

Rapid Communication**First records of three new lizard species and a range expansion of a fourth lizard species introduced to Aruba**

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OPEN ACCESS**Abstract**

The Caribbean islands are becoming a hotspot for the spread of non-native reptiles. Consistent with this trend, we provide the first documentation of three new lizard species discovered on Aruba, *Anolis gingivinus* (Cope, 1864), *Anolis cristatellus* (Duméril and Bibron, 1837), and *Hemidactylus frenatus* (Duméril and Bibron, 1836). In addition, we provide an updated distribution on Aruba for a previously introduced lizard species, *Anolis porcatatus* (Gray, 1840). All four species were identified phenotypically in the field and identifications were confirmed with genetics. Like most non-native lizards in the Caribbean, they tend to use anthropogenic habitats, and their impacts on Aruba's resident species are not known.

Key words: Anole, *Anolis cristatellus*, *Anolis gingivinus*, *Anolis porcatatus*, Caribbean, gecko, *Hemidactylus frenatus*

Introduction

The Caribbean region is emerging as a hotspot for the spread of non-native reptile species (Powell et al. 2011; Powell and Henderson 2012). Two lizard groups, anoles (species from the genus *Anolis*) and geckos (species from the infraorder Gekkota), are among the most prominent of introduced reptiles on Caribbean islands (Helmus et al. 2014; Perella and Behm 2020). Anole and gecko species are frequently introduced accidentally through the live plant trade, but may also be introduced intentionally as pets (Kraus 2009). The Caribbean region is also a reptile biodiversity hotspot and most islands have unique endemic anole and gecko species (Myers et al. 2000; Hedges 2011). Therefore, identifying newly introduced gecko and anole species, including their introduction pathways and ecological impacts, is a conservation priority.

Aruba has 10 previously recorded introductions of non-native reptile species, including two anoles, *Anolis sagrei* (Duméril & Bibron, 1837) and *A. porcatatus* (Gray, 1840), and four geckos, *Gonatodes albogularis* (Duméril & Bibron, 1836), *G. antillensis* (Lidth de Jeude, 1887), *G. vittatus* (Lichtenstein & Martens, 1856), and *Hemidactylus mabouia* (Moreau de Jonnés 1818),

making it one of the most invaded islands in the Caribbean (van Buurt 2005, 2011). Aruba is also one of the most economically connected islands in the region which likely explains the high rate of introductions (Powell et al. 2011; Helmus et al. 2014). Interestingly, unlike most Caribbean islands, the natural habitat in Aruba is quite arid and may be difficult for species to invade if they are not adapted to those conditions. Many of the non-native species may be using anthropogenic habitat that receives irrigation subsidies.

Given the high rate of introductions and harsh natural conditions, we searched anthropogenic habitat on Aruba for non-native reptile species. Here, we report the first observations of two new non-native anole and one non-native gecko species on Aruba. In addition, we report a range expansion of a non-native anole species that was previously observed at only one location on Aruba. To better understand the context and potential impacts of the four introduced species we documented on Aruba, we compiled information from the literature regarding the timing, introduction pathway, and ecological impacts of those four species in their introduced ranges within the Caribbean region.

Materials and methods

Our methods were aimed at finding, recording, and confirming the identity of new introduced reptile species on Aruba. We conducted a survey of Aruba on December 30, 2018 and January 7–9, 2019 which targeted habitats with a high likelihood of having new non-native species based on previous discoveries (e.g., resort gardens; Odum and Van Buurt 2009). Introduced lizards that were sighted during surveys were captured by hand and/or photographed when possible. For most captured individuals, sex, age, SVL, and mass were recorded, a photograph was taken, and a non-lethal tissue sample (tail tip) was collected for DNA analyses. All samples were assigned an identification code (Sample ID) unique to this study (Supplementary material Table S1). Gecko species were found during evening surveys conducted between 19:00 and 23:00 h, and anole species were found during daytime surveys conducted between 8:00 and 18:00 h.

