Conservation Genetics of the Common Snapping Turtle (*Chelydra serpentina*)

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Abstract: *Previous studies of relationships among the subspecies of snapping turtles* (Chelydra serpentina) *based on morphological and osteological characters have been inconclusive. We investigated relationships among the four currently recognized subspecies using restriction endonuclease fragment patterns of mtDNA and protein electrophoresis. Sixteen six-based recognizing restriction endonucleases yielded 90 variable fragments that define 11 different haplotypes. Individuals of the two North American subspecies,* C. s. osceola *and* C. s. serpentina, *are closely related, differing by a maximum of 0.5% sequence divergence. The Central American subspecies,* C. s. rossignonii *and* C. s. acutirostris, *are more distinct, both from each other (a minimum of 1.7% sequence divergence) and from the North American samples (an average of 4.45% sequence divergence). The degree of allozymic variation among the four subspecies was found to be limited and could not be used to diagnose the four recognized subspecies. The mtDNA data presented here support the species-level distinctness of* C. s. rossignonii *and* C. s. acutirostris *from each other and from a* C. s serpentina-C. s. osceola *complex. The recognition of three distinctive groups of* Chelydra *rather than one widespread polytypic species has important conservation implications because it focuses attention on the poorly known middle and South American species.*

La conservación genética de la tortuga *Chelydra serpentina*

Resumen: *Estudios previos sobre las relaciones entre las subespecies de la tortuga* Chelydra serpentina, *basados en caracteres morfológicos y osteológicos no han sido concluyentes. En el presente estudio investigamos la relación entre cuatro subespecies reconocidas en la actualidad usando patrones de fragmentos de endonucleasas de restricción de ADN mitocondrial y electroforesis de enzimas. Dieciseis endonucleasas de restricción que reconocen 6 bases proveyeron 90 fragmentos variables que definen 11 haplotipos diferentes. Los individuos de dos especies de Norte América,* C. s. osceola *y* C. s. serpentina, *están relacionados estrechamente, difiriendo en un máximo de 0.5% de divergencia de secuencias. Las especies de América Central,* C. s. rossignonii *y* C. s. acutirostris, *son mas distintas entre sí (con un mínimo de 1.7% de divergencia de secuencias) y distintas del complejo* C. s. serpentina-C. s. osceola. *El reconocimiento de tres grupos distintivos de* Chelydra *en lugar de una especie politípica de amplia distribución tiene importantes implicaciones para la conservación, debido a que enfoca la atención en las especies menos conocidas de Meso y Sud América.*

Introduction

It has become evident that taxonomic decisions can influence estimates of biodiversity (Collins 1991) and the direction of conservation activities (Daugherty et al. 1990). Collins (1991) was specifically concerned with "diluting the species concept" and "obfuscating the evolutionary diversity" of taxa by the overuse of the subspecies category for allopatric and morphologically distinct populations. But application of the evolutionary species concept (Wiley 1981) as advocated by Frost and Hillis (1990) does not exclude nonmorphological evidence. Avise (1989) highlighted the significance of molecular genetic techniques in systematics and its relevance to

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conservation. For example, genetic data helped Daugherty et al. (1990) clarify the taxonomic status of species and subspecies of the tuatara (*Sphenodon*) and reach the conclusion that conservation efforts had been ineffective in preventing the demise of unique populations and an entire subspecies because of the taxonomically opaque concept of a monotypic *S. punctatus*. Lovich and Gibbons (1996) used this example and others to accent the difficulties presented to conservation efforts by "covert" species—unacknowledged taxa hidden by insufficient study or a confused taxonomy.

Systematists have begun to make other specific recommendations for incorporation of taxonomic and phylogenetic information into conservation planning. Vane-Wright et al. (1991) has proposed an objective measure of taxonomic diversity for prioritizing conservation efforts. The measure they promote contains information on both taxonomic rank (phylogenetic position) and species diversity. Stiassny (1994) has suggested how the relative position of taxa in a clade can be used to identify geographic areas of high priority for conservation.

Chelydra serpentina is the sole survivor of a genus with a long fossil history in the Tertiary, and *Chelydra* is considered the basal clade among three monotypic genera of Gaffney's (1975) Chelydridae. In addition, Gaffney (1984*a*, 1984*b*) has hypothesized that the Chelydridae is the basal clade among extant cryptodires, a subordinal-level group accounting for approximately 80% of living turtle species.

