

Tracing a toad invasion: lack of mitochondrial DNA variation, haplotype origins, and potential distribution of introduced *Duttaphrynus melanostictus* in Madagascar

Vences, Miguel; Brown, Jason L.; Lathrop, Amy; Gonçalo, Rosa M.; Cameron, Alison; Crottini, Angelica; Dolch, Rainer; Edmonds, Devin; Freeman, Karen L.M.; Glaw, Frank; Grismer, Lee; Litvinchuk, Spartak; Milne, Margaret M.; Moore, Maya; Solofo, Jean F; Noël, Jean; Nguyen, Truong Q.; Ohler, Annemarie; Randrianantoandro, Christian; Raselimanana, Achille P. ; van Leeuwen, Pauline; Wogan, Guinevere O.U.; Ziegler, Thomas; Andreone, Franco; Murphy, Robert W.

Amphibia-Reptilia

DOI:
[10.1163/15685381-00003104](https://doi.org/10.1163/15685381-00003104)

Published: 01/01/2017

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):

Vences, M., Brown, J. L., Lathrop, A., Gonçalo, R. M., Cameron, A., Crottini, A., Dolch, R., Edmonds, D., Freeman, K. L. M., Glaw, F., Grismer, L., Litvinchuk, S., Milne, M. M., Moore, M., Solofo, J. F., Noël, J., Nguyen, T. Q., Ohler, A., Randrianantoandro, C., ... Murphy, R. W. (2017). Tracing a toad invasion: lack of mitochondrial DNA variation, haplotype origins, and potential distribution of introduced *Duttaphrynus melanostictus* in Madagascar. *Amphibia-Reptilia*, 38(2), 197–207 . <https://doi.org/10.1163/15685381-00003104>

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3 Tracing a toad invasion: lack of mitochondrial DNA variation, 50
4 haplotype origins, and potential distribution of introduced 51
5 *Duttaphrynus melanostictus* in Madagascar 52
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17 **Abstract.** The black-spined toad, *Duttaphrynus melanostictus*, is widespread in South and South-East (SE) Asia, although
18 recent molecular analyses have revealed that it represents a species complex (here called the *D. melanostictus* complex).
19 Invasive populations of this toad have been detected in Madagascar since at least 2014. We here trace the origin of this
20 introduction based on mitochondrial DNA sequences of 340 samples. All 102 specimens from Madagascar have identical
21 sequences pointing to a single introduction event. Their haplotype corresponds to a lineage occurring in Cambodia, China,
22 Laos, Thailand, Vietnam, and some locations of eastern Myanmar and northern Malaysia, here named the SE Asian lineage.
23 Within this lineage, specimens from one location in Cambodia and three locations in Vietnam have the same haplotype as
24 found in Madagascar. This includes Ho-Chi-Minh-City, which has a major seaport and might have been the source for the
25 introduction. Species distribution models suggest that the current range of the Madagascan invasive population is within
26 the bioclimatic space occupied by the SE Asian lineage in its native range. The potential invasion zone in Madagascar is
27 narrower than suggested by models from localities representing the full range of the *D. melanostictus* complex. Thus, an
28 accurate taxonomy is essential for such inferences, but it remains uncertain if the toad might be able to spread beyond the
29 potential suitable range because (1) knowledge on species-delimitation of the complex is insufficient, and (2) the native range
30 in SE Asia might be influenced by historical biogeography or competition.
31 74

32 **Keywords:** amphibia, Anura, black-spined toad, Cambodia, invasive species, Madagascar, Maxent, mitochondrial DNA, risk
33 assessment, species distribution model, Vietnam.
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1 Introduction

2 Invasive, allochthonous species play a major role
3 in the global biodiversity conservation crisis
4 (Clavero and Garcia-Berthou, 2005; Crowl et
5 al., 2008; Keller et al., 2011; Simberloff et al.,
6 2013). The introduction of non-native species
7 to naïve environments has led to the extinction
8 of many native taxa, especially on islands
9 (Reaser et al., 2007). Impacts have involved
10 direct competition and predation, as well as
11 the indirect consequence of non-natives serving
12 as vectors of new parasites and infectious dis-
13 eases (Conn, 2014). Although amphibians are
14 not generally ranked among the most hazardous
15 invasives (Hatcher et al., 2012; Roy, 2016), in
16 some cases the introduction of a non-native am-
17 phibian has harmed endemic faunas. The most
18 prominent case is the cane toad, *Rhinella ma-*
19 *rina*, which still endangers endemic amphibians
20 and possible predators in Australia, although its
21 effects on the native fauna are mixed and not al-
22 ways detrimental (Shine, 2010).

