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A deep divergence and high diversity of mitochondrial haplotypes in an island snake: the case of *Chilabothrus angulifer* (Serpentes: Boidae)

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Abstract. For the needs of proper management, we analysed the mitochondrial haplotype structure of the European *ex situ* population of Cuban boas. The results showed its extraordinary diversity. We sequenced 96 specimens and detected 25 distinct haplotypes. Besides this haplotype diversity, the results revealed a deep divergence among three principal haplogroups. Bayesian estimates of the divergence time (3.57 and 2.26 Mya) suggest that within the currently only recognized species *Chilabothrus angulifer* (Bibron, 1843) there are evolutionary lines whose distance corresponds to or is greater than among some other – taxonomically recognized – species of the genus. This indicates that the Cuban boas represent in fact at least two cryptic subspecies or species. Nevertheless, after considering the current state of the *ex situ* population, given the current knowledge of the phylogeography of Cuban boas and the fact that they inhabit a single large island (and its nearby coastal islands and islets) at present, we recommend to manage the current *ex situ* population as a whole.

Key words. Conservation genetics, population management, *ex situ* population, evolution, phylogeography, adaptive radiation, speciation, boid snakes, Caribbean herpetofauna, Great Antilles.

INTRODUCTION

Terrestrial fauna of the Great Antilles is characterised by an extraordinary high endemism and species diversity. It is due to unique biogeographical history of the Caribbean (cf. Hedges et al. 2019). A local Cretaceous biota was originally exterminated by Chicxulub impact at the Cretaceous--Tertiary boundary (Lyons et al. 2020). In spite of a strong permanent isolation by the Caribbean Sea, this region has been repeatedly colonized from mainland. It was proposed that the immigrant lineages dispersed through "Gaarlandia", the putative incomplete temporary land bridge connecting NE of the South American Continent with precursors of the Great Antilles during Oligocene and Early Miocene periods. Currently, geological data rejected "Gaarlandia" hypothesis and molecular clock suggests that immigration events were not confined to this period (Ali & Hedges 2021 and references herein, but see Philippon et al. 2020). Thus colonization of the Great Antilles should be attributed to events of overwater dispersal (Ali 2012). The earliest vertebrate fossil record is a frog of the genus *Eleutherodactylus* coming from Puerto Rico Oligocene, 29 Mya (Blackburn et al. 2020). Nevertheless, the colonization events were extremely rare and thus current species richness of the Great Antillean region is mainly a result of subsequent speciation within a few immigrant lineages. While the diversity of native mammalian fauna of the Great Antilles has been substantially reduced by recent extinctions, squamate reptiles representing another clade of terrestrial non-volatile vertebrates have remained less affected and may serve as a model for phylogenetic

and population studies of Caribbean endemism. Studies performed in principal endemic clades of squamates as anoles (*Anolis* sensu lato; Losos 2009 and references herein, Cádiz et al. 2018) and iguanas of the genus *Cyclura* (Malone et al. 2000) clearly demonstrated that geographic isolation among large Antillean islands typically corresponds to the deepest divergences. However, both adaptive radiation and geographic speciation contributed to further speciation. The geographic ranges of individual species of squamates are typically restricted to a certain part of the island as it is well documented in the largest island of Great Antilles – Cuba (Glor et al. 2004, Rodríguez Schettino et al. 2010, 2013). Traditionally, four main zoogeographical regions were recognized: Western, Central, Camagüey-Maniabón and Eastern (Estrada & Ruibal 1999). Species richness tend to be associated with higher elevations. Cladistic analysis of endemism revealed a complex pattern with nested areas of endemism concentrated in the Western and Eastern parts of the island (Murray & Crother 2019).

Great Antillean boas of the genus *Chilabothrus* Duméril et Bibron, 1844 belong to the earliest reptilian colonists of the Great Antillean region. Its relatives inhabit the South American continent which was unequivocally revealed as the source area (Tolson 1987, Reynolds et al. 2014a). According to recent molecular phylogenies, a common ancestor of anacondas of the genus *Eunectes* Wagler, 1830 and rainbow boas of the genus *Epicrates* Wagler, 1830 represents a sister clade of *Chilabothrus* (Reynolds et al. 2013, 2014a). Divergence between *Chilabothrus* and *Eunectes* + *Epicrates* was estimated to 30.2 [CI 24.5, 35.8] Mya (Reynolds et al. 2013). A fossil *Chilabothrus stanolseni* (Vanzolini 1952) from Florida 18.5 Mya supports the view that ancestors of current *Chilabothrus* reached Great Antilles as early as in lower Miocene period (Onary & Hsiou 2018). Nevertheless, at that time boids successfully dispersed from South America also to continental Central America as documented by a fossil record of a snake of the genus *Boa* Linnaeus, 1758 from Panama 19.3 Mye, i.e., prior the Great American Faunal Interchange (Head et al. 2012).

Currently, 14 extant species of the genus *Chilabothrus* are recognized (Pyrron et al. 2014, Reynolds & Henderson 2018, Landestoy et al. 2021). Phylogenetic relationships within the genus were studied repeatedly (Sheplan & Schwartz 1974, Tolson 1987, Kluge 1989, Burbrink 2004, Reynolds et al. 2013, 2014a). Molecular studies (Reynolds et al. 2013, Rodríguez-Robles et al. 2015, Landestoy et al. 2021) confirmed five principal clades that diverged in Lower Miocene: (1) Cuban – *Chilabothrus angulifer* (Bibron, 1843), (2) Puerto Rican – *C. inornatus* (Reinhardt, 1843), *C. monensis* (Zenneck, 1898) and *C. granti* (Stull, 1933), (3) Jamaican – *C. subflavus* (Stejneger, 1901), (4) Hispaniolan – *C. ampelophis* Landestoy, Reynolds et Henderson, 2021, *C. fordii* (Günther, 1861), *C. gracilis* Fischer, 1888, and (5) Hispaniola-Bahamian one *C. argentum* Reynolds, Puente-Rolón, Geneva, Aviles-Rodriguez et Herrmann, 2016, *C. exsul* (Netting et Goin, 1944), *C. chrysogaster* (Cope, 1871), *C. schwartzi* (Buden, 1975), *C. striatus* (Fischer, 1856), and *C. strigillatus* (Cope, 1862).

Molecular phylogenies placed *C. angulifer* as a sister taxon of either the other *Chilabothrus* species with divergence time estimated to 21.7 [16.9, 26.0] Mya (Reynolds et al. 2013) or the Puerto Rican clade with divergence time estimated to 15.3 Mya (Pyron et al. 2013, Reynolds et al. 2015, 2016a, Landestoy et al. 2021). In any case, *C. angulifer* represents a phylogenetically deep and distinct lineage of boids. Besides, its evolutionary history, there are multiple phenotypic characters distinguishing this species from its relatives (see also Reynolds et al. 2016a). *Chilabothrus angulifer* is (1) the largest form of the genus *Chilabothrus* (Tolson 1987, Rodríguez-Robles & Greene 1996), (2) with small litter size, (3) extremely large newborns, and (4) heavy maternal investment per offspring (e.g., Tolson 1987, Frynta et al. 2016). A deep divergence of *C. angulifer* from other *Chilabothrus* species and other boids as well as presence of numerous unique morphological, physiological and behavioural characters, makes this island species a good candidate for conservation concern.

Conservation genetics of both wild and *ex situ* populations was thoroughly studied in multiple species of the genus *Chilabothrus*, especially in *C. subflavus* (Tzika et al. 2008, 2009, Newman et al. 2020), *C. inornatus* (Puente-Rolón et al. 2013, Reynolds et al. 2014b, Aungst et al. 2020), *C. monensis* (Reynolds et al. 2015, Rodríguez-Robles et al. 2015), *C. argentum* (Reynolds 2016b) and *C. chrysogaster* (Reynolds et al. 2011). The within-species genetic variation reported by these studies was rather small, well-corresponding to limited population numbers and fragmented distribution range of these endangered species (Harvey & Platenberg 2009, Newman et al. 2016, Tucker et al. 2020). In contrast to a considerable efforts devoted to genetics of these *Chilabothrus* species, *C. angulifer* has remained nearly neglected in this respect. There are just papers concerning chromosomal evolution (Augstenová et al. 2019) and parthenogenesis (Seixas et al. 2020).

The Cuban boa remains the only one of the three largest Cuban reptiles for which a more detailed population and habitat viability assessment (PHVA) has not yet been performed, while the PHVA is available for the Cuban crocodile, *Crocodylus rhombifer* (Soberon et al. 2000) and for the Cuban iguana, *Cyclura nubila* (Rodríguez et al. 2003). The species is listed as Near Threatened in the Cuban National Red List assessment (González et al. 2012), as Least Concern according to the IUCN Red List (Fong et al. 2021). However, the real conservation status of *Chilabothrus angulifer* is not well known (although a number of recent studies have signigficantly expanded our knowledge of this species – e.g. Dinets 2017, Rodríguez-Cabrera et al. 2015, 2016, 2020). The species is also granted international protection under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Appendix II). An important element of species protection is – in accordance with the modern concept of the conservation strategy (the One Plan Approach to Conservation) – adequate perspective management of the *ex situ* population in human care. The conservation breeding is listed among the recommended elements for the protection of Cuban boas in the Cuban National Red List assessment (Rodríguez Schettino 2012).

The effort to keep Cuban boas in human care is old (Rehák, stoodbook data). The Philadelphia Zoo, USA, imported twenty Cuban boas already in the 19th century (from 1876 to 1893) and another ten between 1900 and 1901, and by 1956 another fifteen specimens. At that time, their survival was usually very poor and they did not reproduce (or the reproduction was unsuccessful as in the Berlin Zoo, Germany, where the Cuban boa was also kept at the beginning of the last century). At that time, the *ex situ* population consisted exclusively of specimens imported from the wild. The birth of live young in captivity was achieved in the late 1950s (1958 in the Smithsonian National Zoological Park, USA).

In the 1960s, 1970s and 1980s, owing to close political and economic relationships with Cuba, Cuban boas were repeatedly imported to zoos and private holders in the former Czechoslovakia (e.g. Prague Zoo imported the first Cuban boas directly from Cuba in 1963 and 1965 – Rehák stoodbook data), German Democratic Republic, Soviet Union, Hungary, and Poland. An independent source of imports represented the U.S. Naval Base, Guantanamo Bay, Cuba. From the end of the 1970s, in addition to imports from the wild, the successful captive breeding began to make a significant contribution to the formation of the *ex situ* Cuban boa population in human care (e.g. Huff 1976, Nowinski 1977, Murphy et al. 1978, Vergner 1978, Tolson 1983, Bloxam & Tonge 1981, Vergner 1989, Rehák 1992). Captive propagation of *C. angulifer* (although originally regarded as a difficult species to breed) began to be very successfull despite its small litter size and extremely slow life-history. Cuban boas have become common in zoos, public and also some private collections (Marešová & Frynta 2008).