To confirm the identity of the species found, we sequenced a 231–945 bp region of the mitochondrial cytochrome oxidase *b* or a 441 bp region of the cytochrome oxidase I gene from the tissue samples we collected in the field. Total genomic DNA was extracted using a Chelex extraction protocol (Estoup et al. 1996). DNA amplification of the cytochrome oxidase *b* gene was accomplished using either the primers cytbS1L:F (5' GAAAAACCGC YRTTGTWWTTCAACTA 3') and cytbH15:R (5' ACTGGTTGDCCYCCR ATYCAKGTKAG 3') (Adalsteinsson et al. 2009; McCranie et al. 2020), and/or rGlu-1L:F (5' GAAAAACCRCCGTTGTWATTCAACTA 3') and rPro-1H3:R (5' TWAAAATKCTAGTTTTGGG 3') (Kumazawa and Endo 2004; Surget-Groba and Thorpe 2013). Amplification of the cytochrome oxidase I gene was accomplished with primer pair LCO1490 (5' GGTC

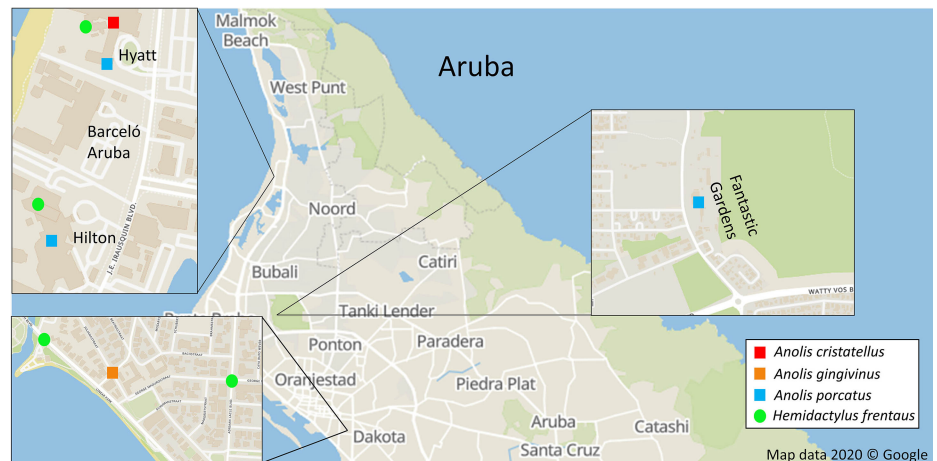


Figure 1. Locations where four introduced lizard species were documented in this study. Northern portion of Aruba showing new locations (enlarged insets) where *Anolis cristatellus*, *A. gingivinus*, *A. porcatus* and *Hemidactylus frenatus* were documented. Exact residential locations where *A. gingivinus* and *H. frenatus* were found are masked for privacy. *A. porcatus* at Fantastico Gardens was confirmed by phenotypic similarity only, not genetics.

CAAATCATAAAGATATTGG 3') and HCO2198 (5' TAAACTTCAGGG TGACCAAAAATCA 3') (Folmer et al. 1994). PCR was performed in volumes of 25 μ L containing 19.2 μ L dH₂O, 2.5 μ L 10X Taq DNA polymerase buffer A (Fisher Scientific), 1 μ L 2.5 mM dNTPs, 0.5 μ L of each 50 μ M primer, 0.3 μ L 5U/mL Taq DNA polymerase, and 1 μ L DNA. When samples had low concentration, up to 5 μ L of template DNA was added, and dH₂O volume was adjusted to keep the total PCR volume at 25 μ L. For our cytochrome oxidase b primers, PCR conditions were 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 48 °C for 30 s, 72 °C for 30 s and a final extension of 72 °C for 5 min. For the cytochrome oxidase I primers, PCR conditions were 4 °C for 1 min, 5 cycles at 94 °C for 40 s, 45 °C for 40 s, 72 °C for 1 min, followed by 35 cycles at 94 °C for 40 s, 51 °C for 40 s, 72 °C for 1 min, and a final hold at 72 °C for 5 minutes (Hebert et al. 2013). DNA sequencing was performed by Genewiz (South Plainfield, NJ). The sequences we obtained were compared to the GenBank nucleotide database using BLAST to confirm species identities.

