MOLECULAR SYSTEMATICS AND BIOGEOGRAPHY OF THE NEW WORLD TURTLE GENERA TRACHEMYS AND KINOSTERNON

A Dissertation
by
DAVID EDWARD STARKEY

Submitted to the Office of Graduate Studies of Texas A&M University in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY

May 1997

Major Subject: Genetics
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May 1997

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ABSTRACT

Molecular Systematics and Biogeography of the New World Turtle Genera

*T*racemys* and *Kinosternon.* (May 1997)

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The order Testudines has evolved to include a diverse array of taxa that inhabit freshwater, marine, and terrestrial ecosystems. The most widely accepted classification scheme divides the turtles into two suborders: Pleurodira and Cryptodira. These suborders are further divided into 13 families with 88 genera and 256 species. Extensive morphological diversity among turtles has led to a vast array of hypothesized phylogenies based on a wide variety of methodologies, however no current phylogeny is universally accepted. The present study addresses turtle relationships using mitochondrial DNA sequence data.

Over 230 taxa representing all extant families were examined for a total of 250,000 basepairs. The mtDNA data support much of the previous research i.e., monophyly of turtle families and a basal diversification into Pleurodires and Cryptodires. However, hypothesized sister relationships between snapping and big headed turtles and either the Old World pond turtles or tortoises and the New World pond turtles are not evident.
Instead, the sequence data supports a close relationship between the Old World pond turtles and tortoises. A novel finding of this study is a delineation between a primitive (Trionychidae and Carettochelyidae) and an advanced clade (all remaining families) within the Cryptodira.

Finally, the biogeography of the broadly distributed New World families Kinosternidae and Emydidae was examined. These two groups were chosen because of the potential insights they may bring to the biogeography of this region. Each family has had North America proposed as their point of origin and, although this study confirms the Emydidae as North American, Central America is confirmed as the point of origin for the Kinosternidae. The radiation of the Emydidae genus *Trachemys* across North, Central, and South America and the Caribbean appears to have occurred rapidly >10 million years ago. The Kinosternidae appear to be much older and migrated northward and southward out of Central America and Mexico. This migration resulted in two expansions into North America and two separate expansions into South America.
For
My Father, Bill
&
In Loving Memory of My Mother, Mary Jo
ACKNOWLEDGMENTS

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CHAPTER I
INTRODUCTION

History of Turtles
The first reptiles appear in the fossil record towards the end of the Paleozoic era of the Pennsylvanian period about 320 million years ago (Carroll, 1969). Reptiles diversified quite rapidly during this era and came to dominate the earth. One of the primary defining features of these reptiles was the anapsid (without openings) skull which is characterized by a solid roof of dermal bone. Another character that allowed for the expansion of this order was the amniotic egg, which freed reptiles from water for reproduction and allowed reptiles to take advantage of places not accessible to their amphibian ancestors. Finally, a third character that allowed reptiles to exploit the land was a tough nonpermeable epidermis.

Reptiles of the present day represent the result of several successful radiations from their Mesozoic ancestors. The Testudines or turtles represent the only surviving anapsid lineage. The other anapsids, the Captorhinidae, appeared in the fossil record throughout the Permian and died out in the late stages of that period. The first turtle to appear in the fossil record was Proganochelys, which has fossils dating to the late Triassic in Germany and Thailand, about 200 million years ago (Romer, 1956; Gaffney, 1975a). Proganochelys possesses many characteristics which leave little doubt that it is in fact, a turtle. The most important of these characters

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are the fusion of the ribs and vertebrae to the dermal bones to form the carapace and the fusion of the pectoral girdle and dermal bones to form the plastron. Interestingly, teeth were also present in the palatines, but absent from the upper and lower jaws. Another Testudinid found from the same general time frame as *Proganochelys* is *Proterochersis*. This is the earliest example of one of the modern lineages, Pleurodira (snake-necks). These genera, along with *Saurischiomes* and *Chelytherium*, have been placed in the gigaorder Proganochelydia with all remaining turtles assigned to the gigaorder Casichelydia, which was to become dominant by the end of the Jurassic (Romer, 1956; Gaffney, 1975a). This indicates that the split between Pleurodires and the other major modern lineage, Cryptodira (hidden-necks), had occurred by approximately 200 million years ago and suggests that *Proganochelys* may be nothing more than an evolutionary side branch. After the appearance of *Proterochersis*, the pleurodires are absent from the fossil record until the appearance of *Platychelys* in the late Jurassic, but occur from there until the present. The Pleurodires can be divided into two subfamilies: Pelomedusidae and Chelidae, and these families have been in existence since the mid-Cretaceous.

The first known cryptodire, *Kayentachelys*, appears in the fossil record by the mid-Jurassic (about 185 million years ago). This turtle possessed characteristics not seen in modern cryptodires, such as teeth in the roof of the mouth. As with the Pleurodires, the Cryptodires do not reappear in the fossil record until the late Jurassic, with the appearance of the Pleisochelyidae and Pleurosternidae. The Cryptodires are present in the fossil record from this point to the present, with the first representatives of the modern groups of Cryptodires appearing in the Cretaceous. The first of
the modern families of turtles, the Cheloniidae (sea turtles), appear in the early Cretaceous and the Baenoids (a semi-aquatic North American family) in the mid-Cretaceous. The Meiolaniids (horned turtles) were another early lineage occurring in the late Eocene of Australia and South America. The remaining modern groups are present by the late Cretaceous, although modern genera do not appear until the Miocene as the early tertiary genera were disappearing. Figure 1 represents the known turtle families (extinct and extant) and their proposed phylogenetic placement (Gaffney, 1984). The phylogenetic relationships of each of the extant families will be discussed in detail later.

The major identifying feature of the turtle is the shell and this defining character has remained virtually unchanged for over 200 million years. A turtle's shell can be divided into two parts, the plastron (lower) and the carapace (upper). The two halves of the shell are joined by bone and/or ligament. The carapace consists of approximately 50 bones, whereas the plastron consists of about six. Each of the bones in the shell is covered by a scute. Scutes and scutal structure have been used extensively in morphological studies to determine phylogeny (e.g., Hutchison and Bramble, 1981). However, several families (Trionychidae and Dermochelyidae) have lost this covering of scutes, with a subsequent reduction of bone in the shell.

Another exceptional feature of the turtle is the migration of the limb girdles inside the rib cage. This has allowed the various families to exploit their respective environments in different ways, e.g., large hind legs in the tortoises for the terrestrial environment, flippers in the sea turtles for swimming and webbing in the toes of some of the semiaquatic turtles. The
Figure 1. Hypothesized relationships of the extinct and extant turtle families based on Gaffney, 1984. Extinct families are indicated by an *.
skull, although still basically the primitive anapsid form, is also highly modified with jaws that have transformed into sharp beaks. Sex determination is also unusual in turtles. In the vast majority of families there are no sex chromosomes and temperature during incubation is the key factor in determining a male versus a female. However in the genera *Siebenrockiella* and *Staurotypus* chromosomes putatively identified as sex chromosomes perform this function (Sites et al., 1979b; Carr and Bickham, 1981; Bickham and Carr, 1983).

Continental Drift and Regional Faunas

There is a vast amount of data to suggest that in the Cretaceous, Gondwanaland was a supercontinent and all the major continents of today were in close proximity (e. g., Fooden, 1972). Gondwanaland began to break apart towards the end of the Cretaceous as modern turtle groups were appearing. By the Paleocene South America and Africa were islands, North America and Eurasia were joined, and Australia and Antarctica were in close proximity creating unique regional faunas.

As a result of continental drift, the configuration of North and South America has changed greatly since the Paleocene. In the Eocene, approximately 50 to 56 million years ago (MYA), the continents were connected through Mexico and Central America. As the Eocene progressed (35 to 40 MYA) and the sea level rose, Central America became isolated as an island. In the Oligocene (23 to 35 MYA) Central America was once again connected to North America and Mexico, while South America was left in isolation. As time progressed into the Miocene (5 to 23 MYA) Central
America was once again insular. By the Pliocene, (<5 MYA), the major land masses of North, Central, and South America were once again connected.

In one theory (e.g., Schuchert, 1955), the history of the Caribbean is closely linked to the New World. In the early Eocene, Cuba and the Dominican Republic were joined as a single island. As the sea levels rose in the late Eocene much of the Caribbean was submerged. In the Oligocene, much of the land mass that would form the Caribbean was above sea level, but isolated. In the Miocene, a similar situation occurred in the Caribbean, however parts of the Dominican Republic were connected to Central America. Finally, in the Pliocene, much greater portions of the Caribbean were above sea level, but occupied positions similar to those of today.

An alternative scenario for the formation of the Caribbean has been proposed (e.g., Case and Holcombe, 1980). It is hypothesized that a proto-Caribbean plate was formed in the Cretaceous and occupied a position in the Pacific, between the North and South American plates. As the plate moved, it passed between the two plates of the Americas during the Jurassic. As the Eocene began, the plate changed directions and moved toward the east (Dengo and Case, 1990). This possibility was first brought to light when it was noticed that much of the deep sea fauna of the Caribbean was similar to that of the Pacific Ocean, rather than that of the Atlantic Ocean (e.g., Singer, 1931).

As the sea levels changed, various portions of what would become the Lesser and Greater Antilles were available for faunal colonization. However, it has also been hypothesized that the alignment of the Greater and Lesser Antilles on the same plate is recent (e.g., Roughgarden, 1995). Specifically, the islands of Cuba and Puerto Rico are thought to be the oldest,
emerging by the Miocene and becoming complete by the Pliocene. Jamaica is also thought to have emerged fully by the Pliocene. There is a great deal of evidence for this hypothesis geologically and faunally. For example, Puerto Rico and Cuba show the highest numbers of endemic species of any islands in the Caribbean (Nichols, 1988). Also, the Cuban lizard Cricosaura has its closest relatives in California, Central America, and Mexico (Hass and Hedges, 1992).

Although there is no doubt that faunal interchange between North, Central, and South America has occurred, there exists much controversy as to when and how. The main problems arise when attempting to identify interchanges which took place before the formation of the Panamanian land bridge in the Pliocene. There are some who argue that intermittent interchanges have taken place since the Cretaceous (e.g., Hershkovitz, 1966). Eisenberg (1989) has argued that reciprocal interchange has taken place, through Central America, since the Mio/Eocene. However, Keast (1972) has argued that this was a unidirectional process, mainly south to north, and that interchange has only occurred since the formation of the land bridge. Reig (1981) has also argued that no matter what the direction, the exchange has been limited to the pre-Pliocene. However, since that time, most would agree that a virtually continuous interchange has linked the faunas of North, Central, and South America (e.g., Webb, 1976; Simpson, 1980).

Stehli and Webb (1985) concluded that based upon reptile, mollusk, and plant data an island arc must have existed in the late Cretaceous/early Paleocene between Central and South America. They concluded that these stepping stone islands are the only way to account for the present-day distributions between North and South America. However, Perfit and
Williams (1989) disagreed and have concluded, based upon mammalian studies, that no dry land connection existed in the late Cretaceous/early Paleocene. Furthermore, Hedges et al., (1992) contended that over water dispersal and extinctions, not an island arc, account for the present-day Caribbean fauna. Finally, Roughgarden (1995) concluded that mammalian data may be of little use in determining the biogeographic history of the Caribbean region. He contended that in the Caribbean, reptiles form the dominant fauna (i.e., Cretaceous-like), whereas in the Pacific the dominant fauna is avian. Roughgarden theorized that this indicates that the Caribbean fauna is ancient and was well established before the major mammalian radiation.

It is evident from many studies that invasions into, and out of, South America have occurred at various times in the past. For example, invasions of primates from Africa into South America occurred and begin to appear in the fossil record of South America towards the end of the Oligocene. Invasions from North America are evidenced by the Artiodactyla (i.e., camels) in the Pliocene. There are also examples of south to north invasions such armadillos, although movements from north to south seem to have occurred with more success. In part, this could be due to Pleistocene glaciations which had little effect in South America, but had major effects in North America, particularly on taxa adapted to tropical Central and South America. In reptiles evidence of this interchange can be seen in the abundance of genera and species in Central American countries such as Panama and South American countries such as Columbia which must have been major "highways" on the route of interchange. For example, Columbia
has the highest reptile genera (16) and species (33) density of any country in South America (Pritchard and Trebbau, 1984).

Turtles are an ancient lineage with an extensive fossil record. This fact should prove extremely useful in attempting to date the times of various expansions into and throughout the New World. Furthermore, as will be discussed, there are an abundance of taxa in North, South, and Central America that will enable these studies to be performed. Turtles, therefore, are an excellent model organism to study the biogeography of the New World.

Introduction to Turtle Families

Perhaps the most problematic Cryptodire family is the Trionychidae or soft shells, an ancient family with fossils dating to the late Jurassic. Turtles of this family are subdivided into two subfamilies: Cyclanorbinae (flapshells) and Trionychinae (softshells) and are found throughout North America, Asia, Africa, and the East Indies (e.g., Iverson, 1991). Although not presently found in South America, there is evidence (plastral bone fragments) that this family occurred there (Auffenburg, 1971, 1974). Furthermore, evidence from the late Pliocene of Venezuela indicates the family may have reached South America a second time (Wood and Patterson, 1973).

Trionychids are currently separated into six genera (e.g., Pritchard, 1979). These genera are characterized by having long flexible necks, leathery soft skin, a proboscis, and a large and flexible scuteless carapace. There is almost universal agreement that the Trionychidae are closely related to Carettochelyidae and a branch of the Cryptodires, but the relationship of
these taxa to other cryptodires is still problematic (e.g., Wermuth and Mertens, 1961; Pritchard, 1967; Bickham et al., 1983).

The pig-nose turtle (*Carettochelys insculpta*) in the monotypic family Carettochelyidea is found in New Guinea. Although now found only in the Fly River of New Guinea, this family was once more widespread, as fossils attributed to *Carettochelys* have been found in North America and Europe (Ernst and Barbour, 1989). This family shares characteristics with other families which has led to much taxonomic confusion. For example, *Carettochelys* has flippers like Cheloniidae (sea turtles), bony plates like those seen in the Testudinidae and a leathery plastron and carapace like those in the Trionychidae.

The Platysternidae are found throughout China and Thailand. The family is currently recognized as monotypic with five subspecies. These turtles are rather small in size as adults, but are characterized by having a head too large to withdraw into the shell and a tail as long as the carapace. The fossil record is incomplete, but fossils recognized as *Platysternon* have been found in Cretaceous formations of Southeast Asia (Romer, 1956).

Turtles of the family Emydidae occur throughout the Americas, Europe, and Asia and fossils have been found dating back to the Cretaceous of North America. The family contains 33 genera and approximately 100 species (Iverson, 1992). Currently, the Emydidae are divided into two subfamilies: Emydinae and Batagurinae (e.g., McDowell, 1961). The Emydinae is a moderately large subfamily with 10 genera and 35 species, while the Batagurinae is slightly larger with 23 genera and 59 species (Iverson, 1992).
Emydids inhabit fresh, brackish, and terrestrial ecosystems. There is a vast array of sizes within the family from the 10 cm *Clemmys muhlenburgi* to the 60 cm *Batagur baska*. The Emydinae are New World in origin with the exception of *Emys*, whereas the Batagurinae are Old World with the exception of *Rhinoclemys*. Emydines and Batagurines are characterized by having small skulls, a low domed carapace and a well developed plastron. However, aside from geographic distribution, there are several morphological features of the cervical vertebrae and scutes that define each subfamily. For example, Batagurids have a single articulation between the 5th and 6th cervical vertebrae, a suture between the 12th marginals and the last vertebral scute is over the suprapygal. In the Emydids, the articulation is double, there is no suture and the last scute is over the pygal. Several differences in skull and limb structure are the main differences between this family and their proposed sister group the Testudinidae. For example, Testudinids have narrower frontal bones, no splenial bone, no mesoplastron, and limbs that are highly modified for walking and/or digging.

