Copeia, 1986(2), pp. 285–294

Biochemical Comparisons and Phylogenetic Relationships in the Family Kinosternidae (Testudines)

MICHAEL E. SEIDEL, JOHN B. IVERSON AND M. DALE ADKINS

Electrophoretic relationships among 18 species, representing all four genera of kinosternid turtles, were defined by phenetic and cladistic analyses. Relatively little protein variation was observed among most species of *Kinosternon* from Mexico and Central America, except *K. sonoriense* and *K. leucostomum*. *K. bauri* and *K. subrubrum*, which inhabit the eastern United States, do not appear to be sister taxa to other *Kinosternon* species. Biochemical results, supported by previous karyotypic data, indicate that *K. bauri* and *K. subrubrum* are more closely allied to *Sternotherus* than to congeners; and that *Kinosternon* is a paraphyletic taxon. Electromorphic data support the thesis that Kinosterninae is a monophyletic subfamily divergent from *Claudius* and *Staurotypus*.

THE New World family Kinosternidae (Gray) is an assemblage of four genera: *Claudius* Cope and *Staurotypus* Wagler in the subfamily Staurotypinae and *Sternotherus* Gray and *Kinosternon* Spix in the Kinosterninae. The two species of *Staurotypus* (*S. salvini, S. triporcatus*) and the monotypic *Claudius angustatus* are highly aquatic inhabitants of Mexico and northern Central America. *Sternotherus* contains four aquatic species (*S. carinatus, S. depressus, S. minor, S. odoratus*) distributed over eastern United States. The highly polytypic genus *Kinosternon* is partitioned into 15 species (*K. acutum, K. alamosae, K. angustipons, K. bauri, K. creaseri, K. dunni, K. flavescens, K. herrerai, K. hirtipes, K. integrum, K. leucostomum, K. oaxacae, K. scorpioides, K. sonoriense, K. subrubrum*), which inhabit a variety of mesic to xeric environments from central South America throughout most of Central America and the United States. Two *Kinosternon* species (*K. bauri* and *K. subrubrum*) inhabit eastern United States in broad sympatry with *Sternotherus* and at least three species (*K. scorpioides, K. leucostomum* and *K. acutum*) are sympatric with the staurotypines in Mexico, Belize and Guatemala.

During the past decade morphological relationships have been extensively studied in the genus *Kinosternon* by Iverson (1978, 1979, 1981), Iverson and Berry (1979), Berry (1978), Berry and Iverson (1980), Berry and Legler (1980) and in *Sternotherus* by Iverson (1977) and Seidel and Lucchino (1981). Crenshaw (1962) and Frair (1972) presented serological comparisons among kinosternids and karyotypic relationships were analyzed by Bull et al. (1974), Killebrew (1975) and Sites et al. (1979). Seidel et al. (1981) described phylogenetic relationships in *Sternotherus* based on electrophoretic data. Hutchinson and Bramble (1981) and more recently Bramble et al. (1984) constructed a cladogram of kinosternid genera based on scute homologies and plastral kinesis. Here we analyze electrophoretic data both cladistically and phenetically (as in Sites et al., 1984) to test their proposed phylogeny and further define specific and intergeneric relationships within the Kinosternidae. We proceed on the assumption that Kinosternidae is a monophyletic family based on cranial (Gaffney, 1975, 1984) and plastral scute (Hutchinson and Bramble, 1981) homologies.

MATERIALS AND METHODS

Two hundred and ninety-seven turtles, representing 18 species and all four genera of Kinosternidae, were collected (mostly by JBI) from the United States, Mexico, Guatemala, Belize, Honduras, El Salvador, Panama and Guyana. Turtles were killed with chloroform and tissues were immediately removed and stored at −60 C until analyzed. Carcasses were prepared as skeletons or fluid preserved and deposited in the Florida State Museum, or in collections of J. B. Iverson or M. E. Seidel.

Extracts were prepared from heart, skeletal muscle, liver and kidney in Tris-EDTA-NADP buffer (pH 6.8) or distilled water following Selander et al. (1971). For horizontal starch-gel electrophoresis, 13 protein systems were analyzed following the technique of Selander et al. (1971), but with buffer modifications of Seidel and Lucchino (1981) and Seidel et al. (1981). Electrophoretic migrations were compared to

TABLE 1. ELECTROMORPH (ALLELE) FREQUENCIES FOR POLYMORPHIC LOCI IN KINOSTERNIDAE. Sample sizes are in parentheses. Electromorphs are designated a–d in order of decreasing anodal mobility. Gel buffer and biochemical staining procedures follow Selander et al. (1971), with modifications of Seidel and Lucchino (1981) and Seidel et al. (1981). Enzymes examined, corresponding Enzyme Commission numbers in parentheses (Nomenclatural Committee of the International Union of Biochemistry, 1979) and loci are as follows: malate dehydrogenase—supernatant (1.1.1.37) S-Mdh; isocitrate dehydrogenase (1.1.1.42) Icdh; phosphogluconate dehydrogenase (1.1.1.44) Pgdh; glycerol-3-phosphate dehydrogenase (1.2.1.2) G-3-pdh; glucosephosphate isomerase (5.3.1.9) Gpi; superoxide dismutase (1.15.1.1) M-Sod-A; aspartate aminotransferase (2.6.1.1) M-Aat-A; lactate dehydrogenase (1.1.1.27) Ldh-B; phosphoglucomutase (2.7.5.1) Pgm; xanthine dehydrogenase (1.2.1.37) Xdh-A; glutamate dehydrogenase (1.4.1.2) Gtdh-A.

