Everglades Cooperative Invasive Species Management Area

21st Annual Summit

Development of multiplex digital PCR assays for use in eDNA monitoring of invasive species in Florida

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Target Organisms

Snakes

- Burmese python
- North African Python
- Boa constrictor
- Rainbow boa

Lizards

- Asian water monitor
- Nile monitor
- Argentine black and white tegu
- Gold tegu
- Red tegu

Fishes

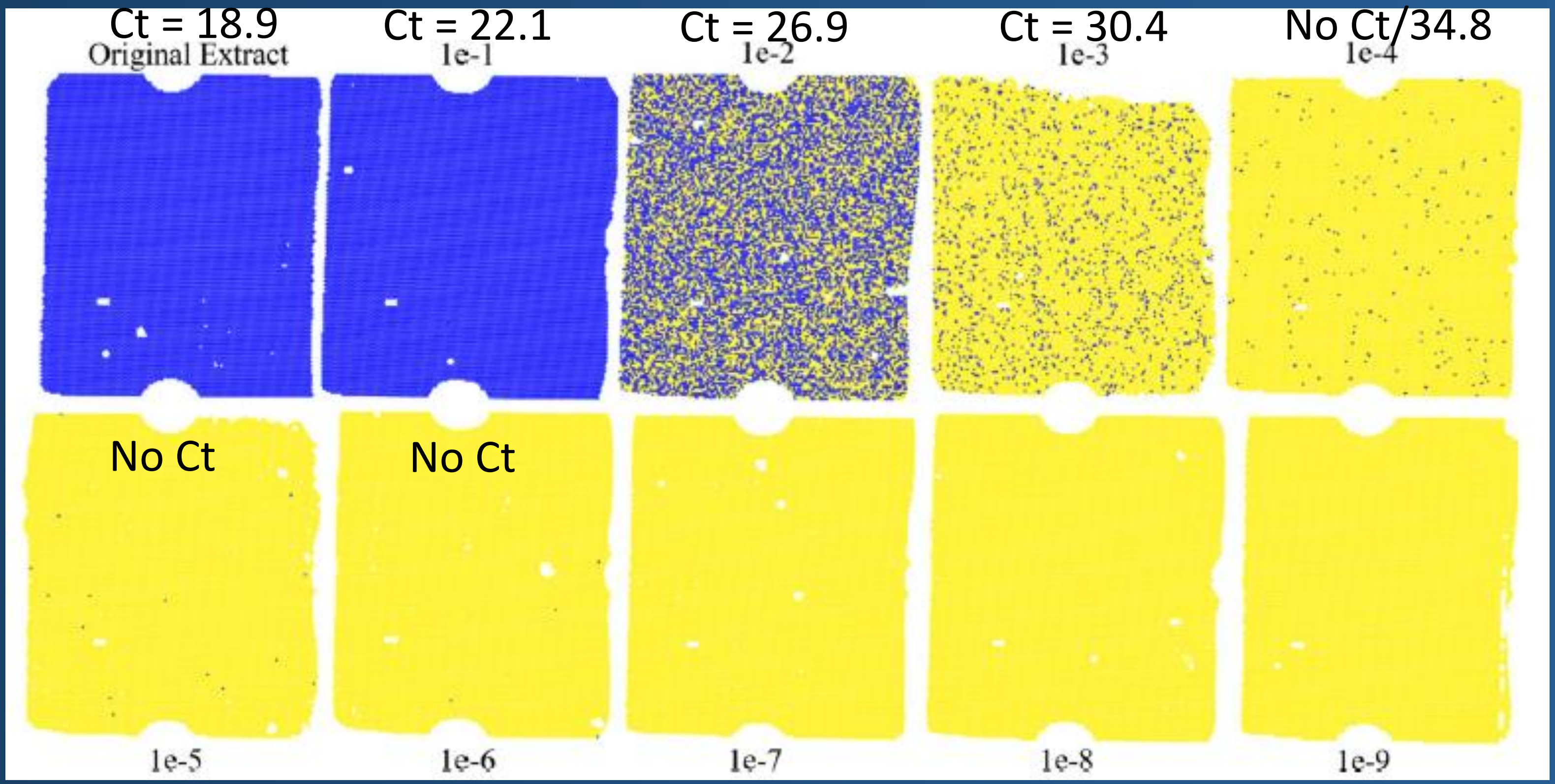
- Asian swamp eel
- Bullseye snakehead

Crocodilians

- Spectacled caiman
- American crocodile
- American alligator

Digital PCR (dPCR)