Results and discussion

Anolis cristatellus

On January 7–8, 2019, we conducted a survey of the Hyatt Regency gardens (Noord, Aruba) (Figure 1) and discovered an anole species that differed phenotypically from the Aruba-native, *Anolis lineatus* (Daudin, 1802). Larger individuals (presumably males) had large, bright yellow dewlaps sometimes with orange margins (Figure 2A, B) and had mottled brown dorsal coloration (Figure 2D). Smaller individuals (presumably females and juveniles) had smaller orange-yellow dewlaps (Figure 2C) and their dorsal coloration was mottled brown with a light dorsal stripe (Figure 2E). We recorded 23 individuals of this new anole species across the front (street-side)

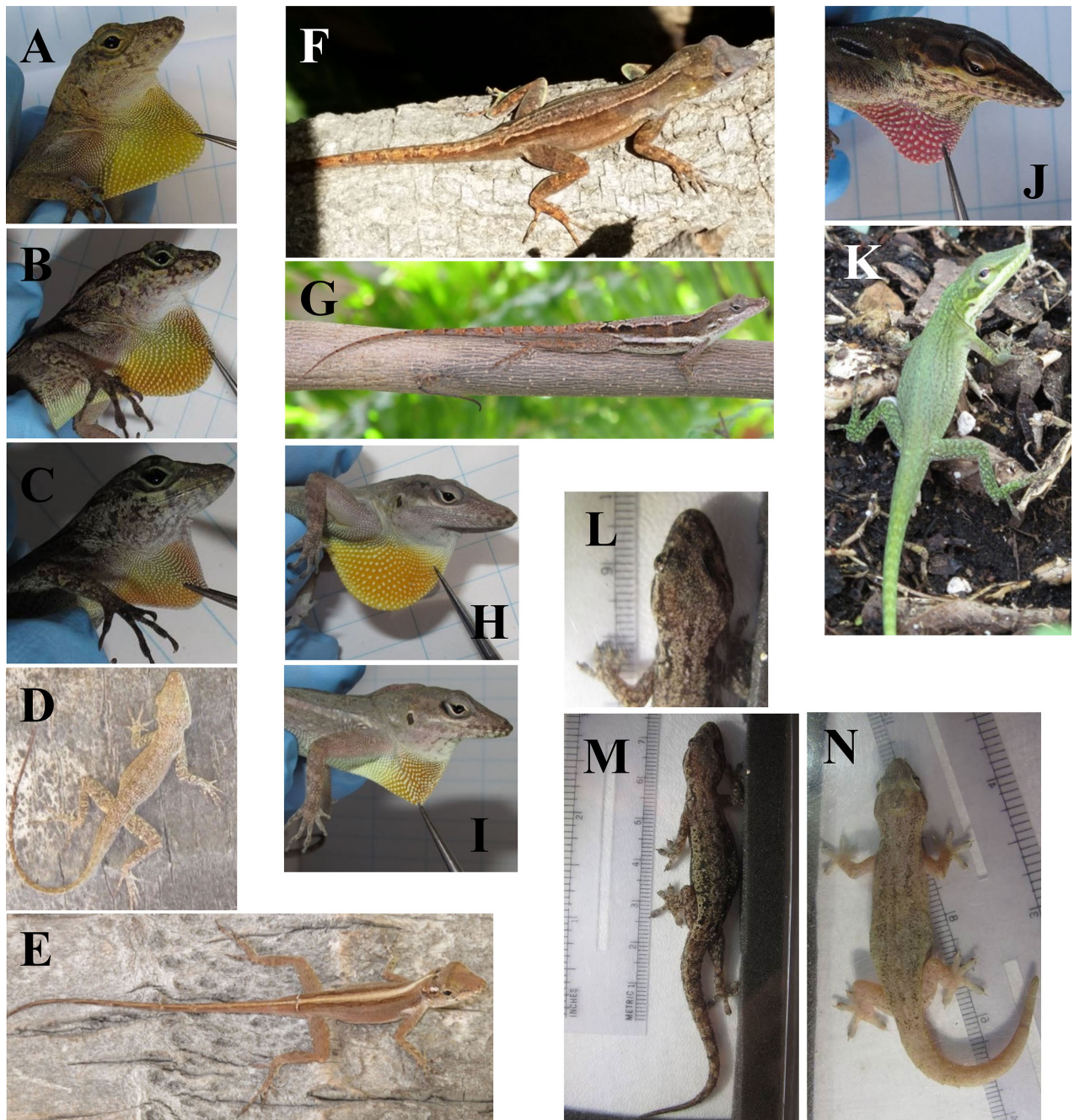


Figure 2. Photos of four introduced lizard species documented on Aruba. A. Male *Anolis cristatellus* (ANOL-AW-19-01, Table S1) with bright yellow dewlap; B. Male *A. cristatellus* (ANOL-AW-19-02) with dewlap similar to 2A but with orange and yellow coloration; C. Female *A. cristatellus* (ANOL-AW-19-03) with small dewlap; D. *A. cristatellus*, likely male with mottled brown dorsal pattern; E. *A. cristatellus*, likely female with mottled brown pattern and a dorsal light-colored stripe; All *A. cristatellus* individuals (A–E) were found in vegetation in the Hyatt garden; F. *A. gingivinus* with grey dorsal stripe continuing into chevrons on the tail; G. *A. gingivinus* with light-colored mid dorsal stripe; H. Male *A. gingivinus* (ANOL-AW-19-10) with large dewlap; I. *A. gingivinus* (ANOL-AW-19-06), likely female, with small dewlap; All *A. gingivinus* individuals (F–I) were found in residential gardens; J. *A. porcatus* (ANOL-AW-19-11) with brown dorsal pattern and pinkish dewlap, found in the front garden of the Hyatt; K. *A. porcatus* (ANOL-AW-19-12) with green dorsal pattern found at Fantastic Gardens (photo: J.E. Behm); L. and M. *H. frenatus* (HEFR-AW-18-03) with dark dorsal pattern and missing its right eye (enlarged in L.); N. *H. frenatus* (HEFR-AW-18-02) with light dorsal pattern. All photos taken by M.R. Helmus unless otherwise noted. Exact photo locality information is in Table S1.

and back (beach-side) gardens of the Hyatt. Most individuals were perched on tree trunks or thinner stems of mixed woody and herbaceous vegetation. We collected dewlap photographs, length, weight, and tissue

Anolis cristatellus

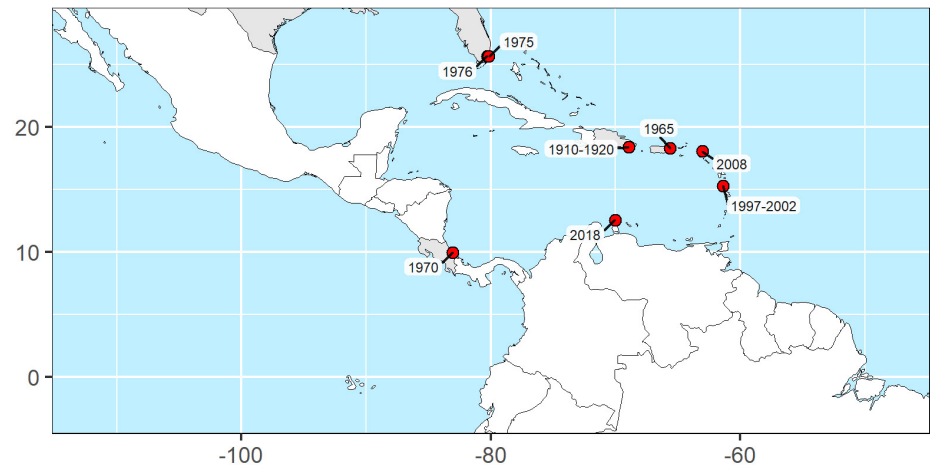


Figure 3. Spread of *Anolis cristatellus* across the greater Caribbean region. Time-series of locations where *A. cristatellus* has been documented based on the earliest likely years of introductions. For the Dominican Republic and Dominica, only a range of dates pinpointing the introduction year is available. See Table S2 for the full list of location names and coordinates.

samples from two large (presumably male) and three small (presumably females or juveniles) individuals (Table S1).

