PUBLICATIONS
Structure and molecular evolution of Cytochrome-b among the freshwater turtle genera Pangshura and Batagur

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ABSTRACT

An in-silico study has been performed for 3D structure prediction and evolutionary profile of Cytochrome-b from the highly endangered Southeast Asian turtle species under the genera Pangshura and Batagur (Testudines: Geoemydidae: Pangshura and Batagur). The analyses were performed using the sequence data of Cytochrome-b, extracted from Protein Knowledgebase (UniProtKB). The study represents the application of comparative modelling method for protein 3D structure prediction. The evolutionary analyses were conducted in MEGAS by Maximum Likelihood, Neighbour-Joining and Minimum Evolution methods. The predicted structures of Cytochrome-b have 20-23 helices, 40-43 helix-helix interacts, 14-15 beta turns, 4-6 gamma turns. The structures were found statistically significant at 95% confidence level and the same were deposited to Protein Model Database (PMDB). Bootstrap test (1000 replicates) was performed to validate the phylogenetic tree. Two distinct clades were observed in the final tree. The results showed that the Cytochrome-b of P.sylhetensis evolved much earlier than the other three species of Pangshura. Further, it reveals that P.smithii and P.tentoria are the sister species followed by P.tecta and P.sylhetensis their successive sister-taxa. Distinctness within the sub-species of P.tentoria is not clear in the evolutionary data of Cytochrome-b protein. The computational models of Cytochrome-b could be of use for further evaluation of molecular mechanism of function. The present study also provides an indispensable groundwork for future conservation and genetic analysis of other turtle genera.

KEYWORDS: Chelonian, Conservation, Cytochrome-b, Geoemydidae, in-silico

The turtle family Geoemydidae includes highly endangered South-east Asian turtle group, mostly are of freshwater turtles (Van Dijk et al., 2000; IUCN, 2006). Most geoemydids are freshwater turtles. The group also occupies a wide range of habitats, from highly aquatic (Batagur and Pangshura) to highly terrestrial (Geoemyda). The genera Pangshura and Batagur are characterized by more or less well defined sexual dimorphism (Ernst et al., 2000; Das, 2001). Batagur [maximum shell lengths 48–58 cm; (Ernst et al., 2000)] is comparatively larger than Pangshura [maximum shell lengths 20–26.5 cm; (Ernst et al., 2000)] and Das (2001) placed them into two distinct genera. Understanding differentiation of these turtles at molecular level would significantly contribute to more powerful conservation formulation. The present study focuses on the structure and molecular evolution of Cytochrome-b, an integral membrane protein of approximately 400 amino acid residues (Esposti et al., 1993). Although, there is an availability of sequence information for Cytochrome-b from genera Pangshura and Batagur, yet there is scare structural and evolutionary information available. Therefore, the biochemistry and molecular mechanism of their functions are yet to be fully understood. In silico analysis has been performed for 3D structure prediction and evolution-
ary profile of Cytochrome-b from the highly endangered Southeast Asian turtle genera *Pangshura* and *Batagur* (Testudines: Geoemydidae: *Pangshura* and *Batagur*) in order to identify their structural and evolutionary properties and to test whether the evolution of the Cyochrome-b protein within each genus correspond well with distinct clades.

**Materials and methods**

**Acquisition and alignment of sequences**

*In silico* analysis was carried out on the sequence information of Cytochrome-b protein extracted from Protein Knowledgebase (UniProtKB). A total of 27 homologous sequences belonging genera *Pangshura* (4 species) and *Batagur* (6 species) along with our group sequence *Lissemys punctata* to test the phylogeny were acquired both by database keyword search and by BLASTp (Altschul et al., 1997) and FASTA (Pearson, 1991) searches are listed in Table 1. The sequences were simultaneously aligned using CLUSTAL-W (Higgins et al., 1994) and Modeller (Fiser et al., 2000) programs.

**Three-dimensional structure prediction**

Comparative (Homology) modeling based on the 3D coordinates of pdb ID 1BCC Chain C (Oxidoreductase Cytochrome Bc1 Complex From Chicken X-Ray Diffraction) were conducted by using Modeller9v2 program (Marti-Renom et al., 2000). The final 3D structures for Cytochrome-b were evaluated (Giorgetti et al., 2005) by ERRAT (Colovos and Yeates, 1993) and ProCheck (Laskowski et al., 2003).

**Molecular Phylogenetic analysis**

Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011). The evolutionary history was inferred by using three different methods namely the Maximum Likelihood (Jones et al., 1992), Neighbor-Joining (Saitou and Nei, 1987) and Minimum Evolution (ME) methods (Rzhetsky and Nei, 1992). The tree is predicted to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling, 1965) and are in the units of the number of amino acid substitutions per site. The analysis involved 27 amino acid sequences. All positions containing gaps and missing data were eliminated. The Maximum Likelihood tree was searched with the highest log likelihood (-1792.5671) value. When the number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise BIONJ method with MCL distance matrix was used. In Neighbor-Joining method, the optimal tree with the sum of branch length = 0.35969843 is considered. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm (Nei and Kumar, 2000) at a search level of 0. The Neighbor-joining algorithm (Saitou and Nei, 1987) was used to generate the initial tree.

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**Table 1.** Currently recognized species and subspecies of *Pangshura* and *Batagur* analyzed in the present study and associated UniProtKB Accession No (According to Fritz and Havas, 2007)

