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Phylogeography of olive ridley turtles (*Lepidochelys olivacea*) on the east coast of India: implications for conservation theory

K. SHANKER,*‡ J. RAMADEVI,† B. C. CHOUDHURY,* L. SINGH† & R. K. AGGARWAL†
**Wildlife Institute of India, Dehradun, India*, †*Centre for Cellular and Molecular Biology, Hyderabad, India*

Abstract

Orissa, on the east coast of India, is one of the three mass nesting sites in the world for olive ridley turtles (*Lepidochelys olivacea*). This population is currently under threat as a result of fishery-related mortality; more than 100 000 olive ridleys have been counted dead in the last 10 years in Orissa. In general, the globally distributed olive ridley turtle has received significantly less conservation attention than its congener, the Kemp's ridley turtle (*L. kempi*), because the latter is recognized as a distinct species consisting of a single endangered population. Our study of mitochondrial DNA haplotypes suggests that the ridley population on the east coast of India is panmictic, but distinct from all other populations including Sri Lanka. About 96% of the Indian population consisted of a distinct 'K' clade with haplotypes not found in any other population. Nested clade analysis and conventional analysis both supported range expansions and/or long-distance colonization from the Indian Ocean clades to other oceanic basins, which suggested that these are the ancestral source for contemporary global populations of olive ridley turtles. These data support the distinctiveness of the Indian Ocean ridleys, suggesting that conservation prioritization should be based on appropriate data and not solely on species designations.

Keywords: ancestral source population, conservation, indel, mitochondrial DNA haplotypes, olive ridley turtle, phylogeography

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Introduction

Sea turtles, once widely distributed and populous, have been globally affected by a variety of direct and indirect threats; many local populations have already been extirpated and several have declined drastically (Limpus 1995; Pritchard 1997). Olive ridley turtles are considered to be the world's most abundant sea turtles, largely because of the presence of a few exceptionally large aggregations in Pacific Mexico and Costa Rica and on the east coast of India (Pritchard 1997; Pandav *et al*. 1998). In contrast, the closely related Kemp's ridley turtle (*Lepidochelys kempi*) is composed of a single population, largely restricted to the Gulf of Mexico (Marquez 1994; Pritchard 1997). The decline of Kemp's ridleys from over 40 000 in the 1940s to a few hundred in the 1980s led to a major rescue effort, involving millions of dollars (Woody 1986). Despite the collapse and decline of many olive ridley populations (Limpus 1995) the focus of conservation attention has remained on Kemp's ridleys on the basis that they are a separate species.

In recent years, molecular genetic techniques have been used to infer taxonomic distinctiveness and to make conservation decisions (Avise 1989). For marine turtles, genetic data has been used both to justify species level classifications, as in the case of ridleys (Bowen *et al*. 1991; Dutton *et al*. 1996), as well as to argue against them in the case of green turtles (*Chelonia mydas*) and East Pacific green turtles, also known as black turtles (*Chelonia agassizi*) (Dutton *et al*. 1996; Karl & Bowen 1999). Following decades of debate on the taxonomic status of the ridleys, molecular

Correspondence: R. K. Aggarwal. Fax: + 91 40 27160591/27160311; E-mail: rameshka@ccmb.res.in
‡Present address: K. Shanker, Ashoka Trust for Research in Ecology & the Environment, Bangalore, India.
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data were used to argue for the distinctiveness of the Kemp's ridley as a separate species (Bowen *et al*. 1991, 1993; Dutton *et al*. 1996). The phylogeography of ridley turtles has also been much debated and the hypothesis of vicariant separation of the two species by the formation of the Isthmus of Panama, and a recent colonization of the Atlantic via the Cape of Good Hope (Pritchard 1967) has gained support from molecular data (Bowen *et al*. 1998).

Ridley turtles are particularly known for their synchronous mass nesting or 'arribadas'. Though olive ridley turtles are still widely distributed and abundant, a large proportion of the population breeds synchronously at only a few rookeries (Pritchard 1997). Olive ridley turtles nest sporadically throughout the Indian Ocean, but major nesting sites are along the east coast of India (Kar & Bhaskar 1982). Since its discovery in 1974, over 100 000 turtles have been reported to nest during arribadas at Gahirmatha in Orissa, while tens of thousands nest at Devi River mouth and Rushikulya (see Shanker *et al*. 2004; for a review). However, this Indian population currently suffers severe fishery-related mortality, with over 100 000 dead turtles recorded along the Orissa coast over the last 10 years (Pandav 2001; B. Mohanty, personal communication).

In this context, we undertook the first genetic study of olive ridley turtles on the east coast of India. Here, we examine the relationship of Indian olive ridley turtles to global populations of ridleys to understand their phylogeography and taxonomic status and to assist in determining conservation priorities.

Materials and methods

Study area

Olive ridley turtles nest sporadically along the entire east coast of India, but many beaches are affected by urbanization and development, and a large proportion of nests are depredated by feral animals (Kar & Bhaskar 1982). Orissa, on the east coast of India, has a coastline of 480 km, which is largely sandy and suitable for nesting, apart from the Balasore coast north of Gahirmatha that is shallow and muddy (Fig. 1). Gahirmatha (21° N, 87° E) is the northernmost of the arribada beaches, and is part of the Bhitarkanika Wildlife sanctuary, at the mouth of the rivers Brahmani and Baitarani. Nesting currently occurs on islands, which are fragments of a spit that broke away from the mainland

