Rapid Communication

The first record of the Asian common toad *Duttaphrynus melanostictus* Schneider, 1799 in Abu Dhabi, United Arab Emirates

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Abstract

We report the first record of the Asian common toad *Duttaphrynus melanostictus* (Schneider, 1799) in Abu Dhabi emirate, United Arab Emirates and the Arabian Peninsula. A total of six individuals were collected in 2016 from habitat within and immediately surrounding the Al Wathba Wetland Reserve (AWWR), a wetland near Abu Dhabi city. The most likely introduction pathway is from accidental stowaways in containers and transport of plant products. Mitochondrial DNA (mtDNA) analysis confirmed the species identification and based on comparison with recent phylogeographic studies found the individuals fell within the south-east Asian mainland lineage comprising toads from Cambodia, China, Vietnam, Thailand and Northern Malaysia. The mtDNA haplotypes recovered from these individuals were novel and likely represent a previously unsampled populations from this lineage. A public awareness campaign was carried out with relevant stakeholders around the reserve and follow up surveys are being carried out since 2016 and no further individuals have been recorded during these surveys and it is assumed that this early intervention prevented *D. melanostictus* from establishing within the wetland.

Key words: alien species, introduced

Introduction

Native to Asia, the Asian common toad *Duttaphrynus melanostictus* (Schneider, 1799) has a widespread distribution throughout the South East Asia region, from Pakistan through to southern China and south into Indonesia (van Dijk et al. 2004).While many Asian amphibians have suffered declines linked to widespread deforestation across this region (Rowley et al. 2010), *D. melanostictus* has thrived, and is often found associated with human settlements and disturbed agricultural landscapes (Wogan et al. 2016). This commensal relationship with humans has facilitated the dispersal of *D. melanostictus* well beyond its native distribution. It has now established alien populations on several tropical islands, including Bali, where it was first recorded in the 1950s and has subsequently spread and established throughout Indonesia and New Guinea (Church 1960; Kraus 2009). Established alien populations are also

found on the Andaman and Nicobar Islands, and the species has also established in Timor Leste around 1999 (Kaiser et al. 2001; Trainor 2009; O'Shea et al. 2015). Outside of Asia *D. melanostictus* has established populations in the Maldives (Kraus 2009) and recently in Madagascar (Kolby et al. 2014; Moore et al. 2015). Individual stowaways are being increasingly recorded in countries such as South Africa, Australia and New Zealand (Kraus 2009; Measey et al. 2017) arriving via air and shipping pathways (Tingley et al. 2018) and where the species is considered a serious threat to biodiversity (Csurhes 2016; Tingley et al. 2018). Similar to the invasive cane toad *Rhinella marina*, *D. melanostictus* is a habitat and dietary generalist, highly fecund (females can produce up to 40,000 eggs per clutch) and secretes poison from glands on its back (Csurhes 2016; Marshall et al. 2018). This combination of life history traits means when *D. melanostictus* is introduced into a new environment it can survive in a diverse range of novel habitats, outcompete native species for food and potentially predate on them making it a highly successful and destructive invader (Kraus 2009). In fact, Measey et al. (2016) listed *D. melanostictus* as the third most scoring alien amphibian in the world considering environmental and economic impacts. The introduction of the most detrimental amphibian to Australia, the related *R. marina,* has resulted in devastating biodiversity losses and even with significant resources allocated to its control, e.g. more than AUD $20 million between 1986 to 2009 (Tingley et al. 2017), it continues its damaging spread across Australia. Similarly, it is feared that without serious intervention the same impacts could be seen in the mega diverse habitats of Madagascar with the recent introduction of *D. melanostictus* (Kolby et al. 2014; Moore et al. 2015; Marshall et al. 2018). Suspected to have arrived around 2010, a 2014 survey found it to have established around Madagascar's main port Toamasina with a surrounding range thought to be to be at least 108 km^2 (Moore et al. 2015), a follow up survey carried out by Licata et al. (2019) documented a fivefold expansion in the area occupied by this invader. A recent investigation by Marshall et al. (2018) also highlighted concerns that Malagasy predators would be vulnerable to the toxins secreted by *D. melanostictus* demonstrating the immediate need to invest in eradication of this species to avoid any similar threat to UAE's native biodiversity (Andreone 2014).

