

Phyloforensics in Action: Genetic Identity and Island Provenance of an Illegally Trafficked Philippine Monitor Lizard

The development of molecular tools for assessing relatedness among individuals, populations, and species has led to novel inferences across these hierarchical groupings. In addition to the empirical study of natural populations, such tools have become increasingly useful for regulating global wildlife trade and combating the illegal trafficking of wildlife and its products (e.g., Gentile et al. 2013; Ledoux et al. 2016; Brandis et al. 2018). Rapidly-evolving mitochondrial DNA sequences have been widely employed to identify traded species (Palsbøll et al. 2006; Rosen and Smith 2010), highlight populations under threat of over-harvesting (Wasser et al. 2008; Welton et al. 2013), identify putative new species (Grismer and Grismer 2010; Siler et al. 2014; Welton et al. 2014), and guide population assessments of heavily trafficked species (Cerling et al. 2016). The monetary cost of these methods continues to decline, making them more broadly accessible to the conservation community, especially in developing or emerging countries where endemic biodiversity faces some of the most severe pressure from illegal harvesting (Murray-Dickinson et al. 2017).

Monitor lizards (*Varanus* spp.) are traded globally for their skin and meat, as well as for the pet trade (Shine et al. 1996; Shine and Harlow 1998; Fa et al. 2000; Stuart 2004; Fidenci and Maran 2009; Pernetta 2009; Welton et al. 2012, 2013; Koch et al. 2013). All species in the genus are recognized by the Convention on the International Trade in Endangered Species (CITES), and are listed in appendices I or II (The CITES Appendices 2018). The Philippine archipelago is home to 12 species of *Varanus* (but see Bayless and Adragna 1997; Grismer 2011; Welton et al. 2013), with the three frugivorous species in the subgenus *Philippinosaurus* (*V. bitatawa*, *V. mabintang*, and *V. olivaceus*) and the eight species in the subgenus *Sotosaurus* (*V. bangonorum*, *V. cumingi*, *V. dalubhasa*, *V. marmoratus*, *V. nuchalis*, *V. palawanensis*, *V. rassmuseni*, *V. samarensis*) listed in CITES Appendix II. The International Union for Conservation of Nature (IUCN) has yet to assess all species of Philippine *Varanus*, but those that have been are classified as Least Concern (*V. cumingi* and *V. marmoratus*), Near Threatened (*V. nuchalis*), Vulnerable (*V. olivaceus*), or Endangered (*V. mabintang*) (IUCN 2019). The sole non-endemic

species is *V. rudicollis*, which has not been documented in the Philippines since its original description (Gray 1845; but see Welton et al. 2013).

Of the endemic Philippine water monitors, *Varanus nuchalis* is perhaps one of the most phenotypically distinct in terms of external morphology. This taxon is characterized by the presence of enlarged dorsal nuchal scales, and has been documented on most of the islands within the Western Visayan Region (Cebu, Guimaras, Negros, Panay, Siquijor) and the Romblon Island Group (Romblon, Sibuyan, Tablas) of the central Philippines, as well as Masbate and Ticao islands of the Bicol Region (Diesmos and Gaulke 2009; Fig. 1, Blue). In addition, biotic surveys of the central Philippines have indicated the presence of highly melanistic phenotypes on a number of islands, resulting in these populations being even more unique (see below) when compared to most Philippine congeners. As noted previously, *V. nuchalis* is included in CITES Appendix II and is considered Near Threatened by the IUCN (2019). However, it must be noted that the most recent assessment for this species was in 2007 (not published until 2009), at which time its populations were deemed to be decreasing, due predominately to over-collecting for domestic and/or international bush meat, skins, and the pet trade (Diesmos and Gaulke 2009). There are presently a number of protected areas scattered throughout the range of *V. nuchalis* (though many of these represent marine reserves), some of which provide ideal habitat for water monitors—mangrove forests and swamps (Gaulke 1992a,b). Notably though, such protected areas are absent from Masbate, Sibuyan and Tablas islands.

In the Philippines, legal protections for *Varanus nuchalis* are afforded under the Wildlife Resources Conservation and Protection Act (Republic Act No. 9417), which prohibits unauthorized collection, and domestic and international trade of Philippine wildlife. In the United States, the unauthorized importation of wildlife is prohibited under the Lacey Act (18 USC §42–43, 16 USC §3371–3378).

Phyloforensic tools have previously been applied to Philippine *Varanus*, inferring the taxonomic identity of animals encountered in black market trade centers in the Philippines, and assessing the validity of sample origins as reported by traders (Welton et al. 2013). Welton et al. (2013) demonstrated, not surprisingly, differential harvest pressure of traded water monitors, with those populations geographically proximate to the capitol city of Manila being more prevalent among trade samples than others, often in contrast to the reported origins of such samples. Additionally, that work first identified the non-monophyly of the “widespread” northern Philippine water monitor, *V. marmoratus*. Subsequent taxonomic work resulted in the recognition of two additional Philippine water monitor species, *V. bangonorum* and *V. dalubhasa* (Welton et al. 2014).

In April of 2017, US Fish and Wildlife Service Special Agents were notified about an in-bound mail package from the Philippines that was intercepted and found to contain live monitor lizards. Upon confiscation, a monitor lizard from the

LUKE J. WELTON

Boston Scientific and BTG International Inc., 615 Arapeen Drive, Suite #105, Salt Lake City, Utah 84124, USA; e-mail: lwelton80@gmail.com

IAN RECCHIO

Los Angeles Zoo and Botanical Gardens, Department of Animal Care/Herpetology; 5333 Zoo Drive, Los Angeles, California 90027, USA

PERRY L. WOOD JR.

