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Additional Research and Taxonomic Resolution of Salamanders (Amphibia: Caudata) from the Mio-Pliocene Gray Fossil Site, TN

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Additional Research and Taxonomic Resolution of Salamanders (Amphibia: Caudata) from the Mio-Pliocene Gray Fossil Site, TN

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by
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Keywords: *Ambystoma*, Spelerpini, Gray Fossil Site, Miocene, Pliocene

ABSTRACT

Additional Research and Taxonomic Resolution of Salamanders (Amphibia: Caudata) from the Mio-Pliocene Gray Fossil Site, TN

by

Hannah E. Darcy

The Gray Fossil Site (GFS), a Mio-Pliocene (4.5 – 7 Ma) locality in the southern Appalachians, boasts the most diverse pre-Pleistocene salamander fauna in North America: *Desmognathus* sp., *Plethodon* sp., *Notophthalmus* sp., a Spelerpinae-type plethodontid, and *Ambystoma* sp. Because greater taxonomic resolution can result in more precise paleobiological interpretations, additional salamander specimens, including cranial bones, were studied here. ETMNH 8045 is a nearly complete articulated ambystomatid that appears most like *Ambystoma maculatum* on the basis of single-row dentition, vomerine diastema, and vertebral proportions. ETMNH 18219 is an isolated vomer most similar to those seen in Plethodontidae and Rhyacotritonidae. The extent of the dentigerous row and the presence of a postdentigerous process are consistent with modern *Pseudotriton* and *Gyrinophilus*. If these taxa, or species of similar ecolocical preferences, occurred around the site, it seems unlikely that they co-inhabited the sinkhole lake that formed the Gray Fossil Site. *Pseudotritoin* and terrestrial *Gyrinophilus* require years to complete the aquatic larval stage; presence could further support the perennial lake hypothesis. Modern *A. maculatum* breed preferentially in vernal pools, and confirmation of this species could suggest seasonal wetlands in the area.

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CHAPTER 1

INTRODUCTION

Salamanders belong to the order Caudata, one of three extant orders of the class Amphibia. Caudates differ from the orders Anura (Frogs and Toads) and Gymnophiona (Caecilians) in having well-developed tails as adults (Duellman and Trueb 1994). All salamanders have permeable skin and require habitats with high humidity or moisture as well as moderate temperature to maintain body temperature and hydration (Duellman and Trueb 1994). Environmental requirements are more extreme in taxa that respirate exclusively subcutaneously (such as members of the family Plethodontidae, the Lungless Salamanders). Salamanders are the only tetrapods with lungless representatives; the order is also unique in exhibiting obligate neotenic species (reaching sexual maturity while retaining larval characteristics) (Trueb 1993). Currently ten extant caudate families are recognized: Hynobiidae (Asiatic Salamanders), Cryptobranchidae (Hellbenders and Giant Salamanders), Salamandridae (Newts), Plethodontidae (Lungless Salamanders), Rhyacotritonidae (Torrent Salamanders), Amphiumidae (Congo Eels), Ambystomatidae (Mole Salamanders), Proteidae (Mudpuppies, Waterdogs, and Olms), Dicamptodontidae (Pacific Giant Salamanders), and Sirenidae (Sirens) (Frost et al. 2006). In addition, two extinct salamander families are known from the North American fossil record: Scapherpetontidae (Late Cretaceous to Early Eocene) and Batrachosauroididae (Late Cretaceous to Late Miocene) (Holman 2006). Rhyacotritonidae is the only known salamander family that does not have fossil representatives (Holman 2006).

Salamanders are the least well-known of the three extant amphibian orders in terms of cranial diversity and development (Trueb 1993). Due to the variety of life histories occurring in this order, few unifying cranial characters are identified. Four of the nine families are comprised exclusive of taxa that retain larval characters as adults: Sirenidae, Amphiumidae, Proteidae, and Cryptobranchidae, while some members of Plethodontidae, Ambystomatidae, and Dicamptodontidae are obligate paedomorphic (Trueb 1993). *Ambystoma tigrinum,* a facultative neonate, occasionally presents an aberrant cranial morphology associated with a canabalistic lifestyle (Pedersen 1993). Only Hynobiidae and Salamandridae lack obligate paedomorphs, though facultative neoteny is known in some populations of salamandrids (Trueb 1993).

In general, caudate skulls are characterized by an open temporal region, large orbit lacking a posterior margin, absence of a cheek, and incomplete upper jaw (Trueb 1993). Salamanders are the only amphibians exhibiting a four-faceted articulation of the exoccipitals with the atlas (first cervical vertebra). They are additionally distinguished from anurans and Caecellians in having a jaw articulation lying well anterior to the posterior limit of the skull (Trueb 1993). Hilton produced some of the first osteological descriptions of ambystomatids, in addition to *Dicamptodon* (Hilton 1946), *Hydromantes* (Hilton 1945a), *Typhlomolge* (Hilton 1945b), and *Haideotriton* (Hilton 1945b; Hilton 1945c). Detailed reviews of cranial morphology include those of Wake and Özeti (1969) on salamandrids, Tihen (1958) on ambystomatids as well as *Rhyacotriton* and *Dicamptodon,* Wake (1966) on plethodontids, and Larsen (1963) on various neotenic and transforming taxa.

Five salamander families are known from the Mio-Pliocene (8 to 4 Ma) fossil record of North America, including the last known occurrence of the extinct Batrachosauroididae (Holman 2006). Ambystomatidae is well represented, including the extinct species: *Ambystoma kansense*, *A. hibbardi*, *A. minshalli,* and *A. priscum,* as well as the extant species *A. maculatum* and *A. tigrinum* (Holman 2006). An atlas and a trunk vertebra of *Peratosauroides problematica,* the last known species of Batrachosauroididae, was found in California (Naylor 1981). Several genera of

Plethodontidae are represented in the Mio-Pliocene: *Aneides* and *Batrachoseps* from California (Clark 1985; Holman 2006), a *Plethodon*-like plethodontid from Texas (Parmley 1989); an unidentified plethodontid from the Pipe Creek Sinkhole in Indiana (Farlow et al. 2001); and *Desmognathus* sp., two morphotypes of *Plethodon* sp., and an unidentified member of the subfamily Spelerpinae from the Gray Fossil Site (GFS) in Tennessee (Boardman and Schubert 2011). Salamandridae is represented by *Notophthalmus* sp. vertebrae at the GFS (Boardman and Schubert 2011) and *Taricha* sp. trackways in Kansas (Peabody 1959). Florida has produced two modern genera of Sirenidae, *Siren* and *Pseudobranchus* (Estes 1981; Holman 2006).

The Gray Fossil Site in northeastern Tennessee (Fig. 1.1) has yielded the most diverse pre-Pleistocene salamander fauna of North America. In their review of salamander vertebrae from the GFS, Boardman and Schubert (2011) identified *Ambystoma* sp. (both adult and neotenic individuals), *Notophthalmus* sp., *Desmognathus* sp., a *Spelerpinae*-type plethodontid, and two forms of a *Plethodon*-type plethodontid. Their findings present the earliest record of Plethodontidae and Ambystomatidae east of the Mississippi River, the first fossil record of *Desmognathus,* and the only North American Mio-Pliocene body fossil of a salamandrid. A wooded-pond environment interpretation of the GFS is supported by this assemblage. In the nearly 15 years since its discovery, the GFS has yielded an extraordinary diversity of taxa and has been proposed as a Lagerstätten (Wallace et al. 2014). Age constraints of the rhino *Teleoceras* and the short-faced bear *Plionarctos* date the GFS to approximately 4.5 – 7 Ma, or latest Miocene – early Pliocene (Wallace and Wang 2004), and is consistent with a Late Hemphillian North American Land Mammal Age fauna (Parmalee et al. 2002). During a period of expanding grasslands over much of the mid-continent, the GFS presents a unique opportunity to study what appears to be a forest refugium (DeSantis and Wallace 2008). Additionally, the GFS is the only Mio-Pliocene fossil site in the southern Appalachians.

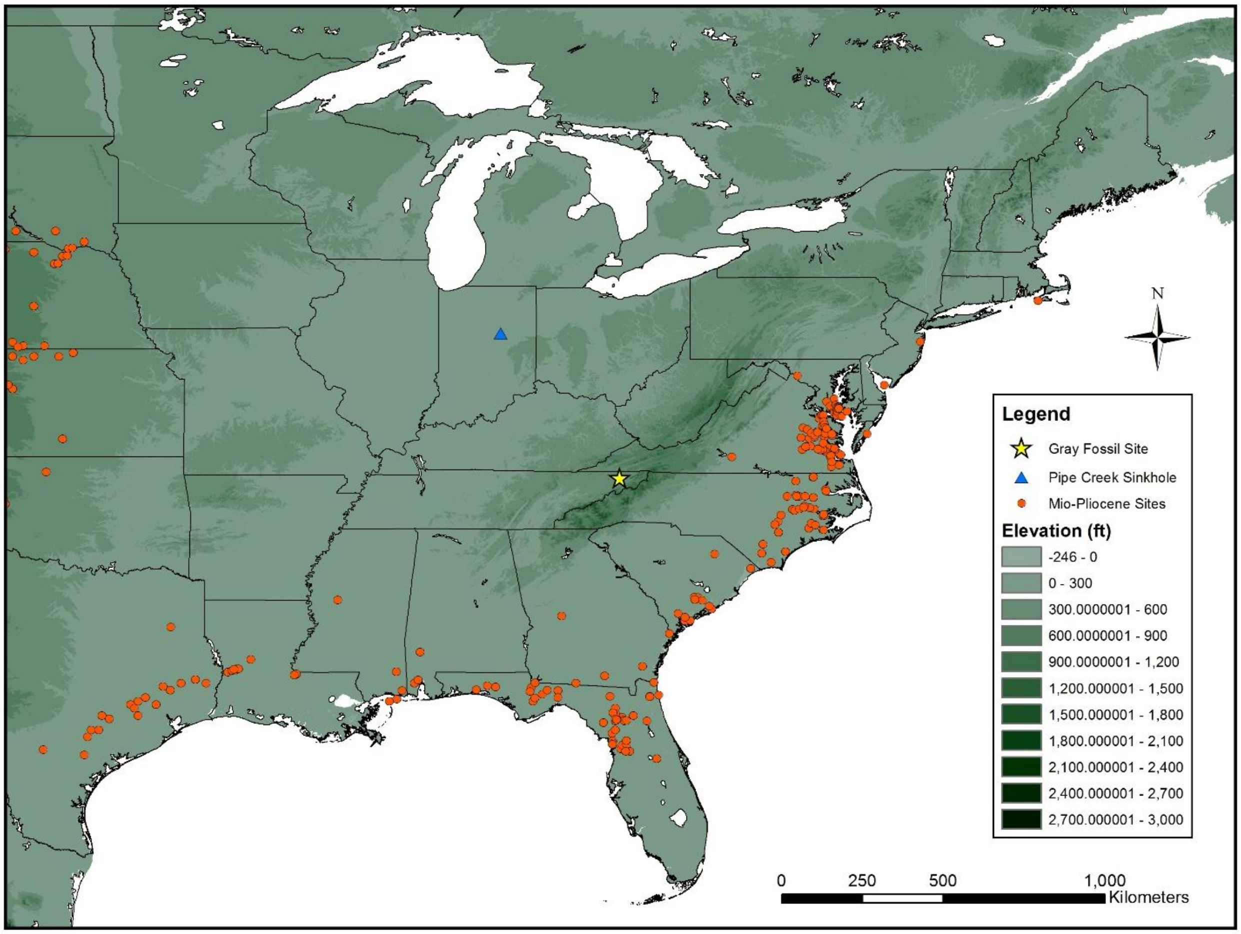

Figure 1.1. Mio-Pliocene (4 – 8 Ma) fossil sites of the eastern United States, including marine and terrestrial sites. The Gray Fossil Site (yellow star) and the Pipe Creek Sinkhole (blue triangle) are the only inland Mio-Pliocene sites in eastern North America. Modified from Peters and McClennen (2015).

While vertebrae are the most commonly recovered salamander fossil due to their relative robustness, identification is usually limited to the generic level due to a lack of distinguishing interspecific characteristics (Wake 1966; Holman 2006). Claims of species level identification must be regarded with healthy skepticism due to the historical trend among paleoherpetologists to consider only locally-occurring species in their identification of lower vertebrates (Bell et al. 2010). Even generic level identifications are not always feasible on the basis of trunk vertebrae, as is the case in the Plethodontidae subfamily Hemidactyliinae (see Boardman and Schubert 2011). In contrast, vomerine morphology provides generic resolution of Hemidactyliinae, including species level identification of *Gyrinophilus porphyriticus*, *Stereochilus marginatus*, and *Eurycea spelaea* (Chapter 3; Wake 1966).

Other cranial elements potentially hold taxonomic value, and with improvements in microfossil screening it is becoming increasingly important for paleontologists to recognize non-dentigerous, non-mammalian elements (Bell and Mead 2014). Quadrate morphology has proven useful in squamate taxonomy (Evans 2008). However, *Triturus cristatus*, a salamander superspecies, has a variable quadrate (Ivanovic et al. 2008), and allopatric populations of *Plethodon cinereus* utilizing different prey sources display differences in the posterior region of the skull (Maerz et al. 2006). Sympatric *Plethodon hoffmani* and *P. cinereus* have the most pronounced differences in their squamosal length to dentary length ratio, due to the squamosal's role in the jaw-closing musculature (Adams and Rohlf 2000). Within Spelerpini, burrowing salamanders including *Gyrinophilus porphyriticus*, *Pseudotriton montanus*, and *Pseudotrion ruber* have more robust snouts than species of the genus *Eurycea*, which prey on surface insects and have more gracile skulls (Martof and Rose 1962). *Gyrinophilus porphyriticus* can be distinguished from *Pseudotriton* species by the fusion of the premaxilla as well as having more elongate nasals (Martof and Rose 1962). Species of *Ambystoma* differ in parasphenoid morphology (Tihen 1958).

Previous identifications of GFS salamanders were made utilizing vertebrae (Boardman and Schubert 2011). Multiple species of mole salamanders coexist today throughout much of the eastern United States (Duellman and Sweet 1999) and multiple species could be represented. In the current study, a nearly articulated specimen of *Ambystoma*, including cranial material, provides greater resolution of at least one *Ambystoma* specimen. Additionally, an isolated vomer indicates the presence of *Gyrinophilus* (or a closely related form) at the site. Both specimens provide insight on the potential paleoecology of the GFS.

CHAPTER 2

AN ARTICULATED *AMBYSTOMA* FROM THE MIO-PLIOCENE GRAY FOSSIL SITE, TENNESSEE

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Abstract

The Gray Fossil Site (GFS), a Mio-Pliocene (4.5 – 7 Ma) locality in the southern Appalachians, boasts the most diverse pre-Pleistocene salamander fauna in North America including representatives of three families: Plethodontidae (*Desmognathus* sp., *Plethodon* sp., and a Spelerpinae-type plethodontid), Salamandridae (*Notophthlamus* sp.), and Ambystomatidae (*Ambystoma* sp., both neotenic and terrestrial). All previous records of GFS salamanders are isolated vertebrae. Here, a nearly-complete articulated *Ambystoma* specimen is presented. Cranial characters (including dentition) and vertebral proportions are utilized in identification. The specimen appears most like the modern species *Ambystoma maculatum*. The GFS is interpreted as a permanent pond due to the presence of *Alligator* sp., large bodied *Rana* sp., and neotenic *Ambystoma* sp.; however, modern *A. maculatum* preferentially breed in vernal pools and wetlands. Confirmation of the articulated specimen as *A. maculatum* could suggest seasonal wetlands in the region, in addition to the permanent pond.

Keywords: *Ambystoma*, Miocene, Pliocene, Crania, Paleoecology

1. Introduction

Ambystomatidae (Amphibia, Caudata) is a monogeneric family of mole salamanders (*Ambystoma*) with a wide distribution in North and Central America (Campbell, 1999; Duellman and Sweet, 1999). Mole salamanders rarely occur beyond regions receiving less than 500 mm annual precipitation (Duellman and Sweet, 1999). Many species of *Ambystoma*, particularly the wide-spread *A. tigrinum*, display the ability to remain neotenic when environmental factors necessitate, and a few are obligate neonates. Neotenic individuals retain larval characteristics including external gills and finned tailed at sexual maturity. Ambiguity may exist as to whether a species is an obligate neonate or that only neotenic specimens have been recovered, such as the fossil species *A. kansense* (Holman, 2006). Here is discussed a fossilized articulated specimen from eastern Tennessee.

When transformed, adults present the following characters that unify the family: prootic and exoccipital fused; stapes present, often fused to skull; lateral wall of nasal capsule incomplete; lateral narial fenestra present; posterior wall of nasal capsule complete; septomaxilla present; naso-lacrimal duct present; nasals present, not articulating medially; prefrontals present; lacrimal absent; premaxillae separate, with pars dorsalis long and separates nasals; quadratojugal absent; angular fused with prearticular; coronoid absent; pterygoid present; palatopterygoid and metapterygoid absent; basitrabecular process present; hyobranchial I and ceratobranchial I separate; ceratobranchial II absent; dentition pedicellate (Trueb, 1993).

1.1 Systematics

Historically, *Ambystoma* species were split among three subgenera, *Ambystoma*, *Linguaelapsus*, and *Bathysiredon*, as well as the genus *Rhyacosiredon* (Tihen, 1958). Thirty-two extant species of *Ambystoma* are currently recognized (Amphibia Web, 2015 http://amphibiaweb.org), grouped into the two subgenera, *Ambystoma* and *Linguaelapsus* (Beneski and Larsen, 1989). *Ambystoma* (*Linguaelapsus*) consists of *A. annulatum, A. cingulatum*, *A. mabeei,* and *A. texanum*. The remaining species belong to the subgenus *Ambystoma*. The monophyly of *Linguaelapsus* is supported by osteology, but is questionable when examined molecularly (Shaffer, Clark, and Kraus, 1991). A former genus accommodating the species *Rhyacosiredon rivularis* has since been synonomized with *Ambystoma*, and the species renamed *Ambystoma rivulare*. (Reilly and Brandon, 1994).

Less formal groupings of *Ambystoma* species were created by Tihen (1958) to describe vertebral proportions (Table 1). The "*A. mexicanum* group" includes extant species *A. mexicanum* and *A. lermaensis* as well as the extinct *A. kansense*. The "*A. tigrinum* group" includes living species *A. tigrinum, A. amblycephalum, A. bombypellum, A. granulosum, A. hibbardi*, *A. ordinarium, A. rosaceum*, and *A. velasci*, and the extinct *A. hibbardi*. The "*A. opacum* group" includes *A. opacum*, *A. talpoideum,* and the extinct *A. tiheni*. The "*A. maculatum* group" includes *A. maculatum*, *A. jeffersonianum*, *A. laterale*, *A. gracile, A. macrodactylum,* and the extinct species *A. minshalli* and *A. priscum*. *Linguaelapsus* species can be placed into two groups based on vertebral proportions: one consisting of *A. mabeei* and *A. annulatum* and another consisting of *A. texanum* and *A. cingulatum*. Most of these groupings do not agree with modern genetic phylogenies (Schaffer, Clark, and Kraus, 1991). *Ambystoma talpoideum* and *A. gracile* are alternatingly placed as the most basal ambystomatid (Kraus, 1988; Schaffer, Clark, and Kraus, 1991).

