

## Diets, habitat preferences, and niche differentiation of Cenozoic sirenians from Florida: evidence from stable isotopes

Bruce J. MacFadden, Pennilyn Higgins, Mark T. Clementz, and Douglas S. Jones

**Abstract.**—Cenozoic sediments of Florida contain one of the most highly fossiliferous sequences of extinct sirenians in the world. Sirenians first occur in Florida during the Eocene (ca. 40 Ma), have their peak diversity during the late Oligocene–Miocene (including the widespread dugongid *Metaxytherium*), and become virtually extinct by the late Miocene (ca. 8 Ma). Thereafter during the Pliocene and Pleistocene, sirenians are represented in Florida by abundant remains of fossil manatees (*Trichechus* sp.). Stable isotopic analyses were performed on 100 teeth of fossil sirenians and extant *Trichechus manatus* from Florida in order to reconstruct diets (as determined from  $\delta^{13}\text{C}$  values) and habitat preferences (as determined from  $\delta^{18}\text{O}$  values) and test previous hypotheses based on morphological characters and associated floral and faunal remains. A small sample ( $n = 6$ ) of extant *Dugong dugon* from Australia was also analyzed as an extant model to interpret the ecology of fossil dugongs.

A pilot study of captive manatees and their known diet revealed an isotopic enrichment ( $\epsilon^*$ ) in  $\delta^{13}\text{C}$  of 14.0‰, indistinguishable from previously reported  $\epsilon^*$  for extant medium to large terrestrial mammalian herbivores with known diets. The variation in  $\delta^{18}\text{O}_{\text{V-SMOW}}$  reported here is interpreted to indicate habitat preferences, with depleted tooth enamel values ( $\approx 25\text{‰}$ ) representing freshwater rivers and springs, whereas enriched values ( $\approx 30\text{‰}$ ) indicate coastal marine environments. Taken together, the Eocene to late Miocene sirenians (Protosireniidae and Dugongidae) differ significantly in both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  from Pleistocene and Recent manatees (Trichechidae). In general, *Protosiren* and the fossil dugongs from Florida have carbon isotopic values that are relatively positive (mean  $\delta^{13}\text{C} = -0.9\text{‰}$ ) ranging from  $-4.8\text{‰}$  to  $5.6\text{‰}$ , interpreted to represent a specialized diet of predominantly seagrasses. The oxygen isotopic values (mean  $\delta^{18}\text{O} = 29.2\text{‰}$ ) are likewise relatively positive, indicating a principally marine habitat preference. These interpretations correlate well with previous hypotheses based on morphology (e.g., degree of rostral deflection) and the known ecology of modern *Dugong dugon* from the Pacific Ocean. In contrast, the fossil and extant *Trichechus* teeth from Florida have relatively lower carbon isotopic values (mean  $\delta^{13}\text{C} = -7.2\text{‰}$ ) that range from  $-18.2\text{‰}$  to  $1.7\text{‰}$ , interpreted as a more generalized diet ranging from  $\text{C}_3$  plants to seagrasses. The relatively lower oxygen isotopic values (mean  $\delta^{18}\text{O} = 28.1\text{‰}$ ) are interpreted as a more diverse array of freshwater and marine habitat preferences than that of *Protosiren* and fossil dugongs. This study of Cenozoic sirenians from Florida further demonstrates that stable isotopes can test hypotheses previously based on morphology and associated floral and faunal remains. All these data sets taken together result in a more insightful approach to reconstructing the paleobiology of this interesting group of ancient aquatic mammalian herbivores.

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### Introduction

Extant sirenians are fully aquatic mammalian herbivores that constitute the Order Sirenia. One of the two modern sirenian families, the Dugongidae, with an extant monotypic genus of hypergrazer, the dugong (*Dugong dugon*), is widely distributed in coastal marine

waters throughout the subtropical and tropical Pacific and Indian Oceans (Walker 1975; Husar 1978a). The second family, the Trichechidae, consists of a single genus, *Trichechus*, the manatee, a mixed-feeding herbivore with three species distributed throughout rivers, estuaries, and coastal marine areas of the tropical and subtropical Atlantic Ocean. Of relevance to the present study, the manatee native to Florida is referred to *T. manatus latirostris*, the West Indian Manatee (Walker

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1975; Husar 1978b). Despite this limited modern diversity and disjunct distribution, which is further threatened by human impact, sirenians have had a rich and fascinating macroevolutionary history of diversification and adaptation over the past 50 million years since their split from the basal Tethytheria (a clade also including proboscideans [McKenna and Bell 1997]).

One of the richest and most extensive fossil records of sirenians occurs in the nearshore, shallow marine and estuarine sediments exposed throughout Florida (Hulbert 2001), and this sequence is critical to understanding the diversification of this order. The systematics, morphology, and adaptive hypotheses concerning these sirenians have been treated extensively in earlier descriptions (e.g., Simpson 1932; Reinhart 1976) and in a series of more recent papers by Domning (e.g., 1988, 1989a,b, 1990, 1994, 1997a).

In addition to developing a modern systematic framework for the Sirenia, Domning (e.g., 1981, 2001a) has developed hypotheses about the diet and ecology of extinct dugongs and manatees based on those of modern sirenians, morphological correlates in the fossil record, and associated geological and paleontological evidence. Domning (2001a) defines the "aquatic megaherbivore adaptive zone" and explains how different sirenians partition food resources and other aspects of individual species niches. Modern dugongs are predominantly grazers, feeding on seagrasses (Hydrocharitaceae and Potamogetonaceae), although they also eat algae when seagrasses are limited or unavailable. These sirenians are characterized by bunodont (bulbous-cusped) cheek teeth with limited tooth replacement and a high degree of rostral deflection (Fig. 1E), which indicates the use of the downturned snout for digging up seagrasses, including their rhizomes. This high degree of rostral deflection (up to 70° [Domning 1977]) can be traced back into the fossil record to the middle Eocene, and for extinct dugongs is likewise interpreted as an adaptation to feeding on ancient seagrasses. Paleontological evidence suggesting the long-standing sea cow and seagrass plant-animal coevolutionary interrelationship first comes from the middle

Eocene of Florida (Ivany et al. 1990), demonstrating very well preserved seagrass beds (with *Thalassia*-like taxa similar to those of the Caribbean basin today) associated with fossil sirenian bones referable to *Protosiren* sp. (Domning et al. 1982; Hulbert 2001).

In contrast to the specialized seagrass grazing of dugongs, manatees are considerably more generalized mixed-feeders, with *Trichechus manatus latirostris* reported to consume over 60 plant species in Florida (Ames et al. 1996). In addition to seagrasses, the diet of *Trichechus* can consist of an array of freshwater and marshy vegetation, including a considerable proportion of true grasses (Poaceae) such as *Distichlis*, *Spartina*, *Paspalum*, and *Panicum* (Domning 1982, 2001a). The rostral deflection angle of *Trichechus* is relatively weak (<40°; Fig. 1F; Domning 2001a). With the exception of the earliest trichechid *Potamosiren magdalenensis* from the middle Miocene of Colombia (Domning 1997b), other manatees have relatively short-crowned teeth that are shed and continually replaced "conveyor-belt"-style from back to front throughout ontogeny. This continuous source of teeth is interpreted as an initial adaptation to feeding on abrasive grasses (Domning 1982, 1997b, 2001b), although more recently manatees have adapted to a more diverse herbivorous diet.

Within the past decade, stable isotopes have become increasingly useful in reconstructing the paleoecology and paleoenvironment of extinct vertebrates. The original literature on this subject is expanding rapidly (see, e.g., Koch 1998 for recent review). These geochemical studies have allowed (1) testing of previously existing hypotheses based on more traditional (i.e., morphological) data, and (2) reconstruction of various aspects of aut- and synecology of vertebrate species and communities not previously discernable with conventional data. Studies of stable carbon isotopes preserved in tooth enamel carbonate have mostly concentrated on terrestrial vertebrates and communities in an attempt to reconstruct ancient herbivore diets and their feeding adaptations on C<sub>3</sub> or C<sub>4</sub> plants. Stable oxygen isotopes, which have been used extensively in ancient marine ecosystems, have been more difficult to apply to terrestrial set-

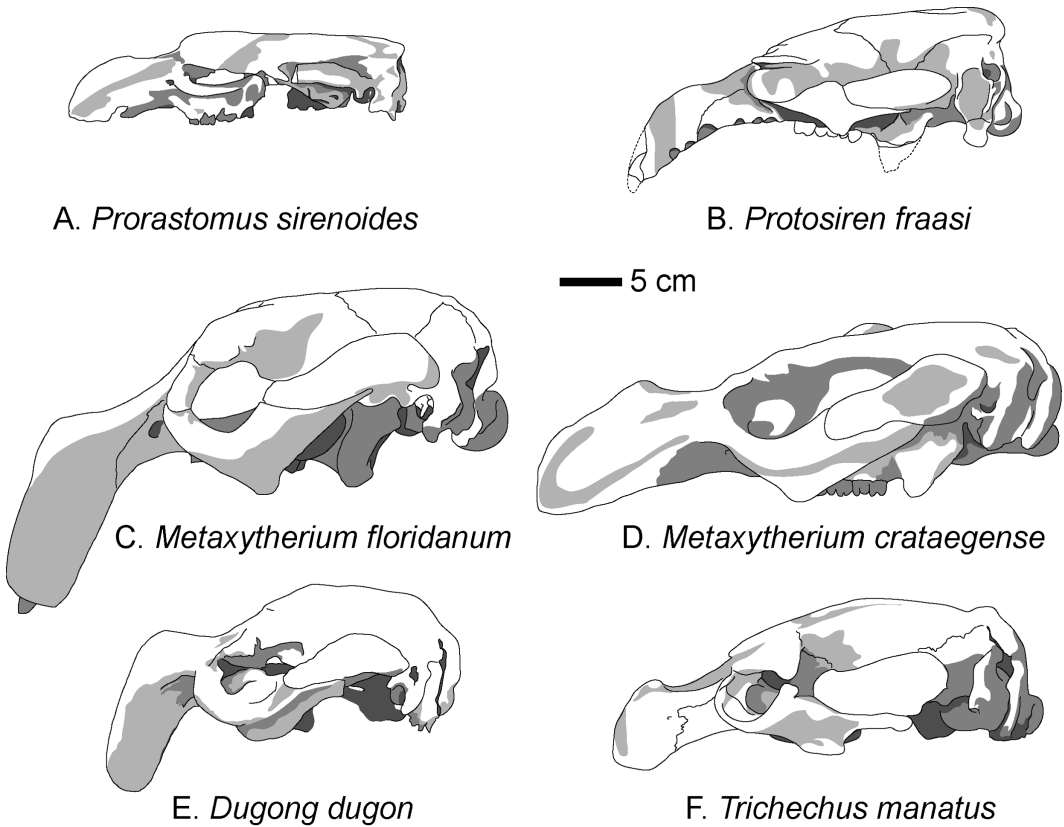


FIGURE 1. Comparisons of degree of rostral deflection in extant and fossil sirenians. A, Primitive morphology in *Prorastomus sirenoides* from the middle Eocene of Jamaica (adapted from Savage et al. 1994). B, *Protosiren fraasi* from the middle Eocene of Egypt (adapted from Domning 2001b). C, High degree of rostral deflection in *Metaxytherium floridanum* from the early Miocene Lower Bone Valley Formation, Florida (adapted from Domning 1988). D, Relatively little rostral deflection seen in *Metaxytherium crataegense* from the middle Miocene of Florida (adapted from Simpson 1932). E, Extant *Dugong dugon*. F, Extant *Trichechus manatus*.

tings because of the high degree of variability of meteoric waters and the many factors influencing the ultimate oxygen isotopic composition of tooth enamel carbonate. Some recent studies have used stable oxygen isotopes to interpret evolutionary transitions between terrestrial versus marine habitats, e.g., the adaptive radiation in Eocene whales (Roe et al. 1998).

Current hypotheses about the evolution of diets and related functional adaptations of extinct sirenians have been extensively described in the existing literature (see Domning references cited above). Of particular relevance here, stable isotopes of carbon and oxygen can be used to test the following hypotheses about the diets and habitats of extinct sirenians of Florida:

1. The relative degree of rostral deflection indicates dietary adaptations, with increased flexure indicating a specialized seagrass diet.
2. Like modern dugongs, extinct Florida protosirenids and dugongs inhabited relatively marine waters, whereas Florida manatees have inhabited a broader range of aquatic environments, including coastal marine as well as freshwater, inland rivers.

#### Sequence of Cenozoic Sirenians from Florida

Taken together, all four sirenian families are represented in the nearshore marine and continental deposits of Florida (Table 1). Although mention will be made of other sirenians, this discussion will principally focus on

TABLE 1. Fossil and extant Sirenia known from Florida. Taxa analyzed during the present study are presented in boldface, with the number of individual bulk samples analyzed in parentheses (also see Appendix). (Compiled from Domning 1994, 2001a and Hulbert 2001).

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Order Sirenia
†Family Prorastomidae
Genus and species indeterminant
†Family Protosirenidae
<b><i>Protosiren</i> sp.</b> , middle Eocene (Clairbornian); ?early Oligocene (4)
Family Dugongidae
†Subfamily Halitheriinae
<i>Metaxytherium</i> sp., very late Oligocene
<b><i>Metaxytherium crataegense</i></b> , late early to middle Miocene (3)
<b><i>Metaxytherium floridanum</i></b> , middle to late Miocene (23)
Subfamily Dugonginae
Undescribed small dugongid, middle Miocene
<i>Crenatosiren olseni</i> , very late Oligocene to very early Miocene
<i>Dioplotherium manigaulti</i> , very late Oligocene to middle Miocene
<i>Dioplotherium allisoni</i> , early Pliocene
<b><i>Corystosiren varguezi</i></b> , early Pliocene (1)
Undescribed genera and species, Pliocene
Family Trichechidae
<b><i>Trichechus</i> sp.</b> , ?late Pliocene, early to late Pleistocene (45)
<b><i>Trichechus manatus latirostris</i></b> , West Indian Manatee, the late Pleistocene (Rancholabrean) to Recent (18 wild, 6 captive)

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those taxa for which stable isotopic results are presented below. This discussion is separated into temporal intervals that represent the available isotopic samples, and not natural phylogenetic groups.

