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SYSTEMATIC AFFINITIES OF *NOTROPIS TOPEKA* (TOPEKA SHINER) INFERRED FROM SEQUENCES OF THE CYTOCHROME *b* GENE.—The systematic affinities of the Topeka shiner, *Notropis topeka* [Nomenclature used in this paper follows recommendations of the Committee on Names of Fishes (Robbins et al., 1991) and Cross and Collins (1992) by retaining usage of *N. topeka* and *N. stramineus* over *N. tristis* and *N. ludibundis*, respectively (Mayden and Gilbert, 1989)], a cyprinid fish confined to parts of the Missouri, Mississippi, and Arkansas river drainages, are essentially unknown (Gilbert, 1980). Bailey (1959) suggested *N. topeka* might be related to *N. anogenus* or *N. ortenburgeri*, two North American cyprinids whose phyletic affinities also are largely unknown. Cross and Collins (1975) noted that *N. topeka* most closely resembles the sand shiner, *N. stramineus*, whereas Coburn (1982) suggested that *N. topeka* could be related to members of the genus *Cyprinella*. Mayden (1989) placed *N. topeka* in a large polytomy basal to a clade he referred to as the *Notropis*-like shiners. This polytomy also included (among others) *N. stramineus* and *N. procne*.

As part of a broader study of the cyprinid genus *Notropis*, we compared 512 homologous DNA bases from the mitochondrially encoded cytochrome *b* (cyt *b*) gene of *N. topeka* with those from seven other cyprinid species endemic to North America. The purpose of the study was to assess whether *N. topeka* shares phyletic affinities with *Cyprinella*, *N. stramineus*, or neither. Species examined in addition to *N. topeka* included two species of *Cyprinella* (*C. lutrensis* and *C. venusta*), four species of *Notropis* (*N. chrosomus*, *N. procne*, *N. stramineus*, and *N. volucellus*), and *Notemigonus crysoleucas*. The two species of *Cyprinella* are representative of the major lineages within the genus (Mayden, 1989). Subgenera or species-groups to which these species of *Notropis* are currently assigned are as follows: *N. chrosomus* (*Hydrophlox*), *N. procne* and *N. stramineus* (*N. procne* species-group), and *N. volucellus* (*N. volucellus* species-group) (Snelson, 1971; Mayden, 1989; Coburn and Cavender, 1992). *Notemigonus* is a monotypic genus placed in a different clade from other endemic North American cyprinids (Cavender and Coburn, 1992).

Methods.—Specimens examined in the study were obtained primarily by seine from natural populations. Collection localities and voucher material are listed in Material Examined. Specimens of *Notemigonus* were obtained from a bait shop in Bryan, Texas. Whole DNAs were prepared using phenol:chloroform extraction and ethanol precipitation and used directly for amplification of target DNA sequences via polymerase chain reaction (PCR) (White et al., 1989). Unbalanced (asymmetric) primer ratios were used to acquire single-stranded DNA (Gyllensten and Erlich, 1988), and single-stranded template DNA was sequenced using standard dideoxy chain-termination methods (Sanger et al., 1977). Sequence was acquired from an interior region of the cyt *b* gene using the cyt *b* primers LC and HB. The primer sequences were as follows, where the letters L and H refer to strand designation (light and heavy, respectively): LC (5′-ATACATGCCAACGGAGCATC-3′) and HB (5′-AGTCCTCGTTGTTTTGAGGTGT-G-3′).

Maximum-parsimony analysis (including 500 bootstrap replications) of unweighted, unordered character-state data was carried out using Phylogenetic Analysis Using Parsimony (PAUP) version 3.1.1 (Swofford, 1993). The shape of the distribution of all tree lengths was assessed using the g_1 statistic (Hillis, 1991; Huelsenbeck, 1991). Ratios of transition to transversion changes (from pairwise comparison) were calculated using Molecular Evolutionary Genetics Analysis (MEGA) version 1.01 (Kumar et al., 1993). Estimates of nucleotide sequence divergence among pairwise comparisons of species were calculated using the Jukes and Cantor (1969) method with the DNA distance (DNADIST) program in version 3.4 of the Phylogenetic Inference Package (PHYLIP) of Felsenstein (1991). A phenogram was generated from these estimates using the unweighted pair group method using arithmetic averages (UPGMA) in PHYLIP. The potential for saturation of nucleotide sequence variation within the data set was evaluated by plotting transition to transversion ratio and the number transitions or transversions at each codon position relative to percent nucleotide sequence divergence (Moritz et al., 1992).