With the political changes at the turn of the eighties and nineties of the last century, imports from nature came to an almost complete end. The future of the *ex situ* population in human care began to depend almost exclusively on captive breeding. It become clear that zoos must focus on the long-term perspective management of the *ex situ* population. At the initiative of the Pra-

gue Zoo and the Amphibian and Reptile Taxon Advisory Group of the European Association of Zoos and Aquaria (chaired by I. Rehák), the *Studbook for Cuban Boa* has started (the first issue – Rehák 1994, the European Studbook for Cuban boa is maintained and continuously updated in a computerized form – I. Rehák, studbook keeper) and the European Endangered Species Programme (newly the EAZA Ex situ Programme) for Cuban boa was proposed. Consequently, the Cuban boa EEP – coordinated by Ivan Rehák/Prague Zoo – was approved by the EAZA and launched in 1993.

The contemporary European zoo population may represent descendants of many founders originated in multiple localities across Cuba and its neighbouring small islands. Consequently, mitochondrial lineages from European zoos may be viewed as more or less representative population sampling of Cuban population. Now, more than thirty years after the end of imports from the wild (after 1989, only one import from the wild is registered in the European studbook – Rehák, studbook data) to Europe and several generations of captive breeding, however, founding individuals already died (at present, only the last two specimens collected from the wild are still alive – Rehak, studbook data) and records about their geographic origin are available only in handful exceptional cases.

The aim of this paper was to provide a first insight to conservation genetics of *C. angulifer.* For this purpose, we described and analysed mitochondrial genetic variation within a population of *C. angulifer* kept in European zoos and associated private holders. The sampling was performed two decades ago during the first years of this millennium. We addressed two main questions. (1) What can the variability estimated within *ex situ* population tell us about variability and historical

Fig. 1. Adult female of *Chilabothrus angulifer* bearing haplotype UD4 (Haplogroup I).

Fig. 2. Subadult female of *Chilabothrus angulifer* bearing haplotype F8 (Haplogroup II).

demography of wild populations? (2) Is there molecular evidence that founders of this *ex situ* zoo population were variable enough to provide a good prospect for further maintenance of this population?

MATERIAL AND METHODS

Sampling

We analysed 96 new samples of *C. angulifer* (Figs 1–3) a and one new sample of *C. inornatus* (see Table 1). The geographic origin of the sampled specimens or their maternal ancestors was certain in just few cases representing localities across the Cuba island (Fig. 4).

The sampling, sequencing and preliminary analyses were carried out during the years 2002–2009. We decided to rely on non-invasive sampling causing no harm and minimizing stress in sampled specimens. Thus, we selected buccal swabs as a source of DNA. We sampled specimens kept by European zoological gardens and collaborating private breeders. We aimed to include putative founders or their maternal descendants (daughter, granddaughter etc.). In order to avoid multiple sampling of the same maternal lineage, we inspected pedigree data if available.

DNA Extractions and Sequencing

We sequenced two mitochondrial genes, combined the new sequences with previously published data (Campbell 1997, Rivera et al. 2011, Reynolds et al. 2013), and supplemented them with additional sequences from GenBank (Phylogenetic analyses).

The two genes included 1133 base pairs (bp) of cytochrome b (CYTB) and 814 bp of NADH dehydrogenase subunit 4 (ND4) gene, partial cds; tRNA-His and tRNA-Ser genes. These genes were chosen according to their phylogenetic information content in previous studies with the same taxonomic scope and availability of sequences. Genomic DNA was extracted from 96% ethanol-preserved buccal swab with NucleoSpin Tissue kit (Macherey-Nagel) according to manufacturer's protocol for buccal swab isolation. Extracted DNA was stored at –18 °C until used as a template for Polymerase Chain Reaction (PCR, Sambrook et al. 1989). Individual markers were amplifed by PCR using the same combination of primers like in previous studies. The entire CYTB gene 1113 bp long was amplified using primers L14910 (5'-GAC

Fig. 3. Adult male of *Chilabothrus angulifer* bearing haplotype 93 (Haplogroup III).

CTG TGA TMT GAA AAC CAY CGT TGT-3') and H16064 (5'-CTT TGG TTT ACA AGA ACA ATG CTT TA-3') and conditions from Burbrink et al. (2000). For ND4 gene we used a modified primer pair (L) ND4 (5'-TGA CTA CCA AAA GCT CAT GTA GAA GC-3') and (H) Leu (5'-TRC TTT TAC TTG GAT TTG CAC CA-3') (Keogh et al. 2008) and conditions from Arevalo et al. (1994).

Fig. 4. Map of Cuba island with locality indication (1 – Sierra Maestra, 2 – Nicaro, 3 – Trinidad, 4 – Matanzas, 5 – Viñales).

Table 1. List of samples collapsed to 25 haplotypes according 1059 bp long allignment of cyt b with description of three haplogroups. The samples names were created by using unique code helping to identify the zoological garden or breeder, where the samples were collected (AA – Aalborg Zoo, Denmark; B – Budapest Zoo, Hungary; BAR – Barcelona Zoo, Spain; BOJ – Bojnice Zoo, Slovakia; BRF – Burford Zoo, UK; BRS – Bristol Zoo, UK; C – Colchester Zoo, UK; CH – Kharkiv Zoo, Ukraine; CHS – Chester Zoo, UK; KAL – Kaliningrad Zoo, Russia; L – Lisbon Zoo, Portugal; PL – Plock Zoo, Poland; R – Rotterdam Zoo, Netherlands; RG – Riga Zoo, Latvia; RO – Rostock Zoo, Germany; TOR – Toruň Zoo, Poland; U – Zoo Ústí nad Labem, Czech Republic; rest of samples came from Prague Zoo and collaborating private holders, Czech Republic). If available, the geographic origin of the sampled specimen or its matriline is indicated in parentheses (in bold); H = haplogroup, N = number of sequences

H	name	N	samples (locality)	CYTB	ND4
I	UD4	2	K1, UD4	OL158846	OM648935
I	UHO	4	F9, S101, UD3, UHO	OL158848	OM648937
I	ML1	1	ML1	OL158847	OM648936
I	R703	1	R703	OL158849	OM648938
I	17	9	17, 18, 77, BN1, E42, EX1, F3 (**Viñales**), U1, U4	OL158850	OM648939
I	P1	5	P1, P5, PR1, PR2, PR6	OL158851	OM648940
I	E43	1	E43	OL158852	OM648941
II	14	7	14, 15, EA, KHN0, M14, M18, ST1	OL158855	OM648944
II	U3	29	16, AA3, B333, BAR2, BLL, BOJ3, BR4, BR5, BR7, BR6, BRF156, BRS1, BRS2, BRS3, C1, C2, F16, F17, DR115, DR117, DR118, KAL1, KAL0, L6504, M19, MD114, RG4, U2, U3	OL158854	OM648943
II	KP1	8	P2, P3, P4, PR3, PR4, PR5, KP1, KT0	OL158858	OM648947
II	F8	1	F8	OL158856	OM648945
II	78	1	78	OL158860	OM648949
II	MAT1	1	MAT1 (**Matanzas**)	OL158853	OM648942
II	BRF157	1	BRF157	OL158857	OM648946
II	BRF158	1	BRF158	OL158859	OM648948
III	M2	1	M2	OL158867	OM648956
III	TRI	1	TRI (**Trinidad**)	OL158865	OM648954
III	KSC	3	CH1, KSC (**Sierra Maestra**), RG3	OL158866	OM648955
III	93	4	93 (**Nicaro**), TO0, TO1, UH1	OL158862	OM648951
III	PLB	5	PLB, PLA, PLC, TOR0L, TOR1L	OL158863	OM648952
III	RO1	1	RO1	OL158861	OM648950
III	KN1	1	KN1 (**Nicaro**)	OL158864	OM648953
III	H	2	DR116, H	OL158868	OM648957
III	AA1	5	AA1, AA2, BAR3, BAR1, CHS1	OL158869	OM648958
III	BRF155	1	BRF155	OL158870	OM648959

Phylogenetic and demographic analyses

We prepared four data sets for phylogenetic analyses performation – CYTB alignment 1059 bp long, ND4 alignment 814 bp long and two alignments combining CYTB and ND4 sequences of total lenght 1874 bp containing sequences downloaded from GenBank used as outgroup (*C. fordii* – KP746467, KP746549; *C. striatus* – KP746472, KP746555; *C. monensis* – KP746425, KP746430, KP746507, KP746512; *C. inornatus* – KC819501, KC819502, KP746552, 746553; *C. exsul* – KC329926, KC329959; *C. chrysogaster* – KC329925, KC329958; *C. subflavus* – KC329948, KC329973; *Epicrates cenchria* – KC329950, KC329974; *Epicrates maurus* – KC329951, KC329976; *Eunectes murinus* – KC329952, KC329977; *Eunectes notaeus* – KC329953, JN967256).

The estimates of evolutionary divergence over sequence pairs between haplotypes of *C. angulifer* population were conducted using the maximum composite likelihood model implemented in MEGA6 (Kumar et al. 2018, Tamura et al. 2013). The relationship within population of *C. angulifer* was represented by using the Median-Joining network approach (Bandelt et al. 1999) in the program Population Analysis with Reticulate Trees (PopART; Leigh & Bryant 2015). Two haplotype networks were worked out – for alignment containing all 96 CYTB sequeces of *C. angulifer* and for alignment ND4 containing one sequence of each CYTB haplotype.

Phylogenetic reconstructions were conducted using Bayesian analysis (BA), maximum likelihood (ML) and Bayesian interference (BI) for alignments combining the CYTB and ND4 sequences. Bayesian analysises (BA) of both multilocus

alignments (CYTB, ND4) were performed using MrBayes v3.2.6 (Huelsenbeck et al. 2001, Ronquist et al 2012). The best-fit models of sequence evolution were selected under MrModeltest 2.3 (Nylander 2004). The six partitions were set following way: three for CYTB and three for ND4 gene (CYTB: GTR+I+G pos1, GTR+I+G pos2, GTR+I+G pos3; ND4: GTR+I+G pos1, GTR+I+G pos2, GTR+I+G pos3). Two independent runs of BA were conducted with a random starting tree and run for 20,000,000 generations, with trees sampled every 1000 generations and with 25% burn-in. The ML analysis of multilocus alignment (CYTB, ND4) was performed in IQ-TREE (Nguyen et al. 2015) using the online web interface W-IQ-TREE (Trifinopoulos et al. 2016). The best substitution model for both genes was selected automatically by ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE. The best model of nucleotide substitution for the partition by gene scheme was identified as follows (CYTB: TIM2e+G4 pos1, TN+F+I pos2, TIM2+F+G4 pos3; TIM2+F+G4 pos1, HKY+F+I pos2, HKY+F+G4 pos3).