The documented detrimental impacts of *D. melanostictus* mean any novel record of this species requires swift confirmation and removal of individuals prior to population establishment. This study reports the first detection of the Asian common toad *Duttaphrynus melanostictus* in the United Arab Emirates (UAE), the surveys carried out to document the extent of the introduction, as well as the use of genetic analysis to confirm the species identity and compare to recently published data (Wogan et al. 2016) to narrow down the region form which these individuals may have originated.

Materials and methods

The United Arab Emirates (UAE) lies on the northern part of the Arabian Peninsula bordered by the Arabian Gulf to the north, Saudi Arabia to the west and south and Oman to the East with a landmass of approximately 83,000 km^2. The UAE is located in one of the world's most hyper-arid regions where summer temperatures exceed 50 °C and rainfall is extremely low with an average annual rainfall of 74.9 mm over 45 years (Sherif et al. 2014). Consequently, this region has very low amphibian diversity, with only two native species; the Arabian toad *Sclerophrys arabica* (Heyden, 1827) and Dhofar toad *Duttaphrynus dhufarensis* (Parker, 1931). These two species are distributed in the more mesic regions of the Hajar mountain chain and its drainage areas in Northern UAE and Oman.

Within the Abu Dhabi emirate there is a network of 13 terrestrial protected areas and one main wetland reserve known as the Al Wathba Wetland Reserve (AWWR) which is a ~ 5 km^2 site composed of a mosaic of habitats including; wetland, sand dunes and sand sheets with dwarf scrub cover. This site was listed on Ramsar in 2013 (www.rsis.ramsar.org/ris/2142) and in 2018 was nominated in the IUCN Green List of Protected and Conserved Areas (UNEP-WCMC 2018). The AWWR is comprised primarily of brackish water with some freshwater pools, and these are fed with excess treated water from a nearby sewage treatment plant. Bordering the wetlands are various industries and the most likely pathway is via contaminant on plants (transport of plant products) and through shipping containers (accidental stowaways in containers) as per the Convention on Biological Diversity (CBD) pathways categorization (Harrower et al. 2018).

In March 2016, the Environment Agency – Abu Dhabi (EAD) received a report that a suspected alien toad had been caught in the wetlands. There are no naturally occurring toads in this part of Abu Dhabi. As a result, survey methods designed to identify toads within the wetland were initiated. These surveys are carried out monthly in the winter months (November–March) and every two months during the summer months (April–October) and consist of nocturnal spotlight surveys along areas where the toads had been initially sighted, with monitoring of waterbodies for toad calls (this is more intensively carried out in the cooler winter months) and is ongoing. Diurnal surveys are carried out in heavily vegetated areas and wetland staff have been trained to monitor for toad/tadpole/spawn sightings as well as to identify mating calls.

Initial identification of the toads was carried out using online taxonomic keys and a photograph was circulated on the ALIENS mailing list of the IUCN Invasive Species Specialist Group. After preliminary identification indicated these individuals were alien and not either of the two amphibian species native to the UAE, all individuals were humanely euthanized via placing them in a fridge (3–4 °C). When no movement was detected after a

few hours the individuals were placed in the deep freeze section to freeze the animals (Shine et al. 2015). Tissue samples for genetic analysis were taken from these individuals and stored. Tissue sub-samples from 3 individuals were subsequently sent to the Australian Centre for Wildlife Genomics at the Australian Museum (Sydney, Australia) for DNA based species identification, as well as comparison with phylogeographic data generated by Wogan et al. (2016) to investigate if region of origin could be determined. DNA extractions were carried out using the Isolate II Genomic DNA kit (Bioline, Meridian BioScience) using the manufactures standard protocol for purifying total DNA from cultured cells and human or animal tissues. One region of mitochondrial DNA (mtDNA) which encompassed; partial cytochrome *c* oxidase subunit III gene, complete tRNA glycine, complete NADH dehydrogenase subunit 3 gene and partial tRNA arginine; and one nuclear region; the partial SOX9 gene were amplified by Polymerase Chain Reactions (PCRs). Primers and PCR conditions were as outlined in Wogan et al. (2016). PCR products were purified using ExoSAP-IT (Thermofisher Scientific) and Sanger sequencing was carried out on an AB3730xl Sequencer at the Australian Genome Research Facility, Sydney. Sequences were quality checked and edited with reference to chromatograms using Sequencher v 5.2.4 (Gene Codes Corporation, AnnArbor, MI, USA).