- Highly sensitive endpoint detection.
- Absolute quantification.
- Functionally replicating PCR reactions 20,000 times on a single sample.



Digital PCR (dPCR)

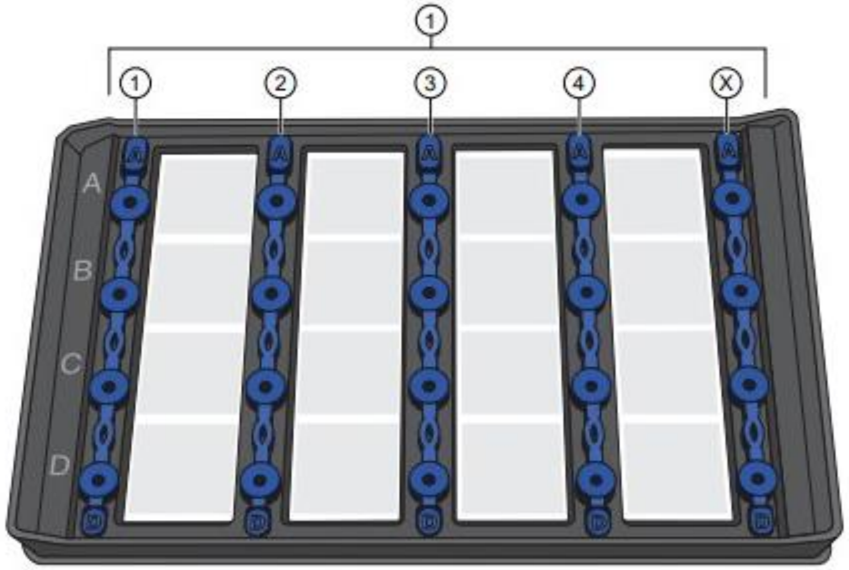
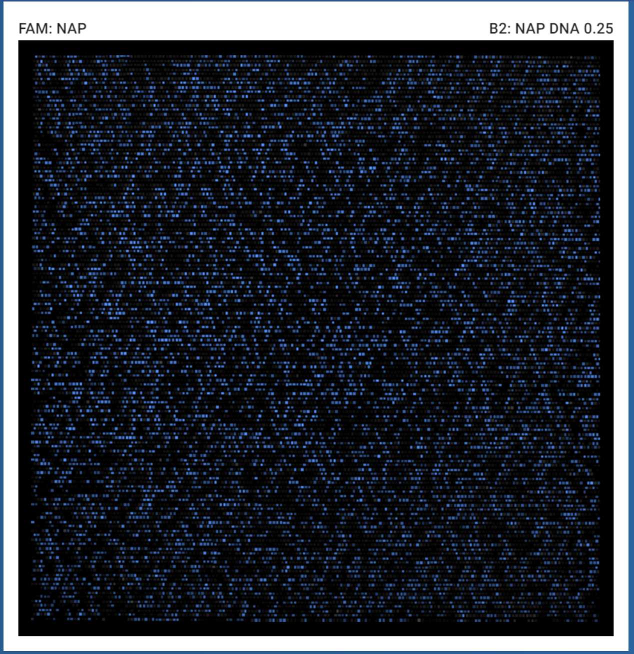