The common snapping turtle is exemplary of a group of long–lived vertebrates in which life-history parameters constrain populations from responding to sustained harvesting of adults and juveniles (Congdon et al. 1994). In spite of a long history of harvesting over the species range, *C. serpentina* is still a prominent component of many North American freshwater ecosystems. *Chelydra serpentina* has one of the greatest latitudinal ranges of any New World reptile, distributed nearly continuously from southern Canada through the eastern two-thirds of the United States and from southeastern Mexico to pacific Ecuador (Iverson 1992). It has also been introduced into the western United States. Four subspecies are recognized in all recent taxonomic references (Wermuth & Mertens 1977; Pritchard 1979; Gibbons et al. 1988; Iverson 1992), distributed as follows: *C. s. serpentina*, of Canada and the eastern United States; *C. s. osceola*, of peninsular Florida; *C. s. rossignonii*, distributed from southeastern Mexico to Honduras; and *C. s. acutirostris*, ranging from the Honduras-Nicaragua border region to Ecuador (Feuer 1966, Gibbons et al. 1988, Iverson 1992). The range of *Chelydra s. serpentina* is geographically separated from that of *C. s. rossignonii* by a gap extending from southern Texas through northeastern Mexico. *Chelydra s. osceola* and *C. s. serpentina* are known to intergrade in northern Florida (Feuer 1971). Most of Central America is poorly collected, so any possible gap between the ranges of *C. s. rossignonii* and *C. s. acutirostris* remains undocumented (Feuer 1966). All four subspecies bear a strong resemblance to each other, the distinguishing characteristics being subtle shell morphometric parameters, epidermal tuberculation, and skull features.

Other authors have suggested that the subspecies are barely worth recognition and that they should be viewed as merely local demes or populations of the widespread *C. serpentina* (Carr 1952; Medem 1977). This view was supported by Frair (1972), who did not detect any electrophoretic mobility differences in serum proteins from the two North American subspecies, although his Figure 1 indicates some differences between the proteins of the North American and Central American subspecies. At the other extreme, Richmond (1958) and Ernst and Barbour (1972) suggested that one of the subspecies, *C. s. osceola* warranted full species recognition.

In contrast to the wide distribution and abundance of the North American subspecies, the Central American

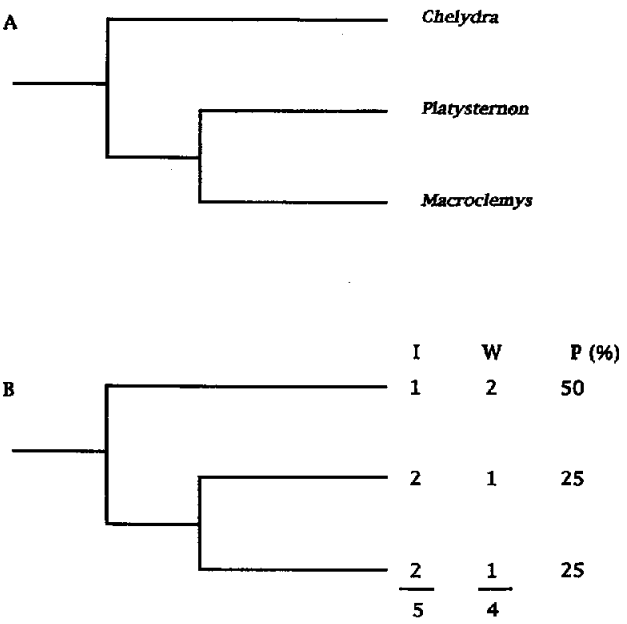

Figure 1. Hypothesized phylogenetic relationships among the three monotypic genera of the Chelydridae (Gaffney, 1975) (A) and the topology in A *annotated with taxonomic information content (I), standardized taxonomic weight (W), and the percentage (P) contribution of each W to the total cladogram's taxonomic weight (Vane-Wright et al., 1991) (B). I equals the number of taxonomic groups to which each terminal taxon belongs. W is calculated by dividing the total cladogram's information content by individual I values, then standardizing these weights by dividing by the lowest weight so that the smallest W equals one. P values may not sum to 100% due to rounding error.*

subspecies appear more restricted in these respects. Several workers have commented on the scant distribution records and restricted range in countries from Mexico to Panama (Schmidt 1946; Duellman 1963; Smith & Smith 1980; Moll & Dodd 1985; R.C. Vogt, personal communication), although Medem (1977) remarked that the species was "not uncommon" in Pacific Colombia. Given that so little is known of population sizes, ecology, or even distribution of the Neotropical subspecies of *C. serpentina*, not much commentary on the conservation status of those populations is possible, but the taxonomic status of this polytypic species is relevant to the setting of conservation priorities.