Results and discussion.—Nucleotide sequences of 512 homologous bases from the cyt *b* gene of the eight species are given in the Appendix. GENBANK accession numbers for cyt *b* sequence of each species are given in Material Examined. Excluding *Notemigonus* (used as outgroup) the number of variable bases was 154, with 12, 0, and 142, occurring at the first, second, and third codon positions, respectively. The

TABLE 1. TRANSITION/TRANSVERSION RATIOS AND ESTIMATES OF NUCLEOTIDE SEQUENCE DIVERGENCE. Transition to transversion ratios (above diagonal) and corrected estimates of nucleotide sequence divergence (below diagonal) were calculated using methods described in text.

	1	2	3	4	5	6	7	8
1. *Notemigonus*	—	1.43	1.48	1.49	1.17	1.50	1.35	1.26
2. *C. lutrensis*	23.26	—	3.33	3.42	3.14	3.78	3.14	3.38
3. *C. venusta*	22.46	17.10	—	3.21	2.86	3.89	3.50	2.44
4. *N. chrosomus*	19.35	18.60	17.60	—	3.24	4.08	3.47	3.25
5. *N. procne*	20.38	20.38	18.85	15.64	—	5.50	4.60	2.19
6. *N. stramineus*	20.12	19.10	19.61	14.21	10.96	—	5.67	2.04
7. *N. topeka*	21.15	20.38	20.12	16.61	11.87	8.28	—	2.30
8. *N. volucellus*	19.61	20.63	19.10	14.68	14.44	15.16	16.61	—

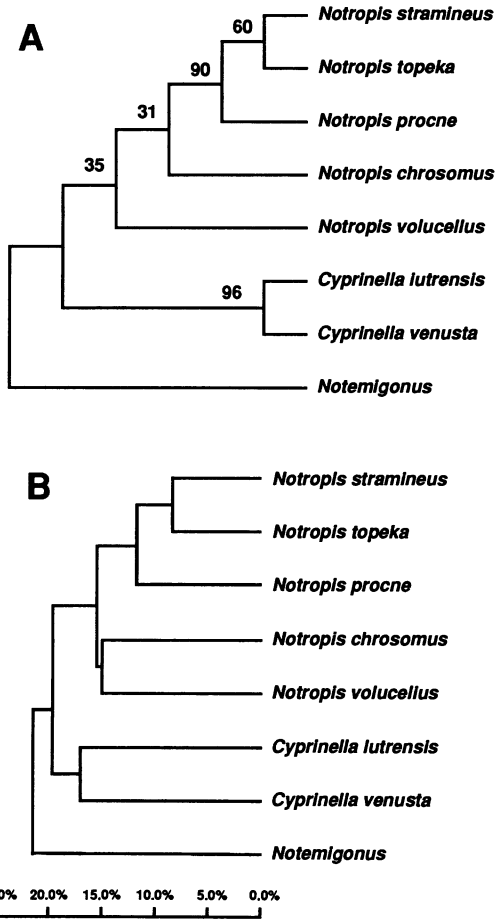


Fig. 1. (A) Phylogenetic tree derived from maximum-parsimony analysis of 512 homologous bases of the cyt *b* gene from six species of *Notropis* and two species of *Cyprinella*. The tree was rooted with *Notemigonus*. Numbers above branches indicate percentage of 500 bootstrap replicates that support each node. Branch lengths are not accurate representations of the number of character state changes. (B) UPGMA phenogram generated using transformed nucleotide-sequence-divergence estimates.

number of phylogenetically informative bases was 109, with 7, 0, and 102, occurring at the first, second, and third codon positions, respectively. Estimates of sequence divergence between species-pairs in the ingroup ranged from 8.28–20.63%, and the transition to transversion ratio ranged from 2.04–5.67 (Table 1).

