Great divergence among haplotypes from Cuba as a challenge for conservation strategies of rock iguanas of the genus *Cyclura*

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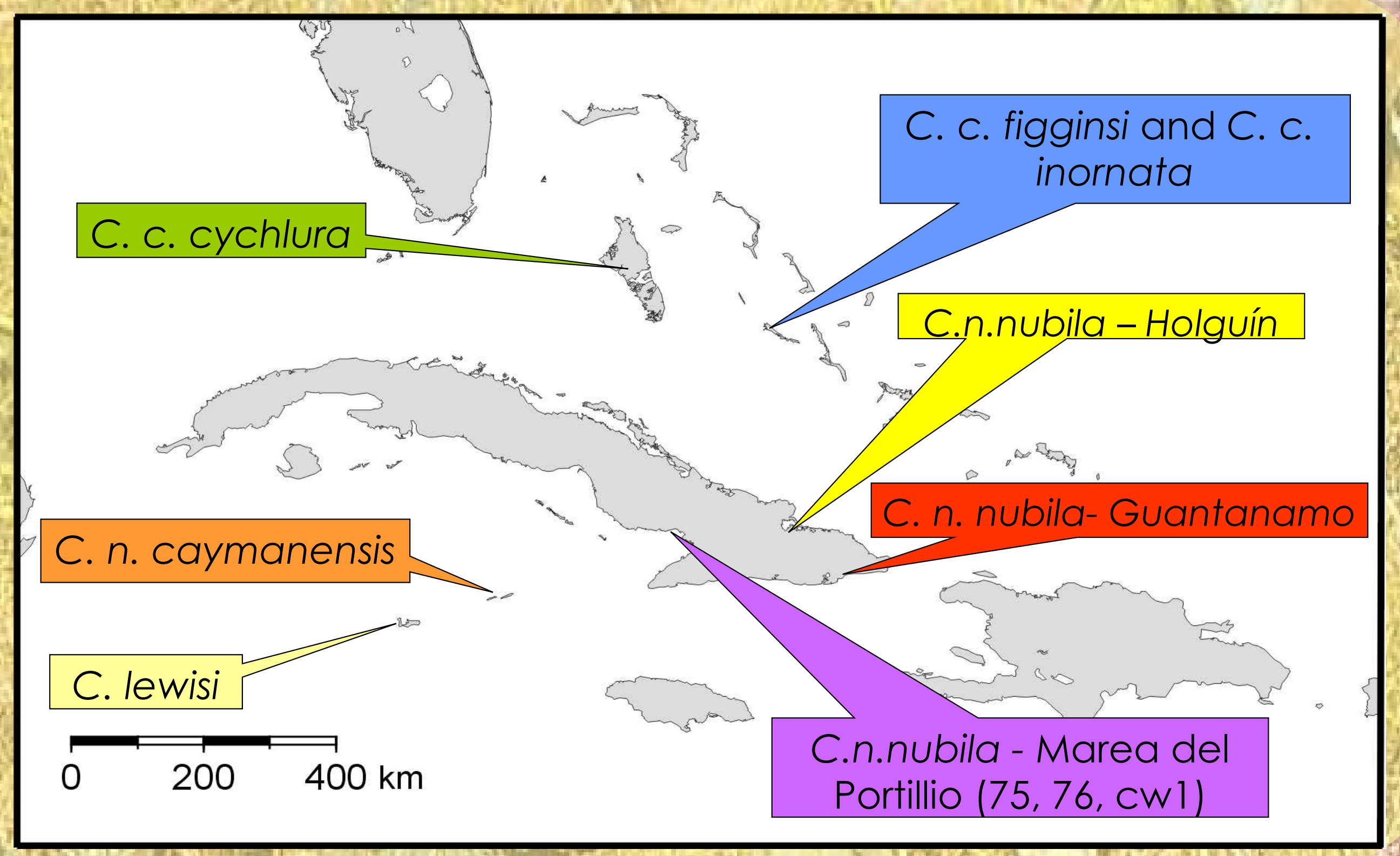
Introduction