The Emydinae is largely North American in distribution, and it has been hypothesized that Emydids originated in North America and reached South America and Europe via land bridges in the late Pliocene (Simpson, 1943). However, waif dispersal has also been put forth as a means of colonization into South America (Darlington, 1957). Although Batagurids are distributed in both the Old and New Worlds, the main species diversity is found in Asia and this has been proposed as their point of origin (Pritchard and Trebbau, 1984). The Emydines are thought to have originated from Batagurid stock and three possible ancestral stocks have been proposed.
1) *Siebenrockiella*, 2) *Rhinoclemmys*, or 3) an offshoot of another early Batagurid (Bickham and Baker, 1976b). Currently, this question has not been answered satisfactorily and it is hoped this study can address this question.

The Testudinidae, or tortoises, occur virtually worldwide, with a prevalence in Africa. This family is not as large as the Emydidae, containing 12 genera and 50 species (Iverson, 1992). Testudinids are known in the fossil record as far back as the Eocene. It has been hypothesized that the Testudinidae arrived in South America during the Tertiary (Oligocene), via waif dispersal from Southeast Asia (Simpson, 1943; Darlington, 1957).

Turtles of this family are almost exclusively terrestrial and they can attain great size, particularly the insular forms. For example, *Geochelone nigrita* (Santa Cruz) can reach 130 cm and 185 kgs. This family is commonly grouped with the Emydidae, but this is not a universally accepted conclusion. The relationships of the Batagurinae, Emydinae, and Testudinidae have been thoroughly analyzed without reaching a consensus.

Karyotypically, the Emydinae are distinct with a diploid number of 50, while the Batagurinae and the Testudinidae have a diploid number of 52.

Furthermore, it has been proposed that the Batagurids are ancestral to the Testudinids and that the Emydids and the Testudinids originated from the Batagurids in the old world (Simpson, 1943; Darlington, 1957).

Sea turtles are found in all the world’s tropical and temperate oceans. There are five genera usually separated into soft and hard-shelled families Dermochelyidae and Cheloniidae (e.g., Pritchard, 1971). The hard-shells are further subdivided into two tribes containing seven genera. They are characterized by having several features unique to the more primitive forms including a non-retractile head and limbs, and a completely roofed-over
skull. Fossils attributed to the Cheloniidae date to the Cretaceous in Europe and the Miocene and Eocene from Japan, Australia, and North America (Tachibana, 1979; Limpus, 1987; Ernst and Barbour, 1989), while Dermochelyid fossils date to the Eocene (Pritchard and Trebbau, 1984).

The Kinosternidae or mud turtles are found throughout the Americas. This family is characterized by having a reduced, hinged plastron, family members usually do not attain great size. Two genera, *Kinosternon* and *Sternotherus*, are generally included in the Kinosterninae, while two additional genera *Staurotypus* and *Claudius* are placed in the Staurotypinae (Bickham and Carr, 1983; Seidel, Iverson, and Adkins, 1986; Iverson, 1991). The Staurotypinae differ from the Kinosternids in that they are restricted to Central America, and do attain great size (Sites et al., 1979a).

It has been proposed that the Kinosternids are late Pliocene arrivals to South America having originated either in North or Central America (Berry and Legler, 1980; Iverson, 1991; Ernst, Lovich, and Barbour, 1994). The fossil history of the family dates to the Oligocene in North America with fossils attributed to living species dating to the Pliocene/Pleistocene (Fichter, 1969).

The sole representative of the family Dermatemydidae is the Central American River Turtle, *Dermatemys mawii*, which as the name implies is limited to Central America. It is characterized by having a smooth flat carapace, a large plastron, and a thick shell. This family was once quite widespread as material attributed to *Dermatemys* has been found in North America, Asia, and Europe. The family is also quite old with fossils dating to the Cretaceous of Asia, its possible place of origin.

The Chelydridae or snapping turtles are found from southern Canada as far south as Columbia, having arrived there via land bridges in the late
Pliocene (Duellman, 1979). Snapping turtles are characterized by large heads and very muscular limbs. Fossils attributed to Chelydridae are known from the Cenozoic (Pliocene) of North America (Galbreath, 1948; Hibbard, 1963). Fossils attributed to the extant genus *Macroclemmys* have been dated to the Miocene (Zangerl, 1945).

The other suborder of turtles, the Pleurodira, has not been studied in the same detail as the Cryptodires. It is believed that this suborder is an ancient one, having become established by the end of the Cretaceous. Africa is the possible point of origin for the Pelomedusids, because fossils are known to exist there from the early Cretaceous (de Broin et al., 1974). This indicates the subfamily may once have been more widespread and could have possibly originated in the Northern Hemisphere. No matter the point of origin, this subfamily was very successful by the end of the Cretaceous as fossils have been found throughout North and South America, Africa, and Europe (Romer, 1966; Gaffney, 1975b).

Although it seems the Chelidae have always occupied a range similar to their present distribution, their point of origin is more in question. Eocene fossils attributed to *Hydromedusa* have been found in South America, a proposed point of origin (Warren, 1969). However, tertiary (Miocene) fossils attributed to the Chelidae have been found in Australia (Gaffney, 1979). The former scenario agrees with Simpson (1943) who has proposed that the Chelids reached Australia via Asia from South America. However, Darlington (1957) proposed dispersal via sea routes to explain the distribution. Finally, dispersal via Antarctica has been suggested and, if true, would indicate that this is an ancient family (Dalziel et al., 1973; McGowan, 1973; Sclater and Fisher, 1974). Today Pleurodires are found throughout

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South America, Africa, and Australia. The Pelomedusids range throughout South America, Africa, and Madagascar, while the Chelids are restricted to South America and Australia.

Familial Systematics

The 18th century was the beginning of many types of systematic analyses, including the contributions of Linnaeus who first classified turtles into one genus *Testudo*, with fifteen species (Linnaeus, 1766). This, like much early work, focused on shared structural characters and resulted in many conclusions we now know to be erroneous (Gaffney, 1984). The next major contributions were those of Dumeril and Bibron, who respectively classified a pleurodire, *Chelus*, as a sister group to all other turtles and coined the terms Pleurodira and Cryptodira to differentiate between the different methods of neck retraction (Dumeril, 1806; Dumeril and Bibron, 1835).

The latter part of the 19th century saw many schemes of classification recognizing the Pleurodires as a natural group. However, the Cryptodires were still not classified as a natural family, due in large part to their vast morphological diversity. This period of history also encompassed the beginnings of evolutionary thought which was reflected in turtle systematics by attempts to delineate the turtle ancestor. Among the earliest attempts was that made by Edward Drinker Cope who thought he had found the ancestral condition in the leatherback, *Dermochelys coriacea* (Cope, 1871). This resulted in Cope defining two families, Athecae and Thecae. *Dermochelys* was placed in the Athecae and proposed to be a sister group to all other turtles. This hypothesis was incorporated into many subsequent
classifications (e.g., Boulenger, 1889; Hay, 1908), however, the proposed sister group status of *Dermochelys* was refuted when it was shown that leatherbacks were closely allied with other sea turtles (Nick, 1912). However, Cope's view of the Dermochelid relationship to other turtles was prevalent until the middle of the 20th century.

Among the major works of the 20th century examining familial relationships are those by Williams (1950), Pritchard (1967), and Gaffney (1975). Williams (1950) was among the first to extensively use the superfamily/family designations in his phylogeny. For many, this work is the starting point in any consideration of family level systematics. Pritchard (1967) incorporated many of Williams' ideas into a phylogeny that elaborated on the relationships of both extinct and extant turtles. Gaffney (1975) was among the first to employ Hennigian methods to analyze turtle relationships.

These studies examined the relationships using morphology, however, a variety of methodologies including serology and karyology have also been utilized. The problem, however, has not been resolved and the three methods of analysis have generated slightly different family groupings (Figure 2). The main points generated by each methodology will be discussed below.

Gaffney and Meylan (1988) used morphological analysis to recognize four super-families: Testudinioidea, Chelonidea, Chelydridea, and Trionychidea (Figure 2). They agree with Bickham and Carr (1983) regarding the Cheloniidae, but differ with Williams (1950) who recognized each in a separate superfamily. Also, their Testudinioidea is limited to the Emydidae and Testudinidae. Furthermore, they recognize the Chelydridae and
Figure 2. Proposed relationships of the extant turtle families. The first hypothesis (a) is based on Gaffney and Meylan, 1988. The second hypothesis (b) is based on Chen and Mao, 1981 and Friar, 1972. The third hypothesis (c) is based on Bickham and Carr, 1983.
Chelidae

Carettochelyidae

Trionychidae

Dermatemydidae

Kinosternidae

Dermochelyidae

Cheloniidae

Chelydridae

Emydidae

Testudinidae

Figure 2-Continued
Figure 2-Continued
Platysternidae as a superfamily, Chelydridea. Finally, they combine the Trionychidae, Carettochelyidae, Kinosternidae, and Dermatemydidae into a superfamily, Trionychidea.

Serological studies have primarily used hemoglobin fingerprinting and allozyme analyses, and, Friar (e.g., 1964, 1979) has worked extensively in this area. His findings have supported many of Williams' conclusions, while differing on several points (Figure 2). First, the Cheloniidae is allied with the Emydidae and the Chelydridae, while Williams sees each as a separate superfamily. Secondly, the Emydidae are aligned with the Platysternidae, all contraindicated by Williams Testudinoidea. Thirdly, in this analysis the Trionychidea were not found to be similar to either the Pleurodires or Cryptodires.

Bickham and Carr (1983) studied turtle families using karyotypic analysis. Three superfamilies are recognized in their classification: Cheloniioidea, Testudinoidea, and Trionychidea (Figure 2). The main differences between this and Williams include the placement of the Chelydridae and the Kinosternidae as subfamilies within Testudinoidea. Also, the Staurotypinae are recognized at the family level. Furthermore, they recognize the Cheloniidae and Dermochelyidae as a single superfamily, Cheloniidea. They follow a similar pattern in combining the Trionychidea with the Carettochelyidea into a superfamily, Trionychidea.

Although these classifications differ significantly, there are several common features. For example, Cheloniidea, Trionychidea, and Testudiniidea are common to all, but differ in their limits (see also Romer, 1966; Mlynarski, 1976). There have also been questions regarding whether or not the Trionychidea deserve the same status as the Pleurodires and
Cryptodires (Lindholm 1929; Mertens et al., 1934). Also, the relationships of the Batagurids, Testudinids and Emydids have not been resolved. The Chelydridae also remain a phylogenetic enigma as does the Platysternidae (e.g., Bickham and Carr, 1983; Gaffney and Meylan, 1988). Furthermore, the Kinosternidae has also been problematic in that it has been assigned to various families (e.g., Albrecht, 1967). Finally, it is apparent from the diversity of classifications that there are problems with convergent evolution and character polarity.

It is believed that a different method of analysis is needed to satisfactorily answer this question. Therefore, I have chosen to address this question using mtDNA sequence analysis of the ND4-Leucine tRNA region. This method has proven useful for other groups at the family and genera level (Arévalo et al., 1994; Forstner et al., 1995). It is believed this study will rectify many of the problems previously seen to provide a phylogenetic hypothesis for turtle families that can, in turn, provide a framework for an in depth analysis of the North American families.

Systematics of North American Turtles

*Trachemys*

The previous discussion suggests that the majority of turtle families predate the breakup of Laurasia and that only two groups appear to have originated in the New World in relatively recent times: the Kinosternidae and Emydinae. The primary focus of this research is biogeography in the New World, therefore the Kinosternidae and Emydidae were chosen as representative models to study this process.
The Emydinae represent the primary Testudinid lineage present in North America (~40 of 95 species, Iverson, 1992). Figure 3 presents the hypothesized generic relationships for the family. Fossil evidence indicates the family was once more widespread in Europe and Asia, and the oldest fossils that can be attributed to the family come from Paleocene deposits in Saskatchewan (Russell, 1934) and Eocene deposits in Europe and North America (Romer, 1956). The Emydinae are subdivided into two tribes (McDowell, 1964; Bramble, 1974). The Emydini, with a more primitive morphology, contains the genera *Clemmys, Emydoidea, Emys,* and *Terrapene.* The Deirochelyini, with a more derived morphology, contains the genera *Chrysemys, Deirochelys, Graptemys, Malaclemys, Pseudemys,* and *Trachemys.*

All Emydids are limited to North America except for *Emys* which is found in Europe, and *Trachemys* which occupies North, Central, and South America and the Caribbean. *Trachemys* thus becomes the most important group for studies of New World biogeography. Within *Trachemys,* the vast majority of species/subspecies belong to the *scripta* group and are designated based on morphological characters. The *scripta* group contains one species with sixteen subspecies that range from North to South America. Morphologically all *Trachemys* are very similar, and are characterized by elongate oval carapaces with strong posterior serrations, a slight vertebral keel, absence of serrations or cusps in the upper jaw, a rounded lower jaw, and three phalanges in the fifth toe (Weaver and Robertson, 1967; Obst, 1985). All of the characters mentioned above have also been used to separate *Trachemys* from the closely related genera *Pseudemys,* and *Chrysemys.*
Figure 3. Hypothesized relationships of the Emydidae based on Gaffney and Meylan, 1988 and Seidel and Adkins, 1987.
Few fossil skulls have been conclusively identified as *Trachemys*. This has made it extremely difficult to determine the fossil history of the genus because so much of the taxonomy has been based upon cranial characters (McDowell, 1964). However, several putative *Trachemys* ancestors have been identified and dated: *Trachemys inflata* (Miocene), *T. hilli* (Miocene) and *T. idahoensis* (Pliocene). Weaver and Robertson (1967) assigned *T. platymarginata* to the *Trachemys* complex and Jackson (1988) reported many cranial features of *T. platymarginata* were similar to *T. scripta* and identified this as a probable ancestor of the group. The only other potential member of the *scripta* group so far identified is *T. idahoensis* (Gillmore, 1933). Gillmore had associated *T. idahoensis* with *Pseudemys rubriventris*, based upon jaw structure. However, when cranial features were examined in conjunction with patterns of distribution, it was determined that *T. idahoensis* was more closely related to the *scripta* group (Zug, 1969). Jackson (1988) concluded that *T. idahoensis* represented a widespread Pliocene *Trachemys*, and that *T. idahoensis* could not be the ancestor to the modern *Trachemys* line as it appears too late in the fossil record and shows many of the diagnostic characters of present day *scripta*. *T. hilli* and *T. inflata* have been proposed as probable ancestors, but too little fossil material exists for these taxa to make accurate assessments. Jackson (1988) noted further that *T. idahoensis* samples from Idaho to Texas resemble modern *T. s. elegans*, whereas *T. idahoensis* from Florida resemble modern *T. s. scripta*. Therefore it appears that these forms had become established by the early Pleistocene.

There is virtually no information in the fossil record regarding the origins of the remaining *Trachemys*. Pregill (1981b) did report a plastral
fragment attributed to *Trachemys* from the late Pleistocene in Puerto Rico; however, it must be remembered that remains of terrestrial vertebrates are virtually absent from the Caribbean and this may reflect a lack of suitable conditions for fossilization rather than the lack of their existence (Poinar and Cannatella, 1987). A single late Pleistocene record (Gazin, 1957) from Panama has been the only report from outside North America supporting *T. scripta* as a recent invader of MesoAmerica (Moll and Legler, 1971; Pritchard and Trebbau, 1984).

