

Research Article

Integrating early detection with DNA barcoding: species identification of a non-native monitor lizard (Squamata: Varanidae) carcass in Mississippi, U.S.A.

Robert N. Reed^{1,*}, Matthew W. Hopken², David A. Steen³, Bryan G. Falk¹ and Antoinette J. Piaggio²

¹U.S. Geological Survey, Fort Collins Science Center, Fort Collins, Colorado, USA

²USDA/APHIS National Wildlife Research Center, Fort Collins, Colorado, USA

³Department of Biological Sciences, Auburn University, Auburn, Alabama 36849, USA

*Corresponding author

E-mail: reedr@usgs.gov

Received: 30 October 2015 / Accepted: 27 April 2016 / Published online: 5 May 2016

Handling editor: Vadim Panov

Abstract

Early detection of invasive species is critical to increasing the probability of successful management. At the primary stage of an invasion, invasive species are easier to control as the population is likely represented by just a few individuals. Detection of these first few individuals can be challenging, particularly if they are cryptic or otherwise characterized by low detectability. The engagement of members of the public may be critical to early detection as there are far more citizens on the landscape than trained biologists. However, it can be difficult to assess the credibility of public reporting, especially when a diagnostic digital image or a physical specimen in good condition are lacking. DNA barcoding can be used for verification when morphological identification of a specimen is not possible or uncertain (i.e., degraded or partial specimen). DNA barcoding relies on obtaining a DNA sequence from a relatively small fragment of mitochondrial DNA and comparing it to a database of sequences containing a variety of expertly identified species. Herein we report the successful identification of a degraded specimen of a non-native, potentially invasive reptile species (*Varanus niloticus*) via DNA barcoding, after discovery and reporting by a member of the public.

Key words: invasive reptile, Mississippi, Nile monitor, *Varanus niloticus*, early detection

Introduction

The number of established populations of non-native reptile species in the United States has steadily increased during recent decades (Kraus 2009; Krysko et al. 2011), and some may exert significant negative impacts on native biodiversity (e.g., Mazzotti et al. 2015; McCleery et al. 2015). Early detection of incipient populations of non-native species may facilitate rapid management actions that result in eradication (Simberloff 2003; Vander Zanden et al. 2010). Alternatively, if a non-native population is detected after it is firmly established, eradication becomes less likely and more expensive (Simberloff et al. 2005).

For many invasive species, early detection is confounded by low detectability, which is the probability that an organism will be observed if present. Many taxa are characterized by low detection

probability, particularly reptiles and amphibians (Christy et al. 2010; Mazerolle et al. 2007; Durso et al. 2011; Dorcas and Willson 2013). Detection probabilities are often conditional on abundance and/or density; as a result even one or a few observations of species characterized by low detectability may actually be evidence of an incipient population.

Unfortunately, many initial reports of non-native species are unverified by professional biologists and thus of questionable significance. Low-quality observations include layperson observations without photographic or other evidence, or low-quality evidence (e.g., bad photos, degraded specimens) from which confident identifications cannot be made. This uncertainty may lead to inaction by land managers and others contemplating intervention, and possibly to a missed opportunity for containment or eradication.

In recent years, DNA barcoding has emerged as an accurate and increasingly inexpensive means of



Figure 1. Partial carcass of a large-bodied lizard, later identified as *Varanus niloticus* using molecular methods, as found in Jackson County, Mississippi, USA. Paper for size reference measures 10.2×15.2 cm. Photograph by B. Baucom.

identifying species (Hebert et al. 2003; Moritz and Cicero 2004; Hebert and Gregory 2005; Kress et al. 2015) that may be useful in early detection when identifiable material is unavailable (e.g., because the specimen is too damaged; Armstrong and Ball 2005; Darling and Blum 2007). The process of DNA barcoding is simple: a specified DNA locus is sequenced (e.g., cytochrome oxidase subunit I (COI); Hebert et al. 2003) and compared to a reference library (Hebert et al. 2003; Moritz and Cicero 2004; Kress et al. 2015). Barcoding is not without its difficulties, including poor sequencing results from degraded tissue (Hajibabaei et al. 2006; Meusnier et al. 2008), incomplete reference libraries (Ekrem et al. 2007; Boykin et al. 2012a), or uncertainty over how species limits correspond to sequence similarity thresholds (Boykin et al. 2012b). In many cases, however, barcoding is an efficient means for confident species identification. Herein, we report on the successful identification of a degraded specimen of a potentially invasive reptile after it was discovered and reported by a member of the public.