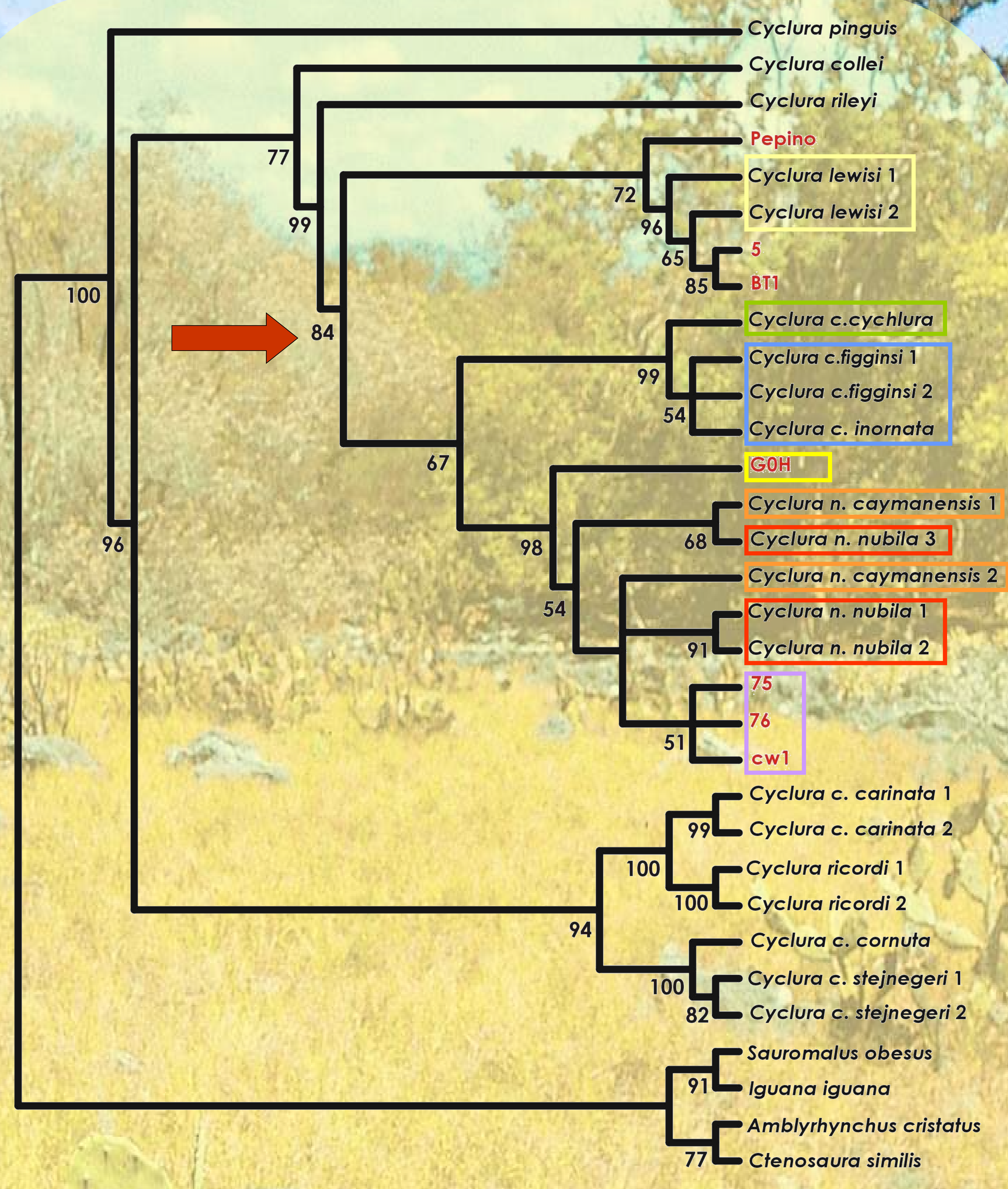
There were described many iguanas from the genus *Cyclura* in the Caribbean region. These iguanas are characterized by high degree of endemism and are basically limited to a single species or subspecies per island. Therefore they are now target group for conservation biology. It has been shown recently that forms living on the Bahamas and the Cayman Islands are closely related to the Cuban *Cyclura n. nubila* (Malone et al. 2000). The goal of this project was to find out whether Cuban populations form a single monophyletic group of haplotypes or on the contrary are from their common ancestor derived also the haplotypes of above-mentioned island forms. In the latter case would the knowledge of the phylogeography within Cuba be essential for understanding the history and conservation importance of endangered island populations.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	*Amblyrhynchus cristatus*	-																														
2	*Sauromalus obesus*	0,125	-																													
3	*Ctenosaura similis*	0,116	0,122	-																												
4	*Iguana iguana*	0,128	0,102	0,123	-																											
5	*Cyclura pinguis*	0,152	0,146	0,146	0,147	-																										
6	*C. nubila lewisi* 1	0,153	0,149	0,152	0,154	0,099	-																									
7	*C. nubila lewisi* 2	0,157	0,149	0,155	0,155	0,103	0,004	-																								
8	*C. carinata carinata* 1	0,151	0,160	0,161	0,157	0,116	0,098	0,098	-																							
9	*C. carinata carinata* 2	0,153	0,160	0,162	0,158	0,118	0,097	0,097	0,011	-																						
10	*C. collei*	0,160	0,163	0,154	0,166	0,113	0,077	0,082	0,111	0,113	-																					
