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Population structure of an endemic vulnerable species, the Jamaican boa (*Epicrates subflavus*)

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Abstract

The Jamaican boa (*Epicrates subflavus*; also called Yellow boa) is an endemic species whose natural populations greatly and constantly declined since the late 19th century, mainly because of predation by introduced species, human persecution, and habitat destruction. *In-situ* conservation of the Jamaican boa is seriously hindered by the lack of information on demographic and ecological parameters as well as by a poor understanding of the population structure and species distribution in the wild. Here, using nine nuclear microsatellite loci and a fragment of the mitochondrial cytochrome *b* gene from 87 wild-born individuals, we present the first molecular genetic analyses focusing on the diversity and structure of the natural populations of the Jamaican boa. A model-based clustering analysis of multilocus microsatellite genotypes identifies three groups that are also significantly differentiated on the basis of *F*-statistics. Similarly, haplotypic network reconstruction methods applied on the cytochrome *b* haplotypes isolated here identify two well-differentiated haplogroups separated by four to six fixed mutations. Bayesian and metaGA analyses of the mitochondrial data set combined with sequences from other Boidae species indicate that rooting of the haplotypic network occurs most likely between the two defined haplogroups. Both analyses (based on nuclear and mitochondrial markers) underline an Eastern vs. (Western + Central) pattern of differentiation in agreement with geological data and patterns of differentiation uncovered in other vertebrate and invertebrate Jamaican species. Our results provide important insights for improving management of *ex-situ* captive populations and for guiding the development of proper *in-situ* species survival and habitat management plans for this spectacular, yet poorly known and vulnerable, snake.

Keywords: conservation genetics, *Epicrates subflavus*, Jamaica, Jamaican Yellow Boa, population structure

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Introduction

The Jamaican (or 'Yellow') Boa (*Epicrates subflavus*) is endemic to Jamaica and is the island's largest native terrestrial predator (Fig. 1). Although it remained abundant from the arrival of the first Europeans in Jamaica in the 1500s until the 19th century (Gosse 1851), the condition of the species deteriorated dramatically in the following years. Rats (*Rattus rattus* and *Rattus norvegicus*) reached Jamaica with the earliest European ships and rapidly developed into a large population causing highly significant destruction in sugar cane fields (then the main commercial product of the island). In an attempt to control this pest, several exogenous species were deliberately introduced in the island: a carnivorous ant from Cuba (*Formica omnivora*), the cane toad (*Bufo marinus*), and the European ferret (*Mustela putorius*). These efforts failed to control the *Rattus* populations. Subsequently, nine small Asian mongoose (*Herpestes javanicus*) individuals were brought to Jamaica in

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Fig. 1 A wild-caught mature Jamaican boa from Cockpit Country. Photographed by M. C. Milinkovitch.

1872 to initiate a population that was expected to 'extirpate the whole race of the vermin' (Lewis 1949). Ten years later, the result was disastrous: mongooses had indeed a significant impact on the rat population but also devastated multiple native avian and reptilian species (Lewis 1949). For example, two species of ground-nesting birds, the Jamaican petrel (*Pterodroma caribbaea*) and Jamaican paura-que (*Siphonorhis americana*), and two reptiles, the Jamaican giant galliwasp (*Celestus occiduus*) and Jamaican racer (*Alsophis ater*), are believed to have been extirpated by the mongoose. Similarly, populations of the Jamaican boa have been declining dramatically ever since the introduction of mongooses (Grant 1940; Oliver 1982; Gibson 1996). Anecdotal observations further suggest that Jamaican boas may be vulnerable to the toxic bufogenins secreted by cane toad (S. Koenig, unpublished data).

Introduced species such as mongooses, cane toads, and cats are not the only threat to Jamaican boas. Indeed, despite the fact that the snake species may actually be beneficial (rats are a component of the boa's diet in forest edge/farm habitat), most Jamaican people have a traditional antipathy to reptiles in general and to snakes in particular, such that snakes are usually killed on sight. The snakes are mistakenly considered venomous and are sometimes viewed as evil creatures in this often strongly religious culture. In addition, human activities contribute to the deterioration of the local flora and fauna. Only 8% of the island's land cover is considered minimally disturbed (classified as closed broadleaf forest with minimal human disturbance), while an additional 23% encompasses 'disturbed' forest degraded by various types of human activities, ranging from small-scale agriculture to open-pit bauxite mining (Forestry Department 2001). Such activities, along with conversion of forest for urban development and tourism, have greatly fragmented the remaining natural habitat.

In-situ conservation of the Jamaican boa is seriously hindered by the lack of information on demographic and ecological parameters as well as by a poor understanding of the population(s) distribution and structure. Given the secretive nature of the Jamaican boa and the difficulties to perform fieldwork in its natural habitat, the latest *in situ* surveys were performed in the 1980s (Oliver 1982) and 1990s (Gibson 1996) on the basis of questionnaires rather than on direct observations. These studies confirmed the localized and patchy distribution of the species and its disappearance from multiple localities. *Epicrates subflavus* is considered today a 'Vulnerable' species by IUCN (Red List; www.iucnredlist.org). Given the signs of decline of the natural population(s), a captive breeding programme was initiated in 1976 at the Durrell Wildlife Conservation Trust (Jersey, UK) and was rationalized in 2002 by incorporating pedigree information into a European Endangered species Programme (EEP) involving 14 member institutions of the European Association of Zoos and Aquaria (EAZA; www.eaza.net). Recently, using nuclear and mitochondrial DNA markers, we identified parental allocation errors and ambiguities in the EEP studbook, and assessed the genetic diversity and levels of inbreeding of the current captive population (Tzika *et al*. 2008). Combining measures of relatedness derived from multilocus genotypes with practical parameters such as age of animals and localization of host institutions, we proposed a scheme of mating groups that would produce minimal inbreeding in the captive population of the Jamaican boa (Tzika *et al*. 2008).

