PSEUDO-CRYPTIC SPECIATION AND ADAPTIVE MORPHOLOGY
IN THE PALEONTOLOGICAL RECORD:
EXAMPLES FROM THE COCCOLITHOPHORE BRAARUDOSPHAERA
AND THE AQUATIC BELOSTOMATID INSECT TRIASSONEPA

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ABSTRACT OF THE THESIS

Pseudo-Cryptic Speciation and Adaptive Morphology in the Paleontological Record: Examples from the Coccolithophore *Braarudosphaera* and the Aquatic Belostomatid Insect *Triassonepa*

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Evolution is the process by which species change through time, and understanding the evolutionary processes that occur today allows us to infer the evolutionary history of organisms from the distant past. In this thesis, the evolutionary processes and adaptations of two distantly related organisms that inhabit different environments were studied.

Chapter one investigated the size variation in pentaliths of *Braarudosphaera bigelowii*, a marine haptophyte that is abundant in the Lower Paleocene Zumaia section of northern Spain, and explored the idea that the variability in pentalith size represents pseudo-cryptic speciation. A cluster analysis in RStudio (mclust package) yielded two size groups, which were interpreted as two unique pseudo-cryptic species: form X (< 4.5 μm) and form Y (≥ 4.5 μm). In addition, a sudden increase in *B. bigelowii* abundance occurred near the Paleocene C27n/26r magnetic reversal and formed a “peak acme” zone
of approximately four meters. Isotopic analyses of organic matter revealed that the onset of this acme coincided with an abrupt negative excursion (3.5‰) in $\delta^{13}C_{\text{org}}$. Biostratigraphic correlation between the Zumaia section and the Danian/Selandian boundary section at Qreiya, Egypt confirmed that this isotopic shift correlates with a hyperthermal event (Latest Danian Event), and suggests that changes in the climate may have affected the abundance of $B. \text{bigelowii}$ at Zumaia.

Chapter two described a new species of aquatic belostomatid insect, $Triassonepa solensis$, from the Upper Triassic, lacustrine Cow Branch Formation that outcrops along the Virginia/North Carolina border. It was placed in a new genus due to the unique structure of its raptorial foreleg, in which the tarsus is elongate and opposed to the tibia + femur. Due to the exclusive preservation of terrestrial adult insects and surface-dwelling aquatic insects, in addition to the geochemistry of the deposit, it is likely that the Cow Branch Formation was a saline, alkaline, rift valley lake.
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General Introduction

Evolution, the process that causes genetic, morphological, and/or behavioral changes in populations through time, occurs on all scales and in all environments, from the microscopic oceanic plankton, to the largest of terrestrial animals. In order to understand evolution, we must first define a “species concept.” Biologists define species as groups of reproductively isolated, interbreeding individuals. With the advent of genetic analytical techniques, biologists are now able to use DNA in conjunction with behavioral patterns to distinguish between living species. However, defining an extinct species is much more difficult, as using behaviors or genetic information is not possible. Paleontologists must instead define species based on shared similarities in morphology. Fortunately, paleontologists can also use the evolutionary processes occurring today in order to better understand the evolutionary history of the organisms that lived in the distant past. In this thesis, I examine evolutionary processes and adaptations in organisms from two different kingdoms of life and living in different environments.

*Braarudosphaera bigelowii* is a planktonic, marine, unicellular “protist” (Division Haptophyta) that appeared in the Early Cretaceous, became extremely abundant at specific times, as during the Oligocene, and is still present in the modern ocean, although usually rare. Belostomatids are cosmopolitan, predatory, freshwater insects (Kingdom Animalia; Phylum Arthropoda) with a fossil record that extends back to the Late Triassic.

In the first chapter, I explore the idea that the large variability in pentalith size of *Braarudosphaera bigelowii* during the Early Paleocene represents pseudo-cryptic speciation. For this I have analyzed the radii of pentaliths from 59 samples from the well-known Zumaia section in northern Spain. Recent genetic analyses have shown that a
single “species” can contain a number of unique genotypes, meaning that the “species” actually consists of a suite of related species. If these species are morphologically identical, they are termed “cryptic species,” whereas if they possess slight morphologic differences that allow their recognition independent of genetics, they are termed “pseudo-cryptic species” (Amato, 2010). The pentalith radius of B. bigelowii can range from 1 µm in living specimens (Takano et al., 2006; Hagino et al., 2009), to almost 16 µm in Oligocene specimens (Steinmetz and Stradner, 1984). Takano et al. (2006) suggested that this size variability might be due to pseudo-cryptic speciation, rather than intraspecific variability. A later genetic analysis by Hagino et al. (2009) determined that within a group of 15 B. bigelowii specimens, there were five unique genotypes that corresponded to pentalith size. This work established that pseudo-cryptic speciation occurs in living B. bigelowii. Based on findings of Hagino et al. (2009), chapter one of this thesis investigates whether pseudo-cryptic speciation also occurred in this species in the past by examining the differences in pentalith size of the Early Paleocene B. bigelowii from Zumaia.

In the second chapter, I describe a new species of aquatic belostomatid insect from the Upper Triassic Cow Branch Formation, and use the species assemblage preserved in the deposit to infer the paleoenvironmental conditions. The Cow Branch Formation is a fossiliferous black shale that outcrops on the border of Virginia and North Carolina. It is a lacustrine deposit that contains numerous plant, fish, and aquatic reptile fossils (Olsen et al., 1978). Most importantly, it preserves a diverse assemblage of insect fossils, many of which are the oldest members of their families.
Insects have a very long geological record that extends back to at least the Devonian, but a recent phylogenomic study has pushed this date back even farther to the Early Ordovician (479 Ma, Kjer et al., 2014). Freshwater insects appear much later in the geological record, in the Upper Triassic Cow Branch Formation, at approximately 225 Ma. A species of belostomatid, herein named *Triassonepa solensis*, is one of the most common insects preserved in this deposit. I discuss the morphology of this aquatic insect and the behavioral characteristics that can be inferred from its morphology.

The insect assemblage preserved in the Cow Branch Formation is quite diverse, with a total of 11 insect orders recovered to date. Most of these insects are aquatic, surface-dwellers (Belostomatidae, Notonectidae) or terrestrial adults (e.g., Hemiptera, Coleoptera, Diptera, Blattodea, Orthoptera). Based on this species assemblage, in addition to the geochemical conditions reported by Liutkus et al. (2010), I discuss the probable paleoenvironmental conditions of the Cow Branch Formation.
Chapter 1

INFERRED PSEUDO-CRYPTIC SPECIATION IN THE COCCOLITHOPHORE SPECIES BRAARUDOSPHAERA BIGELOWII (GRAN AND BRAARUD) DURING THE EARLY PALEOCENE (DANIAN)

Abstract

Pseudo-cryptic speciation occurs commonly in living oceanic plankton. In the coccolithophores, recent genetic studies have revealed that *Braarudosphaera bigelowii* has a number of unique genotypes that correlate well with pentalith size, indicating that this coccolithophore “species” actually contains multiple pseudo-cryptic taxa. In order to look for evidence of pseudo-cryptic speciation in the past, we measured the radii of 2800 *B. bigelowii* pentaliths from the *Braarudosphaera*-rich limestones that contain the GSSP for the Lower/Middle Paleocene (Danian/Selandian) boundary at Zumaia, Spain. Analysis of the radius measurements using the Expectation-Maximization algorithm yielded two size groups, herein termed form X (<4.5 µm) and form Y (≥ 4.5 µm). This result suggests the possibility of at least two pseudo-cryptic species in the Early Paleocene taxon *B. bigelowii*. In addition, there is a sharp increase in overall *B. bigelowii* abundance near the Paleocene magnetochron C27n/26r reversal in the Zumaia section, forming a “peak acme” zone that spans approximately four meters. Isotopic analyses of organic matter show that an abrupt negative excursion of 3.5‰ in δ¹³Corg coincided with the onset of the *B. bigelowii* acme. Biostratigraphic correlation between this section and the Danian/Selandian boundary section at Qreiya, Egypt confirmed that this isotopic shift correlates with a hyperthermal event (Latest Danian Event), and suggests that *B. bigelowii* abundance may have been affected by changing climatic conditions.
1.1. Introduction

As genetic analytical techniques continue to improve, reports of cryptic and pseudo-cryptic species are steadily increasing. Whereas cryptic species are morphologically identical, pseudo-cryptic species exhibit slight morphologic differences that allow their recognition independent of genetics (Amato, 2010). A number of recent molecular studies have shown that pseudo-cryptic speciation is common in coccolithophores (e.g., Geisen et al., 2002; Sáez et al., 2003; de Vargas et al., 2004; Sáez and Lozano, 2005; Hagino et al., 2009), as well as in other oceanic plankton (e.g., Amato and Montresor, 2008; Morard et al., 2009; Amato, 2010; Lundholm et al., 2012).

*Braarudosphaera bigelowii* is an extant coccolithophore species with a long geological record that extends back to the Lower Cretaceous (Berriasian Stage) (Perch-Nielsen, 1985; Bown et al., 1998; Burnett, 1998). Over its 100 million year (Myr) life span, this species has remained morphologically unchanged, with the exception of pentalith size (e.g., Stradner, 1960; Švábenická, 1999), which has ranged from ~1 µm (living cells, Hagino et al., 2009) to ~15.8 µm (Oligocene pentaliths, Steinmetz and Stradner, 1984) in radius. Takano et al. (2006), who first hypothesized pseudo-cryptic speciation in living *B. bigelowii*, also inferred pseudo-cryptic speciation in Turonian *B. bigelowii* based on the two size forms reported from the Bohemian Cretaceous Basin by Švábenická (1999). It is possible that this variation in pentalith size is evidence of pseudo-cryptic speciation through time. More recently, a genetic analysis of 15 living cells of *B. bigelowii* from the seas surrounding Japan revealed five distinct genotypes that correlate well with morphological differences in pentalith size (Takano et al., 2006; Hagino et al., 2009).
This recent discovery of widespread pseudo-cryptic speciation among coccolithophore taxa has profound implications for the study of coccoliths preserved in sediments, as many of the described “species” are possibly suites of cryptic or pseudo-cryptic species. Sáez et al. (2003) and Geisen et al. (2004) alluded to the possibility of pseudo-cryptic speciation in the fossil record, but this phenomenon has not been formally considered as a mechanism for large morphological variations in extinct coccoliths. Instead, these morphological differences are often interpreted as ecologic responses (e.g., *Reticulofenestra* spp., Young, 1990; Beaufort, 1992). Based upon this idea, we explore the possibility that the broad variations in size of *B. bigelowii* pentaliths during the Early Paleocene, as recorded in the Aitzgorri Limestone Formation (Basque Country, Spain), represent pseudo-cryptic speciation rather than intraspecific variability.

### 1.2 Materials and Methods

1.2.1. The section: Location, Lithology, and Age

The material studied here is from the limestones and marlstones in the vicinity of the GSSP (Global Boundary Stratotype Section and Point) for the Danian/Selandian boundary at Zumaia, Spain (Latitude: 43°17'57.1” N, Longitude 2°15’39.6” W; Fig. 1). We have examined a 17.1 m thick interval comprised of 13.9 m of *Braarudosphaera*-rich limestones with marly intercalations (Aitzgorri Limestone Formation), overlain by 3.2 m of mostly grey marlstones (Itzurun Formation). The Danian/Selandian boundary (astronomical age of 61.641 ± 0.040 Ma, Dinarès-Turell et al., 2010) corresponds to the formational contact between the upper Danian *Braarudosphaera*-rich limestones and the lower Selandian marlstones. These strata were deposited in the bathyal zone, at a water
depth of approximately 1000 meters (Pujalte et al., 1998; Bernaola et al., 2007). A summary of the lithology, sedimentology, and bathymetry of the section can be found in Schmitz et al. (2011).