The clouded fossil taxonomy is not the only problem evident in *Trachemys*. *Trachemys* has been placed in synonymy with *Pseudemys*, as a genus separate from *Pseudemys* and *Chrysemys* and as a composite genus with *Pseudemys* and *Chrysemys* (Agassiz, 1857; Cope, 1875; Boulenger, 1889). These different alignments result from a vast array of character states that are shared by these three genera (Table 1). The determination of homology versus homoplasy has been extremely difficult for this suite of characters.

McDowell (1964) using cranial, lower jaw, and foot morphology came to the conclusion either that there should be a composite genus of these three genera or that they should each be classified separately. McDowell also concluded that *Malaclemys* was a descendent of *Trachemys*. Dryden (1985), also using cranial characters, found *Pseudemys, Chrysemys,* and *Trachemys* to bemonophyletic, as was a group containing *Malaclemys* and *Graptemys*. In contrast, Pritchard and Trebbau (1984) proposed *Trachemys* to be ancestral to *Pseudemys*. Seidel and Smith (1986) examined these genera and presented evidence that *Trachemys* is a monophyletic group. Their findings showed that many of the characters used to define this grouping were also present in *Graptemys*. In addition to groupings with *Pseudemys* and
### Table 1
Comparison of selected character states in Emydine turtles.
Presence or absence of a character are indicated by a + or -, respectively.

<table>
<thead>
<tr>
<th>Character</th>
<th>G/M</th>
<th>T</th>
<th>C</th>
<th>P</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>cranium short and deep(^a)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>mandible flattened ventrally</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>narrow alveolar jaw surface</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>median keel (^b)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>female carapace &lt; 250mM</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>female ~ 2x male</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>phalanges on fifth toe &lt;3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>plica media, spade shaped (^c)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>lactate dehydrogenase, slow (^d)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>herbivorous (^e)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**Shared Characters** \(^g\)

| Trachemys       | 19 |
| Chrysemys       | 7  | 12 |
| Pseudemys       | 12 | 17 | 9  |

**Synapomorphies**

| Trachemys       | 9  |
| Chrysemys       | 1  | 2  |
| Pseudemys       | 9  | 9  | 3  |

(Key: \(^a\) McDonald, 1964, \(^b\) Ernst and Barbour, 1972; Dobie, 1981, \(^c\) Zug, 1969 \(^d\) Vogt and McCoy, 1980, \(^e\) Jackson, 1988, \(^f\) Seidel and Jackson, 1990, \(^g\) All character polarity determined relative to Deirochelys, G=Graptemys, M=Malaclemys, T=Trachemys, C=Chrysemys, P=Pseudemys, and D=Deirochelys.)
Chrysemys, Trachemys has been shown to share characteristics with Graptemys (sawbacks) and Malaclemys (terrapins) and as a result has been aligned with these genera (e.g., Zug, 1971). Phenetic comparisons between these genera have noted 9 synapomorphies between Trachemys and Graptemys and 19 shared character states (Table 1). Similar analyses have noted 9 synapomorphies between Pseudemys and Trachemys and 17 shared character states (e.g., McDowell, 1964; Zug, 1971; Ernst and Barbour, 1972).

Biochemical analyses have also been used to examine the Deirochelyini. Vogt and McCoy (1980) studied lactate dehydrogenase isozyme polymorphisms and found no differences between Trachemys and Graptemys, whereas unique bands appeared in Pseudemys and Chrysemys. However, most electrophoretic studies have only confirmed the assumptions of close generic affinities within the Deirochelyini complex (Friar, 1982; Seidel and Adkins, 1987). The subspecific relationships are equally as problematic as no character has been found that can accurately assess subspecies. However, due to a vast amount of morphological variation these subspecific designations have persisted. The most widely accepted hypothesis of the generic relationships of Trachemys is shown in Figure 4. This hypothesis will be examined in detail in the Discussion section.

**Kinosternon**

The other family to be used for insights in New World biogeography is the Kinosternidae (the mud and musk turtles). In particular, I will focus on the relationships of the genera Kinosternon and Sternotherus (now classified as the Kinosterninae) with those of the genera Claudius and
Figure 4. Hypothesized relationships for the genus *Trachemys*. After Seidel, 1988. a=North America, b=Caribbean, and c=Central/South America.
Staurotypus, now classified as the Staurotypinae (Iverson, 1992; Bickham and Carr, 1983).

The family Kinosternidae as currently recognized contains two extinct genera (Xenochelys and an unnamed genus) and four extant genera (Sternotherus, Kinosternon, Claudius, and Staurotypus). The genus Claudius is monotypic (Claudius angustatus), whereas Staurotypus contains two species (Staurotypus tripocatus and S. salvini). These genera are found in Mexico, Guatemala, and El Salvador. The genus Sternotherus is composed of four species found mainly in the Eastern United States. Kinosternon is composed of fifteen species with several recognized subspecies and ranges across the United States, Central, and South America. There are many instances of sympatry throughout the family's range, for instance K. baurii and K. subrubrum occur in broad sympatry with Sternotherus. Recent literature has placed Sternotherus within Kinosternon (Seidel et al., 1986; Gaffney and Meylan, 1988; Ernst and Barbour, 1989; Iverson, 1991, 1992), however, this view is not as yet universally accepted (Stebbins, 1985; Conant and Collins, 1991).

The fossil history of the family is unclear. Hutchison and Bramble (1981) hypothesized that Kinosternids originated in the Paleocene from Hoplochelys. Recent discoveries indicate primitive members or relatives of the family were present in the late Cretaceous of Northeastern Montana (Hutchinson and Archibald, 1986). The primitive genus Baltemys, upon morphological analysis, has been shown to resemble Staurotypus and Xenochelys, and via similar analyses, has been shown to be similar to extant Kinosternids (Hutchinson, 1991). These data have been used to infer that Kinosternon and Sternotherus diverged prior to the early Eocene. Bramble
et al. (1984) and Dunn (1940) have used these data to propose a
functional/evolutionary series of Kinosternids in North America from
with this assessment of synonymy between Kinosternon and Sternotherus,
however, he also saw evidence that many reversals would have to have
taken place to completely join the two genera. Hutchinson proposed that
Kinosternon is paraphyletic and the genus Thyrosternon (Agassiz, 1857)
should be used for K. baurii and K. subrubrum.

The high Central American species diversity and the presence of the
Staurotopinae and Dermatemys in Central America has led some workers to
hypothesize a Central American origin of the family (Savage, 1966; Berry and
Legler, 1980). However, this hypothesis has generally been disregarded by
recent authors (e.g., Iverson, 1992). As currently interpreted, the fossil
evidence indicates a pre-Pleistocene divergence for Staurotypus and
Claudius. The two subfamilies are proposed to have an early Eocene
divergence with the Kinosternon and Sternotherus split occurring
sometime in the Paleocene.

There has been little consensus emerging from attempts to classify the
members of the Kinosternidae. The first was Siebenrock (1907) who
proposed Staurotypus as a sister group to Kinosternon. This early attempt
was followed by Stejneger (1923, 1925, 1941), Hartweg (1934, 1938) and Legler
(1965). The vast majority of work on the Kinosternidae has been based on
examinations of morphology and/or proteins (Friar, 1972; Seidel et al., 1986).
Seidel et al. (1986) attempted a cladistic and phenetic analysis of the family
based on electrophoretic data and concluded that Kinosternon is paraphyletic
with respect to Sternotherus and that the Eastern United States Kinosternon
were more similar to *Sternotherus* than to the remaining Kinosternids. Iverson (1988) examined the neural bone patterns of the family, and while finding the characters subject to homoplasy, used them in conjunction with other characters to conclude that *Sternotherus* is not monophyletic, nor is it the sister taxon to *Kinosternon*.

Iverson (1991) reexamined the data of Seidel et al. (1986), along with several morphological characters, i.e., pelvic, carapace and plastral measurements, using *Staurotypus, Claudius*, and *Xenochelys* as the outgroups. This analysis also concluded that *Sternotherus* was not monophyletic and that many of the similarities seen in *Kinosternon* and *Sternotherus* were due to convergence. One criticism of Seidel's work had been the use of a binary rather than a multistate coding system (Buth, 1984). Iverson rectified this by employing multistate analysis. The results of the analysis corroborate the findings of Seidel et al. (1986) that: 1) *Sternotherus* is not monophyletic, 2) *K. baurii* and *K. subrubrum* are more closely related to *Sternotherus* than to other Kinosternons, and 3) *Kinosternon* is paraphyletic. Iverson also concluded that there were several differences when comparing the binary versus multistate trees: 1) *K. sonoriense* is sister to *K. hirtipes* in binary coding but sister to *K. scorpioides* in multistate coding, and 2) *S. odoratus* is sister to all other *Sternotherus* with binary coding but sister to only *S. minor* and *S. depressus* in multistate coding. These results suggest that both *Kinosternon* and *Sternotherus* are monophyletic. It was also found that there was no unique character that could be used to define the genus *Sternotherus*.

These studies, along with several others, have been used to formulate an array of hypotheses regarding this family, including: 1) *K. carinatum* is
the most primitive member of the subfamily, Kinosterninae (Zug, 1966), 2) 
*K. baurii* and *K. subrubrum* may be sister taxa and closely related to 
*Sternotherus* (Seidel et al. 1986), 3) *K. herrerai* is the most primitive 
*Kinosternon* (Bramble et al. 1984), 4) the Scorpioides group (*K. acutum, 
almosae, creaseri, integrum, oaxacae, and scorpioides*) is monophyletic 
(Iverson, 1988) and 5) *Kinosternon* is paraphyletic with respect to 
*Sternotherus* (Seidel et al., 1986). The 2nd and 5th hypotheses are rejected by 
Iverson (1991). The rejection of these hypotheses suggests that the 
similarities seen in the Eastern United States *Kinosternon* and *Sternotherus* 
are due to convergence. This analysis also demonstrated the need for a more 
robust data set to analyze the family.

Karyotypic analysis has also been used to examine the family. Sites et 
al., (1979b) found similar G-banding patterns in *K. baurii, K. subrubrum,* and 
*Sternotherus.* They used this to suggest a common ancestry. Bickham and 
Carr (1983) also examined the family and found that the Staurotypinae had a 
2n=54, whereas the Kinosterninae had a 2n=56. This analysis resulted in 
their elevation of the Staurotypinae to familial status, although this view is 
not generally accepted (e.g., Gaffney and Meylan, 1988). There have been 
several hypotheses put forth to explain the morphological diversity in the 
family. For example, it is hypothesized that molluscivory led first to an 
increase in head size and a decrease in plastron size and ultimately to a 
hinge allowing the head to withdraw (Bramble et al., 1984; Iverson, 1991). 
Environmental factors have also been used to explain the diversity. The 
choice of an aquatic habitat could have led to convergence in many of the 
taxa, as well as leaving relic populations as sea levels dropped e.g., *K. hirtipes* 
and *K. integrum* (Iverson, 1991). These various analyses have resulted in an
array of hypothesized relationships within the family which are presented in Figures 5 and 6.

Mitochondrial DNA

The method of analysis employed in this study is sequence analysis of mtDNA. It has been shown that mtDNA in reptiles is similar to other vertebrates in that it possesses a highly conserved gene order and rapid rates of substitution (Brown, 1983, 1985). Furthermore, it has been shown that different regions of the mitochondrial genome are evolving at different rates (e.g., Brown, 1983; Miyamoto and Boyle, 1989). This allows studies to be tailored to the appropriate level, i.e., ordinal or species level (Hedges et al., 1991; Disotell et al., 1992; Lamb and Avise, 1992). The use of mtDNA will also allow historical biogeography to be inferred while allowing the evolutionary history of the group to be proposed (Avise et al., 1979). Sequence analysis can also be used to determine the approximate rate of evolution for the group (Moritz et al., 1987). Therefore, the proposed research, along with other studies currently being conducted, will allow the comparison of rates of evolution in diverse vertebrate lineages. The present study will examine the relationships of the turtle families at the molecular level using mtDNA and then focus on the Emydidae and the Kinosternidae to evaluate the origin and subsequent diversification of these New World families. It is hoped that analysis of mtDNA will clarify many of the relationships within these families, as well as shedding light on their biogeographic history.
Figure 5. Hypothesized relationships of the Kinosternidae based on Seidel et al., 1986.
Figure 6. Hypothesized relationships of the Kinosternidae based on Iverson, 1991.
CHAPTER II
MATERIALS AND METHODS

The taxa examined in this study are listed in the Appendix. They include all twelve recognized families and 72 of 89 recognized genera (Iverson, 1992). The extent of the taxonomic coverage is summarized in Table 2. The analysis of the genus *Trachemys* employs all 6 of the currently recognized species and 17 of the 23 recognized subspecies. The coverage of *Kinosternon* is similar with 16 of the 18 currently recognized species and 9 of 24 recognized subspecies (Iverson, 1992). Results from familial analyses were used to determine the appropriate outgroups for intrafamilial studies.

Blood was isolated from each individual and stored in Blood Storage Buffer (100 mM Tris pH 8.0, 100 mM Na$_2$EDTA, 10 mM NaCl, and 1% SDS) until needed. DNA was extracted from fresh/frozen tissue or whole blood using the proteinase K protocol of Maniatis et al. (1982), as modified by Hillis and Davis (1986). Final DNA pellets were redissolved in 20-1,000 μls of water and stored at 4°C. High molecular weight DNA of sufficient quantity for the Polymerase Chain Reaction (PCR) was extracted from 10-20 μls of blood and resuspended in a total volume of 500 μls.

The primers used in PCR amplification were obtained from Arévalo et al., (1994). The primers ND4 and Leucine were chosen because they show a high degree of conservation within turtle sequences and had been shown to be phylogenetically informative in squamates (Arévalo et al., 1994; Forstner et al., 1995). This region corresponds to positions 11,150 to 12,146 of the Bovine genome (Anderson et al., 1982). A 992 basepair (bp) fragment of mtDNA is amplified by these primers and contains the last 246 codons of the...
Table 2

Extent of familial coverage based on the number of genera, species, or subspecies used in the present study.

<table>
<thead>
<tr>
<th>Family</th>
<th>Genera</th>
<th>Species</th>
<th>Subspecies</th>
</tr>
</thead>
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<td>Bataguridae</td>
<td>20\ text{a} / 23\ text{b}</td>
<td>21/59</td>
<td>2/31</td>
</tr>
<tr>
<td>Carettochelydae</td>
<td>1/1</td>
<td>1/1</td>
<td>-</td>
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<td>Chelidae</td>
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<td>2/2</td>
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<td>2/2</td>
<td>2/2</td>
<td>1/4</td>
</tr>
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<td>1/1</td>
<td>-</td>
</tr>
<tr>
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<td>1/1</td>
<td>-</td>
</tr>
<tr>
<td>Emydidae</td>
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<td>33/36</td>
<td>27/61</td>
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<td>1/10</td>
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<td>1/1</td>
<td>1/5</td>
</tr>
<tr>
<td>Testudinidae</td>
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<td>15/39</td>
<td>4/28</td>
</tr>
<tr>
<td>Trionychidae</td>
<td>5/14</td>
<td>6/23</td>
<td>3/11</td>
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<td><strong>71/88</strong></td>
<td><strong>124/256</strong></td>
<td><strong>50/178</strong></td>
</tr>
<tr>
<td>% <strong>Total</strong></td>
<td><strong>81%</strong></td>
<td><strong>49%</strong></td>
<td><strong>28%</strong></td>
</tr>
</tbody>
</table>

(Key: \text{a} is the number of taxa used in this study and \text{b} is the total number of taxa.)
ND4 gene and the tRNAs Histidine, Serine, and Leucine. PCR and sequencing primers used in this study are listed in Table 3 and their approximate positions in the mitochondrial genome are shown in Figure 7.