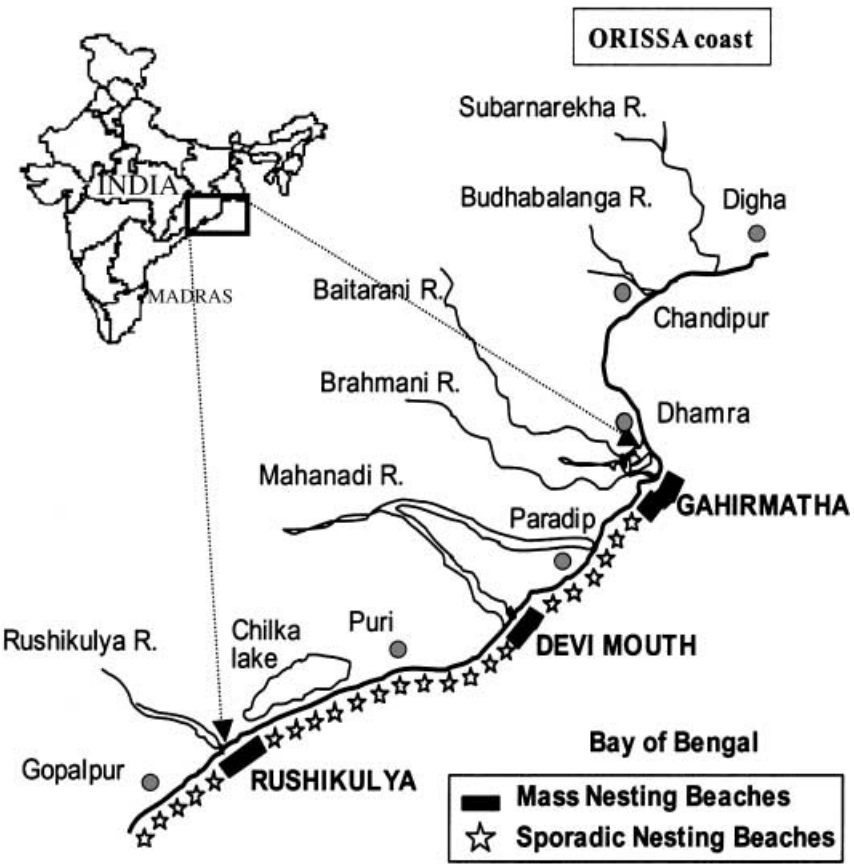

Fig. 1 A map of India showing the principal nesting sites along the east coast. Sporadic nesting of olive ridley sea turtles occurs along both east and west coasts, while the three mass nesting sites are in Orissa. Gahirmatha, the northernmost site, has recorded the largest mass nesting, with > 100 000 nesting turtles (Pandav *et al*. 1998). Figure not to scale.

in 1989 (Pandav 2001). Devi River mouth (20° N, 86° E) is located north of Puri, and this rookery was discovered in 1981 (Kar & Bhaskar 1982). Nesting at Devi River mouth occurs on the mainland as well as on sand bars that are highly dynamic and change from year to year. Rushikulya (19° N, 85° E), located 320 km south of Gahirmatha, is the southernmost of the arribada rookeries; nesting occurs along a stretch of 4 km immediately north of the Rushikulya River mouth (Pandav *et al*. 1998). The natural beach vegetation on the sand dunes includes psammophytes such as *Ipomea pescaprae*, *Spinifex littoreus*, *Gisekia phranacoides* and *Hydrophylax maritima*. Besides this, mangroves occur near the Gahirmatha and Devi rookeries. However, the coast is dominated by extensive *Casuarina* plantations, which are detrimental to nesting habitats (Pandav *et al*. 1998). Olive ridley turtles also nest along the east coast south of Orissa, totalling a few thousand nests per year. Madras, in northern Tamil Nadu, is about 1000 km south of the Rushikulya, and about 500 km north of the northern tip of Sri Lanka.

Sampling

Olive ridley turtles were sampled between February and April 1999, at all three mass nesting sites in Orissa — Gahirmatha, Devi River Mouth and Rushikulya — and at Madras, ~2000 km south of Orissa. Samples were collected from nesting females at all sites in Orissa, and from mating pairs (eight pairs), which were captured at sea off the Gahirmatha coast using a locally designed net. At Madras, samples were collected from hatchlings at a local turtle hatchery. Clutches were sampled within 2 weeks to avoid re-sampling nesting turtles, and only one hatchling per nest was used in the analysis. Muscle and skin samples were collected from the shoulder of adults and from dead hatchlings and stored in 90% ethanol.

DNA extraction, PCR amplification and sequencing

Genomic DNA was isolated by homogenization of tissue samples in lysis buffer (50 mM Tris–HCl pH 8.0, 10 mM ethylene diamine tetraacetic acid pH 8.0, 100 mM NaCl, 2% sodium dodecyl sulphate), followed by overnight incubation at room temperature in the presence of proteinase-K (150 μg/mL homogenate), deproteinization with 5 M sodium perchlorate, extraction twice with chloroform–isoamyl alcohol (24 : 1), and precipitation with 2 volumes of chilled ethanol. After DNA extraction, an approximately 400-base-pair sequence of the mitochondrial control region was amplified using the turtle-specific primers HDCM-1 (Allard *et al*. 1994) and TCR-5 (Norman *et al*. 1994) for 81 samples. The target mitochondrial sequences were amplified by polymerase chain reaction (PCR) using approximately 50 ng of template genomic DNA in 15–20 μL reaction volume containing: 5 pmol of each primer, 150 μM dNTP, 1.5 mM $MgCl_2$, 0.1 M KCl, 20 mM Tris–HCl, and 0.5–1.0 U of Ampli*Taq* Gold polymerase (Perkin Elmer). The PCR profile comprised an initial denaturation of 10 min at 95 °C (to activate the Ampli*Taq* gold polymerase), followed by 35 cycles of: 94 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min; and 72 °C for 5 min. After an agarose check gel to confirm amplification, the PCR products were treated with exonucleaseI and shrimp alkaline phosphatase from the 'PCR product presequencing kit' (United States Biochemical) to remove the unused primers and free nucleotides. Subsequently, the purified PCR products (~100 ng) were sequenced for both strands using the Big dye terminator ready reaction kit (Perkin Elmer) and the original primers (HDCM-1, TCR-5). The sequencing PCR cycle conditions were: 30 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Extended products were purified by alcohol precipitation followed by washing with 70% ethanol. The processed samples were then dissolved in loading dye and sequenced using an automated DNA sequencer ABI Prism 377 or ABI Prism 3700. Sequences were edited using the AUTO-ASSEMBLER software package for further analysis.