BEAST v.2.6.6. (Bouckaert et al. 2014) was used for the BI analysis, the two partitions were set. The GTR model was selected as the best model of nucleotide substitution by jModelTest v.2.1.6. (Darriba et al. 2012) for both partitions, the CYTB and ND4. Site and clock models were unlinked across partitions. Strict clock model was used for all partitions. According to Reynolds et al. (2013), we set the prior of *Chilabothrus* root node to 21.7 Mya (SD=1.8).

Polymorphisms within *C. angulifer* population were worked out by the statistic software DnaSP v6 6.12.03 (Rozas et al. 2017) which estimated the following: haplotype diversity (h), segregating sites (S), nucleotide diversity (π), and Tajima's D, Fu & Li's F*, Fu & Li's D*, and Fu's FS tests. According to Russell et al. (2005), high values of h and π indicate a constant large population size. However, a low value of π and high value of h signifies a recent expansion. To estimate population dynamics through time we have constructed a model in BEAUti, we have run Markov chain Monte Carlo simulations with 30 million iterations and 10 million burn-ins using the GTR model and molecular clock with setting a rate 0.02 per 3 million years in BEAST (Rodríguez-Robles et al. 2015). We have analysed the outputs in TRACER v1.7.1 and displayed them as Bayesian skyline plot drawing the effective population sizes over time.

RESULTS

We sequenced cyt b in 96 specimens of *Chilabothrus angulifer*. We identified 25 distinct haplotypes (Table 1). While 13 of them were detected exclusively in a single specimen, the remaining ones were found in multiple individuals (2, 2, 3, 4, 4, 5, 5, 5, 7, 8, 9 and even 28 times).

Median-Joining Network (MJN) revealed a presence of three clearly distinct main groups, further referred to as Haplogroup I, Haplogroup II, and Haplogroup III (Fig. 5). Maximum uncorrected p-distances among CYTB haplotypes belonging to different groups were 0.0220 (mean = 0.0183), 0.0340 (0.0228) and 0.0293 (0.0277), for I vs II, I vs III and II vs III, respectively. Corresponding values for within group comparisons were 0.0121 (0.0076), 0.0083 (0.0033) and 0.0293 (0.0101), for groups I, II and III, respectively (Table 2).

Bayesian spline-plot of this dataset revealed a long-term stability of effective population size during the last three millions of years followed by a recent decline (Fig. 6). Population parameters estimated separately for each haplogroup, as well as for pooled ones, are provided (Table 3). These parameters are congruent with relative stability of the population numbers in the past. The only exception represents Haplogroup II exhibiting negative values of parameters indicating recent population expansion (significant at $P<0.05$).

Table 2**.** Estimates of evolutionary divergence over sequence pairs between haplotypes of *Chilabothrus angulifer* population. The mean number, minimum, and maximum of base substitutions per site overall sequence pairs within each group is shown. Analyses were conducted using the maximum composite likelihood model implemented in MEGA6 (Tamura et al. 2013); alignment 1059 bp of CYTB; H = haplogroup

H	I			II			III		
	min	max	average	min	max	average	min	max	average
I	0.000628	0.012102	0.007595	–	–	–	–	–	–
II	0.016076	0.022047	0.018310	0.000627	0.008270	0.003298	–	–	–
III	0.024066	0.034391	0.028801	0.023397	0.329260	0.027666	0.000627	0.029300	0.010094

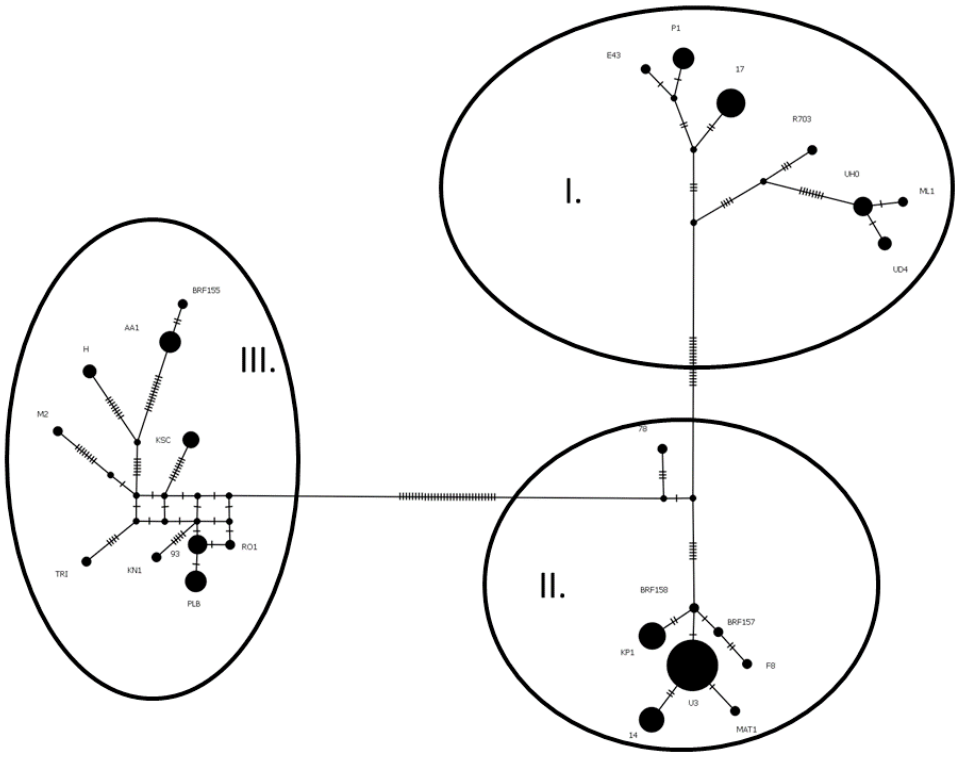

Fig. 5. Median-joining haplotype network computed using CYTB 1059 bp alignment containing all 96 samples of *Chilabothrus angulifer*, the size of the circle is proportional to the frequency of haplotypes and the mutational steps are indicated on the branches.

In addition, we sequenced ND4 gene in representatives of each CYTB haplotype. This proved presence of 19 ND4 haplotypes. The resulting network pattern of ND4 sequences (Fig. 7) clearly resembled that of CYTB one (Fig. 5).

In order to recover phylogenetic relationships among *C. angulifer* haplotypes, we run Bayesian analysis. The analysed alignment contained both examined mitochondrial genes for all 25 *C. angulifer* haplotypes and outgroups. The results corroborated that the groups II and III form well-supported monophyletic clades (posterior probability = 1). In contrast to this, the group I splits into two lineages. One of them has a sister relationship to a clade including both remaining groups (II+III), while the other represents a basal most offshoot of *C. angulifer* tree (Fig. 8).

Next, we examined an alignment (CYTB and ND4) including two representatives of each *C. angulifer* haplogroup, other *Chilabothrus* species and outgroups (*Eunectes* and *Epicrates*). We employed Bayesian and Maximum Likelihood approaches. The results of both computation methods were virtually the same and confirmed the above described topology (Fig. 9).

Finally, we run BEAST and constructed time-calibrated tree to estimate timing of divergence among *C. angulifer* haplogroups (Fig. 10). In contrast to the previous analyses, it placed group

Table 3. Demographic characteristics for the *Chilabothrus angulifer* based on the 1059 bp mitochondrial CYTB alignment. Sequences: number of individuals sequenced (Ns), number of segregating sites (S), number of haplotypes (H), haplotype diversity (h), nucleotide diversity (π), Fu & Li's (F&L) F*, Fu & Li's (F&L) D*, Fu's FS, Tajima's D

clade	Ns	S	H	h	Π	F&L *F**	F&L *D**	Fu's *Fs*	Tajima's *D*
all samples	96	62	24	0.876	0.0270	1.0500	1.0986	2.075	0.5806
I	23	25	7	0.791	0.0083	1.9853	0.6739	5.368	1.3448
II	49	19	8	0.613	0.0075	–3.2485	–3.3104	–0.373	–1.6527
III	24	57	10	0.891	0.0126	–0.2474	–0.2320	4.380	–0.1647

III as a sister of groups I+II. The last common ancestor of all *C. angulifer* haplogroups was estimated to 3.57 (95% CI: 2.37–4.82) Mya, while the split between the group I and II to 2.26 (95% CI: 1.43–3.09) Mya.

DISCUSSION

Haplotype diversity

We sampled 96 individuals and detected presence of 25 mitochondrial haplotypes in examined captive population of *C. angulifer*. Although, we tried to avoid sampling of close maternal relatives, we repeatedly found multiple occurrence of the same haplotype coming from the same institution (cf. Table 1). It is likely, that we sampled multiple maternal descendants of the same founder in some cases. Thus, we even underestimated haplotype diversity among the founders of

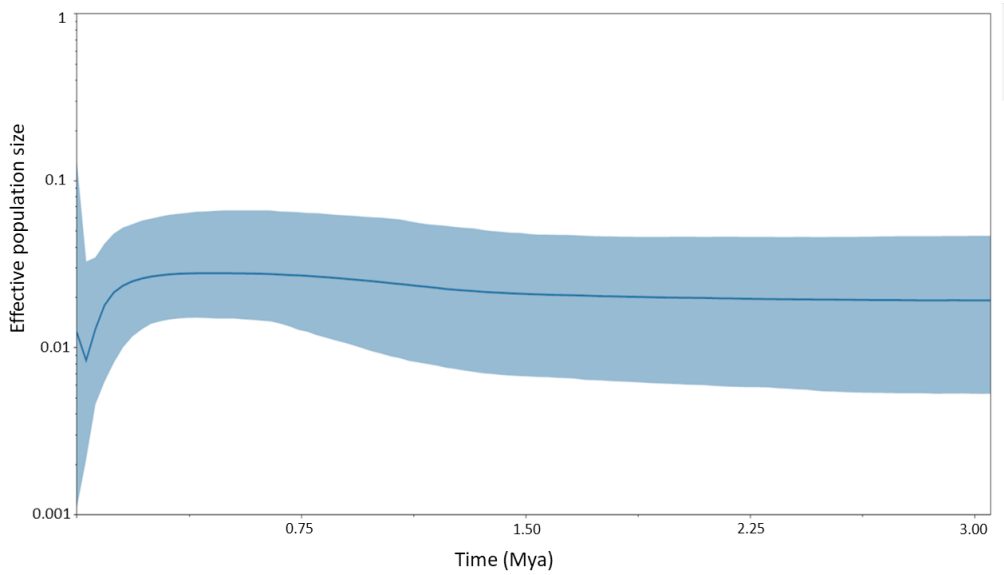

Fig. 6. The Bayesian skyline plot (BSP) of sequence variability in CYTB 1059 bp long for the *Chilabothrus angulifer* population, visualizing the effective population sizes over time.