The Zumaia section studied here spans magnetochrons C27r (partim) to C26r (partim). The C27r/n and C27n/26r magnetic reversals are recorded near the base of the section (Dinarès-Turell et al., 2003), at meters 1.87 and 6.42, respectively. Based on calcareous nannofossil biostratigraphy (Schmitz et al., 2011), the section encompasses Zones NP4 and NP5 (Martini, 1971) and Zones NTp7a through NTp9 (Varol, 1989). It was deposited during the late Early and early Middle Paleocene and represents a duration of approximately 1.10 Myr based on Gradstein et al. (2012; see Appendix A). Using the ages of the C27r/n and C27n/26r boundaries, we determined a sedimentation rate of ~1.54 cm/kyr for magnetochnozone C27n, from which we extrapolated the ages of the samples, including the age of the bottom (~62.54 Ma) and the top (~61.44 Ma) of the section. We calculated an age of 61.58 Ma for the NP4/NP5 biozonal boundary, which differs by ~160 kyr from the age of ~59.95 Ma given by Agnini et al. (2014). This discrepancy may be due to a difference in the taxonomic concept of Fasciculithus tympaniformis, for which the lowest occurrence (LO) is sometimes difficult to locate. For instance, Agnini et al. (2007, p. 223) stated that there is some uncertainty in the precise placement of the LO of F. tympaniformis at ODP Site 1262 due to “the presence of transitional forms between F. tympaniformis and other fasciculith species.” Likewise, Steurbaut and Sztrákos (2008) have shown that, contrary to popular opinion, F. tympaniformis is not the first representative of the genus Fasciculithus. Therefore, its location at Zumaia may not be exactly correlative with its location at ODP Site 1262.
(Agnini et al., 2007; Agnini et al., 2014), and we tentatively use 61.44 Ma as the age of the top of the Zumaia section (18.67m).

1.2.2. Techniques and Methods
Fifty-nine samples (Z1-Z59) of sedimentary rocks taken at intervals varying between 0.09 and 0.76 meters are analyzed here (Fig. 2). These are the same samples as those studied by Bernaola et al. (2009). Forty-three of these samples are from the Braarudosphaera-rich limestones of the Aitzgorri Limestone Formation, which contain a ~13.9 m Braarudosphaera bigelowii acme zone (Bernaola et al., 2009). The remaining sixteen samples are from the lower Itzurun Formation.

1.2.2.1 Micropaleontological Analysis
All samples were prepared on standard smear slides, which were analyzed using a Zeiss Axioplan 2 Imaging microscope. We determined the relative abundance and preservation ratio (PR) of Braarudosphaera bigelowii within each sample by making a count along a single horizontal transect through the middle of each slide where material was most abundant. This ensured minimum bias due to sorting during the preparation of the smear slides. Relative abundance was established by counting the number of pentaliths encountered along the transect and converting it to the number of pentaliths per square millimeter (as in Backman and Shackleton, 1983). All pentaliths with at least two segments were counted. Since two-segmented pentaliths were more abundant than three-segmented ones, the total number of broken pentaliths was divided by two to prevent the “double-counting” of a single specimen. Because determining preservation in regard to the state of recrystallization is subjective (Plate 1), we only took fragmentation into
account. Therefore, the PR was estimated using the ratio of whole to broken pentaliths counted within the transect.

A quantitative morphometric study was conducted on *Braarudosphaera bigelowii* pentaliths within the *Braarudosphaera* acme zone defined by Bernaola et al. (2009; meters 1.60–15.26; Z1–Z43). We focused on meters 6.42–15.26 (Z16–Z43), the interval with the greatest abundance of pentaliths. One hundred pentaliths were photographed per sample in this interval, using an AxioCam HRc camera at 1600X magnification, for a total of 2800 pentaliths. In order to compare our data with those of other authors (i.e., Hagino et al., 2009), the side length and radius of each photographed pentalith were measured using ImageJ software. The side length was measured by summing the lengths of the two outer edges of a single segment (Fig. 3). However, due to recrystallization, which first affected the corners and caused a rounding effect, the edges of the pentaliths tended to be irregular, causing uncertainty in the measurements. We remedied this problem by measuring the radius along the suture between adjacent segments. Because these two measurements have a linear relationship expressed by the equation $y = 0.7018x + 0.039$ (where $x$ is side length and $y$ is radius) as shown in Fig. 4, the radii can be compared to the side length measurements of other authors. Therefore, we chose to use the more reliable radius measurements throughout this study.

Model-based cluster analysis was performed in RStudio on the radii of all pentaliths using the Expectation-Maximization (EM) algorithm (mclust package; Fraley and Raftery, 2002; Fraley et al., 2012) in order to determine the presence of statistically significant size groupings.
In order to constrain the age of the *Braarudosphaera* acme, we conducted a biostratigraphic study of *Diantholitha* and *Lithoptychius* species (Plate 2), in which the total number of specimens was counted per slide. These taxa were chosen for study because they have proven useful for long distance correlation between upper Danian sediments (Aubry et al., 2011; Monechi et al., 2013).

1.2.2.2 Geochemical Analysis

We conducted isotopic analyses of organic carbon (δ\(^{13}\)C\(_{\text{org}}\)) and total nitrogen (δ\(^{15}\)N\(_{\text{TN}}\)), plus the carbon/nitrogen ratio (C/N) in an attempt to determine potential causes of variability in the abundance of *Braarudosphaera bigelowii* at Zumaia. Each sample was crushed into a powder using a mortar and pestle and carbonate was removed using 25% HCl. The residue was washed, dried, and weighed in a tin capsule. The analyses were performed at Rutgers University using a Eurovector Elemental Analyzer coupled with a GVI Isoprime100 CF-IRMS. The carbon isotope composition was normalized against NBS 22 (Coplen et al., 2006) and a secondary sediment standard. Organic carbon and total nitrogen concentrations were determined using the major ion peak areas and an acetanilide calibration line. Analysis of δ\(^{15}\)N\(_{\text{TN}}\) was also performed in a similar process using standards IAEA N-1 and IAEA N-3 (Qi et al., 2003). However, because some nitrogen can be bound within the carbonate lattice (e.g., Ren et al., 2009), δ\(^{15}\)N\(_{\text{TN}}\) was measured on bulk sediment with an ascarite trap to remove CO\(_2\).
1.3 Results

1.3.1 Abundance and Preservation of *Braarudosphaera bigelowii*

Pentalith abundance is highly variable throughout the section and ranges from 0 pentaliths per square millimeter at meters 16.31 and 17.96 (Z48 and Z57) to 42.9 pentaliths/mm$^2$ at meter 7.04 (Z18) (Fig. 2). The average abundance throughout the section is 8.1 pentaliths/mm$^2$. At the base of the section (meter 1.60; Z1), they are abundant, with 20.6/mm$^2$. This number then decreases to an average of 4.2/mm$^2$ between meters 1.87 and 6.13 (Z2 and Z15). Between meters 6.42 and 10.52 (Z16 and Z29), abundance oscillates from very few to greater than 40 pentaliths/mm$^2$, with an average of 21.9/mm$^2$. This interval of approximately 260 kyr (62.22−61.96 Ma; Appendix A) is herein termed the “peak” *Braarudosphaera* acme zone. Above this peak acme, abundance drops significantly. Between meters 10.78 and 15.26 (Z30 and Z43), there are some intervals of greater abundance, but pentaliths do not exceed a density of 10.7/mm$^2$. Beginning at meter 15.48 (Z44; Itzurun Formation), there are very few pentaliths present in each sample and their abundance decreases to ≤ 1 per mm$^2$.

The preservation ratio (PR), quantified as the ratio of whole to broken pentaliths per square millimeter, is variable throughout the section. The lower part of the section, meters 1.60 to 10.52 (Z1 to Z29), contains a higher proportion of whole to broken pentaliths (average ratio of 1.4), while the upper part, meters 10.78 to 18.67, (Z30 to Z59), has a lower proportion of whole pentaliths (average ratio of 0.8). The PR tends to correlate well with abundance (Fig. 2). Especially within the peak acme zone, samples with a greater abundance tend to have a higher PR, while samples with a low abundance
tend to have a lower ratio. For example, meter 7.04 (Z18) has 274 whole and 138 broken pentaliths, while meter 7.40 (Z19) has 35 whole and 40 broken pentaliths.

1.3.2 *Braarudosphaera bigelowii* Pentalith Size

The Zumaia pentaliths vary broadly in size, with radii ranging from 1.4 to 10.6 µm. Plotting count against radius yields a bimodal distribution (Fig. 5) with the more prominent peak centered at ~3.0 µm and the smaller peak centered at ~6.5 µm. The EM method of model-based clustering (Fraley and Raftery, 2002; Fraley et al., 2012) was used to determine the number of statistically significant size groupings present within *Zumaia B. bigelowii*. This program uses Bayesian Information Criterion (BIC) values as a means for model selection, favoring the model with the largest value (Fraley and Raftery, 1999). The EM analysis yielded BIC values of −10,362.900, −8,496.393, and −8,487.693 for one, two, and three groups, respectively. The three-group model had the largest BIC value, with cutoffs between the size groupings at 2.5 and 4.5 µm. However, its BIC value was very close to that of the two-group model, which had a cutoff value of 4.5 µm. Due to the continuous pentalith size variation and lack of clear morphological differences between groups, we cannot support the three-group model within the parameters of this study. Thus, we chose the two-group model with a cutoff value of 4.5 µm, which is also consistent with the bimodal distribution of pentalith radii. The smaller form, here termed form X, includes all specimens smaller than 4.5 µm in radius and constitutes approximately 82 percent of the assemblage. The larger form, here termed form Y, includes all specimens greater than or equal to 4.5 µm in radius and represents the remaining 18 percent of the assemblage.
Both forms co-occur throughout the interval; form X is consistently abundant while form Y varies in abundance, as shown by the violin plot in Fig. 6. Form Y is less common between meters 6.42 and 11.83 (Z16 and Z32), with an average of 11%, but its abundance is generally higher between meters 12.12 and 15.26 (Z33 and Z43) with an average of 30%. However, the abundance of form Y in certain upper samples (e.g., meters 13.03, 14.68; Z36, Z41) is comparable to that in the lower part of the interval (12% and 11%, respectively). Meter 12.36 (Z34) has the greatest percentage (66%) of form Y, while meters 7.40, 10.78 and 11.83 (Z19, Z30 and Z32) have the smallest percentage (3% each). Meters 12.68 and 13.72 (Z35 and Z38) have about equal percentages of both forms (47% and 48% form Y, respectively). Only meter 12.36 (Z34) has more form Y than form X specimens (66% form Y).

1.3.3 Species Distribution within the *Braarudosphaera* Acme Zone

The ranges of *Lithoptychius* and *Diantholitha* species (Fig. 2) were determined for biostratigraphic correlation and dating of the *Braarudosphaera* acme zone (See Aubry et al., 2012). The first fasciculith assignable to *Lithoptychius* occurs at meter 4.08 (Z10; Plate 2, Fig. 7), and is tentatively assigned to *L. collaris*. *Lithoptychius* fasciculiths occur fairly consistently above this level, but poor preservation hampers confident species identification. The first specimen reasonably assignable to *L. varolii* was recovered from meter 4.83 (Z11; Plate 2, Figs. 9, 12). *Lithoptychius* spp. become very abundant at meter 15.48 (Z44), while *Braarudosphaera* simultaneously become scarce. Coccoliths assignable to *Diantholitha* occur just below the peak acme at meter 3.43 (Z8) and their highest occurrence (HO) at meter 7.04 (Z18) is shortly above the onset of the peak acme.
of *B. bigelowii* (meter 6.42; Z16). Two lone specimens of *Diantholitha* found at meters 10.31 and 10.52 (Z28 and Z29) are considered reworked.

