



Genetic and shell-shape analyses of *Orlitia borneensis* (Testudines: Geoemydidae) reveal limited divergence among founders of the European zoo population

KLÁRA PALUPČÍKOVÁ¹, BARBORA SOMEROVÁ¹, TOMÁŠ PROTIVA¹, IVAN REHÁK²,
PETR VELENSKÝ², PAVEL HULVA¹, DANNY GUNALEN³ & DANIEL FRYNTA^{1,4}

¹Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, CZ-12844, Prague 2, Czech Republic. E-mail: klpr@post.cz

²Prague ZOO, U Trojského Zámku 3, CZ-171 00 Prague 7, Czech Republic. E-mail: ophis@fiscali.cz

³Ex-situ Captive Breeding Centre, Jalan Raya Hankam no. 49. Bekasi – Indonesia. E-mail: almusa@cbn.net.id

⁴Corresponding author. E-mail: frynta@centrum.cz

Abstract

The Malaysian Giant Turtle (*Orlitia borneensis*) is a poorly known turtle with rapidly decreasing numbers in nature in spite of its strong protection on paper. Most individuals of this species kept in European zoos and included in captive breeding programs are confiscated from the illegal trade for food consumption and their geographic provenance is unknown. This study was aimed to assess genetic and phenotypic variation of the founders of this captive population. We sequenced the mitochondrial cytochrome *b* gene and found 23 haplotypes. We constructed a haplotype network and examined demographic changes by Bayesian skyline plots of the effective population size. The maximum sequence divergence was less than 1.5% and the phylogenetic structure of the haplotypes was supported poorly. A close genetic similarity among sampled turtles was further confirmed by sequencing the nuclear R35 gene, while the geometric morphometrics of the shell-shape were likewise similar. Thus, the examined captive population of *O. borneensis* may be further treated as a single conservation unit.

Key words: Ex situ breeding, genetic variability, cytochrome *b*, nuclear gene R35, phylogeography, population expansion

Introduction

With the ongoing Asian Turtle Crisis (Cheung & Dudgeon 2006) we are now facing the reality of decreasing numbers of many species of turtles, especially in the family Geoemydidae. This situation is a result of a combination of habitat destruction and targeted exploitation of turtles to meet the demand from Chinese markets for use in traditional medicine and especially for meat (Zhou & Jiang 2008; Chen *et al.* 2009). Proper taxonomy is an important prerequisite to efficient conservation. It is especially important to detect hidden, but possibly deep genetic variation in rare species without recognized subspecies that originally inhabited extensive geographic ranges and that are now restricted to scattered refugees, as it is the case for one of the most charismatic South Asian freshwater turtle species, *Orlitia borneensis* (Gray 1873). The local Indonesian name of this species is Kura Tuntong or Biuku. According to IUCN, *O. borneensis* is listed as Endangered A1d+2d range-wide, and was specifically considered Endangered (A1cd) in Indonesia and Vulnerable (2cd) in West Malaysia. Because it can reach up to 80 cm carapace length (Ernst & Barbour 1989), it is a preferred target of collectors and thus the numbers of individuals of this species in nature are drastically decreasing in spite of its strong legal protection on paper (e.g., Indonesian Law PP No. 7 1999).

It was attempted to develop individual countermeasures to solve this problem. In Indonesia, commercial harvesting of turtle species for food consumption is strictly regulated by a system of quota. Such harvesting is permitted exclusively to licensed foods traders and concerns a few turtle species that are believed to be still abundant (*Amyda cartilaginea*, *Dogania subplana*, *Cuora amboinensis*, *Cyclemys* spp.). Harvesting of the protected species like *O. borneensis* is strictly prohibited. Thus, all *O. borneensis* found outside the country (except those legally