23 Another representative of the true toads (fam-
24 ily Bufonidae), the black-spined toad (*Dut-*
25 *taphrynus melanostictus*), is also an invasive
26 species. According to current taxonomy, this
27 toad is widespread in South and South-East
28 (SE) Asia (AmphibiaWeb, 2016) and has been
29 introduced to Borneo, Sulawesi and Seram, and
30 additional Indonesian islands (Church, 1960;
31 Inger and Stuebing, 2005; Wogan et al., 2016).

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48 It has also been introduced to Madagascar
49 where its presence has been recorded since
50 2014, but might date back at least to 2010 (An-
51 dreone et al., 2014; Crottini et al., 2014; Kolby,
52 2014; Kull et al., 2014; Moore et al., 2015).
53 Recent molecular analyses (Hasan et al., 2014;
54 Wogan et al., 2016) have revealed a deep ge-
55 netic structure within *D. melanostictus*, suggest-
56 ing that this species is, in fact, a largely un-
57 resolved complex of several allopatric species
58 (herein termed the *D. melanostictus* complex).
59 As the various species of this complex inhabit
60 distinct bioclimatic niches, their potentials of
61 invasion likely differ. Thus, understanding the
62 identity and source of invasive populations is
63 critical for risk assessment and management.

64 The introduction of *D. melanostictus* to
65 Madagascar has received much attention from
66 the conservation community because it may
67 constitute a serious threat to the unique biota
68 of this island (Andreone et al., 2014; Crottini et al.,
69 2014; Kolby, 2014; Moore et al., 2015; Pear-
70 son, 2015). Madagascar harbors a unique na-
71 tive amphibian fauna, with over 300 described
72 species in four families (Hyperoliidae, Mantel-
73 lidae, Microhylidae, Ptychadenidae) and 100%
74 endemism at the level of native species (Glaw
75 and Vences, 2007; AmphibiaWeb, 2016). Cur-
76 rently, habitat destruction is the main factor
77 threatening numerous species of Madagascan
78 frogs (Andreone et al., 2005). The amphibian
79 chytrid fungus has been recorded from a small
80 proportion of specimens (Bletz et al., 2015), yet
81 no known disease-related declines like those af-
82 fecting amphibians in other parts of the globe
83 are known (Berger et al., 1998; Vredenburg et
84 al., 2010; Fisher et al., 2012). Threats to the
85 unique Madagascan amphibian fauna through
86 the introduction of foreign pathogens via in-
87 vasive toads, as well as the threats of com-
88 petition or predation, are of serious concern
89 (Brown et al., 2016). Competition for reproduc-
90 tion sites seems to be an issue as well, given
91 that this species has a high rate of fecundity
92 with up to nine thousand eggs per clutch (Cai,
93 1979; Van Leeuwen, personal observation; sup-
94

plementary table S1). Further, it uses all available water bodies including ponds and streams (Ngo and Ngo, 2013) that native amphibians usually inhabit. Given the toxicity of the secretions of bufonid toads, *D. melanostictus* might also pose threats to native Madagascan predators, including the many frog-eating snakes, birds and mammals (Glaw and Vences, 2007). As far as known, this invasion involves the area around the city of Toamasina only (Moore et al., 2015), but further spread is to be expected as the species has already been observed among bananas transported by trucks (R. Dolch, personal observation).

The current risk assessment is based on knowledge from the complete distribution area of the *D. melanostictus* complex (e.g., McClelland et al., 2015; Moore et al., 2015), and the available species distribution model (Pearson, 2015) estimates the potential distribution range in Madagascar using records from the entire range of the complex. For a more precise understanding of the geographical invasive potential of the species, it is crucial to identify the source lineage (species) of the Madagascan invasion and then to base invasion predictions on knowledge of its ecological requirements.

Herein, we identify the origins and potential spread of *D. melanostictus* in Madagascar as part of the risk assessment of this toad invasion. We (1) use mitochondrial DNA (mtDNA) sequences to assess the extent of genetic variation of the toad in Madagascar and exclude the possibility of independent introductions from multiple sources, (2) compare the mtDNA data with an expanded data set of homologous sequences from SE Asia to identify the origin of the introduction, and (3) apply species distribution models to assess the bioclimatically suitable range and the invasive potential of the toad in Madagascar.

Methods

Sample collection

We analysed DNA sequences of 340 samples of *Dutaphrynus melanostictus*. We first performed an exploratory

phylogenetic analysis (supplementary fig. S1) of some sequences of Madagascan samples combined with the comprehensive set of sequences of Wogan et al. (2016), plus a comparison with a few sequences from India. Together these samples were representative of a large portion of the entire range of the *D. melanostictus* complex. The Madagascan samples were nested within a particular mitochondrial clade, marked in green color in the figures of Wogan et al. (2016). Within this clade, the Madagascan samples belonged to a major subclade occurring in SE Asia, which we herein call the ‘SE Asian lineage’ and on which we focused further sampling and analysis. The rationale of restricting the analysis to this subclade is given in supplementary fig. S1.