### 1.3.4 Geochemical Analysis

Total nitrogen ($\delta^{15}N_{TN}$) can be used as an indicator of paleoceanographic conditions. However, trends in the nitrogen data are difficult to discern because many samples have too little nitrogen for detection (Fig. 2). The $\delta^{15}N_{TN}$ ranges from 4.9‰ at meter 10.78 (Z30) to 6.7‰ at meter 8.42 (Z22), with an average value of 6.1‰.

The Carbon/Nitrogen (C/N) ratio can be used to determine the source of the organic matter in an environment. The C/N ratio at Zumaia varies minimally throughout the section, ranging from 5.5 at meter 8.42 (Z22) to 17.2 at meter 3.78 (Z9), with an average value of 9.8 (Fig. 2). It has slightly elevated values (average of 15.1) between meters 3.16 and 5.81 (Z7 and Z14), but is otherwise fairly stable.

Organic carbon isotopes are often used as an indicator of past productivity. The $\delta^{13}C_{org}$ record at Zumaia is highly variable in the lower part of the section but stabilizes in its upper part (Fig. 2). The values range from $-31.2$ to $-26.9$‰, with the minimum occurring at meter 6.13 (Z15) and the maximum at meter 3.16 (Z7). There is an increasing trend from meter 1.87 to 3.16 (Z2 to Z7), followed by a 2.1 meter interval with variable $\delta^{13}C_{org}$ values. A steep negative excursion of 3.5‰ occurs between meters 5.81 and 6.13 (Z14 and Z15). The remaining samples are relatively stable between $-30.2$ and $-28.5$‰.
1.4 Discussion

1.4.1 Pentalith Abundance and Preservation Patterns

The preservation ratio tends to correlate well with abundance (Fig. 2). Especially within the peak acme zone, samples with a greater abundance tend to have a higher preservation ratio. This could imply that low abundance is actually a result of increased fragmentation. If most of the pentaliths within a sample had been broken into isolated segments, they would not have been counted in this study, and abundance would appear lower. If this is the case, it is possible that the peak acme zone may be larger and less variable than proposed here, perhaps explaining the large acme zone described by Bernaola et al. (2009).

1.4.2 Pentalith Size Comparisons

In order to examine pentalith size variation through time, we compare the size of B. bigelowii pentaliths from the upper Lower Paleocene Aitzgorri Limestone Formation (this study), with those preserved in 1) Turonian deposits from Bohemia (Czech Republic; Švábenická, 1999), 2) Recent sediments from the South China Sea (Fernando et al., 2013) and 3) pentaliths in situ on coccospere recovered from living plankton in the seas surrounding Japan (Takano et al., 2006; Konno et al., 2007; Hagino et al., 2009).

The Early Paleocene pentaliths differ from the others in two significant aspects. First, they have a much wider range in size, and second, size variation is continuous, with no discrete groupings as reported in previous studies.

The Paleocene pentaliths at Zumaia consist of two size groups that range from 1.4 to 10.6 µm in radius (Fig. 7). The Turonian samples also contain two size groups
Švábenická, 1999), but their size range is much smaller than that of the Paleocene ones. While the number of specimens and methods of measurement were not described by Švábenická (1999), Takano et al. (2006) calculated their side lengths, which we, in turn, converted to radius. The “small form” ranges from 2.3 to 3.7 µm and overlaps with our form X. The Turonian “normal form” ranges from 5.5 to 6.4 µm and overlaps in size with the smaller end of the range of form Y.

The Paleocene pentaliths also differ from Recent (sub-fossil) and living pentaliths. The Recent pentaliths collected from surface sediments of the Western South China Sea by Fernando et al. (2013) compare well with the size groupings of pentaliths on living cells analyzed by Takano et al. (2006) and Hagino et al. (2009), as could be expected. Pentaliths on living cells ranged from 1 to 6.4 µm in radius (Takano et al., 2006; Konno et al., 2007; Hagino et al., 2009). Based on both size and genotype, Hagino et al. (2009, figure 3) distinguished four size classes and five pseudo-cryptic species within living B. bigelowii from the seas surrounding Japan: “small form” (<1.7 µm in radius), “Intermediate form-A” (2.8–3.8 µm; includes Genotypes I and II), “Intermediate form-B” (3.8–5.1 µm; includes Genotype III) and “Large form” (> 5.3 µm; includes Genotypes IV and V). The smallest pentaliths on living cells are slightly smaller than the smallest pentaliths of Paleocene form X (1.0 µm versus 1.4 µm). However, the maximum size of Paleocene form Y is considerably larger than the largest pentaliths found on living B. bigelowii cells (10.6 µm versus 6.4 µm). Approximately 60% of the Zumaia pentaliths are of sizes recorded in living cells by Hagino et al. (2009; Table 1). The size range of form X overlaps with most of their “small form,” all of “Intermediate form-A,” and part of “Intermediate form-B;” while form Y overlaps with part of “Intermediate form-B,” all
of their “Large form,” and extends much beyond the upper size limit of the living cells. It is possible that the full size range of living *B. bigelowii* has not been sampled, whether in terms of intermediate or extreme values. In fact, both Konno et al. (2007) and Fernando et al. (2013) reported pentaliths with sizes in between those of the “small form” and “Intermediate form-A,” which Fernando et al. (2013) termed “small form B.” It is possible that “small form B” is actually a continuation of “Intermediate form-A” that was undetected in previous studies due to sampling location, scarcity, or other biological/ecological factors. However, with regard to the full amplitude of size difference, a survey of the literature has not revealed any reports of living *B. bigelowii* of a comparable size to (or larger than) the upper size range of form Y (Aubry, 2013).

When the size distributions of *B. bigelowii* from all four time intervals are compared, it is clear that the size of the Early Paleocene pentaliths differs markedly from those of the other samplings in both range and continuity (Fig. 7). The Paleocene pentaliths are the most varied in size, ranging from about as small as the smallest of the living to almost twice as large as the largest of the living. Despite these differences, the abundance patterns related to size are the same, with the larger forms always being less common. Hagino et al. (2009; fig. 3) reported only five specimens of the “Large form” making up 2.8% of their sample. Likewise, Fernando et al. (2013) found only a single specimen (1/303 or 0.3%) that corresponds to the “Large form.” The Zumaia material also contains proportionally fewer large pentaliths than small ones, with a ratio of 426/2800 (~15%) equivalent to or larger than the “Large form” of Hagino et al. (2009). However, due to the differing size ranges of the “Large form” (living) and form Y
(Paleocene), these two morphotypes may represent different genotypes and are more likely discrete products of pseudo-cryptic speciation.

Another difference concerning the distribution of pentalith size during the Early Paleocene and at Present (0 Ma; =living + Recent) must also be considered. The size distribution of pentaliths in Present *B. bigelowii* is discontinuous (Fig. 7). A relatively large gap in size between the small and intermediate forms (1.1 µm in Hagino et al., 2009; 0.8 µm in Fernando et al., 2013) occurs between Present small and intermediate forms. A slightly larger gap of 1.8 µm also occurs among the Turonian pentaliths, although between larger fractions. In contrast, the size distribution was continuous in the Paleocene, with no such gap. Two scenarios could account for the continuous size distribution of Paleocene pentaliths. Because form Y is scarce in the lower part of our record but becomes more abundant in its upper part, it is possible that we are witnessing the beginning of the divergence of form Y from form X. Alternatively, the observed pattern may indicate changes in forcing of some ecological factor that selected for a broad range of sizes (morphotypes) in a single species.

In order to eliminate sampling bias as a cause for the difference in size distribution between Present and Paleocene pentaliths, we compare the number of pentaliths measured in each study (Table 2). A total of 2800 isolated Paleocene pentaliths were measured in this study. Konno et al. (2007) and Hagino et al. (2009) measured one pentalith on each of 19 and 181 living coccospheres, respectively. In addition, Fernando et al. (2013) measured 303 Recent isolated pentaliths. This is a total of 503 Present pentaliths.
Two assumptions can be made in order to compare Present and Paleocene pentaliths. On the one hand, it can be assumed that all of the measured isolated pentaliths are from different coccospheres. In this case, our sample size is much larger than that for the Present since the 503 measured Present specimens equate to approximately 18% of the 2800 Paleocene specimens. Therefore, the lesser size variability in the Present *B. bigelowii* could be affected by their comparatively smaller sample size. However, because large pentaliths are quite rare in Present *B. bigelowii*, it is unlikely that their current size range is as large as that of *B. bigelowii* from Zumaia. On the other hand, it can be assumed that the isolated pentaliths originated from the same coccospheres. Because each coccosphere is composed of 12 pentaliths, an equivalent of 233 Paleocene coccospheres (this study), and 25 Recent coccospheres (Fernando et al., 2013) would have been measured. In this case, our data of 233 Paleocene coccospheres would compare well with the data of the 225 Present coccospheres (19 from Konno et al., 2007, 181 from Takano et al., 2006 & Hagino et al., 2009, and 25 from Fernando et al., 2013), and the sample size would not affect the data. However, it is more likely that the true number of measured coccospheres is somewhere in between these two estimates. Thus, the Paleocene sample size is probably somewhat larger than that for the Present, which could be a factor contributing to our size range that is both continuous and considerably larger.

In order to determine the full size range of *Braarudosphaera bigelowii* through time, we measured pentalith radii in photos of specimens published over time and compiled in a recent database (Aubry, 2013; Fig. 8). Despite a lack of systematic investigations of *B. bigelowii* size, a few pentaliths as large and larger than those of
Paleocene form Y have been documented throughout the Cenozoic. For instance, Early Eocene sediments of coastal Tanzania produced a pentalith ~9.2 µm in radius (Bown, 2005). Miocene deposits of West Africa, Austria, and Slovenia, have preserved a number of large pentaliths ranging from ~7.2–10.3 µm (Martini, 1969; Stradner and Fuchs, 1980; Bartol et al., 2008). Nishida (1971) documented a single large pentalith (8.3 µm) from the Early Pliocene of Japan, and Stradner (1973) recorded one of the same size from the early Quaternary of the Tyrrhenian Sea. A pentalith with an ~11.7 µm radius was illustrated from the Upper Paleocene-Lower Eocene of California (Bramlette and Sullivan, 1961), while the largest known pentalith would seem to be one with a radius of ~15.8 µm recorded from the Lower Oligocene of the Eastern South Atlantic Ocean (Steinmetz and Stradner, 1984). From these data, the size difference between the largest known Cenozoic (Early Oligocene) pentalith and the largest pentalith from Zumaia is 5.2 µm, revealing that *B. bigelowii* had not yet reached its maximum size at the end of the Early Paleocene. Moreover, pentaliths more than twice as large as the largest measured on a living cell were secreted during the Early Oligocene. In fact, this extreme size difference between past and extant pentaliths had already been noted by Deflandre (1950), who observed that the diameter of a pentalith from the Lower Eocene of the Aquitaine Basin (France) was larger than the diameter of a living cell of *B. bigelowii*.

Conversely, small Cenozoic *B. bigelowii* pentaliths seem to be uncommonly reported in the literature (cf. Aubry, 2013; Fig. 8), and very few are as small as the living ones reported by Hagino et al. (2009; 1.0 µm) and Fernando et al. (2013; 1.3 µm). As a reminder, the smallest pentaliths measured from Zumaia had a radius (measured along sutures) of 1.4 µm. Stradner (1960) first mentioned small pentaliths (less than 10 µm in
diameter) from the Miocene, and classified them as *B. bigelowii* subspecies *parvula* (though later authors did not use this name). Three small specimens were reported from Tanzania: Bown (2005) recorded a ~1.3 µm specimen from the Eocene and a ~1.6 µm specimen from the Late Paleocene, and Dunkley Jones et al. (2009) reported a ~2.2 µm specimen from the Late Eocene-Early Oligocene. Although there seems to be little or no record of very small pentaliths between the Early Oligocene and the Present, *B. bigelowii* pentaliths have rarely been documented from Neogene deposits.