Here, using nine nuclear microsatellite loci and a fragment of the mitochondrial cytochrome *b* gene, we present the first molecular genetic analyses focusing on the structure and diversity of the natural population(s) of the Jamaican boa. Our results provide insights and guidance for an objective management of the species through both *in-situ* and *ex-situ* approaches.

Materials and methods

Sample collection and species-specific molecular markers

In March 2006, we collected samples (blood, scale clips, and shed skin) in Jamaica from 46 individuals, some of which having been maintained (but not bred) in captivity by zoos, conservation institutions, or private individuals. We also incorporated samples from (i) 36 individuals previously collected on the island in 1995 (Gibson 1996), and (ii) 5 of the wild-born individuals that have been used as the founders of the captive breeding programme initiated by the Durrell Wildlife Conservation Trust (Jersey, UK). Importantly, all 87 individuals were wild-born and captured opportunistically during the last three decades, such that it is very unlikely that they are particularly closely related. We processed all 87 samples for molecular analysis, but reliable information on their geographical origin was available for only 38 sampled individuals (Table 1).

Table 1 Geographical origin of the samples processed in our study

Parish	Samples
St Thomas	7
St Mary	3
St Ann	12
Trelawny	9
Westmoreland	5
St Elizabeth	1
St Catherine	1
Subtotal	38
Unknown	49
Total	87

Blood samples were stored in lysis buffer (100 mM Tris, 100 mM EDTA, and 2% SDS), whereas shed skin and scale clips were placed in 95% ethanol solution. High-molecular weight DNA was extracted from all samples using the DNeasy Tissue Kit (QIAGEN) with the appropriate protocol according to the manufacturer for each type of tissue. DNA was resuspended in Tris buffer (10 mM Tris–HCl at a pH 8) and stored at –20 °C.

A microsatellite-enriched genomic DNA library cloned in a no–background vector (*StabyCloning*™ kit, Delphi Genetics) of *Epicrates subflavus* was constructed as described in (Tzika *et al*. 2008), and nine polymorphic nuclear microsatellite loci (with 2-, 3-, or 4-bp repeats) were selected on the basis of polymorphism levels and size of amplification products. All samples were genotyped using three multiplex polymerase chain reactions (Multiplex PCR kit; QIAGEN) targeting each two to four different loci (Table 2). The 5′-end of each reverse primer was marked with one of four possible fluorochromes whereas a 7-base tail was added to the 5′-end of the forward primer to improve genotyping consistency and allele binning (by forcing + A alleles; Brownstein *et al*. 1996). Primers and PCR conditions are given in (Tzika *et al*. 2008) and PCR products were separated by electrophoresis using an ABI 3730 sequencer (Applied Biosystems).

Using a previously available *E. subflavus* cytochrome *b* sequence (NCBI U69803), we designed primers for the amplification of a 647-bp fragment. Primers and PCR conditions are given in Tzika *et al*. (2008). PCR products were purified, cycle sequenced on both strands, and run on an ABI 3730 sequencer.

Clustering of individuals

A Bayesian model-based clustering method (Pritchard *et al*. 2000) for inferring population structure and assigning individuals to populations was used as implemented in STRUCTURE version 2.1 (Falush *et al*. 2003). Based on allele frequencies, individuals are assigned [through the use of a Markov chain Monte Carlo (MCMC) simulation] a membership coefficient for each of K populations. Two of the models implemented in STRUCTURE version 2.1 were used: (i) the 'no admixture model', which assumes that each individual purely originates from one of the K populations, and (ii) the 'admixture model', a more flexible approach allowing for mixed ancestry of individuals. As we do not know if migration occurs among groups, we tested both the use of correlated and of independent allele frequencies. The latter assumes that the allele frequencies in each population are independent draws from a distribution whereas the former assumes that frequencies in the different populations are likely to be similar (due to migration or shared ancestry). We performed five independent runs for each value of K ranging from 1 to 6 with 10^7 iterations and a burn-in of 50 000. The number of populations best fitting our data set was defined both using log probabilities [$\Pr(X \mid K)$] and ΔK, as described in (Evanno *et al*. 2005). The latter statistics compares the rate of change in the log probability of data between successive K and the corresponding variance of log probabilities. Membership coefficients were represented graphically using the software DISTRUCT (Rosenberg 2004).

Analyses were also performed with another Bayesian method for the inference of population genetic structure as implemented in BAPS version 4.14 (Corander *et al*. 2004). We initially used individuals as sampling units and performed five runs of 50 000 iterations each, and a burn-in of 10 000. In addition, an admixture analysis was performed with the same program on the partitions inferred from the STRUCTURE analyses. In this case, admixture coefficients are assigned to each individual-cluster pair and a P value is computed for assessing the significance of admixture for each individual.