Our sampling of the SE Asian lineage included 81 samples from Wogan et al. (2016), one additional sequence (without precise locality) from GenBank, and 258 newly generated sequences. The new sequences were obtained from samples collected in Cambodia (9 samples), China and Taiwan (19), Malaysia (1), Thailand (4), Vietnam (123), and Madagascar (102). Most of the Madagascan samples (100) were collected at eight localities in the vicinity of the port and city of Toamasina, which represented most of the current range of the species in March 2015. Two additional samples came from the same general area but with no precise locality information.

Molecular analyses

DNA was extracted from tissue samples or buccal swabs using a standard salt protocol (Bruford et al., 1992). We amplified an approximately 480 bp fragment of mitochondrial DNA that encodes part of the genes encoding complete cytochrome oxidase c subunit III (*COIII*), tRNA glycine (tRNA-Gly), NADH dehydrogenase subunit 3 (*ND3*), and part of tRNA arginine by PCR (94°C 45 s, 49°C 30 s, 72°C 1 min) for 35 cycles, using the primer pair L-COXIII (5′-CCGCATGATACTGACACTT-3′) and Arg-HND3III (5′-AACTGTCTTTTGGACTAGC-3′) of Stuart et al. (2006). Chromatograms were checked and sequences corrected where necessary by using CodonCode Aligner (CodonCode Corp.). Alignment used MEGA7 (Kumar et al., 2016). After trimming sequences on both sides, we retained 347 bp corresponding to 69 bp of tRNA-Gly and 278 bp of *ND3*; this fragment was subsequently referred to as ND3. All new sequences were submitted to GenBank (accession numbers KY823030-KY823289; see supplementary table S2 for a detailed list). We reconstructed a maximum-likelihood tree in MEGA 7 under a general-time-reversible substitution model with gamma-shape parameter and a proportion of invariable sites, and used the obtained tree as a basis for building a haplotype network in HaploViewer (Salzburger et al., 2011).

Species distribution models

Species distribution models (SDMs) were generated in MaxEnt 3.3.3k (Phillips et al., 2006), as implemented in SDMtoolbox 1.1c (Brown, 2014). We used museum records (downloaded from GBIF.org), data from collaborators, and

1 data from our own fieldwork to compile occurrence records,
 2 which then were vetted for spatial and taxonomic accuracy.
 3 The list of GBIF localities was curated and we excluded
 4 localities outside the known range of the species or with-
 5 out precise coordinates, and pre-1950 records without re-
 6 cent confirmation. We randomly selected one of multiple
 7 occurrence records per species within a 10-km radius using
 8 SDMtoolbox (Brown, 2014). All models used the 19 stan-
 9 dard bioclimatic variables representing spatial patterns of
 10 precipitation and temperature, at a 30 arc-second resolution
 11 (available at www.worldclim.org) (Hijmans et al., 2005;
 12 supplementary table S3). Models were built with occurrence
 13 points based on two datasets: (1) a large portion of the en-
 14 tire range of *D. melanostictus* (comprising various candidate
 15 species; Wogan et al., 2016) and (2) a subset consisting of
 16 the spatial locations of members of the SE Asian lineage, in-
 17 cluding only genetically verified occurrence records. Back-
 18 ground points were sampled from an adaptive convex hull
 19 with a 100-km buffer drawn around the occurrence local-
 20 ities ($\alpha = 3$, done in SDMtoolbox). The final dataset for
 21 species distribution modelling was composed of 324 and 90
 22 records for the full dataset and the SE Asian lineage dataset,
 23 respectively.

24 To parameterize the SDMs, we evaluated the perform-
 25 ance of various combinations of five feature classes (lin-
 26 ear; linear and quadratic; hinge; linear, quadratic and hinge;
 27 and linear, quadratic, hinge, product and threshold), and 10
 28 regularization multipliers (from 0.5 to 5, in increments of
 29 0.5) (Shcheglovitova and Anderson, 2013). We evaluated
 30 the performance of SDMs built under each combination
 31 of model parameters through a geographically structured
 32 k-fold cross-validation. Thus, the occurrence records were
 33 partitioned into k-equal geographically clustered subsam-
 34 ples, where $k = 3$, and the models were trained with two of
 35 the groups and then evaluated with the excluded group un-
 36 til all group combinations were run. Model fit was assessed
 37 through measurement of the omission rate, area under the
 38 curve (AUC), and model feature class complexity (Brown,
 39 2014). After optimum model parameters were determined
 40 (those leading to the lowest omission rate, highest AUC,
 41 and lowest complexity, in the order listed), a final SDM was
 42 built with all occurrence sites. The best-fit SDM built on
 43 the entire dataset was parameterized with a linear feature-
 44 class and a regularization multiplier of 3. The best-fit model
 45 for the SE Asian lineage dataset was parameterized with a
 46 regularization multiplier of 3 and linear, quadratic, hinge,
 47 product and threshold features classes. The best resulting
 SDMs for the full dataset and SE Asian lineage dataset (of
 150 SDMs with alternative parameter combinations for each
 dataset) showed high predictive accuracy (average omission
 rates 0.211 and 0.087, respectively) and average AUCs of
 0.987 and 0.767, respectively.