Though we cannot assume that the full size range of *B. bigelowii* in today’s plankton has been sampled, our current pentalith measurements and the review of size discussed above support the observation of Aubry (2007) of a long-term trend of decreasing size in coccolithophores through the Neogene. Pentaliths had begun to diversify in size as early as the Turonian, reaching their maximum size during the Oligocene. It would seem that during the Neogene, their size decreased and reached their minimum range in modern populations.

1.4.3 Species Distribution

This study places the LO of the genus *Lithoptychius* (LO of *L. collaris*) approximately 1.5 meters below that previously reported for Zumaia (Monechi et al. (2013; LO at meter 5.53 meters, sample Z13). It also places the LO of *Diantholitha* spp. approximately 1.75 meters below that reported by Monechi et al. (2013; LO at 5.19 meters; Z12). We attribute this discrepancy to a methodological difference in which we inventoried the entire smear slide, whereas Monechi et al. (2013, p. 30) state “only qualitative analyses were performed to accurately establish the succession of fasciculith
bioevents.” We note that Bown (2016, p. 29, table 1) assigns *Diantholitha mariposa* and *D. spp.* to Biozone NP5 and the Selandian Stage. This is based on the co-occurrence of multiple specimens of these taxa (as illustrated in op. cit., plate 8, figs. 1-40) at the stratigraphic level labeled 7-1/46 in core TD site 27 (Tanzania) with “questionable specimens attributable to” and “that resemble” *Fasciculithus tympaniformis* (op. cit. p. 2 and 3), of which the LO defines the base of Zone NP5 (Martini, 1971). The specimen illustrated in plate 8, figure 3 from sample TD Site 27/7-1, 46 is not representative of *F. tympaniformis*. Taken collectively, *Diantholitha* species have a very narrow stratigraphic range associated with the late Danian radiation of the fasciculiths (Aubry et al., 2012). This range is restricted to a small interval in the upper part of Zone NP4 and to lowermost Chron C26r, whether at ODP Site 1262, Zumaia, or elsewhere (Aubry, 2015) and they constitute reliable stratigraphic markers to identify the late Danian hyperthermal event as we show below.

1.4.4 Geochemical Analyses

1.4.4.1 Total Nitrogen

Total nitrogen (∆15TN) is often used as an indicator of paleoceanographic conditions. In nitrate-limited surface waters of the ocean, ∆15TN values closely match those of deep ocean nitrate that is mixed into the surface ocean (Tesdal et al., 2013). The ∆15TN of deep ocean nitrate during the Paleocene was approximately the same as that of today (L. G., unpublished data) with values of about 5‰ (Sigman et al., 2000). Though many samples had too little nitrogen for detection, the ∆15TN values of Zumaia whole rock ranged from 4.9‰ at meter 10.78 (Z30) to 6.7‰ at meter 8.42 (Z22), with an
average value of 6.1‰ (Fig. 2). Because these values are very close to those of deep ocean nitrate, this suggests that nitrate was quantitatively consumed and was therefore a limiting nutrient (Altabet and Deuser, 1985; Wada and Hattori, 1991). Thus, the nitrogen isotope values recorded at Zumaia suggest that the surrounding ocean was oligotrophic during the interval of Chrons C27r to C26r discussed here.

1.4.4.2 C/N Ratio

The C/N ratio is a good proxy for determining the source of organic matter because it usually varies between 4 and 10 in phytoplankton-derived organic matter, whereas it is usually greater than 20 in land plants (Meyers, 1994). Additionally, this ratio has remained constant through time for a given source. The C/N ratio at Zumaia is fairly stable, but has slightly elevated values (average of 15.1) between meters 3.16 and 5.81 (Z7 and Z14). These higher values in the lower part of the section may indicate a mix of terrestrial- and marine-derived inputs. Around the Chron C27n/26r magnetic reversal, the C/N ratio decreases (average of 8.8), indicating that the organic matter source was marine. This could indicate less continental runoff or water deepening, but evidence for either scenario has not been reported at this time.

1.4.4.3 Organic Carbon

Organic carbon isotopes can be used, among other things, as a proxy for past productivity. Due to biological fractionation during photosynthesis, photosynthetic carbon has a negative $\delta^{13}C_{\text{org}}$ value relative to the carbon source. This value increases (i.e., become more positive) when the growth rate increases, as occurs in high productivity regions of the ocean, but decreases if there is an increase in pCO$_2$ (Hayes, 1993; Rau et al., 1996). The most striking event in the Zumaia section is a negative shift of 3.5‰
between meters 5.81 and 6.13 (Z14 and Z15), which is correlative with the magnetochronozonal C27n/26r boundary. From the same section, Arenillas et al. (2008) reported an isotopic excursion of <1‰ in carbonates (CIE-DS1, in their figure 3) spanning an interval of ~2.5 meters, with the onset located about 1 meter above the magnetochronozonal C27n/26r boundary. Considering that both isotopic records are correlated to the same magnetostratigraphic record (Dinarès-Turell et al., 2003), straight correlation between isotope stratigraphy and magnetostratigraphy (Fig. 9) would indicate that the δ¹³C-org and δ¹³C-carb record different isotopic excursions, one (δ¹³C-org) at the C27n/26r magnetic reversal, and the other (δ¹³C-carb) in early magnetochron C26r. However, this is an unlikely scenario. We note that the interval between the top of magnetochronozone C27n and the formational contact between the Aitzgorri Limestone Formation and the Itzurun Formation reported by Arenillas et al. (2008) is ~12 meters thick, while the thickness of the same interval in our study is ~9 meters (as in Schmitz et al., 2011). We propose that this difference of three meters explains the discrepancy in the stratigraphic location of the two excursions, and consider that the excursion in δ¹³C-carb reported by Arenillas et al. (2008) and the one in δ¹³C-org presented here represent the same event.

Other authors have also reported negative δ¹³C-carb events in the late Danian (around the C27n/26r magnetic reversal) from other sites. This includes the Latest Danian Event (LDE) at Qreiya, Egypt (Bornemann et al., 2009), the “Top Chron C27n Event” at ODP Legs 198 and 208 in the North Pacific and South Atlantic Oceans, respectively (Westerhold et al., 2008; 2011), and the δ¹³C-carb excursion at ODP Site 761B offshore of Northwestern Australia (Quillévééré et al., 2002). Westerhold et al. (2011) suggested that
all of these events, including CIE-DS1 (Arenillas et al., 2008) at Zumaia, represent a single, global, hyperthermal event. However, there has been some controversy concerning the LDE at Qreiya. Originally termed the “Neo-duwi event,” (Guasti et al., 2005) it was first identified as a biotic excursion in which the foraminiferal species *Neoeponides duwi* moved into deeper waters following a change in sea level (Speijer, 2003). Bornemann et al. (2009) reported $\delta^{13}C_{\text{carb}}$ excursions of up to $-2\%$ in the Neo-duwi event beds of three separate Egyptian sections, which they interpreted as evidence of the LDE at Qreiya. Subsequent isotopic analysis of the Neo-duwi beds also sampled at Qreiya found a negative $\delta^{13}C_{\text{carb}}$ excursion occurring simultaneously with a positive $\delta^{13}C_{\text{org}}$ excursion, which Aubry et al. (2012) interpreted as evidence of regional terrestrial input rather than a global hyperthermal event as suggested by Bornemann et al. (2009).

Using biostratigraphic correlation between the Zumaia and Qreiya sections, we are able to show that the $\delta^{13}C_{\text{carb}}$ excursion at Qreiya is correlative with the $\delta^{13}C_{\text{org}}$ excursion at Zumaia (Fig. 10). Due to overprinting of the magnetic signature of the Upper Paleocene-Lower Eocene succession at Qreiya (Kent and Dupuis, 2003), this section can only be correlated to the Zumaia section using biostratigraphy. The occurrence of the short-ranged species of *Diantholitha* in both sections allows us to determine the likely position of the Chron C27n/26r magnetic reversal at Qreiya, and consequently determine if the isotopic excursion within the Neo-duwi beds occurred simultaneously with the excursion at Zumaia. We correlate the *Diantholitha*-bearing interval between meters 3.43 and 7.04 (Z8 and Z18) at Zumaia with the *Diantholitha*-bearing interval between meters 7.2 and 9.9 at Qreiya (Aubry et al., 2011; 2012). At Zumaia, the LO of *Diantholitha* spp. occurs in lower magnetochronozone C27n (meter 3.43; Z8) and its HO in lowermost
magnetochronozone C26r (meter 7.04; Z18). Since the C27n/26r magnetic reversal occurs in the uppermost part of the range of *Diantholitha* spp., we can predict the position of the magnetic reversal at Qreiya to also occur in the uppermost part of its range, at approximately 9.4 meters (Aubry et al., 2012).

We can, in turn, correlate the positions of the isotopic excursions relative to the magnetochronology. The isotopic excursion at Zumaia occurred slightly before the magnetic reversal (latest Chron C27n; meter 6.13; Z15). By correlation, the excursion at Qreiya would have had to occur slightly below the magnetic reversal at meter 9.4, which it does, beginning approximately at meter 9.25. Thus, we conclude that the isotopic excursions at Zumaia and Qreiya were synchronous, and we reaffirm that the Neo-duwi event represents a local expression of the LDE at Qreiya. This allows us, in turn, to infer enhancement of the hydrological cycle (and associated increase in terrestrial input) during the LDE as a result of increased warmth, as initially shown by Schmitz and Pujalte (2003; 2007) for the Paleocene-Eocene Thermal Maximum (PETM). Neither the isotopes nor the C/N ratio measured at Qreiya by Aubry et al. (2012) provided clear evidence of a late Danian hyperthermal, unlike their joint signature in the deep-water (bathyal) section of Zumaia. Instead, the outer neritic section of Qreiya provided evidence of increased terrestrial input and runoff.

Based on this revision, the onset of the *Braarudosphaera bigelowii* peak acme is correlated with the Danian/Selandian hyperthermal event, as shown by the steep negative shift in $\delta^{13}C_{org}$ between meters 5.81 and 6.13 (Z14 and Z15) immediately preceding the onset of the peak acme (meter 6.42; Z16). This suggests that the peak acme of *B. bigelowii* was triggered by sudden climatic warming. However, one would assume that a
hyperthermal-triggered acme event would terminate at the conclusion of the isotopic excursion. This is not what occurs at Zumaia; instead, the peak acme extends much beyond the end of the $\delta^{13}C_{org}$ excursion. This raises the question of whether there is a causal relationship between the two events: the close stratigraphic proximity of the isotopic excursion and the onset of the peak acme could be merely coincidental.

1.4.5 Evolutionary History

At Zumaia, form X was consistently abundant throughout late Chron C27n to early Chron C26r; form Y was also present throughout, but did not become abundant until ~61.84 Ma (early Chron C26r; Fig. 11). We propose four possible explanations for this pattern:

A. A small population of larger specimens of form X was present throughout the 1.1 Myr interval represented by our section due to random genetic mutation (Fig. 11A). Form Y became abundant at ~61.84 Ma when it diverged from form X. This occurred approximately 120 kyr after the end of the peak acme.

B. A speciation event occurred at some time prior to the peak acme and the larger specimens found in the lower samples are the few remnants of a large form (form W) from that earlier speciation event (Fig. 11B). Form Y became abundant after a second speciation event (~61.84 Ma), where it diverged from form X. This would be evidence of iterative evolution.

C. Form Y diverged from form X at some time prior to the peak acme but its abundance was diluted by form X within this acme (Fig. 11C). This caused form Y to appear uncommon during Chron C27n and early Chron C26r. After
the peak acme had ended, form Y became more abundant, giving the illusion of a speciation event at ~61.84 Ma.

