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Studies of Ecology and Morphology in the plethodontid salamander genus Batrachoseps

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Studies of Ecology and Morphology in the plethodontid salamander genus *Batrachoseps*

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy
in Ecology, Evolution, and Marine Biology

by

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June 2016

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DEDICATION

This dissertation is dedicated to my parents Christine and Joseph, and to my grandparents, Mary and John, and Ruth and Jerome.

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Evolution of interspecific morphological diversity within a clade is one of the fundamental patterns studied in evolutionary biology. These morphological differences may reflect adaptation to different environmental conditions and influence patterns across biogeographical spatial scales and macroevolutionary time scales. Using a multivariate measure of morphological shape and a well-studied phylogenetic hypothesis for the plethodontid salamander genus *Batrachoseps*, I revealed the strong correlation between morphology and species range size, and tested ecological hypotheses that might explain this relationship. Morphological evolution in *Batrachoseps* can be generally described as variation on the theme of elongation, a pattern also seen in several families of lizards such as Scincidae and Gymnophthalmidae. In *Batrachoseps* the most elongate forms appear wormlike with highly reduced limbs. Generalized forms of *Batrachoseps* are similar in proportion to other small salamanders in the family Plethodontidae, such as *Plethodon*.

To investigate whether morphological differences reflect a difference in habitat use, a pair of closely related species were studied where their ranges overlap (Chapter 1). Broad partitioning of habitat was the rule with the wormlike *B. nigriventris* occupying low to middle elevation sites (600-1200 meters) and the more generalized *B. stebbinsi* occupying middle to high elevation sites (1100-1800 meters). These species showed considerable overlap in their use of microhabitat in terms of temperature, moisture, and cover objects. Despite their ecological similarity *B. nigriventris* has a much larger species range (34,000 km^2) than *B. stebbinsi* (1,300 km^2). This is consistent with the strong correlation between

evolution of species range size and evolution of elongation in *Batrachoseps* (Chapter 2). Species range size in wormlike species is an order of magnitude larger than more generalized forms. Overall the evolution of elongation in *Batrachoseps* is a strong and significant predictor of the evolution of large range size. This pattern is not achieved by wormlike forms evolving to occupy wider elevational ranges or more abundant low elevation habitat. Large range size is an important metric for predicting the probability of species extinction. Finding traits that predict extinction is of central interest to the study of conservation biology and macroevolution.

A previously proposed hypothesis posited that evolution of wormlike morphology in *Batrachoseps* is a response to dry, seasonal, and unpredictable precipitation patterns. Individuals with small diameters could more easily retreat to subsurface refugia when surface conditions are inhospitable. These small salamanders depend upon seasonal precipitation events for surface activity to carry out basic life history activities. When surface conditions are moist *Batrachoseps* feed, breed, and re-hydrate after long periods of inactivity below the surface. I found a strong correlation between evolution of wormlike morphology and evolution to persist in more seasonal habitats (Chapter 3). Dry season precipitation and precipitation seasonality were significantly correlated with morphological evolution in *Batrachoseps*. This result suggests that wormlike morphology is advantageous in habitats where food resources and mating opportunities are only available periodically. All *Batrachoseps* appear to occupy similar microhabitat but persistence in highly seasonal habitats with low levels of dry season precipitation is associated with evolution of elongate morphology.

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CHAPTER 1

Evidence that elevation gradients and temporal partitioning, not behavioral interactions, structure resource use in sympatric salamanders (Plethodontidae: *Batrachoseps*)

Abstract

Range overlap between ecologically similar species can lead to interspecific competition for resources. Within the area of range overlap competition can be avoided through differences in habitat use. Sympatric competitors can partition resources spatially or temporally, and syntopic species may mediate resource use through agonistic behavior. In western North America 22 species of salamander in the genus *Batrachoseps* (Plethodontidae) are distributed from northern Baja California in Mexico to the Columbia River in northern Oregon. Range overlap between species is relatively rare which has led to the hypothesis that competitive exclusion occurs at range boundaries. In this paper I test this hypothesis using microhabitat data and behavioral trials for two species of sympatric *Batrachoseps* that are closely related and morphologically dissimilar. *Batrachoseps nigriventris* is wormlike with highly reduced limbs, while *B. stebbinsi* has proportions similar to members of the genus *Plethodon*. Within the nearly 600 km^2 of range overlap it appears that sympatry occurs in a narrow elevation band from 1000-1200 meters. In this area the wormlike *B. nigriventris* is found at lower elevations, 500-1200 meters (SD 201), while *B. stebbinsi* was found from 1000-1800 meters (SD 200). Both species occur across a wider range of elevation in nearby allopatric populations. When these species occur at the same site evidence for temporal partitioning was found. Early in the season when microhabitat was cool (7.9°C, SD 0.5) and moist (74% saturated, SD 8.2) *B. stebbinsi* was present, later in the same year *B. nigriventris* was found under the same cover objects under drier (53% sat., SD 16.8) and warmer (11.4, SD 0.5) microhabitat conditions. Interspecific agonistic behavior was virtually absent from arena trials regardless of resident or intruder status. Within the area of range overlap between *B. nigriventris* and *B. stebbinsi* broad differences in habitat use limits sympatry to relatively narrow bad of elevation. At sympatric sites temporal partitioning of resources was observed. Agonistic behavior does not appear to mediate resource competition between these species. Data presented here is consistent with a hypothesis of competitive exclusion at range boundaries in *Batrachoseps*.

Introduction

Range overlap of ecologically similar species affords researchers an opportunity to study mechanisms of coexistence. Understanding coexistence of ecologically similar species is a central focus of ecology (Schoener, 1974; Zobel, 1997; Chesson, 2000). This coexistence is often explained by resource partitioning, although some recent work has suggested that coexistence is possible through aggregation and reproductive interference (Roukolainen and Hanski, 2016). Evidence for coexistence via spatial or temporal partitioning of resources has been found for a diverse array of species (Ross, 1986; Tillman, 1994; Stratton et al., 2000). In several guilds of terrestrial salamanders both spatial and temporal partitioning of resources has been documented (Fraser, 1976; Krzysik, 1979; Toft, 1985; Werner, 2014). When species are sympatric, agonistic behavior can evolve as a way to mediate competition for resources, most often food and shelter sites (Camp, 1999). In this paper I use presence-only microhabitat data and behavioral assays to answer four questions about the biology of Slender Salamanders (Plethodontidae: *Batrachoseps*): How are resources partitioned in sympatry to allow coexistence? Are *Batrachoseps* ecologically identical? Are microhabitat conditions independent from general seasonal and spatial trends? Does agonistic behavior mediate resource use in Slender Salamanders?

With over 400 described species, Plethodontidae (Caudata) is the most speciose family of salamanders (Wake, 2009). Most Plethodontids are fully terrestrial and complete their life cycle without an aquatic larval stage. All Plethodontids in western North America are fully terrestrial. These species avoid desiccation by limiting surface activity to during, and immediately after, precipitation events. Basic life history behaviors, feeding and reproduction are carried out during surface activity (Hendrickson, 1954; Cunningham, 1960; Maiorana, 1974). In many temperate habitats of western North American precipitation is highly seasonal. This pattern limits feeding and reproductive activity, and has the potential to influence life history evolution. Cover objects at the surface allow Plethodontids to remain in suitable microclimates between precipitation events (Maiorana, 1976; Grover, 1998). Once these sites have warmed or dried to the point of being unsuitable the salamanders retreat to suitable microhabitat away from the surface.

In far western North America members of the genus *Batrachoseps* (Plethodontidae) can be found under surface cover objects after fall, winter, and spring rains (Hendrickson, 1954; Cunningham, 1960). 22 species of *Batrachoseps* have been described, 21 of which occur in the state of California; a single species is found from central Oregon to the Columbia River (Figure 1). All *Batrachoseps* are small and slender, and sympatry where ranges contact is usually absent or narrow (Wake, 2006). Niche conservatism is the general rule in the Plethodontidae, so minimal overlap in ranges of *Batrachoseps* species, along with shared traits in their basic biology, has led to the interpretation that they are ecologically identical (Jockusch and Wake, 2002; Kozak and Wiens, 2006; Wake, 2006; Kozak and Wiens, 2010). Testing the hypothesis of ecological similarity using field data has received no formal attention.

Several authors have conducted ecological field studies of *Batrachoseps* but all have been focused on single species; Hendrickson (1954) and Maiorana (1978) on *B. attenuatus*, and Cunningham (1960) on *B. major* are the most thorough recent studies. Previous field notes on ecology come from many authors (Camp, 1915; Campbell, 1931; Brame and Murray, 1968; see also summaries in Lannoo, 2005). There are several factors contributing to the lack of multi-species studies on *Batrachoseps*. Sympatry is relatively rare and is often in areas of limited accessibility. Large swaths of *Batrachoseps* habitat in California are privately owned and/or accessible only by dirt road. Accurate identification of species in the field is also an issue in cases where cryptic wormlike species come into contact (Yanev, 1978; Jockusch and Wake, 2002; Wake, 2006). In some instances morphological similarity necessitates genotyping for accurate identification, which is often impractical for field studies. Examples include *B. attenuatus* and *B. gavilanensis* in Santa Cruz and *B. gavilanensis* and *B. nigriventris* in the southern Diablo Range (Jockusch and Wake, 2000; 2002). A final hurdle to overcome is detectability. *Batrachoseps* generally occur in seasonal and somewhat unpredictable environments (Maiorana, 1976). For this reason they are often unpredictable in their near-surface activity.

Sympatry between *Batrachoseps* species is rare in general, and is virtually absent in closely related species (Jockusch and Wake, 2002; Jockusch et al., 2012; Jockusch et al., 2014). One exception is an area of range overlap in the southern Sierra Nevada between the widely distributed *B. nigriventris* and the narrowly distributed *B. stebbinsi* (Figure 1). Both species are members of the nigriventris group (Figure 2). Morphologically these species represent the extremes of the subgenus *Batrachoseps* (Figure 2). *Batrachoseps nigriventris* is wormlike with highly reduced limbs while *B. stebbinsi* has proportions similar to generalized members of the genus *Plethodon*, such as the eastern Red-backed salamander*, P. cinereus* (Stebbins, 1949; Burton and Likens, 1975). The two species overlap in the Tehachapi Mountains, in an area of approximately 600 km^2. Historical records and anecdotal data note sympatry at two sites and syntopy at one of these (Jockusch and Wake, 2005; Hansen and Wake, 2005a) but survey work in the area has been limited. Historical records for the more robust *B. stebbinsi* are restricted to 850-1100 meters in elevation in the area of range overlap (Hansen and Wake, 2005; R. W. Hansen, pers. comm.; S. S. Sweet, pers. comm.). However there are several sources of error and conflicts in these data from misidentification to inaccurate georeferencing. Historical *B. nigriventris* records showed a much broader elevation range, from 169-1250 meters. Similar errors complicate this dataset. For example the record at 169 meters was reassigned to 388 meters after consulting the collector's field notes (R. W. Hansen, pers. comm.). From these records it appeared that *B. stebbinsi* was restricted to a narrow band of elevation and that *B. nigriventris* completely overlapped its distribution. This biogeographical pattern set the stage to gather data on interspecific differences in habitat and resource partitioning between these closely related, ecologically similar species.

There are many ways to quantify a species' niche using a variety of variables and niche definitions (Grinnell, 1927; Hutchinson, 1958; Wake et al., 2009). In this paper I focus on the Grinnellian niche (Grinnell, 1927) and use microhabitat data to quantify niche use. I seek to quantify the Grinnellian niche by using microhabitat data on cover type, cover size, microhabitat moisture, and microhabitat temperature (Grinnell, 1927). The goal is to understand how resources are partitioned in sympatry and quantify how ecologically similar these two species are. Given the low vagility of *Batrachoseps* and their ability to persist in small, discrete patches of habitat, microhabitat data seem most appropriate for understanding the true nature of habitat utilized by *Batrachoseps* (Hendrickson, 1954; Cunningham, 1960; Yanev et al., 1981; Jockusch et al., 2012). Past studies of Plethodontids in general and *Batrachoseps* in particular have shown temperature, moisture, and cover object use to be important in dictating surface activity patterns (Hendrickson, 1954; Maiorana, 1978; Grover, 1998). Elevation is also important for habitat partitioning in Plethodontids and since *B. nigriventris* and *B. stebbinsi* co-occur in a mountainous region the influence of elevation was also considered in the analysis.

The basic biology of small terrestrial salamanders necessitates an activity pattern that allows them to remain in microhabitat with a relatively narrow temperature range and high moisture content (Spotila, 1972; Feder, 1978; 1982). Microhabitat conditions are influenced by climatic trends on larger temporal and spatial scales but individuals can insulate themselves from these trends behaviorally. The degree to which microhabitat specialists are insulated from larger trends is an interesting aspect of studying microclimate. Understanding the degree to which microhabitat specialists respond behaviorally to general climate trends is important for discerning the biological meaning of species distribution models that utilize data at a larger spatial scale than the home range of a single animal (Kearney and Porter, 2004). In the study area, day of year (DOY) is positively correlated with increased temperature and decreased surface moisture, as winter gives way to spring, and spring to summer (Maiorana, 1976). The study area also has a positive west-to-east elevation gradient from the valley foothills into the Tehachapi and Piute mountains, which run southwest to northeast. There is also a weak but positive south-to-north elevation gradient as the Tehachapi and Piute mountains give way to the taller Sierra Nevada. Understanding how much influence spatial heterogeneity (elevation, latitude, longitude) has on salamander microhabitat conditions can give insight on the behavioral responses of small vertebrates to general spatial and temporal trends (Figure 3). This insight is relevant to biogeographers and conservationists who often use distribution modeling techniques in a uniform way, discounting the potential nuances of species-specific behavioral responses (Early and Sax, 2011; Wright et al., 2013). If these responses differ, particularly in the face of rapid climate change, probability of extirpation could be quite different from one species to the next.

Interspecific agonistic behavior has been reported between ecologically similar salamanders that are found in sympatry (Nishikawa, 1985; Nishikawa, 1987; Camp, 1999). Salamanders in the family Plethodontidae have served as model systems for the study of agonistic and territorial behavior in vertebrates (Jaeger, 1984; Mathis, 1990; Camp, 1999; Mathis and Britzke 1999). The bulk of this research involves the genus *Plethodon* from eastern North America but studies of western *Plethodon*, *Aneides*, and *Ensatina* have also found interspecific and intraspecific agonistic behavior to be present (Staub, 1993; Wiltenmuth, 1996; Wiltenmuth and Nishikawa, 1998). To my knowledge behavioral interactions have not been reported for *Batrachoseps*. Due to its biogeography *B.*

nigriventris appears the most likely species to display agonistic behavior toward congeners. Its range abuts or overlaps with that of seven other species in the genus. It also appears to have expanded its range since the tectonic events described in Wake (2006), and displaced members of the *pacificus* group. Aggression has been shown to facilitate range expansion in some birds (Duckworth, 2007), and *Plethodon glutinosus*, which is widely distributed, is more aggressive than its sympatric, narrowly-distributed relatives (Marvin, 1998; Marshall et al., 2004). Relatively high levels of aggression in *B. nigriventris* would be consistent with the pattern seen in the *P. glutinosus* complex.

In order to understand how habitat and resources are partitioned in the area of range overlap between *B. nigriventris* and *B. stebbinsi* I conducted field surveys to collect microhabitat data. These data were combined with behavioral assays that tested for agonistic behavior. Using the combination of these data I hoped to better understand the spatial, temporal, and behavioral dynamics that are present when species ranges overlap in *Batrachoseps*. Variables used to detect a broad pattern of habitat are latitude, elevation, and watershed. If spatial partitioning is present in sympatry I expect the two species of *Batrachoseps* in this study to be found in close proximity but under different microhabitat conditions. Microhabitat variables considered are microhabitat temperature, microhabitat moisture, cover objects size and cover object type. A pattern of spatial partitioning should be detectable by differences in microclimate or cover object use by the sympatric species. With temporal partitioning a difference in surface activity patterns is expected. Day of year (DOY) is used as a temporal variable to test for temporal partitioning. To test the hypothesis that *Batrachoseps* are ecologically identical, or at least highly similar, microhabitat data from across the study area were used (Figures 1 and 3). If *Batrachoseps* are ecologically homologous and are found in sympatry or syntopy, interspecific agonistic behavior may have evolved, similar to *Plethodon* (Nishikawa, 1985; Ovaska, 1993). Behavioral trials were used to test for agonistic behavior. The goal is to understand interspecific ecological dynamics of *Batrachoseps* in sympatry.

Methods

Field surveys for terrestrial salamanders were conducted from 2010-2013 along riparian corridors across the range of *B. stebbinsi* from latitude 34.86 North to latitude 35.38 North. This encompasses the Tehachapi Mountains, where *B. stebbinsi* and *B. nigriventris* are sympatric, as well as the greater Caliente Creek watershed north of the Tehachapi Mountains (Figure 3). Surveys were conducted from late winter to early summer depending on accessibility and local conditions. Slender Salamanders are active near the surface during and immediately after rains or until the substrate becomes dry (Hendrickson, 1954; Cunningham; 1960; Maiorana, 1978). Survey protocol utilized daytime visual encounter surveys (Scott and Crump, 1994) where natural cover objects were turned over and then replaced to their original position. Microhabitat data were recorded at the point of encounter for any salamander species found. In the study area this included three salamander species, all from the family Plethodontidae: *B. nigriventris*, *B. stebbinsi*, and *Ensatina eschscholtzii croceater*. Data from all three species was used to show trends in sampling across season and elevation and to give a more complete picture of microhabitat use by Plethodontids. Data from *E. e. croceater* was not used in all comparisons as the focus of this study is the comparison between *B. nigriventris* and *B. stebbinsi*. The following data were recorded at point of encounter: GPS coordinates and elevation (Garmin eTrex Legend) corrected to 10 meter accuracy in Google Earth, substrate temperature (Extech 42510 handheld IR thermometer); size of cover object; type of cover object; dominant overstory species. Substrate type and soil moisture category (1-4) were determined using ribbon the test (Klocke and Fischbach, 1984; Schoeneberger, et al., 2002; Miles and Broner, 2006). Soil moisture values were derived from soil moisture category by applying a univariate distribution to each category based on soil type and corresponding approximate moisture content index in the following manner: Category 1 (0-25%); 2 (25-50%); 3 (50-75%); 4 (75-100%) (Klocke and Fischbach, 1984; Miles and Broner, 2006). Analysis of variance tests were conducted for the microclimate variables soil moisture and substrate temperature. Outliers were removed using the non-parametric Tukey method (Thompson, 2008). General spatial and temporal variables considered were year and day of year (DOY) as temporal variables, and elevation, latitude, longitude, and watershed for spatial variables. Analysis of habitat variables was conducted in R (R core team, 2014). Univariate and bivariate tests included linear regression (lm), Pearson correlation (cor.test), and analysis of variance (lm and p.adjust) tests. All of these tests were completed using the ‘stats’ package (R core team, 2014).

Data used in the analysis are presence-only for all Plethodontid salamanders discovered during field work in the Tehachapi and Piute mountains from 2010-2013. Sampling attempted to capture elevation gradients across the study area although sampling itself was largely opportunistic. Access to habitat was denied or restricted by some property owners. Nearly all of the known area of range overlap between *B. nigriventris* and *B. stebbinsi* occurs on the 970 km^2 of land owned by the Tejon Ranch Company. Survey access on Tejon Ranch was limited to sites within their Conservation Lands, and further restricted within

Conservation Areas to riparian corridors. Plethodontid salamanders are likely to occur outside of riparian areas. The lone historical record of syntopy between *B. nigriventris* and *B. stebbinsi* is outside of the Tejon Ranch Conservation Area, and could not be surveyed. All three species were found outside of riparian areas on other properties. For this reason any habitat differences between species are likely to be conservative. Figure 3 shows the watersheds (hydrologic units) sampled for this study.

Agonistic behavior

Behavioral trials were conducted in enclosures in the lab. Two resources were present in each enclosure to try and elicit agonistic behavior. Trials were conducted to test for interspecific agonistic behavior between *B. nigriventris* and *B. stebbinsi*. These trials used 12 individuals of *B. nigriventris* and 6 individuals of *B. stebbinsi*. *Batrachoseps stebbinsi* is listed as a State Threatened Species (CDFW, 2011) and so collecting of this species is tightly regulated. All individuals of both species were collected from within the geographic range of *B. stebbinsi*, although the *B. stebbinsi* used were from outside the area of range overlap. Individual salamanders were held in 40 cm x 15 cm plastic enclosures for 14 days prior to trials. To maintain appropriate microhabitat enclosures were lined with damp paper towels and temperature was controlled at 15-17.5^{o} C (Hendrickson, 1954; Cunningham, 1960; Jockusch, 1997). Enclosures contained two resources likely to be limiting for *Batrachoseps*, food and cover. Leading up to the experiment, each enclosure was supplied with 10-15 pinhead crickets a week. Cover was provided by a 50ml plastic vial, 29.4 mm diameter and 114.3 mm in length, wrapped in a damp paper towel and placed in the enclosure. Diurnal lighting was provided by lights in lab and indirect sunlight. Salamanders were conditioned to red tinted light at night in anticipation of the experiment. Three trials were held on the first night and the remaining nine trials were held on the next night. All trials were held at night as both species are thought to be nocturnal. The enclosure of the resident animal was placed under the camera for observation then the intruder was introduced to the enclosure. The individuals were allowed to acclimate for 5 minutes and then data collection began. Individuals attempting to escape the enclosure were redirected back into the enclosure with a tap on the rostrum or placed back in the center of the enclosure.