The most parsimonious tree from maximum-parsimony analysis (Fig. 1A) contained 349 steps (CI = 0.572, RI = 0.421). Support was strongest for monophyly of the two species of *Cyprinella*; monophyly of a clade that includes *N. stramineus*, *N. topeka*, and *N. procne*; and a sister-species relationship between *N. stramineus* and *N. topeka*, relative to *N. procne*. Comparisons with other *Notropis* taxa (e.g., *N. atherinoides*, *N. texanus*) do not significantly alter conclusions of

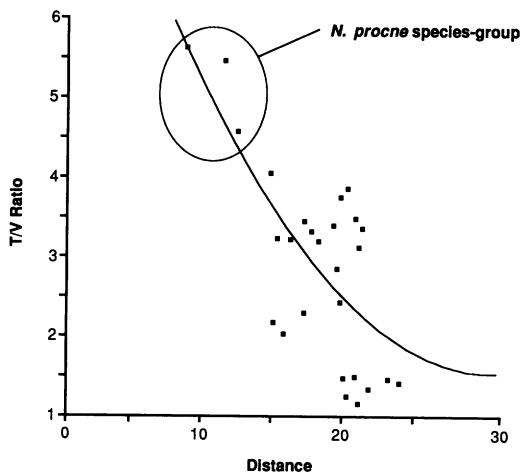
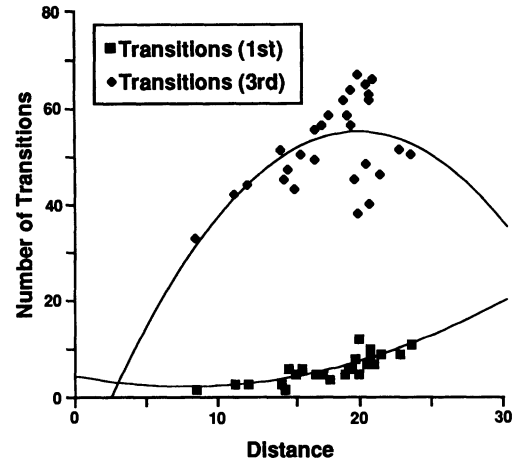


Fig. 2. Plot of transition to transversion (T/V) ratio relative to nucleotide sequence divergence. Distances along the horizontal axis are in percent nucleotide sequence divergence as estimated with the Jukes and Cantor (1969) one-parameter model. This model does not utilize an a priori assumption of T/V ratio.



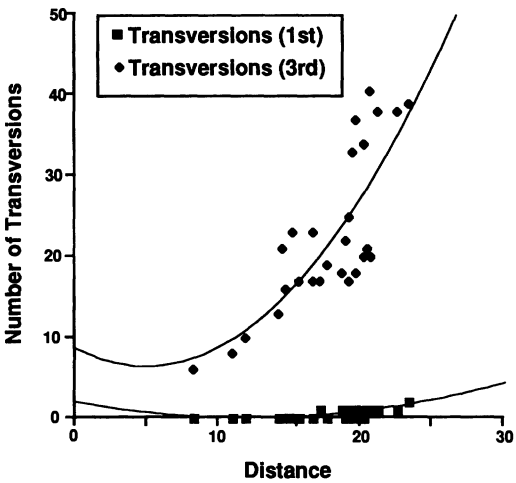


Fig. 3. Plots of the number of transistions and transversions at the first and third codon positions relative to percent nucleotide sequence divergence. Nucleotide sequence divergence was calculated using the method of Jukes and Cantor (1969). No second position transitions were present in the data set. One second position transversion was present in comparisons between *Notemigonus* and other taxa.

the analysis (unpubl. data). The estimated g_1 for the distribution of all tree lengths (ingroup taxa only) was -0.763 ($P < 0.01$). The significant left-skewness indicates that the phylogenetic signal is strong, at least in some portion of the data set. High transition to transversion ratios (4.60—5.67) and bootstrap values (60% and 90%) suggest that saturation of nucleotide substitutions has not occurred within the *N. procne* species-group. Transition to transversion ratios between members of the *N. procne* species-group are elevated relative to comparisons between more distantly related taxa (Fig. 2), lending additional support to the informativeness of cyt *b* sequences within the *N. procne* species-group. When base substitutions are subdivided by codon position and substitution type, saturation of nucleotide substitutions is evident only among divergent taxa in third position transitions (Fig. 3). Third position transistions show evidence of saturation at divergences greater than about 15%, which is greater than the maximum divergence of 11.87% within the *N. procne* species-group. Relatively low transition to transversion ratios and bootstrap values for other taxa in the analysis suggest that more distant relationships indicated by maximum-parsimony analysis are not robust. The topology of the phenogram (Fig. 1B) is congruent with maximum-parsimony analysis in clustering *N. procne*, *N. stramineus*, and *N. topeka*. It differs in clustering *N. volucellus* and *N. chrosomus*.

