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# Possible Past Hybridization Among *Desmognathus ochrophaeus* in Canadice Lake: An Ecological Survey Exploring Desmognathine Salamanders and the Competitive Exclusion Hypothesis in the Western Finger Lakes of New York State

Aisha L. Gadson  
alg6794@rit.edu

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**Possible Past Hybridization Among *Desmognathus ochrophaeus*  
in Canadice Lake: An Ecological Survey Exploring  
Desmognathine Salamanders and the Competitive Exclusion  
Hypothesis in the Western Finger Lakes of New York State**

by

Aisha L. Gadson

A Thesis Submitted in Partial Fulfillment of the  
Requirements for the Degree of Master in Environmental Science

Department of Environmental Science

College of Science

Rochester Institute of Technology  
1 Lomb Memorial Drive, Rochester, NY, 14623

Presented: August 12, 2016

Final Edits Completed: December 2016

**Committee Approval:**

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Paul Shipman, Ph.D.

Date

Associate Professor, Thesis Advisor

---

Larry Buckley, Ph.D.

Date

Head of the Thomas H. Gosnell School of Life Sciences, Associate Professor, Committee  
Member

---

Elizabeth Hane, Ph.D.

Date

Associate Head of the Thomas H. Gosnell School of Life Sciences, Associate Professor,  
Committee Member

## **Table of Contents**

<u>Page Number</u>	<u>Content</u>
Page 4	Abstract
Page 5	Introduction
Page 14	Methodology
Page 19	Results
Page 26	Discussion
Page 30	References
Page 33	Appendices

## **Abstract**

Dusky Salamanders are a varied group of sister taxa found in Northeastern North America. Two species, *Desmognathus fuscus* and *Desmognathus ochrophaeus*, have geographically overlapping ranges. In addition, they are very morphologically similar, share many niche requirements, and are found in extremely similar or even the same salamander communities. These shared traits have been posited to arise from the influence of niche conservatism during their species' evolutions. Also, despite their physical similarities they are historically found not to hybridize on a large scale, nor have ever had a full population merge recorded. Therefore, it appears that the community ecology of these species does not seem to follow the ecological theory of Competitive Exclusion. This study consists of both an ecological and genetic survey to determine if there are any variables that separate both populations observed in the field. The hypothesis is that there will be a low instance of hybridization in both populations, and that there will be ecological differences associated with population densities where they overlap. Also, where they do overlap, I predicted that there was an observable benefit to both species that overrides their need for competition of resources. The results of this study were that the two distinct morphological groups observed shared identical haplotypes in the mitochondrial gene tested, showing a single population. In addition, it was also concluded that there was no statistical difference in the measured ecological variables for both morphologies, thus failing my hypothesis by both measures. This study took place in the Western Finger Lakes (NY) basin, within the wetland/stream around Canadice Lake.

## **Introduction**

### *Overview:*

Community ecology is a discipline that describes the interactions, resources, and history of all species living within an ecosystem. Primarily an amalgamation of biogeography, ecology, and phylogenetics, these disciplines together are used to describe the mechanisms of how each co-existing species within a community came to be in its' most current population state (Webb, 2002). In traditional ecology, species living together are known to interact on an individual level which then eventually accumulate up to the population level. Within a total community however, this is not enough to describe the relationships of whole populations interacting with each other. Such studies require ecological interactions and evolutionary processes, both past and current, to be observed in equal weight (McPeck, 1996).

This can be extremely difficult to achieve on both a theoretical and experimental basis, for a variety of reasons. The primary reason is the sheer magnitude of variables needed to study even one of the numerous species in a community. The most common variable groups include resources, behaviors, ecological history, morphology, environmental states, and species interactions (McPeck, 1996). This can be overwhelming to collect and analyze, and these are merely those variables for each species within the entire system. Known as the proximate approach, these are the variables working on community structure within what can be considered a closed system (Losos, 1996). Although it is easier to consider a community a closed system, neighboring systems are not mutually exclusive. Called the ultimate approach, this methodology encompasses the history and effects that make different communities distinct from each other (Losos, 1996). Variables can include the morphological, population, and intraspecific differences a species has in different communities (Losos, 1996). Another reason for the difficulties in studying community ecology is the multi-disciplinary approach that must occur to get an overall assessment of an ecosystem. The knowledge and skill base encompassing both classic field collection techniques as well as modern genetics and molecular technology is large and can be difficult to interpret together. In addition, due to modern genetics, past knowledge of phylogeny is shifting and changing. Both

the increasing accuracy of evolutionary analyses and the study of microevolution has only increased the diversification of taxa in the world (McPeck, 1996).

These difficulties in studying Community Ecology is the reason I chose to focus on a singular aspect of the field with only two species. The concept I chose to study was the Competitive Exclusion Hypothesis, an ecological concept derived from the observations of Charles Darwin whilst studying related species and their niches (Violle, 2011). Known also as the phylogenetic limiting similarity hypothesis, Competitive Exclusion can be described as the tendency of closely related species having greater niche similarity leading to greater competition for resources (Violle, 2011). Per Violle (2011), this is a hypothesis that is commonly referenced and taught, but not often studied on its own merits. Related to this concept is another hypothesis labeled as Niche Conservatism, which describes tendency of a species to keep in alignment with their ancestral ecological characteristics (Wiens, 2005). This retention of ancestral traits has been directly linked to allopatric speciation, giving reason for higher similarities between sister taxa that occur geographically closer (Wiens, 2005).

The organisms that I studied for this concept were two related species in the *Desmognathus* genus, named *Desmognathus fuscus fuscus* and *D. ochrophaeus*. They, and other *Desmognathus* taxa, developed through allopatric speciation during the last ice age (Kozak, 2006). Both *D. fuscus* and *D. ochrophaeus* have ranges overlapping in the Mid-Atlantic East Coast of the United States. Within this region, studies have recorded the presence of both species within the same locality (Karlin 1981, Rissler 2002). This trend has also been noticed anecdotally by several local herpetologists and naturalists in the Finger Lakes region, NY (Personal Communication, Paul Shipman). In terms of the competitive exclusion hypothesis, these overlapping populations seem to coexist without the usual effects of interspecific competition. This could potentially be explained by high gene flow between the populations. However, documented instances of hybridization tend to be rare, although evidence of cross-back genes has been shown at rates of over 25% (Karlin, 2005). This shows that there is likely some unknown benefit to their interactions, or that they do not share microhabitats within a larger locale.

With these observations in mind, there were two main goals to this study. The first was to provide an ecological and genetic survey that would determine the prevalence of each species being found together within the same sites as well as any hybridization events. The second was to document how closely related the two species were in terms of niche placement by analyzing the correlation of ecological features to individuals within a species. The genetic analysis was for authenticating the phenotypic catalog of each species, as well as determining the presence of hybridization among the observed overlaps in population. The gene in question was Cytochrome Oxidase B, and was used as a ‘barcoding’ to achieve this. Accuracy in identification was significant for testing my hypotheses because both species here are extremely similar not only in appearance, but in their macro-genetic makeup as well.

I hypothesized the existence of an ‘ecological gradient’ associated with population density; that there are two genetically separate, but overlapping populations within a community with a low amount of hybridization in those overlap areas. In addition, should they overlap, I predicted there will be an observable benefit to both species that overrides the negative effects of interspecific competition for resources. The term population here describing a unique group of individuals marking a single species, with distinct ecological traits.

#### *Review of D. fuscus, D. ochrophaeus, and the Competitive Exclusion Hypothesis:*

The plethodontid salamanders in the *Desmognathus* genus have many overlapping traits and ecological requirements after diverging due to allopatric speciation (Rissler, 2003). With the phylogenetic lineage of Amphibia: Urodela: Plethodontidae: *Desmognathus*, salamanders in this genus are morphologically distinguished from other dusky species by a bright post ocular stripe, slightly larger back legs, a bright dorsal stripe and spot patterning, and a snout with straight jaw (Conant 1998). Most are a combined insectivore and carnivore, consuming insects, larvae, and even other salamanders (Sites Jr., 1978). Reproductively, eggs are internally fertilized and oviposited underneath shaded, defensible areas near littoral systems. Such areas include moss, rocks, leaves, logs, and hardened soil deposits (Hom 1987). Some parental care in the form of nest guarding by females is common as well (Hom 1987).