Table 1. Vertebral ratios of *Ambystoma* forms. After Tihen (1958: Table 1, p. 19).

Form	Ratio of centrum length to centrum width at anterior end	Ratio of combined zygapophyseal width to zygapophyseal length
A. mexicanum group	1.9-2.2	1.3-1.6
A. tigrinum group	1.8-2.3	1.3-1.7
A. opacum group	2.0-2.6	1.3-1.5
A. maculatum group	2.2-2.9	1.1-1.4
A. mabeei and *A. annulatum*	2.3-2.7	1.0-1.3
A. texanum and *A. cingulatum*	1.9-2.3	1.0-1.3

Tihen (1958) separated his "*A. mexicanum* group" from the species of the "*A. tigrinum* group" on the basis of the former's obligate paedomorphosis; however, both groups are "virtually indistinguishable morphologically." A group consisting of *Ambystoma tigrinum,* its species complex members, and its closest relatives retains monophyly (Shaffer, Clark, and Kraus, 1991; Shaffer and McKnight, 1996), and will be referred to as the "*A. tigrinum* + *A. mexicanum* group" throughout this work. Tihen notes the following characters of his "*A. tigrinum* group": trunk vertebrae are relatively short and broad; premaxillary spines tend towards short and broad; parasphenoid typically straight-sided, with only slightly concave sides, posterior expansion reduced or absent; diastema between vomerine and palatal teeth absent; vomer lacks postdentigerous process; choanae without lateral bony border; os triangulare tend to be longitudinal. Holman (2006) adds that the vomerine tooth series lacks a diastema at the level of the choana.

Additionally, Tihen (1958) included the subgenus *Linguaelapsus* within *Ambystoma*, which will be referred to here as the "*Linguaelapsus* group." This group consists of *Ambystoma texanum, A. barbouri, A. annulatum, A. cingulatum,* and *A. bishopi,* as well as the extinct *Ambystoma schmidti,* and *A. hibbardi* (Tihen, 1958; Shaffer, Clark, and Kraus, 1991; Holman, 2006). As defined here, the "*Linguaelapsus* group" is united by the following characters: premaxillary spines typically long and narrow, with ventral lamina or thickening above dentigerous ramus; tongue with plicae branching from median groove; polystichous tooth arrangement (multiple tooth rows) on all dentigerous elements; palatal teeth lacking; annular otoglossal cartilage absent; dentary of adults lacks promiment lingual flange; 13 to 15 costal grooves (Tihen, 1958). *Ambystoma mabeei* is excluded due to the very limited development of the premaxillary ventral lamina, monostichous tooth arrangement, and presence of palatal teeth; additionally, it has vertebral proportions more similar to those seen in the "*A. maculatum* group" (Tihen, 1958). The "*Linguaelapsus* group" is poorly supported by combined morphological and genetic data, and is rejected by purely genetic data (Schaffer, Clark, and Kraus, 1991). Despite its poor support, this group is retained as a shorthand for *Ambystoma* species with polystichous tooth arrangements on all dentigerous elements (Tihen, 1958).

Holman (2006) allied the extant forms *Ambystoma minshalli* and *A. priscum* with Tihen's (1958) "*A. maculatum* group." *Ambystoma minshalli* has an extensively developed flange or crest, continuous with the spine, along the posterodorsal surface of the tibia; posterior zygapophyses always extend farther posteriorly than the neural spine; odontoid process of atlas somewhat narrower than most other *Ambystoma* (Holman, 2006). *Ambystoma priscum* trunk vertebrae have a deeply notched and posteriorly produced posterior end of the neural arch; dorsal border of neural arch very straight (Holman, 2006). Among the living members of the group, *A. laterale*, *A. jeffersonianum*, *A. gracile*, and *A. maculatum* share the following traits: vertebrae elongate; premaxillary spines longer and narrower than in "*A. tigrinum* group"; parasphenoid sides concave, definite alate expansion posteriorly; diastema between vomerine and palatal teeth wide, occasionally lacking in *A. maculatum*; vomer lacks postdentigerous process; choannae with partial lateral bony border; os triangulare tending to be transverse (Tihen, 1958). *Ambystoma gracile* trunk vertebrae possess a neural arch that extends posteriorly past the postzygapophyses, while the neural arch ends anterior to the posterior extent of the postzygapophyses in *A. laterale*, and *A. jeffersonianum*. *Ambystoma maculatum* has a variable neural arch length, and the relative position of the terminal end of the neural arch to the postzygapophyses can vary in a single individual (ETVP 7196, FB 1483). *Ambystoma laterale* and *A. jeffersonianum* are morphologically indistinguishable, with a postzygapophyseal area relatively narrower than in *A. maculatum* (Holman, 2006).

Tihen (1958) established an "*Ambystoma opacum* group" consisting of *A. opacum* and *A. talpoideum* on the basis of parasphenoid with concave sides and alate expansion posteriorly; diastema between vomerine and palatal tooth series; vomer lacks postdentigerous process; choannae with partial lateral bony border; os triangulare tending to be transverse; premaxillae bear greater resemblance to those of "*A. tigirinum* group" than to "*A. maculatum* group"; vertebral proportions intermediate between "*A. tigrinum* group" and "*A. maculatum* group." Additionally, *A. opacum* and *A. talpoideum* are unique among *Ambystoma species* in possessing an epipleural process on the first rib (Kraus, 1988). *Ambystoma opacum* and *A. talpoideum* vary in vertebral proportions, with the postzygapophyses of *A. opacum* reaching further beyond the end of the neural spine than in *A. talpoideum*. Holman (2006) allied the extinct *A. tiheni* with the group, but with neural arch more depressed than in *A. opacum* and *A. talpoideum*; foramina on ventral surface of centrum obsolete or absent; end of centrum less widely flared; transverse processes usually more robust.

2. Paleontological Background

2.1 Mio-Pliocene Record of *Ambystoma*

Fossil localities Miocene in age or younger have produced the majority of mole salamander fossils (Holman, 2006). *Ambystoma tiheni*, an extinct species from the Late Eocene of Saskatchewan, Canada, is the only exception (Holman, 1968). Two extinct ambystomatids are known from the Miocene: *A. minshalli* and *A. kansense*. *Ambystoma minshalli* is reported from the Middle to Late Miocene (medial Barstovian NALMA, late Barstovian NALMA, and medial Hemphillian NALMA) (Holman, 2006). *Ambystoma kansense* is an extinct species from the Late Miocene, Hemphillian NALMA, of Kansas (Estes, 1981). Among known extant species, *A. maculatum* is identified from the Late Miocene (Clarendonian NALMA) of Kansas (Holman, 1975). *A. tigrinum* has been indentified from the Late Miocene (Clarendonian) of Nebraska (Voorhies, 1990) and Kansas (Holman, 1975). The Pliocene record includes one extinct ambystomatid species, *A. hibbardi*, from Kansas (Tihen, 1955). Pliocene *Ambystoma tigrinum* have been recorded from Texas, Nebraska, Kansas, Arizona, Idaho, and New Mexico (Holman, 2006). *Ambystoma opacum* is recorded from the Blancan NALMA of Texas (Rogers, 1976), and *Ambystoma maculatum* from the Blancan of Nebraska (Rogers, 1984). Both records of Pliocene *A. opacum* and *A. maculatum* exist to the west of their current range. Species-indeterminate records of *Ambystoma* are common and usually consist of fragmentary vertebrae, with only one record from southeastern Florida existing outside the current range of the genus (Holman, 2006).

2.2 The Gray Fossil Site

The Gray Fossil Site (GFS) in northeastern-most Tennessee is the only Mio-Pliocene fossil locality in the Appalachian region of the eastern United States. Fossiliferous sediments are up to 39 m thick and cover roughtly 1.8-2.0 ha (Wallace and Wang, 2004; Nave et al., 2005). Finely laminated clays, silts, and fine sands with occasional gravel lenses indicate a small lake or pond formed from a paleosinkhole within the Cambrian/Ordovician Knox Group Dolostone (Wallace and Wang, 2004; Shunk et al., 2006; DeSantis and Wallace, 2008; Hulbert et al., 2009). Erosion of the less resistant bedrock has generated reversed topography (Wallace and Wang, 2004; Shunk et al., 2006). Age constraints of the rhinoceros *Teleoceras* and the short-faced bear *Plionarctos* date the GFS to approximately 4.5-7 Ma, or latest Miocene – early Pliocene (Wallace and Wang, 2004), and is consistent with a Late Hemphillian NALMA fauna (Parmalee et al., 2002).

The GFS has yielded the most diverse pre-Pleistocene salamander fauna in North America, consisting of *Ambystoma* sp., *Notophthalmus* sp., *Desmognathus* sp., a Spelerpinae-type plethodontid, and two morphotypes of a *Plethodon*-type plethodontid (Boardman and Schubert, 2011). Identifications were made using isolated vertebrae. *Ambystoma* vertebrae include both adult and neotenic forms, determined by the degree of closure of the notochordal canal. Utilizing phylogenetic bracketing, the GFS *Notophthalmus* sp. supports the wooded-pond environmental interpretation of DeSantis and Wallace (2006, 2008).

3. Materials and Methods

An articulated specimen currently housed in the East Tennessee Museum of Natural History collections (ETMNH 8045) was recovered in 2009 in an area of the GFS known as the "Elephant Pit" when a removed block of dark, organic rich clay was split along a plane of weakness. Specimen was on two slabs, one of which was left intact. Skull elements from the second slab were disarticulated for storage. Butvar-98 consolidant was utilized to preserve the intact slab.

Comparative collections from the East Tennessee State University Vertebrate Paleontology Laboratory (ETVP) were utilized in fossil identification. Vertebral characters follow those by Tihen (1958) and Holman (2006). Cranial characters follow those by Tihen (1958). Characters of the premaxilla, vomer, trunk vertebrae, and tibia were utilized in identification. Though parasphenoids have taxonomic value (Tihen, 1958), this was not recovered.

Preliminary observations allowed for an initial refinement of identification. Comparisons made with the literature regarding the extinct family Batrachosauroididae suggests ETMNH 8045 does not belong to this family on the basis of vertebral characters (Holman, 2006). Among the living families, Ambystomatidae, Plethodontidae, Salamandridae, and Sirenidae were viable possibilities for ETMNH 8045 due to the presence of spinal nerve foramina posterior to the transverse processes of trunk vertebrae (save the first trunk vertebra) (Edwards, 1976). ETMNH 8045 trunk vertebrae lacks the V-shaped posterior expansion of the neural arch and sharp hemal keel diagnostic of Sirenidae. Preliminary identification as Ambystomatidae resulted from initial examination of isolated, associated trunk vertebrae.

Vertebral proportions described in Table 1 and as outlined by Tihen (1958) were used in taxonomic comparisons. Ratios of centrum length to centrum width at anterior end of trunk vertebrae 1 – 4 were utilized. Zygapophyseal proportions were not included due to inadequate preservation of isolated vertebrae and obscuring orientation of articulated vertebrae. Measurements were recorded utilizing Syncroscopy Auto-Montage 3D imaging software. Centrum length could be only roughly approximated for trunk vertebra 2. Centrum proportions of mid-trunk vertebrae are more appropriate for taxonomic identification than the first three trunk vertebrae (Tihen, 1958), but all measurements are reported.

4. Systematic Paleontology

Order Caudata Oppel, 1811

Suborder Salamandroidea Noble, 1931

Family Ambystomatidae Hallowell, 1856

Genus *Ambystoma* Tschudi, 1838

Ambystoma cf. *A. maculatum*

Figures 1 – 18

Referred specimens. 2 premaxillae, 1 left maxilla, 1 left dentary, 1 fragmentary left vomer, 1 right quadrate, 1 right squamosal, 1 left pterygoid, 1 right occipital, 1 atlas, 1 first trunk vertebra, 1 second trunk vertebra, 1 third trunk vertebra, 15 articulated trunk vertebrae, 4 caudal vertebrae, 5 ribs, 2 humeri, 2 ilia, 1 ischiopubis, 2 femora, 1 tibia, 1 fibula (ETMNH 8045).

5. Results

5.1. Cranial Elements

Paired premaxillae (Fig. 2.1) articulate for the majority of the ascending process. Ascending processes relatively broad and flat. Posterior ends of ascending processes broken. Monostichous (single row) dentition. Posterolaterally-directed medial fossa opening present posterior to the dentigerous ridge on the ventral surface.

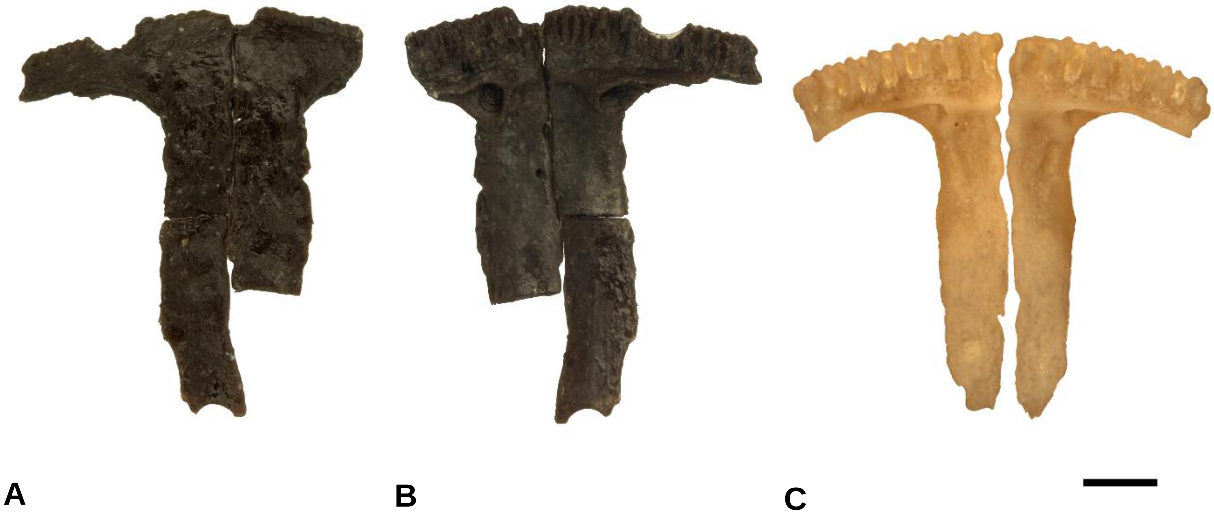

Figure 2.1. Paired premaxillae of ETMNH 8045 in **A**. dorsal and **B**. ventral views. Compare with **C**. premaxillae of modern *Ambystoma maculatum* (FB 1483) in ventral view. Top of page is anterior. Scale bar = 1 mm.

Left maxilla (Fig. 2.2) broken at anterior and posterior ends of the dentigerous row. Monostichous dentition. Ascending process relatively broad. What remains of the dentigerous row is more than twice the height of the bone. A ridge of bone extends perpendicular to the maxilla dorsal to the dentigerous row.

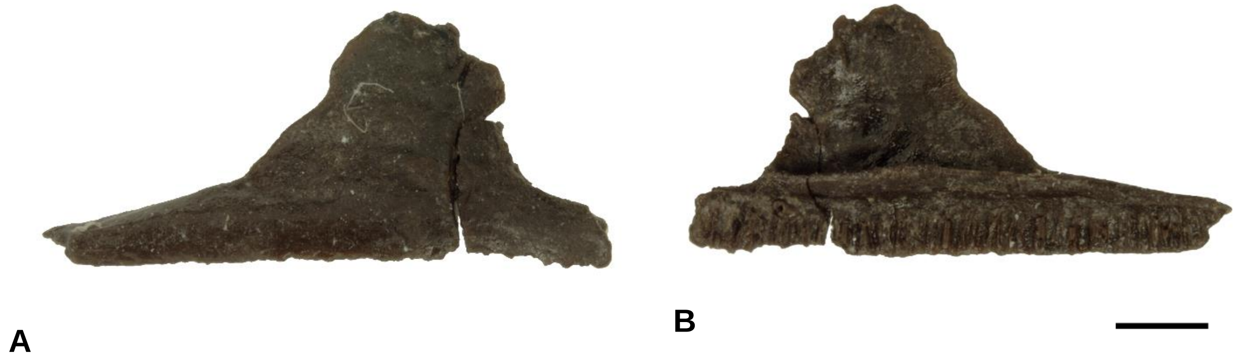

Figure 2.2. Left maxilla of ETMNH 8045 in **A**. lingual and **B**. labial views. Top of page is dorsal. Scale bar = 1 mm.

Dentary fragment (Fig. 2.3) from a more anterior portion of the mandible. Monostichous dentiton. Slight curvature in dorsal view. In occlusal view, the lingual flange approaches the dentigerous row anteriorly, but is broken posteriorly to anterior extent of the dentary. In lingual view (Fig. 3.3 A), the lingual flange nearly contacts the ventral extent of the dentary posteriorly, and approaches the dentigerous row anteriorly.

Figure 2.3. Left dentary of ETMNH 8045 in **A**. lingual, **B**. labial, and **C**. occlusal views. **A-B**. top of page is dorsal; **C**. top of page is labial. Scale bar = 1 mm.

What remains of the vomer (Fig. 2.4) is the lateral most extent of the dentigerous row. The body is triangular in shape, broader medially and coming to a point laterally. A posterior process bears the dentigerous row. This process is curved posteriorly, and ends before either medial or lateral margin of the bone. Dentigerous row is monostichous. Lacks teeth medially, possibly indicating a diastema.

Figure 2.4. **A.** Fragmentary left vomer of ETMHH 8045 in posteroventral view. Note beginning of diastema (arrow). Compare to **B**. left vomer from *Ambystoma laterale* (JIM 0835) in ventral view. Top of page is anterior. Scale bar = 1 mm.

Right Quadrate – (Fig. 2.5) Triangular in lateral view and broad ventrally; anterior margin concave; dorsal end broken; ventral edge articulates with the mandible and is concave; long, rectangular in medial view, with a large, rounded ventral process that is flattened medially. Ventroanterior procces broken off. Damage to ventroposterior expansion.