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species/Sub species</th>
<th>UniProtKB Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pangshura</td>
<td><em>Pangshura snuthn smithn</em> (Gray, 1863)</td>
<td>A7WMA7</td>
</tr>
<tr>
<td></td>
<td><em>Pangshura snuthn pallipes</em> (Moll, 1887)</td>
<td>A7WMB0</td>
</tr>
<tr>
<td></td>
<td><em>Pangshura sylhetensis</em> (Jerdon, 1870)</td>
<td>A7WMB5, A7WMB6, A7WMB7</td>
</tr>
<tr>
<td></td>
<td><em>Pangshura tecta</em> (Gray, 1831)</td>
<td>A7WMC0, A7WMC2, A7WMC4, A7WMC6</td>
</tr>
<tr>
<td></td>
<td><em>Pangshura tentora tentora</em> (Gray, 1834)</td>
<td>A7WMD1, A7WMC7</td>
</tr>
<tr>
<td></td>
<td><em>Pangshura tentora circumdata</em> (Mertens, 1969)</td>
<td>A7WMD5, A7WME1</td>
</tr>
<tr>
<td>Batagur</td>
<td><em>Batagur dhongola</em> (Gray, 1835)</td>
<td>A7WMA1, Q70M67</td>
</tr>
<tr>
<td></td>
<td><em>Batagur kachuga</em> (Gray, 1831)</td>
<td>A7WMA3</td>
</tr>
<tr>
<td></td>
<td><em>Batagur trivittata</em> (Duméril &amp; Bibron, 1835)</td>
<td>A8Y844</td>
</tr>
<tr>
<td></td>
<td><em>Batagur borneanus</em> (Schlegel and Muller, 1845)</td>
<td>A7WMA90, A7WNH1</td>
</tr>
<tr>
<td></td>
<td><em>Batagur affinis</em> (Cantor, 1847)</td>
<td>A7WNG4, C9X3V9</td>
</tr>
<tr>
<td>Out group</td>
<td><em>Lissemys punctata</em> (Bonnaterre, 1789)</td>
<td>B6RAK9</td>
</tr>
</tbody>
</table>

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6
Results and discussion

The predicted 3D Structures of Cytochrome-b

The model of Cytochrome-b for all three subspecies of *P. tentoria* have 20 helices, 41 helix-helix interacts, 15 beta turns, 5 gamma turns. In the sub species of *P. smithii*, Cytochrome-b have 23 helices, 43 helix-helix interacts, 14 beta turns, 6 gamma turns. *P. tecta*, *P. sylhetensis*, *Batagur dhongoka*, *B. kachuga* and *B. baska* Cytochrome-b have 24 helices, 40 helix-helix interacts, 15 beta turns, 4 gamma turns (Fig. 1 & 2). Procheck verification proved that the models are of good quality as judged by Ramachandran Plot (Fig. 3) (Ramachandran & Sasisekharan, 1968). The overall Quality factors predicted by ERRAT verification programme for the predicted 3D structures of Cytochrome-b are more than 95% (Fig. 4). After fruitful verification of the coordinate files, the structures were successfully deposited to PMDB Protein Model Database (Tiziana et al., 2006) of University of Rome and now available for download. Each 3D structure of Cytochrome-b has been assigned an unique PMDB ID for the coordinate entry.

![Figure 1. The predicted 3D structures of Cytochrome-b displayed by UCSF Chimera (A. Pangshura, B. Batagur)](image)

**Figure 1.** The predicted 3D structures of Cytochrome-b displayed by UCSF Chimera (A. Pangshura, B. Batagur)

![Figure 2. Secondary structure assignment of the predicted three-dimensional model of Cytochrome-b.](image)

**Figure 2.** Secondary structure assignment of the predicted three-dimensional model of Cytochrome-b.
Figure 3. Ramachandran analysis of the backbone dihedral angles PSI (y) and PHI (f) for the final structure of Cytochrome-b protein (from *P. syilheisenis*) Red region represents the most favored region, yellow = allowed region, light yellow = generously allowed region, white = disallowed region [ProCheck].

Overall quality factor**: 99.407

Figure 4. ERRAT verification for the overall Quality factors of the predicted 3D structures of Cytochrome-b
A. *Pangshura* (99.407), B. *Batagur* (97.329)
Evolution of Cytochrome-b among the genera Pangshura and Batagur

All tree-building methods revealed Cytochrome-b of Pangshura as perfectly supported monophylum with bootstrap or posterior probability values of more than 70% (Fig. 5 A-C). There were a total of 344 positions in the final dataset. The results are highly consistent with earlier molecular genetics study involving 12S rRNA genes (Shaffer et al., 1997) that within Batagur and Pangshura, all species correspond with well-supported clades. However, the evolutionary tree of Cytochrome-b supports the fact that P. tecta is the closest relative of P. sylhetensis, while P. tecta, P. smithii and P. tentoria are found as sister groups. Batagur dhongoka is found to be an intermediate species of the two genera that belongs to genus Batagur. Distinctness of the subspecies within P. tentoria is badly supported by the evolutionary data of Cytochrome-b protein.

The analysis involved 27 amino acid sequences. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985).

A. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (Jones et al., 1992). The tree with the highest log likelihood (-1792.5671) is shown.

B. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.35969843 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method (Zuckerkrandl and Pauling, 1965).

C. The evolutionary history was inferred using the Minimum Evolution method (Rzhetsky and Nei, 1992). The optimal tree with the sum of branch length = 0.35969843 is shown. The evolutionary distances were computed using the Poisson correction method (Zuckerkrandl and Pauling, 1965).

The equality of evolutionary rate between sequences A (A7WMB5 sylhetensis) and B (A7WMA3 kachuga), with sequence C (B6RAK9 Lissemys punctata) used as an outgroup in Tajima's relative rate test (Tajima, 1993). The χ² test statistic was 0.00 (P = 1.00000 with 1 degree[s] of freedom) There were a total of 345 positions in the final dataset. There were 277 identical sites and 11 divergent sites in all three sequences. Unique differences in sequence A and B are 7, while in sequence C is 43.

The structure Cytochrome-b can be helpful in structural biology for further investigations on allocation of amino acid residues in each fold, prediction of active sites, molecular mechanism of function and structure based phylogeny. The structures of Cytochrome-b were found to be statistically significant by the structure verification programs. Sequence search vs existing PDB entries revealed that the predicted structure has higher fold similarities with PDB IDs 1bcc (C, Cytochrome bc1 complex from chicken) (75%) and 2bcc (C, Stigmatellin-bound cytochrome bc1 complex from chicken) (75%). The present analysis corroborate that the genus Pangshura is monophyletic. The modeling of Cytochrome b of genus Pangshura gains importance for the structural biology and even to the conservation genetic research from several angles.

