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A novel colour morph of *Sphaerodactylus sabanus* Cochran 1938 from Sint Eustatius

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Abstract. Reptiles often display substantial intraspecific variation in dorsal colouration and pattern. Such variation is often, but not always, genetically or geographically structured. In particular, the *Sphaerodactylus* geckos of the Caribbean display considerable intraspecific variation in dorsal patterning. Here, we describe a previously unreported colour and pattern morph of the Saint Kitts Bank Geckolet, *Sphaerodactylus sabanus*, restricted to the island of Sint Eustatius. We surveyed dorsal patterns throughout the range of *S. sabanus* using both field surveys and museum collections. For field samples, we sequenced the mitochondrial *12S* gene to confirm the identity of each specimen and to examine genetic structuring of pattern morphs. Additionally, we compared the relative ratios of each morph across the four main islands within its range: Saba, Sint Eustatius, Saint Kitts, and Nevis. Although relative abundances of each dorsal pattern varied across islands, we found no evidence of either geographic or genetic structure related to dorsal pattern. Thus, *Sphaerodactylus sabanus* may provide a compelling system to study alternative drivers of intraspecific colour and pattern variation.

Keywords. Caribbean, Lesser Antilles, Reptile, Gecko, Sphaerodactylidae, Intraspecific Variation

Introduction

Native to Central America and the Caribbean, several species of *Sphaerodactylus* exhibit dramatic variation in dorsal colour and patterns throughout their ranges (e.g., King, 1962; Thomas, 1964; Thorpe et al., 2008; Daza et al., 2019). The reason for intraspecific colour and pattern variation within *Sphaerodactylus* is unclear, although it appears to be a widely occurring phenomenon across the region. This variation has previously been the basis for descriptions of subspecies within some Caribbean *Sphaerodactylus*, although pattern variation often does not correspond with mitochondrial genetic structure or geography (Thorpe et al., 2008; Surget-Groba and Thorpe, 2012; but see Daza et al., 2019).

Sphaerodactylus sabanus Cochran 1938 is native to the islands of the Saint Kitts Bank (Sint Eustatius, Saint Kitts, and Nevis) and Saba. Formal descriptions of *S. sabanus* (Cochran, 1938; King, 1962; Schwartz and Henderson, 1991; Malhotra and Thorpe, 1999; Powell et al., 2005) have characterised variation in dorsal colour as generally light to dark brown, with some individuals displaying orange head colouration especially when patterned. Dorsal patterning within *S. sabanus* can vary from dorsal spots or lines to uniform colour. Head patterning similarly varies from spots and lines to no pattern, but also vermiculation. When present, patterning generally occurs across the length of the dorsum, though pattern configuration may gradually shift between the head, trunk, and tail (Fig. 1A). This extensive variation in dorsal patterning does not correlate with geography and all forms are present throughout the range. *Sphaerodactylus sabanus* does display some geographically structured variation in eye colouration with individuals from Saba having brown irises and those on the Saint Kitts Bank having black irises with yellow-golden pupillary rings.

In January 2019, we collected an individual *S. sabanus* displaying a pattern and colouration which

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to our knowledge is previously undescribed within the literature. However, individuals displaying this pattern appear to be present in the commercial pet trade (personal observation). The specimen collected from The Quill (17.47887°N, 62.96839°W; MVZ: HERP:292734) on Sint Eustatius had off-white and dark brown vermiculation on the head extending fully through the throat. The head pattern abruptly transitioned to a uniformly coloured trunk at the neck (Fig. 1B, C). The dorsum was uniform dull yellow and the venter was uniform white. Previous descriptions have noted vermiculation in specimens of *S. sabanus,* however, formally described specimens maintain patterning throughout the body rather than an abrupt transition to uniform yellow trunk colouration. Besides colour and pattern, this individual retained all other diagnostic traits of *S. sabanus* (28 mm snout-vent length [SVL], 27 pre-sacral vertebrae, keeled throat scales, and black iris with yellow pupillary ring). Here, we described this novel colour morph of *S. sabanus* and quantify variation in dorsal patterning throughout their range.

