

A report on the hybridization between two species of threatened Asian box turtles (Testudines: *Cuora*) in the wild on Hainan Island (China) with comments on the origin of ‘*serrata*’-like turtles

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Ten new turtle taxa were described from pet trade specimens from China since the 1980s (see Fritz and Obst, 1998; Fritz and Obst, 1999; Parham et al., 2001 for a review). Specimens similar to one of these taxa, *Cuora serrata* Iverson and McCord, 1992 (originally *Cuora galbinifrons serrata*, elevated by Fritz and Obst, 1997), were shown to be hybrids of male *Cuora mouhotii* (Gray, 1862; formerly *Pyxidea*, but see Stuart and Parham, 2004) and females of *Cuora galbinifrons* Bourret, 1939 or *Cuora bourreti* Obst and Reimann, 1994 (Parham et al., 2001; and Stuart and Parham, 2004).

The ‘*Cuora serrata*’-like specimens studied by Parham et al. (2001) and Stuart and Parham (2004) originated from the pet trade and so lack reliable collection data. Here we report the discovery of a ‘*C. serrata*’-like turtle from near a remote village in Hainan, China (Nanmao, Qiongzong County; fig. 1). Nanmao is within 100 km of the type region of *Cuora serrata* (“Tainhfen, 100 km E of Tungfang [= Dong-

fang in fig. 1]”) reported by Iverson and McCord (1992).

What actual locality was meant by ‘Tainhfen’ is unclear. De Bruin and Artner (1999) could not locate ‘Tainhfen’ on any map while on Hainan and it is unfamiliar to the authors of this report that have lived on Hainan for years (HS, JW, SG, BF). ‘Tainhfen’ might refer to ‘Tan Hsien’ (an old name for Danzhou in fig. 1) as there is a major ~100 km east-trending road that connects these two towns, but Danzhou is a major city with an area of 3235 km² that includes many towns and villages. There are no turtles in central Danzhou, but our surveys indicate that *Mauremys sinensis* (formerly *Ocadia sinensis*, see Feldman and Parham [2004] and Spinks et al. [2004]) and *Mauremys mutica* can still be found in the surrounding terrain (Shi, unpublished data). We have not encountered any *Cuora* in the regional trade. Our uncertainty with this locality underscores a reliability problem common to many Asian turtle localities based on food or pet trade specimens (Fritz and Obst, 1998; Fritz and Obst, 1999; Parham and Li, 1999; Parham et al., 2001; Fong et al., 2002). The turtle reported here is also from the trade, but the circumstances of its discovery (see below) lend credence to the accuracy of its locality.

While staying at a Hainan Normal University field station near Nanmao village (19°00′057″N; 110°06′467″E), one of us (BF) was alerted

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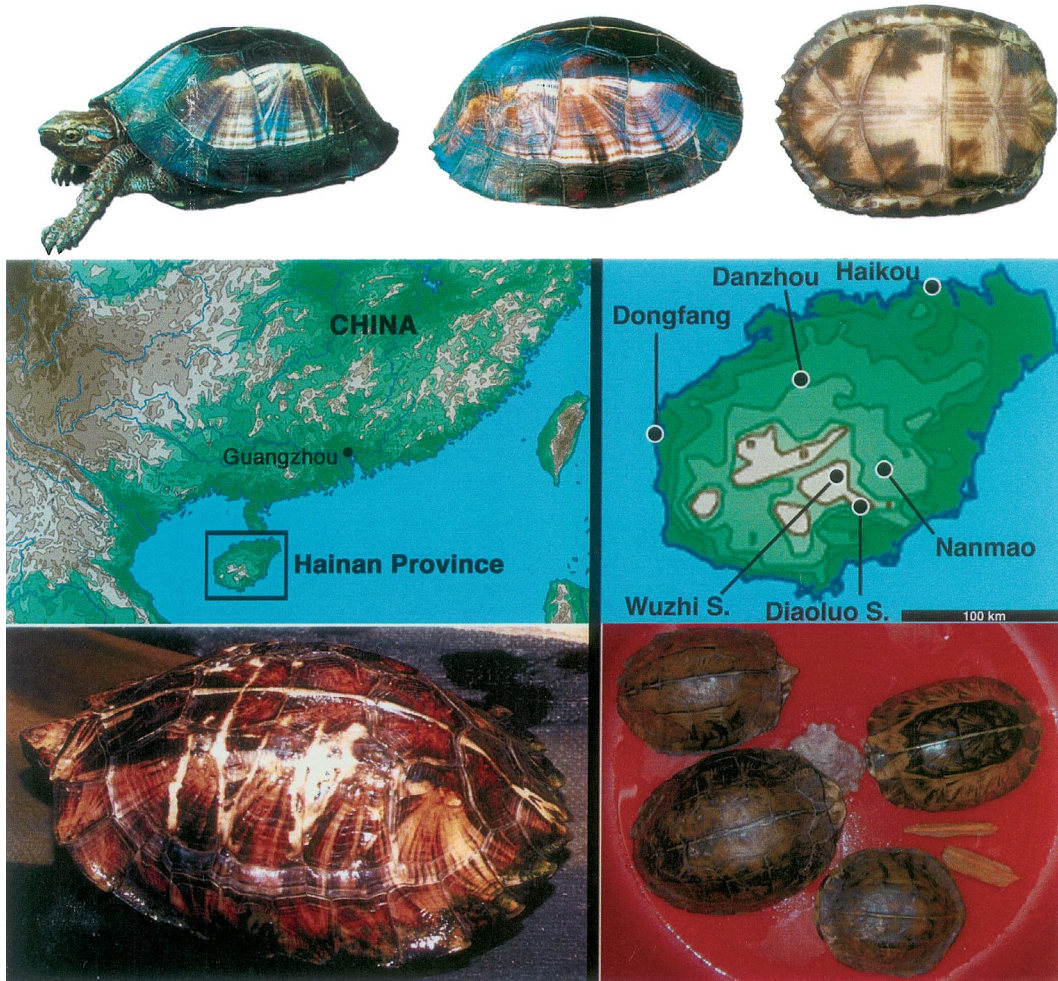