42 *Assessing shared climate space in different parts of the* 43 *species' distribution*

44 We measured shared climate space using the method and R
 45 scripts of Broennimann et al. (2012). This entailed gener-
 46 ating principal component analyses of all climate variables
 47 from study areas and corresponding occurrence localities.

Specifically, we plotted the climate space occupied by three
 groups of *D. melanostictus*: (1) all known localities, (2) lo-
 calities of the SE Asian lineage in its native (Asian) range,
 and (3) Madagascar localities of the SE Asian lineage. In
 addition, we characterized the climate space of two study ar-
 eas: the entire climate space of Madagascar and the climate
 space accessible to all known localities of *D. melanostic-
 tus* (light grey outer shape in fig. 2D-F) depicted by climate
 within a 200-km buffer drawn around an adaptive convex-
 hull (ACH) of all the occurrence localities ($\alpha = 3$, done in
 SDMtoolbox), which resulted in separate buffered polygons
 for localities in Madagascar and SE Asian localities. For the
 three groups of *D. melanostictus*, we calculated Warren's
 D statistic and tested for niche equivalency (n of sims =
 100) and niche similarity (n of sims = 1000) among groups
 to assess how the three groups shared climate. Warren's D
 statistic was used to assess an overall match between the
 occupied climate space and to determine if an inference of
 the climate characteristics of one distribution could have
 been made from the other. The niche equivalency measure
 statistically tested if the climate space occupied among the
 two groups' ranges were equivalent in terms of specific cli-
 mate space and total breadth of climates shared by each
 distribution. Lastly, the niche similarity tested if the over-
 lap between two ranges differed from the climate space in
 one range and the climate space selected at random from
 the other range. The niche similarity test addressed whether
 or not the environmental space occupied in one range was
 more similar to the one occupied in the other range than
 would be expected by chance. If the value was significant
 this meant a majority of the climate space occupied in one
 range was identical to the climate space, or a subset of it,
 occupied by the other distribution. This directional test was
 performed in both ways.

48 **Results**

49 All new sequences of ND3 from the target re-
 50 gion in SE Asia belonged to the SE Asian lin-
 51 eage of *D. melanostictus*. The haplotype net-
 52 work for the 340 sequences of this lineage
 53 (fig. 1) contained 63 distinct haplotypes. For
 54 convenience and easier graphical representa-
 55 tion, we divided these into *ad hoc* haplogroups
 56 H1-H4, which were colored differently in fig. 1.
 57 These main haplogroups were differentiated
 58 from each other by at least three mutational
 59 steps, but H4 (blue) was more distinct, differing
 60 by at least 14 steps from all other haplogroups.
 61 All 102 samples from Madagascar had identi-
 62 cal sequences and we assigned them to a unique
 63 subcategory H1a, although they differed by only
 64 a single mutational step from the most common
 65 haplotype in H1.