The purpose of our study is a preliminary assessment of genetic divergence of mtDNA and allozymes among population samples from the four currently recognized subspecies of the common snapping turtle. Hedrick and Miller (1992) specifically suggested that these two molecular genetic techniques may complement one another in investigations of population differentiation. Our goal is to evaluate the current taxonomy in light of these genetic data and, in turn, to determine the significance of this taxonomy for setting conservation priorities.

Methods

Material Examined

Institutional abbreviations for voucher specimens follow the recommendation of Leviton et al. (1985). The letter in parentheses refers to the specimen samples in Tables 1, 3, 4. *Chelydra serpentina acutirostris*. Ecuador: Esmeraldas, Concepcion, Finca la Esperanza, USNM 281873 (A), USNM 281874 (B). *Chelydra serpentina rossignonii*. Mexico: Veracruz, vicinity of Tlacotalpan, USNM 291913 (C); vicinity of Los Tuxtlas Mountains, USNM 291914 (D). *Chelydra serpentina osceola*. Florida: Orange County, 2 miles N Oviedo, USNM 322767 (E); Seminole County, 4 km NE Oviedo, CM 129873 (H), Lake Jessop, CM 129874 (I); Dixie County, 4 km NW Shamrock, CM129875 (J). Pet trade, TCWC 70359 (F). *Chelydra serpentina serpentina*. Illinois: Union County, Shawnee National Forest LaRue-Pine Hills Ecological Area, Otter Pond, USNM 322768 (L); Wabash County, near Mt. Carmel, no specimen (M); Williamson County, no specimen (N); White County, Norris City, no specimen (Q), Norris City Reservoir, INHS 10758 (R), INHS 10759 (S). Missouri: Mississippi County, 1.6 km SW Illinois state line on route 60-62, INHS 10760 (K). Oklahoma: Cleveland County, pond on University of Oklahoma campus TCWC 69619 (O), TCWC 69651 (P). Pet trade, TCWC 70360 (G).

Mitochondrial DNA

Total genomic DNA was extracted from 19 snapping turtles that included representatives from the geographic range of each of the four commonly recognized subspecies (Table 1). The DNA was digested with the following 16 hexanucleotide-recognizing restriction enzymes: *Apa*I, *Apa*LI, *Bam*HI, *Bc*II, *Bgl*II, *Eco*RI, *Hin*dIII, *Hpa*I, *Kpn*I, *Nde*I, *Pst*I, *Pvu*II, *Sca*I, *Stu*I, *Xba*I, *Xmn*I. Fragments of the digested DNA were separated by electrophoresis through agarose and transferred to a support membrane by Southern blotting (Southern 1975). The

Table 1. Collecting locality and composite haplotypes for the 19 *C. serpentina* sampled for mtDNA.

Sample	*Subspecies*	*Location*	*Composite haplotype**
A	*C. s. acutirostris*	Ecuador: Esmeraldas	AAAAAAAAAAAAAAA
B	*C. s. acutirostris*	Ecuador: Esmeraldas	ABAAAABAAAAABBAA
C	*C. s. rossignonii*	Mexico: Veracruz	AAABBBABABAACAAB
D	*C. s. rossignonii*	Mexico: Veracruz	ACABBBCCABAADCAB
E	*C. s. osceola*	FL: Orange Co.	ADBCCCBDBCABEBBC
F	*C. s. osceola*	Pet Trade	ADBCCCBDBCABEBBC
G	*C. s. serpentina*	Pet Trade	ADBCCCBDBCABFBBC
H	*C. s. osceola*	FL: Seminole Co.	BDBCCCBDBCABEBBC
I	*C. s. osceola*	FL: Seminole Co.	ADBCCCBDBCABEBBC
J	*C. s. osceola*	FL: Dixie Co.	ADBCCCBDBDABEBBC
K	*C. s. serpentina*	MO: Mississippi Co.	ADBCCCBABCABEBCC
L	*C. s. serpentina*	IL: Union Co	ADBCCCBDBCABEBCC
M	*C. s. serpentina*	IL: Wabash Co	ADBCCCDDBCABEBCC
N	*C. s. serpentina*	IL: Williamson Co.	ADBCCCDDBCABEBCC
O	*C. s. serpentina*	OK: Cleveland Co.	ADBCCCBDBCABEBCC
P	*C. s. serpentina*	OK: Cleveland Co.	ADBCCCBDBCABEBCC
Q	*C. s. serpentina*	IL: White Co	ADBCCCDDBCABEBCC
R	*C. s. serpentina*	IL: White Co.	ADBCCCBDBCABEBBC
S	*C. s. serpentina*	IL: White Co.	ADBCCCBDBCABEBBC

