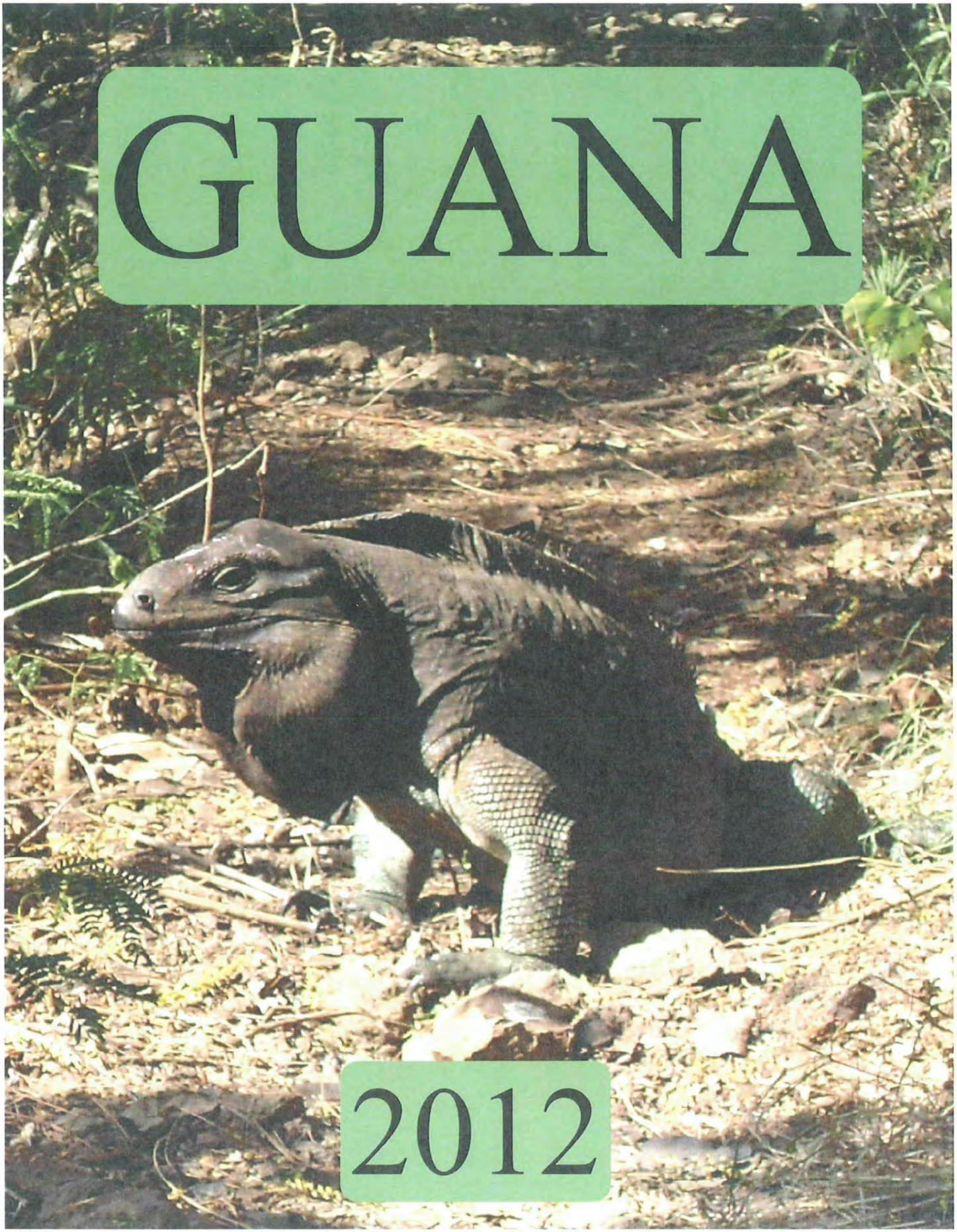
GUANA
2012

The Conservation Agency

Exploration, Education, and Research

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18 May 2013

ANNUAL REPORT FOR 2012

It has been a busy and productive year! COVER, p. 1

Work continues apace on major insect papers: roaches, stick-insects, beetles, and bugs. Lots more next year!

Sincerely, Ship

James Lazell, Ph.D.
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COVER

The Stout Iguana is probably the largest native New World lizard. It was originally endemic to the Greater Puerto Rico Bank of islands, from Puerto Rico itself east to Anegada. It is always touted as delicious and was extirpated from all of its range except Anegada by about 1700. Beginning in 1984, we brought Stout Iguanas from Anegada to Guana. Today Guana has the largest population in the world, thought to exceed 300 individuals.

However, I have always moaned and complained about competition between feral sheep and Stout Iguanas. Turns out I had good reason to worry: see our first article, p. 2.

Photo by Becki Perkins. Cover design by Ben Skopper.

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IRCF REPTILES & AMPHIBIANS
CONSERVATION AND NATURAL HISTORY

Non-overlapping Distributions of Feral Sheep (*Ovis aries*) and Stout Iguanas (*Cyclura pinguis*) on Guana Island, British Virgin Islands

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Abstract.—Stout Iguanas (*Cyclura pinguis*) remain one of the most critically endangered reptiles in the world. Factors contributing to that status include habitat loss, predation by introduced species, and competition with introduced herbivores. On Guana Island, British Virgin Islands, the presence of feral sheep (*Ovis aries*) has been a hypothesized detriment to iguanas. Using motion sensitive cameras, we documented the distribution of feral sheep on Guana Island in 2010. We also quantified the impact of feral sheep on ground vegetation by comparing plant abundance at long-term sheep exclosures and areas where sheep were absent to areas where sheep were present. Finally, we compared sheep distribution to iguana distribution on the island. The co-occurrence of sheep and Stout Iguanas was less than expected, indicating possible competition. Although we detected no difference in vegetative cover between areas where sheep were present and absent, the long-term exclosures showed that the exclusion of sheep allowed the abundance of many plant species to increase. Our data support the hypothesis that feral sheep are altering the abundance of ground-level vegetation and limiting iguana distribution on the island.

Five principal factors contribute to species endangerment: Natural causes, over-hunting, introduced predators, non-predatory invasives, and habitat alteration (Fisher et al. 1969). Hunting, predator introduction, and habitat alteration have received considerable attention in both the popular and scientific press. The more subtle but no less profound effects of non-predatory invasive species such as herbivores have received less attention. Introduced herbivores may outcompete native species for resources or negatively affect them by altering the habitat (Lowney et al. 2005). Herbivorous reptiles appear to be particularly sensitive to the effects of introduced herbivorous mammals. As an example, Cuban Ground Iguanas (*Cyclura nubila*) now compete with deer (*Odocoileus* spp.) and feral goats (*Capra hircus*) at Guantanamo Bay, Cuba (Roca and Sedaghatkish 1998). That competition forced iguanas to move farther while foraging and juveniles to disperse greater distances and suffer greater mortality. Similarly, Stout Iguanas (*C. pinguis*) altered their diet and declined in numbers in response to feral livestock grazing on Anegada Island, British Virgin Islands (BVI; Mitchell 1999). Feral livestock

Fig. 1. The distribution of Stout Iguanas (*Cyclura pinguis*) and feral sheep on Guana Island (British Virgin Islands) is largely disjunct. Photograph by Robert Powell.

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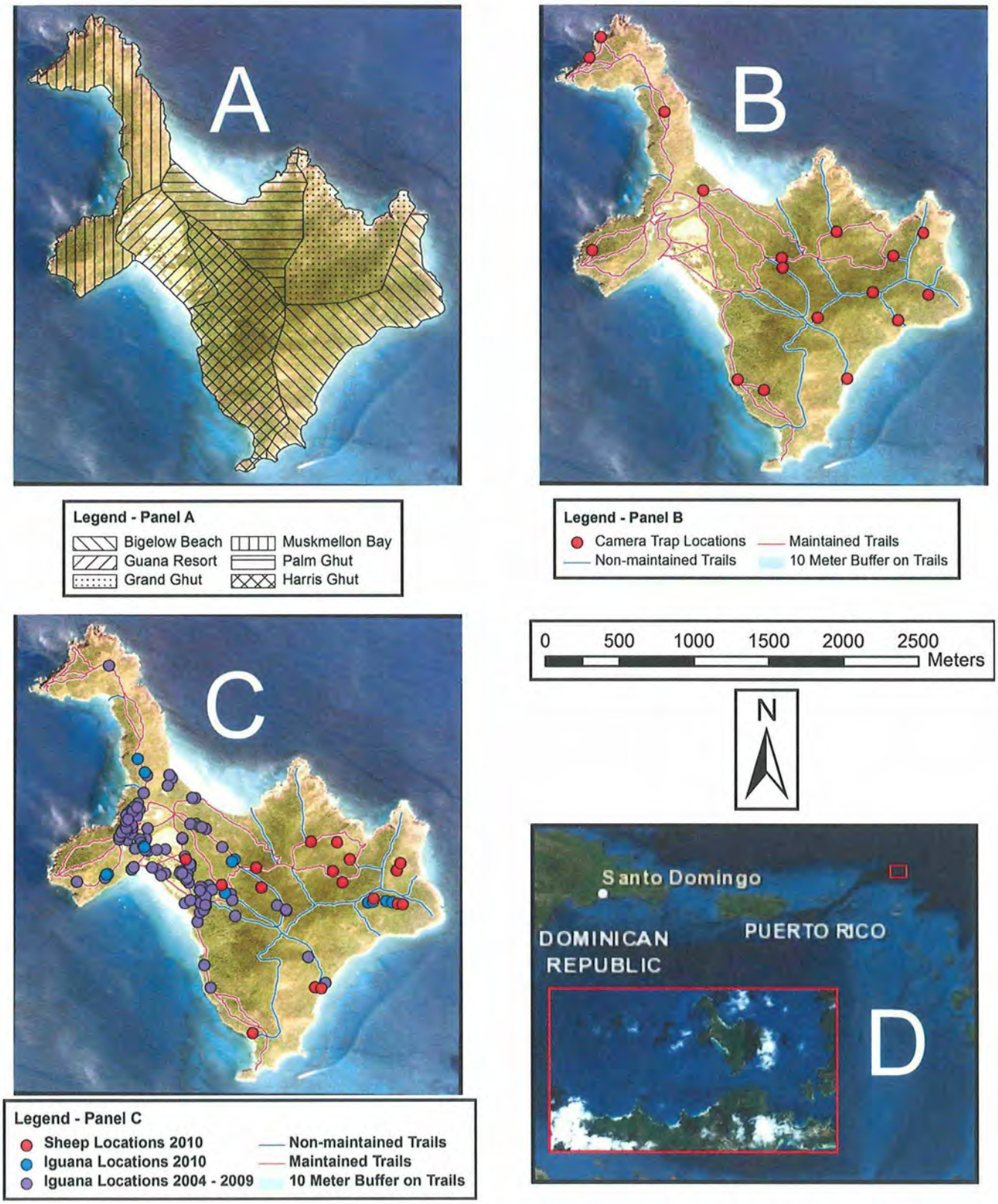

Fig. 2. Sampling effort of: (A) Subdivision of Guana Island into 6 units; (B) locations of cameras traps (red dots) along the trail system (red = maintained trails, blue = non-maintained trails, light blue = 10 m buffer of all trails) of Guana Island; (C) 2010 locations of encountered sheep (red dots), 2010 locations of encountered iguanas (blue dots), 2004–2009 locations of iguanas; (D) location of Guana Island within the greater Caribbean region. Note: scale bar for figures A, B, and C only.

Table 1. Ground cover composition at locations where sheep were and were not detected. "Green vegetation" represents pooling of all living plant material.

		Mean ± SD*	n**	%***
Sheep Absent	Green Vegetation	1.05 ± 3.55	25	4
	Litter	73.76 ± 18.56	25	74
	Rock and Soil	15.60 ± 15.60	25	22
Sheep Present	Green Vegetation	2.93 ± 7.69	50	12
	Litter	73.64 ± 14.43	50	74
	Rock and Soil	7.32 ± 9.33	50	14

* Mean ± SD of encounters of each ground cover type per 100 sample points.

** Number of forest floor photos analyzed. Each photo had 100 sample points.

*** Percentage of cover type with all samples pooled.

also has been shown to be responsible for negative effects on other species of rock iguanas (Lemm and Alberts 2012).

The Stout Iguana is listed as Critically Endangered and Endangered by the IUCN (2004) and the U.S. Fish and Wildlife Service (1999), respectively. By the 1980s, Stout Iguanas were known to occur only on Anegada Island, where they were in rapid decline (Mitchell 1999). Concern for the species' persistence prompted the translocation of eight individuals from Anegada to Guana Island, BVI. A decade later, Goodyear and Lazell (1994) found that the Guana population (Fig. 1) was persisting, but had not achieved an island-wide distribution. Goodyear and Lazell (1994) suggested that competition with feral sheep (*Ovis aries*), still found on Guana Island despite several eradication attempts (Lazell 2005), might have been the cause of the limited expansion by Stout Iguanas. The iguana population has grown considerably (Perry and Mitchell 2003), but a disjunction between Stout Iguana and sheep distributions appears to remain (Anderson et al. 2010). Further, previous researchers have noted the existence of a browse line where sheep are common (G. Perry and C. Boal, pers. obs.). Nonetheless, no concerted effort has previously been made to compare the distributions of the iguana and sheep on the island. We therefore sought to quantify the distribution of both Stout Iguanas and feral sheep on Guana Island to determine if the two species' distributions are indeed non-overlapping. In addition, we sought to quantify the impacts of sheep browsing on island vegetation. Effects of sheep on the vegetation would provide a mechanistic explanation to support the hypothesis that feral sheep are negatively influencing iguana distributions.

Methods

Guana Island is a privately owned 340-ha island located less than 1 km north of Tortola, BVI (Fig. 2D). The island functions as a resort, although much of it is undeveloped, mostly free of human disturbance, and covered in dry tropical forest. Lazell (2005) provided a detailed overview of the island's natural history.

We subdivided Guana Island into six units (Fig. 2A) using ArcGIS 9.2 (ESRI 2006, Redlands, California). Four of the six units (Bigelow Beach, Grand Ghut, Harris Ghut, and Palm Ghut) are natural watersheds. The Guana Resort was defined as the area of the island receiving heavy human traffic. The remainder of the island was pooled into the Muskmellon Bay unit. We created a digital model of Guana Island consisting of 309 100 x 100-m grid cells (Fig. 3A). Steep terrain prevented us from sampling 168 of the 309 grid cells (Fig. 3B), and we do not consider these areas further. Based on field observations (see below), each grid cell was coded as having sheep, iguanas, neither, or both. The amount of overlap between sheep and iguanas was determined by comparing the number of grid cells with occurrence of both species to what would be expected (i.e., joint probability) from the portion of cells occupied by sheep and by iguanas.

We used seventeen motion sensitive cameras (Reconyx model RM30, Holmen, Wisconsin) to passively sample feral sheep and Stout Iguanas. In October 2010, within 10 m of the existing trail system of the island (Fig. 2B), we used a random number generator to determine possible camera placements. The number of camera locations placed in each of the six pre-determined units was determined by the relative size of each unit: Bigelow Beach, Grand Ghut, and Muskmellon Bay each received four cameras, Palm Ghut received three cameras, and Harris Ghut received two cameras (Fig. 2B). We did not place any cameras within the Guana Resort unit, as island staff informed us that the level of human traffic precludes the occurrence of sheep. Cameras were attached to trees 1 m above ground, orientated to provide the least

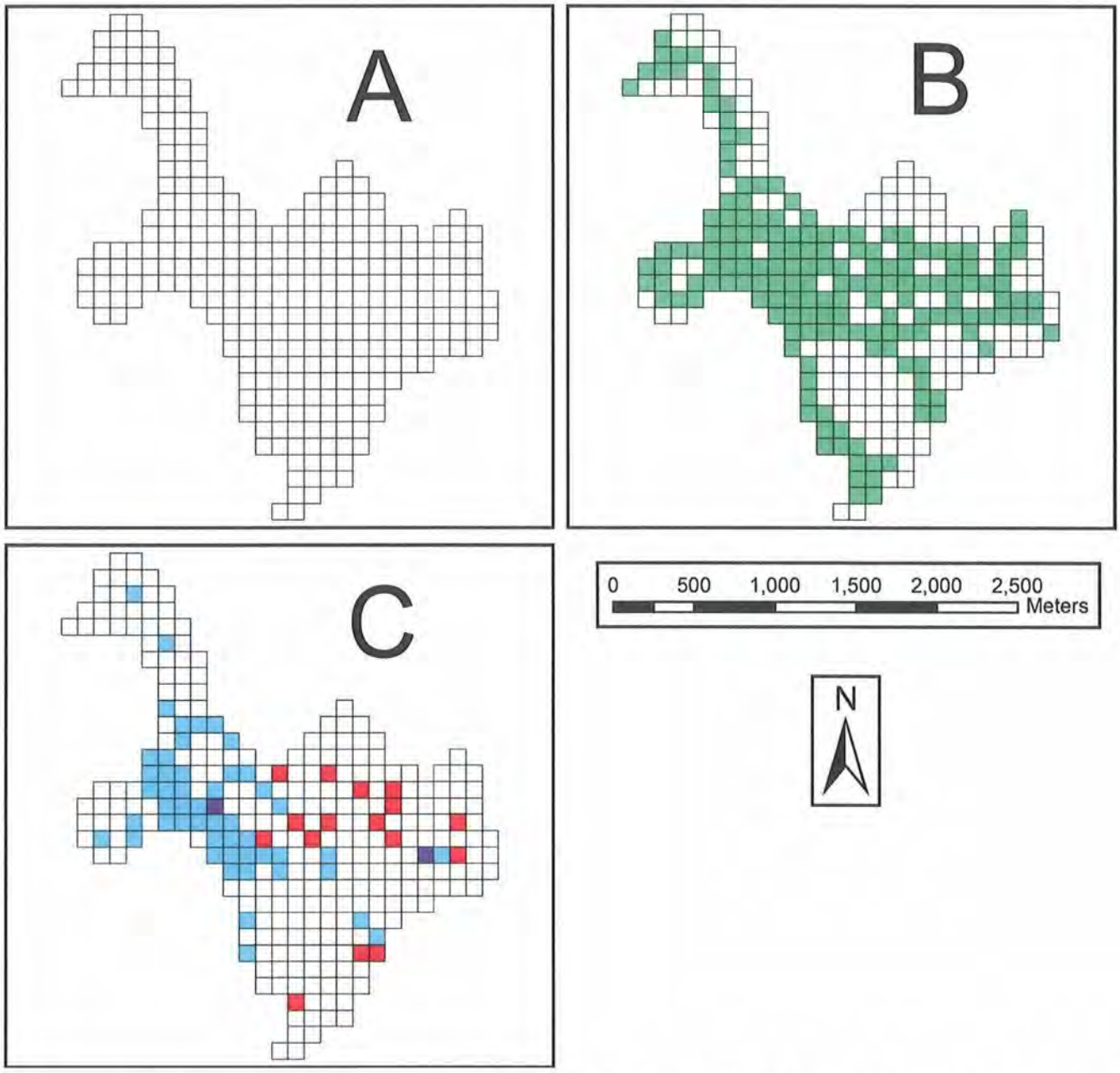

Fig. 3. (A) Subdivision of Guana Island into 309 100 x 100 m cells; (B) sampled cells (green); (C) sampled cells containing sheep (red), iguanas (blue), and sheep and iguanas (purple).

obstructed view, and programmed to record for three days. They then were moved to a new, pre-determined location. Additionally, we recorded the locations of chance encounters of sheep and iguanas during repeated hikes throughout the island. Indirect evidence of sheep presence, such as scat and sound, also were recorded. For iguana distributions, we incorporated all 159 previously recorded (2004–2009) locations (G. Perry, unpubl. data).

We assessed vegetation density by measuring vertical vegetative visual obstruction (hereafter, visual obstruction) at camera-trap locations. Using a 1-m Robel pole (Robel et al. 1970) segmented into 10-cm bands, we recorded visual obstruction at a distance of 1.8 m from the pole in each of the cardinal directions to the nearest 25%. To quantify ground cover, we took digital photographs of the forest floor at the pole location and 1.8 m from it in each of the cardinal directions. Photographs were taken from a height of 1 m. We analyzed photographs using SamplePoint (Booth et al. 2006), which superimposes 100 regularly spaced points on each photograph. At each point we recorded the cover type: Vegetation, litter, or open soil/rock. Wet conditions, such as those experienced by the BVI in the months just before

Table 2. Common plant species inside and outside of exclosures on Guana Island.

Species*	Common name**	Family	Growth habit***
Amyris elemifera	Sea Torchwood	Rutaceae	TR/SH
Bursera simaruba	Gumbo Limbo	Burseraceae	TR/SH
Capparis spp.	Caper	Capparaceae	TR/SH
Eugenia spp.	—	Myrtaceae	TR/SH
Guapira fragrans	Black Mampoo	Nyctaginaceae	TR/SH
Krugiodendron ferreum	Leadwood	Rhamnaceae	TR/SH
Macfadyena unguis-cati	Catclaw Vine	Bignoniaceae	VI
Opuntia repens	Roving Pricklypear	Cactaceae	SS/SH
Tragia volubilis	Fireman	Euphorbiaceae	VI/FB

* Taxonomy from Lazell (2005)

** Common names from USDA NRCS (2013)

*** Growth habit from USDA, NRCS (2013). FB = forb/herb, SH = shrub, SS = subshrub, TR = tree, VI = vine

our study (G. Perry, unpubl. data), can produce high plant densities regardless of browsing by feral sheep. Additionally, sheep are likely to be attracted to locations where vegetation is available. Thus, simple comparisons of locations with and without sheep could provide uninformative results. We therefore supplemented our findings with numbers obtained from two fenced sheep exclosures on the island and their paired, un-fenced control sites. These exclosures were established in 1997–98 and the abundance of nine plant species was measured following establishment and again in 2004 and 2010 (Table 2). They thus provide a long-term comparison of how sheep could be affecting the vegetation.

We used chi-square tests (Zar 2010) to examine differences between ground cover where sheep were present and absent. To examine differences in visual obstruction, we used *t*-tests to compare values recorded at each 10-cm band of the Robel pole in areas where sheep were present to the corresponding segment where sheep were absent. All statistical analyses were performed with R 2.13.0 (R Development Core Team 2011).

Results

Our cameras recorded sheep at five locations and a single iguana at one location (Fig. 2C). During hiking, we encountered sheep and iguanas (Figs. 4–6) at 12 and 53 other locations, respectively. Of the 168 grid cells sampled, we detected iguanas only in 28.6% (n = 48) of cells, we detected sheep only in 9.5% (n = 16) of cells, and we detected both iguanas and sheep in 1.2% (n = 2) of cells (Fig. 3C). Neither we nor previous researchers detected iguanas within the Grand Ghut watershed, which had the greatest number of sheep detections (Fig. 2C). The observed co-occurrence of iguanas and sheep was less than half the value expected based on the probabilities of sighting either species (2.7% or 5 cells).

At camera-trap locations, ground cover differed significantly between areas where sheep were and were not detected ($\chi^2 = 187.16$, df = 2, $p < 0.001$). The litter component of ground cover did not vary between areas where sheep were and were not detected, but the proportion of green vegetation and rock and soil did, with a greater percentage of green vegetation being observed in areas where sheep were detected (Table 1). Visual obstruction did not significantly differ between locations where sheep were and were not detected by cameras (Fig. 7; $p > 0.05$ in all cases).

Of the nine woody and herbaceous plants monitored in and outside of the exclosures, four species (*Amyris elmifolia*,

Fig. 4. Feral sheep were most often detected by camera traps at night and only on the eastern side of the island.

Fig. 5. Immature Stout Iguanas were most commonly encountered near the Guana Resort. This individual was marked with white paint to facilitate identification during a concurrent study. Photograph by Ben Skipper.

Bursera simarubra, *Capparis* spp., and *Tragia volubilis*) clearly increased in abundance when sheep were excluded (Fig. 8). Two other species (*Krugiodendron ferreum* and *Macfadyena unguis-cacti*) displayed stronger increases in abundance inside exclosures compared to outside, although some overlap in standard deviations exists (Fig. 8). *Eugenia* spp. and *Guapira fragrans* abundance seemed less affected by the exclosures, although trends show both increasing inside the exclosures (Fig. 8). One species, *Opuntia repens*, remained approximately stable over the 10-year observation period inside the exclosures, but declined sharply outside of exclosures. No monitored species declined in the exclosures when compared to control plots.

Discussion

Since their re-introduction almost 30 years ago, Stout Iguanas have established a self-sustaining population on Guana Island (Goodyear and Lazell 1994, Perry and Mitchell 2003, Anderson et al. 2010). However, prior researchers (Goodyear and Lazell 1994, Anderson et al. 2010) hypothesized that competition with feral sheep for available browse may limit iguana distribution on the island. Our data support this hypothesis. Iguanas and sheep are much less likely to co-occur than would be expected, suggesting that occurrence of sheep in some of the eastern portions of the island precludes iguana presence. We did encounter several iguanas (both adults and juveniles) at the eastern end of the island, where they had not previously been seen. We believe this represents a wider search effort, but it could represent an expansion of the population compared to the surveys of Goodyear and Lazell (1994) and Anderson et al. (2010).

A possible explanation for the lack of overlap between iguanas and sheep, consistent with Mitchell's (1999) observations on Anegada and studies of other species in the genus

Fig. 6. Large, mature Stout Iguanas were rarely encountered far from the Guana Resort. Photograph by Rebecca Perkins.

Cyclura (Lemm and Alberts 2012), is reduction in available forage for iguanas due to browsing by feral sheep. Although previous researchers (W. Anderson, pers. comm.) have observed a prominent browse line in areas occupied by sheep, we detected no difference in visual obstruction between areas with and without sheep detections. Possibly, the 1.8-m distance from which we recorded visual obstruction was insufficient to assess accurately the effects of browsing. More importantly, perhaps, Guana Island received above-average precipitation in the months before our study (G. Perry, pers. obs.), which could have allowed the vegetation to recover from browsing pressure. Guana Island experienced drought in 2009, which could have rendered the effects of browsing more pronounced, whereas in 2010, high rainfall may have rendered signs of browsing unobservable. Consistent with that interpretation, browse damage was obvious again in 2011, another dry year (G. Perry, pers. comm.).

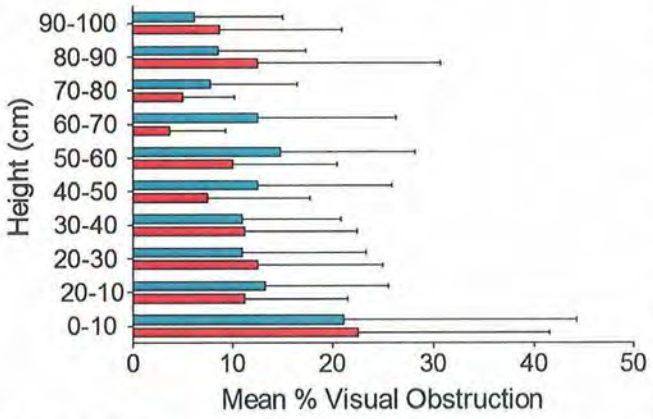

Fig. 7. Mean (± SD) percent visual obstruction measured of vegetation at camera trap locations, red bars indicate camera trap locations where sheep were detected; blue bars indicate areas where sheep were not detected.

We did not find differences in visual obstruction between camera-trap locations where sheep were and were not documented. Somewhat counterintuitive is that camera-trap locations where sheep were detected had a greater proportion of green vegetation than those where sheep were not detected. However, such differences might not be unexpected for two reasons. First, our study was conducted during a wet spell, when vegetation is relatively lush and regrowth is rapid. Second, sheep are likely to be attracted to available forage or avoid areas denuded of vegetation, and thus may preferentially be found at locations with more remaining vegetation. Our comparisons of sheep exclosures to un-enclosed control

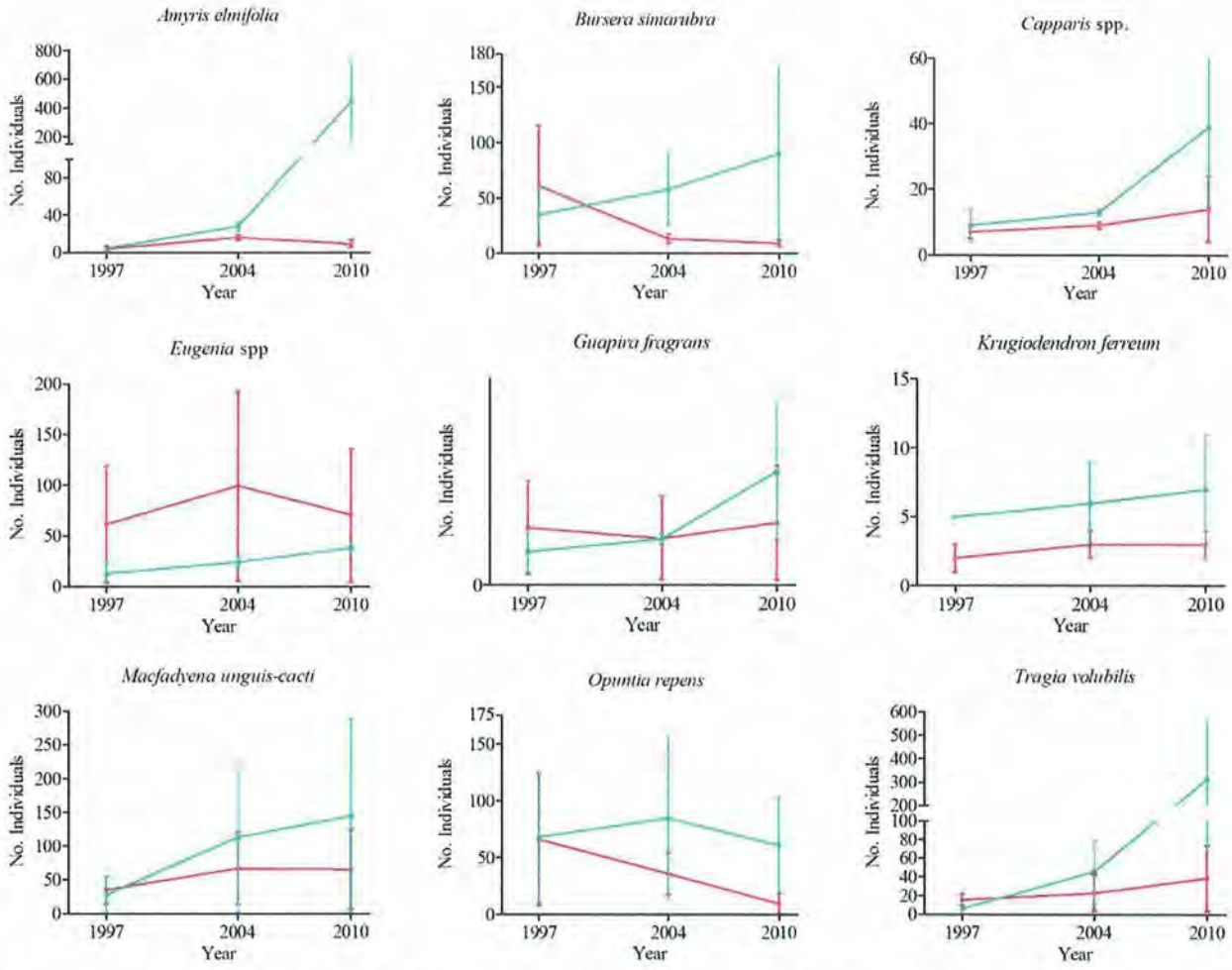

Fig. 8. Mean (± SD) number of individuals of nine plant species monitored from 1997/1998 to 2010. Blue lines represent plants within exclosures. Red lines represent plants outside of exclosures.

plots provided further evidence. We saw marked increases in four plant species, weaker increasing trends in another four species, and no declines inside exclosures.

Our study supports previous suspicions (Goodyear and Lazell 1994, Anderson et al. 2010) that feral sheep limit the distribution of Stout Iguanas on Guana Island. This is a source of concern, as the Guana population is one of the largest populations of the species and its survival may be critical to the long-term existence of *C. pinguis*. Although our short-term assessment of vegetation (assessments at camera-trap locations) did not reveal clear differences in vegetative structure in areas where sheep were and were not detected, assessments at the long-term exclosures did indicate that exclusion of sheep can have a positive effect on the vegetative community. Further exclusion of feral sheep through removal would likely be beneficial to Stout Iguanas by providing an opportunity for more complete expansion of the current distribution into the eastern half of the island. Sheep removal also could be of value to the island's vegetation, some of which is of significant conservation value (Procter and Fleming 1999, Lazell 2005). Other species that depend on the vegetation, such as invertebrates and birds, also could be affected positively by such management practices.

Acknowledgements

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Literature Cited

Alberts, A. 2000. *West Indian Iguanas: Status Survey and Conservation Action Plan.* World Conservation Union, Cambridge, UK.

Anderson, W.M., G.E. Sorensen, J.D. Lloyd-Strovas, R.J. Arroyo, J.A. Sosa, S.J. Wulff, B.D. Bibles, C.W. Boal, and G. Perry. 2010. Distribution and habi-

tat use by the critically endangered Stout Iguana (*Cyclura pinguis*) on Guana Island, British Virgin Islands. *Reptiles & Amphibians* 17:158–165.

Booth, D.T., S.E. Cox, and R.D. Berryman. 2006. Point sampling digital imagery with 'SamplePoint.' *Environmental Monitoring and Assessment* 123:97–108.

Fisher, J., N. Simon, and J. Vincent. 1969. *Wildlife in Danger.* Viking Press, New York.

Goodyear, N. and J. Lazell. 1994. Status of a relocated population of endangered *Iguana pinguis* on Guana Island, British Virgin Islands. *Restoration Ecology* 2:43–50.

IUCN. 2004. *Red List of Threatened Animals.* International Union for the Conservation of Nature and Natural Resources, Gland, Switzerland.

Lazell, J. 2005. *Island: Fact and Theory in Nature.* University of California Press, Berkeley.

Lemm, J. and A.C. Alberts. 2012. *Cyclura: Natural History, Husbandry, and Conservation of West Indian Rock Iguanas.* Academic Press, London, UK.

Lowney, M.S., P. Schoenfeld, W. Haglan, and G.W. Witmer. 2005. Overview of impacts of feral and introduced ungulates on the environment in the eastern United Stated and Caribbean, pp. 64–81. In: D.L. Nolte and K.A. Fagerstone (eds.), *Proceedings of the 11th Wildlife Damage Management Conference.* Wildlife Damage Management, Internet Center for DigitalCommons@ University of Nebraska - Lincoln.

Mitchell, N. 1999. Effect of introduced ungulates on density, dietary preferences, home range, and physical condition of the iguana (*Cyclura pinguis*) on Anegada. *Herpetologica* 55:7–17.

Perry, G. and N. Mitchell. 2003. Guana and Necker island population assessments 2002. *Iguana* 10:49.

Procter, D. and L.V. Fleming (eds.). 1999. *Biodiversity: The UK Overseas Territories.* Joint Nature Conservation Committee Peterborough, UK.

R Development Core Team. 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria (http://www.R-project.org/).

Robel, R.J., J.N. Briggs, A.D. Dayton, and L.C. Hulbert. 1970. Relationships between visual obstruction measurements and weight of grassland vegetation. *Journal of Range Management* 23:295–297.

Roca, E. and G. Sedaghatkish. 1998. *Rapid Ecological Assessment. U. S. Naval Station Guantanamo Bay, Cuba.* The Nature Conservancy, Bethesda, Maryland.

USDA, NRCS. 2013. The PLANTS Database. National Plant Data Team Greensboro, North Carolina (http://plants.usda.gov).

U.S. Fish and Wildlife Service. 1999. Endangered and Threatened Wildlife and Plants 50 CFR 17.11 and 17.12. Division of Endangered Species, U.S. Fish and Wildlife Service, Washington, D.C. (http://www.fws.gov/endangered/wildlife.html).

Zar, J.H. 2010. *Biostatistical Analysis.* 5th ed. Prentice Hall, Upper Saddle River, New Jersey.

DRAFT

Effects of perch diameter and hindlimb length on clinging performance in *Anolis* lizards from the British Virgin Islands

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Abstract

Introduction

Morphology affects many aspects of performance in *Anolis* lizards including running, jumping, biting and clinging (reviewed in Losos 2009). Previous studies of clinging performance in *Anolis* show a positive relationship between toepad size and clinging ability on smooth, flat surfaces (e.g., Irschick et al. 1996; Elstrott and Irschick 2004). Larger lamellae, the expanded subdigital scales that comprise the toepad, contain more setae, the microscopic structures that adhere to surfaces through van der Waals forces (Autumn and Peattie 2002; Autumn et al. 2002), and more setae generate more force. Because anoles in nature use substrates that vary in roughness and diameter, other aspects of morphology may influence clinging ability in addition to lamellae. Studies of other lizard species indicate claws, limbs, tendons, and toes also influence clinging performance (Zani 2000; Tulli et al. 2009, 2011). For example, in a diverse group of 68 species of New World lizards including some *Anolis* species, Zani (2000) found wider toes and more lamellae increased clinging performance on smooth surfaces, whereas thinker claws and shorter toes were better suited for rough substrates. How aspects of morphology other than toepads affect clinging ability in *Anolis* warrants further study.

In *Anolis* lizards, limb length shows a strong relationship with locomotor performance with longer-limbed species running faster on broad substrates (Losos 2009). This relationship changes across perch diameters with sprint speed decreasing on narrower perches for most species and longer-limbed species showing a greater decrease in sprint speed (Losos and Sinervo 1989; Spezzano and Jayne). As with locomotor performance, clinging ability likely varies with perch diameter and hindlimb length. *Anolis* species that use broader diameter perches tend to have larger toepads (Macrini et al. 2003). A better understanding of this morphology-performance relationship is critical for evaluating the functional significance of morphological variation (Losos 1990; Wainwright 1994; citations). Trait variation must translate into performance variation for natural selection to operate, and ecologically relevant measures of performance are important for evaluating adaptive hypotheses (Arnold; Irschick 2003).

In this study, we measure clinging performance of three *Anolis* species on using an ecologically relevant, whole-organism approach. Given the relationship between limb length and locomotor performance in *Anolis* lizards (e.g., Losos and Sinervo 1989; Macrini and Irschick 1998), we are interested if a similar relationship exists between limb length and clinging performance and if this relationship differs between perch diameters. We predict based on previous work in other lizards that clinging force will increase on narrower perches (Losos et al. 1993) and for longer hindlimbs (citations). An understanding of how variation in a trait affects multiple measures of performance, and therefore multiple potential avenues of natural selection, is critical to interpreting adaptive hypotheses.

Methods

Study System

We collected three species of *Anolis* lizards for this study on Guana Island, British Virgin Islands, in October 2012. All three species are distributed throughout the Greater Puerto Rican Bank from mainland Puerto Rico to the U.S. and British Virgin Islands. On Guana Island, these species occur in natural forest and disturbed areas, such as gardens, orchards, and landscaping around buildings and trails (Lazell 2005). They are diurnally active and mostly insectivorous lizards. *Anolis cristatellus* is a small to medium sized arboreal lizard in the 'trunk-ground' ecomorph category (Williams 1983; Losos 2009). It is typically active on the ground and on tree trunks up to 2 m high, and shows strong sexual size dimorphism with males being larger than females (Butler). *Anolis stratulus* is a 'trunk-crown' ecomorph and on average perches higher on trunks than *A. cristatellus.* This species is smaller than *A. cristatellus* and sexual size dimorphism is weak with males and females of similar body size. *Anolis pulchellus* is a 'grass-bush' ecomorph that perches somewhat lower than the other two species often using denser vegetation. Males and females are similar in body size. Both *A. cristatellus* and *A. pulchellus* have relatively long limbs for their body size compared to the shorter limbed *A. stratulus* (Losos 2009).

Habitat use and Morphology

Most lizards encountered during this study were perched on vegetation, although all species were observed on the ground at times. Based on their ecomorph category, we predicted these three species would differ in their structural habitat use with *A. stratulus* using the highest and *A. pulchellus* the lowest perches, respectively, and *A. pulchellus* using narrower perches compared to both *A. cristatellus* and *A. stratulus*. We measured perch height and perch diameter for every undisturbed, adult lizard observed.

For each individual used in the performance trials, we recorded species and sex, and measured hindlimb length and snout-vent length (SVL) with a ruler to the

nearest 0.5 mm. Hindlimbs were measured from the insertion of the limb into the body wall to the tip of the claw, and SVL from the tip of the snout to the cloaca.

Performance

We measured the force necessary to pull a lizard off of a perch. Field-caught lizards were fitted with a harness around their midsection located half way between their fore- and hindlimbs. Lizards were positioned so their limbs were wrapped around the perch rather than held close to their body. This increased maximal clinging force in practice trials and likely better replicates the posture of a lizard attempting to hold on to a perch during a storm or a predation attempt. We attached the harnessed lizard to a digital force gauge (Extech Model 475040; accuracy 0.01 newtons), which we set on peak force mode. We then moved the perch and lizard at a constant speed away from the force gauge such that the perch and lizard were perpendicular to the sensing head of the force gauge. We tested each lizard on perches of two diameters using smooth dowels of 12 and 33 mm. Each lizard was tested at least three times on each diameter to determine maximal force. Trials in which lizards did not perform or clearly underperformed were discarded (Losos et al. 2002). The same person (JJK) conducted all trials to maintain consistency. The highest force measurements on each of the two diameters were used in subsequent statistical analyses.

Statistical analyses

We did not include *A. pulchellus* in statistical analyses due to its small sample size (n=6). All variables were log-transformed prior to analyses. Perch height and diameter use differences between species, sexes, and their interaction were evaluated using separate ANOVAs. We tested for a difference in hindlimb length between species, sexes, and their interaction using analysis of covariance (ANCOVA) with SVL as a covariate. The three-way interaction involving species, sex, and SVL was nonsignificant and removed from the final model. Because we used the same individuals to measure clinging force on both 12 and 33 mm diameter dowels, we used repeated-measures ANCOVA with hindlimb length as a covariate. This model tested for between-subjects effects of species (*A. cristatellus* and *A. stratulus*) and sex (male and female) as well as their interaction, within-subject effects of diameter (12 and 33 mm), and interactions between diameter and species, sex, and species by sex. Interactions involving the covariate hindlimb length were nonsignificant and removed from the final model except for the diameter by hindlimb length interaction.

We also conducted a size-adjusted analysis using the residuals from the hindlimb length-SVL regression to determine if relative hindlimb length (as opposed to overall size) affects clinging performance. Given differences between species and sexes in the relationship between hindlimb length and SVL (Tables 1 and 2), we calculated residuals separately for each species-sex combination. We used these residuals in separate repeated measures ANCOVAs to test for a within-subject effect of diameter (12 and 33 mm).