*Eocene to Early Oligocene (40–35 Ma).*—The earliest and most primitive sirenian group, Family Prorastomidae, although better known from the early to middle Eocene of Jamaica (Savage et al. 1994; Domning 2001c), is represented by extremely fragmentary material (vertebrae) from the late Eocene of Florida that was not amenable to stable isotopic analysis. Prorastomidae are, so far as is known, characterized by a relatively flat rostral morphology (Fig. 1A) indicating little of the deflection seen in other sirenians. Savage et al. (1994) speculated that *Prorastomus* was a selective browser feeding on floating and emergent aquatic plants, and to a lesser degree, seagrasses.

The Family Protosirenidae is somewhat better represented in Florida relative to the Prorastomidae, and an intimate association with fossil seagrasses has been observed. Ivany et al. (1990) described a diverse assemblage of well-preserved seagrass communities from Dolime Quarry, middle Eocene Avon Park

Formation of central Florida (Fig. 2). These seagrasses consist of *Thalassodendron* and *Cymodocea*, genera believed to be very closely related to the common Florida seagrass, *Thalassia* (Ivany et al. 1990). Sirenian fossils referred to *Protosiren* sp. are found in direct association with these seagrasses and a plant-herbivore interaction is implied. In contrast to the more primitive condition seen in *Prorastomus*, Florida *Protosiren* sp. is characterized by a rostral deflection of 35–40° (Domning et al. 1982) (Fig. 1B), implying increased foraging on seagrasses.

It also should be noted that during the late Oligocene through early Miocene, several dugongid species were present in Florida (Hulbert 2001), including three (*Metaxytherium* sp., *Crenatosiren olseni*, and *Dioplotherium manigaulti*) that coexisted at some localities (Domning 1989a). Although diverse, teeth for these sirenians are both rare and difficult to identify taxonomically. Consequently these extinct dugongids were unavailable for isotopic analysis.

*Middle to Late Miocene (ca. 17–8 Ma).*—By far the most common Miocene sirenian in the world was *Metaxytherium* (Fig. 1C,D), and this also was the case in Florida (Domning 1988).

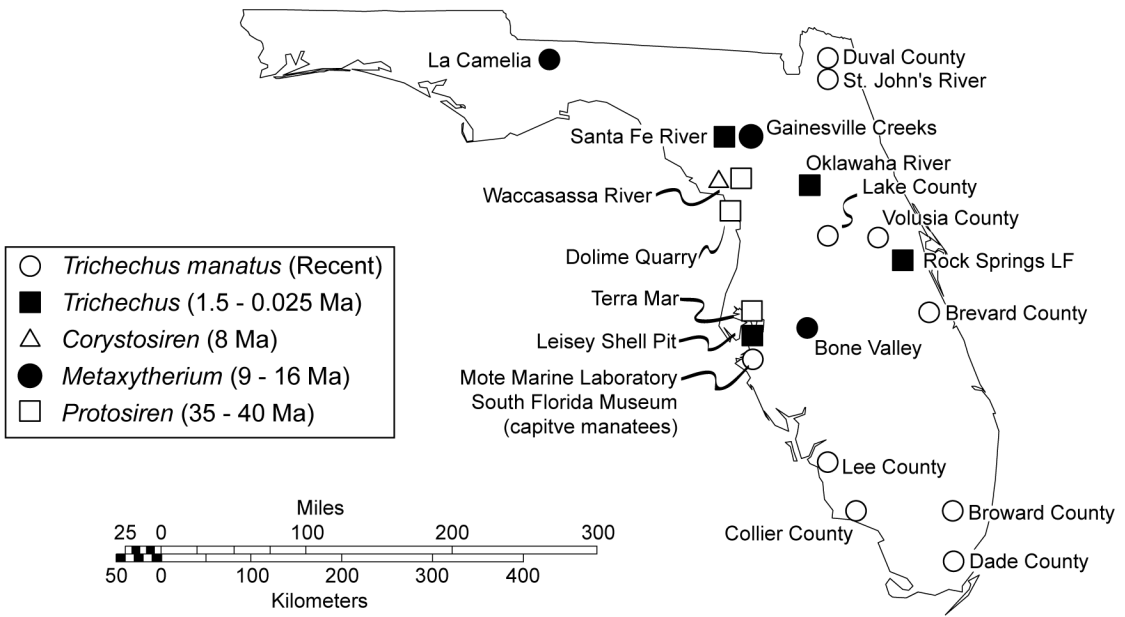


FIGURE 2. Map of localities discussed in text, including those from which specimens were sampled for this study.

The oldest records of this genus analyzed here are from the early part of the middle Miocene, including (1) the La Camelia Mine, Hawthorne Formation in the Florida panhandle (Bryant 1991); and (2) lowermost units of the Bone Valley phosphate mines of central Florida (Fig. 2). Both of these localities are Barstovian land mammal age (sensu Tedford et al. 1987; this North American Land Mammal "Age" [NALMA] is estimated to range from about 16 to 11.5 Ma). These oldest specimens are referred to *Metaxytherium crataegense* (Fig. 1D) (Domning 1988; Bryant 1991; Hulbert 2001). Thereafter, the extensive open-pit phosphate mines of central Florida contain abundant sirenian remains in the Lower Bone Valley Formation, which Domning (1988) concluded are all referable to *Metaxytherium floridanum* (Fig. 1C). These fossils come from the later part of the middle Miocene Agricola Fauna and, according to land mammal biochronology, are early Clarendonian NALMA in age (Morgan 1994), ca. 12 Ma. Thereafter, dugongs occur rarely in younger deposits in the Bone Valley district, from localities such as Nichols Mine, which are probably early Hemphillian in age (Hulbert 1988). In north Florida, various creeks in the Gainesville area contain dugong remains of a form slightly differ-

ent from the Bone Valley *Metaxytherium* that Domning (1988) refers to *M. cf. floridanum*. *Metaxytherium floridanum* is characterized by a relatively high degree of rostral deflection ( $\approx 75^\circ$ , Fig. 1C), suggesting specialization on seagrasses (Domning 1988).

*Late Miocene/Early Pliocene (7–4 Ma).*—The Miocene/early Pliocene was a pivotal time in Florida sirenian evolution. Unfortunately the fossil record of this interval is notably lacking. The diversity seen during the late Oligocene and early Miocene drops, and dugongs are apparently not well represented after the middle Miocene. The latest Miocene–early Pliocene (late Hemphillian) Upper Bone Valley Formation (ca. 5 Ma), which produces extensive interbedded terrestrial and marine mammalian faunal assemblages (the Palmetto Fauna [Morgan 1994; Hulbert 2001]), demonstrates a conspicuous absence of dugongs, probably representing a local near extinction of sirenians. Two notable exceptions to the total extirpation of Florida sirenians during the late Miocene include an early Hemphillian occurrence of *Corystosiren varguei* (also see Domning 1990) and undescribed late-surviving dugongids (Domning 2001a; Hulbert 2001) from the late Miocene and early Pliocene. It is indeed unfortunate that the fossil re-

cord of late Miocene–early Pliocene dugongids is not better represented, because this is the time that most dugongids became extinct in the Atlantic and Caribbean, and the trichechids presumably radiated from South America into this biogeographic province. This poor record of sirenian evolutionary history in the Atlantic/Caribbean region has resulted in speculative hypotheses either about the competitive superiority of manatees relative to dugongs or, if they did not indeed coexist and compete, about the manatees filling the aquatic megaherbivore niche vacated by the regional extinction of dugongs in the Atlantic Ocean (Domning 1982, 2001a).

*Early Pleistocene Through Recent (1.5 Ma to the Present).*—So far as can be determined from the available fossil record, the Trichechidae probably arose in South America and is first represented by *Potamosiren magdalensis* from the middle Miocene La Venta Fauna of Colombia (Domning 1997b). The dental morphology of *P. magdalensis* indicates that the teeth were not replaced continually during ontogeny as in more-derived manatees. Domning (1982, 1997b) suggests that the diet of this most primitive known manatee consisted of softer vegetation but did not include the abrasive true grasses (Poaceae) known to be part of the diet of the extant manatee *Trichechus manatus*.

Although there have been suggestions that manatees are first known in the middle Pliocene (Blancan NALMA) of northern Florida about 2.5 million years ago (Domning 1982), for example from the Santa Fe River, more recent interpretations suggest that this faunal assemblage is mixed with Pleistocene (Rancholabrean) mammals; therefore, a definitive older, middle Pliocene, date cannot be determined unambiguously (R. C. Hulbert personal communication 2001). The first definitive, in situ record of Florida manatees is represented by *Trichechus* sp. from the 1.5-Ma Irvingtonian Leisey Shell Pit south of Tampa (Morgan and Hulbert 1995). Having been derived from a South American ancestor (Domning 1982, 1997b), the extinct Florida fossil manatees are morphologically close to both *T. manatus* and the West African *T. senegalensis*, and may represent a distinct species. Pleistocene and Holocene Florida manatees are characterized by

horizontally replaced teeth that are continually shed during ontogeny. These teeth are very abundant in river localities from northern Florida of this age. Domning (1982, 2001a) suggests that late Pleistocene *Trichechus* sp. from Florida does not represent temporally successive populations, but may instead represent a sequence of genetically independent, multiple dispersal events into this region. Modern Florida manatees, *Trichechus manatus*, are characterized by a moderate degree of rostral deflection (Fig. 1F), indicating some seagrass preference, although this species is known to have a generalized diet (Ames et al. 1996; Domning 1982, 2001a). Modern manatees are known to exist in a wider range of habitats than dugongs, with the former ranging from full marine on one hand to freshwater rivers on the other (Husar 1978b).

In summary, the sequence of fossil sirenians in Florida, although discontinuous, covers some very important stages in the evolution of this order. As will be demonstrated below, isotopic analysis of specimens from this sequence offers some interesting results and insights relative to previous hypotheses about ancient diets and habitat preferences.

### Stable Isotopes, Plant Foods, and Habitat Reconstruction

Within the past few decades, stable isotopes have become widespread as proxies for interpreting and reconstructing the paleoecology of extinct vertebrates (e.g., Koch 1998). Carbon and oxygen isotopes will be used here to reconstruct, respectively, the diet and habitat of sirenians from Florida. The conventional delta ( $\delta$ ) notation is used here for carbon ( $\delta^{13}\text{C}$ ) and oxygen ( $\delta^{18}\text{O}$ ), where  $\delta$  (parts per mil, ‰) =  $[(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , and  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{18}\text{O}/{}^{16}\text{O}$ . The data from the unknown (fossil) are compared relative to internationally accepted standards, which for  $\delta^{13}\text{C}$  is V-PDB (Pee Dee Belemnite) and for  $\delta^{18}\text{O}$  reported here relative to V-SMOW (Standard Mean Ocean Water, both following Vienna convention, hence “V-” [e.g., Coplen 1994]).

*Carbon ( $\delta^{13}\text{C}$ ) and Ancient Diets.*—Plants fractionate carbon with one of three different photosynthetic pathways. Most plants use the Calvin Cycle, in which carbon is incorporated

into three-carbon chain compounds, hence the term "C<sub>3</sub>." C<sub>3</sub> plants include trees; shrubs; cool-growing-season, high-altitude and high-latitude grasses; and many aquatic plants, and represent about 85% of the world's plant biomass. Tropical and temperate grasses, which account for about 10% of terrestrial plant biomass, fractionate carbon into four-carbon chain compounds (as an intermediate product of photosynthesis), and hence the term "C<sub>4</sub>" plants. Of relevance here, grasses that live along the land/water interface photosynthesize carbon by using either the C<sub>3</sub> or C<sub>4</sub> pathway depending upon species. The third photosynthetic pathway, CAM (Crassulacean Acid Metabolism), exemplified by Cactaceae (cactus) and Euphorbiaceae (euphorbia), constitutes the remaining percentage of plant photosynthesis (Ehleringer et al. 1991). In view of the fact that CAM plants do not constitute a part of the known diet of sirenians, particularly in Florida, this photosynthetic pathway is not considered further. In terrestrial ecosystems, C<sub>3</sub> plants have a mean  $\delta^{13}\text{C}$  value of  $-27\text{‰}$  and a broad range of  $-32\text{‰}$  to  $-23\text{‰}$ , whereas C<sub>4</sub> plants have a mean  $\delta^{13}\text{C}$  of  $-13\text{‰}$  with a narrower range of  $-15\text{‰}$  to  $-10\text{‰}$  (Dienes 1980; Farquhar et al. 1989; Boutton 1991). Most fully marine plants (including seagrasses) fractionate carbon by using the C<sub>3</sub> pathway, although because the carbon source in the oceans can be derived from bicarbonate as well as atmospheric CO<sub>2</sub>, the  $\delta^{13}\text{C}$  values of C<sub>3</sub> marine plants can be more enriched than their terrestrial counterparts (Boutton 1991; Keeley and Sandquist 1992; Hemminga and Mateo 1996). The  $\delta^{13}\text{C}$  of coastal aquatic plants is more complicated because of the multiple sources of carbon from which plants draw and fractionate (Boutton 1991; Keeley and Sandquist 1992).