D. Form Y did not diverge from form X. Instead, both forms diverged from a common ancestor (Fig. 11D). Both forms were present at Zumaia throughout the interval between 62.54 Ma and 61.44 Ma, but form Y became abundant at ~61.84 Ma, approximately 120 kyr after the peak acme of form X had ended.

Scenario A seems the least likely of the four possibilities because large pentaliths are present in appreciable numbers in latest Chron C27n and earliest Chron C26r (during the peak acme) and are also present below the studied interval (latest Chron C27r and Chron C27n; 62.54–62.24 Ma). Therefore, it is unlikely that large pentalith size was caused by random genetic mutations and instead, that the larger form is a well-established, morphologically stable, pseudo-cryptic species.

Perhaps scenario B, in which form Y is the result of a pseudo-cryptic speciation event at or slightly prior to 61.84 Ma, is a better explanation. The larger specimens that lived before the speciation event at ~61.84 Ma would be the few remnants of an earlier speciation event (form W) due to their low abundance. In this scenario, the abundance of form X would have created a large genetic pool with increased genetic variability, allowing for the divergence of form Y slightly before the end of the acme. This new form Y would have proliferated after the peak acme of form X had ended (~120 kyr later), possibly due to the opening of a new ecological niche. This is an example of iterative morphologic evolution, a phenomenon that has been shown to occur in the plankton today (e.g., de Vargas et al., 2004). This scenario cannot be excluded because, at this time,
we cannot determine whether form Y and the earlier form W are the same pseudo-cryptic species.

Scenario C is very similar to scenario B; it differs only in the absence of iterative morphologic evolution. The large pentaliths that occur throughout the peak acme are members of form Y, not an earlier large form (form W). In this scenario, the abundance of form Y is diluted by the peak acme of form X, and form Y only becomes abundant at ~61.84 Ma, after the peak acme has ended (~120 kyr later). These two scenarios seem equally plausible, given that a larger form is present in appreciable numbers both before and throughout the peak acme.

Scenario D is similar to scenario C, except that it proposes that form Y did not diverge from form X, but instead, both forms diverged from a common ancestor. Because large pentaliths are already present by ~62.54 Ma, we cannot determine the ancestry of form Y. Therefore, we also cannot exclude the possibility of scenario D.

Regardless of which scenario led to the proliferation of form Y at Zumaia, the sudden abundance of *B. bigelowii* would have increased the genetic pool, thereby increasing chances for genetic mutation. These mutations, in turn, could have allowed for greater environmental tolerances, and prompted phylogenetic divergence and pseudo-cryptic speciation. However, since the proliferation of form Y did not occur until ~61.84 Ma, approximately 400 kyr after the isotopic excursion, we see no evidence for a hyperthermal-induced divergence. Instead, its sudden abundance at ~61.84 Ma might have been caused by some other ecologic shift within the environment that allowed it to outcompete smaller form X. Such changes in environmental conditions have been demonstrated to affect the dominance of different pseudo-cryptic species of living
cocolithophores. Though on a much shorter timescale, the abundances of pseudo-cryptic species of *Calcidiscus leptoporus* have been shown to oscillate seasonally. *Calcidiscus leptoporus* subsp. *quadriperforatus* remains fairly constant throughout the year, while *C. leptoporus* subsp. *leptoporus* dominates the population during cool, mesotrophic conditions (Sáez et al., 2003). There is also a possibility that a similar scenario occurs in living and recent *B. bigelowii* populations. Hagino et al. (2009) reported only three “extraordinarily large” specimens (5.65, 5.72, and 6.36 µm in radius) around Japan, two from Furue Bay and one from the Yatsushiro Sea. In addition, Fernando et al. (2013) only reported one specimen (5.35 µm in radius) equivalent to the large form from a single site in the South China Sea (on the Sunda Shelf), far from the sites investigated in their study. In both instances, the larger cells inhabited areas different from most of the smaller ones, which may indicate differing environmental or chemical preferences between the pseudo-cryptic species of *B. bigelowii*. This idea of geographic separation leads to an alternative scenario to explain the sudden proliferation of form Y at Zumaia. It is possible that form Y evolved during the acme of form X, but in a different setting. *Braarudosphaera* was abundant in the North Atlantic and the North Sea Basin during the late Danian, and an acme zone occurs in the Danish Basin (Clemmensen and Thomsen, 2005; Bernaola et al., 2009). It is possible that form Y evolved in the North Sea and migrated to Zumaia (Fig. 12), which would give the illusion of its sudden divergence at ~61.84 Ma. It is also possible that the large form dubbed form W herein, belongs to pseudo-cryptic form Y. If so, several transient incursions occurred in the Zumaia area until the form finally became permanently established there. Future studies investigating the size of *B. bigelowii* in the North Sea and at intermediate sites between the Danish Basin and Zumaia, could
determine if migration was, in fact, the mechanism by which form Y became abundant after ~61.84 Ma in the Zumaia section.

1.5 Conclusions

This chapter has examined the size variation in late Early Paleocene *Braarudosphaera bigelowii* from the vicinity of the GSSP for the Danian/Selandian boundary at Zumaia, Spain. The size distribution of the late Early and early Middle Paleocene *B. bigelowii* pentaliths differs distinctly from that of living specimens in both range and continuity, with a range almost twice that of the living specimens, and no discreet gaps in size. In addition, the Expectation-Maximization method of model-based cluster analysis yielded two size groupings based on pentalith radius: form X (< 4.5 µm) and form Y (≥ 4.5 µm). It is likely that these two forms are pseudo-cryptic species, though it is difficult to confirm this with morphologic analysis alone.

This study also reaffirms the presence of the LDE (Latest Danian Event) at Zumaia, and revealed a correlation between this hyperthermal event, as shown by the negative excursion in organic carbon isotopes, and the peak acme of *B. bigelowii*. While pentalith abundance increased dramatically at the onset of the hyperthermal, pentalith size was unaffected. It is therefore unlikely that the proliferation of form Y in the upper part of the section was climate-induced.

This is the first study to examine size in non-living, Cenozoic *Braarudosphaera bigelowii* and additional work is necessary to accurately reconstruct the evolutionary history of the taxon. We offer possible explanations for the abundance patterns observed in forms X and Y at Zumaia, but at this time, it is not possible to determine the most
likely scenario. Though we cannot indisputably establish that forms X and Y are unique pseudo-cryptic species without genetic analysis, this study demonstrates the potential of morphometric analysis to assist in the documentation of pseudo-cryptic speciation in the past.
1.6 References


Bown, P.R., 2016. Paleocene calcareous nannofossils from Tanzania (TDP sites 19, 27 and 38). *Journal of Nannoplankton Research, 36*: 1–32.


Qi, H., Coplen, T.B., Geilmann, H., Brand, W.A. and Böhlke, J.K., 2003. Two new organic reference materials for $\delta^{13}$C and $\delta^{15}$N measurements and a new value for the $\delta^{13}$C of NBS 22 oil. Rapid Communications in Mass Spectrometry, 17(22): 2483–2487.


1.7 Table Captions

Table 1.
Comparison of the number of Zumaia pentaliths that correspond to each size class of Takano et al. (2006) and Hagino et al. (2009).

Table 2.
Comparison of the number of isolated pentaliths and pentaliths in situ on coccospheres measured in each study. Numbers in parentheses are the conversions; numbers without parentheses are the original numbers of pentaliths measured in each study.
1.8 Figure Captions

Figure 1.
Location map of areas discussed in text. Circle (Z): Zumaia. Pentagons: locations of *B. bigelowii* discussed herein, 1: Danish Basin (Clemmensen and Thomsen, 2005); 2: Bohemian Cretaceous Basin (Švábenická, 1999); 3: South China Sea (Fernando et al., 2013); 4: Seas around Japan (Takano et al., 2006; Hagino et al., 2009); 5: Bering Sea (Konno et al., 2007). Stars: locations of the LDE, 6: Qreiya, Egypt (Bornemann et al., 2009); 7: Walvis Ridge, ODP Leg 208 (Westerhold et al., 2011); 8: ODP Site 761B (Quillévéré et al., 2002); 9: Shatsky Rise, ODP Leg 198 (Westerhold et al., 2011). Map created using GeoMapApp.

Figure 2.
Summary log of the Zumaia section including sample position, species ranges, *B. bigelowii* abundance & preservation ratio (PR), and isotope stratigraphy. Bars in the “Samples” column represent individual samples and are spaced according to their relative heights within the original section. Numbered sample IDs (i.e. Z1, Z2, etc.) are the same as those used in Bernaola et al. (2009); the letter IDs (i.e. B, C, etc.) were used in Bernaola et al. (2002). The “Ht” column shows the heights used in this study, which were calculated from the lithologic column of Bernaola et al. (2009; figure 3) using GraphClick software. The “Species Ranges” column shows the ranges of *Diantholitha* and *Lithoptychius* species. Their abundance in each sample is given as follows: V- Very abundant; A- Abundant; C- Common; F- Few; R- Rare (see legend). Total *Diantholitha* and *Lithoptychius* specimens were counted per slide. However, samples with very abundant *Lithoptychius* were quantified by extrapolating from the count per one vertical transect through the middle of the slide. For *Diantholitha* species, x indicates the presence of a species. If the specimen was very recrystallized and difficult to identify to species level, cf is used instead. The “*B. bigelowii* Abundance and PR” column illustrates their abundance in number per square millimeter, and the PR of each sample by the size of the circle. A larger circle equates to a greater ratio of whole to broken pentaliths, while a smaller circle indicates a smaller ratio. The smallest circle approaches a ratio of 0 (see legend). The “Isotope Stratigraphy” column gives C/N, $\delta^{13}$Corg (V-PDB) % and $\delta^{15}$N$_{TN}$ (V-PDB) % values. Samples with two points were run twice. Samples with no $\delta^{15}$N$_{TN}$ value contained too little nitrogen to be detected. The position of the Danian/Selandian boundary follows Schmitz et al. (2011). The biozonation follows Martini (1971).

Figure 3.
Pentalith measurements were made on the least recrystallized segment per specimen. Radius was measured along the suture between adjacent segments (dashed line); side length was calculated by summing the short and long outer edges of the segment (solid lines).
Figure 4.
Relationship between side length and radius for *B. bigelowii* pentaliths at Zumaia. The regression line equation allows for comparison between the radii measured in this study and the side lengths of pentaliths measured by other authors.

Figure 5.
Distribution of pentalith radii at Zumaia. The large peak represents form X; the smaller peak represents form Y. The cutoff value between the two forms is 4.5 µm based on the model-based cluster analysis performed with the mclust package in RStudio.

Figure 6.
Violin plot of pentalith radius versus height in the section. The vertical spread indicates the abundance of that radius measurement in the sample. Points are jittered to prevent overplotting. The black line represents the mean of each sample. Histograms show the percentages of forms X and Y in each sample. The sample with the arrow (Z34) is the only one that contains a greater proportion of form Y than form X.

Figure 7.
Comparison of *B. bigelowii* radius measurements for the Living, Recent, late Danian, and Turonian. n is the number of specimens measured in each study. Names of size classes are homogenized after Hagino et al. (2009). Numbers located at size class boundaries are the radii measurements converted from original side lengths using the equation in Figure 4. The upper size limit (3.7 µm) of the Turonian small form was converted from the original side length value given by Takano et al. (2006), due to a discrepancy in its value later reported by Hagino et al. (2009).

Figure 8.
Comparison of *B. bigelowii* sizes through the Cenozoic. The lower bar represents the size range (in radius) of *B. bigelowii* from Zumaia; the upper bar represents that of the living as reported by Takano et al. (2006), Konno et al. (2007), and Hagino et al. (2009). Black pentagons represent the largest sizes and white pentagons represent the smallest sizes found in the literature.