For each trial an intruder was introduced from their enclosure to the enclosure of the resident animal. Interspecific agonistic behavior was scored during the trials. Each 30 minute trial except for one (technical issues) was recorded using digital video. These videos were reviewed and used to finalize behavioral scores. Trials were coded for overtly aggressive or submissive behavior using the method of Camp (1999). Each of the 6 *B. stebbinsi* was used once as a resident and once in the role of intruder. Each of the 12 *B. nigriventris* was only used once, in the role of either resident or intruder. Resident animals remained in the enclosure where they had lived for the previous 14 days and the intruder was introduced into this enclosure. Data from this experiment represents a total of 6 hours of observation of interspecific behavior of *Batrachoseps*. Given the slow pace of movements by *Batrachoseps* the videos were viewed at 4x actual speed to enhance visibility of movements. Counts of previously described salamander behaviors (Camp, 1999) were taken from videos. Each behavior was first categorized as neutral, aggressive, or submissive then rated on a 0-6 scale. A score of 6 represents the maximal aggressive or submissive score for a behavior. A score of 0 indicates a neutral behavior. Behavior count data were totaled into four groups that represented the species name, *B. stebbinsi* or *B. nigriventris*, and their role in the trial, Intruder or Resident. Given the small total number of animals in the study and the small number of observed overt behavior, significance of results were tested using Fisher's exact (Fisher, 1935; R core team, 2014). Two tests were performed the first compared the behavior of resident animals across species. The second test compared the behavior of Intruder animals across species.

Results

Field surveys yielded 297 animals across all years of sampling, 65 *B. nigriventris*, 119 *B. stebbinsi*, and 116 *Ensatina*. Of these, 208 had valid data for habitat sampling and once outliers were removed 199 records were used for analysis, 43 *B. nigriventris*, 75 *B. stebbinsi*, and 81 *Ensatina*. The range of microhabitat temperature measured, 0.2-15.6 °C, is similar to field data reported for field temperature of Plethodontids in the literature (Feder and Lynch, 1982). These data greatly expand the record of thermal microhabitat use for both *Batrachoseps* and *Ensatina* (Feder et al., 1982). Soil moisture values ranged from 18% saturation to 100% saturation. Cover objects ranged from large logs with an estimated area of 280 m^2 to cover as small as 72 cm^2. In general low elevation sites dry out earlier in the season than high elevation sites. For this reason survey effort generally tracked availability of suitable microclimate conditions across the survey season, leading to a positive correlation between elevation and day of year (DOY), Pearson correlation = 0.43, $p\text{-value} < 0.0001$.

Coexistence: Evidence for both spatial and temporal partitioning was found despite small sample sizes. Within the area of range overlap true sympatry and syntopy are only likely to occur in a narrow band of elevation between 1050 and 1250 meters. Partitioning along the elevation gradient appears to be a general rule in the area of range overlap. *Batrachoseps stebbinsi* was found at elevations from 1070-1800 meters (µ = 1286, SD=200) and *B. nigriventris* from 619-1233 meters (µ = 896, SD= 201). Within the area of range overlap between the two *Batrachoseps*, *B. stebbinsi* proved very difficult to find (n=16). Co-occurrence was documented in just three watersheds, El Paso Creek, Tunis Creek, and Grapevine Creek (Figure 3). Both substrate temperature and soil moisture showed considerable overlap but also a statistically significant difference between *B. nigriventris* and *B. stebbinsi* (Figure 4). Microhabitat occupied by *B. stebbinsi* was significantly colder, µ = 7.2 °C compared to *B. nigriventris* µ = 9.9 °C. This trend is partially but not completely predicted by a modest correlation between elevation and microhabitat temperature overall in the area of range overlap, $R^2 = 0.14$, Pearson correlation = -0.37, p-value < 0.0001. *Batrachoseps nigriventris* were found in drier microhabitats, µ = 54% saturated, compared to 69% for *B. stebbinsi*. This pattern is not significantly correlated with elevation, $R^2 = 0.03$, p-value = 0.18. *Batrachoseps nigriventris* used significantly larger wood cover objects on average, µ = 2500 cm^2, *B. stebbinsi* µ = 1000 cm^2, although overlap in these values is again considerable (Figure 6). Use of rock and wood cover in sympatry was similar for *B. nigriventris*, 7 rock and 36 wood, and *B. stebbinsi*, 3 rock and 13 wood (Fisher exact test p-value = 1). A single case of temporal partitioning of resources was observed during field surveys. A site at 1070 meters elevation in Tunis Creek was survey in March of 2010 and seven *B. stebbinsi* were found under a variety of log and rock cover. During a resurvey two months later three *B. nigriventris* were found under many of the same cover objects and no *B. stebbinsi* were seen. Microhabitat temperature had increased significantly, from 7.9 °C to

11.4 °C (R^2= 0.9, p-value < 0.0001), and soil moisture levels had dropped significantly from 74% saturated to 50% saturated (R^2= 0.54, p-value = 0.02).

Niche difference: In the data overall, which includes *B. stebbinsi* and *Ensatina* data from outside the area of range overlap, strong niche differences between *Batrachoseps* were species were absent. Species differences accounted for just 6% of the variation in microhabitat temperature and 8% of the difference in microhabitat moisture. A small but significant difference was found between the two *Batrachoseps* in both temperature and moisture. *Batrachoseps nigriventris* tends to be found in drier and warmer microhabitats, but the overlap was considerable (Figure 5). Species differences explained 20% of the variation in cover object size. *Batrachoseps stebbinsi* used cover objects that were significantly smaller than cover used by *B. nigriventris* or *Ensatina* (Figure 5). The difference in cover object size between *Batrachoseps* can be at least partially explained by the relative use of large wood cover by *B. nigriventris*. *Batrachoseps nigriventris* was found under wood cover 83% of the time, whereas only 36% of *B. stebbinsi* were found under wood cover, a statistically significant difference (p-value = 0.04). Cover type explains about 23% of variation in cover size overall. Wood cover surveyed was significantly larger than rock cover (R^2 = 0.23, p-value < 0.0001).

Microhabitat independence: Due to the orientation of the mountain ranges in the study area, which run southwest to northeast, latitude and longitude are highly correlated, Pearson correlation = 0.8, p-value < 0.0001. As mentioned earlier elevation is weakly correlated with latitude (Pearson correlation = 0.2, p-value = 0.02), and solidly correlated with longitude (Pearson correlation = 0.5, and < 0.0001). Mean elevation where salamanders were found within each watershed is not correlated with latitude (Pearson correlation = -0.07, p-value = 0.8) but is correlated with longitude (Pearson correlation = 0.5, p-value = 0.001). Overall spatial variation had only modest effects on microhabitat variables. Elevation is significantly correlated with substrate temperature (Pearson correlation = -0.38, p-value < 0.0001) and with soil moisture (Pearson correlation= 0.25, p-value = 0.0003). Longitude also showed a modest but highly significant correlation with cover object size (Pearson correlation= -0.32, p-value < 0.0001). Similarly there was only modest distinction between the microhabitat used across watersheds, despite mean differences in spatial variables. Of the 16 watersheds in the survey area, five were significantly different from the remaining eleven in substrate temperature, and two differed from the remaining fourteen in soil moisture (Figure 7). Weak to modest correlations were also the rule in species- specific correlations between habitat variables and spatial variables. The strongest of the significant correlations was the negative correlation between temperature and elevation in the *B. nigriventris* data, Pearson correlation = -0.43, p-value = 0.004. The temporal variable day of year (DOY) was a poor predictor of microhabitat temperature, moisture, and cover object use (Table 1). These patterns held when looking at species-specific data as well. Interannual variation showed a significant warming trend in the data overall (Figure 8). Temperature of microhabitat sampled showed a slight increase between 2010 (μ=5.8 °C) and 2011 (μ=6.8 °C), then a significant increase the following year, 2012 (μ=9.9 °C), and was warmest in 2013 (μ=11.5 °C). This pattern temperature pattern held within all species. No other strong relationship was found between year and microhabitat variables.

Linear model

Of the five variables considered for the simple linear model only longitude was found to be sufficiently strongly correlated with other variables to warrant removal. The two strongest correlations between these variables are between longitude and latitude, Pearson correlation = 0.8, p-value < 0.0001, and longitude and elevation, Pearson correlation = 0.47, p-value < 0.0001. For this reason longitude was removed from consideration in the model and latitude remains as the only purely spatial variable in the model. Model performance was fairly weak overall. The model performed best at predicting substrate temperature. For the combined data from all three species $R^2 = 0.47$ (p-value < 0.0001) and was highly significant. Prediction power for species-specific data showed variability for the temperature data. The model performed especially well predicting temperature for *B. nigriventris*, $R^2 = 0.71$ (< 0.0001) and fairly well for *Ensatina*, $R^2 = 0.64$ (<0.0001). Model performance was much weaker with the *B. stebbinsi* data, $R^2 = 0.34$ (<0.0001). Model performance was much weaker with substrate moisture, although all values were significant below $\alpha = 0.05$. Predictive power for soil moisture was again strongest with *B. nigriventris*, $R^2 = 0.27$ (Table 2). The model showed limited power to predict cover object size overall and within species. Cover object size was predicted best by data from *B. stebbinsi*, $R^2 = 0.28$ (0.0001). See Appendix A for full report of variable correlations.

Aggressive interactions

Overt aggressive or submissive behaviors were almost completely lacking in behavioral trials for *B. nigriventris* and *B. stebbinsi*. The dramatic biting, head butting, and chase behaviors that have been observed in *Plethodon* from eastern North America were absent (Jaeger et al., 1982; Camp, 1999). Overt aggressive behavior was observed for 197 seconds out of six hours of observation. All of the overt aggressive behavior observed was in the lowest category, level 1 (Camp, 1999). The majority of the 197 seconds of aggressive behavior was a 175 second all trunk raised (ATR) behavior by a *B. nigriventris*. Regardless of their role as resident or intruder all individuals from both species displayed a lack of agonistic behavior. Individuals frequently stayed in close proximity to each other or sought refuge together during the trials. The other interactions coded as level 1 overt behaviors were both jump behaviors by intruders upon being touched by the resident individual. No escalation in aggression by either *B. nigriventris* or *B. stebbinsi* followed these jump behaviors. No significant difference in agonistic behavior was detected between species in their roles as residents (p-value = 0.27) or intruders (p-value = 1) using Fisher's exact test.

Discussion

The hypothesis presented by previous authors that *Batrachoseps* can be generally considered ecologically identical, is consistent with the data in this study. Three resource variables were considered but all important to the basic biology of Plethodontid salamanders, temperature, moisture, and cover object use. Despite their morphological differences *B. nigriventris* and *B. stebbinsi* showed a great deal of overlap in the niche they occupied in terms of microhabitat temperature, moisture, and sizes of cover objects used. Across the study area *B. stebbinsi* was found under rock cover more often than *B. nigriventris*, but where their ranges overlap, and habitat is more likely to be similar, no difference was found in type of cover used. Sympatry appears to be quite limited where their ranges overlap as *B. nigriventris* utilizes lowland habitat, and *B. stebbinsi* is found primarily in upland habitat. The two species are found in sympatry at middle elevations, 1000-1200 meters, in the Tehachapi Mountains.

Within the area of range overlap between *B. nigriventris* and *B. stebbinsi* I found evidence that resource competition is mediated in three ways. Significant differences were found in elevation of habitat used by the two species. The more wormlike *B. nigriventris* is generally found at lower elevations, from 500 to 1200 meters, and *B. stebbinsi* at higher elevations, from 1000 to 1800 meters. Sympatry within the overlap of elevational ranges of these taxa is mediated, at least in part, by temporal partitioning, as well as partitioning among diverse habitats at similar elevation. In the single case of sympatry observed in this study resource competition appeared to be mediated by temporal partitioning. Habitat is heterogeneous at a given elevation within the study area. Among the highest elevation records for *B. nigriventris* in the area of range overlap is one from Cummings Valley at 1196 meters in an open woodland of deciduous oak (*Quercus lobata*) with an understory of grass (Poaceae) and sage (*Artemis* sp.). This open habitat is in stark contrast to another record for *B. nigriventris* at similar elevation (1233 meters) which is in a relatively dense canopy of evergreen oak (*Q. chrysolepis*) and deciduous buckeye (*Q. californicus*). Historical records from outside the area of range overlap show *B. nigriventris* occurring as high as 2000 meters on Mount Pinos, and *B. stebbinsi* at low elevations, to 580 meters on Caliente Creek (Hansen and Wake, 2005; 2005a). This pattern is consistent with a hypothesis of habitat divergence in the area of range overlap, driven by ecological similarity and competition for resources (Dickman, 1986). A better understanding of the dynamics over fine spatial and temporal scales is still lacking. Future surveys should concentrate on sites where both species occur, and can be readily found. These should be surveyed across fine temporal and spatial scales to understand differences in activity pattern and response to both habitat and microhabitat conditions. The El Paso Creek, Tunis Creek, and Grapevine Creek watersheds each contain sites that would be ideal for this sort of work.

Results presented here provide a framework for comparative studies of other instances of *Batrachoseps* range overlap and contact at range boundaries. Most examples of contact or sympatry in *Batrachoseps* involve *B. nigriventris*. The range of *B. nigriventris* abuts or overlaps with seven species of *Batrachoseps*, including *B. stebbinsi*. The extent of spatial and temporal partitioning may differ in other instances of sympatry. Understanding how these dynamics differ between species pairs would be useful to understand in greater detail. Several traits may show a significant relationship with partitioning. These include phylogenetic relatedness, morphological similarity, and heterogeneity of habitat. Sympatry has been documented repeatedly between *B. nigriventris* and *B. minor* but no published study has sought to tease out the presence of agonistic behavior, or the effects of resource partitioning over small temporal and spatial scales (Sweet, 2015). Field data (S. S. Sweet, pers. comm.) from habitat where *B. minor* and *B. nigriventris* are sympatric shows a pattern in microclimate use similar to *B. nigriventris* and *B. stebbinsi*. The more robust *B. minor* occupied microhabitat that is slightly cooler and wetter than *B. nigriventris*. In sympatry *B. minor* occupies steeper, more heavily shaded sites with a dense understory of poison oak and other shade-adapted shrubs, on E and NE-facing slopes with bedrock outcropping or just below the surface. Where the two co-occur *B. minor* is surface-active between 6 and 10 C, *B. nigriventris* mostly above 9 C, to about 15 C; *B. minor* withdraws from the surface 1-2 days before *B. nigriventris* as the leaf litter dries out (S. S. Sweet, pers. comm.). The nature of the sympatry between *B. incognitus* and *B. nigriventris* appears similar to the condition with *B. minor* but is less well-documented. Range overlap in the Los Angeles Basin between *B.*

nigriventris and *B. major* is quite extensive, but here too a comprehensive interspecific comparison is lacking. Building a better understanding of the role of elevation gradients, resource partitioning, and behavior in *Batrachoseps*, is necessary to bring understanding of this group closer to that of well-studied *Plethodon* in eastern North America.

Spatial and temporal partitioning of resources in sympatry does not appear to be mediated by interspecific agonistic behavior in *Batrachoseps*. Overt aggressive or submissive interactions were virtually absent in behavioral trials of *B. nigriventris* and *B. stebbinsi*. Each enclosure where the species interacted contained two valuable resources, food and cover. In several trials the two species took shelter under the cover object together. Interspecific sharing of cover objects has been observed by other researchers (R. W. Hansen, pers. comm.), although it was not observed during my field work. This suggests that the results observed in behavioral trials are consistent with behavior in nature. Two improvements could be made to the behavioral trials conducted for this experiment. First would be to use a larger sample of *B. stebbinsi*. This is difficult considering its protected status but is potentially feasible. A second improvement would be to use *B. stebbinsi* from populations within the area of range overlap. Due to collection restrictions and the general difficulty of finding *B. stebbinsi* in this area I was forced to use allopatric *B. stebbinsi*. For these individuals *B. nigriventris* is surely unfamiliar and *B. nigriventris* may never have interacted with these populations historically. It could be argued that *B. nigriventris* is likely to be the more aggressive species, considering their larger species range size, and this pattern was not observed. Neither species showed any consistent agonistic response. There is still the possibility that northern *B. stebbinsi* do not elicit an agonistic behavioral response from

B. nigriventris as they are different enough to be unfamiliar. In light of all the evidence however, this does not seem likely.

Lack of interspecific aggression in *Batrachoseps* stands in stark contrast to sympatric and ecologically similar *Plethodon* from North America (Nishikawa, 1985; Ovaska, 1993; Camp, 1999). It would be interesting to know whether differences in resource abundance and predictability are associated with observed differences in agonistic behavior. Within the framework of the general theory of economic defensibility (Brown, 1964), aggressive behavior is expected to evolve when it consistently brings individuals access to resources. Aggression is predicted to be highest when resources are both highly in abundance and predictably available. Aggression should be weak when resource density is high but predictability is low. Aggression is predicted to be absent if resource density is low (Dyson-Hudson and Smith, 1978). For *Batrachoseps*, surface activity, which is when both mating and feeding occur, is limited and somewhat unpredictable (Maiorana, 1976). Results of behavior trials suggest that time spent defending resources from competitors is too costly to have evolved as an evolutionary stable strategy (Smith and Price, 1973). It is unclear whether a difference in resource abundance alone would predict the higher levels of aggression see in *Plethodon*. Genetic differences may also play a role, and even within *Plethodon* levels of aggression show variability (Nishikawa, 1987; Ovaska, 1993). The framework developed by Brown (1964) and Dyson-Hudson and Smith (1978) should allow for hypothesis testing and comparison of *Plethodon* and *Batrachoseps*. Reports of interspecific aggression in tropical Plethodontids, likely a high abundance and high predictability resource system, are lacking. Niche partitioning and extremely high diversity (Wake, 1987; Wake, 2009) may allow species to largely avoid direct resource competition in these systems. However a lack of published research in this area prevents a firm conclusion about agonistic behavior in tropical Plethodontids guilds (T. J. Papenfuss, pers. comm.).

Differences in the power of a linear model to predict microhabitat across species are suggestive, particularly with respect to substrate temperature. The model performed quite well in predicting substrate temperature in *B. nigriventris* habitat, $R^2 = 0.71$, compared with results for *B. stebbinsi*, $R^2 = 0.38$. Although there was little interspecific difference in DOY for the two *Batrachoseps*, several outliers for *B. nigriventris* were found much later in the season than any *B. stebbinsi* (Figure 9). Field work focused on detecting surface activity pattern, would uncover whether *B. stebbinsi* insulate themselves, to a greater degree, from general warming trends across season and geography.

Utilizing the field data gathered in the study in an ecological framework presented several challenges. Data gathered in an opportunistic manner from exploratory surveys has the potential to carry biases that undermine statistical analysis. Microhabitat was sampled across elevation throughout the season, even though microhabitat values were recorded only when a salamander was present. I posit that trends in the data such as the positive correlation between elevation and DOY reflects a true shift in the presence of suitable microhabitat for Plethodontids salamanders, and *Batrachoseps* in particular. Several limitations also exist with respect to gathering microhabitat data in a consistent manner. The most consistent and trusted microhabitat data are substrate temperature, which can now be easily and quickly measured using an infrared thermometer. Temperature results were consistent and reliable under a variety of conditions so long as the detector was clean. Measurement of available microhabitat moisture was more challenging. I settled on the soil squeeze test because it is rapid and does not require collection and transport of soil for each animal found. It does carry two significant drawbacks, a lack of precision and incompatibility with substrate that is not soil, such as rock or woody debris. Electronic moisture meters, both large and small, are now available and if properly calibrated could return more precise data on the moisture available in these microhabitats. Another potential option is to use a moisture data logger at the point of capture by placing it under the cover object for a designated amount of time. Measuring the size of cover objects themselves is relatively straightforward but documenting cover object use contains some bias. I reported here that the largest cover objects were wood (logs). This sample is biased because it is much easier (and safer!) to move large woody cover than large rock cover.

Tables

Table 1: Predictive power of individual habitat variables used in linear model on microhabitat variables.

	Elevation	Latitude	Year	DOY
Temperature	**R^2= 0.14** **Pearson = -0.38** **p-value < 0.0001**	R^2= 0.004 Pearson = 0.06 p-value = 0.4	**R^2= 0.37** **Pearson = -0.61** **p-value < 0.0001**	R^2= 0.004 Pearson = -0.06 p-value = 0.4
Moisture	**R^2= 0.06** **Pearson = 0.25** **p-value = 0.0003**	R^2= 0.0003 Pearson = 0.02 p-value = 0.8	R^2= 0.002 Pearson = 0.05 p-value = 0.51	R^2= 0.01 Pearson = 0.1 p-value = 0.2
Object size (Log 10)	R^2= 0.0008 Pearson = 0.03 p-value = 0.7	**R^2= 0.04** **Pearson = -0.2** **p-value = 0.006**	R^2= 0.009 Pearson = -0.009 p-value = 0.19	R^2= 0.008 Pearson = 0.09 p-value = 0.2

Table 2: Results from a simple linear model to predict microhabitat variables using spatial and temporal variables. Predictive power for substrate temperature is much stronger for *B. nigriventris* than for *B. stebbinsi*. Variables used: Latitude, Elevation, DOY, and Year.