Network estimations

We used the statistical parsimony approach, as implemented in the TCS software (Clement *et al*. 2000); the median-joining network approach (Bandelt *et al*. 1999), as implemented in NETWORK version 2.0 (www.fluxus-engineering.com/sharenet.htm); and the union of most parsimonious trees method (UMP; Cassens *et al*. 2005a), as implemented in COMBINETREES version 1.0 (www.ulb.ac.be/sciences/ueg/html_files/softwares.html) to estimate genealogical relationships among individuals based on their cytochrome *b* sequences. The statistical parsimony approach works as follows: first, the absolute distance matrix for all pairwise comparisons of haplotypes is computed; second, a so-called 'parsimony limit' (i.e. the uncorrected distance above which the parsimony criterion is violated with more than 5% probability; Templeton *et al*. 1992) is calculated; third, all connections are established among haplotypes starting with the smallest distances and ending when either all haplotypes are connected or the distance corresponding to

	Name	Repeat type	*N*	Size range (bp)	GenBank no.
Set 1	μsat 1	$(AGAT)_n$	10	309–345	EU138906
	μsat 3	$(TCCA)_n$	3	186–194	EU138907
	μsat 10	$(TCAT)_n$	7	391–415	EU138908
	μsat 24	$(GAAG)_n$	11	263–303	EU138909
Set 2	μsat 11	$(AT)_n(AC)_n$	4	263–275	EU138910
	μsat 16	$(AC)_n$	2	196–204	EU138911
	μsat 36	$(CTTC)_n(CTTT)_n(CT)_n$	19	314–392	EU138912
Set 3	μsat 13	$(ATAG)_n$	9	187–219	EU138913
	μsat 30	$(CTT)_n$	2	307–310	EU138914

Table 2 Microsatellite loci used in this study. The nine loci are separated into three multiplexing PCR sets. For each locus we list: its name, the number of alleles (*N*) and range of allele sizes (range) observed in the natural populations, and the GenBank Accession no of the genomic clone sequence on which the primers (available in Tzika *et al*. 2008) have been designed

the parsimony limit has been reached. The median-joining network approach first combines all minimum-spanning trees into a single network, then uses the parsimony criterion to infer additional intermediate haplotypes and reduce overall tree length. The UMP method combines all connections from all maximum parsimony trees into a single reticulated graph and merges branches, node haplotypes, and branch haplotypes, sampled or missing, that are identical among different trees. As the placement and number of loops constructed by this algorithm can depend on the order with which connections are compared among trees, the result with the lowest number of cycles is selected among the 10 graphs produced with 10 different orders of connection comparisons. See Cassens *et al*. (2005a) for a discussion on the relative merits and limitations of the different network estimation approaches.

Genetic diversity

For the microsatellite data, CONVERT (Glaubitz 2004) was used to detect private alleles, that is alleles present in one population and not shared with any other. The following statistics were calculated using FSTAT version 2.9.3 (Goudet 1995): the mean number of alleles per locus (averaged across the nine loci) and per population, and the allelic richness per locus and per population according to the smallest number of individuals typed in each case. ARLEQUIN version 3.1 (Excoffier *et al*. 2005) was used to (i) test for heterozygote deficiency at each microsatellite locus and each population using a Hardy–Weinberg test based on Markov chain iterations (Guo & Thompson 1992); (ii) test for the lack of genetic linkage within each possible pair of loci using a likelihood-ratio statistic, whose distribution is obtained by a permutation procedure (Slatkin & Excoffier 1996); and (iii) compute mitochondrial haplotypic (*H*) and nucleotide (π) diversities of each population.

F-*statistics*

F-statistics within and among the groups inferred from the STRUCTURE analyses were calculated using ARLEQUIN version 3.1. For mtDNA data, genetic differentiation among groups was estimated by computing F_{ST} values for each pair of populations, based on pairwise genetic distances among haplotypes. For the microsatellite data, an allele-size permutation test was first used in order to clarify if allele sizes are informative with respect to population differentiation (Hardy *et al*. 2003), as implemented in SPAGEDI (version 1.2) (Hardy & Vekemans 2002). A statistically significant greater value of R_{ST} vs. pR_{ST} (the latter being computed after allele-size permutation) would suggest that mutations contributed to genetic differentiation and that the mutation process follows at least partially a stepwise mutation model (SMM), whereas a nonsignificant result (R_{ST} not significantly > than pR_{ST}) would suggest that allele size is not informative for population differentiation because either the mutation process is not stepwise-like and/or mutations had not contributed to differentiation. In the latter case, F_{ST} provides a better estimate of gene flow than R_{ST} does.

Phylogeny inferences

The phylogeny of Boidae has been previously investigated on the basis of mitochondrial cytochrome *b* DNA sequences in combination with either morphological data (Burbrink 2005) or DNA sequence fragments of five nuclear genes (Noonan & Chippindale 2006). To test the monophyly of the *E. subflavus* species and root the haplotypic network, we extended the cytochrome *b* data set by including the new haplotypes generated from the samples presented here. Using *Epicrates cenchria* (accession U69779) as the outgroup species, and the available sequences of other *Epicrates* species (accessions U69776, U69781, U69782, U69786, U69787, U69792, U69799, U69803), Bayesian phylogeny inference was performed using MRBAYES 3.0b4 (Huelsenbeck & Ronquist 2001). The general time reversible (GTR) nucleotide substitution model with gamma-distributed rate variation across sites and estimated proportion of invariable sites (i.e. the optimal model selected by MODELTEST version 3.06; Posada & Crandall 1998) was applied separately to three data partitions corresponding to the first, second, and third codon positions. Two independent runs were performed,