Auburn University, Department of Biological Sciences; 101 Rouse Life Sciences, Auburn, Alabama 36849, USA

RAFE M. BROWN

University of Kansas Biodiversity Institute, Division of Herpetology, 1345 Jayhawk Boulevard, Lawrence, Kansas 66045, USA

In collaboration with U.S. Fish & Wildlife Service, Office of Law Enforcement

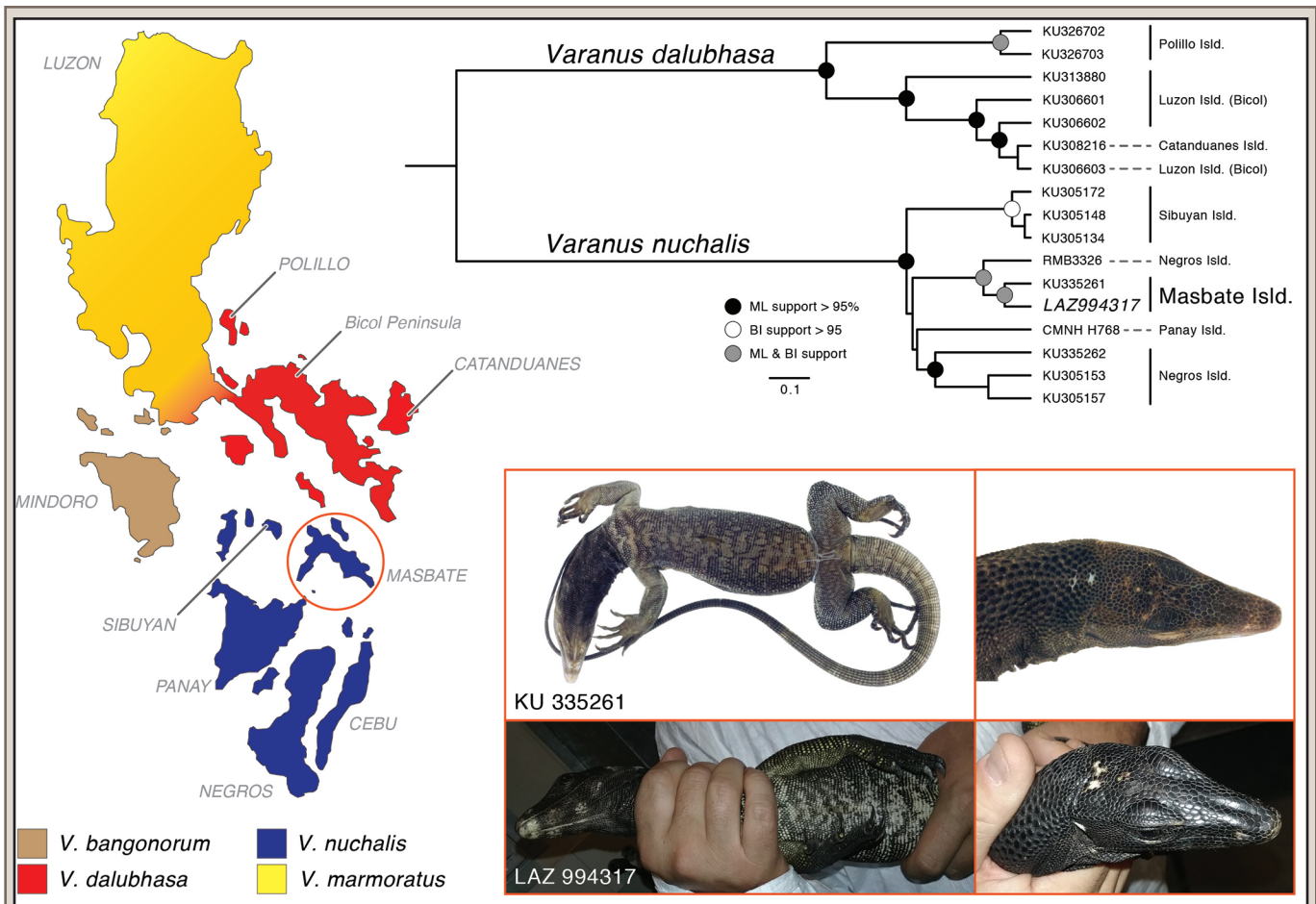


Fig. 1 Map of the northern Philippines with the recognized distributions of Philippine water monitors (left) and the inferred provenance of LAZ 994317 (circled); phylogenetic inference of the identity and relationship of LAZ 994317 to vouchered lineages of water monitors in the central and northern Philippines (top right); and photographic comparison of the phenotype of LAZ 994317 and the only known vouchered specimen from Masbate Island, KU 335261 (bottom right).

package was then transferred for holding to the Los Angeles Zoo (LAZ 994317). One of us (IR) made an initial identification of the specimen as *Varanus nuchalis* on the basis of external morphology. Non-destructive genetic samples were taken from the animal (blood draw and scale clipping) and sent to the Biodiversity Institute at The University of Kansas (KU) for molecular analysis to confirm identification and attempt to identify the specific island of origin. Given the availability of known-locality genetic resources and associated preserved voucher specimens from most of the relevant island populations across the natural range of this species present in natural history collections (Welton et al. 2013), we applied direct comparisons of mtDNA variation in natural populations to that observed in the confiscated specimen.