The present evolutionary study on Cytochrome-b protein provides a stable phylogenetic hypothesis for all Pangshura species, with the suggestion that P. smithii and P. tentoria as sister species followed by P. tecta and P. sylhetensis as their successive sister-taxa. Molecular analysis is now a standard tool in taxonomic and phylogenetic studies, focusing mostly on genes in the mitochondrial genome (Caccone et al., 1999) or DNA loci in the nuclear genome (Cao et al., 2000). The present study provides an indispensable groundwork for future molecular analyses at the protein level. The choice of molecular data is crucial for phylogenetic analyses and molecular studies can now be tailored specifically for particular phylogenetic groups and/or questions (Lamb and Lydeard, 1994).
Figure 5. A-C Evolutionary relationship of Cytochrome-b among Pangshura and Batagur
Conclusion
The present study can be used as an additional method for identification of species as well as for identification of unknown samples with unusual appearances and could be made available for the identification of confiscated specimens. The predicted 3D structures presented here can serve as a guide for the allocation of amino acid residues involved in each fold, which is important for further investigations on molecular mechanism of functions. The molecular evolutionary analysis underline that further sampling is in dire need for developing effective conservation strategies. Pangshura represent distinct genera with four well supported species. However, distinctness within the three currently recognized sub-species of P.tentoria is not clearly visible by the evolutionary analysis of Cytochrome- b protein. Much is still to be learned about how the protein can manipulate a sequence of base pairs in such a peculiar way that results in a fully functional organism.

Acknowledgements
The authors gratefully acknowledge the Department of Biotechnology, Govt. of India for financial support to establish the Bioinformatics Infrastructure Facility (BIF)

References


Introduction
The State of Assam is located between 24.3° N and 28° N and 89.5° E and 96.1°E and with an area of 78,438 km² representing 2.4 percent of the India’s landmass. The turtle fauna of Assam is diverse comprising of Pangshura sylhetensis (Jerdon, 1870) commonly known as Assam roofed turtle. It is a small turtle differing from other species of Pangshura in having 13 pairs of marginal scutes, and attaining a carapace length of 200 mm and is endemic to northeastern region of India and Bangladesh. According to IUCN (2007), it is one of the Asia’s Most Endangered Tortoises and Freshwater Turtles. The species was first reported from the Khasi hills. Study of population status, distribution pattern in an area can be used to formulate conservation strategies and taxonomic relationship among the species. This paper reports the present status and distribution of P. sylhetensis in Darrang, Sonitpur and Udalguri districts of Assam.

Analysis of zoogeography of Pangshura sp. provides information and ranges of their natural distribution and boundaries that helps to find out the endemic status of the species. Endemism constitutes an important feature while formulation of conservation strategies. Therefore distribution of the species and their habitat associations have been attempted in this study.

The study sites
The study was conducted in five sites viz. Kuruwa, Kulshi, of Darrang and Udalguri districts, Biswanath ghat, Gomirighat and Jia Bharali River in Sonitpur district of Assam, India (Table-1).

Table-1: The Survey Areas

<table>
<thead>
<tr>
<th>Survey area</th>
<th>Geographical location</th>
<th>Type of vegetation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Jia Bharali River</strong></td>
<td>26°55'20.22&quot;N 92°50'27.12&quot;E</td>
<td>Semi evergreen forest</td>
</tr>
<tr>
<td><strong>Biswanath ghat</strong></td>
<td>26°46'30.74&quot;N 93°32'04.86&quot;E</td>
<td>Evergreen and Semi evergreen forests</td>
</tr>
<tr>
<td><strong>Gomirighat</strong></td>
<td>26°44'47.93&quot;N 93°38'45.45&quot;E</td>
<td>Evergreen and Semi evergreen forests</td>
</tr>
<tr>
<td><strong>Kuruwa ghat</strong></td>
<td>26°13'32.79&quot;N 91°46'39.74&quot;E</td>
<td>Semi evergreen forest</td>
</tr>
<tr>
<td><strong>Kulshi River</strong></td>
<td>26°45'30.74&quot;N 92°02'51.38&quot;E</td>
<td>Evergreen and Semi evergreen forests</td>
</tr>
</tbody>
</table>
Methods
The study was conducted in different representative ecozones of northern part of the River Brahmaputra valley of Assam during January 2004 - December 2007. Extensive surveys in 3 districts of Assam, India were made in different seasons. Surveys were also done in Buffer Zones of Protected areas considered important by earlier workers.

The survey for *Pangshura sylhetensis* habitat was done using quadrats; transects, museum records and interviews with local people using photo sheets and questionnaires.

The study area was censused one to three times per day between 7a.m. to 5 p.m. in winter, premonsoon and postmonsoon season. Searching for nests was carried out by walking an 1.5 Km transect in 2 hrs. Local fishermen gave information about nest and eggs of *P. sylhetensis*.

During survey period most turtles were marked with red paint on the plastron and released in the same area. For identification of the species Das (1995) was followed.

Live turtles were collected and without causing any injury, measured for curved carapace length (CCL), straight carapace length (SCC) curved carapace width (CCW) and shell height (SH). Scute and bone terminology used by Zangerl (1969) was followed.

Dead specimens collected from the study sites were preserved and measured for curved carapace length (CCL), straight carapace length (SCL), curved carapace width (CCW), shell height (SH) and body weight.

Results and discussion
A total of 136 representatives of *P. sylhetensis* belonging to the family Geoemydidae have been collected from study sites and released after investigation (Plate 4A and 4B). Eight dead specimens of *P. sylhetensis* were collected from Biswanath ghat and Gomirighat.

The present field survey has identified the following important habitats for *Pangshura sylhetensis* in Darrang, Udalguri and Sonitpur districts of Assam—

**Darrang district:** Poska phukhuri near Barampur, Dighirpar phukhuri near Arjuntal, Marasuti of Mangaldoi river near Karimchowka, Beel near Shiv temple of Kuruwa ghat, Beel near Ganesh Kuwari of Dumuni chowki, Khalihoi beel, Rowmari, Chereng and Mowamari beel, Dova near areng of Darrang district.

**Udalguri district:** Dhansirighat, Mara-dhansirighat, Pond of Rawta charali, Kachubeel, beel near Orang National Park, Namkhala bazaar area of Udalguri district.

**Sonitpur district:** Nameri National Park, beel near Beseria village, near Gabharu river, Gahigaon wetland, Gohpur wetland of Sonitpur district of Assam.

These observations resulted from the catch of 136 turtles during the study period (male 81 and female 55) (Table-2). Further, 50 juvenile male's and 20 juvenile females were observed during this study. This confirms the suitability of this habitat as having a breeding populations. Habitat destruction and over exploitation have threatened the survival of this species. Indian population of *P. sylhetensis* suffered a 90% decrease in the last decade and IUCN justifiably declared this species as endangered (IUCN, 2007). But present investigation documents a healthy population of *P. sylhetensis* in Nameri National Park, Biswanath ghat, Gomirighat and Kuruwa ghat areas, probably due to the level of protection and awareness among locals.