As mentioned above, the modern manatee *Trichechus manatus* eats more than 60 species of aquatic (marine/freshwater) plants and these span a wide range of  $\delta^{13}\text{C}$  values, approximately  $-3\text{‰}$  to  $-35\text{‰}$  (Ames et al. 1996). Nevertheless, the diet of *Trichechus manatus* from Florida consists predominantly of seagrasses (mostly *Thalassia*, *Syringodium*, *Halodule*, *Halophila*, *Diplanthera*, and *Ruppia* [Ames et al. 1996]), which have a mean  $\delta^{13}\text{C}$  that

mostly falls into a narrow range between  $-10\text{‰}$  and  $-11\text{‰}$  (Boutton 1991; Hemminga and Mateo 1996), and *Hydrilla*, with a  $\delta^{13}\text{C}$  of  $-13\text{‰}$  to  $-26\text{‰}$  (Ames et al. 1996). It is important to note here that *Hydrilla* is a non-native species in Florida, introduced in the 1950s (Langeland 1990), and was not a major dietary component for any of the sirenians examined in this study. The remaining plant species in the diet of *T. manatus* from Florida include (Ames et al. 1996) terrestrial C<sub>3</sub> and C<sub>4</sub> grasses, macroalgae, mangroves, cattail (*Typha* sp.), and water hyacinth (*Eichhornia*), which together range widely in their  $\delta^{13}\text{C}$  values (about  $-5\text{‰}$  to  $-35\text{‰}$ ), but all of these are in relatively minor quantities.

When isotopes of carbon are incorporated into skeletal tissues, there is an isotopic enrichment ( $\epsilon^*$ ) relative to the plant foods (i.e., <sup>13</sup>C is selectively incorporated into skeletal tissues, resulting in a higher  $\delta^{13}\text{C}$  value). For medium to large terrestrial modern mammals,  $\epsilon^*$  is 14.1‰ (Cerling and Harris 1999), although little was previously known about this enrichment in marine mammals (also see discussion below).

*Oxygen ( $\delta^{18}\text{O}$ ) and Habitat Preference.*—In contrast to the relatively straightforward interpretation of carbon uptake and fractionation, and corresponding  $\delta^{13}\text{C}$  values of tooth enamel carbonate, understanding the array of factors affecting the isotopic fractionation of oxygen in the environment and how oxygen is then metabolized by mammals is more complex. Thus, the inherent assumptions about interpreting the source and history of oxygen, and how it can be used to interpret ancient environments, requires multiple assumptions that are difficult to unambiguously control. It is well known that the fractionation of oxygen isotopes in the environment is a function of temperature, and thus the classic use of  $\delta^{18}\text{O}$  as a paleothermometer. However, because mammals maintain a constant body temperature, variation in enamel  $\delta^{18}\text{O}$  values is not just a result of temperature, but is influenced by physiological processes within the body and the  $\delta^{18}\text{O}$  composition of various environmental sources of oxygen.

In terrestrial mammals, the two principal sources of oxygen are contained in the ani-

mals' drinking water and in plant water. These two categories of ingested water frequently have different isotopic values within the same ecosystem that can introduce a significant amount of variation in  $\delta^{18}\text{O}$  values among individuals in a population. Likewise, a variety of physiological processes, including metabolic rate, sweating, panting, and excretion, can alter the magnitude and fractionation of oxygen fluxes entering and leaving a mammal's body water pool (Bryant and Froelich 1995; Kohn 1996). These factors further increase the  $\delta^{18}\text{O}$  values among individuals and potentially complicate intraspecific and interspecific comparisons of  $\delta^{18}\text{O}$  values.

Fortunately, among aquatic species, the factors affecting body water  $\delta^{18}\text{O}$  values are greatly reduced. Observations of captive marine species suggest that the majority (>98%) of oxygen flux is the result of passive diffusion of environmental water across the skin (Hui 1981; Andersen and Nielsen 1983). Therefore, the  $\delta^{18}\text{O}$  value of an aquatic mammal's body water should correlate strongly with the  $\delta^{18}\text{O}$  value of the environmental waters in which it lives (Yoshida and Miyazaki 1991), and populations of aquatic mammal species living in waters of relatively homogeneous isotopic composition should yield low  $\delta^{18}\text{O}$  variation among individuals. The  $\delta^{18}\text{O}$  value of an aquatic mammal's body water is therefore labeled by the  $\delta^{18}\text{O}$  value of the water in which it lives, and populations migrating between waters of different  $\delta^{18}\text{O}$  values should exhibit higher variation in  $\delta^{18}\text{O}$  values than populations remaining in a single, localized water body (Clementz and Koch 2001).

Ultimately, the ingested oxygen is in isotopic equilibrium with body tissues, including the hydroxylapatite of teeth. Tooth enamel accretes during a small portion of an animal's life span, on the order of months (depending upon factors such as size). Enamel  $\delta^{18}\text{O}$  values thereby serve as an archive of fluctuations in a mammal's body water  $\delta^{18}\text{O}$  values during the time of tooth mineralization (Bryant and Froelich 1995; Kohn 1996). This temporal variation within a tooth can introduce additional differences among individual  $\delta^{18}\text{O}$  values based upon the amount of enamel that is sampled. The  $\delta^{18}\text{O}$  measured from tooth enamel of

modern mammals therefore represents the end result of all of these potential sources of variation. A similar interpretation is made for fossil teeth, with the additional consideration of possible secondary alteration of the initial  $\delta^{18}\text{O}$  signature during fossilization. Despite the possibility of diagenesis and earlier claims to the contrary (particularly for fossil bone [Schoeninger and DeNiro 1982]), tooth enamel  $\delta^{18}\text{O}$  has been used for paleoenvironmental interpretations (e.g., Bocherens et al. 1996; Cerling and Sharp 1997; and MacFadden et al. 1999).

Mean tooth enamel  $\delta^{18}\text{O}$  values of modern and fossil sirenians are used as a proxy for reconstructing their habitat preferences through time, based upon the following model for  $\delta^{18}\text{O}$  of environmental water. Modern seawater has a global average  $\delta^{18}\text{O}_{\text{V-SMOW}}$  value of 0.0‰ (by definition) and is relatively homogeneous worldwide (Hoefs 1997). Nevertheless, some of the greatest variations in seawater  $\delta^{18}\text{O}$  are observed in marginal marine settings where they result from the combined effects of factors such as evaporation, continental runoff, upwelling, and ocean currents. Except in estuarine environments, measurements indicate that the shallow marine coastal waters of coastal Florida are slightly enriched in  $^{18}\text{O}$  compared with the global mean (Swart et al. 1996). Seawater from St. Augustine Beach (used as an internal standard in the Stable Isotope Laboratory of the Department of Geological Sciences, University of Florida) has a mean  $\delta^{18}\text{O}$  of 0.7‰. This supports the observation of Swart et al. (1996) that the  $\delta^{18}\text{O}$  of Florida ocean water lies between 0 and 1‰ and is strongly influenced by the strength of the Loop Current output from the Mississippi River as well as by evaporation (Ortner et al. 1995).

In contrast, Florida freshwater settings generally exhibit relatively depleted  $\delta^{18}\text{O}$  values. Most rivers display a range of  $\delta^{18}\text{O}$  values that generally decrease with increasing distance inland from the coast as a result of evaporation and fractionation during precipitation (Coplen and Kendall 2000). The isotopic composition of precipitation, a major contributor to the riverine water budget, is highly variable across Florida but generally averages about



–3‰ (Swart et al. 1989). Springs represent another important source of water for many Florida rivers and are frequently sought out by manatees during the colder months of the year. Year-round monitoring of  $\delta^{18}\text{O}$  during 1998–99 at a typical spring (Hart Springs) located in north-central Florida recorded relatively depleted values; mean  $\delta^{18}\text{O} = -4.0\text{‰}$ , with monthly values ranging between  $-3.6\text{‰}$  and  $-4.4\text{‰}$  (Jones and Quitmyer unpublished data). A U.S. Geological Survey analysis of six Florida rivers from different geographic regions within the state revealed mean  $\delta^{18}\text{O}$  values ranging from  $-0.1$  to  $-3.9\text{‰}$  (Coplen and Kendall 2000). In a seventh river system, the Econlockhatchee from central Florida, the mean  $\delta^{18}\text{O}$  was  $-2.0\text{‰}$  (Gremillion 1994).

Not all of Florida's freshwater environments, however, are characterized by low  $\delta^{18}\text{O}$  values. For instance, the large standing body of water that makes up the Everglades is isotopically enriched in  $^{18}\text{O}$  (0 to 2‰) as a result of sustained evaporation (Meyers et al. 1993; Swart et al. 1996). Fortunately, such exceptions do not appear to play a major role in the environmental life history of Florida sirenians, past or present.

It is well documented that the  $\delta^{18}\text{O}$  value of global seawater has varied throughout the Cenozoic from  $-1.2\text{‰}$  during the "greenhouse" world of the Paleocene and early Eocene, to modern values consistent with the late Cenozoic "icehouse" (Zachos et al. 2001). New geochemical proxies for seawater paleotemperatures have allowed investigators to assess the relative contributions of temperature and ice volume to the global  $\delta^{18}\text{O}$  record of marine carbonates and thereby reconstruct seawater  $\delta^{18}\text{O}$  evolution during the Cenozoic (Lear et al. 2000; Billups and Schrag 2002). On a regional scale, the local  $\delta^{18}\text{O}$  variations along Florida's ancient coasts and rivers remain poorly constrained throughout the Cenozoic. However, these factors do not diminish the robustness of the present-day model; i.e., the  $\delta^{18}\text{O}$  values of freshwater are depleted relative to seawater, and hence enamel  $\delta^{18}\text{O}$  values of freshwater sirenians (see below) are lower than for their marine counterparts. We assert that this model can be applied throughout the Cenozoic, particularly when anchored to the global sea-

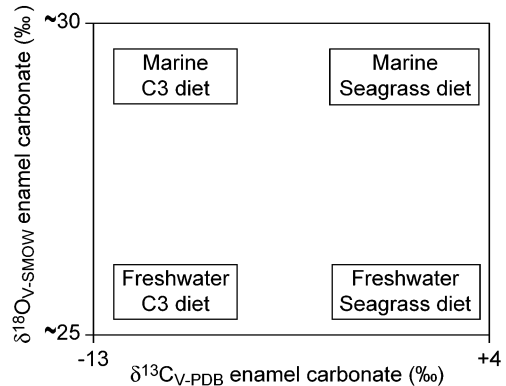


FIGURE 3. Simple box model of potential  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  end-member values of tooth enamel used to interpret, respectively, the diets and habitats of extinct Florida sirenians.

water  $\delta^{18}\text{O}$  curve (Lear et al. 2000; Billups and Schrag 2002), and can be used in the reconstruction of ancient habitat preference. We therefore use it to differentiate marine and freshwater habitats for fossil sirenians from Florida.

A simple conceptual model with four theoretical end-members (Fig. 3) is used below to interpret the carbon and oxygen isotopic data for the fossil sirenians from Florida. These data are also compared with those from modern sirenians with known diets and habitat preferences. The abscissa is calibrated by end-members ranging from a specialized seagrass feeder (tooth enamel  $\delta^{13}\text{C} \approx 4\text{‰}$ ) to a predominantly  $\text{C}_3$  feeder (tooth enamel  $\delta^{13}\text{C} \approx -13\text{‰}$ ). The ordinate is calibrated by end-members ranging from individuals living in relatively freshwater habitats (tooth enamel  $\delta^{18}\text{O} \approx 25\text{‰}$ ) to those inhabiting marine seawater ( $\delta^{18}\text{O} \approx 30\text{‰}$ ). Thus, starting in the upper right-hand corner of the model, individuals with relatively positive  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  end-member values are interpreted to feed mainly on seagrass and live in principally marine habitats. A modern analog for this is the dugong (*Dugong dugon*) from the Pacific Ocean (Husar 1978a). In the lower right-hand corner, individuals with relatively positive  $\delta^{13}\text{C}$  and negative  $\delta^{18}\text{O}$  values would be interpreted to feed mostly on seagrass and mainly live in freshwater habitats. This combination of diet and habitat is rare in nature because seagrasses are primarily marine, but perhaps

was represented in the past. In the lower left hand corner, individuals with relatively negative  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values are interpreted to feed principally on  $\text{C}_3$  aquatic plants and live in predominantly freshwater habitats. Populations of the extant West Indian manatee (*Trichechus manatus*) from Florida are known to trend towards this kind of diet and habitat (Husar 1978b; Ames et al. 1996). In the upper left-hand corner, individuals with more negative  $\delta^{13}\text{C}$  and more positive  $\delta^{18}\text{O}$  values would be interpreted to have a diet of mainly  $\text{C}_3$  plants (i.e., excludes seagrasses) and live in marine habitats. A potential sub-Recent analog within the Sirenia for this marine  $\text{C}_3$  feeder end-member is the Steller's Sea Cow (*Hydrodamalis gigas*) from the North Pacific Ocean that principally ate kelp (mean  $\delta^{13}\text{C}$  of  $-19\text{‰} \pm 1\text{‰}$  [compiled from Hobson et al. 1994; Simenstad et al. 1993]) and was hunted to extinction in 1768 (Domning 1999).

### Materials and Methods

*Stable Isotope Sample Acquisition (Teeth, Food, and Water).*—Six naturally shed teeth of live, captive Florida manatees collected from the bottom of their enclosures were borrowed for analysis from the Mote Marine Laboratory, Sarasota ("Hugh" or "Buffett"; also see Ames et al. 1996), and from the South Florida Museum ("Snooty") in accordance with a U.S. Fish and Wildlife Service permit. Hugh and Buffett, two male manatees, were born at the Miami Seaquarium (Hugh, 28 June 1984; Buffett, 16 May 1987) and have spent their entire lives in captivity. Although both manatees are nearly the same length (298 and 313 cm respectively), Buffett is much heavier, weighing about 660 kg to Hugh's 535 kg (D. Colbert personal communication 2001). Because Hugh and Buffett share the same enclosure, it is not possible to determine from which individual the shed teeth were derived. Snooty is another male manatee that has also spent his entire life in captivity. He is the oldest manatee in captivity and was born 21 July 1948, at the Miami Aquarium. Snooty weighs about 455 kg (C. Audette personal communication 2001). Manatee food and water were collected from their tanks for isotopic analysis.