Locus	Electromorph	Character	*Kinosternon bauri*	*K. subrubrum*	*K. herrerai*	*K. hirtipes*	*K. sonoriense*	*K. flavescens*	*K. alamosae*	*K. integrum*	*K. scorpioides*	*K. oaxacae*	*K. acutum*	*K. leucostomum*	*Staurotypus triporcatus*	*Claudius angustatus*	*Sternotherus odoratus*	*S. carinatus*	*S. depressus*	*S. minor*
S-Mdh			(9)	(4)	(5)	(31)	(5)	(34)	(5)	(51)	(20)	(7)	(1)	(19)	(6)	(1)	(45)	(12)	(10)	(20)
	a	1	0.78	—	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—	0.50	0.50	0.50
	b	2	0.22	1.00	—	—	—	—	—	—	—	—	—	—	—	—	1.00	0.50	0.50	0.23
	c	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.27
Icdh			(6)	(4)	(10)	(29)	(5)	(24)	(5)	(49)	(20)	(8)	(1)	(17)	(2)	(1)	(45)	(12)	(10)	(20)
	a	4	—	—	—	—	—	—	—	—	—	—	—	—	—	1.00	0.39	—	—	—
	b	5	1.00	0.50	0.50	0.50	0.80	0.56	0.50	0.51	0.50	0.50	0.50	0.68	1.00	—	0.61	—	—	—
	c	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.00	1.00	1.00
	d	7	—	0.50	0.50	0.50	0.20	0.44	0.50	0.49	0.50	0.50	0.50	0.32	—	—	—	—	—	—
Pgdh			(2)	(4)	(9)	(31)	(4)	(34)	(5)	(51)	(20)	(6)	(1)	(18)	(6)	(1)	(45)	(12)	(10)	(20)
	a	8	—	—	—	—	—	0.02	—	—	—	—	—	—	—	—	—	—	—	—
	b	9	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
G-3-pdh			(9)	(3)	(9)	(26)	(5)	(33)	(3)	(45)	(18)	(7)	(1)	(16)	(4)	(1)	(45)	(12)	(10)	(20)
	a	10	—	—	—	0.15	0.40	—	—	—	—	—	—	—	—	—	0.01	0.50	1.00	1.00
	b	11	—	—	0.72	0.65	0.30	0.73	0.50	0.69	0.94	1.00	1.00	0.78	0.50	1.00	0.49	0.50	—	—
	c	12	1.00	1.00	0.28	0.20	0.30	0.27	0.50	0.31	0.06	—	—	0.22	0.50	—	0.50	—	—	—
Gpi			(8)	(4)	(6)	(29)	(3)	(29)	(5)	(48)	(16)	(1)	(1)	(15)	(2)	(1)	(45)	(12)	(10)	(20)
	a	13	0.06	—	0.50	0.50	0.67	0.48	0.50	0.50	0.59	0.50	0.50	0.37	—	0.50	—	—	—	—
	b	14	0.94	1.00	0.50	0.50	0.33	0.52	0.50	0.50	0.41	0.50	0.50	0.63	1.00	0.50	1.00	1.00	1.00	1.00
M-Sod-A			(6)	(3)	(1)	(6)	(3)	(21)	(3)	(43)	(11)	(4)	(1)	(3)	(5)	(1)	(45)	(3)	(3)	(6)
	a	15	—	—	—	—	—	—	—	—	—	—	—	—	0.40	—	—	—	—	—
	b	16	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	0.60	1.00	1.00	1.00	—	—
	c	17	—	—	—	—	—	—	—	0.02	—	—	—	—	—	—	—	—	—	—
	d	18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.00	1.00

TABLE 1. CONTINUED.

Locus	Electromorph	Character	*Kinosternon bauri*	*K. subrubrum*	*K. herrerai*	*K. hirtipes*	*K. sonoriense*	*K. flavescens*	*K. alamosae*	*K. integrum*	*K. scorpioides*	*K. oaxacae*	*K. acutum*	*K. leucostomum*	*Staurotypus triporcatus*	*Claudius angustatus*	*Sternotherus odoratus*	*S. carinatus*	*S. depressus*	*S. minor*
M-Aat-A			(9)	(4)	(8)	(31)	(5)	(33)	(5)	(51)	(20)	(7)	(1)	(19)	(6)	(1)	(45)	(12)	(10)	(20)
	a	19	—	—	—	—	—	—	—	0.18	—	—	—	—	0.83	1.00	—	—	—	—
	b	20	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.82	1.00	1.00	1.00	1.00	0.17	—	0.78	1.00	1.00	1.00
	c	21	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.22	—	—	—
Ldh-B			(9)	(4)	(9)	(31)	(5)	(32)	(5)	(49)	(20)	(6)	(1)	(18)	(6)	(1)	(45)	(12)	(10)	(20)
	a	22	—	—	—	—	—	—	—	0.02	—	—	—	1.00	—	—	—	—	—	—
	b	23	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	—	1.00	1.00	—	1.00	1.00	1.00
	c	24	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.00	—	—	—
Pgm			(5)	(3)	(9)	(17)	(4)	(27)	(3)	(43)	(12)	(6)	(1)	(17)	(4)	(1)	(45)	(12)	(10)	(20)
	a	25	—	—	—	—	—	—	—	—	—	—	—	—	1.00	1.00	—	—	—	—
	b	26	—	—	—	0.09	—	0.11	—	0.02	—	—	—	0.47	—	—	—	—	—	—
	c	27	1.00	1.00	1.00	0.91	1.00	0.89	1.00	0.98	1.00	1.00	1.00	0.53	—	—	0.98	1.00	1.00	1.00
	d	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.02	—	—	—
Xdh-A			(9)	(4)	(6)	(30)	(5)	(27)	(5)	(51)	(20)	(6)	(1)	(18)	(6)	(1)	(45)	(3)	(3)	(6)
	a	29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.50	0.50
	b	30	—	—	0.80	0.73	1.00	0.83	1.00	0.64	0.82	0.50	1.00	1.00	1.00	1.00	1.00	1.00	—	—
	c	31	1.00	1.00	0.20	0.27	—	0.17	—	0.36	0.18	0.50	—	—	—	—	—	—	0.50	0.50
Gtdh-A			(9)	(4)	(8)	(31)	(4)	(34)	(5)	(50)	(20)	(6)	(1)	(19)	(6)	(1)	(45)	(3)	(3)	(6)
	a	32	—	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—	—	—	—
	b	33	1.00	—	—	—	—	—	—	—	—	—	—	—	—	—	1.00	1.00	0.50	0.50
	c	34	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.50	0.50