Template DNA, along with a negative control (no DNA) and a positive control (Sceloporus DNA), was amplified in 100 μl reactions for 35 cycles. All amplifications used AmpliTag DNA polymerase (Perkin Elmer-Cetus) and a Perkin Elmer-Cetus thermal cycler using the following protocol: denaturing of DNA for 30 s at 94° C, primer annealing for 60 s at 50° C and primer extension for 2 minutes at 72° C with a final extension of ten minutes at 72° C. The makeup of each reaction was as follows: 1 to 5 μl of DNA template (50 ng), 10 μl each of Taq 10× buffer (Promega) and 15 mM MgCl₂, 1 μl of DMSO, 1 μl of primer stock (10 pmol/μl), 0.5 μl of Taq DNA polymerase (1 unit), 1 μl 10 μM DNTPs with 75 μl of sterile water and a 50 μl overlay of mineral oil.

After PCR, 8-10 μl of amplified product, along with lambda Hind III digested size standard, was separated by electrophoresis on 1% agarose minigels. Gels were stained with ethidium bromide and visualized by fluorescence under ultraviolet light. Depending upon yield, 2-5 μl of PCR product was ligated to 0.1 micrograms (μgs) of pBluescript plasmid that had been digested with Eco RV and had dT overhangs added as per Marchuk et al. (1991). Ligations were transformed into DH5α competent cells via Maniatis et al. (1982). Cultures were grown for one hour at 37° C and plated on LB media containing ampicillin, Xgal, and IPTG to select recombinants.

Recombinants were selected and grown overnight at 37° C. The potential positive DNA from clones was then prepared via standard alkaline
### Table 3

List of primers and locations of each used in the present study.

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Primer Sequence 5'-3'</th>
<th>Direction</th>
<th>Reference Positiona</th>
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<td>gacctcttatcaaaaacact</td>
<td>Forward</td>
<td>11,299-11,318</td>
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<tr>
<td>T1-Bat</td>
<td>ccctctacccattataaat</td>
<td>Forward</td>
<td>11,317-11,336</td>
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<tr>
<td>T1-podoc</td>
<td>acargcctaactgctacgcaac</td>
<td>Forward</td>
<td>11,363-11,388</td>
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<tr>
<td>T1-p</td>
<td>tcaatctgctagccaaac</td>
<td>Forward</td>
<td>11,369-11,388</td>
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<tr>
<td>TDF1</td>
<td>ctaacycaacacacctaataat</td>
<td>Forward</td>
<td>11,378-11,403</td>
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<tr>
<td>TR-podoc</td>
<td>gctagrcagaagagtaatgtaatg</td>
<td>Reverse</td>
<td>11,476-11,499</td>
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<td>Turtle 1 Kino</td>
<td>tagcaacacaayaataagcaagcaacc</td>
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<td>TR</td>
<td>taataayagttcggctgatg</td>
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<tr>
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<td>Forward</td>
<td>11,740-11,763</td>
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<td>TF-ery</td>
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<td>11,750-11,775</td>
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<td>Reverse</td>
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<tr>
<td>LEU</td>
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<td>Reverse</td>
<td>12,040-12,065</td>
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<td>PII</td>
<td>atactttactgtgattgca</td>
<td>Reverse</td>
<td>12,048-12,068</td>
</tr>
</tbody>
</table>

(Key: a The reference position in the Bovine Mitochondrial genome.)
Figure 7. Selected primers used in this study and the relative locations of each in the mitochondrial genome.
lysis procedures (Maniatus et al., 1982). All clones were sequenced from double stranded plasmid DNA using Sequenase II (U.S.B.) and the Seq 2.0 protocols following the Sanger method (Sanger et al., 1977). A series of six primers were used to read both strands of each clone (see Table 3). Autoradiographs were read manually and sequences aligned manually, and with the aid of CLUSTAL (Higgins and Sharp, 1988, 1989).

The availability of automated sequencing technology greatly facilitated data collection in the latter part of this study. Cycle sequencing was performed directly on PCR products, which significantly decreased the amount of work involved. For cycle sequencing, products of the appropriate size were purified using the QIAquick PCR purification kit. This process removes any excess primers and polymerase. Three to five μls of purified product were run on 0.8% agarose gels along with an equal amount of 0.2 μg/μl pGEM3Zf(+) ds control template to determine the correct concentration to use in sequencing reactions.

Sequencing reactions were performed using the Applied Bio-Systems, Incorporated (ABI) Dideoxy termination cycle sequencing kit in conjunction with an ABI 373A automated sequencer. The kit uses a strategy similar to that employed previously, except four dye-labeled dideoxynucleotides are used in a termination mix which also contains 10x Taq Buffer and Taq polymerase. The reactions contain 400 ng of DNA template (2-10 μls), 8 μls of Termination mix, 1 μl of primer (10 pmol/μl) and sterile water to reach the final volume of 20 μls. A 30 μl overlay of mineral oil was added to eliminate evaporation during PCR. The sequencing profile was as follows: 25 cycles at 93° C for 30 s, 50° C for 15 s, and 60° C for 4 minutes.
Sequenced products were cleaned on Centri-sep columns containing 0.05 grams of SEPHADEX-G50 to remove excess primer and dye. Cleaned reactions were dried under vacuum and stored at -80° C until needed. Samples were resuspended in loading buffer as per manufacturers instructions (ABI, 1993) and analyzed on an ABI 373A automated DNA sequencer. Both strands of the DNA were read from each analyzed product and compared to determine the sequence of any ambiguous positions. Manual proofreading of the chromatograms was also used in cases where the sequence could not be determined by the aforementioned methods. A consensus was then reached and used to identify each basepair in question.

Aligned sequences were analyzed using the PAUP* v 4.0d52 (Swofford, 1996). Analyses were performed using three methodologies; Neighbor Joining (NJ), Maximum Parsimony (MP), and Maximum Likelihood (MLE). Random addition sequences of 10 replicates were utilized in MP analysis. MLE analysis utilized the default settings in PAUP. Bootstrap (BP) and Jackknife (JE) replicates of 500 resamplings were performed for both MP and NJ analytical techniques to determine the strength of support for each clade in the resulting phylogenies (Efron and Gong, 1983; Felsenstein, 1985; Hillis and Huelsenbeck, 1992).

The use of multiple outgroups was employed to identify the correct phylogeny (Farris, 1982). Random addition sequences and random starting trees were utilized in parsimony analyses, where appropriate, to ensure that a global optimum was reached. Positional and transition/transversion weighting schemes were employed (see below). It has been suggested that phylogenies based on single genes may not accurately reflect evolution of the terminal taxa (i.e., Nei, 1986). However, at the family/genera level, lineage
sorting and gene versus species tree complications should not be a factor (Pamillo and Nei, 1988). Nei (1991) concluded the methodologies of parsimony, distance, and maximum likelihood are equally likely to determine the correct phylogeny and all were used in this analysis, where possible.

The number of taxa used in the family study was such that computer run times were prohibitive for MP and MLE analyses. Therefore, ten subsets of the original data set created by randomly sampling taxa within each subfamily were used in all but NJ analyses (Table 4). This analysis, coupled with the breadth of sampling, provided an interesting opportunity to evaluate the impact of taxonomic sampling on the recovered phylogeny of turtle families. Subsequent analyses deleted the third nucleotide position in all data sets to determine the signal strength at this position.

The chart option of MacClade 3.0 was used to examine the data for transition/transversion (ts/tv) and positional bias (Madison and Madison, 1992). This option counts the number of specified changes i.e., ts versus tv. These data were then standardized and used in the weighting schemes presented below. This analysis determined that there was a ts/tv bias, and that it was different between the data sets. Li and Grauer (1991) have shown that phylogenetic analyses can be compromised as a result of these types of bias. Therefore, the following weighting schemes were employed. The familial analysis employed a 10:23 ts/tv weighting scheme, the Kinosternon analysis employed a 10:37 ts/tv weighting scheme and the Trachemys analysis employed a 10:31 ts/tv weighting scheme. There were no significant differences found in ts/tv bias when tRNAs were deleted from the data set.
Table 4
List of taxa used in each of the 10 analyses of this study.

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<td>G. elegans</td>
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<td>G. spengleri</td>
<td>R. funerea</td>
</tr>
<tr>
<td></td>
<td>C. amboinensis</td>
<td>C. borneoensis</td>
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<tr>
<td></td>
<td>R. funerea</td>
<td>H. spinosa</td>
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<td>D. <em>r.</em> miaria</td>
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<td>C. mydas (p)</td>
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<td>C. <em>serpentina</em></td>
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<td>D. <em>mawii</em></td>
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<td>C. <em>angustatus</em></td>
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<td>K. <em>baurii</em></td>
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Table 4-Continued

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Positional analysis of the ND4 gene revealed, as expected, differential rates of evolution across the codon positions. Unlike ts/tv bias, there were no differences found between the three data sets in rates of evolution of 1st, 2nd, or 3rd nucleotide positions. Therefore, the same weighting scheme (10:25:4) was utilized in all three analyses. Saturation was also assessed by calculating the uncorrected p distance between taxa separately for the 1st, 2nd, and 3rd positions in the ND4 gene and plotting versus time from estimated dates in the fossil record. Key dates have been discussed in the Introduction and are summarized in Table 5. This assessed the level of phylogenetic signal remaining at each codon position for different levels of divergence. The differences calculated for the 1st and 2nd positions were then utilized to calibrate a molecular clock for the ND4 gene and to date some of the key events in the New World turtle radiation.
Table 5

Major radiations of turtles known from the fossil record and the approximate time of each occurrence. \( \text{my} = \text{million years} \).

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CHAPTER III
RESULTS

Families

There are twelve families of extant turtles and all are represented in the present study. Several insertion/deletion events were found to be unique among the families. For example, the Batagurid *Siebenrockiella crassicolis* has a codon inserted at position 124 of the ND4 gene. All Batagurids are missing a codon at position 735 and the poly-adenylated stop codon "t" at position 743, which is replaced by TAA at position 742 in the ND4 gene. Testudinids have an insertion at positions 898-914 between the Serine and Leucine tRNAs. The Chelidae, Pelomedusidae, and Trionychidae all are missing part of arm 1 of the Serine tRNA at positions 846-850. The Pelomedusidae and Chelidae also have codons missing at positions 216, 617, and 743 of the ND4 gene.

There are also several unique features within the Kinosternidae. For example, the genera *Claudius* and *Staurotypus* are missing codons at positions 738 and 741 of the ND4 resulting in an early stop codon, AGA at position 733, and a variable insert at position 744. Furthermore, they also have extra sequence at positions 898-914 between the Serine and Leucine tRNAs. The Kinosternidae are also missing codons at positions 735, 738, and 741 of the ND4 gene and possess the early stop codon at position 733 and have a variable gap at position 744 between the ND4 gene and the Histidine tRNA.
The patterns of divergence at each codon position versus time are shown in Figure 8. At the third position, this region of the ND4 gene has become saturated. It appears that the third position would not be useful for taxa older than about 10 million years. At the 2nd codon position, the ND4 gene has not become saturated over the timespan of this study. This codon position has a linear slope for all divergence times and would be useful for all taxonomic levels. The first codon position appears to be reaching saturation, but does not appear saturated at this time and would also be informative in this analysis. These results indicate that the first and second positions of the ND4 gene are still evolving at rates that are appropriate for use in a molecular clock across a broad range of divergence times.

In the analysis of the total data set both distance (performed using the Kimura 2-parameter distance measure) and parsimony methods were utilized. Maximum likelihood, however, was not computationally feasible for 232+ taxa. The relationships between the families of the Cryptodira have been a major problem since Williams' (1950) classification. Figure 9 represents the results of the NJ analysis for the complete data set and is presented as a phylogram. Figure 10 shows a reduced taxon set (for clarity) and the relationships of the families as determined in the NJ analysis are presented as a cladogram. The numbers on each branch indicate the support for each clade in the BP or JE analyses. There is strong support for the monophyly of the Pleurodiran families Pelomedusidae and Chelidae. Within the Pelomedusidae, there does appear to be a sister relationship between Podocnemis, Erymnochelys, plus Peltoscephalus and Pelusios and Pelomedusa, but the two groups are not resolved as distinct families.
Figure 8. Plot of the first (o), second (x), and third (Δ) positions versus known divergence times from the fossil record. These data were used in the calibration of a molecular clock for this study.
Figure 9. Neighbor Joining analysis of the total data set presented as a phylogram. Branch lengths indicate the amount of divergence between taxa. In the present phylogram, the family/taxa names are not as important as the overall topology. Family relationships will be clarified in subsequent cladograms.
Figure 10. Results of the Neighbor Joining analysis of the total data set with all positions weighted equally. The first number indicates the Bootstrap support and the second indicates the Jackknife support. Species diversity has been removed for clarity.
In this analysis there appears to be an advanced clade of Cryptodires containing the Kinosternidae, Chelydridae, Cheloniidae, Dematemydidae, Dermochelidae, Testudinidae, Bataguridae, Emydidae, and Platysternidae. This topology is extremely well supported in all types of analyses using all data and only slightly less well supported when 3rd positions are excluded. This result would lend support to Chen and Mao (1981) and Friar (1983) who found similar results in their respective serological and immunological analyses.

There were several other interesting findings, including the placement of the Carettochelyidae outside of the Trionychidae, and the alignment of the Dermatemydidae and the Kinosternidae with the Chelydridae, exclusive of the Platysternidae. The Chelydridae have been consistently assigned to a sister group relationship with the Platysternidae (see previous discussion). However, this result is not evident in this analysis. The sister group to the Chelydridae would appear to be the Kinosternidae, a result suggested by Williams (1950). Furthermore, it is evident that while the Emydidae, Testudinidae, and Bataguridae may all be sister clades, there is no evidence of a close relationship between the Bataguridae and the Emydidae.

Bootstrap (BP) and Jackknife (JE) analyses were performed on the complete data set and the values are indicated on the appropriate branches of Figure 10. Five hundred replicates of each analysis were performed to determine the strength of signal in the data set. Overall, the support for each clade did not differ between these two analyses.

Not surprisingly, Trionychids take the basal position within the Cryptodira. There is good Bootstrap support (78%) for a basal position for the
softshells and there is also extremely good support (96%) for including the Cyclanorbinae and the Trionychinae, as a single family. Surprisingly, there is reasonable BP and JE support for the finding that the Carettochelyidea should not be aligned in a superfamily with the Trionychidae. The relationships of the remaining Pleurodire families could not be resolved in the NJ analysis, however, their distinctiveness as an advanced clade is well supported in both the BP and JE analyses (93%).

The remaining families group into six clades and each will be discussed below. There is good support for a clade containing the Dermatemydidae, the Kinosternidae, and the Chelydridae (70%). The sister relationship between the Dermatemydidae and the Staurotypidae plus the Kinosternidae has extremely good support (100%). Bickham and Carr (1983) erected the Staurotypidae to include *Staurotypus* and *Claudius*. However in no analysis do the Staurotypidae form a sister clade to any group but the Kinosternidae. This strongly supports their inclusion within the Kinosternidae. As mentioned previously, there has been a great deal of evidence for the Dermatemydidae as the sister group to the Kinosternidae. Contrary to Bickham and Carr (1983), the sister group relationship of the Dermatemydidae and the Staurotypinae to the Kinosternidae has extremely high support in the BP and JE analyses (100%). Furthermore, there appears to be at least three clades present within the Kinosternidae. One of these well defined clades contains the taxa that are currently recognized as *Sternotherus* (i.e., Conant and Collins, 1991).

The Platysternidae have no consistent place within the phylogeny. It is hoped that increasing the number of individuals and the scope of the subspecific coverage may change this result by breaking this long branch
(Figure 9). However, at the present time the sister relationship of this family cannot be resolved.