Species	Temperature	Moisture	Object Size
B. nigriventris	**R^2= 0.71** **p-value < 0.0001** **AIC= 183**	**R^2= 0.27** **p-value= 0.02** **AIC=380**	**R^2= 0.22** **p-value= 0.046** **AIC= 58**
B. stebbinsi	**R^2= 0.34** **p-value < 0.0001** **AIC= 396**	**R^2= 0.21** **p-value= 0.002** **AIC= 635**	**R^2= 0.28** **p-value= 0.0001** **AIC= 96**
Ensatina	**R^2= 0.64** **p-value < 0.0001** **AIC= 367**	**R^2= 0.20** **p-value= 0.002** **AIC= 681**	R^2= 0.12 p-value= 0.053 AIC= 113
All species	**R^2= 0.47** **p-value < 0.0001** **AIC= 977**	**R^2= 0.083** **p-value= 0.002** **AIC= 1725**	R^2= 0.04 p-value= 0.08 AIC= 323

FIGURES CONTINUE ON FOLLOWING PAGE

Figures

Figure 1: Map on right, A), shows ranges for 21 described species of *Batrachoseps* in California. A single species occurs exclusively in Oregon, to the north. Ranges for the focal species of this study are shaded on the map and designated with an asterisk on the phylogenetic tree, B). Map showing species range overlap for *B. nigriventris* and *B. stebbinsi*. Range polygons from California Dept. of Fish and Wildlife (CDFG, 2008).

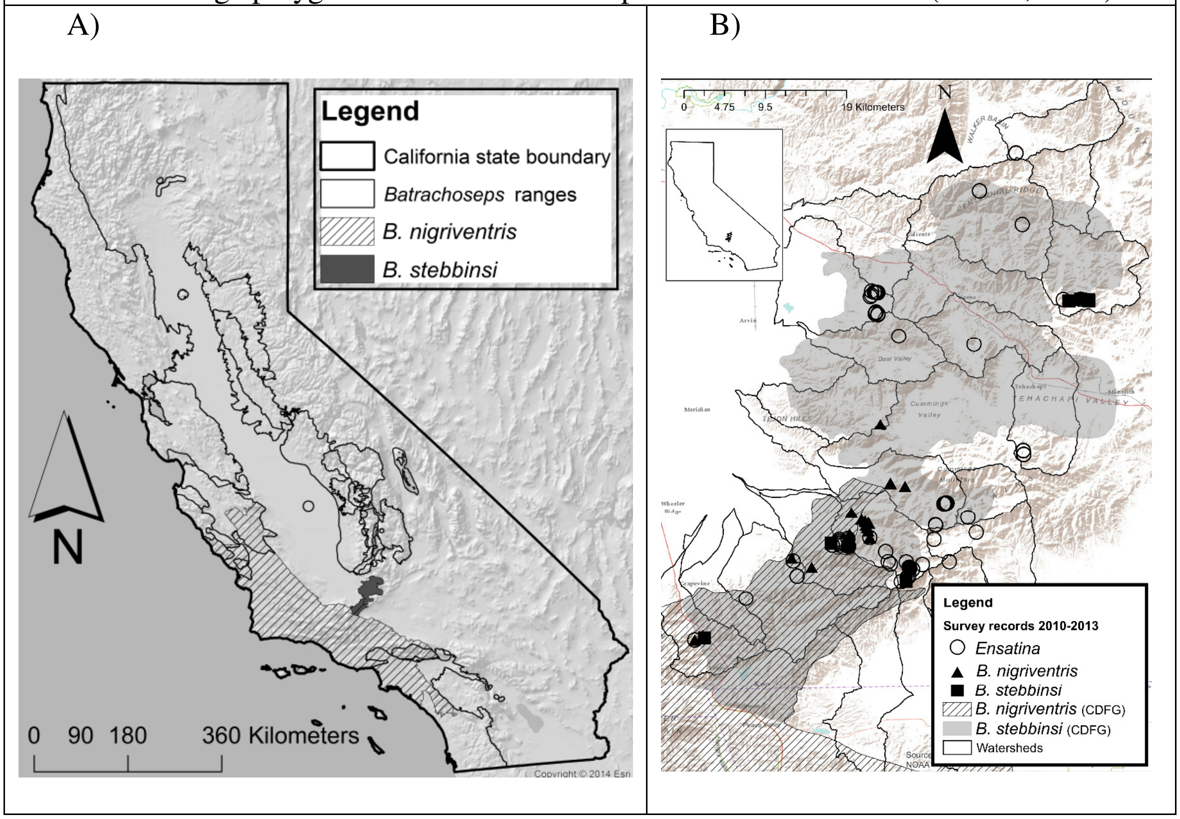

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Figure 2: A) Shows R. C. Stebbins' illustrations of the three salamanders in the study show their morphological diversity (Stebbins, 2003). From left to right: *B. nigriventris*, *B. stebbinsi*, and *Ensatina eschscholtzii croceater*. B) Is phylogeny of all described *Batrachoseps* species adapted from Jockusch and others (2014).

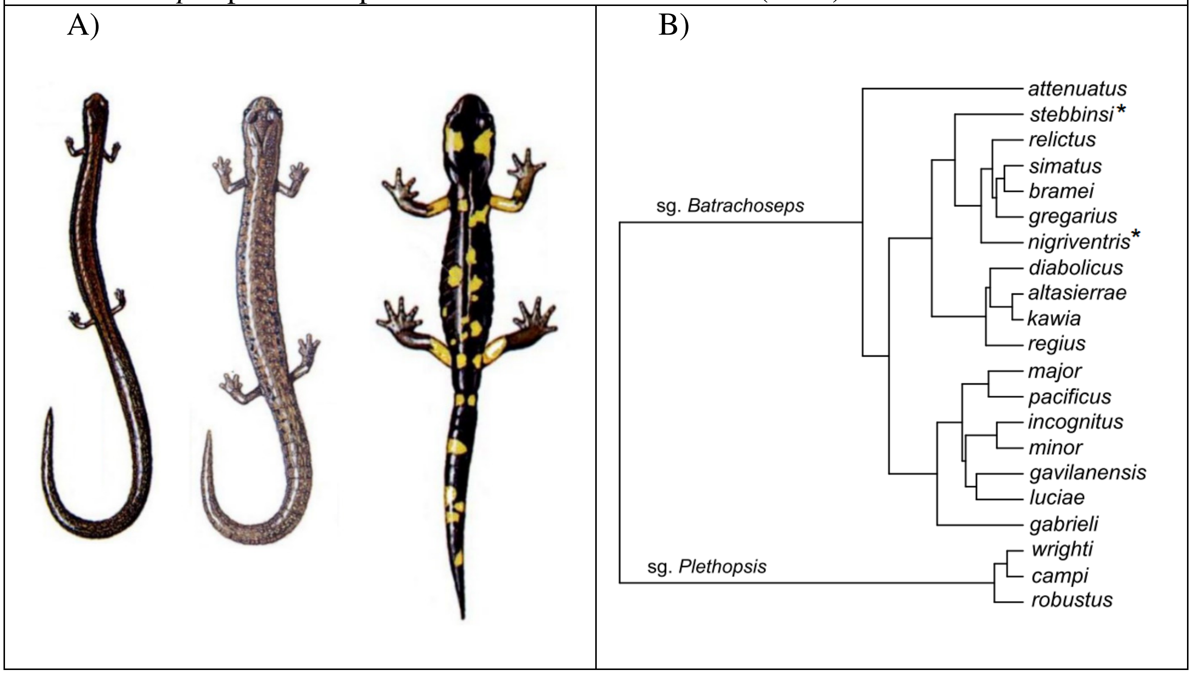

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Figure 3: Maps of study area showing A) watershed names, and B) salamander species found in each watershed during the study. N = *B. nigriventris*, S = *B. stebbinsi*, E = *Ensatina*

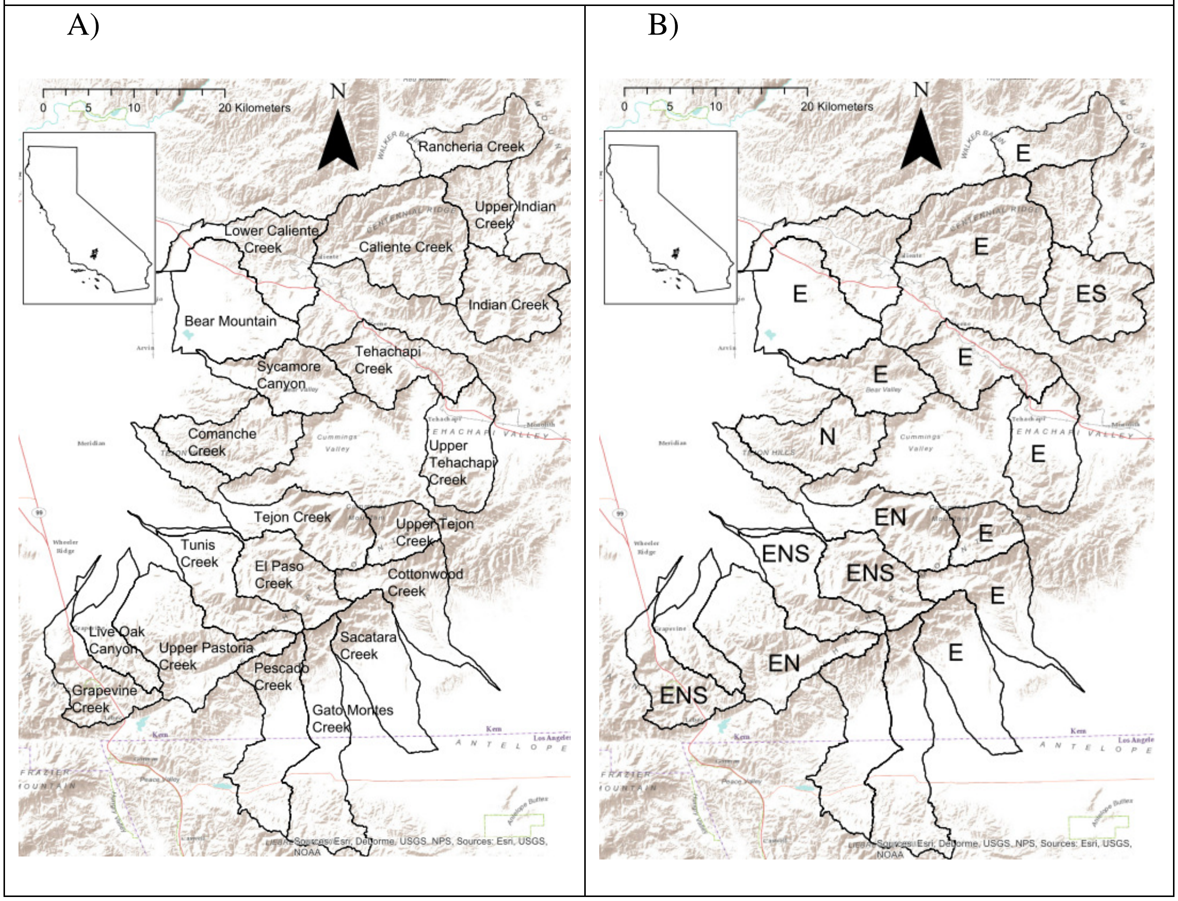

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Figure 4: ANOVA plots for sympatric populations of *B. nigriventris* and *B. stebbinsi*. A significant difference in elevation between species suggests spatial partitioning of resources ($R^2 = 0.44$, p-value < 0.0001). Microclimate showed a great deal of overlap and a weak but significant difference between species, with *B. nigriventris* found in warmer and drier microhabitat.

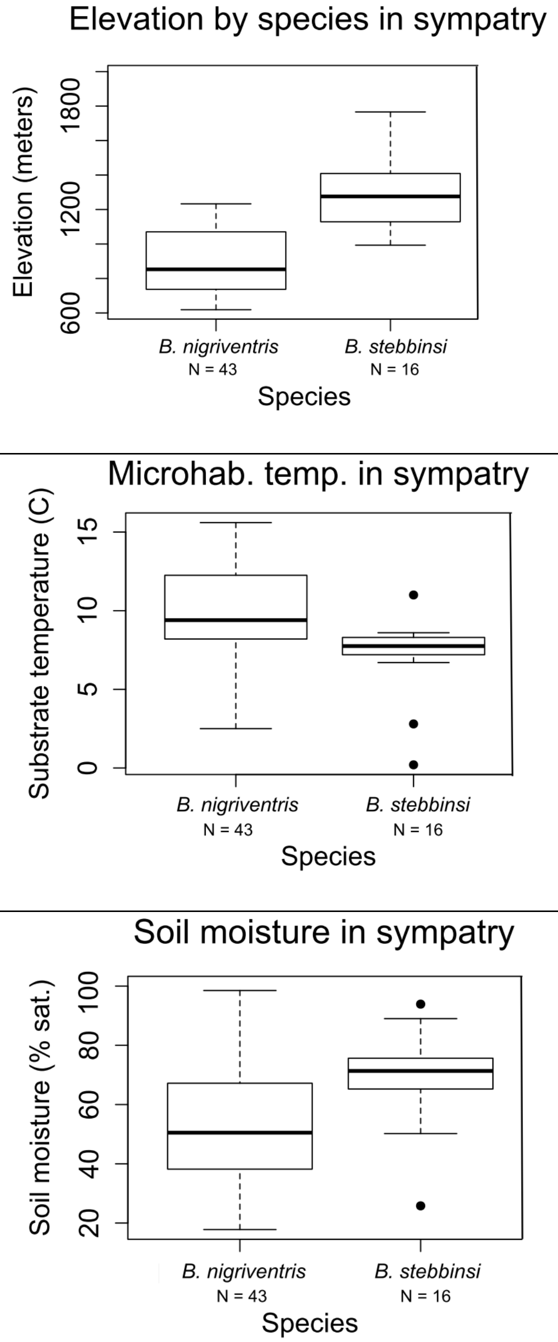
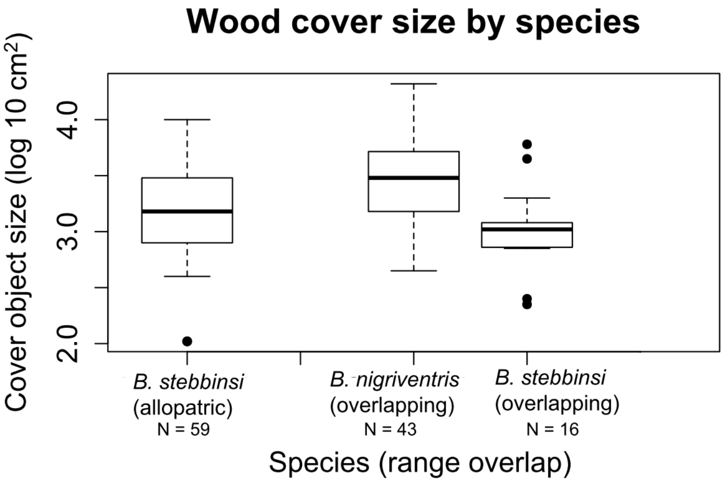


Figure 6: Cover object size used by different populations of *Batrachoseps* in the study area. A) Wood cover used by *B. nigriventris* is significantly larger than wood cover used by both overlapping and allopatric populations of *B. stebbinsi*. B) Rock cover size did not differ between the two species. No significant difference was found in the size of either rock or wood cover used by the two *B. stebbinsi* populations.

A)



B)

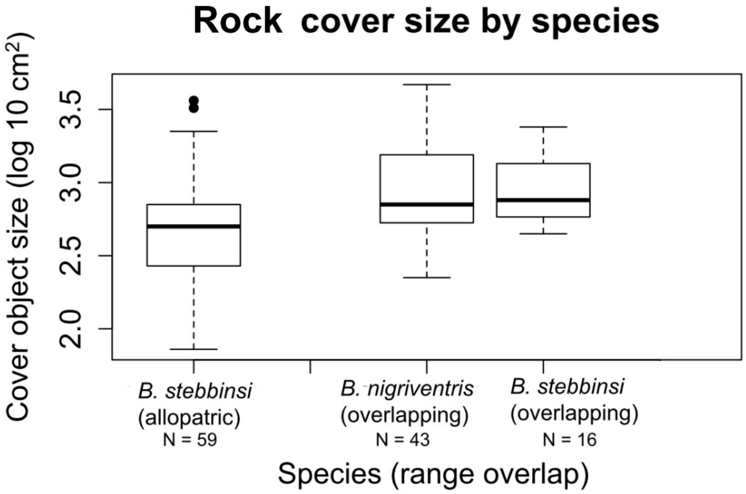


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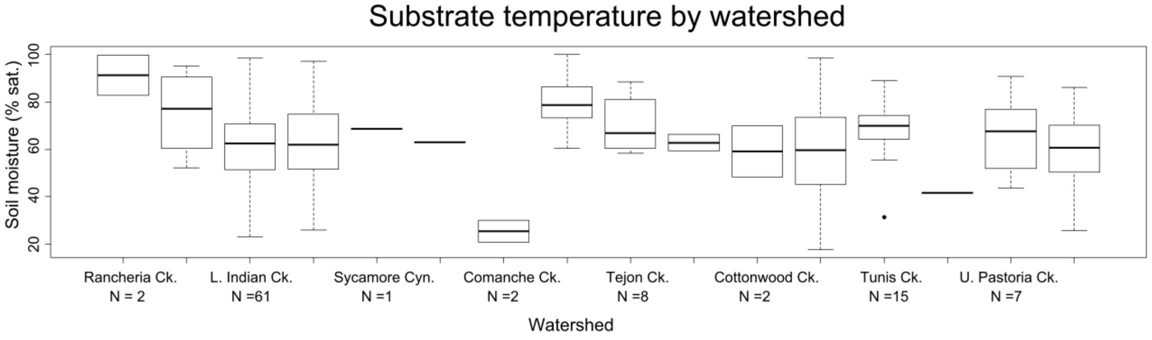
Figure 7: Microclimate variation by watershed. Watersheds are listed from highest latitudes (left) to lowest (right). Latitude did not appear to affect either microclimate variable is a consistent manner.



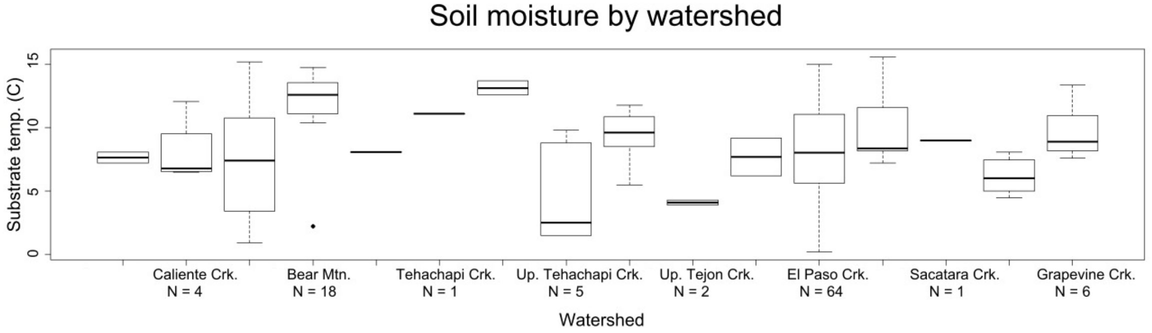
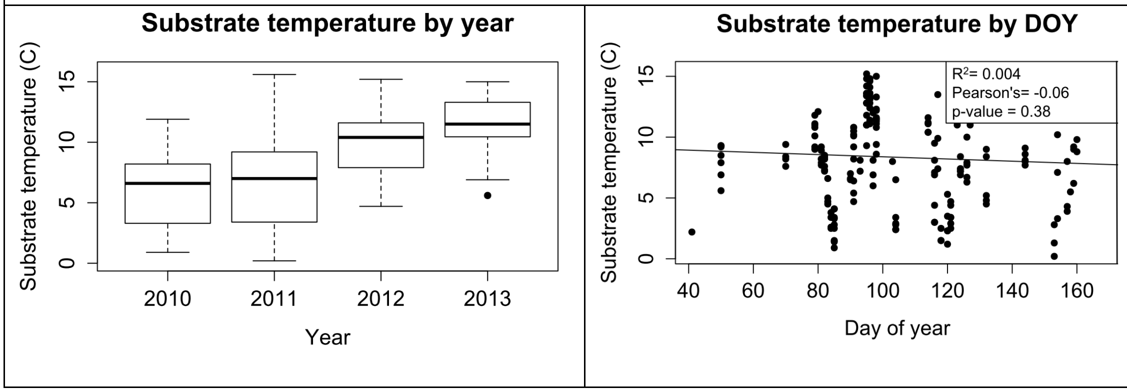
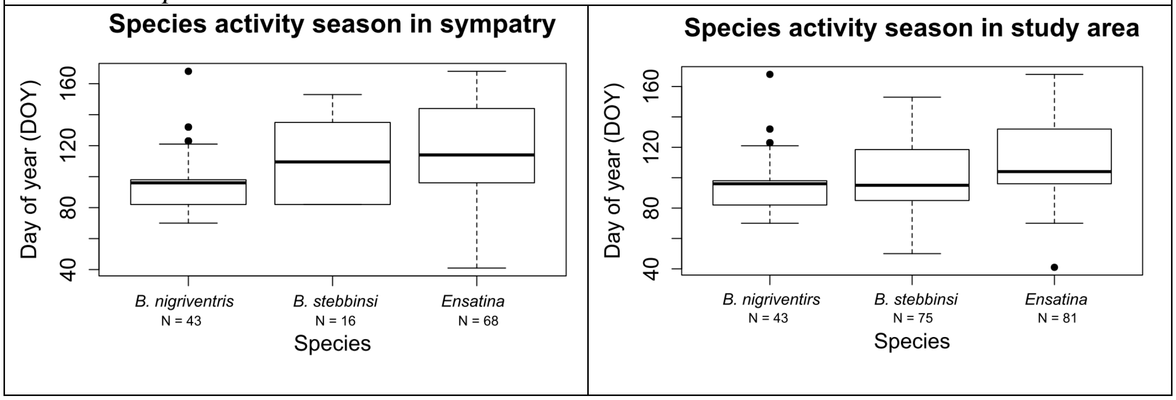


Figure 8: Temporal trends in microhabitat substrate temperature. Microhabitat sampled in 2012 was significantly warmer than 2010 and 2011, and 2013 was significantly warmer than all previous years. Other microhabitat variables did not show any significant response to year or DOY.