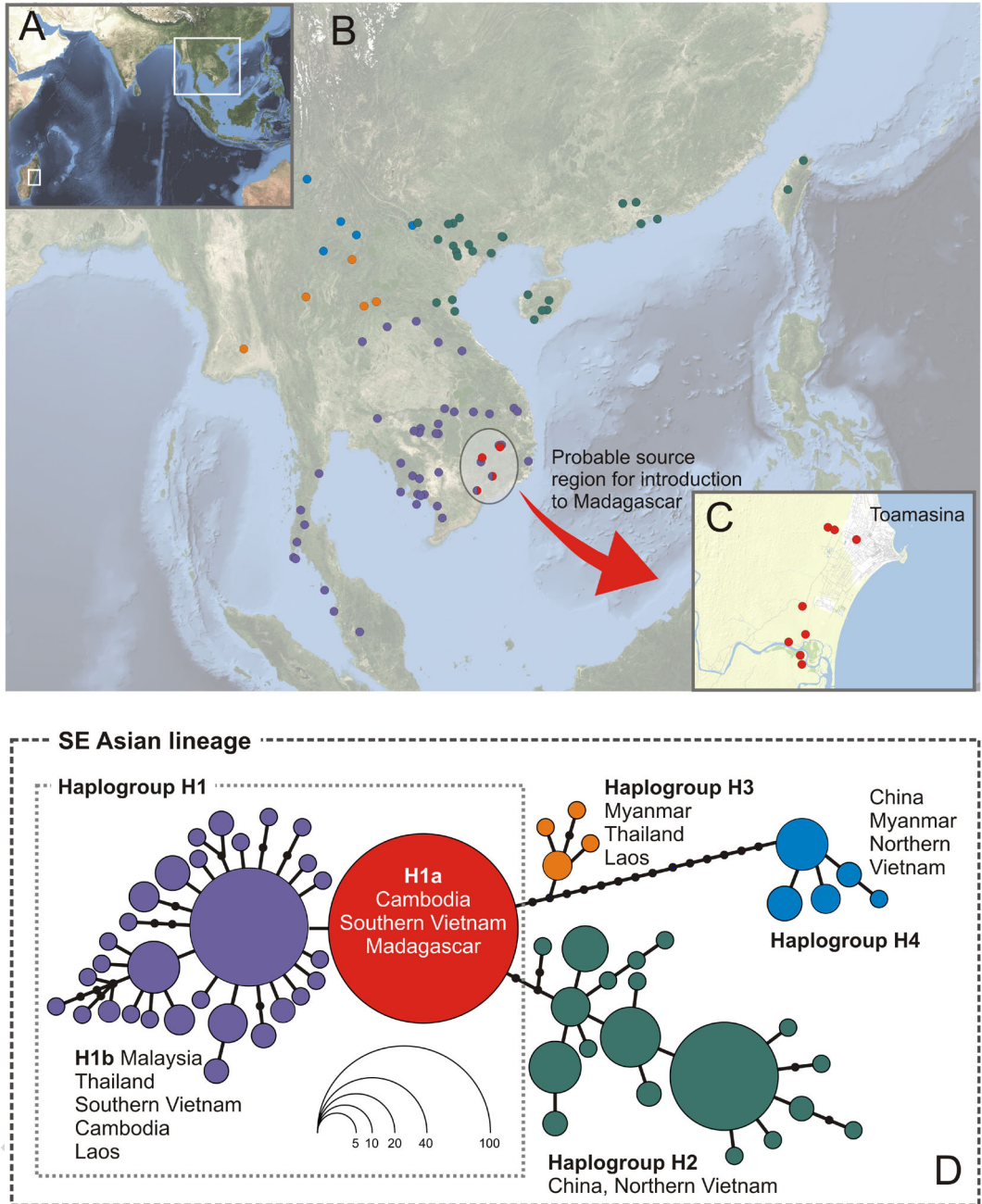


Figure 1. Sampling localities and mitochondrial haplotype network of *Duttaphrynus melanostictus*. (A) Overview map showing in white rectangles the areas highlighted in map (B) showing South-East Asia, and map (C) showing a part of eastern Madagascar where the invasive toad is currently present. Dots on the map are collection localities for samples used in the genetic analysis. Colors of the dots correspond to the haplotype network (D). The network was reconstructed from 347 bp of the mitochondrial region encoding ND3 from 340 samples, all corresponding to a single mitochondrial subclade of Wogan et al. (2016), herein called the SE Asian lineage. Haplogroups H1-H4, including subcategories H1a and H1b, belong to the SE Asian lineage; they were *ad hoc* defined for convenience, and do not correspond to equally differentiated units. The circle in map B defines the area in Cambodia and Vietnam where specimens with H1a were found, which is the only haplotype detected in Madagascar.