Phylogenetic placement of the Abu Dhabi samples was carried out with comparison to the data generated by Wogan et al. (2016) and was carried out by conducting a Bayesian Inference and Maximum likelihood analysis using the most appropriate model of evolution, determined using MEGA version 6, HKY+G. Bayesian inference was carried out using MrBayes 3.2.6 (Ronquist et al. 2012), Metropolis-Coupled Markov Chain Monte Carlo sampling was used to calculate posterior probability using default settings for priors. Two independent analyses ran simultaneously with four chains per run (1 cold, 3 hot), for 1 million generations sampling every 100 generations to obtain 10,000 sampled trees. Tracer 1.5 (Rambaut and Drummond 2009) was used to check for chain convergence and adequate Effective Sample Size (> 200). Maximum Likelihood was carried out using MEGA version 6 (Tamura et al. 2013).

A haplotype network of the south-east Asian mainland lineage only was performed using PopART (Leigh and Bryant 2015) using the statistical parsimony TCS method (Clement et al. 2002).

Results

In total six *Duttaphrynus melanostictus* (4 adults and 2 sub-adults, Figure 1) were identified and removed from along the northern part of the wetland reserve between March and October 2016. These toads were located within or just immediately outside the boundary fence (Figure 2). Surveys are ongoing,

Figure 1. Examples of the individuals removed from the Al Wathba Wetland Reserve. The first individual was collected on 5th March 2016 (left) and smaller juvenile was collected on the 25th July 2016 (right). Left photo by Ahmed Al Dhaheri, right photo by Pritpal Soorae.

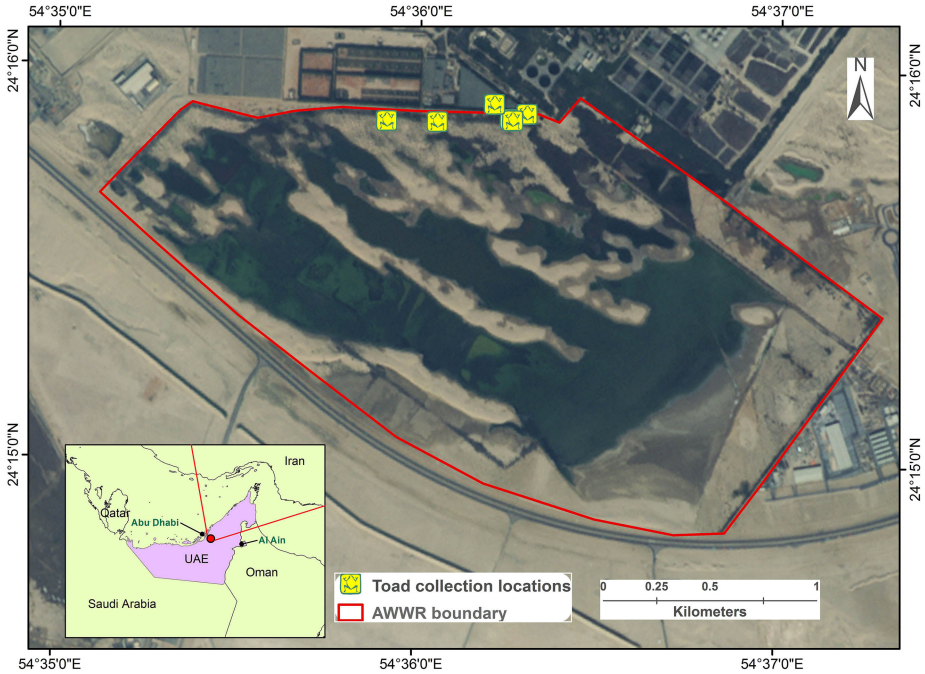

Figure 2. The location of the Al Wathba Wetland Reserve in Abu Dhabi emirate, UAE. The locations of where the toads were recorded are indicated along the northern boundary of the reserve.

but no new incursions have been identified since the initial six animals were removed.

