island did not preclude investigation but special areas of investigation needed cutting and it was difficult to lay out an accurate grid in the tall grass. Next year we hope to have both of these situations remedied before we arrive. The time for investigation is short at Guana, and,

while the archeologists are prepared to spend time in physical efforts, extra effort cuts into the time for conduct of scientific investigation.

The pre-Columbian site at Guana Island offers a chance to investigate numerous questions in prehistoric Caribbean archaeology. We will discuss only two or three here. One goal of the archaeological investigation has been to discover the village lay-out and/or structure at the site. Peter Siegel, a professor at Montclair College and an archaeologist of some stature, has suggested that early Saladoid villages were laid out in a horse-shoe shaped pattern that, over time, became circular in configuration. Evidence from the Tutu site in St. Thomas, tends to support this position. Miguel Rodriguez, a Puerto Rican archaeologist who is also well respected, has found evidence that some possibly Saladoid sites in shoreline locations may have been laid out in a linear fashion. So far, the evidence is inconclusive, and we hoped that investigations at Guana Island would shed light on the question.

Based on a admittedly ethnographic model, Siegel also links Saladoid village structure to an egalitarian society. The author of this report, does not agree with this position, but there is much work to be done to find counter evidence. It was hoped that Guana Island might supply an example of such evidence. However, the site at Guana Island appears to be a post-Saladoid site, and the post-Saladoid period (see my IACA paper-Trinidad, 2005) was a time of cultural change. Also it must be remembered that Guana Island is a small off-shore island and may not be representative of the bigger picture. However, it would be a "first" in Caribbean archaeology if we could establish that among changes at the end of the Saladoid period was a change in village structure.

There are many possible interpretations of prehistoric land use at Guana Island, and, so far, the prehistoric site at Guana does not lend itself to a simple or a single interpretation. It is possible that Guana Island was not permanently settled, or was settled for one or more short periods and used for something else other times. During the prehistoric period, Guana Island may have been used for outings of various durations for various purposes. If the two artifacts that appear to be portions of ball belts are truly such, it is possible that. At some time, there was a ball court at Guana, and the site at Guana Island was used as a meeting place for feasting, dancing etc. when games were held.

It may be that the island was never permanently settled. Other prehistoric artifacts found at Guana, such as cotton whorls, however, tend to suggest permanent settlement. We hope to find post holes or other evidence of permanent structures. The soils are poor for post hole preservation and although we have identified several candidates for postholes, more evidence is needed. There are some other hindrances to investigations as well. These include the now ubiquitous fire ants, which not only poison investigators but also churn up soils, making radiocarbon dating even more difficult. Numerous historic and modern disturbances to the site also have either mixed the stratigraphically deposited remains, or removed portions of the site from investigation. Nevertheless, we feel that, with perseverance, we will be able to establish the nature of pre-Columbian usage of the site on the flat at Guana Island.

Prior to 2006, we had found one area of concentrated midden. This is in the new donkey pen or old garden area which was newly scraped in 2003, revealing evidence of the midden. In 2003, this midden was partially excavated and also partially shovel tested. Shovel testing of the midden continued in 2004 and 2005. Testing results suggested that the midden varies in thickness and in abundance of artifacts. The reason for this requires further investigation. It may be that individual houses were in the midden area (just west of it), or it may be that the refuse disposal system was such that the deposits vary in intensity.

The evidence for a major disposal area at one end of the site is strong. The orchard area, unfortunately, has been severely impacted by planting and other efforts and it will be impossible to learn anything further from this area. Over time, the orchard has yielded many artifacts, mostly pottery, which have been collected. The whereabouts of the collection

that formerly was in the library is currently unknown. In 1988, the present author saw, that there were deep furrows excavated in the southwest corner of the orchard area (presumably for insertion of a water line). Atop the furrows were great number of potsherds. Unfortunately, time did not allow further investigation of this area, which had been totally disturbed. But the evidence seems to suggest that the entire far eastern end of the flat was a disposal area for the prehistoric site. Logically, this is not unlikely, since near the hill, the area was subject to flooding and to rock fall and was not habitable. It would be a good area for disposal of refuse. However, there is the possibility, also, that the eastern part of the site (just west of the midden) was the most densely occupied area of the site, and therefore the greatest amount of refuse emanated from the part of the site that was the most populous. That is was the "best" location on the site is possible since it was the closest to potable water. The terrain today suggests that it is also possible that the village plan was circular, with the south part of the original donkey pen as the "open space" or "central courtyard" in the center of the village. There may also have been more than one period of prehistoric occupation and/or more than one prehistoric settlement on the flats at Guana.