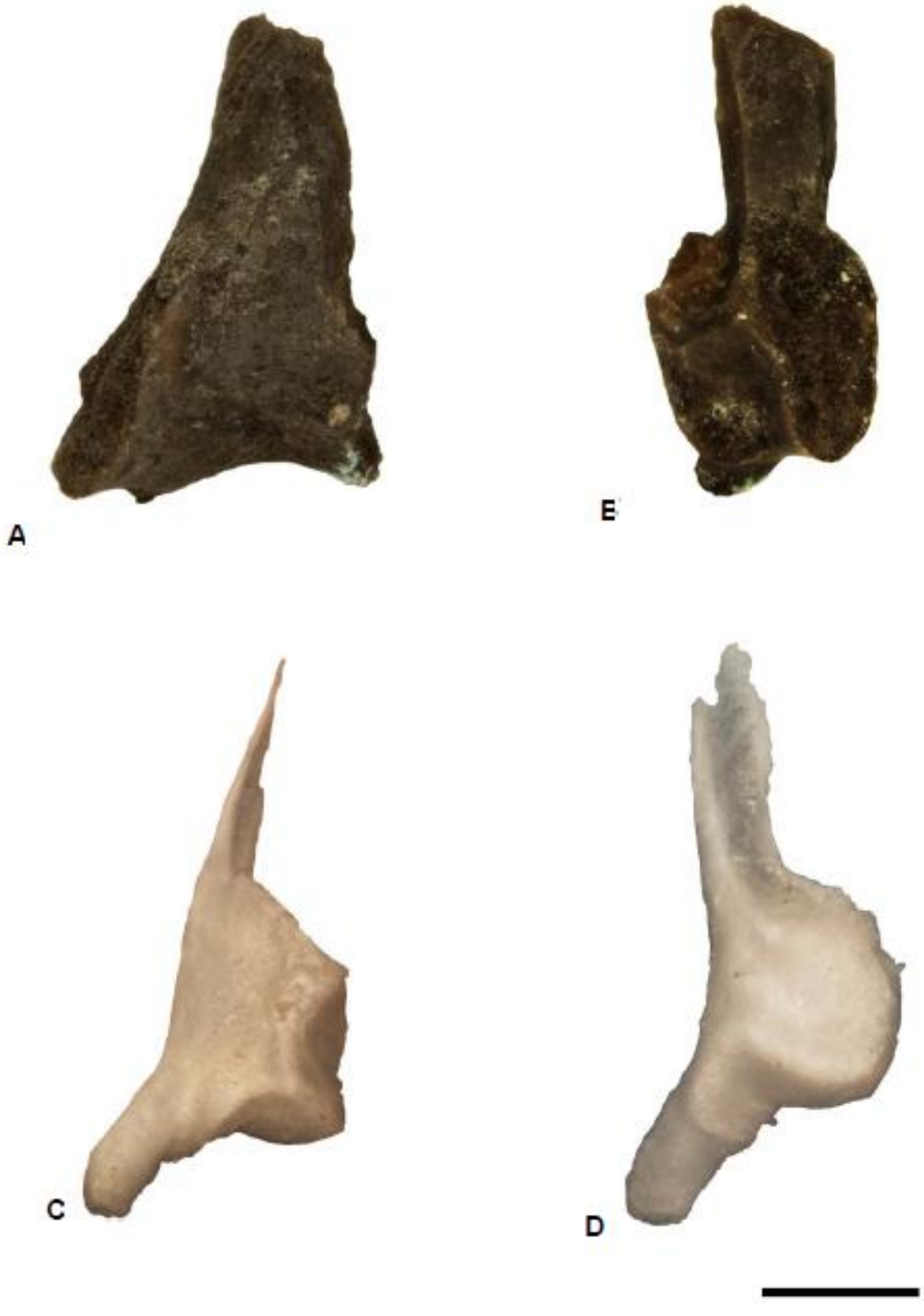

Figure 2.5. Right quadrate of ETMNH 8045 in lateral (1) and posteromedial (2) views. Compare with similar views (3-4) of unbroken right quadrate from modern *Ambystoma maculatum* (#3141). Top of page is dorsal. Scale bar = 1 mm.

Right Squamosal – (Fig. 2.6) Long and thin; damage restricted to thinner anterior margin; dorsal process extends posteriorly and is long and narrow; a crest originates on anterior margin midway down the bone and runs posterodorsally to end in a laterally-extending triangular process. Medial side bears grooves where squamosal contacts the quadrate. Dorsal suture region mostly intact; ventral edge broken.

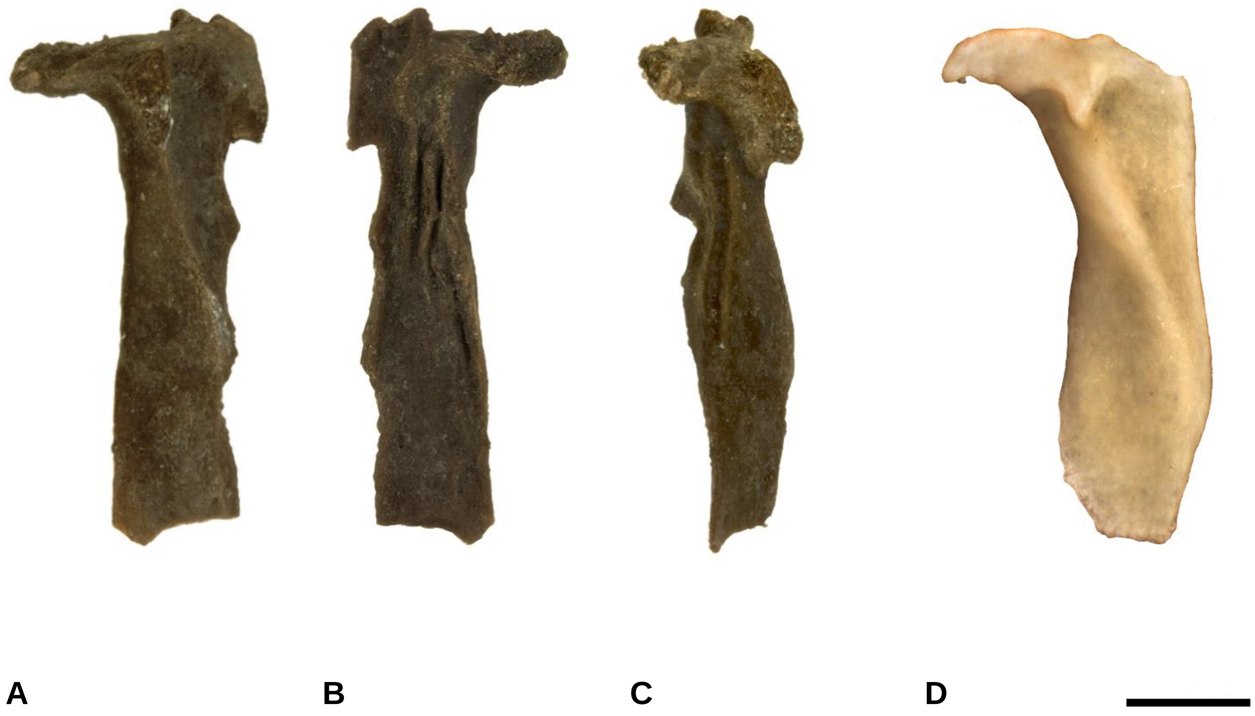

Figure 2.6. Right squamosal of ETMNH 8045 in **A**. lateral **B**. medial **C**. and posterior views. Compare to **D**. undamaged right squamosal of *Ambystoma maculatum* (FB 1483) in lateral view. Top of page is dorsal. Scale bar = 1 mm.

Left Pterygoid – (Fig. 2.7) Fragmentary, with anterior and lateral processes broken; pterygoids are normally L-shaped. Thicker portion of anterior process unbroken. A canal is evident in dorsal view, along the medial and posterior margin of the bone. Canal obscured by a thickening of the bone medial and posterior to the canal. Posteromedial margin rounded, unlike in *A. maculatum*. Thickening is triangular where the canal curves laterally.

Figure 2.7. Left pterygoid of ETMHH 8045 in **A**. dorsal view. **B**. right pterygoid of *Ambystoma maculatum* (FB 1483). Top of page is anterior. Scale bar = 1 mm.

Left Prefrontal – (Fig. 2.8) Bone is flat, long, and triangular. Posterior margin is straight, and bone tapers to a point anteriorly, where it deflects medially to accommodate the nasals laterally.

Figure 2.8. Left prefrontal of ETMNH 8045 in dorsal view. Top of page is anterior. Scale bar = 1 mm.

Nasals – (Fig. 2.9) Both nasals present. Triangular in shape with sides nearly equal in length. Slight concavity posterolaterally.

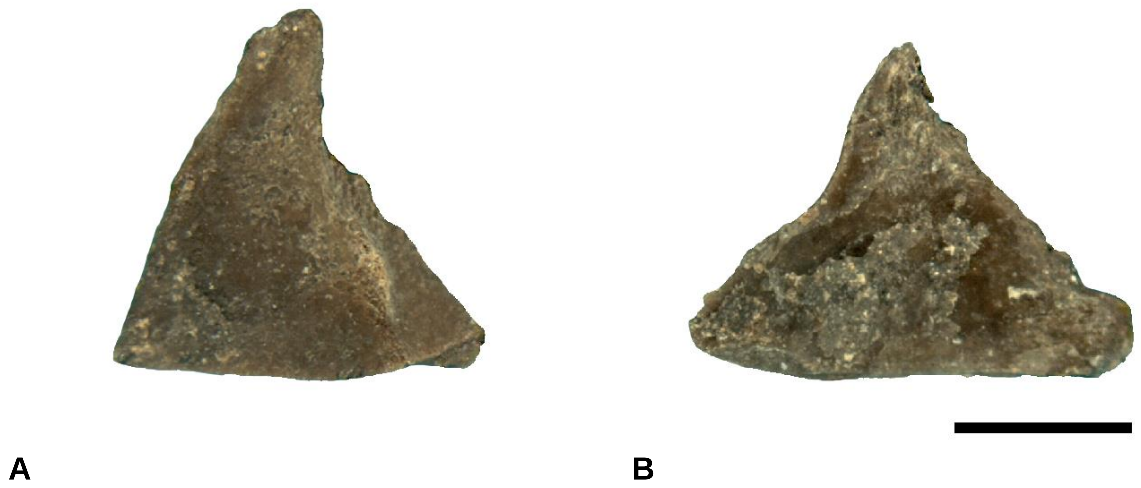

Figure 2.9. Nasals of ETMNH 8045 in **A**. dorsal and **B**. ventral vieww. Scale bar = 1 mm.

Right Otic Capsule – (Fig. 2.10) Generally ovoid bone. Vestibular foramen large and subrounded. Lateral surface convex. Posteromedially margin concave.

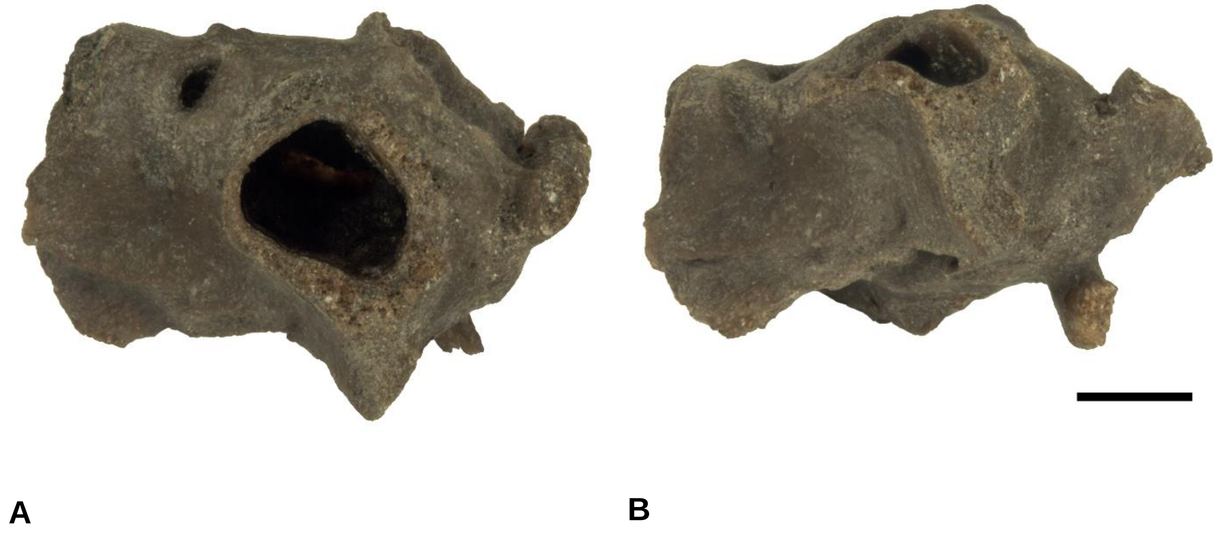

Figure 2.10. Right otic capsule of ETMNH 8045. **A**. ventral view; top of page is posterior. **B**. posterolateral view; top of page is dorsal. Scale bar = 1 mm.

5.2. Vertebral Elements

Atlas – Atlas (Fig. 2.11) large and robust, with non-faceted odontoid process widely separating the atlantal cotyles. Atlantal cotyles roughly circular and project posterolaterally. Posterior cotyle circular. Neural canal triangular and bordered by a thick neural arch. Neural arch elevated posteriorly approximately 45 degrees. Hyperapophysis tall and domed. Right postzygapophyseal articular facet teardrop-shaped; left postzygapophyseal articular facet damaged. Spinal nerve foramina situated posterolaterally and dorsally to the midline of the anterior cotyles (Edwards, 1976).

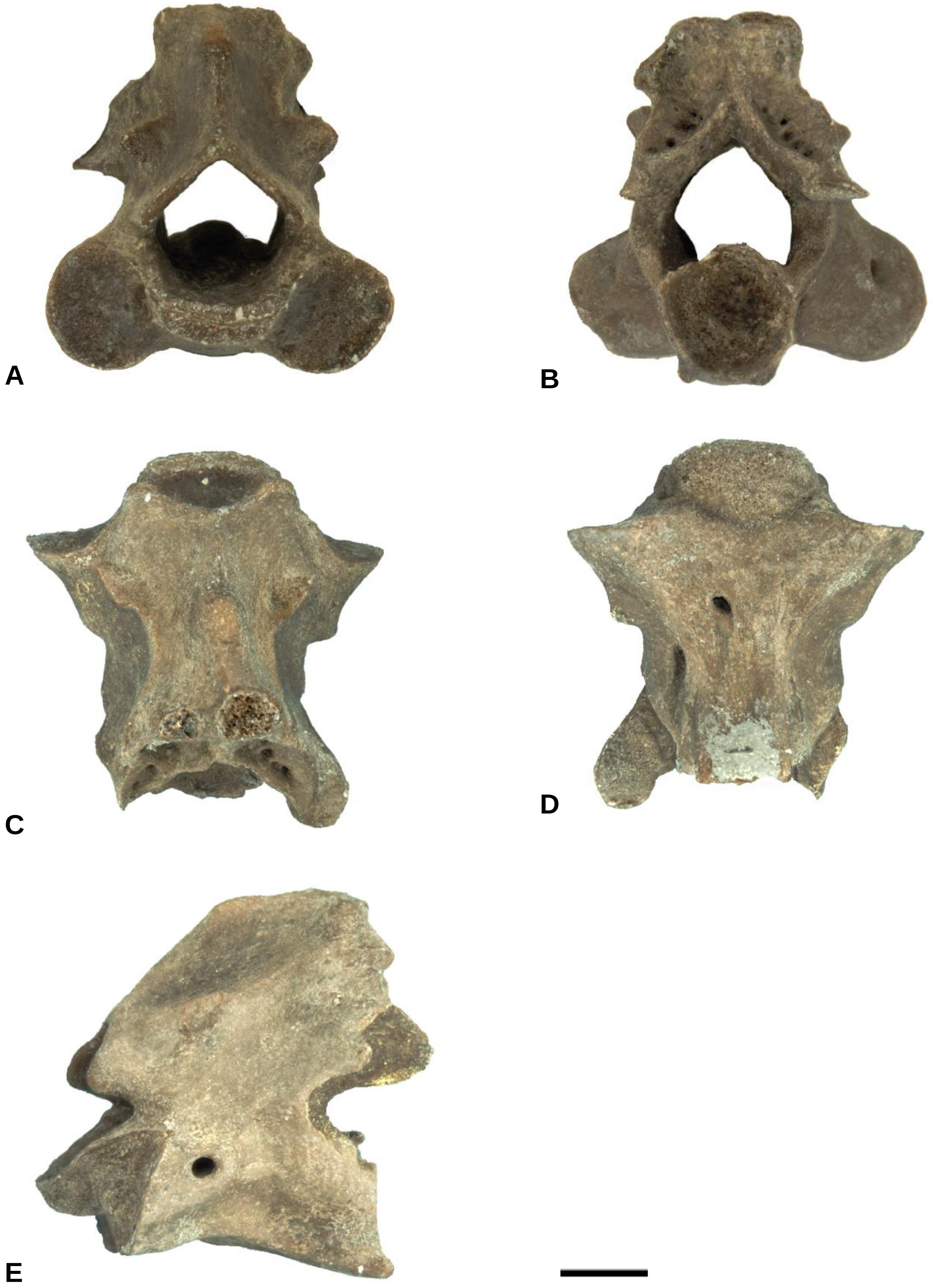

Figure 2.11. Atlas of ETMH 8045 in **A**. anterior, **B**. posterior, **C**. dorsal, **D**. ventral, and E. left lateral views. Scale bar = 1 mm.

First Trunk Vertebra – Posterior and anterior cotyles circular (Fig. 2.12). Neural canal large and somewhat triangular, constricted dorsally. Neural arch rises along its entire length less than 45 degrees. Hyperapophysis broad and does not extend beyond the posterior margin of the postzygapophyses. Prezygapophyses oval-shaped and elongated, elevated posteriorly. Left postzygapophysis oval-shaped and elevated posteriorly; right postzygapophysis missing. Posterior centrum too damaged to determine the relative posterior extent of the postzygapohpysis. Transverse processes robust. Parapophysis and diapophysis both originating slightly anterior to the middle of the centrum and projecting posteriorly. Large vascular foramina present at base of the parapophysis. Right transverse processes absent, and ventral damage is indistinguishable from a spinal nerve foramina.