Materials and Methods

Field surveys.—In January 2019, we *ad hoc* surveyed three islands within the range of *S. sabanus* and captured 13 individuals: Sint Eustatius (N = 7), Saint Kitts (N = 1), and Nevis (N = 5) (Fig. 2). Our surveys included three of the four major islands within the range of *S. sabanus* only excluding Saba. We opportunistically sampled individuals throughout the islands and recorded their dorsal pattern as either patterned throughout ('patterned'), vermiculate head and uniform body ('vermiculate-head'), or uniform colouration ('uniform'). We classified uniform individuals as those with an orange or brown dorsal wash and lacking any pattern. By contrast, patterned individuals were those that displayed any patterning on the trunk and tail irrespective of head patterning. Although dorsal pattern variation is highly variable (King, 1962), we did not differentiate between various dorsal patterns (i.e. spots, stripes, mottling, and vermiculation) when classifying animals as patterned. Both adult and juvenile animals were included because dorsal pattern is consistent

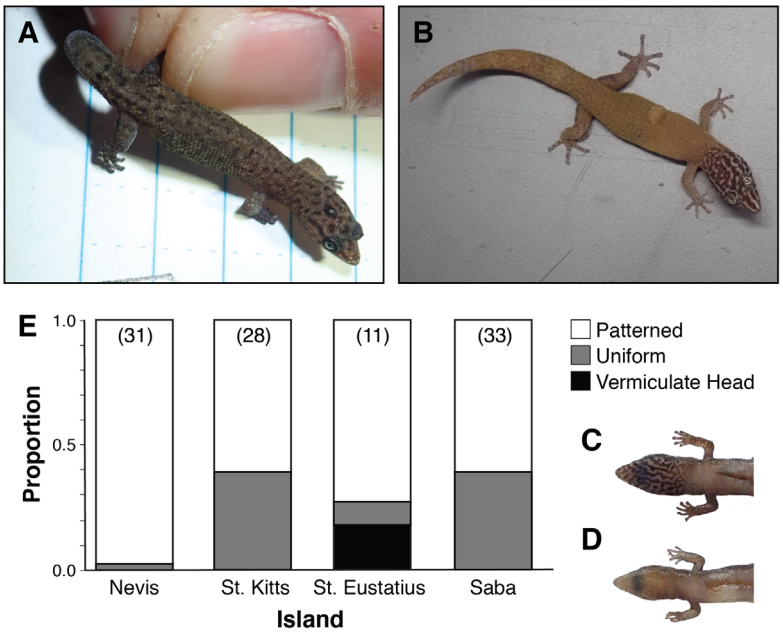

Figure 1. (A) Representative fully patterned individual from Sint Eustatius in dorsal view. (B) Dorsal photograph of live specimen MVZ:HERP:292734 described in this manuscript displaying the vermiculate head-uniform body phenotype. (C) Ventral throat photograph of a vermiculate head-uniform body individual. Vermiculation extends fully into the throat. (D) Ventral throat photograph of a fully patterned individual displaying uniform throat coloration. (E) Proportion of sampled individuals from each island in each pattern classification: patterned throughout, uniform throughout, or vermiculate head-uniform body. Sample sizes are in parentheses and include both museum and field surveys.

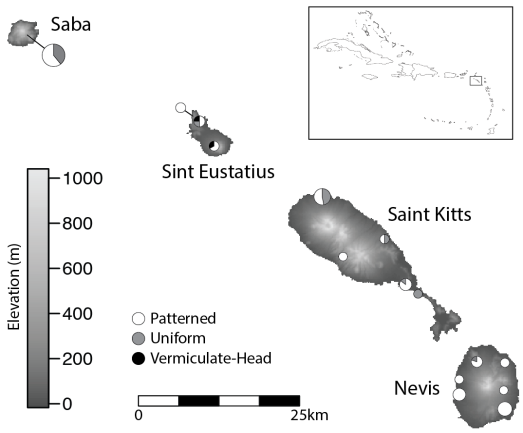

Figure 2. Sampling map for individuals examined in this study from the Lesser Antillean islands of Saba, Sint Eustatius, Saint Kitts, and Nevis including both field collected and museum specimens. Circle charts represent the relative proportion of each pattern classification from a given sampling locality and are scaled to sample size.

between life stages and we did not differentiate between sexes due to lack of sexual dichromatism (Schwartz and Henderson, 1991). A subset of voucher specimens from our field surveys are housed either at the UC Berkeley Museum of Vertebrate Zoology or were donated to the Ministry of Agriculture, Marine Resources, Cooperatives, Environment and Human Settlement, Government of Saint Kitts and Nevis. To confirm the species identity of the vermiculate-head individual, we x-rayed the field-collected specimen and counted the number of pre-sacral vertebrae, a diagnostic trait in *Sphaerodactylus* geckos (King, 1962).