11	*C. cornuta cornuta*	0,153	0,158	0,157	0,158	0,118	0,092	0,091	0,095	0,092	0,110	-																				
12	*C. cornuta stejnegeri* 1	0,152	0,161	0,157	0,160	0,121	0,089	0,088	0,095	0,092	0,110	0,009	-																			
13	*C. cornuta stejnegeri* 2	0,152	0,161	0,157	0,160	0,121	0,089	0,088	0,095	0,092	0,110	0,010	0,001	-																		
14	*C. cychlura cychlura*	0,150	0,149	0,155	0,152	0,105	0,028	0,028	0,101	0,100	0,084	0,092	0,089	0,089	-																	
15	*C. cychlura figginsi* 1	0,153	0,151	0,160	0,157	0,107	0,029	0,029	0,102	0,098	0,083	0,095	0,093	0,093	0,006	-																
16	*C. cychlura figginsi* 2	0,151	0,149	0,160	0,154	0,107	0,029	0,029	0,102	0,098	0,085	0,093	0,091	0,091	0,008	0,004	-															
17	*C. cychlura inornata*	0,152	0,150	0,159	0,155	0,106	0,028	0,028	0,101	0,097	0,084	0,094	0,092	0,092	0,004	0,001	0,003	-														
18	*C. nubila caymanensis* 1	0,160	0,151	0,154	0,158	0,110	0,028	0,028	0,101	0,100	0,091	0,098	0,094	0,094	0,027	0,028	0,029	0,027	-													
19	*C. nubila caymanensis* 2	0,155	0,145	0,153	0,157	0,106	0,025	0,025	0,097	0,096	0,087	0,093	0,088	0,088	0,023	0,025	0,026	0,023	0,006	-												
20	*C. ricordi* 1	0,150	0,151	0,164	0,153	0,112	0,092	0,092	0,043	0,040	0,112	0,092	0,091	0,089	0,096	0,100	0,100	0,098	0,098	0,093	-											
21	*C. ricordi* 2	0,151	0,152	0,163	0,154	0,112	0,089	0,089	0,043	0,040	0,110	0,093	0,092	0,091	0,096	0,100	0,100	0,098	0,096	0,091	0,003	-										
22	*C. rileyi group*	0,163	0,151	0,152	0,157	0,107	0,035	0,035	0,102	0,100	0,075	0,096	0,093	0,093	0,036	0,035	0,035	0,034	0,039	0,036	0,097	0,095	-									