DNA was successfully extracted from all samples, however due to degradation of the tissue samples only the 468 bps mtDNA fragment consistently amplified from all samples. Two mtDNA haplotypes were amplified from the three toads analyzed (Genbank accession numbers MN854049, MN854050), these two haplotypes only differed by 1 bp, which was confirmed by duplicate sequencing. The results of a BLAST query on Genbank returned *Duttaphrynus melanostictus* as the top 100 matches (83–100% query coverage; 95.50–99.57% Percent Identity), for all three samples. Many of these matches were to sequences generated from the vouchered *Duttaphrynus melanostictus* by Wogan et al. (2016), thus confirming

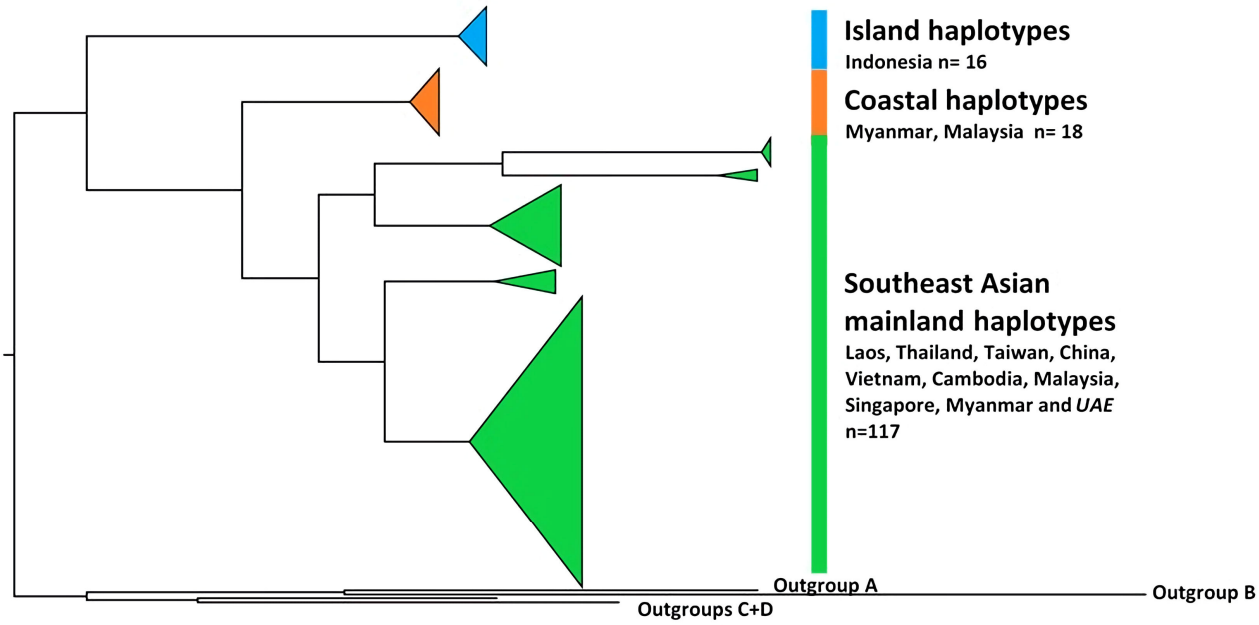

Figure 3. Phylogenetic tree based on Bayesian Inference of 468 bp of mitochondrial DNA, the haplotypes generated from the UAE *Duttaphrynus melanostictus* samples were compared to the ND3 sequences accessioned in Genbank by Wogan et at. 2016 (Genbank accession numbers KU183339–KU183490) and several outgroups A) *Phrynoidis asper* (KU183328), B) *Ingerophrynus macrotis* (KU183335), C) *Bufo pageoti* (KU183330) and D) *Duttaphrynus crocus* (KU183333).

the species identification determined via taxonomic keys and online resources. Bayesian Inference and Maximum Likelihood analyses were carried out using the mtDNA data generated in this study with comparison to the "ND3" data generated by Wogan et al. (2016). Both Bayesian Inference and Maximum Likelihood analysis recovered trees with similar topology as each other and as with the larger mtDNA and nuclear DNA dataset used by Wogan et al. (2016) albeit with lower resolution between the lineages. The haplotypes of the Abu Dhabi toads clustered with the lineage identified by Wogan et al. (2016) as being widely distributed across the south-east Asian mainland encompassing toads collected from Cambodia, China, Laos, Thailand, Vietnam, and some locations of eastern Myanmar and northern Malaysia (see Figure 3). The haplotype sequences recovered from Abu Dhabi toads were not an identical match with any previously published haplotypes, however based on the phylogenetic trees and haplotype network clustered with haplotypes primarily found within Thailand, and one from Laos, suggesting they may be from populations that has yet to be sampled within this mainland lineage within Thailand or Laos (see Figure 4).

