The Mitochondrial DNA Control Region Comparison Studies of Four Hinged Turtles and its Phylogentic Significance of the Genus Cuora Sensu Lato (Testudinata: Geoemydidae)

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ABSTRACT

The complete sequences of the mitochondrial DNA (mtDNA) control region (CR) of Cistoclemmys flavomarginata, Cistoclemmys galbinifrons, Cuora aurocapitata and Cyclemys atripons were amplified by long-polymerase chain reaction (Long-PCR). The lengths were 1207 bp, 1722 bp, 1379 bp and 980 bp, respectively. Combining with the CR sequence of Pyxidea mouhotii (DQ659152), we compared the CR structure, and identified three functional domains (TAS, CD and CSB) in which the conservation sequences (TAS, CSB-F, CSB-1, CSB-2 and CSB-3) were also successfully identified according to their homology to those of other turtles. These 5 turtles have the identical CSB-2 and CSB-3 sequences, and 4 of them have the same CSB-1 sequence while there is one base transversion (T → A) in Cy. atripons. We analyzed the variable number of tandem repeat (VNTR) sequences or microsatellites at the 3' end of CR. The motifs of tandem repeats (7 types) are made up of 2-8 nucleotides, and the copy numbers are from 4 to 48. All of the 5 turtles except Cy. atripons have the “TATTATAT” repeats and are ended by TA. The results of CR structure analysis displayed that the Cuora, Cistoclemmys, and Pyxidea have many similarities, but differ from Cyclemys. With Indotestudo elongate (DQ080043) and Indotestudo forstenii (DQ080044) as outgroups, using the CR sequences (1123bp) excluded the microsatellites at the 3' end of CR, we constructed the molecular phylogenetic trees using the MP, ML and BI methods. The results showed that there was a strong support to the monophyly of the Cuora group consisting of Cuora, Cistoclemmys and Pyxidea, which has a close relationship with Mauremys and Chinemys but far from Cyclemys, which are consistent with the analysis of the CR structure of the 5 turtles.

Key words: Geoemydidae, Cuora group, mitochondrial DNA control region, microsatellites, phylogeny.

INTRODUCTION

The control region (CR) is the most important noncoding region in the mitochondrial DNA (mtDNA), which flanked by tRNAPro and tRNAPhe genes. Variation within the control region has been shown to consist not only of nucleotide substitutions but also length variation due to short insertion/deletion events and differing numbers of tandem repeated sequences...
within its 5′ and 3′ ends (Ludwig et al., 2000). Despite this high level of sequence variation, several common features have been characterized and found to be similar among vertebrates which suggest that the CR sequences can be divided into three domains: termination associated sequence (TAS) domain, central conserved (CD) domain and conserved sequence block (CSB) domain (Sbisa et al., 1997) and these domains contain the origin and termination of heavy strand (H-strand) replication and the promoters for both heavy and light strand (L-strand) transcription (Clayton, 1984; Wong and Clayton, 1985). TAS domain is usually highly variable, while CD domain is typically less variable and contains several areas of highly conserved sequences. Like TAS domain, CSB domain has also been shown to be highly variable and often contains variable number of tandem repeat (VNTR) sequences or microsatellites, which can be used as special molecular markers and provide significantly referenced value into molecular phylogenetics, species identification as well as genetic diversity and conservation (Zhang et al., 2008).

Compare with mammalian, fish, aves and other vertebrates, the studies of turtles CR structure are quiet few, which have been reported only 5 species so far (Zardoya and Meyer, 1998; Zhang et al., 2008; Yan et al., 2008). The studies heretofore show that most turtles have one CR except Platysternon megacephalum (Peng et al., 2006; Parham et al., 2006a) and Malacocheirus tornieri (Parham et al., 2006b) which have two CR. In P. megacephalum, the two control region sequences are highly similar for the initial (5′) 800 bp (TAS and CD domains), but their 3′ regions of the approximate 300 bp are quite different. Thirteen 17 bp tandem repeats and four 10 bp tandem repeats are found in control region I, but no repeat regions are found in control region II. It seems that the concerted evolution has not operated on the region with tandem repeats, perhaps due to acquisition or loss of functional roles (Peng et al., 2006). In M. tornieri, the two control regions are divergent and only the 1683 bp one adjacent to tRNAPro appears functional (corresponds to other turtle control regions in GenBank). The other control region (2017 bp) does not correspond well to any sequence of other turtles (Parham et al., 2006b).

The turtle family Geoemydidae (Bataguridae) is the largest, most diverse family of turtles, with approximately 60 species in the world (Spinks et al., 2004; Bour, 2008; Rhodin et al., 2008) in which the species of kinetic plastron with a hinged between the pectoral and the abdominal is observed in 5 geoemydine genera: Cuora, Cistoclemmys, Pyxidea, Cyclemys and Notochelys (Honda et al., 2002). These turtles are well known as Asian “box turtles”. During the past few decades, their taxonomy and phylogenetic position are still entangled despite numerous studies derived from morphological, chromosomal, and molecular analyses (Bramble, 1974; Hirayama, 1984; Carr and Bickham, 1986; Yasukawa et al., 2001; Honda et al., 2002; Stuart and Parham, 2004). Based on a similar mechanism for closing the anterior part of the shell, Bramble (1974) hypothesized that Cuora sensu lato (as consisting of Cuora sensu stricto and Cistoclemmys), Pyxidea, Cyclemys which all have plastral hinge structure were probably derived from a Hoesemys-like ancestor and therefore all 4 genera could be combined into Hoesemys complex. Furthermore, he assumed that Cuora and Cistoclemmys had closest affinity, so many authors regard Cistoclemmys as a synonym of Cuora (Fritz and Obst, 1997). Hirayama (1984), on the basis of morphological characters, concluded that there is a distant location between Cistoclemmys and Cuora, and a closest relationship of the former with Geoemyda and Pyxidea. Wu et al. (1998) using 12S rRNA gene sequence indicated that Cuora and Pyxidea is sister group, hinge structure in Geoemydidae were probably not derived from one common ancestor. Honda et al. (2002) using 882 bp sequences of combined 12S and 16S rDNA, reconstructed phylogenetic relationships within the genus Cuora and found that the monotypic Pyxidea was nested within Cuora, so they regarded Cistoclemmys and Pyxidea as a synonym of Cuora. This recommendation has been followed by some recent authors (Stuart and Parham, 2004; Spinks et al., 2004). However, the relationships among Cuora, Cistoclemmys, Pyxidea and Cyclemys are always controversial, further studies on the phylogenetic relationships of the hinged turtles (the Asian box turtles) are warranted and interesting.

Owing primarily to the lack of selective constraints