

**PHYLOGENETIC RELATIONSHIPS AMONG THE
ASIAN TORTOISES OF THE GENUS *INDOTESTUDO*
(REPTILIA: TESTUDINES: TESTUDINIDAE)**

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(with two text-figures)

ABSTRACT.—Based on variation in 1094 bp of the mitochondrial cytochrome *b* gene among members of the genus *Indotestudo* from southern Asia (*I. elongata*), south-western India (*I. travancorica*), and eastern Indonesia (*I. forstenii*), *I. travancorica* is most similar genetically to *I. elongata* (3.7% divergence), and strongly divergent from *I. forstenii* (5.5 to 5.9%). Individuals of *I. forstenii* with and without a nuchal scute differ genetically at less than 0.7%. Our analysis offers no support for the hypothesis that Indonesian populations of *I. forstenii* represent introductions of *I. travancorica* from India. The recognition of three nominal species (*I. elongata*, *I. forstenii* and *I. travancorica*) in the genus is thus warranted.

KEY WORDS.—*Indotestudo*, *I. elongata*, *I. forstenii*, *I. travancorica*, turtle, systematics, DNA.

INTRODUCTION

Schlegel and Müller (1840: 30) first described *Testudo forstenii* from Halmahera Island, in the Molucca Islands (now Maluku) in eastern Indonesia and it is now known only from the islands of Halmahera and Sulawesi. Although Schlegel and Müller did not explicitly designate a type specimen, Hoogmoed and Crumly (1984) determined that the description was based on a specimen (RMNH 3811) in the Rijksmuseum van Natuurlijke Historie (at present Nationaal Natuurhistorisch Museum) in Leiden. Blyth (1853: 639) described *Testudo elongata* from “Arakan” (= Rakhine Yoma, a mountain range along coordinates ca. 18-21° N, 93-95° E) in western Myanmar (Burma), apparently based on four syntypes (ZSI 796, 798, 799, and 800) in the

collection of the Zoological Survey of India in Calcutta (Das et al., 1998). Fifty-four years later, Boulenger (1907:560; pl. I-II) described *Testudo travancorica* from the Western Ghats of south-western India, based on two specimens in the British Museum (Natural History) (now, The Natural History Museum, London, BMNH 1906.7.18.6-7).

Lindholm (1929) first recognized the distinctiveness of *Testudo elongata* and designated it as the type species of his new subgenus *Indotestudo* (genus *Testudo*), but he did not mention the placement of the other two species. However, Williams (1952) supported the recognition of the subgenus *Indotestudo* (under *Testudo*), and included *elongata*, *forstenii*, and *travancorica* therein. Williams (p. 220 in Loveridge and Wil-

TABLE 1: Uncorrected (“p”) distance matrix for samples of *Indotestudo* and outgroup taxa.

Species	<i>picta</i>	<i>agassizii</i>	<i>emys</i>	<i>elongata</i>	<i>travancorica</i>	<i>forstenii</i> A
<i>Chrysemys picta</i>	–					
<i>Gopherus agassizii</i>	0.156	–				
<i>Manouria emys</i>	0.170	0.129	–			
<i>Indotestudo elongata</i>	0.170	0.138	0.142	–		
<i>Indotestudo travancorica</i>	0.172	0.141	0.138	0.037	–	
<i>Indotestudo forstenii</i> A (with nuchal)	0.174	0.144	0.138	0.060	0.055	–
<i>Indotestudo forstenii</i> B (without nuchal)	0.174	0.144	0.136	0.062	0.059	0.005

liams, 1957) partitioned the all-inclusive tortoise genus *Testudo*, placing *elongata*, *forstenii* and *travancorica* in the subgenus *Indotestudo* of the genus *Geochelone*, the arrangement followed by Auffenberg (1974). Bour (1980) subsequently elevated *Indotestudo* to full generic rank (including the same three species), a position supported by the cladistic analyses of Crumly (1982, 1984), and adopted by nearly all subsequent authors.

Although the monophyly of *Indotestudo* (whatever its rank) has not been questioned for 50 years, Auffenberg (pers. comm.; mid-1970's) and Pritchard (1979: 319) first speculated that the Indonesian populations of *I. forstenii* might represent an introduction of *I. travancorica* from India. Hoogmoed and Crumly (1984) examined specimens of all three species of *Indotestudo*, could not distinguish *I. forstenii* from *I. travancorica*, synonymized the two taxa under the older name *I. forstenii*, and could not reject the hypothesis that *I. forstenii* represented an introduced population of *I. travancorica*. This arrangement has been followed by nearly all subsequent authors (e.g., Groombridge and Wright, 1982; Das, 1991, 1995); however, Bour (in David, 1994: 87) and McCord et al. (1995) speculated that this synonymy might be premature.