Checking species identities using BLAST confirmed all 5 individuals sampled were *A. cristatellus* (Duméril and Bibron, 1837) (percent similarities to best matches in GenBank: 95.88%–98.79%, Table S1). We used DnaSP 6.0 (Rozas et al. 2017), to identify mitochondrial haplotypes as a coarse indication of the founding population of individuals. In particular, if all individuals sequenced shared the same haplotype, it may indicate a single, small founding event. Across our 5 individuals, we identified three mitochondrial haplotypes (486 bp). Three individuals, ANOL-AW-19-01, ANOL-AW-19-04 and ANOL-AW-19-05 shared the same haplotype, and the remaining two individuals had unique haplotypes. Total nucleotide divergence among haplotypes ranged from $K = 0.03498$ to $K = 0.05967$. The presence of several haplotypes may indicate multiple introductions of *A. cristatellus* to the Hyatt or a single introduction of a genetically variable population. No *A. cristatellus* individuals were spotted on the publicly accessible properties neighboring the front and back gardens of the Hyatt. Given the relatively large size of this established population, we are confident that *A. cristatellus* has been at the Hyatt since at least 2018. In addition, because its distribution appears to be restricted to the resort garden, we suspect it was likely introduced accidentally with imported landscaping materials for the garden as suggested for other introduced anole species in Aruba (Odum and Van Buurt 2009).

Anolis cristatellus is native to the Puerto Rican bank including the island of Puerto Rico and some of the cays surrounding Puerto Rico (Powell and Henderson 2012). *Anolis cristatellus* is spreading across the Caribbean region with seven introductions across mainland and island locations (Figure 3, Table S2). Its first reported island introduction was to the

Dominican Republic at some point between 1910 and 1920 likely during the building of a sugar mill (Williams 1977). It has more recently been reported on Dominica in 2002 (likely introduced between 1997 and 2002) (Malhotra et al. 2011) and St. Martin in 2008 (Breuil et al. 2009). In these island populations, *A. cristatellus* appears to be established in anthropogenic habitats such as hotel gardens, parks, and industrial complexes (Fitch et al. 1989; Breuil et al. 2009; Malhotra et al. 2011). In addition, there is evidence indicating it can be competitively dominant to other established anoles in its introduced range to the point of likely displacing the native *A. oculatus* (Cope, 1879) in Dominica (Daniells et al. 2008; Dufour et al. 2018) and *A. cybotes* (Cope, 1862) in the Dominican Republic (Fitch et al. 1989). In Florida, established populations of *A. cristatellus* are due to the pet trade (Krysko et al. 2011), while introductions to the Dominican Republic and Dominica were likely unintentional with cargo (Williams 1977; Malhotra et al. 2011).

Anolis gingivinus

On December 30, 2018, we observed a second new anole species in a residential area of Oranjestad (Figure 1) and conducted a survey on January 8, 2019, and confirmed it differed phenotypically from *A. lineatus*. Individuals were olive-brown with a sharp, light-colored mid-dorsal stripe and some individuals also had a pronounced gray dorsal stripe continuing into chevrons on their tails (Figure 2F, G). Larger individuals (presumably males) had large yellow-orange dewlaps (Figure 2H) while smaller individuals (presumably females or juveniles) had smaller, dull-colored dewlaps (Figure 2I). In total we found 34 individuals mostly perched in trees and potted plants across four adjacent private residences with the highest concentration of individuals at a residence with many outdoor potted plants. We collected tissue samples from five individuals: two larger males, and three smaller individuals, presumably females or juveniles. Identifying species using BLAST revealed all five individuals were *Anolis gingivinus* (Cope, 1864) (96.55%–97.67% similarity, Table S1). Our haplotype analysis indicated a single haplotype (291 bp) shared by all five individuals. Given the genetic similarity of the individuals and their restricted geographic distribution, it is likely that this was a single introduction event.