METHODS

Data collection.—Genomic DNA extractions were generated using blood and scale clippings, via the Promega Maxwell® RSC Tissue DNA Kit and Blood Kit on the Promega Maxwell® RSC extraction robot. Blood was drawn and stored on Watman FTE cards, with subsequent preparation for extraction comprised of isolation of a 1 cm² section of sampled filter card, followed by three consecutive washes of the sample section with 200 µl of FTA Purification Reagent (each wash consisting of sample incubation

at room temperature for 5 minutes), and then lysis following the standard protocol for Maxwell Blood Kits. Scale clippings were taken from dorsal caudal scales, and lysed following the relevant Maxwell protocols, but with an overnight, incubated lysing at 55°C. Extractions were carried out using the Maxwell recommendation and robot presets for each sample type, with post-extraction quantification using a Promega Quantus Fluorometer, following the manufacturer's protocol. Polymerase chain reactions (PCR) were carried out for all extracts following the protocol of Welton et al. (2013), and targeting the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene. Amplified PCR product was then visualized on a 1.0% agarose gel, with viable PCR product being sent to GENEWIZ® for sequencing. Raw sequences were cleaned, and contigs of complimentary strands were generated and then aligned with those from Welton et al. (2013) in Geneious™ version v6.1.8 (Drummond et al. 2011) using the MAFFT v7.017 (Kato and Kuma 2002) plugin under default settings. We calculated the correct amino acid reading frame for the protein-coding region and confirmed the absence of stop codons in Mesquite v3.04 (Maddison and Maddison 2015). Novel sequence data is deposited in GenBank under the accession number MN792994.

Data analysis.—We partitioned ND2 into 1st, 2nd, and 3rd codon positions and treated them as separate partitions to estimate the best-fit models of molecular evolution in IQ-TREE (Nguyen

et al. 2015) using the Bayesian Information Criterion (BIC). We implemented a Maximum Likelihood (ML) phylogenetic analysis using IQ-TREE (Nguyen et al. 2015) and assessed nodal support using 1000 bootstrap pseudoreplicates via the ultrafast approximation algorithm (Minh et al. 2013). All nodes receiving bootstrap values (UF) of ≥ 95 were considered significantly supported (Minh et al. 2013). The Bayesian Inference (BI) analysis was implemented in BEAST v2.4.6 (Bouckaert et al. 2014) using bModelTest (Bouckaert and Drummond 2017) to simultaneously estimate and explore model substitutions across space, estimate model parameters, and generate a phylogeny. The BI analysis was implemented for 50 million generations while sampling every 5000 generations. The log file was visualized in Tracer v1.6 (Drummond et al. 2012) to ensure that all parameters and effective samples sizes reached stationarity (accepted ESS values ≥ 200). We then used TreeAnnotator v1.8.0 (Drummond et al. 2012) to estimate a maximum clade credibility tree using mean heights. We considered all posterior probabilities (PP) ≥ 0.95 as strongly supported (Huelsenbeck et al. 2001; Wilcox et al. 2002).

RESULTS

Extractions of DNA across sample types resulted in moderate yields, with two blood extractions yielding 10 and 11 ng/ μ l, and two scale extractions yielding 10 and 14 ng/ μ l. Our final alignment length of the ND2 region totaled 1039 bp. The models of molecular evolution selected from the IQTREE analyses estimated that TN+F+G4 was the best-fit model for all three partitions. The bMODELTEST selected the unnamed model 123141 (which is nested between the TN93 and TVM models) for the 1st, 2nd and 3rd codon positions, and had the highest posterior support (20.12, 19.99, and 20.29 %, respectively).

Phylogenetic inferences yielded a well-resolved topology. *Varanus nuchalis* and *V. dalubhasa* were inferred as sister taxa with each species recovered as a well-supported monophyletic lineage (similar to Welton et al. 2013). The confiscated animal, LAZ 994317, was recovered nested within the *V. nuchalis* clade, sister to a single vouchered sample from Masbate Island, Philippines (KU 335261, Fig. 1). This affinity was well-supported in both ML and BI inferences, and we interpret this finding as strong support for a Masbate Island origin of LAZ 994317. However, it remains a possibility that LAZ 994317 originated from Ticao Island, or from an undocumented but closely related population (see below).

DISCUSSION

Molecular evidence corroborates the morphology-based identification of LAZ 994317 as *Varanus nuchalis*, and infers a close affinity to natural populations inhabiting Masbate Island, Philippines. Comparisons of photos in life of LAZ 994317, and those of KU 335261 (Fig. 1, preserved) further support this relationship. In addition to the characteristic enlarged nuchal scales of the species, LAZ 994317 also exhibits the highly melanistic phenotype that has been documented in populations of *V. nuchalis*. Our results represent the first known occurrence of illegal trade of a phenotypically unique population of *V. nuchalis*, likely originating from Masbate Island. To date, this population represents one of two known occurrences of melanism among Philippine water monitors (Gaulke 1991, 1992a,b; see below). However, such populations have been documented in the non-Philippine water monitors *V. salvator komaini* (Nutaphand

1987) and *V. togianus* (Koch et al. 2007). Additionally, melanism is present among populations of *Varanus* inhabiting multiple islands in the Sula region of Indonesia (Weijola and Sweet 2010). Coincidentally, one of the three known species of endemic Philippine frugivorous monitor lizards, *V. mabitang*, also exhibits a melanistic phenotype. However, *V. mabitang* occurs only in the West Visayan region of the Philippines, being confined to Panay Island.