The position of the Cheloniidae also cannot be resolved with the current data set. Furthermore, in spite of strong morphological support, the molecular support for this clade is fairly weak (62%) and may indicate saturation of mtDNA at this ancient divergence. In contrast, while there is only weak support for the Dermochelyidae (the leatherbacks) as the sister to all other sea turtles, there is good support for the monophyly of the Cheloniids and strong support for relationships within the Cheloniidae in all data analyses. These data support Dutton et al. (1996) who show a sister relationship between the Leatherbacks and hard-shelled sea turtles. There is slightly less support in the MP analysis when 3rd positions are deleted, but all other analyses are robust to positional weighting.

The Testudinidae have good support overall (89%), and a relationship with Batagurids is found in the NJ tree, but this is not supported by BP or JE analyses. It appears that the genus Geochelone, as currently recognized, is not valid. The Batagurinae also have extremely good support at the familial level (95, 97%) and it does not appear that the Batagurinae are paraphyletic as Hirayama (1984) proposed. The Emydidae are also well supported (100%), although, as with the entire advanced clade, their position relative to the other families cannot be determined. However, there is extremely good support for the two tribes currently recognized in the Emydinae (100%). The relationship among the Batagurids, Testudinids, and Emydids has been examined as thoroughly as any, within the extant Cryptodires. All possible combinations of relationship have been proposed for these three families. This analysis indicates that all are monophyletic, but the only relationship...
that is consistently found is between the Bataguridae and the Testudinidae. The Emydidae are unresolved relative to the other families in the advanced clade. The relationships within this family will be discussed in greater detail when the *Trachemys* results are discussed.

The total data set was also analyzed using MP to determine concordance with the NJ analysis. Initially, 10 random addition sequences were tried, using a seed equal to one and the MULPARS option to retain all equally parsimonious solutions. However, this analysis proved impossible using existing technologies due to excessive computer run times (>10 days), and a new tactic was tried. In this second analysis, the number of random addition sequences was increased to 20, but the MULPARS option on PAUP was inactivated. This was done to expedite the search by only saving one tree at each addition and branch swapping step, instead of all equally parsimonious trees. The possibility of finding a suboptimal tree was lessened by the increase in the number of random addition sequences.

As a result of this search, two trees were found of 6,153 steps with a Consistency Index=0.23 (CI) and an Retention Index=0.81 (RI). The only difference between the two was the position of the Cheloniidae. These trees were used as the starting point for another heuristic search. However, this search was performed with the MULPARS option active. The strict consensus tree is presented in Figure 11. The MP and NJ searches found most major familial relationships to be the same, although the relationships between families did differ slightly. BP analysis was performed, JE analysis however, was not due to time constraints, but the topology evident in the MP analysis is similar to that seen in the NJ analysis and would strengthen this result.
Figure 11. The strict consensus of two trees of 6,153 steps resulting from a Maximum Parsimony analysis of the total data set. The consensus tree had a Consistency Index equal to 0.232 and a Retention Index equal to 0.807. The numbers on each branch represent Bootstrap values. Species have been removed for clarity.
There were several differences between the NJ and MP analyses. For example, in the NJ analysis there is a sister relationship between the Bataguridae, Testudinidae, and the Cheloniidae. Whereas, in MP analysis, the Bataguridae and the Testudinidae form a sister group, but this clade is unresolved relative to others of the "advanced" group, including the Cheloniidae. Furthermore, in the MP analysis the leatherbacks do not show a sister relationship with the other sea turtles. In this analysis, the leatherbacks form a sister clade with the Platysternidae. However, in both analyses, the sister relationships between the Chelydridae, the Dermatemydidae, and the Kinosternidae is confirmed, although in MP this group is sister to the Emydids. The relationships within the Emydinae are also confirmed in comparisons between these analyses, as is the relationship between the Carettochelyidae and the Trionychidae. Other differences are evident in these trees, but will not be discussed at this time as they apply only at the generic level. Therefore, the major differences between the analyses are relationships of the Platysternidae, the Carettochelyidae, and the Cheloniidae. These discrepancies all occur in branches showing weak BP and JE support in NJ analyses and may result from a failure to find the most parsimonious solution in the MP analysis or from biases affecting the MP analysis which will be investigated further using weighting schemes. However, as previously stated, many of these problematic relationships would be expected to be unresolved in BP and JE analyses.

Upon the removal of the third positions of the ND4 gene a NJ analysis produces a tree with much the same resolution of major clades but largely unresolved within families. The results of BP and JE analysis are seen in Figure 12. A MP analysis was attempted but once again was unable
Figure 12. Cladogram resulting from a Neighbor Joining analysis of the total data set, with third positions removed. The first value indicates Bootstrap support and the second indicates Jackknife support. Species have been removed for clarity.
to be performed due to time constraints and the size of the data set. As in the Total analysis, there is very good support for the Trionychidae (96.95%). However, there is also moderate support for including the Carettochelyidae within this clade (68.72%). As before, there is good support for the distinction of the primitive and advanced clades (83.84%). In this analysis, there are seven clades instead of six as seen in the previous analyses, as there is no BP or JE support for a relationship between the Chelydridae and the Kinosternidae. There is better support for the Cheloniidae at 84% without third positions versus 61% with third positions and slightly less support for the Testudinidae 61% versus 89%. The results from this analysis indicate that there is signal at third positions as increased resolution with BP and JE support is provided when these positions are included. However, there are large differences in rates of change at the different codon positions, and in some cases a weighting scheme should be employed to correct for this positional bias.

After these initial analyses, it was determined that the size of the data set (>230 OTUs) was hampering analyses of familial relationships, i.e., most analyses required more than 5 days on a 132 mHz PowerMac computer. As a result, the taxa were divided by family and analyzed in 10 separate data sets using NJ, MP, and MLE. Bootstrap and JE analyses were also performed where appropriate. Taxa were chosen to include multiple, nonoverlapping generic representatives of all major turtle groups and any overlap in taxa is primarily due to monotypic or ditypic families such as the Platysternidae. As a result, these analyses offer an excellent opportunity to assess the impact of taxonomic sampling on phylogenetic analyses. Table 6 lists the frequency out of the 10 analyses that each specified clade was recovered by each method.
### TABLE 6

Results of the analysis of the 10 data sets using Neighbor Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (MLE) with Bootstrap (BP) and Jackknife (JE) support.

<table>
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<tr>
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of analysis. The clades examined represent the most often cited familial relationships (see Bickham and Carr, 1983; Gaffney and Meylan, 1988). The MP analysis resulted in trees ranging from 2,654 to 2,793 steps with CI's between 0.34 and 0.40 and RI's between 0.40 and 0.43. This would seem to indicate that taxon sampling was affecting both the tree length and the level of homoplasy in the data set. While most trees produced by the 10 analyses were relatively similar in overall structure, there were some notable exceptions (Figure 13). MLE analysis was also performed on each of the 10 data sets. These results again are summarized in Table 6. In general, the findings between MLE, NJ, and MP were very similar, with MLE and NJ finding similar phylogenies most often. However, several differences were evident between these methodologies, i.e., there is significantly less MLE support for a superfamily containing the Trionychidae and the Carettochelyidae, (NJ=7, MP=6 and MLE=3). There is MLE support, however, for Carettochelys as part of the primitive clade, but as the most advanced member (NJ=3, MP=2, and MLE=7).

Initial analyses support the monophyly of each of the major families (NJ=10, MP=7-10, and MLE=10). This result was confirmed in BP and JE analyses with both NJ and MP. The only families lacking strong support in every analysis include the Cheloniidae (MP) and the Trionychidae (MP). In the NJ analysis, the monophyly of the sea turtles was well supported (NJ=10, BP=8, JE=9). However, MP analysis showed the monophyly of the family less often, and with very little support in BP and JE analyses (MP=8, MPBP=1, and MPJE=1). The Trionychidae have strong support in the NJ analysis (NJ=10, NJBP=10, and NJJE=10). However, in the MP analysis there was less support (MP=7, MPBP=6, and MPJE=6). The Carettochelyidae have always
Figure 13. Cladograms representing the diversity of topologies obtained in the analyses using the 10 data sets.
been a problematic family. The majority of phylogenies put forth have placed *Carettochelys* with the Trionychidae. In both preliminary analyses, this relationship was not well supported. The MP analysis finds this clade (MP=7, MPBP=3, and MPJE=3) slightly more often than does NJ (NJ=6, NJBP=2, and NJJE=2). The alternative MP and NJ phylogenies put *Carettochelys* in a basal position relative to the advanced clade, although this placement is never supported by either BP or JE analysis.

There have been various hypotheses put forth as to the relationships of turtle families (see Chapter 2 and Discussion). The most often examined relationship is between the Emydidae, Testudinidae, and Bataguridae. Our molecular analyses show little or no support for the Emydids as a sister clade with either the Testudinids or the Batagurids (Table 6). Emydids are usually found in a sister relationship to a clade containing these families, or are unresolved. The Batagurids and Testudinids form a sister group in the majority of analyses (NJ=10, MP=9, and MLE=10), but there is uneven support for this clade in the BP and JE analyses (NJBP=6, NJJE=3, MPBP=5, MPJE=5).

There is overwhelming support for the Dermatemydidae as the sister group to the Kinosternids (NJ, MP, MLE, and all BP and JE=10). There is strong support for the monophyly of a group containing the Chelydrids and the Kinosternids+Dermatemydids (NJ=9, MP=9, and MLE=10). This relationship is also very well supported in BP and JE analyses (NJBP=8, NJJE=8, MPBP=7, and MPJE=8). However, a strong affinity between the Chelydridae and the Platysternidae, as has been proposed by Gaffney and Meylan, 1988, is almost never evident (NJ=0, MP=1, MLE=0; never BP or JE
support). Contra Bickham and Carr (1983), these analyses never place the Staurotypinae outside the Kinosternidae.

There appear to be several major trends evident in these analyses. The first is the delineation of a primitive and an advanced Pleurodire group. This relationship has strong support in both analyses. Secondly, there is much disagreement as to the placement of the Platysternidae. This family occupies all possible positions in these analyses: as a basal member of the advanced clade or internally in this clade in a variety of places. However, there is no BP or JE support for any scenario.

The ten data sets were subsequently reexamined with the third codon position deleted. The results of this analysis are presented in Table 7. As in the previous analyses, there is good support for the monophyly of all families, except in the MP analysis for the Trionychidae and the Cheloniidae. These analyses also show excellent support for the sister relationship of the Carettochelyidae and the Trionychidae (NJ, MP, and MLE=10). They do not support the previously seen relationship between the Bataguridae and the Testudinidae (NJ=6, MP=2, and MLE=3), or that of the Chelydridae and the Kinosternidae (NJ=2, MP=5, and MLE=7). It is obvious from this analysis that removing third positions eliminates resolution at terminal branches, while increasing the resolution of basal branches.

Positional weighting and ts/tv bias weighting schemes were attempted, but not completed on the total data set due to time constraints. However, these analyses were performed on the Emydids and Kinosternids. The results of these analyses are presented below.
TABLE 7
Results of the analysis of the 10 data sets with the third positions deleted using Neighbor Joining (NJ),
Maximum Parsimony (MP), and Maximum Likelihood (MLE) with Bootstrap (BP) and Jackknife (JE) support.

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<th>NJJE</th>
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<th>MPBP</th>
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Emydidae

Initially, 112 taxa were examined to determine the phylogeny of the Emydidae. A composite outgroup of 2 Batagurids (Geoemyda spengleri and Rhinoclemys funerea) and 2 Testudinids (Geochelone nigra microphyes and Gopherus berlandieri) was used in this analysis.

The initial analysis included all characters weighted equally and used both MP and NJ. A NJ phylogram is presented in Figure 14. A MP cladogram is presented in Figure 15. There are several interesting findings in this initial analysis. The overall phylogeny obtained differs significantly from the generally accepted hypotheses (e.g., Gaffney and Meylan, 1988). The initial clade, the Emydini, possesses several interesting relationships. For example, the genera Emys and Emydoidea appear as sister taxa, a position generally accepted until they were moved to different genera by Loveridge and Williams (1957). Furthermore the genus Terrapene appears sister to a clade containing all other members of the Emydini. Finally, the genus Clemmys resolves as paraphyletic with respect to Emys, Terrapene, and Emydoidea, a result suggested by both Burke et al. (1996) and Bickham et al. (1996).

The Deirochelyini also reveal several interesting relationships. The genus Deirochelys was once considered a sister to Emydoidea (e.g., McDowell, 1964), however, these data do not show this relationship, and concur with Gaffney and Meylan (1988). This genus takes the basal position as the most primitive member of the Deirochelyini. Also, the majority of published phylogenies have grouped Trachemys with Pseudemys, and Chrysemys (e.g., Gaffney and Meylan, 1988), even though morphological studies indicate this relationship may not be valid (e.g., Seidel and Smith,
Figure 14. Neighbor Joining phylogram representing the modified Trachemys data set.
Figure 15. Cladogram resulting from a Maximum Parsimony analysis of the *Trachemys* data set. The first number indicates Bootstrap support and the second indicates Jackknife support for each relationship. Species have been removed for clarity.
The molecular data support Seidel and Smith (1986) and indicate that
the sister relationship is between *Trachemys, Graptemys,* and *Malaclemys.*
This relationship is further strengthened by the fact that *Trachemys* is the
only Emydid genus to have crossed a salt water barrier (during colonization
of the Caribbean) and *Malaclemys* is the only brackish water Emydid.
Finally, the sister relationship between *Pseudemys* and *Chrysemys* is upheld
in the present analysis.

The genus of most importance to this study in terms of New World
biogeography, within the Emydidae, is *Trachemys* and the relationships of
the genus will be examined below. The basal taxa within the Emydidae in
this initial analysis is *T. s. emolli.* Two other clades are resolved subsequent
to this, the first contains the temperate and Caribbean *scripta* along with *T.
dorbigni.* The other clade contains all MesoAmerican taxa. However, these
results were not seen with BP or JE analyses, this “starburst” topology is
evident throughout the subsequent analyses and would seem to indicate a
rapid radiation and rule out insufficient data as the cause of this topology.

The Emydids were also analyzed using MP. Initial attempts to analyze
this data set were unsuccessful due to the number of taxa and the minute
differences between the sequences from conspecific individuals. Based on
the family level analysis, *Graptemys* and *Malaclemys* were chosen as
outgroups. The data set was subsequently reduced to 60 taxa by deleting all
taxa but *Trachemys, Graptemys,* and *Malaclemys.* Within *Trachemys,* all
taxa with identical sequences were deleted to further simplify the analysis.

The reanalysis using NJ results in an essentially unchanged
phylogeny and BP and JE analysis again result in the starburst phylogeny
evident in the previous analysis. In this analysis, the basal clade within
*Trachemys* contains the temperate *scripta* (*elegans, gaigeae, scripta, and troosti*) in keeping with Emydids as a group with a temperate North American origin. In this clade only *T. s. gaigeae* falls out as distinctive. The next clade resolved contains *T. s. emolli*, with the Caribbean taxa (*T. decussata, T. decorata, T. terrapen, and T. stejnegeri*) emerging next. *T. dorbigni* is resolved subsequently as a sister to the Mexican/South American taxa.

Once again, there is over 90% support for *T. s. gaigeae, T. s. yaquia* and *T. decussata* as basal members within their respective clades. The MP analysis resulted in 252 trees of 266 steps with a CI=0.78 and an RI=0.94. Although, BP and JE analyses differ slightly by weakly supporting the alignment of *T. s. emolli* in a clade with the MesoAmerican *scripta* and *T. s. dorbigni* with the U. S. *scripta*, the starburst topology was also seen here (Figure 16).

