

## Genetic evidence for multiple paternity in the critically endangered Cuban crocodile (*Crocodylus rhombifer*)

Yoamel Milián-García<sup>1,2,3,\*</sup>, Evelyn Jensen<sup>2</sup>, Sheila Ribalta Mena<sup>1</sup>, Etiam Pérez Fleitas<sup>4</sup>, Gustavo Sosa Rodríguez<sup>4</sup>, Leiter Guerra Manchena<sup>4</sup>, Georgina Espinosa López<sup>1</sup>, Michael Russello<sup>2,\*</sup>

<sup>1</sup>Facultad de Biología, Universidad de La Habana, calle 25 # 455, e/ J e I, Plaza Vedado, 10400 La Habana, Cuba

<sup>2</sup>Department of Biology, University of British Columbia, Okanagan Campus, 3333 University Way, Kelowna, V1V 1V7, British Columbia, Canada

<sup>3</sup>Centro de Investigaciones Marinas, Universidad de La Habana, calle 16 #114, e/ 1<sup>ra</sup> y 3<sup>ra</sup>, Miramar, Playa, 11300 La Habana, Cuba

<sup>4</sup>Empresa Nacional para la Protección de la Flora y la Fauna, calle 42 y 514 Esq.7 Ave, Miramar, La Habana, Cuba

### Supplementary Material

**Supplementary Movie 1 (as separate file).** Cuban crocodiles (*Crocodylus rhombifer*) mating in the breeding pen (C22) at the Zapata Swamp captive breeding facility. Video by YMG.



**Figure S1.** Mound nest built (upper) and guarded (lower) by a female Cuban crocodile in the breeding pen (C22) at the Zapata Swamp breeding facility. Images by YMG.



**Figure S2.** Tail marking scheme used at the Zapata Swamp captive breeding facility. Images by YM

**Table S1.** Genotypes for each individual within the best configuration from the sibship reconstructions in the five clutches, including the mother observed with guarding behavior for each nest. The discrepancies in the genotypes obtained between the hatchlings and the mother per locus are highlighted in grey.

Group	ID	Cj16	Cj18	Cj20	Cj35	Cj109	Cj119	Cj127	Cj131	CU5-123
1	N-6_199	163/163	224/234	174/176	166/166	/	199/211	327/335	240/262	231/231
1	N-6_200	163/163	224/234	176/176	166/166	/	211/221	335/335	258/262	231/231
2	N-6_202	159/163	224/234	174/174	166/184	/	227/231	327/373	232/258	221/221
3	N-6_212	159/169	230/234	176/176	166/166	/	199/225	327/335	240/258	221/221
4	N-6_198	163/193	230/234	174/176	166/166	/	211/225	335/335	262/262	221/221
4	N-6_201	163/193	230/234	176/182	166/166	/	199/225	327/335	248/262	221/231
4	N-6_203	163/163	230/234	174/176	166/166	/	211/225	327/335	240/262	221/231
4	N-6_204	163/163	226/234	174/176	166/166	/	211/211	327/335	248/262	221/231
4	N-6_205	163/163	226/234	176/182	166/166	/	211/225	327/335	248/262	221/221
4	N-6_206	163/193	230/234	174/176	166/166	393/393	199/211	327/335	240/248	221/221
4	N-6_207	159/163	230/234	176/182	166/166	/	211/211	335/335	262/262	221/221
4	N-6_208	159/193	226/234	174/176	166/166	/	199/225	327/335	262/262	221/231
4	N-6_209	159/193	226/234	174/176	166/166	/	199/225	327/335	248/262	221/221
4	N-6_210	159/193	226/234	174/176	166/166	/	199/225	327/335	248/262	221/231
4	N-6_211	163/163	226/234	176/182	166/166	/	199/225	327/335	240/262	221/231
4	N-6_213	163/193	230/234	176/182	166/166	/	199/225	327/335	240/262	221/221
4	N-6_214	159/163	230/234	176/182	166/166	393/393	199/211	327/335	248/262	221/231
4	N-6_215	163/193	226/234	176/182	166/166	/	199/225	335/335	240/262	221/221
4	N-6_216	159/193	230/234	174/176	166/166	/	199/211	327/335	262/262	221/231
Most likely female	C22051	159/163	234/234	176/176	166/166	387/393	211/225	327/335	240/262	221/231