Figure 9.
δ13Corg (this study) and δ13Ccarb (Arenillas et al., 2008) in the Zumaia section. Because of the discrepancy between sample heights in the two studies, the isotopic excursions do not align. The rightmost column shows our aligned interpretation of the two events, illustrating the fact that they likely represent the same excursion.

Figure 10.
Biostatigraphic correlation between the Zumaia and Qreiya sections. The gray band shows the range of *Diantholitha* spp. The yellow band highlights the Neo-duwi beds, in which the isotopic excursions occur at Qreiya. Isotope data and *Diantholitha* spp. ranges at Qreiya adapted from Aubry et al. (2012). Note that *D. magnolia* is referred to as *D. hemisphaerica* by this author.
**Figure 11.**
Four proposed explanations for the abundance pattern of form Y. A) Genetic mutation allowed for a small number of large pentaliths to be present at all times. B) The large pentaliths present in the lower part of the interval are remnants of an earlier speciation event; form Y evolved later (~61.84 Ma). C) Form Y evolved before the peak acme and is present throughout the interval, but is diluted by the abundance of form X. Its sudden abundance at ~61.84 Ma gives the illusion of a speciation event at this level. D) Form Y did not diverge from form X. Instead, both forms evolved from a common ancestor. Sample ages are given in Appendix A.

**Figure 12.**
Migration as an alternative hypothesis to explain the abundance of form Y. Form Y may have evolved in the North Sea during the peak acme of form X at Zumaia. It would have then migrated to Zumaia, giving the appearance of a speciation event at meter ~61.84 Ma. The sizes of late Danian pentaliths from the North Sea have not yet been reported.
1.9 Plate Captions

Plate 1.
Range of preservation of *Braarudosphaera bigelowii* pentaliths. Preservation quality decreases from left to right. 1, 3, 5: form X; 2, 4, 6: form Y. Scale bars are 5 µm.

Plate 2.
Table 1

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Figure 1
Figure 3
Figure 4

\[ y = 0.7018x + 0.039 \]
\[ R^2 = 0.94 \]
Figure 5

![Graph showing the distribution of microorganisms by radius and side length. The graph is divided into two categories: form X and form Y. The x-axis represents the radius in micrometers (μm), ranging from 1 to 11, while the y-axis represents the number of specimens. The graph includes a legend indicating the distinction between form X (pink) and form Y (light blue). Additionally, there is a smaller inset diagram illustrating the S.L. and P.L. (possibly referring to surface and profile length).]
Figure 6
Figure 7

Living (0 Ma)
Small form
Intermediate form-A
Intermediate form-B
Large form

Recent (>0 Ma)
Small form
Intermediate form-A
Intermediate form-B

Paleocene
L. Danian
(~61.5 Ma)
Form X
Form Y

Cretaceous
Turonian
(~91 Ma)
Small form
Normal form

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Figure 9

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Aligned Isotope Stratigraphy

- This Study
- Arenillas et al. (2008)
Figure 10
Figure 11
Plate 2
Chapter 2

THE OLDEST PREDACEOUS WATER BUGS (INSECTA: HETEROPTERA: BELOSTOMATIDAE) FROM THE TRIASSIC COW BRANCH FORMATION OF VIRGINIA/NORTH CAROLINA

Abstract

A new genus and species of predaceous water bugs, *Triassonepa solensis* n. gen. n. sp., is described from the Triassic Cow Branch Formation of Virginia and North Carolina (USA) based on approximately 36 adult specimens and 51 nymphs. This species is the oldest known member of the extant family Belostomatidae. It is placed in a new genus based on the unique structure of the raptorial foreleg, in which the tarsus is elongate and opposed to the tibia + femur. The fossil record of this family is reviewed and the paleoenvironmental implications of the species assemblage preserved in the Cow Branch Formation are discussed.

2.1 Introduction

The Heteroptera, or sucking bugs, have a long fossil record, potentially spanning back to the Permian. The first putative Heteropteran from this Period is *Paraknightia magnifica* Evans, 1943 from New South Wales (Evans, 1950). However, the first definitive Heteropteran, *Arlecoris louisi* Shcherbakov, 2010, was recently described from the lowermost Middle Triassic (lower Anisian) of the northern Vosges Mountains of France. This species is also the earliest member of the infraorder Nepomorpha Popov, 1968, a group containing the majority of truly aquatic heteropterans (Belostomatidae Leach, 1815, Nepidae Latreille, 1802, Naucoridae Leach, 1815, Corixidae Leach, 1815, and Notonectidae Leach, 1815). The Nepomorpha have the best fossil record of all Heteroptera (Grimaldi and Engel, 2005), no doubt because of their aquatic habits, but the
fossil record of belostomatids is not yet well studied. Modern Belostomatidae are medium- (9 mm) to very large-sized (110 mm adult body length) swimming bugs, which are efficient predators, grabbing prey with raptorial forelegs and siphoning out liquid content with a sharp beak. The internal tissues of their prey are liquefied by injecting potent and painful salivary secretions.

The earliest described belostomatid fossils are from the Jurassic, when the family first diversified. The oldest of these is *Odrowazicoris polonicus* Popov, 1996, an isolated wing in Hettangian-age beds of the Holy Cross Mountains of Poland. *Tarsabedus menkei* Popov et al., 1994, *Lethonectes naucoroides* Popov et al., 1994, and *Mesonepa* sp. were described from other Lower Jurassic deposits, in Dorset, England (Whalley, 1985). The only Middle Jurassic species yet known, *Aenictobelostoma primitivum* Polhemus, 2000, is from the Todilto Formation of New Mexico. Three species are known from the Upper Jurassic Solnhofen Formation of Germany: *Mesobelostomum deperditum* Germar, 1839, *Mesonepa primordialis* Germar, 1839, and *Stygeonepa foersteri* Popov, 1971 (Lakshminarayana, 1984). A fourth Upper Jurassic species, *Nettelstedtia breitkreutzi* Popov et al., 2000, was described from an isolated wing in Nettelstedt, Germany.

Though belostomatids are widespread in Cenozoic sediments, many are still undescribed. A Paleocene specimen was reported from the Paskapoo Formation of Central Alberta, Canada (Mitchell and Wighton, 1979); two unnamed Lower Eocene specimens were reported from the Fur Formation of Denmark (Larsson, 1975; Rust and Ansorge, 1996). Wedmann (2000) mentioned a specimen from the Upper Oligocene Enspel Formation of Westerwald, Germany. *Propoissonia beskonakensis* Nel and Paicheler, 1992 was described from an Oligocene-Miocene diatomite in Turkey. In addition, the extant genus *Lethocerus* Mayr, 1853 became fairly diverse during in the Miocene, including *L. sulcifemoralis* Riha and Kukalová, 1967, and *L. turgaicus* Popov, 1971 from the Oligocene-Miocene of the Czech Republic and the Miocene of Russia, respectively. Two additional Middle Miocene species were described from the Shanwang Formation of Shandong Province, China: *Manocerus stagnans* Zhang, 1989 and *Diplonychus microcephalum* Zhang and Zhang, 1994 (formerly genus *Sphaerodema*). Finally, two modern species, *Lethocerus americanus* Leidy, 1847 and *Belostoma bakeri* Montandon, 1913 are reported to occur in Upper Pleistocene asphalt deposits of California (Miller, 1983).

Today, belostomatids have a worldwide distribution, although the majority of species are found in the tropics. They are represented by three subfamilies consisting of nine genera and approximately 146 species (Schuh and Slater, 1995). The subfamily Belostomatinae Lauck and Menke, 1961, is by far the largest group within Belostomatidae and contains six genera: *Abedus* Stål, 1862, *Appasus* Amyot and Serville, 1843 (Polhemus, 1995), *Belostoma* Latreille, 1807, *Diplonychus* Laporte, 1833, *Hydrocyrius* Spinola, 1850, and *Limnogeton* Mayr, 1853. The subfamily Horvathiniinae

The current belostomatid phylogeny is based on morphology and reproductive behaviors: (Lethocerinae, (Horvathiniinae (Belostomatinae))) (Lauck and Menke, 1961; Mahner, 1993; Smith, 1997). Lethocerinae is the most basal taxon because it retains many characters of Nepidae (‘water scorpions’), the sister group to Belostomatidae. In addition, all species of Lethocerinae are emergent-brooders, meaning that their eggs are deposited on emergent vegetation and attended to by the males. In contrast, the more derived members of Belostomatinae are back-brooders, in which eggs are deposited on the backs of their male mates. Horvathiniinae is placed between Lethocerinae and Belostomatinae because of its more intermediate morphological characteristics. Brooding behavior has not been observed in this group and its phylogenetic position is less certain (Lauck and Menke, 1961).

The relationships within subfamily Belostomatinae are mostly resolved based on morphology with the exception of the genus *Limnogeton*. Its position has been questioned due to its lack of raptorial forelegs and natatorial mid and hind legs. Lauck and Menke (1961) noted the possibility that these characters may indicate a more basal position within Belostomatidae. However, it has been placed within subfamily Belostomatinae because it exhibits back-brooding behavior (Voelker, 1968) and this position was supported in a recent phylogenetic analysis of Nepomorpha (Brożek, 2014).
This paper describes the earliest known species of family Belostomatidae, *Triassonepa solensis* n. gen. n. sp., from the Upper Triassic Cow Branch Formation of southern Virginia and northern North Carolina. Based on both the species assemblage within the formation and the known habitats of modern belostomatids, probable chemical and environmental conditions of the deposit are discussed.

### 2.2 Materials and Methods

#### 2.2.1 Locality and Material

The fossils described in this study are from the Cow Branch Formation of southern Virginia and northern North Carolina, an Upper Triassic (Carnian/ lower Norian, 230–220 Ma) deposit outcropping in the former Solite Quarry where over 30 Van Houten cycles are preserved. Three cycles have yielded insect fossils, but one cycle has produced the majority of the insects in this formation (Olsen et al., 1978; Fraser et al., 1996). The Cow Branch Formation is significant because it preserves the oldest fauna of freshwater insects, which are preserved as thin, two-dimensional, silvery films in a matrix of very fine-grained, black shale. Preservation is often excellent, with many specimens fully articulated and microscopic details visible. In addition to 11 orders of insects (Fraser and Grimaldi, 2003; Grimaldi et al., 2005), the Solite Quarry has produced numerous amphibious reptiles (*Tanytrachelos ahyinis* Olsen, 1979), a gliding reptile (*Mecistotrachelos apeoros* Fraser et al., 2007), fish fossils, dinosaur footprints, and many plant species (Olsen et al., 1978).
2.2.2 Methods

Specimens were viewed using a Nikon SMZ1500 microscope, fitted with a fiber optic ring light. This non-directional, diffuse light source was necessary to illuminate the silvery, carbonized film by which the insects are preserved. In order to examine the minute details, specimens were wet with 70% ethanol to increase contrast between the fossil and the black shale matrix. Photographs were taken using two separate setups. Specimens viewed at the American Museum of Natural History (AMNH) were photographed with a Nikon 16MP camera and Nikon Elements NIS software on a Nikon SMZ1500 stereomicroscope. Specimens housed at the Virginia Museum of Natural History (VMNH) were photographed with a Canon 6D DSLR camera using Canon Utility 2 software. All specimen measurements were taken using ImageJ software. Total lengths of adults were measured from the anterior margin of head to the distal margin of the 7th abdominal segment, in order to exclude the 8th abdominal segment, which protrudes to varying degrees in different specimens.

Most of the material examined in this study is housed at the Virginia Museum of Natural History (VMNH), Martinsville, Virginia, USA; some specimens are deposited in the American Museum of Natural History (AMNH), New York, USA.
2.3 Systematic Paleontology

Suborder HETEROPTERA Latreille, 1810
Infraorder NEPOMORPHA Popov, 1968
Superfamily NEPOIDEA Latreille, 1802
Family BELOSTOMATIDAE Leach, 1815

Genus *Triassonepa* new genus

*Type species.* *Triassonepa solensis* n. sp., by present designation.