Methods

The partial carcass of an unidentified large-bodied lizard was discovered by a member of the public (B. Baucom) in a wooded area of Jackson County, Mississippi, U.S.A. (Latitude 30.6413, Longitude -88.4111) in December 2014. The partial carcass was degraded and rotting, with only the tail, a portion of the pelvic region, and one leg present (Figure 1), and was not definitely identifiable to species. The specimen was frozen in preparation for molecular analysis.

We used DNA barcoding to identify the specimen to species. A tissue sample from the degraded carcass was acquired from the skin and underlying tissue. Sixteen additional tissue samples from *V. niloticus* were obtained from invasive populations in Cape Coral ($n = 10$) and Palm Beach County ($n = 6$; both cities are in Florida, U.S.A.) to use as references. These animals were collected by local agencies as part of ongoing removal programs. We extracted DNA from these samples using a DNeasy[®] Blood

and Tissue Kit (Qiagen Inc., Germany). The isolation and purification process was automated using a QIAcube (Qiagen Inc., Germany) following the “Purification of Total DNA from Animal Tissues” protocol. We targeted the COI barcoding region because it is commonly used for reptile identification (Vences et al. 2012), and used primers from Nagy et al. (2012). PCRs were performed in 25 μ L reactions using illustra PuRe Taq Ready-To-Go PCR Beads (GE Healthcare Life Sciences, United Kingdom) with 2 μ L of 50–100 ng of genomic DNA, 0.4 μ M of each primer, and 21 μ L of water. All PCRs were conducted using a Mastercycler® Gradient (Eppendorf, Germany). The thermal profile for amplification was an initial denaturation at 94°C for 3 min, followed by 40 cycles of 94°C for 40 s, 48.5°C for 30 s, and 72°C for 60 s, and a final extension of 72°C for 7 min. Exo-SAP-IT® (Affymetrix, USA) was used for purification of amplified products following manufacturer’s instructions. We performed cycle sequencing reactions with 1.0 μ L of purified PCR product, 1.0 μ M primer, 0.25 μ L BigDye® v3.1, and 2.275 μ L of 5x buffer (Life Technologies, USA) in 10 μ L reactions, and sequenced the PCR products on an ABI 3130xl genetic analyzer (Life Technologies, USA) using manufacturer recommended settings. We visualized and edited sequences in Sequencher 5.3 (Gene Codes, USA). We compared our sequences to the database of the National Centre for Biotechnology Information through a BLAST search (National Library of Medicine 2015). We additionally compared the sequences to other COI barcode sequences stored in the Bar Code of Life Database (BOLD™; Ratnasingham and Hebert 2007). We compared the DNA sequence from the carcass tissue sample to the 16 samples collected from Florida using the custom BLAST algorithm implemented in GENEIOUS v. 8.1.7.

Results

We successfully amplified 613-bp of COI sequence from the tissue sample. The consensus sequence returned a > 97% match to *Varanus niloticus* (Nile monitor) in both the BLAST (KJ19299.1 from Africa; query coverage = 100% and identity = 98%) and the same sequence in BOLD™ (97.5%) reference libraries (accessed June 18, 2015). There were 15 base differences (all transitions) between the sequences. There were 41 *Varanus* species with COI sequences in the BLAST database, including 4 *V. niloticus* sequences as well as all six exotic species that have been documented in Florida (Early Detection and Distribution Mapping System 2015).

The BLAST database also includes COI sequences for the closely related *Varanus exanthematicus*, (Vidal et al. 2012). For the BLAST results the top 4 hits included all 4 *V. niloticus* sequences (query coverage = 95–100% and identity = 91–98%). The next best match was to *V. salvator* (query coverage = 98% and identity = 84%). We accessioned the tissue at the American Museum of Natural History (AMNH R-500160) and deposited the sequence in GenBank (KT630582).

We found a single haplotype in all 16 samples from Florida (GenBank Accession BankIt1904722 *Varanus niloticus* KU954526) and the COI sequence from the carcass was a 99.8% match to this haplotype. The Florida haplotype was also compared to both the BLAST and BOLD™ reference libraries (accessed March 8, 2016). The Florida haplotype matched GenBank accession number KJ19299.1 (query coverage = 96% and identity = 97%) and the same sequence in BOLD (97%). There was a single base change (C/T) between the carcass and the Florida haplotype.