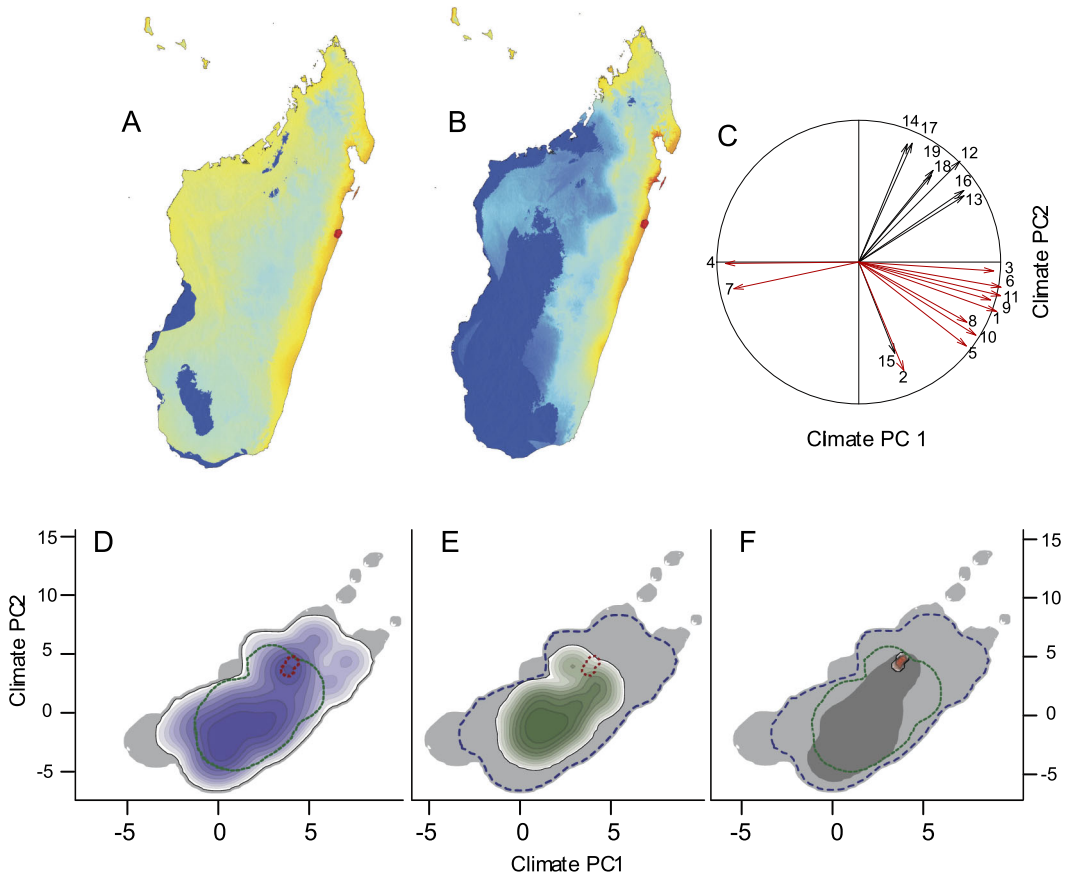


Figure 2. Potential distribution of *Duttaphrynus melanostictus* in Madagascar as predicted by species distribution models derived from (A) a large portion of the entire Asian range of the *D. melanostictus* complex in Asia, and (B) the range of the SE Asian lineage only. Warmer colors indicate a higher prediction. Red dots show the currently known range in the Toamasina area. (C) Correlation circle depicting the relationships among the 19 bioclimatic variables (supplementary table S3) throughout SE Asia and Madagascar. Variables representing temperature are in reddish. PC1 and PC2 axes of a principal component analysis explain 52.2% and 25.6% of the variation, respectively. Lower row of graphs shows the climate space accessible to *D. melanostictus* (light grey), and represents climate sampled within a 200-km buffer drawn around an adaptive convex-hull (ACH) of all the occurrence localities ($\alpha = 3$, done in SDMtoolbox), which resulted separate buffered polygons for localities in Madagascar and SE Asia. The climate space occupied by (D) all Asian localities (purple), (E) Asian localities of the SE Asian lineage (green), and (F) the Madagascar localities (red); dark grey shape in F is climate space occupied by all of Madagascar. Dotted green line represents the bioclimatic space shaded green in graph E; purple dashed line represents the purple shaded space in graph D; red dotted line represents the space of the Madagascar samples shaded red in graph F.

Haplogroups H1-H4 had distinct geographic distributions (fig. 1). Haplogroup H1 (including H1a) occupied all of the southern part of the range (Cambodia, southern Vietnam, parts of Laos and Thailand, and ranging into northern Malaysia). Haplogroup H2 mostly occurred in southeastern China and northern Vietnam. Haplogroup H3 occupied the northern parts of Laos and Thailand and Myanmar. The strongly divergent haplogroup H4 was from localities in

Myanmar, China, as well as northern Laos and northern Vietnam.

The SDM based on localities of the entire *D. melanostictus* complex predicted a wide range of bioclimatically suitable habitat in Madagascar (fig. 2A and supplementary fig. S2), including all eastern and northern coastal lowlands of Madagascar as well as vast areas in the west and north-west of the island. Moderately suitable bioclimate was also present in

1 parts of the eastern mid-elevation rainforest. In
2 contrast, an SDM based only on localities in the
3 native range of the SE Asian lineage (fig. 2B and
4 supplementary fig. S3) resulted in a distinctly
5 more restricted distribution of climatically suit-
6 able areas in Madagascar. The suitable habitat
7 was confined to coastal lowland areas in eastern
8 and northern Madagascar, with low prediction
9 values for the south-eastern and western parts
10 of the island, and for mid- and high-elevations.

11 Our principal component analysis of climate
12 data accessible by *D. melanostictus* character-
13 ized 77.8% of the climatic variation in the first
14 two components (explaining 52.2% and 25.6%
15 of the variation, respectively). A correlation cir-
16 cle (fig. 2C) showed the relative loadings of
17 each bioclimatic variable on the first two princi-
18 pal components and suggested that many of the
19 bioclimatic variables characterize very similar
20 spatial patterns and climatological information.
21 Caution was taken in assuming they were bi-
22 ologically equivalent, as principal components
23 maximized variance among all input variables,
24 which was likely driven by coarse spatial pat-
25 terns associated with elevation and latitude. In
26 other words, the 22.2% variation not charac-
27 terized in the first two components was likely
28 largely comprised of the unique portion of cli-
29 mate space restricted to individual bioclimatic
30 variables.