K. flavescens and *S. odoratus, S. minor* or *S. carinatus* standards. Presumptive alleles were designated a, b, c, d on the basis of decreasing anodal mobility of their products (electromorphs). Genetic similarities and distances between species were described by the technique of Rogers (1972). Phenetic analysis included calculation of Rogers' genetic coefficients and complete linkage cluster analysis (BMDP1M.2; Dixon, 1981) based on Rogers' genetic distances. Cladistic analysis was accomplished by treating enzyme electromorphs (presumptive alleles) as characters with two states, present (1) or absent (0), in a taxon. These binary-coded characters were analyzed by Phylogenetic Analysis Using Parsimony (PAUP algorithm written by D. L. Swofford, Illinois Natural History Survey) which may produce multiple, completely resolved trees. Character polarities were based on out-group comparisons and trees were rooted with *Claudius, Staurotypus* or a composite outgroup of both genera.

General banding patterns (profiles) of tissue proteins were analyzed by isoelectric focusing, which provided additional data for comparisons among kinosternines. Extracts of kidney-heart, liver and skeletal muscle, prepared as in starch gel electrophoresis, were applied to ultrathin (0.1mm) polyacrylamide gels (Serva, 1983/84 catalog). Homogenates from skeletal muscle were focused in an ampholyte gradient pH 5–7 and proteins from liver and kidney-heart were focused in the pH 4–7 range. Gels were placed in a custom cooling cell (2C) and focused for 3 hr at constant power (2.0 watts, maximum 1700 volts) using an LKB 2103 power supply and Bio-Rad electrodes. Immediately after focusing, gels were fixed in trichloroacetic acid and stained with SERVA Blue W (a triphenylmethane dye). Gels were scored for presence or absence of bands and comparisons were made only among turtles analyzed on the same gel.

Results

Eleven of the 13 protein systems assayed by starch-gel electrophoresis were polymorphic (Table 1). Alcohol dehydrogenase and general protein (muscle) were monomorphic. Coefficients of genetic similarity and distance (Rogers' S and D) are presented in Table 2. Low similarities ($S < 0.65$) were found when comparing *Staurotypus* or *Claudius* to species of *Sternotherus* and eastern United States *Kinosternon* (*K. bauri* and *K. subrubrum*). Higher S values were obtained comparing *K. bauri* to *K. subrubrum* (0.83) and *Claudius* to *Staurotypus* (0.82). Other than *K. leucostomum* and possibly *K. sonoriense*, the southern United States and Central American *Kinosternon* (*K. herrerai, K. hirtipes, K. flavescens, K. alamosae, K. integrum, K. scorpioides, K. oaxacae, K. acutum*) form a homogeneous genetic complex ($S = 0.92–0.98$) which is as genetically distinct from congeners *K. bauri* and *K. subrubrum* ($S = 0.64–0.79$) as it is from *Claudius* and *Staurotypus* ($S = 0.70–0.80$). These relationships are illustrated in Fig. 1 by a phenogram based on Rogers' genetic distances. The eastern United States *Kinosternon* cluster with *Sternotherus* while *Claudius* and *Staurotypus* fall somewhat closer to Central American *Kinosternon.* Relatively large distances separate species of *Sternotherus* from each other and *K. leucostomum* from the other Central American *Kinosternon.*

It was evident that eight of the Central American *Kinosternon* (including southwestern United States) were nearly identical for those protein systems examined. For cladistic analysis a binary-coded matrix (Table 3A) was constructed by consolidating taxa sharing more than 30 electromorphic character states (and/or having high genetic similarity, $S \geq 0.92$) into single groups and analyzed by PAUP. This produced seven resolved trees from which a strict consensus tree (Rohlf, 1982) was computed (Fig. 2). Although the resulting cladogram is polytomous, *K. leucostomum, K. sonoriense* and other Central American *Kinosternon* form a monophyletic complex, similar to their phenetic clustering in Fig. 1, which does not include *K. bauri* and *K. subrubrum.* A second matrix (Table 3B) was formed by consolidating congeneric species (except *K. bauri* and *K. subrubrum*) to further analyze intergeneric relationships and phylogenetic position of the eastern U.S. *Kinosternon.* For these data PAUP produced only one cladogram (Fig. 3) which depicts a monophyletic origin for the Kinosterninae, and consistent with the phenetic results, *K. bauri* and *K. subrubrum* appear as a sister group to *Sternotherus.*

Cladistic divergence between eastern U.S. *Kinosternon* and Central American congeners (Fig. 2) results from the presence of an S-Mdh (b) synapomorphic allele (product) in the former, shared by *K. bauri, K. subrubrum* and *Sternotherus* and absence of plesiomorphs G-3-pdh (b) and Xdh-A (b) which are present in all other kinosternids except *S. minor* and *S. depressus* (Table 1). *Claudius* and *Staurotypus* are symple-