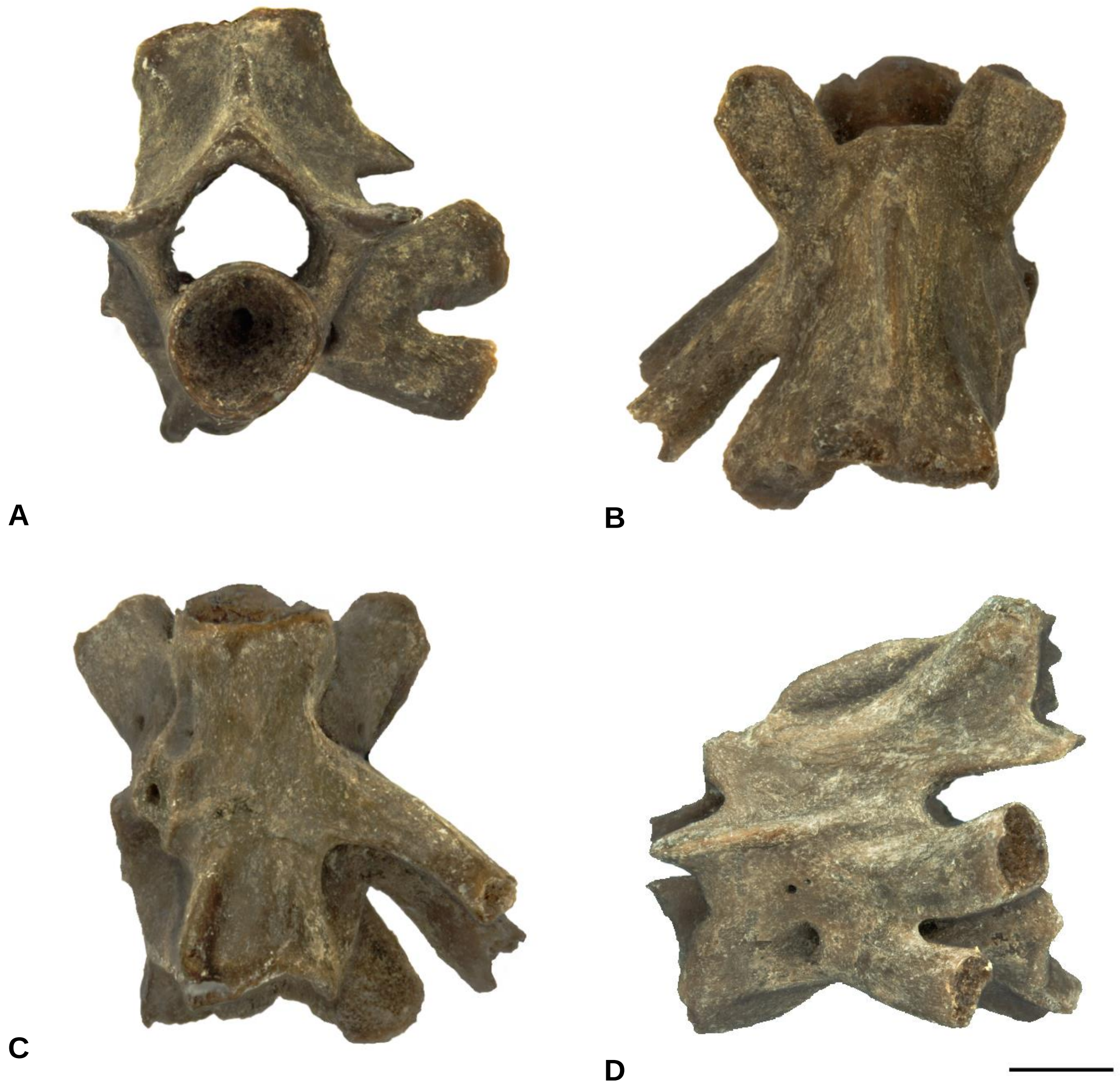

Figure 2.12. First trunk vertebra of ETMNH 8045 in **A**. anterior, **B**. dorsal, **C**. ventral, and **D**. left lateral views. Scale bar = 1 mm.

Second Trunk Vertebra – Anterior cotyle circular, posterior cotyle too damanged to determine shape (Fig. 2.13). Neural canal large, canal opening appearing somewhat triangular. Neural arch horizontal anteriorly and rises slightly posteriorly. Prezygapophysis extends anterior to the anterior margin of the centrum in lateral view. Inter-prezygapophyseal neural arch margin too damaged to determine shape. Both postzygapophyses absent. Right prezygapophyseal absent; left prezygapophyseal articular facet narrow and ovoid, slightly elevated anteriorly. Transverse processes robust, parapophysis originating near the middle of the centrum, with the diapophysis originating posterior to the parapophysis; both processes project posteriorly. Large vascular foramina present at the base of the parapophyses, evident on the undamaged left side. Spinal nerve foramina present left of the centrum at the base of the parapophysis.

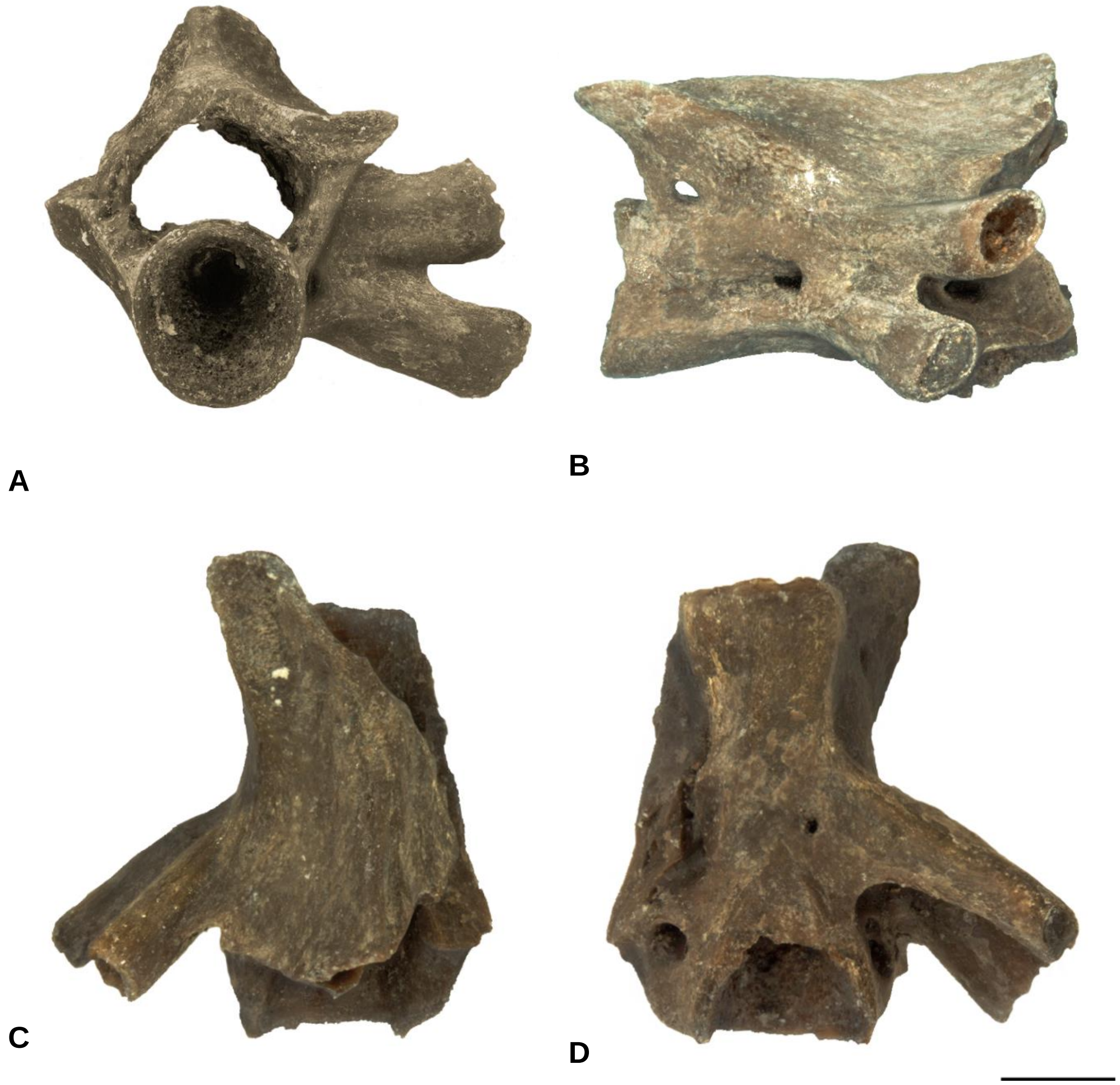

Figure 2.13. Second trunk vertebra of ETMNH 8045 in **A**. anterior, **B**. dorsal, **C**. ventral, and **D**. left lateral views. Scale bar = 1 mm.

Third Trunk Vertebra – Posterior and anterior cotyles circular (Fig. 2.14). Neural canal roughly circular. Neural crest rises along the entire length of the vertebra, less than 45 degrees from the horizontal. Neural crest does not extend beyond the posterior margin of the postzygapophyses. Rib-bearing processes do not extend posteriorly of the centrum in lateral view. Processes fused less than half their length. Amphicoelous. Prezygapophyses do not extend anteriorly to the anterior margin of the centrum.

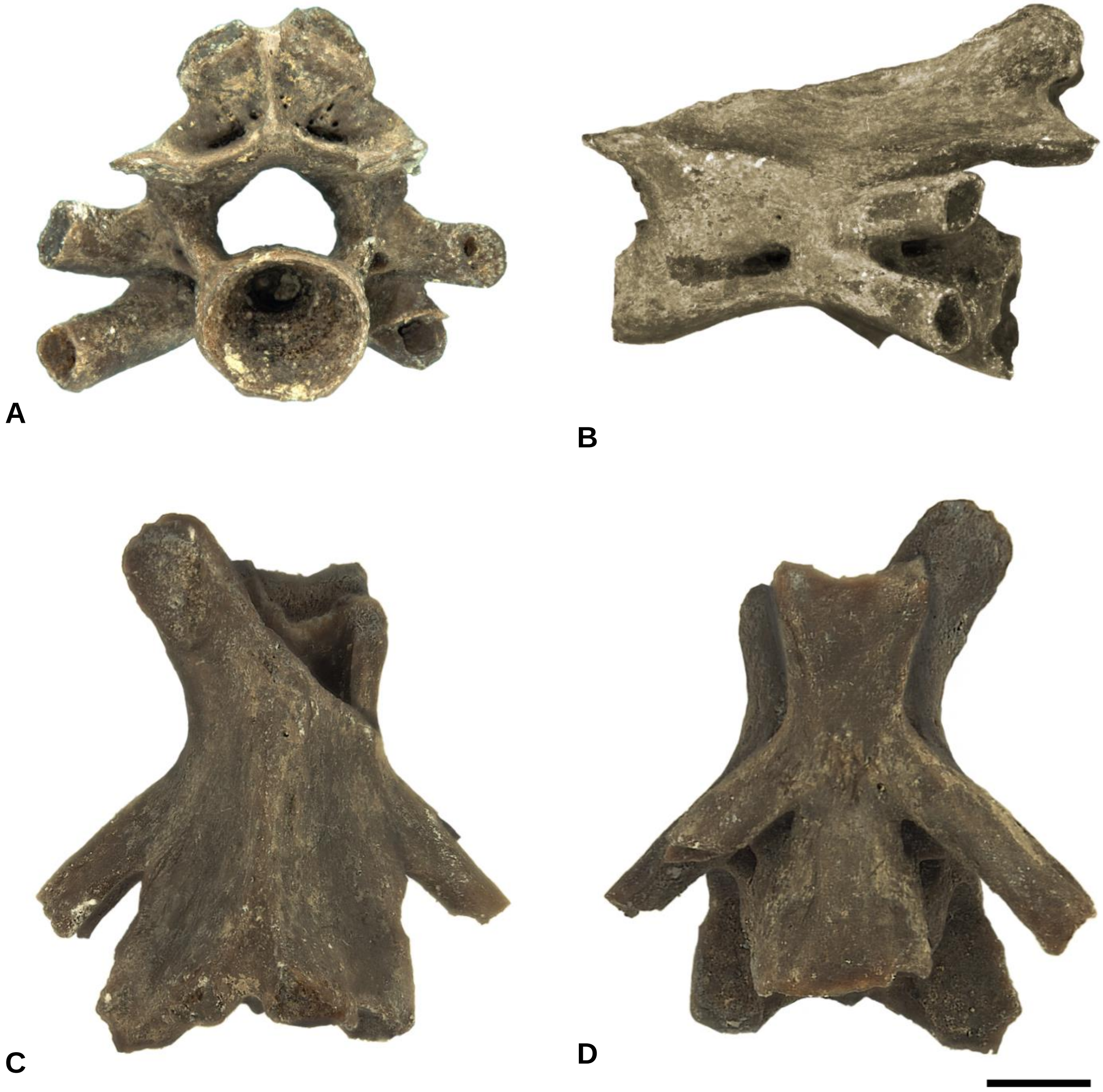

Figure 2.14. Third trunk vertebra of ETMNH 8045 in **A**. posterior, **B**. left lateral, **C**. dorsal, and **D**. ventral views. Scale bar = 1 mm.

Trunk Vertebrae – 13 mid-trunk vertebrae are preserved in matrix on one of the blocks (Fig. 2.15), 12 of which are articulated in series. Ventral surfaces are presented. Vertebrae amphicouelous. Transverse processes long, less robust, and posteriorly oriented, not extending beyond the posterior margin of the centrum. Single spine nerve foramina present posterior to the diapophyses. Prezygapophyses oval-shaped. First trunk vertebrae in articulated series has horizontally-oriented prezygapophyses and a circular anterior cotyle that has anterior basapophyses. Neural canal is flattened.

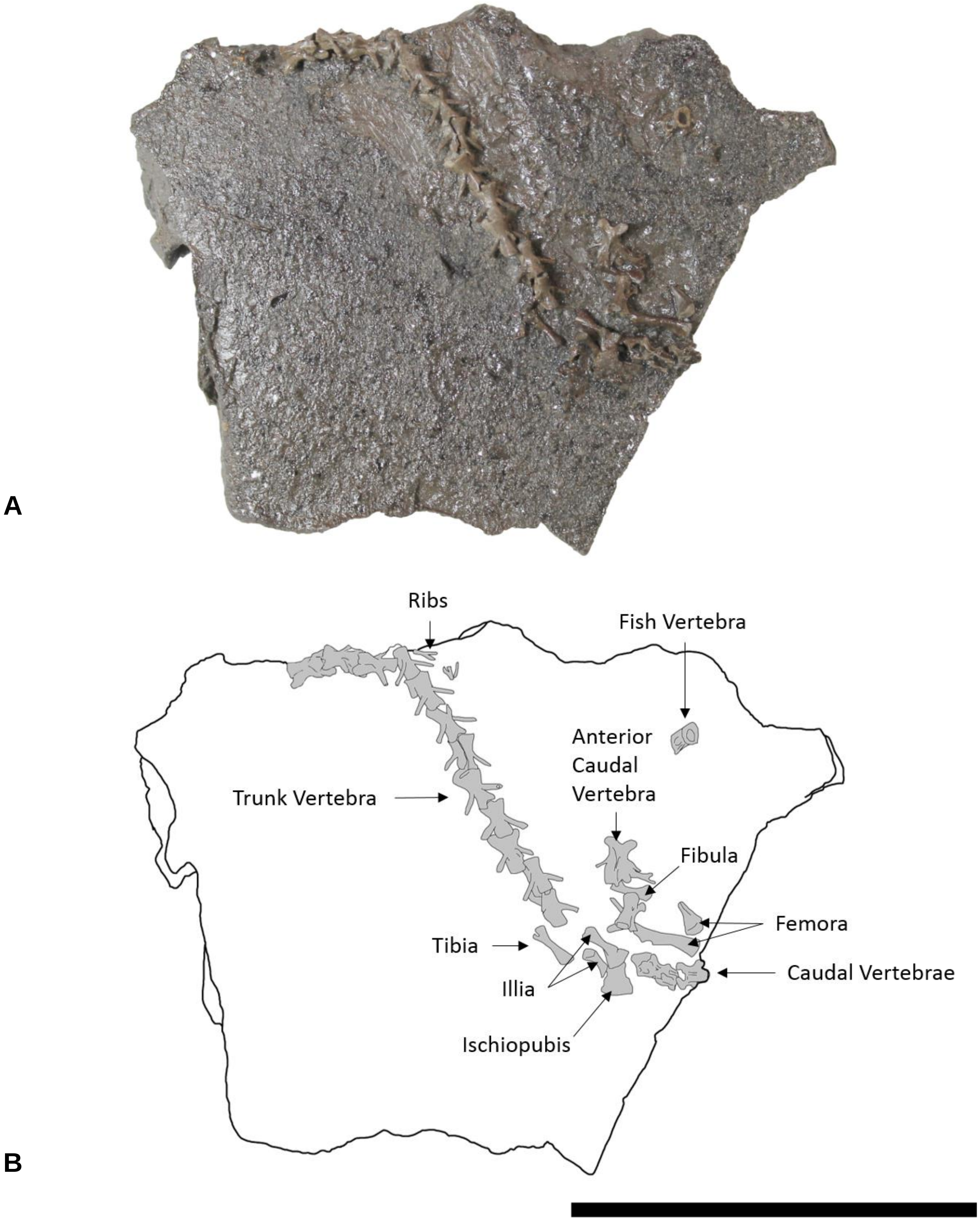

Figure 2.15. **A**. Intact block of ETMNH 8045, containing vertebral, pelvic, and rib elements, as well as an associated fish vertebra. **B**. Sketch of intact block. Top of page is anterior. Scale bar = 5 cm.

Table 2. Measurements of trunk vertebrae 1 – 4 centra and their calculated proportions.

Trunk Vertebra	Centrum Length (μm)	Centrum Width (μm)	Centrum Length/Width
1	3531.62	1516.48	2.328827284
2	4074.45	1476.44	2.759644821
3	4274.40	1516.48	2.818632623
4	4407.71	1552.32	2.83943388

Sacral Vertebra – Somewhat displaced from the articulated vertebral column, and presents the dorsal surface (Fig. 2.15 sv). Distinct from other trunk vertebrae in having an elongate neural arch that extends posteriorly past the postzygapophyses and transverse processes projecting more posteriorly. Left postzygapohysis and left transverse processes missing. One displaced rib obscures the right transverse processes and partially obscures the right postzygapophysis. Prezygapohpyseal articular facets circular. Two facets apparent at the posterior margin of the neural arch.

Caudal Vertebrae – Two anterior caudal vertebrae are preserved with the articulated specimen (Fig. 2.15 cv). Not elongate compared to the larger trunk vertebrae. Two heamal arches apparent on the ventral surface. No narrowing of the centrum midway along its length. Pre- and postzygapophyses narrow. Transverse processes greatly reduced.

Two isolated caudal vertebrae are associated with the specimen (Fig. 2.16). Both have too much damage to the transverse processes to determine their robustness or posterior extent. One (Fig. 2.16 A-C) vertebra preserves the prezygapophyses, which are narrow and elongate. The neural arch is constricted midway along the column, but the centrum does not display this constriction. The neural spine is upswept posteriorly and extends posteriorly beyond the remains of the postzygapophyses. Centrum damaged anteriorly and posteriorly. Ventral surface of the centrum is smooth. The other vertebra (Fig. 2.16 D-F) does not display this constriction. The postzygapophyses are short and narrow. The neural spine is not upswept, though it may be damaged. The centrum narrows midway along its length and bears a narrow haemal arch.