Discussion

These records of *Duttaphrynus melanostictus* from Abu Dhabi, UAE, established via morphological and molecular identification, are the first confirmed record for this species from the Arabian Peninsula. Given there are no known established populations of *D. melanostictus* that could naturally disperse to the UAE, and the fact this species is human commensal and often documented dispersing as a stowaway via air and shipping pathways

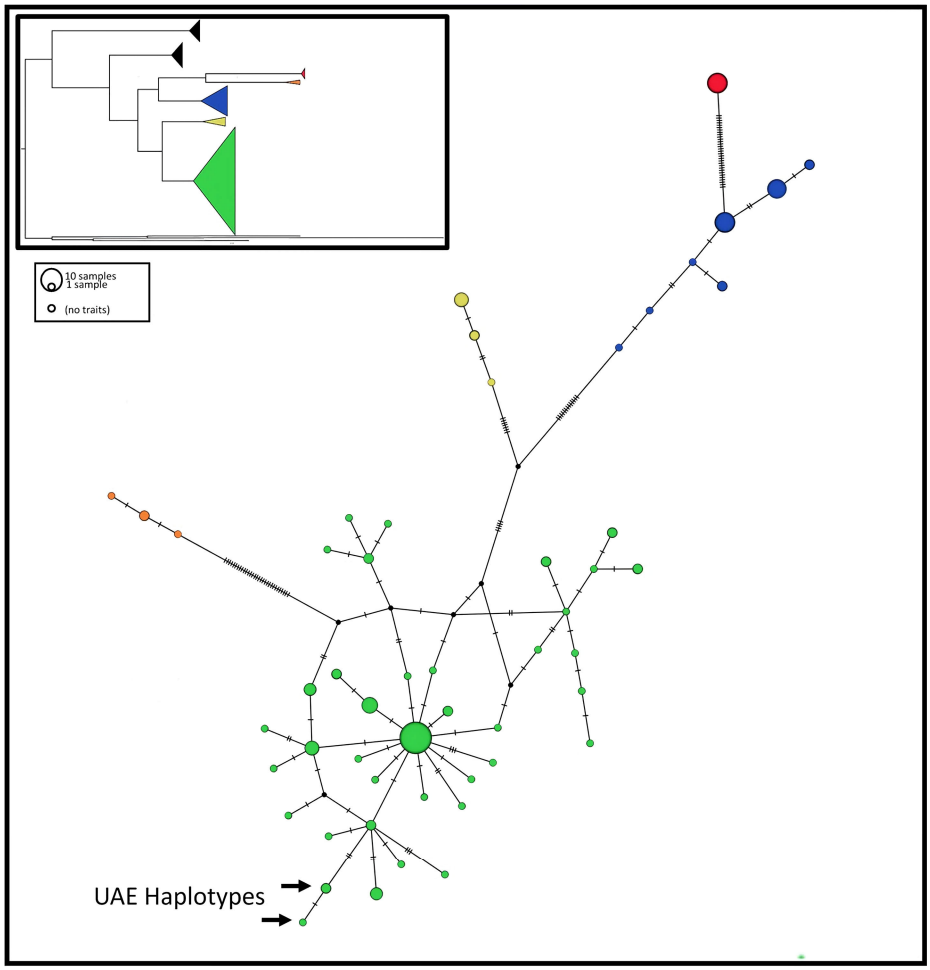

Figure 4. A statistical parsimony TCS method haplotype network of the south-east Asian mainland lineage only. Haplotypes are colored based on the sub-lineages within the south-east Asian mainland (as per the phylogenetic tree insert), red: haplotypes from Singapore and Malaysia; blue: Myanmar; yellow; China, Myanmar and Laos; orange: Myanmar; green; Laos, Thailand, Taiwan, China, Vietnam, Cambodia, Malaysia and UAE.