Results

As predicted by their ecomorph category, *A. stratulus* perched higher than *A. cristatellus*, and within each species males perched higher than females (Tables 1 & 2). *Anolis stratulus* perched on broader vegetation compared to *A. cristatellus*, but there was no difference in perch diameter between males and females (Tables 1 & 2). Although not included in these statistical analyses, *A. pulchellus* used lower and narrower perches compared to the other two species, as expected for a grass-bush ecomorph species (Table 1). The relationship between hindlimb length and SVL varied between species and sexes, and *A. cristatellus* had relatively longer hindlimbs compared to *A. stratulus* and males had longer hindlimbs than females (Fig. 1, Table 3). The few *A. pulchellus* sampled had relative hindlimb lengths similar to *A. stratulus* (Fig. 1).

Clinging force differed significantly between species and diameters (Table 4). Across all species-sex combinations, lizards generated greater force when tested on the narrower 12-mm dowel. Furthermore, male *A. cristatellus* generated substantially greater clinging forces compared to the other species and female *A. cristatellus* (Fig. 2). The relationship between clinging force and hindlimb length differed between diameters, being more similar for all species-sex combinations at 12 mm as compared to 33 mm (Table 4, Figs. 3-4). On the broader dowel diameter, clinging force remained relatively low up to a hindlimb length of approximately 40 mm at which point the increase in clinging force per unit of hindlimb length increased substantially. Only male *A. cristatellus* had hindlimb lengths in this range. Significant diameter by species and diameter by sex interactions were also detected, supporting the large difference in clinging force found between male *A. cristatellus* and the other groups. Hindlimb length in these previous analyses could be an indicator of an overall size effect and not hindlimb length *per se*. Size-adjusted analyses showed a relationship between clinging force and relative hindlimb length for *A. stratulus* only, and the relationship between clinging force and relative hindlimb length differed by diameter for male *A. stratulus* (Table 5).

Discussion

Clinging force differs by diameter, hindlimb length, sex, and species for the *Anolis* lizards in this study. When on the 33-mm dowel, clinging force is reduced on average 31% for male *A. cristatellus*, 46-48% for female *A. cristatellus* and *A. stratulus*, and 59-68% for *A. pulchellus* (Fig. 2). Clinging ability is greater in general with longer hindlimbs, for males, and for *A. cristatellus* (Figs 2-4), but these relationships are confounded. For example, male *A. cristatellus* have much longer hindlimbs compared to the other groups (Fig. 1). When adjusted for body size, relative hindlimb length significantly affects clinging force in *A. stratulus* only (Table 5). Thus, the relationship between clinging force and hindlimb length in *A. cristatellus* may reflect variation in overall size or some unmeasured traits, such as toepad size or number of lamellae.

Most individuals experienced a reduction in clinging force on the broader diameter dowel (Fig. 2). It was only the largest male *A. cristatellus* that showed little difference in clinging ability between dowel diameters. When extrapolating to diameters larger than those used here and more similar to the perches used by these species in nature (Table 1), we would expect clinging ability to decrease further. But why then do lizards occupy such broad perches when clinging performance is so poor? There are at least two explanations. First, broad perches are mostly trees with rough bark in nature; thus, claws become a more important factor in clinging ability (Zani 2000). Most previous studies on *Anolis* isolate the performance of toepad lamellae on smooth surfaces (Irschick et al. 1996; Elstrott and Irschick 2004). Whether claws, and possibly lamellae, improve clinging performance on rough substrates that *Anolis* lizards typically use has not been tested directly. Second, locomotor performance may be more important than clinging ability when on broad perches. In contrast to clinging force, sprint speed increases with perch diameter (Losos and Sinervo 1989; Marcrini and Irschick 1998). A similar trade-off exists between clinging ability and sprint speed with perch diameter in two species of chameleons (Losos et al. 1993). A better understanding is needed for how morphological variation affects these two measures of performance in *Anolis* lizards, and whether habitat use and behavior influence performance ability in nature (Irschick and Losos). The selective pressures influencing these morphology-performance-habitat use relationships are not well known (Elstrott and Irschick 2004; Losos 2009). Clinging ability could be related to numerous factors including aggressive interactions, predation pressure, risk of falling, use of smooth surfaces, and tropical storms. Disentangling these potential selective forces, and the contribution of morphological trait variation to clinging performance, is a challenging task.

A previous study showed *A. cristatellus* had about twice the toepad area and clinging force of *A. pulchellus*, which had the weakest clinging ability out of the 12 *Anolis* species in that study (Elstrott and Irschick 2004). Unfortunately, *A. stratulus* was not included in that study, but in this study, *A. stratulus* had greater clinging force compared to female *A. cristatellus* (Figs. 2 & 3) despite having relatively shorter hindlimbs over a similar range of body sizes (Fig. 1). Claws are unlikely to contribute substantially to clinging force on the smooth dowels used in this study, but larger toepads or more lamellae could account for the performance difference observed here. Toepad area data are not available for *A. stratulus*, but the other trunk-crown species on Puerto Rico, *A. evermanni*, has larger toepads and greater clinging ability compared to *A. cristatellus* (Elstrott and Irschick 2004). Future studies should evaluate the effect of multiple morphological traits on clinging performance. In fact, Irschick et al. (1996) found approximately 50% of the variation in clinging ability among diverse pad-bearing lizards remained unexplained after removing the effects of body size, indicating that factors in addition to toepad area influence clinging ability.

Clearly, studies are needed to simultaneously evaluate the contribution of toepad lamellae, toe and limb morphology, and claws to clinging performance, and if the relative importance of these morphological variables varies on different substrate types (e.g., roughness and diameter). Furthermore, traits may be subject to multiple selective forces that vary over time and space. Therefore, a thorough understanding of the multiple ways in which a trait functions in an ecological context is needed.

Acknowledgements

Gad Perry, Skip Lazell
...the Falconwood Foundation through a grant to The Conservation Agency."

References

Autumn K, Peattie A. 2002. Mechanisms of adhesion in geckos. *Integrative and Comparative Biology* 42: 1081–1090.

Autumn K, Sitti M, Liang YCA, Peattie AM, Hansen WR, Sponberg S, Kenny TW, Fearing R, Israelachvili JN, Full RJ. 2002. Evidence for van der Waals adhesion in gecko setae. *Proceedings of the National Academy of Sciences, USA* 99: 12252–12256.

Elstrott and Irschick 2004

Irschick DJ, Austin CC, Petren K, Fisher RN, Losos JB, Ellers O. 1996. A comparative analysis of clinging ability among pad-bearing lizards. *Biological Journal of the Linnean Society* 59: 21–35.

Irschick DJ 2003 Measuring performance in nature: implications for studies of fitness within populations. Integrative and Comparative Biology 43:396-407.

Losos JB. 1990a. Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecological Monographs* 60: 369–388.

Losos et al. 1993

Losos et al. 2002

Losos 2009.

Macrini TE, Irschick DJ, Losos JB. 2003. Ecomorphological differences in toepad characteristics between mainland and island anoles. *Journal of Herpetology* 37: 52–58.

Spezzano and Jayne

Tulli MJ, V. Abdala, and F.B. Cruz. 2011. Relationships among morphology, clinging performance and habitat use in Liolaemini lizards. J Evol Biol 24:843-855.

Zani P.A. 2000. The comparative evolution of lizard claw and toe morphology and clinging performance. J Evol Biol 13:316-325.

Zani PA. 2001. Clinging performance of the western fence liz- ard, *Sceloporus occidentalis. Herpetologica* 57: 423–432.

Table 1. Sample sizes and means (± SD) for morphological measurements and structural habitat use of the three *Anolis* species separated by sex.

Species	Sex	N	SVL (mm)	Hindlimb Length (mm)	N	Perch Height (cm)	Perch Diameter (mm)
cristatellus	Male	24	59.7±6.9	47.2±5.7	28	117.6±63.7	88.0±78.1
cristatellus	Female	32	45.2±5.2	33.8±3.8	38	65.6±38.3	58.3±55.0
pulchellus	Male	3	41.7±3.2	29.8±1.9	3	34.3±1.2	23.0±15.7
pulchellus	Female	3	40.3±2.1	27.8±1.3	3	18.7±15.4	6.7±2.9
stratulus	Male	24	45.9±2.4	30.3±1.3	24	127.9±59.5	121.2±75.8
stratulus	Female	20	41.8±1.4	27.0±1.0	10	122.6±79.4	99.1±52.9

Table 2. Results of ANOVAs testing for differences between species and sexes in a) perch height and b) perch diameter for *A. cristatellus* and *A. stratulus*.

Factor	*F*	df	*P*
a) Perch height (R^2 = 0.1903)			
Species	4.3571	1, 62	0.0410
Sex	4.1640	1, 62	0.0456
Species by Sex	2.0636	1, 62	0.1559
b) Perch diameter (R^2 = 0.1619)			
Species	11.6193	1, 96	0.0010
Sex	1.6473	1, 96	0.2024
Species by Sex	0.2728	1, 96	0.6026

Table 3. Results of an ANCOVA using snout-vent length (SVL) as a covariate to explore factors affecting differences in hindlimb length for *A. cristatellus* and *A. stratulus*.

Factor	*F*	df	*P*
Species	161.41	1, 88	< 0.0001
Sex	46.77	1, 88	< 0.0001
Species by Sex	3.34	1, 88	0.0711
SVL	43.83	1, 88	< 0.0001
Species by SVL	10.90	1, 88	0.0014
Sex by SVL	4.94	1, 88	0.0287

Table 4. Results of a repeated-measures ANCOVA using hindlimb length as a covariate to explore factors affecting differences in clinging force at two perch diameters: 12 and 33 mm.

Factor	*F*	df	*P*
Between subjects:			
Species	13.76	1, 90	0.0004
Sex	2.25	1, 90	0.1375
Species x Sex	3.30	1, 90	0.0724
Hindlimb Length	77.30	1, 95	< 0.0001
Within subjects:			
Perch Diameter	20.33	1, 95	< 0.0001
Perch Diameter x Species	11.48	1, 95	0.0010
Perch Diameter x Sex	4.73	1, 95	0.0321
Perch Diameter x Species x Sex	0.29	1, 95	0.5929
Perch Diameter x Hindlimb Length	16.59	1, 95	< 0.0001

Table 5. Results of repeated-measures ANCOVAs using the residuals of hindlimb length by SVL regressions to test for a difference in clinging force between the two perch diameters (12 and 33 mm). We conducted separate analyses for each species-sex combination because species and sexes differed in their relationship between hindlimb length and SVL (Table 3).

Factor	***F***	**df**	***P***
a) *cristatellus* - female			
Between subjects:			
Relative Hindlimb Length	2.41	1, 27	0.1324
Within subjects:			
Perch Diameter	100.49	1, 27	< 0.0001
Perch Diameter by Relative Hindlimb Length	0.65	1, 27	0.4285
b) *cristatellus* - male			
Between subjects:			
Relative Hindlimb Length	0.03	1, 22	0.8598
Within subjects:			
Perch Diameter	28.10	1, 22	< 0.0001
Perch Diameter by Relative Hindlimb Length	0.17	1, 22	0.6846
c) *stratulus* - female			
Between subjects:			
Relative Hindlimb Length	8.18	1, 18	0.0104
Within subjects:			
Perch Diameter	42.67	1, 18	< 0.0001
Perch Diameter by Relative Hindlimb Length	0.01	1, 18	0.9560
d) *stratulus* - male			
Between subjects:			
Relative Hindlimb Length	1.84	1, 20	0.1900
Within subjects:			
Perch Diameter	106.63	1, 20	< 0.0001
Perch Diameter by Relative Hindlimb Length	4.81	1, 20	0.0403

Figure Legends

Figure 1. The relationship between snout-vent length and hindlimb length for three species of *Anolis* lizards. Lines on the plot indicate significant linear relationships calculated separately for each species-sex combination.

Figure 2. Differences in mean (± SD) clinging force by species, sex and dowel diameter. See Table 2 for statistical results.

Figure 3. The relationship between hindlimb length and clinging force on two dowel diameters: a) 12-mm and b) 33-mm. Lines on the plot indicate significant linear relationships calculated separately for each species-sex combination.

Fig. 1

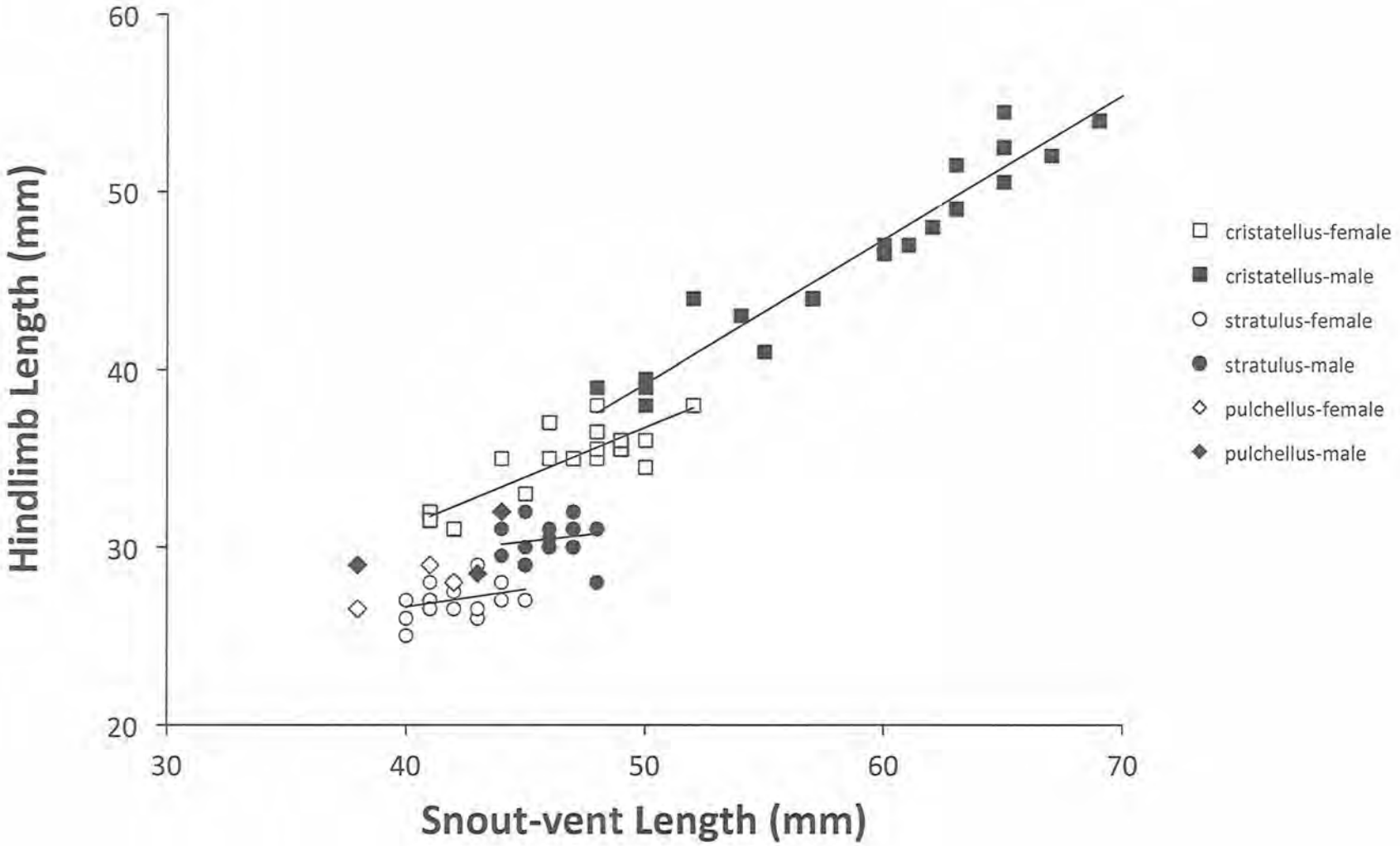

Hindlimb Length (mm)
60
50
40
30
20
30
40
50
60
70
Snout-vent Length (mm)
cristatellus-female
cristatellus-male
stratulus-female
stratulus-male
pulchellus-female
pulchellus-male

Fig. 2

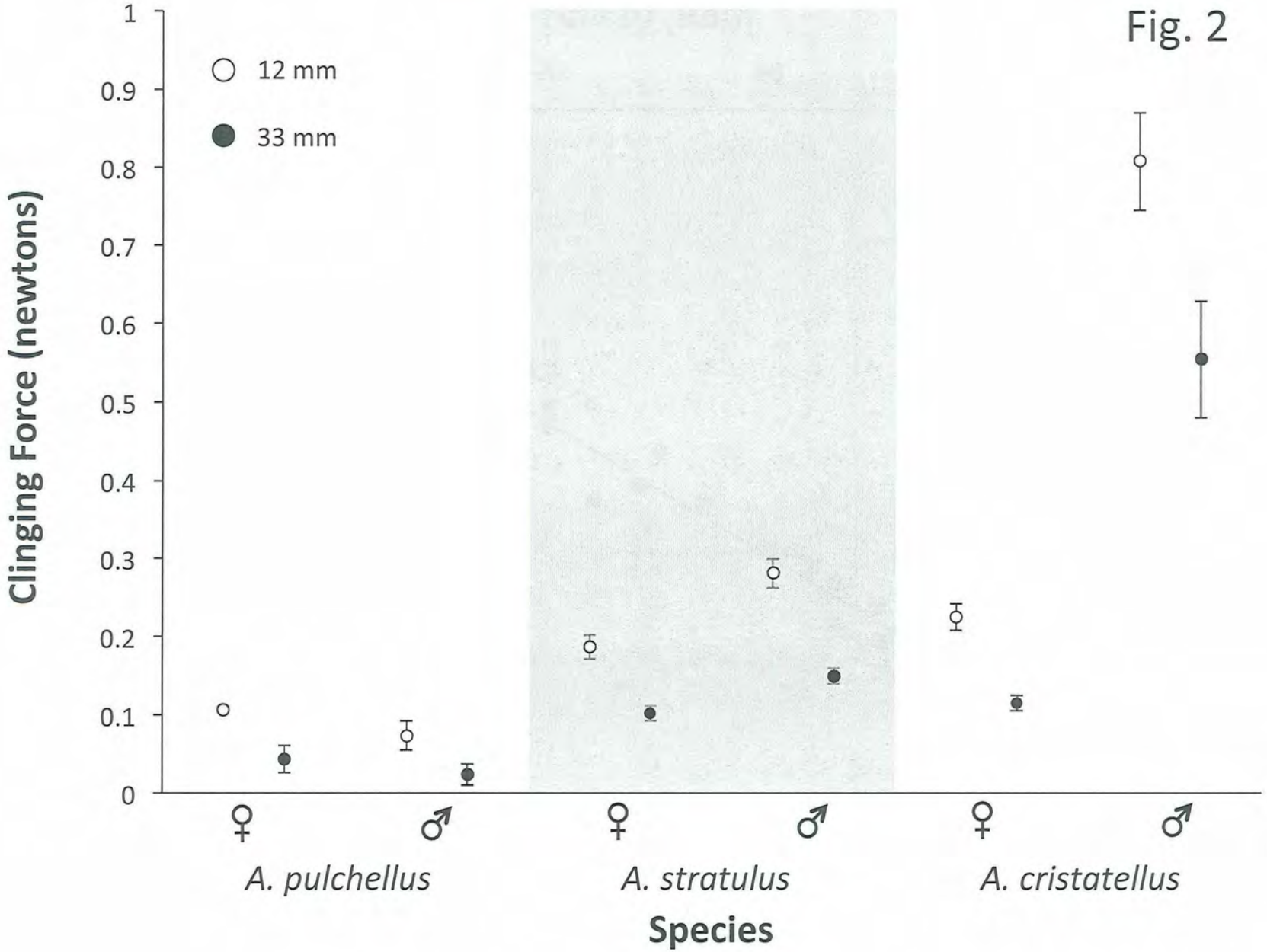

Clinging Force (newtons)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
12 mm
33 mm
♀
♂
♀
♂
♀
♂
A. pulchellus
A. stratulus
A. cristatellus
Species

Fig. 3a

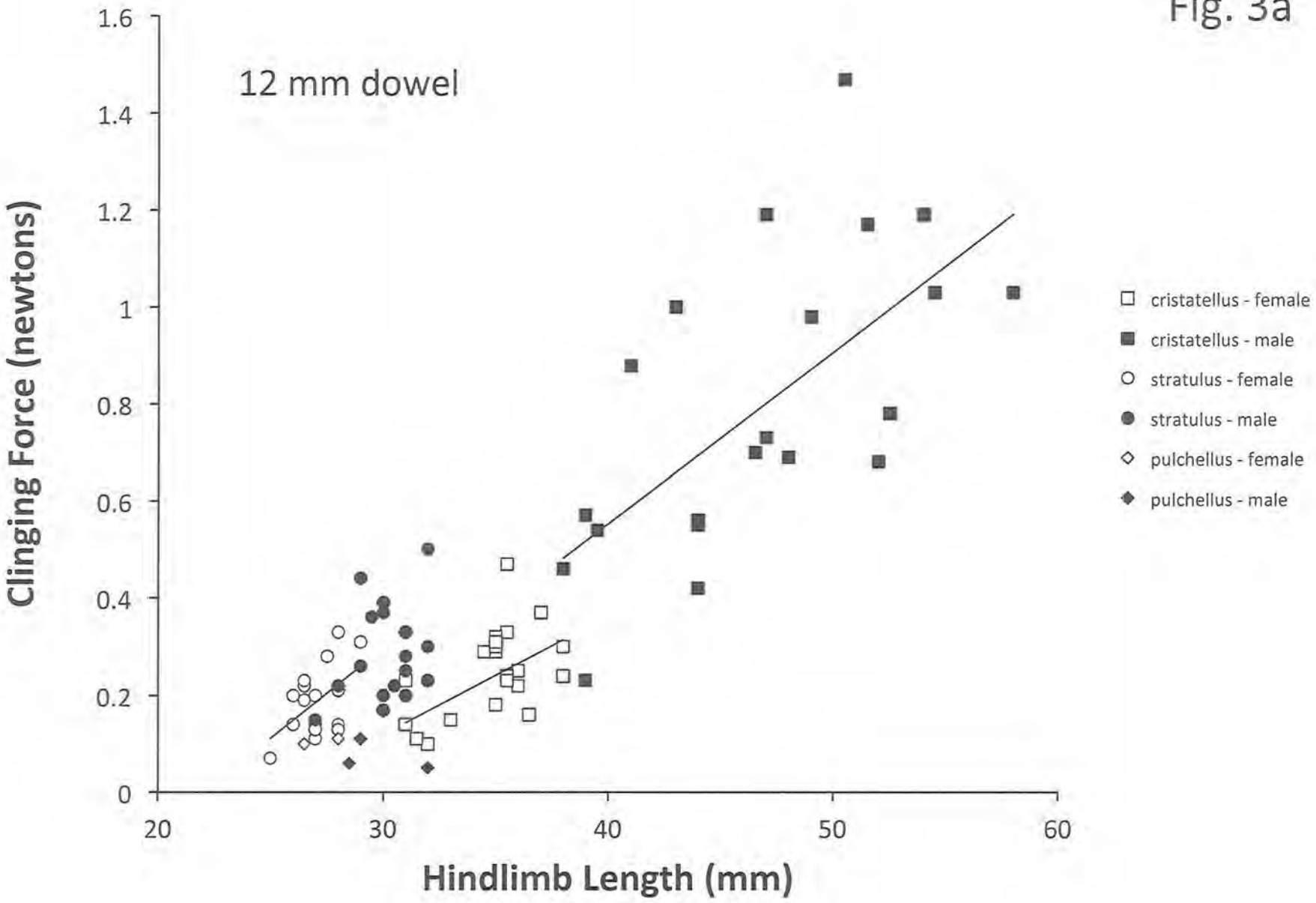
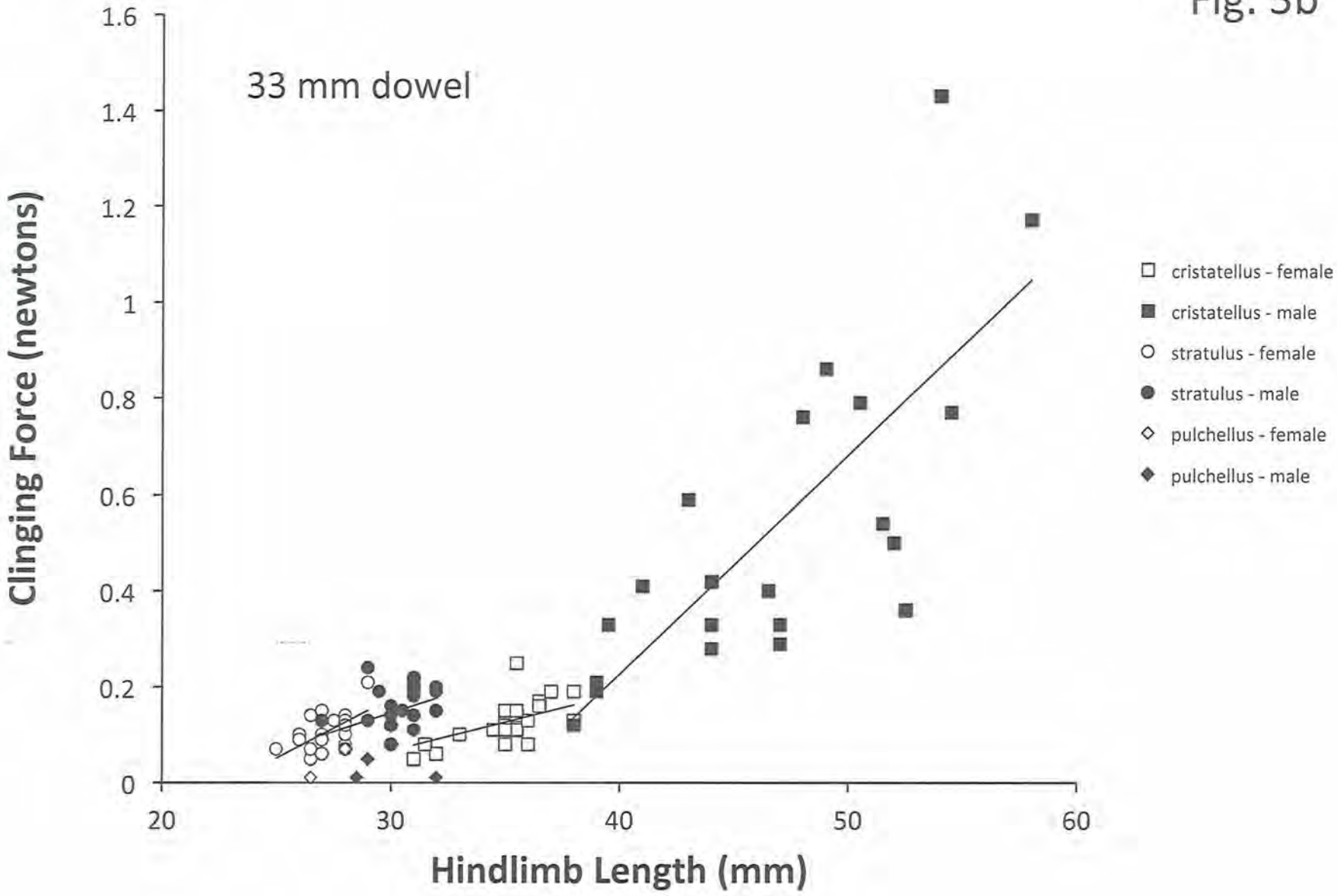

12 mm dowel
Clinging Force (newtons)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
20
30
40
50
60
Hindlimb Length (mm)
cristatellus - female
cristatellus - male
stratulus - female
stratulus - male
pulchellus - female
pulchellus - male

Fig. 3b


33 mm dowel
Clinging Force (newtons)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
20
30
40
50
60
Hindlimb Length (mm)
cristatellus - female
cristatellus - male
stratulus - female
stratulus - male
pulchellus - female
pulchellus - male

Proposal - Guana Island 2013

Based on preliminary results (see Table 1 and enclosed manuscript), I propose to continue the two projects initiated in 2012. First, sampling for the phylogeographic analysis of the three common *Anolis* lizard species (*A. cristatellus, A. stratulus, A. pulchellus*) found on the Greater Puerto Rican bank. *Anolis cristatellus* and *A. stratulus* are well sampled on a few islands (e.g., Guana), but *A. pulchellus* is not well sampled on any island. Molecular genetic data will be collected for this project in collaboration with colleagues at Harvard University and the University of Massachusetts-Boston. Second, I propose to expand the project on clinging force, which found a positive relationship between clinging force and hindlimb length and an effect of substrate diameter on clinging force. I will test how different aspects of morphology (lamellae, claws, limbs) contribute to clinging performance on different substrates (substrate diameter and roughness).

Table 1. Tissue samples collected for three *Anolis* lizard species by island and site in the British Virgin Islands in October 2012.

Island	Anegada	Beef	Cooper	Guana	Guana	Guana	Guana	Jost Van Dyke	Little Thatch
Site				Garden of Eden	Long Man's Point	Orchard	Palm Ghut		
Species									
cristatellus	5	6	7	39	7	29	5	6	7
stratulus	0	0	1	38	3	15	4	1	2
pulchellus	2	0	0	5	0	5	0	0	0

Island	Moskito	Norman	Peter	Salt	Scrub	Tortola	Tortola	Tortola	Virgin Gorda	**Total**
Site						Sage Mt.	Road Town	West End		
Species										
cristatellus	7	12	5	7	4	11	7	1	1	**166**
stratulus	1	4	2	0	0	0	6	0	1	**78**
pulchellus	0	0	0	0	0	0	0	0	0	**12**

MOLECULAR ECOLOGY

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Sea level, topography and island diversity: phylogeography of the Puerto Rican Red-eyed Coquí, *Eleutherodactylus antillensis*

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Abstract

Quaternary climatic oscillations caused changes in sea level that altered the size, number and degree of isolation of islands, particularly in land-bridge archipelagoes. Elucidating the demographic effects of these oscillations increases our understanding of the role of climate change in shaping evolutionary processes in archipelagoes. The Puerto Rican Bank (PRB) (Puerto Rico and the Eastern Islands, which comprise Vieques, Culebra, the Virgin Islands and associated islets) in the eastern Caribbean Sea periodically coalesced during glaciations and fragmented during interglacial periods of the quaternary. To explore population-level consequences of sea level changes, we studied the phylogeography of the frog *Eleutherodactylus antillensis* across the archipelago. We tested hypotheses encompassing vicariance and dispersal narratives by sequencing mtDNA (*c.* 552 bp) of 285 individuals from 58 localities, and four nuDNA introns (totalling *c.* 1633 bp) from 173 of these individuals. We found low support for a hypothesis of divergence of the Eastern Islands populations prior to the start of the penultimate interglacial *c.* 250 kya, and higher support for a hypothesis of colonization of the Eastern Islands from sources in eastern Puerto Rico during the penultimate and last glacial period, when a land bridge united the PRB. The Río Grande de Loíza Basin in eastern Puerto Rico delineates a phylogeographic break. Haplotypes shared between the PRB and St. Croix (an island *c.* 105 km south-east of this archipelago) likely represent human-mediated introductions. Our findings illustrate how varying degrees of connectivity and isolation influence the evolution of tropical island organisms.

Keywords: Caribbean Sea, gene flow, human-mediated introductions, island biogeography, isolation, phylogeography, Puerto Rican Bank, quaternary, West Indies

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Introduction

Ecological and evolutionary studies of island biotas provide fundamental perspectives on speciation, adaptive radiation, community assembly and biogeography (Losos & Ricklefs 2009). Factors such as isolation, topography, island area and island age contribute to biogeographic patterns (MacArthur & Wilson 1967; Triantis *et al.* 2008; Whittaker *et al.* 2008), with historical fluctuations of sea level hypothesized to play an important role in shaping insular biomes (e.g. Heatwole & MacKenzie 1967; Carine 2005). Quaternary (2.6 mya—present) climatic oscillations changed eustatic sea levels more than 100 m (Waelbroeck *et al.* 2002; Bintanja *et al.* 2005; van Daele *et al.* 2011), altering the size, number

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and degree of isolation of islands worldwide. During interglacial periods, rising sea levels fragmented and reduced the size of terrestrial habitats, leading to population isolation and smaller population sizes, which facilitated evolutionary divergence or local extirpation (Pregill & Olson 1981). As sea levels lowered during glacial periods, terrestrial areas expanded, potentially connecting previously isolated populations and allowing colonization of new areas. Although some studies detected high levels of gene flow (e.g. Malone *et al.* 2003; Heaney *et al.* 2005) and low levels of endemism (e.g. Heatwole & MacKenzie 1967; Rand 1969) in tropical archipelagoes with a history of land-bridge connections, others inferred absence of gene flow between populations and high endemism, despite opportunities for dispersal (e.g. Esselstyn & Brown 2009; Lim *et al.* 2011). This contrast implies that the role of fluctuating sea levels on diversification in tropical insular systems is complex.

We explore the genetic consequences of cyclical sea level fluctuations in the Puerto Rican Bank (hereafter PRB), an archipelago in the eastern Caribbean Sea (Fig. 1a, b) comprised of the main island of Puerto Rico (8768 km^2; maximum elevation 1338 m) and the 'Eastern Islands' (east of 65.6°W; *c.* 340 km^2; maximum elevation 500 m), which include Vieques, Culebra, the Virgin Islands (St. Thomas, St. John, Tortola, Virgin Gorda and Anegada) and more than 180 associated small cays. Whereas low sea level during glacial periods led to a land bridge between Puerto Rico and the Eastern Islands, higher sea level during interglacial periods separated the Eastern Islands from each other and from Puerto Rico (Heatwole & MacKenzie 1967; Renken *et al.* 2002). Indeed, Puerto Rico and the Eastern Islands form distinctive biogeographic clusters (Hedges 1999), suggesting limited gene flow between these regions, despite the periodic emergence of a land-bridge connection.

Distinctive phylogeographic patterns inferred for species in Puerto Rico and the Eastern Islands suggest disparate evolutionary histories. Previous mtDNA studies inferred long-term persistence and diversification in topographically diverse Puerto Rico (e.g. Velo-Antón *et al.* 2007; Rodríguez-Robles *et al.* 2008, 2010; Jezkova *et al.* 2009; Barker *et al.* 2011), with sea level fluctuations promoting speciation in the Eastern Islands (Brandley & de Queiroz 2004; Oneal *et al.* 2010). However, those studies did not examine population-level responses across the archipelago. We herein use multiple loci to examine demographic phenomena (Edwards & Beerli 2000; Hudson & Turelli 2003) and assess the effect of inundations on PRB populations of the Red-eyed Coquí, *Eleutherodactylus antillensis* (Anura: Eleutherodactylidae) Reinhardt and Lütken 1863. This widespread (sea level to 1220 m), predominantly arboreal frog occurs in most larger islands of the PRB (except for Anegada; Henderson & Powell 1999) and is ideal for testing alternative biogeographic scenarios. Because anurans are intolerant of saltwater (Balinsky 1981; Duellman & Trueb 1994), dispersal of *E. antillensis* among islands during high sea level stands has probably been limited, although there are reported instances of frogs crossing salt-water barriers via rafting (Measey *et al.* 2007).

We formulate and test two competing hypotheses to elucidate the relative roles of persistence and dispersal in structuring genetic diversity in a widespread species across a climatically dynamic tropical archipelago. The 'Ancient Eastern Islands Isolation Hypothesis' proposes

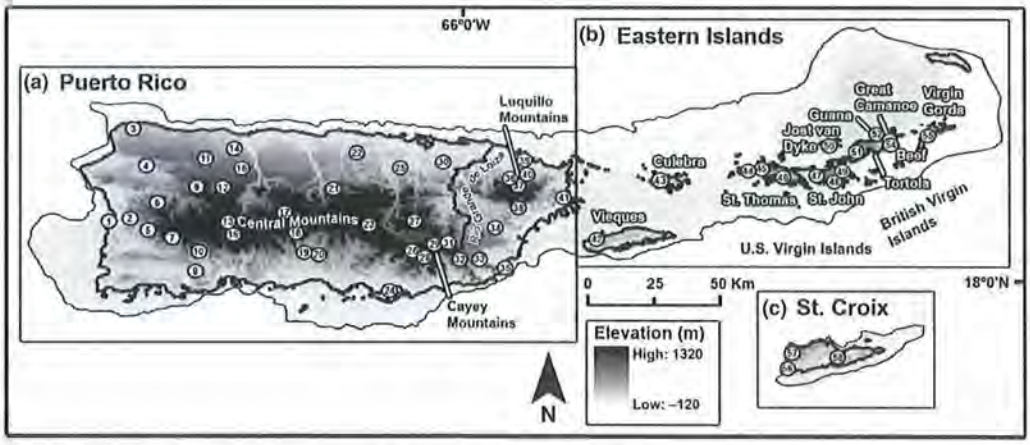

Fig. 1 Map of the Puerto Rican Bank (a and b) and St. Croix (c) illustrating the topography of the islands and the approximate geographic location of the sampling localities of *Eleutherodactylus antillensis* (see Table S1, Supporting Information, for specific locality data). The outermost line in a, b and c indicates the approximate land configuration at maximum sea level (–120 m; Siddall *et al.* 2003) lowering during the Last Glacial Maximum (*c.* 26.5–19 kya), and the thicker line depicts the current extent of land area.

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that *E. antillensis* persisted in isolation in the Eastern Islands since before the start of the penultimate interglacial *c.* 250 kya (Dutton *et al.* 2009), despite the emergence of a land-bridge connection uniting the PRB during glacial periods between the penultimate interglacial (190–245 kya; Dutton *et al.* 2009), last interglacial (119–130 kya; Siddall *et al.* 2003; Hearty *et al.* 2007; van Daele *et al.* 2011) and Holocene interglacial (*c.* 0–12 kya; Waelbroeck *et al.* 2002; Siddall *et al.* 2003; van Daele *et al.* 2011). This hypothesis, which predicts deep divergence between populations in the Eastern Islands and Puerto Rico, is based on previous work suggesting that vicariance due to Pliocene or Pleistocene changes in sea level promoted speciation between Puerto Rican and Eastern Islands taxa (Brandley & de Queiroz 2004; Oneal *et al.* 2010). In contrast, the 'Eastern Dispersal Hypothesis' proposes that *E. antillensis* colonized the Eastern Islands from sources in eastern Puerto Rico subsequent to the penultimate interglacial via a land bridge. This hypothesis is based on the premise that in Caribbean land-bridge archipelagoes, topographically complex islands may have lower extinction rates than smaller, lower-elevation islands and act as sources of migrants to these areas during periods of low sea level (Rand 1969; Losos 1996). The Eastern Dispersal Hypothesis predicts relatively recent gene flow (shallow divergence) from populations in eastern Puerto Rico (the putative source area) towards the Eastern Islands populations, with Eastern Islands' samples nesting within clades from eastern Puerto Rico. Prevailing east-to-west ocean currents in the PRB region would have hindered eastward dispersal to the Eastern Islands during brief periods (*c.* 11–18 kyrs; Hearty *et al.* 2007; Dutton *et al.* 2009) of high sea level during at least three interglacial periods since *c.* 250 kya, but comparatively long periods of low sea level (Siddall *et al.* 2003; Dutton *et al.* 2009; Rohling *et al.* 2009) produced a land bridge between eastern Puerto Rico and the Eastern Islands that would have facilitated eastward dispersal. We focus on how sea level fluctuations during the last two glacial–interglacial periods shaped genetic diversity in *E. antillensis*, because these events may have produced changes in the size, configuration and isolation of terrestrial habitats (Siddall *et al.* 2003; Dutton *et al.* 2009; van Daele *et al.* 2011) that impacted the connectivity of populations in the PRB (Heatwole & MacKenzie 1967).

We also assess the presence of *E. antillensis* on St. Croix, an island *c.* 105 km southeast of the PRB (Fig. 1c) that has never had a direct land connection with this archipelago (Gill *et al.* 1989). Although St. Croix has a relatively high proportion of endemics (Heatwole & MacKenzie 1967; Brandley & de Queiroz 2004), several species on this island became established following unintentional human-mediated introductions from sources in the PRB (Platenberg 2007). We test the hypothesis that the *E. antillensis* population on St. Croix originated from a recent human-mediated introduction to this island (Grant & Beatty 1944). Because the Ancient Eastern Islands Isolation Hypothesis and the Eastern Dispersal Hypothesis address natural historical processes in the PRB, understanding the extent to which human transport may have shaped the distribution of *E. antillensis* is critical.

Materials and methods

Sampling, DNA sequencing, haplotype phasing and sequence alignment

We sampled 285 individuals from 58 localities (avg. 4.6 individuals per locality, range 2–5; Fig. 1a–c; Table S1, Supporting Information) at elevations of 0–981 m from 12 of the 13 islands where *Eleutherodactylus antillensis* occurs (the exception being Great Thatch). A subset of specimens at each locality (except for Virgin Islands National Park, St. John) was euthanized and deposited at the Museum of Southwestern Biology (MSB), University of New Mexico, Albuquerque, or the Museum of Vertebrate Zoology (MVZ), University of California, Berkeley. Tissue samples (liver, thigh muscle and/or toe clips) are from the Division of Genomic Resources, MSB, and the Rodríguez-Robles laboratory at the University of Nevada, Las Vegas.

We included 285 *E. antillensis* in analyses of the mitochondrial (mtDNA) control region (CR) (*c.* 552 bp). Nucleotide sequences from the nuclear DNA (nuDNA) intron-spanning loci β-crystallin (intron 1; CRYBA, *c.* 192 bp), myosin heavy chain (putative flanking intron of exon 36; MYH, 463 bp), rhodopsin (intron 1; RH1, 596 bp) and ribosomal protein L9 (intron 4; RPL9int4, 382 bp) were obtained from 173 specimens representing three randomly chosen individuals from each of the 58 localities (except for Great Camanoe, from which only two samples were available). DNA was extracted in a total volume of 30 μL using a modified CTAB/PVP (cetyl trimethylammonium bromide/polyvinylpyrrolidone)—chloroform/isoamyl alcohol DNA extraction technique (Stewart & Via 1993; M. Perdue, unpublished data). Polymerase chain reactions (PCRs) contained *c.* 50 ng of template DNA, 10 μM of each primer, 10 mM dNTP, 25 mM $MgCl_2$, 2 μL of 10× polymerase reaction buffer and 0.20 units of AmpliTaq Gold *Taq* polymerase (Applied Biosystems, Foster City, CA, USA) and were adjusted to a final volume of 15 μL with ddH_20. Sequencing reactions were conducted using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and cleaned with ethanol precipitation. Primer descriptions and PCR

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conditions are provided in the Supporting Information (Table S2, Supporting Information).