TABLE 2. GENETIC COEFFICIENTS (ROGERS, 1972) OF SIMILARITY (S) BELOW DIAGONAL AND DISTANCE (D) ABOVE DIAGONAL FOR PAIRED COMBINATIONS OF KINOSTERNID SPECIES. Calculations were based on polymorphic (Table 1) and monomorphic loci.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. *Kinosternon bauri*	—	.17	.26	.27	.26	.27	.26	.27	.29	.26	.30	.36	.36	.48	.28	.23	.31	.31
2. *K. subrubrum*	.83	—	.22	.22	.26	.23	.21	.22	.24	.21	.25	.34	.37	.45	.30	.30	.34	.35
3. *K. herrerai*	.74	.78	—	.02	.08	.02	.03	.03	.02	.04	.03	.15	.26	.24	.33	.25	.38	.38
4. *K. hirtipes*	.73	.78	.98	—	.08	.03	.04	.04	.04	.04	.05	.15	.27	.24	.34	.25	.37	.37
5. *K. sonoriense*	.74	.74	.92	.92	—	.08	.06	.10	.08	.11	.08	.17	.25	.26	.33	.24	.38	.38
6. *K. flavescens*	.73	.77	.98	.97	.92	—	.04	.04	.04	.06	.05	.13	.25	.24	.33	.26	.39	.39
7. *K. alamosae*	.74	.79	.97	.96	.94	.96	—	.06	.05	.07	.04	.15	.23	.24	.30	.24	.39	.39
8. *K. integrum*	.73	.78	.97	.96	.90	.96	.94	—	.05	.05	.07	.17	.26	.24	.34	.28	.39	.38
9. *K. scorpioides*	.71	.76	.98	.96	.92	.96	.95	.95	—	.03	.02	.16	.28	.23	.35	.26	.39	.39
10. *K. oaxacae*	.74	.79	.96	.96	.89	.94	.93	.95	.97	—	.04	.18	.30	.24	.37	.28	.37	.37
11. *K. acutum*	.70	.75	.97	.95	.92	.95	.96	.93	.98	.96	—	.14	.27	.20	.34	.24	.40	.40
12. *K. leucostomum*	.64	.66	.85	.85	.83	.87	.85	.83	.84	.82	.86	—	.29	.29	.34	.33	.49	.49
13. *Staurotypus triporcatus*	.64	.63	.74	.73	.75	.75	.77	.74	.72	.70	.73	.71	—	.18	.40	.37	.49	.48
14. *Claudius angustatus*	.52	.55	.76	.76	.74	.76	.76	.76	.77	.76	.80	.71	.82	—	.47	.39	.55	.55
15. *Sternotherus odoratus*	.72	.70	.67	.66	.67	.67	.70	.66	.65	.63	.66	.66	.60	.53	—	.22	.42	.43
16. *S. carinatus*	.77	.70	.75	.75	.76	.74	.76	.72	.74	.72	.76	.67	.63	.61	.78	—	.20	.22
17. *S. depressus*	.69	.66	.62	.63	.62	.61	.61	.61	.61	.63	.60	.51	.51	.45	.58	.80	—	.02
18. *S. minor*	.69	.65	.62	.63	.62	.61	.61	.62	.61	.63	.60	.51	.52	.45	.57	.78	.98	—

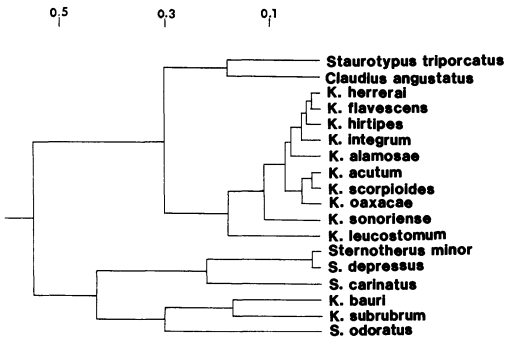

Fig. 1. BMDP1M cluster analysis of 18 kinosternid species based on genetic distance values (Rogers, 1972).

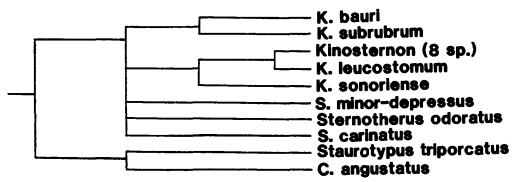

Fig. 2. Strict consensus tree (derived from the PAUP algorithm) expressing cladistic relationships among kinosternid species based on binary coding of 34 electromorph products (Table 3A). The consistency index is 0.65 and length is 49. The tree is rooted with a "composite" outgroup of *Claudius* and *Staurotypus*. Additional trees rooted with *Claudius* or *Staurotypus* produced identical topologies for the kinosternines.

siomorphic for the unique allele-product Pgm (a) and for M-Aat-A (a) found elsewhere only in *K. integrum*. Other apomorphies appear in *K. hirtipes, K. sonoriense* and *Sternotherus* (G-3-pdh, a); *K. integrum* and *K. leucostomum* (Ldh-B, a); and *K. hirtipes, K. flavescens, K. integrum* and *K. leucostomum* (Pgm, b). Apomorphic characters (electromorphs) which separate genera are identified in Fig. 3 and Table 4.

The unexpected phenetic and suggested phylogenetic position of *K. bauri* and *K. subrubrum* prompted additional comparisons to *Sternotherus* and other *Kinosternon*. Tissue proteins from two *S. odoratus*, one *K. bauri*, two *K. sonoriense* and one *K. leucostomum* were analyzed for general banding pattern variation by isoelectric focusing and results are presented in Fig. 4. In liver and kidney-heart, high resolution was obtained for bands near the cathode (isoelectric points = pH 6–7) whereas both cathodal and anodal bands were clearly resolved in skeletal muscle. In liver, the overall profile of electromorphs for *K. bauri* shows similarities to both *K. sonoriense* and *S. odoratus* (Fig. 4A). However, *K. bauri* (4) has a unique banding pattern in zone I and *K. leucostomum* (1) shows no distinct electromorph in that zone. Two bands of high intensity in zone II are shared by all three *Kinoster-*

TABLE 3. MATRICES FOR CLADISTIC ANALYSIS OF 34 BINARY-CODED ENZYME CHARACTERS (COLUMNS) OF KINOSTERNID TURTLES. Characters are listed in Table 1.