Figure 1. Top: A '*Cuora serrata*'-like turtle from Nanmao, Hainan Province China (Photos by HS). Middle: maps showing localities named in the text. Bottom left: A '*Cuora serrata*'-like turtle from the living collection of Jim Barzyk (Photo by JFP) showing scars. Bottom right: '*Cuora serrata*'-like turtles for sale in the back rooms of the Qing Ping live animal market of Guangzhou, Guangdong Province, China (Photo by JFP).

to the capture of an unusual *Cuora* (fig. 1) on June 3rd, 2003 by one of the local turtle trappers (Mr. Yongjun Lu). The specimen was caught in an area of mixed vegetation including bamboo, trees, and shrubs (18°59'839"N; 110°06'622"E). The canopy cover is about 80% and the forest floor is scattered with large stones. Some of these stones are surrounded by burrows that are presumed to be utilized by terrestrial turtles. According to YL, turtle trappers often set their traps near such holes. The trap that caught the unusual specimen was placed

in this manner. The unusual turtle was captured along with a typical-looking *C. mouhotii*.

According to YL, he has never seen a '*serrata*'-like turtle before (despite years of trapping in the region). The specimen shares characters with *C. mouhotii* (a flat-topped, posteriorly-serrated carapace, an angular plastron that is not solid black) and *C. galbinifrons* (kinetic plastron, carapace coloration). It is similar to some hybrid specimens sold as '*C. serrata*' in the pet trade (Iverson and McCord, 1992; Parham et al., 2001; Stuart and Parham, 2004). When captured, the unusual *Cuora* weighed 400 g, had

a carapace length of 12.88 cm, a carapace width of 9.63 cm, a carapace height of 5.96 cm, and a plastron length of 12.23 cm. It is currently in the living collection of Hainan Normal University under the supervision of HS. We sequenced the mtDNA from the 'serrata'-like turtle from Nanmao to confirm it was a hybrid of *C. mouhotii* and female *C. galbinifrons*.

We compare a piece of the Nad-4 mitochondrial gene of the Nanmao 'C. serrata'-like turtle (MVZ 241561) with sympatric *C. mouhotii* and *C. galbinifrons* and a larger data set of *C. galbinifrons* and related taxa from Stuart and Parham (2004). Tail tips were taken from this specimen as well as sympatric *C. mouhotii* ($n = 1$; MVZ 241562) and *C. galbinifrons* ($n = 2$; MVZ 241559, MVZ 241560) and preserved in 70% Ethanol for genetic study. The photographic voucher information and GenBank accession numbers for this study can be found in Appendix 1. The amplification and sequencing methods are identical to those given in Parham et al. (2001) except that we used the Nad-4 primers from Stuart and Parham (2004). We sequenced 841 basepairs of the mitochondrial genome that included the final 678 bp of the Nad-4 gene (+163 bp of adjacent tRNAs). Sequences were aligned by eye and analyzed using the parameters and complete data matrix (40 OTUs, 310 informative sites) given in Stuart and Parham (2004). The new *C. mouhotii* sequence was generated in case the 'serrata'-like turtle had a *C. mouhotii* mother, but was not included in the phylogenetic analysis. This specimen (MVZ 241562) differed from a *C. mouhotii* from nearby Wuzhi Shan (MVZ 230482; AF 348287; Parham et al., 2001) by a single third position base pair change (position 264 is a T instead of a C). Abbreviations: AF, GenBank accession numbers; FMNH, Field Museum of Natural History specimen number; MVZ, Museum of Vertebrate Zoology specimen number.

The sequence from the 'serrata'-like turtle from Nanmao is identical to that of sympatric *C. galbinifrons* (fig. 2). This haplotype is identical to a specimen of *C. galbinifrons* from Diaoluo Shan (MVZ 230466, AF 348291; Parham et al., 2001), but is distinct from those of five other 'serrata'-like specimens (FMNH 261572, AY 354267; FMNH 261573, AY 364625; FMNH 261578, AY 364622; MVZ 230511, AF 348294; MVZ 230628, AY 363626; MVZ 230629, AF348295) sequenced by Parham et al. (2001) and Stuart and Parham (2004). In light of its *C. mouhotii* characters, the 'serrata'-like turtle is probably a hybrid of between a male *C. mouhotii* and a female *C. galbinifrons*.