Data analysis

The GenBank database (National Center for Biotechnology Information, USA: NCBI Home page http://www.ncbi.nlm.nih.gov) was searched for similar sequences using a BLAST search. Mitochondrial DNA sequences of Kemp's ridleys and other marine turtles were downloaded from the database for phylogenetic comparisons. Other olive ridley turtle sequences were downloaded from the database and obtained from Bowen *et al*. (1998) for population comparisons. Population genetic statistics 'within the Indian population' and 'between Indian and global populations' were computed using analysis of molecular variance (AMOVA) in ARLEQUIN version 2.0 (Schneider *et al*. 2000). The Kimura two-parameter model (Kimura 1980) was used to calculate sequence divergences. Wright's fixation index of population subdivision (conventional F_{ST}) was calculated to test for differences in haplotype frequencies (Weir & Cockerham 1984) as well as Φ statistics (Excoffier *et al*. 1992), which incorporate information on nucleotide differences between haplotypes. The significance values of the statistics were computed using a nonparametric permutation approach with 1000 permutations of the original data matrices to generate null distributions of the test statistics (Schneider *et al*. 2000). Maternal gene flow was estimated between pairs of populations using the relationship $Nm = {}^1/_2(1/F_{ST} - 1)$, where Nm is the effective number of females that migrate between populations per generation (Takahata & Palumbi 1985). Haplotype diversity (h) and nucleotide diversity (π), were also calculated using ARLEQUIN version 2.0 (Schneider *et al*. 2000). Phylogenetic trees were constructed using gamma-corrected genetic distance estimates, derived using the

Kimura two-parameter model, that take into account unequal nucleotide frequencies and transitions/transversion rates. Medium joining, maximum parsimony, maximum likelihood and neighbour-joining trees were generated using software packages NETWORK (Bandelt *et al*. 1999) and PHYLIP ver. 3.6 (Felsenstein 1989, 2004). In each case, the related taxon *Caretta caretta* was used as outgroup to root the trees, input data were randomized (with a random seed number and 10 replicates) and a search was made for the best tree. In addition, in the case of maximum parsimony and maximum likelihood, other parameters used in phylogenetic analysis were: global rearrangements, empirical base frequencies and one category of substitution rates. Support for nodes found on the shortest tree after global rearrangements was assessed by bootstrap analysis followed by 'majority rule consensus tree' construction as implemented in PHYLIP ver. 3.6. A gene tree was also constructed using TCS 1.1.3 (Clement *et al*. 2000), based on the method of Templeton *et al*. (1992). This was followed by nested clade analysis to separate and test the genetic structure due to recurrent gene flow and historical events. A cladogram structure was developed based on the mitochondrial control region haplotypic data (Templeton *et al*. 1992; Templeton & Sing 1993; Crandall 1996; Templeton 1998) and used for nested contingency analysis using the GEODIS 2.0 program (Posada *et al*. 2000), along with the geographical data quantified as D_c (geographical spread of a particular clade), D_n (distribution of a given clade relative to the sister clades). This analysis tested the association of clades with geographical locations, as well as the significance of the distances (D_c and D_n) and interior-tip contrasts. The statistical significance of these measures was determined using random permutation tests which simulate the null hypothesis of a random geographical distribution for all clades within a nesting category given the marginal clade frequencies and sample sizes per locality. Inferences from this analysis were based on the key of Templeton (1998). Geographical distances between populations were measured as the straight line distances between sampling sites across the ocean, and via known migratory routes between oceans (i.e. around the southern tip of Africa and through southeast Asia).

Evolutionary effective population size can be estimated from observed genetic diversity, as equivalent to the time (in generations) to shared ancestry (Avise *et al*. 1988). The molecular clock for turtle mitochondrial DNA is believed to be several fold slower than the conventional rate for other vertebrate groups (cf. Dutton *et al*. 1999). The sequence divergence estimates calculated for different turtle species is approximately 1.2–2.4% per million years (Myr; Encalada *et al*. 1996). N_{ef} (Effective population size) was estimated from theta (θ), where $\theta = 2 \times N_{ef} \times \mu$ (where μ = mutation rate/site/generation). The parameter θ was estimated using the programs FLUCTUATE and MIGRATE (Beerli & Felsenstein 2001).