It remains unclear how widespread melanism is in Philippine water monitors. In addition to Masbate Island, this phenotype is also present in the *Varanus nuchalis* population from the adjacent island of Ticao (M. Gaulke, pers. comm.). Although genetic samples are currently unavailable, populations of *Varanus* from Masbate and Ticao are likely to be closely related given their close proximity and presumed intermittent connectedness during sea level low-stands over the past several million years (Brown and Diesmos 2009; Brown et al. 2014). This connectedness between land masses is inferred from the shallow sea (Masbate Pass) separating the two islands—an area that would have been exposed when sea levels dropped to below the ~120 m isobathymetric depth (Heaney 1985, 1986; Brown and Diesmos 2001). Similarly, there are no records of *Varanus* from Burias Island, though this landmass shares a more similar geological history with the Bicol Peninsula of Luzon and was likely never connected to Masbate and/or Ticao by exposed shallow sea beds. However, to date there are no known herpetological surveys of Ticao or Burias islands that have resulted in comparative material. Vouchered samples from Sibuyan Island, despite being geographically proximate to Masbate, do not exhibit this melanistic form, but rather have a coloration similar to the other documented populations of *V. nuchalis*—dark dorsum ground color overlain by a series of faint yellow-gold to white ocelli and speckles arranged in transverse bands.

As recently as 2016, an illegal shipment of Philippine water monitors, packaged in socks and trafficked in audio speakers, was confiscated by U.S. customs officials (The United States Department of Justice 2019). Although samples from that confiscation were unavailable for phyloforensic analyses, these instances highlight the need for improved monitoring of wildlife trade in the Philippines. Despite the absence of this species from recent assessments of internet-based trade (see Canlas et al. 2017), this confiscation in particular may indicate trade-based knowledge about the distribution of the melanistic phenotype in the Philippines—such animals, due to their relative rarity, can often command higher prices (several thousand U.S. dollars versus a few hundred for the “normal” phenotype; LW, pers. obs.). It remains unclear if the confiscation of LAZ 994317 represents an isolated or opportunistic incidence of international trade violation (and a CITES violation), or is indicative of a more pervasive problem (e.g., an established trade route). Such confiscations highlight the need for continued scrutiny of trade conduits, both traditional (importers and exporters) and more modern (e.g., social media). We recommend increased monitoring of this population in particular, given its documented presence in illicit trade markets, and presumed monetary value relative to other Philippine and Southeast Asian species. Additional *in situ* studies are sorely needed to fully assess the health and population trends in *Varanus nuchalis* and its constituent populations, and it is only with such studies that the relative magnitude and impacts of trade on this species can be adequately characterized. Similar studies are also warranted

to fully assess the impact of trade on a number of other endemic Philippine species, such as Sailfin Dragons (*Hydrosaurus pustulatus*; Siler et al. 2014), and freshwater turtles (e.g., *Cuora amboinensis* and *Siebenrockiella leytensis*; Diesmos et al. 2008) which share habitat preferences with water monitors and which are also collected and traded illegally.

The international trade in wildlife and its products is estimated to generate upwards of more than US \$100 billion annually (Nellemann et al. 2016). Impacts of this trade can be detrimental to source populations (Reed and Rodda 2009; Lyons and Natusch 2011; Wittemyer et al. 2014; Auliya et al. 2016), but can also have detrimental effects when traded live animals and plants escape captivity or are deliberately released into non-native environments (Doherty et al. 2016; García-Díaz et al. 2016). Additional impacts arise through the transmission of pathogens and disease (Karesh et al. 2005), as in the case of numerous strains of *Batrachochytrium* fungi (e.g., *B. dendrobatidis* and *B. salamandrivorans*) that threaten amphibians across the globe (O'Hanlan et al. 2018). Recent time-calibrated analyses of genetic data from fungal strains around the world led O'Hanlan et al. (2018) to implicate the international trade in amphibians as the likely source of global transmission of the pathogen. The potential threat of such internationally-transplanted pathogens have led some government agencies to put an embargo on certain species (e.g., U.S. Fish and Wildlife Service, European salamanders; Bean 2016) in an effort to proactively combat threats to native biodiversity. Unfortunately, the impacts of invasive species are not limited to amphibians. Indeed, summaries and analyses of documented species extinctions within major vertebrate groups have revealed that invasive species have been the leading cause of extinction for species of birds, the second leading cause of extinction for North American and global species of fishes, and the second leading cause of extinction for species of mammals (Miller et al. 1989; Burbridge and Manly 2002; Blackburn et al. 2004; Harrison and Stiassny 2004; McPhee and Flemming 2004; Clavero and Garcia-Berthou 2005; BirdLife International 2017). Although many of these extinctions have occurred among species with reduced ranges due to island endemism, Pimentel et al. (2005) estimated that even in the United States, ~42% of species that were recognized as Threatened or Endangered were at risk primarily due to the impacts of invasive species. Not only do invasive species pose a direct risk to endemic biodiversity through competition and/or predation, but there is also an indirect economic impact from invasive species that has been estimated at more than US \$120 billion per year (Pimentel et al. 2005), a figure that is likely much higher today. The only invasive reptile for which economic impacts have been assessed is the Brown Treesnake (*Boiga irregularis*), the introduction of which to the U.S. has resulted in damage and mitigation costs of at least US \$5.6 million per year (Pimentel 2007). Although introductions of species like the Brown Treesnake can be unintentional, the international trade (legal or illegal) in wildlife provides a direct, active opportunity for the transmission and establishment of such species (Souviron-Priego et al. 2018). Indeed, the legal pet trade alone has now become the primary pathway by which invasive reptiles and amphibians arrive in non-native environments (Stringham and Lockwood 2018).

