Biochemical Systematics and Ecology 54 (2014) 279–284

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Genetic diversity and population differentiation of the Chinese soft-shelled turtle (*Pelodiscus sinensis*) in three geographical populations

Xingjiang Bu, Luo Liu, Liuwang Nie*

Life Science College, Anhui Normal University, The Provincial Key Lab of the Conservation and Exploitation Research of Biological Resources in Anhui, Wuhu 241000, China

ARTICLE INFO

Article history:
Received 10 October 2013
Accepted 21 February 2014
Available online

Keywords:
Pelodiscus sinensis
Microsatellite
Genetic diversity
Population differentiation

ABSTRACT

The Chinese soft-shelled turtle (*Pelodiscus sinensis*) is one of the most important economical chelonians in the world. To understand the genetic variations of the Chinese soft-shelled turtle in China, 62 individuals were sampled from three localities and 18 polymorphic microsatellite loci tested were used to detect genetic diversity and population structure. Results showed that the genetic diversity of the wild *P. sinensis* was high. Except for the Wuhu populations, the majority of microsatellite loci are not deviation from Hardy–Weinberg equilibrium in the other two populations. AMOVA analysis indicated that genetic variations occurred mainly within populations (97.4%) rather than among populations (2.6%). The gene flow estimates (*Nm*) among three geographic populations demonstrated that strong gene flow existed ($Nm > 1$, mean 6). The present study supported that different habitats, breed turtles escaped, multiple paternity and long evolutionary history may be responsible for the current genetic diversity and differentiation in the wild Chinese soft-shelled turtle.

1. Introduction

The Chinese soft-shelled turtle *Pelodiscus sinensis* is favored for its medicinal and nutritional value and has been consumed as food for a long period of time (Altherr and Freyer, 2000). However, in recent decades, the wild populations of Chinese soft-shelled turtles have sharply declined throughout their range due to unregulated harvest, environmental pollution and other human disturbances in the wild, resulting in the disappearance of the turtle in many places (Que et al., 2007). Currently, it has been listed as a vulnerable species in the China Red Data Book of Endangered Animals (Zhao, 1998) and the IUCN List category in 2000 (http://www.iucnredlist.org).

Knowledge on the genetic diversity and population differentiation of wild populations is essential for effective protection measures (Osentoski et al., 2002). The high levels of genetic diversity can facilitate adaptation to changing environmental conditions through natural selection (Alacs et al., 2007). Until now studies on *P. sinensis* mainly focused on nutrition, captive breeding, artificial hatching and reproductive physiology (Du and Ji, 2003; Zhu, 2009; Yasumasu et al., 2010). However, little has been known about the situation of this species in the wild environment. Based on morphological differences, Li et al.

* Corresponding author. Tel.: +86 553 3822885; fax: +86 553 3869571.
E-mail addresses: lwnie@mail.ahnu.edu.cn, lwniegene@163.com (L. Nie).

http://dx.doi.org/10.1016/j.bse.2014.02.022
0305-1978/

(1997) analyzed fifteen morphological characters of *P. sinensis* from Shaoxing, Nanjing, Chaohu and Qingdao areas of East China, finding no difference between Nanjing and Chaohu populations, while significant differences between (Nanjing, Chaohu) and Shaoxing, particulaly in Qingdao populations.

In the present study, the collected samples of *P. sinensis* were different in morphology between the Yangtze River and the Yellow River Basin populations. For example, abdominal color of adult turtle from the Yangtze River and the Yellow River Basin is white and yellow, respectively. Whether these morphological differences coincide with genetic differentiation or not need be further verified.

Molecular markers are effective tools for detecting genetic diversity and population differentiation (Whitehead et al., 2003; Liang et al., 2012). Among different types of molecular markers, microsatellite markers have been widely used in the study of animal genetic diversity because of their codominant, wide distribution, high polymorphism, easy detection, etc. (Attard et al., 2010; Ahlering et al., 2011). Here, the genetic diversity and population differentiation of three geographic populations of *P. sinensis* were investigated using 18 microsatellite loci.