FIGURES CONTINUE ON FOLLOWING PAGE

Figure 9: Box plot figures show days of year (DOY) that each species was found, A) in sympatry, and B) overall. No significant difference was found between the two *Batrachoseps* in either case.



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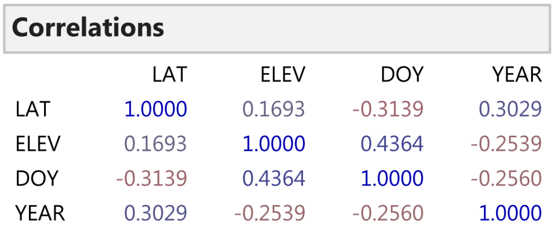
Appendix A

Correlation report for variables used in linear model, all species combined.

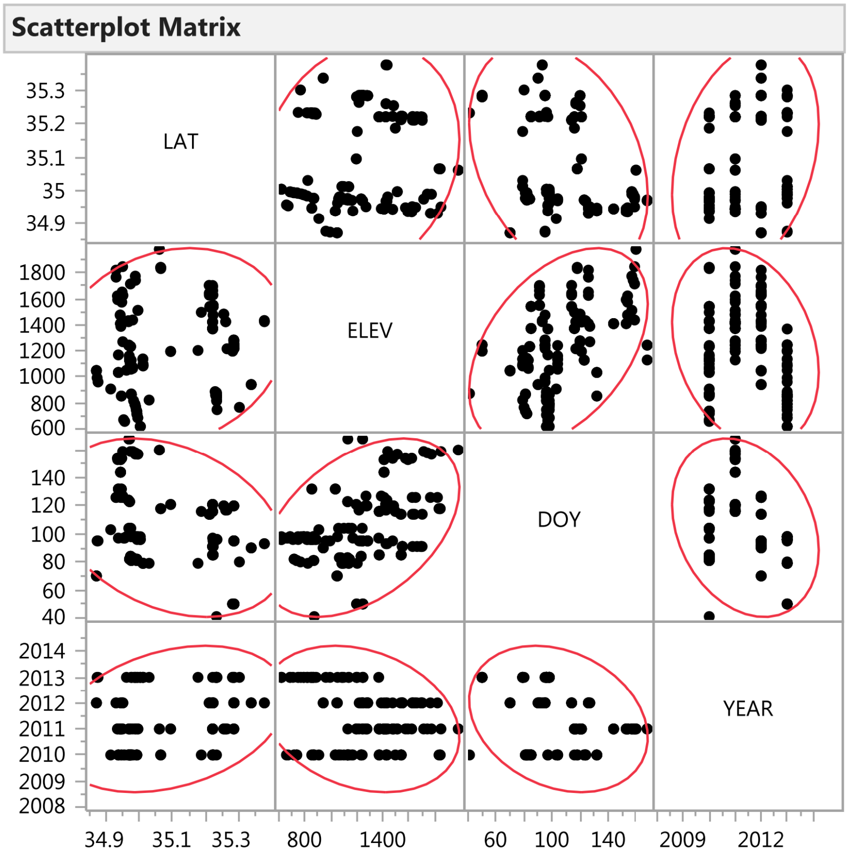
Correlation reports produced in JMP 11 (SAS, 2013).

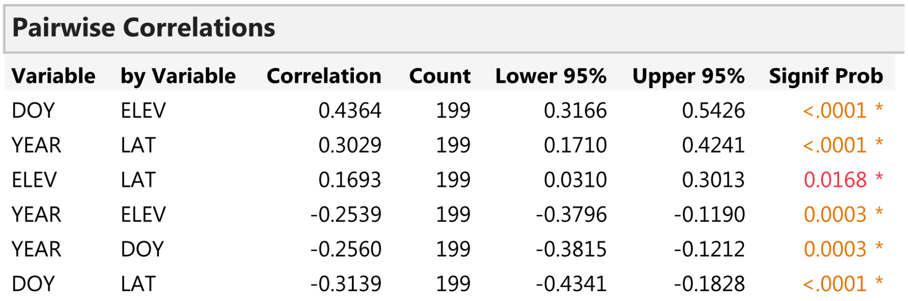


Correlations

	LAT	ELEV	DOY	YEAR
LAT	1.0000	0.1693	-0.3139	0.3029
ELEV	0.1693	1.0000	0.4364	-0.2539
DOY	-0.3139	0.4364	1.0000	-0.2560
YEAR	0.3029	-0.2539	-0.2560	1.0000

Scatterplot Matrix





Pairwise Correlations

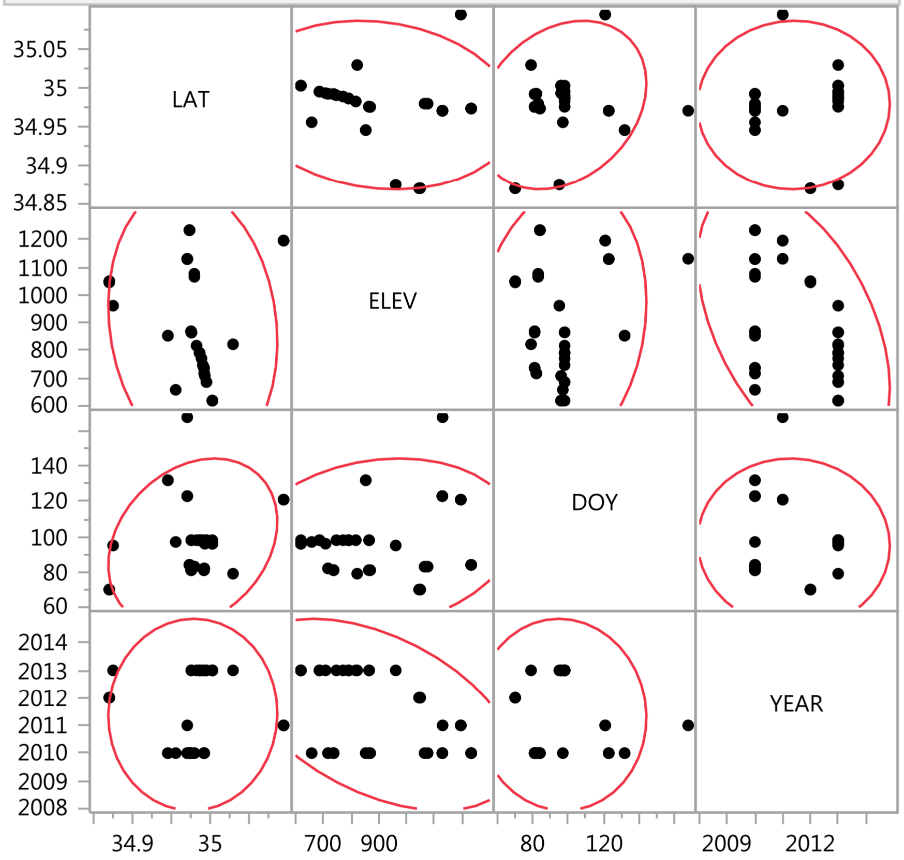
Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
DOY	ELEV	0.4364	199	0.3166	0.5426	<.0001 *
YEAR	LAT	0.3029	199	0.1710	0.4241	<.0001 *
ELEV	LAT	0.1693	199	0.0310	0.3013	0.0168 *
YEAR	ELEV	-0.2539	199	-0.3796	-0.1190	0.0003 *
YEAR	DOY	-0.2560	199	-0.3815	-0.1212	0.0003 *
DOY	LAT	-0.3139	199	-0.4341	-0.1828	<.0001 *

Correlation report for variables used in linear model, *B. nigriventris*. Correlation reports produced in JMP 11 (SAS, 2013).

Correlations

	LAT	ELEV	DOY	YEAR
LAT	1.0000	-0.1317	0.2571	0.0131
ELEV	-0.1317	1.0000	0.1602	-0.4672
DOY	0.2571	0.1602	1.0000	-0.0307
YEAR	0.0131	-0.4672	-0.0307	1.0000

Scatterplot Matrix



Pairwise Correlations

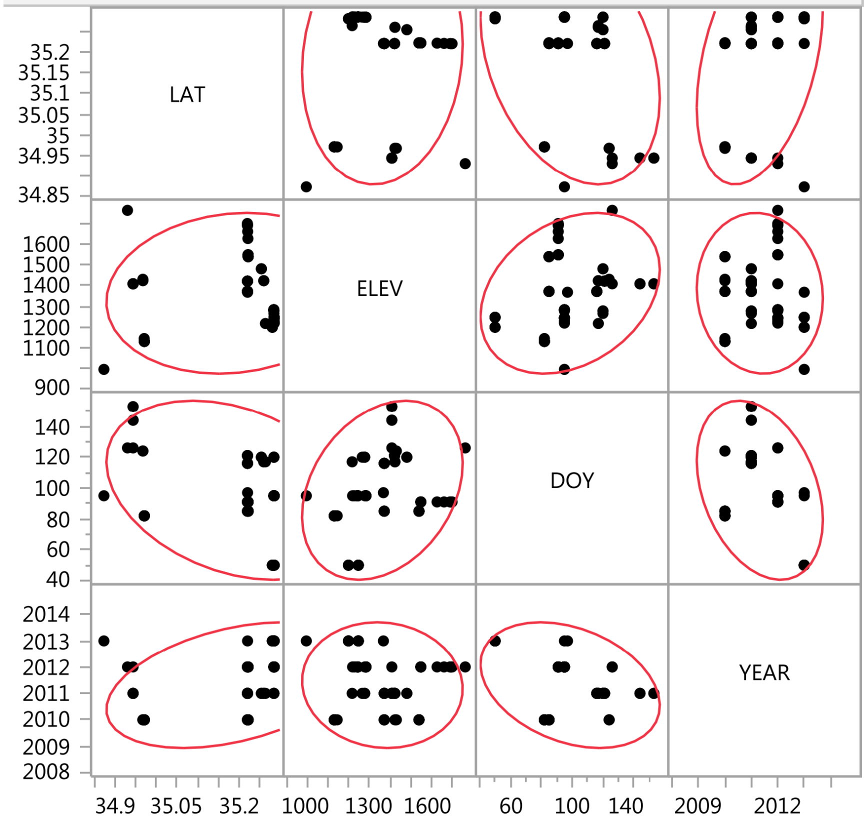
Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
ELEV	LAT	-0.1317	43	-0.4156	0.1755	0.3997
DOY	LAT	0.2571	43	-0.0469	0.5175	0.0961
DOY	ELEV	0.1602	43	-0.1472	0.4394	0.3048
YEAR	LAT	0.0131	43	-0.2884	0.3122	0.9335
YEAR	ELEV	-0.4672	43	-0.6731	-0.1942	0.0016 *
YEAR	DOY	-0.0307	43	-0.3281	0.2721	0.8449

Correlation report for variables used in linear model, *B. stebbinsi*. Correlation reports produced in JMP 11 (SAS, 2013).

Correlations

	LAT	ELEV	DOY	YEAR
LAT	1.0000	0.1097	-0.3168	0.3910
ELEV	0.1097	1.0000	0.2936	-0.0631
DOY	-0.3168	0.2936	1.0000	-0.3299
YEAR	0.3910	-0.0631	-0.3299	1.0000

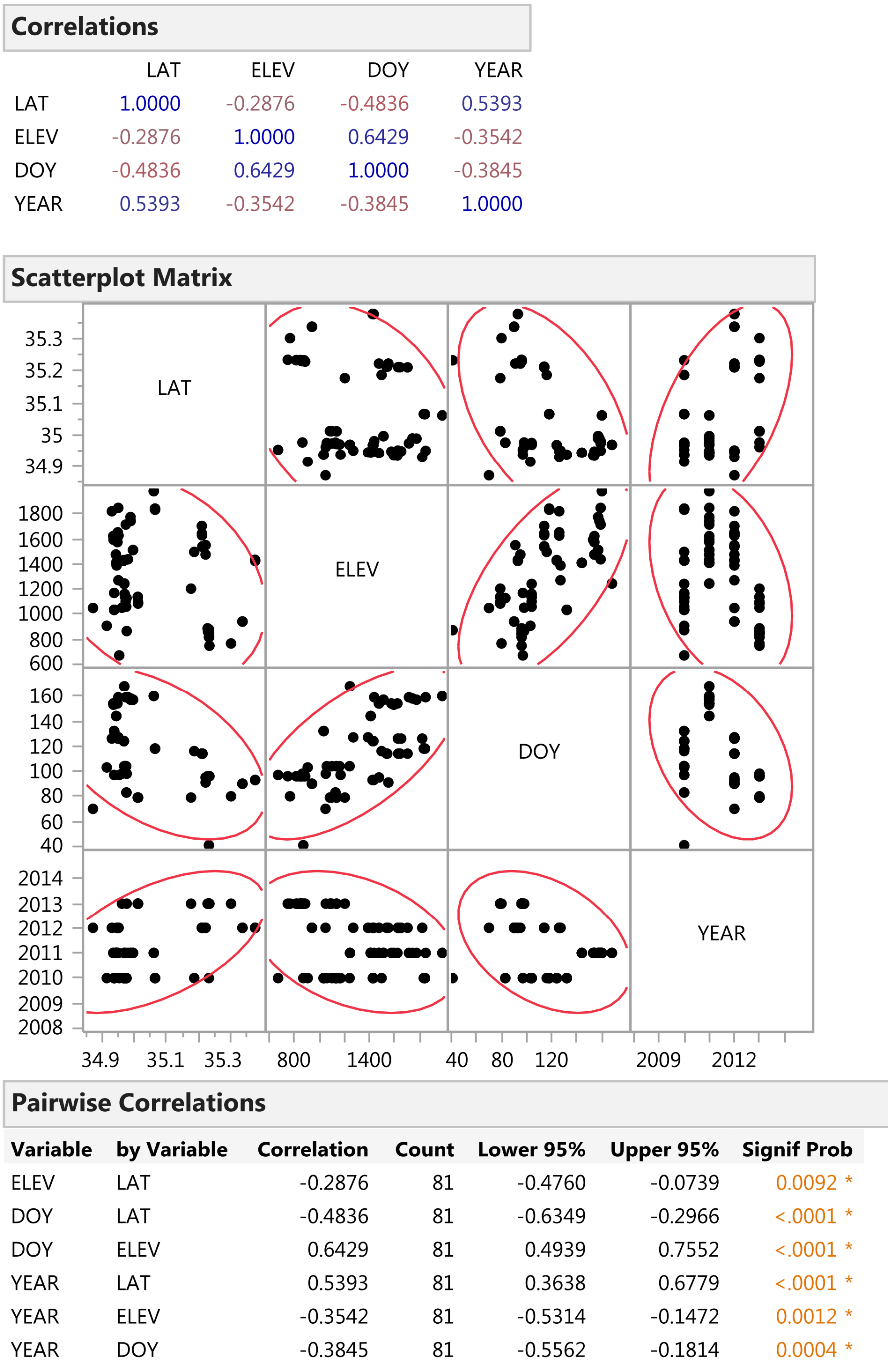
Scatterplot Matrix



Pairwise Correlations

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
ELEV	LAT	0.1097	75	-0.1203	0.3284	0.3490
DOY	LAT	-0.3168	75	-0.5073	-0.0968	0.0056 *
DOY	ELEV	0.2936	75	0.0714	0.4880	0.0106 *
YEAR	LAT	0.3910	75	0.1801	0.5676	0.0005 *
YEAR	ELEV	-0.0631	75	-0.2860	0.1662	0.5906
YEAR	DOY	-0.3299	75	-0.5181	-0.1113	0.0038 *

Correlation report for variables used in linear model, *Ensatina*. Correlation reports produced in JMP 11 (SAS, 2013).



Correlations

	LAT	ELEV	DOY	YEAR
LAT	1.0000	-0.2876	-0.4836	0.5393
ELEV	-0.2876	1.0000	0.6429	-0.3542
DOY	-0.4836	0.6429	1.0000	-0.3845
YEAR	0.5393	-0.3542	-0.3845	1.0000

Scatterplot Matrix

Pairwise Correlations

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
ELEV	LAT	-0.2876	81	-0.4760	-0.0739	0.0092 *
DOY	LAT	-0.4836	81	-0.6349	-0.2966	<.0001 *
DOY	ELEV	0.6429	81	0.4939	0.7552	<.0001 *
YEAR	LAT	0.5393	81	0.3638	0.6779	<.0001 *
YEAR	ELEV	-0.3542	81	-0.5314	-0.1472	0.0012 *
YEAR	DOY	-0.3845	81	-0.5562	-0.1814	0.0004 *

CHAPTER 2

Morphology is a strong predictor of species range size in a Slender Salamanders (Plethodontidae: *Batrachoseps*)

Abstract

Species range size is a fundamental unit in the study of species biology. Range size not only reflects a species' ecology on the biogeographic scale but is a predictor of extinction. Identifying traits that predict extinction is relevant to both macroevolution and conservation biology. In this paper I examine the relationship of species range size and morphology in the salamander genus *Batrachoseps* (Plethodontidae). I also use historical occurrence data to test whether the elevational range occupied, or the median elevation occupied by a species, is correlated with morphology. Among the 22 species in *Batrachoseps* there is a large amount of variation in range size, from 1 km^2 to ca. 50,000 km^2. This variation is strongly and significantly correlated with variation in species morphology. This significant finding describes a pattern where an increase in species range size is accompanied by the evolution of elongation, which in the extreme results in wormlike morphology. The strong correlation between morphology and range size is not the result of wormlike forms occupying a broader breadth of elevation than expected, which would be consistent with the niche breadth hypothesis. There was also no significant difference found in the median elevation occupied by wormlike and more generalized forms. Wormlike species of *Batrachoseps* have larger species ranges but this trend is not driven by their occupation of a wider breadth of habitat, at least on the biogeographic scale.

Introduction

Identifying factors that predict species range size is fundamental to the study of biogeography and has wider implications due to the documented negative correlation between range size and extinction risk (Purvis et al., 2000; Saupe et al., 2015). Finding species traits that predict extinction is important in both macroevolution and conservation science (Lips et al., 2003; Van Allen et al., 2012). This topic is particularly timely given that we are currently experiencing a sixth mass extinction (Barnosky et al., 2011; McCallum, 2015). Among vertebrates the Amphibia appears to be particularly vulnerable on a global scale (Pounds et al., 2006; Stuart et al., 2004; Wake and Vredenberg, 2008). Amphibians have seen dramatic declines in abundance, extirpation of populations, and whole species extinction (Lips et al., 2003; Knapp, 2004; Briggs et al., 2005; Finlay and Vredenberg, 2007). During this current era of rapid climate change an increased probability of extinction is likely for amphibians with small range sizes that occupy a narrow breadth of elevation (Blaustein et al., 1994; Wake and Vredenburg, 2008). This narrow specialization makes species vulnerable to climate impacts or other habitat disturbance across small spatial scales (Thomas et al., 2004; Wake and Vredenburg, 2008; Maxcy and Richardson, 2012; Olson and Kluber, 2012). In this study I take a macroevolutionary perspective and examine the relationship between morphological evolution, range size, and elevation occupied by all species in the fully terrestrial salamander genus *Batrachoseps* (Plethodontidae). Results are relevant for the conservation of these species, and will identify whether morphological evolution has resulted in species traits that increase the probability of persistence across evolutionary time scales (Vrba, 1984).

Among extant taxa the salamander genus *Batrachoseps* (Plethodontidae) from western North America is a good candidate for the study of macroevolutionary patterns. It is an old group, ca. 50 my, with variation in both morphology and range size (Stebbins and Lowe, 1949; Brame and Murray, 1968; Jockusch et al., 2012; Shen et al., 2015). Morphology in *Batrachoseps* is represented in Figure 1, which illustrates variation on the theme of the elongate body type. Several species resemble generalized plethodontids, such as those in genus *Plethodon* (Stebbins and Lowe, 1949). Other *Batrachoseps* display the suite of traits associated with elongation in vertebrates: reduced limbs and increase in number of trunk vertebrae (Gans, 1975; Gans, 1986; Jockusch, 1997). Elongation is achieved through a narrowing of the head and body compared to their congeners (Brame and Murray, 1968; Stebbins, 2003). Narrowing of the head and body is a path to elongation that doesn't require a change in absolute length (Gans, 1975).