*Diagnosis.* As for type species, by monotypy.

*Etymology.* The genus name is a combination of the prefix Triasso-, for the Triassic Period from which the genus is derived, and -nepa, a standard suffix used for the superfamily Nepoidea.

*Occurrence.* Former Solite Quarry C, Eden, Rockingham County, North Carolina, USA (36° 32' 29.6556" N, 79° 40' 12.8424" W); Carnian/Norian, Upper Triassic, Cow Branch Formation.

*Remarks.* *Triassonepa* differs from all other known extinct and extant genera of Belostomatidae by the structure of the foreleg, in which the tarsus is elongate and opposed to the tibia + femur.

*Triassonepa solensis* new species

Figures 1-2, Plates 1-3

*Holotype.* VMNH 94671, male, Fig. 1.

*Etymology.* The specific epithet is named after the former Virginia Solite Corporation quarries, from which the specimens were recovered.
**Diagnosis.** Body elongate. Head small, largely hidden beneath pronotum dorsally, with triangular clypeus. Pronotum small with concave anterior and posterior margins. Hemelytra with prominent claval suture; dense reticulations on apical third. Prothoracic legs raptorial, with elongate tarsus opposed to tibia. Metathoracic tibia and tarsus with dense setal fringe on inner (mesal) margin. Abdomen with seven visible segments (segments 2–8) and five visible pairs of spiracles.

**Occurrence.** Former Solite Quarry C, Eden, Rockingham County, North Carolina, USA (36° 32' 29.6556" N, 79° 40' 12.8424" W); Carnian/Norian, Upper Triassic, Cow Branch Formation.

**Description of Adults.** The description is based on the ~36 adult specimens collected to date (Pl. 1). Body elongate; total length (anterior margin of head, excluding clypeus, to apex of 7th abdominal segment) 10.6–14.1 mm (mean 12.1). **Head:** Small, largely hidden beneath pronotum dorsally, with triangular clypeus. Eyes, antennae, and mouthparts not visible. Ocelli, if present, not visible. **Thorax:** Pronotum small, 2.5–3.5 mm in width, 0.8–1.1 mm in length, with concave anterior and posterior margins. Mesonotum width approximately 1.6X pronotum width; possible pigmentation preserved on pronotum and mesonotum. Scutellum about as wide as pronotum, but with indistinct margins. Hemelytra with fine crenulations on proximal two-thirds, reticulations on distal third; clavus and claval suture distinct; venation as shown in Figure 2 (also Pl. 2.4). Hindwing, if present, not preserved. Prothoracic legs raptorial (Pl 2.1); femur wide at base (~1 mm), narrowed distally, partially obscured by body; tarsus elongate and opposed to tibia, tibia and tarsus of similar width, tibia length approximately 1.1X length of tarsus; tarsal claw
not visible. Mesothoracic legs slender, with no setae or fringe apparent; tibia approximately 1.3X length of entire tarsus. Metathoracic legs slender, with dense setal fringe on inner (mesal) margin of tibia and tarsus (Pl. 2.6); few specimens with double setal fringe on tarsus (Pl. 2.8–2.9); femur partially obscured by body; tibia length approximately 1.2X tarsus length; tarsus with two segments. **Abdomen**: Broad, often obscured by hemelytra; length (7.4–8.6 mm) and width (5.4–6.3 mm) vary, likely due to diagenetic alteration; abdominal segments 2–8 visible; dorsum with five pairs of spiracles visible on segments 3–7. Spiracle diameter ranges from 0.26 to 0.40 mm (mean 0.34). Terminalia variable, but with lateral paddle-like lobes; pair of short processes between the lobes that resemble respiratory tubes; two specimens (VMNH 94671, Pl. 2.12, holotype; VMNH 94672, Pl. 1.4) with more elongate terminalia and two distal articulated appendages, possibly claspers.

*Description of nymphs.* Body oval, narrowed at anterior and posterior ends. Head small, often not preserved, no features discernable. Thorax roughly trapezoidal with dividing line between lateral halves; wing pads not visible. Prothoracic and mesothoracic legs not preserved. Few specimens with metathoracic tarsus preserved; setal fringe present, as in adults; two specimens (e.g., VMNH 54155, Pl. 3.4) with double setal fringe; claws, if present, not visible. Abdomen roughly triangular; six segments visible (segments 3–8).

*Remarks.* Specimen VMNH 94671 was chosen as the holotype because of the excellent preservation of its legs (Fig. 1). It is the only specimen yet found with a preserved foreleg, and one of only two specimens with a preserved mesothoracic leg. Though this specimen
has a narrower abdomen and lacks the stoutness of the other specimens, this difference in overall shape can likely be attributed to diagenesis due to its morphologic similarity to the other specimens. Additionally, it is the largest specimen (14.10 mm), but it is of a similar size to that of the only other male specimen (VMNH 94672, 13.49 mm). This may indicate that males of this species were larger than the females, which is unusual in the Heteroptera.

_Triassonepa solensis_ n. gen n. sp. is the earliest known member of the family Belostomatidae. Unfortunately, all adult specimens are preserved in dorsal aspect, concealing many morphological characters. This is interesting because preservation of the ventral surface is quite common in other belostomatid fossils (i.e., _Sinobelostoma liui_, Chou and Hong, 1989; _Lethocerus vetus_, Nel and Waller, 2006). _Triassonepa solensis_ ranges in length from approximately 10.6–14.1 mm, placing it on the small side of the range of modern belostomatids (9–110 mm; Schuh and Slater, 1995).

The head of _Triassonepa solensis_ is poorly preserved in almost all specimens, preventing a detailed description at this time. The only feature often visible is the triangular clypeus. Neither compound eyes nor ocelli are preserved, though the absence of ocelli may be expected since modern belostomatids do not possess them (Lauck and Menke, 1961). The absence of preserved antennae is also expected given that the antennae of modern belostomatids are small and concealed within grooves beneath their heads (Schuh and Slater, 1995). In addition, due to the exclusive preservation of the dorsal surface, no mouthparts are visible.

The legs of _Triassonepa solensis_ provide some interesting characters for comparison with modern members of the Belostomatidae. One unique character of this
species is the structure of its prothoracic legs (Pl. 2.1). Though only preserved in a single specimen, the foreleg of *T. solensis* has an elongate tarsus that opposes the tibia. In contrast, modern belostomatids have a thin tibia + tarsus opposed to the femur (Pl. 2.2–2.3). This raptorial foreleg appears to be less specialized than those of modern belostomatids. Another difference between *T. solensis* and modern belostomatids is the possession of setae on the legs. Modern belostomatids have setae on both the mesothoracic and metathoracic legs, but it appears that the mesothoracic legs of *T. solensis* lack these setae. This might suggest that instead of using these legs for swimming, they may have instead used them in conjunction with the forelegs to capture and hold prey. However, only three mesothoracic legs on two specimens have been recovered to date, indicating that the absence of these setae is not yet definitive. *Triassonepa solensis* does, however, possess a setal fringe on the metathoracic legs (Pl. 2.6, 2.8–2.10), indicating that they were specialized swimmers. It is interesting to note that a few specimens (including two nymphs) appear to have a double setal-fringe (Pl. 2.8–2.9). It is unclear at this time whether this double-fringe is a result of the position in which they were preserved, or if these specimens are a distinct species. Measurements of body length, abdomen width, and tarsal length have shown that these two specimens have similar proportions to specimens with a single fringe, and therefore may be the same species.

The hemelytra of *Triassonepa solensis* also provide good features for comparison to modern taxa. The hemelytra do not appear to cover the entire abdomen of any specimen, but this is likely an artifact of preservation (Pl. 2.4). Because the apical parts of modern heteropteran hemelytra are thin and membranous, it is unlikely that these regions
would have been preserved. In addition, the abdominal spiracles of *T. solensis* are located on its dorsal surface, which is typical of insects that breathe underwater via a plastron (a thin film of air held beneath the wings and used as a physical gill). In order for *T. solensis* to use a plastron, as is done by many modern aquatic insects including belostomatids (e.g., *Abedus herberti*, Goforth and Smith, 2012), its hemelytra would need to fully cover its spiracles (i.e., to the edges of the abdomen).

The 8th abdominal segment of *Triassonepa solensis* contains two lateral, paddle-shaped lobes and two medial processes resembling respiratory tubes (Pl. 2.11). It is morphologically quite similar to the eighth abdominal segment of female naucorids (particularly *lyocoris exclamationis*, as illustrated by Lee, 1991). This suggests that: (1) *T. solensis* occupies a very basal position within Belostomatidae, and (2) the majority of the preserved specimens were likely female. However, two specimens (VMNH 94671, Pl. 2.12, holotype; VMNH 94672, Pl. 1.4) possess terminalia with a slightly different structure. The 8th abdominal segments of these specimens are more elongate and possess two apical, articulated appendages that resemble claspers. It is therefore likely that these two specimens are males.

Immature *Triassonepa solensis* were separated into instars based on total body lengths (Pl. 3). Because the head was not often preserved, there is a minor amount of uncertainty in some of the measurements. A total of 51 nymphs were measured from the anterior margin of the head to the apex of the abdomen, yielding five size classes (Fig. 3): Instar I, 1.6–2.3 mm (mean 2.0); Instar II, 2.6–3.4 mm (mean 3.0); Instar III, 4.0–5.2 mm (mean 4.6); Instar IV, 6.2–7.7 mm (mean 6.9); Instar V, 9.5–10.3 mm (mean 9.9). Each instar was approximately 1.5X larger than the preceding one. Modern belostomatids also
have five instars and show a similar growth ratio of 1.2–1.5 with each successive instar (Tables 1–2). These ratios correspond to Dyar’s Rule, which states that an insect instar is approximately 1.4X the size of its previous instar.

2.4 Discussion

2.4.1 Belostomatid Habitats

Due to the presence of swimming fringes on the hind legs of *Triassonepa solensis*, it is reasonable to assume that this species had similar physiology and behaviors to modern belostomatids. Understanding the habitats of these modern belostomatids has implications for determining the nature and chemistry of ancient ‘Lake Solite.’

Belostomatids live in a wide variety of habitats, but are most commonly found in shallow bodies of water with marginal vegetation. Kashian and Burton (2000) reported *Lethocerus* sp. from the wetlands of northern Lake Huron in areas dominated by sedges. Belostomatids also occur in many of India’s small freshwater lakes. Majumder et al. (2013), for example, found two genera (*Lethocerus* and *Diplonychus*) living in the marginal vegetation of manmade, urban lakes in Tripura, northeastern India. *Diplonychus rusticus* was collected from both Pocharam Lake in southeastern India (Deepa and Rao, 2007), and Loktak Lake of northeastern India (Takhelmayum and Gupta, 2011). Loktak Lake is unique in its possession of phumdis, floating islands composed of vegetation, organic matter, and soil, amongst which belostomatids live. Belostomatids have also been collected from arid wetlands such as Bañado Carilauquen in west-central Argentina (Scheibler and Ciocco, 2013). These semi-permanent wetlands are located near a shallow, saline lake, though the wetlands themselves have negligible salinity.
In addition to shallow lakes and wetland environments, belostomatids inhabit the marginal areas of deep lakes, such as Lake Victoria in Kenya (Muli and Mavuti, 2001; Orwa et al., 2015). Three species were identified there: Diplonychus sp. (formerly genus Sphaerodema), Hydrocyrius sp., and Lethocerus niloticus. Similarly to Loktak Lake, Diplonychus sp., and Hydrocyrius sp. were found to inhabit the lake’s floating hyacinth mats (Orwa et al., 2015).