Figure 2.16. Isolated caudal vertebrae. One caudal vertebra in **A**, dorsal, **B**. ventral, and **C**. right lateral views. Another caudal vertebra in **D**. dorsal, **E**. ventral, and **F**. left lateral views. Scale bar = 1 mm.

Ribs – No articulations between the vertebral transverse processes and the bicapitate ribs are evident. Ribs are preserved in close association with trunk vertebrae in the intact block (Fig. 2.15 r) as well as in isolation (Fig. 2.17). An epipleural process is not evident on any ribs observed; however, this feature is seen only on the first rib in *Ambystoma opacum* and *A.*

talpoideum, and, given the relatively poor preservation of the anterior elements of ETMNH 8045, the first rib may not have been recovered.

Figure 2.17. **A-E**. Isolated ribs from ETMNH 8045. Top of page is proximal. Scale bar = 1 mm.

5.3. Limb Girdle Elements

Pectoral Girdle –Both humeri present as isolated elements with different degrees of preservation. Left humerus (Fig 2.18 A-B) has a better preservation of the proximal end, retaining the crista dorsalis humeri but not the crista ventralis humeri and is broken distally such that the radial condyle, ulnar condyle, lateral epicondylus, and olecranon fossa are absent. In the right humerus (Fig. 2.18 C-D), the proximal crests are absent but the base of the radial and ulnar condyles, as well as the trochlear groove, are preserved. No other anterior limb elements are preserved.

Figure 2.18. Isolated humeri of ETMNH 8045. Left humerus, **A**. extensor and **B**. flexor sides. Right humerus, **C**. extensor and **D**. flexor sides. Top of page is proximal. Scale bar = 1 mm.

Pelvic Girdle – Right ilium is present in the intact block, displaying the ventral surface (Fig. 2.15 il). Still articulated with an ischiopubis (Fig. 2.15 ip). Elements of both femora are present in the intact block (Fig. 15, f). One is broken about half way down the shaft. The other is evident by a femoral head. Both bear a trochanter oriented proximally. Tibia preserved in the intact block (Fig. 15, t). Lacks an extensively developed flange or crest, continuous with the spine, along the posterodorsal surface, which is characteristic of the extinct species *Ambystoma minshalli*. One fibula is present (Fig. 15, fb).

6. Discussion

ETMNH 8045 has the following characters of taxonomic significance: monostychous dentition on all tooth-bearing elements; diastema on vomerine tooth series at the level of choana; metamorphosis evident by development of a septum in vertebral notochord; no vertical lamina on ventral surface of premaxilla; vertebral proportions approximating Tihen's (1958) "*A. maculatum* group"; odontoid processes on atlas more rounded; posterior neural arch more depressed than in *A. opacum* and *A. talpoideum*; neural arch lacking significant notching; tibia lacking expanded flange; dorsolateral surface of neural arch projects downward; ventral spinal nerve foramina on centrum present.

Relationship to the "*A. tigrinum* + *A. mexicanum* group" can be rejected due to the presence of a diastema on the vomerine tooth series at the level of the choana. ETMNH 8045 is monostichous, as opposed to members of the subgenus *Linguaelapsus* in which every dentigerous element is polystichous (except in *A. mabeei*). Trunk vertebrae more closely resemble the "*A. maculatum* group" than the "*A. opacum* group" in terms of centrum proportions as well as vertical extent of the neural arch. For these reasons, all species but *A. maculatum*, *A.*

macrodactylum, and *A. mabeei* are rejected as representing the GFS articulated specimen. ETMNH 8045 compares most favorably with *A. maculatum*, the Spotted Salamander. Definitive identification is withheld until specimens of *A. macrodactylum* and *A. mabeei* can be examined in depth.

Determination of species allow for one of three possible paleogeographical interpretations. Modern *Ambystoma macrodactylum* is found today from the Pacific Coast of North America through the Cascades and Sierra Nevadas into the Intermontane Plateaus. Fossil remains of *A. macrodactylum* at the GFS would be a significant eastward expansion of their current range. *Ambystoma maculatum* inhabits the area from the Interior Lowlands to the Laurentian Uplands, including the interior highlands, the Appalachian Highlands, and the Atlantic Coastal Plain; it is found near the GFS today. *Ambystoma mabeei* is found only on the Atlantic coastal plain (Duellman and Sweet, 1999). Other coastal plain organisms have been identified at the GFS, including *Alligator*, indicating a warmer paleoclimate (Schubert and Wallace, 2006).

6.1. Inferences about GFS

Limited inferences can be made about the GFS based on the morphology of ETMNH 8045. Closure of the notochordal canal in trunk vertebrae support the interpretation of the specimen as a terrestrial adult, and development of the premaxillae are consistent with adult, transformed *Ambystoma*. It can be inferred from the number of trunk vertebrae that the living animal likely would have had 14 costal grooves, based on Highton's (1957) observation that there are two more trunk vertebrae in *A. mabeei* than there are grooves. Costal grooves cannot be used to aid identification due to Lindsey's (1966) demonstrated that trunk vertebrae counts of

Ambystoma gracile correlate with ambient temperature during development; however, once formally identified, ETMNH 8045 may allow for paleotemperature estimates based on trunk vertebrae count.

Spotted Salamanders (*Ambystoma maculatum*) and the closely related Blue-Spotted Salamander complex (*A. laterale-A. jeffersonianum* complex) are not known to readily breed in permanent pools of water due to predation of eggs and larvae by fish, typically utilizing ephemeral wetlands or vernal pools (Turtle, 2000). However, semi-permanent ponds that dry frequently enough to exclude fish are also suitable (Turtle, 2000). Spotted Salamanders will spend the majority of the year in upland forests near the breeding pond, to which the salamanders will show high fidelity (Petranka, 1998; Windmiller, 1996). During non-breeding months, Spotted Salamanders may range as far as 1 km away from the breeding pond (Homan et al., 2004). Given the fossil specimen's favorable comparison with *A. maculatum*, and following Schubert and Wallace (2006) in utilizing phylogenetic bracketing to infer paleoecology, ephemeral aquatic habitats or wetlands were likely present within 1 km of the GFS.

However, most evidence suggests a permanent pond environment for the GFS. Boardman and Schubert (2011) reported neotenic *Ambystoma*, a phenotype that necessitates pond permanence. Non-existence of mud cracks in examined strata does not support frequent drying. Additionally, terrestrial spelerpinae-type plethodontids such as cf. *Gyrinophilus* may require up to 5 years to complete their larval phase (Bruce, 1980; Chapter 3). ETMNH 8045 is associated with a fish vertebra (Fig. 2.15), so fish presumably coexisted with this individual.

Two possible explanations exist. Ephemeral ponds may have been present in the immediate vicinity of the GFS. Modern *A. maculatum* may travel as much as 1 km from their breeding ponds, so the presence of *Ambystoma* cf. *A. maculatum* does not contradict an interpretation of the GFS as a permanent pond. Alternatively, the permanent pond may have been suitable for breeding due to the lack of large-bodied predatory fish. However, literature on Spotted Salamander breeding habits is not overly specific on what fish species or size classes prey upon salamander eggs and larvae, and smaller fish are known from the GFS. Small fish may be sufficient deterrents to salamander breeding. Therefore, the presence of ephemeral ponds in the near vicinity of a permanent pool seems most likely.

If ETMNH 8045 represents an ephemeral-pond breeding Mole Salamander, at least two species of *Ambystoma* likely coexisted at the GFS. Neotenic vertebrae reported by Boardman and Schubert (2011) indicate breeding suitability of the permanent pond for at least one other species of *Ambystoma.* Sympatry of Mole Salamanders is known throughout the Eastern United States (Duellman and Sweet, 1999). Further studies investigating linear measurements and proportions of the numerous isolated trunk vertebrae available (number citation from boardman thesis) could statistically hypothesize the number of sympatric *Ambystoma* morphotypes.

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CHAPTER 3

(1) CRANIA OF PLETHODONTIDAE AND RHYACOTRITONDIAE

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(2) Summary

Salamanders may display two general vomerine morphotypes that correspond with either aquatic or terrestrial feeding. In Lungless Salamanders (Plethodontidae), most skull elements are fairly conservative among species with similar life histories. Therefore, vomerine morphology may be useful in determining aspects of ecology. An isolated vomer recovered from the Mio-Pliocene (4.5 – 7 Ma) Gray Fossil Site (GFS) in eastern Tennessee, USA, possesses characters that are found in two salamander families: Plethodontidae and Rhyacotritonidae. Plethodontidae has a nearly cosmopolitan distribution, occurring mainly in North, Central, and South America, with isolated populations in Italy and South Korea. In contrast, Rhyacotritonidae is restricted to the Pacific Northwest of the United States. Characters of the vomerine dentigerous row seen in ETMNH 18219 compare most favorably with those of terrestrial *Gyrinophilus*. Both a principal component analysis and discriminant analysis utilizing digitized landmarks of Plethodontidae and Rhyacotritonidae representatives support identification as cf. *Gyrinophilus*. The presence of a terrestrially-feeding plethodontid supports the reconstruction of the GFS as sufficiently humid to support salamander populations. ETMNH 18219 lends further support to the interpretation of

the GFS as a perennial pool, assuming the fossil taxa required three to five years to complete the aquatic larval phase, similar to the modern *G. porphyriticus*.

(3) Keywords: vomer, Gray Fossil Site, Spelerpini, *Rhyacotriton, Gyrinophilus*, Miocene, Pliocene

(4) Introduction

The skull of the lungless salamanders, Plethodontidae, is characterized by the absence of a pterygoid bone in adults and the presence of large patches of paravomerine teeth and nasolabial grooves (Min et al., 2005). About two-thirds of the approximately 675 living species of salamanders belong to this family (Amphibiaweb.org, January 2015). Lunglessness has freed the hyobranchium and its musculature from the task of force-pump breathing, allowing the development of elaborate tongue projection mechanisms in many taxa, particularly the Bolitoglossinae (Lombard and Wake, 1976). About 85% of plethodontid species are direct-developing (Marks, 2000). The Southern Appalachian Mountains of the eastern United States are an area of high salamander endemism and home to the greatest diversity of the plethodontids *Plethodon, Desmognathus,* and *Eurycea* species (Duellman and Sweet, 1999, p. 67). Estimates on total salamander biomass in forested regions of the Southern Appalachians average 1.65 kcal m^{-2} (dry weight), greater than that of all other predators combined (Hairston, 1987). Additionally, plethontid salamanders have been shown to contribute significantly to carbon sequestration, due in part to their predation of invertebrates and the relative scarcity of salamander predators (Hairston, 1987; Best and Welsh, 2014).

Rhyacotritonidae is a monogeneric family of salamanders with uncertain affinities to other families within Salamandroidea. The family is distinguished from all others in that adult males possess unique, square-shaped glands lateral and posterior to the vent (Good and Wake, 1992). Additionally, *Rhyacotriton* possesses an epihyal, a character shared only with some *Ambystoma* (Ambystomatidae); operculum lacking, shared only with some derived hynobiids; lacks paedomorphic characters including gills and lidless eyes; some populations are the only transformed salamanders to lack nasal bones, and nasals are never fully formed; distinguished from all but dicaptodontids (Dicamptodontidae) in that spinal nerves exit intervertebrally presacrally but through a ventral foramina postsacrally (Edwards, 1976). Originally described as a species of *Ranodon* (Gaige, 1917), most considered the *Rhyacotriton* to be a member of the family Ambystomatidae since the work of Dunn (1920). Tihen (1958) isolated the genus into the ambystomatid subfamily Rhyacotritoninae. Later placed under the subfamily Dicamptodontinae (Regal, 1966), Edwards (1976) elevated Dicamptodontinae to the familial level. *Rhyacotriton* was elevated to familial level by Good and Wake (1992) when phylogenetic analysis including cranial, vertebral, soft-tissue, and genetic characters failed to support a *Rhyacotriton* + *Dicamptodon* clade. Osteological characters of *Rhyacotriton* include an elongate premaxillary nasal process, unossified medial nasal center, lacrimal absent in adults, angular absent, spinal nerves that exit intervertebrally presacrally and through ventral foramina postsacrally (Good and Wake, 1992). Today the genus is confined to the Pacific Northwest and consists of four species identified by external coloration (Good and Wake, 1992).

MODERN DISTRIBUTIONS

While the majority of modern plethodontids inhabit North, Central, and South America, two genera are found in Eurasia. *Hydromantes* includes species in Europe (Italy and France, subgenera *Atylodes* and *Speleomantes*) and California (subgenus *Hydromantes*). *Karsenia koreana* is a recently discovered species from South Korea (Min et al., 2005). *Karsenia koreana* was first reported by Min, et al. (2005) from montane woodlands in southwestern Korea. Externally resembling Western North American *Plethodon*, *K. koreana* differs from *Plethodon* in having distal tarsals 4 and 5 arrangement seen only in *Aneides* and *Chiropterotriton*; *K. koreana* differs from *Aneides* and *Chiropterotriton* by having a paired premaxilla (Min et al, 2005). Mitochondrial genome analyses support a sister-taxon relationship of *Hydromantes* and *Karsenia koreana* (Vieites et al., 2011; Pyron and Wiens, 2011); this clade is thought to be the remnant of a formerly wider distribution that originated in Western North America and dispersed across the Bering Land Bridge prior to the Miocene (Wake, 2013).

The Southern Appalachians of eastern North America are a biodiversity hotspot of Lungless Salamanders, where three genera of plethodontids have their highest occurrence. Of the 26 species of *Plethodon*, 9 occur only in this region. Seven of 14 *Desmognathus* species and 2 of 7 *Eurycea* species are endemic (Duellman and Sweet, 1999). All members of Spelerpini (*Eurycea*, *Gyrinophilus, Pseudotriton, Stereochilus*, and *Urspelerpes*) occur in either the Appalachians, Allegheny Plateau, Piedmont, or Atlantic Coastal Plain (Duellman and Sweet, 1999). Most Lungless Salamander species belong to the superfamily Bolitoglossinae, which inhabit Central and South America. The family represents a relatively recent radiation, and is currently understood to have arisen from a Western North American lineage (Wake 2013).

FOSSIL RECORD

Currently the only record of fossil plethodontids outside of the Americas is a trunk vertebrae of *Hydromantes* from the Middle Miocene of Slovakia (Venczel and Sanchíz, 2005). It is thought to be a remnant of a larger clade derived from Western North American salamanders, a clade that includes the newly-discovered Korean plethodontid, *Karsenia koreana* (Wake, 2013). A fossil assigned to Plethodontidae has been recovered from Pleistocene sediment from Santa Cruz Nuevo, Mexico (Tovar et al., 2014).

California has yielded the majority of North America's pre-Pleistocene plethodontid fossils. Peabody's (1959) description of late Miocene trackways of *Batrachoseps* near Columbia, California is the first record of a plethodontid salamander earlier than the Pleistocene. The Lower Micoene (Arikareean) Cabbage Patch Formation of Montana has yielded *Plethodon* and *Aneides* fossils (Tihen and Wake, 1981). Two plethodontids, *Aneides lugubris* and *Batrachoseps* sp., have been reported from the upper Mehrten Formation (Hemphillian, latest Miocene) of the western Sierra Nevada foothills of California; *Batrachoseps* is also known from the Pinole Tuff (Hemphillian) in the San Francisco Bay area (Clark, 1985). California's Hemphillian record also includes trackways of *Batrachoseps relictus* (Peabody, 1959; Wake, 1966; Brame and Murray, 1968; Petranka, 1998). The Gray Fossil Site is unique in yielding the earliest known plethodontid record east of the Mississippi River, including a "*Plethodon*-type plethodontid" with two morphotypes, *Desmognathus* sp., and a Spelerpinae-type plethodontid (Boardman and Schubert, 2011). A slightly older eastern site, the Pipe Creek Sinkhole of Indiana, has an unidentified plethodontid (Farlow et al., 2001). In contrast to the plethodontid record, Rhyacotritonidae is unknown in the fossil record (Holman, 2006). This is surprising given the numerous fossil sites in California that have yielded other salamander remains, yet those localities may be representing community structures that were not utilized by *Rhyacotriton*.

PREVIOUS WORK ON CRANIAL OSTEOLOGY OF PLETHODONTIDAE AND RHYACOTRITONIDAE

Buckely et al. (2010), in describing the osteology of *Karsenia koreana*, noted the conservative nature of plethodontine skulls over vast periods of time and across vast geographic ranges, with most species sharing a ‘common composition.’ Trueb (1993) summarized the general cranial characters for adult plethodontids: fused prootic/exoccipital; operculum fused to stapes, united to otic capsule; lateral wall of nasal capsule incomplete; lateral narial fenestra present; posterior wall of nasal capsule complete; naso-lacrimal duct present; Jacobson’s organ present; medial articulation of nasals absent; lacrimal absent; pars dorsalis of premaxilla long and separates nasals; premaxillary dentition present; quadratojugal absent; angular fused with prearticular; coronoid absent; articular absent; pterygoid abset; metapterygoid absent; basitrabecular process present; hyobranchian I and ceratobranchial I separate; ceratobranchial II absent; dentition pedicellate. Maxillae, septomaxillae, prefrontals, and stapes may or may not be present (Trueb, 1993). The most complete review of plethodontid osteology available is that by Wake (1966). Tihen (1958) includes *Rhyacotriton* in his comprehensive review of ambystomatids. Good and Wake (1992) include osteology in their review of the genus *Rhyacotriton*.

Much has been written comparing the dorsoanterior cranial elements of *Pseudotriton* and *Gyrinophilus*. Cope (1869, p. 108) established *Gyrinophilus* as a genus distinguished from *Pseudotriton* based on the former’s fused premaxillae, also noting that *Gyrinophilus* differed in possessing nasal bones separated from each other as well as a prootic-squamosal crest. Dunn (1926) added an additional character for *Gyrinophilus*: the prefrontals do not border the nares, as they do in *Pseudotriton*. Grobman (1959) analyzed the premaxillae, nasals, prefrontals, and prootic-squamosal crests of both genera, and found that all previously established characters distinguishing the two only apply to older adult individuals. He suggested synonomizing *Gyrinophilus* with *Pseudotriton*. Martof and Rose (1962) support the validity of *Gyrinophilus*. They found that *Pseudotrion* skulls have greater density and that the anterior elements (premaxilla, prevomer [vomer], and maxilla) are more closely joined together. *Gyrinophilus* skulls are more elongate and pointed, and the posterior end of the skull is approximately 11% narrower. They assert that even though both genera have premaxillae fused anteriorly as larvae (with those of *Gyrinophilus* separating at metamorphosis), *Gyrinophilus* premaxillae bear nasal processes that never fuse, while *Pseudotrtion* always have fused nasal processes. They interpret the greater flexibility and elongation of *Gyrinophilus* skulls as an adaptation for eating other salamanders, while *Pseudtotrion* have robust skulls for digging and feeding on earthworms, insects, and relatively smaller salamanders.