Our understanding of evolutionary relationships in *Batrachoseps* has undergone significant change since the 1970s (Yanev, 1978; Jockusch et al., 1998, Jockusch et al., 2001). A basal split in the genus (Figure 2) recognizes two subgenera (Stebbins and Lowe, 1949; Yanev, 1978). Subgenus *Plethopsis*, called the robust clade, consists of three species and sg. *Batrachoseps*, called the attenuate clade, contains 19 described species (Jockusch and Wake, 2002; Jockusch et al., 2014). The great majority of species have been described in the last twenty years through discovery of new populations and the use of genetic techniques which uncovered cryptic diversity (Brame and Murray, 1968; Yanev, 1978; Marlow et al., 1979; Jockusch et al., 1998; Jockusch et al., 2001; Jockusch et al., 2002; Wake et al., 2002; Jockusch et al., 2012; Jockusch et al., 2014). 6thThe pattern of genetic divergence within the two subgenera is markedly different with the three extant *Plethopsis* having diverged relatively recently, within the last 5 million years, while divergence in sg. *Batrachoseps* began ca. 20 mya (Jockusch et al., 2014; Shen et al., 2015). Despite deep genetic divergence, species from across the phylogeny have evolved similar shapes (Figure 2).

Body size is often the trait used to explore biogeographic relationships but other gross morphological measures, such as shape, appear to be more variable, and hence more relevant, in the evolutionary history of certain groups (Sweet, 1980; Liow, 2004). It is possible to use data from fossil or modern taxa in macroevolutionary study and each has advantages and drawbacks. The study of extinct groups requires an accurate fossil record and inference regarding phylogenetic relationships and ecological context of the organisms (Gould, 1967; Saupe et al., 2015). For modern taxa extinction probability must be inferred but DNA data can be used to create accurate phylogenies and species ecology can be studied first-hand (Foufopoulos and Ives, 1999; Purvis et al., 2000; O'Grady et al., 2004). An additional advantage of using extant taxa is that the types of species which do not leave a robust fossil record can be studied. Among modern taxa extant but old clades, with well-understood phylogenies should be good candidates for inquiry into extinction risk during the current sixth mass extinction. Documenting traits in extant taxa that are associated with extinction risk or resistance is valuable for conservation efforts and for comparing patterns in the current mass extinction with the patterns seen in previous mass extinction events (Purvis et al., 2000a). A focus on groups with well-studied phylogenies allows correlations between extinction risk and species level traits to be understood in an evolutionary context and phylogenetic influence to be accounted for (Pagel, 1999).

Species range size is one of the fundamental units used by researchers to understand species biology (Gaston, 2003 and sources therein). It is straightforward to see how having a large range would insulate a species from extinction due to background processes such as demographic stochasticity or the catastrophic events that precipitate mass extinctions (Gould et al., 1977; Lande, 1988; Lande, 1993).The distribution of range size within clades generally resembles an exponential or log normal distribution. This result is due to few species having large ranges and many more have small to medium-sized ranges (Willis 1922; Webb and Gaston, 2005). This pattern has been shown in many groups including trees (*Pinus*), birds (Aves), Lizards (Lacertilia), and butterflies (Lepidoptera) among others (Gaston, 1990; Anderson and Marcus, 1992; Brown et al., 1996; Gaston, 1996; Gaston and He, 2002). Species range size has been identified as a reliable predictor of extinction probability so finding traits that predict range size among related species is important to both conservation biology and macroevolution. (Gaston and Blackburn, 1996; Saupe et al., 2015). Species range size has consistently been found to be a strong predictor of extinction risk (Purvis et al., 2000; Thomas et al., 2004; Feeley et al., 2007; Saupe et al., 2015), and the realized niche occupied by a species has also been shown to be significant (Saupe et al., 2015). Sky island species illustrate how climate change, and realized niche along an elevation gradient can interact to produce a relict species, vulnerable to extinction (Van Devender et al., 2013; DeChaine and Martin, 2005; Shepard and Burbrink, 2008).

Several explanations have been proposed for the consistent pattern of range size distributions across a diversity of taxa. These hypotheses include stochastic models, biogeographic history, current species traits, and phylogenic influence (Jablonski, 1986; Lande, 1993; Gaston and He, 2002; Gaston, 2003; Böhning-Gaese et al., 2006). Stochastic models have been proposed by ecologists and evolutionary biologists to explain range size distributions and the related extinction probability (Saether et al., 1998; Gaston and He, 2002). Stochastic explanations center on the impact of “random” catastrophic events, environmental stochasticity and demographic stochasticity (Gould et al., 1977; Lande, 1993; Saether et al., 1998; Gaston and He, 2002). Biogeographic hypotheses attempt to explain range size distributions using differences in latitude, longitude, or size of biogeographic region (Stevens, 1989; Pagel et al., 1991; Bönhing-Gaese et al., 2006). Ecological hypotheses include the niche breadth hypothesis (Brown, 1984) and the resource abundance hypothesis (Venier and Fahrig, 1996). These ecological explanations should be reflected in trait adaptation among species. Current species traits have frequently been tested for a correlation with species range size. Species traits that are considered important include morphology, chiefly body size or shape, and behavioral traits such as territoriality and dispersal ability (Gaston and Blackburn, 1996; Holt et al., 1997; Munguia et al., 2008; Lee et al., 2013; Morales-Castilla et al., 2013). Both species traits and range size itself have been shown to be heritable to varying degrees (Jablonski, 1986; Waldron, 2007). For this reason the influence of phylogeny on species traits and range size should be accounted for if correlations between traits and range size are to be understood (Harvey and Pagel, 1991).

A pattern of morphological features predicting range size, and hence extinction risk, provide evidence that microevolutionary processes, such as adaptation, and not macroevolutionary process, such as catastrophic events and stochasticity, can drive extinction patterns through time (Gould, 1985; Simmons, 2002). To be clear adaptation could not be for range size in itself but adaptation to conditions on a microevolutionary scale could insulate a lineage from extinction over macroevolutionary time. When a strong trait-range size is found, further investigation is needed to understand what is driving this pattern. Correlation between body size and range size has been investigated frequently and across a diversity of organisms (Gaston and Blackburn, 1996; Diniz-Filho and Balestra, 1998; Pyron, 1999; Murray and Dickman, 2000; Roy et al., 2001; Reed, 2003; Geraghty et al., 2007). Some taxonomic groups show variation in body size while body shape remains relatively consistent. Many guilds of predators show near geometric scaling, while in other groups size is relatively consistent and species show variation in shape (Sweet, 1980). Studies investigating the association of shape with ecological and evolution patterns has a long history but have increased with the wider availability of multivariate statistical methods (Adams and Rohlf, 2000; Adams et al., 2004). Body size differences are often tied to first principles of life history and physiological constraints (Schmidt-Nielsen, 1975). For this reason body size and surface to volume ratio appear to have similar evolutionary importance across a diverse array of animals (Gould, 1966; Schmidt-Neilsen, 1972; Blueweiss et al., 1978). Evolution of shape may be connected to first principles in a similar way, or be tied to more specific conditions, such as movement ecology in a specific habitat (Losos, 1990), or adaptation to predator-prey regime (Walker, 1997). Connecting biological mechanisms across the temporal spectrum from ecology to microevolution to macroevolution has been a goal in biology for some time (Simmons, 2002; Pianka and Sweet 2005). The use of biogeographic patterns as macroevolutionary metrics allows the testing these types of hypotheses using extant taxa.

In terrestrial vertebrates (Tetrapoda) body elongation has evolved many times (Gans, 1986; Carroll, 1996). Evolution of body elongation in the Tetrapoda is dramatic in that it is accompanied by a reduction in size or total loss of the defining character of the group, the feet (poda). Several adaptationist hypotheses have been proposed to explain the evolution of elongation in tetrapods. Locomotor adaptation in elongate vertebrates has been proposed for shifts to fossorial habitat, structurally complex habitat, loose sand habitat, and aquatic habitat (Bergman and Irshick, 2009). Adaptive life history hypotheses propose fecundity selection on the females of small vertebrates, including *Batrachoseps* (Griffith, 1990; Jockusch, 1997). Most extant species of elongate tetrapods are found in the Order Squamata, the lizards, snakes, and amphibisbaenians (Vitt and Caldwell, 2014). Elongation is found among extant amphibians in aquatic salamanders and terrestrial salamanders (Parra-Olea and Wake, 2001), and in the limbless caecilians (Gymnophonia) (Wells, 2007; Zhang and Wake, 2009). Terrestrial salamanders with elongate morphology are primarily semi-fossorial species in the family Plethodontidae (Duellman and Treub, 1986; Parra-Olea and Wake, 2001). These genera include *Batrachoseps*, Phaeognathus, *Oedipina*, and *Pseudoeurycea* (Parra-Olea and Wake, 2001; AmphibiaWeb.org).

Genetic divergence and speciation in *Batrachoseps* appears to be strongly influenced by geologic and climate history (Wake, 2006). This is unsurprising for a small and low vagility amphibian (Hendrickson, 1954; Cunningham, 1960). Standard length in adult *Batrachoseps* ranges from 3.5- 6 cm (Stebbins, 2003). Annual movement by an individual *Batrachoseps* is on the order of 10 meters (Hendrickson, 1954; Cunningham, 1960). Surface activity is generally limited to times during and after precipitation, although some populations may be continuously active at the margins of springs, seeps, or small watercourses (Hendrickson, 1954; Maiorana, 1978; Yanev et al., 1981). Across much of the range of *Batrachoseps* precipitation events are strongly seasonal and activity pattern follows this seasonal pattern (Maiorana, 1975; Maiorana, 1976). Reproduction in *Batrachoseps* is fully terrestrial so dispersal of larval stages along watercourses is not a factor. This fully terrestrial biology can result in highly localized populations and high levels of genetic diversity across small spatial scales (Martinez-Solano et al., 2007; Martinez-Solano et al., 2012). Species range size along with morphology shows considerable variation in *Batrachoseps* (Stebbins, 2003). Distribution of range size in clades of taxa generally resembles a log-normal or negative binomial distribution (Willis 1922; Gaston, 1990; Anderson and Marcus, 1992; Brown et al., 1996; Gaston, 1996; Gaston and He, 2002; Webb and Gaston, 2005). Morphological shape differences are less dramatic than disparity in range size but are considerable and have been acknowledged by researchers for some time (Stebbins and Lowe, 1949; Brame and Murray, 1968). By using a robust phylogenetic hypothesis (Jockusch et al., 2014) it is now possible to understand these morphological differences in an evolutionary context. This is important in salamanders, and plethodontid salamanders in particular, as homoplasy is common and often dramatic (Wake, 2009; Wake, 2014).

Ecological differences between species of *Batrachoseps* are thought to be negligible (Jockusch and Wake, 2002; Wake, 2006). Despite this perception there are examples of species range contact and replacement in the presence of an elevation gradient. Several of these examples involve wormlike species occupying lowland habitat adjacent to generalized species in upland habitat (Figure 3). In southern California wormlike forms *B. major* and *B. nigriventris* occupy lowland habitat adjacent to *B. gabrieli* in the Transverse Ranges (Figure 3A). In the southern Sierra Nevada *B. gregarius*, *B. simatus*, and *B. relictus* replace each other sequentially along an elevation gradient, according to their morphology (Figure 3B). This pattern is suggestive but is not universal. Two wormlike forms *B. nigriventris* and *B. gregarius* achieve relatively high elevations (> 1800 meters), even in sympatry with generalized forms. Occasionally the pattern is flipped, usually in coastal habitat, with a strong marine influence on climate. Examples of this include *B. luciae* (generalized) and *B. gavilanensis*, or *B. nigriventris* and the undescribed B. sp. nov. Arguello (Jockusch et al., 2014). Due to the inconsistency of a clear pattern and the general importance of elevation in organizing biological communities (Sundqvist et al., 2013), a formal comparative analysis is warranted.

Investigating the relationship between elevation and species range size has implications not only for understanding the heterogeneous geography in the ranges of these species, but also for identifying species that are at particular risk for extinction. In low vagility species like *Batrachoseps* emigration to suitable habitat that is disjunct from the species current range is unlikely. For this reason plethodontid salamanders in general are at risk for isolation and extirpation due to climate shifts. Two dramatic examples occur in the southwestern North America where relict populations of *Plethodon neomexicanus* and *Aneides hardii* are isolated on mountain peaks, surrounded by harsh and dry lowland habitat (Whitford, 1968; Reagan, 1972). Species most at risk for extinction due to this pattern will have small species ranges, occur at high elevation, and have a small elevation buffer between habitat they currently occupy and the maximum elevation of habitat in their species range.

In this study I report the interaction between morphological evolution and species range size in a phylogenetic context. I will discuss the findings in the context of macroevolutionary patterns and extinction probability. Maiorana (1976) presented a hypothesis that more wormlike forms of *Batrachoseps* are adapted for arid, unpredictable, and highly seasonal environments. Jockusch (1997) found that within species of *Batrachoseps* the number of trunk vertebrate increased further inland, as climate became more arid. If wormlike forms are associated with arid and seasonal environments we might expect these forms to dominate during the current interglacial cycle in western North America (Duellman and Sweet, 2000). I tested for the ability for morphology, shape, to predict extinction probability, as range size. I used phylogenetic independent contrasts to account for the role of trait heredity (Felsenstein, 1985). Results are interpreted as the summation of events at multiple biological levels across long time periods, and inform our understanding of both macroevolutionary phenomena and conservation efforts aimed at predicting extinction probability (Hunter, 1998).

Two mechanisms proposed to explain range size disparity between species are the niche breadth hypothesis and the resource abundance hypothesis (Brown, 1984; Venier and Fahrig, 1996; Gaston and Spicer, 2008). Here I will test for differences in elevation of habitat as a metric for niche and resource use. Median range size elevation will indicate whether a species occupies relatively abundant lowland habitat or in less abundant upland habitat. Elevation range of species occurrence will be used as a proxy for niche breadth. The correlation between shape and these two variables, median elevation and elevation range, will be analyzed in a Phylogenetic Independent Contrast framework.

A final consideration on the probability of extinction will be the amount of buffer a species has to occupy higher elevation habitat within its range. This range shift buffer will be measured as the difference between the highest elevation where a species is known to occur and the highest elevation habitat available in its range. In Mesoamerica many plethodontid salamanders are endemic to narrow bands of elevation (Wake and Lynch, 1976; Wake, 1987). This narrowness and specialization makes species vulnerable to climate impacts or other habitat disturbance across small spatial scales (Blaustein et al., 1994; Wake and Vredenburg, 2008). Measuring a species' buffer against elevation range shift should be useful in identifying species most at risk of extinction of extirpation due to climate-driven range shifts.

Methods

Species Range Size

Three range size metrics were considered in this study, latitudinal extent, published maps of range polygons (CDFG, 2008; Evelyn and Sweet, 2012); and range area polygons calculated from point data using the alpha hull method (Burgman and Fox, 2003). Measures of range size have been formally classified as either measuring Extent Of Occurrence (EOO) or Area Of Occupancy (AOO) (Gaston, 1991). EOO measures are used here since comprehensive surveys across the range are not available for all species of *Batrachoseps*. Latitudinal extent was calculated by subtracting the smallest latitude value from the largest latitude value found in museum records (Herpnet, 2014). Published range maps came from state California's wildlife management agency (CDFG, 2008), and a recent US Forest Service report (Evelyn and Sweet, 2012). *Batrachoseps wrighti* is found only in Oregon. For *B. wrighti* published species range size was taken from Clayton and Olsen (2009). *Batrachoseps aridus* has the smallest range of any *Batrachoseps*, and published maps were found not to reflect its actual distribution based on published reports (Hansen and Wake, 2005). A range polygon for *B. aridus* was drawn in Google Earth Pro (Google Inc., 2015) based on the published range description in Hansen and Wake (2005). Concave hull polygons were calculated using species locality data (Herpnet.org) and the α-hull method of

Burgman and Fox (2003). Five levels of α were considered: 2, 2.5, 3, 3.5, and 4, this method is preferred over the convex hulls because it is less likely to overestimate range size (Burgman and Fox, 2003). The α-hull method has two advantages over published maps because it is not limited by political boundaries and assumptions are more explicit since the polygon is based on verified species localities. The performance of published maps, latitude extent, and α-hull polygons were compared. All range size measures were log 10 transformed and tested for normality using the Shapiro-Wilk method (R Core Team, 2014).

Museum records used in spatial analysis were reviewed for obvious spatial errors and errors due to taxonomic changes in *Batrachoseps* not reflected in the museum records. Erroneous records were corrected or removed before range size was calculated. The most common taxonomic corrections were the assignment of multiple species to *B. attenuatus* after the recommendation of Hendrickson (1954); the assignment of multiple species to *B. relictus* after the recommendation of Brame and Murray (1968); and the assignment of multiple species from the *pacificus* clade to *B. pacificus* prior to the publication by Jockusch and others (1998). Museum records for *B. major* from the San Joaquin Valley and Baja California Sur were removed based on the recommendation of a recent population genetic study for this species (Martinez-Solano et al., 2012). San Joaquin Valley populations are known to be introduced by humans (E. L. Jockusch, pers. comm.). Additional records were added from the UC Santa Barbara Museum collection for novel localities of *B. nigriventris* in and around Santa Barbara County. Additional records were added to *B. stebbinsi* based on 4 years of field study by the author (USFWS, 2011). Coordinates of historical *B. stebbinsi* were corrected based on interviews and field notes with the original collectors (S. S. Sweet, T. Papenfuss, and R. W. Hansen pers. comm.).

Elevation

Elevation values used in analysis are from the National Elevation Model raster dataset (Gesch et al., 2002). This dataset was sampled in two ways to create the dataset used here. Species range area polygons (see above) were used to sample this elevation dataset in ArcMap 10.2.2 (ESRI, 2014). These data were used to calculate the elevation of habitat available within each species range. Point locality records for each species (see above) were also used to sample elevation data in ArcMap 10.2.2 (ESRI, 2014). Elevation samples using the point data represent the elevation of habitat where the species is known to occur. These point data elevation samples were used to calculate a summary statistic for the elevation of a species range (median), and the elevation range where it occurs. Total elevation buffer is calculated as the difference between the lowest habitat where a species is known to occur and the highest elevation habitat available within the range polygon of that species. Range shift buffer is calculated as the difference between the highest elevation habitat where a species is known to occur and the highest elevation habitat available within its species range polygon (α hull 3.5). Finally a sample of all elevation available to *Batrachoseps* was created to put results in context. To sample these elevation data a polygon was created to cover the extent of the published range polygons (CDFG, 2008; CDFW, 2014) and α-hull polygons (α = 3.5) using the merge tool in AcMap 10.2 (ESRI, 2014). The IUCN range for *B. wrighti* was also added as no published GIS range polygon could be found from state or regional entities (Hammerson and Bury, 2004; IUCN, 2015).

Morphology

A single continuous variable, shape, was created to quantify interspecific differences in morphological shape independent of absolute size (Jockusch et al., 2012). SHAPE was created using 9 linear morphological measures from each of the 22 described species in *Batrachoseps* (Figure 1). To compare variation in shape, not just differences in size, residuals of the regression of each variable against standard length (SL) were analyzed. In salamanders SL is measured from the tip of the snout to the caudal end of the vent. Correlations between residuals were calculated, and the forelimb-length residual was excluded because of its high correlation ($R^2 = 0.90$) with the hind limb-length residual. Principal Component Analysis (PCA) of the correlation matrix of the remaining seven residuals, as implemented by the prcomp command in R (R CORE TEAM, 2014), was used to capture the main axes of morphological variation. This type of shape analysis is an accepted method for quantifying animal shape in plethodontid salamanders (Jockusch et al., 2012). Linear measurements used were standard length, SL, which is the length from the tip of the snout to the posterior angle of the vent, head width, snout–gular fold length, axilla–groin length, chest width behind the forelimbs, forelimb length, hind limb length, maximum foot width and head depth behind the eyes (Jockusch et al., 2012). These measurements were performed on preserved museum specimens for all 19 species of subgenus *Batrachoseps*, see Table 1. All morphological measurements for the 19 members of sg. *Batrachoseps* were gathered by Iñigo Martinez-Solano (Jockusch et al., 2012). Measurements for the three members of sg. *Plethopsis* were gathered by the author.

Phylogenetic Independent Contrasts

Range has been shown to be a heritable trait in several studies (Jablonski, 1986; Waldron, 2007) so phylogenetic independent contrasts (Felsenstein, 1985) were used account for the role of phylogeny in correlation tests between range size and both morphology and elevation. The species tree used in PIC was the most recent published phylogeny for *Batrachoseps* (Jockusch et al., 2014). This phylogeny was constructed using 5 nuclear genes (Jockusch et al., 2014). Two species of sg. *Batrachoseps* were excluded from PIC analysis. The undescribed species nov. “Arguello” was excluded pending formal description. The federally endangered *B. aridus* was excluded from the species tree due to a lack of nuclear genetic data (Jockusch et al., 2014). PIC analysis was performed using the R packages ape (Paradis et al., 2014) and caper (Orme, 2013). R code for analysis of continuous variables was adapted from the APE tutorial for PIC on continuous traits (http://www.r-phylo.org/mediawiki/index.php?title=HowTo/Phylogenetic_Independent_Contrasts&action=edit). The code used for this analysis is available in Appendix A.

Distributions of shape, the log 10 range size metrics, and elevation range were found to be not significantly different than normal using the Shapiro-Wilk method (R core team, 2014). The distribution of median elevation values was log 10 transformed since the raw data are significantly different than normal. Pearson product moment correlations were used to quantify the direction and strength of the phylogenetically corrected interaction between morphology and range size (R Core Team, 2014).