We edited sequences using SEQUENCHER 4.5 (GeneCodes) and deposited them in GenBank (accession numbers JN385299–JN38696; Table S1, Supporting Information). For heterozygote individuals polymorphic at more than one position, the gametic phase was inferred using PHASE 2.1 (Stephens *et al.* 2001; Stephens & Donnelly 2003). We collapsed multi-base insertion–deletions (indels) in RPL9int4 into single-base polymorphisms for phasing analyses. Five independent runs from different starting seeds were run for 1000 iterations, with a single thinning interval and 100 burn-in iterations. When PHASE could not reconstruct haplotypes with a posterior probability of ≥ 0.85, PCR fragments were cloned for a minimum of five clones per sample using the pGEM-T-Easy Vector Systems kit (Promega). Finally, we repeated the PHASE analyses using known haplotypes. We aligned sequences in MAFFT 6 (Katoh *et al.* 2002, http://mafft.cbrc.jp/alignment/server/). To avoid disproportionate weighting of multi-base indels, we collapsed multi-base indels in the RPL9int4 alignment into single-base polymorphisms for analyses in which indels were included (see below). Alignments were deposited in DRYAD (doi:10.5061/dryad.hc257).

Neutrality, recombination and genetic diversity in Eleutherodactylus antillensis

We assessed selective neutrality of each locus with the Hudson–Kreitman–Aguade (HKA) test (Hudson *et al.* 1987), with 10 000 coalescent simulations in HKA (J. Hey, http://genfaculty.rutgers.edu/hey/software), and by using Fu's F_S test (Fu 1997) with 10 000 coalescent simulations in ARLEQUIN 3.1 (Excoffier *et al.* 2005). To investigate recombination in nuDNA loci, we conducted RDP (Martin *et al.* 2005), Bootscan (Salminen *et al.* 1995; Martin *et al.* 2005), Geneconv (Padidam *et al.* 1999), Chimaera (Posada & Crandall 2001), MaxChi (Maynard Smith 1992) and SiScan (Gibbs *et al.* 2000) tests in RDP 3 (Martin *et al.* 2010). Recombination was also inferred by calculating the pairwise homoplasy index [Φ_w] (Bruen *et al.* 2006) in SPLITSTREE 4.11.3 (Huson & Bryant 2006). We estimated percentage of fragment length containing segregating sites [S(%)] and nucleotide (π) diversity for each locus in ARLEQUIN. Indels were included in these analyses.

Phylogenetic and network analyses

To test predictions of group structure in Puerto Rico and the Eastern Islands, we performed maximum parsimony, maximum likelihood and Bayesian analyses of CR haplotypes and each nuDNA locus separately. Phylogenies for CR, CRYBA and MYH were rooted with the closely related *Eleutherodactylus brittoni* and *E. cochranae* (Hedges *et al.* 2008). We lacked RH1 and RPL9int4 sequences for *E. brittoni*, and therefore used *Eleutherodactylus portoricensis* as the second outgroup for these data sets. Because CR evolves rapidly and divergent outgroups can confound phylogenetic analysis due to homoplasy (Brandley *et al.* 2009), we also used midpoint rooting (Hess & De Moraes Russo 2007) for this locus. We constructed parsimony trees and generated nonparametric bootstrap values using PHYLIP 3.68 (Felsenstein 1993). Nodal support was evaluated using 1000 bootstrap replicates, and we used CONSENSE (Felsenstein 1993) to obtain a 50% majority rule consensus tree. Phylogenetic trees were constructed with MEGA 5.05 (Tamura *et al.* 2011) using the maximum likelihood method with 1000 bootstrap replicates and applying the model of nucleotide substitution (CR–GTR + Γ; CRYBA–HKY; MYH–HKY; RH1–HKY; RPL9int4–HKY + I + Γ) for the data that encompasses the model chosen by AIC in MODELTEST 3.7 (Posada & Crandall 1998). We employed a Bayesian approach to phylogenetic reconstruction implemented in MRBAYES 3.1.2 (Huelsenbeck & Ronquist 2001) using these same models of nucleotide substitution. We ran four chains for 20 000 000 generations each, with default parameters as starting values and sampling every 1000 generations, and repeated the analysis twice to ensure consistency. We discarded the first 5000 trees as burn-in, and checked for stationarity and convergence of the chains with the software TRACER 1.5 (Rambaut & Drummond 2007). Bayesian posterior probabilities (BPP) were obtained from the 50% majority rule consensus of the remaining trees. Indels were included in the maximum parsimony analysis, but excluded in the maximum likelihood and Bayesian analyses. We generated a maximum parsimony network for CR haplotypes with NETWORK 4.2 (http://www.fluxus-technology.com) and created geographic visualizations of nuDNA haplotypes using PHYLOGEOVIZ 2.4.4 (Tsai 2011). Indels were included in the NETWORK and PHYLOGEOVIZ analyses.

Genetic structure

To assess genetic structuring in *E. antillensis*, we estimated the number of populations (K) and incorporated sampling locality data in a spatially explicit framework to infer the location of genetic discontinuities among those populations. MtDNA has a higher mutation rate and faster coalescence time than nuDNA introns, and thus we analysed these two marker classes separately to compare patterns of spatial structure inferred from each. To define groups for additional hypothesis testing procedures using MIGRATE and DIYABC (see below),

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we analysed the five loci together. Indels were included in these analyses. To explore spatial genetic structure in CR, we conducted a spatial analysis of molecular variance (SAMOVA) in SAMOVA 1.0, which defines groups of populations that are geographically homogenous and maximally differentiated from each other (Dupanloup *et al.* 2002). Using GENELAND 3.2.4, we inferred groups of *E. antillensis* based on nuDNA, and on mtDNA and nuDNA combined, with and without a spatially explicit model (Guillot *et al.* 2005b, 2008), and thus conducted four different analyses. GENELAND infers the number of populations using individual multilocus genotypes and, when provided with coordinates, the spatial location of genetic discontinuities among those populations (Guillot *et al.* 2005a). For nuDNA, each variable site was encoded as an allele. Including mtDNA in GENELAND analyses violates assumptions of Hardy–Weinberg equilibrium within populations and linkage equilibrium between loci, but encoding each mtDNA sequence as a single haplotype is not statistically problematic (G. Guillot, personal communication). For each of the four GENELAND analyses, we examined $K = 1$–10 distinct groups (five replicates each), with 250 000 Metropolis-coupled Markov chain Monte Carlo iterations recorded every hundredth step after an initial 10% burn-in, and applied the uncorrelated allele frequency model. We accepted the modal K value from the posterior distribution as the most probable number of groups (Guillot *et al.* 2005b). To assess diversity across the range of *E. antillensis*, we used spatial group assignments from GENELAND (see Results; Fig. 2) and estimated the number of haplotypes (h), haplotype diversity (H_d), nucleotide diversity (π) and Fu's F_S (Fu 1997) with 10 000 coalescent simulations for each group using ARLEQUIN. Indels were included in these analyses.

Testing alternative models of gene flow

We constructed gene flow models in accordance with predictions of a priori hypotheses and assessed the models using Bayesian inference in MIGRATE 3.2.7 (Beerli & Felsenstein 2001; Beerli 2006). The model selection procedure inferred parameters using coalescent theory (Kingman 1982), and then ordered models by their Bayes factors (Beerli & Palczewski 2010). We evaluated two models that differed in migration rates between populations in Puerto Rico and the Eastern Islands (Table 1). The Ancient Eastern Islands Isolation Hypothesis predicts an absence of gene flow between populations in Puerto Rico and the Eastern Islands (Model 1), whereas the Eastern Dispersal Hypothesis predicts gene flow from populations in eastern Puerto Rico towards Eastern Islands populations (Model 2). The sampling sites from the PRB were pooled into three groups (western Puerto Rico, eastern Puerto Rico and Eastern Islands) based on results of the GENELAND analyses of all loci (see Results; Fig. 2). We allowed for gene flow in either direction between western and eastern Puerto Rico in the two models. Indels were excluded from these analyses.

Settings in MIGRATE were specified to promote convergence and efficiently sample search space. We applied Felsenstein's (1984) model of nucleotide substitution, with mutation rates estimated from the data and allowed to vary. To promote convergence, we applied uniform prior distributions for Θ and M and used slice sampling. Prior distributions of Θ (mean = 0.08 and range 0–0.8) and M (mean = 50 000 and range 0–100 000) were selected after conducting preliminary runs with broad prior distributions (P. Beerli, personal communication), and then modifying the priors in subsequent runs if posterior distributions were not

Fig. 2 Results of the GENELAND analysis of *Eleutherodactylus antillensis* based on all loci (mtDNA control region and four nuDNA introns). A map of estimated group membership, indicated by different shades of grey (left panel), and a histogram of posterior probability values for each value of K (number of populations; right panel) is presented. Groups correspond to those indicated in Fig. 3 and Tables 1–5 as follows: western Puerto Rico (black), eastern Puerto Rico (grey) and Eastern Islands (white). The location of the Río Grande de Loíza in eastern Puerto Rico is indicated.

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Table 1 Models of gene flow used for testing the Ancient Eastern Islands Isolation Hypothesis and the Eastern Dispersal Hypothesis in MIGRATE 3.2.7

	Ancient Eastern Islands Isolation Hypothesis Model 1 	Eastern Dispersal Hypothesis Model 2 
All samples		
Bézier-corrected log marginal likelihood	−5180	−4986
Log Bayes factor	−194	0
Rank of model	2	1
Excluding potentially introduced samples		
Bézier-corrected log marginal likelihood	−4932	−4773
Log Bayes factor	−159	0
Rank of model	2	1

The Bézier-corrected log marginal likelihood, log Bayes factor and rank of each model are indicated. WPR, western Puerto Rico, EPR, eastern Puerto Rico, EIS, Eastern Islands.

contained within the bounds of the prior distribution. After a burn-in period of 100 000 generations, we sampled every 100 steps from one long chain (50 000 steps). To calculate Bézier-corrected log marginal likelihoods, we applied thermodynamic integration under a static heating scheme (Beerli & Felsenstein 2001) of four chains (temperatures 1.0, 1.5, 3.0, 10^4). To efficiently sample search space when calculating parameters, we applied the randomtree index, set the replicate index to 20 and combined chains from all replicates. This simulation was repeated five times to ensure consistency. In subsequent runs, we changed the random number seed and used Bayesian estimates of Θ and M from the previous run as starting values (Beerli & Felsenstein 2001). We calculated the natural log Bayes factor following Beerli & Palczewski (2010).

Testing alternative models of divergence

Using DIYABC 1.0.4.46 (Cornuet *et al.* 2010), we performed coalescent simulations (Hudson 1990) in an approximate Bayesian computation (ABC) framework to explore the history of divergence in populations of *E. antillensis* in the PRB. We generated a divergence model for each hypothesis. Both models of divergence included eastern and western Puerto Rican groups (see GENELAND Results; Fig. 2), and only differed in the timing of divergence of the Eastern Islands group (Fig. 3). Indels were excluded from these analyses.

The priors for all demographic parameters had uniform distributions that were bound by specified minimum and maximum values (Table 2). Each model incorporated an ancestral effective population size (N_a, prior distribution: 10–1 000 000) and an effective population size for populations in western Puerto Rico (N_1, prior distribution: 10–6 000 000), eastern Puerto Rico (N_2, prior distribution: 10–5 000 000) and the Eastern Islands (N_3 and N_4). We set the prior distribution for the effective population size of Eastern Islands populations lower in the divergence model for the Eastern Dispersal Hypothesis (N_4, prior distribution: 10–2 000 000) than in the divergence model for the Ancient Eastern Islands Isolation Hypothesis (N_3, prior distribution: 0–4 000 000), because spatial expansion into the Eastern Islands by a small number of founder individuals may have lowered the effective population size there. We adjusted the upper boundary for the prior distribution for effective population size parameters to the geographic size of eastern Puerto Rico and the Eastern Islands relative to western Puerto Rico because genetic variation may be lower in smaller populations (Frankham 1996), and the values are reasonable with respect to the estimated density of adult male *E. antillensis* per hectare on Guana Island (Ovaska 2005) and the large effective population sizes attained by other eleutherodactyline frogs (Crawford 2003a; Barker *et al.* 2011). The divergence model for the Ancient Eastern Islands Isolation Hypothesis depicts the Eastern Islands group diverging from western and eastern Puerto Rican groups between the start of the penultimate interglacial and the early middle Pleistocene (t_1, prior distribution: 250 000–500 000 generations), and the western and eastern Puerto Rican groups diverging between the Holocene interglacial and the early middle Pleistocene (t_2, prior distribution: 1–500 000 generations; Fig. 3). We used the 'set condition' option to impose the rule that $t_1 > t_2$ because the Ancient Eastern Islands Hypothesis predicts deep divergence between Puerto Rican and

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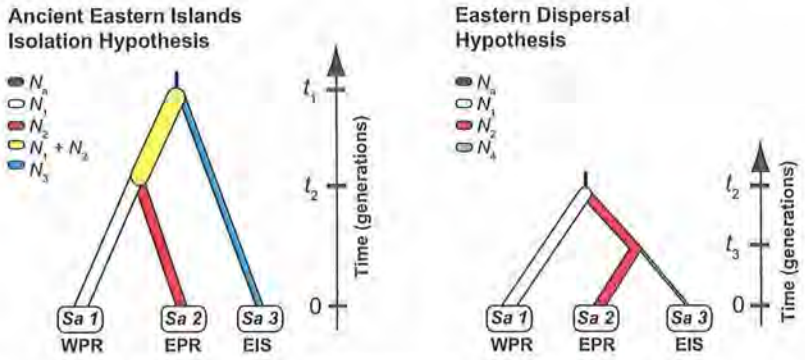

Fig. 3 The two models of divergence for *Eleutherodactylus antillensis* tested in DIYABC 1.0.4.46 differ in the estimated divergence time for Puerto Rican and Eastern Islands populations and the effective population size of the Eastern Islands populations. The divergence model for the Ancient Eastern Islands Isolation Hypothesis depicts the Eastern Islands group diverging from western and eastern Puerto Rican groups between the start of the penultimate interglacial and the early middle Pleistocene (t_1 prior distribution = 250 000–500 000 generations), despite the emergence of a land-bridge connection between this region and Puerto Rico during the penultimate and last glacial periods. The divergence model for the Eastern Dispersal Hypothesis depicts relatively shallow divergence (t_3 prior distribution: 1–250 000 generations) between Eastern Islands and Puerto Rican groups due to colonization of the Eastern Islands from populations in eastern Puerto Rico (the putative source area) during the penultimate and last glacial periods, which resulted in a small effective population size in the Eastern Islands. In both models, the eastern and western Puerto Rican groups began diverging between the Holocene interglacial and the early middle Pleistocene (t_2 prior distribution: 1–500 000 generations). Time is number of generations, with a generation length of 1 year. *Sa* 1 comprises samples from western Puerto Rico (WPR); *Sa* 2 comprises samples from eastern Puerto Rico (EPR); and *Sa* 3 comprises samples from the Eastern Islands (EIS), based on the GENELAND analysis. The ancestral effective population size (N_a) and effective population size for WPR (N_1), EPR (N_2) and EIS (N_3 and N_4) groups are indicated. Times and population sizes are not to scale.

Eastern Islands populations. In contrast, the divergence model for the Eastern Dispersal Hypothesis depicts the western Puerto Rican group diverging from the eastern Puerto Rican group between the Holocene interglacial and the early middle Pleistocene (t_2), and the Eastern Islands and eastern Puerto Rican groups diverging after *E. antillensis* colonized the Eastern Islands via a land bridge between the Holocene interglacial and the penultimate interglacial (t_3, prior distribution: 1–250 000 generations). We imposed the rule that $t_2 > t_3$ for this model because the Eastern Dispersal Hypothesis predicts shallow divergence between eastern Puerto Rican and Eastern Islands populations. Time was number of generations, with a generation length of 1 year, based on observations that *E. antillensis* may reach reproductive maturity in the subsequent wet season after hatching (Ovaska 2005), and on studies of the closely related Puerto Rican Coquí, *Eleutherodactylus coqui* (Stewart & Woolbright 1996). We conducted preliminary runs with broad prior distributions and modified the priors in subsequent runs if posterior distributions were not contained within the bounds of the prior distribution.

Obtaining meaningful estimates of historical demographic parameters (e.g. gene flow, divergence times, effective population size) depends in part on the accuracy of estimates of mutation rates for individual loci (Hey & Nielson 2004). The lower boundary for the CR mutation rate was set to $\mu = 0.96 \times 10^{-8}$ nucleotide substitutions per site per year (sub/s/y), based on estimates for nucleotide substitution rates for mtDNA in eleutherodactyline frogs (Crawford *et al.* 2007). Because this rate was estimated from coding regions of mtDNA, which may evolve more slowly than CR, we estimated an upper boundary for CR by inferring its rate relative to a coding mtDNA locus, cytochrome *b* (cyt *b*), using sequences collected from *E. portoricensis* (Barker *et al.* 2011) (cyt *b* accession numbers—HM229960–HM229995; CR accession numbers —HM229817, HM229820, HM229826, HM229828, HM229832, HM229838, HM229845, HM229846, HM229849, HM229857, HM229860, HM229862, HM229870, HM229871, HM229876, HM229881, HM229884, HM229885, HM229896, HM229898, HM229899, HM229910, HM229911, HM229919, HM229923, HM229926, HM229931-HM229933, HM229935, HM229938, HM229940, HM229941, HM229944, HM229945, HM229955). Mutation rates of a particular marker can vary across species (Nabholz *et al.* 2009; Lanfear *et al.* 2010), but we did not collect a comprehensive cyt *b* data set for *E. antillensis* because preliminary work indicated that CR had higher levels of variation. We compared the overall mean mtDNA CR and cyt *b* genetic distances (*p*-distances) among individuals of *E. portoricensis* using MEGA 5.05 and determined that the nucleotide substitution rate of mtDNA CR was *c.* 4.2 times that of cyt *b*. Based on an estimated coding mtDNA

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Table 2 Prior distributions used for the coalescent simulations (performed in DIYABC 1.0.4.46) of models of genetic divergence for *Eleutherodactylus antillensis* groups in western Puerto Rico (WPR), eastern Puerto Rico (EPR) and the Eastern Islands (EIS)

Symbol	Description	Prior distribution
Demographic parameters		
N_a	Ancestral effective population size	Uniform (10, 1×10^6)
N_1	Effective population size of WPR group	Uniform (10, 6×10^6)
N_2	Effective population size of EPR group	Uniform (10, 5×10^6)
N_3	Effective population size of EIS group in divergence model for Ancient Eastern Islands Isolation Hypothesis	Uniform (10, 4×10^6)
N_4	Effective population size of EIS group in divergence model for Eastern Dispersal Hypothesis	Uniform (10, 2×10^6)
t_1	Divergence time between the start of the penultimate interglacial and the early middle Pleistocene	Uniform (250.0, 500.0)
t_2	Divergence time between the Holocene interglacial and the early middle Pleistocene	Uniform (0.001, 500.0)
t_3	Divergence time between the Holocene interglacial and the penultimate interglacial	Uniform (0.001, 250.0)
Mutation rate parameters		
μ, mitochondrial DNA	Mean mutation rate (per site per generation)	Uniform (0.96×10^{-8}, 4.1×10^{-8})
	Individual locus mutation rate (per site per generation)	Gamma (0.96×10^{-8}, 4.1×10^{-8}) Hasegawa, Kishino and Yano (HKY) model, % invariant sites = 83, shape of gamma = 0.73
μ, autosomal diploid DNA	Mean mutation rate (per site per generation)	Uniform (6.0×10^{-10}, 4.1×10^{-9})
	Individual locus mutation rate (per site per generation)	Gamma (6.0×10^{-10}, 4.1×10^{-9}) Hasegawa, Kishino and Yano (HKY) model, % invariant sites = 0, shape of gamma = 2

We used uniform prior distributions for demographic parameters. Divergence time is in thousands of generations. We used the 'set condition' option to impose the rule that $t_1 > t_2$ for the model of genetic divergence based on the Ancient Eastern Islands Isolation Hypothesis, because this hypothesis predicts deep divergence between Puerto Rican and Eastern Islands populations. In contrast, we imposed the rule that $t_2 > t_3$ for the model of genetic divergence for the Eastern Dispersal Hypothesis, because this hypothesis predicts shallow divergence between eastern Puerto Rican and Eastern Islands populations. Prior distributions for the mutation rate (μ) were drawn from a uniform distribution across loci, and individual locus mutation rates were then drawn from a gamma distribution to allow for rate heterogeneity across sites. Models of nucleotide substitution are provided.

rate of 0.96×10^{-8} sub/s/y, we estimated that CR evolves at a rate of 4.1×10^{-8} sub/s/y per lineage. This estimate is refined from an earlier study of genetic diversification in *E. portoricensis* (Barker *et al.* 2011). In two *E. antillensis* individuals for which both CR and cyt *b* sequences were available, CR had approximately three times as much variation as cyt *b*, and therefore we believe that a rate of 4.1×10^{-8} sub/s/y for CR is appropriate for this species. The minimum (6.0×10^{-10} sub/s/y) and maximum (4.1×10^{-9} sub/s/y) values of μ for the nuDNA introns were based on a lower boundary estimate of anuran nuDNA (Crawford 2003a), and a tenfold slower rate than our upper boundary for mtDNA (Sheldon *et al.* 2000; Crawford 2003b), respectively.

We simulated 1 000 000 data sets for the Ancient Eastern Islands Isolation Hypothesis and the Eastern Dispersal Hypothesis and used the number of haplotypes, number of segregating sites, average number of pairwise differences and Tajima's *D* as summary statistics to compare observed and simulated data sets. We selected these summary statistics based on their performance in previous ABC studies (Hickerson *et al.* 2006; Beaumont 2008). The 1000 and 10 000 simulated data sets with summary statistics most similar to the observed data were identified through the direct and logistic regression rejection steps of the ABC algorithm and used for ABC estimation of parameters.

We evaluated the appropriateness of each model of genetic divergence and its associated parameters and performed model-checking computations as empirical verifications of the performance of the ABC procedure. To check that at least one combination of and priors produced simulated data sets similar to our observed data, we used the 'Pre-evaluate scenario-prior combinations' option in DIYABC that involved a principal component analysis (PCA) of the first 100 000 simulated data sets (Cornuet *et al.* 2010). As part of the model-checking procedure, we conducted PCA on test quantities

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obtained with the model-posterior combination from the most strongly supported model, together with 10 000 pseudo-observed data sets (Cornuet *et al.* 2010). We evaluated the 'goodness-of-fit' of each model by using the 'Confidence in scenario choice' option in DIYABC and simulated 500 data sets per model with parameter values drawn from the same prior distributions as the models. From these results, we calculated the proportion of times that the most strongly supported model did not have the highest posterior probability when it was the true model (type I error), as well as the proportion of times that the least supported model had the highest posterior probability when it was not the true model (type II error). Because these error rates are computed from Bayesian posterior probabilities, they are not strict type I and type II errors in a classical frequentist framework, whereby the null hypothesis is only completely rejected or completely supported (Bertorelle *et al.* 2010).

Results

Neutrality, recombination and genetic diversity in Eleutherodactylus antillensis

Nucleotide variation in the five loci was consistent with neutral expectations (HKA test, $\chi^2 = 6.64$, d.f. = 4, $P = 0.16$), but significantly negative values of Fu's F_S for CR ($F_S = -25.66$, $P = 0.0002$) and RPL9int4 ($F_S = -23.16$, $P = 0.0002$) indicate selection or recent population expansion (Fu 1997). We did not detect recombination in the nuDNA data sets. The mtDNA CR data set contained 98 haplotypes with 49 variable positions; seven were single-base pair indels. The four nuDNA loci totalled *c.* 1633 bp and exhibited varying levels of polymorphism (Table 3), with CRYBA the least variable (five variable positions; one a single-base pair indel), and RPL9int4 the most variable (22 variable positions; two single-base pair indels and one-five-base pair indel). MYH and RH1 did not contain indels.

Phylogenetic and network analyses

Phylogenetic analyses of CR revealed a shallow, largely unresolved topology. We present the results of the phylogeny rooted with outgroup taxa (Fig. S1, Supporting Information) because it was not appreciably different from the mid-point rooted phylogeny. Only a single clade (which contained three haplotypes) was well supported across maximum parsimony, maximum likelihood and Bayesian analyses, and therefore most CR haplotypes form a basal polytomy. Phylogenetic analyses of nuDNA loci showed varying degrees of resolution, but each indicated that most genetic structuring

Table 3 Descriptive statistics for the five loci used in this study

			Western Puerto Rico						Eastern Puerto Rico						Eastern Islands					
Locus	L (bp)	S (%)	π	N	h (# priv)	H_d	π	Fu's F_S	N	h (# priv)	H_d	π	Fu's F_S	N	h (# priv)	H_d	π	Fu's F_S
mtDNA																		
CR	552	49 (8.9)	0.0065	140	51 (45)	0.933	0.0055	−26.379*	63	27 (19)	0.939	0.0063	−16.183*	67	30 (27)	0.953	0.0060	−21.721*
nuDNA																		
CRYBA	192	5 (2.6)	0.0019	84	4 (1)	0.146	0.0020	−0.774	39	4 (1)	0.376	0.0031	−0.145	41	3 (1)	0.180	0.0010	−1.318
MYH	463	9 (1.9)	0.0017	84	5 (2)	0.115	0.0004	−4.033*	39	6 (2)	0.594	0.0018	−1.711	41	5 (2)	0.653	0.0023	0.522
RH1	596	8 (1.3)	0.0018	84	4 (1)	0.623	0.0021	2.684	39	6 (3)	0.503	0.0013	−1.490	41	4 (1)	0.534	0.0010	−0.090
RPL9int4	382	22 (5.8)	0.0065	84	13 (7)	0.500	0.0034	−4.564	39	29 (20)	0.944	0.0096	−16.302*	41	9 (3)	0.499	0.0042	−1.309

Sequence length (L, including alignment gaps; bp, base pairs), number of segregating sites (S), with percentage of sequence length in parentheses, and nucleotide diversity (π) are shown. The following statistics are reported for western Puerto Rican, eastern Puerto Rican and Eastern Islands populations of *Eleutherodactylus antillensis*: sample size (N), number of haplotypes (h), with number of private haplotypes in parentheses, haplotype diversity (H_d), nucleotide diversity (π) and Fu's F_S values. Significant F_S values at $P < 0.02$ are indicated with an asterisk.

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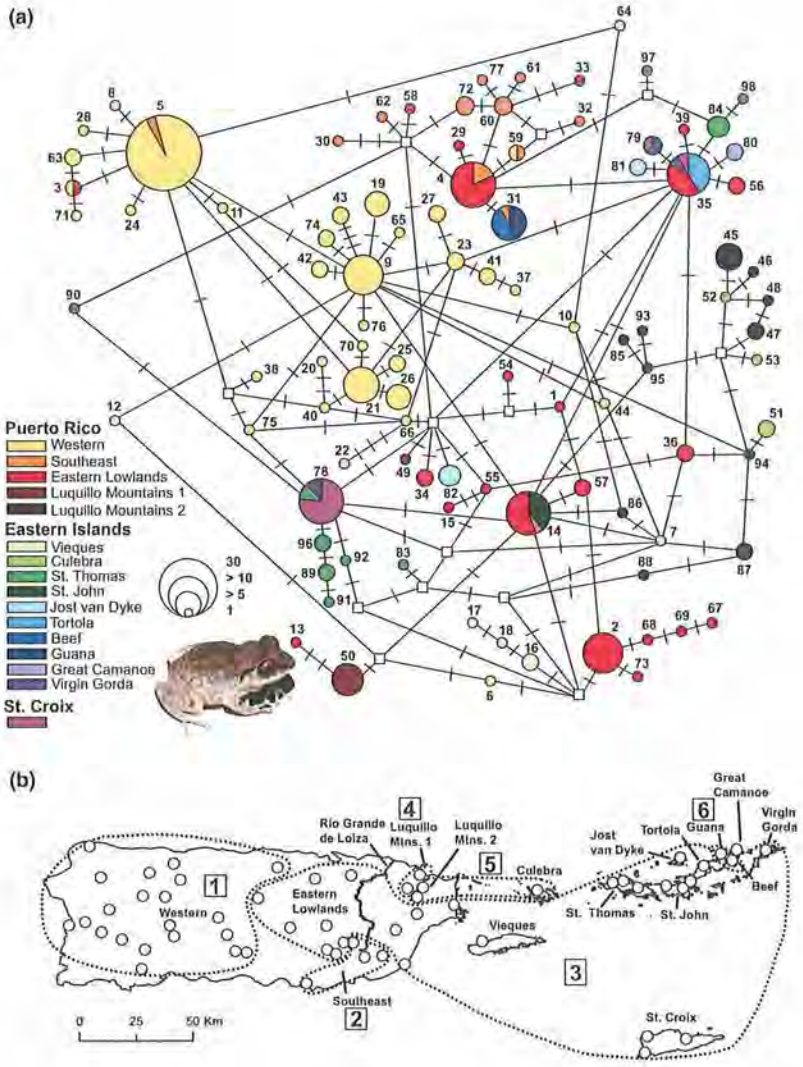

Fig. 4 (a) Maximum parsimony network representing the relationships among haplotypes of the mtDNA control region (CR) of 285 individuals of *Eleutherodactylus antillensis* (inset image; photograph by David Dennis). Indels were included in this analysis. Circles represent unique haplotypes; hatch marks depict single mutations; empty squares indicate missing (i.e. extant unsampled or extinct ancestral) haplotypes. Circle size is proportional to haplotype frequency, and the colour of each circle indicates the individual island from which the haplotype was sampled, except for Puerto Rico, where circles are coloured according to SAMOVA results. Numbers designating haplotypes correspond to those in Table S1 and Fig. S1 (Supporting Information). (b) Genetic partitions of populations of *E. antillensis* identified by the SAMOVA analysis of the mtDNA CR for $K = 6$, where K is the number of predefined groups. Groups are depicted with dashed lines. When $K = 6$, the first group comprises all but 10 localities west of the Río Grande de Loíza in Puerto Rico (Western); the second group contains four localities in south-eastern Puerto Rico (Southeast); the third group consists of 12 eastern Puerto Rican localities (Eastern Lowlands), St. Croix and all Eastern Islands, except Culebra, Beef and Guana; the fourth group is made up of two localities in the Luquillo Mountains region (Luquillo Mountains 1); the fifth group comprises two localities in the Luquillo Mountains region (Luquillo Mountains 2) and Culebra; and the sixth group contains populations from Beef and Guana.

in *E. antillensis* occurs within Puerto Rico (Fig. S2, Supporting Information). Most Eastern Islands samples fall within an unresolved polytomy (CRYBA) and/or within clades that also contain Puerto Rican samples (MYH, RH1, RPL9int4), although most of these clades were not well supported across all three analyses. The MYH and RH1 phylogenies recovered a well-supported clade containing three or fewer Eastern Islands samples across all three analyses.

The maximum parsimony network revealed that three CR haplotypes are shared between Puerto Rico and the Eastern Islands (H14, H31, H35; Fig. 4a). We

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ran a MIGRATE and DIYABC analysis that excluded samples with these CR haplotypes to account for the possibility of human transport of *E. antillensis* in the PRB (see Discussion). Most Eastern Islands populations contain shallowly differentiated private CR haplotypes. All three CR haplotypes in St. Croix (H35, H78, and H79) are shared with populations from Virgin Gorda, Puerto Rico, St. Thomas, and/or Tortola. Three CRYBA haplotypes, five MYH haplotypes, four RH1 haplotypes and nine RPL9int4 haplotypes occur in the Eastern Islands, but fewer than half of those are endemic to the region. Endemic nuDNA haplotypes are usually restricted to a single-island population (e.g. CRYBA, MYH, RH1; Fig. S3, Supporting Information) and occur at low frequencies. There are no unique nuDNA haplotypes in St. Croix.

Genetic structure

Spatial genetic structure inferred from CR, nuDNA loci and all five loci combined identified a distinctive group of *E. antillensis* in western Puerto Rico, but varied with respect to eastern Puerto Rico and the Eastern Islands. SAMOVA analyses of CR did not identify the precise number of populations (K) displaying the highest differentiation among groups (F_{CT}), because F_{CT} values increased as K increased. Some groups contained a single population when $K \geq 7$, indicating that group structure was declining, and thus we present results for $K = 6$ (c.f. Heuertz *et al.* 2004; Fig. 4b). GENELAND analyses with and without a spatially explicit model inferred three groups ($K = 3$) for nuDNA and for the loci combined. The spatial location of genetic discontinuities among populations was identical in analyses of nuDNA and the combined five loci and depicted one group located west of the Río Grande de Loíza in Puerto Rico, a second one comprising all but three sites east of the Río Grande de Loíza, and a third one occurring in the Eastern Islands and St. Croix (Fig. 2).

Genetic parameters for populations in western Puerto Rico, eastern Puerto Rico and the Eastern Islands varied across loci. For CR, populations from eastern Puerto Rico had fewer private haplotypes than those from western Puerto Rico and the Eastern Islands (Table 3). Populations from all three groups had significantly negative values of Fu's F_S for CR. For the four nuDNA loci, individuals from the Eastern Islands had fewer haplotypes than those from eastern Puerto Rico (Table 3). Populations in eastern Puerto Rico had significantly negative values of Fu's F_S for RPL9int4, whereas populations in western Puerto Rico had significantly negative values of Fu's F_S for MYH.

Assessing alternative models of gene flow and divergence

MIGRATE produced consistent parameter estimates across replicates and provided stronger support for the Eastern Dispersal Hypothesis (Model 2) than for the Ancient Eastern Islands Isolation Hypothesis (Model 1; Table 1). The migration rate (M) of *E. antillensis* from populations in eastern Puerto Rico into Eastern Islands populations was significantly larger than zero, and the scaled effective population size (Θ) of the eastern Puerto Rican group was significantly larger than that of the western Puerto Rican and Eastern Islands groups (Table 4). Overlapping 95% confidence intervals (CIs) indicated that differences in the median M of the groups were not significant. Analyses excluding potentially introduced samples produced similar results.

The DIYABC analysis supported the Eastern Dispersal Hypothesis (posterior probability = 0.97; 95% CI: 0.95–0.98), with estimated divergence dates of 52.4 kya (95% CI: 2.7–207.0 kya) between eastern Puerto Rican and Eastern Islands groups, and of 89.4 kya (95% CI:

Table 4 Scaled effective population sizes (Θ) and scaled migration rates (M) estimated from the Bayesian MIGRATE 3.2.7 analysis (Model 2) for all loci of western Puerto Rican (WPR), eastern Puerto Rican (EPR) and Eastern Islands (EIS) populations of *Eleutherodactylus antillensis*

	Θ			M		
	WPR	EPR	EIS	EPR to WPR	WPR to EPR	EPR to EIS
All samples	0.0061 (0–0.0112)	0.0500 (0.0204–0.0754)	0.0077 (0.0008–0.0142)	383 (0–1100)	983 (0–1867)	1583 (300–2833)
Excluding potentially introduced samples	0.0059 (0–0.0108)	0.0707 (0.0372–0.0828)	0.0143 (0.0044–0.0254)	417 (0–1200)	2217 (633–3933)	1283 (133–2300)

Values are presented as the median of 20 replicate MIGRATE runs per data set, with the 95% confidence intervals in parentheses.
$\Theta = N_e\mu$ for mtDNA and $4N_e\mu$ for nuclear DNA, where N_e = effective population size, and μ = mutation rate per site per generation.
$M = m/\mu$, where m = immigration rate.

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Table 5 Posterior probabilities (*PP* [(95% CI]) and parameter estimates of models of genetic divergence for *Eleutherodactylus antillensis* in Puerto Rico and the Eastern Islands tested in DIYABC 1.0.4.46

		Demographic parameters							Mutation parameters	
Hypothesis	*PP*	N_a	N_1	N_2	N_3	N_4	t_1	t_2	t_3	Mitochondrial DNA μ	Autosomal diploid DNA μ
Ancient Eastern Islands Isolation											
All data	0.03	4.3×10^5	2.8×10^6	3.4×10^6	2.2×10^6		270.0	208.0		1.1×10^{-8}	7.4×10^{-10}
	(0.02–0.05)	(1.3×10^5	(1.3×10^6	(1.6×10^6	(1.0×10^6		(251.0 –428.0)	(13.0–418.0)		(9.7×10^{-9}	(6.0×10^{-10}
		–8.8×10^5)	–4.9×10^6)	–4.8×10^5)	–3.6×10^6)					–2.2×10^{-8})	–2.9×10^{-9})
Excluding potentially	0.10	4.0×10^5	1.2×10^6	1.2×10^6	4.4×10^5		347.0	282.0		1.1×10^{-8}	1.5×10^{-9}
introduced samples	(0.06–0.13)	(8.9×10^4	(4.6×10^5	(4.0×10^5	(1.6×10^5		(254.0–489.0)	(31.4–461.0)		(9.7×10^{-9}	(6.0×10^{-10}
		–8.8×10^5)	–2.6×10^6)	–3.2×10^6)	–1.4×10^6)					–2.4×10^{-8})	–4.0×10^{-9})
Eastern Dispersal											
All data	0.97	2.5×10^5	2.5×10^6	3.6×10^5		1.3×10^6		89.4	52.4	2.3×10^{-8}	2.1×10^{-9}
	(0.95–0.98)	(7.4×10^4	(9.2×10^5	(1.7×10^6		(4.8×10^5		(19.2–311.0)	(2.7–207.0)	(9.7×10^{-9}	(6.0×10^{-10}
		–7.2×10^5)	–5.4×10^6)	–4.9×10^6)		–2.0×10^6)				–3.8×10^{-8})	–4.0×10^{-9})
Excluding potentially	0.90	3.5×10^5	2.1×10^6	2.0×10^6		4.7×10^5		220.0	114.0	1.3×10^{-8}	1.4×10^{-9}
introduced samples	(0.87–0.94)	(1.0×10^5	(7.7×10^5	(7.2×10^5		(9.8×10^4		(30.4–473.0)	(0.8–241.0)	(9.7×10^{-9}	(6.0×10^{-10}
		–8.4×10^5)	–5.3×10^6)	–4.7×10^6)		–1.7×10^6)				–2.9×10^{-8})	–3.9×10^{-9})

Median parameter estimates (95% CI) for the following demographic parameters are given below for each model: ancestral effective population size (N_a); effective population size for western Puerto Rican (N_1), eastern Puerto Rican (N_2) and Eastern Islands (N_3 and N_4) groups; t_1 = divergence time (in thousands of generations) between the start of the penultimate interglacial and early middle Pleistocene; t_2 = divergence time (in thousands of generations) between the Holocene interglacial and the early middle Pleistocene; and t_3 = divergence time (in thousands of generations) between the Holocene interglacial and the penultimate interglacial. Median parameter estimates for mutation rate (μ) parameters are also provided.

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19.2–311.0 kya) for the split between western and eastern Puerto Rican groups (Table 5). The eastern Puerto Rican group had a larger estimated effective population size (N_2) than those in western Puerto Rico (N_1) and the Eastern Islands (N_4), but the overlapping 95% CIs indicated that these differences were not significant. The Ancient Eastern Islands Isolation Hypothesis had much lower support (posterior probability = 0.03; 95% CI: 0.02–0.05). Pre-evaluation of models showed that the observed data set was surrounded by simulated data sets for each model, indicating that the simulations produced data sets similar to the observed one (Fig. S4, Supporting Information). Additionally, the observed data set was surrounded by many simulated pseudo-observed data sets of the Eastern Dispersal Hypothesis model (Fig. S5, Supporting Information), reflecting a good fit of the model-posterior combination to the pseudo-observed data set (Cornuet *et al.* 2010). In assessing the goodness-of-fit of models, the type I error rate was 0.24, and the type II error rate was 0.26. Analyses excluding potentially introduced samples had higher support for the Eastern Dispersal Hypothesis (posterior probability = 0.90; 95% CI: 0.87–0.94) than for the Ancient Eastern Islands Isolation Hypothesis (posterior probability = 0.10; 95% CI: 0.06–0.13) and produced estimated divergence dates deeper than those inferred in the analysis with all samples (Table 5). When we analysed the Eastern Dispersal Hypothesis model with a larger upper boundary for the prior distribution of divergence time parameters (10–1 000 000 generations), the estimated divergence between eastern Puerto Rican and Eastern Islands groups was 48.4 kya (results not shown). This finding demonstrated that prior distribution of divergence time parameters used for hypothesis testing was sufficiently large, and provided further support for a scenario in which *E. antillensis* populations in the Eastern Islands began diverging from those in eastern Puerto Rico during the last glacial period.

Discussion

Sea level changes and colonization of the Eastern Islands by Eleutherodactylus antillensis

The dynamic history of the PRB left an indelible mark on its biota (Heatwole & MacKenzie 1967). Sea level changes significantly altered the size and degree of isolation of terrestrial habitats in this archipelago (Heatwole & MacKenzie 1967; Renken *et al.* 2002). Brief periods (*c.* 11–18 kyr) of high sea level stands fragmented the Eastern Islands region to an extent similar to its current configuration (Fig. 1b) at least three times in the past 250 000 years (Dutton *et al.* 2009; Muhs *et al.* 2011). Longer periods of low sea level stands predominated during the past five glacial periods (Rohling *et al.* 2009), uniting the PRB into a single landmass. The hypotheses that motivated this study represent competing models for population-level responses to sea level changes. The Ancient Eastern Islands Isolation Hypothesis predicts deep divergence between populations of *E. antillensis* in the Eastern Islands and Puerto Rico, because effectively no migration occurred between these regions when a land bridge united the PRB during the penultimate and last glacial periods. In contrast, the Eastern Dispersal Hypothesis predicts relatively shallow divergence between populations in eastern Puerto Rico (the putative source area) and those in the Eastern Islands, because individuals from Puerto Rico colonized the Eastern Islands via a land-bridge connection between these regions during the penultimate and last glacial periods.