Museum surveys.—To improve our sampling, we examined museum specimens of *S. sabanus* collected from all four major islands within its range: Saba (N = 33), Sint Eustatius (N = 4), Saint Kitts (N = 27), and Nevis (N = 26). All examined materials were housed at either the Smithsonian Institution's National Museum of Natural History (Washington, DC) or the California Academy of Sciences (San Francisco, CA). Non-melanic colour is generally lost in formalin preserved specimens, thus, we scored individuals only by their dorsal pattern without respect to colouration. As with field surveys we included both adult and juvenile individuals from both sexes. To test if the relative proportions of each dorsal pattern differed across islands, we performed a chi-squared test of independence across our combined field and museum surveys.

Genetic analysis.—Recent work has shown that some instances of colour polymorphism in *Sphaerodactylus* species actually represent multiple distinct species (Daza et al., 2019). To confirm the identity of *Sphaerodactylus sabanus* we collected during our field surveys, we sequenced *12S* ribosomal RNA (*12S*) for 12 individuals. One individual from Sint Eustatius could not be sequenced. As outgroups, we included an individual *Sphaerodactylus fantasticus* collected from Montserrat and *S. sputator* collected from Sint Eustatius. Sequences are accessioned under GenBank MT683095-68310. In brief, we polymerase chain reaction (PCR) amplified a 375 base pair (bp) fragment of mitochondrial 12S ribosomal RNA using primers H1478 (5'-TGACTGCAGAGGGTGACGGGCGGTGT GT-3') and L1091 (5'-AAAAAGCTTCAAACTGG GATTAGATACCCC-ACTAT-3') (Kocher et al., 1989). PCR conditions included: denaturation at 94 °C – 2 min, 35 cycles (denaturation at 94 °C – 45 s, annealing at 53 °C – 30 s, extension at 72 °C – 1 min), and a final extension at 72 °C for 1 min. We subsequently purified amplicons using ExoSAP-IT (Applied Biosystems) and conducted cycle sequencing using our amplifying primers and BigDye v 3.1. We then precipitated the cycle sequence products with ethanol (125 mM EDTA precipitation) and ran the samples on an ABI 3730 automated DNA sequencer (Applied Biosystems). Sequences were edited using Geneious 9.1.8 (Biomatters) and aligned using the MUSCLE (Edgar, 2004) algorithm.

We constructed a *12S* gene tree in BEAST2 (Bouckaert et al., 2014) running two MCMC chains for 10,000,000 generations and sampling every 1,000 generations discarding 10 percent of trees as burn-in. We determined HKY+Γ as the best fit model of evolution for *12S* using the Bayesian information criterion (BIC) in JModelTest2 (Darriba et al., 2012). To assess convergence, we visually examined likelihood traces and effective sample sizes (ESS) of parameters in Tracer v1.6.0 (Rambaut et al., 2014).

Results and Discussion

Description.—The live specimen collected from The Quill on Sint Eustatius (MVZ:HERP:292734) had off-white and dark brown vermiculation on the head extending fully through the throat. The head pattern abruptly transitioned to a uniformly-coloured trunk at the neck. Below the neck, the dorsum was uniform dull yellow and the venter was uniform white (Fig. 1B,

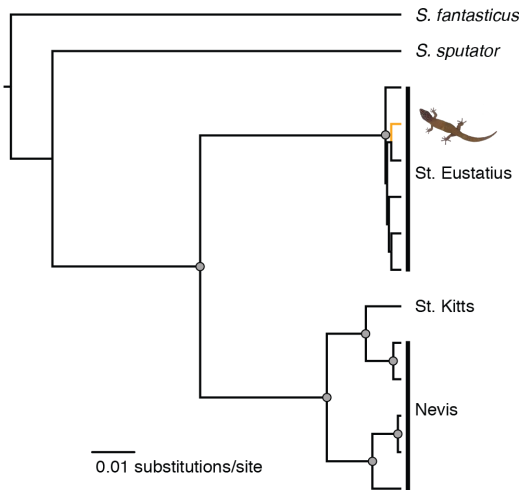

Figure 3. 12S mitochondrial phylogeny of field collected *S. sabanus* from Sint Eustatius, Saint Kitts, and Nevis. We did not collect genetic samples for individuals from Saba. Outgroup sequences of *S. fantasticus* and *S. sputator* are included. Nodes with posterior probabilities greater than 0.95 denoted by grey circles. Scale shown in substitutions per site. Individual (MVZ: HERP:292734) displaying the novel vermiculate-head pattern described here denoted in orange and by image.