Figure 8 **MAP plate with MAP plate gasket strips in place**

① MAP plate gasket strips on columns 1–4 and column X



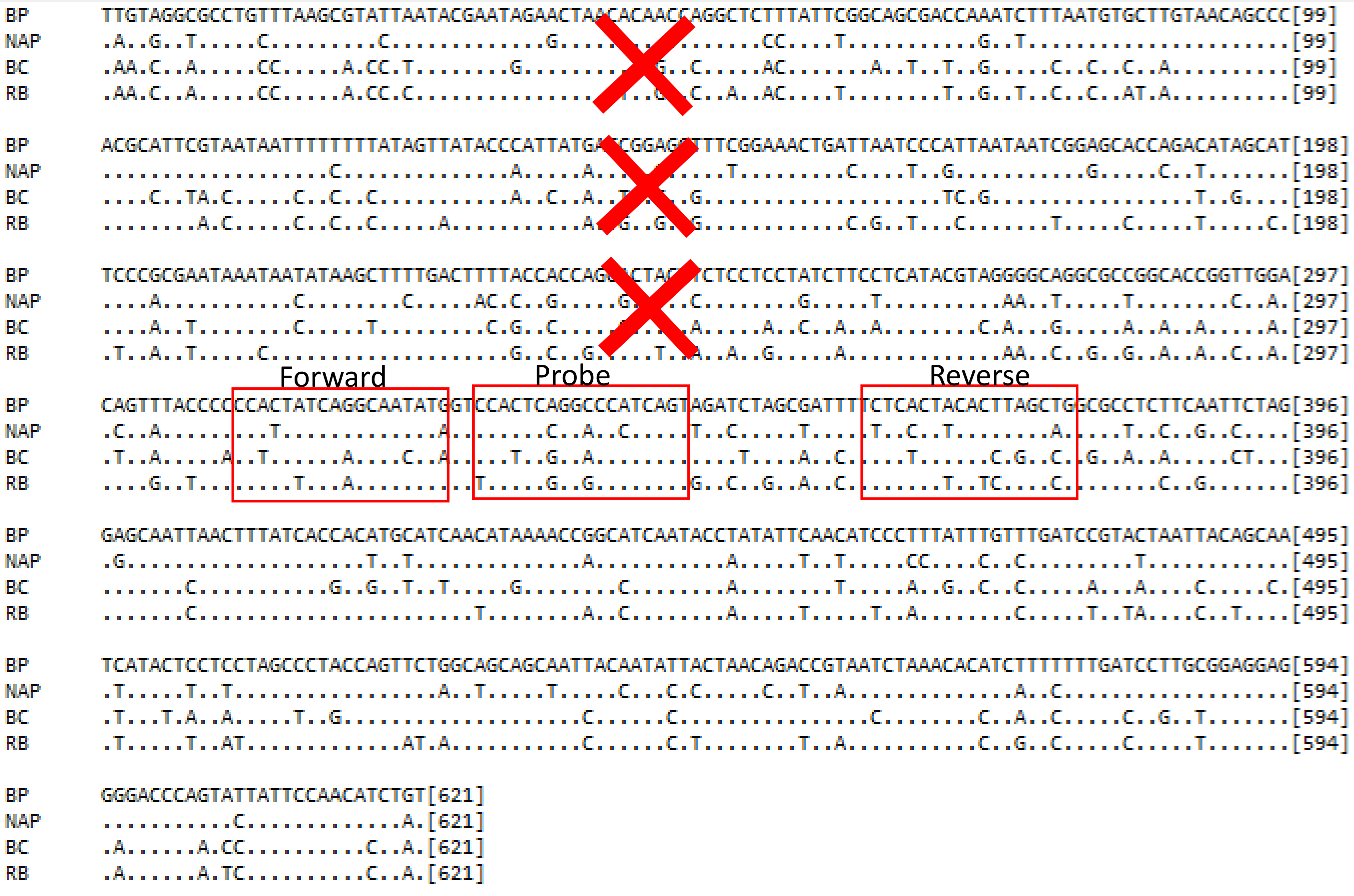
Four open channels and one reference dye

- FAM
- VIC
- JUN
- ABY
- ROX - reference

Cytochrome *c* oxidase subunit I (COI) barcoding region

- LCO1490/HCO2198 primers from Folmer et al. 1994 used to generate a ≈600 bp product.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol., 3: 294-299. *Link: https://bit. ly/2x9R9WL*.