These data support the hypothesis that *N. topeka* is related to *N. stramineus* and belongs in the *N. procne* species-group. This species-group is comprised minimally of *N. albizonatus*, *N. mekistocholas*, *N. procne*, *N. stramineus*, and *N. uranoscopus* (Snelson, 1971; Burr and Mayden, 1981; Warren et al., 1994). Only *N. stramineus* and *N. topeka* are found west of the Mississippi River, suggesting that *N. topeka* may represent a regional derivative of the far more widespread *N. stramineus*. Our hypothesis differs from that of Coburn (1982) who suggested that *N. topeka* might have close affinities to the genus *Cyprinella*. Using cyt *b* sequences, placement of *N. topeka* as sister to the *C. lutrensis* and *C. venusta* clade increases the tree length by 22 steps. Our hypothesis also differs from Mayden's (1989) placement of *N. topeka*, *N. stramineus*, and *N. procne* in a polytomy basal to a clade that includes *Cyprinella* and other *Notropis*-like shiners. Placement of these three species basal to the remaining species increases the cyt *b* tree length by five steps. Of future interest will be acquisition of cyt *b* sequence from *N. anogenus* and *N. ortenburgeri*, two cyprinids suggested by Bailey (1959) to be relatives of *N. topeka*.

Material examined.—Collection localities (drainages) of material examined are listed below. Catalog numbers of voucher specimens deposited in the Texas Cooperative Wildlife Collections (TCWC) at Texas A&M University and the Kansas Museum of Natural History (KU) are given in parentheses; the GENBANK accession number for the cyt *b* sequence acquired from each species is given in brackets. The GENBANK accession number for *Notemigonus* cyt *b* sequence is U01318. Institutional abbreviations are as listed in Leviton et al. (1985). *Cyprinella lutrensis*—Coleto Creek (Guadalupe River), Victoria County, Texas (TCWC

7253.01) [U01319]; *C. venusta*—Choccolocco Creek (Coosa River), Calhoun County, Alabama [U01320]; *N. chrosomus* —Gurley Creek (Black Warrior River), Blount County, Alabama (TCWC 7248.01) [U01321]; *N. procne*—Eno River (Neuse River), Durham County, North Carolina (TCWC 7249.01) [U01336]; *N. stramineus*—South Canadian River (Arkansas River), Pottowatomie County, Oklahoma (TCWC 7250.01) [U01325]; *N. topeka*—Mill Creek (Kansas River), Wabaunsee County, Kansas (KU 22636) [U01326]; and *N. volucellus*—Cahaba River (Cahaba River), Bibb County, Alabama (TCWC 7251.01) [U01327].

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APPENDIX. NUCLEOTIDE SEQUENCES OF THE CYT *B* GENE OF EIGHT SPECIES OF NORTH AMERICAN CYPRINIDS. The sequence corresponds to positions 322–833 of the cyprinid cytochrome *b* gene (Schmidt and Gold, 1993).

Notemigonus	CTT	TAT	AAA	GAG	ACC	TGA	AAC	ATT	GGT	GTA	GTA	TTA	TTC	CTC	CTA	GTT	ATA	ATG	ACA
N. chrosomus	...	..C	..G	...	...	...	..T	...	..G	..C	..T	C..	C.T	..T	...	...	...	...	...
N. volucellus	..G	..C	...	...	...	...	...	...	...	...	..C	C..	C..	..T	T..	..A	...	...	..C
N. stramineus	...	...	..G	...	...	...	...	..C	..G	..T	..C	C..	C..	..T	..G	...	...	...	...
N. procne	...	...	...	...	...	...	...	...	..A	..T	..T	C.T	C.T	...	T..	..A	...	..A	...
N. topeka	...	...	..G	...	...	...	..T	...	..G	..T	..C	C..	C..	..T	..G	...	..G	...	...
C. venusta	..C	...	...	..A	...	...	..T	...	..A	..G	..T	C..	C.T	...	..C	...	...	..A	..G
C. lutrensis	A..	...	..G	...	...	...	..T	...	..A	..C	..C	C..	C.T	...	..C	..G	...	..A	...