Results

Haplotypic diversity of the mitochondrial control region

Among the 81 samples sequenced in our study, a total of eight haplotypes were observed (Table 1). These included three haplotypes, J, K and N, that were previously reported from Sri Lanka, Malaysia, Australia and Pacific Costa Rica (Bowen *et al*. 1998), while the remaining five were recorded for the first time. Haplotype K was the most abundant haplotype observed in 69 (85.2%) individuals (Table 1). In contrast, haplotypes N and J were found only in one and two individuals from Madras and Gahirmatha, respectively. Similarly, all the new haplotypes i.e. K-1 to K-5 (AF513542–47), were observed in only one to four individuals and at one to three sites per haplotype. The five new haplotypes observed in the Indian samples were affiliated with the most abundant haplotype K, which differs significantly at many nucleotide positions from all other earlier reported haplotypes (Bowen *et al*. 1998). Notably, three individuals, one from Madras and two from Gahirmatha, were found to have haplotypes N and J, respectively, which differs from all other closely related Indian haplotypes (K clade, Fig. 2) by more than 11 nucleotide changes. These rare haplotypes have earlier been documented only from Pacific Costa Rica and the West Pacific (Bowen *et al*. 1998). In the analysed samples, the haplotype diversity ($h = 0.27$) and nucleotide diversity ($\Pi = 0.003 \pm 0.002$) were low, as has generally been observed in ridley turtles. Notably, one male from Gahirmatha offshore waters belonged to K-1, a haplotype that was otherwise found only in Madras.

Table 1 Frequency distribution of haplotypes at different sites off the east coast of India

Area/Haplotype	N	J	K	K-1	K-2	K-3	K-4	K-5	Total
Gahirmatha — offshore	—	—	14	1	—	—	1	—	16
Gahirmatha — nesting	—	2	18	—	—	1	—	1	22
Devi Mouth	—	—	12	—	—	1	—	—	13
Rushikulya	—	—	12	—	—	2	—	—	14
Madras	1	—	13	1	1	—	—	—	16
Total	1	2	69	2	1	4	1	1	81
Proportion	0.012	0.025	0.852	0.025	0.012	0.049	0.012	0.012	

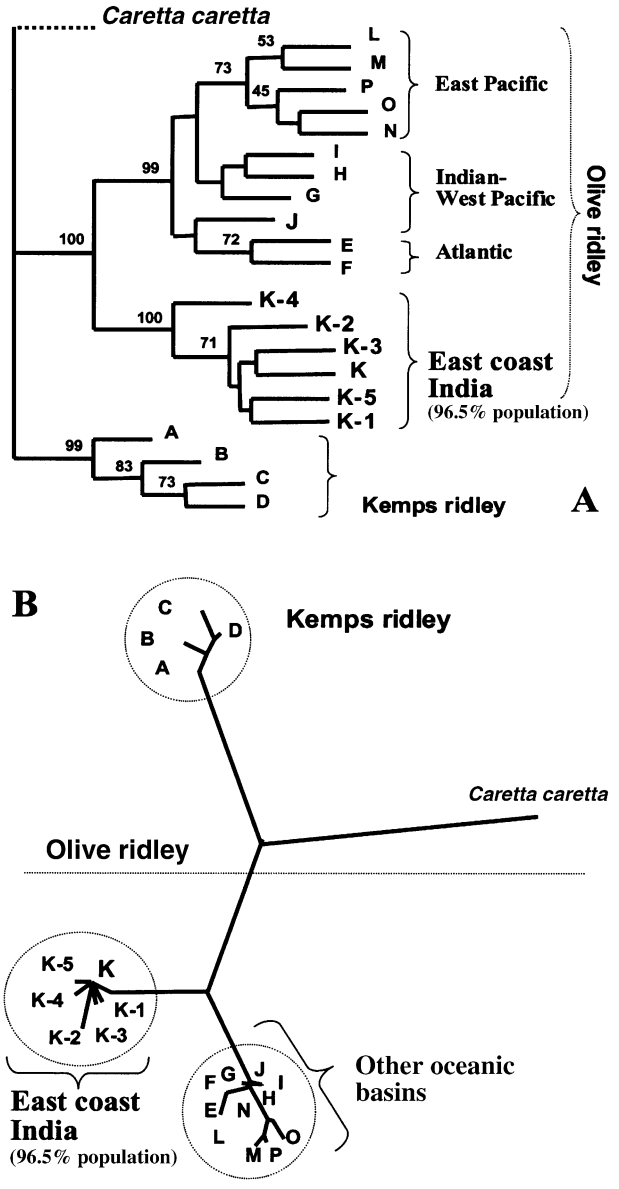

Fig. 2 Phenograms/gene trees based on mitochondrial control region haplotype diversity, showing the relationships between olive ridley populations in different oceanic basins. (A) Maximum likelihood (with bootstrap values) tree; note the haplotypes representing Indian population form a coherent cluster (with strong bootstrap support) distinct from all other olive haplotypes and nearest to the Kemp's haplotypes. (B) Neighour-joining tree: note that both the Kemp's and Indian ridley populations appear as distinct separate clusters whereas, all other olive populations together form the third cluster. The analysis was performed using software package PHYLIP ver. 3.6. The trees were rooted using the *Caretta caretta* (AF-374399) as outgroup taxon.

Population structure

There were no significant differences in the haplotype frequencies between sites in Orissa ($\Phi_{ST} = -0.06-0.04$ and $F_{ST} = -0.06-0.01$; $P > 0.05$). Similarly, there were no differences in haplotype distribution between Orissa and Madras, further south along the coast ($\Phi_{ST} = -0.01-0.04$ and $F_{ST} = -0.037$ to -0.012; $P > 0.05$). However, the Indian populations were distinct from the adjacent Sri Lankan population ($\Phi_{ST} = 0.34-0.46$ and $F_{ST} = 0.12-0.20$; $P < 0.05$).