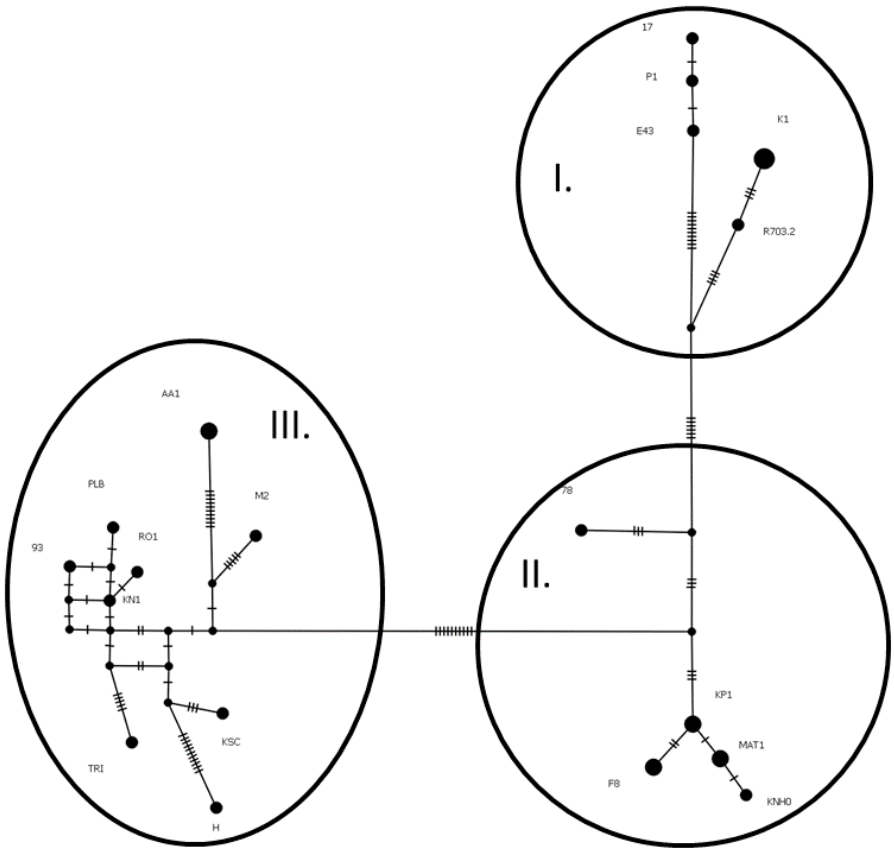

Fig. 7. Median-joining haplotype network computed using ND4 814 bp alignment containing all 96 samples of *Chilabothrus angulifer*, the size of the circle is proportional to the frequency of haplotypes and the mutational steps are indicated on the branches.

the examined captive population. This clearly supports the view that haplotype diversity in the source natural populations of this endangered snake was extremely high.

Divergence among haplogroups

The deepest divergence among principal haplogroups of *C. angulifer*, we report here (3.57 Mya, 2.37–4.82), suggests that they split already in the Pliocene period. This estimate is of comparable magnitude as those previously reported between clearly distinct species of the genus *Chilabothrus* belonging to the same major clade of this genus (for these clades see under Introduction). Specifically, divergences within *ampelophis-fordii*, *exsul-schwarzi-argentum-striatus-strigillatus* and *monensis-granti* clades are possibly even more recent (cf. Landestoy et al. 2021). Of course, it can be assumed that in widely distributed Cuban boas inhabiting a relatively large island (and its nearby coastal archipelagos) with a complicated geological history, the genetic structure and phylogeography will be much more complicated, making the interpretation of detected divergences much more difficult and complex than in the case of isolated congeneric species from

small islands or islets. Current advanced genomic studies in continental rattlesnakes of the genus *Crotalus* showed complex evolutionary history of these snakes. Episodes of temporal allopatry accompanied by genetic drift and divergent selection were repeatedly followed by secondary contacts with pervasive gene flow even between anciently diverged lineages (Schield et al. 2015, 2017, 2018, 2019).

Sequence divergence of mitochondrial genes among mainland species of boas tend to be higher than among the haplogroups of *Chilabothrus angulifer*, e.g., *Epicrates* (Passos & Fernandes 2008, Rivera et al. 2011), *Boa* (Hynková et al. 2009), *Corallus* (Colston et al. 2013), *Eryx* (Eskandarzadeh et al. 2020a, b), *Acrantophis* (Vences & Glaw 2003), and *Candoia* (Austin 2000). There are, however, multiple exceptions, e.g., sequence divergence between the South American *Epicrates cenchria* and its sister species *E. maurus* with predominantly Central American range (Rivera et al. 2011) is very close to that between *Chilabothrus angulifer* haplogroups III and I+II. Similarly, geographically localized haplogroups within *Boa imperator* exhibiting distinct parapatric geographic ranges may be viewed as cryptic species or at least subspecies (Suárez-Atilano et al. 2014, Card et al. 2016). Island populations of *B. imperator* are challenging. The discordance between very small sequence divergence and parallel change in morphological and developmental traits reminds us that magnitude of the adaptive evolution is not necessarily proportional to expired time (Boback 2006, Boback & Siefferman 2010, Green 2010, Bushar et al. 2015, Card et al. 2019). In pythons, the snakes bearing adaptive strategies comparable to that of boas (Esquerré & Keogh 2016), we can found multiple examples demonstrating above discussed phenomena (Rawlings et al. 2008, Esquerré et al. 2020).

Hybridization between snakes belonging to different mitochondrial clades

Sequence divergence in mitochondrial genes can be used as a predictor of ability to hybridize (Jančúchová Lásková et al. 2015a). Although, the 3% genetic divergence among haplogroups of *C. angulifer* is comparable with that among distinct species of boids and pythons (see above), it is still much smaller than that between the most distant species of squamates that are still able to interbreed and produce fertile hybrids (Jančúchová Lásková et al. 2015b, for a review see

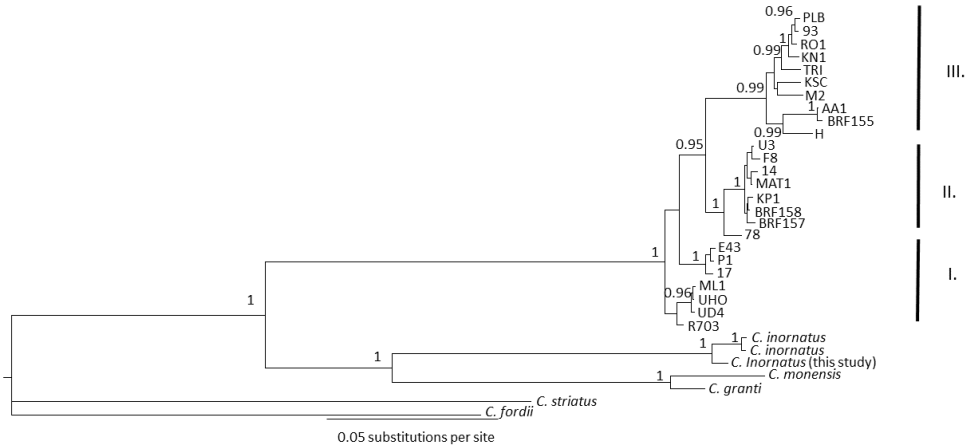

Fig. 8. Bayesian tree computed from multilocus alignment (CYTB and ND4 genes). Statistical support of the nodes is expressed as a posterior probability (posterior probabilities >0.95 are shown)

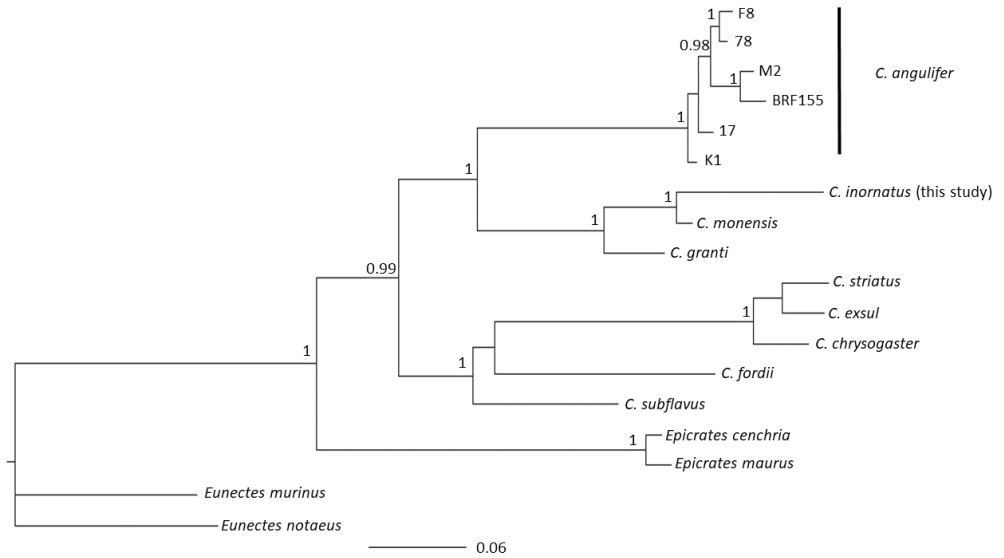

Fig. 9. Bayesian MCMC consensus tree from the concatenated and partitioned 2-gene dataset. Nodes with posterior probabilities >0.95 are shown, while numbers indicate posterior probabilities at nodes with lower support are without number marking. Refer to Table 1 for more information on tip labels.

Jančúchová Lásková et al. 2015a). In captive *C. angulifer*, we already proved fertility of hybrids between individuals belonging to different haplogroups (unpublished data).

Why are the haplogroups so divergent within a single island

We report here a surprisingly deep divergence among mitochondrial haplogroups of *C. angulifer*. This requires persistence of maternal lineages within this species for a long period of last few millions of years. According to the phylogeographic and coalescent theory (cf. Avise 2000), this may be explained either by (1) an extremely large and stable population size, or by (2) spatial subdivision of the species into multiple isolated populations, each locally maintaining a certain haplogroup.

The first hypothesis, requiring an extremely large population size, is compatible with the view that prior colonization of the island by humans, *C. angulifer* belonged to principal top terrestrial predators of Cuba. Compared to "warm blooded" predators, the ratio between biomass of predators and their prey is much higher in the case of "cold-blooded" predators like snakes. Thus, we cannot exclude that *C. angulifer* was present in high densities and distributed throughout the territory of this large island. Although recent records of *C. angulifer* are absent in some zones of the island (Rodríguez Schettino et al. 2013), the historical distribution range could be fairly continuous. Although, *C. angulifer* was repeatedly reported from several Pleistocene localities (cf. Syromyatnikova et al. 2021 and reference herein), we may only speculate about real population size of *C. angulifer* in the Pleistocene or Pre-Colombian periods. Our Bayesian-spline estimating demography for a quasi-unstructured population revealed that except very recent decline the population was stable during the last three millions of years.