If we can find evidence of individual houses, this would assist in the investigation of village structure. The disposal pattern appears to be similar to that at Magens Bay in St Thomas. The Magens Bay site was occupied from the late Saladoid through the Taino periods and unfortunately has not been very well investigated or recorded. Even though Irving Rouse named the ceramic style that followed the Saladoid in St Thomas, "Magen Bay/Salt River I and II, (comparable to the Monserrate, Santa Elena and Esperanza styles in Puerto Rico) there is not a single reliable date for the Magens Bay site. The ceramic styles so far found on Guana Island appear similar to the Monserrate and Santa Elena styles of eastern Puerto Rico. These, in turn, are similar to ceramic styles found in St John by Ken Wild, NPS archaeologist (see Righer, Wild and Lundberg in *Late Ceramic Age Societies in the Eastern Caribbean* edited by Andre Delpuech and Corinne L. Hofman, 2004). There does not appear to be a late, or Esperanza/Taino phase at Guana Island.

Test pitting and the 2006 investigations have uncovered substantial artifactual remains outside the main midden area, but, so far, these concentrations do not match in volume, those found in the new donkey pen area. They may be indications of individual house structures or individual events. Further analysis and investigation are needed.

A second goal of the archaeological investigations at Guana is to find evidence of those fauna that were present during the prehistoric period (see my 1989 article "The natural resource potential of historic and prehistoric archaeological sites." In: CRM Bulletin, National Park Service, October 1989; and a paper presented in 1986, "The natural resource potential of archaeological and historical sites: a case for their inclusion in Natural and Protected Areas Management Programs." Paper presented at a Workshop on Coastal and Marine Protected Areas of the Lesser Antilles, and published in the Proceedings of the Agricultural Extension Service of the University of the Virgin Islands. This information should help with investigation of habitats through time at Guana and, with the exception of extinct fauna, assist with restoring the original fauna to the island.

In the past, reportedly, shovel test investigations in 1987 yielded remains of monk seal and turtle. The remains were lost and we are attempting to find more evidence of monk seal, possibly tortoise and any other animals that were present on Guana during the pre-Columbian period that man utilized the island. This year, we were assisted by Dr. Samuel Turvey who specializes in extinct mammals of the Caribbean. His expertise is expected to add a lot to our findings.

An overall goal of the project, of course, is to learn as much as possible about the prehistoric occupation of Guana Island. We hope to learn about the natural resources that were available to the human occupants, and how they were used, as well as the social and cultural nature of the inhabitants.

In 2006, Ellen MacLean and I laid out the grid in the same location as previously. Our datum is buried in the woods and hopefully safe. Since I was recovering from an operation and since my anticipated crew did not arrive, in order not to unnecessarily strain the resources at Guana, I spent a week in St Thomas with Ellen. During that time I purchased materials for Guana, including a shovel which proved to be much needed. I also brought over materials that had been stored in Ellen's cellar.

The following week, Josh Kehrburg arrived. Before that time, Mano kindly cut grass in areas where we needed to excavate units and Tom Willard removed sod from a 2 x 2 meter unit at 45N/17W that we anticipated excavating. Based on initial investigation in 2005, this unit was expected to yield midden remains. When Josh arrived, we began excavation. Numerous artifacts were recovered and one probable post hole was found. Since a companion post hole could not be located, it is uncertain whether this post hole represents a domicile or was a post of another sort.

The major portion of a ceramic pot was found projecting from the south wall of an extension to the 2 x 2 meter unit. In general, many ceramic sherds were recovered but the bone remains were sparse.