Nests of *P. sylhetensis* were observed only in March/April at Biswanath ghat and Kuruwa ghat with 6-8 eggs per nest. During this study period, three new habitats were located and their physiochemical parameters were recorded.

Table-2: The abundance of *P. sylhetensis* in the study sites.

<table>
<thead>
<tr>
<th>Year</th>
<th>JB</th>
<th>GG</th>
<th>BG</th>
<th>KG</th>
<th>KR</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>2005</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>Shell</td>
</tr>
<tr>
<td>2006</td>
<td>14</td>
<td>8</td>
<td>10</td>
<td>6</td>
<td>Shell</td>
</tr>
<tr>
<td>2007</td>
<td>18</td>
<td>8</td>
<td>12</td>
<td>8</td>
<td>Shell</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>28</td>
<td>34</td>
<td>24</td>
<td>-</td>
</tr>
</tbody>
</table>

JB: Jia Bharali River
GG: Gomirighat
BG: Biswanath Ghat
KG: Kuruwa Ghat

(total 50 specimens: 30 M and 20 F)
(total 28 specimens: 17 M and 11 F)
(total 34 specimens: 20 M and 14 F)
(total 24 specimens: 14 M and 10 F)

Our survey shows that every year during the period from the first week of October to first week of February, a huge numbers of eggs of *P. sylhetensis* are collected by the inhabitants (particularly by children) and sold in the local market. Other reasons for the decline of the species are illegal slaughtering for meat, egg perdition, water pollution and human disturbances (Rao, 1985 and Gupta, 2000). The killing of adult female freshwater turtles and incidental catch of breeding adults has probably increased over the past few years. The nest poachers can easily identify the nests and dig out the eggs and sell these in the local markets.

Earlier, *P. sylhetensis* was recorded from Kukurmara and Chandubi beels of Kamrup district; Kalathua village of Sibsagar district; Rupali Bhumuk of Manas National park; Barpeta district, Kundil river of Sodia; Banko beel of Dibru-Saikhowa Wild Life Sanctuary; Lohit river near Saikhowaghat, Tinsukia district; Ghilamara of Lakhimpur district and Nameri National Park, Sonitpur district of Assam. Cachar district of Assam, Garo, Khashi and Naga hills.

Moll (1986) had reported *P. sylhetensis* from Cherrapunji (Khasi hills) and Garo hills of Meghalaya, Cachar dist. of Assam. Coudhury (1995) reported the new locality of *P. sylhetensis* in Sadiya, subdivision of Tinsukia district, Kalathua village of Sibsagar district, Assam. Choudhury et al. (1997) described the distribution and diet of *P. sylhetensis* in Assam. They conducted a survey between March 1995 to August 1996 in Kamrup district, Assam, north eastern India to ascertain the distribution of the species. They collected *P. sylhetensis* from Kukurmara river and Chandubi beel located in the south western part of Kamrup district. Praschag and Fachbach (2001) reported *P. sylhetensis* from Nameri National Park of Assam, India. This species has been reported from various locations from the Northeastern region in the past (Tikadar and Sharma 1985, Choudhury 1995, Choudhury et al. 1997).

Conclusion
The survey shows that vast areas of their original habitats have been lost. Amongst all the study sites surveyed, Jia Bharali seems to be an ideal home for *P. sylhetensis*. Other reasons for the decline of the species are illegal slaughtering for meat, poaching of egg, water pollution and by-catch. Killing of adult freshwater turtles and incidental catch of breeding adults has intensified over the past few years. Hence, there is an urgent need to sensitize and educate the people about the significance of chelonians and their conservation.

Acknowledgements
We thankfully acknowledge the Forest Officials, Government of Assam for the accord of necessary permission and help during the field work. We are also thankful to Rufford Small Grants Foundation, U.K and UGC, NERO, Guwahati for financial assistance.
References


Plate 4

(A): Major rivers of Assam along with the study sites (red colored) and (B): Map of Assam along with collection sites (red colored)
Checklist of turtle fauna so far recorded from northeast India

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Received: 15 February 2009; Revised and Accepted: 20 October 2009

ABSTRACT

The paper reports 23 turtle species so far recorded from Northeast region (NER) of India, belonging to 3 families namely Geoemydidae (15 species), Trionychidae (6 species) and Testudinidae (2 species). The freshwater turtle population is declining due to wanton killing and destruction of their natural habitat. Out of 23 species found in NER, 6 species namely Pangshura sylhetensis, Pyxidea mouhotii, Nilssonia nigricans, Chitra indica, Manouria emys and Indotestudo elongata are listed by IUCN as the most endangered species of Asia. Chitra indica is on the new list of the 25 most endangered turtle species of the world.

KEY WORDS: Checklist, conservation, N.E. India, Tortoises, Turtles.

The northeastern region of India covering an area of 2, 62,179 km$^2$ (7.6% total area of the country) is a biologically highly diverse area and one of the most important biodiversity hot spots of the world. The region is at the conjunction of the Himalaya and Indo-Burma biodiversity hotspots. It is regarded as one of the major centre of turtle diversity (Das, 1990). However, the region has been poorly explored scientifically with regard to surveys, conservation and monitoring of turtle fauna. The turtle and tortoise diversity of northeast India is the highest in the country and 23 of 29 species are found in this region (Das, 1995; Ernst et al., 2000; Das, 2001; Praschag & Gemel, 2002). However, the lack of recent field information has created a gap in conservation and management of turtle species in the region. The present paper reports a checklist of turtles and tortoises from the northeastern states which are recorded in the field work during the last four years.

Materials and methods

Field surveys were carried out by the Centre for Wildlife Research and Conservation Action (CWRCA), Department of Zoology, Gauhati University, Assam during January 2005-December 2008 using three different methods including interviews of peoples using photo sheets of turtles, trappings using nets with the help of fishermen and visual encounter surveys (VES). Fishermen, traders, collectors and field workers of respective states were interviewed to learn about their distribution, habitat and extent of exploitation. Literature searches also carried out for the detailed information. During the survey, both living specimens and any signs of their presence including dead specimens were noted. For identification of the species, Das (1995) was followed. The following measurements were taken using the dial vernier callipers: straight line carapace length (CL), carapace width (CW), plastron length (PL) and shell height (SH). Subsequently the live specimens were released in their habitat.