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BP      TTGTAGGCGCCTGTTTAAGCGTATTAATACGAATAGAACTAACACAACCAGGCTCTTTATTCGGCAGCGACCAAATCTTTAATGTGCTTGTAACAGCCC[99]
NAP     .A..G..T.....C.........C.............G..................CC....T...........G..T.....................[99]
BC      .AA.C..A.....CC.....A.CC.T.........G.........G..C.....AC.......A..T..T..G.....C..C..C..A..........[99]
RB      .AA.C..A.....CC.....A.CC.C...................T..G..C..A..AC....T........T..G..T..C..C..AT.A..........[99]

BP      ACGCATTCGTAATAATTTTTTTTATAGTTATACCCATTATGACCGGAGCTTTCGGAAACTGATTAATCCCATTAATAATCGGAGCACCAGACATAGCAT[198]
NAP     ..................C.............A.....A...........T.........C....T..G..........G.....C..T.......[198]
BC      ....C..TA.C.....C..C..C...........A..C..A..T.....G..................TC.G...............T..G....[198]
RB      ........A.C.....C..C..C.....A...........A..G..G..G...........C.G..T...C.......T.....C.....T.....C.[198]

BP      TCCCGCGAATAAATAATATAAGCTTTTGACTTTTACCACCAGCCTACTCTCCTCCTATCTTCCTCATACGTAGGGGCAGGCGCCGGCACCGGTTGGA[297]
NAP     ....A..........C........C.....AC.C..G.....G.....C.........G.....T..........AA..T.....T........C..A.[297]
BC      ....A..T.......C.....T.........C.G..C.........A.....A..C..A..A........C.A...G.....A..A..A.....A.[297]
RB      .T..A..T.....C................G..C..G.....T..A..A..G.....A...........AA..C..G..G..A..A..C..A.[297]
                      Forward                Probe                           Reverse
BP      CAGTTTACCCCCCACTATCAGGCAATATGGTCCACTCAGGCCCATCAGTAGATCTAGCGATTTTCTCACTACACTTAGCTGGCGCCTCTTCAATTCTAG[396]
NAP     .C..A.........T.............A.......C..A..C......T..C.....T.....T..C..T.........A.....T..C..G..C.....[396]
BC      .T..A.....A..T......A.....C..A....T..G..A.............T....A..C.....T......C.G..C..G..A..A.....CT...[396]
RB      ....G..T..........T...A...........T.....G..G........G..C..G..A..C........T..TC....C........C..G.......[396]

BP      GAGCAATTAACTTTATCACCACATGCATCAACATAAAACCGGCATCAATACCTATATTCAACATCCCTTTATTTGTTTGATCCGTACTAATTACAGCAA[495]
NAP     .G..................T..T..............A..........A.....T..T.....CC....C..C.........T............[495]
BC      .......C..........G..G..T..T.....G........C.........A........T.....A..G..C..C.....A...A....C.....C.[495]
RB      .......C.......................T........A..C.........A.....T.....T..A........C.....T..TA....C..T....[495]

BP      TCATACTCCTCCTAGCCCTACCAGTTCTGGCAGCAGCAATTACAATATTACTAACAGACCGTAATCTAAACACATCTTTTTTTGATCCTTGCGGAGGAG[594]
NAP     .T.....T..T..................A..T.....T......C...C.C.....C..T..A.............A..C....................[594]
BC      .T...T.A..A.....T..G.....................C......C................C........C..A..C.....C..G..T........[594]
RB      .T.....T..AT.............AT.A...........C......C.T.........T..A...........C..G..C.....C.....T........[594]