Although, slow life history of *C. angulifer* may also contribute to extension of coalescence time (but see Rodríguez-Cabrera et al. 2016 reporting early sexual maturation in natural population),

the first hypothesis is not in accord with a strong phylogeographic structure and/or endemism reported in other Cuban reptiles (see below for rock-iguanas).

The second hypothesis might be supported by the fact that Cuba consists from multiple continental fragments, originally representing separate islands. During the Lower Middle Miocene (14–16 Mya), there were at least four main islands, i.e., Western, West Central, East Central and Eastern islands that have been joined only recently. The Western island was the most distant from the others. Formation of Cuba in its current form has been completed in Pliocene, roughly 4 Mya ago (see Iturralde-Vinent 2006). Therefore, estimated divergence time among *C. angulifer* haplogroups (3.57 Mya) is not old enough to be compatible with the scenario suggesting a secondary contact of haplogroups initially evolved in isolation on precursor islands. Nevertheless, as the confidence interval of our estimate is too wide (2.37–4.82 Mya), this scenario cannot be ruled out entirely.

Putative subdivision of *C. angulifer* populations after final formation of the island during Pliocene is more consistent with our estimates of divergence time among three principal haplogroups. We have no evidence about permanent geographic barrier dividing the island during the Pliocene-Quaternary period, however, lowland parts of the island were periodically inundated during the interglacial maxima. This process temporally separated the area into three or even more pieces. On the contrary, the Isla de la Juventud would merge with mainland Cuba when the sea level would drop by about 18 m, while during the last glacial maximum (ca. 20,000 years ago)

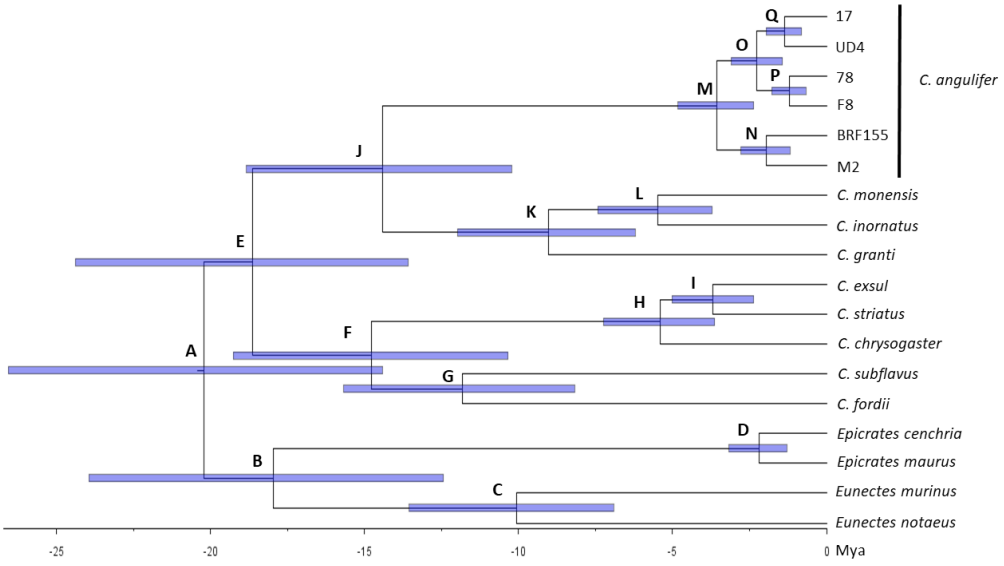

Fig. 10. Time-calibrated species tree for the clade *Chilabothrus*, *Epicrates* and *Eunectes*. Nodes are labelled with letters and 95% HPD intervals are shown. Estimated divergence times in million years, [95% HPD] are following: A – 20.0, [14.4, 26.6]; B – 17.7, [12.4, 24.0]; C – 9.9, [6.9, 13.6]; D – 2.1, [1.3, 3.2]; E – 18.4; F – 14.6 [10.3, 19.3]; G – 11.7 [8.2, 15.7]; H – 5.3 [3.6, 7.2]; I – 3.6 [2.4, 5.0]; J – 14.2 [10.2, 18.8]; K – 8.9 [6.2, 12.0]; L – 5.4 [3.7, 7.4]; M – 3.5 [2.4, 4.8]; N – 1.9 [1.2, 2.8]; O – 2.2 [1.4, 3.1]; P – 1.2 [0.7, 1.8]; Q – 1.3 [0.8, 2.0]. Except nodes B (0.99), E (0.95) and L (0.93) all posterior probabilities are 1.00.

the sea level was about 125 m lower than it is today (Fairbanks 1989, Tolson & Henderson 1993, Poore et al. 2000, Steadman & Franklin 2017).

A strong male-biased dispersal was reported in many snake species (e.g., Rivera et al. 2006, Keogh et al.2007, Dubey et al. 2008, Pernetta et al. 2011, Folt et al. 2019). This mechanism may contribute to a limited flow of maternally inherited mitochondrial genes and consequent geographically limited distribution of haplotypes. Therefore, an isolation by distance should be also considered, besides a true geographic barrier. A study performed in continental coral snakes recently demonstrated that spatial sorting of their mitochondrial haplotypes can be attributed to genetic surfing caused by stochastic and demographic processes rather than to allopatric divergence (Streicher et al. 2016).

In general, all scenarios involving the second hypothesis predict presence of a clear geographic distribution pattern of the haplogroups. Below we discuss evidence supporting this prediction.

Geographic distribution of the haplogroups

We sampled captive population, decades after last imports from wild. Thus, we rely on only anecdotic records about the precise geographic origin of examined haplotypes.

The sample belonging to the maternal descendant of the founder coming from Viñales in the Western part of Cuba is the only clearly localized sample of the Haplogroup I comprising 7 haplotypes (23 individuals). The most common origin of imported specimens of *C. angulifer* were probably provinces surrounding the capital Havana. Multiple founders possessing haplotypes belonging to a haplogroup II, which is the most represented in our sampling (8 haplotypes, 48 individuals), probably come from this part of the island, nevertheless we can be sure about it in just one case (locality Matanzas). Among 10 haplotypes (24 individuals) belonging to a haplogroup III, only four are of known geographic origin. All of them come from Central (Trinidad) and Eastern Cuba (Nicaro and Sierra Maestra). Another record of Haplogroup III from Eastern part of Cuba represents CYTB sequence (accession No. KC329922) belonging to our haplotype AA1 which was reported from Guantánamo by Reynolds et al. (2013)**.** This may suggest, that Haplogroup III is distributed predominantly in the Central-Eastern parts of the Island, while Haplogroups I and II in the Western areas (see below for a comparison with Cuban rock iguanas).

Although, it is likely that *C. angulifer* haplogroups follow the above suggested geographic pattern, we have no sufficient data to finally prove this hypothesis and further research in the wild is needed to solve this problem.

A comparison with phylogeography of other species

Cuban rock-iguana (*Cyclura nubila*) belongs to the most charismatic species of squamates inhabiting Cuba. Because its large body size and similar history on the Great Antilles, this species provides a reliable comparison with *Chilabothrus angulifer*. Similarly, as in *C. angulifer*, there is a deep divergence among mitochondrial haplotypes of this iguana (Frynta et al. 2010). Recently, Shaney et al. (2020) sampled multiple populations of *Cyclura nubila* and demonstrated that each geographically defined population of *C. nubila* is characterized by an exclusive group of closely related haplotypes. Moreover, the haplotypes coming from the western and eastern parts of Cuba form mutually clearly distinct clades exhibiting parapatric distribution. Each of these clades further splits into several local haplogroups. The split between these principal clades is deep, they estimated their divergence to 6.8 Mya (Shaney et al. 2020). Nevertheless, confidence interval of their estimate (4.2–9.7 Mya) partly overlaps that we computed for a split between major mitochondrial clades (I+II versus III) of *Chilabothrus angulifer* (2.37–4.82 Mya). Thus, the divergence patterns recovered in these two charismatic reptilian species may reflect the same underlying process.

A deep split between Western and Eastern clades of CYTB (5.2%) was also reported in endemic rodents of the genus *Capromys*. Nevertheless, as a result of more rapid substitution rate in rodents, a divergence time between these clades was estimated just to 1.1 My (Upham & Borroto-Páez 2017). Interestingly enough, geographic distribution of these *Capromys* clades follows almost precisely the pattern reported in *Cyclura nubila* (Shaney et al. 2020).

Phylogenetic relationships among the haplogroups I, II and III

The topologies recovered by MrBayes and maximum likelihood proved that haplogroups II and III are monophyletic and mutually exclusive. These methods, however, failed to support monophyly of Haplogroup I and placed lineages belonging to this group as sister clades of the remaining haplotypes belonging to groups II and III (Figs 8 and 9). In contrast to this topology, a time-calibrated tree produced by BEAST reveal basal split between Haplogroup III and the clade including mutually sister clades formed by haplogroups I and II. We prefer the topology of the time-calibrated tree (Fig. 10) because of following reasons: (1) The divergence between C. *angulifer* and its closest relatives of the the Puerto Rican clade (15.3 Mya) is much longer compared to the deepest split between *C. angulifer* haplogroups (3.57 Mya). Thus, finding a proper root of the haplotype tree is really difficult and uncertain. (2) The split between groups III and I+II is in accord with the haplotype networks, (3) In the analyses placing haplogroup III with II, the branch leading from their common ancestor to the Haplogroup III is long.

Conservation genetics of European *ex situ* population

On one hand, we reported the deep splits among principal mitochondrial haplogroups and thus we have to expect that natural populations of *Chilabothrus angulifer*, similarly like those of the Cuban rock-iguanas (*Cyclura nubila*), are divided into multiple conservation units according to coalescence of their genes (cf. Shaney et al. 2020). After several generations of captive breeding (the generation time for *C. angulifer* is about 11.0 years, the oldest captive born Cuban boa, who contributed to the current European population with offsprings, is a female born in 1973 – Rehák, studbook data) and interbreeding among *C. angulifer* originated from multiple populations, pure--bred lineages are rare and thus not sufficient for establishing viable population. Such an attempt, reflecting our finding, that Cuban boas represent at least two different subspecies or species (depending on the criteria and applied species concept). would require derivation of new *ex situ* populations from the wild (as pointed out already by Rehák 2006, 2008).

On the other hand, zoo populations of the reptiles are typically extremely small (Marešová & Frynta 2008, Frynta et al. 2010) and thus suffer from inbreeding rather than outbreeding depression. The number of maternally unrelated founders of the European zoo population reflected by haplotype diversity reported in this study is large. It clearly confirms the view that the number of founders and their genetic variability at the beginning of this millennium were large enough to create a viable *ex situ* population of this endangered snake species. Nevertheless, an initial expansion, outbreeding, maintenance at a sufficient population size and regular breeding are required to prevent loss of genetic variation and viability of the population.