Pritchard (2000) recently reopened the controversy by examining the morphology of all three species of *Indotestudo*. He concluded that *I. travancorica* was distinguishable from *I. forstenii* and *I. elongata*, and hence should be resurrected from the synonymy of *I. forstenii*. However, neither Pritchard nor any other author has hypothesized a phylogenetic relationship

among the populations of *Indotestudo*. Therefore, in order to address the taxonomic and evolutionary relationships among these species from a molecular perspective, we examined mtDNA sequence variation in these and related tortoises.

MATERIALS AND METHODS

Blood samples were obtained from two pet trade specimens of *Indotestudo forstenii* in the collection of A. Weinberg, and known to have come from Sulawesi (McCord, pers. comm.), one with a nuchal (hereafter, *I. forstenii* A) and one without (hereafter, *I. forstenii* B); a captive specimen of *I. travancorica* from Chalakudy (10° 06' 23"N; 76° 09' 53"E), Kerala State, south-western India, now in the live collection of the Centre for Herpetology at the Madras Crocodile Bank Trust, which will be deposited in the collection of the Zoological Survey of India, upon its death; and a pet trade specimen of *I. elongata*, believed to have come from Myanmar, in the live collection of WPM. Tissue samples from *Manouria emys* (considered as representing the most “primitive” living genus of tortoises; Crumly, 1982, 1984), *Gopherus agassizii* (representing another early tortoise genus), and *Chrysemys picta* (an emydid turtle; Gen Bank accession #5902189) were included as our outgroup.

Whole genomic DNA was extracted from blood via SDS/protease K digestion, followed by phenol/chloroform extraction (Shaffer et al., 1997). Most (1094 of 1140bp) of the mitochondrial cytochrome *b* gene was amplified using Taq-mediated PCR and sequenced on an ABI 377 (Applied Biosystems, Foster City, CA) auto-

matic sequencer using primers developed by PQS and HBS (available on request). Overlapping sequences within individual samples were aligned and edited using SeqED (Applied Biosystems), and complete sequences were aligned across samples using ClustalW (Thompson et al., 1994). Alignments were unambiguous, with no insertions or deletions present. All sequences will be deposited in Gen Bank. Phylogenetic analyses were conducted using PAUP* 4.0b3a (Phylogenetic Analysis Using Parsimony; provided by David L. Swofford; www.lms.si.edu/PAUP).

RESULTS AND DISCUSSION

Within the 1094 bp fragment of the cytochrome *b* gene, 152 nucleotide sites were phylogenetically informative and 788 were constant across all taxa. An heuristic search of equally weighted characters (with 100 bootstraps) using maximum parsimony produced two shortest trees differing only in the placement of two of the outgroup taxa. (Fig. 1). An analysis by the neighbour-joining method (HKY 85 model; 100 bootstraps; unweighted characters) produced identical results (Fig. 1). Maximum likelihood analysis of the same data produced a slightly different arrangement of the ingroup taxa (Fig. 2).

Although these analyses suggest some ambiguity in the placement of *I. elongata*, *I. travancorica* is clearly more similar genetically to *I. elongata* (3.7% distant; Table 1) than to *I. forstenii* (5.5 to 5.9%). In addition, the terminal position of *forstenii*, its high divergence from its congeners, and the low variation within *I. forstenii* (even between individuals with versus without a nuchal scute) all argue that *Indotestudo forstenii* represents a distinct species native to Indonesia. Furthermore, there is no genetic support (nor historical data) for the hypothesis that *I. forstenii* represents an introduced population of *I. travancorica*. As a consequence, we follow Pritchard (2000) in recommending that *Indotestudo travancorica* be resurrected from the synonymy of *I. forstenii*. Three nominal species in the genus *Indotestudo* are therefore recognisable: *I. elongata* from the mainland of southern and south-eastern India, *I. forstenii*

from the islands of Sulawesi and Halmahera in eastern Indonesia and *I. travancorica* from the Western Ghats of south-western India.

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FIGURE 1: Strict consensus tree of phylogenetic relationships among species of the genus *Indotestudo* based on parsimony and neighbour-joining analyses of the cytochrome *b* gene sequence data. Numbers above nodes are bootstrap percentages for the parsimony analysis; numbers below are bootstrap percentages for the neighbour-joining analysis. Length = 430 steps; consistency index = 0.83. Branch length is proportional to number of nucleotide changes by the neighbour-joining analysis.

FIGURE 2: Strict consensus tree of phylogenetic relationships among species of the genus *Indotestudo* based on maximum likelihood analyses of the cytochrome *b* gene sequence data. Branch length is proportional to the number of nucleotide substitutions per site.