BP      GGGACCCAGTATTATTCCAACATCTGT[621]
NAP     ..........C.............A.[621]
BC      .A......A.CC..........C..A.[621]
RB      .A......A.TC..........C..A.[621]
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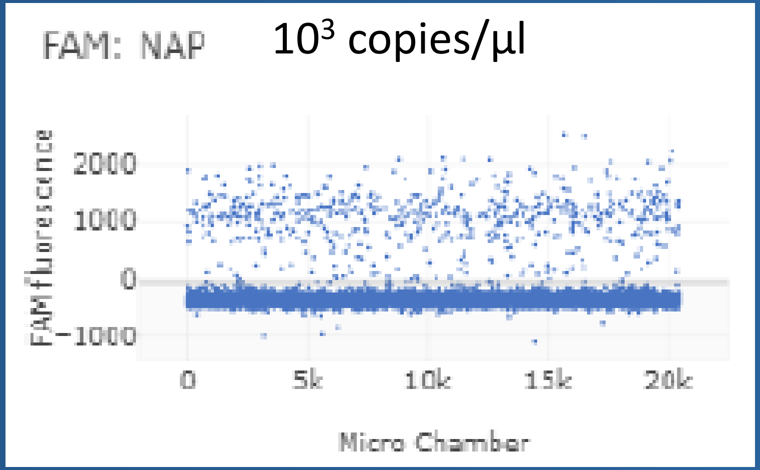
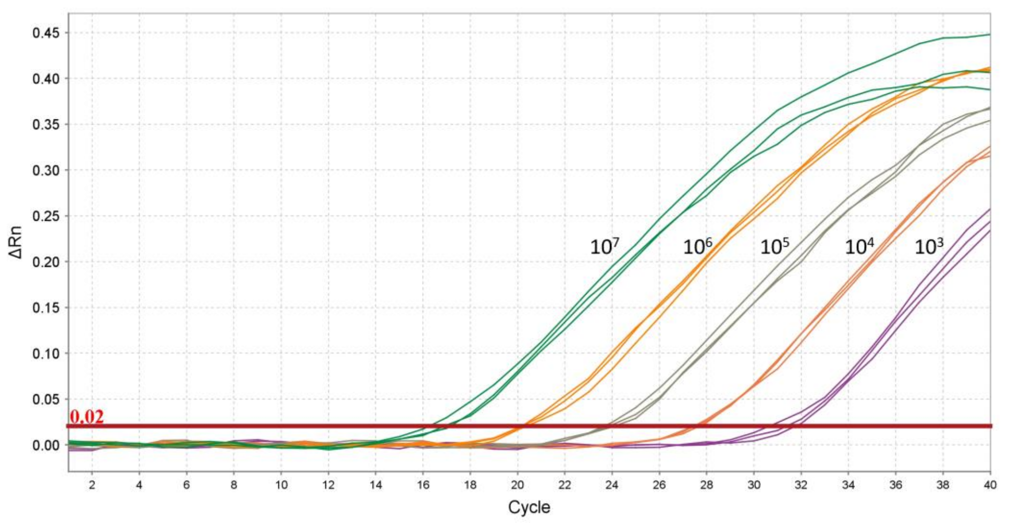
Plasmid Standards

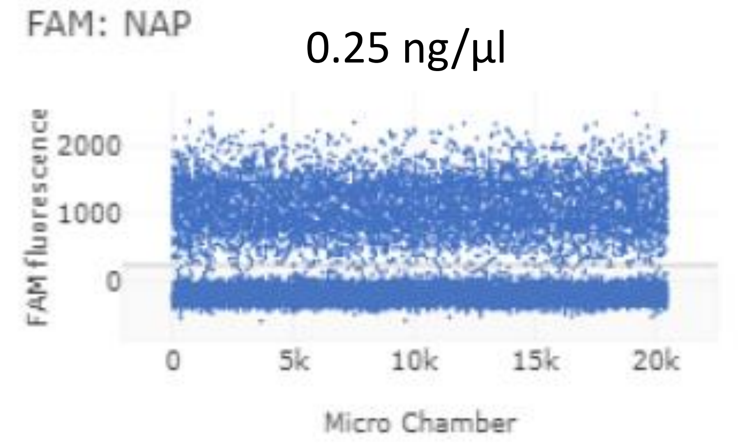
	Assay			
	Boa constrictor (BC)	Epicrates cenchria (RB)	Python sebae (NAP)	Python bivittatus (BP)
Species	Avg. Ct±SE	Avg. Ct±SE	Avg. Ct±SE	Avg. Ct±SE
Boa constrictor	19.29±0.04	No Ct	No Ct	No Ct
Epicrates cenchria	No Ct	18.50±0.44	No Ct	No Ct
Python sebae	No Ct	No Ct	18.58±0.09	No Ct
Python bivittatus	No Ct	No Ct	No Ct	16.30±0.11