Recent population of Cuban boas in zoos (as well as in private collections) consists mostly of captive born animals originated of founders imported from wild – especially in the 1970s and 1980s (Rehák, studbook data). The species is currently mainly found in European institutions, with just a few others in Asia and North America, so the management at the European level (European *ex situ* population) is a convenient option. The current population is descended from at least of fifteen founders, but any more accurate number is no possible to calculate because more than 80% of specimens have unknown pedigree. For the same reason, a more detailed population assessment, including demographic and genetic analysis, cannot be performed, population projections

cannot be accurately created and thus important sources for the well based establishing of the Long-term Management Plan for Cuban boas in human care are missing. Currently, 233 living Cuban boas (68 males, 80 females and 85 of undetermined sex – held in 63 cooperating institutions) are registered in the European studbook for Cuban boas (Rehák, studbook data), which is a sufficient number for management (the current population size may be able to minimize random demographic and catastrophic events for the long-term) and at the same time a number requiring adequate management. We do not have any data to suggest reduced fitness, survival or reproductive problems in connection with crossbreeding.

At the same time, we consider that although the divergence among some populations of Cuban boas is greater than that of some other, taxonomically recognized, species (see above), it is important to note that most of these species are isolated island species "doomed" to evolve independently, whereas in the case of Cuban boas, the individual evolutionary lines previously formed during Cuba's complex geological history, currently inhabit a single island (with the surrounding nearby coastal archipelagos), and it can be assumed that their future evolution is likely to be associated with the unavoidable hybridization.

In conclusion, we therefore recommend the management of the existing *ex situ* population of Cuban boas in human care as a whole, as a single unit. At least until a more accurate picture of the phylogeography of Cuban boas, the geographical distribution of their individual evolutionary lines, their natural hybridization and the possible existence of hybrid zones is available.

Limitations of the study

Our study is based on genetic samples from captive animals. Thus, we relied solely on maternally inherited mitochondrial genes. As the examined individuals were mostly descendants of individuals coming from different regions of Cuba, the utility of biparentally inherited nuclear genes was greatly limited. Moreover, sex chromosomes of *C. angulifer* are not clearly differentiated (Augstenová et al. 2019), which prevented us to employ Y-chomosome. Therefore, further genetic examination of wild populations of *C. angulifer* including genomic approach is urgently needed to complete the picture.

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Author contributions. IR and DF designed, conceived and supervised the research; IŠ performed laboratory work, curation of the data and preliminary phylogenetic analyses; BOS performed final analyses and graphic presentation of the results; IR, DF and OŠ collected the samples; DF and IR funding acquisition; DF, IR and BOS wrote the paper.

REFERENCES

Ali J. R. 2012: Colonizing the Caribbean: is the GAARlandia land-bridge hypothesis gaining a foothold? *Journal of Biogeography* **39**: 431–433.

Ali J. R. & Hedges S. B. 2021: Colonizing the Caribbean: New geological data and an updated land-vertebrate colonization record challenge the GAARlandia land-bridge hypothesis. *Journal of Biogeography* **48**: 2699–2707.

Arbona E. M. 2016: The Cuban boa, Chilabothrus angulifer (Squamata: Boidae) on Jardines de la Reina archipelago. *Poeyana* **503**: 64–65.

Arevalo E., Davis S. K. & Sites Jr J. W. 1994: Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the Sceloporus grammicus complex (Phrynosomatidae) in central Mexico. *Systematic Biology* **43**: 387–418.

Austin C. C. 2000: Molecular phylogeny and historical biogeography of Pacific island boas (Candoia). *Copeia* **2000**: 341–352.

Avise J. C. 2000: *Phylogeography*: *The History and Formation of Species*. Cambridge: Harvard University Press, x+447 pp.

Augstenová B., Mazzoleni S., Kostmann A., Altmanová M., Frynta D., Kratochvíl L. & Rovatsos M. 2019: Cytogenetic analysis did not reveal differentiated sex chromosomes in ten species of boas and pythons (Reptilia: Serpentes). *Genes* **10**: 934.

Aungst E. R., Puente-Rolón A. R. & Reynolds R. G. 2020: Genetic diversity in the US ex situ populations of the endangered Puerto Rican boa, Chilabothrus inornatus. *Zoo Biology* **39**: 205–213.

Bandelt H., Forster P. & Röhl A. 1999: Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**: 37–48.

Blackburn D. C., Keeffe R. M., Vallejo-Pareja M. C. & Vélez-Juarbe J. 2020: The earliest record of Caribbean frogs: a fossil coquí from Puerto Rico. *Biology Letters* **16**(20190947): 1–6.

Bloxam Q. & Tonge S. 1981: A comparison of reproduction in three species of Epicrates (Serpentes, Boidae) maintained at the Jersey Wildlife Preservation Trust. *Dodo*, *Journal of the Jersey Wildlife Preservation Trust* **18**: 64–67.

Boback S. M. 2006: A morphometric comparison of island and mainland boas (Boa constrictor) in Belize. *Copeia* **2006**(2): 261–267.

Boback S. M. & Siefferman L. M. 2010: Variation in color and color change in island and mainland boas (Boa constrictor). *Journal of Herpetology* **44**: 506–515.

Bouckaert R., Heled J., Kühnert D., Vaughan T., Wu C. H., Xie D., Suchard M. A., Rambaut A. & Drummond A. J. 2014: BEAST 2: a software platform for Bayesian evolutionary analysis. *Public Llibrary of Science Computational Biology* **10**(4; e1003537): 1–6.

Burbrink F. T. 2004: Inferring the phylogenetic position of Boa constrictor among the Boinae. *Molecular Phylogenetics and Evolution* **34**: 167–180.

Burbrink F. T., Lawson R. & Slowinski J. B. 2000: Mitochondrial DNA phylogeography of the polytypic North American rat snake (Elaphe obsoleta): a critique of the subspecies concept. *Evolution* **54**: 2107–2118.

Bushar L. M., Reynolds R. G., Tucker S., Pace L. C., Lutterschmidt W. I., Odum R. A. & Reinert H. K. 2015: Genetic characterization of an invasive Boa constrictor population on the Caribbean island of Aruba. *Journal of Herpetology* **49**: 602–610.

Cádiz A., Nagata N., Díaz L. M., Suzuki-Ohno Y., Echenique-Díaz L. M., Akashi H. D., Makino T. & Kawata M. 2018: Factors affecting interspecific differences in genetic divergence among populations of Anolis lizards in Cuba. *Zoological Letters* **4**: 1–12.

Campbell B. N. 1997: *Hic Sunt Serpentes*: *Molecular Phylogenetics and the Boidae* (*Serpentes*: *Booidea*). Unpubl. PhD Dissertation. Otawa: Queens University.

Card D. C., Adams R. H., Schield D. R., Perry B. W., Corbin A. B., Pasquesi G. I., Row K., Van Kleeck M. J., Daza J. M., Booth W., Montgomery C. E., Boback S. M, & Castoe T. A. 2019: Genomic basis of convergent island phenotypes in boa constrictors. *Genome Biology and Evolution* **11**: 3123–3143.

Card D. C., Schield D. R., Adams R. H., Corbin A. B., Perry B. W., Andrew A. L., Pasquesi G. I. M., Smith E. N., Jezkova T., Boback S. M., Booth W. & Castoe T. A. 2016: Phylogeographic and population genetic analyses reveal multiple species of Boa and independent origins of insular dwarfism. *Molecular Phylogenetics and Evolution* **102**: 104–116.

Colston T. J., Grazziotin F. G., Shepard D. B., Vitt L. J., Colli G. R., Henderson R. W., Bonatto S., Zaher H., Noonan B. E. & Burbrink F. T. 2013: Molecular systematics and historical biogeography of tree boas (Corallus spp.). *Molecular Phylogenetics and Evolution* **66**: 953–959.

Darriba D., Taboada G. L., Doallo R. & Posada D. 2012: jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772–772.

Dinets V. 2017: Coordinated hunting by Cuban boas. *Animal Behavior and Cognition* **4**: 24–29.

Dubey S., Brown G. P., Madsen T. & Shine R. 2008: Male-biased dispersal in a tropical Australian snake (Stegonotus cucullatus, Colubridae). *Molecular Ecology* **17**: 3506–3514.

Esquerré D. & Keogh S. J. 2016: Parallel selective pressures drive convergent diversification of phenotypes in pythons and boas. *Ecology Letters* **19**: 800–809.

Esquerré D., Donnellan S., Brennan I. G., Lemmon A. R., Moriarty Lemmon E., Zaher H., Grazziotin S. G. & Keogh J. S. 2020: Phylogenomics, biogeography, and morphometrics reveal rapid phenotypic evolution in pythons after crossing Wallace's line. *Systematic Biology* **69**: 1039–1051.

Eskandarzadeh N., Rastegar-Pouyani N., Rastegar-Pouyani E., Zargan J., Hajinourmohamadi A., Nazarov R. A., Sami S., Rajabizadeh M., Nabizadeh H. & Navaian M. 2020a: A new species of Eryx (Serpentes: Erycidae) from Iran. *Zootaxa* **4767**: 182–192.

Eskandarzadeh N., Rastegar-Pouyani N., Rastegar-Pouyani E., Todehdehghan F., Rajabizadeh M., Zarrintab M., Rhadi F. A. & Kami H. G. 2020b: Revised classification of the genus Eryx Daudin, 1803 (Serpentes: Erycidae) in Iran and neighbouring areas, based on mtDNA sequences and morphological data. *Herpetological Journal* **30**: 2–12.

Estrada A. R. & Ruibal R. 1999: A review of Cuban herpetology. Pp.: 31–62. In: Crother B. I. (ed.): *Caribbean Amphibians and Reptiles*. San Diego & London: Academic Press, 495 pp.

Fairbanks R. G. 1989: A 17,000-year glacio-eustatic sea level record: influence of glacial melting rates on the Younger Dryas event and deep-ocean circulation. *Nature* **342**: 637–642.

Folt B., Bauder J., Spear S., Stevenson D., Hoffman M., Oaks J. R., Woods J. L. Jr., Jenkins C., Steen D. A. & Guyer C. 2019: Taxonomic and conservation implications of population genetic admixture, mito-nuclear discordance, and male-biased dispersal of a large endangered snake, Drymarchon couperi. *Public Library of Science One* **14**(3; e0214439): 1–21.

Fong A. 2021. Chilabothrus angulifer. *The IUCN Red List of Threatened Species 2021*: e.T7815A18979599. https://dx.doi.org/10.2305/IUCN.UK.2021-2.RLTS.T7815A18979599.en. Downloaded on 30 October 2021.

Frynta D., Rehák I. & Starostová Z. 2010: New haplotypes of Cyclura nubila nubila from Cuba changed the phylogenetic tree of rock-iguanas: a challenge for conservation strategies? *Amphibia-Reptilia* **31**: 134–143.

Frynta D., Marešová J., Landová E., Lišková S., Šimková O., Tichá I. & Fuchs R. 2011: Are animals in zoos rather conspicuous than endangered*? Nova Science* **2011**: 1–41.