Notemigonus	GCC	TTT	GTC	GGC	TAC	GTT	CTG	CCA	TGA	GGA	CAA	ATG	TCT	TTC	TGA	GGT	GCC	ACC	GTA
N. chrosomus	...	...	..A	...	..T	..A	..C	...	..G	..G	...	...	..C	...	...	..G	...	...	..T
N. volucellus	...	...	..A	...	..T	..A	..C	...	..G	...	...	..A	...	...	..G	..C	...	...	..T
N. stramineus	...	...	..G	..T	...	..A	..T	...	..G	..C	...	...	..C	...	...	...	...	...	..T
N. procne	...	...	..A	..T	..T	..A	..C	...	...	..G	...	..A	..C	...	...	...	...	...	..T
N. topeka	...	...	..A	..T	...	..A	..C	...	...	..T	...	...	..C	...	...	...	...	...	..T
C. venusta	...	...	..A	...	...	..A	..C	...	..G	..T	..G	..A	...	..A	...	..G	..T	..G	..C
C. lutrensis	...	...	..G	...	...	..A	..C	..C	...	..T	...	...	...	...	...	...	...	..A	..T

Notemigonus	ATT	ACA	AAC	CTT	CTT	TCA	GCA	GTG	CCC	TAC	ATA	GGG	GAC	ACC	CTT	GTA	CAG	TGA	ATC
N. chrosomus	...	...	..T	..C	..A	...	..G	..A	..T	..T	...	..A	...	...	...	..C	..A	...	..T
N. volucellus	...	...	..T	...	..A	...	...	...	...	..T	...	...	...	...	...	..C	..A	...	..T
N. stramineus	...	..C	...	...	..A	...	...	..A	...T	..T	...	..A	..T	...	..A	..G	..A	...	..T
N. procne	...	..C	..T	...	..G	...	...	..C	..T	..T	...	..A	..T	...	..A	..G	..A	...	..T
N. topeka	...	..C	...	...	..A	...	...	..C	..T	..T	...	...	..T	...	..A	..G	..A	...	..T
C. venusta	...	...	..T	...	..C	...	...	..A	..T	...	..G	..A	..T	...	..A	...	...	...	..T
C. lutrensis	...	..G	...	...	..C	...	...	..A	..T	...	..G	...	..T	...	..G	..C	..A	..G	...

Notemigonus	TGA	GGT	GGC	TTC	TCA	GTT	GAC	AAC	GCA	ACC	CTC	ACA	CGG	TTC	TTC	GCA	TTC	CAC	TTC
N. chrosomus	...	..G	...	..T	...	..A	..T	..T	...	..A	..G	...	..A	..T	..T	..C	...	...	...
N. volucellus	..G	..A	..A	..T	...	..A	..T	...	...	..A	T.G	...	..A	...	...	..C	...	...	...
N. stramineus	..G	..A	...	..T	..G	..A	..T	...	...	..A	..A	...	..A	...	...	..C	...	...	...
N. procne	...	..A	...	..T	...	..A	..T	...	...	..A	T.A	...	...	...	...	..C	...	...	...
N. topeka	...	..A	...	..T	..G	..G	..T	...	..G	..G	T.G	...	..A	...	...	..C	...	...	...
C. venusta	...	..A	...	..G	..G	..A	...	...	..G	...	..G	..C	..A	...	..T	..C	...	...	...
C. lutrensis	..G	..A	..T	..T	..G	..A	...	..T	...	..G	..A	..G	...	...	..T	..C	...	...	...