23	*C. nubila nubila* 1	0,160	0,149	0,157	0,159	0,110	0,027	0,027	0,101	0,100	0,091	0,098	0,094	0,094	0,026	0,027	0,028	0,026	0,010	0,007	0,096	0,094	0,036	-								
24	*C. nubila nubila* 2	0,159	0,148	0,155	0,158	0,109	0,026	0,026	0,100	0,098	0,089	0,097	0,093	0,093	0,025	0,026	0,027	0,025	0,009	0,006	0,095	0,093	0,035	0,001	-							
25	Pepino	0,160	0,150	0,157	0,159	0,106	0,018	0,018	0,104	0,103	0,085	0,091	0,086	0,086	0,026	0,027	0,027	0,026	0,027	0,023	0,098	0,096	0,032	0,026	0,025	-						
26	75	0,154	0,144	0,152	0,154	0,105	0,023	0,023	0,096	0,095	0,086	0,094	0,089	0,089	0,022	0,023	0,025	0,022	0,009	0,003	0,092	0,089	0,032	0,006	0,004	0,022	-					
27	76	0,155	0,145	0,153	0,155	0,106	0,025	0,025	0,095	0,094	0,087	0,095	0,091	0,091	0,023	0,025	0,026	0,023	0,010	0,004	0,093	0,091	0,034	0,007	0,006	0,023	0,001	-				
28	cw1	0,157	0,144	0,154	0,154	0,107	0,026	0,026	0,098	0,097	0,088	0,096	0,092	0,092	0,022	0,023	0,025	0,022	0,011	0,006	0,094	0,092	0,035	0,008	0,007	0,025	0,002	0,003	-			
29	5	0,157	0,149	0,155	0,155	0,102	0,007	0,004	0,103	0,102	0,082	0,094	0,092	0,092	0,030	0,031	0,031	0,030	0,030	0,027	0,096	0,094	0,037	0,029	0,028	0,020	0,026	0,027	0,028	-		
30	G0H	0,158	0,147	0,154	0,157	0,107	0,021	0,021	0,101	0,097	0,086	0,092	0,087	0,087	0,020	0,019	0,020	0,018	0,009	0,006	0,096	0,094	0,030	0,008	0,007	0,020	0,004	0,006	0,007	0,023	-	
31	BT1	0,157	0,149	0,155	0,155	0,101	0,006	0,003	0,102	0,101	0,081	0,093	0,091	0,091	0,029	0,030	0,030	0,029	0,029	0,026	0,095	0,093	0,036	0,028	0,027	0,019	0,025	0,026	0,027	0,001	0,022	-
32	*C. nubila nubila* 3	0.162	0.152	0.157	0.158	0.111	0.030	0.030	0.104	0.103	0.093	0.101	0.096	0.096	0.028	0.031	0.032	0.030	0.010	0.016	0.102	0.100	0.041	0.018	0.017	0.029	0.015	0.016	0.017	0.032	0.012	0.031