Group	ID	Cj16	Cj18	Cj20	Cj35	Cj109	Cj119	Cj127	Cj131	CU5-123
1	N-9_143	159/175	234/234	174/174	166/166	393/393	225/225	327/335	240/262	221/223
1	N-9_160	159/175	234/234	174/182	166/166	393/393	225/225	327/335	240/258	221/221
2	N-9_144	159/159	234/234	174/182	166/166	387/393	225/225	327/335	258/262	221/223
2	N-9_152	159/175	234/234	174/182	166/166	393/393	225/225	333/335	258/262	221/223
2	N-9_165	159/175	234/234	174/174	166/166	387/393	225/225	335/335	258/262	221/221
3	N-9_136	159/175	234/234	174/174	166/166	393/393	211/211	335/335	258/262	221/223
3	N-9_139	159/159	234/234	174/174	166/166	393/393	211/227	335/335	240/258	221/221
3	N-9_140	159/175	234/234	174/174	166/166	387/393	211/227	335/335	258/262	223/223
3	N-9_141	159/175	234/234	174/182	166/166	387/393	211/211	327/335	240/258	221/221
3	N-9_145	159/175	234/234	174/182	166/166	387/393	211/211	335/335	240/258	221/221
3	N-9_146	159/159	234/234	182/182	166/166	393/393	211/227	327/327	240/240	221/223
3	N-9_148	159/175	234/234	174/182	166/166	393/393	211/225	327/335	258/262	221/221
3	N-9_149	159/175	234/234	174/182	166/166	387/393	211/227	327/335	240/262	221/221
3	N-9_150	159/175	234/234	182/182	166/166	387/393	211/227	327/335	240/258	223/223
3	N-9_151	159/159	234/234	174/182	166/166	393/393	211/227	327/335	240/258	221/221
3	N-9_153	159/159	234/234	174/182	166/166	393/393	211/211	327/327	240/240	221/223
3	N-9_156	159/175	234/234	174/182	166/166	387/393	211/225	327/335	240/240	221/223
3	N-9_157	159/175	234/234	182/182	166/166	387/393	211/225	335/335	258/262	221/221
3	N-9_158	159/175	234/234	174/174	166/166	387/393	211/211	335/335	240/258	221/221
3	N-9_161	159/159	234/234	182/182	166/166	387/393	211/227	327/327	240/262	223/223
3	N-9_162	159/175	234/234	174/182	166/166	393/393	211/225	335/335	258/262	221/221
3	N-9_163	159/175	234/234	182/182	166/166	393/393	211/211	327/335	240/258	221/223
3	N-9_167	159/159	234/234	174/182	166/166	387/393	211/227	335/335	258/262	221/221
3	N-9_170	159/175	234/234	174/182	166/166	/	211/227	327/327	240/240	221/221
3	N-9_171	159/175	234/234	174/174	166/166	387/393	211/211	327/335	240/240	221/223
3	N-9_172	159/175	234/234	174/182	166/166	393/393	211/211	327/335	240/258	221/221
3	N-9_173	159/159	234/234	174/182	166/166	387/393	211/225	335/335	258/262	221/221

3	N-9_174	159/159	234/234	174/182	166/166	387/393	211/211	327/335	240/262	221/221	
3	N-9_175	159/159	234/234	174/182	166/166	393/393	211/225	327/327	240/240	223/223	
4	N-9_169	159/175	234/234	182/182	166/166	387/393	225/225	327/327	240/240	223/223	
4	N-9_S-N	159/159	234/234	182/182	166/166	387/393	225/225	327/327	240/240	223/223	
Most likely female		C22023	163/163	224/232	174/174	0/0	393/393	209/225	327/335	258/262	223/223