**Each letter in the haplotype represents a different restriction pattern for the following restriction enzymes:* Apa*I*, Apa*LI*, Bam*HI*, Bc*II*, Bg*III*, Eco*RI*, Hind*III*, Hpa*I*, Kpn*I*, Nde*I*, Pst*I*, Pvu*II*, Sca*I*, Stu*I*, Xba*I*, Xmn*I*.*

blots were hybridized with radioactively labeled mtDNA of *C. serpentina* that was cloned into pUC-19 (NEB, Beverly, Massachusettes). The DNA was visualized by means of autoradiography. Specific details of these techniques can be found in Hillis and Davis (1986).

The resulting fragment patterns were analyzed using the computer program RESTSITE (Miller 1990). Percent nucleotide sequence divergence was estimated for all pairs of individuals using the fragment approach (Nei & Li 1979), and standard errors were calculated via jackknifing. Restriction sites were inferred from fragment patterns for all samples of *C. s serpentina* and *C. s. osceola*.

Allozymes

Muscle and liver tissues were dissected from each specimen. Each tissue sample was immersed in an equal volume of chilled 0.01 M Trizma base, pH = 7.0 and disrupted by a high-speed mechanical homogenizer. Homogenates were centrifuged for 20 minutes at 5° C. Supernatant was collected and stored at −78° C. Supernatant fractions were absorbed onto wicks of Whatman no. 3 filter paper and subjected to electrophoresis in 12.5% horizontal starch gels. One of five buffer systems was used to resolve the products of 27 enzyme loci (Table 2). Enzyme names and numbers are those recommended by the International Union of Biochemists (1984), except for peptidases, which are identified by substrate. Staining protocols were modified from Selander et al. (1971), Harris and Hopkinson (1978), and Buth and Murphy (1980). Alleles at each locus were assigned alphabetical designations, with the most anodally migrating electromorph designated as allele *a*.

Results

The 16 restriction enzymes produced an average of 56 fragments per individual. A total of 107 different fragments was observed, 17 of which were found in all 19 individuals. The 90 variable fragments define 11 different haplotypes that fall into three main groups (Table 1). The mtDNAs of the nominate subspecies and *C. s. osceola* are similar, differing at only three restriction fragments (equal to one restriction site). In contrast, *C. s.*

Table 2. Allozyme loci surveyed for 19 specimens of *C. serpentina*.

Enzyme	*Enzyme commission number*	*Locus*	*Buffer system**
Adenylate kinase	2.7.4.3	Ak	C
Adenosine deaminase	3.5.4.4	Ada	H
Aspartate aminotransferase	2.6.1.1	M-Aat	B,J
Aspartate aminotransferase	2.6.1.1	S-Aat	B,J
Creatine kinase	2.7.3.2	Ck-A	C
Esterase			
substrate			
alpha-napthyl acetate	——	Est	F
Fumarate hydratase	4.2.1.2	Fum	C
Glucose-6-phosphate dehydrogenase	1.2.1.49	G6pdh	A
Glucose-6-phosphate isomerase	5.3.1.9	Gpi-A	G,F
Isocitrate dehydrogenase	1.1.1.42	M-Icdh	B
Isocitrate dehydrogenase	1.1.1.42	S-Icdh	B
Lactate dehydrogenase	1.1.1.27	Ldh-A	C,E
Lactate dehydrogenase	1.1.1.27	Ldh-B	C,E
Malate dehydrogenase	1.1.1.37	M-Mdh	H
Malate dehydrogenase	1.1.1.37	S-Mdh	H
Malate dehydrogenase (NADP)	1.1.1.40	M-Me	E
Malate dehydrogenase (NADP)	1.1.1.40	S-Me	E
Mannose-6-phosphate isomerase	5.3.1.8	Mpi	A
Peptidases			
substrates			
L-phenylalanyl-L-proline	——	Pep-1	D,E
L-leucyl-L-alanine	——	Pep-2	D,G
L-Leucylglycylglycine	——	Pep-3	D
Phosphogluconate dehydrogenase	1.1.1.44	Pgdh	B
Phosphoglucomutase	5.4.2.2	Pgm	I
Purine nucleoside phosphorylase	2.4.2.1	Pnp	C,E
Superoxide dismutase	1.15.1.1	Sod-1	A
Superoxide dismutase	1.15.1.1	Sod-2	A,G
Triose-phosphate isomerase	5.3.1.1	Tpi	A,G