Eighteen tooth samples of the extant West

Indian Manatee, *Trichechus manatus*, from Florida cataloged in the skeletal collection of the Florida Museum of Natural History Mammal Collection (abbreviation "UF M"; Appendix) were analyzed in accordance with the U.S. Fish and Wildlife Service permit. With the exception of one specimen from La Venta, Colombia (see further discussion below), all isotopic analyses of fossil sirenians are from Florida, with 67 specimens from the Florida Museum of Natural History Vertebrate Paleontology Collection (abbreviation "UF") and nine specimens from the private fossil collection of John Waldrop (abbreviation "JW") of Lake Wales, Florida. Residual sample powders of the JW specimens used for isotopic analysis are archived in the UF collection.

Tusk samples of six modern dugongs were collected from populations within the Torres Straits off the coast of Australia by the School of Tropical Environment Studies and Geography at James Cook University in Queensland, Australia. This dugong population was selected as a modern analog because it has been studied extensively by researchers at James Cook University, providing a long record of observational data on the ecology of this population (Marsh et al. 1984; Nietschmann 1984; Marsh 1986; Marsh and Saalfield 1991). These studies confirm that dugongs are strictly marine mammals specializing on seagrasses with only minor amounts of marine algae in their diet. Most importantly, no records suggest any habitation of freshwater sites or inclusion of freshwater plants in their diets.

*Isotope Sample Preparation, Analyses, Standardization, and Conventions.*—Bulk samples were mechanically drilled from teeth, by using either a Foredom<sup>™</sup> variable speed drill or a Dremel<sup>™</sup> tool. Sirenians other than dugongs (see below) were sampled by collecting a small sample of powdered enamel from some point along the tooth. The exact sample location was based on tooth preservation, ease in sampling, and minimizing destruction of diagnostic morphology. Serial samples were removed from one *Metaxytherium* tooth, one captive *Trichechus* tooth, and two fossil *Trichechus* teeth to assess the isotopic variation along the length of a tooth. Dugong tusks (*Dugong*

*dugon*) from Australia were sectioned longitudinally and bulk samples were collected by drilling the edge of the tusk across several successive growth bands. This method allowed homogenization of several years of growth and provides a robust estimate of the average isotopic values for each individual. Sample sizes prior to preparation were characteristically about 100–300 mg for off-line extraction and 10–25 mg for on-line extraction.

Following standard protocol (e.g., as described in MacFadden and Cerling 1996), samples were pulverized by using a mortar and pestle, then treated with either NaOCl or 30% H<sub>2</sub>O<sub>2</sub> overnight to remove any superficially bound organic compounds and then with 1 N or 0.1 N acetic acid (some were also buffered following recommendations in Koch et al. 1997). For off-line extractions (done at the University of Utah), approximately 50–100 mg of the treated powders was reacted with H<sub>3</sub>PO<sub>4</sub> for 40–48 hours at 25°C. The evolved CO<sub>2</sub> samples were then purified and extracted following standard cryogenic laboratory procedures (McCrea 1950). Previous analyses of tooth enamel carbonates have demonstrated the presence of a contaminant, probably SO<sub>2</sub> (e.g., MacFadden et al. 1996), that will significantly affect the carbon and oxygen isotopic values of the tooth enamel carbonate sample. Accordingly, all off-line enamel CO<sub>2</sub> samples were collected in sample tubes containing Ag wool and then heated for at least 24 hours at 50°C. This essentially removes this contaminant from the purified sample. Off-line extracted samples were measured on a Finnigan mass spectrometer in the Department of Biology SIRFER laboratory at the University of Utah.

For on-line extractions, approximately 1 mg was weighed and loaded into an Isocarb extraction carousel (University of California-Santa Cruz) or individual reaction vessels on a multiprep device at the UF. The samples were reacted with H<sub>3</sub>PO<sub>4</sub> (for varying times and temperatures, depending on instrument and inlet system used) and then purified and extracted for analysis on either a VG Prism (UF) or a Micromass Optima mass spectrometer (UCSC).

The diet of the captive manatees used in this study consists principally of romaine lettuce.

Hugh and Buffett also are fed a large quantity of kale. Other foods added to supplement their diet are relatively minor (<1% each). Samples of all foodstuffs consumed by these manatees were collected for isotopic analysis. All samples were dried in an oven and then each was pulverized. The carbon isotopes for each of these organic samples were measured with the Carlo Erba CNS Analyzer in the University of Florida Department of Geological Sciences. Water samples were collected by dipping samples from the surface of the tank and also directly from the fill spigot for each of the two tanks housing captive manatees. Water samples were equilibrated with CO<sub>2</sub> at 45°C for 5 hours before CO<sub>2</sub> is drawn into and measured by the VG Prism mass spectrometer at the University of Florida.

In all cases, unknown sample analyses were calibrated to either internal laboratory standards (TEC-CC, Carrera Marble, MEme, or UCSC elephant enamel) or NBS-19, and ultimately back to the V-PDB or V-SMOW standards (Coplen 1994). Analytical precision for each mass spectrometer is characteristically better than about 0.1‰ for δ<sup>13</sup>C and 0.2‰ for δ<sup>18</sup>O.

## Results and Discussion

*Isotopic Fractionation in Modern Sirenian Food, Water, and Tooth Enamel Carbonate.*—Previous studies have shown that tooth enamel δ<sup>13</sup>C values of mammalian herbivores are enriched relative to ingested plant foods. For mammals in general, this isotopic enrichment has been stated to be approximately 12–14‰ (e.g., Koch et al. 1992) for medium to large mammalian herbivores. Body size may be a contributing source of variation. Ambrose (1998) found that small mammals, i.e., lab rats, have a significantly lower mean isotopic enrichment of 9.4‰. Using extensive analytical data from a wide variety of extant medium to large mammalian herbivores with known diets, Cerling and Harris (1999) calculated that the mean isotopic enrichment (ε\*) for tooth enamel carbonate relative to diet is 14.1 ± 0.5‰. As most previous studies have dealt with terrestrial mammalian herbivores, little is known about the corresponding ε\* for marine mammals, including sirenians. Ames et al. (1996) analyzed

TABLE 2. Stable isotopic composition ( $\delta^{13}\text{C}_{\text{v-PDB}}$ ) of manatee food and isotopic enrichment ( $\epsilon^*$ , sensu Cerling and Harris 1999) for Hugh/Bufgett and Snooty.

Sample number	Material	Amount	$\delta^{13}\text{C}$ (‰)
<i>Mote Marine Laboratory, Hugh/Bufgett</i>			
PH 01-06	Romaine	96 heads/day/2 manatees	-27.8
PH 01-07	Kale	12 heads/day/2 manatees	-30.7
PH 01-08	Monkey biscuit	12-16/day	-21.3
PH 01-09	Carrot	$\ll 1\%$	-27.8
PH 01-10	Apple	$\ll 1\%$	-24.7
PH 01-11	Beet	$\ll 1\%$	-26.7
		weighted mean <sup>1</sup> =	-27.9
<i>South Florida Aquarium, Snooty</i>			
PH 01-12	Romaine	200 lbs/day/2 manatees	-27.8
PH 01-13	Romaine	200 lbs/day/2 manatees	-28.1
PH 01-14	Monkey chow	3%	-19.8
PH 01-15	Elephant chow	$\ll 1\%$	-26.5
PH 01-16	Apple	$\ll 1\%$	-24.4
PH 01-17	Broccoli	$\ll 1\%$	-25.7
PH 01-18	Sweet potato	$\ll 1\%$	-23.7
		weighted mean <sup>2</sup> =	-27.4

Isotopic enrichment between food and tooth enamel carbonate  $\delta^{13}\text{C}$ :

$\delta^{13}\text{C}$  values (Appendix) used to calculate mean enrichment  $\epsilon^*$  include Hugh/Bufgett (5 samples) and Snooty (1 sample) each subtracted from weighted mean of food source above yields isotopic enrichment of 14.0‰.

<sup>1</sup> Approximate  $\delta^{13}\text{C}$  for average daily intake of Hugh and Bufgett, where for total diet, romaine = 85%, kale = 11%, monkey biscuit = 3%, and all other foods  $\approx 1\%$ .

<sup>2</sup> Approximate  $\delta^{13}\text{C}$  for average daily intake of Snooty, where for total diet, romaine = 96%, monkey biscuit = 3%, and all other foods  $\approx 1\%$ .

the  $\delta^{13}\text{C}$  values of known plant food and soft tissues (liver, kidney, blubber, and skin) of Hugh and Bufgett, then at the Lowry Park Zoo, Tampa (later moved to Mote Marine Laboratory), but no teeth were analyzed during that study.

Given the lack of existing data from which to model the  $\epsilon^*$  of modern sirenians, and then by inference, fossil sirenians from Florida, known food and naturally shed teeth of Hugh/Bufgett and Snooty were analyzed. A potential source of error is introduced from teeth that were shed and collected from the bottom of the manatee's tanks prior to the time that we actually collected the plant foods for isotopic analysis. Therefore, the assumption is that the carbon isotopic values of the diet incorporated into the teeth were relatively constant over the time interval between which the teeth were mineralized and shed and the diet was analyzed. Information provided by the animal keepers (also see Ames et al. 1996) indicated that the diet remained constant, consisting principally of romaine lettuce (>96%), with the remaining 4% including kale, carrots, apples, beets, broccoli, sweet potato, and commercially available monkey biscuits and elephant chow. Our analytical results (Table 2)

indicate that the mean  $\delta^{13}\text{C}$  value of ingested foodstuffs was -27.9‰ for Hugh/Bufgett and -27.4‰ for Snooty, both of which are close to the mean  $\delta^{13}\text{C}$  value for  $\text{C}_3$  terrestrial plants (Dienes 1980; Farquhar et al. 1989; Boutton 1991). Using five teeth from Hugh/Bufgett and one tooth from Snooty (Appendix), and with the known isotopic composition of the food, we report here the calculated isotopic enrichment ( $\epsilon^*$ ) of 14.0‰. This is essentially the same value determined for terrestrial mammalian herbivores (14.2‰ [Cerling and Harris 1999]), and is the model used to interpret the isotopic enrichment between fossil sirenians from Florida and their ancient diets.

Eight water samples, representing either tap fill or tank water, were analyzed from the enclosures containing the captive manatees to attempt a calculation of an enrichment factor between  $\delta^{18}\text{O}$  of environmental water and tooth enamel. For Hugh/Bufgett the mean fill water  $\delta^{18}\text{O}$  is -2.1‰ ( $n = 2$ ) and -1.0‰ ( $n = 2$ ) for the tank water. For Snooty the mean fill water is -0.3‰ ( $n = 2$ ) and 0.0‰ ( $n = 2$ ) for the tank water. Despite the fact that these water samples are nonsaline, their isotopic values lie close to expected values for marine water. We interpret the slightly more positive tank

TABLE 3. Stable isotopic values indicating intra-tooth variation in teeth of fossil and extant Florida sirenians.

Approximate position from base of enamel, mm (proportion with respect to tooth height)	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)
<i>Metaxytherium floridanum</i> , UF JW F6912, Bone Valley-Kingsford-0, ca. 10 Ma		
14.5 mm (0.86)	-0.4	30.1
11.5 mm (0.64)	-1.1	28.6
8.5 mm (0.47)	-1.4	28.9
4.5 mm (0.25)	-1.6	27.7
Mean, Standard Deviation, range	-1.1, 0.5, 1.2	28.8, 1.0, 2.4
Hugh or Buffett, <i>Trichechus manatus</i> , captive, UF uncataloged, Recent		
5.5 mm (0.61)	-15.0	27.0
3.5 mm (0.39)	-14.4	26.9
1.5 mm (0.17)	-13.5	26.8
Mean, Standard Deviation, range	-14.3, 0.8, 1.5	26.9, 0.1, 0.2
<i>Trichechus</i> sp., Oklawaha River I, UF uncataloged, ca. 0.025 Ma		
7.5 mm (0.83)	1.3	31.0
6 mm (0.67)	1.2	30.4
4.5 mm (0.50)	1.1	30.2
3 mm (0.33)	1.5	30.3
1.5 mm (0.17)	2.6	32.2
Mean, SD, range	1.5, 0.6, 1.5	30.8, 0.8, 2.0
<i>Trichechus</i> sp., Oklawaha River I, UF uncataloged, ca. 0.025 Ma		
8 mm (0.98)	-11.0	30.5
6.5 mm (0.72)	-11.7	30.0
4.5 mm (0.50)	-10.2	30.4
3 mm (0.33)	-9.7	30.5
1.5 mm (0.17)	-8.2	30.9
Mean, Standard Deviation, range	-10.1, 1.4, 3.5	30.4, 0.3, 0.9

water to represent the effect of evaporation. Given the small sample size and ambiguous results, these data are not used either to represent a good model for the  $\delta^{18}\text{O}$  of Florida natural freshwater or to calculate an enrichment factor between the  $\delta^{18}\text{O}$  of tooth enamel relative to environmental water.

*Isotopic Variation along the Sirenian Tooth Crown.*—Most specimens analyzed during this study consisted of one bulk sample removed from an individual tooth. Recent studies have shown that isotopic variation along the crown of modern and fossil mammal teeth can be used to explore climatic and dietary variation over the period of time during which the tooth mineralizes (e.g., Bryant et al. 1996; Cerling and Sharp 1997). The actual time of mineralization of individual sirenian teeth is poorly known (D. Domning personal communication 2003), but judging from eruption sequence (Domning and Hayek 1984) and tooth development in other medium-sized mammals (references in Bryant et al. 1996), this probably occurs over a period of many

months to about a year in manatees and other sirenians.

To understand isotopic variation in Florida sirenians, we selected four teeth for serial sampling. Three of these represent *Trichechus* (one extant captive and two late Pleistocene specimens) and the fourth represents Miocene *Metaxytherium* (Table 3). We selected the teeth from the captive individuals (Hugh/Buffett), realizing that these manatees are fed on an isotopically monotonous diet and live in an enclosure with an isotopically homogeneous water source. We therefore predicted relatively little variation in tooth enamel  $\delta^{13}\text{C}$  or  $\delta^{18}\text{O}$ . In contrast, the late Pleistocene *Trichechus* from the Oklawaha River and Miocene *Metaxytherium* should demonstrate isotopic variation representing diets and habitat of wild populations as archived within individuals.