Observations

The recorded chelonians were photographed and the sex of the specimens were attempted to identify though it is difficult morphologically. However, the bigger sizes of the soft-shell turtle were female in Nilssonia nigricans, Nilssonia hurum, Chitra indica, Batagur kachuga, Pangshura sylhetensis. It has been observed that generally the females are being caught and killed due to their size and the small sized males are
released into the water, thereby sex ratio imbalance has appeared as major threat. Moreover, the hunting of the animals and destruction of eggs (Figure 1) are the major threats for this little known aquatic creature.

![Figure 1. Freshwater turtle eggs rescued from poachers in Assam (Photo by Chittaranjan Baruah)](image-url)

**Recommendations**

Lack of recent field information has created a gap in conservation activities and status evaluation of almost all turtle species. Immediate adoption of conservation measures is essential for the conservation of endangered turtle genetic resource available in this region.

As a part of the conservation of genetic diversity, the following studies are proposed:

(i) survey of the different turtle species, (ii) reproductive cycle of endangered species, (iii) study on allozyme variation and molecular phylogeny to analyze the polymorphism and evolutionary history, (iv) breeding in captivity, and (v) generate awareness against killing and need of conservation through community participation.

**Conclusion**

Habitat loss and over exploitation for meat is considered persistent threat to chelonians of the region (Das, 1995; Choudhury, 1995). Gupta and Guha (2002) reported tradition and conservation of turtle in NE India. Gupta (2000) reported certain areas rich in freshwater chelonian diversity of Northeastern India including Assam. Out of 23 species found in northeast India, 6 species namely Pangshura sylhetensis, Pyxidea mouhotii, Nilssonia nigricans, Chitra indica, Manouria emys and Indotestudo elongata are listed by IUCN (2007) as most endangered species of Asia. The soft-shell turtle *Chitra indica* needs special attention as it is on the new list of the 25 most endangered turtle species of the world. As a part of the conservation of turtle diversity, we emphasize on some important research and conservation initiatives.

**Acknowledgements**

The authors are thankful to Dr. Kartik Shanker, Centre for Ecological Sciences, IISc, Bangalore; Dr. Shailendra Singh, Scientist, IUCN-TSA/ Madras Crocodile Bank Trust and Dr. Peter Praschag, Am Katzelbach 98; 84 Graz, Austria for assisting the turtle research in the northeast India.

**References**


Praschag, P. and Gemel, R. 2002. Identify of Black soft-shell turtle *Aspideretes nigricans* (Anderson, 1875) with remarks on related species (Reptilia: Testu-
Table 1. A consolidated list of chelonians found in different states of the region, based on field survey and referred literatures, have been listed in the following table.

<table>
<thead>
<tr>
<th>Family</th>
<th>Common Name</th>
<th>Taxon</th>
<th>AP</th>
<th>AS</th>
<th>NL</th>
<th>MN</th>
<th>MZ</th>
<th>ML</th>
<th>TR</th>
<th>IUCN Status</th>
</tr>
</thead>
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<tr>
<td>Geoemydidae</td>
<td>Assam roofed turtle</td>
<td>Pangshura sylhetensis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>EN</td>
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<tr>
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<td>Pangshura tecta</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>LR/LC</td>
</tr>
<tr>
<td>Geoemydidae</td>
<td>Indian tent turtle</td>
<td>Pangshura tentori</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>LR/LC</td>
</tr>
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<td>Geoemydidae</td>
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<td>Pangshura smithi</td>
<td>+</td>
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<td>-</td>
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<td>LR/NT</td>
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<td>Keeled box turtle</td>
<td>Psyses souhota</td>
<td>+</td>
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<td>+</td>
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<tr>
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<td>Tricarinate hill turtle</td>
<td>Melanochelys tricarina</td>
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<td>Melanochelys truga</td>
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<td>LR/NT</td>
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<td>Geoemydidae</td>
<td>Indian eyed turtle</td>
<td>Morena petersi</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>VU</td>
</tr>
<tr>
<td>Geoemydidae</td>
<td>Asian giant softshell turtle</td>
<td>Pelochelys bibron</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
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<td>Geoemydidae</td>
<td>Red-crowned roofed turtle</td>
<td>Batagur cachuga</td>
<td>?</td>
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<td>-</td>
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<td></td>
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<td>Geoemydidae</td>
<td>Three-striped roof turtle</td>
<td>Batagur dhongoka</td>
<td>+, ?</td>
<td>+</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td></td>
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</tr>
<tr>
<td>Geoemydidae</td>
<td>Crowned river turtle</td>
<td>Hardella thuris</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>VU</td>
</tr>
<tr>
<td>Geoemydidae</td>
<td>spotted pond turtle</td>
<td>Geoemydina hamiltoni</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td></td>
<td></td>
<td>VU</td>
</tr>
<tr>
<td>Geoemydidae</td>
<td>Asian leaf turtle</td>
<td>Cyclemys gemeli</td>
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<td>-</td>
<td>-</td>
<td>+</td>
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<td>Geoemydidae</td>
<td>South Asian box turtle</td>
<td>Cuora ambozensis</td>
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<td></td>
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<td>VU</td>
</tr>
<tr>
<td>Trionychidae</td>
<td>Black soft-shell turtle</td>
<td>Nilssoma nigricans</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>VU</td>
</tr>
<tr>
<td>Trionychidae</td>
<td>Indian peacock soft-shell turtle</td>
<td>Nilssoma hurum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>VU</td>
</tr>
<tr>
<td>Trionychidae</td>
<td>Gangetic soft-shell turtle</td>
<td>Nilssoma gangesicus</td>
<td>+</td>
<td>+</td>
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<td>VU</td>
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<tr>
<td>Trionychidae</td>
<td>Narrow-headed softshell turtle</td>
<td>Chitra indica</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Trionychidae</td>
<td>Indian flap-shelled turtle</td>
<td>Lissimus punctata</td>
<td>+</td>
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<td>-</td>
<td></td>
<td></td>
<td></td>
<td>LR/LC</td>
</tr>
<tr>
<td>Trionychidae</td>
<td>Asian soft-shell turtle</td>
<td>Amyda cartilaginea</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>VU</td>
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<tr>
<td>Testudinidae</td>
<td>Elongated tortoise</td>
<td>Indotestudo elongata</td>
<td>+</td>
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<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td>VU</td>
</tr>
<tr>
<td>Testudinidae</td>
<td>Asian brown tortoise</td>
<td>Manouria emys</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>EN</td>
</tr>
</tbody>
</table>