**A: amine-citrate (Clayton & Tretiak 1972); B: tris-citrate I; C: tris-citrate II; D: tris-HCl; E: tris-maleate; F: lithium hydroxide; G: Poulik (B-G, Selander et al. 1971); H: tris-citrate (Whitt 1970); I: Tris-borate (Ridgeway et al. 1970); J: Tris-borate (Wilson et al. 1973).*

rossignonii differs from *C. s. acutirostris* by a minimum of 10 restriction fragments, and these two differ from *C. s. serpentina* and *C. s. osceola* by a minimum of 45 restriction fragments. The percentage of sequence divergence and number of fragment differences between all pairs of individuals are given in Table 3.

Nineteen of 27 loci were monomorphic. Genotypic arrays for the eight polymorphic loci are presented in Table 4. The genetic diversity for seven of the eight polymorphic loci is characterized by the presence of rare alleles in only one or two specimens. Even though sample sizes are acknowledged as small, the limited degree of genetic differentiation among the North American samples suggests that further studies of allozymic variation for the Central and South American populations may not be warranted.

Discussion

Boulenger (1902) and Schmidt (1946) listed six morphological characters that distinguish the North American from the Central American subspecies of *Chelydra serpentina*. In contrast, Feuer (1966) hypothesized that *C. s. serpentina* and *C. s. acutirostris* were closely related to each other. He also suggested a close relationship between *C. s. rossignonii* and *C. s. osceola*. These conclusions were based on shared osteological features and characteristics of the gular barbels and neck tuberculation. Medem (1977) reviewed most of these morphological characters and concluded that some of them were too variable to be considered diagnostic. The mitochondrial data presented here support a *rossignonii-acutirostris* pair distinctive from a *serpentina-osceola* pair. Although the sample size is small, the present data also support the separation of *C. s. rossignonii* from *C. s. acutirostris*.

The distinctiveness of the *C. s. rossignonii* and *C. s. acutirostris* mtDNA from that of the other two subspecies is accentuated by the similarity of the mtDNA haplotypes among the North American samples, which is surprisingly high in view of the geographic distance between collection sites. Estimates of nucleotide sequence divergence among all of the North American specimens ranges from 0 to 0.5% (Table 3), with standard errors as large as the estimates in most cases. In comparison, the minimum divergence between *C. s. acutirostris* and *C. s. rossignonii* is 1.7% and that between either Central American subspecies and the North American subspecies averages 4.5%. This is a surprising degree of differentiation given the putative conspecificity of these four subspecies. Bowen et al. (1991) and Avise et al. (1992) presented evidence for a variety of turtle taxa suggesting a mtDNA divergence rate of 0.2–0.4% per million years, compared to the conventional expectation of 2% divergence per million years between a pair of lineages (Brown et al. 1979). If this slower mtDNA divergence rate were assumed in a comparison among specimens of *C. serpentina*, then the minimum 1.7% divergence evidence between *C. s. rossignonii* and *C. s. acutirostris* predicts a time since these groups shared a common ancestor of 4.25–8.5 million years and a time of divergence between the North and Central American subspecies pairs of 11.125–22.25 million years (4.45% mean divergence). Although tentative, these divergence time estimates are suggestive of

Table 3. Percentage of sequence divergence (above diagonal) and number of restriction-fragment differences (below diagonal) between all pairs of individuals.*