The stable isotopic data for the captive *Trichechus*, i.e., Hugh/Buffett, show relatively little variation in both  $\delta^{13}\text{C}$  (range 1.5‰) and  $\delta^{18}\text{O}$  (range of 0.2‰). This would be expected given both the monotonous diet (Table 2) and

source of water used for the tank enclosure. The data for the fossil specimens demonstrate more variation in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  as might be predicted from diversity in diets and probable habitats. The dugongid *Metaxytherium* is generally believed to have had a specialized diet, corroborated by the narrow range (1.2‰) of  $\delta^{13}\text{C}$  values. The corresponding  $\delta^{18}\text{O}$  values show a 2.4‰ range, indicating some slight variability in habitat, ranging from relatively marine (30.1‰) to more mixed, or freshwater (27.7‰). The late Pleistocene *Trichechus* specimens indicate a wide range of diets among individuals (a mean of 1.5‰ indicates seagrasses feeding in one individual, whereas a mean of -10.1‰ indicates a diet of principally  $\text{C}_3$  plants in the other). The mean  $\delta^{18}\text{O}$  values of 30.8 and 30.4‰ suggest relatively marine environments and the narrow range (2.0 and 0.9‰) suggests that the manatees inhabited these environments for the time that the teeth were mineralized. Several major points are evident from the data from the serial analyses: (1) the small amount of isotopic variation predicted from the captive manatee fed on a monotonous diet and living in a constant source of water is corroborated; (2) the extinct dugongid and fossil manatee *Trichechus* demonstrated more isotopic variation, but this is no more than 1.5‰ for  $\delta^{13}\text{C}$  and 2‰ for  $\delta^{18}\text{O}$ . This suggests that the far greater degree of total isotopic variation (22.3‰ for  $\delta^{13}\text{C}$  and 9.2‰ for  $\delta^{18}\text{O}$ ; Table 4, Appendix) seen in the bulk sample data presented elsewhere in this paper cannot be accounted for within the demonstrated limits of individual isotopic variation that would be expected during the time interval when the tooth mineralized.

*Stable Isotopes, Ancient Diets, and Habitat Preferences.*—To determine differences in habitat as represented by  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  data, we used ANOVA to compare four groups of tooth enamel data for Florida sirenians: (1) Eocene-early Oligocene *Protosiren* ( $n = 4$ ); (2) Miocene *Metaxytherium*, including all samples of *M. crataegense*, *M. floridanum*, and *M. cf. floridanum* ( $n = 26$ ); (3) extinct ?Plio-Pleistocene *Trichechus* sp. ( $n = 45$ ); and (4) modern wild *T. manatus* ( $n = 18$ ) (Table 4; see raw data in Appendix). One specimen, *Corystosiren varguezii* (UF 18826), from the early Hemphillian Waccasas-

sa River, was not analyzed as part of the ANOVA because its statistical "group" represents a sample size of one. The captive manatees ( $n = 6$ ) also were not analyzed in this ANOVA. The ANOVA results indicate significant differences in the four statistical populations, both for  $\delta^{13}\text{C}$  ( $n = 93$ ,  $df = 89$ ,  $F = 12.2$ ,  $F_{\text{critical}} = 2.71$ ,  $p \ll 0.001$ ) and  $\delta^{18}\text{O}$  ( $n = 93$ ,  $df = 89$ ;  $F = 9.0$ ,  $F_{\text{critical}} = 2.71$ ,  $p \ll 0.001$ ). The statistical differences in both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  are therefore interpreted to have paleobiological significance related to, respectively, diets and habitat preferences.

*Extant Sirenians: Wild Populations and Captive Specimens of Trichechus manatus and Wild Dugong dugon.*—Carbon isotopic values for Recent, wild manatees have a mean  $\delta^{13}\text{C}$  of -5.7‰ and broad range between -13.9‰ and 0.5‰ (Table 4), indicating a mixed diet. The corresponding  $\delta^{18}\text{O}$  mean is 29.1‰ with a range between 27.3‰ and 31.2‰, indicating a mixture of freshwater to marine habitats. These data demonstrate that modern *Trichechus manatus* from Florida fills a broad ecological niche ranging from marine with a seagrass diet, to freshwater with a  $\text{C}_3$  diet (Figs. 3, 4). Captive *Trichechus* marks an end-member, admittedly unnatural for a freshwater,  $\text{C}_3$  feeder (Fig. 3), although some specimens approach marine  $\delta^{18}\text{O}$  values owing to the probable enrichment of water in  $^{18}\text{O}$  from high evaporation rates in the shallow tanks.

For modern wild Australian dugongs, the mean  $\delta^{13}\text{C}$  of 1.2‰ and narrow range (-0.6‰ to 1.7‰) indicate a specialized seagrass diet (Table 4, Fig. 4), as is also empirically known from studies of dugong diets (Walker 1975; Husar 1978a; Marsh et al. 1984; Neitschmann 1984). Likewise, the mean  $\delta^{18}\text{O}$  of 29.8‰ and narrow range (29.6 to 30.1‰) suggest a predominantly marine environment. These values for water and diet can be assumed to represent an end-member, given that dugongs generally are strictly marine and predominantly eat seagrasses (Fig. 3).

The  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values from tooth enamel carbonate indicate that Recent dugongs and manatees demonstrate a broad range of isotopic variation that correlates with known diets and habitat preferences of these extant sirenians. Along with the conceptual model

TABLE 4. Univariate statistical data (*n*, mean, standard deviation, range) for different subsets of bulk sample stable carbon and oxygen isotopic data (as discussed in the text) for the fossil and extant sirenians from Florida. See Appendix for raw data.

Group	<i>n</i>	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)
Eocene–early Oligocene <i>Protosiren</i>	4	3.4, 1.0, 1.9 to 4.1	30.2, 1.6, 28.3 to 32.2
All <i>Metaxytherium</i>	26	-1.7, 4.1, -13.1 to 2.8	29.3, 1.6, 25.1 to 31.0
<i>M. crataegense</i>	3	-1.4, 1.5, -3.0 to -0.1	28.0, 2.2, 25.4 to 29.6
Agricola <i>M. floridanum</i>	8	-1.0, 1.9, -4.8 to 0.9	29.1, 2.3, 25.1 to 30.7
Gainesville <i>M. cf. floridanum</i>	5	0.1, 1.0, -1.0 to 1.2	30.7, 0.3, 30.4 to 31.0
Later Bone Valley <i>M. floridanum</i>	10	-3.4, 6.1, -13.1 to 2.8	29.0, 0.5, 28.3 to 29.9
All Florida Protosirenidae and Dugongidae	31*	-0.9, 4.3, -13.1 to 5.6	29.2, 1.9, 23.0 to 32.2
?Plio-Pleistocene <i>Trichechus</i> sp.	45	-7.7, 5.5, -18.2 to 1.7	27.7, 1.6, 24.7 to 32.1
Leisey	3	-6.2, 2.3, -7.7 to -3.5	27.8, 1.7, 26.5 to 29.7
Santa Fe River sites	25	-9.9, 5.3, -18.2 to 1.7	27.3, 1.6, 24.7 to 32.1
Rock Springs	5	-5.9, 7.3, -14.3 to 0.1	29.1, 1.1, 28.1 to 30.4
Oklawaha River	12	-4.4, 3.5, -11.4 to -0.3	27.7, 1.6, 25.8 to 30.4
Recent, wild <i>Trichechus manatus</i>	18	-5.7, 5.1, -13.9 to 0.5	29.1, 0.9, 27.3 to 31.2
All Florida fossil and wild <i>Trichechus</i>	63	-7.2, 5.4, -18.2 to 1.7	28.1, 1.6, 24.7 to 32.1
Captive <i>Trichechus manatus</i>	6	-13.8, 0.9, -14.9 to -12.3	26.5, 1.4, 25.1 to 28.5
Wild <i>Dugong dugon</i>	6	1.2, 0.9, -0.6 to 1.7	29.8, 0.2, 29.6 to 30.1

\* Includes one specimen of late Miocene *Corystosiren varguezii*, UF 18826 (Appendix).

(Fig. 3), these data are used below as the reference model of extant analogs to interpret the diets and habitat preferences of fossil sirenians from Florida. (These data are also plotted in the background of Figures 5–7 along with those for fossil sirenians as a means of interpreting the paleoecology of the extinct groups.)

*Eocene-Oligocene Protosiren sp. (ca. 40 to 35 Ma).*—The primitive dugongid *Protosiren* is relatively rare in the middle Cenozoic limestones of Florida. Only four individual tooth specimens were available for isotopic analyses. The three Eocene *Protosiren* species and one early Oligocene cf. *Protosiren* species have a combined mean  $\delta^{13}\text{C}$  value of 3.4‰ with a



FIGURE 4. Plot of  $\delta^{13}\text{C}$  versus  $\delta^{18}\text{O}$  for all modern sirenians sampled in this study. All measurements were made from tooth enamel carbonate. Different ecologies grade between modeled end-members of ecological variation. Modern *Dugong* (white diamonds) is known to be a strictly marine, seagrass feeder, and captive *Trichechus* (black diamonds) are strictly freshwater,  $\text{C}_3$  feeders. Wild *Trichechus* (gray diamonds) is known to live in either habitat, i.e., ranging between the two end-members.

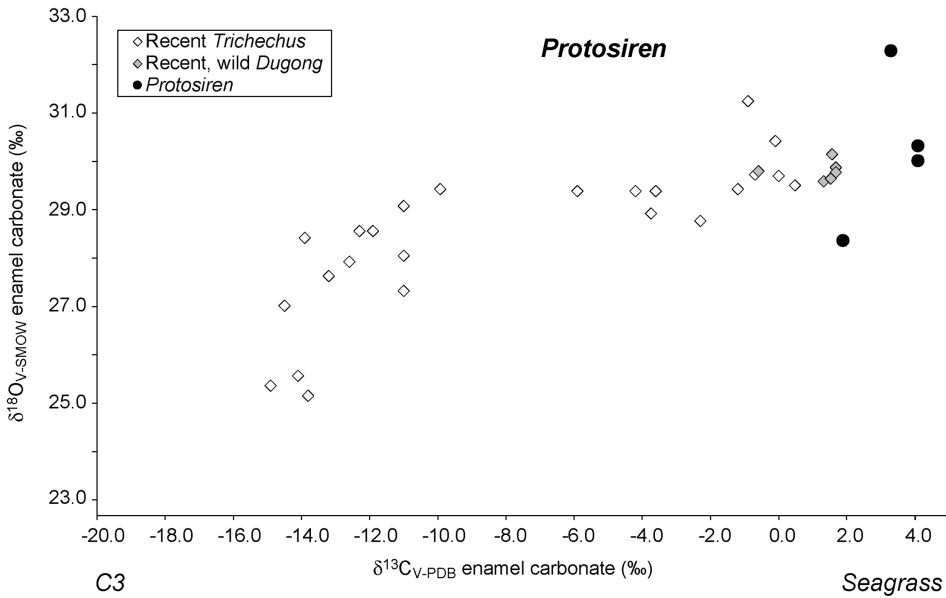


FIGURE 5. Plot of  $\delta^{13}\text{C}$  against  $\delta^{18}\text{O}$  for all teeth of *Protosiren* sampled in this study. Data from recent sirenians are also plotted for comparison. All measurements were made from tooth enamel carbonate.

narrow observed range (Table 4, Fig. 4). Given the isotopic enrichment factor calculated for sirenians of 14.0‰, these data indicate that Florida *Protosiren* sp. fed on vegetation with a mean isotopic value of about  $-11\text{‰}$ , suggesting that their diet consisted principally of seagrasses. These results support previous observations (Ivany et al. 1990) of a direct association with *Thalassia*-like seagrasses and *Protosiren* sp. from the Dolime Quarry, middle Eocene Avon Park Formation of northern peninsular Florida (Fig. 2).

The mean  $\delta^{18}\text{O}$  value of 30.2‰ (range 28.3–32.2‰) for these same four *Protosiren* specimens indicates an environmental water source enriched in  $^{18}\text{O}$ , an observation made all the more significant in light of the relatively depleted global ocean  $\delta^{18}\text{O}$  interpreted for Eocene seawater (Zachos et al. 2001). In comparison with the data for recent sirenians, *Protosiren* plots near the values for modern dugongs (Fig. 5), suggesting that *Protosiren* lived and fed in environments similar to that of modern dugongs. The isotopic data indicate these early sirenians were principally marine (also like extant dugongs) and confirm previous hypotheses that early during their history, dugongs specialized on seagrasses (e.g., Domning 1981, 2001a; Ivany et al. 1990). Al-

though a specialized diet and habitat are indicated from the isotopic data, this interpretation may be an artifact of the small sample size available for analyses. Well-preserved crania of Florida *Protosiren* do not exist, but if they did, it is predicted that they would have a relatively well developed flexure consistent with a seagrass diet (Domning 2001a), probably similar to *Protosiren fraasi* from the middle Eocene of Egypt (Fig. 1B).