31 All tests of niche equivalency were signifi-
32 cant ($p < 0.01$). They rejected the null hypoth-
33 esis of niche equivalence among the different
34 groups of localities included in the SDM anal-
35 yses (supplementary table S4). Values of War-
36 ren's D statistic ranged from moderately high
37 (0.59) between all populations against Madag-
38 ascar and SE Asian lineage to being very
39 low between Madagascar and the two larger
40 datasets (0.03, in both instances) and between
41 SE Asian lineage and the other two dataset
42 (0.02, in both instances). These values charac-
43 terized niche overlap, with complete overlap be-
44 ing represented by 1 and no overlap being 0. The
45 tests of niche similarity confirmed that climates
46 occupied by the entire dataset and the SE Asian
47

lineage dataset were very similar (but not iden- 48
tical) and differences were significant in both 49
directions ($p = 0.002$); these calculations ex- 50
cluded localities from Madagascar. These tests 51
also revealed that the climate occupied by the 52
Madagascan population was similar to that of 53
the native SE Asian lineage ($p = 0.046$), 54
but not vice versa ($p = 0.563$). In part, this 55
was due to the very small climatic space occu- 56
pied by Madagascan populations, which nested 57
well within climate space of SE Asia (fig. 2D- 58
F). A similar pattern was observed between 59
the Madagascan population and the complete 60
dataset ($p = 0.012$ and $p = 0.559$, respec- 61
tively). Thus, the two larger datasets represented 62
similar, broad climates (fig. 2D, E) whereas the 63
current Madagascan localities represented a cli- 64
mate space similar to a small portion of the cli- 65
mates occurring in the native range of the SE 66
Asian lineage. 67

68 Discussion 69

70 Our study uses a conservation genetic approach 71
to assess the diversity and origin of popula- 72
tions of *D. melanostictus* introduced into Mada- 73
gascar. Our molecular analyses, based on mi- 74
tochondrial DNA gene sequences, allow for 75
two main conclusions: (1) introduced Madagas- 76
can toad populations are genetically uniform 77
for mtDNA and are likely to have originated 78
from an introduction of a few individuals from 79
a single source population; (2) the mitochon- 80
drial data pinpoint the source population for the 81
introduction to a limited region in Cambodia 82
and Vietnam that includes Ho Chi Minh City, a 83
town with a major seaport. Unfortunately, anal- 84
yses do not include samples from the greater 85
Bangkok area in Thailand, another seaport from 86
which substantial trade with Madagascar takes 87
place. However, given the distribution of haplo- 88
types, it is unlikely that haplotype H1a occurs 89
in or around Bangkok. Thus, Madagascan toads 90
probably have their origin from Vietnam (or 91
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Cambodia), and possibly from the greater Ho-Chi-Minh-City area. Future analyses can further test this hypothesis via fine-scale nuclear genetic markers.

The invasive toads sampled from Madagascar assign to a part of the *D. melanostictus* complex from SE Asia that probably represents a species distinct from other lineages of the complex that occur in Indonesia and Myanmar (Wogan et al., 2016). However, the taxonomy of this complex is far from being resolved. We here apply a very strict definition of this lineage and exclude from analysis other mitochondrial subclades of the *D. melanostictus* complex that might be conspecific with the SE Asian lineage (e.g., from Myanmar and Malaysia; Wogan et al., 2016). In addition, Wogan et al. (2016) did not include populations from South Asia (especially India) in their multigene study, and, thus, the identity of these requires future study. Although this taxonomic uncertainty might affect our geospatial modelling, it does not invalidate the identification of the source region for the invasive populations in Madagascar.

The haplotype network (fig. 1) serves to visualize the associations of the main haplotype groups within the SE Asian lineage, but it does not fully represent and objectively analyze the biogeographic history of the *D. melanostictus* complex in Asia. On one hand, the network is void of evolutionary direction (Kong et al., 2015), and on the other hand other lineages within the *D. melanostictus* complex that occur in the southern- and western-most parts of the area (Wogan et al., 2016) are not included in the network. In addition, our *ad hoc* haplotype groups contain geographically structured variation not considered herein.

The simplified representation shows that the haplotype H1a of Madagascar is part of a larger assemblage of other, distinct H1 haplotypes (fig. 1). Therefore, H1a is unlikely to occur naturally far outside its identified range that encompasses one Cambodian and three localities in southern Vietnam: (1) a logging concession in Cambodia, 1 sample; (2) the Saigon Zoo and

Botanical Gardens (Ho-Chi-Minh-City, Vietnam), 8 samples; (3) Yok Don National Park (Vietnam), 1 sample; and (4) Cat Tien National Park (Vietnam), 1 sample (details in supplementary table S5).