Taxa																																		
A.																																			
Kinosternon bauri	1	1	0	0	1	0	0	0	1	0	0	1	1	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	1	0
K. subrubrum	0	1	0	0	1	0	1	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	0
Kinosternon (8)	1	0	0	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	0	0	1	1	0	0	1	1	1	0	0
K. sonoriense	1	0	0	0	1	0	1	0	1	1	1	1	1	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	1	0	0
K. leucostomum	1	0	0	0	1	0	1	0	1	0	1	1	1	1	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	1	0	1	0	0
Staurotypus	1	0	0	0	1	0	0	0	1	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	0	0	0	1	0	1	0	0
Claudius	1	0	0	1	0	0	0	0	1	0	1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0
Sternotherus odoratus	0	1	0	1	1	0	0	0	1	1	1	1	0	1	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0	1	0	0	1	0
S. carinatus	1	1	0	0	0	1	0	0	1	1	1	0	0	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0
S. minor-depressus	1	1	1	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	1	0	1	1
B.																																		
K. bauri-subrubrum	1	1	0	0	1	0	1	0	1	0	0	1	1	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	1	0
Kinosternon	1	0	0	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	0	0	1	1	0	0	1	1	1	0	0
Staurotypus	1	0	0	0	1	0	0	0	1	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	0	0	0	1	0	1	0	0
Claudius	1	0	0	1	0	0	0	0	1	0	1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0
Sternotherus	1	1	1	1	1	1	0	0	1	1	1	1	0	1	0	1	0	1	0	1	1	0	1	1	0	0	1	1	1	1	1	0	1	1

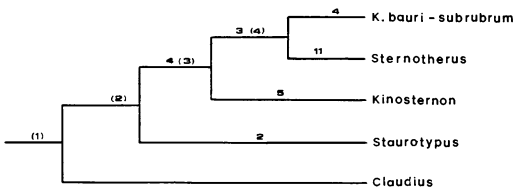

Fig. 3. Cladistic relationships among genera of kinosternid turtles expressed by the PAUP algorithm based on binary coding of 34 electromorph products (Table 3B). The tree is rooted with *Claudius* as the outgroup. Additional trees rooted with *Staurotypus* or a "composite" outgroup produced identical topologies for the kinosternines. The consistency index is 0.88 and length is 33. The numbers refer to the number of characters defining each branch of the tree, while numbers in parentheses are stem numbers. The characters defining each stem or branch leading to a terminal taxon are given in Table 4.

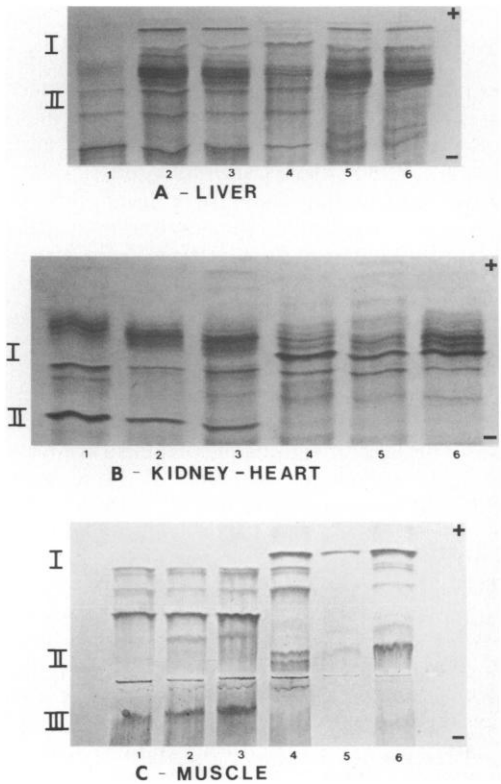

Fig. 4. Electrophoregram of tissue proteins from *K. leucostomum* (1), *K. sonoriense* (2, 3), *K. bauri* (4) and *S. odoratus* (5, 6) separated by isoelectric focusing.

non (1–4) and a third band appears in *K. leucostomum*. In kidney-heart, the electromorph pattern of *K. bauri* appears more similar to *S. odoratus* than to the *Kinosternon* (Fig. 4B). *K. bauri* (4) and *S. odoratus* (5, 6) share a unique, dense band in zone I and lack a similar band shared by *Kinosternon* (1–3) in zone II. Although generally alike, a unique pattern in zone I distinguishes *K. leucostomum* (1) from *K. sonoriense* (2, 3). In skeletal muscle, electromorphs of *K. bauri* are again more similar to *S. odoratus* than to congeners (Fig. 4C). This is based on shared bands in zones I and II which are absent from *K. sonoriense* (2, 3) and *K. leucostomum* (1) and presence of dense bands in zones II and III absent from *S. odoratus* (5, 6) and *K. bauri* (4). *K. leucostomum* (1) lacks a second band in zone II which is present in both individuals of *K. sonoriense* (2, 3). The two specimens of *S. odoratus* (5, 6) had identical patterns in muscle, although the staining intensity of one (5) is faded. In general the results from isoelectric focusing support the findings from starch-gel electrophoresis. *K. bauri* shows greater likeness to *S. odoratus* than to Central American congeners and *K. leucostomum* is distinct but similar to *K. sonoriense*.