*Middle to Late Miocene Metaxytherium* (Barstovian to ?early Hemphillian, ca. 16–9 Ma) and *Corystosiren* (early Hemphillian, ca. 8 Ma).—Samples of *Metaxytherium* were analyzed from four different localities during this study (Table 4, Appendix). The mean carbon isotopic values and very small observed ranges for three of these samples, i.e., Barstovian *M. crataegense* (mean  $\delta^{13}\text{C} = -1.4\text{‰}$ ), early Clarendonian *M. floridanum* from the Bone Valley Agricola mine (mean  $\delta^{13}\text{C} = -1.0\text{‰}$ ), and late Clarendonian *M. cf. floridanum* from the Gainesville creeks (mean  $\delta^{13}\text{C} = 0.1\text{‰}$ ) indicate that these *Metaxytherium* were specialized feeders with a diet predominantly consisting of seagrasses. Most plot very close to modern dugongs (Fig. 6). The carbon isotopic data for Clarendonian/early Hemphillian *M. floridanum* from the later Bone Valley sequence sug-



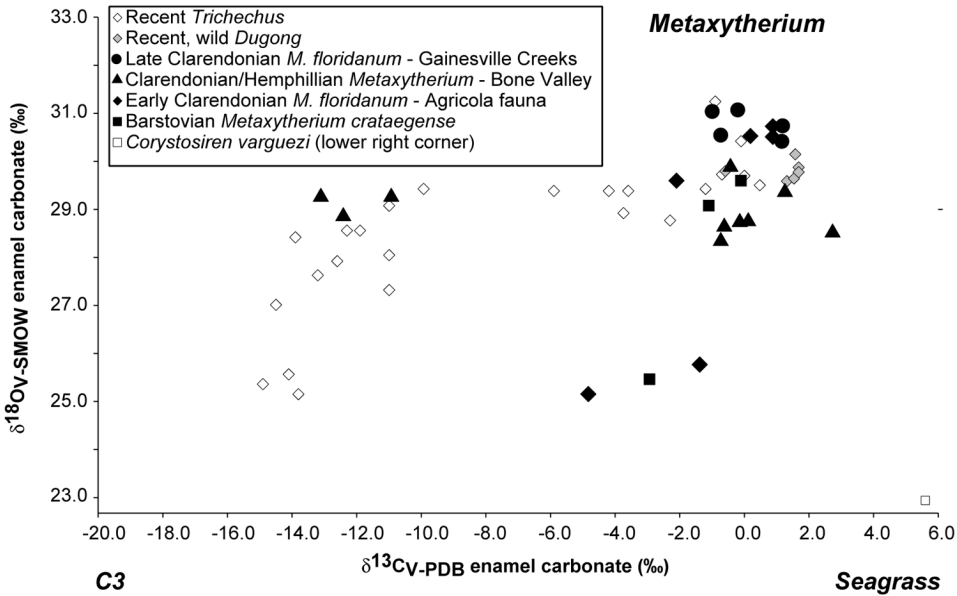


FIGURE 6. Plot of  $\delta^{13}\text{C}$  against  $\delta^{18}\text{O}$  for all teeth of *Metaxytherium* sampled in this study. *Metaxytherium* data are separated according to locality and/or age of the fossils. Data from recent sirenians are also plotted for comparison. All measurements were made from tooth enamel carbonate.

gest a more diverse diet for this fourth suite of samples. The mean  $\delta^{13}\text{C}$  is more negative (mean  $\delta^{13}\text{C} = -3.4\text{‰}$ ) than for the preceding samples of *Metaxytherium* and the observed range is very interesting. Seven of the specimens have  $\delta^{13}\text{C}$  values close to  $0\text{‰}$  (Fig. 6), indicating a diet of seagrasses. This is consistent with previous hypotheses about the diet of *Metaxytherium* based on its dentition and high degree of rostral deflection (e.g., Domning 1988, 2001a); (Fig. 1C). However, the remaining three specimens, with  $\delta^{13}\text{C}$  values of  $-10.9$  (JW F7502),  $-12.4$  (JW F7407E), and  $-13.1\text{‰}$  (JW F7405A2) (Fig. 6, Appendix) indicate a significantly different diet. Using the isotopic enrichment of  $14.0\text{‰}$  presented above, and taking the mean for these three specimens ( $-12.1\text{‰}$ ), then the mean value of the ingested plant foods would be  $-26.1\text{‰}$ , indicating a diet consisting predominantly of  $\text{C}_3$  plants. From the isotopic results alone, and in the absence of other corroborating evidence, it is not possible to further identify what specific kinds of  $\text{C}_3$  plants these *Metaxytherium* individuals ate when their tooth enamel mineralized.

The oxygen data for all four *Metaxytherium*

samples yield a mean  $\delta^{18}\text{O}$  of  $29.3\text{‰}$  (Table 4). Relative to the extant *Trichechus* specimens analyzed here, these data indicate an isotopically enriched water source, suggesting a principally marine habitat. The majority of the data, as indicated by the large grouping of individual points between approximately  $28\text{‰}$  and  $31\text{‰}$  (Fig. 6), support this interpretation. However, three data points, i.e.,  $25.4\text{‰}$  (UF 36446) for *M. crataegense* from the Barstovian Palmetto mine, and  $25.1$  and  $25.8\text{‰}$  (UF 206653, 206655) for *M. floridanum* from the early Clarendonian Agricola Fauna, are among the lowest  $\delta^{18}\text{O}$  values observed during this study and are similar to those for the captive manatees known to live in full freshwater (Fig. 5).

Given the morphology of other fossil dugongs, and known diet and habitat preferences of extant *Dugong dugon*, it is perhaps not surprising that for the most part, *Metaxytherium* from the Miocene of Florida was a specialized seagrass feeder and lived in marine waters. Exceptions to this general pattern include (1) three specimens indicating a diet of  $\text{C}_3$  plants; and (2) three other specimens living in more freshwater habitats, although grazing predominantly on seagrasses.

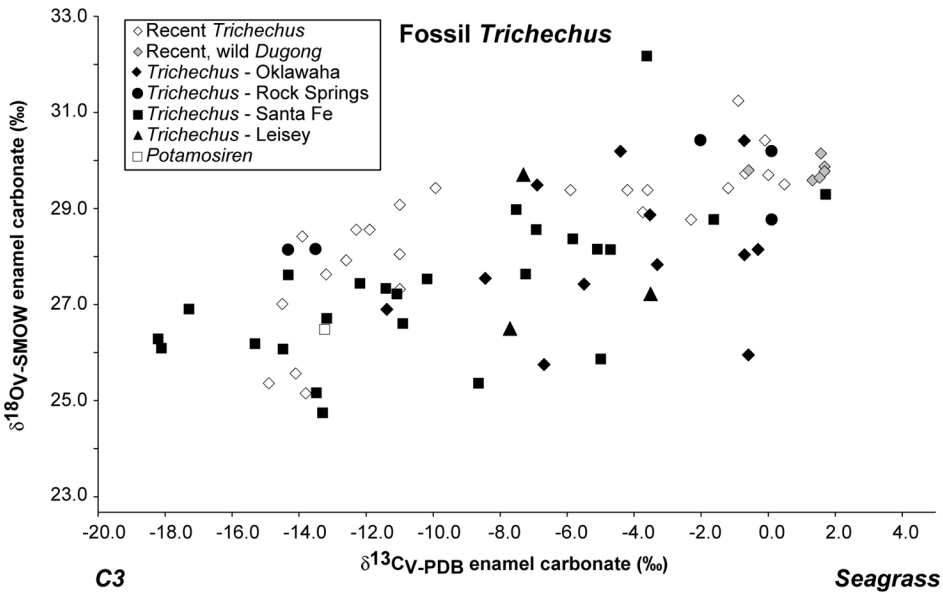


FIGURE 7. Plot of  $\delta^{13}\text{C}$  versus  $\delta^{18}\text{O}$  for all fossil teeth of *Trichechus* sampled in this study. Fossil *Trichechus* are separated according to the locality from which the fossil was collected. Data from recent sirenians are also plotted for comparison. All measurements were made from tooth enamel carbonate.

*Corystosiren* is a late-surviving dugong at a time when sirenian fossils are exceedingly rare in Florida. A single specimen of *C. varguezii* from the early Hemphillian Waccasassa River yielded a relatively enriched  $\delta^{13}\text{C}$  value of 5.6‰ and a very depleted  $\delta^{18}\text{O}$  value of 23.0‰ (Fig. 6 [lower right corner], Appendix). These data suggest that this dugong was feeding on seagrasses, but living in predominantly freshwater. If so, then this ecological niche is not commonly found in other fossil or extant dugongs. It is obvious that a single data point may not be representative of the overall autecology of this species. Unfortunately, more specimens of the rare *C. varguezii* are not available for additional isotopic analyses.

?Late Pliocene and Pleistocene *Trichechus* sp.—The manatees (Family Trichechidae) are believed to have originated in South America during the middle Tertiary (Domning 1982), with the earliest fossil record of this sirenian family represented by *Potamosiren magdalenis* (Domning 1997b) from the late middle Miocene (ca. 10 Ma) of Colombia. A single specimen (INGEOMINAS IGM 250927; isotopic sample BJMacF 96-70) of this early manatee from the La Venta Fauna yielded a  $\delta^{13}\text{C}$  value of -13.3‰ (Fig. 7), indicating that this indi-

vidual was feeding not on seagrasses (as was suggested because of its thick enamel), but rather on  $\text{C}_3$  vegetation. This same sample yielded a  $\delta^{18}\text{O} = 26.3\text{‰}$ , suggesting a principally freshwater habitat.

Representatives of the Family Trichechidae apparently dispersed into Florida either during the late Pliocene or early Pleistocene. The first definite, in situ occurrence of manatees, referred to here as *Trichechus* sp., which is probably a species different from *T. manatus* (Domning 1982; Hulbert 2001), comes from the 1.5 Ma Irvingtonian Leisey Shell Pit (Fig. 2). The mean  $\delta^{13}\text{C}$  value of -6.2‰ for Leisey *Trichechus* sp. indicates a mixed diet and the mean  $\delta^{18}\text{O}$  of 27.8‰ indicates mixed freshwater and marine habitats (Table 4).

A large sample suite ( $n = 25$ ) comes from several localities along the bed of the Santa Fe River in northern Florida. Given the age span of the associated mammalian fauna, these fossils almost certainly represent a temporally mixed assemblage. The age range might be as old as late Pliocene (middle Blancan), but it surely spans much of the Pleistocene, including the late Blancan and Irvingtonian NALMAs. These samples may represent a mixing of genetically independent populations that

migrated into this region at different times (Domning 1982, 2001a). Some localities along the Santa Fe River have better temporal resolution than others. Santa Fe River localities are divided for the purposes of plotting when a more precise age may be estimated, but all are considered together in the statistical analysis. The isotopic data have a mean  $\delta^{13}\text{C}$  value of  $-9.9\text{‰}$ , but the range is perhaps more interesting (Table 4, Fig. 7). At one end of the spectrum, the minimum  $\delta^{13}\text{C}$  value of  $-18.2\text{‰}$  is exceedingly depleted and suggests an individual feeding predominantly on  $\text{C}_3$  plants that lived in closed-canopy communities (approximately  $-32\text{‰}$ ; [van der Merwe and Medina 1991]). At the other end of the spectrum,  $\delta^{13}\text{C}$  values as enriched as  $1.7\text{‰}$  indicate individuals feeding on seagrasses, a principal food source similar to that of modern *Trichechus manatus* from Florida.

Fossil manatees were analyzed from two other late Pleistocene sites, Rock Springs and the Oklawaha River. In contrast to the temporal and possible genetic mixing that characterize the Santa Fe River specimens, these other sites seem relatively homogeneous, with Rock Springs being about 125,000 years old (Wilkins 1983) and the Oklawaha being latest Rancholabrean judging from its associated fauna (MacFadden personal observation 2001). The Rock Springs sample demonstrates a broad range of  $\delta^{13}\text{C}$  values from  $-14.3\text{‰}$  to  $0.1\text{‰}$  that appears to represent a bimodal distribution of values clustering at either extreme (Table 4, Fig. 7). This is interpreted as some individuals feeding on  $\text{C}_3$  plants whereas others were feeding on seagrasses. The Oklawaha sample likewise has a broad range of  $\delta^{13}\text{C}$  values, from  $-11.4$  to  $-0.3\text{‰}$ , suggesting both mixed feeding as well as some specialization on seagrass.

Taken together, the  $\delta^{18}\text{O}$  data for fossil and extant wild *Trichechus* sp. from Florida indicates a considerable spread and continuous variation from  $24.7\text{‰}$  to  $32.1\text{‰}$  (Table 4, Fig. 7). These data are interpreted to indicate a range of habitats from full freshwater, presumably peninsular rivers and springs, to full marine waters. This range of habitat preferences is similar to that of extant *Trichechus manatus*.

*Temporal Variation and Evolution of Diet and Habitat.*—To understand temporal variation and patterns in the stable isotopic data for the samples of fossil and extant Florida sirenians presented in this paper, we analyzed the mean and individual  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  data with respect to time interval or zone. It should be noted that some of the time intervals or zones containing sirenian data are approximate and their exact ages as plotted represent intervals in which ages are estimated by stage of evolution biochronology within a particular NALMA. As such, these age determinations should be considered approximate (to within  $\pm 10^5$  to  $10^6$  years for the pre-Pleistocene data and  $10^5$  to  $10^4$  years for Pleistocene data). Also, there are two important intervals in which isotopic samples were rare and consequently not available for this study (ca. 30 to 20 Ma) or it seems that extinct sirenians were exceedingly rare in the Florida fossil record (ca. 5 to 2 Ma).

The tooth enamel carbon isotopic data for Eocene and Oligocene (before 30 Ma on Fig. 8) *Protosiren* yield relatively enriched  $\delta^{13}\text{C}$  values interpreted to represent a specialized seagrass diet. Likewise, most of the *Metaxytherium* specimens are relatively enriched in  $\delta^{13}\text{C}$ , also indicating a specialized seagrass diet, consistent with the previously observed morphological adaptation of a high degree of rostral deflection (e.g., Domning 1977, 2001b). Three specimens of *Metaxytherium* that are relatively negative in  $\delta^{13}\text{C}$  indicate individuals feeding on a principally  $\text{C}_3$  diet. A single specimen of *Corystosiren* with a  $\delta^{13}\text{C}$  value of  $5.6\text{‰}$  is the most enriched of all values analyzed in this study and likewise suggests a specialized seagrass diet. The mean  $\delta^{13}\text{C}$  values for fossil and extant wild *Trichechus* are more negative, and the individual data points have a broader range of  $\delta^{13}\text{C}$  values indicating a more diverse diet relative to *Protosiren*, *Metaxytherium*, or extant *Dugong*.