The two Dusky salamander species, *Desmognathus fuscus fuscus*, and *Desmognathus ochrophaeus*, are two species groups that are comparable in physiology, niche requirements, and breeding habits. There have been previous studies performed on both the speciation of taxa under the *Desmognathus* umbrella (Rissler, 2003), as well as studies on the topic of hybridization between these species (Karlin 1981). The former, through parsimonious tree analysis, demonstrated that both species are just that, separate species with other sister taxa between them (Rissler, 2003). I have confirmed this through use of parsimonious bootstrapping, with the genetic information from NCBI's GenBank (Appendix III). Hybridization, as mentioned above, is very rare although some long standing mixed communities have shown evidence of past interbreeding (Karlin, 2005). This would imply that there is some yet unknown behavioral or temporal reason for high rate of reproductive isolation.

Both species are conditionally streamside species, with mating migrations to their home spawning sites at nearby lakes or ponds. Foraging for both species encompasses aquatic and terrestrial habitats, and prey diversity is very high for them as well, at most times of the year (Sites Jr., 1978). Although there is sexual selection within populations, there is little to no monogamy, as evidenced by the prevalence of females storing their previous partner's genetic material for future fertilization (Arnold, 1993). Per Jaeger (1993), since these species are streamside classed in an area with high prey abundance, these species are not extremely territorial.

Despite being less territorial than other salamanders, aggressive behavior can be found within both species; biting is a common aggressive behavior, both on an individual level, as well as an interspecies level (Jaeger, 1993). These behaviors can also become prevalent during the brooding stage after clutch laying. Like others in their genus, both species have been shown to have egg-bearing females guarding the clutch during initial development. The females will protect the clutch by reducing foraging to fend off predators after their eggs. Then, either just before or at hatching, the females abandon the now mobile offspring (Jaeger, 1993).

The subspecies *Desmognathus fuscus fuscus*, commonly known as the northern dusky salamander, is currently classed as the most prevalent of 2 subspecies as known under the *Desmognathus*

*fuscus* umbrella (Bonnett, 2002, Crother 2003). Physical traits include bright spotting and a sharply keeled tail (Conant, 1998). Their geographical range extends for most of the Eastern Seaboard of the US [excluding Florida], and as far west as the Mississippi river (Conant 1998). They can be considered to prefer stream-side and moist terrestrial areas (Rissler 2003). The species history for these salamanders is extremely complex. At one point, there were three subspecies labeled under the umbrella of *D. fuscus*; *D.f. fuscus*, *D.f. conanti*, and *D.f. santeetlah*. However, in 1996, *D.f. conanti* was elevated to species level by Titus and Lawson (Crother, 2003), and adapted the previous umbrella common name of ‘spotted dusky salamander’.

The other species, *Desmognathus ochrophaeus* or the mountain dusky salamander, has a range that is mostly restricted to the Appalachian & Smoky Mountains, but reaching through to Upstate New York as well. They are commonly found in mountain springs, but can also be found in lower elevations. Like *D. fuscus*, they prefer moist terrestrial areas (Rissler 2003). *D. ochrophaeus* can be characterized by its dual dorsal line patterning, equally proportioned limbs, oval tail, and a snout that has irregular ridging at the mouth cavity opening (Conant 1998). *Ochrophaeus* reproductive habits are very much like those of the spotted dusky salamander (Hom 1987).

Having no known major isolating mechanism, similar reproductive habits, and physiology, hybridization between the two species is possible. Studies on this phenomenon show that in areas of population overlap hybridization and backcrossing incidents do occur (Karlin 1981). It must be noted that evidence of backcrossing was much stronger than that for true hybrids, as individuals of true heterozygosity for the species-specific traits tested were not found. This study also concluded that though there was hybridization and backcrossing between both species in overlap areas, unique genetic variations existed that are species-specific across all populations (Karlin 1981). Many questions attempt to discover why sister taxa can be genetically distinct, have long-standing non-hybridizing populations, and yet, still share many niche qualities. The prevailing hypothesis is phylogenetic niche conservatism, which can be defined as two populations of the same species, diverging in allopatric speciation with the goal of maintaining their ‘ancestral’ niche qualities (Kozak, 2006). It is an idea that describes some of the

mechanisms involved in vicariate isolation in regions where ideal habitats are interspersed with unfavorable conditions over time (Kozak, 2006). The example environment described by Kozak (2006) is mountainous regions, where species that are elevation dependent can have interspersed areas within and out of the tolerable range. Due to the large amount of mountainous and riverine habitat that stretches along the geographic range for this genus, it has been posited that dusky salamanders are a prime example for this phenomenon. As time passed, the ancestral populations for dusky salamanders became more isolated and fragmented. Each individual population then became driven to preserving 'their niches', which starts being reflected in the population as new traits and genetic variants (Kozak, 2006). Then, when reintroduced in fringe populations, there was enough change in behavior and/or mating mechanisms were enough to reduce hybridization or crossing-back for both subsets.

With these observations in mind, it becomes important to view my two species as unique populations, with a small amount of genetic flow if local ranges overlap. The reasons for non-hybridization are most likely to be temporal isolation, sexual isolation [both pre- and post-zygotic], or minor environmental preferences that reduces the likelihood of physical interaction (Arnold, 1993). It is possible that full populations merges could occur, but behavioral preferences for breeding likely keep most individuals from *D. fuscus* and *D. ochrophaeus* from interacting enough to make that happen. The question then is; that with little gene flow to combat negative interactions, but overlapping local populations, how is it possible that they can maintain stable populations without succumbing to the pressure of the competitive exclusion hypothesis?

To explore this, the hypothesis of competitive exclusion must be studied. As noted before, it is a mechanism that describes the population interactions between two species that share similar niche requirements (Violle, 2011). This concept is considered important in that the competition for resources must eventually geographically exclude the species population that is least successful in acquiring or holding on to vital necessities, and as such is a mechanism that ultimately will make cohabitation within a community impossible (Violle, 2011). It is important to note that the resource being competed for must be usable by individuals of either species, even in the absence of their competitor (Means, 1975). This means

that the competition happening is not influenced by habitat engineering by one of the community competitors. With that restriction in mind, many population studies have still been known to follow such a trend, whether grouped together in one geographic locale or in overlapping neighboring communities. However, not many have rigorously tested the progression, strength, or overall veracity of competitive exclusion in regards to multiple types of population interactions (Violle, 2011). Thus, when populations that did not adhere to this concept (whether in part or fully) this hypothesis began to receive greater scrutiny and experimentation.

When discussing *D. fuscus* and *D. ochrophaeus*, there are many documented instances of finding both species in the same local area but as distinctly separate populations. *Desmognathus* overall are unique and have been the basis of interspecific study for a long time. This is due to high number of species each with distinct behaviors and phenotypes, and yet many similar habitat requirements (Rissler, 2004). This phenomenon has been noted in more recent years with other species types as well, including species of plants (Tillman, 2007). These observations can be described as populations of closely related species are being prevalently found in the same community, as opposed to a wide distribution of genetically varied organisms (Violle, 2011). Called phylogenetic clustering, this distribution pattern is becoming more and more observed in natural systems. As opposed to phylogenetic over dispersion, which is representative of a community with non-related populations filling different niches (Violle, 2011). The development of phylogenetic clustering can be linked to several ecological hypotheses due to unique interactions between a specific species and one or more other species. One such hypothesis is known as habitat partitioning, in which the macro habitat can be divided into different yet overlapping microhabitats that form a gradient of species populations (Hairston, 1980). This gradient can be derived from several physical characteristics, such as terrestrial to aquatic, high to low elevations, and shaded to exposed areas. A studied example of this are grass communities that live along a nitrogen gradient, due to high competition for this limiting nutrient (Wedin, 1993). The degree of partitioning in these plant communities depended on several direct variables, including the strength of the competitors and seasonal patterns (Wedin, 1993). As noted before, high levels of competitive exclusion in the overlapping areas

still can occur, but it is not the only mechanism in the shaping of these population distributions (Hairston, 1980; Violle, 2011).