Discussion

We applied DNA barcoding to a degraded sample that could not be conclusively identified by visual examination, and were able to identify it as *V. niloticus*. It is unknown whether our report of *V. niloticus* in Mississippi represents an incipient population or the fortuitous observation of a single escaped or released captive. Native to sub-Saharan Africa, *V. niloticus* is a large-bodied (to >2 m total length; Lenz 2004) generalist predator that consumes a wide variety of invertebrate and vertebrate prey (Dalhuijsen et al. 2014). According to the Law Enforcement Management Information System database maintained by the U.S. Fish and Wildlife Service, a total of 130,521 *V. niloticus* individuals were imported into the United States between 1999 and 2014. This level of trade represents nontrivial potential for sustained propagule pressure and source of incipient populations. Several invasive populations of *V. niloticus* are present in Florida (from the vicinities of Cape Coral, West Palm Beach, and Homestead; Enge et al. 2004; Early Detection and Distribution Mapping System 2015). These are the only introduced populations known globally. The close genetic similarity between the carcass we identified in Mississippi and the samples from the invasive range in Florida suggests they are closely related and/or originating from a similar area of the native range. Therefore this may have been an individual that escaped or was released from captivity.

In South Africa, *V. niloticus* is present at latitudes exceeding 33°, suggesting tolerance of relatively cool winters (Alexander and Marais 2007). The specimen reported herein was found at 30°N. We offer no predictions about establishment potential at these latitudes in the United States, except to suggest that a robust species distribution model based on the native range could inform establishment risk for the United States north of the currently established populations in Florida.

A member of the public reported the initial observation of the dead specimen, and this represents a successful early detection of a potentially invasive species. For species with low detectability such as *V. niloticus*, vigilance by members of the public is one of the best means of early detection. To be successful, the general public must be aware of the importance of early detection and reporting, and there is a need for continued outreach by scientists and resource managers to ensure that citizens are aware of the reporting needs and mechanisms for invasive species. In this case, reporting the sighting was prompted by the reporter having recently seen news coverage of research being conducted on invasive tegu lizards.

Our results highlight how DNA barcoding can facilitate the detection of invasive species. This approach has already been successfully applied to early detection of other difficult-to-identify species or specimens (e.g., invertebrate diapausing eggs, Briski et al. 2011; morphologically cryptic flatworms, Justine et al. 2015), and can be combined with environmental-DNA sampling for passive invasive-species monitoring (Dejean et al. 2012; Piaggio et al. 2014). As the completeness of the database for sequence comparison is critical to successful barcoding, expanding reference libraries such as BLAST and BOLD™ to include all or most potentially invasive species (Boykin et al. 2012a) will facilitate efficacious integration of DNA barcoding and invasive species science.

Acknowledgements

Brenda Baucom reported finding the lizard carcass. J. Wood provided tissue samples from Florida. This project was supported by funding from the Invasive Species Program of the U.S. Geological Survey and the National Wildlife Research Center Wildlife Genetics Project. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. C.M. Romagosa kindly provided information on international trade in *V. niloticus*. D. Griffin assisted with laboratory procedures. The manuscript was greatly improved by comments from C.M. Romagosa and two anonymous reviewers.