Sample	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*	*N*	*O*	*P*	*Q*	*R*	*S*
A		0.6	1.7	2.4	4.1	4.1	4.3	4.1	4.1	4.0	3.6	3.9	4.2	4.2	3.9	3.9	4.2	4.2	4.2
B	10		2.4	2.6	3.5	3.6	3.7	3.5	3.5	3.4	3.1	3.4	3.6	3.6	3.4	3.4	3.6	3.6	3.6
C	25	33		0.9	5.0	5.1	5.3	5.0	5.0	5.0	4.5	4.8	5.1	5.1	4.8	4.8	5.1	5.1	5.1
D	33	35	14		5.3	5.4	5.6	5.3	5.3	5.3	4.8	5.1	5.4	5.4	5.1	5.1	5.4	5.4	5.4
E	53	49	60	62		0.1	0.3	0.0	0.0	0.1	0.3	0.1	0.3	0.3	0.1	0.1	0.3	0.3	0.3
F	54	50	61	63	3		0.5	0.1	0.1	0.3	0.5	0.3	0.4	0.4	0.3	0.3	0.4	0.4	0.4
G	55	51	62	64	6	9		0.3	0.3	0.5	0.3	0.1	0.3	0.3	0.1	0.1	0.3	0.3	0.3
H	53	49	60	62	0	3	6		0.0	0.1	0.3	0.1	0.3	0.3	0.1	0.1	0.3	0.3	0.3
I	53	49	60	62	0	3	6	0		0.1	0.3	0.1	0.3	0.3	0.1	0.1	0.3	0.3	0.3
J	52	48	59	61	3	6	9	3	3		0.5	0.3	0.5	0.5	0.3	0.3	0.5	0.5	0.5
K	49	45	56	58	6	9	6	6	6	9		0.1	0.3	0.3	0.1	0.1	0.3	0.3	0.3
L	52	48	59	61	3	6	3	3	3	6	3		0.1	0.1	0.0	0.0	0.1	0.1	0.1
M	55	51	62	64	6	9	6	6	6	9	6	3		0.0	0.1	0.1	0.0	0.0	0.0
N	55	51	62	64	6	9	6	6	6	9	6	3	0		0.1	0.1	0.0	0.0	0.0
O	52	48	59	61	3	6	3	3	3	6	3	0	3	3		0.0	0.1	0.1	0.1
P	52	48	59	61	3	6	3	3	3	6	3	0	3	3	0		0.1	0.1	0.1
Q	55	51	62	64	6	9	6	6	6	9	6	3	0	0	3	3		0.0	0.0
R	55	51	62	64	6	9	6	6	6	9	6	3	0	0	3	3	0		0.0
S	55	51	62	64	6	9	6	6	6	9	6	3	0	0	3	3	0	0	

**Sample localities are described in Table 1.*

Table 4. Genotypic arrays for eight polymorphic loci sampled among 19 specimens of *C. serpentina*.

*Sample**	*Taxon*	*Ak*	*a-Est*	*Gpi-A*	*M-Icdh*	*S-Icdh*	*Pep-1*	*Pnp*	*Tpi*
A	*C. s. acutirostris*	b/b	b/b	a/b	b/b	b/b	b/b	b/b	b/b
B	*C. s. acutirostris*	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b
C	*C. s. rossignonii*	b/b	b/b	b/b	a/a	a/a	b/b	a/b	b/b
D	*C. s. rossignonii*	b/b	b/b	b/b	a/a	a/a	b/b	b/b	b/b
E	*C. s. osceola*	b/b	—	b/b	a/a	a/a	b/b	b/b	b/b
F	*C. s. osceola*	b/b	—	b/b	a/a	a/a	b/b	a/a	b/b
G	*C. s. serpentina*	b/b	b/b	b/b	a/a	a/a	b/b	a/b	b/b
H	*C. s. osceola*	b/b	b/b	b/b	a/a	a/a	b/b	b/b	b/b
I	*C. s. osceola*	—	a/a	b/c	a/a	a/a	b/b	b/b	b/b
J	*C. s. osceola*	b/b	b/b	b/b	a/a	a/a	b/b	b/b	b/b
K	*C. s. serpentina*	b/b	b/b	b/b	a/a	a/a	b/b	a/a	b/b
L	*C. s. serpentina*	a/b	b/b	b/b	a/a	a/a	b/b	a/a	b/b
M	*C. s. serpentina*	b/b	b/b	b/b	a/a	a/a	b/b	a/b	b/b
N	*C. s. serpentina*	b/b	b/b	b/b	a/a	a/a	b/b	a/a	b/b
O	*C. s. serpentina*	b/b	b/b	b/b	a/a	a/a	a/a	a/b	a/b
P	*C. s. serpentina*	b/b	b/b	b/b	a/a	a/a	a/a	a/b	b/b
Q	*C. s. serpentina*	b/b	b/b	b/b	a/a	a/a	b/b	a/a	b/b
R	*C. s. serpentina*	b/b	b/b	b/b	a/a	a/a	b/b	a/b	b/b
S	*C. s. serpentina*	b/b	b/b	b/b	a/a	a/a	b/b	a/b	b/b

**Sample localities are described in Table 1.*

an extraordinarily long separation among lineages of a supposed single species.