VOMERINE MORPHOLOGY OF SALAMANDERS

Within the salamander skull, three main regions of adult dentition exist: marginal teeth consisting of the premaxillary, maxillary, and dentary teeth; vomerine teeth; and parasphenoid teeth, with parasphenoid teeth arising embryologically from the posterior end of the vomerine tooth row from which it may or may not separate (Lawson et al., 1971). In adulthood, the vomer bone bears the vomerine teeth (Fig. 3.1).

The vomer is broadly triangular in most salamanders, with an anterior margin articulating with the premaxilla and maxilla and a medial articulation between the vomers near the anterior extent of the parasphenoid (Trueb 1993, page 301). Cryptobranchid vomers completely articulate medially; most other salamanders posess an antero-medial fenestra between the premaxillae and vomers (Trueb 1993 pg 301). Cryptobranchids and "hynobiids" lack a preorbital process supporting the posterior margin of the choana. Ambystomatids have poorly developed preorbital processes (Tihen, 1958). Salamandrids and most plethodontids have well-developed preorbital processes (Trueb, 1993).

Salamandrids and plethodontids are characterized by elaboration of their vomerine dentition, associated with terrestrial life zones (Trueb, 1993, pg. 308; Vasilyan and Böhme, 2012). The dentigerous process of the vomer is elongate and extends posteriorly to the otic region in salamandrids, while the dentigerous row of plethodontids often have an anterior transverse portion, and the posterior region can expand into an elaborate palatal tooth patch (Trueb, 1993, pg. 308).

Vomerine morphology is closely linked with ontogenesis. In general, larvae and paedomorphic lineages bear teeth on the anterior portion of the vomer parallel to the maxillary-premaxillary tooth row (Xiong et al., 2014). Kraus (1988) found that among Salamandroidea species, the anterior palatal teeth are comprised of vomerine teeth as well as pterygoid teeth, and during metamorphosis the tooth-bearing portion of the pterygoid breaks from the rest of the bone and fuses with a lateral extension of the vomer, forming the preorbital process. Kraus also notes that vomerine teeth extend to the medial border of the choana in *Rhyacotriton olympicus, Dicamptodon ensatus, Dicamptodon. copei, Desmognathus quadramaculatus, Pseudotriton ruber, Gyrinophilus porphyriticus, Hemidactylum scutatum,* and some *Ambystoma* species. Teeth do not extend to the choana in *Tylototriton verrucosus, Pleurodeles waltl, Taricha granulosa*, and the Ambystomatidae species *A. annulatum* and *A. cingulatum*; the preorbital process dissolves medially and disappears soon after metamorphosis in *A. annulatum* and *A. texanum* (Kraus, 1988). Hynobiid salamanders have a gently curving vomerine tooth row as larvae that develops more curvature posteromedially during metamorphosis; adults in paedomorphic populations retain the larval shape (Xiong et al., 2014). Modern cryptobranchids retain the larval vomerine morphology as paeodmorphic adults, but the fossil *Aviturus exsecratus* metamorphoses and bears a sharply-curved vomerine dentition on the posterior edge of the vomer (Vasilyan and Böhme, 2012). Plethodontid salamanders exhibit a similar ontogenetic pattern (Wake, 1966).

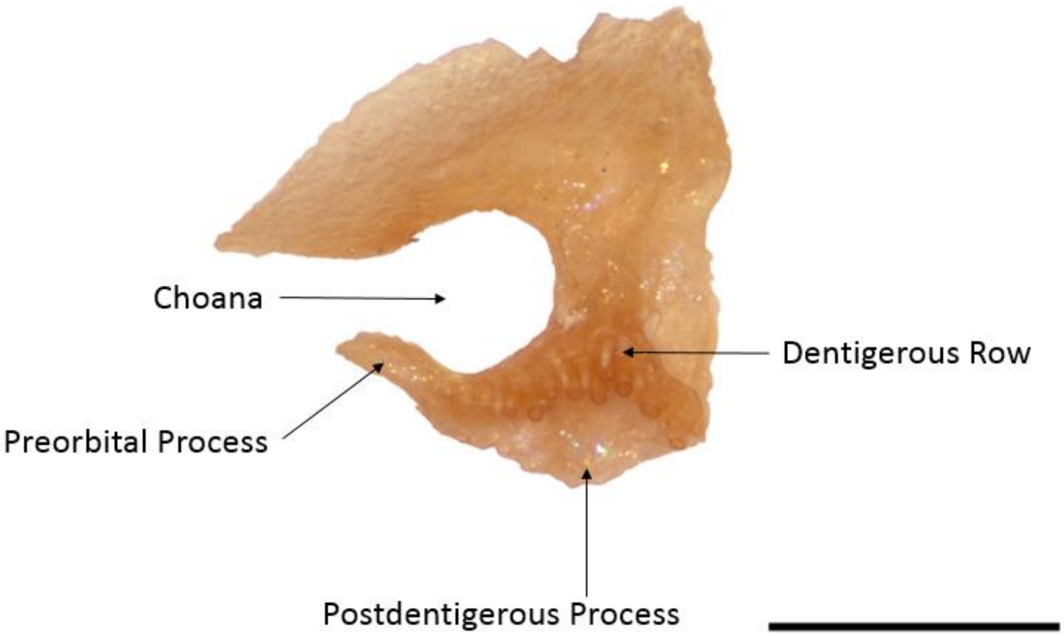

Figure 3.1. Features of the salamander vomer, *Rhyacotriton variegatus* NVPL 6982. Right vomer, palatal view. Top of page is anterior. Scale bar = 1 mm.

In his monograph on the osteology and evolution of plethodontid salamanders, Wake (1966, p. 20-22) identified three morphotypes among plethodontid vomers: a Hemidactyliine pattern, a Plethodonine pattern, and a pattern I refer to as the Desmognathine group (Figs. 3.2-3.5; Wake, 1966, Figure 8). He included in the Hemidactyliine group *Gyrinophilus, Pseudtotriton, Stereochilus, Eurycea, Typhlotriton,* and *Hemidactylium*. Within the Plethodonine group were *Plethodon, Ensatina, Aneides, Hydromantes, Batrachoseps, Bolitoglossa, Oedipina, Pseudoeurycea, Chiropterotriton, Parvimolge, Lineatriton* and *Thorius. Desmognathus* and *Phaeognathus* belong to the Desmognathine group.

Figure 3.2. Representative vomers showing the **A**. Hemidactyliine pattern (*Gyrinophilus porphyriticus*, NCSM 82389), **B**. Plethodonine pattern (*Plethodon yonahlossee*, JIM 0794), and **C**. Desmognathine pattern (*Desmognathus quadramaculatus*, JIM 0811). Right vomers, palatal view. Top of page is anterior. Scale bars = 1 mm.

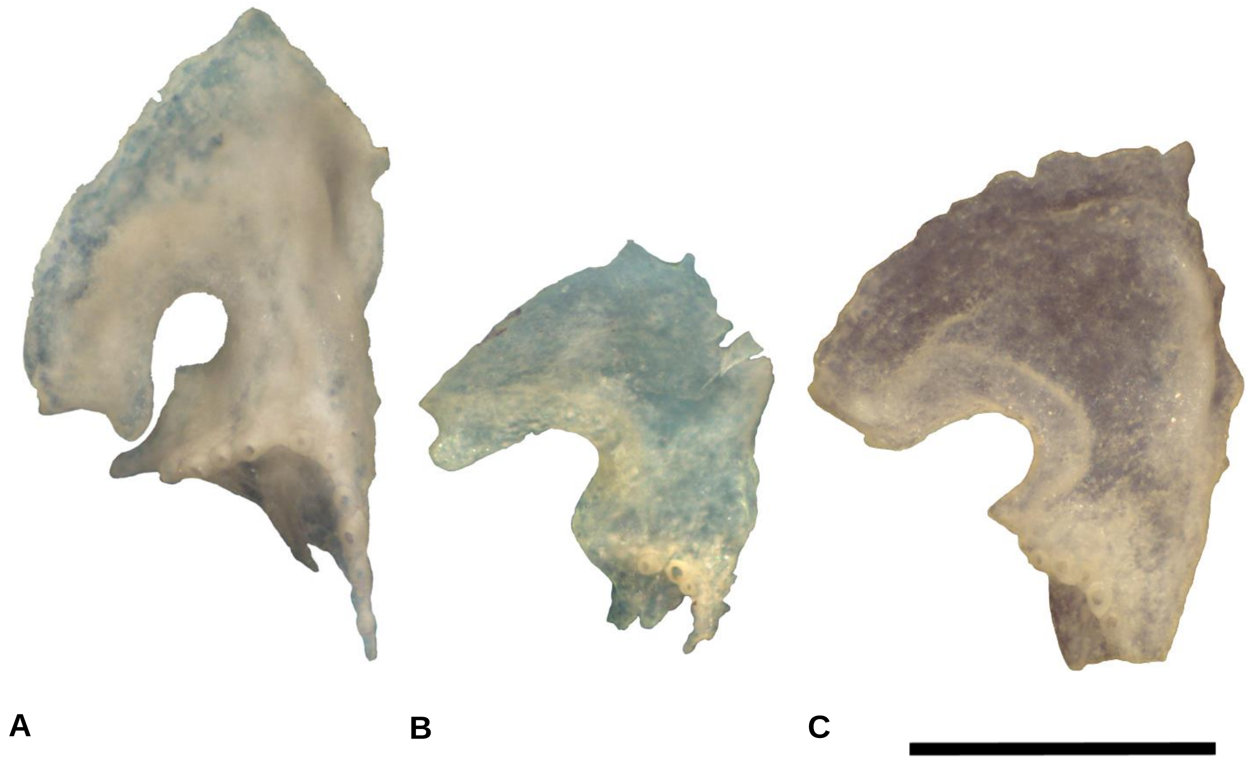

Figure 3.3. Additional representative vomers with the Hemidactyliine pattern. **A**. *Stereochilus marginatus* ETVP 2905, **B**. *Eurycea bislineata bislineata* JIM 0799, and **C**. *Eurycea cirrigera* DCP 4510. Right vomers, palatal view. Top of page is anterior. Scale bars = 1 mm.

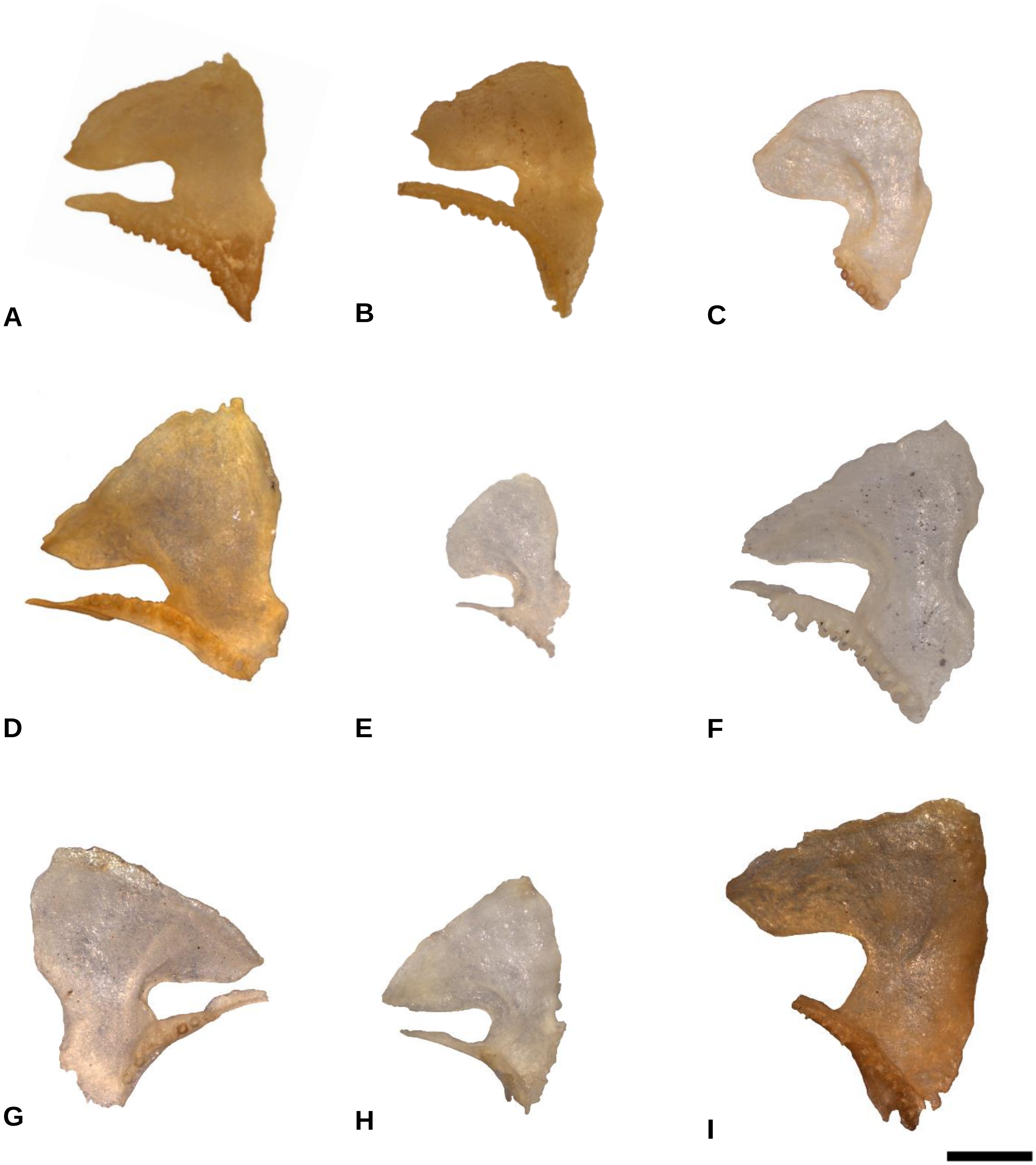

Figure 3.4. Additional representative vomers with the Plethodonine pattern. **A**. *Hydromantes genei* JIM 1146 and **B**. *H. italicus* JIM 1163, **C**. *Aneides ferreus* NVPL 6957, **D**. *P. yonahlossee* JIM 0794, **E**. *P. neomexicanus* NVLP 6967, **F**. *P. glutinosus glutinosus* JIM 0786, **G**. *P. jordoni* BWS 946, **H**. *P. dunni* NVPL 6976, **I**. *Pseudoeurycea belli* (uncataloged). Top of page is anterior. Scale bar = 1 mm.

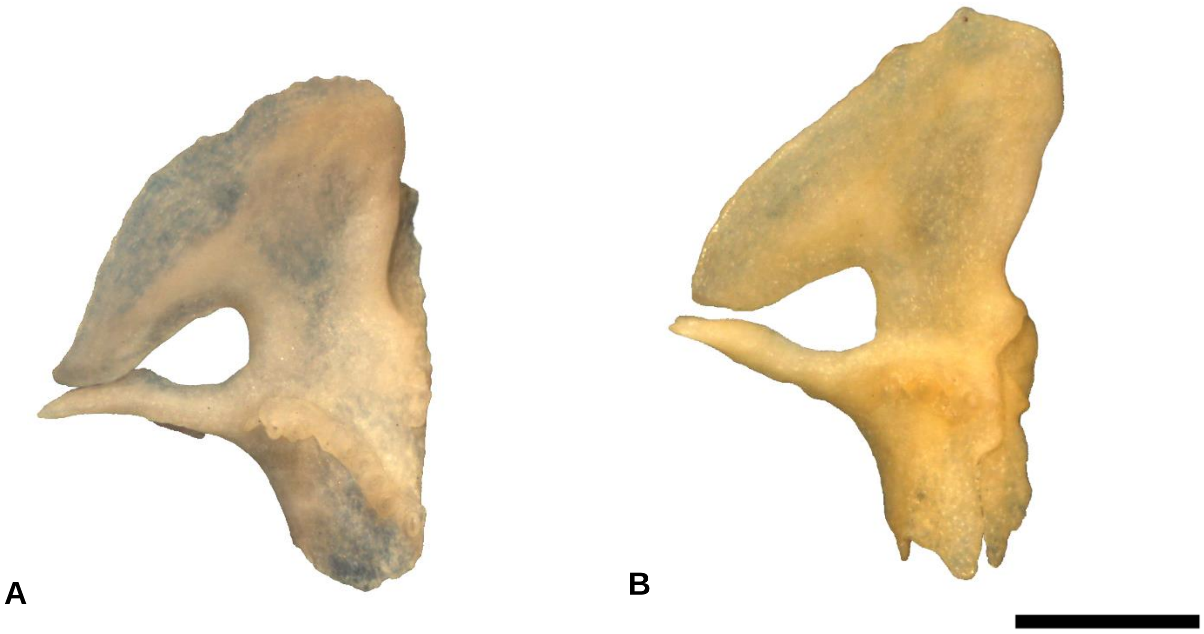

Figure 3.5. Additional representative vomers with the Desmognathine pattern. **A**. *Desmognathus brimleyorum* ETVP 2904 and **B**. *Desmognathus monticola* JIM 0808. Top of page is anterior. Scale bar = 1 mm.

Hemidactyliine-pattern vomers have bony posteriolateral growth (postdentigerous process); open and moderately sized fontanelles; preorbital process present primitively; tooth series “sharply arched”. *Gyrinophilus, Pseudotriton, Stereochilus*, and *Eurycea spelaea* (*Typhlotriton spelaeus*) share additional characteristics: well developed preorbital processes, extending beyond lateral margins of internal nares, not extending beyond vomerine body marings; anterior and posterior portions of tooth series continuous; vomerine tooth sries originates on preorbital process, proceeds anteriomedially, turning sharply almost at the midline to proceed posterolaterally. In *Stereochilus marginatus*, lateral margins of the vomerine body projects a little posteriorly beyond the preorbital process, drawn into spinous posterolateral processes, diagnostic of the genus; preorbital processes directed strongly posterolaterally, not overlapped by the body process. In *Eurycea spelaea* (*Typhlotriton spelaeus*), the vomerine tooth series is very sharply arched in old adults; posterior portion of each series curves back on itself as it leaves the vomer proper; the “body of the vomer is unusual in the genus and bears a posteriorly directed process that fomrs the lateral margins of the nares.” Other *Eurycea* species have short preorbital process that do not reach the lateral edges of the internal nares. *Hemidactylium* has a slender, toothless preorbital process that extends to lateral margins of internal nares; anterior tooth series arch anteromedially, though less so than other members of the group. Genera with this morphotype belong to two closely related tribes, Hemidactyliini (*Hemidactylium*) and Spelerpini (today containing *Eurycea, Gyrinophilus, Haideotriton, Pseudotriton, Stereochilus,* and *Urspelerpes*), both of which belong to the subfamily Hemidactyliinae (Wake, 2012).