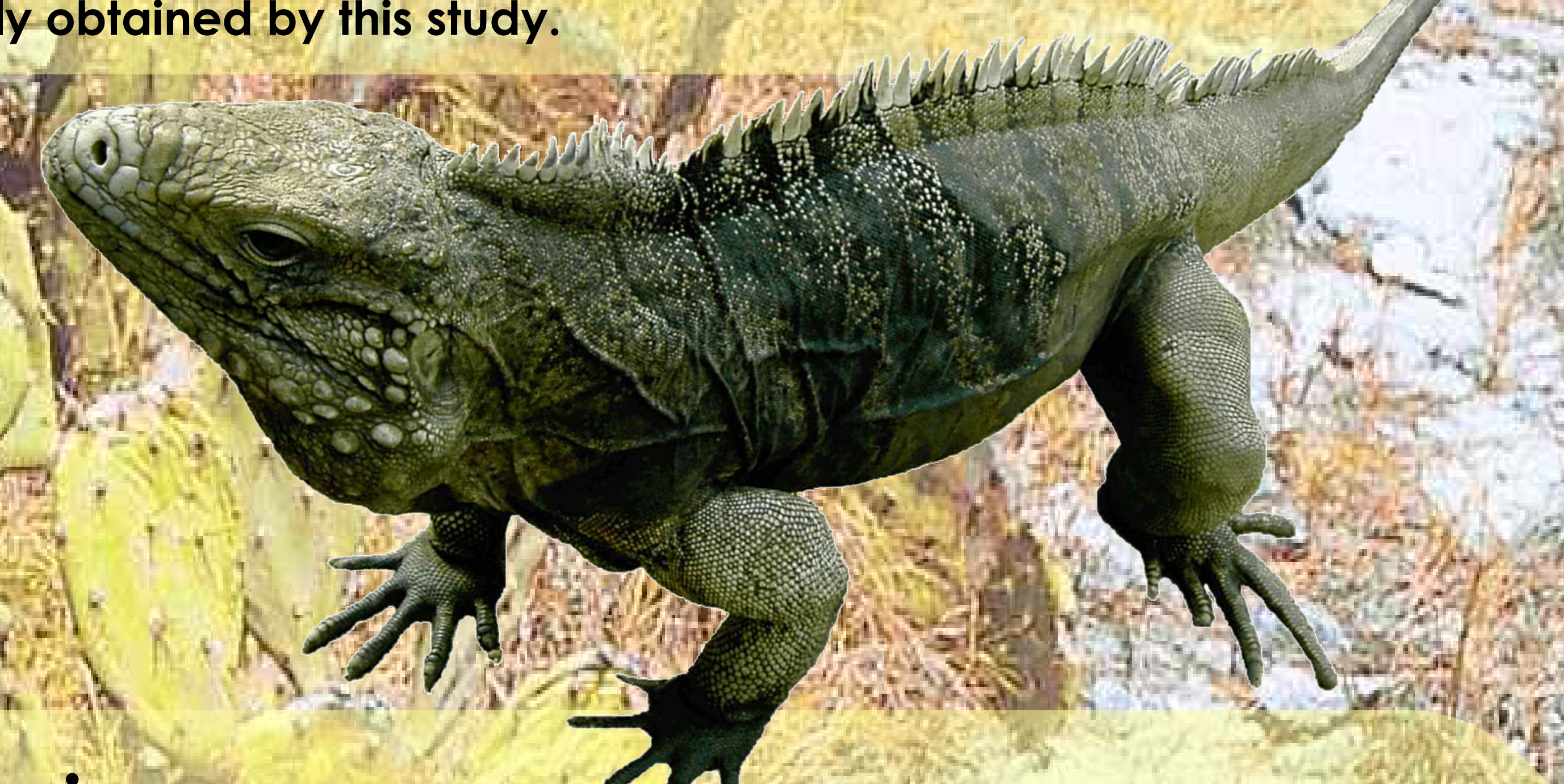
Tab1: Table of p-distances within genus *Cyclura* and outgroups. An approximate molecular clock for studied locus is 1.29 million years for every 1% sequence divergence (Malone *et al.* 2000).