each with four chains for 10^6 generations, and trees were sampled every 100 cycles. Convergence onto the stationary distribution was verified by checking if the average standard deviation of split frequencies was below 0.05 between two independent runs. Bayesian posterior probabilities were estimated by building the majority-rule consensus tree among the 7000 last sampled trees (3000 first samples discarded as 'burn-in'). We also conducted 250 replicated metaGA searches (Lemmon & Milinkovitch 2002) using the software METAPIGA 1.0.2b (www.ulb.ac.be/sciences/ueg/html_files/softwares.html) with probability consensus pruning among four populations, using a HKY + I model. The metaGA (Lemmon & Milinkovitch 2002) is an evolutionary computation heuristics (i.e. implementing a set of operators that mimic processes of biological evolution) that vastly improves the speed and efficiency with which maximum-likelihood trees are found and yields a probability index for each branch. It has been suggested that a metaGA search with a finite number of populations provides an estimate of the posterior probability distribution of possible trees. The 1000 resulting trees were therefore used to compute a majority-rule consensus tree and calculate posterior branch support (PBS) values. Given that the dynamic of searching tree space using the metaGA is quite different from that using Metropolis-coupled Markov chain Monte Carlo (implemented in MRBAYES 3.0b4), high support values generated by the two above methods increases the confidence in the validity of that support.

Results

All of the 87 sampled individuals were successfully sequenced for the target cytochrome *b* fragment (sequences were deposited at GenBank under accession nos EU138894–EU138905) and genotyped for the nine polymorphic microsatellite loci.

Clustering of individuals using microsatellite data

As the exact geographical origin of many (58%) of the sampled Jamaican boa individuals is unknown or uncertain (many of the individuals sampled in zoos or at privates were not associated to reliable information on their exact location of capture), the STRUCTURE analysis was particularly relevant because it allows clustering of the individuals without the need for a priori geographical information. Figure 2(a) shows the average and variance of log-likelihood among five independent runs for one to six populations using an admixture model and correlated allele frequencies among populations (Falush *et al*. 2003), and Fig. 2(b) shows the corresponding distribution of ΔK values (Evanno *et al*. 2005). Both statistics suggest that a clustering of individuals in three groups best fits the data. Analyses using the 'no admixture model' and a larger burn-in (500 000) yielded very similar results (data not shown). Most individuals are consistently assigned to a single group with a high probability (Fig. 3a). Visual inspection of the population assignment values indicates that only five individuals (indicated with an asterisk in Fig. 3a) out of 87 have variable group assignments among the five independent runs ($K = 3$). The average q value (approximating the proportion of the genotype belonging to the assigned population) across the 87 individuals of 0.89 is compatible with expectations from simulated data sets (Latch *et al*. 2006). When, a posteriori to the STRUCTURE analysis, we examined the localities some of the sampled individuals are known to originate from (Table 1 and Fig. 3b), we noticed that all individuals assigned to the first and second clusters (blue and red) were known to originate from localities at the western and central parts of the island, whereas all individuals known to come from St Thomas parish (at the most eastern part of Jamaica) are assigned to the third cluster (yellow).

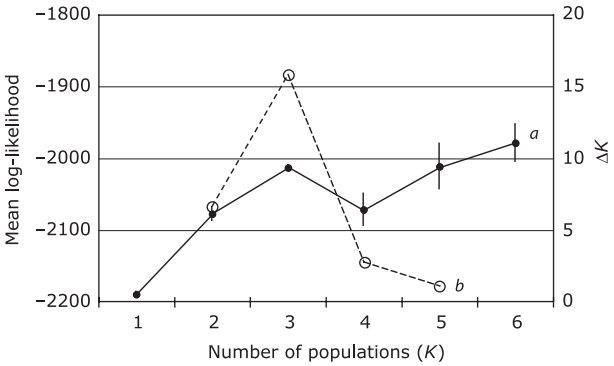

Fig. 2 Identification of the number of populations best fitting the microsatellite data. (a) Mean log-likelihood and associated variance (across five independent runs) computed with STRUCTURE (under the 'admixture and correlated allele frequencies among populations' model) for each value of K ranging from 1 to 6. (b) Value of ΔK as a function of K; the modal value of ΔK points to the most likely number of partitions (Evanno *et al*. 2005).

The analyses performed with BAPS at the individual level failed to detect a K value that best describes our data set; the log-likelihood constantly rises as the assumed number of populations is increased, reaching a point where some groups include only one individual. Analyses using more generations (10^6) yielded the same problem. On the other hand, the BAPS admixture analyses based on the three partitions inferred by STRUCTURE provide a good support for that clustering: only five individuals (indicated with circles in Fig. 3a) are associated with significant admixture ($P < 0.05$), meaning that they are assigned to a cluster with a probability significantly lower than those of the other individuals assigned to the same cluster. Three of these five individuals showed assignment problems with STRUCTURE as well (see above).