To date, the impact of invasive monitor lizards has yet to be fully characterized in the United States. A number of species have been documented in south Florida, including the Savannah Monitor (*Varanus exanthematicus*), Nile Monitor (*V. niloticus*), and Asian Water Monitor (*V. salvator* spp.) (University

of Florida, Department of Wildlife Ecology & Conservation 2007; University of Georgia Center for Invasive Species and Ecosystem Health 2018), though only *V. niloticus* has been shown to have established breeding populations in the state (Johnson and McGarrity 2017). The extent of these and similar species' capability to expand their non-native ranges into North America is unknown. However, recent work on the invasive Tegu lizards (Jarnevich et al. 2018), in which distribution models utilizing natural occurrence data were used to assess suitable habitat in the United States, highlights the need for such studies in the future. In addition to habitat suitability, the ecological impact of invasive monitor lizards requires further investigation in order to more fully realize these species' impacts on native biota.

In this study, we utilized mitochondrial DNA to verify the taxonomic status, and identify the likely island origin of a confiscated Philippine water monitor lizard. Our results represent the first known occurrence of illegal trade of the phenotypically unique population of *Varanus nuchalis* occurring on Masbate Island, Philippines. Despite the absence of this species from recent assessments of internet-based trade (see Canlas et al. 2017), its confiscation highlights the need for continued scrutiny of trade conduits, both traditional (importers and exporters) and more modern (e.g., social media). We recommend increased monitoring of this population in particular, given its now known presence in illicit trade markets, and presumed monetary value relative to other Philippine and Southeast Asian species. Additional *in situ* studies are sorely needed to fully assess the health and population trends in *V. nuchalis* and its constituent populations, and it is only with such studies that the relative magnitude and impacts of trade on this species can be adequately characterized.

Acknowledgments.—We thank Mark Auliya for comments on previous drafts of this manuscript. We also thank the Biodiversity Monitoring Bureau (BMB) of the Philippine Department of Environment and Natural Resources for facilitating this and other such studies. Initial fieldwork was supported by: NSF DEB 0804115 and 0743491, Fulbright and Fulbright-Hayes Fellowships to C. Siler, and KUBI Panorama Grants. All necessary land-access, research, collection, transport, export, and import permits, including relevant CITES documentation for reference samples, were acquired and submitted by the authors and the University of Kansas Biodiversity Institute.

Author contributions.—LJW conceived the research, collected and analyzed data, and wrote the manuscript. IR conceived the research, collected data, and provided revisions to previous manuscript drafts. PLW collected and analyzed data and provided revisions to previous manuscript drafts. RMB conceived and funded the research and provided revisions of previous manuscript drafts.

LITERATURE CITED

- AULIYA, M., S. ALTHERR, D. ARIANO-SANCHEZ, E. H. BAARD, C. BROWN, R. M. BROWN, J.-C. CANTU, G. GENTILE, P. GILDENHUIS, E. HENNINGHEIM, J. HINTZMANN, K. KANARI, M. KRVAVAC, M. LETTINK, J. LIPPERT, L. LUISSELLI, G. NILSON, T. Q. NGUYEN, V. NIJMAN, J. F. PARHAM, S. A. PASACHNIK, M. PEDRONO, A. RAUHAUS, D. R. CORDOVA, M.-E. SANCHEZ, U. SCHEPP, M. VAN SCHINGEN, N. SCHNEWEISS, G. H. SEGNIAGBETO, R. SOMAWEERA, E. Y. SY, O. TURKOZAN, S. VINKE, T. VINKE, R. VYAS, S. WILLIAMSON, AND T. ZIEGLER. 2016. Trade in live reptiles, its impact on wild populations, and the role of the European market. *Biol. Conserv.* 204:103–119.
- BAYLESS, M. K., AND J. A. ADRAGNA. 1997. Monitor lizards in the Philippine Islands: a historical perspective (Sauria: Varanidae). *Asia Life Sci.* 6:39–50.