Plethodonine-pattern vomers bear tooth rows that reach their anterior extent on the preorbital process, not near the midline as in the Hemidactyliine-pattern; no posteriolateral vomerine growth (postdentigerous process); preoribtal processes relatively slender, extend to at least the lateral margins of the internal nares (except in *Batrachoseps, Thorius,* some species of *Aneides* [*A. ferreus, A. flavipunctatus, A. lugubris*], and some species of *Chiropterotriton* [*C. bromeliacia, C. dimidiatus,* and *C. nasalis*]). Preorbital process varies, “virtually absent’ in most *Batrachoseps* species, while extending beyond lateral margin of vomerine body in *Ensatina*. The Plethodonine-pattern is represented in the plethodontid tribes Aneidini (*Aneides*), Ensatinini (*Ensatina*), Hydromantini (*Hydromantes* and *Karsenia*), Batrachosepini (*Batrachoseps*), Bolitoglossini (*Bolitoglossa, Bradytriton, Chiropterotriton, Cryptotriton, Dendrotriton, Ixalotriton, Nototriton, Nyctanolis, Oedipina, Parvimolge, Pseudoeurycea,* and *Thorius*), and Plethodontini (*Plethodon*) (Wake, 2012).

In the Desmognathine configuration, the vomerine teeth are in a short, arched series that does not extend onto the preorbital process. Anterior teeth may be lost in larger individuals of *D. monticola* and *D. quadramaculatus*. In *Phaeognathus*, the tooth row is relatilvey long and straight, with slight anteriolateral curvature, located much more posteriorly than in other plethodontids. Tooth presence is variable in *Leurognathus*. *Desmognathus* and *Phaeognathus* belong to the tribe Desmognathini, in the subfamily Plethodontinae (Wake, 2012).

The tribes Batrachosepini, Bolitoglossini, Hemidactyliini, and Spelerpini make up the subfamily Hemidactyliinae. The subfamily Plethodontinae is comprised of Aneidini, Desmognathini, Ensatinini, Hydromantini, and Plethodontini (Wake, 2012). Based on the occurrence of a Plethodonine pattern of vomerine morphology in both subfamilies, one may suppose it is the primitive state for the group.

Vomers with tooth rows that reach their anteirormost point more medially than the preorbital process, teeth that extend onto a preoribtal process, and a postdentigerous process are also seen in species of *Rhyacotriton*.

THE GRAY FOSSIL SITE

The GFS has the earliest fossil record of salamanders in the Appalachian Mountains and possesses the most diverse pre-Pleistocene salamander fauna on the continent. Four plethodontids were previously identified from the GFS on the basis of vertebrae: two morphotypes of *Plethodon* sp. (designated Type A and Type B, on the basis of atlases), *Desmognathus* sp., and a member of the subfamily Spelerpini (on the basis of double spinal nerve foramina) (Boardman and Schubert, 2011). This is the earliest report of *Desmognathus*, which is estimated to have split from other plethodontids around 7 Ma (Chippindale et al., 2004; Tihen and Wake, 1981).

The GFS displays a faunal and floral connection to two main regions: East Asia and Western Europe. Taxa today found primarily in Asia that have been recovered from the GFS include the red panda (Wallace and Wang, 2004) and Asian *Vitis* grapes (Gong et al., 2010). A European Badger has also been recovered from the GFS (Wallace and Wang, 2004). Recently, a third influence on the site has come to light. Mead, et al. (2012) described *Heloderma* osteoderms from the GFS. Modern *Heloderma suspectum* and *H. horridum* ranges extend from the hot, dry Sonoran desert to the tropical coast of Guatemala (Beck, 2005), though they are most common in tropical deciduous forests (Beck, 2005). In addition to *Alligator*, *Heloderma* remains at the GFS indicate a warmer climate when deposition occurred.

(5) Main Body

MATERIALS AND METHODS

FOSSIL COLLECTION AND IDENTIFICATION

Microfossil remains at the GFS are regularly collected by wet screen sieving using 1.7 mm mesh box screens. Recovered bone is picked under a dissecting microscope and sorted by class and order. Initial identifications were made under a light microscope utilizing modern specimens either housed at or loaned to East Tennessee State University. Collections utilize include those from the East Tennessee State University Vertebrate Paleontology Laboratory (ETVP), East Tennessee State University Neogene Vertebrate Paleontology Laboratory (NVPL), North Carolina Museum of Natural History (NCSM), and from the personal collections of Blaine W. Schubert (BWS), Jim I. Mead (JIM), and Dennis C. Parmley (DCP). Characters utilized to identify the fossil as either Plethodontidae or Rhyacotritonidae include the posterior situation of the tooth row, extension of the tooth row onto the preorbital process, and a postdentigerous process.

TAXON SELECTION

With three significant sources of influence, any study on GFS material must consider taxa beyond those that occur in the region today. Historically there has been a tendency for paleoherpetologists to make identifications based on the local, modern fauna and proceed to comment on biogeography (Bell et al., 2010). In order to avoid this circular reasoning, any taxa with similar characters to the fossil ETMNH 18219 is included. In particular, *Rhyacotriton* species possess vomers superficially similar to the fossil specimen as well as to Wake's Hemidactyliine vomer morphotype. *Rhyacotriton* vomers (Fig. 3.1) have a vomerine tooth row on the posterior end of the bone that extends onto the preorbital process and have a postdentigerous process. Because *Rhyacotriton* has never been found in the fossil record, the timing and locating of their origin, as well as their former extent, are unknown.

MORPHOLOGICAL DATA AND ANALYSIS

Landmarks were utilized to capture the shape of the vomerine tooth row in relation to the medial point of inflection of the choana as well as the anterior and posterior extent of the medial edge of the bone. Not all taxa possess a postdentigerous process, so no landmarks were placed in that region. Representatives of all three plethodontid vomer morphotypes identified by Wake (1966) are included. *Rhyacotriton* is included due to its similarity to Hemidactyliine in having a tooth-bearing preorbital process and a postdentigerous process. Anterior features, including the lateral extent of the anterior process as well as the relative mediolateral position of the choana, were excluded as they would not aid in fossil identification. Additionally, the postorbital process is excluded due to lack of homologous structure on all taxa.

Vomers were photographed using a Lexar microscope camera with the bone oriented such that the medial margin of the bone was parallel to the vertical axis of the view finder. Landmarks were digitized using tpsDIG2 software (Rohlf, 2013a). All points were considered in the same dataset, appended using tpsUtil (Rohlf, 2013c) and Procrustes superimposed using tpsSuper (Rohlf, 2013 b). IBM SPSS statistical software (version 21) was used to conduct a principal component analysis (PCA) and a discriminant analysis.

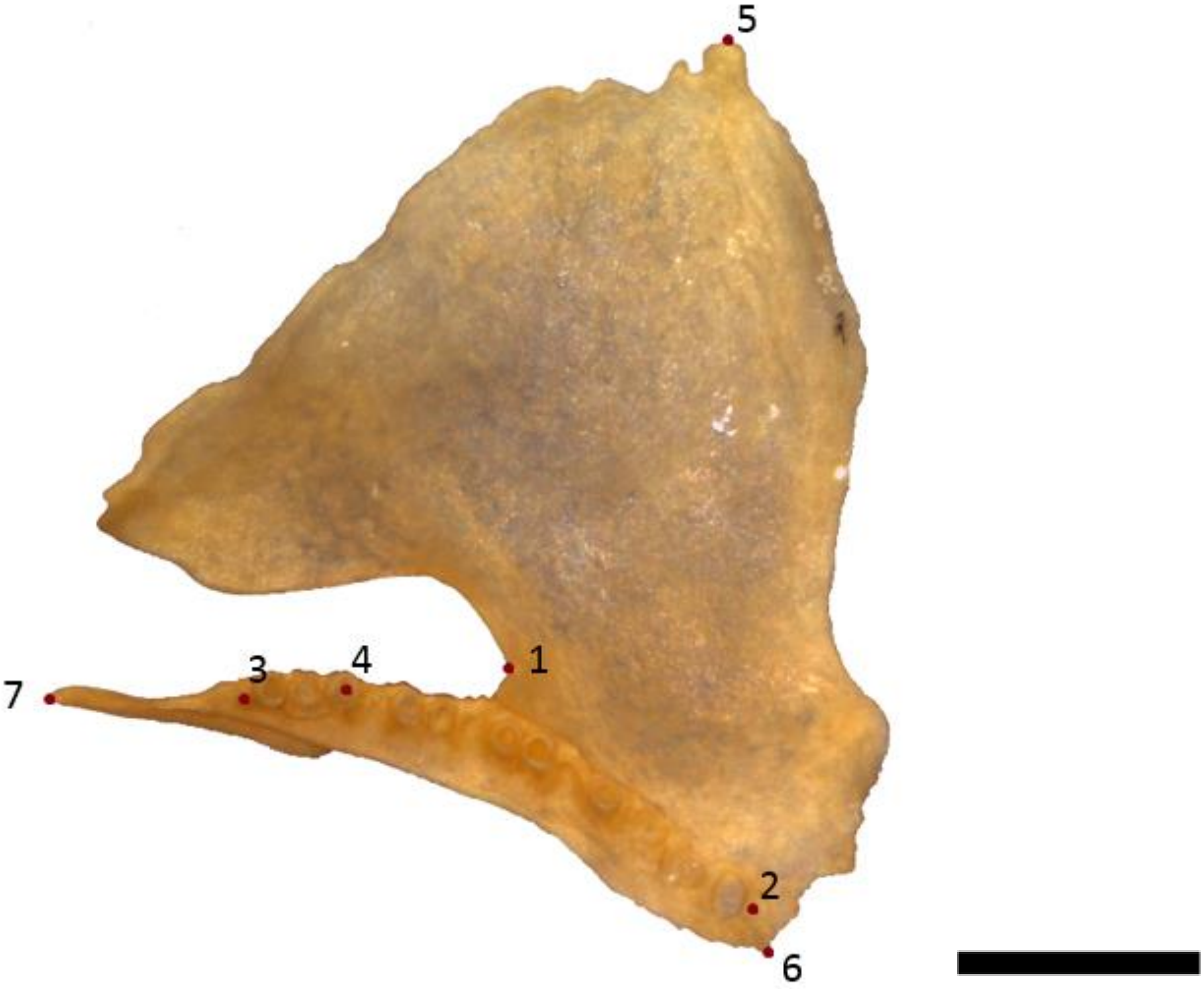

Figure 3.6. Placement of landmarks used in the study, on *Plethodon yonahlossee* (JIM 0794). **1** Medial point of inflection of choana margin, **2** Posterior extent of tooth row, base of tooth pedicelle, **3** Lateral extent of tooth row, base of tooth pedicelle, **4** Anterior-most extent of tooth row, base of tooth pedicelle, **5** Anterior extent of anterior process, **6** Posterior extent of medial edge, **7** Lateral point of inflection of preorbital process.

RESULTS

DESCRITPTION OF FOSSIL

ETMNH 18219 (Fig. 3.7) is a right vomer with the following distinguishing characters: a preoribtal process, a dentigerous row that extends onto the preorbital process, and a postdentigerous process. Ten tooth pedicelles without crowns remain. Medial margin approximately 2.3 mm anteroposteriorly. Anterior process appears broken anteriolaterally, though this is often poorly ossified in recent specimens.

Figure 3.7. ETMNH 18219, a right vomer from the Gray Fossil Site, in palatal view. Top of page is anterior. Scale bar = 1 mm.

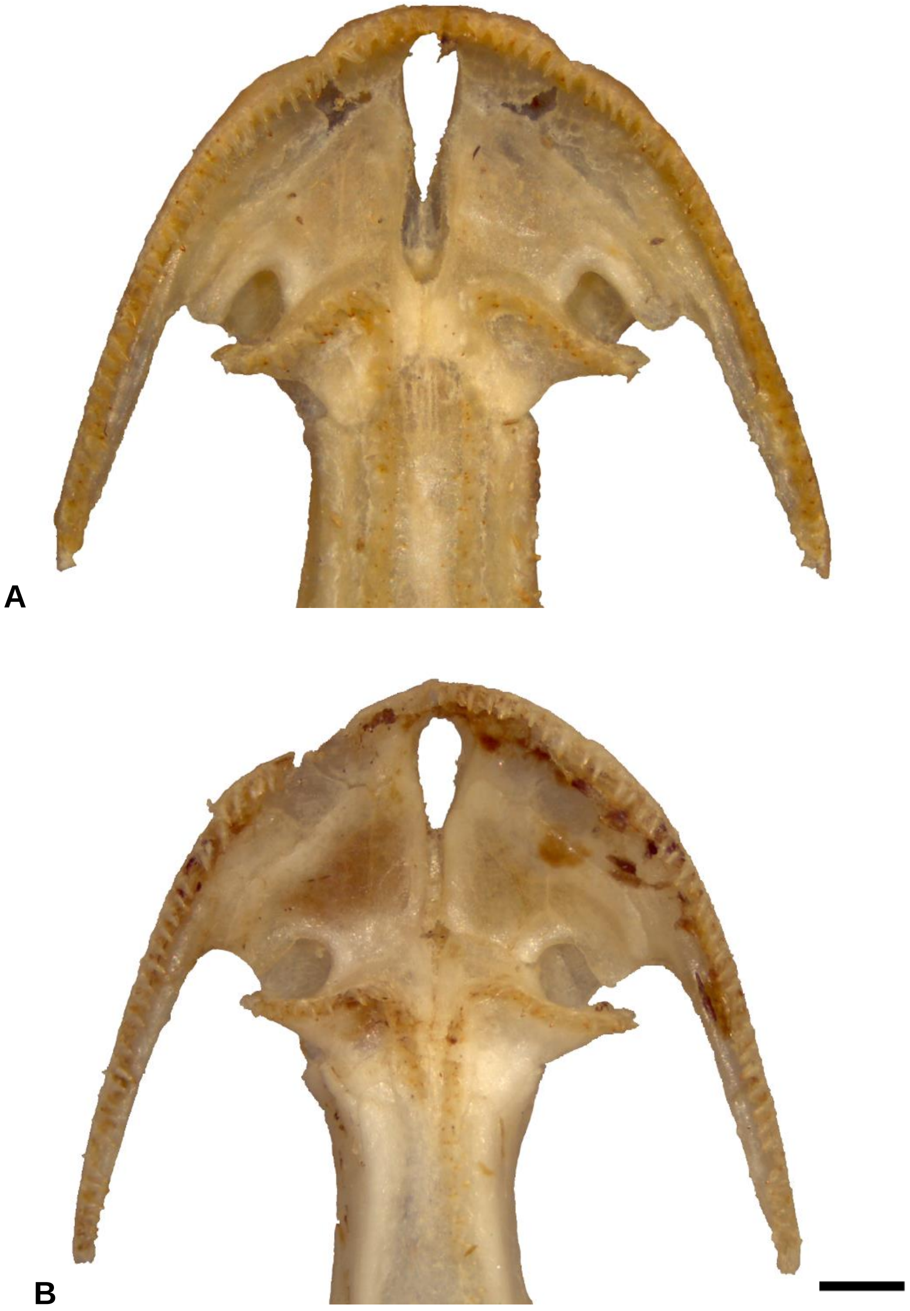

Figure 3.8. Palatal views of **A**. male and **B**. female *Gyrinophilus porphyriticus* (NCSM 82390 and NCSM 82389), demonstrating possible sexual variation in vomerine morphology. Top of page is anterior. Scale bar = 1 mm.

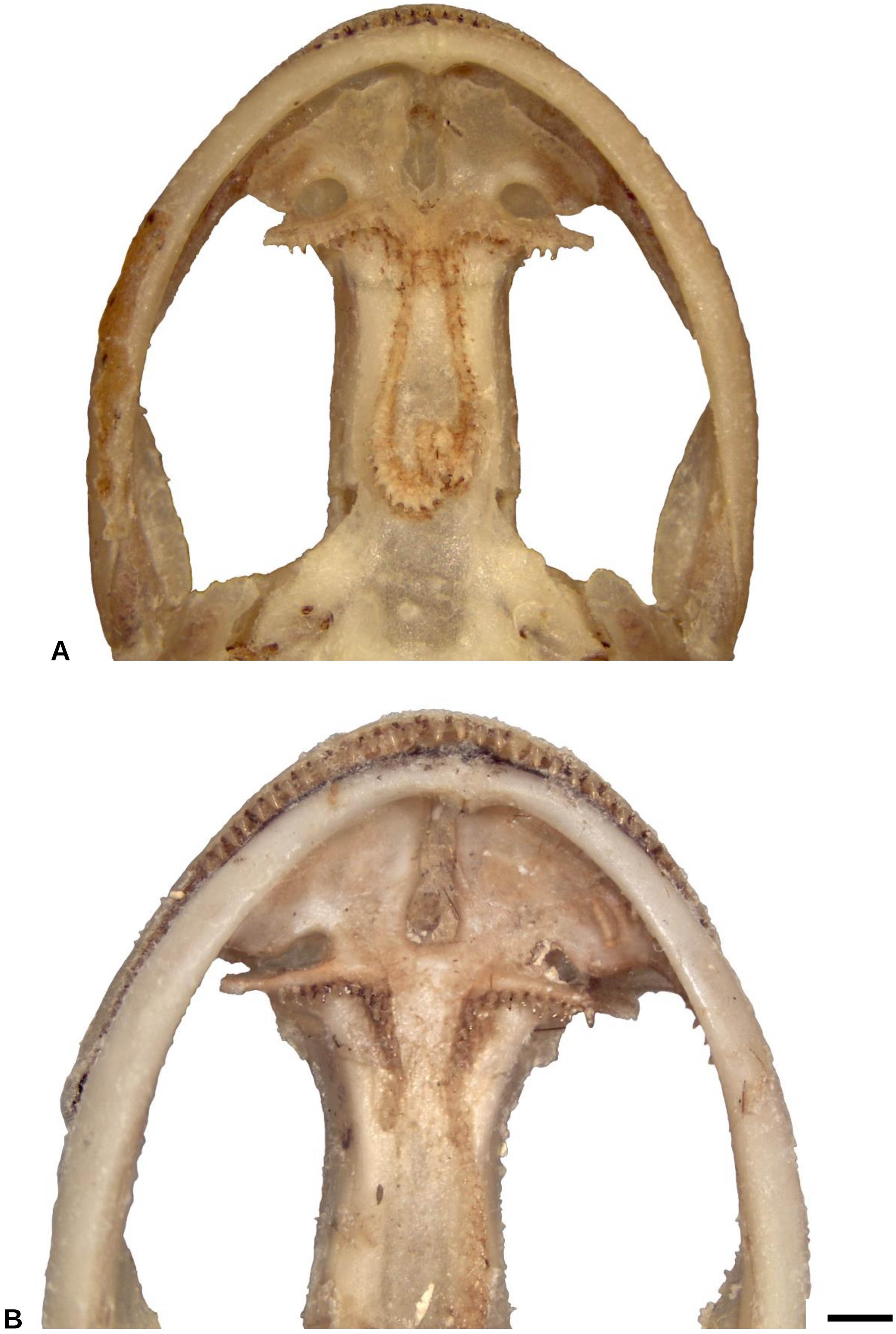

Figure 3.9. Palatal view of *Pseudotriton ruber*. **A**. NCSM 82393 and **B**. #35. Top of page is anterior. Scale bar = 1 mm.