It has been noted that divergences based on mtDNA are not always in agreement with those based on other characters such as nuclear DNA or morphological measurements. Two explanations have been given for disagreement between mtDNA gene trees and species trees. First, discordance can arise from differential introgression of a foreign mtDNA type following hybridization; second, it can result in differential retention of ancestral mtDNA polymorphisms—in other words lineage sorting (Nigel & Avise 1985). Because there are no known taxa with which *C. serpentina* can hybridize, the first explanation can be ruled out as a source of the mtDNA divergence observed among the different subspecies of snapping turtles. Lineage sorting could be responsible for the observed divergences, but it requires that the ancestral *C. serpentina* population had intrapopulational sequence divergence of at least 5.6% (the maximum observed in this study). This level of variation is unlikely given the general slowdown in rates of mtDNA evolution reported for other turtle species (Avise et al. 1992) and is closer to interpopulational and interregional divergences cited in the same paper.

In contrast, the allozyme data do not diagnose any of the four named subspecies. The failure to discover significant allozymic variation among the four groups of *Chelydra* studied here is similar to Seidel and Lucchino's (1981) study of the North American aquatic turtles *Sternotherus minor, S. carinatus*, and *S. depressus*. They did not discover any unique allozyme alleles for either *S. carinatus* or *S. depressus*, even though these species could be diagnosed morphologically. Seidel et al. (1981) studied the aforementioned three species of *Sternotherus* and *S. odoratus* for 25 presumptive genetic loci, 18 of which were polymorphic. They did not discover any unique alleles for *S. depressus* and reported fixed differences among the four species only for *S. odoratus*.

The mitochondrial and allozyme data reported here do not distinguish the peninsular Florida populations from other North American populations. Richmond (1958), Feuer (1966), and Medem (1977) identified morphological differences between *C. s. serpentina* and *C. s. osceola* that should not be dismissed. But when one of us (J. L. Carr) attempted to evaluate some of the superficial anatomical characters, definition of some features presented problems, and there is no description of ontogenetic variation for these features. Further understanding of the relationship between the nominal North American taxa of *C. serpentina* will require more-extensive studies of variation for the "diagnostic" features and more-intensive studies of genetic variation within the species' extensive range and across the potential zone of intergradation identified by Feuer (1971).

Given our genetic data, we advocate the elevation of the two Neotropical subspecies of *C. serpentina* to full species status. The North American *C. serpentina* is allopatric to both Neotropical taxa, and there is a high level of mtDNA divergence across the North-Central American gap. The two Neotropical forms are also distinctly different in their mtDNA makeup, but their ranges are too poorly known to conclude whether or not there is a distributional gap separating them or if they are parapatrically distributed (Feuer 1966). We believe that recognition of both *C. rossignonii* and *C. acutirostris* is the most informative taxonomic hypothesis possible at this time. Our data do not illuminate genetic relationships

between the nominate form and *C.* s. *osceola* so we suggest continued use of *C. s. serpentina* and *C. s. osceola* for the common and Florida snapping turtles respectively.

The recognition of three snapping turtle species where there was previously one illustrates the implications of taxonomy for conservation efforts. As suggested by Collins (1991) and Lovich and Gibbons (1996), the use of trinomens can "hide" the actual species-level diversity within a higher taxon, diminishing the potential evolutionary and ecological significance of distinctive biotic entities. In the case of *Chelydra*, subspecies allocations disguised the significance of two tropical snapping turtle species and their contribution to overall genetic diversity. Because so many conservation-related activities are centered on the species, the distinctive tropical snappers may have been neglected, as was the fate of unacknowledged *Sphenodon* taxa (Daugherty et al. 1990). In addition, recognition of three *Chelydra* species affects the taxonomic diversity index, one measure proposed for setting conservation priorities (Vane-Wright et al. 1991).

The measure proposed by Vane-Wright et al. (1991) incorporates information on both species diversity and taxonomic position in a cladistic hypothesis of relationships. Gaffney (1975) has hypothesized cladistic relationships among the three genera of the family Chelydridae (Fig. 1a). At the time, the three genera were each considered monospecific (*Chelydra serpentina, Macroclemys temmincki*, and *Platysternon megacephalum*). Given this cladogram and only three species, *Chelydra* (the genus and the species) contributes 50% of the total diversity in the family (Fig. 1b). With the new conception that includes three species of *Chelydra* as an unresolved trichotomy (Fig. 2a), the total information content of the cladogram is minimally doubled (from 5 to 10), and *Chelydra* (the three species together) contributes 60% of total family diversity (Fig. 2b). A hypothetical situation in which the cladogram for the family has been completely resolved is illustrated in Fig. 2c, with the attendant consequences for the taxonomic index indicated in Fig. 2d. In this case, as would be the case whenever species are added to a basal clade, the increased diversity has changed the diversity component of the index in such a way that the percentage contribution to total diversity of each terminal taxon is diminished from 25–50% down to 20% (Fig. 2b), but the basal clade as a whole (*Chelydra*) has increased its share of total diversity from 50% up to as much as 60% (Fig. 2b). The same should be true if indices are calculated at the family level for all cryptodires; the value for the Chelydridae will increase compared to that of the other families when individual species values are summed. Haiduk and Bickham (1982) hypothesized that *Platysternon* was not a member of the Chelydridae, leaving the family with only two genera. This alternative hypothesis does