TABLE 4. AUTAPOMORPHIC CHARACTERS FOR TAXA AND SYNAPOMORPHIC CHARACTERS FOR STEMS ENUMERATED IN PAUP TREE (FIG. 3). Rows of numbers to the right of each taxon/stem correspond to the electromorph character numbers in Table 1. Positive numbers indicate presence of characters, negative numbers indicate absence.

Taxa/stems	Derived character states
Staurotypus	−13, +15
Stem (3)	+10, −25, +27, +31
Kinosternon	+7, +8, +17, +22, +26
Stem (4)	+2, −19, +33
Sternotherus	+3, +4, +6, −13, +18, +21, +24, +28, +29, −32, +34
K. bauri-subrubrum	+7, −10, −11, −30

Discussion

In spite of ecological and morphological diversity in Central American and SW United States *Kinosternon* (Berry, 1978; Iverson, 1976, 1981; Iverson and Berry, 1979; Seidel and Reynolds, 1980), electrophoretic (genetic) similarities suggest that they are a monophyletic complex of closely allied species. Phenetic analysis of protein data indicates that *K. leucostomum* and *K. sonoriense* are more genetically distinct than the other eight species. Rogers (1984) points out that often distinct morphological species differ only in allelic frequencies, justifying the application of phenetic procedures (Rogers, 1972) that make full use of frequency data. The lack of significant protein variation among *K. acutum* and members of the *K. scorpioides* species group including *K. scorpioides, K. integrum, K. alamosae* and *K. oaxacae* (sensu Berry, 1978; Iverson, 1981), is not surprising considering their close morphological and zoogeographic relationships (Berry, 1978; Iverson, 1976). Similarly, present electrophoretic results support the morphological and zoogeographic data (Iverson, 1981; Siebenrock, 1907) in recognizing *K. hirtipes, K. flavescens* and *K. herrerai* as related species. However, the absence of electrophoretic evidence for monophyly of *K. hirtipes* and *K. sonoriense* is surprising in light of their parapatry and similar morphology (Iverson, 1981). Phylogenetic relationships among the Mexican and Central American *Kinosternon* based on scute morphometry are presently being analyzed (Iverson, in prep.).

Present results and previous analyses of serum proteins (Frair, 1972) and shell morphology (Lamb, 1983) leave little doubt that the two eastern United States *Kinosternon* (*K. bauri* and *K. subrubrum*) are closely allied. However their genetic distance from other congeners (Fig. 1) is equal to or greater than the distance observed between genera. Phenetic analysis of electrophoretic data indicates that *K. bauri* and *K. subrubrum* are more closely allied to *Sternotherus*, with which they are broadly sympatric, than to other *Kinosternon* with which they are mostly allopatric. Moreover, *K. bauri* and *K. subrubrum* do not appear to be sister groups to other *Kinosternon* species (Figs. 2 and 3), which suggests that *Kinosternon* is a paraphyletic taxon. These somewhat surprising relationships are further supported by karyotype data (Sites et al., 1979) which indicates that the G-band positive heterochromatin of *K. bauri* and *K. subrubrum* is more similar to *Sternotherus* than to the Central American *Kinosternon* (*K. scorpioides*). Sites et al. (1979) suggest that the eastern United States *Kinosternon* and *Sternotherus* shared a common ancestor. Crenshaw (1962) found that *K. bauri* and *S. odoratus* have a single serum albumin band whereas *K. scorpioides* has two bands. Allocation of *subrubrum* and *bauri* to *Kinosternon*, sensu stricto (Holbrook, 1842; Carr, 1952; and others; but see Boulenger, 1889; Siebenrock, 1907; and Romer, 1966) has been based on kinesis of the posterior plastral lobe (double hinged plastron). However, Bramble et al. (1984) question the value of this character in generic diagnosis by noting that *K. herrerai* exhibits no more structural evidence of posterior lobe kinesis than does *Sternotherus*. Considering the variability of plastral morphology in the Kinosternidae, it seems possible that the dual kinetic condition in *K. bauri* and *K. subrubrum* is a derived homoplasy which evolved (perhaps from a sternotherine-like ancestor) in response to temporary aquatic habitats in the eastern-southeastern United States. Based on protein morphology, shell kinesis and karyotypes, we conclude that the present generic concepts of *Sternotherus* and *Kinosternon* be questioned and place *Sternotherus* in the synonymy of *Kinosternon*. Additional morphological work (Iverson, in prep.) specifically addresses this interpretation.

Cladistic analysis of electrophoretic data indicates that the subfamily Kinosterninae is monophyletic, divergent from *Claudius* and *Staurotypus* which perhaps are sister taxa. This is consistent with paleontological evidence (Mlynarski, 1976) and phylogenetic theory based on plastron morphology (Bramble et al., 1984) and karyotypic data (Bickham, 1981; Bickham and Carr, 1983). In spite of the apparent value of electrophoretic comparisons based on small or single samples (Gorman and Renzi, 1979; Sites et al., 1984), we presently refrain from drawing any definite conclusions regarding the Staurotypinae because our analysis includes only one specimen of *Claudius*. Biochemical comparisons (in progress) utilizing *Dermatemys* as an outgroup will further test subfamilial partitioning of Kinosternidae.

Specimens examined.—Museum acronyms follow Duellman et al. (1978); other abbreviations are JBI and MES (private collections of John B. Iverson and Michael E. Seidel, respectively) and JI (field numbers of specimens to be deposited in

Florida State Museum Collection, University of Florida, UF). Tissues used in isoelectric focusing are marked with asterisks. Specimens are listed by state only for Mexico and the United States.

Claudius angustatus—Belize: CM 91084.

Staurotypus triporcatus—Belize: CM 91085–89. Oaxaca: JI 81329.