Anolis gingivinus is endemic to the Anguilla and Sombrero island banks in the Caribbean and is found on the larger islands of Sombrero, Anguilla, St. Barthélemy, and St. Martin as well as on most of the smaller surrounding islands and cays. From our literature search, we could not find reports of introduced populations of *A. gingivinus* anywhere globally, indicating this population in Aruba could be the first. In its native range, it appears to tolerate anthropogenic habitat well (Hodge et al. 2011), yet has strong competitive interactions with the ecologically similar congener, *A. wattsi pogus* (Boulenger, 1894), on St. Martin (Pacala and Roughgarden 1982).

Anolis porcatius

On January 8, 2019, we conducted a survey of the Hilton Resort (previously named the Radisson Hotel) garden given that it was reported as the introduction location for *A. porcatius* (Gray, 1840) and *A. sagrei* (Duméril and Bibron, 1837) in 2008 (Odum and Van Buurt 2009; van Buurt and Debrot 2012). We found 14 individuals phenotypically resembling *A. porcatius* in the greener, more irrigated vegetation in the garden, and no individuals resembling *A. sagrei*. No individuals were collected at the Hilton.

During our previous survey of the Hyatt where we found *A. cristatellus*, we found three individuals resembling *A. porcatius* in both the front and back gardens on the south side of the property, which is the side closest to the Hilton. The Hilton and Hyatt are separated by the Barceló Aruba Resort (Figure 1) which we did not search. We collected a tissue sample from one individual, likely a male, with a large, pinkish dewlap (Figure 2J) at the Hyatt and BLAST confirmed it was *A. porcatius* (97.73% similarity, Table S1). On January 9, 2019, we conducted a survey of a plant nursery, Fantastic Gardens (Figure 1), and found three individuals that were phenotypically similar to *A. porcatius*. We took a photograph and measurements of one smaller individual, likely a juvenile (Figure 2K), but genetic analyses confirming its species identity were not possible. The previous known range of *A. porcatius* on Aruba was limited to the Hilton Resort. Our finding at the Hyatt indicates an expansion of its range, possibly due to natural dispersal from the Hilton to the Hyatt given the proximity of the properties. The finding of an individual resembling *A. porcatius* at Fantastic Gardens which is ca. 4 km from the Hilton likely indicates either a second introduction of *A. porcatius*, or a stepping-stone introduction assisted by humans within Aruba.

Anolis porcatius is native to Cuba and has been introduced to Florida and the Dominican Republic within the Caribbean region. In the Dominican Republic, *A. porcatius* was first observed in 1970, but is thought to have been introduced in 1955 during the World's Fair given its high concentration in the area where the fair was held in Santo Domingo (Williams 1977; Powell et al. 1990). In comparison, *A. porcatius* was introduced much earlier to Florida in 1904 likely with cargo (Barbour 1904; Krysko et al. 2011). Both introduced populations of *A. porcatius* appear to use anthropogenic habitat and are found in urban areas (Powell et al. 1990; Kolbe et al. 2007). In Florida, *A. porcatius* hybridizes with native *A. carolinensis* (Voigt, 1832) (Kolbe et al. 2007; Wegener et al. 2019), but other impacts of *A. porcatius* on resident anoles in its invaded range are not well-documented.

Hemidactylus frenatus

On the evening of December 30, 2018, we conducted a survey of the area surrounding Queen Wilhelmina Park in Oranjestad and found one juvenile

and four adult geckos across several locations. We obtained tissue samples from two of the adults and genetic analyses confirmed that they were *Hemidactylus frenatus* (Duméril and Bibron, 1836) (100% similarity, Table S1). The two adults were both males of different color morphs, and the darker one was missing an eye (Figure 2L–N). The two *H. frenatus* sampled shared the same haplotype (889 bp).