Results

Phylogenetic independent contrasts (PIC) show a strong and significant correlation between species range size and morphology. The degree of elongation in more wormlike morphology strongly predicts larger species range size. When all species of *Batrachoseps* are included (except *B. aridus*, see Methods) the Pearson correlation coefficient is -0.75, p-value = 0.0001 (Table 2, Figure 4). This strong and highly significant correlation occurs within both subgenera in *Batrachoseps* (Table 3). Within subgenus *Batrachoseps* the Pearson correlation coefficient of PIC tests is -0.71 (p-value = 0.001). Subgenus *Plethopsis* contains only three species but the most attenuate (*B. wrighti*) has the largest range. PIC methods are not valid for just three taxa but conventional correlation tests show a strong negative correlation (Pearson coefficient = -0.998, p-value = 0.04).

A strong positive correlation was found between the size of species ranges produced by all three methods considered (α-hull 3.5, latitude extent, and published maps), see Table 4.

Shape and median elevation

Median elevation shows a significant and modest positive correlation with shape in the genus *Batrachoseps* (Table 2). Phylogenetic independent contrast tests found a Pearson correlation = 0.45, p-value=0.04 (Figure 5). However when only the subgenus *Batrachoseps* is considered this correlation is weak and not significant, Pearson correlation= 0.19, p-value=0.47 (Table 3). This result provides weak, mixed results for the resource abundance hypothesis.

Shape and elevation range

Evolution of wormlike morphology is not correlated with the use of a wider breadth of elevation (Table 2). Pearson correlation results were weak and not significant (Figure 5). This indicates that wormlike species with large ranges species do not use a wider breadth of habitats, with respect to elevation. This result is inconsistent with the niche breadth hypothesis.

Elevation buffers

Three species tallied an elevation shift buffer of 0 meters (Table 1). Two of these species, *B. aridus* and *B. relictus*, have exceedingly small ranges, published range < 150 km^2. A third species, *B. stebbinsi*, has a published range of 1,327 km^2. Three other species with small ranges have range shift buffers < 250 meters: *B. minor* (232 m), *B. incognitus* (180 m), and *B. pacificus* (168 m).

Discussion

This study identified a strong correlation between morphological shape and range size in the salamander genus *Batrachoseps* (Plethodontidae). In *Batrachoseps* evolution of wormlike morphology, where animals become more slender with reduced limbs, is strongly correlated with large species range size. The correlation is robust to the method used for calculating species range size. The strong correlation was also robust to the species included in the analysis as the two subgenera sg. *Plethopsis* and sg. *Batrachoseps*, separately show strong correlations in the same direction. Small species range size in *Batrachoseps* is not the result of habitat loss due to recent anthropogenic activities. However, anthropogenic activities, such as habitat alteration for land development, will have a greater impact species with small ranges, since a larger proportion of the total species population will be affected. Rapid climate change is underway (Molina et al., 2014), which is likely to carry a greater impact for species with small ranges. The distribution of range size in *Batrachoseps*, and its correlation with morphology, indicates a trend over evolutionary time scales.

Despite the apparent role of elevation in structuring contact zones between *Batrachoseps*, no consistent significant relationship was found between elevation range, or median elevation, and morphology (Table 2). These results indicate that as a whole *Batrachoseps* species do not achieve larger ranges by occupying a wider breadth of elevation, or by disproportionately occupying habitat in the most abundant elevations. These results run contrary to a biogeographic application of the niche breadth hypothesis (Brown, 1984) which predicts species with large range to utilize a wider breadth of resources, and the resource abundance hypothesis (Venier and Fahrig, 1996). Within sg. *Batrachoseps* alone a modest and significant trend was seen, consistent with the niche breadth hypothesis (Table 3). However this modest and inconsistent result indicates that morphological adaptation to occupying wider elevation range is not likely to be driving larger biogeographic patterns. Median elevation of species occurrence is not significantly correlated with morphology in the genus as a whole, or in *sg. Batrachoseps* (Table 2, 3). Results of elevation tests indicate that the evolution of wormlike morphology in *Batrachoseps* does not allow a wider use of habitats and is not for adaptation to lowland habitat specifically. This finding is consistent with the hypothesis that *Batrachoseps* are ecologically homologous and that range boundaries are driven by vicariance due to tectonic, climatic, and hydrologic events (Jockusch and Wake, 2002; Wake, 2006).

Three species were found to have range shift buffers of 0 meters. These species are diverse in the location of their ranges, which highlights both the effectiveness and limitation of the range shift buffer metric. *Batrachoseps aridus* is a member of the phylogenetically complex *pacificus* group (Martinez-Solano et al., 2012). It is known to occur in two desert canyons with steep walls and springs that provide enough subsurface moisture, enough to support small groves of Mexican Fan Palm (*Washingtonia robusta*), Cottonwood (*Populus fremontii*), and willow (*Salix* sp.). Upland habitat above these canyons is open desert scrub. *Batrachoseps aridus* appears to be a classic case of a relict species, stranded in small, discrete patches of habitat, and surrounded by hostile conditions. In contrast *B. relictus* is a sky island species, restricted to Breckenridge Mountain in the southern Sierra Nevada. Historical records indicate that it was present at middle elevations, 600-700 meters, in the 1970s but all recent records are from elevations near 2000 meters. While *B. aridus* lacks a range shift buffer because it is surrounded by unsuitable habitat, *B. relictus* has no habitat at all to shift into. *Batrachoseps stebbinsi* has been enigmatic for researchers due to its disjunct species range, and patchy occurrence within its range. In the northern part of its range it has been found as low as 580 meters and as high as 1745 meters. That second number takes it to the top of the highest peaks within its northern range. In its southern range, the Tehachapi Mountains, *B. stebbinsi* is again found near the apex of the tallest peaks. A shortcoming of the range shift metric used here is that it does not reveal how much area is available in upland habitat, a number which could vary a great deal depending on where a species range is located. It also does not account for movement upslope and outside of a species current range, likely an important consideration for species found in habitat along foothills. Despite these drawbacks this type of metric provides relevant information on extinction probability for low vagility species like *Batrachoseps*.

Despite some confusion in species definitions among *Batrachoseps* the conclusions presented in this paper hold (Martínez-Solano et al., 2012; Jockusch et al., 2014). Nearly 80% of the area where *Batrachoseps* are found is held by wormlike forms. This morphology is present in groups which diverged from each other tens of millions of year ago (Shen et al., 2015). The mechanism for wormlike species occupying such large ranges is not adaptation to elevation range, or to habitats at the most abundant elevations. Species with small ranges, and particularly those with small range shift buffers, are at the highest risk for extinction. These species mostly have a more robust and generalized morphology. This morphology should be advantageous within their current ranges but a firmer grasp on what is driving morphological evolution is needed. Finding an answer to how selection is driving evolution of wormlike morphology in *Batrachoseps*, be it through effects on life history, locomotion, or physiology, has implications for the conservation of these species and morphological evolution in general (Gans, 1975; Maiorana, 1976; Jockusch, 1997; Cardillo et al., 2005).

Results presented in this paper also reflect the degree to which all *Batrachoseps* are microhabitat specialists. Species are found at high elevation, low elevation, and even in deserts, so long as suitable microhabitat is present. The degree to which this microhabitat specialization drives biogeographic patterns is important to understand when evaluating habitat suitability at coarse spatial scales. Current spatial modeling techniques often utilize a common set of climatic variables for a wide range of species (Wright et al., 2013). The value of this technique for low vagility microhabitat specialists is questionable. This appears to be particularly true as habitat conditions grow harsher, which is the case with current climate trends. In harsh environments local hydrology and geomorphic structure appear to be important factors in driving habitat suitability (Hansen and Wake, 2005).

Range size metrics, published range maps, latitude extent, and α-hull calculation were highly correlated in this study (Burgmann and Fox, 2003). Each appears to carry certain advantages. For data poor species latitude extent appears to be a reasonable option for testing biogeographical hypotheses. This is particularly true in a region that is dominated by north-to-south geographic features such as the mountain ranges, oceanic coastline, and inland valleys of western North America. The potential for bias and imprecision inherent in many published range polygons was noticeable when comparing them to α-hull results. Polygons created using the α-hull method were more likely to exclude arid valleys that lacked historical records for *Batrachoseps*. Published maps often include these features within the polygon. For this reason the α-hull method may be a good alternative for calculating range size for species with an intermediate to large number of data points. Selecting an appropriate α level is important, 3.5 was used in this study, and testing whether there is a level of α that works well across different spatial scales and with different amounts of data points would be useful. There were areas where the α-hull method did not have enough data points to accurately portray the species range in the opinion of experts (E. L. Jockusch, pers. comm.. S. S. Sweet, pers. comm.). If not used for explicit range size calculations the α-hull method appears to be a useful starting point for calculating a range size.

TABLES AND FIGURES ON FOLLOWING PAGES

Tables

Table 1: Morphology, range size, and elevation data for all species of *Batrachoseps*. Species are ordered from smallest to largest species range.

Species	Shape	Published range size	Alpha hull range size	Elevation range (meters)	Elevation shift buffer (meters)
B. aridus	4.50	96 km^2	0.93 km^2	687 m	0 m
B. relictus	2.93	132 km^2	67 km^2	1526 m	0 m
B. simatus	2.49	210 km^2	58 km^2	733 m	361 m
B. bramei	3.89	231 km^2	128 km^2	658 m	279 m
B. minor	2.99	238 km^2	17 km^2	135 m	232 m
B. pacificus	4.59	507 km^2	408 km^2	429 m	168 m
B. incognitus	2.98	726 km^2	217 km^2	648 m	180 m
B. campi	7.70	935 km^2	481 km^2	1998 m	504 m
B. altasierrae	2.31	1,023 km^2	641 km^2	1364 m	539 m
B. regius	3.06	1,303 km^2	86 km^2	403 m	903 m
B. stebbinsi	5.32	1,327 km^2	501 km^2	1309 m	0 m
B. kawia	2.50	1,358 km^2	811 km^2	1478 m	1158 m
B. gabrieli	5.05	1,510 km^2	318 km^2	867 m	755 m
B. luciae	2.68	1,702 km^2	1,945 km^2	1027 m	451 m
B. robustus	6.62	2,958 km^2	2,028 km^2	1250 m	336 m
B. diabolicus	2.20	8,091 km^2	2,028 km^2	475 m	723 m
B. gavilanensis	1.50	10,399 km^2	12,912 km^2	985 m	700 m
B. gregarius	0.25	12,388 km^2	9,817 km^2	1582 m	585 m
B. wrighti	4.89	12,912 km^2	13,092 km^2	1616 m	1474 m
B. major	1.88	22,594 km^2	23,988 km^2	1315 m	1852 m
B. nigriventris	1.02	34,356 km^2	32,659 km^2	2282 m	367 m
B. attenuatus	0.69	48,753 km^2	50,816 km^2	1650 m	358 m

TABLES CONTINUE ON FOLLOWING PAGE

Table 2: Correlation and regression results on for morphological, range size and elevation data. Results for uncorrected and phylogenetic independent contrasts are included.

	Shape by variable	PIC Shape by PIC variable
Lat extent (log 10)	Pearsons: -0.40 $R^2 = 0.16$ p-value = 0.07	**Pearsons: -0.72** **$R^2 = 0.52$** **p-value = 0.0004**
Pub range (log 10)	Pearsons: -0.40 $R^2 = 0.16$ p-value = 0.07	**Pearsons: -0.79** **$R^2 = 0.62$** **p-value < 0.0001**
Alpha 3.5 (log 10)	Pearsons: -0.36 $R^2 = 0.13$ p-value = 0.11	**Pearsons: -0.75** **$R^2 = 0.57$** **p-value = 0.00012**
Elevation (log 10 median)	**Pearsons: 0.497** **$R^2 = 0.2474$** **p-value = 0.02177**	Pearsons: 0.39 $R^2 = 0.15$ p-value = 0.09
Elevation range	Pearsons: -0.027 $R^2 = 0.0007$ p-value = 0.91	Pearsons: -0.33 $R^2 = 0.11$ p-value = 0.16

Table 3: Correlation results for the two subgenera in *Batrachoseps*. Both standard and Phylogenetic Independent Contrast (PIC) results are shown for sg. *Batrachoseps*. Subgenus *Plethopsis* has too few species for PIC tests.

	Shape by variable *sg. Plethopsis*	Shape by variable *sg. Batrachoseps*	PIC Shape by PIC variable *sg. Batrachoseps*
Lat extent (log 10)	**Pearsons: -0.999** **$R^2 = 0.998$** **p-value = 0.03**	**Pearsons: -0.68** **$R^2 = 0.47$** **p-value = 0.002**	**Pearsons: -0.71** **$R^2 = 0.5$** **p-value = 0.001**
Pub range (log 10)	**Pearsons: -0.998** **$R^2 = 0.996$** **p-value = 0.04**	**Pearsons: -0.64** **$R^2 = 0.41$** **p-value = 0.004**	**Pearsons: -0.75** **$R^2 = 0.57$** **p-value = 0.0005**
Alpha 3.5 (log 10)	**Pearsons: -0.998** **$R^2 = 0.997$** **p-value = 0.04**	**Pearsons: -0.64** **$R^2 = 0.41$** **p-value = 0.004**	**Pearsons: -0.71** **$R^2 = 0.51$** **p-value = 0.001**
Elevation (log 10 median)	Pearsons: 0.85 $R^2 = 0.73$ p-value = 0.35	Pearsons: 0.26 $R^2 = 0.07$ p-value = 0.31	Pearsons: 0.19 $R^2 = 0.03$ p-value = 0.47
Elevation range	Pearsons: 0.27 $R^2 = 0.07$ p-value = 0.83	**Pearsons: -0.49** **$R^2 = 0.24$** **p-value = 0.04**	**Pearsons: -0.54** **$R^2 = 0.29$** **p-value = 0.03**

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Table 4: All three techniques for quantifying species range size were highly correlated.

	Lat extent	Pub range
Published range	**Pearsons: 0.9252** **$R^2 = 0.8559$** **p-value < 0.0001**	**-**
Alpha hull 3.5	**Pearsons: 0.9609** **$R^2 = 0.9233$** **p-value < 0.0001**	**Pearsons: 0.9492** **$R^2 = 0.901$** **p-value < 0.0001**

FIGURES ON FOLLOWING PAGES

<u>Figures</u>

Figure 1: Shape variation for all 22 described species of *Batrachoseps*. Shape value shown as normal density distribution based on mean and standard deviation. Images above curves demonstrate how scores relate to morphological differences (from Stebbins, 2003). Dashed lines indicate that shape score does not include the tail, defined as posterior to the ventral opening. Asterisk under apex of curve signifies three members of sg. *Plethopsis*. Images from Stebbins (2003) appear courtesy of Houghton-Mifflin.

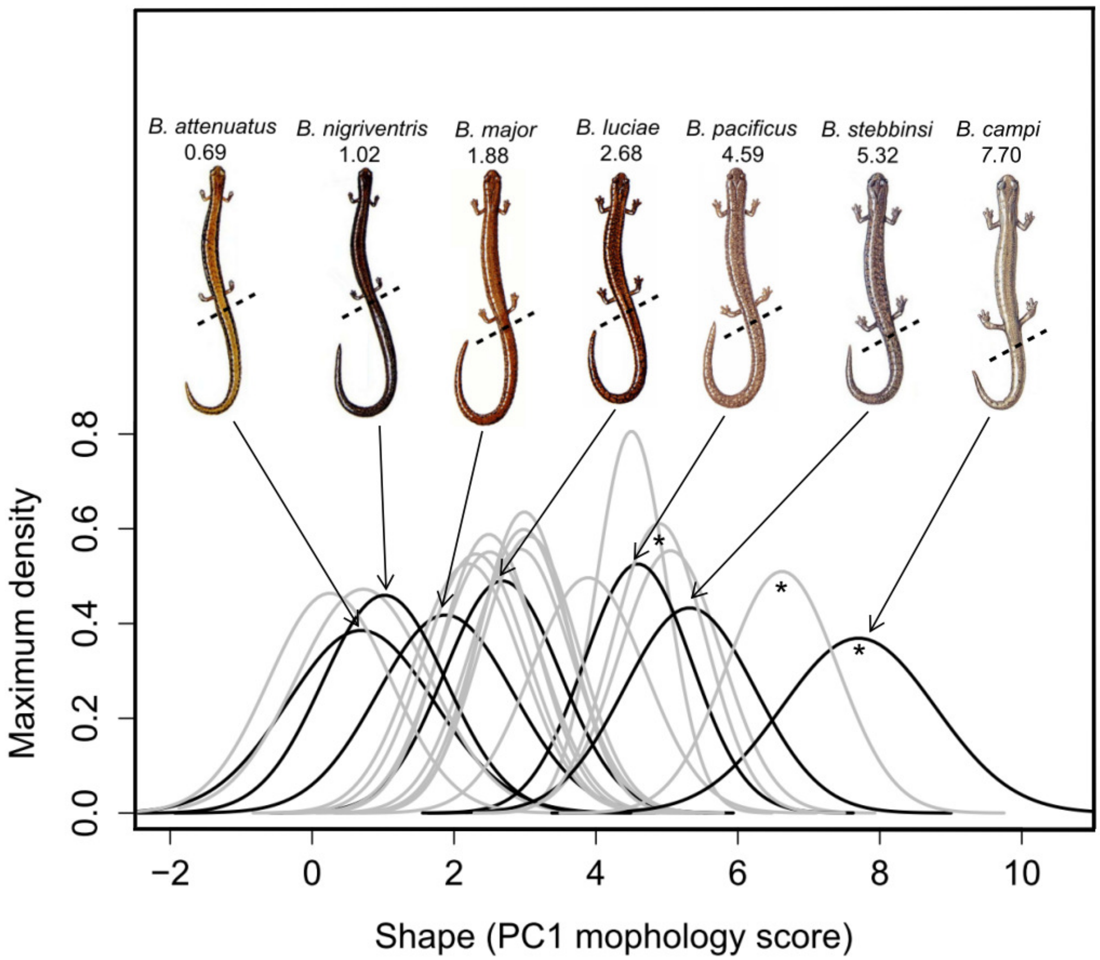

Figure 2: Most recent published phylogeny based on 5 nuclear genes (Jockusch et al., 2014). Numbers in parentheses are shape scores. High scores indicate robust species with generalized morphology. As numbers get smaller the degree of elongation increases. Species are considered wormlike if their shape score is in the 25th percentile and below (≤ 2.1) see text for details. A single species, *B. aridus*, I excluded due to lack of nuclear genetic material (Jockusch et al., 2014).

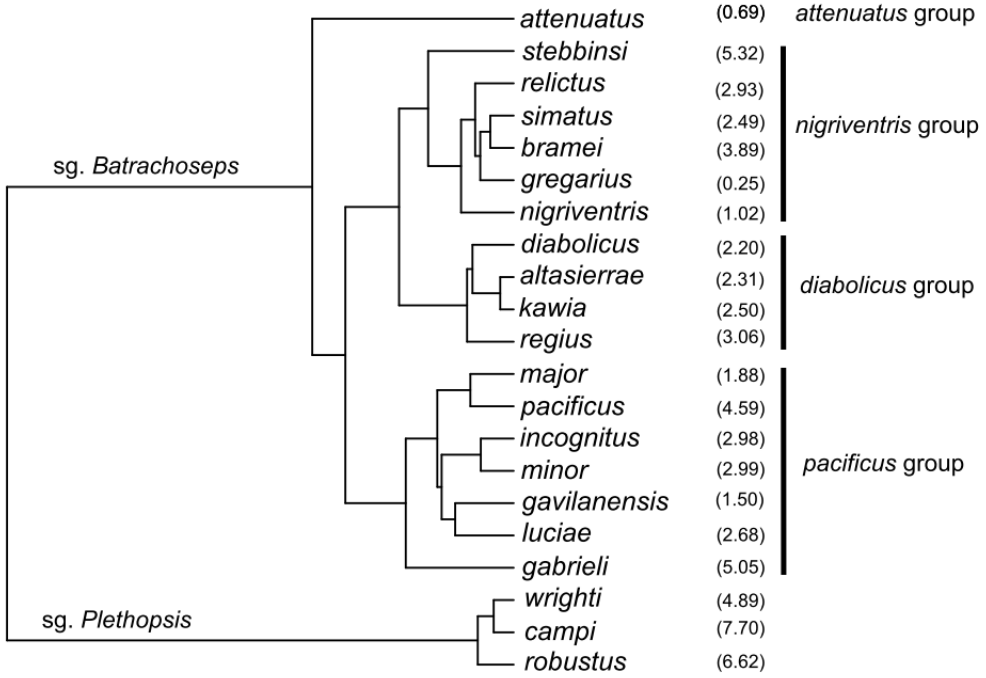

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Figure 3: Distribution of species range size in *Batrachoseps* resembles an exponential distribution where most species have small and medium-sized ranges and a few have very large ranges. When log 10 transformed this distribution is not significantly different than normal distribution. Results were similar for all three range size metrics considered. See results section for details.