Another link in the potential influence of phylogenetic clustering is the hypothesis of facilitative interactions, a mechanism described as an interspecies interaction that is beneficial to both parties in some manner (Violle, 2011). There are many potential benefits to these behaviors, including resources protection from non-related salamanders and shared predation burdens. This mechanism is a good descriptor for the observation that in closely related salamander populations, the presence of more than one closely related, yet partially morphologically distinct species aids in overall predation protection from one population being overexploited by predators (Jaeger, 1993). It can be likened to vast schools of fish having their genetic pool being protected by sheer numbers rather than avoidance techniques. In this case, it is postulated that because of the similarity in size and coloration predators for them will not favor one population over the other, thus granting protection to both species in terms of genetic diversity loss. However, these predator and prey relations can contrast (even while occurring simultaneously) the notion of facilitative interactions as well.

Predator relationships are one of the most basic interactions in ecosystems. They shape populations on both ends of the relationship, as well as populations of other species that interact with either end. Traditionally direct predator cascades are easier to describe, but may not fully actually describe all the influences it can have on targeted populations. They also can be influenced by other relationship types. One such complex cascade involves one predator with two related preferred prey types in one community. This seems easy enough at first; if there is equal preference, then chance shapes the prey populations. If there is one prey more favored, then the non-favored prey population blooms due to more niche freedom. However, this process is heavily influenced both the behaviors of prey species, and their interactions with each other (Hileman, 1992). Anti-predator mechanisms and predator avoidance mechanisms are two approaches that have been studied in *D. ochrophaeus* as one of two prey species specifically. The former describes how to escape an encounter, and the latter describes how to avoid the encounter. Depending on the preferred mechanism, two prey species that are related can force the other

into different interactions with predators (Hileman, 1992). One example is that if both species prefer to avoid a predator by hiding yet one species is better at doing so, then their competition and behavior can shift both populations regardless of the predator's preference (Hileman, 1992). This example is one of many permutations on how the three species interacting with each other can have drastic population consequences.

Another cascade that is related to the one above involves solely *Desmognathus* salamanders. It involves the idea that related species can be both competitors and predators/prey within the same community (Wollrab, 2013). This is due to the drastically ontogenetic size shifts that can occur among related and geographically linked species. The genus of *Desmognathus* salamanders have an extremely varied adult body size, and many are opportunistic feeders. Thus, when two species with similar juvenile sizes have disparate adults' sizes, they can go from direct competitors to being in a predator prey relationship (Wollrab, 2013). This interaction, again, is one of the many types of predation relationships that can occur within related populations. Also, it is to be noted that once again, the results of a single mechanism can be influenced by others, and they can have a profound impact in varying ways on the populations within a community (Chase, 2002).

From all the examples provided, the hypotheses above can interplay or contrast with one another in varying degrees to influence the distribution of populations. These ecological influences can be defined into two archetypes; niches assembly and dispersal assembly. The former refers to all the mechanisms that involves direct species interaction, such as competition, predation, beneficial cooperation previously discussed. The latter emphasizes the dispersal of individuals that eventually give rise to genetically diverse populations, like niche conservatism, allopatric, and sympatric speciation (Rissler, 2004). From this, I can deduce that any population interactions are the sum of both types niche development. Not to the effect that every sub-category of each archetype has a hand in every population distribution, but that the interaction of the categories of niche shaping is what is critical to the distribution of species. Chase (2002) describes the inherent interaction between predation and competition, and that not only can both affect populations to varying degrees, but there may be minute mechanisms that further impact the

influence of both. He also describes how bias can force a conclusion in how much impact a mechanism truly has on a community. Thus, circling back to the hypothesis that competitive exclusion is not the dominating mechanism in all population structures but rather a part of the influences that shapes the community. It also can be concluded that competition may even be a much smaller impact than previously realized in previously studied interactions (Chase 2002; Violle 2011).

## **Methodology**

### **Field data collection:**

I surveyed for streamside salamanders and collected ecological data along the small tributaries located around Canadice Lake, NY, from Fall 2015 through Spring 2016. The ecological survey performed was critical for determining if there was a difference in niche requirements between these two species. To describe the potential community structure of this *Desmognathine* complex, there are three likely formations; two populations separated by niche with minimal overlap, two populations minimally separated by niche with lots of overlap, or one population that has no niche separation and completely overlaps the same area. The hypothesis from earlier, postulating the existence of two species exhibiting minimal negative consequences from competitive exclusion whilst occupying most of the same space, concurs with the second category out of this list. This survey was divided into two parts; field collection and genetic analysis. The former was sub-divided into ecological data and species capture data.

### ***Survey Locations:***

The locations of collection took place in eight stream systems around Canadice Lake, found in the Finger Lakes of New York State. This lake, along with all others west of Seneca lake, are known as the Western Finger Lakes. This region is well within the range of both *D. fuscus* and *D. ochrophaeus*, and populations have previously been documented here (Conant, 1998). They all are glacial lakes with a Northerly drainage pattern (Wemett, 2001). This was due to the reversal of pre-glacial drainage patterns. The area was gouged from centuries of ice movement, and then became filled depressions after the

glaciers receded (Wemett, 2001). Canadice in specific is characterized by a mixture of forested and swamp ecosystems surrounding the lake, with lake bound stream complexes running through the trees. They usually consist of shale ledges, and shale and/or bedrock basins. There was also a prevalence of limestone mixed into the bedrock of both the aquatic and terrestrial portions of this area.

Each potential stream for collection around the lake were based on the geophysical characteristics that these species prefer; shallow clear stream bed systems, moderately free flowing water with the chance for small pools, and areas where rocks or debris stand above the moving water for perching opportunities. In addition to searching for these characteristics, locations were chosen by a visual encounter method with ease of access from the main lake road by foot being the predominant criterion.

Four streams were surveyed in Fall on the West-facing side of Canadice lake, while the other four were surveyed in early Spring on the East-facing side. All streams were selected from the closest path bordering the lake and are marked on the map located in Appendix V. The spring surveys started after the first sustained thaw, and were limited match to the fall counts for comparative purposes. Within each location, approximately every 10 meters was noted as a new sample site. The determination of a new sample site is either an abrupt shift in the physical qualities within the location or reaching the end 10m since the last site data was recorded. This was to collect accurate local data to correlate individuals to microhabitat data. The search method for collecting the salamanders was a visual encounter method, going in perpendicular lines across the river bed. Stones and other stream debris was investigated as potential cover microhabitats for the target species. Each site within a locale was sampled for the following parameters; GPS Coordinates (One per location, coordinates found in appendix IV), percent leaf coverage/organic cover, and substrate composition, which can be described as the sum percentage of cobble (rocks) and boulders, gravel, and sand. Also measured was percent leaf litter cover, deepest stream depth, widest stream width, widest stream bed width, deepest bank depth, total dissolved solids (ppm), pH of water, and the temperature of both the ground and water.

All parameters were collected once per site, with exception of those to denoted as location only. Any parameter that did not require a percentage or measuring stick was measured in accordance to the



directions provided by each test or equipment. All parameters with a percent coverage involved were determined out of 100%, either in whole or a sum-total, and was determined by the interpretation of the sample collector. The geological makeup of the studied section came from direct observation, based upon the notes of previously made survey maps of the area (Muller, 1986). The substrate sizes were separated into three categories; sand, gravel, and rock/boulder, correlating to the ISO international scale for soil material and mass. See Appendix I for the ISO chart to see size versus naming conventions.

#### *Species Data:*

Using a visual encounter search method, individual salamanders were captured, measured, and sampled for tissue by clipping the tip of the tail. The clipping of the tail not only provided a genetic sample but also ensured that individual salamanders were not resampled. All salamanders were immediately released at their original site of capture after sampling.

Each specimen had a correlating identification number, with *D. fuscus* having the designation A0000, *D. ochrophaeus* having the designation B0000, undetermined or juvenile dusky salamanders with C0000, and 'other' salamander species with D0000. Each collector was designated a number that correlates to the first number in the sequence, for example A1000. Meaning that collector number 1 found a specimen of the species *D. fuscus*. The last three numbers refer to the identification order of the specimens found, no matter which species was determined. These identification numbers will be used to both track tissue samples and demographic correlations to specific parameters.