BP and JE analyses show similar results to the NJ analysis although, while all major *Trachemys* clades have good support, there is no support for relationships between the groups. The results indicate a starburst radiation in *Trachemys*. Support for *T. s. gaigeae* being distinct from the remaining temperate taxa is very good, with little distinguishing this remaining group. The Mexican subspecies from the gulf coast of Mexico (*T. s. cataspila*) to Venezuela (*T. s. chichiriviche*) are virtually identical. The only MesoAmerican form that appears distinct is *T. s. yaquia*, from the Pacific coast of Mexico. *T. s. emolli* and *T. s. dorbigni* are again distinct from all remaining *Trachemys*, though their positions remain unresolved. The remaining clade contains all the Caribbean taxa, with the Cuban taxa forming a sister clade to all others.
Figure 16. Neighbor Joining analysis of the modified *Trachemys* data set. The first value indicates the Bootstrap support and the second indicates the Jackknife support for each branch.
The data set was subsequently analyzed using NJ and MP with the 3rd codon position deleted. The NJ analysis revealed a loss of any resolution in the phylogeny. The MP search found 584 trees of 112 steps with a CI=0.84 and an RI=0.95. As with the NJ analysis, the resolution on the tree was poor with the exception of the MesoAmerican taxa, and is not presented here.

As an alternative to losing all information in 3rd positions, a positional weighting scheme of 10/25/4 was employed to examine 1st, 2nd, and 3rd positions. The resulting tree is similar to that of the previous analysis, but with BP analysis many of these branches collapsed. Figure 17 show the resultant topology and BP support. The starburst topology evident in the previous analyses is also seen here. *T. s. emolli* and *T. dorbigni* are unresolved, *T. s. yaquia* is the only MesoAmerican *scripta* resolved, and *T. s. gaigeae* is the only distinct temperate *scripta*. The Antillean taxa are resolved in a similar manner to that seen in the previous analyses. A simple heuristic search was performed to examine the effect of ts/tv bias. Upon analysis of the data set using the chart option of MacClade 3.0 it was determined that the appropriate ratio was 10:31. A MP search found 10 trees of 4,045 steps. This analysis recovered all of the major groups seen in previous analyses. For example, *T. s. gaigeae* forms a sister clade to the remaining temperate *scripta*. The Antillean taxa group in a similar manner with *T. decussata* forming a sister clade to all remaining Caribbean *Trachemys*. *T. s. dorbigni* and *T. s. emolli* are again unresolved, but sister to the MesoAmerican *scripta*. Within this MesoAmerican clade, the only taxon that is resolved is *T. s. yaquia*. Had BP and JE analyses been possible it is believed that this tree would have been similar to that seen in previous analyses and is therefore not presented here.
Figure 17. Cladogram resulting from a Maximum Parsimony search of the *Trachemys* data set using a weighting scheme of 10:25:4 for first, second, and third positions. Values indicate Bootstrap support.
Kinosternidae

The initial NJ and MP analyses included all characters weighted equally. Although there were minor differences in the relationships within the major groups, the composition of the major clades are nearly identical. A NJ phylogram is presented as Figure 18. A NJ cladogram is shown in Figure 19. When the Chelydridae were used as the outgroup, both analyses confirmed the sister group relationship of the Staurotypinae to the Kinosternidae. In addition, the NJ analysis delineated three main groups within the *Kinosternon* with strong BP and JE support (Figure 19).

The basal clade contains: *K. leucostomum, K. herrerai, K. creaseri, K. acutum,* and *K. dunni,* all taxa with ranges across Central America, South America, and southern Mexico. The two derived clades contain: 1) all taxa formerly recognized as *Sternotherus,* and 2) the temperate *Kinosternon* plus the Mexican species. *K. f. arizonense* is distinct from all other temperate *Kinosternon,* forming a sister group to them, with *K. f. flavascens* more similar to these taxa than to *K. f. arizonense.* The basal lineage of this group is *K. alamosae* which, in conjunction with the position of *K. f. arizonense,* may indicate the Pacific coast of Mexico as the point of origin for this group. Therefore, these initial results suggest that North America has been invaded twice, once from the east through Texas (*Sternotherus*) and once from the west through Arizona (temperate *Kinosternon*).

MP analysis utilized ten random additions with a seed equal to one and the MULPARS option on PAUP active. The resulting search found 566 equally parsimonious trees of 1,180 steps, with a CI=0.511 and an RI=0.842 and a strict consensus is shown in Figure 20. BP and JE analyses were also performed with conditions identical to those in previous analyses, except the
Figure 18. Neighbor Joining phylogram representing the *Kinosternon* data set.
Figure 19. Neighbor Joining analysis of the *Kinosternon* data set. The first value indicates the Bootstrap support and the second indicates the Jackknife support for each branch.
Figure 20. Maximum Parsimony analysis of the *Kinosternon* data set. The first value indicates the Bootstrap support and the second indicates the Jackknife support for each branch.
number of BP replicates was reduced to 150 to expedite the search. The major clades were identical to those found in the NJ analysis, however, there were minor differences evident in the species relationships. For example, the NJ analysis shows a clade containing *K. leucostomum* #P and *K. dunni*, while in the MP analysis the relationships between these taxa and the remaining members of the initial clade are not resolved. A similar lack of resolution is seen within *Sternotherus*, and with *K. f. arizonense*, and *K. alamosae*.

One difference evident between the NJ and MP analyses is the level of support for the basal position of the initial Mexican group. This group and their basal position receives strong support in NJ analysis (92-100%), but much less support in seen in the MP analysis (51-68%). The composition in this clade, however, is identical. There is a marginal amount of support for the relationship of the remaining Kinosternids. Within the remaining taxa, there is very good support for *Sternotherus* (97%), although except for a clade containing *K. carinatum* and *K. depressum* the relationships within this group remain unresolved. There is also good support for the difference between the U. S. and the remaining Kinosternids (85%) in the BP analysis, however, this is weakly supported in the JE analysis (62%). Unlike NJ analyses, the position of *K. f. arizonense* remains unresolved in MP analysis. In the remaining U. S. and Mexican taxa relationships are similar to that seen previously, but with limited BP and JE support.

When third positions were deleted from the data set, NJ and MP show nearly identical topologies. The removal of this position significantly decreased the level of support in both BP and JE analyses and while the same major clades are evident, support is only weak to moderate. All major
clades and their relationships are identical to those seen in previous analyses, and are not presented here. However, it is important to note that this analysis does support a Central American origin for the Kinosternidae and multiple invasions into North America.

A simple heuristic search was performed to examine the impact of ts/tv bias using a relative weighting of 37:10. This search found 669 trees of 18,938 steps (Figure 21). The major clades seen in previous analyses and their relationships were still evident in this analysis. A positional weighting scheme of 10/25/4 was also utilized in this analysis and resulted in 40 trees of 2,720 steps. Once again, the results are similar that found in the previous analysis, however, there are a few exceptions including the placement of *K. leucostomum* #P and *K. dunni* outside the initial Mexican clade. Also, *K. alamosae* is resolved in a basal position to the temperate *Kinosternon*, not with the MesoAmerican taxa. However, BP and JE have not been completed for either analysis due to the enormous computer time required and thus the level of support for these results cannot be assessed.

Overall, the results from the family level analysis indicate that there is good support for the monophyly of each major family. There is good support for the inclusion of the Carettochelyidae with the Trionychidae and the placement if these families basal to an advanced Cryptodire clade. The sister relationship of the Bataguridae and Testudinidae is well supported, but there is no support for any relationship of these taxa to the Emydidae. The Cheloniidae is also well supported within the advanced clade. Finally, the relationship between the Chelydridae and the Kinosternidae is extremely well supported, as is the monophyly of *Sternotherus* within the Kinosternidae. There is extremely strong support for a rapid radiation
Figure 21. A strict consensus of the *Kinosternon* data set containing 669 trees of 18,938 steps generated using a transition/transversion ratio of 10:37.
within *Trachemys* with North American origins and two reinvasions of South America and the Caribbean. The Kinosternids resolve into two distinct subfamilies: the Staurotypinae and the Kinosterninae. The Staurotypinae, *Claudius* and *Staurotypus*, are restricted to Central America. The Kinosterninae also have their basal clades in Central America, with multiple invasions occurring into North and South America from this Central American point of origin.
CHAPTER IV
DISCUSSION AND CONCLUSIONS

Families

The two major suborders of turtles, Pleurodira and Cryptodira, evolved early in the history of turtles. It has been proposed that each suborder is monophyletic (e.g., Gaffney and Meylan, 1988). For the Pleurodira this has interesting implications based on present-day patterns of distribution. Generally, the Pleurodira are separated into two families, the Chelidae and the Pelomedusidae. The Chelidae have a range limited to Australia and South America, whereas the Pelomedusidae range across Africa and South America. Gaffney and Meylan (1988) support a paraphyletic Chelidae, with the Australian genus *Chelodina* sister to the South American genus *Hydromedusa*. In a more recent work, Shaffer et al. (1997), using Cytochrome b, 12S rDNA, and 115 morphological characters, find that the South American genera are monophyletic (BP=58%) as are the Australian genera (BP=69%).

The work of Gaffney and Meylan (1988) supports the paraphyly of this subfamily, and thus suggests that the ancestor of the Chelidae was present before the split of South America and Australia, about 55 million years ago. Shaffer et al. (1997) support a monophyletic South American and Australian group. This finding would support a more recent divergence, although they give no date for this split. Our analysis sheds no light on the question of the paraphyly versus monophyly of the Chelidae, as our sample of South American genera is limited to *Hydromedusa*. However, our results do suggest that the split was older than 55 million years ago, though no exact
date could be determined due to saturation of our molecular clock after about 100 million years.

Due to their present-day distribution, the Pelomedusidae are thought to be ancestral to the Chelidae. This would seem to indicate an ancient origin for the Pelomedusidae, perhaps Gondwanan. Another fact that lends support to the hypothesis of the Pelomedusidae being ancestral to the Chelidae is the inability of the Pelomedusids to cross salt water barriers. Gaffney and Meylan (1988) have defined two major clades within the Pelomedusidae. The first contains the genera *Podocnemis, Peltocephalus,* and *Erymnochelys,* there is some support for defining these genera as the Podocnemidae (see Introduction). The second contains the genera *Pelomedusa* and *Pelusios,* which would be retained within the Pelomedusidae. Shaffer et al. (1997) support the restricted Podocnemidae (BP=93%) and the Pelomedusidae (BP=100%). A time of origin for this split, however, is not hypothesized, but would have to predate the split of the Australian and South American Chelids. Our data would support this delineation within the Pelomedusidae (Figure 10). However, as with the Chelid split, we cannot accurately date the split within the Pelomedusidae. Therefore, the only conclusions that can be reached at this time are that this is ancient suborder and that it is perhaps much older than previously supposed.

The Cryptodires present similar challenges in defining a phylogeny that includes the soft shelled (Trionychidae and Carettochelyidae) and hard shelled (e.g., Testudinidae and Emydidae) families. The Trionychidae and Carettochelyidae appear to be the most ancestral clade of the Cryptodiran suborder. Their common morphology has resulted in the inclusion of these
two families, along with the Dermatemydidae and Kinosternidae, within the superfamily Trionychoidea (Gaffney, 1984; Fon-Yi et al., 1989). The molecular data support the sister relationship of Trionychidae and Carettochelyidae, and as seen in Table 6, this result is stronger when 3rd positions are excluded. This is a logical result since these are ancient families and the third codon position is saturated by 100 million year ago (Figure 8). The range of the Trionychidae across Africa, Asia, and North America would also support this as an ancient superfamily. Our results support the findings of much of the morphological work and the chromosomal work of Bickham and Carr (1983) in placing this superfamily as a sister to all remaining Cryptodires. These studies, along with the present study, also refute any relationship between the Trionychidae and the Dermatemydidae and Kinosternidae (see Figure 10 and Table 6).

In contrast to most recent morphological phylogenies (e.g., Gaffney et al., 1991) the Chelydridae have very good support as a member of a clade that also includes the Dermatemydidae and the Kinosternidae (Figure 10). This result is quite interesting, and from the biogeography of extant forms is more logical than the proposed close relationship between the Chelydridae and the Platysternidae, as the Chelydridae and Kinosternidae are both exclusively New World and occupy similar ranges throughout this region. Furthermore, fossils attributed to Chelydrids and Kinosternids are mainly New World, whereas, Platysternid fossils are limited to Asia. Therefore, biogeographically, it makes more sense to envision these families evolving in the New World, probably after the breakup of Laurasia about 70 million years ago. It is also interesting to note that both the Chelydridae and Kinosternidae have limited distributions in South America. If Central

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American Chelydrids could be obtained, it would be interesting to determine their age relative to North and South American lineages and the timing of their invasion into South America relative to that of the Kinosternidae.

In the most recent molecular and morphological analysis of turtle phylogenies, the sister relationship of the Platysternidae and the Chelydridae is maintained, BP=90% using 12S rDNA, cytochrome b, and their morphological data set (Shaffer et al., 1997). Furthermore, there has been much morphological support for a superfamily containing the Kinosternidae, Dermatemydidae, Trionychidae, and Carettochelyidae. This relationship is not supported here and is also not supported by Shaffer et al. (1997). If these relationships were valid it would imply that these were very old families predating the breakup of Gondwanaland. However, the Trionychidae and Carettochelyidae are always basal to the advanced clade, of which the Kinosternidae and the Dermatemydidae are a part.

Another historically problematic area in turtle systematics is the relationship of the Testudinoidea. This superfamily contains the Bataguridae, Testudinidae, and the Emydidae. The Bataguridae contains many mono/ditypic genera, whereas the Emydidae contain genera that are extremely polytypic. This fact could indicate that the Emydidae have diversified more recently, and in most hypotheses the Bataguridae are considered ancestral to the Testudinidae and the Emydidae, due in part to the patterns of distribution of the Bataguridae and the Emydidae. This would indicate that these families were at least Laurasian in age.

It has been shown that Testudinids are capable of overwater dispersal across vast expanses of salt water, so this family could have evolved at a time later than the Emydidae and the Bataguridae and still exhibit a
worldwide distribution. However, our results agree with Shaffer et al. (1997) in defining a relationship between only the Bataguridae and the Testudinidae, and do not support an affinity of either with the Emydidae. This would also imply that the vast majority of similarities between the families would be due to shared primitive characteristics or convergence, and not synapomorphies.

The Cheloniidae group as a superfamily and the present analysis supports the study of Dutton et al. (1996) regarding the monophyly of this family. The Cheloniidae appears derived and is contained within the radiation that encompasses all Cryptodiran families, except the Trionychidae and the Carettochelyidae. Given the extreme age of fossil sea turtles (known from 125 million years ago), this implies a very ancient radiation of all Cryptodiran families.

The molecular data resolve many of the higher order relationships among turtles. However, there is no resolution within the advanced Cryptodire group. Shaffer et al. (1997) found a similar result with approximately 1,600 base pairs of sequence data and over 100 morphological characters and pointed out several possible explanations: 1) the wrong suite of characters was sampled, 2) an insufficient number of characters were sampled, and 3) the characters are correct and truly reflect the phylogeny. Shaffer et al. (1997) argue that the 3rd alternative is correct because of the short internal branch lengths within the Cryptodira and similar genetic distances between the families within this group. We also find short internal branch lengths and similar genetic distances between members of the Cryptodiran group and support Shaffer et al. (1997) in their hypothesis of a rapid radiation. The timing of this radiation based on the molecular clock,
Figure 8, concurs with the date proposed by Shaffer et al. (1997) and results in the hypothesis that the advanced Cryptodires radiated rapidly about 100 million years ago. The best overall hypothesis of Testudinid relationships resulting from this study is presented in Figure 22.

Emydidae

The Emydidae are one of the most successful Chelonian lineages comprising approximately 14% of the extant species. The Emydidae are a North American group with only the genera *Emys* and *Trachemys* expanding beyond. The subdivision of the Emydids into the subfamilies Dierochelyini and Emydini, as defined by Seidel and Adkins (1987), is well supported in this analysis. Within the Emydini, 2 lineages are found, one containing *Terrapene* and the other all remaining genera (*Clemmys, Emys,* and *Emydoidea*). Within the Deirochelyinae, the genus *Deirochelys* represents an early radiation followed by the 2 main lineages (*Trachemys, Graptemys,* and *Malaclemys*) and (*Pseudemys* and *Chrysemys*).