AP = Arunachal Pradesh; AS = Assam; NL = Nagaland; MN = Manipur; MZ = Mizoram; ML = Meghalaya; TR = Tripura.
EX = Extinct; EW = Extinct in the Wild; CR = Critically Endangered; EN = Endangered; VU = Vulnerable; LR/nt = Lower Risk/Near Threatened; LR/nc = Lower Risk/Least Concern; LR/cd = Lower Risk/Conservation Dependent; DD = Data Deficient
Figure 2. Photographs of turtle fauna found in Northeast India. **A. Geoemydidae:** (a) Pangshura tecta, (b) P. sylhetensis (Photo: Peter Praschag), (c) P. smithii smithii, (d) P. tentoria, (e) P. tentoria flaviventer, (f) Hardella thurjii, (g) Melanochelys tricarina, (h) Cuora amboinensis, (i) Cyclemys daniata. **B. Trionychidae:** (j) Amyda cartilaginea, (k) Aspideretes gangeticus, (l) Nilssonia nigricans, (m) Aspideretes hurum, (n) Chitra indica, (o) Lissemys punctata. **C. Testudinidae:** (p) Manouria emys emys (Photo: Arindam Pachani), (q) Indutestudo elongate (Photo by Ashok Kr. Mallik).
Status and conservation of Assam roofed turtle *Pangshura sylhetensis* in the Brahmaputra floodplain, Assam, India

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ABSTRACT

This paper reports the present status and ongoing efforts aimed at conservation of the Assam roofed turtle *P. sylhetensis* along with other freshwater turtles in the floodplain wetlands and rivers of Assam. Efforts have been made to educate people about the significance of chelonian species and its conservation and to start a participatory conservation programme on endangered turtle. An *in situ* hatchery was set up in a riverine island in the river Brahmaputra, Assam. Attempt was made to protect a few clutches of eggs in 50 wild nests of which 21 (109 eggs) for *P. sylhetensis*, 21 nests (178 eggs) for *P. tentoria*, 2 nests (24 eggs) for *Nilssonia nigricans* and 1 nest (10 eggs) for *Nilssonia gangetica*. Additionally, more than 300 rescued eggs from mixed species (*P. sylhetensis*, *P. smithii*, *P. tecta* and *P. tentoria*) were also translocated to the other end of the same hatchery. However, success of 50% hatchlings for *P. sylhetensis*, 10% for *N. nigricans* and 8% for *N. gangetica* were recorded. But the eggs rescued from the poacher could not produce a single hatchling, might be due to wrong orientation. This model experiment has evoked an excellent response amongst the community and may be useful for conservation.

KEY WORDS: Abundance, conservation, Geoemydidae, zoogeography

Northeast India lies at the convergence of the Himalaya and Indo-Myanmar biodiversity hotspots (Shrestha, 2001). Regarding the testudine fauna of northeast India, few scientific surveys or conservation initiatives have been undertaken to date. The region however, has recently been recognized as a tortoise and freshwater turtle priority conservation area (Bhulmann *et al.* 2009). The islands (Chars) of the river Brahmaputra in Assam provide important habitats (e.g nesting and basking sites) for many species of freshwater turtles, including the Assam roofed turtle *Pangshura sylhetensis* (Jerdon, 1870). This species is restricted to the northeast Indian states of Assam, Meghalaya, Arunachal Pradesh, Manipur, Nagaland, West Bengal and Bangladesh. Recently it has been reported from the adjacent Bhutan (Ahmed *et al.* 2009). It is considered ‘endangered’ (IUCN, 2010), listed as a CITES Appendix II species, and within India receives protection under the Indian Wildlife (Protection) Act, 1972: Schedule I (highest protection category).

It is a small (carapace length up to 20 cm), omnivorous and mostly nocturnal species, inhabiting slow flowing floodplain rivers, streams with woody areas in lowland foothills, forest and oxbow lakes, typically with stands of reed grasses and aquatic macrophytes, as it basks communally on logs on water (Choudhury *et al.* 1997; Ahmed *et al.* 2009). Analysis of zoogeography of *Pangshura sp.* provides information and ranges of their natural distribution and boundaries that helps to find out the endemic status of the species. Study of population status, distribution pattern in an area can be used to formulate conservation strategies and taxonomic relationship among the species. This paper reports
the present status of *P. sylhetensis* in Darrang, Sonitpur and Udalguri districts of Assam. Several conservation initiatives undertaken in Assam for the conservation of Assam roofed turtle in particular, and other turtles and tortoises in general. These include field surveys to evaluate the status and threats to Assam roofed turtle population, instigation of an *in situ* egg protection scheme, and community awareness and participatory programme to educate local people about the need of conservation and sustainability.

**Materials and methods**

**Status, distribution and threat assessment**

Field surveys were carried out from 2006 to 2009 in five forested riverine areas of Assam (Table 1, Fig.1). Surveys were undertaken by TCRP team members following the methods of quadrate sampling; transect methods, museum survey with the help of the local guides. Poachers, traders and collectors were interviewed to learn more about the distribution and habitats and conservation status of the turtle population.

**Conservation actions**

The Assam roofed turtle conservation programme has been initiated in Assam by the ‘Turtle Conservation and Research Program’ (TCRP), a volunteer network, based at Guwahati, Assam. Conservation activities have been initiated, as outlined in the ‘Conservation Action Plan for Endangered Turtles and Tortoises of India’ (CFH/MCBT 2006).

**Table 1.** The study area for *Pangshura sylhetensis* in Assam, 2006-2009.

<table>
<thead>
<tr>
<th>Survey area</th>
<th>Longitude</th>
<th>Latitude</th>
<th>Forest type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jia Bharali River</td>
<td>26°35'20.22&quot;N</td>
<td>92°50'27.12&quot;E</td>
<td>Semi evergreen forest</td>
</tr>
<tr>
<td>(Nameri National Park)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biswanath Ghat</td>
<td>26°46'30.74&quot;N</td>
<td>93°32'04.86&quot;E</td>
<td>Evergreen and semi-evergreen forests</td>
</tr>
<tr>
<td>(Kaziranga National Park)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gomringhat</td>
<td>26°44'47.93&quot;N</td>
<td>93°38'45.45&quot;E</td>
<td>Evergreen and semi-evergreen forests</td>
</tr>
<tr>
<td>Kurwa Ghat</td>
<td>26°13'32.79&quot;N</td>
<td>91°46'39.74&quot;E</td>
<td>Semi evergreen forests</td>
</tr>
<tr>
<td>Kulshi River</td>
<td>26°45'30.74&quot;N</td>
<td>92°02'51.38&quot;E</td>
<td>Evergreen and semi-evergreen forests</td>
</tr>
</tbody>
</table>

Figure 1. Map showing the tributaries of the river Brahmaputra in Assam along with the study sites (red colour)
A series of group discussions were carried including local representatives of riparian village communities and their views have been taken into consideration in preparing and implementing the future conservation strategies. Primary objectives are: 1) to undertake field surveys to assess status, distribution and threats to turtle populations; 2) to undertake education programmes to raise awareness amongst local communities about the plight of river turtle population; 3) to encourage community participation in conservation projects, and 4) to develop *in situ* egg protection schemes.