C). Besides colour and pattern, this individual retained all other diagnostic traits including 28 mm SVL, 27 pre-sacral vertebrae, keeled throat scales, and black iris with yellow peripheral ring. Our *12S* phylogeny confirmed that the described individual is a *S. sabanus* nested within other individuals sampled from Sint Eustatius (Fig. 3) and shared a haplotype with a fully patterned individual collected from The Quill. In total, we recovered 36 segregating sites across *S. sabanus 12S* sequences.

The only other individual we examined from existing museum collections that matched the vermiculate head-uniform body pattern was an adult collected from Sint Eustatius in 1987 (Venus Bay: USNM 509287). Venus Bay is located in Boven National Park on the northern end of Sint Eustatius, whereas our field-collected individual was collected at The Quill National Park on the southern end of the island separated by approximately 4.8 km (Fig. 2). The vermiculate-head phenotype represents 18% or 2 out of the total 11 individuals observed from Sint Eustatius (Table 1). Although we lack sampling from the developed central region of the island, the disjointed phenotype is likely distributed throughout Sint Eustatius. No individuals from Saint Kitts (N = 28), Nevis (N = 31), or Saba (N = 33) exhibited the vermiculate-head phenotype suggesting that its occurrence is restricted to the island of Saint Eustatius. While it is possible that this pattern occurs on Saint Kitts, Nevis and Saba at low frequencies but was not detected, we surveyed approximately 2-3 times more individuals on each of Saint Kitts, Nevis, and Saba compared to Sint Eustatius. Thus, our data suggest that this morph is either exceptionally rare or absent on the other islands. Geographic restriction of phenotypes is common in *Sphaerodactylus* geckos, although such traits are often fixed for a given locality unlike what we observed with dorsal colouration in *S. sabanus*. For example, *S. fantasticus* exhibit geographically structured dorsal colouration (Thorpe et al., 2008) and iris colouration phenotypes are sorted by islands in *S. sabanus* with brown irises restricted to Saba (Schwartz and Henderson, 1991; Powell et al., 2005).

Despite variable dorsal patterning across fully patterned individuals, to our knowledge, there are no references in the literature of head patterning extending to the throat even when vermiculation is present. In contrast to previous descriptions of *S. sabanus*, which noted only that the throat was uniform white or yellow in all cases (Cochran, 1938; King, 1962; Schwartz and Henderson, 1991; Powell et al., 2005), we observed some vermiculate pattern extending into the throat of all individuals with vermiculate heads irrespective of trunk colour or pattern. Still, this throat patterning is less defined in fully patterned compared to vermiculate-uniform individuals (Fig. 1C, D). Additionally, throat patterning gradually transitions to a uniform venter in individuals with full dorsal patterning. Full extension of head patterning to the throat and abrupt transition to a uniform venter was observed only in vermiculate-uniform individuals. A similar vermiculate head phenotype that abruptly transitions to separate trunk

Table 1. Proportion of sampled individuals from each island (N) classified into three pattern categories: vermiculate head-uniform body, uniform colouration throughout, and patterned throughout.

Island	N	Vermiculate-Head	Uniform	Patterned
Saba	33	0	0.39	0.61
Sint Eustatius	11	0.18	0.09	0.73
Saint Kitts	28	0	0.39	0.61
Nevis	31	0	0.03	0.97

patterning has been observed in other sphaerodactylid geckos including constitutively in *Sphaerodactylus macrolepis* (Daza et al., 2019) and polymorphically in *S. fantasticus* (King, 1962; Thomas, 1964), *Gonatodes rozei* (Rivero-Blanco and Schargel, 2012), and *Gonatodes ocellatus* (Murphy, 2018).

Whereas the vermiculate-head phenotype was observed only on Sint Eustatius, the uniform colour phenotype occurs on every island. Uniform individuals are more common on St. Kitts and Saba making up 39% of all surveyed individuals on both islands compared to 3% on Nevis and 9% on Saint Eustatius (Table 1; Fig. 1E). Although there are differences in the relative proportions of different dorsal pattern phenotypes across islands (chi-squared test of independence: $\chi^2 = 32.31$, $d.f. = 6$, $P < 0.001$), these phenotypes did not correspond to genetically distinct mitochondrial lineages (Fig. 3). Gene flow between colour morphs is common in *Sphaerodactylus* geckos. Even in *Sphaerodactylus* species in which colour morphs are geographically structured, the underlying genetic structure is often not congruent with phenotypic structure (Thorpe et al., 2008; Surget-Groba and Thorpe, 2012). Thus, we propose that *Sphaerodactylus* geckos are a compelling study system for investigating the evolution of intraspecific pattern and colour variation.

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