Our multilocus phylogeographic analysis of *E. antillensis* revealed genetic signatures largely supporting the Eastern Dispersal Hypothesis. These signatures include: (i) Eastern Islands populations share some CR haplotypes with those in eastern Puerto Rico (Fig. 4a); (ii) Eastern Islands populations group with several eastern Puerto Rican populations in SAMOVA analyses of CR (Fig. 4b); (iii) Eastern Islands populations share most nuDNA haplotypes with Puerto Rican populations (Table 3 and Fig. S3, Supporting Information); (iv) highest support for a model where gene flow occurs from populations in eastern Puerto Rico towards Eastern Islands populations (Table 1); and (v) highest support for a model in which eastern Puerto Rican and Eastern Islands populations diverged subsequent to the start of the penultimate interglacial *c.* 250 kya (Table 5). Absence of a divergent Eastern Islands clade in phylogenies inferred from mtDNA and nuDNA loci (Figs S1 and S2, Supporting Information), low support for a model in which gene flow does not occur between eastern Puerto Rico and Eastern Islands populations of *E. antillensis* and low support for a model in which eastern Puerto Rican and Eastern Islands populations diverged prior to the start of the penultimate interglacial do not support the Ancient Eastern Islands Isolation Hypothesis. However, we cannot rule out the possibility that *E. antillensis* was present in the Eastern Islands prior to the penultimate interglacial. These populations may have become extinct, and the region recolonized by founders from eastern Puerto Rico. Alternatively, Eastern Islands populations may have persisted in isolation prior to the penultimate interglacial, and experienced gene flow with eastern Puerto Rican populations across the land bridge during the last two glacial periods. We did not find strong evidence for admixture in Eastern Islands populations, such as elevated genetic variation and strong differentiation (Kolbe *et al.* 2004). However, inferring admixture events for

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weakly differentiated populations by relying on a limited marker set can be challenging (Corander & Marttinen 2006).

The phylogenies constructed from mtDNA and nuDNA lacked detailed resolution, but did not conform to predictions of the Eastern Dispersal Hypothesis. Low levels of sequence divergence probably explain why most CR haplotypes and individual samples for each nuDNA locus form a basal polytomy. In phylogenies inferred from MYH, RH1 and RPL9int4, many Eastern Islands samples nested in Puerto Rican clades, but those clades were not well supported across all three analyses and they contained individuals from both eastern and western Puerto Rico (Fig. S2, Supporting Information). This lack of resolution likely indicates shallow levels of isolation across the PRB.

Our estimated divergence date of 52.4 kya (95% CI: 2.7–207.0 kya) between eastern Puerto Rican and Eastern Islands populations suggests that isolation occurred during the last glacial period. Lower sea level during the last glacial period *c.* 118–12 kya (Waelbroeck *et al.* 2002; Siddall *et al.* 2003; van Daele *et al.* 2011) united Puerto Rico and the Eastern Islands. However, increasingly xeric environments in the coastal lowlands (Renken *et al.* 2002) could have promoted isolation of *E. antillensis* following establishment in the Eastern Islands. Small, arid islands in the PRB do not support populations of *E. antillensis*, and drought is associated with lower population densities of these frogs (Ovaska 2005). Sediment records from lowland Guatemala provide evidence for a moist climate *c.* 200–85 kya (Mueller *et al.* 2010) that shifted towards drier conditions *c.* 48 ± 23 kya (Hodell *et al.* 2008), and a high-resolution pollen record from north-eastern Venezuela suggests that dry forests and savanna predominated in Caribbean lowlands *c.* 28–68 kya (González *et al.* 2008; Hessler *et al.* 2010). Data for the PRB are lacking for these time periods, but an analysis of eolianite deposits from the lowlands of the PRB indicates aridity during the Last Glacial Maximum *c.* 26.5–19 kya (Renken *et al.* 2002). In the Greater Antilles (Cuba, Hispaniola, Jamaica, Puerto Rico) and in the PRB in general (Higuera-Gundy *et al.* 1999; Renken *et al.* 2002; Hodell *et al.* 2008), increasing rainfall following the Last Glacial Maximum led to an expansion of mesic forests that may have increased population connectivity, although a concomitant rise in sea level (Peltier 2002) would have reduced dispersal opportunities in the PRB. Large confidence intervals surrounding the estimated divergence between eastern Puerto Rican and Eastern Islands groups prevent us from inferring whether xeric conditions during glacial periods, or salt-water barriers during interglacials, were primarily responsible for initiating population divergence in *E. antillensis*.

Spatial expansion of *E. antillensis* into the Eastern Islands may have left a signature of lower genetic diversity and a smaller effective population size in the founder populations, but estimates of these parameters were either inconsistent across loci, or the differences between them were not significant. For the four nuDNA markers, individuals from the Eastern Islands had fewer haplotypes than those from eastern Puerto Rico, but a similar result was not inferred for CR. A higher mutation rate and faster coalescence time of mtDNA, combined with increased isolation of populations in the Eastern Islands since sea levels rose *c.* 14–11 kya (Heatwole & MacKenzie 1967), may explain the large number of CR haplotypes in this region. The establishment of populations from independent source populations (Kolbe *et al.* 2004) and/or a high population growth rate following a founder event(s) may also limit the loss of genetic diversity following spatial expansion (Austerlitz *et al.* 1997). Eastern Islands populations of *E. antillensis* have a smaller scaled effective population size (Θ) than populations in eastern Puerto Rico (Table 4), a finding that is consistent with a history of spatial expansion. DIYABC estimated the effective population size of the Eastern Islands group (N_4) to be smaller than that of the eastern Puerto Rican group (N_2), but the slightly overlapping 95% CIs suggest that these differences may not be significant (Table 5). Our ability to distinguish between complex population genetic models (e.g. incorporating admixture or changes in population size), and to reduce error rates associated with measuring confidence in scenario choice, and with the variance of divergence times, effective population size and migration rates may improve with additional independent loci (Beerli & Palczewski 2010; Cornuet *et al.* 2010; Robert *et al.* 2011).

In the GENELAND analyses of nuDNA and of all loci, populations of *E. antillensis* in the Eastern Islands formed a distinctive group (Fig. 2), a finding consistent with previous work showing that species in this region form distinctive biogeographic clusters (Hedges 1999). Nevertheless, levels of differentiation between Puerto Rican and Eastern Islands groups of *E. antillensis* are shallower than in the codistributed lizard *Anolis cristatellus* (Brandley & de Queiroz 2004) and in species of *Amphiacusta* ground crickets (Oneal *et al.* 2010). The latter likely persisted in isolation in the Eastern Islands over several glacial–interglacial cycles. Indeed, endemic species of plants (e.g. *Solanum conocarpum*; Acevedo-Rodríguez 1996), frogs (*Eleutherodactylus lentus* and *E. schwartzi*; Henderson & Powell 1999), lizards (e.g. *Anolis ernstwilliamsi*, *A. roosevelti*, *Sphaerodactylus parthenopion* and *Mabuya macleani*; Lazell 1983; Henderson & Powell 1999; Mayer & Lazell 2000), amphisbaenians (e.g. *Amphisbaena fenestrata*; Henderson & Powell

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1999) and snakes (e.g. *Typhlops richardii*; Henderson & Powell 1999) survived in the Eastern Islands throughout multiple glacial–interglacial cycles. Despite this high endemism in the Eastern Islands region, most species occur on multiple islands, and therefore *single-island* endemism is low (Heatwole & MacKenzie 1967).

Diversification of Eleutherodactylus antillensis *within Puerto Rico*

Larger patches of suitable habitat, greater topographic and ecological diversity (Ewel & Whitmore 1973), and less pronounced inundations potentially lowered extinction rates in Puerto Rico relative to the Eastern Islands, and/or promoted diversification in multiple isolated populations on this large island (Rand 1969; Losos 1996). In fact, Puerto Rico contains the highest species richness (Figueroa Colón 1996) and largest proportion of endemics in the PRB (Heatwole & MacKenzie 1967; Hedges 1999). Mountainous regions in Puerto Rico harbour distinctive phylogroups of frogs and lizards (Velo-Antón *et al.* 2007; Rodríguez-Robles *et al.* 2010; Barker *et al.* 2011), and likely shaped genetic diversity in *E. antillensis* by providing mesic refugia during particularly arid glacial periods. Most well-supported clades in the *E. antillensis* nuDNA phylogenies are comprised of individuals from Puerto Rico (Fig. S2, Supporting Information), where group structure is highest (Figs 2 and 4b).

Our analyses of group structure suggest that a historical dispersal barrier for *E. antillensis* occurs in the lowlands west of the Río Grande de Loíza in Puerto Rico (Figs 2 and 4b). Eastern Puerto Rican and western Puerto Rican populations began diverging 89.4 kya (95% CI: 19.2–311.0 kya), coinciding with a shift in Caribbean climate to drier hydrologic conditions and the onset of wet–dry oscillations (Hodell *et al.* 2008; Mueller *et al.* 2010). In addition to promoting isolation between eastern Puerto Rican and Eastern Islands populations, xerophytic vegetation in the lowlands of the PRB during at least some portions of the last glacial period may have also inhibited dispersal between populations in eastern and western Puerto Rico. First proposed as a barrier to westward dispersal of the mesophilic Locust Coquí, *Eleutherodactylus locustus* (Rivero & Mayorga 1963), and the Golden Coquí, *Eleutherodactylus jasperi* (Drewry & Jones 1976), the Río Grande de Loíza Basin marks genetic discontinuities in *Eleutherodactylus coqui* (Velo-Antón *et al.* 2007) and the Mountain Garden Lizard, *Anolis krugi* (Rodríguez-Robles *et al.* 2010). In combination with strong, cool winds during the last glacial period (Hodell *et al.* 2008), increased aridity in the highlands connecting the Cayey Mountains (in the south-eastern region of Puerto Rico) to the Central Mountains (Fig. 1a) could have limited dispersal of *E. antillensis* through higher elevation areas as well. The lowlands of western Puerto Rico receive less rainfall than those in eastern Puerto Rico (Daly *et al.* 2003) and may have been particularly xeric during the last glacial period (Renken *et al.* 2002), which may explain why scaled effective population size of *E. antillensis* is significantly smaller in western Puerto Rico than in the eastern part of the island.

Human-mediated introductions of Eleutherodactylus antillensis

Failure to identify a single unique *E. antillensis* haplotype on St. Croix, an island that has never had a direct land connection with the PRB (Gill *et al.* 1989), supports anecdotal evidence of a recent (1937) human-mediated introduction to St. Croix (Grant & Beatty 1944). The GENELAND analysis revealed that St. Croix populations most likely originated from Eastern Islands sources (Fig. 2). Although our data support the natural occurrence of *E. antillensis* in the Eastern Islands, three CR haplotypes (H14, H31, H35; Fig. 4a), all occurring in eastern Puerto Rico, are shared with distant Eastern Islands (St. John, Tortola, Guana, Beef and Virgin Gorda). Incomplete lineage sorting resulting from recent divergence and rapid spatial expansion from eastern Puerto Rico may account for this observation, but these particular shared haplotypes occur within 11 km of a shipping port in Puerto Rico. A nearly ubiquitous presence of *E. antillensis* in residential gardens and plant nurseries, and frequent transport of horticultural materials in the PRB (Platenberg 2007), supports possible accidental transport of individuals and/or eggs in potted plants between islands. Multiple introductions of *E. coqui* and the Cuban Tree Frog, *Osteopilus septentrionalis*, to various Eastern Islands (MacLean 1982; Owen *et al.* 2005) suggest the possibility of human transport of *E. antillensis* in the PRB. The estimated divergence dates between eastern Puerto Rican and Eastern Islands groups, and between western and eastern Puerto Rican groups, are deeper when potentially introduced samples are excluded (Table 5), raising the possibility that human transport of *E. antillensis* has complicated our historical inferences.

In conclusion, environmental change over the past two glacial–interglacial cycles shaped genetic diversity in *E. antillensis* by providing opportunities for colonization via land-bridge connections during periods of low sea level, as well as by creating habitat barriers that promoted isolation between populations in eastern Puerto Rico and the Eastern Islands, and between populations in eastern and western Puerto Rico. Our results support anecdotal evidence of a human-mediated introduction of *E. antillensis* to St. Croix, and therefore also

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illustrate the role of recent events in shaping phylogeographic patterns in this species. Collectively, our findings are consistent with previous studies indicating that glacial land connections promoted dispersal in other Caribbean land-bridge archipelagoes, including the Great Bahama Bank (Malone *et al.* 2003) and the Turks and Caicos Banks (Reynolds *et al.* 2011). Sea level rise of up to 2 m by 2100 (Pfeffer *et al.* 2008) may reduce populations in small, low-elevation islands, increasing their risk of extirpation through demographic stochasticity, environmental stochasticity and catastrophes, loss of genetic heterozygosity and rare alleles, edge effects, and/or human disturbance (Burkey 1995). Because colonization success by over-water dispersal is low for terrestrial species in the Caribbean (Losos 1996), exploring the geographic distribution of distinct lineages in these dynamic insular systems contributes fundamental information for conservation management strategies.

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References

Acevedo-Rodríguez P (1996). Flora of St. John, U.S. Virgin Islands. The New York Botanical Garden Press, Bronx, New York.

Austerlitz F, Jung-Muller B, Godelle B, Gouyon P-H (1997) Evolution of coalescence times, genetic diversity and structure during colonization. *Theoretical Population Biology*, **51**, 148–164.

Balinsky JB (1981) Adaptation of nitrogen metabolism to hyperosmotic environment in Amphibia. *Journal of Experimental Zoology*, **215**, 335–350.

Barker BS, Waide RB, Cook JA (2011) Deep intra-island divergence of a montane forest endemic: phylogeography of the Puerto Rican frog *Eleutherodactylus portoricensis* (Anura: Eleutherodactylidae). *Journal of Biogeography*, **38**, 2311–2325.

Beaumont A (2008) Joint determination of topology, divergence time, and immigration in population trees. In: Simulations, Genetics, and Human Prehistory (eds Matsumura S, Forster P, Renfrew C), pp. 134–154. McDonald Institute for Archaeological Research, Cambridge, Massachusetts.

Beerli P (2006) Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. *Bioinformatics*, **22**, 341–345.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences of the USA*, **98**, 4563–4568.

Beerli P, Palczewski M (2010) Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics*, **185**, 313–326.

Bertorelle G, Benazzo A, Mona S (2010) ABC as a flexible framework to estimate demography over space and time: some cons, many pros. *Molecular Ecology*, **19**, 2609–2625.

Bintanja R, van de Wal RSW, Oerlemans J (2005) Modelled atmospheric temperatures and global sea levels over the past million years. *Nature*, **437**, 125–128.

Brandley MC, de Queiroz K (2004) Phylogeny, ecomorphological evolution, and historical biogeography of the *Anolis cristatellus* series. *Herpetological Monographs*, **18**, 90–126.

Brandley MC, Warren DL, Leaché AD, McGuire JA (2009) Homoplasy and clade support. *Systematic Biology*, **58**, 184–198.

Bruen TC, Philippe H, Bryant D (2006) A simple and robust statistical test for detecting the presence of recombination. *Genetics*, **172**, 2665–2681.

Burkey TV (1995) Extinction rates in archipelagoes: implications for populations in fragmented habitats. *Conservation Biology*, **9**, 527–541.

Carine MA (2005) Spatio-temporal relationships of the Macaronesian endemic flora: a relictual series or window of opportunity? *Taxon*, **54**, 895–903.

Corander J, Marttinen P (2006) Bayesian identification of admixture events using multilocus molecular markers. *Molecular Ecology*, **15**, 2833–2843.

Cornuet J-M, Ravigné V, Estoup A (2010) Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v. 1.0). *BMC Bioinformatics*, **11**, 401.

Crawford AJ (2003a) Huge populations and old species of Costa Rican and Panamanian dirt frogs inferred from mitochondrial and nuclear gene sequences. *Molecular Ecology*, **12**, 2525–2540.

Crawford AJ (2003b) Relative rates of nucleotide substitution in frogs. *Journal of Molecular Evolution*, **57**, 636–641.

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Crawford AJ, Bermingham E, Polanía CS (2007) The role of tropical dry forest as a long-term barrier to dispersal: a comparative phylogeographical analysis of dry forest tolerant and intolerant frogs. *Molecular Ecology*, **16**, 4789–4807.

Daly C, Helmer EH, Quiñones M (2003) Mapping the climate of Puerto Rico, Vieques and Culebra. *International Journal of Climatology*, **23**, 1359–1381.

Drewry GE, Jones KL (1976) A new ovoviviparous frog, *Eleutherodactylus jasperi* (Amphibia, Anura, Leptodactylidae). *Journal of Herpetology*, **10**, 161–165.

Duellman WE, Trueb L (1994) Biology of Amphibians, 2nd edn. The John Hopkins University Press, Baltimore, Maryland.

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, **11**, 2571–2581.

Dutton A, Bard E, Antonioli F, Esat TM, Lambeck K, McCulloch MT (2009) Phasing and amplitude of sea-level and climate change during the penultimate interglacial. *Nature Geoscience*, **2**, 355–359.

Edwards SV, Beerli P (2000) Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution*, **54**, 1839–1854.

Esselstyn JA, Brown RM (2009) The role of repeated sea-level fluctuations in the generation of shrew (Soricidae: *Crocidura*) diversity in the Philippine Archipelago. *Molecular Phylogenetics and Evolution*, **53**, 171–181.

Ewel JJ, Whitmore JL (1973) The Ecological Life Zones of Puerto Rico and the U.S. Virgin Islands. Forest Service Research Paper ITF-18. United States Department of Agriculture, Forest Service, International Institute of Tropical Forestry, Río Piedras, PR, 74 p.

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Felsenstein J (1984) Distance methods for inferring phylogenies: a justification. *Evolution*, **38**, 16–24.

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package), Version 3.5c. Distributed by the Author, Department of Genetics, University of Washington, Seattle.

Figueroa Colón JC (1996) Phytogeographical trends, centers of high species richness and endemism, and the question of extinctions in the native flora of Puerto Rico. In: The Scientific Survey of Puerto Rico and the Virgin Islands: An Eighty-year Reassessment of the Islands' Natural History (ed. Figueroa Colón JC), pp. 89–102. Annals of the New York Academy of Sciences, New York, New York.

Frankham R (1996) Relationship of genetic variation to population size in wildlife. *Conservation Biology*, **10**, 1500–1508.

Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Gibbs MJ, Armstrong JS, Gibbs AJ (2000) Sister-scanning: a Monte Carlo procedure for assessing signals in recombinant sequences. *Bioinformatics*, **16**, 573–582.

Gill IP, Hubbard DK, McLaughlin P, Moore C (1989) Sedimentological and tectonic evolution of Tertiary St. Croix. In: 12th Caribbean Geological Conference (ed. Hubbard DK), pp. 49–71, West Indies Laboratory, Teague Bay, St. Croix.

González C, Dupont LM, Behling H, Wefer G (2008) Neotropical vegetation response to rapid climate changes during the last glacial period: palynological evidence from the Cariaco Basin. *Quaternary Research*, **69**, 217–230.

Grant C, Beatty HA (1944) Herpetological notes on St. Croix, Virgin Islands. *Herpetologica*, **2**, 110–113.

Guillot G, Estoup A, Mortier F, Cosson JF (2005a) A spatial statistical model for landscape genetics. *Genetics*, **170**, 1261–1280.

Guillot G, Mortier F, Estoup A (2005b) GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes*, **5**, 712–715.

Guillot G, Santos F, Estoup A (2008) Analysing georeferenced population genetics data with Geneland: a new algorithm to deal with null alleles and a friendly graphical user interface. *Bioinformatics*, **24**, 1406–1407.

Heaney LR, Walsh JS Jr, Peterson AT (2005) The roles of geological history and colonization abilities in genetic differentiation between mammalian populations in the Philippine archipelago. *Journal of Biogeography*, **32**, 229–247.

Hearty PJ, Hollin JT, Neumann AC, O'Leary MJ, McCulloch M (2007) Global sea-level fluctuations during the Last Interglaciation (MIS 5e). *Quaternary Science Reviews*, **26**, 2090–2112.

Heatwole H, MacKenzie F (1967) Herpetogeography of Puerto Rico. IV. Paleogeography, faunal similarity and endemism. *Evolution*, **21**, 429–438.

Hedges SB (1999) Distribution patterns of amphibians in the West Indies. In: Patterns of Distribution of Amphibians: A Global Perspective (ed. Duellman WE), pp. 211–254. The John Hopkins University Press, Baltimore, Maryland.

Hedges SB, Duellman WE, Heinicke MP (2008) New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. *Zootaxa*, **1737**, 1–182.

Henderson RW, Powell R (1999) West Indian herpetoecology. In: Caribbean Amphibians and Reptiles (ed. Crother BI), pp. 223–268. Academic Press, San Diego, California.

Hess PN, De Moraes Russo CA (2007) An empirical test of the midpoint rooting method. *Biological Journal of the Linnean Society*, **92**, 669–674.

Hessler I, Dupont L, Bonnefille R *et al.* (2010) Millennial-scale changes in vegetation records from tropical Africa and South America during the last glacial. *Quaternary Science Reviews*, **29**, 2882–2899.

Heuertz M, Fineschi S, Anzidei M *et al.* (2004) Chloroplast DNA variation and postglacial recolonization of common ash (*Fraxinus excelsior* L.) in Europe. *Molecular Ecology*, **13**, 3437–3452.

Hey J, Nielsen R (2004) Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilus*. *Genetics*, **167**, 747–760.

Hickerson MJ, Dolman G, Moritz C (2006) Comparative phylogeographic summary statistics for testing simultaneous vicariance. *Molecular Ecology*, **15**, 209–223.

Higuera-Gundy A, Brenner M, Hodell DA, Curtis JH, Leyden BW, Binford MW (1999) A 10,300 ^{14}C yr record of climate and vegetation change from Haiti. *Quaternary Research*, **52**, 159–170.

Hodell DA, Anselmetti FS, Ariztegui D *et al.* (2008) An 85-ka record of climate change in lowland Central America. *Quaternary Science Reviews*, **27**, 1152–1165.

Hudson RR (1990) Gene genealogies and the coalescent process. *Oxford Surveys in Evolutionary Biology*, **7**, 1–44.

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Hudson RR, Turelli M (2003) Stochasticity overrules the "three-times rule": genetic drift, genetic draft, and coalescence times for nuclear loci versus mitochondrial DNA. *Evolution*, **57**, 182–190.

Hudson RR, Kreitman M, Aguadé M (1987) A test of neutral molecular evolution based on nucleotide data. *Genetics*, **116**, 153–159.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, **23**, 254–267.

Jezkova T, Leal M, Rodríguez-Robles JA (2009) Living together but remaining apart: comparative phylogeography of *Anolis poncensis* and *A. cooki*, two lizards endemic to the aridlands of Puerto Rico. *Biological Journal of the Linnean Society*, **96**, 617–634.

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**, 3059–3066.

Kingman JFC (1982) The coalescent. *Stochastic Processes and Their Applications*, **13**, 235–248.

Kolbe JJ, Glor RE, Rodríguez Schettino L, Chamizo Lara A, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, **431**, 177–181.

Lanfear R, Welch JJ, Bromham L (2010) Watching the clock: studying variation in rates of molecular evolution between species. *Trends in Ecology and Evolution*, **25**, 495–503.

Lazell JD (1983) Biogeography of the herpetofauna of the British Virgin Islands, with description of a new anole (Sauria: Iguanidae). In: Advances in Herpetology and Evolutionary Biology. Essays in Honor of Ernest E. Williams (eds Rhodin AGJ, Myata K), pp. 99–117. Museum of Comparative Zoology, Harvard, Cambridge, Massachusetts.

Lim HC, Rahman MA, Lim SLH, Moyle RG, Sheldon FH (2011) Revisiting Wallace's haunt: coalescent simulations and comparative niche modeling reveal historical mechanisms that promoted avian population divergence in the Malay archipelago. *Evolution*, **65**, 321–334.

Losos J (1996) Ecological and evolutionary determinants of the species-area relation in Caribbean anoline lizards. *Philosophical Transactions of the Royal Society, Series B: Biological Sciences*, **351**, 847–854.

Losos JB, Ricklefs RE (2009) Adaptation and diversification on islands. *Nature*, **457**, 830–836.

MacArthur RH, Wilson EO (1967) The Theory of Island Biogeography. Princeton University Press, Princeton, New Jersey.

MacLean W (1982) Reptiles and Amphibians of the Virgin Islands. Macmillan Caribbean, London, Basingstoke.

Malone CL, Knapp CR, Taylor JF, Davis SK (2003) Genetic consequences of Pleistocene fragmentation: isolation, drift, and loss of diversity in rock iguanas (*Cyclura*). *Conservation Genetics*, **4**, 1–15.

Martin DP, Williamson C, Posada D (2005) RDP2: recombination detection and analysis from sequence alignments. *Bioinformatics*, **21**, 260–262.

Martin DP, Lemey P, Lott M, Moulton V, Posada D, Lefeuvre P (2010) RDP3: a flexible and fast computer program for analyzing recombination. *Bioinformatics*, **26**, 2462–2463.

Mayer GC, Lazell JD (2000) A new species of *Mabuya* (Sauria: Scincidae) from the British Virgin Islands. *Proceedings of the Biological Society of Washington*, **113**, 871–886.

Maynard Smith J (1992) Analyzing the mosaic structure of genes. *Journal of Molecular Evolution*, **34**, 126–129.

Measey GJ, Vences M, Drewes RC, Chiari Y, Melo M, Bourles B (2007) Freshwater paths across the ocean: molecular phylogeny of the frog *Ptychadena newtoni* gives insights into amphibian colonization of oceanic islands. *Journal of Biogeography*, **34**, 7–20.

Mueller AD, Anselmetti FS, Ariztegui D *et al.* (2010) Late quaternary palaeoenvironment of northern Guatemala: evidence from deep drill cores and seismic stratigraphy of Lake Petén Itzá. *Sedimentology*, **57**, 1220–1245.

Muhs DR, Simmons KR, Schumann RR, Halley RB (2011) Sea-level history of the past two interglacial periods: new evidence from U-series dating of reef corals from south Florida. *Quaternary Science Reviews*, **30**, 570–590.

Nabholz B, Glémin S, Galtier N (2009) The erratic mitochondrial clock: variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals. *BMC Evolutionary Biology*, **9**, 54.

Oneal E, Otte D, Knowles LL (2010) Testing for biogeographic mechanisms promoting divergence in Caribbean crickets (genus *Amphiacusta*). *Journal of Biogeography*, **37**, 530–540.

Ovaska KE (2005) The frog (*Eleutherodactylus antillensis*). In: Island: Fact and Theory in Nature (ed. Lazell JD Jr), pp. 175–180. University of California Press, Berkeley, California.

Owen J, Perry G, Lazell JD, Petrovic C, Egelhoff J (2005) *Osteopilus septentrionalis* (Cuban Tree Frog). *Herpetological Review*, **36**, 76.

Padidam M, Sawyer S, Fauquet CM (1999) Possible emergence of new geminiviruses by frequent recombination. *Virology*, **265**, 218–225.

Peltier WR (2002) On eustatic sea level history: Last Glacial Maximum to Holocene. *Quaternary Science Reviews*, **21**, 377–396.

Pfeffer WT, Harper JT, O'Neel S (2008) Kinematic constraints on glacier contributions to 21st-century sea-level rise. *Science*, **321**, 1340–1343.

Platenberg RJ (2007) Impacts of introduced species on an island ecosystem: non-native reptiles and amphibians in the U.S. Virgin Islands. In: Managing Vertebrate Invasive Species: Proceedings of an International Symposium, (eds Witmer GW, Pitt WC, Fagerstone KA). National Wildlife Research Center, Fort Collins, Colorado.

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Posada D, Crandall KA (2001) Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology and Evolution*, **16**, 37–45.

Pregill GK, Olson SL (1981) Zoogeography of West Indian vertebrates in relation to Pleistocene climatic cycles. *Annual Review of Ecology and Systematics*, **12**, 75–98.

Rambaut A, Drummond AJ (2007) Tracer, version 1.5. Available at: http://beast.bio.ed.ac.uk/Tracer.

Rand AS (1969) Competitive exclusion among anoles (Sauria: Iguanidae) on small islands in the West Indies. *Breviora*, **319**, 1–16.

Renken RA, Ward WC, Gill IP, Gómez-Gómez F, Rodríguez-Martínez J (2002) Geology and hydrogeology of the Caribbean islands aquifer system of the commonwealth of Puerto Rico and the U.S. Virgin Islands. *U.S. Geological Survey Professional Paper*, **1419**, 1–148.

Reynolds RG, Gerber GP, Fitzpatrick BM (2011) Unexpected shallow genetic divergence in Turks Island boas (*Epicrates c. chrysogaster*) reveals single evolutionary significant unit for conservation. *Herpetologica*, **67**, 477–486.

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Rivero JA, Mayorga H (1963) Notes on the distribution of some Puerto Rican frogs with a discussion on the possible origin of *Eleutherodactylus locustus*. *Caribbean Journal of Science*, **3**, 81–85.

Robert CP, Cornuet J-M, Marin J-M, Pillai NS (2011) Lack of confidence in approximate Bayesian computation model choice. *Proceedings of the National Academy of Sciences of the USA*, **108**, 15112–15117.

Rodríguez-Robles JA, Jezkova T, Leal M (2008) Genetic structuring in the threatened "Lagartijo del Bosque Seco" (*Anolis cooki*) from Puerto Rico. *Molecular Phylogenetics and Evolution*, **46**, 503–514.

Rodríguez-Robles JA, Jezkova T, Leal M (2010) Climatic stability and genetic divergence in the tropical insular lizard *Anolis krugi*, the Puerto Rican 'Lagartijo Jardinero de la Montaña'. *Molecular Ecology*, **19**, 1860–1876.

Rohling EJ, Grant K, Bolshaw M *et al.* (2009) Antarctic temperature and global sea level closely coupled over the past five glacial cycles. *Nature Geoscience*, **2**, 500–504.

Salminen MO, Carr JK, Burke DS, McCutchan FE (1995) Identification of breakpoints in intergenotypic recombinants of HIV type 1 by bootscanning. *AIDS Research and Human Retroviruses*, **11**, 1423–1425.

Sheldon FH, Jones CE, McCracken KG (2000) Relative patterns and rates of evolution in heron nuclear and mitochondrial DNA. *Molecular Biology and Evolution*, **17**, 437–450.

Siddall M, Rohling EJ, Almogi-Labin A *et al.* (2003) Sea-level fluctuations during the last glacial cycle. *Nature*, **423**, 853–858.

Stephens M, Donnelly P (2003) A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *American Journal of Human Genetics*, **73**, 1162–1169.

Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics*, **68**, 978–989.

Stewart NC Jr, Via LE (1993) A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR amplifications. *BioTechniques*, **14**, 748–750.

Stewart MM, Woolbright LL (1996) Amphibians. In: The Food Web of a Tropical Rainforest (eds Reagan DP, Waide RB), pp. 273–320. The University of Chicago Press, Chicago, Illinois.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Triantis KA, Mylonas M, Whittaker RJ (2008) Evolutionary species-area curves as revealed by single-island endemics: insights for the inter-provincial species-area relationship. *Ecography*, **31**, 401–407.

Tsai Y-H E (2011) PHYLOGEOVIZ: a web-based program that visualizes genetic data on maps. *Molecular Ecology Resources*, **11**, 557–561.

van Daele M, van Welden A, Moernaut J, Martínez-Solano I, Beard KH, Parra-Olea G (2011) Reconstruction of Late-Quaternary sea- and lake-level changes in a tectonically active marginal basin using seismic stratigraphy: the Gulf of Cariaco, NE Venezuela. *Marine Geology*, **279**, 37–51.

Velo-Antón G, Burrowes PA, Joglar RL, Martínez-Solano I, Beard KH, Parra-Olea G (2007) Phylogenetic study of *Eleutherodactylus coqui* (Anura: Leptodactylidae) reveals deep genetic fragmentation in Puerto Rico and pinpoints origins of Hawaiian populations. *Molecular Phylogenetics and Evolution*, **45**, 716–728.

Waelbroeck C, Labeyrie L, Michel E *et al.* (2002) Sea-level and deep water temperature changes derived from benthic foraminifera isotopic records. *Quaternary Science Reviews*, **21**, 295–305.

Whittaker RJ, Triantis KA, Ladle RJ (2008) A general dynamic theory of oceanic island biogeography. *Journal of Biogeography*, **35**, 977–994.

This study forms part of B.S.B.'s doctoral research, which focused on integrating molecular genetics, ecological niche modelling and GIS-based landscape analyses to understand how anurans responded to past climate change in the PRB. J.A.R-R. works on the biogeography and behavioural and evolutionary ecology of reptiles and anurans from the PRB. V.S.A. completed her undergraduate Honours Thesis on aspects of the phylogeography of *Eleutherodactylus antillensis*. A.M. was an UnO undergraduate scholar and contributed to data collection and analyses. R.B.W. has a long-standing interest in long-term population dynamics of tropical organisms. J.A.C. is interested in biotic conservation, historical biogeography, evolution and island biology.

Data Accessibility

Sample locations and PCR conditions are uploaded as online Supporting Information. GenBank accession numbers for *Eleutherodactylus antillensis* and outgroup (*Eleutherodactylus brittoni*, *E. cochranae*, *Eleutherodactylus portoricensis*) mtDNA and nuDNA sequences: JN385299–JN38696; JX142403–JX142406; HQ823678; JX157960–JX157962. Final DNA sequence assembly DRYAD (doi:10.5061/dryad.hc257).

Supporting information

Additional Supporting Information may be found in the online version of this article.

Fig. S1 Maximum likelihood tree for 98 unique mtDNA control region haplotypes of *Eleutherodactylus antillensis*.

Fig. S2 Maximum likelihood trees recovered from individual nuclear intron-spanning loci β-crystallin (CRYBA), myosin heavy chain (MYH), rhodopsin (RH1) and ribosomal protein L9 (RPL9int4) for *Eleutherodactylus antillensis*.

Fig. S3 Georeferenced pie diagrams for each sampling locality of *Eleutherodactylus antillensis*.

Fig. S4 Results of a principal component analysis (PCA), performed in DIYABC 1.0.4.46, of the first 100 000 simulated data sets of two divergence models for *Eleutherodactylus antillensis*.

Fig S5 Results of a principal component analysis (PCA), performed in DIYABC 1.0.4.46, of the test quantities obtained with the Eastern Dispersal Hypothesis model-posterior combination, together with 10 000 pseudo-observed data sets.

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Table S1 Catalogue numbers for traditional and photographic voucher specimens deposited in the Museum of Southwestern Biology (MSB), University of New Mexico, Albuquerque, and the Museum of Vertebrate Zoology (MVZ), University of California, Berkeley.

Table S2 Primers used for amplification (amp) and sequencing (seq) of fragments of the mtDNA control region (CR), nuclear intron-spanning loci β-crystallin (CRYBA), myosin heavy chain (MYH), rhodopsin (RH1) and ribosomal protein L9 (RPL9int4) in *Eleutherodactylus antillensis* and outgroup taxa.

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ORNITHOLOGICAL MONITORING AND RESEARCH ON GUANA ISLAND, BRITISH VIRGIN ISLANDS

PROJECT REPORT 2012

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27 April 2013

Photograph by Tom Willard

Bridled Quail Dove

ORNITHOLOGICAL RESEARCH AND MONITORING ON GUANA ISLAND, BRITISH VIRGIN ISLANDS: PROJECT REPORT 2012

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INTRODUCTION

The Caribbean is both a biodiversity hotspot with a rich array of resident species, and an important region for neotropical migrant landbirds during their annual migration from North America to South America. While considerable research has been conducted on avian ecology in the western Caribbean, comparatively little has been conducted in the Virgin Islands or east Caribbean. Thus, ornithological work on Guana Island makes substantive contributions toward a better understanding of the basic ecology and conservation needs resident Caribbean birds and neotropical migrants. Components of avian research on Guana Island are 1) mist-netting and banding neotropical songbirds that migrate through the Caribbean region during the autumn migration, and 2) specific studies focusing on species resident to the island. Here I provide an update and discussion of the results of the 2012 Science Month, a review of research productivity stemming from avian research on Guana Island, and plans for the 2013 Science Month.

RESULTS AND DISCUSSION

Mist-Netting and Migrant Ecology

I operated mist-net activities between 7 and 24 October, 2012 for a total of 342 net hours. During netting, I captured a total of 113 new birds and recaptured 56 birds that had been previously banded for a total of 169 captures at a mean capture rate of 0.49 birds per net hour. I also captured and released several hummingbirds for which I do not have a permit to band. Despite this being similar in terms of net hours and capture effort, we only captured, this is the lowest capture rate since I took over the avian research program on Guana Island in 2003. The next lowest was in 2009 with 0.51 birds per net hour.

Of the birds captured only 29 were neotropical migrant landbirds. Blackpoll warblers accounted for 86% of these, with other migrants being chestnut-sided warbler, a Swainson's thrush, and a barn swallow. We also captured one resident yellow warbler. Due to the low number of migrants, the majority of birds captured were bananaquits, pearly-eyed thrashers, and black-faced grassquits.

Interesting captures were of one Caribbean elania that was originally banded in 2005, recaptured in 2010, and then in 2012, making it at least 7 years old, the second oldest age record for the species on Guana Island and possibly elsewhere. More interesting was the recapture of a bridled quail-dove originally banded as an adult bird in 2007, making it at least 6 years old; this is likely an age record for the species.

I have conducted a 10 year assessment of the data for blackpoll warbler captures, timing of arrival, age ratios, and conditions upon capture. These data have been analyzed and I have prepared a manuscript that is currently submitted to the journal *Condor* for consideration for publication.

Bananaquit Demography

Based on my intensive study of bananaquits, and an incredibly robust recapture data set, I and a colleague were able to develop a model to determine sex of the species even when not in a breeding condition. This was published in late 2012 (Bibles and Boal 2012 below). We are now using that model to estimate sex- and age-specific survival rates for the species and incorporate covariates of weather and climate factors to attempt to understand how global climate change may affect this

species. If bananaquits are a suitable surrogate for other Caribbean birds, this may also allow broader predictions of the impact of climate change on Caribbean birds in general. We will be working on this through the summer of 2013; I anticipate manuscript submission in late 2013.

Mangrove Cuckoo Ecology
The lead field investigator for this project is Tracy S. Estabrook (M.S.). She has conducted annual call-playback surveys for mangrove cuckoos each year for the last several years and we are not attempting to model occupancy and density using the software programs DISTANCE and PRESENCE. Our next step will be devising methods to capture and color-mark this intriguing species in order to monitor site fidelity and survival. Current capture methods of mist-nets are not effective with the species, but we have a new set of traps we will be experimenting with in 2013.

Bridled Quail Doves
Bridled quail doves are a species of substantial conservation concern, and Guana Island probably has the healthiest population of the species per area size of any island in the Caribbean. This makes Guana a very special location for studying the species. Since initiating a focused study on bridled quail doves, I have captured and banded 38 individuals. However, capturing and marking bridled quail doves requires a different approach than mist-netting, and it can be quite challenging. I have been color-banding the species and have been able to get visual recaptures on individuals in subsequent years to note movement patterns and start collecting information on survival. This is completely new information, as very little is known on the species in general. In 2013 I will be bringing a bow net to Guana Island which may allow substantial improvement in trapping success. As this line of research grows, I hope to pursue funding to expand research on the *Geotrygon* genus to include sites in other parts of the BVI, the USVI and Puerto Rico.

Additionally, I continue to collect data to explore the apparent abnormality of the eyes of the bridled quail doves. When I started trapping them I noticed they all appeared to have an unusual iris with what looks like a erosion of the pigment of the iris forward from the pupil (see examples pictures below). This may be normal but previously unreported, or may be due to a genetic bottleneck or other genetic anomaly. My exploration of this phenomenon continues.

Surveys
I conducted an island-wide bird surveys in 2007 – 2009 and pooled it with data from surveys conducted on Guana Island by Arendt (1995) and Wunderle (2001). We have conducted an analysis to compare species presence and abundance among the different study period and have written a manuscript that is currently submitted to the journal *Ornitologia Neotropical.*

PROJECT PRODUCTIVITY
Peer-Reviewed Papers

2006 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.

2007 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* 119:716–720.

2008 Boal, C. W. 2008. Observations of an Antillean crested hummingbird (*Orthoryhncus cristatus*) attacking saddled anoles (*Anolis stratulus*). *Caribbean Journal of Ornithology* 21:48-49.

2008 Boal, C. W. 2008. Predation of a dwarf gecko (*Sphaerodactylus macrolepis*) by a bridled quail dove (*Geotrygon mystacea*). *Caribbean Journal of Ornithology* 21:50-51.

2011 Boal, C.W. 2011. Bridled Quail-Dove (*Geotrygon mystacea*), Neotropical Birds Online (T.S. Schulenberg, Editor). Ithaca: Cornell Lab of Ornithology; retrieved from Neotropical Birds Online: http://neotropical.birds.cornell.edu/portal/species/overview?p_p_spp=177941

2012 Bibles, B.D., and C.W. Boal. 2012. Morphometric-based sexual determination of bananaquits (*Coereba flaveola*). *Ornitologia Neotropical* 23:507-515.

Submitted Boal, C.W. Timing and condition of *en route* migrant blackpoll warblers in the British Virgin Islands. *Condor*

Submitted Boal, C.W., J.M. Wunderle, Jr., and W.J. Arendt. Autumn monitoring of resident avifauna on Guana Island, B.V.I. *Ornitologia Neotropical*

Peer-Reviewed Papers Produced as Part of Graduate Student Course

Names with [G] indicate graduate student author.

2010 Anderson[G], W.M., G.E. Sorensen[G], J.D. Lloyd-Strovas[G], R.J. Arroyo[G], J.A. Sosa[G], S.J. Wulff[G], B.D. Bibles, C.W. Boal, and G. Perry. 2010. Distribution and Habitat Use by the Critically Endangered Stout Iguana (*Cyclura pinguis*) on Guana Island, British Virgin Islands. *Reptiles and Amphibians* 17:5-10.

2013 Skipper[G], B., B. Grisham[G], M. Kalyvaki[G], D. McGaughey[G], K. Mougey[G], L. Navarrete[G], R. Rondeau, C. Boal, and G. Perry. 2013. Non-overlapping distributions of feral sheep (*Ovis aries*) and stout iguana (*Cyclura pinguis*) on Guana Island, British Virging Islands. *IRCF Reptiles and Amphibians* 20:7-15.

Accepted Navarette[G], L., B. Grisham[G], M. Kalyvaki[G], K. McGaughey[G], K. Mougey[G], B. Skipper[G], G. Perry, and C. Boal. **Accepted.** Diurnal activity patterns of black-necked stilts (*Himantopus mexicanus*) during the non-breeding season in the eastern Caribbean. *Journal of Caribbean Ornithology*:

Papers Currently in Development

1. Age and sex-specific survival of bananaquits
2. Using call-playback to survey and monitor mangrove cuckoos

Presentations

2003 Boal, C. W. 2003. Birds of prey in the British Virgin Islands. H. Levity Stoutt College, Roadtown, Tortola, British Virgin Islands.

2005 Boal, C. W. 2005. Avian research on Guana Island: a decade in review. H. Levity Stoutt College, Roadtown, Tortola, British Virgin Islands.