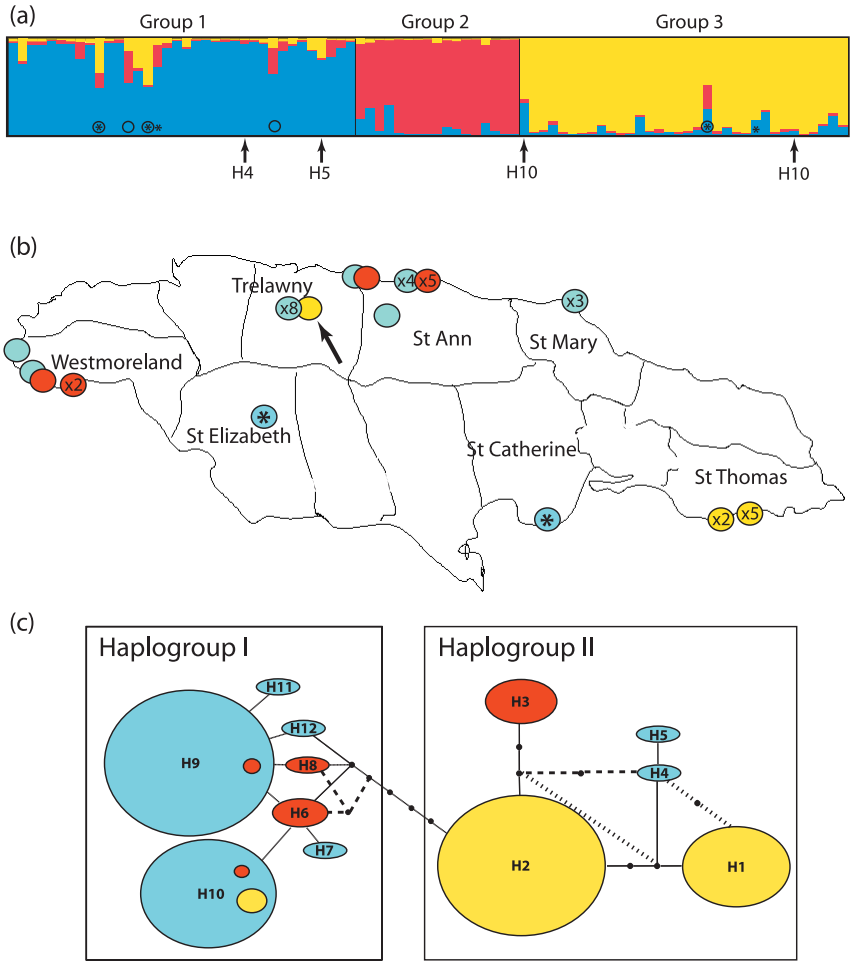

Fig. 3 (a) Membership coefficients inferred with STRUCTURE version 2.1 (Falush *et al*. 2003) and plotted with DISTRUCT (Rosenberg 2004) without using prior population definitions (using 'admixture and correlated allele frequencies among populations' model). Each individual is represented by a column, and membership coefficients are colour coded according to the cluster of origin (blue, group 1; red, group 2, and yellow, group 3). Arrows indicate individuals whose mitochondrial haplotypes unexpectedly belong to haplogroup II (haplotypes 4 and 5 in *c*) or to haplogroup I (haplotype 10 in *c*). Asterisks indicate individuals that have variable population assignments among the five independent STRUCTURE runs ($K = 3$), whereas circles indicate individuals associated with significant BAPS admixture; see text for details. (b) Sampled individuals with known origin placed on a map of Jamaica where the borders of parishes are delimitated; colours correspond to the group (defined by the STRUCTURE analyses in a.) to which they are assigned and the number of individuals is indicated. Asterisks indicate the two individuals (one in St Catherine and the other in St Elizabeth parish) that unexpectedly exhibit a 'haplogroup II' mtDNA haplotype (H4 and H5 in *c)* and the arrow indicates one individual that unexpectedly exhibits a 'haplogroup I' mtDNA haplotype (H10 in *c*). (c) Haplotypic network connecting the 12 newly identified mitochondrial cytochrome *b* haplotypes. The size of the circles is proportional to the number of individuals. Haplotypes are coloured according to the group to which the corresponding individual is assigned on the basis of its multilocus microsatellite genotype (STRUCTURE analysis in a.). The TCS network is shown; dashed and dotted lines indicate additional connections generated by the median-joining and UMP approaches, respectively.

Network estimation

The 87 sequences of the 647-bp cytochrome *b* fragment define 16 polymorphic sites and 12 distinct haplotypes. In concordance with the well-known substitution bias of the animal mitochondrial genome, transitions (ti) outnumber transversions (tv) in pairwise sequence comparisons. The TCS, median-joining, and UMP approaches all inferred the haplotypic network shown in Fig. 3(c). Two haplogroups are well differentiated and are separated by four to six fixed mutations (depending on the resolution of loops in the network). Haplogroup I (Fig. 3c) includes seven haplotypes belonging to individual snakes that are (i) known to originate exclusively from the western and central portions of the island (Fig. 3b), and (ii) assigned to groups 1 (blue) and 2 (red) (as inferred from the corresponding microsatellite data; Fig. 3a). One exception is haplotype 10; although most individuals bearing it are assigned to group 2 (blue) on the basis of their multilocus microsatellite genotype, two individuals exhibiting that haplotype are assigned to group 3