- BEAN, M. J. 2016. Injurious wildlife species; listing salamanders due to risk of salamander chytrid fungus. U.S. Department of the Interior, Federal Register 81:1534–1556.
- BIRDLIFE INTERNATIONAL. 2017. Spotlight on threatened birds. <<http://www.birdlife.org/datazone>> (accessed 15 December 2018).
- BLACKBURN, T. M., P. CASSEY, R. P. DUNCAN, K. L. EVANS, AND K. J. GASTON. 2004. Avian extinction and mammalian introductions on oceanic islands. *Science* 305:1955–1958.
- BOUCKAERT, R., AND A. DRUMMOND. 2017. bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evol. Biol.* 17:1–11.
- , J. HELED, D. KÜHNERT, T. VAUGHAN, C-H. WU, D. XIE, M. A. SUCHARD, A. RAMBAUT, AND A. J. DRUMMOND. 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Comput. Biol.* 10(4):e1003537.
- BRANDIS, K. J., P. B. J. MEAGHER, L. J. TONG, M. SHAW, D. MAZUMDER, P. GADD, AND D. RAMP. 2018. Novel detection of provenance in the illegal wildlife trade using elemental data. *Sci. Rep.* 8:15380.
- BROWN, R., AND A. C. DIESMOS. 2001. Application of lineage-based species concepts to oceanic island frog populations: The effects of differing taxonomic philosophies on the estimation of Philippine biodiversity. *Sill. J.* 42:133–162.
- , AND ———. 2009. Philippines, Biology. In R. Gillespie and D. Clague (eds.), *Encyclopedia of Islands*, pp. 723–732. University of California Press, Berkeley, California.
- , C. D. SILER, C. H. OLIVEROS, J. A. ESSELSTYN, A. C. DIESMOS, P. A. HOSNER, C. W. LINKEM, A. J. BARLEY, J. A. OAKS, M. B. SANGUILA, L. J. WELTON, D. C. BLACKBURN, R. G. MOYLE, A. T. PETERSON, AND A. C. ALCALA. 2014. Evolutionary processes of diversification in a model island archipelago. *Annu. Rev. Ecol. Evol. Syst.* 44:411–435.
- BURBRIDGE, A. A., AND B. F. MANLY. 2002. Mammal extinctions on Australian islands: Causes and conservation implications. *J. Biogeogr.* 29:465–473.
- CANLAS, C. P., E. Y. SY, AND S. CHNG. 2017. A rapid survey of online trade in live birds and reptiles in the Philippines. *TRAFFIC* 29:58–63.
- CERLING, T. E., J. E. BARNETTE, L. A. CHESSON, I. DOUGLAS-HAMILTON, K. S. GOBUSH, K. T. UNO, S. K. WASSER, AND X. XU. 2016. Radiocarbon dating of seized ivory confirms rapid decline in African elephant populations and provides insight into illegal trade. *PNAS* 113:13330–13335.
- CLAVERO, M., AND E. GARCIA-BERTHOUS. 2005. Invasive species are a leading cause of animal extinctions. *Trends Ecol. Evol.* 20:110.
- DIESMOS, A., AND M. GAULKE. 2009. *Varanus nuchalis*. The IUCN Red List of Threatened Species 2009: e.T169767A667, <http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T169767A6671267.en>.
- , R. M. BROWN, A. C. ALCALA, AND R. V. SISON. 2008. Status and distribution of nonmarine turtles of the Philippines. *Chelon. Conserv. Biol.* 7:157–177.
- DOHERTY, T. S., A. S. GLEN, D. G. NIMMO, E. G. RITCHIE, AND C. R. DICKMAN. 2016. Invasive predators and global biodiversity loss. *PNAS* 113:11261–11265.
- DRUMMOND, A. J., B. ASHTON, S. BUXTON, M. CHEUNG, A. COOPER, C. DURAN, M. FIELD, J. HELED, M. KEARSE, S. MARKOVITZ, R. MOIR, S. STONES-HAVAS, S. STURROCK, T. THIERER, AND A. WILSON. 2011. Geneious V5.4. Available from: <http://www.geneious.com/> (accessed 22 June 2017).
- , M. A. SUCHARD, D. XIE, AND A. RAMBAUT. 2012. Bayesian phylogenetics with BEAUTI and BEAST 1.7. *Mol. Biol. Evol.* 29:1969–1973. <http://dx.doi.org/10.1093/molbev/mss075>
- FA, J. E., J. E. GARCIA YUSTE, AND R. CASTELO. 2000. Bushmeat markets on Bioko Island as a measure of hunting pressure. *Conserv. Biol.* 14:1602–1613.
- FIDENCI, P., AND J. MARAN. 2009. Illegal domestic trade of the Philippine forest turtle (*Siebenrockiella leytenensis*) in the Philippines. *TurtleLog: Online Newsletter of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group*. <http://dx.doi.org/10.3854/tln.003.2009>
- GARCÍA-DÍAZ, P., J. V. ROSS, A. P. WOOLNOUGH, AND P. CASSEY. 2016. The illegal wildlife trade is a likely source of alien species. *Conserv. Lett.* 10:690–698.