The tooth enamel oxygen isotopic data for Eocene and Oligocene (before 30 Ma on Fig. 9) *Protosiren* indicate relatively enriched  $\delta^{18}\text{O}$ , which, given the estimated values of global early Cenozoic seawater (Zachos et al. 2001), indicates that *Protosiren* was living principally in marine habitats. This also is the case for the

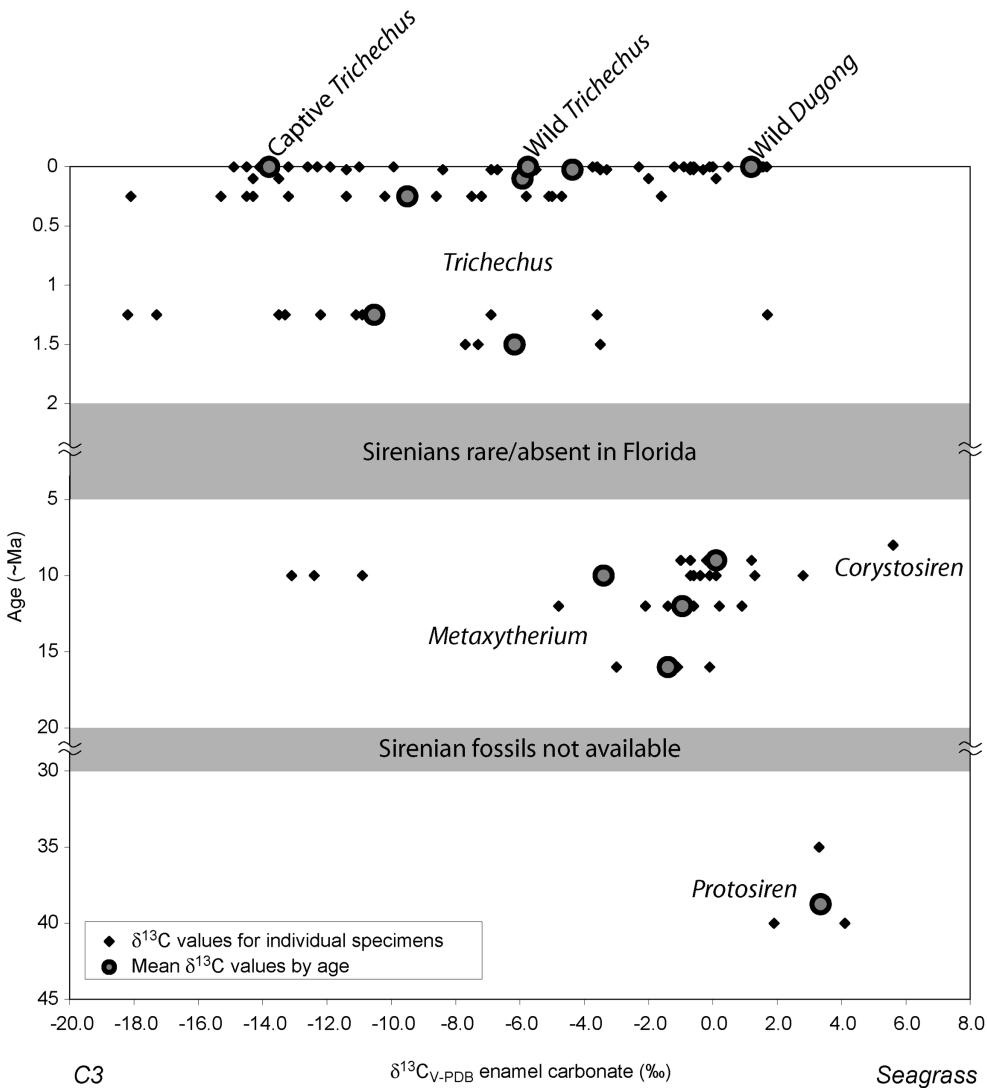


FIGURE 8. Plot of  $\delta^{13}\text{C}$  versus time for the bulk samples of Florida sirenians presented in this paper. This includes mean  $\delta^{13}\text{C}$  values (Table 4) for time horizons or intervals represented by large circles and individual  $\delta^{13}\text{C}$  data points presented (Appendix). Note the change in timescale and intervals for which samples were not available for analysis. A single mean value for age and  $\delta^{13}\text{C}$  has been calculated for all *Protosiren* analyses from the approximately 40 and 35 Ma levels.

Miocene (20 to 5 Ma interval) as represented by the majority of the *Metaxytherium* specimens analyzed. On the other hand, three specimens of *Metaxytherium* (different from the three outliers for the  $\delta^{13}\text{C}$  data described above) and the one analysis for *Corystosiren* are relatively depleted in  $\delta^{18}\text{O}$ . We interpret these four data points to represent individuals living in relatively freshwater environments (although it is possible that these could represent an artifact of diagenesis). For the Pleis-

tocene, as represented by *Trichechus*, the mean values at about 1.5, 1.25, 0.25, 0.1, and 0.025 Ma are slightly less positive than for the Eocene through Oligocene, but the variance around the mean is perhaps more informative and interesting. The broader spread of individual data points is interpreted as a diversification of habitats ranging from full marine to full freshwater (spread of approximately 8‰) for *Trichechus* relative to either *Protosiren* or *Metaxytherium*.

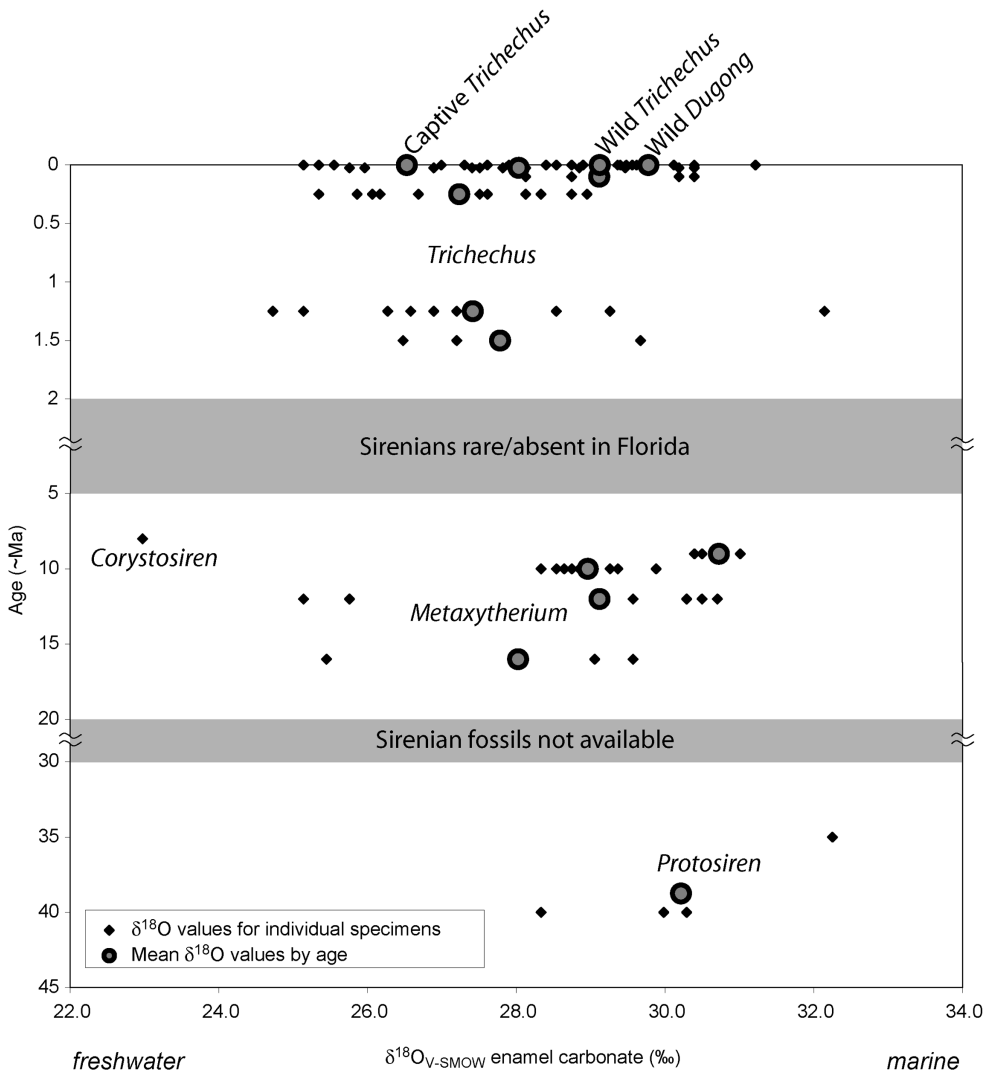


FIGURE 9. Plot of  $\delta^{18}\text{O}$  versus time for the bulk samples of Florida sirenians presented in this paper. This includes mean  $\delta^{18}\text{O}$  values (Table 4) for time horizons or intervals represented by large circles and individual  $\delta^{18}\text{O}$  data points presented (Appendix). Note the change in time scale and intervals for which samples were not available for analysis. A single mean value for age and  $\delta^{18}\text{O}$  has been calculated for all *Protosiren* analyses from the approximately 40 and 35 Ma levels.

**Concluding Remarks**

Fossil discoveries and morphological interpretations over the past several decades have done much to advance our understanding of fossil sirenians, including those from the rich Cenozoic sequence in Florida. The diet of extinct sirenians previously has been interpreted from morphological characters such as the degree of rostral deflection (e.g., Domning 2001a). The habitat preferences of extinct sirenians have been interpreted from associated fossils such as Eocene seagrasses from central

Florida (Ivany et al. 1990) and the presence of shallow marine or estuarine faunas. Stable isotopic analyses of tooth enamel add another line of evidence that allows further testing of these paleoecological hypotheses.

The isotopic data presented here (Table 4) for the Eocene to late Miocene *Protosiren* and dugongs (i.e., extinct species of the Family Dugongidae; Table 1) from Florida differ significantly from those of fossil and wild *Trichechus* for both  $\delta^{13}\text{C}$  ( $T$ -statistic = 5.7,  $T$ -critical = 1.7,  $p \ll 0.001$ ) and  $\delta^{18}\text{O}$  ( $T$ -statistic = 2.8,  $T$ -crit-

ical = 1.7,  $p = 0.006$ ). These isotopic data are interpreted to represent differences in the ecological adaptations of *Protosiren* and extinct Florida dugongs relative to extinct and extant manatees. As a general pattern, *Protosiren* and the extinct Florida dugongs had a more specialized diet of seagrass and a predominantly marine habitat. In contrast, fossil manatees and modern *Trichechus manatus* in Florida have been more generalized feeders, with a broader isotopic range representing diets of  $C_3$  plants to seagrasses. In general, the isotopic data reported here confirmed previous hypotheses based on morphological and associated faunal evidence. There were, however, a few surprises; e.g., *Metaxytherium crataegense* fed on seagrass despite a low degree of rostral deflection, and three specimens of *M. floridanum* principally fed on  $C_3$  plants.

The results presented here indicate that *Protosiren* and extinct dugongs from Florida were relatively conservative in their adaptations, maintaining diet and habitat preferences similar to those of modern *Dugong dugon*. In contrast, *Trichechus* has been considerably more generalized both in diet and habitat preference since it first appeared in Florida about 2 Ma. Both dugongs and manatees have filled the "aquatic megaherbivore niche" (Domning 2001a) or paleoguild (sensu van Valkenburgh 1995). It therefore has been interesting to speculate whether these two sirenian groups competed, or whether the manatees filled an "empty" niche created by the extinction of the late Miocene dugongs. Although the fossils of sirenians in Florida are abundant, the critical time interval, i.e., late Miocene to Pliocene (ca. 10 to 3 Ma) is poorly represented and therefore does not allow testing of the competition versus replacement hypotheses for these aquatic megaherbivores. We suspect, however, that this niche was vacated by dugongs and then filled several million years later by manatees. Our reasons for this relate to the fact that Hemphillian marine/terrestrial mammalian faunas are very well represented in the Florida sequence, including those of the Upper Bone Valley (e.g., Palmetto Fauna [Morgan 1994]). These faunas contain both diagnostic Hemphillian land mammals and a diverse marine mammal fauna (Morgan 1994) but are

conspicuous in their paucity of sirenians. This evidence suggests that other than a few rare occurrences (*Corystosiren varguezii* and undescribed dugongids, sensu Domning 2001a and Hulbert 2001), dugongids had essentially become extinct in Florida by about 5 Ma (i.e., the age of the Upper Bone Valley Formation).

Despite the rich fossil record of sirenians in Florida, it also is unfortunately inadequate during other critical time intervals. The maximum diversity of dugongs in Florida occurred during the late Oligocene and early Miocene with three coexisting taxa, *Metaxytherium* sp., *Crenatosiren olseni*, and *Dioplotherium manigaulti*, as represented at localities such as White Springs in northern Florida (Domning 1989a). Although all three species are considered to have fed principally on seagrasses, judging from degree of rostral deflection, tusk morphology, and estimated body size, Domning (2001a) interprets some niche differentiation for *Metaxytherium* sp., *C. olseni*, and *D. manigaulti*. With regard to diet, these specializations pertain to the size of the seagrass rhizome upon which each dugong species would have fed. If specimens were available for these extinct dugongs, stable carbon isotopes could confirm that they fed upon seagrasses but would not be able to further resolve the hypotheses about specific specialization on different-sized rhizomes.

After dugongids became rare in the late Miocene, there is a roughly 3-Myr gap (ca. 5 to 2 Ma) in which sirenians are virtually absent in Florida, despite the presence of appropriate-aged (Blancan) faunas containing aquatic elements. The available fossil evidence indicates that primitive trichechids originated in the New World, with fossil occurrences of *Potamosiren magdalensis* from the late Miocene La Venta Fauna of Colombia and *Rhibodon* from the Pliocene of Argentina and North Carolina (Domning 1982, 1997b). By the time that *Trichechus* first appears in the Florida sequence by the early Pleistocene, it is similar to *T. manatus*, although Domning (1982) considers the fossil manatee a different, heretofore undescribed species.