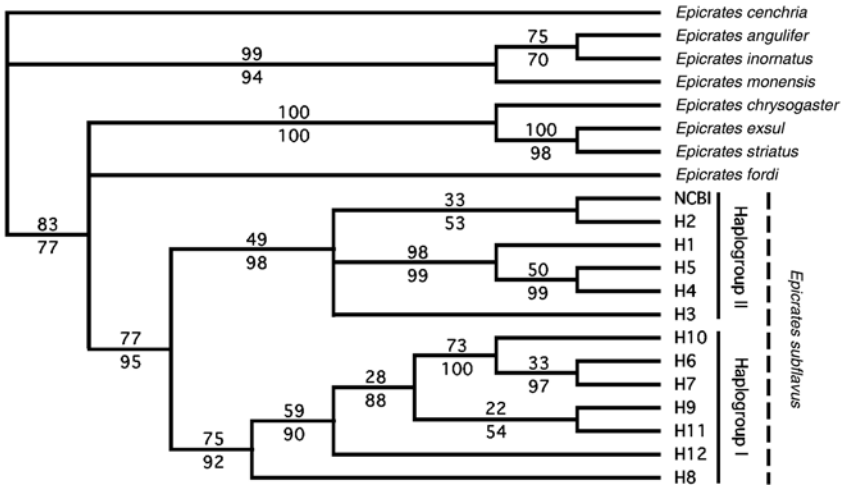

Fig. 4 Phylogenetic position of the Jamaican Boa in the genus *Epicrates*. Bayesian posterior probabilities (Huelsenbeck & Ronquist 2001) and branch support values based on the metapopulation genetic algorithm (Lemmon & Milinkovitch 2002) are given above and below the branches, respectively. The reciprocally monophyletic haplogroups I and II are indicated.

(yellow). Haplogroup II, is even more geographically heterogeneous; although most individuals are assigned to group 3 (yellow) and originate from St Thomas parish at the extreme east of the island, haplotypes H4 + H5 and H3 occur in individuals originating from more western portions of the island and assigned to groups 1 (blue) and 2 (red) on the basis of their multilocus microsatellite genotypes. In other words, although the major split in the haplotypic network is generally consistent with the microsatellite-based group assignment (haplogroup I includes haplotypes occurring mostly in individuals assigned to group 1, whereas haplogroup II includes haplotypes occurring mostly in individuals assigned to group 3), a few individuals exhibit a mixture of an 'eastern' genotype and a 'western-central' haplotype or vice versa. The biological relevance of this pattern is examined in the discussion section.

Phylogeny inference

Bayesian and metaGA analyses of the data set combining previously published cytochrome *b* sequences of Boidae (Burbrink 2005) with the 12 unique cytochrome *b* haplotype of *Epicrates subflavus* identified here indicate that the Jamaican boa forms a monophyletic species (clade supported by a posterior probability of 77% and a metaGA branch support value of 95%, Fig. 4). From our analyses, it was not possible to confirm that *Epicrates fordi*, a boa endemic to Hispaniola, is indeed the sister taxon to the Jamaican boa (Burbrink 2005) because both posterior probability and metaGA branch support values for the placement of that lineage are below 50%. Within the Jamaican boa clade, haplotypes are separated into reciprocally monophyletic 'haplogroup I' and 'haplogroup II', although posterior probabilities supporting the monophyly of the former is higher than that of the latter. In other words, the haplotypic network presented in Fig. 3(c) is most probably rooted either on the branch corresponding to the fixed differences between the two haplogroups, or on a branch within haplogroup II.

Genetic diversity

Table 3 lists the values of statistics computed separately for the different groups identified by our analyses of nuclear and mitochondrial markers. When the three groups inferred by the analysis of nuclear microsatellite data (Fig. 3a) are considered, group 1 (blue) has more alleles and a higher allelic richness than groups 2 and 3. Note also that group 1 is the most differentiated as it exhibits 18 private alleles, compared to only two for each of the two other group. When considering observed and expected heterozygosities, the former are within the range of the latter for the three groups, so no significant hetozygosity deficiency is evidenced. Genetic linkage is detected for 5, 5, and 9 pairs of loci for groups 1, 2, and 3, respectively. Four identical pairs of loci exhibit linkage in two among three populations, but no pair of loci exhibits linkage in all three populations, suggesting that linkage is the result of additional structure within the three inferred groups rather than caused by intrachromosomal linkage.

For mitochondrial cytochrome *b* data, Table 3 indicates that haplogroups I and II have similar haplotypic (*H*) diversities whereas nucleotide diversity (π) is smaller for haplogroup I than for haplogroup II, reflecting the lower sequence divergences among haplotypes within the former than within the latter (as evidenced by the network shown in Fig. 3c). When considering the three groups inferred with the nuclear microsatellite data, haplotypic (*H*) and nucleotide (π) diversities are highest for group 2 (red), a result explained by the approximately equal distribution of group 2 haplotypes in haplogroup I and in haplogroup II.

Table 3 Genetic diversity statistics computed from microsatellite and mitochondrial cytochrome *b* data. Number of alleles, allelic richness (*R*), number of private alleles, observed (H_O) and expected (H_E) heterozygosities, and the number of pairs of linked loci are given for each of the three groups (group 1, group 2, group 3) identified by the STRUCTURE analyses (Fig. 3a), as well as for the combined groups (All). Number of haplotypes, haplotypic (*H*) and nucleotide (π) diversities are given for the two haplogroups (haplogrI and haplogrII) identified in Fig. 3(c)

	All	Group 1	Group 2	Group 3	HaplogrI	HaplogrII
No. of samples	87	36	17	34	46	41
Microsatellites						
No. of alleles	7.44	7	4.33	4.33		
Allelic richness	5.51	5.81	4.33	3.99		
Private alleles	29	18	2	2		
H_O	0.49 (± 0.19)	0.49 (± 0.26)	0.42 (± 0.24)	0.53 (± 0.15)		
H_E	0.64 (± 0.20)	0.59 (± 0.27)	0.57 (± 0.25)	0.63 (± 0.13)		
Pairs of linked loci		5/36	5/36	9/36		
Cytochrome *b*						
No. of haplotypes	12	7	5	3	7	5
H	0.79 (± 0.03)	0.47 (± 0.09)	0.78 (± 0.06)	0.42 (± 0.06)	0.57 (± 0.07)	0.64 (± 0.06)
π (percentage)	0.76 (± 0.41)	0.22 (± 0.15)	0.79 (± 0.45)	0.27 (± 0.18)	0.14 (± 0.11)	0.34 (± 0.21)