(Tingley et al. 2018) these individuals most likely to have arrived in the UAE via these pathways as well. The proximity to nearby commercial industries was the most likely pathway, and these were identified as accidental stowaways in containers and through the transport of plant products (Harrower et al. 2018). The individual *D. melanostictus* then subsequently spread into the AWWR and from the locations they were recorded as shown in Figure 2. Two important factors leading to establishment of alien species in a novel environment are propagule pressure (the number of individuals introduced) and environmental suitability (Hayes and Barry 2008; Tingley et al. 2018). Recent species distribution modelling by Tingley et al. (2018) did not identify the UAE as environmentally suitable for the establishment *D. melanostictus*, whereas other regions of recent invasion, e.g. Madagascar were identified as highly environmentally suitable. Despite this, it is of concern that these individuals were transported unintentionally to one of the few areas in the emirate that could possibly support a population of *D. melanostictus* where water bodies and wetland habitats are present year round.

If *D. melanostictus* were to establish in the AWWR, it could be highly detrimental to some of the species known to inhabit the wetlands, in particular, the invertebrate fauna (Soorae et al. 2019). The species could also possibly spread from AWWR via roadside vegetation, farms or human mediated methods to the city of Al Ain. Al Ain is located on the border with Oman and next to the Hajar Mountain range where environmental conditions are suitable to support the two native amphibian species found in the region, the Arabian toad *Sclerophrys arabica* and Dhofar toad *Duttaphrynus dhufarensis*. As it has been documented in other regions of invasion, *D. melanostictus* could outcompete these native species, both for food and during the breeding season. In some cases, direct predation has been observed where *D. melanostictus* tadpoles have been recorded opportunistically feeding on other tadpoles (Mahapatra et al. 2017, thus endangering any other native species who predate on amphibians (Marshall et al. 2018). Although it is currently unknown whether *D. melanostictus* can establish viable alien populations in these regions in the long term however is unknown, the Dhofar toad. *D. dhufarensis,* is able to aestivate for two years or more to avoid extreme drought conditions in these regions (Soorae et al. 2013), and while *D. melanostictus* can hibernate to avoid temperature extremes, doing so in such extreme aridity has not been documented.

Our molecular analysis carried out identified two haplotypes from the three individuals sequenced, these haplotypes corresponded to the mainland south-east Asian lineage identified by Wogan et al. (2016). This is a widely distributed lineage throughout south-east Asia, with five distinct sub-lineages within it (Figure 3), and while the haplotypes in this study did not exactly match any of the previously sequenced haplotypes, they were most closely related to haplotypes that were found in Thailand, and one from Laos (Figure 4). Our sequence data did fall within the same south-east Asian mainland sub-lineage as sequence data from the recently established population in Madagascar (data not shown). Vences et al. (2017) suggested the Malagasy population originated from either Cambodia or Southern Vietnam, possibly being transported via the major seaport of Ho Chi Minh City. The closer placement of our sequence data to haplotypes that were found in Thailand suggest a potentially different origin of invasion. Thailand has previously been identified by Tingley et al. (2018) as one of the primary origins of stowaway toads entering Australia, in particular via air shipments, and is a likely country of origin for some of the imports arriving at the commercial industry neighboring AWWR.

Rapidly identifying and controlling alien species prior to population establishment is crucial to avoid any significant biodiversity impacts. As regular surveys are being conducted in the areas where *D. melanostictus* were initially recorded, we are confident that *D. melanostictus* has been eradicated, as no further sightings have been recorded. The agency has also

undertaken a public awareness program by educating AWWR personnel in how to visually identify *D. melanostictus* and instigated reporting of any toad signs/sightings to allow for immediate control measures to be put in place by the EAD. Finally, as part of wider alien species monitoring being carried out by EAD, the agency will focus on future awareness programs and management on potential introduction pathways e.g. where cargo imports and movements occur including live plant nurseries and construction equipment imports.