The alignment of *Malaclemys* and *Graptemys* is not surprising, as many authors favor the inclusion of these genera within *Malaclemys*. However, *Trachemys* is usually allied with *Pseudemys* and *Chrysemys*, and the sister group relationship of *Trachemys* to *Malaclemys* and *Graptemys* is somewhat surprising. Several other interesting relationships arise in this analysis, including a close relationship between *Emys* and *Emydoidea*, indicating a single colonization of Europe about 10 to 12 million years ago. Also, the species of the genus *Clemmys* are as distinct from each other as they are from *Emys, Emydoidea, or Terrapene*, a result also evident in Burke et al. (1996) and Bickham et al. (1996). In both of the previous analyses

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Figure 22. Hypothesized familial relationships of the extant families of turtles. The top series of numbers is the Neighbor Joining analysis, with all data included. The bottom series of numbers is the identical analysis, with the third positions deleted. The first number indicates the overall support seen in the Neighbor Joining analysis. The second number indicates the Bootstrap support and the third indicates the Jackknife support. In cases where all values were identical, only that value is presented.
*Terrapene* was found to be a derived clade, whereas in our analysis it is a sister clade to all remaining Emydini. Furthermore, in all three analyses *C. muhlenbergii* and *C. insculpta* are sister taxa, though their relationship to the remaining Emydini differs in each analysis. These analyses show that the relationships within the Emydini have not yet been adequately resolved and are in need of further analysis to define the nature of this subfamily.

As discussed previously, there is little doubt that *Trachemys* is sister to *Graptemys* and *Malaclemys* and, like all Emydids, is North American in origin. Figure 23 shows the distribution of all extant Emydids, while Figure 24 shows the ranges of *Trachemys* as they are currently understood.

The North American origin of the genus leads to the question of when and how, the MesoAmerican and Antillean forms originated. Seidel (1988) examined the Antillean taxa and concluded that the Temperate *scripta* are the basal lineage. The North American complex is usually defined as one group, however some recognize *T. s. gaigeae* as a species (e.g., Dixon, 1987). Seidel hypothesized that the lineage derived subsequent to this was the Antillean taxa and that the physiological tolerances to salt exhibited by the genus would have enabled *Trachemys* to reach South America from the Caribbean. He concluded that the tropical *scripta* are a lineage distinct from the temperate forms and are derived from the Antillean taxa. Seidel examined the proto-Caribbean plate hypothesis as a means of origin for the Caribbean taxa, but concluded that this would have entailed a lengthy parallel evolution between these forms and the temperate *scripta* group. He postulated multiple dispersals into the Caribbean and a reinvasion of Mexico from Cuba, resulting in a paraphyletic Antillean group (Seidel et al., 1986).
Figure 23. The species distribution of the Emydidae. The primary density of Emydids is in the Eastern United States. The density of taxa never exceeds two outside the United States. Also shown is the recent colonization of Europe by the genus *Emys*.
Figure 24. Distribution of the genus *Trachemys* in the United States, Mexico, the Caribbean, Central and South America.
In a competing scenario, Pleistocene North American is proposed to have given rise to the stocks that became the present-day *scripta* (Legler, 1990). Legler hypothesized the central Mexican interior basin as the point of origin for what would become the Mexican/Central American taxa (Smith and Miller, 1986). The ancestral stocks of what would become *T. s. gaigeae* and *T. s. hartwegi* are proposed to have originated in Pleistocene basins created by the Rio Grande and Rio Concho Rivers. Legler (1990) divides all non-North American *Trachemys* into two groups, the Northern (*T. s. taylori, T. s. gaigeae,* and *T. s. hartwegi*) and gulf coast (all remaining taxa) groups, which he proposes originated in the Pleistocene. These ancestors gave rise to stocks that would become *T. s. cataspila* and *T. s. venusta* and eventually migrate to northern Columbia, with almost continuous gene flow. At some point this coastal invasion also resulted in the isolation of *T. s. emolli* in Nicaragua and Costa Rica. Also, the ancestors of *T. s. grayi* are proposed to have crossed the Isthmus of Tehuantepec and become established on the Pacific coast. The remaining Pacific subspecies, *T. s. ornata* and *T. s. yaquila*, are hypothesized to have originated from these initial coastal migrations, via dispersal. The presence of *T. s. hiltoni* in the Rio Fuerte basin of Sonora and Sinaloa, Mexico was an enigma to Legler. He theorized that this subspecies originated from the same stock that gave rise to *hartwegi/gaigeae* some time in the Pleistocene. This stock would later migrate to Pacific coast and became established. *T. s. nebulosa* is the hypothesized sister to *T. s. hiltoni* and is thought to have originated from it by dispersal across the Gulf of California. Legler does not present his findings as a phylogeny, however one can be inferred from his assumptions regarding the historical biogeography of the genus. The most basal lineages

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would consist of either the North American taxa, *gigeae/hartwegi* or the Antillean taxa. The internal branches would be composed of the gulf coast taxa and *T. s. emoli*. The Pacific coast taxa would also be internal with the exact position determined by the time of speciation. *T. s. hiltoni* and *T. s. nebulosa* would either be placed at the base of the tree or at a more internal branch depending upon when these events occurred.

Neither the scenario of Seidel or Legler are supported by the data accumulated in the present study. The validity of Legler’s assumptions regarding the Pacific taxa cannot be addressed, as only *T. s. grayi* and *T. s. yaquia* are included in this study, however, all remaining *scripta* are used. It is apparent that the radiation of *Trachemys* was explosive and not stepwise as Seidel and Legler imply. Furthermore, there are five distinct lineages within *Trachemys*. The first of these is the gulf coast lineage. There is virtually no differentiation between *T. s. cataspila* and *T. s. chichiriviche*, as Legler noted, and this implies that there has been virtually unimpeded gene flow across this entire range from northern Mexico to northern Venezuela. *T. s. emoli* appears to be divergent from other Central American taxa, but the ancestor for this taxon cannot be determined. The Pacific coast subspecies are represented by *T. s. grayi* and *T. s. yaquia*. *T. s. grayi* is nearly identical to all gulf coast taxa, but *T. s. yaquia* is divergent from, but sister to, the gulf taxa. This probably indicates the isolation of northern Pacific taxa in Mexico, including *T. s. hartwegi, T. s. hiltoni, T. s. nebulosa*, and *T. s. ornata*.

South America contains the southernmost *scripta*, as well as *T. dorbigni*. *T. dorbigni* appears to have arisen during the explosive radiation of the genus. The lack of *Trachemys* between the southernmost *scripta* and *T. s. dorbigni* may indicate a lack of adequate sampling or the lack of suitable
habitat. However, based upon this analysis of the South American and MesoAmerican/temperate *scripta*, it is obvious that at least two invasions of South America have occurred. Using the molecular clock in Figure 9, from 20 million years ago to the present, it is possible to date the first at around 8 to 10 million years ago and the second at less than 1 million years ago. Therefore, this study indicates that the genus *Trachemys* appeared in a very short time in the New World and “exploded” across its present range.

Lastly, there is an East to West split in the U. S. separating *T. s. gaigeae* from all remaining temperate *scripta* and similarly an East/West split in Mexico delineating eastern and western Mexico *scripta*.

The Antillean *Trachemys* are also an enigma. The past 50 years have seen a wide variety of hypothesized phylogenies and an equal number of scenarios to account for them. The vast majority of these studies have utilized morphological differences in their classifications. For example, Boulenger (1889) proposed that all Caribbean forms were conspecific with mainland *T. scripta*. Barbour and Carr (1940) described four species as follows: *T. decussata* (Cuba), *T. terrapen* (Jamaica), *T. decorata* (Hispaniola) and *T. stejnegeri* (Hispaniola and Puerto Rico). These authors recognized the affinity between *T. decorata* and *T. stejnegeri* and placed these taxa in the “stejnegeri” group. Conversely, Williams (1956) and Schwartz and Thomas (1975) found only the Jamaican species to be distinct and placed all the remaining taxa in a single species. Wermuth and Mertens (1961) went further and assigned all Caribbean taxa to *T. terrapen*. Schwartz (1978) concluded the only distinct form, not only on Hispaniola, but in the entire Caribbean, was *T. decorata*. Pritchard (1979) concluded, to the contrary, that all the Hispaniolan sliders were *T. decussata*. Seidel and Inchaustegui

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Miranda (1984) saw the Hispaniolan forms in a subspecific relationship. Seidel and Adkins (1987), however, did find variation at several allozyme loci that could be useful in distinguishing the Antillean taxa. Finally, Seidel (1988) agreed with Barbour and Carr (1940) that there is a close affinity between the two, but they are in fact distinct. It is evident from this myriad of hypothesized relationships that to date no universally agreed upon phylogeny has been determined.

The above phylogenetic hypotheses have resulted in various vicariant and dispersal scenarios for the Caribbean. For example T. d. decussata, on the Cayman Islands, was described as a new subspecies, however subsequent analyses showed the subspecies, T. d. decussata and T. d. plana, to be identical (Seidel, 1988). This finding is not unique in that as much as 70% of the Cayman fauna is attributable to dispersal from Cuba (Williams, 1964). Furthermore, there also exists evidence of a large faunal interchange between Hispaniola and Puerto Rico (Gill, 1978; Pregill, 1981a) attributable to vicariant events. It has been hypothesized that eastern Hispaniola and western Puerto Rico were once joined as an island and that eventually the part of the island that would become eastern Hispaniola broke away and joined western Hispaniola (Guyer and Savage, 1986). These examples show that both vicariance and dispersal could have played a role in the evolution of the Caribbean fauna.

Our study indicates that dispersal has played a primary role in the Antilles. The Antillean Trachemys appear during the explosive radiation of Trachemys, with Cuba as the most likely point of origin and expansion to neighboring islands in a stepwise manner. The results of this study suggest two distinct species in the Caribbean: T. decussata and all those remaining.
There is no evidence for a subsequent reinvasion into Central America. The forms present in Central America appear at the same time as those in the Caribbean and developed in parallel to them.

Finally, it is interesting to note that based on the Emydid phylogeny determined by this study that *Malaclemys* (brackish water, coastal specialist) and *Graptemys* (freshwater) are the sister clade to *Trachemys*. This relationship is extremely significant because *Trachemys* is the only genus to have successfully traversed a salt water barrier. In conclusion, a temperate ancestor, in an initial explosive radiation, gave rise to the Caribbean taxa, *T. dorbigni*, *T. s. emolli*, and the MesoAmerican taxa. Possible range contractions or extinctions left *T. dorbigni* and *T. s. emolli* with relictual distributions. Then secondary expansions occurred into Central and South America, resulting in the present distribution of the genus.

**Kinosternidae**

The ranges of the present day Kinosternidae are presented as Figure 25. There is a high density of taxa in Central America and Mexico, and, unlike *Trachemys*, many of the temperate species are sympatric. It will be shown below that *Sternotherus* is distinct from *Kinosternon* and should be recognized in this regard. However, before presenting the conclusions, Iverson's (1991) hypothesis on the evolution of the Kinosternidae will be examined. A North American radiation, from a *Xenochelys*-like ancestor, is proposed to have given rise to a form with a reduced plastron. Radiation of these taxa supposedly resulted in *Sternotherus*, which retained what is thought to be the primitive plastron. As this second clade evolved, a second plastral hinge developed, along with a reduced head, and a tendency towards
Figure 25. Distribution of the present-day Kinosternidae. This figure shows the centers of distribution to be Central America and the Southeastern United States. Species densities range from 6 taxa in these areas to 1 taxa in the Central and Northeastern United States and South America.
plastral enlargement. This second lineage apparently colonized Mesoamerica at least two times. The first colonization led to a lineage with smaller plastrons, including *K. dunni* and *K. angustipons*. The second invasion was by a larger plastral group, which led to the *K. scorpioides* group, but was followed by a plastral reduction in some taxa (Iverson, 1991). Iverson (1991) stated that much of this radiation can be explained by vicariant events as the distributions are exactly parapatric. Figure 26 presents the hypothesized relationships for the Kinosternidae and how they would fit this scenario.

The hypothesis of speciation put forth by Iverson (1991) for Kinosternids, i.e., North America origins, are corroborated by studies of other vertebrates. The North American fossil record suggests that both North and South American Sigmodontine rodents descended from a North American lineage present in the late Miocene (Baskin, 1989). Several hypotheses have been put forth to explain the diversity present in extant sigmodontines including 1) waif dispersal in the early Miocene (Hershkovitz, 1966, 1972), and 2) recent invasions during the great American interchange (Simpson, 1969). These explanations are plausible in taxa capable of overland dispersal. Current investigations of the Emydid genus *Trachemys* also indicate North America as the source for this group. Kinosternids, however, have their sister taxa limited to Central America. This would lend support to Savage (1966) and Berry and Legler (1980) who have suggested that Kinosternids are Central American, not North American, in origin. Furthermore, Kinosternids are primarily bottom walkers, while *Trachemys* is capable of overland dispersal. This fact seems
Figure 26. The radiation out of Central America as shown by the presence of *Dermatemys*, the Staurotypidae and the basal *Kinosternon* lineages in Central America. The remaining *Kinosternon*, and *Sternotherus*, would have radiated from this central location to North and South America.
to point to a more central source of radiation for the family, i.e., Central America.

The molecular data seem to corroborate a Central American origin for the Kinosternidae. This family not only has its closest relatives in Central America, but the phylogenies generated reveal taxa from this region as basal. In our evolutionary scenario, we begin in Central America. The ancestors of the Kinosternidae must have ranged across Central America and southern Mexico. This conclusion is supported by the family that is sister to the Kinosternidae, the Dermatemydidae. The distribution of this family is limited to Veracruz, the Yucatan, and northern Oaxaca in Mexico, Belize, Honduras, and Guatemala. The other subfamily in the Kinosternidae is the Staurotypinae. This subfamily also occupies a range similar to the Dermatemydidae. For example, Claudius ranges from Veracruz, Mexico to Belize, and Guatemala. The genus Staurotypus occupies a very similar range across Mexico and Belize to Guatemala and Honduras (S. triporatus). The other species, S. salvini, occurs in eastern Oaxaca and southern Chiapas, Mexico and southern Guatemala and El Salvador. Figure 26 shows how well an expansion from a Central American origin fits geographic distributions. Therefore, the distributions of the Dermatemydidae and the Staurotypinae, as well as the basal Kinosternids, also occupying a similar range, point to Central America as the point of origin for the family.