Group	ID	Cj16	Cj18	Cj20	Cj35	Cj109	Cj119	Cj127	Cj131	CU5-123
1	N-14_665	159/175	230/230	176/182	166/166	419/419	211/211	335/335	248/262	221/231
2	N-14_667	159/179	234/234	182/182	166/166	387/387	211/227	335/335	248/262	223/231
3	N-14_659	159/175	230/234	182/182	166/166	387/393	211/211	335/335	248/262	223/231
3	N-14_660	159/175	234/234	182/182	166/166	387/393	211/211	335/335	248/262	221/231
3	N-14_661	159/175	230/234	182/182	166/166	387/393	211/211	335/335	248/262	221/231
3	N-14_664	159/179	230/230	176/182	166/166	387/393	211/227	335/335	248/262	223/231
3	N-14_668	159/175	234/234	182/182	166/166	385/419	211/227	335/335	248/262	221/231
3	N-14_669	159/175	234/234	182/182	166/166	387/393	211/211	335/335	248/262	221/231
3	N-14_670	163/179	230/230	174/182	166/166	387/393	221/227	335/335	262/262	223/231
3	N-14_672	159/175	234/234	176/182	166/166	387/393	211/211	327/335	240/262	221/231
4	N-14_662	159/179	230/234	176/182	166/166	387/387	211/211	335/335	248/262	221/231
4	N-14_666	159/175	230/234	176/182	166/166	393/393	211/227	335/335	248/262	221/231
4	N-14_671	159/175	230/234	176/182	166/166	387/387	211/211	335/335	248/262	223/231
4	N-14_673	159/175	234/234	176/182	166/166	387/387	211/227	335/335	248/262	221/231
4	N-14_675	159/179	230/234	176/182	166/166	387/387	211/211	335/335	248/262	221/231
5	N-14_676	159/179	230/234	182/182	166/166	381/419	211/211	335/335	248/262	221/231
6	N-14_663	159/179	230/230	176/182	166/166	393/393	211/211	327/335	240/262	223/231
6	N-14_677	159/175	234/234	176/182	166/166	393/393	211/211	327/335	240/262	223/231
Most likely female	C22084	159/159	230/234	174/182	166/166	387/393	211/211	327/335	240/258	221/221