The species parameters to be sampled were, species identification, note if specimen was found within cover or exposed, cover dimension and type, and ground temperature near cover, Also measured was distance from stream, height above stream, distance from stream bed (only taken if stream width and depth is 0), length of specimen (from snout tip to tail tip )(cm), length of tail (cm), length between limbs (cm), width of the widest portion of the head (cm), any distinguishing markings or details, potential age, and a tissue sample from the tip of the tail (Only in the Fall data collections).

Any measured parameters that required equipment were performed as per the instructions provided by the manufacturers. Any type of debris within the stream bed was searched to find specimen that were hiding beneath cover. The defining characteristic used to identify both target species was tail shape, ovoid or rounded with *D. ochrophaeus*, and keeled for *D. fuscus* (Conant, 1998). With regards to tissue sample collecting, the samples consisted of approximately 5mm to 10mm of tail tissue, removed with an unused, disposable scalpel. The tissue samples were stored in DNA buffer solution in 1.5 mL Eppendorf tubes on ice, until they were frozen at -25 degrees Celsius. The buffer solution was made by the RIT biology department for short term tissue storage before processing.

#### Genetic analysis:

This portion of the experiment deals with the DNA extraction and genetic analysis of the collected salamander tail samples. The tissue samples were suspended in 1.0 milliliters of DNA buffer solution made from the RIT biology department. For the tissue extraction, whether with the long-term samples or the immediate ones, the method from extracting the DNA will be the same. The extraction was performed per the PureLink® genomic DNA mini kit for tissue. The only deviation from kit's directions were the digestion times in the 55° C water bath, which was changed from 3 hours to 24-48 hours. In addition, the maximum amount of elution buffer was used for final extraction from the HiBind column, which equated to 300 microliters.

The next process for the DNA samples to go through was polymerase chain reaction (PCR) for replicating a select mitochondrial gene. The gene being used for analysis was Cytochrome Oxidase B, unit 1 (a.k.a COX1); which is common for all *desmognathus* salamanders as well as other plethodontids. Each sample was labeled numerically, starting with 1 and ending at 32 for most test runs, shifting to 2-33 when a blank was added testing purposes. The listing of numbered samples is in appendix II. Most DNA samples were left at their initial concentrations for the PCR process, but some were diluted to a 9:1 ratio of purified Nano water to DNA (marked with a star on the appendix II list). These were samples and mixed with GoTaq PCR and staining solution, the primers for either three genes, and purified water. The

mixture proportion is as follows; 12.5 microliter of GoTaq, 9.5 microliters of purified nuclease free water, 1.0 microliters of forward COX1 forward primer, 1.0 microliters of reverse COX1 reverse primer, and 1.0 microliters of DNA solution (not added for the blank).

The maximum fluid amount per sample for the process totaled at 25 microliters. The annealing temperature for the PCR was experimented with to achieve the best concentration of the target gene fragment. Overall, the range for the annealing temperatures was 52 degrees Celsius to 58 degrees Celsius, and the selected final temperature was 56 degrees Celsius. The complete temperature cycling settings started with 5 minutes at 95 degrees Celsius. Then 1 minute intervals of 95, the chosen annealing temperature, and 72 degrees Celsius, for 35 cycles. Finally, 72 degrees Celsius for five minutes. The holding temperature after the completed run was set to a DNA safe 4 degrees Celsius. This process was repeated until the bands became distinct by annealing variation and that the blank sample came back negative.

The way that I tested whether the PCR runs were successful was to perform gel electrophoresis with a DNA ladder. This process was repeated as many times as PCR runs were performed. It started with creating the gel. Using the size of gel box as a measure for volume, a gel was made from heating agarose and a liquid solution at a specific percent concentration of solids. I mistakenly used purified water for my initial gel samplings, but switched to 1% LB buffer as the liquid base. Also, I switched from 1% agarose to 2% agarose when I changed ladder types. The number of boxes used varied due to size, but always the minimum to accommodate the number of samples. The gels were then oriented and submerged in the 1% LB buffer. The average voltage range used was 100 - 200 volts, and was determined based on time constraints. The gel was then developed in a 100x dilution of GelRed dye solution for at least 2 hours. This step allowed for the gel to be viewed under ultraviolet fluorescent light to photograph the results.

The final procedures for these genetic samples included the purification of the selected PCR run as well as testing the concentration and purity of DNA within each sample. The purification of the PCR samples was achieved using the E.Z.N.A. Cycle-Pure kit (by Omega®) instructions. Within the confines of the procedures for this part, 30 microliters of elution buffer was used to extract the DNA from the spin

columns. After this step, the purity and concentration of each sample was tested using a nanodrop machine and the accompanying software. This was done twice for each sample. After determining that purity fell into the acceptable nanometer proportion range of 1.6-2.4, the samples were plated and shipped to the GENEWIZ® laboratories for sequencing.

## Results

This study culminated in the creation of a snapshot representing the community of *Desmognathus* salamanders found in the stream complexes around Canadice Lake, NY. Based upon phenotypic identification in the field, I captured 9 *D. fuscus*, and 27 *D. ochrophaeus* (Figure 1). In addition to these target species, I also captured 3 unknown Desmognathine juveniles, and 6 non-Desmognathine salamanders (3 *Plethodon cinereus*, 1 *Gyrinophilus porphyriticus*, and 2 *Ambystoma maculatum*).

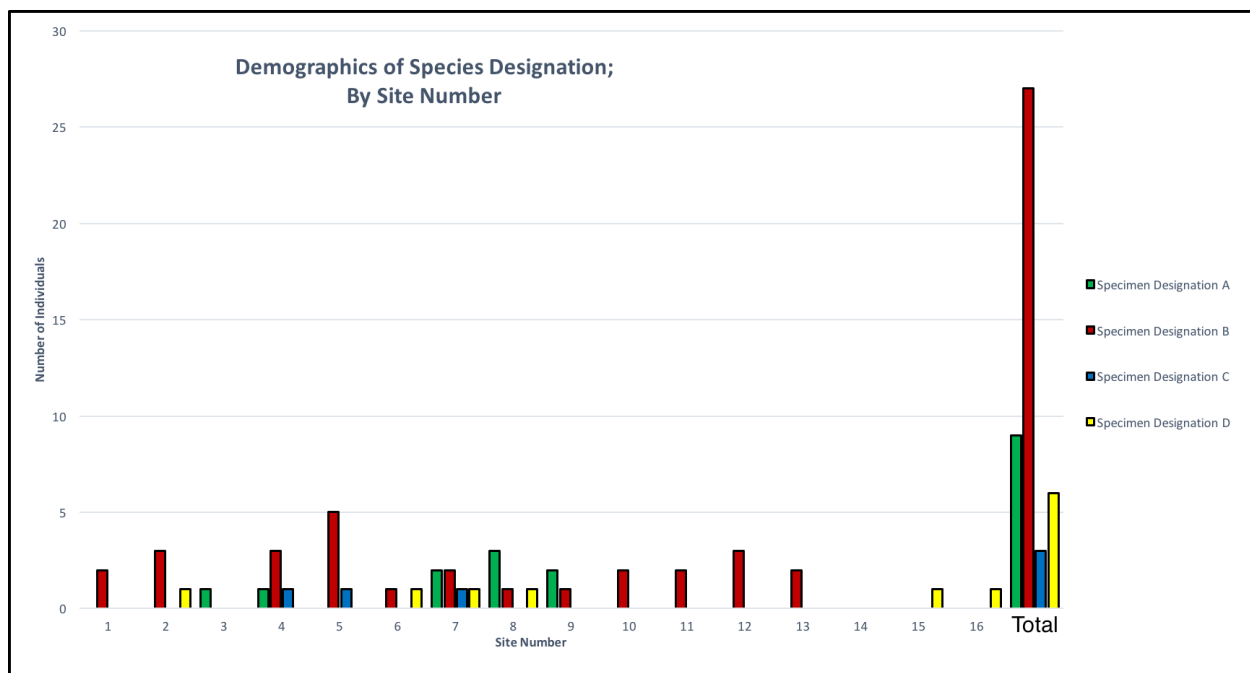


Figure 1: Sampled Populations for Fall and Summer Collection Seasons, by Site Number

Figure 1. All captured species data representatives, as catalogued across seasons one and two (fall and spring respectively). Species designation is as follows: A is *D. fuscus*, B is *D. ochrophaeus*, C is unknown *Desmognathus*, and D is 'other' salamanders. The 'other' salamander species category included 3 *Plethodon cinereus*, a *Gyrinophilus porphyriticus*, and 2 *Ambystoma maculatum*. 38 specimens were

*caught in fall versus 7 in the spring (sites 1-11 and 12-16 respectively). All stream location collections ended if 1) Stream terminated into purely terrestrial habitat; 2) If stream locations became too dangerous to transverse by foot; or 3) If there was adverse weather. All unknown desmognathus salamanders caught were juveniles. Counting of non-desmognathus species was done to get an idea of other present species, but is not included in further analysis.*

The distribution in time of these populations varied as the number of specimen captured in the fall far exceeded that in spring (Figure 1). That being 38 for fall versus 7 in the spring, with each season having the same number of sampling locations (4). The sampling differences may have occurred due to seasonal fluxes, especially as spring collections started just after a late thaw. Another source of differing counts may have come from the fall samples coming from a different side of the lake, which can affect amounts of sun exposure and minor temperature shifts. However, *D. ochrophaeus* was found in both seasons on both sides of the lake, which demonstrated that both sides of the lake could support salamanders with similar niche requirements.