- GAULKE, M. 1991. Systematic relationships of the Philippine water monitors as compared with *Varanus salvator salvator*, with a discussion of possible dispersal routes. *Mertensiella* 15:154–167.
- . 1992a. Taxonomy and biology of the Philippine monitors (*Varanus salvator*). *Philipp. J. Sci.* 121:345–381.
- . 1992b. Distribution, population density, and exploitation of the water monitor in the Philippines. *Hamadryad* 17:21–27.
- GENTILE, G., M. CIAMBOTTA, AND W. TAPIA. 2013. Illegal wildlife trade in Galápagos: Molecular tools help the taxonomic identification of confiscated iguanas and guide their repatriation. *Conserv. Genet. Resour.* 5:867–872.
- GRAY, J. E. 1845. Catalogue of the specimens of lizards in the collection of the British Museum. Trustees of the British Museum, London. xxviii + 289 pp.
- GRISMER, J. L., AND L. L. GRISMER. 2010. Who's your mommy? Identifying maternal ancestors for asexual species of *Leiolepis* Cuvier, 1829 and the description of a new endemic species of asexual *Leiolepis* Cuvier, 1829 from southern Vietnam. *Zootaxa* 2433:47–61.
- GRISMER, L. L. 2011. Lizards of Peninsular Malaysia, Singapore and their Adjacent Archipelagos. Chimaira, Frankfurt. 728 pp.
- HARRISON, I. J., AND M. L. J. STASSNY. 2004. CREO List of Fish Extinctions since AD 1500. American Museum of Natural History, Committee on Recently Extinct Organisms. <<http://creo.amnh.org/pdi.html>> (accessed 15 December 2018).
- HEANEY, L. R. 1985. Zoogeographic evidence for middle and late Pleistocene land bridges to the Philippine Islands. *MORSEA* 9:127–144.
- . 1986. Biogeography of small mammals in SE Asia: estimates of rates of colonization, extinction and speciation. *Biol. J. Linn. Soc. Lond.* 28:127–165.
- HUELSNBECK, J. P., F. RONQUIST, R. NIELSEN, AND J. P. BOLLBACK. 2001. Bayesian Inference of phylogeny and its impact on evolutionary biology. *Science* 294:2310–2314.
- IUCN. 2019. The IUCN Red List of Threatened Species. Version 2019-2. <http://www.iucnredlist.org>
- JARNEVICH, C. S., M. A. HAYES, L. A. FITZGERALD, A. A. YACKEL ADAMS, B. G. GALK, M. A. M. COLLIER, L. R. BONEWELL, P. E. KLUG, S. NARETTO, AND R. N. REED. 2018. Modeling the distributions of tegu lizards in native and potential invasive ranges. *Nat. Sci. Rep.* 8:10193.
- JOHNSON, S. A., AND M. MCGARRITY. 2017. Florida invader: Nile monitor lizard. University of Florida IFAS Extension, WEC293. <<http://edis.ifas.ufl.edu/pdf/FILES/UW/UW33800.pdf>> (accessed 15 December 2018).
- KARESH, W. B., R. A. COOK, E. L. BENNETT, AND J. NEWCOMB. 2005. Wildlife trade and global disease emergence. *Emerg. Infect. Dis.* 11:1000–1002.
- KATO, M., AND M. KUMA. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 30:3059–3066.
- KOCH, A., M. AULIYA, A. SCHMITZ, U. KUCH, AND W. BÖHME. 2007. Morphological studies on the systematics of south east Asian water monitors (*Varanus savator* Complex): Nominotypic populations and taxonomic overview. *Mertensiella* 16:109–180.
- , T. ZIEGLER, W. BÖHME, E. ARIDA, AND M. AULIYA. 2013. Pressing problems: distribution, threats, and conservation status of the monitor lizards (Varanidae: *Varanus* spp.) of Southeast Asia and the Indo-Australian Archipelago. *Herpetol. Conserv. Biol.* 8:1–62.
- LEDoux, J. B., A. ANTUNES, A. HAGUENAUER, M. PRATLONG, F. COSTANTINI, M. ABBATI, AND D. AURELLE. 2016. Molecular forensics into the sea: how molecular markers can help to struggle against poaching and illegal trade in precious corals? *The Cnidaria. Past, Present and Future* 45:729–745. https://dx.doi.org/10.1007/978-3-319-31305-4_45
- LYONS, J. A., AND D. J. D. NATUSCH. 2011. Wildlife laundering through breeding farms: Illegal harvest, population declines and a means of regulating the trade of green pythons (*Morelia viridis*) from Indonesia. *Biol. Conserv.* 144:3073–3081.
- MADDISON, W., AND D. R. MADDISON. 2015. Mesquite: a modular system for evolutionary analysis. Version 3.04, <http://mesquiteproject.org> (accessed 22 June 2017).