References

- Alexander G, Marais J (2007) A Guide to the Reptiles of Southern Africa. Struik Publishers. Cape Town, South Africa, 408 pp
- Armstrong K, Ball S (2005) DNA barcodes for biosecurity: Invasive species identification. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 360: 1813–1823, <http://dx.doi.org/10.1098/rstb.2005.1713>
- Boykin LM, Armstrong K, Kubatko L, De Barro P (2012a) DNA barcoding invasive insects: database roadblocks. *Invertebrate Systematics* 26: 506–514, <http://dx.doi.org/10.1071/IS12025>
- Boykin LM, Armstrong KF, Kubatko L, De Barro P (2012b) Species delimitation and global biosecurity. *Evolutionary Bioinformatics* 8: 1–37
- Briski E, Cristescu ME, Bailey SA, MacIsaac HJ (2011) Use of DNA barcoding to detect invertebrate invasive species from diapausing eggs. *Biological Invasions* 13: 1325–1340, <http://dx.doi.org/10.1007/s10530-010-9892-7>
- Christy MT, Yackel Adams AA, Rodda GH, Savidge JA, Tyrrell CL (2010) Modelling detection probabilities to evaluate management and control tools for an invasive species. *Journal of Applied Ecology* 47: 106–113, <http://dx.doi.org/10.1111/j.1365-2664.2009.01753.x>
- Dalhuijsen K, Branch WR, Alexander GJ (2014) A comparative analysis of the diets of *Varanus albigularis* and *Varanus niloticus* in South Africa. *African Zoology* 49: 83–93, <http://dx.doi.org/10.3377/004.049.0105>
- Darling JA, Blum MJ (2007) DNA-based methods for monitoring invasive species: A review and prospectus. *Biological Invasions* 9: 751–765, <http://dx.doi.org/10.1007/s10530-006-9079-4>
- Dejean T, Valentini A, Miquel C, Taberlet P, Bellemain E, Miaud C (2012) Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology* 49: 953–959, <http://dx.doi.org/10.1111/j.1365-2664.2012.02171.x>
- Dorcas ME, Willson JD (2013) Hidden Giants: Problems associated with studying secretive invasive pythons. In: Lutterschmidt WI (ed), *Reptiles in Research: Investigations of Ecology, Physiology, and Behavior from Desert to Sea*. Nova Biomedical. New York, USA, 527 pp
- Durso AM, Willson JD, Winne CT (2011) Needles in haystacks: Estimating detection probability and occupancy of rare and cryptic snakes. *Biological Conservation* 144: 1508–1515, <http://dx.doi.org/10.1016/j.biocon.2011.01.020>
- Early Detection and Distribution Mapping System (2015) Nile monitor, *Varanus niloticus* (Linnaeus in Hasselquist, 1762). <https://www.eddmaps.org/distribution/viewmap.cfm?sub=18353> (Accessed June 18, 2015)
- Ekrem T, Willassen E, Stur E (2007) A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Molecular Phylogenetics and Evolution* 43: 530–542, <http://dx.doi.org/10.1016/j.ympev.2006.11.021>
- Enge KM, Krysko KL, Hankins KR, Campbell TS, King FW (2004) Status of the Nile monitor (*Varanus niloticus*) in southwestern Florida. *Southeastern Naturalist* 3: 571–582, [http://dx.doi.org/10.1656/1528-7092\(2004\)003\[0571:SOTNMVJ2.0.CO;2](http://dx.doi.org/10.1656/1528-7092(2004)003[0571:SOTNMVJ2.0.CO;2)
- Hajibabaei M, Smith M, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PD (2006) A minimalist barcode can identify a specimen whose DNA is degraded. *Molecular Ecology Notes* 6: 959–964, <http://dx.doi.org/10.1111/j.1471-8286.2006.01470.x>
- Hebert PD, Cywinska A, Ball SL (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B: Biological Sciences* 270: 313–321, <http://dx.doi.org/10.1098/rspb.2002.2218>
- Hebert PD, Gregory TR (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology* 54: 852–859, <http://dx.doi.org/10.1080/10635150500354886>