There were no variables correlating with any specific population (those being phenotypically categorized as *D. fuscus*, *D. ochrophaeus*, and juvenile *Desmognathus*) (Table 1). This was determined by performing multivariable linear regression analyses using Minitab statistical software. The groupings for the regression models were body measurements, site stream and environmental measurements, and specimen stream measurements. Each grouping was performed against the population counts. Having significantly no correlation between the population captured and any measured variable, was demonstrated by p-values of greater than 0.05. Thus I combined phenotypic populations into a single group and described habitat using descriptive statistics. The primary cover type that the captured salamanders were found under were shale and limestone cobble; not organic materials such as wood or leaves as initially thought. The average canopy cover was 76.6%, while leaf litter accounted for an average of 26.9% of stream bed coverage.

Another group of results involved the inorganic substrate composition as well as stream dimensions (Table 1). Rocks/boulders, gravel, and sand had an average of 43.3%, 35.5%, and 21.2%

coverage respectively. Stream dimensions appear to be important as well in key habitat markers having averages of water depth at 0.1m, bed depth at 2.0m, stream width at 0.9m, and finally bed width at 4.0 m. These variables depict a stream where my salamanders prefer extremely shallow, slow flowing, but clear water (evidenced by the average total dissolved solids at 199.4 ppm or approximately 0.02% by volume), with a lot of moist, semi terrestrial space. Such streams also were characterized by a definitive barrier in the form of transition from semi-terrestrial stream bed to a height jump into purely terrestrial landscape. The pH of the water averaged 8.4, but is reasonably explained due to the high prevalence of limestone in the soil and bedrock of the region. Temperatures where the salamanders were found varied by the weather of the region and time of year, the only significance being the water was on average cooler than the ground.

On average, most salamanders were found 0.8m away from the stream itself, and 0.2m above the base of the bed (Table 1). When there was no stream, salamanders were found and average of 0.5m away from the edge of stream bed. None were found exposed or in the water. This posits that between the moving water and the edge of the stream bed is where these individuals preferred to be in. As noted above, there was a predominant cover type, where 37 out of 39 *Desmognathus* salamanders were caught under shale and limestone over any other variety. Cover size averaged an area at 5.6cm<sup>2</sup>, well into the dominating rock/boulder size category.

Table 1: Descriptive Statistics of Ecological Data

*Table 1. Multiple linear regression was performed on all variables against species designation (1 for D. fuscus, 2 for D. ochrophaeus, 3 for unknown Desmognathus). However, no variable from any category showed significant correlation with a specific population ( $p > 0.05$ ).*

Variable	Mean	Range	Standard Deviation
Canopy Cover (% Coverage)	76.6%	50.0	21.0
Geology/Substrate composition (% Coverage)			

Rocks/Boulders	43.3%	55.0	18.0
Gravel	35.5%	80.0	23.8
Sand	21.2%	30.0	9.5
Deepest Stream depth (m)	0.1 m	0.3	0.1
Widest stream width (m)	0.9 m	2.3	0.7
Widest stream bed width (m)	4.0 m	3.8	0.8
Deepest bank depth (m)	2.0 m	3.3	1.1
Leaf litter (% coverage)	26.9%	65.0	17.5
Total Dissolved Solids (ppm)	199.4	263.3	91.0
pH of water	8.4	0.5	0.1
Temperature of both ground and water			
Ground (°F)	14.8°C	29.6	8.0
Water Temp (°C)	13.2°C	17.7	5.1
Cover Area (cm <sup>2</sup> )	5.6 cm <sup>2</sup>	75.2	12.6
Ground temperature near cover (°F)	14.9°C	27.2	6.3
Specimen distance from stream (m)	0.8 m	3.5	0.9
Specimen height above stream (m)	0.2 m	2.5	0.4
Specimen distance from stream bed (m)	0.5 m	1.1	0.5

---

Body proportion measurements were the last set of measurements taken in the field (Table 2). The average length fell at 7.2cm, tail length at 3.9cm, distance between limbs at 2.1cm, and head width was 0.5cm. As with the habitat measurements, I was unable to determine any morphological differences between salamanders phenotypically categorized as either *D. fuscus* or *D. ochrophaeus* as by determined by ANOVA (Table 2). The results from Table 2 do not include the three juvenile/unknown *Desmognathus*, because of inherent size differences from their adult forms. The ANOVA showed no significance in any measured body proportion to either population grouping, which indicated that

salamanders phenotypically categorized as *D. fuscus* or *D. ochrophaeus* were of single morphological group (except for having either keeled or round tails).

Table 2: 1-sided ANOVA Test Comparing Morphological Measurements between Phenotypically Categorized Populations of *D. ochrophaeus* and *D. fuscus*, Found Around Canadice Lake.

*Table 2. This ANOVA was performed along with the Tukey Method, and for all morphological measurements, the null hypothesis was not rejected ( $p \geq 0.05$ ). Species groups were labeled as 1 for *D. fuscus* phenotype and 2 for *D. ochrophaeus* phenotype. The Tukey method paired both populations into a single grouping.*

Variable Tested	Average length (cm)	p-value	Species Group (1)	Species Group (2)
Length of specimen (cm)	7.2cm	0.90	A	A
Length of tail (cm)	3.9cm	0.75	A	A
Length between limbs (cm)	2.1cm	0.40	A	A
Head Width (cm)	0.5 cm	0.56	A	A

The final analyses that were performed were based upon the genetic data collected in fall data set. After sequencing, the accompanying trace files were analyzed for degree of residue and replication. 28 out of 32 samples were found sufficient for usage in using the SeqManPro program (part of the DNASTAR© suite of genetics analysis programs), which is a program that combines the forward and reverse sequences into a complete DNA sequence. The next step was to use the program MegAlign (from DNASTAR©) to align the sequences as well as measure the degree of similarity between the sequences. This was to measure both the percentage of similarity and percent difference between sequences (Figure 2).