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References

Andreone F (2014) Madagascar: Risk review is under way for invasive toad. *Nature* 512: 253, https://doi.org/10.1038/512253c

Church G (1960) The invasion of Bali by *Bufo melanostictus. Herpetologica* 16(1): 15–21

Clement M, Snell Q, Walker P, Posada D, Crandall K (2002) TCS: estimating gene genealogies. *IPDPS* 2: 184, https://doi.org/10.1109/IPDPS.2002.1016585

Csurhes S (2016) Invasive animal risk assessment - Asian spined toad, *Bufo melanostictus*. Department of Agriculture and Fisheries, Biosecurity Queensland, 17 pp

Harrower CA, Scalera R, Pagad S, Schonrogge K, Roy HE (2018) Guidance for interpretation of CBD categories on introduction pathways. CEH Project no. C06225. European Commision, 100 pp

Hayes KR, Barry, SC (2008) Are there any consistent predictors of invasion success? Biological Invasions 10: 483–506, https://doi.org/10.1007/s10530-007-9146-5

Kaiser H, Carvalho VL, Ceballos J, Freed P, Heacox S, Lester B, Richards SJ, Trainor CR, Sanchez C, O'Shea M (2001) The herpetofauna of Timor-Leste: A first report. *Zookeys* 109: 19–86, https://doi.org/10.3897/zookeys.109.1439

Kolby JE, Kraus F, Rabemananjara F, Rabesihanaka S, Rabibisoa N, Rafanomezantsoa J (2014) Stop Madagascar's toad invasion now. *Nature* 509: 563–563, https://doi.org/10.1038/509563a

Kraus F (2009) Alien reptiles and amphibians: a scientific compendium and analysis. Vol. 4. Springer Science & Business Media, 563 pp, https://doi.org/10.1007/978-1-4020-8946-6

Leigh JW, Bryant D (2015) PopART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 6: 1110–1116, https://doi.org/10.1111/2041-210X.12410

Licata F, Ficetola GF, Freeman K, Mahasoa RH, Ravololonarivo V, Fidy, JFSN, Koto-Jean AB, Nahavitatsara ER, Andreone F, Crottini A (2019) Abundance, distribution and spread of the invasive Asian toad *Duttaphrynus melanostictus* in eastern Madagascar. *Biological Invasions* 21: 1615–1626, https://doi.org/10.1007/s10530-019-01920-2

Mahapatra S, Dutta SK, Sahoo G (2017) Opportunistic predatory behaviour in *Duttaphrynus melanostictus* (Schneider, 1799) tadpoles. *Current Science* 112: 1755–1759, https://doi.org/10.18520/cs/v112/i08/1755-1759

Marshall BM, Casewell NR, Vences M, Glaw F, Andreone F, Rakotoarison A, Zancolli G, Woog F, Wüster, W (2018) Widespread vulnerability of Malagasy predators to the toxins of an introduced toad. *Current Biology* 28: R654–R655, https://doi.org/10.1016/j.cub.2018.04.024

Measey GJ, Vimercati G, De Villiers FA, Mokhatla M, Davies SJ, Thorp CJ, Rebelo AD, Kumschick S (2016) A global assessment of alien amphibian impacts in a formal framework. *Diversity and Distributions* 22: 970–981, https://doi.org/10.1111/ddi.12462

Measey J, Davies SJ, Vimercati G, Rebelo A, Schmidt W, Turner A (2017) Invasive amphibians in southern Africa: A review of invasion pathways. *Bothalia-African Biodiversity & Conservation* 47: 1–12, https://doi.org/10.4102/abc.v47i2.2117

Moore M, Solofo Niaina Fidy FJ, Edmonds D (2015) The new toad in town: Distribution of the Asian toad, *Duttaphrynus melanostictus*, in the Toamasina area of eastern Madagascar. *Tropical Conservation Science* 8: 440–455, https://doi.org/10.1177/194008291500800210

O'Shea M, Sanchez C, Kathriner A, Mecke S, Lopes Carvalho V, Varela Ribeiro A, Afranio Soares Z, de Lemos Araujo L, Kaiser H (2015) Herpetological diversity of Timor-Leste: updates and a review of species distributions. *Asian Herpetological Research* 6(2): 73–131

Rambaut A, Drummond AJ (2009) Tracer: MCMC trace analysis tool. 1.5 ed. Edinburgh: Institute of Evolutionary Biology. http://tree.bio.ed.ac.uk/software/tracer/

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542, https://doi.org/10.1093/sysbio/sys029

Rowley JJL, Brown R, Bain R, Kusrini M, Inger R, Stuart B, Wogan G, Thy N, Chan-Ard T, Cao TT, Diesmos A (2010) Impending conservation crisis for Southeast Asian amphibians. *Biology Letters* 6: 336–338, https://doi.org/10.1098/rsbl.2009.0793