Frynta D., Vejvodová T. & Šimková O. 2016: Sex allocation and secondary sex ratio in Cuban boa (Chilabothrus angulifer): mother's body size affects the ratio between sons and daughters. *The Science of Nature* **103**(5–6): 48.

Glor R. E., Gifford M. E., Larson A., Losos J. B., Schettino L. R., Lara A. R. C. & Jackman T. R. 2004: Partial island submergence and speciation in an adaptive radiation: a multilocus analysis of the Cuban green anoles. *Proceedings of the Royal Society of London. Series B*: *Biological Sciences* **271**: 2257–2265.

Green S. E. 2010: *Evolutionary Biology and Conservation of the Hog Island Boa constrictor.* Unpubl. PhD Dissertation. Canterbury: University of Kent, 243 pp.

Harvey D. S. & Platenberg R. J. 2009: Predicting habitat use from opportunistic observations: a case study of the Virgin Islands tree boa (Epicrates granti). *Herpetological Journal* **19**: 111–118.

Head J. J., Rincon A. F., Suarez C., Montes C. & Jaramillo C. 2012: Fossil evidence for earliest Neogene American faunal interchange: Boa (Serpentes, Boinae) from the early Miocene of Panama. *Journal of Vertebrate Paleontology* **32**: 1328–1334.

Hedges S. B., Powell R., Henderson R. W., Hanson S. & Murphy J. C. 2019: Definition of the Caribbean Islands biogeographic region, with checklist and recommendations for standardized common names of amphibians and reptiles. *Caribbean Herpetology* **67**: 1–53.

Huelsenbeck J. P., Ronquist F., Nielsen R. & Bollback J. P. 2001: Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **294**: 2310–2314.

Huff T. A. 1976: Breeding the Cuban boa Epicrates angulifer at the Reptile Breeding Foundation. *International Zoo Yearbook* **16**: 81–82.

Hynková I., Starostová Z. & Frynta D. 2009: Mitochondrial DNA variation reveals recent evolutionary history of main Boa constrictor clades. *Zoological Science* **26**: 623–631.

Iturralde-Vinent M. A. 2006: Meso-Cenozoic Caribbean paleogeography: implications for the historical biogeography of the region. *International Geology Review* **48**: 791–827.

Jančúchová-Lásková J., Landová E. & Frynta D. 2015a: Are genetically distinct lizard species able to hybridize? A review. *Current Zoology* **61**: 155–180.

Jančúchová-Lásková J., Landová E. & Frynta D. 2015b: Experimental crossing of two distinct species of leopard geckos, Eublepharis angramainyu and E. macularius: viability, fertility and phenotypic variation of the hybrids. *Public Library of Science One* **10**(12; e0143630): 1–27.

Kalyaanamoorthy S., Minh B. Q., Wong T. K., Von Haeseler A. & Jermiin L. S. 2017: ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**: 587–589.

Keogh J. S., Webb J. K. & Shine R. 2007: Spatial genetic analysis and long-term mark–recapture data demonstrate male-biased dispersal in a snake. *Biology Letters* **3**: 33–35.

Kluge A. G. 1989: A concern for evidence and a phylogenetic hypothesis of relationships among Epicrates (Boidae, Serpentes). *Systematic Biology* **38**: 7–25.

Kumar S., Stecher G., Li M, Knyaz C. & Tamura K 2018: MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* **35**: 1547–1549.

Landestoy M. A., Reynolds R. G. & Henderson R. W. 2021: A small new arboreal species of West Indian boa (Boidae; Chilabothrus) from southern Hispaniola. *Breviora* **571**: 1–20.

Leigh J. W. & Bryant D. 2015: POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6**: 1110–1116.

Losos J. 2009: *Lizards in an Evolutionary Tree*: *Ecology and Adaptive Radiation of Anoles.* Berkeley: University of California Press, 528 pp.
Lyons S. L., Karp A. T., Bralower T. J., Grice K., Schaefer B., Gulick S. P., Morgan J.V. & Freeman K. H. 2020: Organic matter from the Chicxulub Crater exacerbated the K-Pg impact winter. *Proceedings of the National Academy of Sciences of the United States of America* **117**: 25327–25334.
Malone C. L., Wheeler T., Taylor J. F. & Davis S. K. 2000: Phylogeography of the Caribbean rock iguana (Cyclura): implications for conservation and insights on the biogeographic history of the West Indies. *Molecular Phylogenetics and Evolution* **17**: 269–279.
Marešová J. & Frynta D. 2008: Noah's Ark is full of common species attractive to humans: The case of boid snakes in zoos. *Ecological Economics* **64**: 554–558.
Murphy J. B., Barker D. G. & Tryon B. W. 1978: Miscellaneous notes on the reproductive biology of reptiles. 2. Eleven species of the family Boidae, genera Candoia, Corallus, Epicrates and Python. *Journal of Herpetology* **12**: 385–390.
Murray C. M. & Crother B. I. 2019: Squamate areas of endemism in Cuba as determined by parsimony analysis of endemicity. *Cladistics* **35**: 426–434.
Newman B. C., Henke S. E., Koenig S. E. & Powell R. L. 2016: Distribution and general habitat use analysis of the Jamaican Boa (Chilabothrus subflavus). *South American Journal of Herpetology* **11**: 228–234.
Newman B. C., Henke S. E., Powell R. L. & Rudolph D. C. 2020: Review of the Jamaican boa (Chilabothrus subflavus): Biology, ecology, and conservation management of a vulnerable species. *Herpetological Conservation and Biology* **15**: 390–408.
Nguyen L. T., Schmidt H. A., Von Haeseler A. & Minh B. Q. 2015: IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32**: 268–274.
Nowinski B. 1977: Voortplanting van de Cubaanse boa, Epicrates angulifer, in het terrarium. *Lacerta* **35**: 144–147.
Nylander J. A. A. 2004: *MrModeltest v2*. Uppsala: Uppsala University, Biology Centre. [distributed by the author].
Onary S. & Hsiou A. S. 2018: Systematic revision of the early Miocene fossil Pseudoepicrates (Serpentes: Boidae): implications for the evolution and historical biogeography of the West Indian boid snakes (Chilabothrus). *Zoological Journal of the Linnean Society* **184**: 453–470.
Passos P. & Fernandes R. 2008: Revision of the Epicrates cenchria complex (Serpentes: Boidae). *Herpetological Monographs* **22**: 1–30.
Pernetta A. P., Allen J. A., Beebee T. J. C. & Reading C. J. 2011: Fine-scale population genetic structure and sex-biased dispersal in the smooth snake (Coronella austriaca) in southern England. *Heredity* **107**: 231–238.
Philippon M., Cornée J. J., Münch P., Van Hinsbergen D. J., Boudagher-Fadel M., Gailler L., Boschman L.M., Quillevere F., Montheil L., Gay A., Lebrun J. F., Lallemand S., Marivaux L., Antoin P.-O. & GARANTI Team 2020: Eocene intra-plate shortening responsible for the rise of a faunal pathway in the northeastern Caribbean realm. *Public Library of Science One* **15**(10; e0241000): 1–13.
Poore R. Z., Williams R. S. Jr. & Tracey C. 2000: Sea level and climate. *U. S. Geological Survey Fact Sheet* **002–00**: 1–2 [at https://pubs.usgs.gov/fs/fs2-00/].
Puente-Rolón A. R., Reynolds R. G. & Revell L. J. 2013: Preliminary genetic analysis supports cave populations as targets for conservation in the endemic endangered Puerto Rican boa (Boidae: Epicrates inornatus). *Public Library of Science One* **8**(5; e63899): 1–9.
Pyron R. A., Burbrink F. T. & Wiens J. J. 2013: A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BioMedCentral Evolutionary Biology* **13**: 1–54.
Pyron R. A., Reynolds R. G. & Burbrink F. T. 2014: A taxonomic revision of boas (Serpentes: Boidae). *Zootaxa* **3846**: 249–260.
Rawlings L. H., Rabosky D. L., Donnellan S. C. & Hutchinson M. N. 2008: Python phylogenetics: inference from morphology and mitochondrial DNA. *Biological Journal of the Linnean Society* **93**: 603–619.
Rehák I. 1992: Systematika, chov a rozmnožování hroznýšů rodu Epicrates [Systematics, captive care and breeding of snakes of the genus Epicrates]. *Terarista*, *Praha* **3**: 22–29 (in Czech).
Rehák I. (ed.) 1994: *International Studbook of Cuban Boas*, *Epicrates angulifer. Volume 1*. Prague: Zoological Garden Prague, ii+81 pp.
Rehák I. 2006: *Cuban Boa EEP 2006 Update*. Unpubl. Presentation. Madrid: EAZA Annual Conference 2006.
Rehák I. 2008: *Ex situ Management Programme for Cuban Boas and Cuban Iguanas*. Unpubl. Presentation. Topes de Collantes, Sancti Spiritus, Cuba: VIII Latin-American Congress of Herpetology.
Reynolds R. G. & Henderson R. W. 2018: Boas of the world (superfamily Booidae): a checklist with systematic, taxonomic, and conservation assessments. *Bulletin of the Museum of Comparative Zoology* **162**: 1–58.
Reynolds R. G., Gerber G. P. & Fitzpatrick B. M. 2011: Unexpected shallow genetic divergence in Turks Island Boas (Epicrates c. chrysogaster) reveals single evolutionarily significant unit for conservation. *Herpetologica* **67**: 477–486.

Reynolds R. G., Niemiller M. L., Hedges S. B., Dornburg A., Puente-Rolón A. R. & Revell L. J. 2013: Molecular phylogeny and historical biogeography of West Indian boid snakes (Chilabothrus). *Molecular Phylogenetics and Evolution* **68**: 461–470.

Reynolds R. G., Niemiller M. L. & Revell L. J. 2014a: Toward a Tree-of-Life for the boas and pythons: multilocus species-level phylogeny with unprecedented taxon sampling. *Molecular Phylogenetics and Evolution* **71**: 201–213.

Reynolds R. G., Puente-Rolón A. R., Kolodzaike K. & Butler-Smith T. 2014b: Isolation and characterization of 23 novel polymorphic microsatellite markers from the endangered Puerto Rican boa (Chilabothrus inornatus) using paired-end Illumina shotgun sequencing. *Conservation Genetics Resources* **6**: 107–109.

Reynolds R. G., Puente-Rolón A. R., Platenberg R., Tyler R. K., Tolson P. J. & Revell L. J. 2015: Large divergence and low diversity suggest genetically informed conservation strategies for the endangered Virgin Islands Boa (Chilabothrus monensis). *Global Ecology and Conservation* **3**: 487–502.

Reynolds R. G., Collar D. C., Pasachnik S. A., Niemiller M. L., Puente-Rolón A. R. & Revell L. J. 2016a: Ecological specialization and morphological diversification in Greater Antillean boas. *Evolution* **70**: 1882–1895.