- Assays designed run on standard PCR (gradient).
- PCR products inserted into plasmids and cloned.
- Plasmids isolated and serially diluted.
- Dilutions evaluated by qPCR.



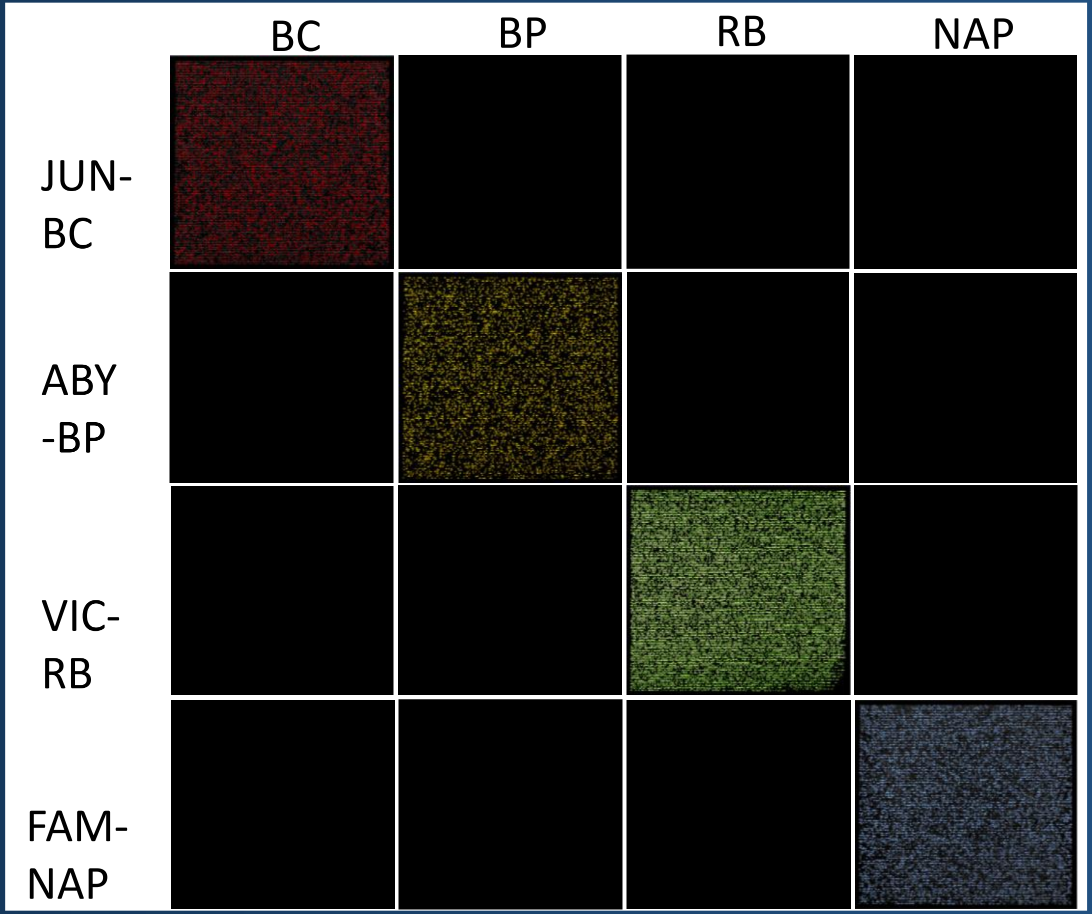
10^3 copies/µl

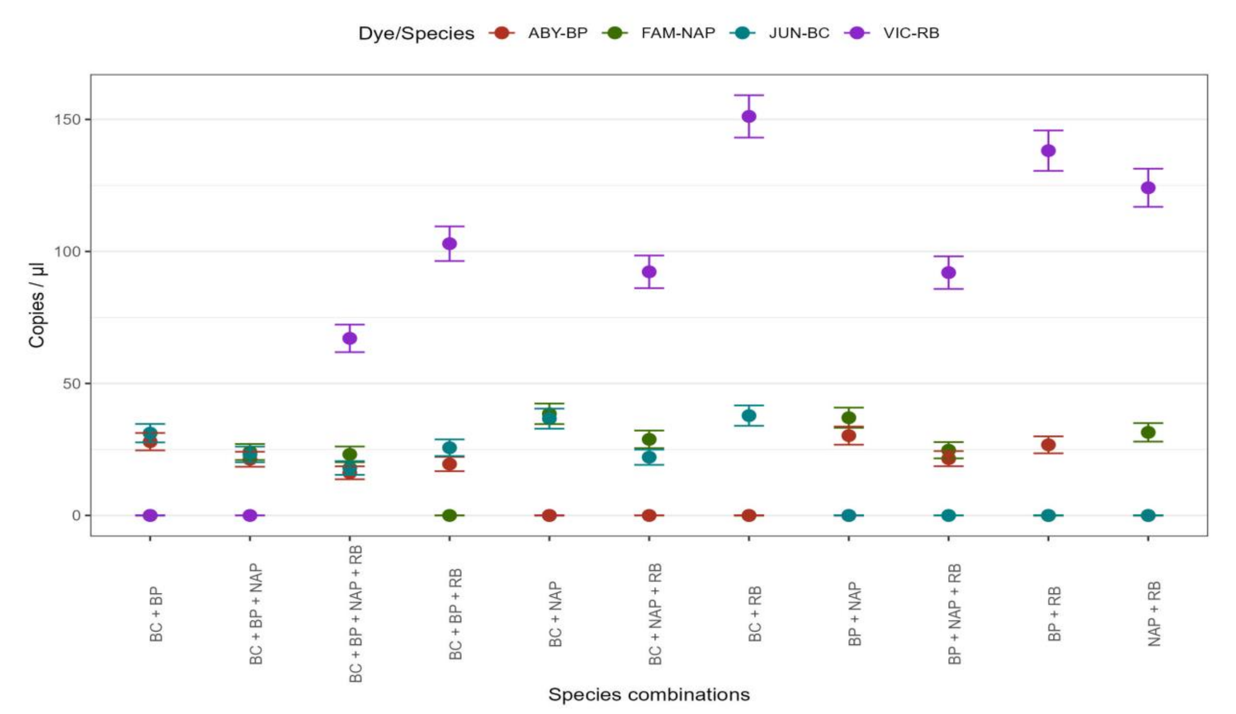




0.25 ng/µl

Assay Validation





Current Status of Assays and Validation

Taxonomic group	COI seq.*	qPCR Validated	Multiplex Validated	eDNA Controls
Snakes	+	+	+	+
Tegus	+	+	-	-
Monitors	+	+	+	-
Crocodilians	+	+	+	-
Fishes	+	+	+	-

* NADH used for gold tegu, could not get COI seq. data with available primers

Future Efforts and Applications

- Evaluate all assays against laboratory controls.
- Evaluate all assays against field controls.
- Evaluate against field samples in areas with suspected or unknown populations of invasives.

Utility for Monitoring Invasive Species

- Can allow for evaluating presence of species over larger areas more cost effectively (evaluate range expansion, overlapping ranges for multiple species).
- Can eliminate false sight identifications in the field/confirm sight identifications.
- Can allow for exploring species interactions over time.
- Assessment of removal efforts/validate absence of species.
- Best tool for early detection (can use preexisting sequence data in GenBank/synthetic DNA for assay development).

Acknowledgments

- South Florida Water Management District
- University of Florida Invasion Science Research Institute

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Questions

Think Locally, Act neighborly

invasive species know no boundaries!