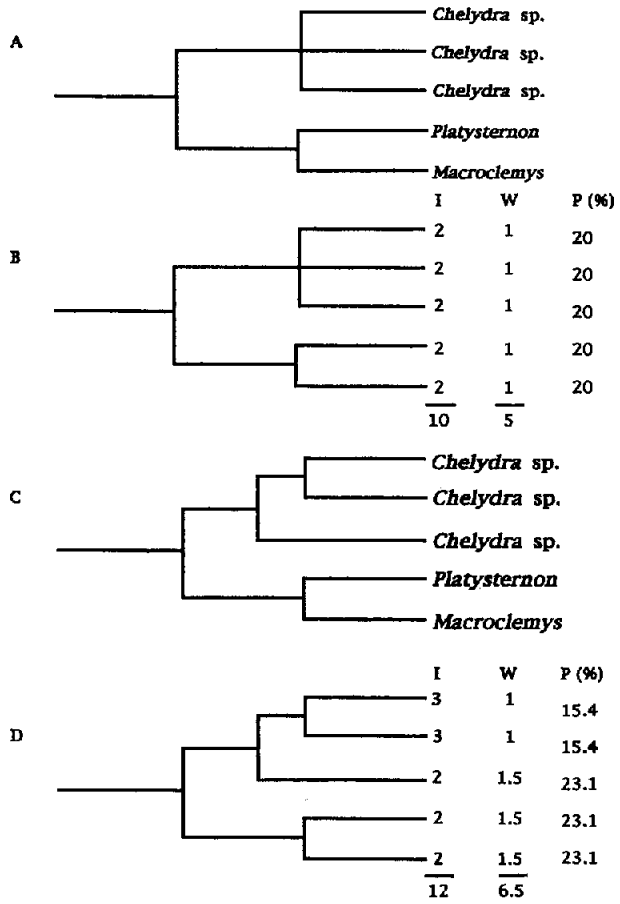

Figure 2. Phylogenetic relationships among the genera of the Chelydridae, with the addition of two species of Chelydra *but with the relationships among the species of* Chelydra *unresolved (A); the topolgy in A annotated with the taxonomic information content (I), the standardized taxonomic weight (W), and the percentage (P) contribution of each standardized taxonomic weight (B); phylogenetic relationships among the genera of the Chelydridae with three species of* Chelydra, *and with the relationships among the species of* Chelydra *resolved (C) (The topology of the species of* Chelydra *is for illustrative purposes only, and we do not endorse this topology as a hypothesis of relationships.); and the topology in C annotated with I, W, and P (D). See Fig. 1 legend for an explanation of the values I, W, and P.*

not change the values of this taxonomic index for *Chelydra* or any of the three species, but the index for *Macroclemys* doubles.

Chelydra serpentina is an example of a widespread polytypic species that is not presently considered of urgent conservation concern (World Conservation Union 1991). Examination of mtDNA from the four nominal subspecies revealed a pattern of genetic differentiation indicative of a multi-species complex. We consider the

recognition of three snapping turtle species to reflect diversity more accurately in the genus *Chelydra*. Now a single, widespread species remains, in North America, but there are two Neotropical species with relatively small ranges. Because *Chelydra* represents the basal clade among the Chelydridae, which is in turn the basal clade among all extant crytodirous turtles (Gaffney 1975, 1984*a*, 1984*b*), the taxonomy within the genus is influential in quantitative measures of taxonomic diversity proposed for use in setting biodiversity conservation priorities (Vane-Wright et al. 1991). The changed taxonomy of *Chelydra* decreases the significance of the individual species but increases the overall generic and familial values. Such measures may become increasingly useful to those trying to evaluate the thoroughness of biodiversity inclusion in protected areas (for examples, see Forey et al. 1994). Increasingly refined species taxonomies have an important role to play as the basis from which these efforts build.

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