Reynolds R. G., Puente-Rolón A. R., Geneva A. J., Aviles-Rodriguez K. J. & Herrmann N. C. 2016b: Discovery of a remarkable new boa from the Conception Island Bank, Bahamas. *Breviora* **549**: 1–19.

Rivera P. C., Gardenal C. N. & Chiaraviglio M. 2006: Sex-biased dispersal and high levels of gene flow among local populations in the argentine boa constrictor, Boa constrictor occidentalis. *Austral Ecology* **31**: 948–955.

Rivera P. C., Di Cola V., Martínez J. J., Gardenal C. N. & Chiaraviglio M. 2011: Species delimitation in the continental forms of the genus Epicrates (Serpentes, Boidae) integrating phylogenetics and environmental niche models. *Public Library of Science One* **6**(9; e22199): 1–13.

Rodríguez R., Berovides V., González A., Pérez E., Matamoros Y. & Miller P. (eds) 2003: *Análisis de la Viabilidad de La Población y del Hábitat de la Iguana Cubana Cyclura nubila nubila*. La Habana: Jardín Zoológico de la Habana, 73 pp.

Rodríguez-Cabrera T. M., Torres J. & Marrero R. 2015: At the lower size limit of snakes preying on bats in the West Indies: The Cuban boa, Chilabothrus angulifer (Boidae). *Reptiles & Amphibians* **22**: 8–15.

Rodríguez-Cabrera T. M., López J. T., Marrero R., Savall E. M. & Ochotorena A. S. 2016a: Sexual maturation in free-ranging Chilabothrus angulifer (Serpentes: Boidae). *Phyllomedusa*: *Journal of Herpetology* **15**: 163–174.

Rodríguez-Cabrera T. M., Marrero R. & Torres J. 2016b: An overview of the past, present, and future of the Cuban boa, Chilabothrus angulifer (Squamata: Boidae): a top terrestrial predator on an oceanic island. *Reptiles & Amphibians* **23**: 152–168.

Rodríguez-Cabrera T. M., Savall E. M., Rodríguez-Machado S. & Torres J. 2020: Trophic ecology of the Cuban boa, Chilabothrus angulifer (Boidae). *Reptiles & Amphibians* **27**: 169–200.

Rodríguez-Robles J. A. & Greene H. W. 1996: Ecological patterns in Greater Antillean macrostomatan snake assemblages, with comments on body-size evolution in Epicrates (Boidae). *Contributions to West Indian Herpetology*: *A Tribute to Albert Schwartz. Contributions to Herpetology* **12**: 339–357.

Rodríguez-Robles J. A., Jezkova T., Fujita M. K., Tolson P. J. & García M. A. 2015: Genetic divergence and diversity in the Mona and Virgin Islands boas, Chilabothrus monensis (Epicrates monensis) (Serpentes: Boidae), West Indian snakes of special conservation concern. *Molecular Phylogenetics and Evolution* **88**: 144–153.

Rodríguez Schettino L. 2012: Epicrates angulifer Cocteau y Bibron, 1843, Majá de Santa María, Cuban tree boa. Pp. 160–154. In: González A. H., Rodríguez Schettino L., Rodríguez A., Mancina C. A. & Ramos I. (eds): *Libro Rojo de los Vertebrados de Cuba*. La Habana: Editorial Academia, 304 pp.

Rodríguez Schettino L., González V. R. & Rodríguez E. P. 2010: Distribución regional y altitudinal de los reptiles de Cuba. *Poeyana* **498**: 11–20.

Rodríguez Schettino L. R., Mancina C. A. & González V. R. 2013: Reptiles of Cuba: checklist and geographic distributions. *Smithsonian Herpetological Information Service* **144**: 1–99.

Ronquist F., Teslenko M., Van Der Mark P., Ayres D. L., Darling A., Höhna S., Larget B., Liu L., Suchard M. A. & Huelsenbeck J. P. 2012: MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.

Rozas J., Ferrer-Mata A., Sánchez-DelBarrio J. C., Guirao-Rico S., Librado P., Ramos-Onsins S. E. & Sánchez-Gracia A. 2017: DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution* **34**: 3299–3302.

Russell A. L., Medellín R. A. & McCracken G. F. 2005: Genetic variation and migration in the Mexican free-tailed bat (Tadarida brasiliensis mexicana). *Molecular Ecology* **14**: 2207–2222.

Sambrook J., Fritsch F. & Maniatis T. 1989: In vitro amplification of DNA by PCR. In: Green M. R. & Sambrook J. (eds): *Molecular Cloning*. *A Laboratory Manual*. New York: Cold Spring Harbor Laboratory Press, 2028 pp.

Schield D. R., Card D. C., Adams R. H., Jezkova T., Reyes-Velasco J., Proctor F. N., Spencer C. L ., Herrmann H-W., Mackessy S. P. & Castoe T. A. 2015: Incipient speciation with biased gene flow between two lineages of the Western diamondback rattlesnake (Crotalus atrox). *Molecular Phylogenetics and Evolution* **83**: 213–223.

Schield D. R., Adams R. H., Card D. C., Perry B. W., Pasquesi G. M., Jezkova T., Portik D. M., Andrew A. L., Spencer C. L., Sanchez E. E., Fujita M. K., Mackessy S. P. & Castoe T. A. 2017: Insight into the roles of selection in speciation from genomic patterns of divergence and introgression in secondary contact in venomous rattlesnakes. *Ecology and Evolution* **7**: 3951–3966.

Schield D. R., Adams R. H., Card D. C., Corbin A. B., Jezkova T., Hales N. R., Meik J. M., Perry B. W., Spencer C. L., Smith L. L., García G. C., Bouzid N. M., Strickland J. L., Parkinson C. L., Borja M., Castañeda-Gaytán G., Bryson R. W. Jr., Flores-Villelae O. A., Mackessy S. P. & Castoe T. A. 2018: Cryptic genetic diversity, population structure, and gene flow in the Mojave rattlesnake (Crotalus scutulatus). *Molecular Phylogenetics and Evolution* **127**: 669–681.

Schield D. R., Perry B. W., Adams R. H., Card D. C., Jezkova T., Pasquesi G. I., Nikolakis Z. L., Row K., Meik J. M., Smith C. F., Mackessy S. P. & Castoe T. A. 2019: Allopatric divergence and secondary contact with gene flow: a recurring theme in rattlesnake speciation. *Biological Journal of the Linnean Society* **128**: 149–169.

Seixas F., Morinha F., Luis C., Alvura N. & dos Anjos Pires M. 2020: DNA-validated parthenogenesis: first case in a captive Cuban boa (Chilabothrus angulifer). *Salamandra* **56**: 83–86.

Shaney K. J., Diaz-Ramirez L. G., Espindola S., Castañeda-Rico S., Berovides-Álvarez V. & Vázquez-Domínguez E. 2020: Defining intraspecific conservation units in the endemic Cuban rock iguanas (Cyclura nubila nubila). *Scientific Reports* **10**: 1–12.

Sheplan B. R. & Schwartz A. 1974: Hispaniolan boas of the genus Epicrates (Serpentes: Boidae) and their Antillean relationships. *Annales of Carnegie Museum* **45**: 57–143.

Soberon R., Ross P. & Seal U. (eds) 2000: *Cocodrilo Cubano* (*Crocodylus rhombifer*) *Análisis de la Viabilidad de la Población y del Hábitat: Libro de Resumen.* Apple Valley: CBSG, 87 pp.

Steadman D. W. & Franklin J. 2017: Origin, paleoecology, and extirpation of bluebirds and crossbills in the Bahamas across the last glacial–interglacial transition. *Proceedings of the National Academy of Sciences of the United States of America* **114** : 9924–9929.

Streicher J. W., McEntee J. P., Drzich L. C., Card D. C., Schield D. R., Smart U., Parkinson C. L., Jezkova T., Smith E. N. & Castoe T. A. 2016: Genetic surfing, not allopatric divergence, explains spatial sorting of mitochondrial haplotypes in venomous coralsnakes. *Evolution* **70**: 1435–1449.

Suárez-Atilano M., Burbrink F. T. & Vázquez-Dominguez E. 2014: Phylogeographic structure of Boa constrictor imperator with emphasis of diversification across the lowlands and mountains of Central America and Mexico. *Journal of Biogeography* **41**: 2371–2384.

Syromyatnikova E., Aranda E. & González S. F. 2021: First insight into the diversity of snakes in the Pleistocene of Cuba. *Acta Palaeontologica Polonica* **66**: 395–407.

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.

Tolson P. J. 1983. Captive propagation and husbandry of the Cuban boa, Epicrates angulifer. Pp. 285–292. In: *Proceedings of the 6th Reptile Symposium on Captive Propagation and Husbandry*. Thurmont, Maryland: Zoological Consortium, Inc.

Tolson P. J. 1987. Phylogenetics of the boid snake genus *Epicrates* and Caribbean vicariance theory. *Occasional Papers of the Museum of Zoology*, *University of Michigan* **715**: 1–68.

Tolson P. J. & Henderson R. W. 1993: *The Natural History of West Indian Boas.* , Somerset, England: R&A Publishing Ltd., Taunton, 125 pp.

Trifinopoulos J., Nguyen L. T., von Haeseler A. & Minh B. Q. 2016: W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* **44**(W1): W232–W235.

Tucker A. M., McGowan C. P., Mulero Oliveras E. S., Angeli N. F. & Zegarra J. P. 2020: A demographic projection model to support conservation decision making for an endangered snake with limited monitoring data. *Animal Conservation* **24**: 291–301.

Tzika A. C., Koenig S., Miller R., Garcia G., Remy C. & Milinkovitch M. C. 2008: Population structure of an endemic vulnerable species, the Jamaican boa (Epicrates subflavus). *Molecular Ecology* **17**: 533−544.

Tzika A., Remy C., Gibson R. & Milinkovitch M. C. 2009: Molecular genetic analysis of a captive-breeding program: the vulnerable endemic Jamaican yellow boa. *Conservation Genetics* **10**: 69–77.

Upham N. S. & Borroto-Páez R. 2017: Molecular phylogeography of endangered Cuban hutias within the Caribbean radiation of capromyid rodents. *Journal of Mammalogy* **98**: 950–963.

Vences M. & Glaw F. 2003: Phylogeography, systematics and conservation status of boid snakes from Madagascar (Sanzinia and Acrantophis). *Salamandra* **39**: 181–206.

Vergner J. 1978: Tři druhy rodu Epicrates z Antilské oblasti [Three species of the genus Epicrates from the Antillean region]. *Akvárium Terárium* **21**: 140–142 (in Czech).

Vergner I. 1989: K ekologii, etologii a rozmnožování hroznýšovce kubánského v teráriu, Epicrates angulifer [On the ecology, ethology and reproduction of the Cuban boa in a terrarium]. *Akvárium Terárium* **32**: 26–31 (in Czech).