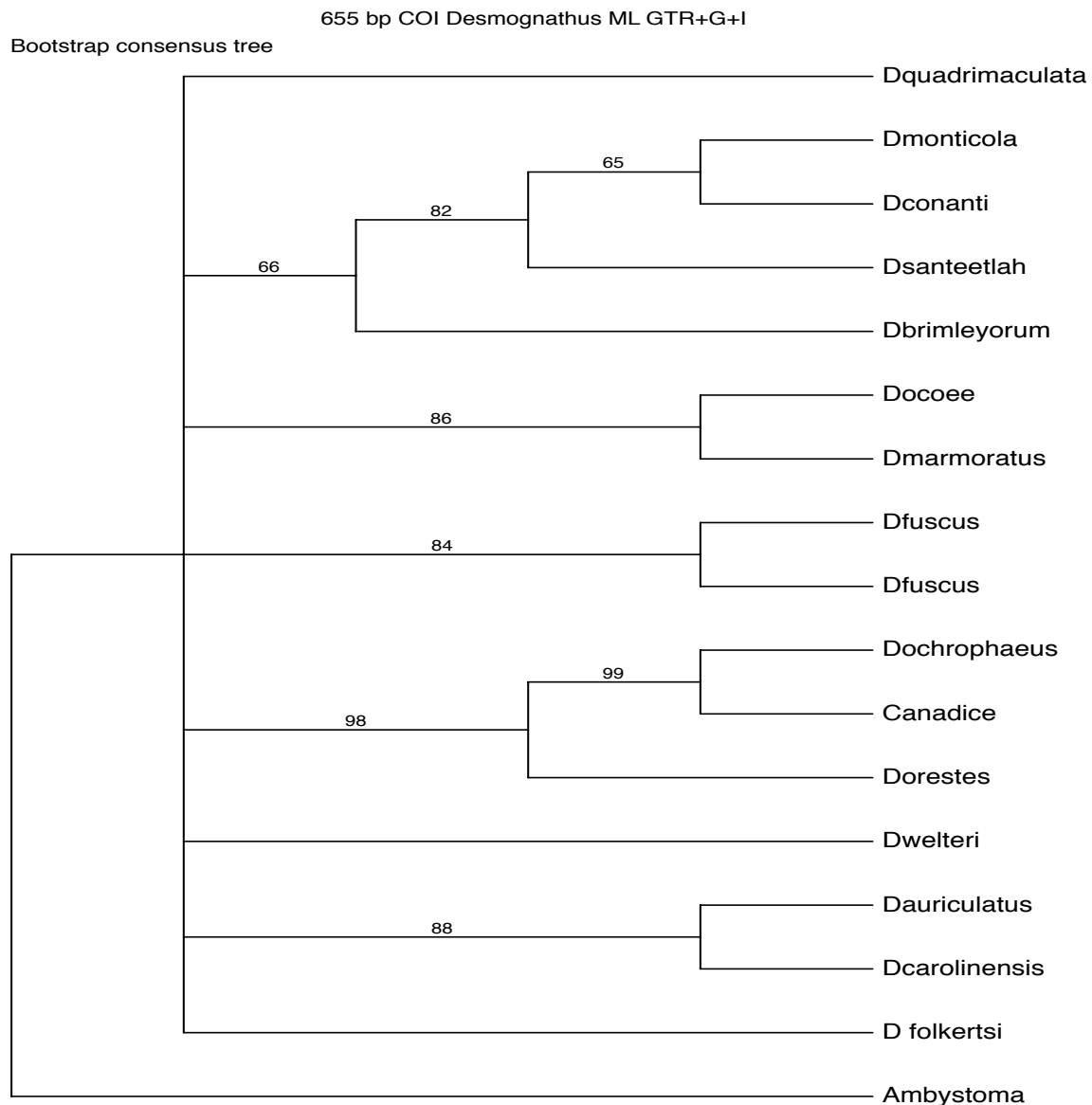


Figure 3: Bootstrap Consensus Tree with *Desmognathus* Salamander representatives

Figure 3. *MegAlign*'s phylogenetic function. The *Ambystoma* genus branch was used as the outlier grouping for this data set. All non-*Canadice* samples were obtained through a BLAST search on GenBank. The numbers along the branches represent the percent strength of the relationship of each branch.

From this phylogenetic tree, the singular mitochondrial haplotype found among the two phenotypic populations around Canadice lake was most related to *Desmognathus ochrophaeus* (Figure 3).

This was reinforced by the percent strength of the bootstrap analysis, which equated to 99%. Their closest relative was *D. orestes*, while *D. fuscus* sits in its own branch. This was very congruent to relationships calculated in the GenBank tree (Appendix III).

## **Discussion**

Overall, these results show that the stream systems around the Canadice Lake system is dominated by a single phenotypically variable (keeled tail versus ovoid tail) *Desmognathus* group, stemming from a maternal lineage from *Desmognathus ochrophaeus*. This study may be the first example of variation in tail shape for populations of *D. ochrophaeus*. Although not demonstrated in this study, this may also be the result of hybridization with another Desmognathine salamander, *D. fuscus*.

This populations' niche is defined by shallow yet flowing stream systems, with a buffer of semi-terrestrial area. Canopy cover was extremely high, and the stream bed had a large abundance of cobble and boulders. Individuals prefer cover when not moving, and access to that cover seems critical in their distribution. Due to the low abundance of any other salamander species, it appears that the desmognathus complex in this community dominates both space and resources. My hypothesis that the presence of both *Desmognathus fuscus* and *Desmognathus ochrophaeus* coincided with minimal competition repercussions is one to be rejected. This being due to the overall lack of presence of two separate niches present in this community as well as the two populations having some gene flow.

In contrast, this study only reinforced the hypothesis of competitive exclusion, due to the dominance of one species complex across an area of similar niche conditions (Violle, 2011). There was a set of ecosystem variables that were consistent with both suspected species' preferences while resources and space appeared to be abundant as well. However, population trends confirmed resource usage was dominated by one local population, rather than two. Population genetics confirmed that as well, as both morphologically different groups did share the haplotype on the gene tested. Despite this correlation, competition cannot be the only pressures shaping this population (Chase, 2002). The presence of other salamander species, though in smaller number at the time of collection, does show that the conditions

needed by this *Desmognathus* group are useable by others (Rissler, 2004). Such species included *Plethodon cinereus* (Red-Backed Salamander) and *Ambystoma maculatum* (yellow spotted salamander) (Figure 1), both of which were found in multiple streams along with our *Desmognathus* salamanders. Though it was only in single occurrences with the more prevalent unidentified grouping. The presence of a much larger predatory species, such as *Gyrinophilus porphyriticus* (Figure 1), indicates the presence of potential local predators. As noted before, salamanders are generally opportunistic feeders, and larger salamanders frequently consume smaller ones (Wollrab, 2013).

My genetic analysis was the most substantial in trying to determine the accuracy of my cataloging, and the relatedness within my local population against established identities. It was confirmed conclusively that *Desmognathus ochrophaeus* was present in the Canadice stream complex. Both the phenotypic and genetic information of those classified in the field as *D. ochrophaeus* matched up together. The biggest conundrum of the study was the specimens categorized as *Desmognathus fuscus* in the field. The phenotypic variation of the tail shapes I observed in the field were very distinct and was used almost exclusively to perform visual identification in the field. The round tail shape *D. ochrophaeus* possesses as opposed to the keel-shape normally described to belong to *D. fuscus* has historically been documented with neither species having any major variations (Conant, 1998). A single *D. ochrophaeus* population possessing both phenotypes would be unusual, however this seems to be the case in my study. There could be the remote possibility that there was cross contamination in the samples sequenced, but the very large number of PCR replications and fact that the actual extractions took place in very small batches make this reasoning extremely unlikely.

The emphasis on the keel shape is significant because it heralds several unique ecological options. One is that a few keel-tailed salamanders were absorbed into a *D. ochrophaeus* population at some point in the past and a few descendants kept the tail trait, along with other significant endemic genetic differences. In such a scenario, that species would likely be *D. fuscus*, due to the complete range overlap that only they of Desmognathine decent share with *D. ochrophaeus* within New York State (Gibbs, 2007). A second hypothesis is that a current *D. fuscus* population of the area had an ancestral

hybridization event with a female *D. ochrophaeus*. Such an event could have led to female hybrids breeding back into that small *D. fuscus* group, giving way to a unique population with primarily *fuscus* nuclear DNA but retaining *ochrophaeus* mitochondrial DNA. The best way to determine which idea is correct would be to either perform protein analysis or nuclear gene sequencing. There is a historical precedent for using protein analysis to test the second hypothesis, as hybridization among *D. ochrophaeus* and *D. fuscus* was explored in this way before (Karlin, 1981). As mentioned above, the rate of hybridization within current communities were extremely low, but there was significant evidence of historical back-crossing in both species (Karlin, 1981). This previous study is why I feel there is more merit in the idea of ancestral gene exchange rather than full hybridization with both species.