F-*statistics*

As shown in Table 4, the data from the nine microsatellite loci reveal a highly significant ($P < 0.001$) differentiation among the three groups; the global F_{ST} is 0.093, indicating relatively low levels of gene flow (2.44 migrants per generation), and the highest differentiation ($F_{ST} = 0.12$) is between group 2 (red) and 3 (yellow). On the other hand, the greatest pairwise F_{ST} value (0.760) using the mtDNA data is associated to the comparison of groups 1 (blue) and 3 (yellow), a result that reflects the major split between Haplogroups I and II. Obviously, the global F_{ST} value rises when haplogroups, rather than groups based on microsatelite data, are considered.

Discussion

Molecular genetic markers can provide valuable insights into the geographical distribution of genetic diversity (and associated causal processes) of species whose observation in nature is difficult because of, for example their secretive behaviour and/or difficulties for accessing their habitat (as exemplified by cetacean species; Milinkovitch *et al*. 2002). As, respectively, about 40% and 20–25% of all vertebrate species are considered vulnerable and endangered (Frankham *et al*. 2004), a comprehensive assessment of population structure is one critical component for their effective conservation. Endemic island species are at even greater risk, as 95% of reptilian species extinctions in the last 400 years have taken place on islands (Frankham *et al*. 2004). *Epicrates subflavus* is a clear representative of such 'high risk' species. This endemic Jamaican snake is very poorly known, not only in terms of its population structure but also of its basic ecology. Given the rapid decline of the Jamaican boa and the constant deterioration of its natural habitat, it is of vital importance to gather objective demographic and population structure data for developing efficient *in situ* and *ex situ* conservation programmes (Tzika *et al*. 2008).

Here, we used both nuclear and mitochondrial DNA markers for the first assessment of the natural population structure of the Jamaican boa. Our analyses of nine nuclear microsatellite loci indicate the existence of three significantly differentiated groups, whereas our network inference analyses of the mitochondrial cytochrome *b* locus generated two significantly differentiated haplogroups. The two types of analyses underline an Eastern vs. (Western + Central) pattern of differentiation (group 1 vs. 3, and haplogroup I vs. II). On the other hand, individuals assigned to group 2 on the basis of their multilocus microsatellite genotypes are dispersed in the two haplogroups.

Various causal factors that can explain the partial discrepancy between the patterns inferred from microsatellite genotypes and that obtained using mitochondrial haplotypes are associated with the different modes of inheritance for the two types of markers. First, in diploid organisms, autosomal markers exhibit a fourfold higher effective population size (hence, experience much less genetic drift) than mitochondrial markers (Birky *et al*. 1989). In agreement with this expectation, genetic differentiation among populations is much less pronounced for the nine autosomal microsatellite loci than for the mitochondrial data (Table 4). Second, contrasting patterns between nuclear and mitochondrial DNA in terms of *F*-statistics can also be interpreted as evidence for male-biased dispersal that homogenizes allele frequencies among populations at biparentally, but not at maternally, inherited genetic markers (O'Corry-Crowe *et al*. 1997; Berube *et al*. 1998; Escorza-Trevino & Dizon 2000; Cassens *et al*. 2005b). Such sex-specific dispersal parameters

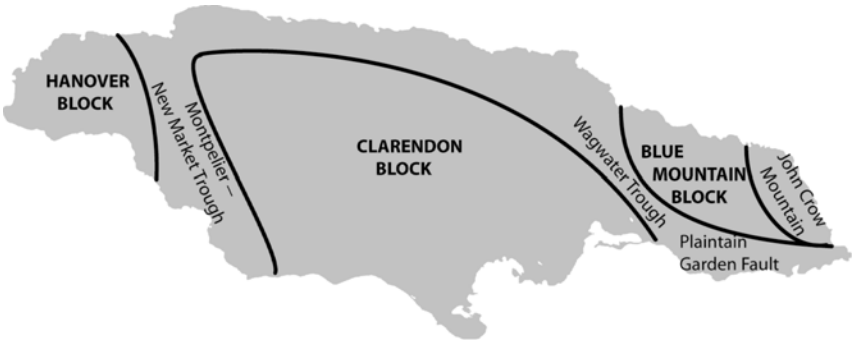

Fig. 5 Simplified structural map of uplift blocks and fault traces of Jamaica (modified after Draper 1987).

	Group 1	Group 2	Group 3
Group 1		0.279	0.760
Group 2	0.095		0.496
Group 3	0.084	0.120	
Global F_{ST} (microsatellites) Gr1/Gr2/Gr3			0.093
Global F_{ST} (microsatellites) Gr1 + Gr2/Gr3			0.074
Global F_{ST} (cyt *b*) Gr1/Gr2/Gr3			0.619
Global F_{ST} (cyt *b*) haplogr1/haplogr2			0.816

Table 4 Genetic differentiation, in terms of pairwise *F*-statistics, among the three groups defined by the STRUCTURE analyses (Fig. 3a). Values computed from mitochondrial DNA haplotypes (cyt *b*) and microsatellite data are given above and below the diagonal, respectively. Global F_{ST} values are also given. All values are highly significant ($P < 0.001$). Results are very similar (and values remain significant) when using only samples of known origin

can also result in incongruent population assignments between nuclear vs. mitochondrial marker-based analyses. Although, no reliable information is available on the spatial ecology and home range of *E. subflavus*, we can extrapolate information available for a closely related species, *Epicrates inornatus* (Puente-Rolon & Bird-Pico 2004; Wunderle *et al*. 2004). Although the home range does not differ significantly between the sexes in this endemic Puerto Rican species, the mean daily and monthly movements recorded for males are greater than those of females. Such a mating system, where males perform active searches of receptive females, and females are philopatric, has been described for other snakes species within and outside the family Boidae (Paquin *et al*. 2006; Rivera *et al*. 2006; Koegh *et al*. 2007).