Notemigonus	CTC	CTG	CCA	TTC	GTC	GTC	GCC	GGC	GCA	ACT	ATC	CTA	CAC	TTA	CTC	TTC	CTA	CAC	GAA
N. chrosomus	..G	T.T	...	...	...	A..	...	..T	...	...	G..	..C	...	...	..A	..T	...	...	...
N. volucellus	..A	T.C	..C	..T	...	A..	..T	..T	...	..C	G.T	..C	...	...	..G	...	T..	...	...
N. stramineus	...	T.C	...	...	...	A.T	...	..T	...	...	G..	..C	...	..G	...	...	T.G	...	...
N. procne	..A	T.C	..G	...	...	A.T	...	..T	...	...	G.G	..C	...	...	...	...	T..	...	...
N. topeka	...	T.T	..G	...	...	A.T	..T	..T	...	..G	G..	..C	...	..G	...	...	T..	...	..G
C. venusta	..A	T.C	...	...	...	A..	..T	..T	...	...	G.T	..T	...	C.G	..T	...	...	...	..G
C. lutrensis	..A	T.T	..C	...	...	A..	..T	..T	...	...	G..	..T	...	C..	..T	..T	..C	..T	...

Notemigonus	ACA	GGA	TCA	AAC	AAC	CCC	GCC	GGA	CTA	AAC	TCG	GAC	GCA	GAC	AAA	ATC	TCT	TTC	CAC
N. chrosomus	...	..G	...	...	...	..T	..T	..C	...	...	..C	...	..G	...	...	...	...	...	...
N. volucellus	...	..G	...	...	...	...	...	..G	T..	...	..C	...	...	...	...	...	...	...	...
N. stramineus	..G	..G	...	...	...	..T	...	..G	...	...	..C	...	...	..T	..G	..T	..C	...	...
N. procne	..G	..G	...	...	...	..T	...	...	T..	...	..C	...	...	..T	...	...	..C	...	...
N. topeka	..G	..G	...	...	...	..T	...	..G	...	...	..C	..A	...	...	...	..T	..C	...	...
C. venusta	...	..G	...	...	...	...	...	...	T..	...	..C	..T	..G	..T	..G	...	..G	...	...
C. lutrensis	..G	..G	...	..T	...	...	...	..G	...	...	..C	...	...	..T	...	...	..C	...	..T

Notemigonus	CCA	TAC	TTC	TCA	TAC	AAA	GAC	CTT	CTT	GGA	TTT	GTG	GTC	ATA	CTA	CTG	GCC	CTC	ACC
N. chrosomus	..C	...	...	..C	...	...	...	..C	...	..C	...	..C	C..	...	...	..A	...	..T	G.A
N. volucellus	..T	...	...	..C	..T	..G	...	..C	...	..C	...	..C	C.A	...	..G	..A	..T	..G	..A
N. stramineus	..C	...	...	..C	..T	..G	...	...	...	..T	...	..T	C..	...	..C	..A	..T	..T	..A
N. procne	..C	...	...	..C	..T	..G	...	...	...	..T	...	..T	C.G	...	..T	..A	..T	...	..G

APPENDIX. CONTINUED.