The haplotype networks constructed using the programs NETWORK and TCS both matched closely (Fig. 3). Nested clade analysis grouped the haplotypes into 13 'one-step' clades, of which only two showed geographical variation, neither of which showed significance. The 'one-step' clades were grouped into five 'two-step' clades, of which four showed geographical variation, but only clades 2-3 ($\chi^2 = 9$, $P = 0.017$) and 2-4 ($\chi^2 = 47$, $P < 0.001$) showed geographical association and significant distance values. These were further grouped into three 'three-step' clades, of which only 3-2 showed geographical association ($\chi^2 = 111$, $P < 0.001$) and two 'four-step' clades, of which clade 4-1 showed significant geographical association ($\chi^2 = 132$, $P < 0.001$). Significant geographical association was also found within the total cladogram ($\chi^2 = 167$, $P < 0.001$) (Fig. 3). The analyses supported range expansion between Sri Lanka and Australia and at a higher level between India's olive ridleys and the other olive ridleys. Long-distance colonization was indicated between (i) Sri Lanka (J haplotype) and the Atlantic; (ii) Sri Lanka with Malaysia/Australia and Costa Rica and (iii) Kemp's and Olive ridleys (Fig. 4). The direction of colonization between Kemp's and olive ridleys could not be determined, because the interior tip status was arbitrarily defined.

Phylogeography

A comparison of all the Indian samples with ridley populations reported earlier from Sri Lanka and other oceanic basins (in Bowen *et al*. 1998) revealed significant differences in haplotype frequencies suggesting that the Indian population is genetically distinct from all other populations with almost no maternal gene flow between these populations (as suggested by the low *Nm* values, Table 2). The phylogenetic analysis revealed the uniqueness of the predominant K haplotypes of the Indian populations compared to those haplotypes found in other ocean basins (Fig. 2). Additionally, a 'sequence signature' of the mitochondrial control region, was identified, which suggests that the Indian population of olive ridley turtles is ancestral to contemporary populations documented from other oceanic basins. The signature sequence is characterized by a '7-bp indel', which is found to be characteristically missing in the haplotype K and all its derivatives that define > 96% of the Indian population. More significantly, the signature motif was also found missing in the most closely related species *Lepidochelys kempi* and four other marine turtle species, namely, *Caretta caretta*, *Chelonia mydas*, *Eretmochelys imbricata* and *Dermochelys coriacea* (Table 3). In comparison, all haplotypes of the olive

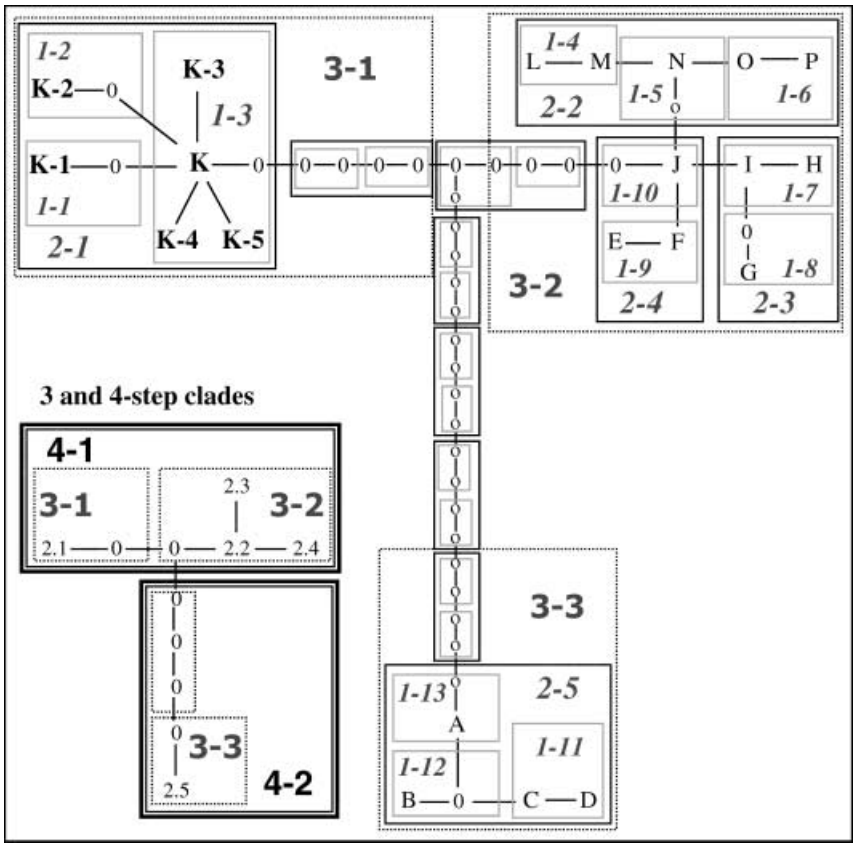

Fig. 3 The nesting cladogram based on haplotypes of the mitochondrial control region of ridley turtles analysed and compared in the present study. Haplotype A to D are of *L. kempi* and E to P, K-1 to K-5 are of olive ridley turtles. The haplotype network structure: thin-lined polygons: one-step clades (1-1-1-13) of haplotypes; dark-lined polygons: one-step clades nested together into two-step clades (2-1-2-5); The higher nesting categories are shown as the network of two-step clades in the bottom left of the Figure. In the step-two clade network, dotted-polygons indicate two-step clades nested together into three-step clades (3-1-3-3, also shown in the main network), whereas double-lined polygons show the three-step clades nested together into two four-step clades (4-1 and 4-2).