The founders that would become the three remaining groups of the Kinosternidae, therefore, appear to have originated in Central America and radiated into northern Mexico and the remaining countries of Central America and into South America. This radiation resulted in a monophyletic Sternotherus, a U. S. Kinosternon clade and a second Mexican
Kinosternon clade. There appear to be two different routes of invasion, one through the eastern U. S. and the other through the western U. S. The eastern U. S. invasion resulted in the Sternotherids, which today are located only in the eastern U. S. The western invasion resulted in the remaining U. S. Kinosternids and a second Mexican clade. For example, the basal member of both the U. S. Kinosternid clade and the second Mexican clade are located in the southwest U. S. or Northwestern Mexico: southwestern Arizona and Sonora, Mexico (K. f. arizonense) and Sonora and Sinaloa, Mexico (K. alamosae). Overall, the U.S. Kinosternon are nearly identical, with only K. f. arizonense distinct. This is similar to the east/west delineation of Trachemys. This second Mexican clade is primarily located on the west coast of Mexico, a situation also evident in Trachemys. There also appear to have been multiple invasions into South America, also seen in Trachemys. Our molecular clock allows us to compare the time frame for the different invasions into South American for Trachemys versus Kinosternon. When South American Kinosternon are compared to their closest Central American relatives it appears that two invasions occurred, exactly as seen in Trachemys. The timing of the invasions, however, is somewhat different. The initial Kinosternid invasion appears around 10 to 12 million years ago, roughly in the same time frame as the first invasion of Trachemys. The second Kinosternid invasion would appear to have occurred about 4 to 5 million years ago. Therefore, we must conclude that Iverson (1991) is incorrect in his hypothesis of a North American origin for the family and conclude that the family is Central American in origin and the similarities Iverson noted were due to convergence and similar choices of habitat.
Conclusions

Overall, the results of this study strengthen many of the familial relationships that have been proposed. For example, there is good support for a superfamily containing the Trionychidae and the Carettochelyidae. Furthermore, there is good support for primitive and advanced clades within the Cryptodira. Within the advanced clade, there is strong support for a sister relationship between the Testudinidae and Bataguridae, as well as for the soft and hard-shelled sea turtles. There is also support for a family containing the Chelydridae, the Dermatemydididae, and the Kinosternidae, inclusive of the Staurotypinae. The families of the advanced clade seem to have arisen at a similar time, giving support to Shaffer et al. (1997) who have hypothesized a rapid radiation for the Cryptodira about 90-120 mya. Figure 27 compares the time of divergence in the two scenarios that have been proposed using immunological distances and paleontology. The results of this study favor the hypothesis put forth by the paleontological data.

The sister relationship of Trachemys with Graptemys and Malaclemys is well supported in this analysis. The diversification of these genera appears to have occurred about 6 to 8 million years ago, a date that would be consistent with those identified for the species diversification within Trachemys. However, when Pseudemys and Chrysemys were compared a slightly older date:, 9 to 11 million years, was obtained. This may indicate that the dates for Trachemys are slightly underestimated.

The North American Trachemys resolve into two groups, with only T. s. gaigeae distinct. The Antillean taxa appear to have a Cuban origin with island hopping used to explain the current diversity. Again, the
Figure 27. The relationships of selected families based on immunological distance data (Chen et al. 1980) or paleontological data (Shaffer et al. 1997) and correlated to the major Eras and Epochs.
MesoAmerican forms are all very similar, with only an east/west split delineating these taxa. *T. s. emolli* must have originated from vicariant events during an initial radiation. *T. dorbigni* remains an enigma, unless evidence can be found of a once continuous distribution throughout South America. However, range contraction could also be used to explain the present-day distributions of *T. dorbigni* and *T. s. emolli*.

The Kinosternidae are Central American in origin. An initial Mexican group colonized much of Central American and the gulf coast of Mexico. These taxa gave rise to three distinct lineages, *Sternotherus*, the U. S. and MesoAmerican *Kinosternon*. There appear to have been two routes of invasion into the U. S. The first was through an eastern corridor and resulted in *Sternotherus*. The second was through the southwest and resulted in the U. S. *Kinosternon* and a second Mexican, primarily Pacific coast, clade. Of these three, the U. S. and MesoAmerican groups are sister, while the sternotherids form a distinct, monophyletic clade. The only resolution within *Sternotherus* is between *K. depressum* and *K. carinatum*. Similarly, there is very little resolution with the U. S. taxa, except the east/west delineation of *K. f. arizonense*. The 2nd Mexican clade is similar in its lack of resolution, but this Pacific clade is distinct from the initial gulf coast clade.

Finally, the invasions into South America seem to have two, or perhaps three origins. The first of these was approximately 8 to 10 million years ago, across a filter bridge, and resulted in colonization of South America by members of the initial Kinosternid clade and the ancestors of *T. dorbigni*. The second would seem to coincide with a drastic drop in sea levels about 4 to 5 million years ago. This would have allowed for a second
Kinosternid invasion. There is evidence of a similar interchange occurring in studies of Sigmodontine rodents (Engel, unpublished 1996). A more recent, perhaps third invasion, has resulted in the *Trachemys* (*T. callirostris* and *T. chichiriviche*) that now occupy Columbia and Venezuela and is evidenced by the nearly complete sequence identity of *Trachemys* throughout Mexico, Central, and South America. These studies together strengthen the idea of multiple invasions, by numerous taxa, into South America across land bridges that arose at different times in the geologic past. Finally, this study supports the hypothesis of two New World families having vastly different geographic centers of origin, Emydids in North America and Kinosternids in Central America, but evolving to occupy similar ranges throughout the New World.
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APPENDIX

LIST OF SPECIMENS USED IN THIS STUDY

(Numbers in parentheses refer to specimen numbers in DES, MF, PD, EEL or JBI databases. DES is Dave Starkey, MF is Mike Forstner, PD is Peter Dutton, EEL is Ed Louis, and JBI is John Iverson).

Cryptodires

Testudinidae


Geochelone pardalis babcocki (DES 103). locale unknown. (Sudan, Angola, Africa and South Africa). pet trade.


Gopherus berlandieri (DES 223). locale unknown. (Southwest U. S. and Northeast Mexico. Scott Davis, supplier.)
Homopus areolatus (EEL 1). Locale unknown. (South Africa).
U. S. Customs seizure.

Homopus signatus cafer (EEL 2,3). Locales unknown. (South Africa). U. S. Customs seizure.

Homopus signatus signatus (EEL 3-6). Locales unknown. (South Africa). U. S. Customs seizure.


Bataguridae

Annamemys annamensis (DES 270). locale unknown.
(Vietnam). pet trade.

Callagur borneoensis (DES 125). locale unknown. (Borneo and Sumatra). Fort Worth Zoo.

Cuora amboinensis amboinensis (DES 261). locale unknown.
(Burma, Thailand, Vietnam, and Indonesia). pet trade.

Geoemyda spengleri spengleri (DES 124). locale unknown.
(Southern China). Fort Worth Zoo.


*Rhinoclemmys areolata* (DES 161). Cozumel Island, Mexico. (Southern Mexico, Belize, Guatemala, and Honduras). Collection of Dennis Uhrig.

*Rhinoclemmys funerea* (DES 260). locale unknown. (Honduras to Panama). pet trade.


**Emydidae**


Clemmys muhlenburgi (DES 89). Maryland. (Georgia to Massachusetts). Al Redmond, supplier.

Deirochelys reticularia miaria (DES 75). Lonoke County, Arkansas. (Southeast U. S.). Kenny Krauter, supplier.


Emys orbicularis orbicularis (DES 108). Italy. (Central Europe). Doug Albert, supplier.

Graptemys barbouri (DES 16). Flint River, Dougherty County, Georgia. (Alabama, Georgia, and Florida). Al Redmond, supplier.

Graptemys caglei (DES 26). Guadalupe River, Comal County, Texas. (Texas). Rick Van Dyke, supplier.

Graptemys flavimaculata (DES 69). Pascagoula River, Jackson County, Mississippi. (Mississippi). Kenny Krauter, supplier.


Graptemys oculifera (DES 26). Pearl River, Lawrence County, Mississippi. (Mississippi and Louisiana). Robert Guthrie, supplier.


Graptemys pseudogeographica kohni (DES 78). Jonesville, Catahoula County, Louisiana. (Texas and Louisiana to Missouri and Illinois). Kernie King, supplier.


Graptemys versa (DES 20). Pedernales River, Gillespie County, Texas. (Texas). Rick Van Dyke, supplier.

Malaclemys terrapin litoralis (DES 150). Brazoria County, Texas. (Texas, gulf coast). Steve Riopelle, supplier.

Malaclemys terrapin pileata (DES 154). St. Bernard County, Louisiana. (Florida to Texas). Steve Riopelle, supplier.

Malaclemys terrapin tequesta (DES 63). Indian River, Brevard County, Florida. (Florida, gulf coast). Kerney King, supplier.
Malaclemys terrapin terrapin (DES 28). Tuckahoe River, Cape May County, New Jersey. (Massachusetts to North Carolina). Kerney King, supplier.

Pseudemys alabamensis (DES 5, 6). Tensaw River, Baldwin County, Alabama. (Alabama). Kerney King, supplier.


Pseudemys concinna hieroglyphica (DES 7, 10). Obion River, Obion County, Tennessee. (Southeast U. S.). Kerney King, supplier.


Pseudemys concinna suwanniensis (DES 8, 9). Suwannee River, Levy County, Florida. (Florida). Kerney King, Supplier.

Pseudemys floridana floridana (DES 2, 12). Lake Marion, Orangeburg County, South Carolina. (North Carolina to Louisiana). Kerney King, Supplier.
Pseudemys floridana penninsularis (DES 103, 119). Lake Okeechobee, Palm Beach County, Florida. (Florida). Jim Watt, supplier.


Pseudemys nelsoni (DES 1, 11). Johns River, Orange County, Florida. (Florida). Kemey King, supplier.


Pseudemys texana (DES 21, 31). Colorado River, Travis County, Texas. (Texas). Kemey King, supplier.


Terrapene ornata ornata (DES 122). Callahan County, Texas. (South Dakota to New Mexico and Louisiana). Leroy Higginbotham, supplier.


Trachemys dorbigni (DES 175,176). Uruguay. (Brazil to Uruguay). Dennis Uhrig, private collection.


Trachemys scripta cataspila (DES 165, 208, RC1, RC2). Mexico. (Northern Mexico). Dennis Uhrig, private collection.


Trachemys scripta elegans (DES 23, 42). Rio Grande River, Cameron County, Texas. (Northern Mexico to New Mexico and Alabama). Roland Perry, supplier.


Trachemys scripta gaigeae (DES 245, 269). Rio Grande River, Doña Ana County, New Mexico. Rio Grande

*Trachemys scripta grayi* (DES 373). locale unknown. (Southeast Mexico and Western El Salvador). Dennis Uhrig, private collection.

*Trachemys scripta scripta* (DES 33). Flint River, Dougherty County, Georgia. (Virginia to Florida). Al Redmond, supplier.


*Trachemys scripta yaquia* (DES 192, 193, 251, 324). locale's unknown. (Sonora, Mexico). Harold Carty, private collection.

Trachemys stejnegeri vicina (DES 187, 188) San Domingo, Dominican Republic. (Hispaniola). Dennis Uhrig, private collection.

Trachemys terrapen (DES 189, 190, 238). Ocho Rios, Jamaica. (Jamaica). Dennis Uhrig, private collection.

Cheloniidae

Caretta caretta (PD Cc 1). locale unknown. (Subtropical, Tropical Oceans). Peter Dutton, supplier.

Chelonia agassizi (PD Ca 1). Michoacan, Mexico. (Eastern Pacific Ocean). Peter Dutton, supplier.

Chelonia mydas (PD Cm 1, Cm 58). Gulf of Mexico. French Frigate Shoals, Hawaii. (All seas). Peter Dutton, supplier.

Eretmochelys imbricata (PD Ei 1). Gulf of Mexico. (Tropical Oceans). Peter Dutton, supplier.

Lepidochelys kempi (PD Lk 1). Rancho Nuevo, Mexico, Gulf of Mexico. (Gulf of Mexico and Northern Atlantic Ocean). Peter Dutton, supplier.


Natator depressus (PD Nd 1). Australia. (Northern Australia and Gulf of Papua). Peter Dutton, supplier.
Dermochelidae

*Dermochelys coriacea* (PD P1, D26). Costa Rica, Pacific Coast.
French Guiana, Atlantic Coast. (All seas). Peter Dutton, supplier.

Platysternidae

*Platysternon megacephalum* (DES 257, 258). locale's unknown.
(China, Burma and Thailand). pet trade.

Chelydridae

*Chelydra serpentina serpentina* (DES 143). Brazos River, Brazos County, Texas. (Southern Canada to Columbia and Ecuador). Scott Davis, supplier.


Kinosternidae

*Kinosternon acutum* (JBI 105). locale unknown. (Southern Mexico, Belize, Guatemala). John Iverson, supplier.

*Kinosternon alamosae* (JBI mtj14). Sonora, Mexico.
(Sonora and Sinaloa, Mexico). John Iverson, supplier.

Kinosternon carinatum (JBI E, MF 466, 467, 559). Glover Creek, McCurtain County, Oklahoma. Lake Pontchartrain, St. John the Baptist Parish, Louisiana. Lake Hamilton, Hot Springs County, Arkansas (Oklahoma, Texas, Mississippi). Mike Forstner and John Iverson, suppliers.

Kinosternon creaseri (JBI l). Quintana Roo, Mexico. (Yucatan, Mexico). John Iverson, supplier.


Kinosternon flavascens flavascens (DES 221, 222, 243, 244, JBI m, MF 635). locale unknown J. D. Murphree Wildlife Refuge, Beaumont, Texas. Gimlet Lake, Garden County, Nebraska. Bracketville, Texas. (Great Plains and Texas to Northern Mexico). Dave
Starkey, Mike Forstner and John Iverson, suppliers.


*Kinosternon integrum* (JBI o). Oaxaca, Mexico. (Tamaulipas and Oaxaca, Mexico). John Iverson, supplier.


Kinosternon scorpioides creuntatum (DES 279, JBI 109). locale’s unknown. (Tamaulipas, Mexico to Honduras). pet trade and John Iverson, supplier.

Kinosternon scorpioides scorpioides (DES 278, 301, MF 634).
Locale’s unknown. (Panama to Brazil and Peru). pet Trade.

Kinosternon sonoriense sonoriense (JBI ksf1). Maricopa County, Arizona. (California and New Mexico to Sonora and Chihuahua, Mexico). John Iverson, supplier.

Kinosternon subrubrum hippocrepis (JBI s, t). Goose Pond, Santa Rosa County, Florida. (Texas, Oklahoma to Mississippi). John Iverson, supplier.

Kinosternon subrubrum steindachneri (DES 280, JBI u). locale unknown. Lee County, Florida. (Florida). Robert Guthrie and John Iverson, suppliers


Claudius angustatus (JBI 102). locale unknown. (Veracruz, Mexico to Guatemala and Belize). John Iverson, supplier.

Staurotypus salvinii (JBI 102, DES 262). locale’s unknown. (Oaxaca and Chiapas, Mexico to Guatemala and El Salvador). pet trade and John Iverson, supplier.
*Staurotypus triporcatus* (JBI 108). locale unknown. (Veracruz, Mexico to Belize, Guatemala and Honduras). John Iverson, supplier.

*Dermatemys mawii* (DES 123, JBI 101). locale's unknown. (Southern, Mexico to Belize, Guatemala and Honduras). Fort Worth Zoo and John Iverson, supplier.

**Trionychidae**


*Apalone spiniferus guadalupensis* (DES 81). Colorado River, Travis County, Texas. (Southeastern Canada to Northern Mexico). Rick Van Dyke, supplier.

*Chitra indica* (MF 853). Depok, Java. (Pakistan to Thailand). Mike Forstner, supplier.


**Carettochelyidae**

Pleurodires

Chelidae

*Chelodina longicollis* (DES 196). locale unknown.

(Southeastern Australia). Fort Worth Zoo.


*Emydura subglobosa* (DES 264). locale unknown.

(Queensland, Australia and New Guinea). pet trade.

*Hydromedusa tectifera* (DES 275). locale unknown. (Brazil, Uruguay and Argentina). pet trade.

*Platemys platecephala* (DES 351). locale unknown. (Northern South America). pet trade.

Pelomedusidae

*Erymnochelys madagascariensis* (DES 211, 212). Madagascar.

(Madagascar). Bill McCord, supplier.


Podocnemis sextuberculata (DES 218). Brazil. (Brazil, Columbia, and Peru). Fort Worth Zoo and Bill McCord, supplier.

Podocnemis unifilis (DES 196, 219). Locale unknown. Amazon Basin, Brazil. (Bolivia, Brazil, Columbia, Ecuador, Guianas, Peru, and Venezuela). Fort Worth Zoo and Bill McCord, supplier.

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