Community awareness: Between April 2006 to May 2010, a total of 11 awareness campaigns (both oral and poster/brochure presentations) have been carried in riparian village communities, including local schools, across Assam. We have also helped to organize and celebrate: i) Endangered Species Day; ii) World Biodiversity Day; and iii) World Turtle Day in different parts of Assam to educate the community in turtle conservation. Several events were organized to involve all the age groups, making them excited about the role of turtles in our environment.

*In situ* egg protection programme: Various sections of the river Brahmaputra were surveyed during April 2006 to December 2009, to identify locations with evidence of nesting turtles (e.g. tracks in silt or sand, nests, predated eggs, and presence of turtles in potential breeding areas). In such localities, local people are being encouraged to protect turtles and their nesting habitats. An *in situ* egg protection programme has been initiated with the participation of local communities.

An island (Char) in the river Brahmaputra within the district Morigaon, Assam was selected for hatchery due its habitat suitability. In October 2009, using old fishing nets and a thorn brush barrier (a defense against jackals, the primary threat to turtle nests in this area), 250 square meters were enclosed by nylon nets with stiff support to create an *in situ* hatchery for enhancing hatching success. The nests were dug at a depth of 24 cm and distance was maintained at 100 cm apart. The hatchery had 10 natural nests of *P. sylhetensis* and received 11 translocated nests of *P. sylhetensis*. A total of 50 nests were protected of which 21 nests (109 eggs) from *P. sylhetensis*, 21 nests (178 eggs) for *P. tentoria*, 2 nests (24 eggs) for *Nilssonia nigricans* and 1 nest (10 eggs) for *Nilssonia gangetica*. Furthermore, more than 300 rescued eggs belonging to *P. sylhetensis*, *P. smithii*, *P. tecta* and *P. tentoria* were translocated between November and January of the year and planted in other end at a depth of 21 cm. The soil temperature was recorded at 28 °C. The relative humidity was at 78-82 % throughout the incubation period. The nests were distanced at 100 cm from one another. Plastic boards displaying clutch number, size and date of collection of each nest were fixed for identification.

**Results and discussion**

**Number of *P. sylhetensis* observed:** A total of 136 *P. sylhetensis* was recorded during the study period (male -81 and female -55) and the habitat parameters were found to supports the suitability of the plains of the Brahmaputra valley. Further, the count of 50 male juvenile and 20 female juvenile during this study period (2006-2009) confirms the suitability of this region as one of the proper breeding ground. Nests of *P. sylhetensis* were observed only during October-February at Biswanath Ghat (Table-1) and Kuruwa Ghat (Table-2) with 6-8 numbers of eggs per nest (Table 2).

**Status, distribution and threat assessment:**
The five major threats to Assam roofed turtle are as follows:-

i) Habitat destruction and pollution - forests and wetlands are declining in area. Deforestation and resultant soil erosion has led to increased siltation of lakes and other wetlands. The deep pools that are the favoured habitats are rapidly becoming shallow and choked with silt, leading to a decline in habitat quality.

ii) Exploitation for food - Indigenous inhabitants consume turtle meat and eggs. The study found that every year from the first week of October to the first week of February, a huge number of turtle eggs are collected by the inhabitants of the Brahmaputra *chars* (Riverine islands) and sold in local markets. The killing of adult female freshwater turtles and incidental catch of adults has created particular pressure on *P. sylhetensis* population (Gupta, 2000).
Table 2. Observations of *P. sylhetensis* in the study sites.

<table>
<thead>
<tr>
<th>Year</th>
<th>Jia Bharali River</th>
<th>Gomirighat</th>
<th>Biswanath Ghat</th>
<th>Kuruwa Ghat</th>
<th>Kulshi River</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>2007</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>6 Shell</td>
<td>Shell</td>
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<tr>
<td>2008</td>
<td>14</td>
<td>8</td>
<td>10</td>
<td>6 Shell</td>
<td>Shell</td>
</tr>
<tr>
<td>2009</td>
<td>18</td>
<td>8</td>
<td>12</td>
<td>8 Shell</td>
<td>Shell</td>
</tr>
<tr>
<td>Total</td>
<td>50 (30 male, 20 female)</td>
<td>28 (17 male, 11 female)</td>
<td>34 (20 male, 14 female)</td>
<td>24 (14 male, 10 female)</td>
<td>-</td>
</tr>
</tbody>
</table>

iii) Pet trade - a heavy illegal pet trade is contributing to declines.

iv) Superstitious beliefs - Hanging a carapace in a cattle-shed is believed to bring good as well as luck, and to keep snakes and burglars away from the premises. We are in the process of trying to dissuade the local people from continuing their customs related to the killing of turtles.

v) Use as ethno-medicine - Both the flesh and eggs of turtles are believed to be a remedy for gout and arthritis, while the carapace of Assam roofed turtle and other turtle species is also used as 'medicine' for other ailments including asthmas. Due to decline of catch, traders are offering increasingly high (very lucrative) prices to tribal hunters and fishermen for carapaces.

The present field survey has identified following potential habitats of *Pangshura sylhetensis* in Darrang, Udalguri and Sonitpur districts of Assam:

**Darrang district:**
- Poska phukhuri near Barampur, Dighirpar phukhuri near Arjantal, Marasuti of Mangaldoi river near Karimchowka, Beel near Shiv temple of Kuruwa ghat, Beel near Ganesh Kuwari of Dumuni chowki, Khalilboi beel, Rowmari, Chereng & Mowamari beel, Dova near areng of Darrang district.