Group	ID	Cj16	Cj18	Cj20	Cj35	Cj109	Cj119	Cj127	Cj131	CU5-123
1	N-33_396	175/175	230/234	174/174	166/166	387/393	227/227	327/335	258/258	221/231
1	N-33_401	175/175	230/234	174/182	166/166	387/393	227/227	327/335	258/258	231/231
2	N-33_406	151/175	224/234	174/176	166/166	387/393	221/225	335/335	258/262	221/231
3	N-33_414	175/175	234/234	174/182	166/166	387/387	225/225	327/327	258/258	223/231
4	N-33_409	159/175	230/234	174/182	166/166	387/393	227/227	327/327	240/262	231/231
4	N-33_413	159/175	230/234	174/182	166/166	387/393	227/227	327/335	258/262	231/231
4	N-33_416	175/175	234/234	174/182	166/166	387/393	227/227	327/327	240/258	231/231
5	N-33_388	159/175	234/234	182/182	166/166	387/393	211/225	327/335	258/258	231/231
5	N-33_389	175/175	230/234	174/174	166/166	387/393	225/225	327/335	258/258	229/231
5	N-33_391	175/175	230/234	174/174	166/166	387/393	211/225	335/335	258/258	231/231
5	N-33_392	159/175	234/234	174/174	166/166	387/393	211/227	327/335	240/262	221/231
5	N-33_394	159/175	230/234	174/182	166/166	387/393	211/227	327/327	240/258	231/231
5	N-33_395	159/175	230/234	174/174	166/166	387/393	211/227	/	258/262	229/231
5	N-33_399	159/175	230/234	174/174	166/166	387/387	211/225	327/335	240/262	221/231
5	N-33_400	159/175	234/234	174/182	166/166	387/393	211/225	335/335	258/262	221/231
5	N-33_402	159/175	234/234	174/182	166/166	387/393	211/225	335/335	258/258	221/231
5	N-33_405	175/175	234/234	182/182	166/166	387/393	225/225	327/335	258/258	231/231
5	N-33_408	159/175	230/234	182/182	166/166	387/393	211/225	327/335	258/258	221/231
5	N-33_410	175/175	230/234	174/182	166/166	387/393	211/227	327/335	258/258	231/231
5	N-33_411	159/175	234/234	174/182	166/166	387/393	211/227	335/335	258/258	221/231
5	N-33_412	175/175	230/234	174/174	166/166	387/393	225/225	327/335	240/258	231/231
5	N-33_417	175/175	234/234	174/182	166/166	387/393	211/227	335/335	258/262	221/231
5	N-33_418	159/175	230/234	174/182	166/166	387/393	225/225	335/335	258/262	231/231
<b>Most likely female</b>	C22071B	175/175	230/234	174/182	166/166	387/387	225/225	327/335	258/262	231/231

Group	ID	Cj16	Cj18	Cj20	Cj35	Cj109	Cj119	Cj127	Cj131	CU5-123
1	N-58_739	163/179	230/232	174/176	166/166	393/393	211/211	335/335	262/262	221/221
2	N-58_741	163/175	224/234	174/176	166/166	387/393	211/221	335/335	258/258	221/231
3	N-58_743	163/179	224/234	174/176	166/166	387/393	211/211	335/335	258/262	221/225
4	N-58_734	163/163	234/234	174/176	166/184	387/393	211/211	327/335	258/258	221/221
4	N-58_735	163/163	234/234	174/176	166/184	387/393	211/211	335/373	232/262	225/231
4	N-58_736	163/163	234/234	174/176	166/184	387/393	227/227	335/373	232/262	221/221
4	N-58_737	163/163	232/234	174/176	166/184	387/393	211/211	335/373	232/262	221/225
4	N-58_738	163/179	234/234	174/176	166/166	393/393	211/211	335/373	232/258	221/221
4	N-58_740	163/179	230/232	174/176	166/166	393/393	211/211	335/335	258/262	221/221
4	N-58_742	163/163	232/234	174/176	166/166	393/419	227/227	327/335	258/262	225/231
4	N-58_744	163/179	234/234	174/176	166/166	393/393	211/211	335/373	232/258	221/221
<b>Most likely female</b>	C22005	159/163	230/234	174/182	166/166	387/393	209/225	327/335	240/240	231/251

**Table S2.** The best configuration from the sibship reconstructions indicating the assigned mother and fathers and the probabilities of those assignments for each *C. rhombifer* nest. The number of hatchlings per nest associated with each father is also indicated.

	Mother (probability)	Fathers (# of offspring, mean probability)					
Nest 6	C22051 (1.00)	C22101 (15, 0.97)	male 1 (1)	male 2 (1)	male 3 (1)	male 4 (1)	
Nest 9	female 1	male 5 (24)	male 6 (3)	male 7 (3)	CB041 (1, 0.66)		
Nest 14	female 2	C22056 (13, 0.96)	male 8 (4)	C22014 (1, 0.40)			
Nest 33	C22071B (0.60)	C22007 (15, 0.75)	male 9 (3)	male 2 (1)	male 5 (1)	male 6 (1)	male 7 (1)
Nest 58	female 3	male 3 (5)	C22102 (4, 1.0)	CB016 (1, 0.21)	male 10 (1)		