Map of Caribbean region with marked distribution of *C. lewisi* and subspecies of *Cyclura nubila* and *C. cychlura* plus localities of Cuban haplotypes. Colours correspond to the frames showing the position of each form on the phylogenetic tree.





The above picture shows consensual phylogenetic tree recovered by MP (tree length is 648 steps; CI=0.6497; RI=0.7877). Numbers are showing branch support by bootstrapping with 1000 pseudoreplicates. Tree topology reconstructed by NJ was nearly similar. ML and BA trees differed in placing *C. rileyi* within "superclade" comprising all Cuban haplotypes and in placing the most distant haplotype of Cuba – Grand Cayman group "Pepino" originating from Cuba (probably from the north-west coast) even to unresolved position on the basis of the entire "superclade". In red are haplotypes newly obtained by this study.



Material and methods

Samples for the DNA analysis were collected in zoos and from private breeders using nondestructive buccal smears. We sequenced fragment of mitochondrial DNA (894 bp region including a part of the ND4 subunit of the NADH gene and the tRNA genes histidine, serin and partially leucin) in 21 iguanas. Newly obtained haplotypes of *Cyclura n. nubila* (tab.2) were merged with previously published ones and with sequences of additional species from genus *Cyclura* as well as with outgroup species. The whole 894-bp region of mtDNA was divided into two partitions: ND4 and tRNA, which were used in partitioned Bayesian analysis.

Distance method of neighbour-joining (NJ) as well as maximum parsimony (MP) and maximum likelihood (ML) analysis were performed in PAUP* version 4.0b10 (Swofford, 2001) and Bayesian analysis (BA) was conducted with MrBayes 3.1 (Huesenbeck and Ronquist, 2001) For MP we conducted heuristic search analysis with 1000 random taxon addition replicates using tree-bisection and reconnection (TBR) branch swapping. The branch support was evaluated using 1000 bootstrap pseudoreplicates (Felsenstein 1985).

Tree search with NJ algorithm was done with Jukes – Cantor distance and 1000 replicates and the final topology was supported by 10000 bootstrap pseudoreplicates. Modeltest 3.7 (Posada and Crandall, 1998) was used to determine models of sequence evolution and parameter settings for the whole region as well as for two data partitions. These models and parameters were set in ML and BA analyses respectively. For ML analysis we used heuristic search with 10 random taxon addition replicates and the TBR. Support for the ML tree topology was assessed by bootstrap analysis with 1152 pseudoreplicates. Two independent runs of the partitioned BA were conducted with a random starting tree and ran for 5x 10^6 generations. Trees from generations before the tree log-likelihoods reached the plateau were discarded using the bun-in command.

haplotype name	locality	origin of samples	number of individuals of the same haplotype
Pepino	Cuba, import (maybe Matanzas)	Prague ZOO	1
5	Cuba, import	Prague ZOO, private breeder	3
BT1	Cuba, import (maybe Baracoa)	private breeder	1
G0H	Cuba,Holguín	private breeder, ZOO Lisboa, Prague ZOO, ZOO Ústí nad Labem	5
75	Cuba, Marea del Portillio	Prague ZOO, ZOO Jihlava, private breeder	9
cw1	Cuba, Marea del Portillio	private breeder	1
76	Cuba, Marea del Portillio	Prague ZOO	1
C. nubila nubila 3	Cuba, Guantanamo	sequence from Sites et al. (1996), GenBank accession no. U66236	1
C. nubila nubila 1	Cuba, Guantanamo	sequence from Malone et al. (2000), GenBank accession no. AF217765	2
C. nubila nubila 2	Cuba, Guantanamo	sequence from Malone et al. (2000), GenBank accession no. AF217766	1

Tab.2: Haplotypes of *C. nubila nubila* and origin and number of found representatives of each haplotype.

Conclusions

The smallest exclusive monophyletic group comprising all haplotypes from Cuba is paraphyletic with respect to *C. cychlura*, *C. n. caymanensis* and *C. lewisi* from the Bahamas and the Cayman Islands.

It seems that above mentioned forms originated from Cuba, where probably this monophyletic group of haplotypes diverged.

The evolutionary history of island forms cannot be explained without detailed analysis of Cuban populations.

The taxa *C. lewisi*, *C. n. caymanensis* and *C. cychlura* do not fulfil requirements of phylogenetic species concept, however, they still may be understood as evolutionary species due to their irreversible isolation.

Aknowledgements
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For references see back side of the handout.