2006 Boal, C. W. 2006. New bird species in the British Virgin Islands: evidence for migration pattern changes? H. Levity Stoutt College, Roadtown, Tortola, British Virgin Islands.

2005 Estabrook, T. S. 2005. Mangrove cuckoos: where the heck are they and what the heck are they doing? H. Levity Stoutt College, Roadtown, Tortola, British Virgin Islands.

2009 Boal, C. W. 2009. Timing and condition of autumn migrant Blackpoll Warblers in the British Virgin Islands. Annual Meeting of the Cooper Ornithological Society, Tucson, AZ, USA.

Invited Seminars

2013 Boal, C.W. 2013. Blackpoll Warblers: a 10 gram bird that is way tougher than you. ANRS Seminar Series, Texas Tech University, Lubbock, TX.

FUTURE WORK

Avian studies during Science Month in 2013:

- Continuation of the banding station to study species diversity, abundance, and ecological aspects of neotropical migrant land birds using Guana Island during autumn migration.

- Continuation and expansion of the mangrove cuckoo study.
  - In 2013 we will experiment with methods to capture mangrove cuckoos. Capturing this species is challenging, but we have designed some new traps that may prove effective. My increasing captures of the species we can color-band select individuals for long-term monitoring of site fidelity and survival. This would provide substantive new information for the very poorly understood species..

- Continuation of the focused study on ecology of bridled quail doves
  - Color-banding and annual monitoring of survival and site fidelity.
  - Expansion to use of short-term radio-transmitters to monitor daily movement patterns and home-range, habitat-use, and roosting locations and behavior.
  - Collection of samples (photographic and genetic samples) to examine possible inbreeding depression as evidenced by eye abnormalities.

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Bananaquit. Adult being banded (lower left), weighed (lower right) and prior to release (top).

Swainson's Thrush. 2012 was the first occurrence of this species in several years.

Blackpoll Warbler. The most common neotropical migrant we capture on Guana Island.

Caribbean Elania. This bird is at least 7 years old.

Tom Willard, an indispensable assistant, preparing to release a bridled quail dove.

Bridled Quail Dove. Two examples of the eye abnormality observed to be common if not ubiquitous among the species on Guana Island.

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MORPHOMETRIC-BASED SEXUAL DETERMINATION OF BANANAQUITS (*COEREBA FLAVEOLA*)

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Resumen. – Morfometría basada en la determinación sexual de la reinita común (*Coereba flaveola*). – La Reinita Común (*Coereba flaveola*) es un passerino común en los trópicos y ha sido una especie conveniente para estudios ecológicos. Esta especie tiene un plumaje sexualmente monomórfico, y no se puede ser sexada, a menos que los individuos esten en condición reproductiva. Esto es problemático para estudios demográficos y comparativos que requieren una determinación precisa de la edad y sexo de los individuos. Aunque los machos son mas grandes que las hembras, existe traslape tanto en cuerda alar como en masa corporal. Usamos datos morfométricos colectados durante mas de ocho años para desarrollar modelos predictivos, basados en una regresión logística para asignar reinitas comunes adultas a sexo. Nuestra modelo ha clasificado 96% de individuos de validación al sexo correcto. Sugerimos que este enfoque puede mejorar los estudios ecológicos de la especie, facilitando la determinación correcta del sexo, independientemente de su estatus reproductivo. Creemos que nuestra modelación es aplicable en otras localidades, pero debido a que existen variaciones a través de la distribución de la especie, los modelos necesitan ser ajustado a las poblaciones locales.

Abstract. – The Bananaquit (*Coereba flaveola*) is a common passerine throughout the tropics and has been a convenient species for ecological studies. This species has sexually monomorphic plumage and cannot be reliably sexed unless in breeding condition. This is problematic for demographic and comparative studies, which are contingent upon accurately aging and sexing individuals. Although male Bananaquits are larger than females, there is overlap in both wing chord and mass. We used morphometric data collected over eight years to develop a predictive model based on logistic regression to assign adult Bananaquits to sex. Our model classified 96% of validation individuals to the correct sex. We suggest that this approach may enhance ecological studies of the species by facilitating correct sex determination independent of breeding status. We believe our modeling approach is applicable elsewhere but, because there may be geographical variation across the species distribution, models will need to be customized to local populations. *Accepted 8 December 2012.*

Key words: Bananaquit, *Coereba flaveola*, monomorphic plumage, morphometry, sex determination, size dimorphism.

INTRODUCTION

Bananaquits (*Coereba flaveola*) are an abundant passerine found throughout much of the new world tropics. Generally, they have small territories and occur at high densities on many Caribbean islands (Wunderle 1984). The diet of Bananaquits has made them an interesting species for studies of sugar preferences and physiological aspects of nectarivory (Mata & Bosque 2004) and competition with other nectarivorous birds (Askins *et al.* 1987). The

species exhibits plumage polymorphism in parts of its range, such as Grenada, and has been closely studied in context of temporal shifts in the morph ratio cline (Wunderle 1981a, 1981b, 1983; MacColl & Stevenson 2003). The extensive variation in plumage and other features, including size, have prompted several studies to document and explain the variation (e.g., Diamond 1973, Prys-Jones 1982), and infer evolutionary history (Seutin *et al.* 1994, Bellemain *et al.* 2008). Despite its abundance and widespread distribution, relatively few studies have examined the basic biology (Biaggi 1955, Gross 1958, Wunderle *et al.* 1987, Wunderle *et al.* 1992) and breeding ecology (Wunderle 1982, 1984) of Bananaquits.

Because they have sexually monomorphic plumage, Bananaquits cannot be reliably sexed unless in breeding condition (i.e., presence of brood patch or cloacal protuberance). However, this is contingent upon banding studies being conducted during the breeding season. Many studies in the tropics are conducted during the migration or wintering season when most resident tropical birds are not in a breeding state (Faaborg *et al.* 1984, Murphy *et al.* 2004, Boal *et al.* 2006). Sophisticated modeling of survival and demography are contingent upon accurately aging and sexing individuals in the marked sample. Failure to account for sex ratio, or removing unsexed individuals from samples, can lead to introduction of bias, weak inference, and erroneous conclusions. Wolfe *et al.* (2009) recently emphasized this need for better quantitative data on gender determination and other characteristics. Here we address these needs for one of the most common Caribbean passerines by analyzing measurements for Bananaquits captured on Guana Island, British Virgin Islands, to determine if the sexes of adult individuals can be differentiated using some standard morphological measurements.

METHODS

We conducted bird surveys on Guana Island (18°30'N, 64°30'W), a small (3 km^2) island located approximately 0.5 km north of Tortola, British Virgin Islands. The British Virgin Islands, along with the U. S. Virgin Islands, are a chain of approximately 76 islands and cays located roughly 150 km east of Puerto Rico that, combined with Puerto Rico, constitute the Puerto Rican Bank (Lazell 2005). Temperature in the British Virgin Islands normally ranges from 28–33°C, with annual mean rainfall for Guana Island estimated at 92 cm (Lazell 2005).

Guana Island is topographically rugged with elevations ranging from sea level to 246 m. It is privately owned and has undergone little development or fragmentation. A resort area occupies approximately 3% of the island; the remainder of the island is a de facto nature preserve. The majority of the island is vegetated with subtropical dry forest (90%) and mesic ghaut forest (5%) (Lazell 2005). The primary native vegetation on Guana Island includes *Tabebuia heterophylla*, *Bursera simaruba*, *Pisonia subcordata*, *Conocarpus erectus*, *Plumeria alba*, *Acacia muricata*, and *Coccoloba uvifera*. *Leucaena leucocephela* is common in disturbed areas. Other introduced species include *Cocos nucifera*, *Tamarinda indica*, and *Delonix regia* (Lazell 2005).

We operated a banding station on Guana Island during each October of 2003–2010. Altogether 12 32-mm mist nets were opened for an average of 422 (± 57 SE) h each year. All nets were placed in subtropical dry forest and in human-altered areas near the island hotel. Nets were located in the same locations along a northeast–southwest ridge on the west side of the island at ca. 70 m a.s.l. each year.

Captured Bananaquits were placed in temporary holding bags and transported to a banding station for processing. We recorded

unflattened wing chord with a stopped wing ruler. We measured mass with an electronic scale accurate to 0.1 g (Ohaus Model CS200). We aged each Bananaquit as adult or juvenile based on plumage (Raffaele *et al.* 1998), and examined adults for evidence of breeding status (i.e., brood patch, cloacal protuberance). To ensure consistency, one person performed all measurements and classifications. We attached an aluminum leg band provided by the U. S. Geological Survey Bird Banding Laboratory and, beginning in 2005, we attached unique combinations of two plastic colored bands to adults.

Preliminary examination of wing chord and mass of known sex Bananaquits led us to suspect we could use these metrics to determine sex of individuals with a high level of confidence. We compared mass and wing chord of all adult Bananaquits, male only, and females only using linear regression. We used these individuals to calculate 95% confidence intervals on mass and wing chord for male and female Bananaquits. We used logistic regression to develop a predictive model for classification of individuals of unknown sex. The logistic model was developed using individuals captured from 2003–2010 for which sex was verified by presence of brood patch or cloacal protuberance. Adult individuals classified as unknown sex were included if they were sexed at a later capture, with the measurements from the initial capture utilized for the analysis. Only one set of measurements was included for each individual to avoid lack of independence of data. We randomly selected 50 known-sex adults, 25 of each sex, for removal from the dataset to use for model validation. Four logistic regression models were run: 1) wing chord only, 2) mass only, 3) wing chord-mass additive model, and 4) wing chord-mass interaction model. The model best fitting the data was chosen using AIC (Burnham & Anderson 2002). Sex of validation individuals was predicted using the best model with a predicted probability of being female = 0.5 indicating females. We then compared the assigned values with the known sex. The model was then applied to all unknown sex individuals for whom we had wing chord and mass measurements.

RESULTS

A total of 519 captures of adult Bananaquits from 2003–2010 represented 304 individuals, of which 285 had both wing chord and mass measured. Of these, 222 birds were field-sexed (131 male, 91 female). Wing chord for all individuals was 57.0 mm (SE = 0.155 mm, range 51–62 mm). Males had larger wing chords (mean = 59.1 mm, SE = 0.114 mm, range 55–62 mm) than females (mean = 54.6 mm, SE = 0.133 mm, range 51–58 mm) (t_{220} = –25.523, $P < 0.0001$). Males also were heavier (mean = 10.2 g, SE = 0.063 g, range 8.0–12.5 g) than females (mean = 9.4 g, SE = 0.086 g, range 6.4–11.8 g) (t_{220} = –8.151, $P < 0.0001$). Wing chord exhibited substantially less variation (male CV = 2.2; female CV = 2.3) than mass (male CV = 7.1; female CV = 8.7). Unknowns generally exhibited the full range of observed wing chords (mean = 56.0 mm, SE = 0.324 mm, range 51–62 mm) and mass (mean = 9.3 g, SE = 0.086 g, range 6.4–11.8). Overlap of sexes was observed with both metrics, with extensive overlap in mass and overlap in the 55–58 mm wing chord range (Fig. 1). In addition, average mass varied considerably between years, ranging from 9.8 g in 2009 to 10.9 g in 2008 for males, and from 8.8 g in 2009 and 9.8 g in 2007 for females. Mass and wing chord exhibited a positive relationship for both sexes (linear regression: male $P = 0.0010$; female $P = 0.0064$), although the relationship was noisy and mass provided little explanation for variation in wing chord (male $R^2 = 0.0815$; female $R^2 = 0.0805$). The relationship appeared to be additive, with no difference in slope of the

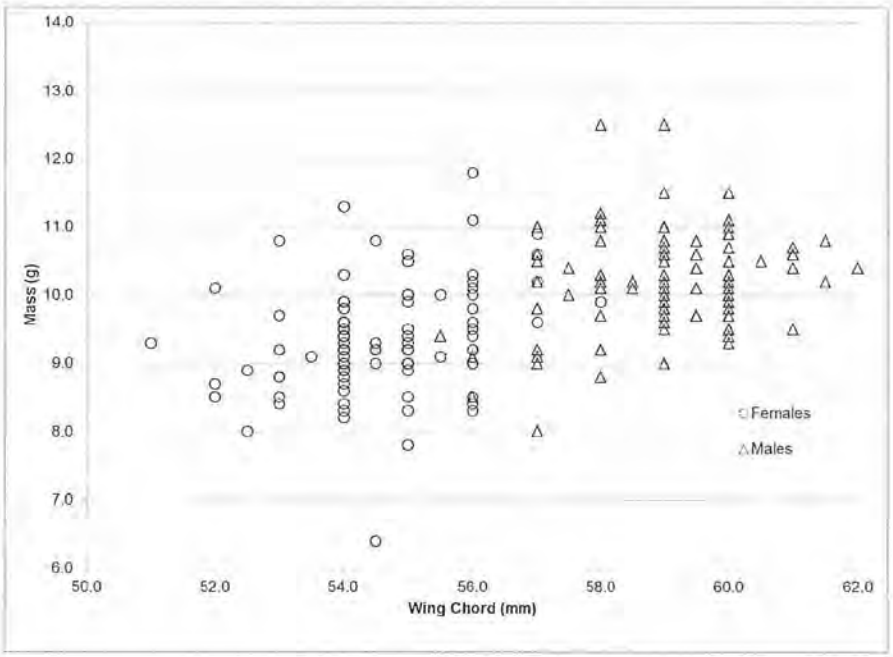

FIG. 1. Scatterplot illustrating mass (g) and wing chord (mm) of adult Bananaquits, by sex, on Guana Island, BVI, 2003-2010. Data from 222 individuals (131 M, 91 F).

relationship between sexes (male slope = 0.516, 95% CI [0.214, 0.817]; female slope = 0.439, 95% CI [0.127, 0.751]), but males had a somewhat higher intercept (male intercept = 53.83, 95% CI [50.74, 56.927]; female intercept = 50.49, 95% CI [47.55, 53.42]).

Measurements from 172 birds in Definitive plumage (106 male, 66 female) were used to perform the logistic regressions. The best model was the Wing Chord + Mass additive model (AIC = 46.715), followed by Wing Chord only (ΔAIC = 1.47), and the Wing Chord * Mass interaction (ΔAIC = 1.955) models (Table 1). The Mass only model performed poorly compared to the other models (ΔAIC = 149.082). The best model provides the probability of the bird being female given the measurements by the equation:

$$Pr\{\text{Female} \mid \text{Mass, Wing Chord}\} = 1 / [1 + e^{[36.8+0.8947(\text{Mass})-2.5749(\text{Wing Chord})]}]$$

The 95% profile likelihood confidence interval on the slope parameter for wing chord (= –2.5749) did not encompass zero (–3.8901, –1.7311), but the confidence interval for mass did include zero (–0.0450, 1.9341), suggesting that wing chord has significant explanatory power in classifying sex. When applied to the validation samples, the model correctly classified 100% of males and 92% of females. The 95% confidence intervals on the probability included 0.5 for six (12%) of the classifications, including one of the two misclassifications. Censoring of individuals for which the 95% confidence interval included any value between 0.4 and 0.6 resulted in 100%

TABLE 1. Ranking of logistic models for determining sex of adult Bananaquits based on measurements of mass (g) and wing chord (mm). Number of parameters (K), Akaike Information Criterion (AIC), difference in AIC (ΔAIC), -2 * ln likelihood (-2 ln), and model (Akaike) weights (w_i) are provided.

Model	K	AIC	ΔAIC	-2 ln	w_i
Wing Chord + Mass	3	46.715	0.000	40.715	0.539
Wing Chord	2	48.185	1.470	44.185	0.258
Wing Chord * Mass	4	48.670	1.955	40.670	0.203
Mass	2	195.797	149.082	191.797	0.000

classification success, but required removal of nine (18%) of the validation individuals. In both failed classifications, the females had larger than average wing chords (both 57.0 mm) and larger than average mass (9.6 and 10.2 g, respectively). Based on this model, adult Bananaquits with wing chords < 55.0 mm can be assumed female, and those with wing chords > 57.5 mm can be assumed males. Within the 55.5–57.5 mm wing chord, mass becomes important with, counter-intuitively, larger mass individuals being females (Fig. 2). The model classified the 63 unknown sex individuals as 29 males and 34 females. Certainty of classification was similar to that for the validation test. Ninety-five percent confidence intervals on the probability of being female included 0.5 for eight (13%) individuals, and included a value between 0.4 and 0.6 for 13 (21%) individuals.

DISCUSSION

Bananaquits exhibit sexual dimorphism in wing chord although some overlap exists. Based on presence of breeding criteria, we observed adult males with wing chords as short as 55 mm, and adult females with wing chords as long as 58 mm. This range of overlap was supported by the results of our predictive model. In a well-developed guide for aging and sexing Bananaquits in Jamaica, Susan Koenig (unpub. data) found individuals with wing chord < 52 mm can be reliably sexed as female, and those > 57 mm can be reliably sexed as males. On Dominica, 89% of individuals with wing lengths less than a median of 62 mm showed evidence of brood patches (Prys-Jones 1982). These data suggest that sexing individuals based only on wing chord is questionable, and requires classifying individuals within the zone of overlap as unknowns, which may compromise results of subsequent demographic analyses. Other measurements likely present the same problem. We found substantial overlap (62% of the observed range) in mass of males and females.

The observed variation in size due to sex suggests that averaging morphometrics in ecological studies of Bananaquits without accounting for sex may be problematic. Diamond (1973) found positive correlations between elevation and several morphometrics (i.e., mass, wing length, and bill length) of unsexed Bananaquits in Jamaica. However, when museum specimens of known sex from Central and South America were measured, the relationship was only significant for wing length and elevation in males (Diamond 1973). Prys-Jones (1982) found a strong positive correlation between wing length and mass of Bananaquits in Dominica without accounting for differences in sex. We observed a similar relationship although mass had little explanatory power for wing chord for either sex (male $R^2 = 0.0815$, female $R^2 = 0.0805$).

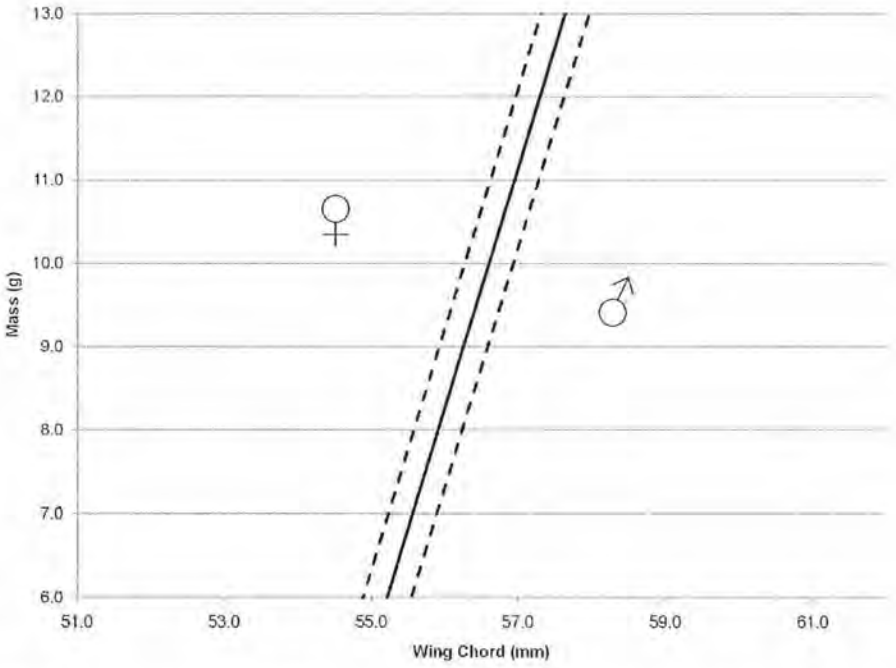

FIG. 2. Assignment of sex of adult Bananaquits based on logistic model using wing chord (mm) and mass (g). Solid line indicates predicted probability of being female = 0.5. Dashed lines indicate predicted probabilities of being female = 0.7 (left) and 0.3 (right).

This weak relationship is probably due to high yearly variation in mass.

Determining sex based on morphometrics may be confounded by not only altitudinal variation in size (Diamond 1973) but also variance in sizes across the species distribution. For example, examining data from disparate locations within the range of the Bananaquit reveals the potential for substantial spatial variation in body size, even though direct comparison is difficult due to differences in measurement technique. In the South and Central America, flattened wing chord of male and female Bananaquits averaged 57.0 mm (N = 64, SE = 0.378, 5.30 CV) and 54.1 mm (N = 25, SE = 0.011, 5.44 CV), respectively (Diamond 1973). In the northeast region of the Caribbean within the BVI, we found males had a much longer average unflattened wing chord of 59.1 mm whereas females only had a slightly larger average wing chord of 54.6 mm than that of mainland females. Southward at the center of the Lesser Antilles, Prys-Jones (1982) reported an overall average wing chord for Bananaquits on Dominica as 60.6 mm (± 3.2 mm). However, Prys-Jones (1982) used maximum chord rather than unflattened chord. Converting unflattened wing chord to maximum chord (Pyle 1997) suggests that average wing chord in the BVI is similar to that on Dominica. Unflattened wing chord measurements used for sexing on Jamaica (male > 57 mm, female < 52 mm; Susan Koenig, unpub. data) sug-

gests that Bananaquits on Jamaica are similar in size to mainland birds.

Ecological studies involving size should either avoid using unsexed individuals or censor individuals of unknown sex. First, averaging morphometrics using unsexed individuals requires the assumption that comparison samples have equivalent sex ratios. For example, when removing unknown sex individuals (22% of 285 individuals), we observed a sex ratio of 0.59 (males vs females) and obtained an average wing chord of 57.3 mm. Using the averages for each sex and assuming a 50:50 sex ratio results in a drop of 0.5 mm (56.8 mm) in the estimated average, which is 5% of the overall range in observed measurements. Second, use of threshold criteria that create a zone of "unknown" create a bias by tending to censor larger individuals of the smaller sex, and vice versa, leading to an apparent larger difference between sexes than exists. In addition, this censoring leads to smaller variance estimates than appropriate, increasing the probability of finding a non-existent difference (i.e., committing Type 1 error). For example, we found that coefficients of variation on wing chord for males and females were 2.2 and 2.3%, respectively, when Bananaquits were sexed based on breeding characters. Had we used a wing chord criteria classifying individuals from 55.0–57.5 mm as unknown sex, the coefficients of variation for males and females would have been 1.7 and 1.4%, respectively.

Errors in classifying sex using our model could have deleterious effects on an analysis. However, we believe that the level of error is very low and unlikely to have significant influence, especially in comparison to the impact of censoring unknown sex individuals from the analysis. In our classification of the 63 individuals of unknown sex, classifying using the 55–57.5 wing chord criteria would have resulted in censoring 28 (44%) individuals from further analysis. Using the conservative approach of censoring individuals in which the probability of being female was approaching 0.5 (e.g., 0.4–0.6) would have only removed 13 individuals (21%) from further consideration, resulting in substantially less bias. We believe the model we have developed is a valid, field-applicable approach for determining sex of non-breeding adult Bananaquits. We suggest adult Bananaquits (i.e., those individuals in Definitive plumage) should be sexed using breeding criteria, if possible, and then classifying the remaining unknown sex individuals using our approach. This approach had a high success rate (96%) in classifying adult individuals that did not exhibit breeding characteristics, and has the potential to significantly reduce the analyss problems associated with having unknown sex individuals within a dataset. We believe our current model is applicable to adult Bananaquits in the British and U. S. Virgin Islands, but have not yet tested it beyond our study island. However, we also suspect it will need to be modified to account for regional variance across the Bananaquit distribution, but see no reason the approach we took to developing morphometric-based sexing criteria could not be applied elsewhere. Development of localized models using this approach and existing morphometric data is currently possible for many portions of the species' range. In addition, incorporation of other metrics, such as tail chord or exposed culmen, may benefit the model and should be explored.

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REFERENCES

Askins, R. A., K. M. Ercolino , & J. D. Waller. 1987. Flower destruction and nectar depletion by avian nectar robbers on a tropical tree, *Cordia sebestena*. J. Field Ornithol. 58: 345–349.

Bellemain, E., E. Bermingham, & R. E. Ricklefs. 2008. The dynamic evolutionary history of the Bananaquit (*Coereba flaveola*) in the Caribbean revealed by a multigene analysis. Evol. Biol. 8: 240

Biaggi, V. 1955. The Puerto Rican honeycreeper (Reinita) *Coereba flaveola* (Bryant). Univ. of Puerto Rico Agricultural Experiment Station, Mayaguez, Puerto Rico, USA. Special Publication, 61 pp.

Boal, C. W., F. C. Sibley, T. S. Estabrook, & J. Lazell. 2006. Insular and migrant species, longevity records, and new species records on Guana Island, British Virgin Islands. Wilson J. Ornithol. 118: 218–224.

Burnham, K. P., & D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. 2nd ed. Springer, New York, New York, USA.

Diamond, A. W. 1973. Altitudinal variation in a resident and a migrant passerine on Jamaica. Auk 90: 610–618.

Faaborg, J., W. J. Arendt, & M. S. Kaiser. 1984. Rainfall correlates of bird population fluctuations in a Puerto Rican dry forest: a nine year study. Wilson Bull. 96: 575–593.

Gross, A. O. 1958. Life history of the Bananaquit of Tobago Island. Wilson Bull. 70: 257–279.

Lazell, J. 2005. Island: fact and theory in nature. Univ. of California Press, Berkeley, California, USA.

MacColl, A. D. C., & I. R. Stevenson. 2003. Stasis in the morph ratio cline in the Bananaquit on Grenada, West Indies. Condor 105: 821–825.

Mata, A., & C. Bosque. 2004. Sugar preferences, absorption efficiency and water influx in a Neotropical nectarivorous passerine, the Bananaquit (*Coereba flaveola*). Comp. Biochem. Phys. A 139: 395–404.

Murphy, M. T., J. Zysik, & A. Pierce. 2004. Biogeography of the birds of the Bahamas with special reference to the island of San Salvador. J. Field Ornithol. 75: 18–30.

Prys-Jones, R. P. 1982. Molt and weight of some land-birds on Dominica, West Indies. J. Field Ornithol. 53: 352–362.

Pyle, P. 1997. Identification guide to North American birds-Part I: Columbidae to Ploceidae. Slate Creek Press, Bolinas, California, USA.

Raffaele, H., J. Wiley, O. H. Garrido, A. Keith, & J. I. Raffaele. 1998. A guide to the birds of the West Indies. Princeton Univ. Press, Princeton, New Jersey, USA.

Seutin, G., N. K. Klein, R. E. Ricklefs, & E. Bermingham. 1994. Historical biogeography of the Bananaquit (*Coereba flaveola*) in the Caribbean region: a mitochondrial DNA assessment. Evolution 48: 1041–1061.

Wolfe, J. D., P. Pyle, & C. J. Ralph. 2009. Breeding seasons, molt patterns, and gender and age criteria for selected northeastern Costa Rican resident landbirds. Wilson J. Ornithol. 121: 556–567.

Wunderle, J. M. 1981a. An analysis of a morph ratio cline in the Bananaquit (*Coereba flaveola*) on Grenada, West Indies. Evolution 35: 333–344.

Wunderle, J. M. 1981b. Movements of adult and juvenile Bananaquits within a morph-ratio cline. Auk 98: 571–577.

Wunderle, J. M. 1982. The timing of the breeding season in the Bananaquit (*Coereba flaveola*) on the island of Granada, WI. Biotropica 14: 124–131.

Wunderle, J. M. 1983. A shift in the morph ratio cline in the Bananaquit on Grenada, West Indies. Condor 85: 365–367.

Wunderle, J. M. 1984. Mate switching and a seasonal increase in polygyny in the Bananaquit. Behaviour 88: 123–144.

Wunderle, J. M., M. S. Castro, & N. Fletcher. 1987. Risk-averse foraging by Bananaquits on negative energy budgets. Behav. Ecol. Sociobiol. 21: 249–255.

Wunderle, J. M., R. A. Cortes, & W. Carromero. 1992. Song characteristics and variation in a population of Bananaquits on Puerto Rico. Condor 94: 680–691.

Population History of the Zenaida Dove (*Zenaida aurita*) in the Antilles: Phylogeography, Contemporary Gene Flow and Morphological Divergence

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Abstract

Caribbean avifaunal biogeography has been mainly studied based on mitochondrial DNA. Here, we investigated both past and recent island differentiation and micro-evolutionary changes in the Zenaida Dove (*Zenaida aurita*) based on combined information from one mitochondrial and 13 microsatellite markers and four morphological characters. This Caribbean endemic and abundant species has a large distribution, and two-subspecies are supposed to occur: *Z. a. zenaida* in the Greater Antilles (GA) and *Z. a. aurita* in the Lesser Antilles (LA). Doves were sampled on two GA islands (Puerto Rico and the British Virgin Islands) and six LA islands (Saint Barthélemy, Guadeloupe, Les Saintes, Martinique, Saint Lucia and Barbados). Although one mitochondrial DNA lineage was detected in GA and another in LA, their level of divergence was too moderate to corroborate the existence of two subspecies. Colonisation of the studied islands appeared to be a recent process. However, both phenotypic and microsatellite data suggest that differentiation is already under way between all of them, despite the existence of limited gene flow. No sex-biased dispersal or isolation by distance was observed. Differentiation for morphological traits was more pronounced than for neutral markers. These results suggest that despite recent colonisation, genetic drift and/or restricted gene flow are promoting differentiation for neutral markers. Variation in selective pressures between islands is also likely to be involved to explain phenotypic differentiation. Overall the results are suggestive of a re-expansion-early differentiation phase, thus providing for the first time evidence for the taxon cycling hypothesis in a non passerine Antillean bird.

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Steven Latta

From: Ricklefs, Robert E. <ricklefs@umsl.edu>
Sent: Thursday, March 28, 2013 11:59 AM
To: Karine Monceau
Cc: Steven Latta
Subject: Acknowledgement

Dear Karine,

The following will work for an acknowledgment for our samples.

"Samples from Guana Island, British Virgin Islands, were made available by Robert Ricklefs and Steven Latta, with support for their collection provided by Fred Sibley, James Lazell and the Falconwood Foundation. Fieldwork in the British Virgin Islands was supported by National Science Foundation grant DEB-0542390."

Best wishes,

Bob

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RESEARCH ARTICLE

ZooKeys

A review of the *Paectes arcigera* species complex (Guenée) (Lepidoptera, Euteliidae)

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Citation: Pogue MG (2013) A review of the *Paectes arcigera* species complex (Guenée) (Lepidoptera, Euteliidae). In: Schmidt BC, Lafontaine JD (Eds) Contributions to the systematics of New World macro-moths IV. ZooKeys 264: 125–163. doi: 10.3897/zookeys.264.3274

Abstract

Five new species of *Paectes* Hübner [1818] related to *Paectes arcigera* (Guenée) (Puerto Rico, U.S. Virgin Islands, British Virgin Islands, Guadeloupe, Dominica, St. Lucia, Trinidad) and *P. longiformis* Pogue (Brazil) are described: *P. asper* **sp. n.** (Florida, Bahamas, Cuba, Cayman Islands, Jamaica, Haiti, Dominican Republic, Puerto Rico, British Virgin Islands, U.S. Virgin Islands, Dominica, Colombia), *P. medialba* **sp. n.** (Argentina), *P. similis* **sp. n.** (Brazil), *P. sinuosa* **sp. n.** (Argentina, Brazil, Paraguay), and *P. tumida* **sp. n.** (Colombia, Guyana, Suriname, French Guiana). Adults and genitalia are illustrated for all species. Taxonomic changes include the **rev. stat.** of *P. nana* (Walker) (Florida, Greater Antilles, Mexico, Guatemala, Galapagos) as a valid species and **revised synonyms** *P. indefatigabilis* Schaus and *P. isabel* Schaus as junior synonyms of *P. nana* instead of *P. arcigera*. New host records for *P. sinuosa* and *P. nana* reared on Brazilian peppertree (*Schinus terebinthifolius* Raddi, Anacardiaceae) are presented. The holotype and female genitalia of *P. obrotunda* (Guenée) are illustrated.

Keywords

Taxonomy, new species, Brazilian peppertree, *Schinus terebinthifolius*, Anacardiaceae, invasive species, new host records

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Introduction

Specimens of a species described as *Paectes longiformis* Pogue were sent to me for identification from scientists at the Biological Control Research and Containment Laboratory, University of Florida, Ft. Pierce, FL. This species is being tested for possible release as a biological control agent of the Brazilian peppertree (*Schinus terebinthifolius* Raddi, Anacardiaceae), an invasive species with severe economic impact. Specimens originated near the airport in Salvador, Bahia, Brazil. Originally thought to be *Paectes obrotunda* (Guenée), it proved to be a new species (Manrique et al. 2012).

In the collection of the USNM there were over 250 specimens identified as *P. obrotunda*. The results of this study showed that these specimens consisted of two described species, *Paectes arcigera* (Guenée) and *Paectes nana* (Walker) and five additional new species that are described here. Taxonomic changes included the revised status of *P. nana* as a valid species and not a synonym of *P. arcigera*. *Paectes burserae* (Dyar) is a syn. n. of *P. nana*. *Paectes indefatigabilis* Schaus and *P. isabel* Schaus, both from the Galapagos Islands, Ecuador, are synonyms of *P. nana* and not *P. arcigera* as previously thought (Poole 1989; Roque-Álbelo and Landry 2011). *Paectes obrotunda* (Guenée) is also referred to the *Paectes arcigera* group.

The *Paectes arcigera* group includes only the species referred to in this paper. Species in this group can be recognized by the elongate free saccular extension in the male genitalia. Including the species in this revision there are 12 species of *Paectes* in North America and 40 species in the Neotropics. Two of these species, *P. nana* and *P. asper* Pogue, occur both in North America and the Neotropics.

Material and methods

Repository abbreviations

Specimens and images were examined from the following collections:

BMNH	The Natural History Museum, London, UK
LAN	Peter J. Landolt collection, Yakima, WA, USA
MGCL	McGuire Center for Lepidoptera and Biodiversity, University of Florida, Gainesville, FL, USA
TDC	Terhune S. Dickel Collection, Ocala, FL, USA
UFPC	Coleção Entomológica Padre Jesus Santiago Moure, Universidade Federal do Paraná, Curitiba, BRAZIL
USNM	National Museum of Natural History, Washington, DC, USA
WSU	Washington State University, Pullman, WA, USA

Dissection of genitalia follows the method of Pogue (2002) except specimens were mounted in Euparal and stained exclusively in Mercurochrome. Male genital morphol-

ogy follows Forbes (1954) and female morphology follows Lafontaine (2004). Terms used in describing forewing morphology follow Lafontaine (2004). Images of adult moths were taken with a Visionary Digital Imaging System using a Canon EOS 5D Mark II camera with a modified K2 long-distance lens and a pulsed xenon flash. Forewing length was measured using a calibrated ocular micrometer from the juncture of the thorax to the apex, including fringe.

Distribution maps (Figs 48–52) were generated using ESRI® ArcMap™ 10.0 (ESRI, Redland, CA). Latitude and longitude coordinates were obtained from the label data or from a localities database that I maintain. The data points were entered into a FileMaker Pro 11.0 v 3 database and then directly assembled as a data layer onto a world map projection using a GCS-WGS-1984 Geographic Coordinate System.

Key to species based on male genitalia

1 Free saccular extension extending above costa (Fig. 29) 2
– Free saccular extension extending below costa (Fig. 31) 6
2 Free saccular extension wide, apex enlarged (Fig. 29) ..... ***P. arcigera***
– Free saccular extension narrow, apex not enlarged (Fig. 30) 3
3 Setae on dorsal surface of valve hairlike, straight (Fig. 30) ..... ***P. longiformis***
– Setae on dorsal surface of valve thick, curved (Fig. 32) 4
4 Lateral margin of valve bearing wide, flat setae on sclerotized ridge (Fig. 32) ..... ***P. nana***
– Lateral margin of valve lacking wide flat setae 5
5 Free saccular extension sinuate; base covered with minute spicules (Fig. 35) ..... ***P. sinuosa***
– Free saccular extension straight, curved near apex; base lacking minute spicules (Fig. 33) ..... ***P. asper***
6 Setae on dorsal surface of valve hairlike, straight; free saccular extension lacking spicules (Fig. 31) ..... ***P. similis***
– Setae on dorsal surface of valve thick, curved; free saccular extension covered with minute spicules 7
7 Base of free saccular extension bulbous, more than twice width of arm below apex (Fig. 36) ..... ***P. tumida***
– Base of free saccular extension gradually narrowing toward apex, not bulbous (Fig. 34) ..... ***P. medialba***

Key to species based on female genitalia

1 Lateral margin of 8th sternite produced into short, triangular projections (Fig. 40) 2
– Lateral margin of 8th sternite smooth, lacking projections (Fig. 37) 6

2 Ductus bursae at juncture with appendix bursae approximately same width as juncture with corpus bursae (Fig. 38) 3
– Ductus bursae at juncture with appendix bursae narrow at juncture with appendix bursae and widens at juncture with corpus bursae (Fig. 41) ..... ***P. medialba***
3 Ostium bursae with a medial, curved, sclerotized bar (Fig. 43) ..... ***P. tumida***
– Ostium bursae without an obvious sclerotized structure (Fig. 38) 4
4 Lateral margin of 8th sternite not well developed, apex pointing laterally (Fig. 38) ..... ***P. longiformis***
– Lateral margin of 8th sternite well developed, apex pointing ventrally (Fig. 40) 5
5 Juncture of appendix bursae and ductus bursae just distal to ostium bursae (Fig. 40) ..... ***P. asper***
– Juncture of appendix bursae at middle of ductus bursae (Fig. 42) ..... ***P. sinuosa***
6 Ostium bursae a round circle (Fig. 39) ..... ***P. nana***
– Ostium bursae a sclerotized band or half-circle 7
7 Ostium bursae a large, heavily sclerotized half-circle shape (Fig. 37) ..... ***P. arcigera***
– Ostium bursae a sclerotized band with narrowed lateral apices (Fig. 46) ..... ***P. obrotunda***

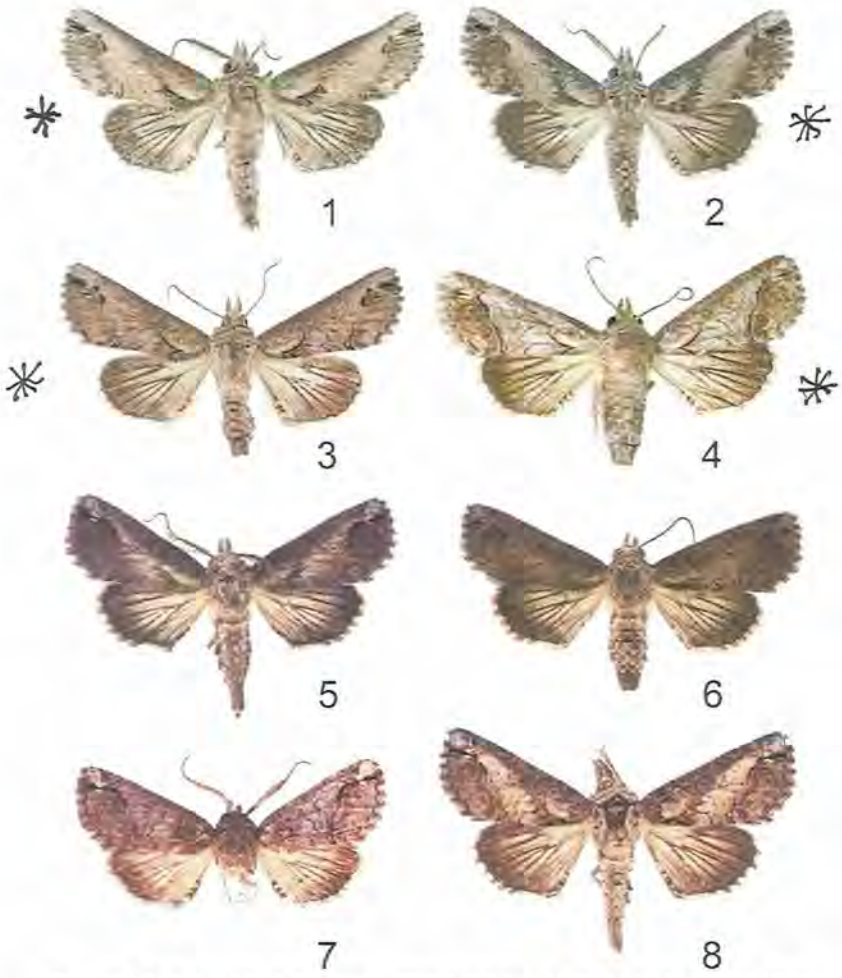
Descriptions

***Paectes arcigera* (Guenée, 1852)**
http://species-id.net/wiki/Paectes_arcigera
Figs 1–4, 29, 37, 48

Ingura arcigera Guenée *in* Boisduval and Guenée 1852: 312.

Type material. St. Thomas: lost. **Neotype:** Dominica. USNM, here designated. This is a confusing group of species that can only be identified reliably by genitalic characters, so to ensure the stability of the name, a male labeled "DOMINICA: Grande Savane, 1 July 1964, O. S. Flint, Jr., genitalia slide male, USNM 135918 [green label]" is designated as neotype for *Ingura arcigera* Guenée, 1852.

Other material examined. All specimens in USNM unless noted (62 males, 49 females). **BRITISH VIRGIN ISLANDS:** Guana Island, 1–14 July 1984 (22 males, 11 females), Genitalia slides m, USNM 135957, 1359980, 135991, 135993, 136010, S.E. & P.M. Miller; Virgin Gorda Island, Virgin Gorda Peak, ca. 400 m, 17–19 July 1986 (4 males, 1 female), Genitalia slide m, USNM 135958, S.E. Miller & M.G. Pogue. **DOMINICA:** same data as neotype (1 male, 1 female), genitalia slide male, USNM 136004, 13 May 1964 (1 male), 14 June 1964 (1 male), 28 Oct. 1966 (2 males), E.L. Todd, 31 Oct. 1966 (2 males), genitalia USNM 136003, E.L. Todd, 1 Nov. 1966 (1male), E.L. Todd; Clarke Hall, 11 Jan. 1965 (1 female), J. F.