The SDM based on the native distribution of the SE Asian lineage suggests that the potential range of the toad in Madagascar is restricted to the lowlands of the eastern and northern coasts (fig. 2B). This constitutes a more restricted area than suggested by models based on the entire Asian range of the *D. melanostictus* complex (Pearson, 2015; fig. 2A). SDMs based on bioclimate can provide useful information for a risk assessment of invasive species, but their spatial predictions must be interpreted with caution (e.g., Broennimann and Guisan, 2008; Sinclair et al., 2010; Elith et al., 2010). In a new environment, an invasive species might be able to occupy novel environmental niches that competitors or predators dominate in their native range, or are inaccessible due to geographical barriers. Using the analytical approach of Broennimann et al. (2012), our analyses ascertain that the bioclimatic space occupied by the toads in Madagascar fully overlaps with that of the SE Asian lineage and of all populations of the *D. melanostictus* complex; thus, it does not contradict our predictive SDM.

Discordance between the all-range SDM and the SDM of the SE Asian lineage is relevant because the favored model predicts a lower probability of invasion of the mid-elevation rainforest areas of eastern Madagascar, which harbor the highest diversity of endemic amphibian and reptilian species (Brown et al., 2016). Furthermore, analyses do not predict highly suitable habitat for the SE Asian lineage (fig. 2B) in several centers of diversity of threatened amphibians (Andreone et al., 2005) and reptiles (Jenkins et al., 2014), such as the highlands of southeastern and north-eastern Madagascar, as well as some massifs in the west. However, conservationists need to be prudent when translating these results into a risk assessment. On one hand, our definition of the SE Asian lineage

1 might be too restrictive. Other populations of
 2 the *D. melanostictus* complex, especially from
 3 Myanmar and Malaysia, might be conspecific
 4 with the SE Asian lineage, and, thus, its cli-
 5 matic envelope might be wider than our mod-
 6 els resolve. On the other hand, the environmen-
 7 tal space of the toad in SE Asia might not be
 8 limited by bioclimate alone but also influenced
 9 by biogeographical barriers or competition, and
 10 the toad might thus invade bioclimates not oc-
 11 cupied in its original SE Asian range when re-
 12 leased from this constraints. Therefore, stochas-
 13 tic, spatially explicit, Individual Based Mod-
 14 els (IBMs) based on data collected from spa-
 15 tial behavioural ecology, abundance estimates,
 16 survival rates, and dispersal capabilities of the
 17 Madagascan toads in their invasive area are
 18 now necessary to ascertain the reliability of
 19 our predictions. These should aim to comple-
 20 ment the bioclimatic variables by including land
 21 cover and geology, as well as involve novel,
 22 mechanistic approaches that consider physio-
 23 logical parameters, and biotic and abiotic in-
 24 teractions (Kearney and Porter, 2009; Evans et
 25 al., 2015). Notwithstanding the potential for im-
 26 provement, our model represents a plausible al-
 27 ternative hypothesis to that based on the entire
 28 range of the *D. melanostictus* complex (Pear-
 29 son, 2015; fig. 2A), which probably includes
 30 multiple species (Wogan et al., 2016). Thus, our
 31 analyses highlight that a solid taxonomic as-
 32 sessment and a careful selection of localities for
 33 model-training are essential for accurately pre-
 34 dicting SDMs and making risk assessments.

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 37 **Acknowledgements.** We are grateful to Gaby Keunecke
 38 and Meike Kondermann for their help with labwork, to
 39 Malagasy authorities for collection (021/14/MEF/SG/DGF/
 40 DCB.SAP/SCB) and export permits (113N-EA04/MG15),
 41 to Junot Ratovondrahona, Jean Marcel Razafimanantsoa,
 42 Jean Christian Rafanomezanjanahary and George Ramaro-
 43 lahy for help with toad surveys and sample collection in
 44 Madagascar, and to Bryan L. Stuart for helpful discussions.
 45 TQN and TZ cordially thank Sinh Van Nguyen (Institute of
 46 Ecology and Biological Resources, Hanoi) for facilitating
 47 the export of tissue samples from Vietnam. Sequencing was
 funded, in part, by NSERC Discovery Grant 3148 to RWM.
 ACr was supported by an Investigador FCT (IF) grant from

the Portuguese “Fundação para a Ciencia e a Tecnologia”
 (IF/00209/2014), and by a grant of the Paris Zoo for work
 in Madagascar.

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*Submitted: December 25, 2016. Final revision received:
April 8, 2017. Accepted: May 2, 2017.
Associate Editor: Sebastian Steinfartz.*