Sherif M, Almulla M, Shetty A, Chowdury, RK (2014) Analysis of rainfall, PMP and drought in the United Arab Emirates. *International Journal of Climatology* 34: 1318–1328, https://doi.org/10.1002/joc.3768

Shine R, Amiel J, Munn AJ, Stewart M, Vyssotski AL, Lesku JA (2015) Is "cooling then freezing" a humane way to kill amphibians and reptiles? *Biology Open* 4: 760–763, https://doi.org/10.1242/bio.012179

Soorae PS, Els J, Gardner D, El Alqamy H (2013) Distribution and ecology of the Arabian and Dhofar Toads (*Duttaphrynus arabicus* and *D. dhufarensis*) in the United Arab Emirates and adjacent areas of northern Oman. *Zoology in the Middle East* 59: 229–234, https://doi.org/10.1080/09397140.2013.841428

Soorae P, Sakkir S, Saji A, Khan SB, Al Zaabi R, Shah JN, Ali A, Al Omari K, Al Dhaheri A, Javed S, Tubati SR, Ahmed S, Alrashidi ZA, Al Dhaheri, S (2019) A Review of the Flora and Fauna in the Al Wathba Wetland Reserve in Abu Dhabi, United Arab Emirates. *Wetlands*, https://doi.org/10.1007/s13157-019-01235-x

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729, https://doi.org/10.1093/molbev/mst197

Tingley R, Ward-Fear G, Schwarzkopf L, Greenlees MJ, Phillips BL, Brown G, Clulow S, Webb J, Capon R, Sheppard A, Strive T (2017) New weapons in the Toad Toolkit: a review of methods to control and mitigate the biodiversity impacts of invasive cane toads (*Rhinella marina*). *The Quarterly Review of Biology* 92: 123–149, https://doi.org/10.1086/692167

Tingley R, García-Díaz P, Arantes CRR, Cassey P (2018) Integrating transport pressure data and species distribution models to estimate invasion risk for alien stowaways. *Ecography* 41: 635–646, https://doi.org/10.1111/ecog.02841

Trainor CR (2009) Survey of a population of black-spined toad *Bufo melanostictus* in Timor-Leste: confirming identity, distribution, abundance and impacts of an invasive and toxic toad. Darwin, Australia: Charles Darwin University, 50 pp, https://core.ac.uk/display/47196141

UNEP-WCMC (2018) Protected Area Profile for Al Wathba from the World Database of Protected Areas. www.protectedplanet.net (accessed December 2018)

van Dijk PP, Iskandar D, Lau MWN, Huiqing G, Baorong G, Kuangyang L, Wenhao C, Zhigang Y, Chan B, Dutta S, Inger RF, Manamendra-Arachchi K, Khan, MS (2004) *Duttaphrynus melanostictus* (errata version published in 2016). Online Database of The IUCN Red List of Threatened Species 2004: e.T54707A86445591 (accessed 20 May 2018), https://doi.org/10.2305/IUCN.UK.2004.RLTS.T54707A11188511.en

van Dijk PP, Iskandar D, Lau MWN, Huiqing G, Baorong G, Kuangyang L, Wenhao C, Zhigang Y, Chan B, Dutta S, Inger RF, Manamendra-Arachchi K, Khan MS (2004) *Duttaphrynus melanostictus* (errata version published in 2016). IUCN Red List of Threatened Species 2016: e.T54707A86445591. www.iucnredlist.org

Vences M, Brown JL, Lathrop A, Rosa GM, Cameron A, Crottini A, Dolch R, Edmonds D, Freeman KL, Glaw F, Grismer LL (2017) Tracing a toad invasion: lack of mitochondrial DNA variation, haplotype origins, and potential distribution of introduced *Duttaphrynus melanostictus* in Madagascar. *Amphibia-Reptilia* 38: 197–207, https://doi.org/10.1163/15685381-00003104

Wogan GO, Stuart BL, Iskandar DT, McGuire JA (2016) Deep genetic structure and ecological divergence in a widespread human commensal toad. *Biology Letters* 12: 20150807, https://doi.org/10.1098/rsbl.2015.0807