When Bob Pederson arrived, he machine scraped two areas where post holes might be anticipated (west of the garden midden) and two additional excavation units were laid out northwest of the first 2 x 2 meter unit. In addition, several test pits were marked for excavation. The hypothesis was that actual house locations would not yield much in the way of artifactual remains and the artifacts that were indeed present would most likely be whole or nearly whole. It was anticipated also that hearths and other activity areas would be present inside and just outside of the houses (this is assuming that houses were similar to most other Caribbean island houses of the period--either open structures of posts and palm thatch or semi-closed structures. Hammocks were hung from posts and there were hearths for cooking. Sometimes men occupied one area of a house, and woman the other. Children played about (it would take an entire book to describe the ethnographic evidence). Midden areas were likely to be located outside of and fairly close to houses. South American ethnographic accounts (see Versteeg and Schinkel, *The Golden Rock Site*, 1992) suggest that there was a "back" of the house--just before the forest--and here the midden was deposited and in these areas, artifacts would be broken, signifying refuse. In the later part of the site at Tutu, burials were grouped outside of houses, almost in cemetery areas. Whether or not caciques (chiefs) were buried off-site in special places is unknown at Tutu. We have not found any prehistoric burials at Guana--another perplexing situation. If indeed, at Guana, the social system was advanced enough that Caciques were present, and if indeed caciques died on Guana, the caves above the site would be a likely location for cacique burial.

Thus, in the hope of finding some evidence of house remains, excavation units were laid out where it was not anticipated that artifacts would be found. Shovel test pits were situated with a view toward recovering animal bone. The second unit, a 1 x 2 meter unit, at 88.50 N/58.50 W, did not yield many artifacts. We did find one large thick pottery piece that may have been part of a water jug. Numerous *Cittarium pica* remains were recovered. Large long bones of a bird (one articulated) MIGHT be remains of the flightless rail, *Nesotrachis debooyi*. These were taken by Sam Turvey to London for identification.

A third excavation unit, laid out at 66N/50.50W did not yield many artifacts or features. It was abandoned for lack of time.

Bob Pederson scraped one area near the former Test 2, where a house was suspected to be located. His scrape, refined to an area 7 meters by 2 meters in area, yielded one feature that was excavated by Pederson and Kehrburg. This feature was puzzling. The dark stain became sand at 19 cmbs and then there was a smaller dark stain at 30 cmbs. The smaller stain extended to 47 cmbs. Results were inconclusive. [Note: This entire area seems to have been deeply disturbed. This is where we took a C-14 sample at some depth and it had an

historic date. Yet, the ceramics are Monserrate in style]

Another scrape was made north of the first and west of the new donkey pen. In this area, shovel testing had indicated that the midden remains were not numerous and it was hoped that a house might be present. This scrape, made at 88.25N/2.5E uncovered an area 5 meters by 2 meters in area but no stains or features were immediately observed. Further work was not conducted before the area had to be backfilled.

Shovel tests were laid out on the 65 North line. Shovel tests east of the original donkey pen were excavated in 2005; and in 2006 the object was to explore the donkey pen and the area to the west of it which rose gently to higher ground near the south shore "road".

STP A was located at 64N/10W. This test was moved one meter to the south because it seemed that the area had been previously tested. The soil became light sand at 51 cmbs.

STP B was made at 64N/15W and moved one meter to the south. An iron spike was found at 15 cmbs. Hooved animals are known to cause severe damage to archaeological sites, so this is not surprising. The soil became sand at 50 cmbs.

STP C was at the northeast edge of the south road at 65N/35W. Artifacts appeared at 27 cmbs and continued to 37 cmbs. Larger sherds and bone were bagged.

STP D was located at 104.990N/53.60W also in the field north of the green tarp. There was a possible glass fragment at 22 cmbs. No midden or cultural material was observed between 22 and 45 cmbs. Total recovered material consisted of one shell, 1 sherd and 1 possible glass fragment.

STP E was located at 120 N/66W (in the field north of the green tarp). Sandy soil begins at 16 cmbs. One weik was found at 26 cmbs. No cultural material was present to 63 cmbs.

STP F was located at 100N/69.40W, just northeast of the road. Tiny sherds and shell were found at 15 cmbs. Cultural material ended at 38 cmbs. Only one large sherd and no bone were found.

STP G was situated at 105.60N/ 73.70W northeast of road. Cultural material extended from 15 cmbs to 40 cmbs. Sherds were bagged, 1 shell preform was found and one bone.