Sternotherus carinatus—Louisiana: MES 51–52; USNM 221783. Mississippi: MES 15–17, 19, 22, 48, 50; UMMZ 172936; USNM 221784.

Sternotherus depressus—Alabama: MES 11, 13, 24–27, 29–31; USNM 221785.

Sternotherus minor—Alabama: MES 21, 32–33, 36–39. Florida: MES 12, 34–35, 40–41, 44–46, 53. Tennessee: MES 9–10, 14, 20.

Sternotherus odoratus—Alabama: MES 508–13, 515–518. Connecticut: AMNH uncatalogued (6). Florida: Jim Berry uncatalogued (4); MES 505–507. Indiana: Sam Reynolds uncatalogued (5). Massachusetts: CM 83016, 83018–24, 83026–27. New York: Sam Reynolds uncatalogued (2). Tennessee: MES 522, 525; UMMZ 172936. West Virginia: MES 503–504, 1691–92*.

Kinosternon acutum—Veracruz: JI 83199.

Kinosternon alamosae—Sonora: JI 80198, 80200, 80206, 80209, 80216.

Kinosternon bauri—Florida: CM 91128–136; JI uncatalogued*.

Kinosternon flavescens—Arizona: JI 81A01–03, 81A09, 81A31. Chihuahua: JI 80233–234, 80238. Durango: JI 80061–063. Nebraska: JI uncatalogued (6). New Mexico: MES 57–60, 64–65, 175, 291, 2 uncatalogued. Sonora: JI 82138, 82195–197. Tamaulipas: JI 79027–029.

Kinosternon herrerai—Veracruz: JI 79039–43, 81054, 81071–74.

Kinosternon hirtipes—Chihuahua: MES 19, 54–56, 62–63, 291. Durango: JI 80044, 80046, 80112, 79184–185. Guanajuato: JI 78178–179. Jalisco: JI 78312–13, 78319–22, 81481. Michoacan: JI 78185–87, 78245, 78247–49, 78270–71, 81453.

Kinosternon integrum—Colima: JI 81490–91. Jalisco: JI 78297–300, 78310–11, 78333–34; MES 305–311, 313–321, 479, 499–500, 532–34, uncatalogued (2). Michoacan: JI 79250, 79284. Oaxaca: JI 79113, 79115–116, 79118. Queretaro: JI 79190–91, 79198. Sinaloa: JI 80177. Sonora: JBI 861–862, 874; JI 82160–61. Tamaulipas: JI 78123–124.

Kinosternon leucostomum—Belize: CM 91093–96, uncatalogued (1). Guatemala: JI uncatalogued (1)*. Honduras: JI uncatalogued (1). Panama: JBI 1049, 1051–52. Veracruz: JI 81115, 81124, 81126, 81128–29, 81158, 81160–61, 81339, 81341.

Kinosternon oaxacae—Oaxaca: JI 79090–92, 79094–95, 79097, 79099–100.

Kinosternon scorpioides—Belize: CM 91097–98. Chiapas: JI 81296–301; MES 295, 297–98. El Salvador: JI uncatalogued (3). Guyana: JBI 1056–57. Tamaulipas: JI 79034–36. Yucatan: JI 81212.

Kinosternon sonoriense—Arizona: JBI 974–976; JI 84135–136*. Chihuahua: JI 80243–44.

Kinosternon subrubrum—North Carolina: MES 294. New Jersey: AMNH 120034–36.

Acknowledgments

Special thanks to R. Lucchino, Utica College, for generously providing equipment and assistance with part of the electrophoretic analysis; and B. Grunbaum, T. Gillis and T. Fenger for valuable technical advice on isoelectric focusing procedures. We thank W. L. Fink, University of Michigan and S. Bauserman, Marshall University, for assistance with computerized data analysis. F. Binder, H. Mills, F. Zain, B. Kahle, D. Tarter and J. Joy generously provided laboratory space or equipment. We thank T. Lamb, J. Dodson, S. Reynolds, M. Klemens, R. Blaney, J. Berry, P. Meylan, B. Lamar, D. Gicca, H. Hidalgo and J. W. Gibbons for providing live specimens. Field work in Mexico was made possible by permits from the Direccion General de la Fauna Silvestre and assistance by C. Babcock McElrath, D. Cortier, D. Gicca, R. Magill, P. Meylan, P. Rosen, D. Takahashi and J. Thorbjarnarson. Iverson's research was supported by the National Science Foundation (DEB-8005586 and BSR-8404462), the American Philosophical Society, Earlham College, the Joseph Moore Museum and the Florida State Museum. Seidel's work was supported by a Marshall University faculty development grant.

Literature Cited

Berry, J. F. 1978. Variation and systematics in the *Kinosternon scorpioides* and *K. leucostomum* complexes (Reptilia: Testudines: Kinosternidae) of Mexico and Central America. Unpubl. PhD. dissert., University of Utah, Salt Lake City, Utah.

———, and J. M. Legler. 1980. A new mud turtle, genus *Kinosternon*, from Sonora, Mexico. Contrib. Sci. Los Angeles Co. Mus. Nat. Hist. 325:1–12.

———, and J. B. Iverson. 1980. A new species of mud turtle, genus *Kinosternon*, from Oaxaca, Mexico. J. Herpetol. 14(4):313–320.

Bickham, J. W. 1981. Two-hundred-million-year-old chromosomes: deceleration of the rate of karyotypic evolution in turtles. Science 212:1291–1293.

———, and J. L. Carr. 1983. Taxonomy and phylogeny of the higher categories of cryptodiran turtles based on cladistic analysis of chromosomal data. Copeia 1983(4):918–932.

Boulenger, G. A. 1889. Catalogue of the chelonians, rhynchocephalians, and crocodiles in the British Museum (Natural History). Trustees of the British Museum, London, United Kingdom.