The possibilities of how the genetics played into the shape of this community leads heavily back into niche conservatism. The role niche conservatism played in shaping this unique community would have to have started when the two species were introduced to each other after the recession of the glaciers. Both the landscape and the climate were drastically shifting causing the geographic barriers that kept isolated but related species apart (Weins, 2005, Wemett, 2001). Since *Desmognathus* salamanders were thought to undergo allopatric speciation due to these barriers in the past, reintroduction would have occurred more frequently as land was uncovered (Rissler, 2003). The creation of the Finger Lakes provided entirely new habitat that was extremely suitable for salamanders, while warmer temperatures would have increased resource abundance. It is reasonable to deduce that in this climate upheaval may have interfered in the reproductive isolation behaviors that current communities experience. It is also reasonable to deduce that niche conservatism would have driven different species following the glaciers northward to the new and desirable habitat. These two suppositions could be the mechanism that describes ancestral hybridization leading to current populations that have mixed morphologies. Again, this reasoning has some merit due to the work of Karlin (1981) showing ancestral evidence of interbreeding between *D. fuscus* and *D. ochrophaeus*.

With these hypotheses and questions in mind, the future potential for research based upon this population may be of interest. The first potential project would be to perform the genetic analysis with

nuclear DNA. This would allow for analysis directly within the boundaries of this experiment with these data. Another potential avenue would be to recollect from the stream site I visited and perform either a protein analysis or again, a nuclear gene comparison. To study the community structure, an ecological survey encompassing several years would be valuable. For instance, I noted in my results that only those of the *D. ochrophaeus* phenotype were collected in the spring season. It would be interesting to see if that was an isolated incident or that perhaps the emergence of the keel-tailed phenotype from hibernation occurs later in the season. If this was true, it could be the reason for *D. ochrophaeus*' higher abundance or potentially be the mechanism that reproductively isolates them from *D. fuscus* if they are indeed two reproductively separate groups. Another goal that a long term ecological survey would accomplish would be to provide a more complete and thorough map of Desmognathine salamanders in the Finger Lakes region.

To conclude, the influences on how populations are shaped and the effects thereof are extremely important to consider when doing any kind of ecological study. Not considering all the variables can limit and even misrepresent what is happening in a system. In contrast, there is also difficulty in not overwhelming the models with extraneous data. The importance of my study within the realm of Ecology is demonstrating how a multidisciplinary view is necessary to study such complicated systems (Chase, 2002). Despite my hypothesis being rejected, it was important to perform because there are incidences where several *Desmognathus* species coincide heavily with others in their genus, and there are unique and isolated populations that have yet to be studied (Carr, 1985). Analyzing which of the two was the case here took a broad perspective to interpret, and more study is needed to further the understanding of how these species interact and form communities.