Still, high pairwise F_{ST} values among populations and the occurrence of a mostly western-central haplogroup vs. a mostly eastern haplogroup, indicate that dispersal patterns (sex specific or not) are insufficient to disrupt this very significant differentiation in the distribution of genetic diversity of the Jamaican boa. This pattern is in close agreement with findings originating from molecular and morphological analyses of other Jamaican species, such as frogs (Hedges 1989), terrestrial crabs (Schubart *et al*. 1998), millipedes (Bond & Sierwald 2002), and beetles (Velez & Feder 2006). The occurrence of such a recurrent (across taxonomic groups) pattern of genetic differentiation correlates with the geological history of Jamaica. In the late Oligocene or early Miocene (15–20 million years ago), the island, which had been fully submerged for 33 million years, re-emerged through uplift of the Caribbean plate (Porter 1990). The first emergent land areas were thick limestone (carbonate) sequences in the north-central region of the Clarendon Block (Fig. 5). Uplift next followed (and continues today) for the eastern Blue Mountain Block, formed of Cretaceous and Lower Eocene clastics and limestone, with an associated subsidence of its southern St Thomas Belt along the Plaintain Garden Fault (James & Mitchell 2004). As the Blue Mountain Block was uplifted, it may have been initially separated from the central Clarendon Block by an older structural feature, the volcanic island-arc of the Wagwater Trough, which developed 65–50 million years ago but which was overlaid with limestone during Jamaica's long submergence. Extreme western Jamaica, the Hanover Block, was likely a third emerging island, separated from the Clarendon Block by the Montpelier-New Market Trough. As uplift continued during the Lower Miocene and Pliocene (10–5 million years ago), the 'islands' of these three blocks coalesced into the present-day form of Jamaica. Of relevance for the Jamaican Boa population, the intrusion of the clastic Blue Mountains between the carbonate John Crow Mountains in the extreme east and the Port Royal Mountains located on the western flank of the Blue Mountains may constitute a major barrier to dispersal, either because of its high elevations (maximum 2256 m) and associated cool, misty climate, or because boas prefer habitats associated with a limestone substrate for as-yet-unknown reasons. In addition to geological events, variations in sea level in the late Pliocene and early Pleistocene period probably

played a significant role in the separation between Eastern and Western Jamaica fauna (Schubart *et al*. 1998).

Jamaican reptilian and amphibian species have been poorly studied in comparison with those in other Caribbean islands (Crombie 1999). As far as the Jamaican boa is concerned, very limited data are available on the species ecology, population densities, habitat usage, and potential morphological differentiation among populations. Given the direct and indirect threats that these animals face, mainly resulting from human activities (persecution, predation by introduced species, natural habitat destruction and fragmentation), the molecular analyses we report here could be particularly important for delineating management units. Significant mtDNA divergence (possibly with reciprocal monophyly) among populations, significant differentiation at nuclear loci, as well as adaptive distinctiveness should all be taken into account for the definition of management units (Crandall *et al*. 2000; DeSalle & Amato 2004; Palsboll *et al*. 2007). Moritz (2002) underlined the importance of protecting historically isolated lineages because they cannot be recovered but also recognized the importance of protecting adaptive features by maintaining the context for selection, heterogeneous landscapes, and viable populations, rather than protecting specific phenotypes (other than those obviously essential for population viability).

Our analysis based on multiple molecular genetic markers is the first attempt to uncover the levels of variability and differentiation of the natural populations of the Jamaican boa. We identify an Eastern vs. (Western + Central) pattern of differentiation demonstrating the importance of managing the Blue and John Crow Mountains separately from the populations of the West-central dry, mesic, and wet limestone forests. These latter habitats do, however, require comprehensive management at the landscape level not only to ensure protection of the full historic ecological range of habitats occupied by boas but also to ensure connectivity among populations across the landscape for the west-central haplogroup I. At present, scant attention has been given to managing remnant forest within a landscape context of ecosystem (and genetic) connectivity; instead, fragments are typically managed as isolated units. Furthermore, and unfortunately, coastal areas are being extensively destroyed and left with increasingly small and isolated patches of forest amidst a sea of development for high-density tourism and urban sprawl. Of concern is also the increasing threat of bauxite mining to Cockpit Country, the largest block of contiguous wet limestone forest on Jamaica and a recognized stronghold for the Jamaican boa (Oliver 1982; Gibson 1996). Open-pit bauxite mining of valleys not only results in an absolute reduction in forest cover but also severely isolates forests left on hillsides. The extensive road network built to access the mining pits further facilitates encroachment by humans for illegal timber extraction, increasing the risk of fatal encounters for boas. The reduction in population size and spatial extent of the Cockpit Country boa population could have profound consequences for the long-term viability of this species in Jamaica.

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