**Udalguri district:**
- Dhansirighat, Mara-dhansirighat, Pond of Rawta charali, Kachubeel, beel near Orang National Park, Namkhalu bazaar area of Udalguri district.

**Sonitpur district:**
- Nameri National Park, beel near Beseria village, near Gahbaru river, Gahigaoa wetland, Gohpur wetland of Sonitpur district of Assam.

*P. sylhetensis* is now found only in a few protected areas, including the Kaziranga, Nagaon and Nameri National Parks in the Brahmaputra River basin. The year 2009 survey records demonstrated increased numbers of *P. sylhetensis* in the Jia Bharali River (Nameri National Park), Biswanath Ghat, Gomirighat and Kuruwa Ghat areas, probably due to habitat conservation as protected area and awareness campaign. Moll (1986) had reported *Pangshura sylhetensis* from Cherrapunji (Khasi hills) and Garo hills of Meghalaya, Cachar district of Assam. Coudhury (1995) reported the new locality of *Pangshura sylhetensis* in Sadiya, subdivision of Tinsukia district, Kolathua village of Sibsagar district, Assam. Choudhury et al. (1997) described the distribution and diet of *Pangshura sylhetensis* in Assam. They conducted a survey between March 1995 to August 1996 in Kamrup district, Assam, north eastern India to ascertain the distribution and biology of the species. They collected *P. sylhetensis* from Kukurumara river and Chandubi beel located in the south western part of Kamrup district. Praschag and Fachbach (2001) reported *Pangshura sylhetensis* from Nameri National Park of Assam, India. Availability of the species in various location of the Northeastern region has been reported (Tikadar and Sharma 1985, Chaudhury 1995, Choudhury et al. 1997). The population of *Pangshura sylhetensis* suffered a 90% decrease in the last decade and IUCN justifiably placed this species as endangered (IUCN, 2007; IUCN, 2010). But present investigation shows increased in numbers of *Pangshura sylhetensis* in Nameri National Park, Biswanath ghat, Gomirighat and Kuruwa ghat areas, probably due to habitat conservation as protected area and awareness campaign.

**Community participation program**

Awareness raising and capacity building programs are initiated among the riparian community in and around the *in situ* egg-protection sites. The acceptable suggestions of the local people are being taken into consideration for egg protection and conservation network development (Baruah and Sharma, 2010). Over 3,000 people from 21 villages attended the 11 awareness campaigns, and 12 local youths have been introduced to field techniques. Three former poachers have been employed as field
assistants in the Indian turtle conservation project. Oral and poster/brochure presentations continue to be conducted among local communities.

i) Endangered Species Day celebration - over 350 persons attended in various events organized as a part of conservation sensitization. The celebration was helpful in community awareness and capacity building for safeguarding the turtle species. This celebration helped to plan for a series of follow-up meetings, in an effort to save turtle diversity of Deepar Beel Ramsar site.

ii) World Biodiversity Day - over 300 persons attended the various events held in 2009 and more than 350 participants including different stakeholders attended in the 2010 celebration. The celebration of the World Biodiversity Day helped to generate dialogue among the local community leaders, the district administration, local researchers, and the forest department as well as with the regional conservation organizations, in an effort to build strong partnerships and networks for a wider turtle conservation program in the region.

iii) World Turtle Day celebration - In this programme, there were more than 450 people including local people of Hajo, distinguish speakers, school children and some women. Protection and conservation was the common motto of all the participants. The programme drew to a close with a Turtle painting competition amongst the school students. Emphasis was also given on the food scarcity for turtles in the temple pond due to overcrowding and concrete construction of the bank. Analysis of physicochemical parameters inferred that it is one of the best breeding ground for its increase, since the water and the soil quality are at optimum level for breeding.

In situ egg protection program
The hatching successes of 50% hatchlings for P. sylhetensis 10% for N.nigricans and 8% for N gangetica were recorded. The number of success was 34 out of the total 50 nests But the eggs rescued from the poacher could not produce a single hatchling might be due to wrong orientation. P. sylhetensis began to hatch at the end part of April or first part of May of the year. The hatchlings were measured and selectively photographed, and released in to the Brahmaputra River, near the hatchery area. Releases were made early in the morning (between 6 and 7 am) or late evenings (between 5 and 6 pm), mainly to reduce heat stress and the risk of depredation. Transformation of some of the eggs was performed for N. nigricans eggs in a secondary hatchery on the bank.

Ongoing conservation activities
Field surveys are being continued in northeast India. A study has been conducted on the status and distribution of P. sylhetensis in the wetlands and rivers of Darrang, Sonitpur and Udalguri districts of Assam. Public awareness campaigns are being continued among the riparian communities in different parts of Assam. We have also created dialogue with regional conservation organizations as well as local researchers, in an effort to build strong partnerships and networks for a wider turtle conservation programme in the region. Training on various aspects of turtle biology and conservation viz survey techniques, egg collection, and hatchery management has been given to several students volunteers.

A software has been developed new computer software to enable a better understanding on the status, distribution patterns and taxonomy of turtle species of north-east India (Baruah & Sharma 2009). It will also help to fulfill the need for an effective identification system to assist in protection enforcement. A poster and brochure are being designed to educate communities in their local language.

Conclusion
Surveys indicate that vast areas of riverine habitats are being degraded or lost in all of the study sites. As well as habitat loss, over-exploitation for food and an illegal pet trade are contributing to the rapid decline in freshwater turtle populations in Assam. Immediate adoption of conservation measures is essential for the survival of many turtle species in Assam and also elsewhere in northeast India. A very important conservation component is to educate local people about the decline of tortoise and freshwater turtle population. As a part of the conservation measures we emphasize the need of the participatory conservation initiatives with further research to find alternative means of supporting livelihoods of the many impoverished rural communities in the region. Future planned initiatives
include further surveys and ecological research along the rivers with potential turtle habitat in northeast India, establishment of captive breeding centres and release of hatchlings into the wild and wider management and protection of nest sites.

Acknowledgments
Our work was technically supported by the Turtle Survival Alliance - India program. We gratefully acknowledge Rufford Small Grant's Foundation, Mohamed bin Zayed Species Conservation Fund and the Conservation Leadership Programme (CLP) for financial support on the endangered turtle conservation program in Northeast India. We thank Shailendra Singh and Kartik Shanker for supporting chelonian research in India. Special thanks go to Peter Praschag, Indranil Das and Saibal Sengupta for their encouragement.

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