N. topeka	..C	...	...	..T	...	..G	...	...	...	..T	..C	..T	C.G	..G	..T	..A	..T	...	..A
C. venusta	..C	...	...	..C	...	...	...	..C	...	..C	..C	..C	T.A	...	...	..A	..T	..T	..A
C. lutrensis	..T	...	...	..C	..T	...	...	...	...	..C	..C	..C	C..	..G	...	T.A	...	..G	..A
Notemigonus	TCC	CTG	GCC	TTA	TTC	TCC	CCT	AAC	CTG	CTA	GGT	GAT	CCA	GAA	AAC	TTT	ACC	CCA	GCA
N. chrosomus	...	..C	A.G	...	...	...	..C	.C.	...	..C	..C	..C	...	...	..T	..C	...	..G	...
N. volucellus	...	..A	A.A	...	..A	..T	..G	.C.	..A	..C	...	..C	...	..G	...	..C	...	..C	...
N. stramineus	...	..A	A.G	...	...	...	...	.CA	..A	..C	...	..C	...	..G	...	..C	...	...	...
N. procne	...	...	A.A	...	..G	...	..C	.CA	..A	..C	...	...	...	...	...	..C	...	..G	..G
N. topeka	...	...	A.G	C..	...	..T	..A	.C.	..A	..C	...	...	...	..G	...	..C	...	...	...
C. venusta	..A	...	A.A	..G	...	...	...	.C.	...	..T	...	..C	...	..G	...	..C	...	..T	...
C. lutrensis	...	...	A.G	..G	...	...	..C	.C.	T..	..T	..A	..C	..G	..G	...	..C	..T	..C	...
Notemigonus	AAC	CCA	CTC	GTA	ACG	CCA	CCA	CAT	ATT	CAG	CCA	GAA	TGA	TAT	TTT	CTA	TTT	GCC	TA
N. chrosomus	...	..G	..G	..C	..C	...	...	...	...	..A	..C	..G	...	..C	..C	T..	...	...	..
N. volucellus	...	...	T.A	..T	..C	..G	...	...	...	...	...	...	...	..C	..C	T..	...	...	..
N. stramineus	...	..C	..G	..T	..C	..C	..G	...	...	...	..C	..G	..G	..C	..C	...	...	...	..
N. procne	...	..C	..G	..C	..C	..C	...	..C	...	...	..C	...	...	..C	..C	..G	..C	...	..
N. topeka	..T	..C	..G	..T	..C	..C	..G	...	...	...	..C	..G	...	..C	..C	..G	...	...	..
C. venusta	..T	..C	..A	..C	..C	...	...	..C	..C	..A	..T	...	..G	...	..C	T.G	...	...	..
C. lutrensis	...	..C	T.A	..C	..A	...	...	..C	..C	...	..T	..G	...	...	..C	T..	..C	..T	..

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AGE AND GROWTH OF LOGGERHEAD TURTLES (*CARETTA CARETTA*) FROM CHESAPEAKE BAY.—Growth rates for loggerhead turtles (*Caretta caretta*) have been calculated from tag-recapture studies of both juveniles (Limpus, 1979; Mendonca, 1981; Bjorndal and Bolten, 1988) and adult females (Frazer and Ehrhart, 1985). These indirect methods have allowed researchers to establish growth curves for sea turtles in the wild (Frazer and Ehrhart, 1985, Frazer, 1987). Growth curves also have been estimated for captive loggerhead turtles (Frazer and Schwartz, 1984; Uchida, 1967), but these rates differ from those reported in the wild. Estimates of age at sexual maturity for captive loggerhead turtles range from 6–20 yr (Caldwell, 1962; Uchida, 1967; Frazer and Schwartz, 1984). Estimates of age at maturity in the wild range from 5–30+ yr (Mendonca, 1981; Limpus, 1979; Table 1).

Previous growth studies of marine turtles could only predict age for a known carapace length. However, Zug et al. (1986) noted growth layers in cross-sections of humerus bones from loggerhead turtles. These layers were postulated to be the product of annual growth, i.e., one growth layer represents one year. Each growth layer consists of a light wide band, presumably representing a fast growth period during spring and summer, followed by a narrow dark band, presumably representing a slow growth period during fall and winter.

The assumption of annual growth layers subsequently was verified by Klinger and Musick (1992) based on immature Chesapeake Bay loggerhead turtles marked with tetracycline (see Frazier, 1985a, 1985b). Immature loggerhead turtles enter Chesapeake Bay during late spring (Lutcavage and Musick, 1985) and feed on a variety of decapod crustacea, mollusks and fish species. When water temperatures drop below 20 C (usually in late Sept. and Oct.), these turtles migrate out of the bay (J. A. Musick, R. A. Byles, R. C. Klinger, and S. A. Bellmund, unpubl., 1984). This seasonal feeding pattern proved useful in correlating carapace length with age (as estimated growth layer number). With these data, we now seek to establish growth curves for the loggerhead turtles that inhabit Chesapeake Bay.

Methods.—Humeri were removed from dead loggerhead turtles that stranded in the Chesapeake Bay from 1983–1985. Cross-sections were histologically prepared according to methods described in Klinger and Musick (1992). Growth layers were counted and measured from the focus of the medullary cavity (core of spongy cancellous bone) to the periosteum (outer layer) on the short axis (radius length). Back calculations of length at age were established using the Fraser-Lee formula (Everhart and Youngs, 1981):

$$L' + C = S'/S\,(L - C)$$