- MCPHEE, R., AND C. FLEEMING. 2004. CREO List of Mammal Extinctions since AD 1500. American Museum of Natural History, Committee on Recently Extinct Organisms. <<http://creo.amnh.org/pdi.html>> (accessed 15 December 2018).
- MILLER, R. R., J. D. WILLIAMS, AND J. E. WILLIAMS. 1989. Extinctions of North American fishes during the past century. *Fisheries* 14:22–38.
- MINH, Q., M. A. T. NGUYEN, AND A. VON HAESELER. 2013. Ultrafast approximation for phylogenetic bootstrap. *Mol. Biol. Evol.* 30:1188–1195.
- MURRAY-DICKSON, G., M. GHAZALI, R. OGDEN, R. BROWN, AND M. AULIYA. 2017. Phylogeography of the reticulated python (*Malayopython reticulatus* ssp.): conservation implications for the world's most heavily-traded snake species. *PLoS ONE* 12:e0182049.
- NELLEMAN, C., R. HENRIKSEN, A. KREILHUBER, D. STEWART, M. KOTSOVOU, P. RAXTIER, E. MREMA, AND S. BARRAT (EDS.). 2016. The rise of environmental crime: a growing threat to natural resources, peace, development and security. United Nations Environment Programme (UNEP). 104 pp.
- NGUYEN, L.-T., H. A. SCHMIDT, A. VON HAESELER, AND B. Q. MINH. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol. Biol. Evol.* 32:268–274.
- NUTAPHAND, W. 1987. Mangkorn Dam black monitor. *J. Thai. Zool. C.* 2(15):51.
- O'HANLAN, S. J., A. RIEUX, R. A. FARRER, G. M. ROSA, B. WALDMAN, A. BATAILLE, T. A. KOSCH, K. A. MURRAY, B. BRANKOVICS, M. FUMAGALLI, M. D. MARTIN, N. WALES, M. ALVARADO-RYBAK, K. A. BATES, L. BERGER, S. BÖLL, L. BROOKES, F. CLARE, E. A. COURTOIS, A. A. CUNNINGHAM, T. M. DOHERTY-BONE, P. GHOSH, D. J. GOWER, W. E. HINTZ, J. HÖGLUND, T. S. JENKINSON, C.-F. LIN, A. LAURILA, A. LOYAU, A. MARTEL, S. MEURLING, C. MIAUD, P. MINTING, F. PASMANS, D. S. SCHMELLER, B. R. SCHMIDT, J. M. G. SHELTON, L. F. SKERRATT, F. SMITH, C. SOTO-AZAT, M. SPAGNOLETTI, G. TESSA, L. F. TOLEDO, A. VALENZUELA-SÁNCHEZ, R. VERSTER, J. VÖRÖS, R. J. WEBB, C. WIERZBICKI, E. WOMBWELL, K. R. ZAMUDIO, D. M. AANENSEN, T. Y. JAMES, M. T. P. GILBERT, C. WELDON, J. BOSCH, F. BALLOUX, T. W. J. GARNER, AND M. C. FISHER. 2018. Recent Asian origin of chytrid fungi causing global amphibian declines. *Science* 360:621–627.
- PALSBOÛL, P. J., M. BÉRUBÉ, H. J. SKAUG, AND C. RAYMAKERS. 2006. DNA registers of legally obtained wildlife and derived products as means to identify illegal takes. *Conserv. Biol.* 20:1284–1293.
- PERNETTA, A. P. 2009. Monitoring the trade: using the CITES database to examine the global trade in live monitor lizards (*Varanus* spp.). *Biawak* 3:37–45.
- PIMENTEL, D. 2007. Environmental and economic costs of vertebrate species invasions into the United States. In G. W. Witmer, W. C. Pitt, and K. A. Fagerstone (eds.), *Managing Vertebrate Invasive Species: Proceedings of an International Symposium*, pp. 1–8. USDA/APHIS Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado. 38.
- , R. ZUNIGA, AND D. MORRISON. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* 52:273–288.
- REED, R. N., AND G. H. RODDA. 2009. Giant Constrictors: Biological and Management Profiles and an Establishment Risk Assessment for Nine Large Species of Pythons, Anacondas, and the Boa Constrictor. USGS Open File Report (OFR), 2009-1202. <http://dx.doi.org/10.3133/ofr20091202>
- ROSEN, G. E., AND K. F. SMITH. 2010. Summarizing the evidence on the international trade in illegal wildlife. *EcoHealth* 7:24–32.
- SHINE, R., AND P. HARLOW. 1998. Ecological traits of commercially harvested water monitors, *Varanus salvator*, in northern Sumatra. *Wildl. Res.* 25:437–447.
- , ———, B. AMBARIYANTO, AND J. S. KEOGH. 1996. Monitoring monitors: a biological perspective on the commercial harvesting of Indonesian reptiles. *Mertensiella* 9:61–68.
- SILER, C. D., A. LIRA-NORIEGA, AND R. M. BROWN. 2014. Conservation genetics of Australasian sailfin lizards: flagship species threatened by coastal development and insufficient protected area coverage. *Biol. Conserv.* 169:100–108.
- SOUVIRON-PRIEGO, L., A. R. MUÑOZ, J. OLIVERO, J. M. VARGAS, AND J. E. FA. 2018. The legal international wildlife trade favours invasive species establishment: the monk and ring-necked parakeets in Spain. *ARDEOLA* 65:233–246.
- STRINGHAM, O. C., AND J. L. LOCKWOOD. 2018. Pet problems: Biological and economic factors that influence the release of alien reptiles and amphibians by pet owners. *J. Appl. Ecol.* 55:2632–2640.
- STUART, B. L. 2004. The harvest and trade of reptiles at U Minh Thuong National Park, southern Vietnam. *TRAFFIC* 20:25–34.
- THE CITES APPENDICES. 2018. CITES Secretariat/UNEP World Conservation Monitoring Centre, <<http://cites.org/eng/app/appendices.php>> (accessed 20 October 2018).
- THE UNITED STATES DEPARTMENT OF JUSTICE. 2019. New Hampshire Man Sentenced For Trafficking In Protected Wildlife. <https://www.justice.gov/usao-ma/pr/new-hampshire-man-sentenced-trafficking-protected-wildlife> (accessed 19 May 2020).
- UNIVERSITY OF FLORIDA DEPARTMENT OF WILDLIFE ECOLOGY & CONSERVATION. 2007. Florida's Lizards, <<http://ufwildlife.ifas.ufl.edu/REDDy/othermonitorlizards.shtml>> (accessed 15 December 2018).
- UNIVERSITY OF GEORGIA CENTER FOR INVASIVE SPECIES AND ECOSYSTEM HEALTH. 2018. Early Detection & Distribution Mapping System. <<http://www.eddmaps.org/florida/distribution/index.cfm>> (accessed 15 December 2018).
- WASSER, S. K., W. J. CLARK, O. DRORI, E. S. KISAMO, C. MAILAND, B. MUTAYOBA, AND M. STEPHENS. 2008. Combating the illegal trade in African elephant ivory with DNA forensics. *Conserv. Biol.* 22:1065–1071.
- WEIJOLA, V. S.-Å., AND S. SWEET. 2010. A new melanistic species of monitor lizard (Reptilia: Squamata: Varanidae) from Sanana Island, Indonesia. *Zootaxa* 2434:17–32.
- WELTON, L. J., C. D. SILER, A. C. DIOSMOS, M. L. L. DIOSMOS, R. D. LAGAT, R. M. CAUSAREN, AND R. M. BROWN. 2012. Genetic identity, geographic ranges, and major distribution records for frugivorous monitor lizards of Luzon Island, Philippines. *Herpetol. Rev.* 43:226–230.
- , ———, C. W. LINKEM, A. C. DIOSMOS, M. L. DIOSMOS, E. SY, AND R. M. BROWN. 2013. Dragons in our midst: Phyloforensics of illegally traded Southeast Asian monitor lizards. *Biol. Conserv.* 159:7–15.
- , S. L. TRAVERS, C. D. SILER, AND R. M. BROWN. 2014. Integrative taxonomy and phylogeny-based species delimitation of Philippine water monitor lizards (*Varanus salvator* complex) with descriptions of two new cryptic species. *Zootaxa* 3881:201–227.
- WITTEMYER, G., J. M. NORTHRUP, J. BLANC, I. DOUGLAS-HAMILTON, P. OMONDI, AND K. P. BURNHAM. 2014. Illegal killing for ivory drives global decline in African elephants. *PNAS* 111:13117–13121.
- WILCOX, T. P., D. J. ZWICKL, T. A. HEATH, AND D. M. HILLIS. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Mol. Phylogenet. Evol.* 25:361–371.