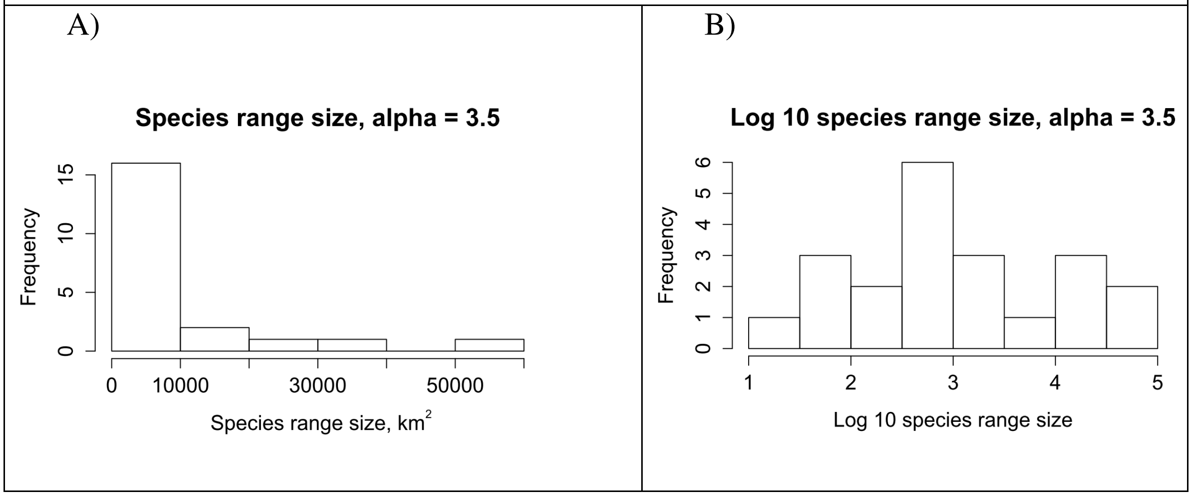

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Figure 3: Two examples of range contact between species along an elevation gradient. In both cases, A & B, species with more generalized morphology are restricted to higher elevations. Wormlike forms by contrast are in lowlands and upland habitat. Elevation range calculated using historical records. Elevation profile from USFS digital elevation model. Salamander images from Stebbins (2003) appear courtesy of Houghton-Mifflin.

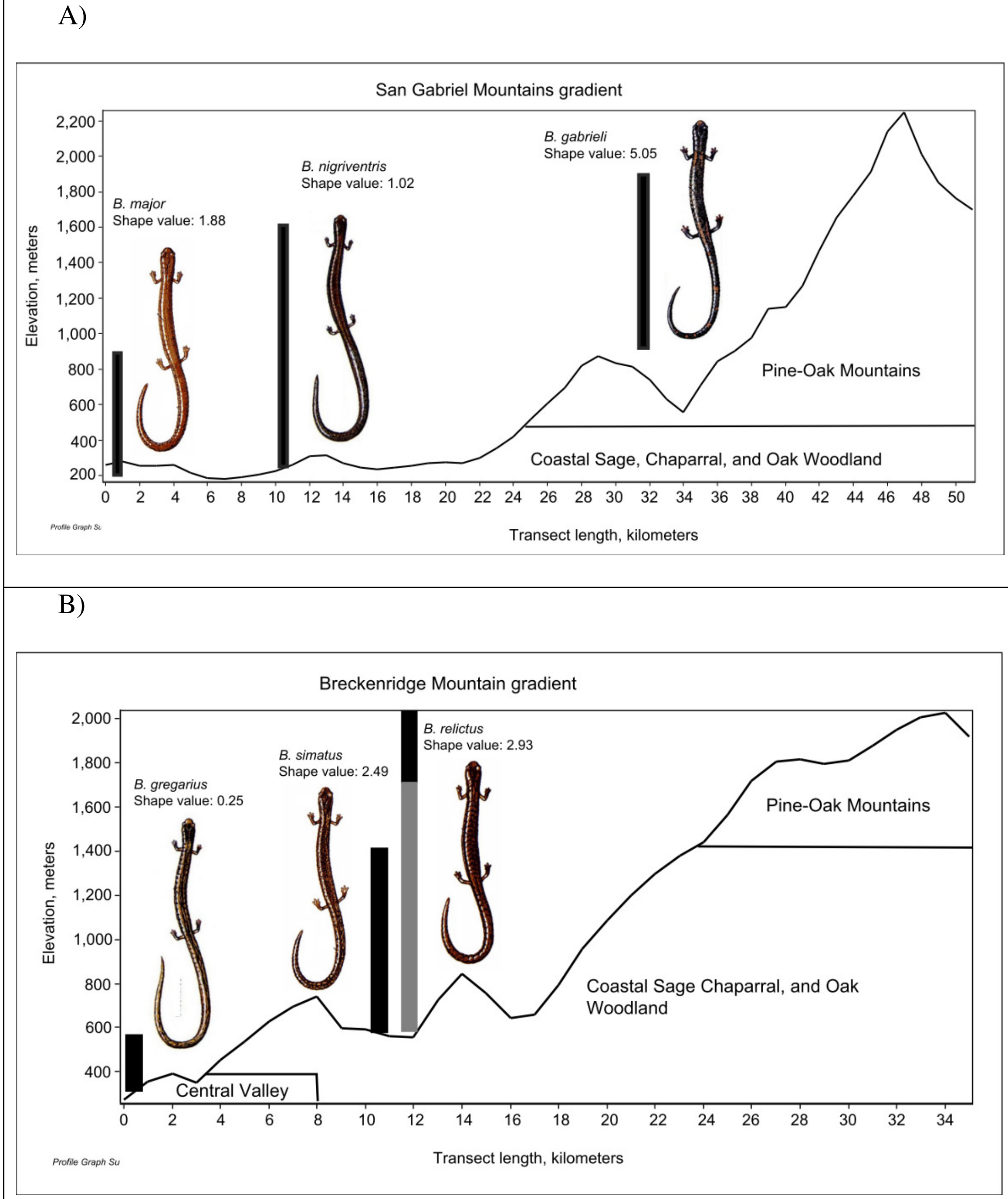


Figure 4: Plots of phylogenetic independent contrasts and their correlation results. A) A strong correlation was found between morphology (shape) and range size. In this plot alpha hull range size was used (see methods for details). Numbers to plot correspond to node labels on species tree (B) for sg. *Batrachoseps*. Species tree based on Jockusch and others (2014).

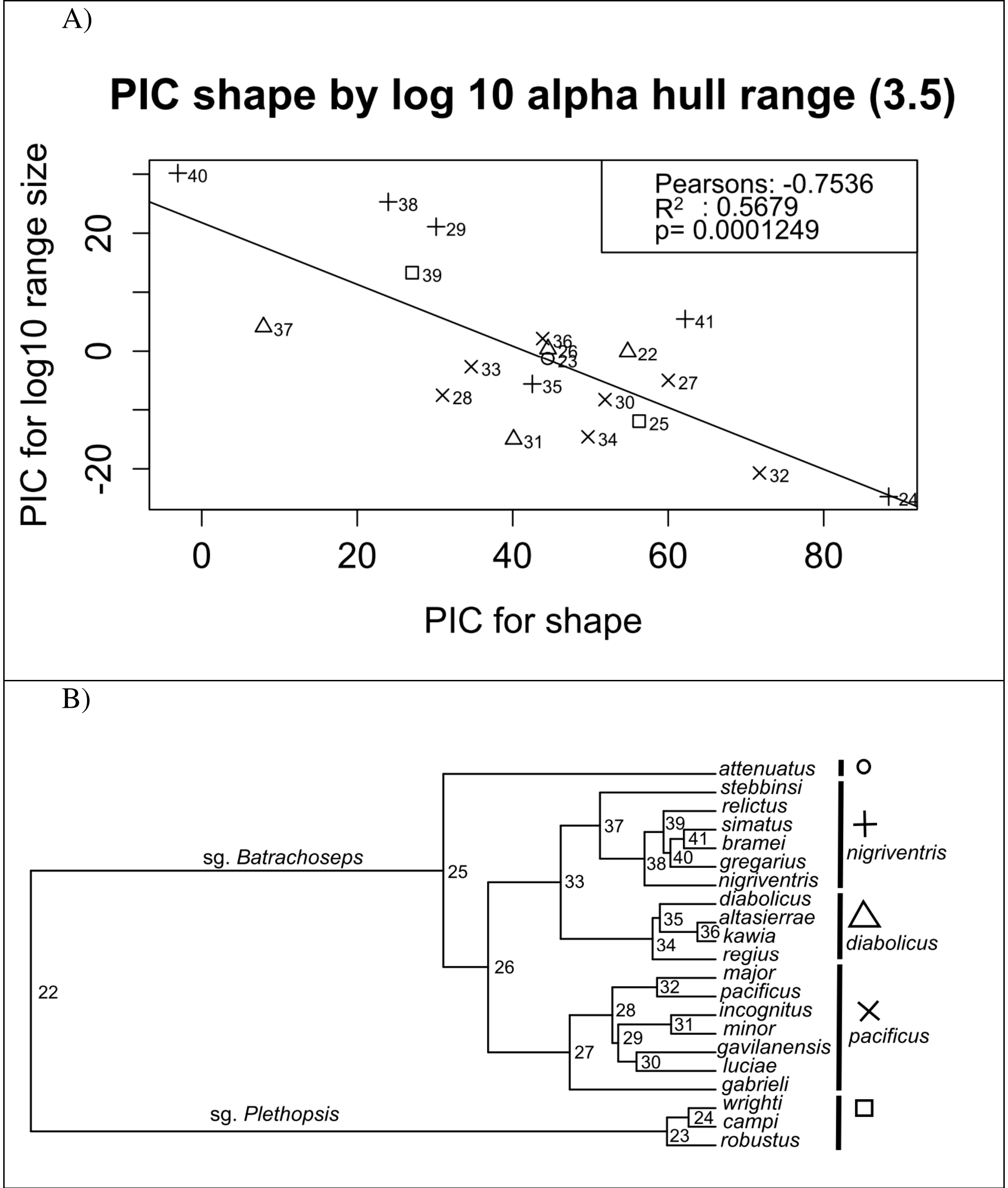
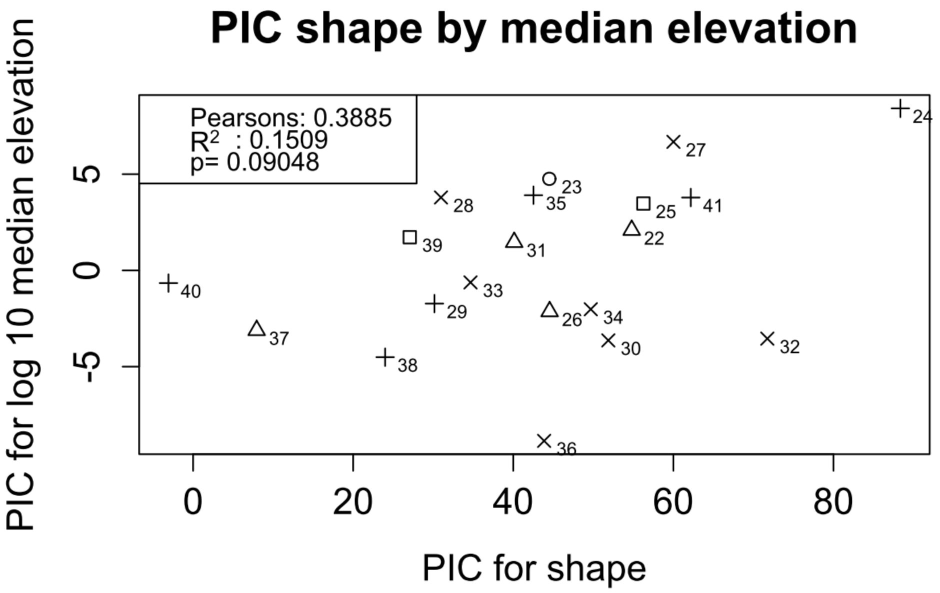
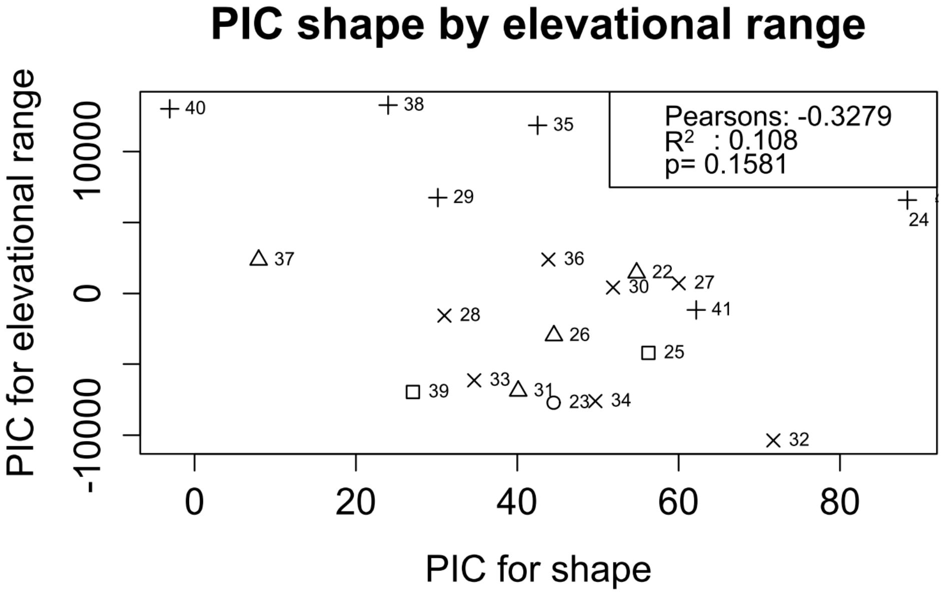


Figure 5: Median elevation also showed a weak and non-significant correlation with shape in *Batrachoseps* as a whole (A), and within sg. *Batrachoseps*. The elevational range occupied by a species has a weak and non-significant correlation with morphology (shape) in *Batrachoseps* as a whole (B). This correlation was modest and significant in sg. *Batrachoseps*, see results section for details. Symbols and labels correspond to species tree in Figure 4.

A)



B)