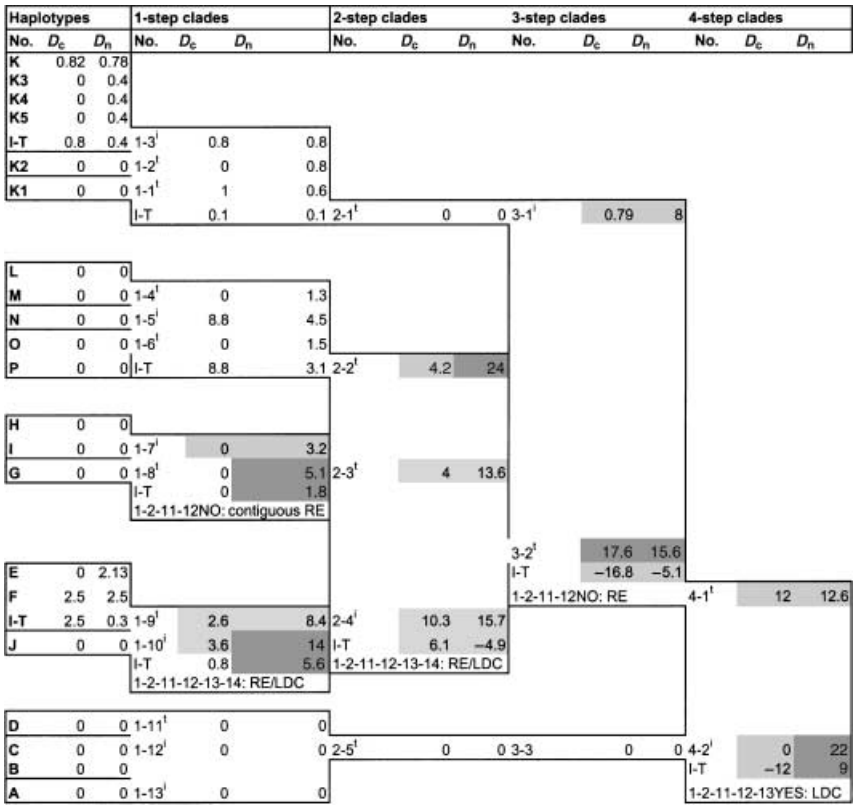

Fig. 4 Results of the nested geographical analysis of the ridley turtle's mitochondrial DNA haplotypes, carried out using GEODIS 2.0. suggesting range expansion (RE) and/or long-distance colonization (LDC) for olive ridley turtles in global basins other than those in Indian waters, and also for overall ridley populations of both the related species. The nested design and designations are as given in Fig. 4. Other annotations used in the figure are: No., either a haplotype or a nested clade; D_c, within-clade distance; D_n, the nested clade distance; I-T: Interior tip contrasts; inference key as per Templeton (1998). Superscripts indicate interior (i) and tip (t) clades. Shading indicates significance of χ^2 test of geographical association of haplotypes (light shading indicates $P < 0.05$, dark shading $P < 0.01$).

Table 2 A comparison of the Indian population of olive ridleys with other ridley populations and the Kemp's ridley (Bowen *et al*. 1998) Φ_{ST}, F_{ST} and *Nm* estimates were calculated using ARLEQUIN version 2.01 (Schneider *et al*. 2000)

	Φ_{ST}*	F_{ST}†	*Nm**	*Nm*†
Kemp's ridley	0.96	0.64	0.02	0.28
Surinam	0.92	0.73	0.04	0.18
Brazil	0.92	0.80	0.04	0.13
Australia	0.90	0.68	0.05	0.24
Sri Lanka	0.58	0.25	0.36	1.53
Costa Rica	0.92	0.59	0.04	0.35

All 'F_{ST}' estimates are significant ($P < 0.05$).
*Estimates based on molecular diversity (Kimura 2P distance method).
†Estimates based on haplotype frequencies (conventional *F*-statistics).

ridley turtles documented from other oceanic basins carry the signature 7-bp insertion (Table 3).

Effective population size and coalescence times

Based on the provisional mtDNA evolutionary rate, the coalescence time for only the K clade comprising ~96% Indian haplotypes would be ~0.2–0.4 Myr (assuming that the individuals are derived from the K haplotype). The coalescence time for all olive ridley turtles was 1.8–3.6 Myr, while all ridleys coalesce at 2.75–5.5 Myr. Generation time can be approximated as age at maturity plus half the reproductive longevity (Pianka 1974). The age at maturity is estimated to be 11–16 years for Kemp's ridley turtles (Zug *et al*. 1997), while reproductive longevity can be up to or more than 20 years for olive ridleys (Pandav & Kar 2000). These studies suggest a generation time of roughly 20 years for the olive ridley turtles, based on which the mutation rate/site/generation is 2×10^{-7}. Based on the estimate of θ, the effective population size (N_{ef}) of the K clade is ~9000 (5500–13 000) and that of the Orissa population is ~19 000 (12 000–27 000).

Discussion

Phylogeography of the ridleys

Olive ridley turtles are found on both sides of the Pacific and Atlantic Oceans and in the Indian Ocean (Pritchard 1997), and are separated from Kemp's ridley by only the Caribbean Sea (Reichert 1993), though they have recently been recorded in Florida, USA (Foley *et al*. 2003). It has been hypothesized that the divergence of the two ridleys was concurrent with the formation of the Isthmus of Panama (Pritchard & Trebbau 1984), and that olive ridley turtles from the Indian Ocean recently colonized the Atlantic Ocean via the Cape of Good Hope (Pritchard 1967).