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## Appendices

### Appendix I: The ISO Soil Material and Size Chart, with Correlating Assigned Numeric Value

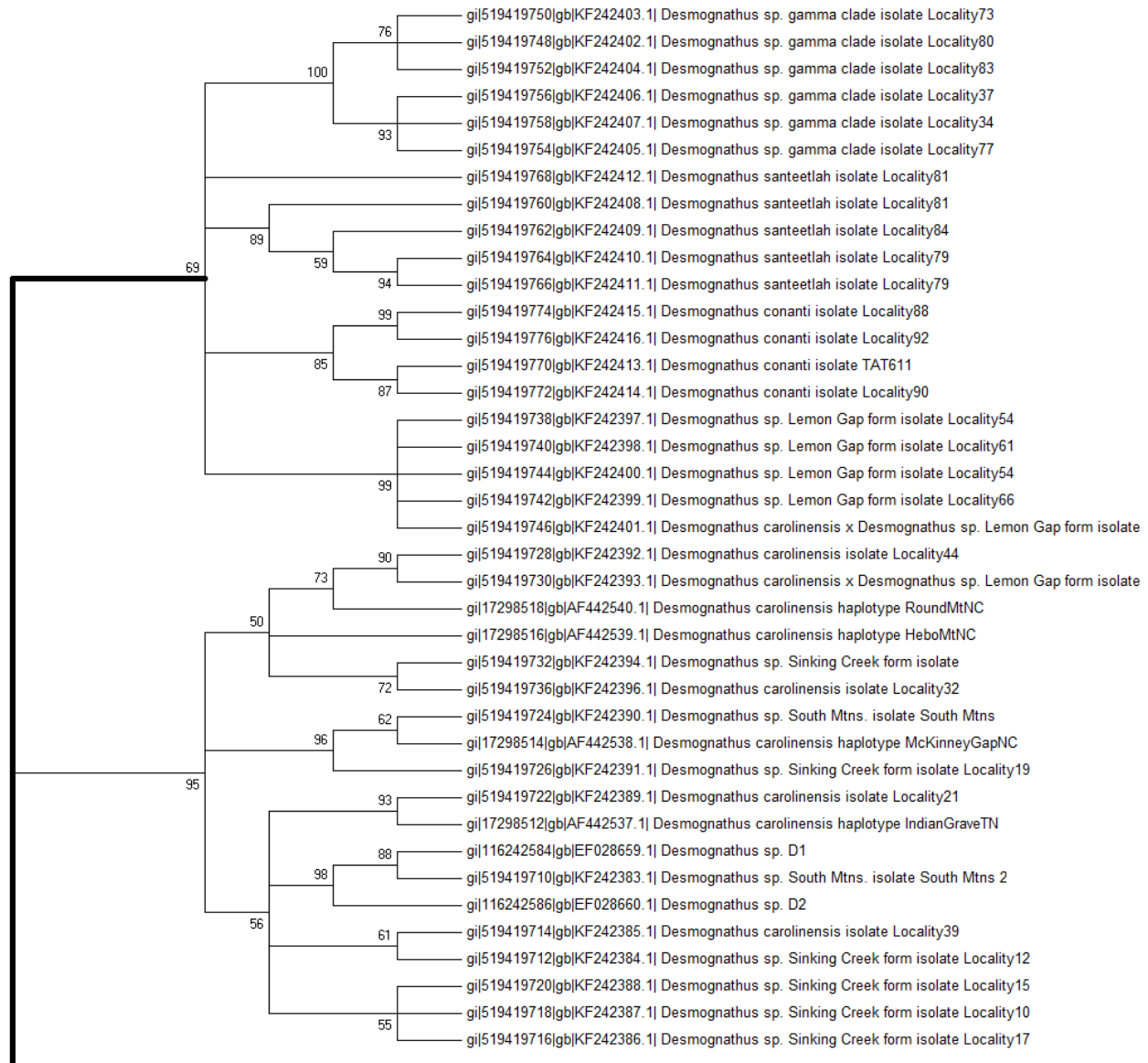
#### **ISO 14688-1<sup>[3]</sup>**

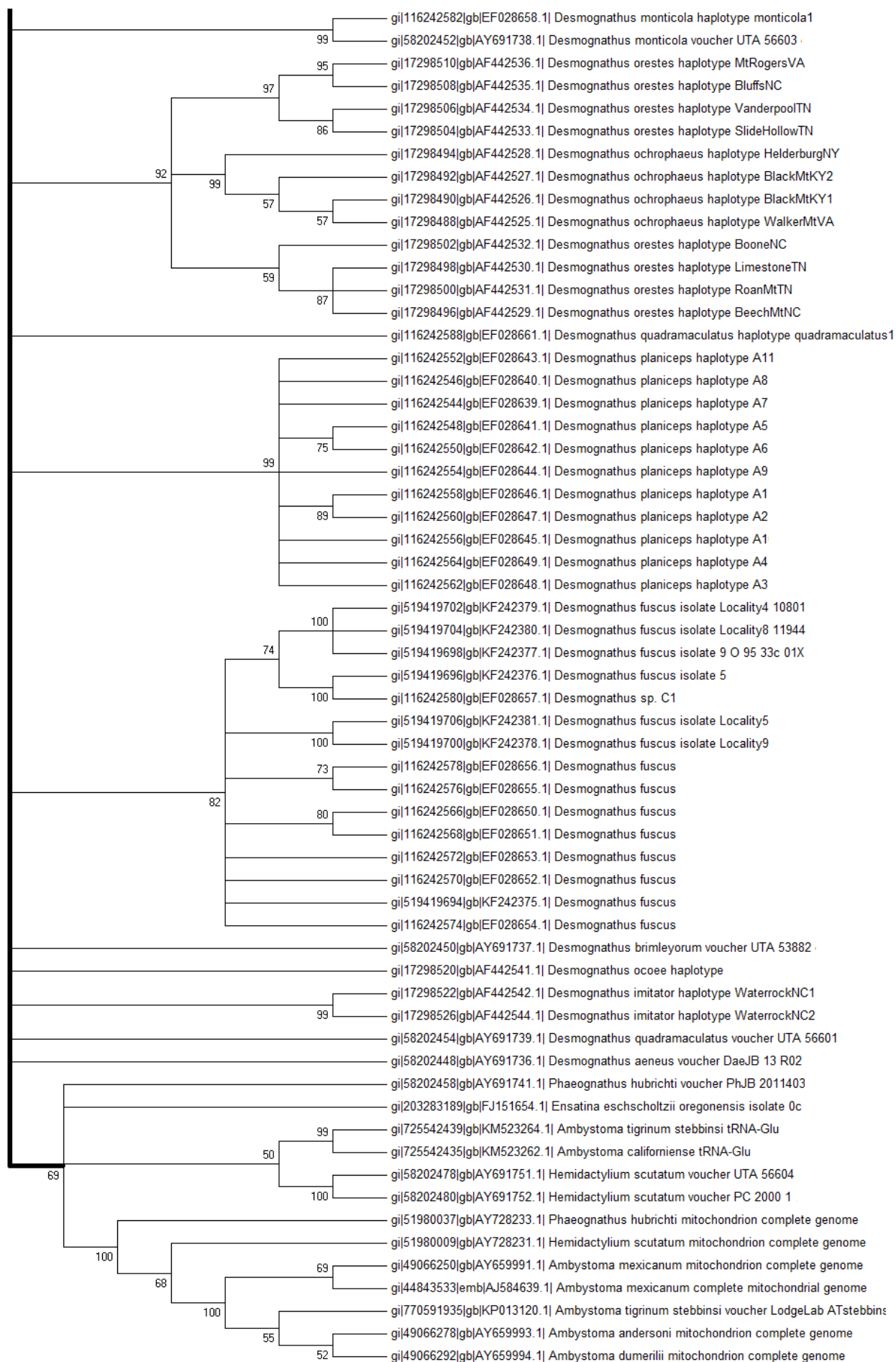
Name				Size range (mm)	Assigned Numeric Value:
Very coarse soil		Large boulder	LBo	>630	<u>1</u>
		Boulder	Bo	200 – 630	<u>2</u>
		Cobble	Co	63 – 200	<u>3</u>
Coarse soil	Gravel	Coarse gravel	CGr	20 – 63	<u>4</u>
		Medium gravel	MGr	6.3 – 20	<u>5</u>
		Fine gravel	FGr	2.0 - 6.3	<u>6</u>
	Sand	Coarse sand	CSa	0.63 - 2.0	<u>7</u>
		Medium sand	MSa	0.2 - 0.63	<u>8</u>
		Fine sand	FSa	0.063 - 0.2	<u>9</u>
Fine soil	Silt	Coarse silt	CSi	0.02 - 0.063	<u>10</u>
		Medium silt	MSi	0.0063 - 0.02	<u>11</u>
		Fine silt	FSi	0.002 - 0.0063	<u>12</u>
	Clay	Cl	≤0.002	<u>13</u>	

Appendix II: **Numeric listing for Tail Sample Clippings, associated with specimen number**

1. Blank	18. B2017
2. B1001	19. B2018
3. B1002	20. A2019*
4. B1003	21. C2020*
5. B2001	22. A2022*
6. B1004	23. B2023*
7. B2004	24. A2025
8. B2005	25. A2026*
9. C2006	26. A2027*
10. B2007	27. A1005
11. A2008	28. A2028*
12. B2009	29. B2029*
13. B2010	30. B2030*
14. B2011	31. B2031
15. B2013	32. B2032
16. B2014	33. B2033
17. B2015	

**Appendix III: Parsimonious Tree of the gene Cytochrome Oxidase 1, using *Desmognathus* sequences from GenBank, NCBI.org**

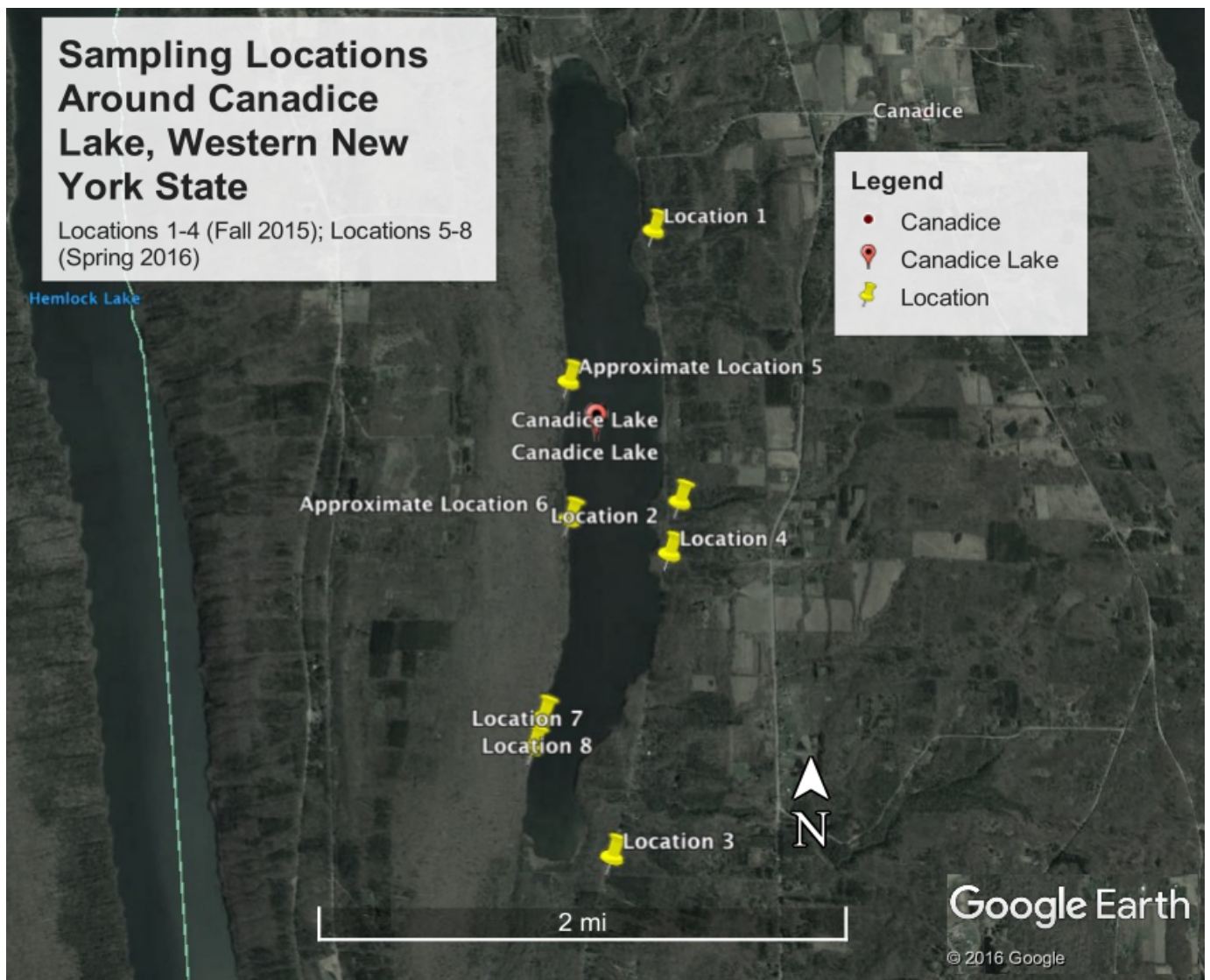




Appendix IV: Location GPS coordinates

Location #	GPS Coordinates
1	N42°43'44.0", W077°33'52.4"
2	N42°42'43.3", W077°33'47.3"
3	N42°41'29.7", W077°34'08.9"
4	N42°42'32.1", W077°33'50.7"
5	GPS signal could not be located, within 2 miles of next GPS signal
6	GPS signal could not be located, within 2 miles of next GPS signal
7	N42°41'51.7", W077°34'30.0"
8	N42°41'57.8", W077°34'27.1"

Appendix V: Collection Map for Canadice Lake, Fall 2015 and Spring 2016



*Appendix V: 2 locations that approximate the points where they are located. Those are locations 6 and 7, due to equipment malfunction during the attempt to capture the GPS coordinates during data collection.*