Figures 1–8. *Paectes* adults. **1** *P. arcigera* ♂, Virgin Gorda Peak, Virgin Gorda Island, British Virgin Islands, 17–19 July 1986, S. E. Miller & M. G. Pogue **2** *P. arcigera* ♂, Grand Savane, Dominica, 1 July 1964, O. S. Flint, Jr. **3** *P. arcigera* ♀, Grand Savane, Dominica, 1 July 1964, O. S. Flint, Jr. **4** *P. arcigera* , Guana Island, British Virgin Islands, 1–14 July 1984, S. E. & P. M. Miller **5** *P. longiformis* ♂, Holotype, nr. Salvador Airport, Bahia, Brazil, March 2010, R. Diaz, V. Manrique & M. Vitorino **6** *P. longiformis* ♀, nr. Salvador Airport, Bahia, Brazil, March 2010, R. Diaz, V. Manrique & M. Vitorino **7** *P. similis* ♂, Holotype, Pernambuco [Recife], Pernambuco, Brazil, Pickel Coll. **8** *P. nana* ♂, nr. San Vicente, Hidalgo, Mexico, 2 July 1965, Flint & Ortiz.

G. Clarke & Thelma M. Clarke, 16 Jan. 1965 (1 male), J. F. G. Clarke & Thelma M. Clarke; 2.2 mi E of Pont Casse, 7 May 1964, O. S. Flint, Jr. (1 male); Roseau, Nov. 1967 (1 female), N.L.H. Krauss; S. Chiltern (1 female), 8–10 Dec. 1964 (1 female), P.J. Spangler; no specific locality, May-June 1905 (1 male, 4 females), Genitalia slide m MGP 1325, E. A. Agar [BMNH], Oct. 1904 (1 male, 3 females), Nov. 1904 (1 female), Apr. 1905 (1 male), E. A. Agar [BMNH], (2 males, 2 females), Genitalia slide m MGP 1324, E. A. Agar [BMNH], (2 males, 6 females) [BMNH]; Portsmouth, 8 Oct. 1956 (1 female), E. Hamblett [BMNH]. **GRENADA:** St. George's Cave, July 18 (1 male, 1 female), genitalia slide male MGP 1321 [BMNH]. **GRENADINES:** Union I., June 1905 (1 male), genitalia slide MGP 1322 [BMNH]. **GUADELOUPE:** Port de Jaray, 14 Sep. 1982 (1 male), B. Lalanne-Cassou. **PUERTO RICO:** Bayamón, 15 Jan. 1933 (1 female), Anderson & Lesesny; Guanica, Fajardo, 29 July 1913 (1 male), E. G. S. Collector; Maricao, Centro Vacacional, Monte del Estado, nr. Maricao, 1–9 Mar. 1971 (1 male), C.P. Kimball; Puerto Rico, Mayaguez, 3–4 Aug. 1955 (1 female), J.A. Ramos; San Juan, June–July 1932 (1 male), Genitalia slide USNM 135929, C.G. Anderson. **ST. LUCIA:** no specific locality, (2 males, 4 females), Branch; (4 males, 3 females), Maj. Cowrie, (2 males, 1 female) [BMNH]; 1 mi NW Soufriere, 18–23 Nov. 1975 (1 male), Genitalia slide USNM 135933, E.L. Todd. **ST. VINCENT:** Bequia I., Sep. 1903 (2 females); windward side, (1 male), H. H. Smith [BMNH]. **TRINIDAD:** No specific locality (1 female), A. Busck. **U. S. VIRGIN ISLANDS:** ST. CROIX: 1 mi W airport, 6–16 July 1967 (1 male), Genitalia slide USNM 42808; Christiansted, 19 Nov. 1941 (1 male), H.A. Beatty; Gallows Point, 9 July 1956 (1 female), genitalia slide USNM 136045, J.G. Coutsis; Orangegrove, W. End, 6–16 July 1967 (1 male), E.L. Todd.

Diagnosis. The only reliable way to distinguish *P. arcigera* from *P. asper* Pogue is by characters in the male and female genitalia. Male genitalia of *P. arcigera* consist of a reduced, fingerlike valve and costa, and a greatly expanded free saccular extension (Fig. 29). In *P. asper* the valve is triangulate, the costa has a truncate apex, and the free saccular extension (Fig. 33) is approximately half the width as in *P. arcigera*. Female genitalia of *P. arcigera* have a large, half-round ostium bursae covered with thorn-like spines and the lateral apices of the eighth sternite are not produced (Fig. 37). In *P. asper*, the ostium bursae is a crescent-shaped invagination covered with fine spicules and the lateral apices of the eighth sternite are produced (Fig. 40).

Redescription. Adults. Sexes dimorphic. **Male.** *Head* – antenna broadly bipectinate to 3/5 length, then filiform; eyes large, globular; vertex with broad scales, cream colored, thin black lines adjacent to scape; frons with broad scales, projecting slightly beyond anterior eye margin, mostly cream colored with a few gray and ferruginous scales, two black dots along eye margin, one ventral to antenna, other dorsal to palp; labial palp porrect, mixture of cream-colored, gray, and ferruginous scales, internal surface white. *Thorax* – prothorax somewhat variable, well-marked specimens cream colored with medial ferruginous band, anterior margin a thin black line, posterior margin gray to black and not as well defined as anterior line; patagium with cream-colored hairlike scales mixed with ferruginous, gray, and black

with subterminal and terminal areas; reniform spot obscure, with only a few pale-ferruginous scales; postmedial line black, a double line from posterior margin to vein M2 then single until merging with black dash between veins R5 and M1 that extends to outer margin; apical spot white; subterminal area brown, veins gray, color extending on to fringe; terminal line a series of black, shallow scalloped lines between veins; fringe brown, gray patches from wing veins resulting in a somewhat checkered appearance; hind wing white, marginal shading dark gray, veins highlighted dark gray, anal fold a white and dark gray striped pattern. *Abdomen* – cream colored scattered with a few pale-ferruginous scales; male eighth segment membranous with a pair of short, sternal, sclerotized bars and a pair of longer, wider, dorsal sclerotized bars; a pair of lateral coremata bearing numerous, fine, elongate setae. *Genitalia* (Fig. 32) – Uncus triangulate, apex recurved and pointed; subscaphium longer than uncus, triangulate, decurved, apex pointed; valve membranous, elongate, narrowed distally, apex round, covered with many elongate setae, basal-dorsal margin sclerotized, with several wide, spine-like setae; costa of valve short, deeply curved, apex produced and rounded, densely covered with elongate setae; sacculus well developed, proximal half fused with valve, distal half free, elongate, curved inward, longer than valve, apex round; saccus triangulate; aedeagus straight, slightly bent at distal third, dorsum in distal third covered with minute spicules; base of vesica a short tube with one flat, elongate cornutus with pointed apex directed posteriorly, vesica ovate, small round diverticulum just distal to flat basal cornutus, apex of vesica with an irregular sclerotized area bearing a short, thumb-like cornutus. **Female.** As in male except: *Head* –antenna filiform; forewing length 9.4–9.9 mm; ground color pale gray; antemedial line black, reduced to a concave line from just below Cu vein to anal vein connected to a convex line from anal vein to posterior margin; basal spot absent; interior of wing from base to postmedial line pale gray with scattered white scales or scales tipped white and only slightly paler than subterminal and terminal areas; medial line black, faint, dentate from just below Cu vein to posterior margin. *Genitalia* (Fig. 39) – Papillae anales truncate, soft, fleshy, covered with numerous setae; ninth sternite covered with minute spicules distally with spicules becoming larger and thicker closer to ostium bursae; anterior apophyses fused with eighth segment; posterior apophyses present; ostium bursae with sclerotized, crescent-shaped large dorsal and small ventral caps; base of ductus bursae, as it emerges from ostium bursae, sclerotized then becomes membranous and striated, after splitting with appendix bursae, ductus bursae narrower and more heavily striated; appendix bursae ovate, membranous; corpus bursae ovate, covered internally with numerous thornlike signa.

Distribution and biology. *Paectes nana* is widespread from Florida through the Greater Antilles, except for Puerto Rico, and from Mexico to Costa Rica; in South America distributed from Venezuela, Colombia, and northern Ecuador (Fig. 50). It has been introduced to the Galapagos Islands (Roque-Álbelo and Landry 2011).

Paectes nana is a native species from Florida that has been reared from Brazilian peppertree in several counties, including Hernando, Lee, Levy, Monroe, and St. Lucie. Larvae that were collected in September and October had a pupal stage from 9–18 days and larvae collected in January and February had a pupal stage from 11–15 days.

Adults probably fly all year with recorded dates from January–March, June–July, September–October, and December. Dyar (1901) stated that larvae of *P. nana* (referred to as *P. burserae*) are common on gumbo-limbo (*Bursera simaruba* (L.) Sarg., Burseraceae). In Costa Rica *P. nana* collecting dates range from May through November and has been reared from *B. simaruba* and *B. tomentosa* (Jacq.) Triana & Planch.

Remarks. *Paectes nana* has two forms. A form that is easily confused with *P. asper* and a more boldly marked form where the antemedial and postmedial lines and marginal dash are heavily marked with black and there are scattered black scales along the forewing posterior margin adjacent to the antemedial line. The holotype of *P. nana* is a heavily marked form.

Paectes asper Pogue, sp. n.

urn:lsid:zoobank.org:act:16EDE70C-D0AF-4CF7-A020-27E4EB09AF8F
http://species-id.net/wiki/Paectes_asper
Figs 12–19, 33, 40, 51

Type material. Holotype male – **CUBA**: Santiago, Collection Wm. Schaus; HOLOTYPE / *Paectes asper* Pogue" [red label]. USNM. Paratypes – (134 males, 85 females). All from USNM unless noted. Same data as holotype (9 males, 9 females) genitalia slide male USNM 135978, genitalia slides female USNM 135977, 135981– 135983; (2 males), genitalia slide male MGP 1314 [BMNH]. **BAHAMAS**: no specific locality (1 male, 1 female) [BMNH]. ABACO ISLANDS: no specific locality, (2 males, 2 females), Mar. 1902 (1 male), genitalia slide male MGP 1313, J.J. Bonhote [BMNH]. ANDROS: Andros Town, 27–29 Jan. 1965 (1 male), genitalia slide USNM 135927, leg. W. U. R. Piath; Mangrove Cay, 11 Jan. 1902 (1 female), J.J. Bonhote [BMNH]. NEW PROVIDENCE: Nassau, (1 female), Col. Jacob Doll.; Nassau I., 8 July 1898 (2 males, 1 female), 14 July 1898 (3 females), J.J. Bonhote [BMNH]. **BRITISH VIRGIN ISLANDS**: Great Camanoe Is., 1/3 mi ESE Cam Bay, 18 Mar. 1974 (1 male), C.L. Remington; Guana Island, North Bay, 0 m, 15–25 July 1986 (1 female), S.E. Miller & M.G. Pogue; Guana Island, 0-80 m, 13–26 July 1986 (1 male), genitalia slide USNM 135931, S.E. Miller & M.G. Pogue; Guana Island, 1–14 July 1984 (11 males, 8 females), Genitalia slides male USNM 135979, 135990, 135992, 136009, genitalia slides female 135998, 136005, 136006, 136007, 9–15 July 1985 (1 female), S.E. and P.M. Miller; Tortola, 14 May 1980 (1 female), 29 May 1980 (1 female), 28 July 1973 (1 female), 23 Oct. 1972 (1 female), Oct. 1972 (3 males), genitalia slides MGP 1319, 1320, 12 Nov. 1973 (1 male), 14 Nov. 1972, (1 female), 18 Nov. 1972 (1 female), J. Lorimer, 5 June 1974 (1 female) [BMNH]. **CAYMAN ISLANDS:** CAYMAN BRAC: behind Stakes Bay, 20 May 1938 (3 females), 21 May 1938 (1 female), 22 May 1938 (1 male), C.B. Lewis, G.H Thompson; N. coast of Stakes Bay, 20 May 1938 (1 male, 1 female), 22 May 1938 (1 male), genitalia slide MGP 1318, C.B. Lewis, G.H Thompson; west end of Cotton-tree Land., 19 May 1938 (1 male), 22 May 1938 (1 male), C.B. Lewis, G.H Thompson [BMNH]. GRAND CAYMAN: east end of East End, 13

Figures 17–24. *Paectes* adults. **17** *P. asper* ♀, Grand Savane, Dominica, 14 June 1964, O. S. Flint, Jr. **18** *P. asper* ♀, 1 mi N Mahaut, Dominica, 12 June 1964, O. S. Flint, Jr. **19** *P. asper* ♀, Haiti **20** *P. medialba* ♂, Holotype, Tucuman, Argentina, R. Schreiter **21** *P. medialba* ♀, Tucuman, Argentina, Mar. 1905, E. Dinelli **22** *P. sinuosa* ♂, Salta, Argentina, Feb. [19]05, J. Steinbach **23** *P. sinuosa* ♂, Suncho Corral, Santiago del Estero, Argentina, J. Steinbach **24** *P. sinuosa* ♀, Sara, Santa Cruz, Bolivia, 450 m, Jan., J. Steinbach.

May 1938 (1 male), 16 May 1938 (1 female), C.B. Lewis, G.H Thompson; Georgetown, (2 males, 2 females), genitalia slide male MGP 1317, A.W. Cardinall; N. coast of North Side, 11 July 1938 (1 female), 14 July 1938 (1 female), 16 July 1938 (1 female),

C.B. Lewis, G.H Thompson; west end of Georgetown, 14 May 1938 (1 female), C.B. Lewis, G.H Thompson [BMNH]. LITTLE CAYMAN: south coast of South Town, 31 May 1938 (2 males), 2 June 1938 (1 male, 1 female), 4 June 1938 (1 female), C.B. Lewis, G.H Thompson [BMNH]. **COLOMBIA:** SAN ADRES, PROVIDENCIA, AND SANTA CATALINA: San Andrés, 300 ft., Apr. 1926 (2 males), genitalia slides MGP 1328, 1351, F.W. Jackson [BMNH]. **CUBA:** no specific locality, (10 males, 2 females), genitalia slide male USNM 42806, genitalia slides female USNM 135962, 135985, Coll. Wm. Schaus, (1 male), Dognin Coll.; no specific locality, (4 males, 4 females), genitalia slide male MGP 1315 [BMNH]. GUANTANAMO: Baracoa, (3 males, 1 female), Aug. Busck Collector, 12 Feb. 1958 (1 male), Genitalia slide USNM 135955, B. Wright. HOLGUIN: Holguin, (2 males, 2 females), H.S. Parrish [BMNH]. LA HABANA: Cayamas, (1 male), E.A. Schwarz. ORIENTE: Santiago, (1 male, 1 female), genitalia slide male MGP 1314, W. Schaus [BMNH], June 1902 (1 male), Nov. 1902 (1 male), W. Schaus [BMNH]. **DOMINICA:** 1 mi N Mahaut, 12 June 1964 (1 female), genitalia slide USNM 136002, O.S. Flint, Jr.; Clarke Hall, 3 June 1964 (1 female), genitalia slide USNM 135984, O.S. Flint, Jr.; Grande Savane, 13 May 1964 (1 female), genitalia slide USNM 135961, 20 May 1964 (1 male, 1 female), genitalia slide male USNM 135975, genitalia slide female USNM 136057, 14 June 1964 (1 female), genitalia slide USNM 135995, 31 Oct. 1966 (1 male, 1 female), genitalia slide male USNM 135994, genitalia slide female USNM 136008, O.S. Flint, Jr.; Macoucheri, 1 Feb. 1965 (1 male), genitalia slide USNM 136058, 12 Feb. 1965 (1 male, 1 female), genitalia slide female USNM 42810, 5 Mar. 1965 (1 male), J.F.G. & Thelma Clarke. **DOMINICAN REPUBLIC:** San Cristobal, 8–9 June 1969 (1 male), genitalia slide USNM 135986, Flint & Gomez. **HAITI**: No specific locality, (2 males, 1 female), genitalia slide male USNM 135928; no specific locality, (2 males, 1 female), genitalia slide male MGP 1322 [BMNH]. **JAMAICA**: no specific locality, (3 males), genitalia slide male USNM 135930; no specific locality, (6 males, 6 females) [BMNH]. ST. ANDREW: Newcastle, (1 male), genitalia slide MGP 1316 [BMNH]. ST. JAMES: Montego Bay, 24 Jan. 1924 (1 male, 1 female), Gillett; Up Camp (1 male) [BMNH]; Kingston, July 17, at electric light, several were taken, Cockerell (1 male). TRELAWNY: Runaway Bay, 28 Mar. 1905 (1 male) [BMNH]. **PUERTO RICO:** no specific locality, (1 male), genitalia slide MGP 1331 [BMNH]. **U.S.A.:** FLORIDA: Miami-Dade Co., Biscayne Bay, (1 male), Collection H.G. Dyar; Coconut Grove, Nov. 1897 (1 male), Roland Thaxter Coll. Florida City, 9 June 1937 (1 female); Miami, (5 males, 1 female), genitalia slide male USNM 136000, genitalia slide female USNM 136001. Monroe Co., Key Largo Key [sic], 13 Dec. 1968 (1 male), genitalia slide MGP 1285, Mrs. Spencer Kemp MGCL, 6 Jan. 1969 (1 female), genitalia slide USNM 136260, Mrs. Spencer Kemp USNM; Bahia Honda State Park, 6 Jan. 1989 (1 male), 17 Jan. 1990 (1 male), 21 Jan. 1996 (1 male, 1 female), 12 Mar. 1989 (1 male), 23 Mar. 1990 (1 male), 29 Mar. 1990 (1 male), 28 Oct. 1988 (1 male), 8 Nov. 1988 (1 male), 29 Dec. 1989 (1 male, 1 female), T.S. Dickel TDC; Long Key State Park, 5 Feb. 1986 (1 male), 16 Feb. 1985 (1 male), 4 Mar. 1994 (1 male), 26 Dec. 1994 (1 male), T.S. Dickel TDC; Key Largo Hammock Botanical State Park, 17 Jan. 1987 (1 male),

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Systematics and faunistics of Grapholitini, 2: *Ethelgoda* HEINRICH, *Ofatulena* HEINRICH, *Cyanocydia* gen. n., and *Metacydia* gen. n.

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ABSTRACT. Neotropical members of four genera of Grapholitini are treated: *Ethelgoda* HEINRICH, *Ofatulena* HEINRICH, *Cyanocydia* gen. n., and *Metacydia* gen. n. Five species are described as new: *Ethelgoda execargae* sp. n., *E. anfracta* sp. n., *E. nymphra* sp. n., *Ofatulena lutherica* sp. n., and *Metacydia polyseta* sp. n.

KEY WORDS: Tortricidae, Grapholitini, Neotropic, new taxa.

INTRODUCTION

The systematics and distribution of Neotropical Grapholitini are poorly known. This paper is one of a series of papers dealing with material from Central and South America. The faunistic data are based on collections primarily from Costa Rica, Brazil and Ecuador. In the course of the studies that comprise this series of papers, new species have been found in almost all known genera, but a few new genera are necessary as well (further papers are in preparation).

All the specimens were collected by the junior author; the types of the newly described species are deposited in the Becker Collection, Camacan, and will eventually be deposited in one of the Brazilian Museums. Some material has been kindly donated to the Institute of Systematics and Evolution of Animals, PAS, Kraków.

The numbers cited from the labels of the type material refer to the numbers in Becker's register book. Abbreviation: GS – genitalia slide.

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RESULTS

Ethelgoda HEINRICH, 1926

Ethelgoda was described for the single Nearctic species *E. texanana* WALSINGHAM. According to the original description "the genus has hardly a single character to define it, yet on the sum of its characters it fits in none of the other genera." Externally, HEINRICH compared it with *Talponia* and stated that "on the male genitalia and abdominal characters it could go in *Grapholitha*". Its female genitalia, "except for the two signa, are those of *Dichroramphа* and its hind wing venation is that of *Ricula*".

Description

Based on the present material we provide the following summary of the genital characters of the genus.

Male genitalia. Socii plate-shaped, coalesced basally, hairless; sacculus angulate, rounded; ventral incision of valva large with straight or slightly convex median part of proximal edge; cucullus rather small with ventral lobe larger than dorsal lobe and caudal edge somewhat convex; aedeagus simple.

Female genitalia. Posterior part of sterigma weakly convex, lateral parts extending, connected with posterior, shallow pocket-like parts of the subgenital sterite; sclerite of antrum, median and proximal parts of ductus bursae developed, signa pair.

Comments

At present the genus includes seven species (four described by RAZOWSKI (2011) distributed from Texas to the Federal District in Brazil). It is close to *Sereda* HEINRICH, 1926 showing some similarities in the shape of the valva. On the other hand, it is related to *Ofatulena* HEINRICH, 1926, which has a similar sterigma-complex and only one signum (see the comments on *Ofatulena*).

Ethelgoda texanana (WALSINGHAM, 1879)

Material examined

Four males and one female from Chiapas, Mexico (Villa las Rosas, 1300 m, 27. VI. 1981 [43483]); British Virgin Island (Guana, X. 1989) and Cuba (Santiago: Siboney, 23. VI. 1990).

Comments

This species has been described from Texas, U.S.A. It is known from Florida, U.S.A., Cuba and Jamaica.

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A taxonomic revision of the genus *Mesophleps* Hübner, 1825 (Lepidoptera: Gelechiidae)

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Abstract

The genus *Mesophleps* Hübner (Lepidoptera: Gelechiidae) is revised; 54 available names (including one unjustified emendation), one junior primary homonym and one unavailable name were considered; type material of 44 previously described nominal species was examined. Nine new species are described: *M. acutunca* sp. nov., *M. bifidella* sp. nov., *M. unguella* sp. nov., *M. gigantella* sp. nov., *M. coffeae* sp. nov., *M. parvella* sp. nov., *M. aspina* sp. nov., *M. truncatella* sp. nov. and *M. undulatella* sp. nov. Two possibly new species are discussed but not formally named for lack of material. Twenty-five new combinations are introduced: *M. safranella* (Legrand, 1965) comb. nov., *M. epichorda* (Turner, 1919) comb. nov., *M. tabellata* (Meyrick, 1913) comb. nov., *M. crocina* (Meyrick, 1904) comb. nov., *M. ochracella* (Turati, 1926) comb. nov., *M. geodes* (Meyrick, 1929) comb. nov., *M. catericta* (Meyrick, 1927) comb. nov., *M. tephrastis* (Meyrick, 1904) comb. nov., *M. cycnobathra* (Lower, 1898) comb. nov., *M. tetrachroa* (Lower, 1898) comb. nov., *M. ochroloma* (Lower, 1901) comb. nov., *M. trichombra* (Lower, 1898) comb. nov., *M. mylicotis* (Meyrick, 1904) comb. nov., *M. macrosemus* (Lower, 1900) comb. nov., *M. apentheta* (Turner, 1919) comb. nov., *M. meliphanes* (Lower, 1894) comb. nov., *M. chloranthes* (Lower, 1900) comb. nov., *M. centrothetis* (Meyrick, 1904) comb. nov., *M. chloristis* (Meyrick, 1904) comb. nov., *M. argonota* (Lower, 1901) comb. nov., *Megacraspedus arnaldi* (Turati & Krüger, 1936) comb. nov., *Aponoea cinerellus* (Turati, 1930) comb. nov., *Pycnobathra acromelas* (Turner, 1919) comb. nov., *Sarotorna mesoleuca* (Lower, 1900) comb. nov., *S. dentata* Meyrick, 1904, comb. nov. One species, *Nothris mesophracta* Turner, 1919, is removed from *Mesophleps* but no current genus is available. Fourteen new synonymies (one genus, 13 species-group taxa) are established: *Bucolarcha* Meyrick, 1929, syn. nov. of *Mesophleps* Hübner, [1825]; *Stiphrostola longinqua* Meyrick, 1923, syn. nov. and *Brachyacma trychota* Meyrick, 1929, syn. nov. of *M. ioloncha* (Meyrick, 1905); *Lipatia crotalariella* Busck, 1910, syn. nov. of *M. adustipennis* (Walsingham, 1897); *Brachyacma epichorda* Turner, 1919, syn. nov. of *M. epiochra* (Meyrick, 1886); *Mesophleps pudicellus* var. *apicellus* Caradja, 1920, syn. nov. and *Mesophleps silacellus* subsp. *calaritanus* Amsel, 1939, syn. nov. of *M. silacella* (Hübner, 1796); *Mesophleps lala* Agenjo, [1961], syn. nov. of *M. corsicella* (Herrich-Schäffer, 1856); *Crossobela barysphena* Meyrick, 1923, syn. nov. of *M. trinotella* Herrich-Schäffer, 1856; *Mesophleps orientella* Nel & Nel, 2003, syn. n. and *Mesophleps gallicella* Varenne & Nel, 2011, syn. nov. of *M. ochracella* (Turati, 1926); *Nothris centrothetis* Meyrick, 1904, syn. nov. and *Nothris chloristis* Meyrick, 1904, syn. nov. of *M. chloranthes* (Lower, 1900); *Mesophleps cinerellus* Turati, 1930, syn. nov. of *Aponoea obtusipalpis* Walsingham, 1905. One genus and one species are recalled from synonymy: *Pycnobathra* Lower, 1901, gen. rev., and *M. ioloncha* (Meyrick, 1905) sp. rev. Lectotypes are designated, in accordance with the *Code*, article 74.7.3, for 14 species: *Gelechia palpigera* Walsingham, 1891; *Paraspistes loloncha* Meyrick, 1905; *Lathontogenus adustipennis* Walsingham, 1897; *Brachyacma epichorda* Turner, 1919; *Nothris crocina* Meyrick, 1904; *Nothris ochracella* Turati, 1926; *Nothris tephrastis* Meyrick, 1904; *Ypsolophus ochroloma* Lower, 1901; *Ypsolophus macrosemus* Lower, 1900; *Nothris centrothetis* Meyrick, 1904; *Nothris chloristis* Meyrick, 1904; *Ypsolophus argonota* Lower, 1901; *Mesophleps arnaldi* Turati & Krüger, 1936, and *Mesophleps cinerellus* Turati, 1930. *Mesophleps* is a widely distributed Old World genus, except for one New World species, with seed-feeding larvae on Cupressaceae, Cistaceae, Cruciferae (Brassicaceae), Leguminosae (Fabaceae), Rubiaceae and doubtfully Dipterocarpaceae.

Key words: Lepidoptera, Gelechiidae, Anacampsinae, *Mesophleps*, revision, new species, combination, synonym, species group, host-plant, world distribution

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Introduction

The genus *Mesophleps* (Gelechiidae, Anacampsinae) comprises 37 species recognized as valid in this paper, but our material indicates that further species remain to be described. The adults are average-sized Gelechiidae with a wingspan of about 10.0–25.0 mm, and their larvae, as far as the biology is known at all, are seed feeders in the fruits and pods of several plant families but predominantly Leguminosae. Until 1990 (Park 1990) the non-European species were not assigned to *Mesophleps* but to a number of different genera, the best known amongst them being *Brachyacma*. It is largely due to the exceptional variation in the wing venation and the scaling of the labial palpus, in conjunction with the almost world-wide distribution, that the genus has been described more than once and now has a formidable list of synonyms. Characters of the venation and the labial palpi were, in the time preceding routine examination of the reproductive organs, both considered to be of prime generic importance. *Mesophleps* is established as monophyletic by at least two synapomorphies: the transverse bands of microtrichia on the anterior margin of abdominal tergites IV–VII (VIII) in both sexes and the 'double' gnathos in the male genitalia. It should be noted as a curiosity that Meyrick (1886: 278), who hardly ever considered the genitalia structures, noticed the uncus and the double gnathos, quite accurately referring to the latter in the original description of *Brachyacma* as 'two short oblique lateral spines'. Once one has a mental picture of the genus it is quite easy to recognize a species as *Mesophleps* by its wing pattern and general aspect. *Mesophleps* is native to the temperate and tropical parts of the Old World (Figs 23, 24). It remains uncertain whether *M. adustipennis* from southern North America, Central and South America (Fig. 25), usually recorded under the name *Brachyacma palpigera*, is a New World endemic or an inadvertent introduction from the Old World.

In the past, '*Brachyacma palpigera* (Walsingham)' has attracted some interest as it was recorded in several parts of the world as a locally important pest of agricultural legumes such as pigeon pea (*Cajanus cajan*), indigo (*Indigofera* spp.), soya bean (*Glycine max*) and others, although we are unaware of any recent reports of serious damage on a more than local scale. In the United States '*B. palpigera*' is known as the soybean webworm moth. Our study has now shown that more than one species is involved and that the New World species is *Mesophleps adustipennis* (Walsingham).

Material and methods

This study is based primarily on the world collection of Microlepidoptera at the BMNH, London, and extensive Chinese material in the collection of NKUM, Tianjin. All material studied is in BMNH, unless otherwise indicated. We have examined type material (holotypes, lectotypes, syntypes or paratypes) of 44 of the 53 nominal species currently placed in or previously associated with the genus *Mesophleps* or one of its synonyms. Type material of eight species could not be traced or was unavailable. The types of several Australian species were examined by KS in ANIC, Canberra, and SAM, Adelaide, Australia, but not dissected.

In the absence of a phylogenetic analysis we have arranged the species-groups and species within them according to morphological similarities that can be expected to reflect in many instances true phylogenetic relationships. The majority of the Australian species belong to a distinct species-group that for logistic reasons could not be dealt with in the same detail as the non-Australian *Mesophleps*. However, to provide at least a rough overview, the types in BMNH of the species described by Edward Meyrick were dissected, the genitalia illustrated and the species likewise arranged according to morphological similarities.

Measurements at the beginning of each species description represent the wingspan, in millimetres, of the smallest and largest specimen examined. In several instances the number of available specimens was limited and the variation in the size of such species may be greater than is indicated by the recorded measurements.

Preparation of the genitalia followed the protocol of Robinson (1976), staining was effected with Mercurochrome or Chlorazol Black E. For a better display of the abdominal sclerites a few abdomina were opened up laterally by cutting with a pair of fine scissors along the spiracular line. In the males there is no need to detach one of the vinculum arms for employing the unrolling technique that was originally developed specifically for Gelechiidae (Pitkin 1986). Viewed laterally, tegumen/uncus and vinculum/saccus in *Mesophleps* overlap each other and are hinged in such a way that they can be opened by 180 degrees so that the result looks as in Fig. 7 [slide no. 15833, *epiochra*] and displays all detail with the same clarity as it would be seen after unrolling. The phallus is extracted, but as it is bulbous great care should be taken not to crush or dent it. It is then mounted in a lateral position.

For the sake of uniformity and for a better view of the entire ductus bursae and the sclerotized antrum, if present, the female genitalia of Gelechiidae should be separated from the abdomen by severing the intersegmental membrane between segments VII and VIII, unless the ostium bursae opens in VII rather than the usual VIII. The bursa copulatrix is then withdrawn from the abdomen and cleaned before the genitalia are permanently mounted on a glass slide in Euparal or an equivalent medium. It is highly undesirable to retain the abdomen and its appendages long-term in fluid because of the great danger of loss, damage or confusion in subsequent examinations (Sattler 1973). In the case of types and important voucher specimens the preservation in a permanent medium on a slide is essential.

The host-plants recorded are based on examined specimens, information taken from the literature is duly referenced whilst dubious or incorrect records are appropriately flagged and discussed. To maintain continuity with earlier literature we follow Robinson et al. 2001 (http://www.nhm.ac.uk/research-curation/research/projects/hostplants/index.html#5) in using the familiar family names Cruciferae and Leguminosae in preference to the recent alternatives Brassicaceae and Fabaceae.

The spelling of locality names primarily follows *The Times Comprehensive Atlas of the World* (2011), unless there were compelling reasons to use other sources. If a modern spelling differs significantly from that on the specimen label, the latter is additionally cited in parentheses, for example Az Zuwaytīnah ('Zuetina'). When localities could only be traced with difficulty, detailed information is given, including the geographical coordinates.

To satisfy the condition of the *International Code of Zoological Nomenclature* (Edn 4), Article 74.7.3, it is stated here that the 14 lectotypes designated in this revision are all established out of taxonomic necessity and to contribute to the stability of the nomenclature.

Abbreviations of institutions

ANIC	Australian National Insect Collection, Division of Entomology, CSIRO, Canberra, Australia.
BMNH	British Museum (Natural History), now Natural History Museum, London, UK.
CAOU	Entomological Laboratory, College of Agriculture, Osaka Prefecture University, Sakaishi, Osaka Prefecture, Japan.
ETH	Eidgenössische Technische Hochschule, Zürich, Switzerland.
MNCN	Museo Nacional de Ciencias Naturales, Madrid, Spain.
MNHN	Muséum national d'Histoire naturelle, Paris, France.
MV	Museum of Victoria, Melbourne, Australia.
NKUM	College of Life Sciences, Nankai University, Tianjin, P.R. China.
SAM	South Australian Museum, Adelaide, Australia.
TM	Transvaal Museum, Pretoria, South Africa.
USNM	U.S. National Museum of Natural History, Washington, D.C., U.S.A.
VOB	Collection Vitor Becker, Camacan, Bahia, Brazil.
ZMUC	Zoologisk Museum, Copenhagen, Denmark.

The systematic position of *Mesophleps*

The current system of the Gelechiidae is still strongly influenced by that first introduced by Meyrick (1925), although a considerable number of refinements and modifications have been made since that time. Meyrick recognized nine groups of genera, each identified by the name of a characteristic genus and roughly equivalent to a current subfamily or tribe. Groups 8 and 9 were subsequently removed from Gelechiidae s. str. and given family status (Symmocidae, Holcopogonidae – both currently subfamilies of Autostichidae – Lecithoceridae and Glyphidoceridae) whilst other groups were given formal rank as subfamilies (or tribes, depending on author) Apatetrinae, Anomologinae (Aristoteliinae), Gelechiinae, Anacampsinae (Palumbininae, Stomopteryginae), Chelariinae (Hypatiminae), and Dichomeridinae, leaving only Meyrick's almost exclusively Australian group 5 (*Protolechia* type) unaccounted for. It is most remarkable that Meyrick, whose classification is based entirely on wing venation and external characters, such as the vestiture of the labial palpus, was able to identify more or less correctly all cur-

rently recognized family-group taxa, except Pexicopiinae (Hodges 1986: 7) which had escaped his notice. The family Gelechiidae in this restricted sense, i.e. excluding the Lecithoceridae, Symmocidae, Holcopogonidae and Glyphidoceridae, is generally accepted as monophyletic, supported by at least two autapomorphies: the laterally articulated gnathos arms in the male genitalia (Hodges 1999: 147) and the subradial retinaculum on the underside of the female forewing (Braun 1924: 246). There is also broad agreement on the existence of more or less well-defined supragenerie groupings within the family; however, there is little agreement as to their appropriate level. For example, Sinev (1992: 156) divided Gelechiidae into nine subfamilies (Gelechiinae, Anacampsinae, Aristoteliinae, Metzneriinae, Teleiodinae, Stomopteryginae, Anomologinae (Apatetrinae), Brachmiinae, Dichomeridinae) whilst Hodges (1999: 147) recognized only four (Physoptilinae, Gelechiinae, Dichomeridinae, Pexicopiinae).

In Meyrick's classification *Mesophleps* and most of its synonyms were placed in group 7 (*Dichomeris* type), probably because some species bear more or less pronounced scale tufts on segment 2 of the labial palpus. This placement was also maintained by Le Marchand (1947: 152, 'Dichomerinae') and in the North American check list (Hodges 1983: 24, *Brachyacma*); however, without its position ever having been specifically discussed, consensus emerged in European check lists that *Mesophleps* was more appropriately placed in Anacampsinae (Bradley 2000: 28), sometimes as Gelechiinae, Anacampsini (Karsholt & Riedl 1996: 120; Leraut 1997: 125). In the most recent arrangement by Ponomarenko (2009: 156–168) the Gelechiidae are divided into the subfamilies Physoptilinae, Anomologinae (tribes Anomologini, Apatetrini, Aristoteliini and Pexicopiini), Gelechiinae (tribes Gelechiini, Gnorimoschemini and Litini), Anacampsinae (tribes Anacampsini and Brachmiini), Dichomeridinae (tribes Dichomeridini, Chelariini and Anarsiini). In her arrangement *Mesophleps* is accommodated in Anacampsinae, tribe Anacampsini; we concur with this placement.

Systematic part

Mesophleps Hübner, [1825]

Mesophleps Hübner, [1825], *Verz. bekannter Schmett.*: 406. Type-species: *Tinea silacella* Hübner, 1796, *Samml. eur. Schmett.* **8**: 37, pl. 17, fig. 117, by subsequent designation: Meyrick, 1925: 168.

Brachyacma Meyrick, 1886, *Trans. ent. Soc. Lond.* **1886**: 278. Type-species: *Brachyacma epiochra* Meyrick, 1886, *ibidem* **1886**: 279, by monotypy. [Synonymized by Park, 1990: 136; Li & Zheng, 1995: 27, 33.]

Lathontogenus Walsingham, 1897, *Proc. zool. Soc. Lond.* **1897**: 87. Type-species: *Lathontogenus adustipennis* Walsingham, 1897, *ibidem* **1897**: 88, by original designation and monotypy. [Synonymized with *Brachyacma* Meyrick by Meyrick, 1925: 168, and *Mesophleps* Hübner by Li & Zheng, 1995: 27, 33.]

Paraspistes Meyrick, 1905, *J. Bombay nat. Hist. Soc.* **16**: 600. Type-species: *Paraspistes ioloncha* Meyrick, 1905, *ibidem* **16**: 600, by monotypy. [Synonymized with *Lathontogenus* Walsingham by Walsingham, 1915: 408, *Brachyacma* Meyrick by Meyrick, 1925: 168, and *Mesophleps* Hübner by Li & Zheng, 1995: 27, 33.]

Chretienia Spuler, 1910, *Schmett. Eur.* **2**: 359. Type-species: *Gelechia oxycedrella* Millière, 1871, *Iconogr. Descr. Chenilles Lépid. inédits* **3**: 177, 193, pl. 118, figs 1–6, by monotypy. [Synonymized by Park, 1990: 136.]

Lipatia Busck, 1910, *Bull. Dep. Agric. Trin.* **9**: 243. Type-species: *Lipatia crotalariella* Busck, 1910, *ibidem* **9**: 244, fig., by original designation and monotypy. [Synonymized with *Lathontogenus* Walsingham by Walsingham, 1915: 408; *Brachyacma* Meyrick by Meyrick, 1925: 168; *Mesophleps* Hübner by Li & Zheng, 1995: 27, 33.]

Stiphrostola Meyrick, 1923, *Exot. Microlepid.* **3**: 25. Type-species: *Stiphrostola longinqua* Meyrick, 1923, *ibidem* **3**: 25, by monotypy. [Synonymized by Park, 1990: 136, as *Stiprotola*, an incorrect subsequent spelling.]

Crossobela Meyrick, 1923, *Exot. Microlepid.* **3**: 34. Type-species: *Crossobela baryspheua* Meyrick, 1923, *ibidem* **3**: 34, by original designation and monotypy. [Synonymized by Park, 1990: 136.]

Xerometra Meyrick, 1925, *Genera Insect.* **184**: 18 [key]. 170. Type-species: *Nothris crocina* Meyrick, 1904, *Proc. Linn. Soc. N.S.W.* **29**: 421 [key], 423, by original designation. [Synonymized by Park, 1990: 136.]

Gnosimacha Meyrick, 1927, *Exot. Microlepid.* **3**: 354. Type-species: *Gnosimacha catericta* Meyrick, 1927, *ibidem* **3**: 354, by monotypy. [Synonymized by Park, 1990: 136.]

Bucolarcha Meyrick, 1929, *Exot. Microlepid.* **3**: 515. Type-species: *Bucolarcha geodes* Meyrick, 1929, *ibidem* **3**: 515, by monotypy. **Syn. nov.**

Uncostriodonta Agenjo, 1952, *Faunula lepid. almeriense*: 87. Type-species: *Mesophleps trinotella* Herrich-Schäffer, 1856, *Neue Schmett. Eur. angrenzenden Ländern* (1): 6, fig. 46, by original designation and monotypy. [Synonymized by Park, 1990: 136, as *Uncostridonta*, an incorrect subsequent spelling.]

Uncostridonta Park, 1990, *Korean J. appl. Ent.* 29: 136, an incorrect subsequent spelling.

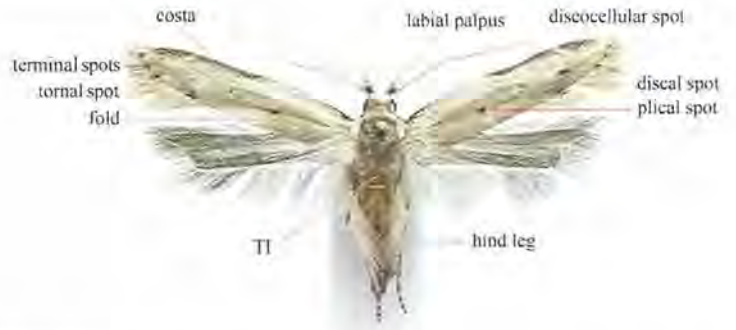

FIGURE 1. Adult of *Mesophleps palpigera* (Walsingham).

Head. Frons evenly convex; ocellus absent; proboscis short, basal half squamose. Antenna two-thirds to four-fifths length of forewing, scape without pecten (except in M. catericta), apical segment often black. Labial palpus variable, recurved, typically segment 2 with dorsal scales raised or fanned out towards apex, 3 shorter than 2, unremarkable; sometimes 2 with sub-triangular forward-directed brush (catericta, tephrastis -group); exceptionally palpus porrect, segment 2 with triangular dorsal brush, 3 very short (geodes).

Wingspan 6.5–26.0 mm. Forewing yellow to yellowish brown, rarely dark brown, grey-brown or grey, frequently with dark costa, particularly in distal half, sometimes with small plical, discal and discocellular spots (Fig. 1). Exceptionally wing distinctly marked with large plical spot, discal obliquely extended to middle of costa, and pair of spots on distal fifth of costa and in tornus often fused to form transverse band (*oxycedrella*). Forewing narrow to broadly elongate-ovate, venation variable. Forewing costa with or without pterostigma between Sc and R_1; R_3 free or stalked with R_{4+5}, common stalk of R_{4+5} about as long as free R_4 and R_5, rarely much longer, M_1 approximated to, connate or stalked with R_{4+5}, CuA_1 separate, approximated to or connate with M_1. Shape of hind wing variable; costa weakly sinuate, costal and dorsal margins diverging, wing distally up to twice its width at base, termen moderately concave beneath apex; or costal and dorsal margins almost parallel, costa gently arched, termen strongly concave beneath sharply produced apex. R_1 absent or joining Sc near base; Rs +M_1 stalked, M_1 arising near CuA_1 or both veins connate (Figs 2–6; Weber 1948: 225, pl. 12, figs 5–7). Frenulum of female composed of three acanthae (in *M. catericta* sometimes only two).