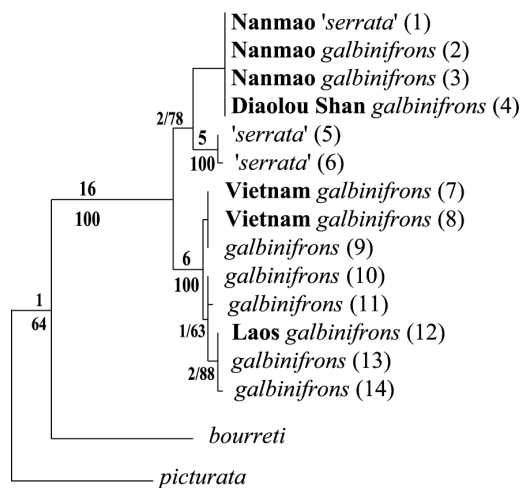


Figure 2. Parsimony phylogram showing the placement of Nanmao 'serrata' relative to other specimens of *C. galbinifrons* (15 non-relevant taxa have been pruned for clarity). The number above each stem is the decay index and the number below each stem is the bootstrap value (based on 1000 non-parametric pseudoreplications). For some nodes these values are given as decay index/bootstrap for space reasons. For voucher information of specimens 1-3 please see Appendix. Specimens 4-14 in this study are specimens 15-25 in Stuart and Parham (2004).

The presence of two distinct *galbinifrons* mt haplotypes in 'serrata'-like turtles (fig. 2) shows that multiple hybridization events must have occurred. Similarly, Stuart and Parham (2004) demonstrated that 'serrata'-like specimens also have two distinct *C. bourreti* haplotypes so there were at least four independent hybridization events that formed 'serrata'-like specimens. Four is a minimum estimate since hybrids with identical mtDNA (e.g., specimens 30 and 31 in Stuart and Parham, 2004) may result from separate hybridization events involving females with identical mt haplotypes. Also, just few 'serrata'-like specimens (six) have been sequenced.

This report supports the hypothesis that a 'serrata'-like turtle can form through hybridization of *C. mouhotii* and *C. galbinifrons* in the wild. However, the origin of pet trade specimens is still unclear. The damaged appearance of some pet trade specimens (fig. 1) has led some to suggest that other 'C. serrata'-like turtles are from the wild. Stuart and Timmins

(2000) show a photograph of a specially trained hunting dog gnawing on a *C. galbinifrons*. Alternatively, the crowded conditions in which farmed turtles are kept (Shi and Parham, 2001), along with human or canine inflicted damage in captivity might also account for their haggard appearance. It is not possible to distinguish whether these wounds were inflicted in the wild or in captivity. Nevertheless, a wild origin for some specimens is supported by a 2001 interview with a pet dealer in the Qing Ping live animal market of Guangzhou. He claimed that he occasionally finds the hybrids mixed in with imported shipments of other wild-caught turtles (especially terrestrial *Cuora*). He buys these turtles (fig. 1) for the 'food' price and then sells them to foreign enthusiasts for a much higher price.

Although some specimens of '*C. serrata*'-like turtles may come from the wild, there are presently no data to suggest that a wild, interbreeding population of '*C. serrata*'-like turtles exists. Instead, the data presented here support the hypothesis that '*C. serrata*'-like turtles do not form a natural species, but result from multiple hybridization events between male *Cuora mouhotii* and females of *Cuora galbinifrons* or *Cuora bourreti* (Parham et al., 2001; Stuart and Parham, 2004). Multiple explanations (including captive breeding) may be necessary to account for all the known specimens (Parham et al., 2001). It is interesting to note that the only member of the *C. galbinifrons* complex that is not presently known to hybridize with *C. mouhotii* is *Cuora picturata* Lehr, Fritz, and Obst 1998 (Stuart and Parham, 2004). *Cuora picturata* is endemic to Southern Vietnam, outside the natural range of *C. mouhotii*, so if a *picturata* '*serrata*' were found it would probably have a captive origin.

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Appendix

Voucher information for specimens used in this study. All turtles were found near the village of Nanmao (see text and fig. 1 for locality). Numbers 1-3 correspond to terminal taxa in fig. 2. 1) Nanmao *Cuora* (*Cuora mouhotii* × *Cuora galbinifrons*), MVZ 241561 (photographic voucher, specimen is presently alive in the collection of Hainan Normal University; GenBank accession # = AY 699013); 2) *Cuora galbinifrons* MVZ 241559 (photographic voucher; GenBank accession # = AY 699014); 3) *Cuora galbinifrons* MVZ 241560 (photographic voucher; GenBank accession # = AY 699015). We also sequenced mtDNA from one *Cuora mouhotii* MVZ 241562 (photographic voucher; GenBank accession # = AY 699016).

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