The stable isotopic analyses were successful in reconstructing the paleobiology of Florida sirenians. As a modern baseline, studies of

captive *Trichechus manatus* were important in establishing a carbon isotopic enrichment value ( $\epsilon^*$ ) of 14.0‰, essentially the same calculated for medium to large terrestrial mammalian herbivores (Cerling and Harris 1999). Likewise, the extant sample of Australian *Dugong dugon* formed a baseline for comparison with extinct Florida dugongs. Carbon isotopic analyses were useful in interpreting the ancient diets of extinct sirenians, as described above. In addition, although there is a complex isotopic source, which is dependent upon several environmental and physiological factors, the resulting  $\delta^{18}\text{O}$  analyzed in tooth enamel can be used to discriminate habitat preferences. For Florida sirenians,  $\delta^{18}\text{O}$  values range from relatively freshwater (depleted  $\delta^{18}\text{O}$ ), representing inland rivers and springs, to full marine (enriched  $\delta^{18}\text{O}$ ), representing shallow, coastal habitats.

Although fossils provide unparalleled clues to ancient life, the fossil record is far from complete, and new discoveries can always advance our understanding of any group. Such is the case with fossil sirenians from Florida. Additional specimens of the late Oligocene–early Miocene diversity, better knowledge of the rare, late-surviving Mio-Pliocene dugongs, and discovery of in situ Pliocene trichechids would all go a long way toward advancing our understanding of the phylogenetic history and paleobiogeography of this interesting group. In addition, the application of stable isotopic analyses of tooth enamel carbonate could be used in conjunction with these future discoveries to further refine our understanding of the diets and habitat preferences of the extinct Sirenia of Florida.

### Acknowledgments

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## Appendix 1

Taxonomic, locality, age, and isotopic data for the tooth enamel carbonate bulk samples of Cenozoic Florida sirenians analyzed during the present study. Ages, in million years (Ma), are approximate mid-points within given intervals. These mid-point ages are not to be taken as precise ages for each specimen within particular interval. Abbreviations: JW, private collection of John Waldrop, Lake Wales, Florida; FGS-V, Florida Geological Survey Collection, now part of UF; UF, University of Florida Vertebrate Paleontology Collection; UF-M; UF Mammal Collection. Following Hoefs (1997),  $\delta^{18}\text{O}$  data calibrated to V-PDB were corrected to V-SMOW (presented below) as follows:  $\delta^{18}\text{O}_{\text{V-SMOW}} = 1.03091 (\delta^{18}\text{O}_{\text{V-PDB}}) + 3(.91)$ .

Museum#	Taxon	Tooth	Locality	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)
<i>Middle Eocene, Clairbornian, ca. 40 Ma</i>					
UF 24806	<i>Protosiren</i> sp.	LM?	Waccasassa River	4.1	30.0
UF 115722	<i>Protosiren</i> sp.	rm3	Dolime Quarry	4.1	30.3
UF 25708	<i>Protosiren</i> sp.	M2 or M3	Waccasassa River	1.9	28.3
<i>Early Oligocene, ca. 35 Ma</i>					
UF uncataloged	cf. <i>Protosiren</i> sp.	tooth frag	Terra Mar	3.3	32.3
<i>Middle Miocene, Barstovian, ca. 16 Ma</i>					
UF 182432	<i>Metaxytherium crataegense</i>	molar	La Camelia	-0.1	29.6
UF 182433	<i>Metaxytherium crataegense</i>	molar	La Camelia	-1.1	29.1
UF 36446	<i>Metaxytherium crataegense</i>	molar	Bone Valley, Palmetto	-3.0	25.4
<i>Middle Miocene, early Clarendonian, ca. 12 Ma</i>					
FGS V-3512	<i>Metaxytherium floridanum</i>	partial tooth	Bone Valley, Agricola Fauna	-0.6	30.3
FGS V-10056	<i>Metaxytherium floridanum</i>	RM3 frag	Bone Valley, Agricola Fauna	0.2	30.5
UF 28829	<i>Metaxytherium floridanum</i>	cheek tooth	Bone Valley, Agricola Fauna	-0.7	30.5
UF 28833	<i>Metaxytherium floridanum</i>	molar frag	Bone Valley, Agricola Fauna	0.9	30.7
UF 206653	<i>Metaxytherium floridanum</i>	molar frag	Bone Valley-4 corners-0	-4.8	25.1
UF 206654	<i>Metaxytherium floridanum</i>	RM3	Bone Valley-4 corners-0	-2.1	29.6
UF 206655	<i>Metaxytherium floridanum</i>	RM3	Bone Valley-IMC-Hopewell	-1.4	25.8
UF uncataloged	<i>Metaxytherium floridanum</i>	molar frag	Bone Valley, Agricola Fauna	0.9	30.5
<i>Late Miocene, Clarendonian/early Hemphillian, ca. 10 Ma</i>					
UF 28816	<i>Metaxytherium floridanum</i>	molar	Bone Valley, Tiger Bay-1	-0.4	29.9
JW F7502	<i>Metaxytherium floridanum</i>	molar	Bone Valley-Kingsford-0	-0.1	28.7
JW F7502	<i>Metaxytherium floridanum</i>	molar	Bone Valley-Kingsford-0	-10.9	29.3
JW F6912	<i>Metaxytherium floridanum</i>	molar	Bone Valley-Kingsford-0	1.3	29.4
JW F6914	<i>Metaxytherium floridanum</i>	molar	Bone Valley-Kingsford-0	2.8	28.5
JW F7502A	<i>Metaxytherium floridanum</i>	molar	Bone Valley-Kingsford-0	0.1	28.7
JW F6914	<i>Metaxytherium floridanum</i>	molar	Bone Valley-Kingsford-0	-0.6	28.6
JW F7407E	<i>Metaxytherium floridanum</i>	molar	Bone Valley, Tiger Bay-0	-12.4	28.8
JW F7405A1	<i>Metaxytherium floridanum</i>	molar	Bone Valley, Phosphoria-0	-0.7	28.3
JW F7405A2	<i>Metaxytherium floridanum</i>	molar	Bone Valley, Phosphoria-0	-13.1	29.3
<i>Late Miocene, late Clarendonian, ca. 9 Ma</i>					
UF 97338	<i>Metaxytherium</i> cf. <i>floridanum</i>	partial tooth	Gainesville creeks	1.2	30.4
UF 115953	<i>Metaxytherium</i> cf. <i>floridanum</i>	partial molar	Gainesville creeks	1.2	30.7
UF 8023	<i>Metaxytherium</i> cf. <i>floridanum</i>	molar	Gainesville creeks	-0.7	30.5
UF 58098	<i>Metaxytherium</i> cf. <i>floridanum</i>	partial tooth	Gainesville creeks	-1.0	31.0
UF uncataloged	<i>Metaxytherium</i> cf. <i>floridanum</i>	molar	Gainesville creeks	-0.2	31.0

## Appendix 1. Continued.

Museum#	Taxon	Tooth	Locality	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)
<i>Late Miocene, early Hemphillian, ca. 8 Ma</i>					
UF 18826	<i>Cystosiren varguezii</i>	incisor	Waccasassa River	5.6	23.0
<i>Early Pleistocene, Irvingtonian, 1.5 Ma</i>					
UF 156878	<i>Trichechus</i> sp.	lm?	Leisey Shell Pit	-7.3	29.7
UF 87227	<i>Trichechus</i> sp.	lm?	Leisey Shell Pit	-3.5	27.2
UF 93273	<i>Trichechus</i> sp.	lm?	Leisey Shell Pit	-7.7	26.5
<i>Late Pliocene–Pleistocene, late Blancan–Rancholabrean, mixed, ca. 1.25 Ma</i>					
UF 182425	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 1B	-3.6	32.1
UF 182426	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 1B	-10.9	26.6
UF 182427	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 1B	-18.2	26.3
UF 182428	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 1B	-12.2	27.4
UF 182429	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 1B	-17.3	26.9
UF 182430	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 1B	-11.1	27.2
UF 182431	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 1B	-6.9	28.5
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 1A	1.7	29.3
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 1A	-13.5	25.1
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 1A	-13.3	24.7
<i>Late Pleistocene, middle Rancholabrean, ca. 0.25 Ma</i>					
UF 10710	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 1	-14.3	27.6
UF 10710	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 1	-7.5	29.0
UF 182418	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 2	-11.4	27.3
UF 182419	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 2	-5.1	28.1
UF 182420	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 2	-1.6	28.7
UF 182421	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 2	-10.2	27.5
UF 182422	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 2	-4.7	28.1
UF 182423	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 2	-7.2	27.6
UF 182424	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 2	-5.8	28.3
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River 4A	-13.2	26.7
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River	-18.1	26.1
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River	-15.3	26.2
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River	-14.5	26.1
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River	-5.0	25.9
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Santa Fe River	-8.6	25.3
<i>Late Pleistocene, Rancholabrean ("Sangamonian"), ca. 0.1 Ma</i>					
UF/FGS V-4404	<i>Trichechus</i> sp.	cheek tooth	Rock Springs LF	0.1	28.7
UF/FGS V-4409	<i>Trichechus</i> sp.	cheek tooth	Rock Springs LF	0.1	30.2
UF/FGS V-4451a	<i>Trichechus</i> sp.	cheek tooth	Rock Springs LF	-14.3	28.1
UF/FGS V-4451b	<i>Trichechus</i> sp.	cheek tooth	Rock Springs LF	-13.5	28.1
UF 48982	<i>Trichechus</i> sp.	cheek tooth	Rock Springs LF	-2.0	30.4

## Appendix 1. Continued.

Museum#	Taxon	Tooth	Locality	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)
<i>Late Pleistocene, late Rancholabrean, ca. 0.025 Ma</i>					
UF 182412	<i>Trichechus</i> sp.	cheek tooth	Oklawaha River 1	-5.5	27.4
UF 182413	<i>Trichechus</i> sp.	cheek tooth	Oklawaha River 1	-8.4	27.5
UF 182416	<i>Trichechus</i> sp.	cheek tooth	Oklawaha River 1	-4.4	30.2
UF 182417	<i>Trichechus</i> sp.	cheek tooth	Oklawaha River 1	-3.5	28.8
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Oklawaha River 1	-0.7	30.4
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Oklawaha River 1	-3.3	27.8
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Oklawaha River 1	-0.3	28.1
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Oklawaha River 1	-0.6	26.0
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Oklawaha River 1	-0.7	28.0
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Oklawaha River 1	-6.7	25.8
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Oklawaha River 1	-6.9	29.5
UF uncataloged	<i>Trichechus</i> sp.	cheek tooth	Oklawaha River 1	-11.4	26.9
<i>Recent (osteological and naturally shed from captive individuals), 0 Ma</i>					
UF M 15110	<i>Trichechus manatus</i>	cheek tooth	Brevard County	-3.6	29.4
UF M 15113	<i>Trichechus manatus</i>	cheek tooth	St. John's River	-0.9	31.2
UF M 15114	<i>Trichechus manatus</i>	cheek tooth	Brevard County	-0.1	30.4
UF M 15115	<i>Trichechus manatus</i>	cheek tooth	Duval County	-1.2	29.4
UF M 15120	<i>Trichechus manatus</i>	cheek tooth	Duval County	-0.7	29.7
UF M 15122	<i>Trichechus manatus</i>	cheek tooth	Volusia County	-11.0	27.3
UF M 15141	<i>Trichechus manatus</i>	cheek tooth	Brevard County	-3.7	28.9
UF M 15160	<i>Trichechus manatus</i>	cheek tooth	Lee County	-11.0	29.1
UF M 15172	<i>Trichechus manatus</i>	cheek tooth	Dade County	0.0	29.7
UF M 15173	<i>Trichechus manatus</i>	cheek tooth	Dade County	-5.9	29.4
UF M 15176	<i>Trichechus manatus</i>	cheek tooth	Dade County	-13.9	28.4
UF M 15195	<i>Trichechus manatus</i>	cheek tooth	Broward County	-11.9	28.5
UF M 15196	<i>Trichechus manatus</i>	cheek tooth	Dade County	0.5	29.5
UF M 15134	<i>Trichechus manatus</i>	cheek tooth	Collier County	-9.9	29.4
UF M 19135	<i>Trichechus manatus</i>	cheek tooth	Lee County	-4.2	29.4
UF M 20602	<i>Trichechus manatus</i>	cheek tooth	Brevard County	-2.3	28.7
UF M 20773	<i>Trichechus manatus</i>	cheek tooth	Lake County	-11.0	28.0
UF M 23993	<i>Trichechus manatus</i>	cheek tooth	Lake County	-12.6	27.9
Hugh or Buffett	<i>Trichechus manatus</i>	U cheek tooth	Captive	-12.3	28.5
Hugh or Buffett	<i>Trichechus manatus</i>	I cheek tooth	Captive	-14.9	25.3
Hugh or Buffett	<i>Trichechus manatus</i>	I cheek tooth	Captive	-13.8	25.1
Hugh or Buffett	<i>Trichechus manatus</i>	I cheek tooth	Captive	-14.1	25.5
Hugh or Buffett	<i>Trichechus manatus</i>	cheek tooth	Captive	-13.2	27.6
Snooty	<i>Trichechus manatus</i>	U cheek tooth	Captive	-14.5	27.0
MD-142	<i>Dugong dugon</i>	tusk	Australia	1.3	29.6
MD-19	<i>Dugong dugon</i>	tusk	Australia	-0.6	29.8
MD-146	<i>Dugong dugon</i>	tusk	Australia	1.7	29.9
MD-16	<i>Dugong dugon</i>	tusk	Australia	1.6	30.1
MD-22	<i>Dugong dugon</i>	tusk	Australia	1.5	29.6
MD-116	<i>Dugong dugon</i>	tusk	Australia	1.7	29.8