Pregenital abdomen. Scales on tergites TI-III of both sexes yellow, rarely yellow colour restricted to TI and II, or all segments unicolourous; scale sockets on denuded TI, TII and sometimes TIII, denser and bigger than on other segments (Fig. 11). Anterior margins of TIV–VII (IV–VIII in males of *M. geodes* and *catericta*) in both sexes with variable, narrow to broad, transverse band of densely set posteriorly-directed microtrichia (Figs 17–22); SII with pair of venulae and pair of short apodemes, sensory setae small, fairly scattered (Fig. 11). Segment VII in female, VIII in male a closed ring, rarely modified (males of *corsicella*, *bifidella*, *gigantella* and *geodes*) (Figs 14–16).

Genitalia ♂ (Figs 7, 8). Uncus variable, pointed, round, oval or sub-rectangular, rarely bilobed (*bifidella*, Fig. 71) or otherwise modified, strongly sclerotized in many species. Gnathos composed of evenly curved transverse band bearing pair of always very strongly sclerotized posterior hooks; rarely gnathos hooks fused at base or merged to one (*tephrastis*-group). Tegumen unremarkable, usually as wide as or slightly wider than uncus, with anterior margin shallowly concave to triangularly excised; pedunculi variable, simple and long, evenly curved, to distally broadened, forked. Diaphragma with groups of sensory setae. Valva narrow, simple, undivided, barely shorter than tegumen; small group of long sensory setae situated at base, near junction with vinculum and pedunculus. Sacculus and juxta merged with vinculum. Arms of vinculum narrow, distally broad, distal margin strongly sclerotized and turned ventrad, beneath exit hole of phallus often notched or excavated. Phallus usually shorter than vinculum, in *albilinella*-group distinctly longer, basal half to two-thirds bulbous, apical half to one-third narrow; with short spur that articulates with juxta part of vinculum frame; ductus ejaculatorius enters anteriorly; bulbus ejaculatorius slightly longer and wider than phallus.

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Shulian Hao) (NKUM); 1 ♂, 3 ♀♀, Guizhou Province, Chishui, 240 m, 21..23.ix.2000 (*Haili Yu*) (NKUM); 3 ♂♂, 2 ♀♀, Guizhou Province, Xishui, 500 m, 29–31.v.2000 (*Yanli Du*) (NKUM); 1 ♂, 1 ♀, Guizhou Province, Daozhen, Dashahe, 1450 m, 23.v.2004, 17.viii.2004 (*Shulian Hao, Yunli Xiao*) (NKUM); 2 ♂♂, Guizhou Province, Jiangkou, Mt. Fanjing, 530 m, 600 m, 27.vii.2001, 2.vi.2002 (*Houhun Li & Xinpu Wang*) (NKUM); 1 ♂, 1 ♀, Shandong Province, Liangshan, Guanli, 12, 19.viii.1995 (*Shijin Li*) (NKUM); 2 ♂♂, 1 ♀, Anhui Province, Yuexi Country, Wenquan, 7, 22.viii.1995 (*Xiangfu Hu*) (NKUM); 1 ♂, 1 ♀, Hong Kong, Tai Po Kau, Forest Reserve, 200 m, viii.1993 (*Kent Li*). **Thailand**: 1 ♂, 3 ♀♀, Khao Yai National Park, 750 m, 15–19.x.1990 (*Bradley & Lewvanich*); 2 ♀♀, *ditto*, headquarters, 720 m, 31.viii–6.ix.1986, 2–4.xi.1988 (*Robinson; Bradley, Lewvanich & Fletcher*); 4 ♀♀ *ditto*, 1200 m, 22.viii.1986 (*Allen*); 1 ♀, Khao Soi Dao Wildlife Sanctuary, 300 m, 19–21.ii.1990 (*Bradley, Fletcher & Lewvanich*); 1 ♂, 2 ♀♀, Doi Suthep-Pui National Park, 1200 m, 1500 m, 9–10.iii.1988, 23–27.x.1990 (*Bradley, Lewvanich & Boonkong; Bradley, Lewvanich & Kuroko*); 2 ♀♀, Chiang Mai, 300 m, 750 m, 9, 11.xii.1985 (*Allen*); 1 ♀, Chiang Mai, Doi Suthep-Pui, 1440 m, 22–23.ix.1986 (*Robinson*); 1 ♀, Chiang Mai, Samoeng/Hang Dong Rd, km 15, 700 m, 5–12.vi.1988 (*Cotton & Kitching*); 1 ♂, Chaiyaphum, Phu Khieo Wildlife Sanctuary, plateau 500 m N of HQ, 900 m, 5–10.xi.1991 (*Kitching & Cotton*); 3 ♀♀, Uthai Thani District, Khao Nang Rum, 400 m, 440 m, 16–18.i, 1.iii, 22.viii.1986, 1.xi.1991 (*Allen*); 1 ♀, Uthai Thani District, Huai Kha Khaeng Wildlife Sanctuary, km 16 HQ to Khao Nang Ram, 1.xi.1991 (*Kitching & Cotton*); 1 ♂, 2 ♀♀, Kanchanaburi, Kwae Yai R., 30 m, 8–9.i.1986 (*Allen*); 1 ♀, Larn Sang National Park, ca 600 m, 22.x.1990 (*Bradley, Lewvanich & Kuroko*); 1 ♀, Chulaphon Dam, 730 m, 12–13.ii.1990 (*Bradley, Fletcher & Lewvanich*). **Brunei**: 1 ♂, Kampong Kapok, 3 km WSW of Muara, edge of mangrove forest, 1 m, i–ii.1992 (*Classey*). **Indonesia**: 1 ♂, 1 ♀, Moluccas, Bacan ('Batian'), viii.1897 (*Doherty*). **Nepal**: 1 ♂, Terai, Dharan, sal [robusta] & secondary forest, 30 m, 14–15.xi.1983 (*Allen*). **India**: 2 ♂♂, 2 ♀♀, Tamkil Nadu, Coimbatore, ex *Indigofera* ('Indigo') pods, 5.iv, 18.viii, 7.ix.1916 (*Rao*); 1 ♂, Karnataka, Kodagu ('Coorg'), Pollibetta, 24.x–16.xi.1915. (*Fletcher*); 1 ♂, Bihar, Pusa, ex seeds of *Parkinsonia aculeata*, 17.xii.1927 (*Rangi*). **Sri Lanka**: 1 ♂, Polonnaruwa District, Aralaganwila (7°46'N, 81°10'E), ex *Cajanus cajan* ('pigeon pea'), 22.ii.1990 (*CIE*); 1 ♂, Puttalam, xi.1904 (*Pole*).

Mesophleps adustipennis (Walsingham, 1897)

(Figs 29, 30, 63, 92, 122)

Lathontogenus adustipennis Walsingham, 1897, *Proc. zool. Soc. Lond.* **1897**: 88. LECTOTYPE ♂, WEST INDIES, Grenada, windward side, Balthazar, 250 ft, 27.iv, (*Smith*) (Walsingham no. 6228; genitalia slide no. 3720, BMNH), here designated [examined]. [Incorrectly synonymized with *palpigera* by Walsingham, 1915: 409.]

Lipatia crotalariella Busck, 1910, *Bull. Dep. Agric. Trin.* **9**: 244, fig. Holotype ♀ [abdomen missing], Trinidad, ex *Crotalaria*, em. 21.v.1910 (*Urich*) (USNM) [examined]. [Incorrectly synonymized with *palpigera* by Busck 1914: 10.] **Comb. nov., syn. nov.**

Brachyacma palpigera (Walsingham, 1891); Forbes 1930: 123; Becker 1984: 50.

Mesophleps adustipennis (Walsingham); Landry & Roque-Albelo 2010: 739, figs 34, 74, 97.

♂, ♀. Wingspan 7.5–18.0 mm. Labial palpus recurved, segment 2 in lateral view distally broader (about twice as wide as at base), dorsally with erectile sub-triangular tuft, dark brown to ochreous brown, distally with white ring, 3 about one-half length of 2, white with dark apex. Antenna with alternating brown and light rings. Forewing greyish white to yellowish brown, distal three-fifths of costa lined with dark brown stripe, stripe interrupted by oblique pale line running from distal fifth towards termen, dorsum often fuscous; black discal, discocellular, plical and three terminal spots present but often indistinct, rarely also tornal spot; discocellular sometimes confluent with tornal to form large shadow.

Genitalia ♂ (Fig. 63). Uncus rounded, almost circular. Gnathos band of moderate width, hooks strong, separated from each other by almost width of uncus. Tegumen with triangular anterior emargination; pedunculi broad, distally truncated or forked. Posterior margin of vinculum medially notched. Phallus with bulbous base and short straight distal portion.

Genitalia ♀ (Figs 92, 122). Papillae anales unremarkable, apophyses posteriores about twice length of apophyses anteriores. Dorso-posterior margin of segment VIII strongly convex medially; ventro-anterior margin sinuous, medially concave. Subostial plate more or less trapezoid, caudal margin weakly concave at ostium bursae, anterior margin straight to very gently curved; sclerotized antrum broad, weakly tapered distally, sometimes slightly exceeding transverse anterior margin of subostial plate; ductus bursae thin, about twice length of apophyses anteriores, entering corpus bursae postero-laterally; corpus bursae elongate ovoid, anteriorly broad, about twice length of ductus bursae; ductus seminalis from cervix bursae.

Remarks. *M. adustipennis* is externally very similar to, and was incorrectly synonymized with *M. palpigera*. Both species differ in segment 2 of the labial palpus which in *adustipennis* has a stronger dorsal tuft, in lateral view is triangular, in cross-section oval and is distally twice as wide as at the base whilst in *palpigera* it is round in cross-section and distally not much thicker than at the base.

In the male genitalia *adustipennis* differs from *palpigera* in the round uncus and shorter, stouter gnathos hooks. The female genitalia of *adustipennis* are almost indistinguishable from those of *palpigera*; the ventro-anterior margin of abdominal segment VIII tends to be more strongly sinuous in the former, but there is some variation in this as in other genitalic characters. No confusion is possible as long as *adustipennis* remains the only *Mesophleps* species in the New World. In the African *M. safranella* the uncus is slightly more elongate and less evenly rounded, the gnathos hooks are slightly shorter and stouter and the distal margin of the vinculum is evenly rounded towards a medial notch whilst in *adustipennis* the vinculum is distally truncated, often has a distinct medial notch and lacks the broad protruding sclerotization that is present in *safranella*.

L. adustipennis was described from an unspecified number of specimens of both sexes originating from the West Indian islands of St Croix, St Thomas (both former Danish, now US Virgin Islands) and Grenada. Labelling of material in the Walsingham collection indicates that in addition to two specimens marked as 'Type ♂' and 'Type ♀' (both from Grenada) there were eleven 'paratypes', five of which (one from St Croix and four from Grenada) are now preserved in BMNH; a further female, St Thomas, 11.ii.1894 (*Hedemann*), is preserved in ZMUC, Copenhagen (Karsholt, pers. comm.). As no holotype was indicated in the original description, all specimens have the status of syntypes and we designate here as the lectotype the specimen originally labelled as the 'Type ♂' (Walsingham no. 6228; genitalia slide no. 3720, BMNH).

L. crotalariella was described from an unspecified number of specimens, sex not stated, from Trinidad (leg. Urich), and there is some slight uncertainty over the number of original specimens. The description mentioned the 'type', a 'cotype' and a third specimen from Nassau, Providence I., British West Indies. *L. crotalariella* was later synonymized with *palpigera* but is in fact a synonym of *M. adustipennis*.

M. adustipennis is the only *Mesophleps* found in the New World and it is not clear whether it really is an element of the native American fauna or an Old World introduction. It could have been inadvertently carried to the New World from India or Africa with some agricultural plant such as pigeon pea or *Crotalaria*. However, we have not been able to identify clear Old World *adustipennis*, with the African *safranella* perhaps being the closest to it.

Biology. Host-plants: *Cajanus cajan* (pigeon pea) (Leonard & Mills 1931: 472), *Crotalaria* (Busck 1910: 244) (Leguminosae, Papilionoideae); *Acacia* ('*Vachellia*') *farnesiana* (Bottimer 1926: 812), *Pithecellobium pallens* (Grimm 1995: 321) (Leguminosae, Mimosoideae). The 'whitish' larva lives in the pods and feeds on the seeds; it pupates in a tough silken cocoon within the pods, usually near one end, and the adult emerges after about ten days. In Puerto Rico a high percentage of the dry pods of pigeon pea examined in June 1930 was infested, but many mature larvae were parasitized by an unidentified *Copidosoma* ('*Paralitomastix*') species (Hymenoptera: Chalcidoidea, Encyrtidae) (Leonard & Mills 1931: 472). In the Galapagos Islands the species has been reared from the fruits of *Prosopis juliflora* and from *Leucaena leucocephala*, the lead tree (Leguminosae, Mimosoideae) (Landry & Roque-Albelo 2010: 740).

Distribution. Mexico, Honduras, Costa Rica, Panama, Cuba, West Indies (Cayman Islands, Jamaica, Puerto Rico, Guana, St Thomas, St Croix, Anguilla, Dominica, Barbados, Grenada, Tobago, Trinidad), Venezuela, Ecuador (Galapagos Islands), Peru, Brazil (Rondônia, Amazonas, Maranhão, Minas Gerais, Espirito Santo, São Paulo). It is here assumed that all literature records of *Brachyacma palpigera* (and its former synonym *crotalariella*) from western and southern parts of the USA, California (Riverside Co., Yolo Co.) (Richers 2004), Texas (Bottimer 1926: 812), Mississippi (MacGown 2004), Florida (Heppner 2004), as well as the Bahamas (New Providence) (Busck 1910: 244), Puerto Rico (Leonard & Mills 1931: 472) and Mexico (Grimm 1995: 321) apply to *M. adustipennis*, the only *Mesophleps* species so far confirmed as occurring in the Western Hemisphere.

Material examined (61 ♂♂, 49 ♀♀, including 13 ♂♂, 11 ♀♀ genitalia preparations)

Mexico: 1 ♀, Tamaulipas, San Fernando, 50 m, 16.viii.1988 (Becker) (VOB), 1 ♀, El Ensino, 250 m, 4–13.viii.1988 (Becker) (VOB); 1 ♀, Campeche, Escárcega, 85 m, 17–21.vi.1981 (Becker) (VOB). **Honduras:** 1 ♂, 1 ♀, iii.1935 (unspecified). **Costa Rica:** 1 ♀, Turrialba, 600 m, vii.1981 (*Becker*) (VOB). **Panama:** 1 ♀, La Chorrera,

v.1912 (*Busck*). **Cuba**: 2 ♂♂, Santiago, Turquino, 470 m, 27–29.vii.1990 (*Becker*) (VOB). **West Indies**: 19 ♂♂, Cayman Islands, 17.iv–26.viii.1938 (*Lewis & Thompson*); 2 ♂♂, 3 ♀♀, Jamaica, Runaway Bay, 27.ii, 24.iii.1905 (*Walsingham*); 1 ♂, Puerto Rico, Guánica, 160 m, 20.viii.1987 (*Becker*) (VOB), 1 ♂, 4 ♀♀, Patillas, 590 m, viii.1987 (*Becker*) (VOB), 1 ♂, 1 ♀, Cayey, 450 m, 2.viii.1987 (*Becker*) (VOB), 1 ♂, 1 ♀, Maricao, 770 m, 12.viii.1987 (*Becker*) (VOB), 2 ♂♂, Carite, 500 m, 17.viii.1987 (*Becker*) (VOB); 11 ♂♂, 6 ♀♀, British Virgin Islands, Guana, 9–23.vii.1987, x.1989 (*Becker*) (VOB); 1 ♂, 1 ♀, US Virgin Islands, St Thomas, 300 m, 25–30.vii.1987 (*Becker*) (VOB), 1 ♂, ('Danish W Indies ') St Croix, 27.iv.1894 (*Gudmann*) (paralectotype of *adustipennis*); 1 ♂, 2 ♀♀, Anguilla, 15.v, 12, 26.viii.1980 (*Parker*); 1 ♂, 1 ♀, Dominica, 1984 (*Plumbley*); 1 ♀, Barbados, Husbands, ex dry pods of *Cajanus cajan*, 4.vi.1973 (unspecified); 1 ♂, Grenada, lectotype of *L. adustipennis* (as above), 5 ♀♀, Grenada, windward side, Balthazar, 27.iv.[1894] (*Smith*) (paralectotypes of *L. adustipennis*); 1 ♂, Tobago, Marden House nr Scarborough, 9.i.1982 (*Cock*); 1 ♀, Trinidad, holotype of *L. crotalariella* (as above), 1 ♀, Trinidad, St. George Co., Curepe, xi.1981 (*Cock*). **Venezuela**: 1 ♀, El Avila, Caracas, 28.ix–3.x.1974 (*Ridout*). **Ecuador**: 2 ♂♂, 5 ♀♀, Galápagos Islands, Santa Cruz (Indefatigable), v.1970 (*Perry & Vries*). **Peru**: 1 ♂, Rio Napo, vi.1920 (*Parish*). **Brazil**: 1 ♂, 2 ♀♀, Rondônia, Cacaulândia, 140 m, 15–18.x.1993, 13–31.xii.1997 (*Becker*) (VOB), 1 ♀, Vilhena, 600 m, 10–13.iv.1996 (*Becker*) (VOB); 1 ♂, Amazonas, Tefé ('Teffe') i.1920 (*Parish*); 1 ♂, Matura, vi.1920 (*Parish*); 1 ♀, Maranhão, Açailândia, 150 m, 19–27.xi.1990 (*Becker*) (VOB); 1 ♀, Minas Gerais, Caraça, 1300 m, 7–10.v.1996 (*Becker*) (VOB), 3 ♀♀, Unaí, 700 m, 7.xi.1982, 3.xi.1983 (*Becker*) (VOB), 1 ♂, Serra do Cipó, 17–19.iv.1991 (*Becker*) (VOB), 1 ♂, Corumbá, 180 m, 23–25.iv.1985 (*Becker*) (VOB), 1 ♀, Corumbá, 600 m, 20–22.iv.1985 (*Becker*) (VOB); 6 ♂♂, Espírito Santo, Linhares, 40 m, 16–18.ix.1991, 20–29.ii.1992, 6.iii.1993 (*Becker*) (VOB); 1 ♀, São Paulo, Bertioga, 5 ♂♂, 15–17.v.1996 (*Becker*) (VOB).

Mesophleps safranella (Legrand, 1965) comb. nov.

(Figs 17, 31, 64, 93, 123)

Gelechia safranella Legrand, 1965, *Mém. Mus. natn. Hist. nat. Paris* (N. S.) (A)37: 74, pl. 5, fig. 4. Holotype ♀, SEYCHELLES: Mahé, Beau Vallon, 1.v.1959 (*Legrand*) (slide no. 8876, Lerault; MNHN, Paris) [examined].

♂, ♀. Wingspan 11.5–14.0 mm. Labial palpus recurved, segment 2 distally broadened by erect dorsal scales, dark brown to black with white distal ring; 3 shorter than 2, white. Antenna with alternating dark and light brown rings. Forewing yellowish brown to ochreous brown, dorsum sometimes darker greyish brown; costa basally with delicate blackish brown edge, distal three-fifths with slightly wider brown to blackish brown stripe interrupted by faint oblique white line running from distal quarter towards termen, black spots variable, discal and plical often smaller than discocellular, tornal small or shadowy; termen with small number of indistinct black spots, usually two or three near apex more distinct.

Pregenital abdomen (Fig. 17). Anterior margins of TIV–VII with broad and straight, transverse band of densely set posteriorly-directed microtrichia.

Genitalia ♂ (Fig. 64). Uncus short, rounded; gnathos band narrow, hooks short, robust, about one-half length of uncus; tegumen with triangular to sub-triangular anterior emargination, pedunculi broad, distally forked; sclerotized posterior margin of vinculum medially concave, sometimes notched, with broad protruding sclerotization. Phallus with bulbous base, more or less evenly tapered towards narrow distal third.

Genitalia ♀ (Figs 93, 123). Dorso-posterior margin of segment VIII slightly convex; ventro-anterior margin weakly sinuous. Subostial plate relatively broad, caudal margin concave at ostium bursae, anterior margin straight; sclerotized antrum relatively broad, funnel-shaped, sometimes slightly exceeding transverse anterior margin of subostial plate; ductus bursae thin, about twice length of apophyses anteriores, entering corpus bursae postero-laterally; corpus bursae elongate ovoid, anteriorly broad, tapered posteriorly, about length of ductus bursae; ductus seminalis from cervix bursae.

Remarks. *M. safranella* can be distinguished with certainty from several other species of the *palpigera*-group only by the genitalia. The male genitalia differ from those of *palpigera*, *ioloncha* and *subhutiana* in the rounded rather than sub-rectangular uncus. The uncus resembles most that of the Neotropical *adustipennis* but in *safranella* it is slightly more elongate and less evenly rounded. Moreover, in *safranella* the gnathos band is narrower and the gnathos hooks are stouter and slightly shorter in relation to the uncus. In the female the dorso-posterior margin of segment VIII is convex but less so than in *palpigera* and *adustipennis*. *M. ioloncha* differs from *safranella* in the more elongate uncus and longer, slimmer gnathos hooks in the male genitalia and the large oval rather than posteriorly tapered corpus bursae in the female.

Biology. Host-plants: *Acacia* sp., *Albizia lebbeck* (Leguminosae, Mimosoideae). The larva lives in the pods.

Distribution. Niger, Benin, Kenya, Malawi, Madagascar, Seychelles.

Material examined (9 ♂♂, 8 ♀♀, including 5 ♂♂, 3 ♀♀ genitalia preparations)

Niger: 1 ♂, Borodarou, ex pod of *Acacia*, 21.i.2000 (*Polaszek & Bal*). **Benin**: 1 ♀, Atcherigbe, ex pod of *Albizia lebbeck*, 18.i.2000 (*Polaszek*). **Kenya**: 2 ♂♂, 4 ♀♀, Kikuyu Ibea, Escarpment, 7500–8500 ft, ix–x.1900 (*Doherty*); 2 ♂♂, 2 ♀♀, Coast, Mwabungu s. l., 3.iv.1999, 19.viii.2000 (*Agassiz*). **Malawi** ('Nyasaland'): 2 ♂♂ [no locality], ex dry seeds of *Albizia lebbeck*, 1.iv.1933 (*Lamborn*). **Madagascar**: 1 ♂, 1 ♀, Diego Suarez, 24, 25.viii.1917 (*Melou*). **Seychelles**: 1 ♂, holotype (as above).

Mesophleps epiochra (Meyrick, 1886) comb. nov.

(Figs 7, 11, 18, 32, 94, 124)

Brachyacma epiochra Meyrick, 1886, *Trans. ent. Soc. Lond.* **1886**: 279. Holotype ♂, FIJI: (*Lucas*) [not traced].

Brachyacma epichorda Turner, 1919, *Proc. R. Soc. Qd* **31**: 163. LECTOTYPE ♀ [not ♂], AUSTRALIA: Queensland, Toowoomba, 31.iii.1916 (*Turner*) (genitalia slide no. G 1618; ANIC), here designated [examined]. [Incorrectly synonymized with *B. palpigera* by Meyrick 1925: 169.] **Comb. nov., syn. nov.**

♂, ♀. Wingspan 8.0–20.0 mm. Labial palpus segment 2 sub-triangular, outer surface ochreous brown, inner surface greyish white, dorsally with large scale tuft, distally white, 3 about one-third to two-thirds length of 2, white, apex dark brown. Antenna greyish white. Forewing ochreous yellow, scattered with ochreous brown scales and white-tipped brown scales especially in apical half; costa with basal two-fifths whitish brown, distal three-fifths ochreous brown, sometimes black, rarely interrupted by oblique white line running from distal sixth towards termen, along termen with some faint dark spots; dark brown discocellular and discal spots present, dark brown plical spot short or long.

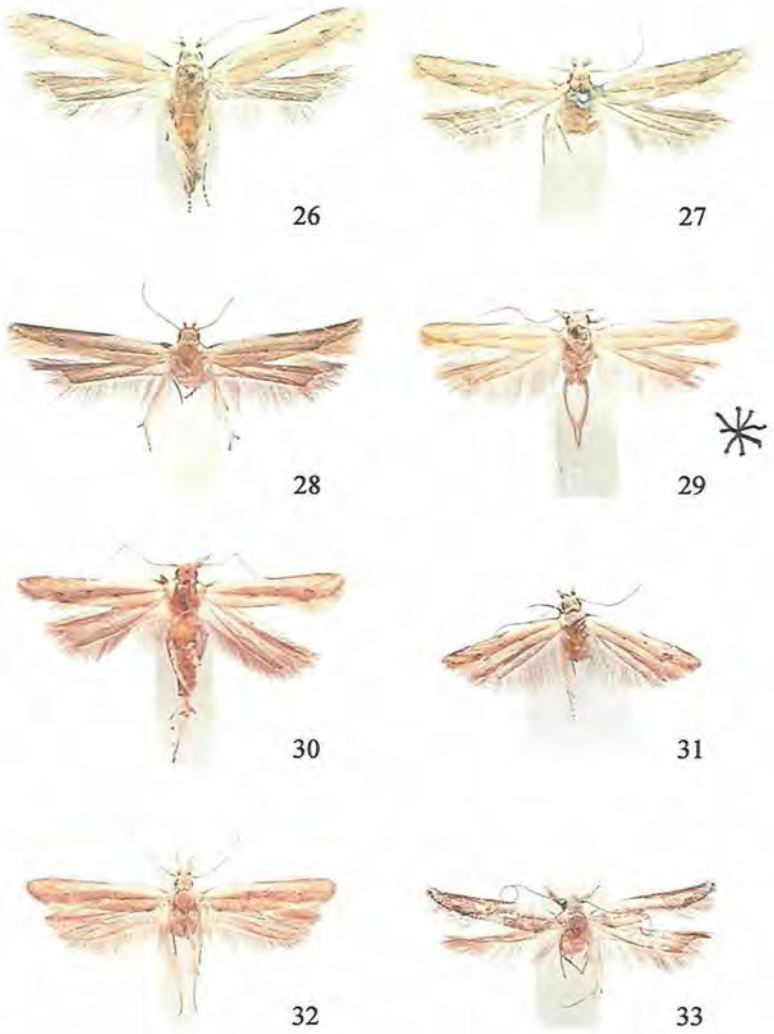
Pregenital abdomen (Figs 11, 18). Scale sockets on denuded TI and TII denser and bigger than on other segments. SII with pair of venulae and pair of short apodemes. Anterior margins of TIV–VII with slightly sinuate transverse band of densely set posteriorly-directed microtrichia.

Genitalia ♂ (Fig. 7). Uncus round, basally constricted, sometimes with short 'neck'; gnathos hooks widely separated at base, slender, pointed, shorter than uncus; valva narrow, widest at middle; tegumen relatively narrow, not exceeding width of uncus; downturned distal part of vinculum about one-third total its length, sclerotization of posterior margin relatively narrow, with distinct median emargination; phallus with basal two-thirds bulbous, distal one-third abruptly narrowed.

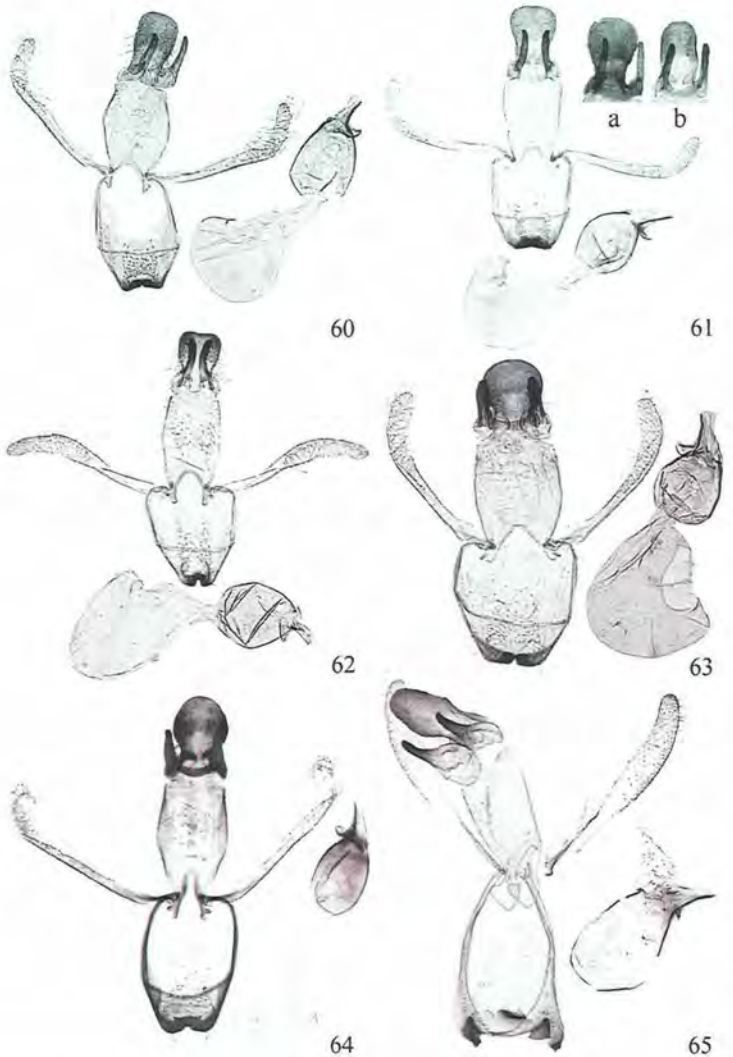
Genitalia ♀ (Figs 94, 124). Ovipositor and segment VIII short, dorso-posterior margin of VIII broadly and evenly convex; apophyses anteriores short, about one-half length of apophyses posteriores; sclerotized antrum very short and broad, more or less trapezoid in outline caudal margin weakly concave; ductus bursae thin, very long, almost 10 times length of apophyses anteriores, entering corpus bursae latero-posteriorly; corpus bursae ovoid, less than one-half length of ductus bursae; ductus seminalis thin, arising from cervix bursae.

Remarks. *M. epiochra* is very close to *M. adustipennis*. Both species are extremely variable in size, but in our samples *adustipennis* lacks the really big specimens as they are found amongst our *epiochra* from New Caledonia and Tonga. The labial palpi of both species are more or less porrect with a short upturned segment 3, but the dorsal brush of 2 appears to be bigger in *epiochra*. However, it is difficult to compare this character because the palpi are easily lost, the dorsal brush can be more or less worn down or the scales are in a more or less erect state. In the male the uncus of *epiochra* is bigger and, when a little flattened in a preparation, may even exceed the width of the tegumen, whilst in *adustipennis* it is not broader. The gnathos hooks are perhaps slightly slimmer in *epiochra* and their apices reach the middle of the uncus whereas in *adustipennis* they are stouter, blunter, and exceed the middle of the uncus. More convincing differences are found in the females where the dorso-posterior margin of segment VIII is strongly convex medially in *adustipennis* but moderately and evenly so in *epiochra*, and the ductus bursae, which in *adustipennis* is about twice the length of the apophyses anteriores whilst it is many times that length in *epiochra*.

B. epiochra was described from the male holotype that appears to be missing. It is not in BMNH and was not seen by KS in SAM, Adelaide, where the Lucas collection is now kept. The identity of *epiochra* is not in doubt as



FIGURES 26–33. Adults of *Mesophleps* spp. 26. *M. palpigera* (Walsingham), ♀, Gabon; 27. *M. ioloncha* (Meyrick), ♀, Java; 28. *M. sublutiana* (Park), ♀, Thailand; 29. *M. adustipennis* (Walsingham), lectotype, ♂, Grenada; 30. *M. adustipennis* (Walsingham), ♀, Galapagos Islands; 31. *M. safranella* (Legrand), ♂, Kenya; 32. *M. epiochra* (Meyrick), ♀, New Caledonia; 33. *M. tabellata* (Meyrick), holotype, ♂, India.



FIGURES 60–65. Male genitalia of *Mesophleps* spp. 60. *M. palpigera* (Walsingham), slide no. 30901; 61. *M. ioloncha* (Meyrick), slide no. 30866, a & b. variation in uncus, slide nos.: a. 30783, b. 30794; 62. *M. sublutiana* (Park), slide no. L03073; 63. *M. adustipennis* (Walsingham), slide no. 30887; 64. *M. safranella* (Legrand), slide no. 30971; 65. *M. tabellata* (Meyrick), slide no. 8544, Clarke.

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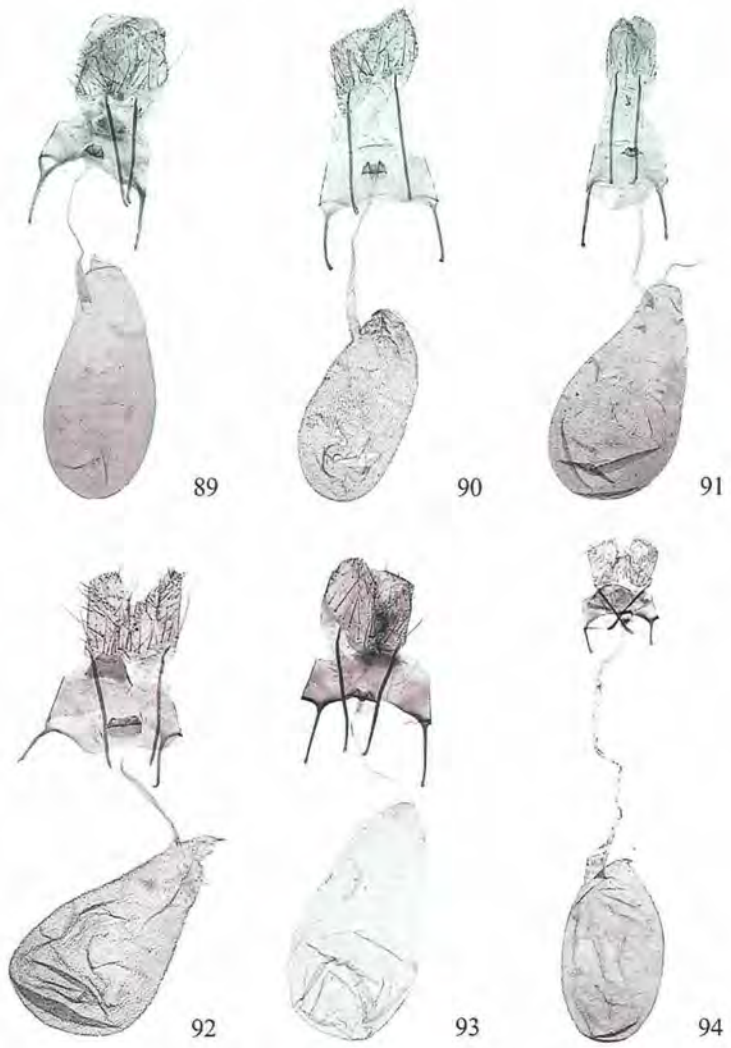
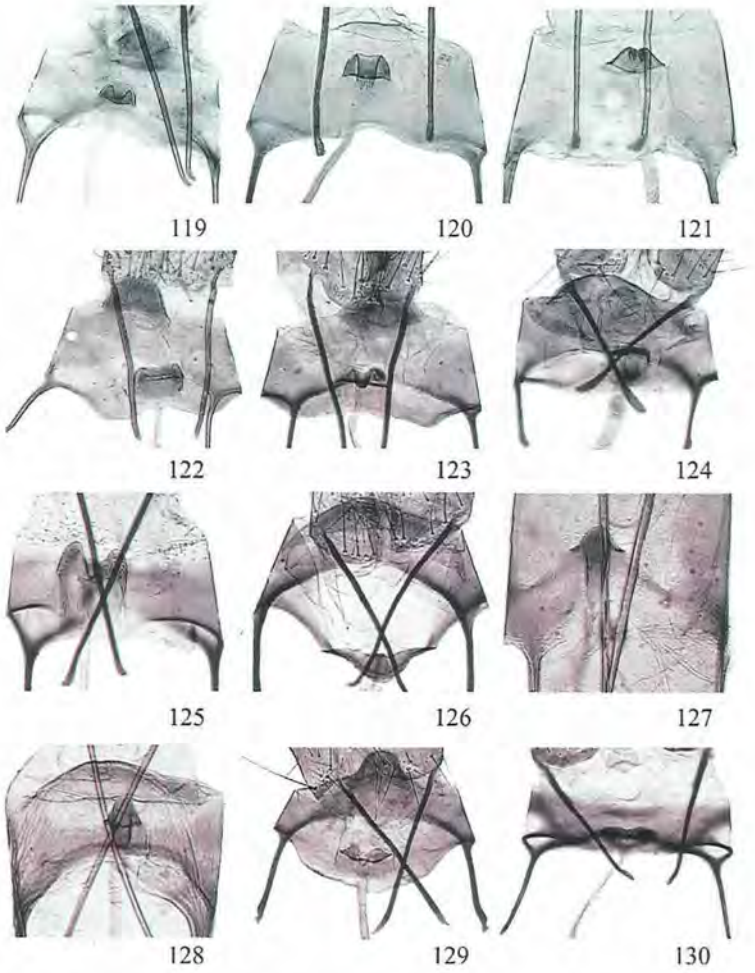


FIGURES 89–94. Female genitalia of *Mesophleps* spp. 89. *M. palpigera* (Walsingham), slide no. 30902; 90. *M. ioloncha* (Meyrick), slide no. 30883; 91. *M. sublutiana* (Park), slide no. 30802; 92. *M. adustipennis* (Walsingham), slide no. 30877; 93. *M. safranella* (Legrand), slide no. 30970; 94. *M. epiochra* (Meyrick), slide no. 30978.



FIGURES 119–130. Female segment VIII of *Mesophleps* spp. 119. *M. palpigera* (Walsingham), slide no. 30902; 120. *M. ioloncha* (Meyrick), slide no. 30883; 121. *M. sublutiana* (Park), slide no. 30802; 122. *M. adustipennis* (Walsingham), slide no. 30877; 123. *M. safranella* (Legrand), slide no. 30970; 124. *M. epiochra* (Meyrick), slide no. 30978; 125. *M. acutunca* sp. nov. paratype, slide no. L03071; 126. *M. crocina* (Meyrick), slide no. 30936; 127. *M. silacella* (Hübner), slide no. 13871; 128. *M. corsicella* (Herrich-Schäffer), slide no. 13809; 129. *M. oxycedrella* (Millière), slide no. 30049; 130. *M. trinotella* Herrich-Schäffer, slide no. 30925.

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ZOOTAXA

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Revision of the New World genus *Peckia* Robineau-Desvoidy (Diptera: Sarcophagidae)

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see p. 64 + 82

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Abstract

The New World and largely Neotropical genus *Peckia* Robineau-Desvoidy, 1830 is revised with a key to all species. *Peckia* is considered a senior synonym of *Guanoxipha* Lehrer, 2012, **n. syn.** and of *Sarcodexia* Townsend, 1892, **n. syn.**, the first one under *Squamatodes* Curran and the latter maintained as a valid subgenus, which here is redefined giving the new generic combinations *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830), **n. comb.** and *P.* (*S.*) *notata* (Lopes, 1935), **n. comb.**; and the new subgeneric affiliations *P.* (*S.*) *aequata* (Wulp, 1895), *P.* (*S.*) *chirotheca* (Hall, 1933), *P.* (*S.*) *dominicana* (Lopes, 1982), *P.* (*S.*) *florencioi* (Prado & Fonseca, 1932), *P.* (*S.*) *roppai* (Lopes & Tibana, 1982) and *P.* (*S.*) *tridentata* (Hall, 1937). *Peckia virgo* (Pape, 1994) is transferred from subgenus *Euboettcheria* Townsend, 1927 to subgenus *Squamatodes* Curran, 1927. *Sarcophaga adolenda* Lopes, 1935 is transferred from its current position in *Peckia* to the genus *Retrocitomyia* Lopes, 1982, **n. comb.** A total of 67 species are recognized and grouped in the subgenera *Euboettcheria*, *Pattonella* Enderlein, 1928, *Peckia* Robineau-Desvoidy, 1830 (*sensu stricto*), *Sarcodexia* and *Squamatodes*. Nine new species are described, viz., *Peckia* (*Euboettcheria*) *santamariae* **n. sp.** (Colombia), *Peckia* (*Euboettcheria*) *cacao* **n. sp.** (Costa Rica), *Peckia* (*Euboettcheria*) *calixtoi* **n. sp.** (Puerto Rico), *Peckia* (*Euboettcheria*) *hernandosi* **n. sp.** (Ecuador), *Peckia* (*Pattonella*) *kladosoides* **n. sp.** (Colombia), *Peckia* (*Peckia*) *cocopex* **n. sp.** (Costa Rica: Cocos Island), *Peckia* (*Peckia*) *sarmientoi* **n. sp.** (Ecuador), *Peckia* (*Peckia*) *rosalbae* **n. sp.** (Colombia) and *Peckia* (*Sarcodexia*) *cocos* **n. sp.** (Costa Rica: Cocos Island). The following new synonymies are proposed as junior synonyms under their respective species: under *Peckia* (*Euboettcheria*) *tridentata* (Hall, 1937) is *Euboettcheria alvarengai* Lopes & Tibana, 1982, **n. syn.**; under *Peckia* (*Peckia*) *chrysostoma* (Wiedemann, 1830) is *Paraphrissopoda hugolopesiana* Lehrer, 2006, **n. syn.**; under *Peckia* (*Peckia*) *pexata* (Wulp, 1895) are *Sarcophaga concinnata* Williston, 1896, **n. syn.**, *Sarcophaga otiosa* Williston, 1896, **n. syn.** and *Paraphrissopoda catiae* Lehrer, 2006, **n. syn.**; under *Peckia* (*Peckia*) *rubella* (Wiedemann, 1830) is *Sarcophaga capitata* Aldrich, 1916, **n. syn.** and under *Peckia* (*Squamatodes*) *trivittata* (Curran, 1927) is *Squamatodes stahli* Dodge, 1966, **n. syn.** Lectotypes are designated for *Sarcophaga aequata* Wulp, 1895, *Sarcophaga concinnata* Williston, 1896, *Sarcophaga otiosa* Williston, 1896 and *Sarcophaga volueris* Wulp, 1895. *Paraphrissopoda alvesia* Lehrer, 2006 is deemed an unavailable name as no depository was given for the putative type material.

Key words: Diptera, Sarcophagidae, *Euboettcheria*, *Pattonella*, *Peckia*, *Sarcodexia*, *Squamatodes*, revision, systematics, Neotropical Region.

Introduction

Peckia Robineau-Desvoidy, 1830 is a New World genus of generally large-bodied flesh flies, many of which breed in vertebrate excrement and carrion, including human corpses (Jirón *et al.* 1983; Salviano *et al.* 1996; Carvalho *et al.* 2000; Reeves *et al.* 2000), and for that reason the genus is considered of forensic and medical importance (Jirón *et al.* 1983; Moura *et al.* 1997; Oliveira-da-Silva *et al.* 2006; Barros *et al.* 2008; Buenaventura *et al.* 2009).