2. Materials and methods

2.1. Sample collections and DNA extraction

A total of 62 Chinese soft-shelled turtle individuals were collected from three different wild populations in which 34 are from Wuhu environs (surrounding rivers), 12 are from Dabie Mountain area (Chang river of Taihu county, near Jiangxi province) in Anhui province, and 16 are from Liaocheng area (surrounding rivers of Chiping county) in Shandong province, China (Fig. 1).

Total genomic DNA for subsequent surveys was extracted from muscle tissue using the standard phenol-chloroform protocol, as described by Sambrook and Russell (2001).

2.2. PCR amplification and genotyping

All turtle samples were screened for variation at each of 18 microsatellite loci (CST16, CST26, CST27, CST28, CST29, CST33, CST34, CST35, CST41, CST42, CST43, CST46, CST47, CST48, CST52, CST54, CST57, and CST61) (Bu et al., 2011). PCR amplifications were performed according to the conditions given in Bu et al. (2011). Each forward primer of the microsatellite loci was labeled with a fluorescent dye at its 5′ end. Amplified products were analyzed on an ABI PRISM 3730 Genetic Analyzer, with LIZ 500 (Applied Biosystems) as the internal size standard. Microsatellite alleles were precisely sized using the software Genemarker (Applied Biosystems) to calculate their number, range and distribution.

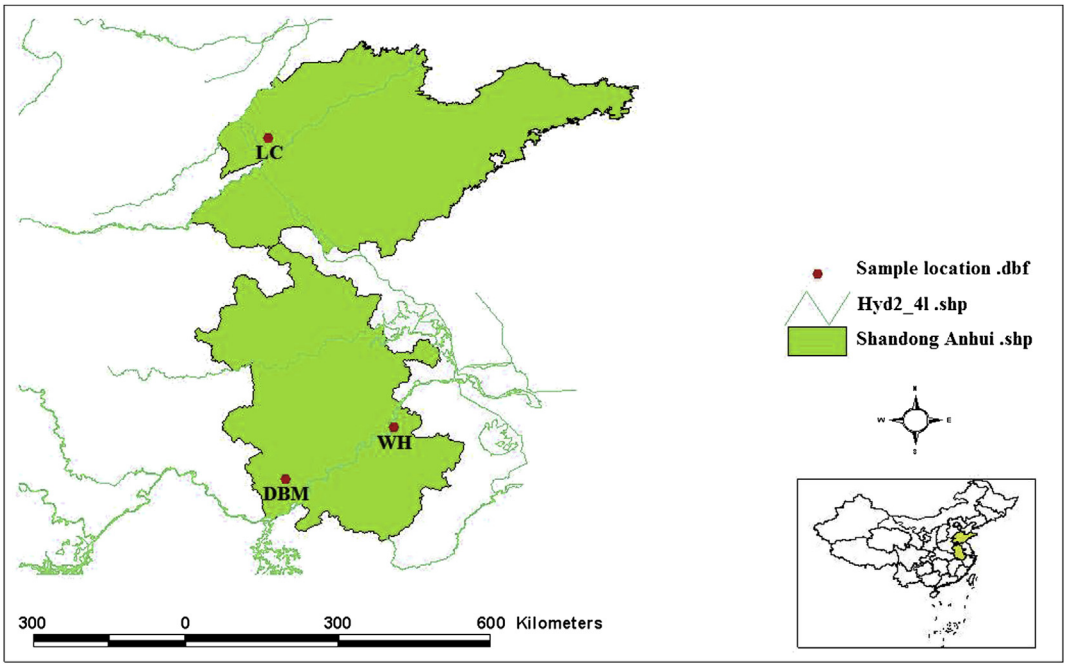

Fig. 1. A map showing the sampled populations of *Pelodiscus sinensis*. LC: Liaocheng; DBM: Dabie Mountain area; WH: Wuhu.