Bramble, D. M., J. H. Hutchinson and J. M. Legler. 1984. Kinosternid shell kinesis: structure, function and evolution. Copeia 1984(2):456–475.

Bull, J. J., R. G. Moon and J. M. Legler. 1974. Male heterogamety in kinosternid turtles (genus *Staurotypus*). Cytogenet. Cell Genet. 13(5):419–425.

Carr, A. F. 1952. Handbook of turtles: the turtles

of the United States, Canada, and Baja California. Cornell University Press, Ithaca, New York.

CRENSHAW, J. W. 1962. Variation in the serum albumins and other blood proteins of turtles of the Kinosternidae. Physiol. Zool. 35:157–165.

DIXON, W. J. 1981. BMDP statistical software. University California Press, Berkeley, California.

DUELLMAN, W. E., T. FRITTS AND A. E. LEVITON. 1978. Museum acronyms. Herp. Rev. 9:5–9.

FRAIR, W. 1972. Taxonomic relations among chelydrid and kinosternid turtles elucidated by serological tests. Copeia 1972(1):97–108.

GAFFNEY, E. S. 1975. A phylogeny and classification of the higher categories of turtles. Bull. Amer. Mus. Nat. Hist. 155:387–436.

———. 1984. Historical analysis of theories of chelonian relationships. Syst. Zool. 33(3):283–301.

GORMAN, G. C., AND J. RENZI, JR. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: Effects of sample size. Copeia 1979(2): 242–249.

HOLBROOK, J. E. 1842. North American herpetology; or a description of the reptiles inhabiting the United States. Vol. I. J. Dobson and Son, Philadelphia, Pennsylvania.

HUTCHINSON, J. H., AND D. M. BRAMBLE. 1981. Homology of the plastral scales of the Kinosternidae and related turtles. Herpetologica 37:73–85.

IVERSON, J. B. 1976. The genus *Kinosternon* in Belize (Testudines: Kinosternidae). *Ibid.* 32(3):258–262.

———. 1977. Geographic variation in the musk turtle *Sternotherus minor.* Copeia 1977(3):502–517.

———. 1978. Distributional problems of the genus *Kinosternon* in the American southwest. *Ibid.* 1978(3): 476–479.

———. 1979. A taxonomic reappraisal of the yellow mud turtle, *Kinosternon flavescens* (Testudines: Kinosternidae). *Ibid.* 1979(2):212–225.

———. 1981. Biosystematics of the *Kinosternon hirtipes* species group (Testudines: Kinosternidae). Tulane Stud. Zool. Bot. 23(1):1–74.

———, AND J. F. BERRY. 1979. The genus *Kinosternon* in northeastern Mexico (Testudines: Kinosternidae). Herpetologica 35:318–324.

KILLEBREW, F. C. 1975. Mitotic chromosomes of turtles. III. The Kinosternidae. *Ibid.* 31(4):398–403.

LAMB, T. 1983. The striped mud turtle (*Kinosternon bauri*) in South Carolina, a confirmation through multivariate character analysis. *Ibid.* 39(4):383–390.

MLYNARSKI, M. 1976. Handbuch der Palaoherpetologie (Encyclopedia of paleoherpetology). Part 7. Testudines. Gustav Fischer Verlag, Stuttgart, West Germany.

NOMENCLATURAL COMMITTEE OF THE INTERNATIONAL UNION OF BIOCHEMISTRY. 1979. Enzyme Nomenclature 1978. Academic Press, New York, New York.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Univ. of Texas Publ. 7213: 145–153.

———. 1984. Deriving phylogenetic trees from allele frequencies. Syst. Zool. 33(1):52–63.

ROHLF, F. J. 1982. Consensus indices for comparing classifications. Math. Biosci. 59:131–144.

ROMER, A. S. 1966. Vertebrate paleontology. 3rd Edition. University of Chicago Press, Chicago, Illinois.

SEIDEL, M. E., AND R. V. LUCCHINO. 1981. Allozymic and morphological variation among the musk turtles *Sternotherus carinatus, S. depressus* and *S. minor* (Kinosternidae). Copeia 1981(1):119–128.

———, AND S. L. REYNOLDS. 1980. Aspects of evaporative water loss in the mud turtles *Kinosternon hirtipes* and *Kinosternon flavescens.* Comp. Biochem. Physiol. 67A:593–598.

———, ——— AND R. V. LUCCHINO. 1981. Phylogenetic relationships among musk turtles (genus *Sternotherus*) and genic variation in *Sternotherus odoratus.* Herpetologica 37(3):161–165.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON AND J. B. GENTRY. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus.* I: variation in the old-field mouse (*Peromyscus polionotus*). Univ. of Texas Publ. 7103:49–90.

SIEBENROCK, F. 1907. Die Schildkrotenfamilie Cinosternidae. Sitzungber. Akad. Wiss. Wien. 116(1): 527–599.

SITES, J. W., JR., J. W. BICKHAM, M. W. HAIDUK AND J. B. IVERSON. 1979. Banded karyotypes of six taxa of kinosternid turtles. Copeia 1979(4):692–698.

———, ———, B. A. PYTEL, I. F. GREENBAUM AND B. A. BATES. 1984. Biochemical characters and reconstruction of turtle phylogenies: Relationships among batagurine genera. Syst. Zool. 33(2):137–158.

(MES, MDA): DEPARTMENT OF BIOLOGICAL SCIENCES, MARSHALL UNIVERSITY, HUNTINGTON, WEST VIRGINIA 25701, AND (JBI): DEPARTMENT OF BIOLOGY, EARLHAM COLLEGE, RICHMOND, INDIANA 47374. Accepted 24 June 1985.