However, the data are inconsistent with this hypothesis, which implies that the haplotypes closest to the Kemp's ridleys should be those in the east Pacific (Bowen *et al*. 1998). In fact, our data clearly show that haplotype K, closest to the Kemp's, is the most frequent (85.2%) in the Indian population that represents one of the biggest ridley populations (possibly half a million turtles in recent times) in the world. Furthermore, haplotype K and related haplotypes in the Indian population carry a '7-bp indel signature', also found in the Kemp's ridley and other marine turtle species,

Table 3 Part of the mitochondrial control region sequence showing the '7-bp indel', the plausible 'ancestral signature motif' in olive ridleys from the Indian Coast, Kemp's ridleys and other related marine turtle species

Turtle sp./haplotypes	Population/ origin	Base positions*															
		435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450
Olive ridley																	
E, F	Atlantic*	T	G	G	T	T	G	C	A	C	G	A	T	A	A	A	T
G, H, I, J	Indian-West pacific*	T	G	G	T	T	G	C	A	C	G	A	T	A	A	A	T
L, M, N, O, P	East Pacific,* India†	T	G	G	T	T	G	C	A	C	G	A	T	A	A	A	T
K, K-1 to K-5	East Coast, India†	T	G	G	T	T	–	–	–	–	–	–	–	A	A	A	T
Lepidochelys kempi																	
A, B, C, D		T	G	G	T	T	–	–	–	–	–	–	–	A	A	A	T
Caretta caretta (AF-374399)‡		T	G	G	T	T	–	–	–	–	–	–	–	A	A	A	T
Chelonia mydas (AF-366259)‡[3]		T	G	G	T	T	–	–	–	–	–	–	–	G	A	A	T
Eretmochelys imbricata (U-22368)‡		T	G	G	T	T	–	–	–	–	–	–	–	A	A	A	T
Dermochelys coriacea (AF-121964)‡		T	G	G	T	T	–	–	–	–	–	–	–	–	A	A	T

*as per Bowen *et al*. (1998).
†Present study (GenBank Accession nos AF314653–55; AF513539–47; AF514311).
‡GenBank accession number.

which suggests that the eastern Indian population may be ancestral to other olive ridley turtles. This is well supported by the phylogenetic analysis (Fig. 2a,b) and broadly by the nested clade approach (which indicated geographical distinctions, range expansion or colonization at the highest nesting level; see Figs 3 and 4). The analysis also suggested the prevalence of long-distance colonization in olive ridley turtles.

Bowen *et al*. (1998), in essence, suggest that a population ancestral to both ridleys was present in the central American region and following a vicariant separation with the formation of the Isthmus of Panama, a population ancestral to olive ridley turtles colonized the Indian Ocean, which thereafter served as a source for the (re)colonizations of the Pacific and Atlantic Oceans. However, an equally (or more) parsimonious explanation is that Indian ridleys and the Kemp's ridleys could be remnants of a global population which was otherwise extirpated following climatic changes prior to and after the closure of the Isthmus of Panama. Apart from geographical separation of populations, the closure of the Isthmus of Panama would have had significant impacts on climate and circulation in the Pacific and Atlantic Oceans (Berggren 1982), leading to the extirpation of populations in these basins. While the climate of the eastern Pacific has not been stable in recent evolutionary time (Kotilainen & Shackleton 1995), the Indian Ocean and north Atlantic have been warmer following the closure of the isthmus (Murdock *et al*. 1997), enabling the survival of populations in these regions. Thus the Indian Ocean region, in particular the distinct Indian population, may have served as a source for ridley re-colonizations following the extirpation of populations in other ocean basins.

There is considerable debate over the timing and effect of the Isthmus of Panama closure (Haug & Tiedemann 1998) as well as the effect of the closure of the Indonesian seaway (Cane & Molnar 2001). The climatic effects of the closure of the Isthmus of Panama may have preceded the actual closure by a million years (Haug & Tiedemann 1998), which is consistent with fossil evidence placing the separation between the two ridley species at about 5 Myr ago (Dodd & Morgan 1992). Given the uncertainty over palaeoclimatic effects and sequence divergence rates, the exact mechanism and timing of separation of the two ridley taxa cannot be determined. However, the recent origin of olive ridley populations in the Pacific and Atlantic from Indian or Indian Ocean populations is clearly supported by the molecular data.

Notably, a single individual of haplotype N (so far documented only from Pacific Costa Rica) was found in Madras and two individuals of haplotype J (found in Indian–West Pacific populations) were found in Gahirmatha. These haplotypes (J, N) could represent recent *trans*-oceanic migrants from the Pacific to the Indian population, which is characterized by the K clade (~96% of the population). In this case, the Orissa population may be principally derived from the K clade, which has an N_{ef} of only ~5500–13 000 females. On the other hand, these haplotypes (J, N) could represent the retention of an ancestral polymorphism in the resident olive ridley population, which colonized the West and Eastern Pacific from the Indian Ocean and is the basis for maternal lineages in those Oceanic basins.

The concept of sources and sinks was first introduced in the context of the utilization of marginal habitats by species (Pulliam 1988; Pulliam & Danielson 1991). However, this concept has not been applied over global spatial scales or evolutionary time scales. We present a scenario where the Pacific and Atlantic Oceans may represent evolutionary marginal habitats or 'sinks' for ridley turtles, while the Indian Ocean serves as the source. It may be important to prioritize source habitats for conservation (Pulliam & Danielson 1991). The application of this concept over such spatial and temporal scales may be relevant to other migratory species with strong dispersal abilities that are able to colonize distant geographical areas. This would reflect in the prioritization of global populations of these species, with source populations gaining precedence over sink populations.