STP H was situated at 11.30N/77.60W just northeast of the road. Cultural material was present from 15-41 cmbs. Small shell fragments occurred. 1 sherd and 1 bone only were recovered.

STP I was located southwest of the road at 105N/80W. This test pit yielded significant remains. It appears to be an area that may not have been plowed or otherwise disturbed. A unit will be excavated here next year (provided it has not been disturbed in the meantime). Cultural material extended from 26 to 59 cmbs. Very large sherds were recovered from 35 and 37 cmbs. Bone and sherds were recovered from all levels. Josh found an *Isolobodon portoricensis* jaw here. We would like to have this jaw AMS dated since, so far, we have not been able to obtain good Carbon-14 dates for the site. If the *Isolobodon portoricensis* should prove to be historic in age this would assist Sam Turvey in his interpretation of the time of extinction of the animal. A large piece of a plate-like vessel was recovered from 22 cmbs. Large portion of a burnished shoulder angular bowl was found at 37 cmbs. A small smooth finished round conch shell disc (about 3 centimeters in diameter) was recovered at 42 cmbs. Large long bones also were recovered. The long bones were sent for analysis to London with Sam Turvey.

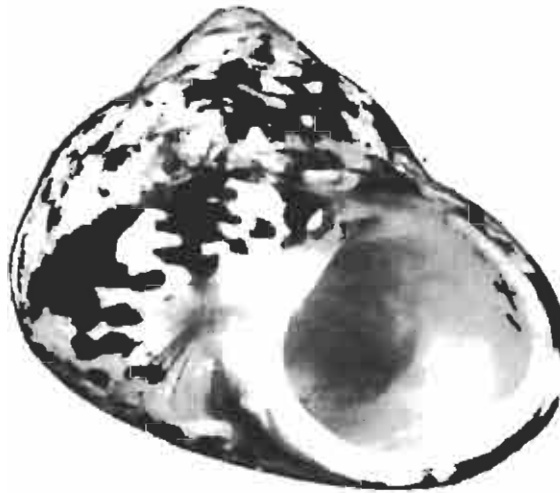
STP J was located at 91N/62.70W, northeast of the road and near the green tarp (88.50N/58.50W).. Cultural material appeared at 15 cmbs and artifacts were most numerous

at about 36 cmbs. Cultural material ended at 40 cmbs. Larger sherds and bone were bagged.

During the research, we discovered that, in general, artifacts in the upper soil layers tended to be smaller in size (2 cm by 2 cm or smaller) and that below about 18 cm below sod, the artifact pieces were larger. Artifacts tended to end between 40 and 60 cmbs, although, in some cases, there were a few large *Cittarium pica* at depths of 70 cmbs, suggesting two occupations. The sizes of the artifacts suggested areas where plowing, planting or other disturbance had occurred in the upper levels of the site. Artifacts below this disturbance were most likely in place and unaffected by surface activities. Certain portions of the site, in general those nearest to the beach, appeared not to have been significantly affected by surface disturbances.

We anticipate continuing our search for bones and for settlement pattern data next year. We hope to re-open Bob Pederson's scrape near 88.25N/2.50E and to excavate a unit at STP I-105N/80W. We also will be sure to mention that the grass and midden need cutting and whatever can be done about fire ants would be appreciated (I seem to be highly allergic to fire ant bites). During the 2006-2007 year the material recovered in the 2006 season will be analyzed. Ceramics have yet to be numbered and sent to Emily Lundberg, but it is anticipated that at least some of this work will be done. There has been no response from Dolores Piperno. We anticipate much information from Sam Turvey. I hope to present another Guana paper at the Jamaica IACA Congress in July of 2007.

Our thanks to Henry and Gloria Jarecki for making our investigations possible, to Skip Lazell for inviting us to Guana Island Science month and having faith in our expertise, to Betsy Carlson for analyzing Guana fish bone from 2003, to David Steadman for his analysis of bone from the site, to Emily Lundberg and Dolores Piperno for agreeing to assist with artifact analysis, to Greg Pregill for his assistance with bone analysis, to Sam Turvey for his continuing assistance with our project, and to Lynford Cooper who assisted with shipment of artifacts for analysis



*Top Shell or Walk*  
(*Cittarium pica*)