On the evening of January 8, 2019, we conducted surveys of the Hilton and Hyatt resort gardens and found an additional three *H. frenatus* at each resort near lights on buildings. We also found several *H. mabouia* (Moreau De Jonnés, 1818) at the Hilton ($n = 3$) and Hyatt ($n > 10$) on buildings in the same areas as *H. frenatus*. No *H. frenatus* individuals were collected during the Hilton or Hyatt surveys. Given its distribution at multiple locations across Aruba, it is difficult to surmise where *H. frenatus* was initially introduced, the introduction pathway, or the year of introduction.

Native to Asia, *H. frenatus* is spreading across the Caribbean region and was recently documented on Curaçao, the island neighboring Aruba to the east (Behm et al. 2019). Because *H. frenatus* phenotypically resembles several other gecko species, such as *H. mabouia* on Aruba, it may go unnoticed when first introduced to new locations, so its actual distribution across the Caribbean may be much greater. In addition, because *H. frenatus* goes unnoticed, its introduction pathway is often unknown, although it is likely transported unintentionally through cargo. It appears to tolerate anthropogenic habitat well and is a dominant competitor against resident gecko species in its introduced range (Perella and Behm 2020).

Caribbean-wide implications

Two of the species we report here, *H. frenatus* and *A. cristatellus*, have sizable non-native ranges within the greater Caribbean region. Because of this, it is difficult to determine the source location of the populations on Aruba. Regardless, these two species appear to be successful colonists and may continue to spread around the Caribbean. In comparison, the population of *A. gingivinus* on Aruba is the only non-native occurrence of the species that has been reported. It was likely introduced from the Anguila bank, possibly from St. Martin if both islands' shared Dutch colonial history indicates continued trade ties, however, other introduction sources are certainly possible. Future work will indicate if the introduction of *A. gingivinus* is a single occurrence or the start of a Caribbean-wide spread, like *A. cristatellus*.

Implications for Aruba

In less than four full days, we documented three new non-native lizards on Aruba. Although we targeted areas with a high likelihood of having introduced species, i.e., large resorts where introductions were previously

documented, and a plant nursery, we also found species in residential areas. This high number of species we found in a short time frame indicates Aruba may benefit from more thorough surveys. While our work revealed these species have established reproductive populations on Aruba, our limited survey does not indicate their full distribution across Aruba. All of these species are exploiting anthropogenic habitat on Aruba, like non-native reptiles elsewhere in the Caribbean (Powell and Henderson 2008; Jesse et al. 2018). Of the species we documented here, we suspect *A. gingivinus* may have the highest likelihood of tolerating the natural habitat conditions based on the conditions of the residential habitat where we found it, but that is pure speculation. More comprehensive surveys are clearly needed to determine the extent of these species' distributions, including whether they use native habitat. During our surveys, we never encountered native gecko or anole species at the same locations where we spotted the newly introduced species. We did record the native anole, *A. lineatus*, at the Hyatt Regency, but they appeared to be relegated to a few palm trees adjacent to the beach and far from the more lush, irrigated gardens where we found *A. cristatellus* and *A. porcatius*. Whether the lack of native species at the locations where we found the introduced species indicates negative competitive interactions between the invaders and native species or an intolerance of developed habitat by the native species remains to be determined. Future work exploring the impact of these non-native lizards on Aruba's ecosystems would be useful. Finally, if reducing the importation of non-native lizard species is a goal for Aruba's government, we suggest thorough inspections of plant imports for lizards and their eggs.

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Authors' contribution

JEB and MRH conceptualized the research, field sampling design and methodology, and collected field data and samples, and secured ethics approval. JEB and GMB processed the samples and conducted the genetic sequence analyses. JEB, GMB, and MRH contributed to draft writing, review, and editing.

Ethics and permits

Authors have complied with institutional and national policies governing the humane and ethical treatment of the study subjects; all samples were collected under the approval of Temple University's Institutional Animal Care and Use Committee (IACUC) protocol number 4614. After consulting with the proper governmental authorities, all research pertaining to this article did not require any research permits. Data regarding these samples are shared in the manuscript.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Sample IDs, sample locations (including GPS coordinates), demographic data, morphological data, and genetic sample information.

Table S2. Locality names, GPS coordinates and years *Anolis cristatellus* was introduced to locations within the greater Caribbean region.

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