The postdentigerous process of *Rhyacotriton variegatus* is broad mediolaterally and thin anteroposteriorly (Fig. 3.1). In contrast, the postdentigerous process of ETMNH 18219 extends more posteriorly and is narrower mediolaterally (Fig. 3.7). In this respect, the fossil more closely resembles members of Spelerpini. *Stereochilus marginatus* and most *Eurycea* species lack an elongate preorbital process (Fig. 3.2). ETMNH 18219 shares with *Gyrinophilus* and *Pseudotriton* a postdentigerous process of similar proportions and a tooth row that extends onto a well-developed preorbital process.

GEOMETRIC MORPHOMETRICS

A discriminant analysis (Fig. 3.10) showed separation of the three Plethodontidae morphotypes identified by Wake (1966) as well as *Rhyacotriton* and the fossil specimen. The first function explained 44.8% of the variance and had an eigenvalue of 2.738. The second function explained 38.7% of the variance with an eigenvalue of 2.431. A third function explained the remaining 15.5% variance with an eigenvalue of 0.949. The first function served to separate the Desmognathine morphotype from all other categories.

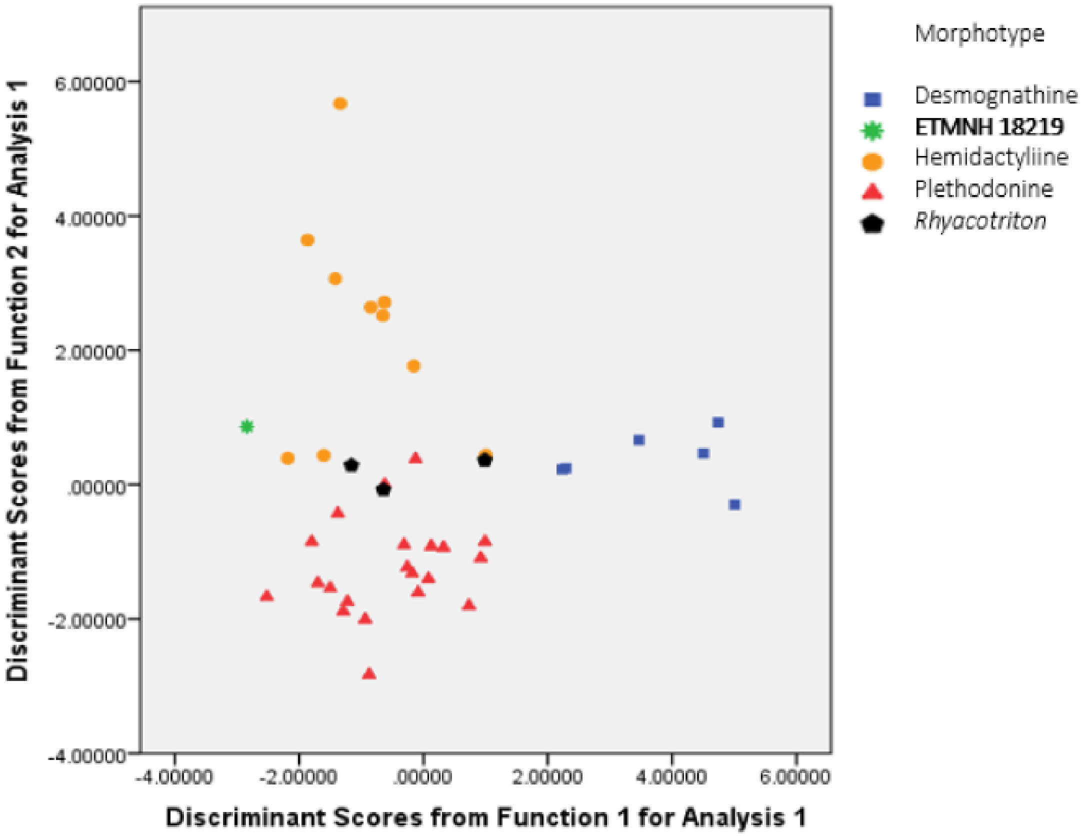

Figure 3.10. Discriminant analysis of the three morphotypes identified by Wake (1966), *Rhyacotriton*, and the fossil taxa ETMNH 18219.

A PCA (Fig. 3.11.) was sufficient to separate Spelerpini genera plus *Rhyacotriton* and the fossil into two distinct groups: one containing *Stereochilus* and *Eurycea* and another with the remaining taxa *Gyrinophilus*, *Pseudotriton*, *Rhyacotriton*, and the fossil. The first component explained 55.098% of the variance with an eigenvalue of 7.714. The second component explained 15.656% of the variance with an eigenvalue of 2.192. Together the two explain 70.754% of the variance cumulatively.

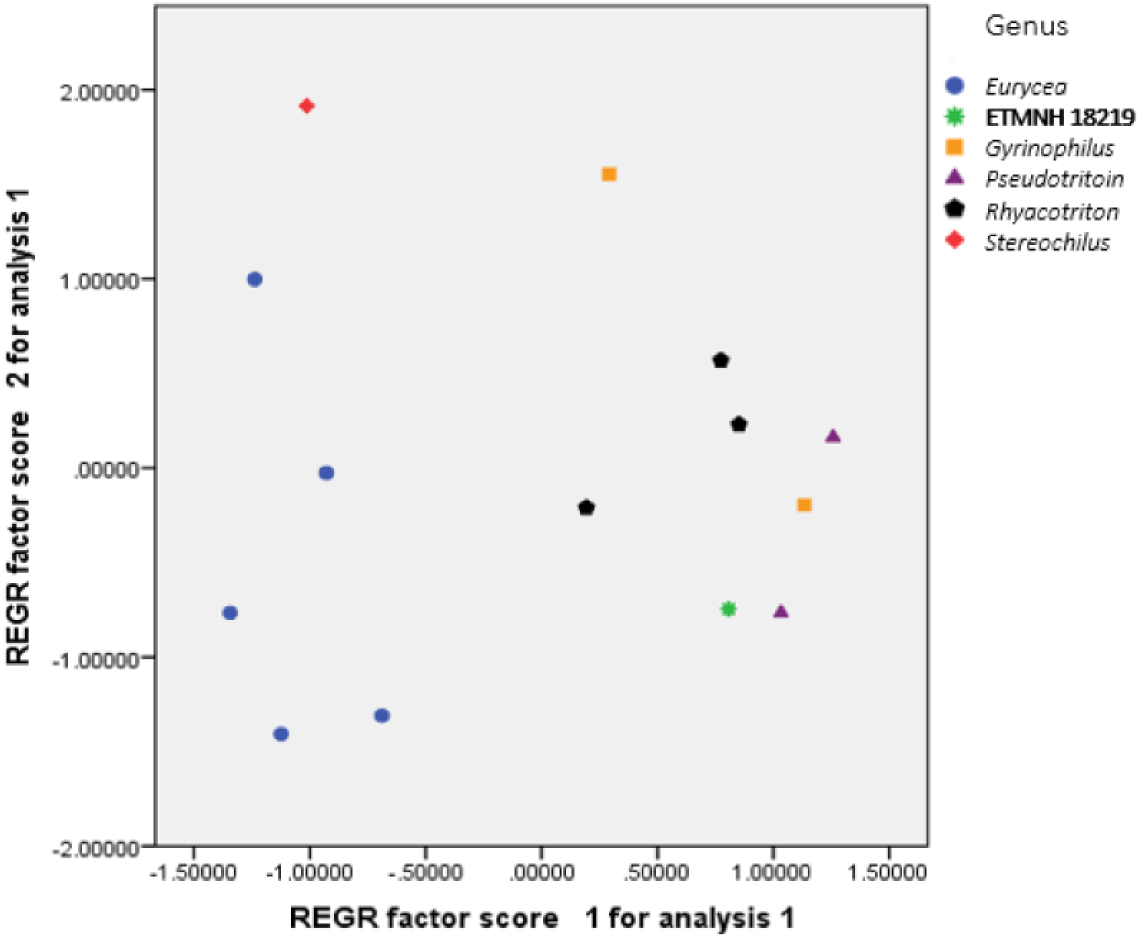

Figure 3.11. PCA of Spelerpini genera (without *Hemidactylium*), *Rhyacotriton*, and the fossil ETMNH 18219. *Stereochilus* and *Eurycea* have a morphotype distinct from the other genera, including the fossil taxa.

DISCUSSION

Geometric morphometric analyses that sought to capture the shape of the vomerine tooth row seem to support Wake's original Plethodontidae morphotypes, even when a distinctive character (the presence of a postdentigerous process) is excluded from study. PCA analysis suggests that ETMNH 18219 is not *Eurycea* or *Stereochilus*. Morphologically, the fossil specimen has a more developed preorbital process than either *Eurycea* (except *E. spelaea*) or

Stereochilus, and this seems to drive the separation seen in the PCA analysis. Wake (1966) notes that *Eurycea spelaea* (*Typhlotriton spelaeus*) possesses a tooth series that extends onto the preorbital process, and without examining this species, *Eurycea* cannot be entirely ruled out as a possibility for ETMNH 18219.

Though this specimen most closely resembles *Gyrinophilus porphyriticus* NCSM 82389, a formal diagnosis cannot be made at this time given the amount of variation seen between male and female *G. porphyriticus*. A more thorough review of Spelerpini cranial morphology is required. Bolitoglossinae requires similar attention, as numerous species have been identified in recent years on the basis of genetic, coloration, or wrist morphology data (including Townsend et al., 2010; Aldemar et al., 2013; Garcia-Gutierrez et al., 2013). One species, *Ixalotriton niger*, appears to share the same vomerine characters seen in ETMNH 18219 (Wake and Johnson, 1989 Fig. 3.2). The possibility remains that ETMNH 18219 represents a unique Eastern North American lineage of salamanders occurring to the south today. In withholding a formal diagnosis, this study seeks to avoid the biases so common in historical herptile fossil descriptions, namely, justifying an identification due to the species' presence in the area today (Bell et al., 2010). Nonetheless, possible implications of a Spelerpini identification are outlined below, given that at least on member of this subfamily is present at GFS (Boardman and Schubert, 2011).

SUPPORT TO ENVIRONMENTAL RECONSTRUCTION OF GFS

Vasilyan and Böhme (2012) identified two vomerine dental arrangements correlated with feeding styles. One arrangement, designated by Vasilyan and Böhme as 'zigzag', is seen in their 'pond-type' salamanders that utilize tongue protraction and use their vomerine teeth to hold onto small terrestrial invertebrates. This morphology is seen in adult, terrestrial hynobiids, plethodontid, and salamandrid salamanders. In contrast, a second arrangement is seen in 'stream-type' salamanders including cryptobranchids and larval forms of other salamanders, in which feeding in running water requires transversely oriented vomerine teeth to prevent prey from escaping the mouth as water is released. The ontogeny of these two dental arrangements was demonstrated in Hynobiidae by Xiong et al. (2014). Species of the aquatic genera *Liua, Batrachuperus, Pachyhynoius*, and *Paradacylodons* have transverse vomerine tooth rows, while terrestrial species within *Hynobius* and *Salamandrella* possess more developed tooth rows that curve posteriorly. Juvenile *Hynobius guabangshanensis* have aquatic vomerine tooth morphologies that transform into the terrestrial pattern when the aquatic larvae metamorphoses.

Convergence in vomerine morphology may only occur on the most basic level, such as when a 'pond-type' salamander becomes paedomorphic and acquires the 'transversely oriented' vomerine teeth seen in the 'stream-type' salamanders. For example, though the general skull proportions of *Karsenia koreana* are more similar to those found in *Plethodon*, their vomerine morphology is conservative, most similar to *Aneides* and *Ensatina* to which *K. koreana* is more closely related (Min et al., 2005).

The presence of a 'pond-type,' post-metamorphic salamander supports the interpretation of GFS as a moist environment that can sustain terrestrial plethodontid populations. *Gyrinophilus porphyriticus* and both species of *Pseudotriton* have an aquatic larval stage and metamorphose. *Stereochilus marginatus* is completely aquatic as an adult without becoming neotenic, living in drainage ditches, small ponds, and calm streams (Hairston, 1987, pg 85). *G. porphyriticus* has the longest larval period of any plethodontid, metamorphosing after three to five years (Bruce, 1980). *Gyrinophilus* at the GFS could indicate the presence of a local perennial pond. Adult

Pseudotriton can be found at ‘considerable distances’ from sources of water; when they do occur near bodies of water, they are quiet, silted ponds (Martof and Rose, 1962). *Gyrinophilus porphyriticus* typically inhabits the rocky substrate that surrounds cool springs and streams (Martof and Rose, 1962). Both *Pseudtoriton* spp. and *Gyrinophilus porphyriticus* tend to burrow, with more robust snouts than those of the insectivorous *Eurycea* (Martof and Rose, 1962).

(6) Concluding Remarks

This study exemplifies the identification power of cranial elements. Whereas vertebral characters were only able to identify a specimen to the subfamily level (Boardman and Schubert, 2011), a tooth bearing cranial bone has led to a generic level classification.

Nonetheless, ruling out most *Eurycea* species as well as *Stereochilus* demonstrates the greater resolution power of cranial material compared to vertebrae. Given the estimated divergence times of plethodontid genera (for example, *Desmognathus* may have diverged from other plethodontids around 7 Ma [Chippindale et al., 2004; Tihen and Wake, 1981]), one would expect a more diverse salamander fauna than has been identified. Current salamander identifications reflect only a fraction of the potential diversity during the Miocene. Only through continued wet screen sieving and microscopic sorting will the full extent of salamander diversity be understood.

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CHAPTER 4

CONCLUSION

Cranial characters facilitate greater taxonomic resolution of fossil salamanders than what can be ascertained from exclusively vertebral characters. Vertebrae do not typically demonstrate discrete features that enable species-level identification; and genus-level identification may not always be possible (Boardman and Schubert, 2011). In the case of ambystomtatids, dental traits including the presence of a diastema on the vomer at the level of the choana, and the number of tooth rows on all dentigerous elements may be of utility (Tihen 1958). Such characters in isolation are unable to discern species, though the latter trait may identify the *Ambystoma* subgenus *Lingulaepsus*. Life stage may be ascertained from either cranial or vertebral development: *Ambystoma* premaxillae become more robust and articulate medially after metamorphosis, and the closure of the notochordal canal of trunk vertebrae indicates both sexual maturity and terrestriality. Cranial characters are more powerful when used in tandem with vertebral characters, as in the case of ETMNH 8045 (Chapter 2). ETMNH 8045 vertebrae have centrum proportions corresponding to Tihen's (1958) "*A. maculatum* group", and the cranial characters of ETMNH 8045 are consistent with and compare favorably with modern *A. maculatum*.

In the case of isolated elements, vertebral comparisons are not inherently necessary for identification. ETMNH 18219, an isolated vomer, demonstrates a morphotype seen in most terrestrial-feeding, 'pond-type' salamanders (Vasilyan and Böhme 2012). This morphotype alludes to an environment suitably moist to support terrestrial salamander populations. Additional characters of the vomer (extent and curvature of the dentigerous process, extent of preorbital process, and presence of postdentigerous process) indicate affinity to Plethodontidae or Rhyacotritonidae. Character analysis, visual comparison, and geometric morphometrics have demonstrated the taxonomic power of the vomer: cf. *Gyrinophilus* adds to Boardman and Schubert's (2011) identification of a Spelerpinae-type plethodontid.

Phylogenetic bracketing utilizing modern species, following Schubert and Wallace (2006), presents two seemingly contrasting interpretations of the GFS. *Ambystoma maculatum* preferentially breed in vernal pools or wetlands to avoid fish that will prey upon salamander eggs and larvae (Turtle 2000). Spotted Salamanders also show high fidelity to their breeding pools, and spend the majority of the year in the upland forests surrounding the ponds (Windmiller 1996; Petranka 1998). However, adults may range as far as 1 km from their pond before migrating back for the breeding season (Homan et al. 2004). Fossil *Ambystoma* cf. *A. maculatum* does not disprove the interpretation of the GFS as a permanent pond environment; rather, the area surrounding the site may have flooded seasonally or held standing wetlands unable to support fish. Additionally, ETMNH 8045 is found in association with a fish vertebrae, and *A. maculatum* may over-winter in larger ponds. ETMNH 18219, the vomer of cf. *Gyrinophilus*, supports a permanent pond interpretation. The modern terrestrial species of *Gyrinophilus, G. porphyriticus*, requires 3 to 5 years to complete the aquatic larval stage.

Further refinement of the GFS salamander fauna is feasible. Boardman and Schubert (2011) identified trunk vertebrae from neotenic *Ambystoma* individuals. Neotenic *A. maculatum* populations are unlikely to become established at the GFS, given the frequency with which small fish fossil are recovered (Wallace, personal commun. 2015). Multiple *Ambystoma* species coexist today throughout much of the eastern United States. Non-vertebral elements that may aid identification of sympatric Mole Salamanders include vomers (which will lack a diastema in *A.*

tigrinum and their closest relatives) and first ribs (which bear an epipleural process in *A. opacum* and *A. talpoideum*) (Holman 2006).

Multiple species of Spelerpinae-type plethodontids may also coexist in the GFS fauna. Modern *Gyrinophilus porphyriticus* are terrestrial predators, while modern *Stereochilus marginatus* are aquatic (though not neotenic) as adults. *Gyrinophilus porphyriticus* and species of *Pseudotriton* burrow to feed and may coexist with insectivorous *Eurycea* (Martof and Rose 1962). Vomers would continue to be a useful element to recover. Premaxillae may also have utility in distinguishing transformed *Gyrinophilus* and *Pseudotrion* (Martof and Rose 1962).

Further work at the GFS will depend heavily upon the continued application of fine-screened sediment processing and microscopic sorting. Vertebrae are sufficiently large and robust to be recovered utilizing crude processing methods. However, identification of non-vertebral elements is more difficult to the unskilled eye and requires specific training of laboratory workers. As the importance of salamander cranial bones becomes more apparent, and their utility in paleoenvironmental reconstructions is demonstrated, these elements will receive their due attention.

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