The genus *Peckia* was described by Robineau-Desvoidy (1830), who originally included five species (Evenhuis *et al.* 2010), and currently it contains 63 described species (Pape 1996; Pape & Andersson 2001). All of

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phallic tube, as sclerotized as the phallic tube. Juxtal lateral plate short with rounded apex in lateral view. Distiphallus with a pair of lateral styli strongly enlarged distally and appearing closed in ventral view. Vesica composed of two plate-like structures connected proximally, with the ventral edge angled in lateral view and each with an acute, hook-shaped apex.

Female. Unknown.

Variable features. The following differences were observed in the paratype with respect to the holotype: Chaetotaxy: dorsocentrals = 0 + 2.

Taxonomic remarks. *Peckia* (*Peckia*) *rosalbae* is close to *P.* (*Peckia*) *nephele*, but differs by the lateral styli appearing closed in ventral view and by the anteriorly acute vesica more sclerotized and smaller than in *P.* (*Peckia*) *nephele*. *Peckia* (*Peckia*) *rosalbae* and *P.* (*Peckia*) *nephele* have lateral styli strongly enlarged distally, whereas these structures are filamentous in other species of the genus. The usual colour pattern of the Sarcophaginae can change, exhibiting a shiny black spot on the thorax posteriorly and on the abdomen in the center.

Biology. Unknown.

Distribution. NEOTROPICAL—Colombia (Magdalena).

Etymology. The specific epithet is given in honour of Rosalba Ruiz Molina, the mother of the first author.

Holotype. Male, **COLOMBIA**, Magdalena, Santa Marta, Hacienda Victoria, 11°07'47.8"N 74°05'42.4"W, 968m, 01.vi.2005 [no collector] (ICN).

Paratype. 1 male, same data as holotype (ZMUC).

Peckia (*Peckia*) *rubella* (Wiedemann)

(Figs. 3, 81, 82) (fig. 98 in Aldrich 1916, figs. 293–295 in Roback 1954)

Sarcophaga rubella Wiedemann, 1830: 357. Antigua ["Antigoa"]. Holotype male, in ZMUC (examined).
Sarcophaga capitata Aldrich, 1916: 209. Puerto Rico, Mayaguez. Holotype male, in USNM (examined). **N. syn.**

Description. Male. Head. Ocellar setae equal to or shorter than postoculars. Outer vertical seta stronger than postoculars. Black orbital setae. Four frontal setae situated below the dorsal limit of the lunule. Genal setae black dorsally, yellow or white ventrally. Occipital setae white or yellow. **Thorax.** Chaetotaxy: acrostichals = 0 + 1, dorsocentrals = 0 + 3 (anterior one shorter), intra-alars = 1 + 2 (anterior one shorter), supra-alars = 2 + 3, basal scutellars = 3. Prosternum and posterior surface of hind coxa with black setae. Black antero-ventral scutellar setae. Two katepisternal setae. Postalar wall with only black setae. Lower calypter with a central dark spot and a fringe of long hair-like setae along outer margin, extending to its posterior margin. Mid femur without a ctenidium. Antero-dorsal surface of mid tibia with 1 median seta and 1 apical seta. Hind tibia antero-dorsally with 1 seta in the basal third, 1 in the middle third and 1 preapical. **Abdomen.** Postero-ventral seams between T3/T4 and T4/T5 parallel. Microtrichosity of the abdomen laterally grey. ST1+3 with only black hair-like setae. Two lateral setae on each side of T4. T5 with grey microtrichosity. Posterior seam of T5 not projected posteriorly and ventrally, and normal setae in postero-ventral area directed posteriorly. **Terminalia.** ST5 orange. Medial margin of ST5 Π-shaped. Inner margins of ST5 arms straight. Inner margins of ST5 arms with a patch of short setae anteriorly and long hair-like setae posteriorly. Syntergosternite 7+8 longer than high in lateral view, orange, without microtrichosity. Epandrium bright orange. Cercus orange proximally and brown or dark brown distally. Cercus in lateral view progressively narrowing towards the apex, with the dorsal margin curved, with a projection at about midlength. Cercal apex acute in lateral view. Surstylus orange, triangular, with a rounded apex, with postero-distal surface less sclerotized (more smooth; lighter and more transparent colour) than the remaining surface. Pregonite triangular, becoming narrower towards the apex, curved in lateral view, with a rounded apex. Postgonite elongated, with a hooked apex. Basi- and distiphallus connected by a desclerotized strip. Juxta without demarcated connection with the phallic tube, as sclerotized as the phallic tube. Juxta with lateral plate, long, convergent in apical view, with a truncated apex in lateral view. Distiphallus with a pair of lateral styli separated, each with a longitudinal cleft, visible in lateral and apical view. Vesica very large, composed of two plate-like structures on each side connected proximally, with microtrichia dorsally.

Distribution. NEOTROPICAL—American Virgin Is., Antigua, British Virgin Is., Panama, Puerto Rico.

Material examined. Antigua: 1 male (*Sarcophaga rubella* Wiedemann, 1830, holotype), Antigoa [Antigua], Mus. Westerm. (ZMUC). **British Virgin Is:** 1 male, Guana Island, 0–80m, 13–26.vii.1986, S.E. Miller & M.G. Poque (ZMUC). **Puerto Rico:** 4 males, Luquillo NatlFor. El Verde, 04–17.xii.1968 (ZMUC); 3 males, Rio Grande, El Verde Station, 3.1km WNW Pico El Yunque, Sierra de Luquillo, 18°19'15"N 65°49'11"W, 355m, 03–06.vi.1996, C. Young, R. Davidson, M. Klingler, W. Zanol, S. Thompson & J. Rawlins (ZMUC); 1 male, Utuado. Bosque Estetal de Rio Abajo, 4.9km W Dos Bocas, 18°19'59"N 66°43'00"W, 17.vi.1996, C. Young, J. Rawlins, R. Davidson, W. Zanol, M. Klinger & S. Thompson (ZMUC).

Peckia (*Peckia*) *sarmientoi* n. sp.

(Figs. 63, 64)

Description. Male. Length. 13.2 mm. **Head.** Ocellar setae equal to or smaller than postoculars. Outer vertical seta of same size as postoculars. Orbital setae black. Four frontal setae situated below the dorsal limit of the lunule in lateral view. Genal setae black dorsally, yellow or white ventrally. First two rows of occipital setae black, others yellow. **Thorax.** Chaetotaxy: acrostichals = 0 + 1, dorsocentrals = 0 + 2 (anterior one shorter), intra-alars = 1 + 2 (anterior one shorter), supra-alars = 2 + 3, basal scutellars = 3. Prosternum and posterior surface of hind coxa with black setae. Antero-ventral scutellar setae black. Two katepisternal setae. Postalar wall with only black setae. Lower calypter with a central dark spot and a fringe of long hair-like setae along outer margin, extending to its posterior margin. Mid femur without a ctenidium. Antero-dorsal surface of mid tibia with a median seta and 1 apical seta. Hind tibia antero-dorsally with 1 seta in the basal third, 1 in the middle third and 1 preapical. **Abdomen.** Postero-ventral seams between T3/T4 and T4/T5 parallel. Posterior seam of T5 not projected posteriorly and ventrally. T5 with golden microtrichosity. Abdomen in lateral view with grey and golden microtrichosity. ST1+3 with yellow and white setae. Two lateral setae on each side of T4. **Terminalia.** ST5 orange. Medial margin of ST5 Π-shaped. Inner margins of ST5 arms straight. Posterior margin of ST5 arms bent dorsally. Inner margins of ST5 arms with a patch of short setae on the median region. Syntergosternite 7+8 is longer than high in lateral view, orange, with golden microtrichosity. Epandrium bright orange. Cercus orange proximally and brown or dark brown distally. Cercus in lateral view progressively narrowing towards the apex, dorsal margin with an undulation of the cuticle at about midlength, with an acute apex. Surstylus orange, triangular, with a rounded apex. Postero-distal surface less sclerotized (more smooth; lighter and more transparent colour) than the remaining surface. Pregonite tongue-shaped, curved with a rounded apex in lateral view. Postgonite elongated, with a hooked apex. Basi- and distiphallus connected by a desclerotized strip. Juxta without demarcated connection with the phallic tube, as sclerotized as the phallic tube. Juxta with lateral plate, long, straight with a rounded apex in apical view. Juxtal lateral plate with spinose outer margin. Distiphallus with a pair of lateral styli separated, each with a longitudinal cleft, visible in lateral view. Vesica composed of two plate-like structures on each side connected proximally.

Female. Unknown.

Taxonomic remarks. *Peckia* (*Peckia*) *sarmientoi* has the juxtal lateral plate with outer margin spinose, a feature only found in this species.

Biology. Unknown.

Distribution. NEOTROPICAL—Ecuador.

Etymology. The specific epithet is given in honour of Carlos E. Sarmiento M. for his significant contributions in systematics that allowed the development of this study, and because his work on the systematics of wasps (Vespidae and Braconidae) and on the use of insects as a model for the study of macroevolutionary patterns have been a source of inspiration for many students.

Holotype. Male, **ECUADOR**, Napo province, Yasuni National Park, Yasuni Research Station, 76°36'W 00°38'S, 3–20m, xi.1998, T. Pape & B. Viklund (NRM).

Peckia (*Peckia*) *satanica* Dodge

(fig. 36 in Dodge 1965a)

Peckia satanica Dodge, 1965a: 489. Bahamas, Cat I. Holotype male, in AMNH (not examined).

Description. Male. Head. Ocellar setae equal to or shorter than postoculars. Outer vertical seta stronger than postoculars. Black orbital setae. Four frontal setae situated below the dorsal limit of the lunule. Gena with only black setae. First row of occipital setae black, others yellow. **Thorax.** Chaetotaxy: acrostichals = 0 + 1,

yellow or light brown setae. Chaetotaxy: acrostichals = 0 + 1, dorsocentrals = 0 + 2, intra-alars = 1 + 2 (anterior one shorter), supra-alars = 1 + 3, basal scutellars = 3. White or yellow and black antero-ventral scutellar setae. Two katepisternal setae. Postalar wall with white or yellow anterior setae. Lower calypter with a central dark spot, with black setae dorsally and a fringe of long hair-like setae along outer margin extending to its posterior margin. Mid femur without a ctenidium. Antero-dorsal surface of mid tibia with 1 median and 1 apical seta. Ventral surface of hind femur with 1 row of setae. Hind tibia with 2 setae in the basal third, 2 in the middle third and 1 preapical on antero-dorsal surface. **Abdomen.** Postero-ventral margins of T3 and to a lesser extent T4 curved posteriorly, so that segment 3 and to a lesser extent segment 4 are of near-equal length dorsally and ventrally. Posterior seam of T5 projected posteriorly and ventrally. Microtrichosity of the abdomen laterally grey and golden. ST1+3 with yellow or light brown hair-like setae. Two lateral setae on each side of T4. T5 with grey microtrichosity. Posterior seam of T5 projected posteriorly and ventrally. Postero-lateral area of T5 with setae directed towards the ventral body region. **Terminalia.** ST5 orange. Medial margin of ST5 Λ-shaped. Inner margins of ST5 arms straight. Inner margins of ST5 arms with a patch of short setae on the anterior region and long hair-like setae uniformly distributed. Syntergosternite 7+8 as long as high in lateral view, orange, with golden microtrichosity. Epandrium bright orange. Cercus orange proximally and brown or dark brown distally. Cercus in lateral view progressively narrowing towards the apex, with the dorsal margin angulated in lateral view. Cercal apex acute in lateral view. Surstylus orange, triangular, with an acute apex. Pregonite subquadrate, straight in lateral view, with an undulated apex. Postgonite elongated, with a hooked apex. Basi- and distiphallus connected by a desclerotized strip. Phallic tube short and straight. Juxta with a demarcated connection with the phallic tube. Juxta without juxtal lateral plate. Juxta entire with a middle line dorsally forming a crest running the full juxtal length. Lateral styli fused and forming only one broad tube. Vesica absent.

Distribution. NEOTROPICAL—Argentina (Misiones), Brazil (Federal District, Goiás, Mato Grosso, Minas Gerais, Pará, São Paulo).

Material examined. Argentina: 1 female (*Squamatodes stahli* Dodge, 1966, holotype), Argentina, Misiones, Eldorado ["Kol. Eldorado, Misiones am Parana"] (ZSM). **Brazil:** 1 male, Districto Federal, Brazilia, xii.1961, H.S. Lopes (ZMUC); 1 male, Minas Gerais, Lassance, 20–31.i.1939, Martins, Lopes & Mangabeira (MNRJ); 1 male, Minas Gerais, Pirapora, Isca Gallina, 20–29.xii.1978, C.B. Carvalho (MNRJ); 1 male, Minas Gerais, Pirapora, 09.ix–10.x.1978, C.B. Carvalho (ZMUC); 1 male, Minas Gerais, Sierra do Cipó, 25.iii.1998, D. Yanega (ZMUC); 1 male, Pará, Santarem, vi.1931, R.C. Shannon (ZMUC).

Peckia (*Squamatodes*) *virgo* (Pape), new subgeneric affiliation

(Fig. 116) (figs. 395–398 in Pape 1994)

Blaesoxipha (*Gigantotheca*) *virgo* Pape, 1994: 40. British Virgin Islands, Necker I. Holotype male, in USNM (not examined).

Description. Male. Head. Ocellar setae stronger than postoculars. Outer vertical seta of same size as postoculars. Black orbital setae. Two frontal setae situated below the dorsal limit of the lunule. Genal setae black anteriorly, yellow or white posteriorly. Occipital setae white or yellow. **Thorax.** Chaetotaxy: acrostichals = 0 + 1, dorsocentrals = 0 + 3 (anterior one shorter), intra-alars = 1 + 2 (anterior one shorter), supra-alars = 2 + 3, basal scutellars = 4. Prosternum and posterior surface of hind coxa with black setae. Black antero-ventral scutellar setae. Three katepisternal setae. Postalar wall with only black setae. Lower calypter with a central dark spot and a fringe of long hair-like setae along outer margin, extending to its posterior margin. Mid femur with a ctenidium. Hind femur with a row of antero-ventral and a row of postero-ventral setae. **Abdomen.** Postero-ventral margins of T3 and to a lesser extent T4 curved posteriorly, so that segment 3 and to a lesser extent segment 4 are of near-equal length dorsally and ventrally. Posterior seam of T5 projected posteriorly and ventrally. Microtrichosity of the abdomen laterally grey and golden. ST1+3 with only black hair-like setae. T5 with grey microtrichosity. Posterior seam of T5 not projected posteriorly and ventrally, and normal setae in postero-ventral area directed posteriorly. **Terminalia.** ST5 orange. Medial margin of ST5 Π-shaped. ST5 arms with acute apophyses anteriorly. Inner margins of ST5 arms straight. Syntergosternite 7+8 longer than high in lateral view, orange, with golden microtrichosity and a crown of setae. Epandrium bright orange. Cercus orange proximally and brown or dark brown distally. Cercus in lateral view progressively narrowing towards the apex, straight with a few setae dorsally.

Surstylus orange, triangular, with an acute apex. Pregonite elongated with a rounded apex. Postgonite elongated, with a hooked apex. Basi- and distiphallus connected by a desclerotized strip. Phallic tube short and straight. Juxta with a demarcated connection with the phallic tube. Juxta without juxtal lateral plate. Juxta entire with a rounded protuberance. Lateral styli fused and forming only one broad tube. Vesica absent.

Distribution. NEOTROPICAL—British Virgin Is., Dominican Republic.

Material examined. British Virgin Is: 1 male, Necker Island, 21.vii.1987 (ZMUC); 1 male, Guana Island, 01–14.vii.1984, S.E. & P.M. Miller (ZMUC). **Dominican Republic:** 1 male, Peravia, 9km S San José de Ocoa, 19.vii.1987, A.L. Norrbom (ZMUC).

Unplaced species-group names

The following names represent nominal species-group taxa that cannot be reliably placed in synonymy but are not expected to represent additional species.

Sarcophaga auribarbata Townsend, 1912: 357. Peru, Piura. Holotype female, in USNM (not examined). Considered by Aldrich (1930: 27) as synonymous with his *Sarcophaga cotyledonea* (= *Peckia* (*Pattonella*) *intermutans*), but treated as a valid species of *Paraphrissopoda* (= *Peckia sensu stricto*) by Lopes (1969: 36).

Sarcophaga incerta Walker, 1853: 324. Jamaica. According to the curator of BMNH, the holotype female is missing. Aldrich (1930) examined this holotype and mentioned that this species "runs to the vicinity of *hillifera* Aldrich", but he still considered himself unable to identify it further. Later, Dodge (1965b) received notes about this holotype from Oldroyd and commented about its poor condition.

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References

Aldrich, J.M. (1916) *Sarcophaga and allies in North America*. Thomas Say Foundation, Entomological Society of America, La Fayette, Indiana, 301 pp. + 16 pls.

Aldrich, J.M. (1930) Notes on the types of American two-winged flies of the genus *Sarcophaga* and a few related forms, described by the early authors. *Proceedings of the United States National Museum*, 78, 1–39 + 3 pls. http://dx.doi.org/10.5479/si.00963801.78-2855.1

Assis-Fonseca, E.C.M. (1953) An important character in the identification of the females of *Sarcophaga* species (Dipt., Calliphoridae). *Journal of Society of British Entomologists*, 4, 167–168.

Bänziger, H. & Pape, T. (2004) Flowers, feces and cadavers: natural feeding and laying habits of flesh flies in Thailand (Diptera: Sarcophagidae, *Sarcophaga* spp.). *Journal of Natural History*, 38, 1677–1694. http://dx.doi.org/10.1080/0022293031000156303

Barros, R.M. de, Penteado-Dias, A.M. & Pujol-Luz, J.R. (2006) Registro de *Peckia* (*Squamatodes*) *trivittata* (Curran) (Diptera, Sarcophagidae) parasitada por *Gnathopleura semirufa* (Brullé) (Hymenoptera, Braconidae, Alysiinae) no cerrado de Brasília, DF. *Revista Brasileira de Entomologia*, 50, 436–438. http://dx.doi.org/10.1590/S0085-56262006000300017

Barros, R.M. de, Mello-Patiu, C.A. de & Pujol-Luz, J.R. (2008) Sarcophagidae (Insecta, Diptera) associados à decomposição

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A review of the genus *Hoplocheiloma* Cresson (Diptera: Micropezidae)

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Abstract

The Caribbean genus *Hoplocheiloma*, which contains eight known species, is reviewed with the redescription of all named species and the description of the following new species: *H. rhytisma, H. dominica, H. jamaica and H. hispaniola*. *Hoplocheiloma notitipenne* Cresson is synonymized with *H. maculosum* (Loew), *H. fabricii* Steyskal is synonymized with *H. totliana* (Gmelin), and *Gymnosphen macropyga* Frey is transferred from *Hoplocheiloma to Calosphen*. All *Hoplocheiloma* species are keyed and illustrated.

Key words: Caribbean, Cresson, Micropezidae, *Hoplocheiloma*

Introduction

Hoplocheiloma is a small and distinctive genus of Taeniapterinae characterized by prominent clypeal setae (a unique feature not found elsewhere in the Micropezidae), and a broadly triangular and bare anal cell (CuP) intermediate in length between the typical tapered anal cell of the Taeniapterini *sensu* Aczel (1951) and the short anal cell of most Grallipezini *sensu* Aczel (1951). The genus is Caribbean in distribution, ranging from Florida and the Yucatan through the Antilles to Dominica. One species (*H. totliana* (Gmelin)) was recorded from Brazil (as *H. fasciata* (Fabricius)) on the basis of a specimen in the Winthem collection in Vienna (Hennig, 1935), but the specimen in question could not be relocated and the record is considered dubious. Another species, *H. perforatum* (Enderlein) was described on the basis of a single specimen simply labeled "Brasil", but all other known specimens of this species are from the Dominican Republic. Other putative records from Central and South America are errors or probable errors, as discussed below. Endemic species are found on the islands of Hispaniola, Montserrat, Dominica, and Jamaica; further narrowly distributed species will likely be found as other Caribbean islands are surveyed. This paper is based on specimens from the British Virgin Islands, (Guana Island), Cuba, Dominica, Dominican Republic, Jamaica, Mexico (Yucatan), Montserrat, Puerto Rico, Saba, St. Kitts, United States (Florida), St. Martin, St. Barthelemy and the United States Virgin Islands (St. Croix, St. Thomas, Tortola, Buck Island).

This review was initially prompted by a request for identifications of specimens taken as part of a survey of Hispaniolan arthropods (Perez-Gelabert, 2008), which exposed the need for a full review of the genus to enable meaningful identifications. Perez-Gelabert (2008) lists only *H. notitipenne* Cresson from Hispaniola; *H. notitipenne* is here synonymized with *H. maculosum* (Loew), and two further species, *H. perforatum* (Enderlein) and *H. hispaniola* n. sp., are here added to the Hispaniolan fauna. Three other new species *H. dominica, H. rhytisma*, and *H. jamaica*, are described from Dominica, Montserrat and Jamaica, respectively.

Material and methods

Notes on morphological terminology: The area of the head posterior to the ocelli is usually divided into distinct regions, and Cresson's (1930) terminology is followed here in referring to the part of the postocciput bearing the outer vertical setae as the paracephalon, and in calling the area mesad of the paracephalon the epicephalon (Fig. 5A). The epicephalon bears the inner vertical setae. The middle area posterior to the ocelli is the postocellar part of the frontal vitta; *Hoplocheiloma* species lack setae (postocellar setae) in this area. Interpretation of the male terminalia follows McAlpine (1998), with the entire visible phallus treated as a distiphallus divided into basal and distal parts. McAlpine (1998) is also followed in naming the wing bands, with the addition of the term "distal band" for a band incorporating the preapical and apical bands. Size is given as body length from head (exclusive of antenna) to abdominal apex excluding deflexed structures, and as wing length; body length is approximate because of shrinkage of dried specimens.

Male and female terminalia were cleared in hot potassium hydroxide and stained, where necessary, with lignin pink. Photographs of live specimens were taken with Nikon 35mm cameras with a 105mm macro lens; pinned specimen images were either shot with the same equipment or compiled from images taken on a Visionary Digital photomicroscope and stacked using CombineZ software.

Sources of specimens and collection acronyms are given in the acknowledgments.

Key to the species of *Hoplocheiloma*

1. Wing distinctly spotted, transverse bands broken up so the wing appears mostly infuscated, with 7 widely separated, clear, circular patches (Fig. 7). Head with only one large seta (inner vertical), other setae reduced or absent (Fig. 6) . . . . . *H. hispaniola* new species. Dominican Republic.
- Wing with a broad discal band crossing middle (Fig. 25). Head with three large setae (inner vertical, orbital and outer vertical, as in Fig. 22) 2
2. Notum mostly black, postpronotum contrastingly reddish. Base of oviscape bare and shining dorsomedially (Fig. 21) 3
- Notum entirely orange to orange-brown, sometimes patterned with silvery grey areas. Pruinose area at base of oviscape surrounding oviscape so there is no dorsomedial shining strip (Fig. 25). 4
3. Wing mostly infuscated, with discal and preapical dark bands separated by clear strips each made up of three more or less coalesced clear circular areas, circular areas usually separated by pigmentation along the wing veins but both clear and dark bands always reaching hind wing margin; apical infuscation distinct (Fig. 14). Anterior notopleural seta at most half as long as posterior seta. Membranous parts of abdomen mostly pale, female abdomen with pleural pigmentation mostly restricted to bands on dorsal half (Fig. 13), male abdomen with pigmentation of segment three circling abdomen and preceded and followed by extensive pale areas . . . . . *H. maculosum* (Loew). Dominican Republic, Cuba.
- Wing bands separated from one another by straight-sided clear strips; dark preapical band parallel-sided, broadly separated from hind margin of wing; apical infuscation indistinct (Figs. 11, 12). Notopleural setae equal in size. Abdominal membranes more extensively pigmented, ventral part of abdomen largely dark. . . . . *H. jamaica* new species
4. Head and notum orange-brown, presutural part of scutum patterned with silvery grey areas. Wing with three distinct pigmented bands and an infuscated tip, outer margin of discal and preapical bands concave at middle (Fig. 25). Hind femur dark at least on basal half, with 1–2 orange distal bands. . . . . *H. perforatum* Cresson. Dominican Republic, maybe Brazil.
- Head and notum orange, at least presutural part of scutum without silvery gray pattern. Hind femur usually orange with narrow dark bands (sometimes uniformly orange-brown). Outer margins of discal band convex or more or less straight, preapical band narrow and more or less straight-edged (*H. totliana*, Fig. 33) or coalesced with apical infuscation to form a composite distal band (*H. ferrugatum*, Fig. 5) 5
5. Wing with discal band sometimes broad but never reaching hind margin of wing, distal band narrow but distinct from apical infuscation (Fig. 27). Epicephalon and paracephalon dull, paracephalon not conspicuously bulging. Main vertical row of katepisternal setae usually darker than weaker anterior golden row. 6
- Discal band very large, taking up most of wing, broadly reaching hind margin of wing and separated from large apical infuscated area (distal band coalesced with apical infuscation, by a narrow transverse pale area (Fig. 5). Epicephalon and paracephalon shining, paracephalon conspicuously bulging (Fig. 5). Main vertical row of katepisternal setae golden, same color as weaker anterior golden row. . . . . *H. ferrugatum* Hennig. Mexico (Yucatan).
6. Pleuron with two prominent dark patches covered with silvery pruinosity, one covering the posterior third of the anepisternum and a less distinct one on the upper katepisternum (Fig. 27). Discal band more or less circular, widely separated from anterior wing margin and with distal face strongly convex. R_{2+3} ending far beyond plane of dm-cu (Figs. 1, 27) 7
- Pleuron uniformly orange. Discal band with distal face almost straight, anterior part reaching wing margin (Fig. 33). R_{2+3} ending at or very slightly beyond plane of dm-cu . . . *H. totliana* (Gmelin) (widely known as *H. fabricii* Steyskal) USA (Florida), Puerto Rico, St. Thomas, Puerto Rico, Cuba, Jamaica, St. Martin (Widespread in Antilles but not known from Hispaniola).
7. Discal spot of wing small, less than half wing width and not approaching R_{2+3} (Fig. 4). Base of arista usually brown . . . . . *H. dominica* new species. Dominica
- Discal spot of wing large, at least half wing width and touching or crossing R_{2+3} (Fig. 27). Base of arista yellow . . . . . *H. rhytisma* new species. Montserrat

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Legs: Fore coxa silvery pruinose and densely covered with white microsetulae on anterior surface, bare and reddish brown on most of posterior surface. Fore femur mostly brown with broad but sometimes diffuse basal and preapical yellow bands. Fore tibia black, tarsomere one of foreleg white, other fore tarsomeres black; mid and hind femora mostly orange-brown with a narrow distal dark band, mid and hind tibiae black, basal two thirds of first tarsomere of mid and hind legs white, tarsomeres otherwise black.

Wing with a distinct circular discal spot, a smaller preapical band crossing R_{4+5} and M, and a small indistinct stigmatal spot; otherwise clear (Fig. 27). R_{2+3} ending far beyond plane of dm-cu, extending beyond dm-cu by approximately the length of the crossvein (Fig. 27).

Abdomen: Abdominal pleuron of both sexes darkly pigmented except for pale mid ventral area and pale bulge on segment two of male. Tergite one brown, other tergites black, tergites 1 and 2 fused but delineated by a band of silvery microsetulae. Tergite 1 with long pale lateral and dorsolateral setae, other tergites with small black setulae only.

Female abdomen: Tergite 7 with a prominent central pale area. Oviscape shining except for basal silvery white densely microsetulose area that is penetrated by a dorsomedial shining strip. Single spermatheca very elongate on an elongate duct as long as the paired spermathecal duct; paired spermathecae small and spherical with a very long, multiply convoluted base (Fig. 28).

Male abdomen: Pleuron two with an elongate-oval setose pleural sac (Fig. 29). Epandrium yellow, contrasting with black pregenital segments. Genital fork long and narrow, arms converging distally with entire inner surface covered with short, stout spines; basal part of fork medially carinate, carina continuous with trough between fused basal halves of arms. Hypandrium with anterior arms fused into a scoop-shaped sclerite. Distiphallus with long, relatively broad base; distal part half as long as basal part, tapered to a point (Fig. 30). Apex of aedeagal apodeme narrow.

Type material. HOLOTYPE (♂, MTEC) and 3 paratypes (1 ♂, 2 ♀, MTEC): MONTSERRAT: Cassava Ghaut, Beattie House, 16°45.91'N62°12.95'W, 011-23.iii.2002, A. Krakower, u.v. light. OTHER PARATYPES: MONTSERRAT: Cassava Ghaut, Beattie House, 13-13.January.2002, at light, M. A. Ivie and K. Marske (1 ♂, MTEC); Cedar Ghaut, 04.viii.2005, G. Martinson, D. Hughley, yellow pan trap (1 ♀, MTEC); Woodlands Riverside House, 140', 10-12.January.2002, Ivie, Marske, Puliafico (1 ♀, MTEC); Centre Hills N.E. of Fleming Spring Ghaut, 750', 19.vi.2000, M. Ivie and K. Guerrero (1 ♀, MTEC).

Comments. *Hoplocheiloma rhytisma* can be distinguished from the closely related *H. dominica* (endemic to Dominica) by its more extensive discal wing spot, which extends to R_{2+3}.

Etymology. From the Greek *rhytisma* (n.) for "patch", referring to the silvery-grey patches on the katepisternum and anepisternum.

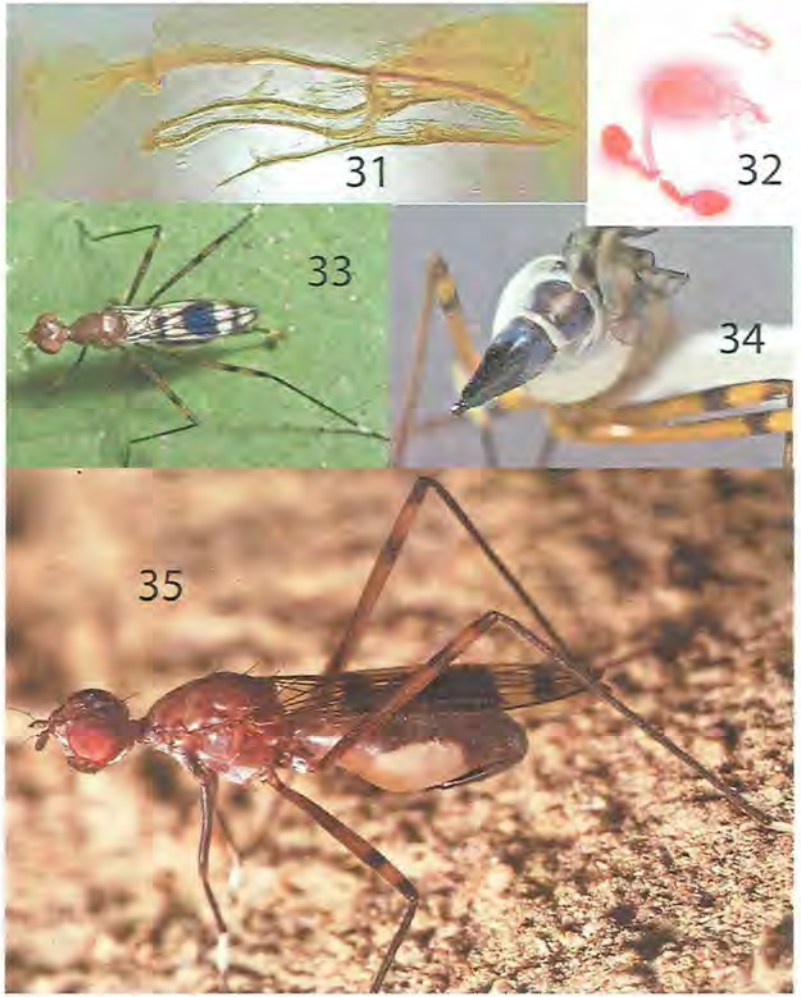
Hoplocheiloma totliana Gmelin

Figs. 31–35

Hoplocheiloma totliana Gmelin, 1790: 2850. nom. nov. for *Musca fasciata* Fabricius 1775 (preoccupied Meuller 1764; see Thompson and Pont 1993).
Hoplocheiloma fabricii Steyskal, 1968: 9; 1775. nom. nov. for *Musca fasciata* Fabricius 1775. New synonym.
Hoplocheiloma fasciata (Fabricius), Hennig 1935: 55 (key, diagnosis)

Body length 7–10mm. Wing length 7–8mm. General colour: Most of head and thorax orange; face yellowish white; proepisternum with a transverse black band just above the long, golden ventral proepisternal setae. Abdominal tergite 1 brown, other tergites darker; depression between fused tergites 1 and 2 black.

Head: Frons uniformly orange except dark brown or black ocellar triangle, frontal vitta broad, dull (microsetulose) and slightly tapered anteriorly; orbital strips subshining; two small frontal setae, a larger orbital and large inner vertical and outer vertical setae. Clypeus uniformly pale orange laterally, middle part with two stout setae and a few small setulae, lateral portions of clypeus covered with fine white setulae. Lunule with a few scattered black setulae, face weakly sclerotized and pale except for dark lower margin (often forming a distinct narrow black transverse band), densely microsetulose. Palpi small, half as long as clypeus; almost parallel-sided, with evenly spaced small black setae. Mentum strongly setose.



FIGURES 31–35. *Hoplocheiloma totliana* (Gmelin). 31, male terminalia; 32, spermatheceae and associated structures; 33, live female dorsal, Cuba; 34, female abdominal apex to show dorsum of oviscape; 35, live female left lateral, Cuba.

Thorax: Pronotum orange except for two distinct black marks along anterior margin. Mesonotum orange with an indistinct presutural silvery transverse band and two indistinct silvery postsutural spots. Both sexes with a uniform row of small acrostichal and dorsocentral setae, 1–3 anterior dorsocentral setae usually enlarged, longer than

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other setae but not conspicuously thickened. Notopleuron with a single large posterior seta and a smaller anterior seta. One large prescutellar dorsal seta only. Main vertical row of katepisternal setae black or dark brown with a weaker anterior row of golden setae, ventral apex of katepisternum with two long thick setae and one or more long thin seta.

Legs: Fore coxa densely covered with white microsetulae on anterior surface, bare and reddish brown on most of posterior surface. Fore femur and tibia dark brown to black, tibia with dense white microsetulae ventrally in distal third, tarsomere one and basal half of tarsomere two of foreleg white, other fore tarsomeres black; mid and hind femora mostly yellowish brown (basal half varies from brown to yellowish brown) with a narrow distal dark preapical band and a dark apex; mid and hind tibiae brown, basal two thirds of first tarsomere of mid and hind legs white, tarsomeres otherwise black.

Wing with a broad discal band with a straight or convex distal edge and similar narrow, parallel-sided preapical and stigmatal bands (reaching anterior margin of wing and usually crossing entire wing although weaker posteriorly) and a very small infuscated apical area; otherwise clear. R_{2+1} ending at or very slightly beyond plane of dm-cu.

Abdomen: Tergite one pale brown, other tergites darker, tergites 1 and 2 fused but delineated by a band of silvery microsetulae. Tergite 2 weakly excavated along posterior margin, with dark posterolateral corners (dark pigment extending on to intersegmental membrane). Tergite three darker than other tergites, blue black at least basomedially, and normally elevated posteriorly and projecting over tergite 4.

Tergite 1 with long pale lateral and dorsolateral setae, other tergites with small black setulae only.

Female abdomen: Pleuron darkly pigmented on dorsal part of segments 2–4, dark area progressively more extensive posteriorly such that the pale area of segments 2–6 appears circular with the posterior margin of the sphere closed by a dark segment 6 and the anterior portion mostly open (Fig. 35). Tergite 7 with a prominent central pale and depressed area. Oviscape shining except for basal silvery white densely microsetulose area broadly encircling base of oviscape (Fig. 34). Single spermatheca with a very elongate body on a very short base separated from thick duct by a spherical swelling. Paired spermathecae cylindrical with bodies slightly tapered and distinctly invaginated at apex; bases very elongate, thick, abruptly narrowed at junction with duct; duct thick, with an apical swelling between duct and constricted part of spermatheeal base (Fig. 32).

Male abdomen: Pleuron with dark areas of segment 5 extending continuously to genital fork, dark areas of segments 2 and 3 narrow, extending down from tergites and separated by wide pale areas (this pattern hard to discern on most pinned specimens). Pleuron without any conspicuous weakened areas or swellings (ie, bulging dome or pleural sac of segment two absent). Epandrium yellow, contrasting with black pregenital segments. Genital fork long and narrow, arms converging distally with entire inner surface covered with short, stout spines; basal part of fork medially carinate, carina continuous with trough between fused basal halves of arms. Hypandrial arms fused anteriorly to form a short bridge. Distiphallus with basal part relatively short and broad, distinctly shorter than distal part; distal part with a broad, parallel-sided transparent shell abruptly ending just before narrow, pointed apex (Fig. 31).

Type material. The Fabricius type (type locality "West Indies) was not examined. Hennig's (1935) concept of this species seems unproblematic and was followed here.

Other material examined. CUBA: Santiago de Cuba, Botanical Gardens, xi.2005, S.A. Marshall (2 ♀, in 95% alcohol, DEBU); Gran Piedra, pan traps behind biological station, 23.xi.2005, S.A. Marshall (1 ♂, DEBU); nr. Victoria de las Tunas, 18.vi.1950, Berg and Link (1 ♀, USNM).BRITISH VIRGIN ISLANDS: Guana Island, 0–80m, 13–26.vii.1986, S.A. Miller and M.G. Pogue (1 ♀, USNM). JAMAICA: (two ♂, no further data, USNM); Try, Duncans, 23.viii.1966, Howden and Becker (one ♀, CNCI). PUERTO RICO: on human manure, August Busk (3 ♂, 2 ♀, USNM); San Juan, 10.vii, 19.x and 26.vi.1953, B.B. Sugarman (4 ♂, USNM); finca Ferguson, nr Mt. El Yunque, 4.iv.1972, L. Knutson (1 ♂, USNM). SABA: Booby Hill, 22.iii.1968, R.S. Miller (1 ♀, MTEC). SAINT BARTHELEMY: 29.vii.1981, R.S. Miller (1 ♂, MTEC). SAINT MARTIN: Paradise Peak,11.ii.1978, S.A. Marshall (one ♀ without an abdomen, CNCI, labeled "*H. fabricii* det Albuquerque" and "female 14". This is presumably the specimen used to illustrate *H. fabricii* female terminalia in Albuquerque (1986), returned to CNC without an abdomen). U. S. VIRGIN ISLANDS: Saint Thomas. 25.xi.1966, Tallia-Muncie (1 ♂, USNM); intercepted on plane from St. Kitts, 11.x.1962 (1 ♂, USNM); Frenchman Bay Estate, 01 and 25.v.1978, 09.ix.1978, 10 and 12.x.1978, M. Ivie (3 ♀, MTEC); College of the V.I., M. Ivie (1 ♀, MTEC), Buck Island Reef N.M., FIT, 340', Z. Hillis (1 ♀, MTEC). TORTOLA: 18–19.viii.1982, R.S. Miller (1 ♀, MTEC). SAINT CROIX: 9.iv.1995, E. McCord, 75-6587 (1 ♀, USNM). UNITED STATES: Florida, Collier Co., Seminole State Park, 28.xii.1978, S.A. Marshall (1 ♂, DEBU); Miami via San Juan, PR and Camaguey, Cuba, ex plane, 14.ii.1946 (1 ♂, USNM); Royal Palm Park, 22.iv.1930, 28.iv.1930, A.L. Melander (1 ♀, 1 ♂ USNM).

Comments. *Hoplocheiloma totliana* is apparently widespread in the Caribbean basin, probably secondarily so because of synanthropic habits (it is associated with human dung and has twice been intercepted in airplanes). The orange body combined with the distinctive wing venation (a fat discal band between similar narrow stigmatal and preapical bands) render it distinctive despite considerable variation in size and thoracic chaetotaxy. Hennig (1935) recorded this species (as *H. fasciata*) from Florida, Cuba, Jamaica and Brazil, with the latter record based on one specimen from the Winthem collection (Vienna). I have been unable to relocate that specimen and all recent records of *H. totliana* are Caribbean.

Species excluded from *Hoplocheiloma*

Hoplocheiloma macropyga (Frey)

Hennig treated *Gymnosphen* Frey, a monotypic genus erected for *G. macropyga* Frey, as a synonym of *Hoplocheiloma* because he considered the generic descriptions used by Frey for *Gymnosphen* and by Cresson for *Hoplocheiloma* to be the same. In fact, the only reliable diagnostic feature for the genus (the clypeal setae) is not mentioned in Frey's description of *Gymnosphen*, and Hennig apparently did not see the type of Frey's species. He provides neither diagnosis nor comments on the species, merely citing Frey and apparently including the species in his key to species on the basis of Frey's description.

Gymnosphen macropyga was described on the basis of a unique specimen from Brazil, deposited in the Finnish Natural History Museum, Helsinki. Photographs of the holotype, kindly provided by Dr. G. Ståhls, show very clearly that the clypeus lacks setae. This species is provisionally moved to *Calosphen* pending review of the *Rainieria/Calosphen* group of genera.

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References

Aczél, M.L. (1951) Morfología externa y división sistemática de las "Tanypezidiformes" con sinopsis de las especies Argentinas de "Tylidae" ("Micropezidae") y "Neriidae" (Dipt.). *Acta Zoologica Lilloana*, 11. 483–589, pls. I–4.

Albuquerque, L. Paes de (1986) *Sistematica das Taeniapterinae da Regiao Neotropical (Diptera – Micropezidae)* PhD thesis. Universidade Federal do Parana, Brazil.

Cresson, E.T. Jr. (1930) Notes on and descriptions of some neotropical Neriidae and Micropezidae (Diptera). *Transactions of the American Entomological Society*, 56, 307–362.

Gmelin, J.F. (1790) Caroli a Linne. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species; cum caracteribus, differentiis, synonymis, locis. Editio decima tertia, aucta, reformata [= Ed. 13.] Vol. 1: Regnum Animale, Pt 5, Pp. 2225–3020.

Hennig, W. (1935) Revision der Tyliden (Dipt., Acalypt.). I. Teil: Die Taeniapterinae Amerikas [concl.]. *Stettiner Entomologische Zeitung*, 96, 27–67.

McAlpine, D. (1998) Review of the Australian stilt flies (Diptera: Micropezidae) with a phylogenetic analysis of the family.