Taxonomy and conservation prioritization

Many species concepts have been proposed over the years (King 1993), but with little consensus (Mallet 1995). Among these, the Biological Species Concept is most widely used but has been heavily criticized (Sokal & Crovello 1970; Mallet 1995), mainly as a result of the arbitrariness of the genetic distance or morphological divergence that is generally used to assign the species status (Frost & Hillis 1990). The conservation of diversity at molecular and organism levels may not be well served when conservation planning is centred around a particular taxonomic level, especially one as contentious as the 'species' (Mallet 1995). This has led to the development of 'management units' and 'evolutionary significant units' (Moritz 1994).

The taxonomy of Kemp's ridley turtles has long been debated (Garman 1880; Dittmars 1936; Carr 1957; Loveridge & Williams 1957). Earlier studies on the distinctiveness of Kemp's ridley turtles were based on comparisons with nearby Atlantic and Pacific populations of olive ridley turtles (see Bowen *et al*. 1991, 1993; Dutton *et al*. 1996), from which they are obviously distant. Despite using Pacific ridleys for comparison, molecular phylogenies based on ND4-Leucine tRNA and control regions of mitochondrial DNA showed a lower sequence divergence between the two taxa of ridleys than between the Pacific and Atlantic populations of green turtles (see Dutton *et al*. 1996) which are still widely classified as a single species. The focus on the distinctiveness of the Kemp's ridley as a species has distracted attention from the diversity within global olive

ridley populations. Even strong proponents of species status for the Kemp's ridley accept that there is little morphological difference between the two ridleys (e.g. Pritchard 1997). However, we do not recommend a reassignment of species status. On the contrary, we suggest that taxonomic assignments can be arbitrary and need to be based on more appropriate data, including molecular, morphological and behavioural diversity towards more effective conservation of diversity at all scales.

Conservationists have used taxonomic distinctiveness as a justification for prioritization (including allocation of money and effort) and this has led to political and geographical bias to the assignment of species names (Karl & Bowen 1999). The designation of one or some populations as a species may subsume the more complex variation in the world-wide population. This could undermine the conservation of the genetic diversity of the taxon and devalue other distinct clusters (or populations), as in this case the Indian ridleys. Using the criteria adopted for other species of sea turtles, Kemp's and olive ridley turtles may not be different species. However, the Kemp's ridley is a distinct monophyletic lineage, and forms a separate cluster from all other ridley populations. The clustering analysis of the molecular data however, also reveals Indian ocean population (particularly the K clade found predominantly in the Indian population) as a distinct cluster whereas all other olive ridley populations together form the third cluster. Both populations contain distinct clades that are not present in other populations. Though the K haplotype is found in the Sri Lankan population, the clade is fully represented only in the Indian population. Hence, both the Kemp's ridley and the Indian ridley should qualify as evolutionarily significant units (ESU), while other ridley populations might be classified as management units within an eastern Pacific ESU.

Using microsatellite markers, we have shown that there is no genetic substructuring in the ridley population along the east coast of India and the samples from Orissa to Madras, the two farthest sampling sites, are part of the same large population (Aggarwal *et al*. 2003). This finding is consistent with tagging studies, which have demonstrated nesting by individuals at multiple rookeries in Orissa (Pandav 2001). Most notable was the distinctiveness of the Indian populations from the adjacent Sri Lankan population (Φ_{ST} = 0.34–0.46 and F_{ST} = 0.12–0.20; $P < 0.05$), considering that ridleys along the entire east coast of India (~1500 km from Madras to Gahirmatha) comprised a single population, while Sri Lanka is only 500 km south of Madras.

Many sea turtle populations have been critically affected by human-related activities, both past and present (Limpus 1995). Leatherback turtles (*Dermochelys coriacea*) in the Pacific may become extinct in less than 50 years (Spotila *et al*. 2000) and hawksbill turtles (*Eretmochelys imbricata*) are also considered to be globally endangered (Meylan & Donnelly 1999). The olive ridley population on the east coast of India is the most important in the region because it includes the largest nesting population in the Indian Ocean, as well as a number of important minor sites. The population faces a number of direct and indirect threats, the most serious of which is fishery-related mortality (Pandav *et al*. 1998; Pandav 2001). Our results indicate that the population on the east coast of India needs to be conserved as a single evolutionary significant and management unit, with attention to both arribada rookeries in Orissa as well as to select sporadic nesting sites along the east coast.

For a holistic approach to conservation, it is necessary to safeguard both past evolutionary history as well as future evolutionary potential (Moritz 1994; Bowen 1999). In this instance, the Indian ridley, as evident from the phylogeography, represents both past history as well as future evolutionary potential. This perspective emphasizes the great need to make conservation decisions on more criteria than mere classification as 'species'. It is suggested that, in future, such prioritization should incorporate actual data on the relationships between populations — be it molecular, morphological or behavioural — rather than rely on species designations. Our data demonstrate how a reliance on species definitions obscures information and leads to gaps in conservation prioritization.

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Dr Kartik Shanker works on the ecology and evolutionary biology of herpetofauna, and in particular on the biology, conservation and genetics of marine turtles in the Indian ocean. Dr Ramesh K. Aggarwal works on the development and use of DNA markers for genetic diversity, conservation genetics and genetic improvement of endangered fauna and flora. Ms. J. Ramadevi is actively involved in DNA markers studies on genetic diversity and conservation genetics of endangered animal species. Mr B.C. Choudhury coordinates the national UNDP sea turtle conservation project and has been involved with the biology and conservation of freshwater and marine turtles for 25 years. Dr Lalji Singh is the pioneer of the DNA typing analysis in India and heads the group on conservation genetics of the endangered animals.