Belostomatids are one of the few groups of aquatic insects that can tolerate agriculture-impacted and polluted water bodies. Belostomatids inhabit the length of the Enfranz River in Ethiopia, from the clean headwaters to the agriculture-dominated mouth (Mehari et al., 2014). Other nepomorphs, such as naucorids and nepids, were only found in the unimpacted, upstream areas. Additionally, Belostoma sp. has been collected in the eutrophic Kipkaren River of Kenya (Aura et al., 2011) and Diplonychus sp. was found to inhabit the margins of a number of polluted Bangalore lakes in southern India (Balachandran and Ramachandra, 2010). Perhaps the most extreme case is the collection of Belostoma sp. from hydrogen-sulfide rich Cueva del Azufre in Tabasco, Mexico (Tobler et al., 2007).

Belostomatids are also known to inhabit temporary environments such as rain pools (Fontanarrosa et al., 2009), agricultural fields (Das and Gupta, 2010), rice paddies (Hendawy et al., 2005), and sinkholes (Blinn and Sanderson, 1989) and are common inhabitants of ephemeral playa lakes in arid environments (Haukos and Smith, 1992). Belostomatids have been collected in the stagnant ‘buffalo-wading pools’ of Tarangire National Park on the savanna of northern Tanzania (D.G., personal observation). Merickel and Wangberg (1981) collected Belostoma flumineum along the shores of two
playas near Lubbock, Texas and Richardson et al. (1972) found one juvenile belostomatid in the Jornada Playa of New Mexico. Adult belostomatids disperse to these ephemeral environments via flight, and as a result, are often found at bright lights during the night.

A few studies have even reported belostomatids inhabiting brackish waters. Angelin et al. (2010) collected *Diplonychus* sp. and *Belostoma* sp. from an estuary in southern India with a salinity of between 4 and 8 parts per thousand (ppt). Siddiqi (2008) reported belostomatids in the marginal areas of India’s Lake Lonar, which is a hyperalkaline, saline, crater lake with a pH of approximately 10.5 (Siddiqi, 2008) and a salinity of up to ~6 ppt (Yannawar and Bhosle, 2013). However, Badve et al. (1993) report that the marginal areas of the lake near the inflow of the freshwater springs have a pH closer to 7.5. It is in these areas that the marshes exist, and it is likely that the belostomatids inhabit these more suitable areas.

2.4.2 Environmental Interpretation of ‘Lake Solite’

Like their modern counterparts, many fossil belostomatids are reported from shallow, lacustrine paleoenvironments (i.e., Grimaldi and Maisey, 1990; Martínez-Delclòs et al., 1995; Prokop and Nel, 2000). However, this contrasts with both interpretations of the paleoenvironment of the Cow Branch Formation. Olsen et al. (1978) first described the environment as a large, deep, chemically stratified lake. This stratification would have produced anoxic bottom waters that prevented bioturbation and therefore allowed for exquisite fossil preservation of delicate insects like midges and tiny hemipterans. Though unusual for a modern belostomatid habitat, other deep lacustrine paleoenvironments have been reported to contain belostomatid fossils. One such deposit,
the Upper Oligocene Enspel Formation of Germany (Poschmann et al., 2010), has produced ten belostomatid fossils, four of which are adult specimens (Wedmann, 2000).

More recent research by Liutkus et al. (2010) proposed that the Cow Branch Formation was a shallow, alkaline, saline, rift valley lake. They presented a number of reasons for this interpretation: 1) dominance of terrestrial and nearshore-dwelling insects and terrestrial vascular plants, 2) exquisite fossil preservation, and 3) presence of dolomite and absence of quartz and zirconium throughout the deposit.

Based on the environmental preferences of modern belostomatids, their abundance in the Cow Branch Formation would indicate a shallow, nearshore paleoenvironment. The tolerance of modern belostomatids for polluted and harsh water conditions suggests they may have also been tolerant to extreme environments such as saline, alkaline, rift valley lakes. Because belostomatids breathe air, they would be unaffected by poor water quality. However, harsh water conditions would affect organisms possessing gill respiration (i.e., Ephemeroptera, Plecoptera, Odonata, Trichoptera, etc.) and the lack of gilled insect nymphs of these orders within the deposit is good evidence for poor water quality. The only gilled insect order reported from the Cow Branch Formation is Diptera (Liutkus et al., 2010). However, this appears to be a misidentification of the enigmatic, gilled, larva-like arthropod, which may actually be a crustacean.

In addition to belostomatids, the insects preserved in the Cow Branch Formation are mostly terrestrial adults from the orders Hemiptera (Sternorrhyncha), Diptera, and Coleoptera. A few other taxa have been found to date, including adult members of Blattodea, Odonata, Orthoptera, Plecoptera (Fraser and Grimaldi, 2003), Thysanoptera
(Grimaldi et al., 2004), Mecopterida (Grimaldi et al., 2005), Amphiesmenoptera, and Neuroptera (D.G., personal observation). This unique assemblage of terrestrial insects further suggests that the water was toxic to gill-possessing, aquatic larvae and other sensitive groups. Moreover, Fraser and Grimaldi (1999) noted the abundance of conchostracans within the insect bed. Modern members of this group are most commonly found in ephemeral, alkaline water bodies (Tasch, 1969).

Liutkus et al. (2010) discussed the exquisite preservation of the insect fossils as evidence for a shallow lake. Most insects are completely articulated, which is quite rare for Triassic fossils (cf., Riek, 1974; Brauckmann and Schlüter, 1993; Shcherbakov et al., 1995; Martins-Neto et al., 2008). The lack of disarticulation indicates very limited postmortem movement. If the lake had been as deep as originally suggested, most insects would have decayed, disarticulated, or have been eaten before settling to the benthic zone. Moreover, there are no fossil fish found in the insect layers, further evidence that the insects were buried in very shallow water.

The geochemistry of the deposit also supports the interpretation of a saline, alkaline lake. Liutkus et al. (2010) reported dolomitic claystone throughout the insect bed, and in modern lakes primary precipitation of dolomite occurs most often in waters with elevated salinity, alkalinity, and with abundant magnesium and calcium (DeDeckker and Last, 1989). Furthermore, the surrounding basin is rich in quartz, making its absence in the Cow Branch deposit significant. In addition, albite is abundant in the deposit (Liutkus et al., 2010), which is proposed to have formed by the reaction of clay, quartz, and sodium under alkaline conditions (van de Kamp and Leake, 1996). Moreover, the deposit is depleted in zirconium. Ayers and Zhang (2005) have demonstrated that this element
dissolves in alkaline conditions. These three geochemical conditions support a saline, alkaline environment during the time of deposition.

Though most fossil belostomatids have been reported from shallow, non-saline, lacustrine environments, Polhemus (2000) reported fossils from a saline environment, the Jurassic Todilto Formation of New Mexico. This formation is interpreted as a paralic, saline playa due to its interfingered marine and continental sediments (Lucas et al., 2000). Though this interpretation is somewhat controversial, the water chemistry in this type of environment may have been similar to that proposed by Liutkus et al. (2010) for the Cow Branch Formation. Vega et al. (2006) gave a second example of a potentially saline belostomatid habitat from the Lower Cretaceous Sierra Madre Formation of southeastern Mexico, which has been interpreted as a brackish marginal lagoon or estuary. Due to the wide environmental tolerances of both fossil and modern belostomatids, the dominance of terrestrial adult insects and lack of aquatic nymphs, the abundance of conchostracans, the exquisite preservation of the Solite fossils, and the geochemistry of the Cow Branch Formation, it is very likely that ‘Lake Solite’ was a shallow, saline, alkaline rift-valley lake.

2.5 Conclusions

This chapter has described a new genus and species of predaceous water bug, *Triassonepa solensis* n. gen. n. sp., from the Upper Triassic Cow Branch Formation of Virginia/North Carolina. It is the oldest known member of the family Belostomatidae. Due to the unique structure of its prothoracic leg, in which the tarsus is elongate and opposed to the tibia + femur, it has been placed in a new genus.
Additionally, this study has discussed the paleoenvironmental conditions of the Cow Branch Formation. Despite its original description as a deep, chemically-stratified lake, new geochemical evidence, in addition to the wide environmental-tolerances of both fossil and modern belostomatids, the dominance of terrestrial adult insects and lack of aquatic nymphs, the abundance of conchostracans, and the exquisite preservation of the Solite fossils, it is very likely that ‘Lake Solite’ was a shallow, saline, alkaline rift-valley lake.
### 2.6 References


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2.7 Table Captions

Table 1.
Mean lengths (in mm) of the five instars of *Triassonepa solensis* (this study), *Lethocerus maximus* (Cullen, 1969), *L. mazzai* (De Carlo, 1962), *Hydrocyrius columbiae* (Miller, 1961), *Belostoma flumineum* (Flosi, 1980), *B. malkini* (Cullen, 1969), and *Abedus breviceps* (Keffer and McPherson, 1988).

Table 2.
Growth ratios of the same species as in Table 1, calculated by dividing instar length by length of the previous instar.
2.8 Figure Captions

Figure 1. 
*Triassonepa solensis* n. gen. n. sp., habitus of holotype, VMNH 94671. Scale bar is 2 mm.

Figure 2. 
Reconstruction of *Triassonepa solensis* showing major morphological characters.

Figure 3. 
Lengths of *Triassonepa solensis* instars and adults. A total of 70 specimens (51 nymphs and 19 adults) were measured. The two largest adult specimens are males.
2.9 Plate Captions

Plate 1.
Photographs of adult specimens of *Triassonepa solensis*: (1) VMNH 49641; (2) VMNH 53881; (3) VMNH 53880; (4) VMNH 94672. Scale bars are 2 mm.

Plate 2.
Morphological comparison of the forelegs, hemelytra, metathoracic legs, and terminalia of *Triassonepa solensis* and extant belostomatid species: (1) foreleg, VMNH 94671, holotype; (2) foreleg, *Belostoma flumineum*; (3) foreleg, *Benacus griseus* (formerly genus *Lethocerus*); (4) hemelytra, VMNH 53881; (5) hemelytra, *Diplonychus urinator sudanensis*; (6) setal fringe on metathoracic leg, VMNH 50230; (7) setal fringe on metathoracic leg, *Belostoma elongatum*; (8) double setal fringe on metathoracic leg, VMNH 90281; (9) double setal fringe on metathoracic leg, VMNH 90279; (10) metathoracic leg, VMNH 94671, holotype; (11) female terminalia, VMNH 90281; (12) male terminalia, VMNH 94671, holotype; (13) terminalia, *Diplonychus urinator sudanensis*. Scale bars are 1 mm.

Plate 3.
*Triassonepa solensis* growth series: (1) Instar I, VMNH 94575a; (2) Instar II, VMNH 50512; (3) Instar III, VMNH 53871; (4) Instar IV, VMNH 54155; (5) Instar V, VMNH 52498. Scale bar is 2 mm, located in photo 1.
Table 1

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<th><em>L. maximus</em></th>
<th><em>L. mazzai</em></th>
<th><em>H. columbiae</em></th>
<th><em>B. flumineum</em></th>
<th><em>B. malkini</em></th>
<th><em>A. breviceps</em></th>
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Figure 1
Figure 3
Plate 3
General Conclusion

This Master’s Thesis has explored the potential of paleontology for interpreting the evolutionary processes that occur in both marine and lacustrine environments. I have presented two examples of what the fossil record can contribute to our understanding of evolution. In chapter one, I demonstrated that an understanding of pseudo-cryptic speciation in living members of the coccolithophore species *Braarudosphaera bigelowii* allowed me to infer that the two size classes of pentaliths from the Zumaia section represent two unique pseudo-cryptic species. In chapter two, I showed that behaviors of a long-extinct insect, *Triassonepa solensis*, can be inferred based on its morphology and an understanding of the behavioral patterns of modern belostomatids. Additionally, I have shown that paleoenvironmental conditions of the Cow Branch Formation can easily be inferred by the life habits of the insects preserved and knowledge of the environmental preferences of their living relatives.