- Justine JL, Winsor L, Barrière P, Fanai C, Gey D, Han AWK, La Quay-Velázquez G, Lee BPYH, Lefevre JM, Meyer JY (2015) The invasive land planarian *Platydemus manokwari* (Platyhelminthes, Geoplanidae): records from six new localities, including the first in the USA. *PeerJ* 3: e1037, <http://dx.doi.org/10.7717/peerj.1037>
- Kraus F (2009) Global trends in alien reptiles and amphibians. *Aliens: The Invasive Species Bulletin* 28: 13–18
- Kress WJ, García-Robledo C, Uriarte M, Erickson DL (2015) DNA barcodes for ecology, evolution, and conservation. *Trends in Ecology & Evolution* 30: 25–35, <http://dx.doi.org/10.1016/j.tree.2014.10.008>
- Krysko KL, Burgess JP, Rochford MR, Gillette CR, Cueva D, Enge KM, Somma LA, Stabile JL, Smith DC, Wasilewski JA (2011) Verified non-indigenous amphibians and reptiles in Florida from 1863 through 2010: Outlining the invasion process and identifying invasion pathways and stages. *Zootaxa* 3028: 1–64
- Lenz S (2004) *Varamus niloticus*. In: Pianka ER, King DR (eds), *Varanoid Lizards of the World*. Indiana University Press. Bloomington, Indiana, USA, 608 pp
- Mazerolle MJ, Bailey LL, Kendall WL, Royle JA, Converse SJ, Nichols JD (2007) Making great leaps forward: Accounting for detectability in herpetological field studies. *Journal of Herpetology* 41: 672–689, <http://dx.doi.org/10.1670/07-061.1>
- Mazzotti FJ, McEachern M, Rochford M, Reed RN, Eckles JK, Vinci J, Edwards J, Wasilewski J (2015) *Tupinambis merianae* as nest predators of crocodylians and turtles in Florida, USA. *Biological Invasions* 17: 47–50, <http://dx.doi.org/10.1007/s10530-014-0730-1>
- McCleery RA, Sovie A, Reed RN, Cunningham MW, Hunter ME, Hart KM (2015) Marsh rabbit mortalities tie pythons to the precipitous decline of mammals in the Everglades. *Proceedings of the Royal Society of London B: Biological Sciences* 282, <http://dx.doi.org/10.1098/rspb.2015.0120>
- Meusnier I, Singer GA, Landry J-F, Hickey DA, Hebert PD, Hajjibabaei M (2008) A universal DNA mini-barcode for biodiversity analysis. *BMC Genomics* 9: 214, <http://dx.doi.org/10.1186/1471-2164-9-214>
- Moritz C, Cicero C (2004) DNA barcoding: Promise and pitfalls. *PLoS Biology* 2: 1529–1531, <http://dx.doi.org/10.1371/journal.pbio.0020354>
- Nagy ZT, Sonet G, Glaw F, Vences M (2012) First large-scale DNA barcoding assessment of reptiles in the biodiversity hotspot of Madagascar, based on newly designed COI primers. *PLoS ONE* 7: e34506, <http://dx.doi.org/10.1371/journal.pone.0034506>
- National Library of Medicine (2015) Basic Local Alignment Search Tool. <http://blast.ncbi.nlm.nih.gov/Blast.cgi> (accessed 15 June 2015)
- Piaggio AJ, Engeman RM, Hopken MW, Humphrey JS, Keacher KL, Bruce WE, Avery ML (2014) Detecting an elusive invasive species: A diagnostic PCR to detect Burmese python in Florida waters and an assessment of persistence of environmental DNA. *Molecular Ecology Resources* 14: 374–380, <http://dx.doi.org/10.1111/1755-0998.12180>
- Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology Notes* 7: 355–364, <http://dx.doi.org/10.1111/j.1471-8286.2007.01678.x>
- Simberloff D (2003) How much information on population biology is needed to manage introduced species? *Conservation Biology* 17: 83–92, <http://dx.doi.org/10.1046/j.1523-1739.2003.02028.x>
- Simberloff D, Parker IM, Windle PN (2005) Introduced species policy, management, and future research needs. *Frontiers in Ecology and the Environment* 3: 12–20, [http://dx.doi.org/10.1890/1540-9295\(2005\)003\[0012:ISPMAF\]2.0.CO;2](http://dx.doi.org/10.1890/1540-9295(2005)003[0012:ISPMAF]2.0.CO;2)
- Vander Zanden MJ, Hansen GJ, Higgins SN, Kornis MS (2010) A pound of prevention, plus a pound of cure: early detection and eradication of invasive species in the Laurentian Great Lakes. *Journal of Great Lakes Research* 36: 199–205, <http://dx.doi.org/10.1016/j.jglr.2009.11.002>
- Vences M, Nagy ZT, Sonet G, Verheyen E (2012) DNA barcoding amphibians and reptiles. In: Kress WJ, Erickson DL (ed), *DNA Barcodes: Methods and Protocols*. Humana Press. New York City, USA, pp 79–107, http://dx.doi.org/10.1007/978-1-61779-591-6_5
- Vidal N, Marin J, Sassi J, Battistuzzi FU, Donnellan S, Fitch AJ, Fry BG, Vonk FJ, de la Vega RCR, Couloux A (2012) Molecular evidence for an Asian origin of monitor lizards followed by Tertiary dispersals to Africa and Australasia. *Biology Letters* 8: 853–855, <http://dx.doi.org/10.1098/rsbl.2012.0460>