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APPENDIX A

```
#CODE FOR RANGE SIZE MORPHOLOGY ANALYSIS
#CHAPTER 2 IN DISSERTATION

library(caper)
library(stats)
#for each set of species
#run these tests
setwd("C:/Users/evelyn/Documents/My Box Files/2013/NICHE
PAPER/Data/Morphology/APE/Ultrametric/Su14/Trim named rooted tree
working/4Aug15")
saldata<-read.csv("Batrac_allspp_elev_noar.csv",
header=TRUE,row.names="SPECIES")
#ecoregions will be treated as discrete variables in PIC and
#non-PIC analyses. This is more biologically valid than
#the approach of Bonihng-Gaese et al., 2006.


saltree<-read.nexus("ForChrisTemp.txt")

#ELJ preferred tree that doesn't have mtDNA
saltree=saltree[[2]]


#trim off the tips I don't want: Arguello and the Plethopsis group
saltree=drop.tip(saltree, "Arguello")
#saltree=drop.tip(saltree, "pacificus")
#saltree=drop.tip(saltree, "campi")
#saltree=drop.tip(saltree, "robustus")
#saltree=drop.tip(saltree, "wrighti")


#range size metrics
lat.ext<-(saldata$LOG10_LATEXT)
pub.range<-(saldata$LOG10_CDFG)
alpha.range<-(saldata$LOG10_A35)

#-----------------------------------------------

#alpha 3.5 against published range

X<-pub.range
Y<-alpha.range

#run Pearson's correlation
pearsons<-cor.test(X, Y)
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
Figure=plot(X, Y,pch = c(16, 22)[saldata$SUBGENUS],xlab="Published Range
Size (CDFG, USFS)",ylab="Log10 Rang Size (sq. km)")
lm(Y~X)

abline(lm(Y~X))
legend("topleft",cex=0.9,legend=c(paste("Pearsons:",cor.estimate),
```

```
paste("R^2:",format(summary(linear)$r.squared, digits=4)),
                                   paste("p=",cor.pval)))
linear<-lm(Y~X)
summary(linear)
#---------------------------------------------------------

#alpha 3.5 against latitude extent
X<-lat.ext
Y<-alpha.range

#run Pearson's correlation
pearsons<-cor.test(X, Y)
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
Figure=plot(X, Y,pch=16,xlab="Latitude Extent",ylab="Log10 Rang Size (sq.
km)")
lm(Y~X)

abline(lm(Y~X))
legend("topleft",cex=0.9,legend=c(paste("Pearsons:",cor.estimate),

paste("R^2:",format(summary(linear)$r.squared, digits=4)),
                                   paste("p=",cor.pval)))
linear<-lm(Y~X)
summary(linear)
#-------------------------------------------------------

#published maps against latitude extent

X<-lat.ext
Y<-pub.range

#run Pearson's correlation
pearsons<-cor.test(X, Y)
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
Figure=plot(X, Y,pch=16,xlab="Latitude Extent",ylab="Published Range Size
(CDFG, USFS)")
lm(X~Y)

abline(lm(Y~X))
legend("topleft",cex=0.9,legend=c(paste("Pearsons:",cor.estimate),

paste("R^2:",format(summary(linear)$r.squared, digits=4)),
                                   paste("p=",cor.pval)))
linear<-lm(Y~X)
summary(linear)



#single linear regression of range size metric and
#predictor variables
#------------------------------------------------------

shape<-(saldata$SHAPE)
```

```
#Linear regression of predictor vars and range size

X<-shape
Y<-alpha.range

Figure=plot(X, Y,pch=16,main= "Shape by alpha hull range size",
xlab="Shape",ylab="Log10 Range Size (alpha=3.5)")
linear<-lm(Y~X)
summary(linear)


abline(lm(X~Y))
legend("topright",legend=c(paste("Pearsons:",cor.estimate),
                           paste("R^2:",format(summary(linear)$r.squared,
digits=4)),
                           paste("p=",cor.pval)))

#-----------------------------------------------------

#-----------------------------------------------------

#Linear regression of predictor vars and range size
#published maps

X<-shape
Y<-pub.range

Figure=plot(X, Y,pch=16,main= "Shape by published range
size",xlab="Shape",ylab="Log10 Range Size (sq. km)")
linear<-lm(Y~X)
summary(linear)


abline(lm(X~Y))
legend("topright",legend=c(paste("Pearsons:",cor.estimate),
                           paste("R^2:",format(summary(linear)$r.squared,
digits=4)),
                           paste("p=",cor.pval)))

#------------------------------------------------------------
#Linear regression of predictor vars and range size
#published maps

X<-shape
Y<-lat.ext

Figure=plot(X, Y,pch=16,main= "Shape by latitude extent of
range",xlab="Shape",ylab="Log10 decimal degrees latitude")
linear<-lm(Y~X)
summary(linear)


abline(lm(X~Y))
legend("topright",legend=c(paste("Pearsons:",cor.estimate),
                           paste("R^2:",format(summary(linear)$r.squared,
digits=4)),
                           paste("p=",cor.pval)))

#-------------------------------------------------------
#now ready to look at predictor variables and range size in a phylogentic
#test
```

```
X<-shape
Y<-alpha.range

#run Pearson's correlation
pearsons<-cor.test(X, Y)
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
Figure=plot(X, Y,pch=16,main= "Shape by log 10 alpha hull range
(3.5)",xlab="shape",ylab="Log10 Range Size (sq. km)")
linear<-lm(Y~X)
summary(linear)


abline(lm(X~Y))
legend("topright",legend=c(paste("Pearsons:",cor.estimate),
                           paste("R^2:",format(summary(linear)$r.squared,
digits=4)),
                           paste("p=",cor.pval)))


plot(alpha.range ~ shape,data=saldata,type="n")
text(alpha.range ~ shape,data=saldata,labels=rownames(saldata),cex=0.5)

#plot(saldata)

regress<-lm(shape ~ alpha.range)
abline(regress)


#calculate contrasts scaled using expected
#variances
Contrastshape <- pic(shape, saltree)
Contrastalpha.range <- pic(alpha.range, saltree)

#view contrasts
Contrastshape
Contrastalpha.range

#to extract the variances as well as the
#contrasts run
Contrastshape.var <- pic(shape, saltree, var.contrasts=TRUE)
Contrastalpha.range.var <- pic(alpha.range, saltree, var.contrasts=TRUE)

#this produces a table with both values
Contrastshape.var
Contrastalpha.range.var

#To avoid scaling the contrasts by the
#variance use
Contrastshape <- pic(shape, saltree, scaled=FALSE)
Contrastalpha.range <- pic(alpha.range, saltree, scaled=FALSE)

Contrastshape
Contrastalpha.range

#to calculate contrasts for several variables
#create a matrix with variables in columns
#then just use apply
```

```
#contrasts.saldata<-apply(saldata, MARGIN=c(3:8), FUN= "pic", saltree)

#####displaying contrasts######
Contrastshape
Contrastalpha.range

#to plot contrasts on appropriate nodes, when
#I have both expected variances and contrasts
#to the object ContrastwingL.var and
#ContrasttarsusL use nodelabels function:
plot (saltree, adj=c(0.1, 0.1), cex=0.6, show.tip.label = FALSE)
nodelabels(round(Contrastshape.var [,1], 3), adj = c(-0.2, -0.25),
cex=0.5, frame="n")
nodelabels(round(Contrastalpha.range.var [,1], 3), adj = c(-0.2, 1),
cex=0.5, frame="n")

###Correlated trait evolution THIS IS WHAT
#I AM DOING WITH predictor variable/alpha.range size
Regressshapealpha.range<-lm(Contrastalpha.range~Contrastshape)

#to see the stats
summary.lm(Regressshapealpha.range)

#run Pearson's correlation
pearsons<-cor.test(Contrastalpha.range.var[,1], Contrastshape.var[,1])
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
#Figure=plot(Contrastshape, Contrastalpha.range, pch=16,xlab="PIC for
shape (m)",ylab="PIC for log10 alpha.range size (sq km)")
#lm(Contrastalpha.range~Contrastshape)

#positivize shape PIC
shape.contrasts<-Contrastshape.var[,1]
shape.plot<-shape.contrasts+40
shape.label<-as.matrix(shape.plot)


#plot the regression
Figure=plot(shape.plot, Contrastalpha.range.var[,1], pch =
c(1,2,3,4,0)[saldata$GROUP],main= "PIC shape by log 10 alpha hull range
(3.5)",xlab="PIC for shape",ylab="PIC for log10 range size")
lm(Contrastalpha.range~Contrastshape)
text(Contrastalpha.range.var[,1]~shape.plot,data=saldata,labels=rownames(s
hape.label),cex=0.5, adj=c(-0.5, 0.7))


linear<-lm(Contrastalpha.range.var[,1]~shape.plot)
summary(linear)

abline(lm(Contrastalpha.range.var[,1]~shape.plot))
legend("topright",cex=0.75,legend=c(paste("Pearsons:",cor.estimate),

paste("R^2:",format(summary(linear)$r.squared, digits=4)),
                                     paste("p=",cor.pval)))
#phylo with node labels
plot (saltree, adj=c(0.1, 0.1), cex=0.5, show.tip.label = TRUE)
#nodelabels(rownames(shape.label[1,], adj = c(-0.2, -0.25), cex=0.5,
frame="n")
#nodelabels(rownames(shape.label), adj = c(-0.1, 0.4), cex=0.3, frame="n")
```

```
#----------------------------------------------------------------------
#----------------------------------------------------------------------




X<-shape
Y<-pub.range

#run Pearson's correlation
pearsons<-cor.test(X, Y)
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
Figure=plot(X, Y,pch=16,xlab="shape",ylab="Log10 Rang Size (sq. km)")
linear<-lm(Y~X)
summary(linear)


abline(lm(X~Y))
legend("topright",legend=c(paste("Pearsons:",cor.estimate),
                           paste("R^2:",format(summary(linear)$r.squared,
digits=4)),
                           paste("p=",cor.pval)))


plot(pub.range ~ shape,data=saldata,type="n")
text(pub.range ~ shape,data=saldata,labels=rownames(saldata),cex=0.5)

#plot(saldata)

regress<-lm(shape ~ pub.range)
abline(regress)


#calculate contrasts scaled using expected
#variances
Contrastshape <- pic(shape, saltree)
Contrastpub.range <- pic(pub.range, saltree)

#view contrasts
Contrastshape
Contrastpub.range

#to extract the variances as well as the
#contrasts run
Contrastshape.var <- pic(shape, saltree, var.contrasts=TRUE)
Contrastpub.range.var <- pic(pub.range, saltree, var.contrasts=TRUE)

#this produces a table with both values
Contrastshape.var
Contrastpub.range.var

#To avoid scaling the contrasts by the
#variance use
Contrastshape <- pic(shape, saltree, scaled=FALSE)
Contrastpub.range <- pic(pub.range, saltree, scaled=FALSE)
```

```
Contrastshape
Contrastpub.range

#to calculate contrasts for several variables
#create a matrix with variables in columns
#then just use apply
#contrasts.saldata<-apply(saldata, MARGIN=c(3:8), FUN= "pic", saltree)

#####displaying contrasts######
Contrastshape
Contrastpub.range

#to plot contrasts on appropriate nodes, when
#I have both expected variances and contrasts
#to the object ContrastwingL.var and
#ContrasttarsusL use nodelabels function:
plot (saltree, adj=c(0.1, 0.1), cex=0.6, show.tip.label = FALSE)
nodelabels(round(Contrastshape.var [,1], 3), adj = c(-0.2, -0.25),
cex=0.5, frame="n")
nodelabels(round(Contrastpub.range.var [,1], 3), adj = c(-0.2, 1),
cex=0.5, frame="n")

###Correlated trait evolution THIS IS WHAT
#I AM DOING WITH predictor variable/pub.range size
Regressshapepub.range<-lm(Contrastpub.range~Contrastshape)

#to see the stats
summary.lm(Regressshapepub.range)

#run Pearson's correlation
pearsons<-cor.test(Contrastpub.range.var[,1], Contrastshape.var[,1])
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
#Figure=plot(Contrastshape, Contrastpub.range, pch=16,xlab="PIC for shape
(m)",ylab="PIC for log10 pub.range size (sq km)")
#lm(Contrastpub.range~Contrastshape)

#positivize shape PIC
shape.contrasts<-Contrastshape.var[,1]
shape.plot<-shape.contrasts+40

#plot the regression
Figure=plot(shape.plot, Contrastpub.range.var[,1], pch = c(16,
22)[saldata$SUBGENUS],main = "PIC shape by log 10 published range size",
xlab="PIC for shape",ylab="PIC for log 10 range size")
lm(Contrastpub.range.var[,1]~shape.plot)

linear<-lm(Contrastpub.range.var[,1]~shape.plot)
summary(linear)

abline(lm(Contrastpub.range.var[,1]~shape.plot))
legend("topright",legend=c(paste("Pearsons:",cor.estimate),
                          paste("R^2:",format(summary(linear)$r.squared,
digits=4)),
                          paste("p=",cor.pval)))
#-----------------------------------------------------------------------
#-----------------------------------------------------------------------
```

```
X<-shape
Y<-lat.ext

#run Pearson's correlation
pearsons<-cor.test(X, Y)
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
Figure=plot(X, Y,pch=16,xlab="shape",ylab="Log10 Rang Size (sq. km)")
linear<-lm(Y~X)
summary(linear)


abline(lm(X~Y))
legend("topright",legend=c(paste("Pearsons:",cor.estimate),
                           paste("R^2:",format(summary(linear)$r.squared,
digits=4)),
                           paste("p=",cor.pval)))


plot(lat.ext ~ shape,data=saldata,type="n")
text(lat.ext ~ shape,data=saldata,labels=rownames(saldata),cex=0.5)

#plot(saldata)

regress<-lm(shape ~ lat.ext)
abline(regress)


#calculate contrasts scaled using expected
#variances
Contrastshape <- pic(shape, saltree)
Contrastlat.ext <- pic(lat.ext, saltree)

#view contrasts
Contrastshape
Contrastlat.ext

#to extract the variances as well as the
#contrasts run
Contrastshape.var <- pic(shape, saltree, var.contrasts=TRUE)
Contrastlat.ext.var <- pic(lat.ext, saltree, var.contrasts=TRUE)

#this produces a table with both values
Contrastshape.var
Contrastlat.ext.var

#To avoid scaling the contrasts by the
#variance use
Contrastshape <- pic(shape, saltree, scaled=FALSE)
Contrastlat.ext <- pic(lat.ext, saltree, scaled=FALSE)

Contrastshape
Contrastlat.ext

#to calculate contrasts for several variables
#create a matrix with variables in columns
#then just use apply
#contrasts.saldata<-apply(saldata, MARGIN=c(3:8), FUN= "pic", saltree)
```

```
#####displaying contrasts######
Contrastshape
Contrastlat.ext

#to plot contrasts on appropriate nodes, when
#I have both expected variances and contrasts
#to the object ContrastwingL.var and
#ContrasttarsusL use nodelabels function:
plot (saltree, adj=c(0.1, 0.1), cex=0.6, show.tip.label = FALSE)
nodelabels(round(Contrastshape.var [,1], 3), adj = c(-0.2, -0.25),
cex=0.5, frame="n")
nodelabels(round(Contrastlat.ext.var [,1], 3), adj = c(-0.2, 1), cex=0.5,
frame="n")

###Correlated trait evolution THIS IS WHAT
#I AM DOING WITH predictor variable/lat.ext size
Regressshapelat.ext<-lm(Contrastlat.ext~Contrastshape)

#to see the stats
summary.lm(Regressshapelat.ext)

#run Pearson's correlation
pearsons<-cor.test(Contrastlat.ext.var[,1], Contrastshape.var[,1])
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
#Figure=plot(Contrastshape, Contrastlat.ext, pch=16,xlab="PIC for shape
(m)",ylab="PIC for log10 lat. ext. (degrees)")
#lm(Contrastlat.ext~Contrastshape)

#positivize shape PIC
shape.contrasts<-Contrastshape.var[,1]
shape.plot<-shape.contrasts+40

#plot the regression
Figure=plot(shape.plot, Contrastlat.ext.var[,1], pch = c(16,
22)[saldata$SUBGENUS],main="PIC shape by latitude extent",xlab="PIC for
shape (m)",ylab="PIC for log10 lat. ext. (degrees)")
lm(Contrastlat.ext.var[,1]~shape.plot)

linear<-lm(Contrastlat.ext.var[,1]~shape.plot)
summary(linear)

abline(lm(Contrastlat.ext.var[,1]~shape.plot))
legend("topright",legend=c(paste("Pearsons:",cor.estimate),
                           paste("R^2:",format(summary(linear)$r.squared,
digits=4)),
                           paste("p=",cor.pval)))

#####---------------------------------
#####---------------------------------

#ELEVATION

#####---------------------------------
#####---------------------------------

#Elevation analysis

#two questions:
```

```
#1) is shape correlated with elevation? (median) PIC

#with alpha range polygons

#elev.med<-(saldata$LOG10_MED_ELEV)
elev.med<-(saldata$LOG10_MED_ELEV)


X<-shape
Y<-elev.med

#run Pearson's correlation
pearsons<-cor.test(X, Y)
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
Figure=plot(X, Y,pch=16,main = "Shape by median elevation of species
occurance", xlab="Shape",ylab="Median elevation (meters)")
linear<-lm(X~Y)
sum.1<-summary(linear)

abline(lm(X~Y))
legend("topright",legend=c(paste("Pearsons:",cor.estimate),
                           paste("R^2:",format(summary(linear)$r.squared,
digits=4)),
                           paste("p=",cor.pval)))


plot(elev.med ~ shape,data=saldata,type="n")
text(elev.med ~ shape,data=saldata,labels=rownames(saldata),cex=0.5)

#plot(saldata)

regress<-lm(shape ~ elev.med)
abline(regress)


#calculate contrasts scaled using expected
#variances
Contrastshape <- pic(shape, saltree)
Contrastelev.med <- pic(elev.med, saltree)

#view contrasts
Contrastshape
Contrastelev.med

#to extract the variances as well as the
#contrasts run
Contrastshape.var <- pic(shape, saltree, var.contrasts=TRUE)
Contrastelev.med.var <- pic(elev.med, saltree, var.contrasts=TRUE)

#this produces a table with both values
Contrastshape.var
Contrastelev.med.var

#To avoid scaling the contrasts by the
#variance use
Contrastshape <- pic(shape, saltree, scaled=FALSE)
Contrastelev.med <- pic(elev.med, saltree, scaled=FALSE)
```

```
Contrastshape
Contrastelev.med

#to calculate contrasts for several variables
#create a matrix with variables in columns
#then just use apply
#contrasts.saldata<-apply(saldata, MARGIN=c(3:8), FUN= "pic", saltree)

#####displaying contrasts######
Contrastshape
Contrastelev.med

#to plot contrasts on appropriate nodes, when
#I have both expected variances and contrasts
#to the object ContrastwingL.var and
#ContrasttarsusL use nodelabels function:
plot (saltree, adj=c(0.1, 0.1), cex=0.6, show.tip.label = FALSE)
nodelabels(round(Contrastshape.var [,1], 3), adj = c(-0.2, -0.25),
cex=0.5, frame="n")
nodelabels(round(Contrastelev.med.var [,1], 3), adj = c(-0.2, 1), cex=0.5,
frame="n")

###Correlated trait evolution THIS IS WHAT
#I AM DOING WITH predictor variable/elev.med size
Regressshapeelev.med<-lm(Contrastelev.med~Contrastshape)

#to see the stats
summary.lm(Regressshapeelev.med)

#run Pearson's correlation
pearsons<-cor.test(Contrastelev.med.var[,1], Contrastshape.var[,1])
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
#Figure=plot(Contrastshape, Contrastelev.med, pch=16,main = "PIC shape by
median elevation range size ", xlab="PIC Shape median elevation",ylab="PIC
median elevation")
#lm(Contrastelev.med~Contrastshape)
#positivize shape PIC

shape.contrasts<-Contrastshape.var[,1]
shape.plot<-shape.contrasts+40

#plot the regression
Figure=plot(shape.plot, Contrastelev.med.var[,1], pch =
c(1,2,3,4,0)[saldata$GROUP],main="PIC shape by median elevation",
xlab="PIC for shape",ylab="PIC for log 10 median elevation")
lm(Contrastelev.med.var[,1]~shape.plot)
text(Contrastelev.med.var[,1]~shape.plot,data=saldata,labels=rownames(shap
e.label),cex=0.5, adj=c(-0.6, 1.1))


linear<-lm(Contrastelev.med.var[,1]~shape.plot)
summary(linear)

#abline(lm(Contrastelev.med.var[,1]~shape.plot))
legend("topleft",cex=0.7,legend=c(paste("Pearsons:",cor.estimate),
                           paste("R^2:",format(summary(linear)$r.squared,
digits=4)),
                           paste("p=",cor.pval)))
```

```
#2) is shape correlated with elevation range? (max-min) PIC

elev.range<-(saldata$RANGE_ELEV)

X<-shape
Y<-elev.range

#run Pearson's correlation
pearsons<-cor.test(X, Y)
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
Figure=plot(X, Y,pch=16,main = "Shape by elevation range of species
occurance", xlab="Shape",ylab="Median elevation (meters)")
linear<-lm(X~Y)
sum.1<-summary(linear)

abline(lm(X~Y))
legend("topright",legend=c(paste("Pearsons:",cor.estimate),
                           paste("R^2:",format(summary(linear)$r.squared,
digits=4)),
                           paste("p=",cor.pval)))


plot(elev.range ~ shape,data=saldata,type="n")
text(elev.range ~ shape,data=saldata,labels=rownames(saldata),cex=0.5)

#plot(saldata)

regress<-lm(shape ~ elev.range)
abline(regress)


#calculate contrasts scaled using expected
#variances
Contrastshape <- pic(shape, saltree)
Contrastelev.range <- pic(elev.range, saltree)

#view contrasts
Contrastshape
Contrastelev.range

#to extract the variances as well as the
#contrasts run
Contrastshape.var <- pic(shape, saltree, var.contrasts=TRUE)
Contrastelev.range.var <- pic(elev.range, saltree, var.contrasts=TRUE)

#this produces a table with both values
Contrastshape.var
Contrastelev.range.var

#To avoid scaling the contrasts by the
#variance use
Contrastshape <- pic(shape, saltree, scaled=FALSE)
Contrastelev.range <- pic(elev.range, saltree, scaled=FALSE)

Contrastshape
Contrastelev.range

#to calculate contrasts for several variables
```

```
#create a matrix with variables in columns
#then just use apply
#contrasts.saldata<-apply(saldata, MARGIN=c(3:8), FUN= "pic", saltree)

#####displaying contrasts######
Contrastshape
Contrastelev.range

#to plot contrasts on appropriate nodes, when
#I have both expected variances and contrasts
#to the object ContrastwingL.var and
#ContrasttarsusL use nodelabels function:
plot (saltree, adj=c(0.1, 0.1), cex=0.6, show.tip.label = FALSE)
nodelabels(round(Contrastshape.var [,1], 3), adj = c(-0.2, -0.25),
cex=0.5, frame="n")
nodelabels(round(Contrastelev.range.var [,1], 3), adj = c(-0.2, 1),
cex=0.5, frame="n")

###Correlated trait evolution THIS IS WHAT
#I AM DOING WITH predictor variable/elev.range size
Regressshapeelev.range<-lm(Contrastelev.range~Contrastshape)

#to see the stats
summary.lm(Regressshapeelev.range)

#run Pearson's correlation
pearsons<-cor.test(Contrastelev.range.var[,1], Contrastshape.var[,1])
cor.estimate<-signif(pearsons$estimate,4)
cor.pval<-signif(pearsons$p.value,4)

#plot the regression
#Figure=plot(Contrastshape, Contrastelev.range, pch=16,main = "PIC shape
by elevation range of species occurance ", xlab="PIC Shape",ylab="PIC
elevation range")
#lm(Contrastelev.range~Contrastshape)

#positivize shape PIC
shape.contrasts<-Contrastshape.var[,1]
shape.plot<-shape.contrasts+40

#plot the regression
Figure=plot(shape.plot, Contrastelev.range.var[,1], pch =
c(1,2,3,4,0)[saldata$GROUP],main= "PIC shape by elevational
range",xlab="PIC for shape",ylab="PIC for elevational range")
lm(Contrastelev.range.var[,1]~shape.plot)
text(Contrastelev.range.var[,1]~shape.plot,data=saldata,labels=rownames(sh
ape.label),cex=0.5, adj=c(-0.75, 0.4))


linear<-lm(Contrastelev.range.var[,1]~shape.plot)
summary(linear)

#abline(lm(Contrastelev.range~Contrastshape))
legend("topright",cex=0.75,legend=c(paste("Pearsons:",cor.estimate),
                           paste("R^2:",format(summary(linear)$r.squared,
digits=3)),
                           paste("p=",cor.pval)))


######----------------------------------
######---------------------------------
```

```
#BUFFER SEE TABLE IN PAPER

######----------------------------------
######----------------------------------

#----------------------------------------------------------------------
#----------------------------------------------------------------------
#Histograms testing for normality
no.log.alpha<-10^alpha.range


hist(shape, main= "Species morphology value, aka Shape (S)");
hist(alpha.range, main= "Log 10 species range size, alpha = 3.5",
xlab="Log 10 species range size");
hist(no.log.alpha, main= "Species range size, alpha = 3.5", xlab="Species
range size, km");
hist(lat.ext, main= "Latitudinal extent of species range");
hist(pub.range, main= "Published range map (CDFG, USFS)");
hist(elev.range, main= "Elevation range from localities");
hist(elev.med, main= "Median elevation from localities");



plot(density(shape));
plot(density(alpha.range));
plot(density(no.log.alpha));
plot(density(lat.ext));
plot(density(pub.range));
plot(density(elev.range));
plot(density(elev.med));

no.log.alpha
## Perform the test
shapiro.test(Shape);
shapiro.test(alpha.range)
shapiro.test(no.log.alpha)
shapiro.test(lat.ext);
shapiro.test(pub.range)
shapiro.test(elev.range)
shapiro.test(elev.med)


## Plot using a qqplot
qqnorm(shape);qqline(shape, col = 2);
qqnorm(alpha.range);qqline(alpha.range, col = 2)
qqnorm(no.log.alpha);qqline(no.log.alpha, col = 2)
qqnorm(lat.ext);qqline(lat.ext, col = 2);
qqnorm(pub.range);qqline(pub.range, col = 2)
qqnorm(elev.range);qqline(elev.range, col = 2)
qqnorm(elev.med);qqline(elev.med, col = 2)
```

CHAPTER 3

Evolution of elongate morphology associated with seasonal habitats in a genus of fully terrestrial salamanders (Plethodontidae: *Batrachoseps*)

Abstract

In the salamander genus *Batrachoseps* two subgenera are deeply divergent genetically and distinguished morphologically by the greater degree of paedomorphosis in sg. *Batrachoseps* compared to sg. *Plethopsis*. Gross morphology in subgenus *Plethopsis* is similar to other small members of Plethodontidae while sg. *Batrachoseps* contains both generalized and wormlike forms. The novel wormlike morphology in sg. *Batrachoseps* has been proposed as the main factor facilitating an ecological shift which allows sg. *Batrachoseps* to persist where sg. *Plethopsis* has not. As with other fully terrestrial salamanders feeding and breeding activity is dependent on moist surface conditions. I used verified locality records and spatial precipitation data to test whether wormlike morphology is associated with environments with periodic food availability; xeric conditions; and unpredictable fluctuations in precipitation within a phylogenetic context. Results indicate that the greater degree of paedomorphosis in sg. *Batrachoseps* is associated with more seasonal (less stable) habitats. Analysis of variance tests between sg. *Batrachoseps* and sg. Plethopsis are strongest for mean values of precipitation seasonality and significant at all annual precipitation quantiles considered, 75%, 90%, 95%, 99%. Within sg. *Batrachoseps* wormlike morphology is significantly correlated with degree of precipitation seasonality and this relationship increases in strength at more extreme quantiles. Pearson correlations increase from -0.55 (p-value = 0.02) at the 75% quantile to -0.8 (p-value = 0.0001) at the

99% quantile. These results indicate that the more paedomorphic sg. *Batrachoseps* in general and wormlike species in particular can persist in areas where surface activity is more seasonal than the habitat of sg. *Plethopsis*.

Introduction

Evolution of snakelike or wormlike body form has occurred repeatedly in vertebrates (Gans, 1975; Carroll, 1997; Martil et al., 2015). Within extant tetrapods the Squamata (lizards and snakes) show the greatest amount of variation in this pattern although the tailed amphibians (salamanders) also exhibit variations on this theme. A suite of traits are generally associated with the evolution of elongation. These include an increase in the number (or less frequently, the length) of trunk vertebrae, a lengthening of the body relative to the width or a narrowing of the body relative to the length, a reduction in the size of the limbs, and a reduction in the number of distal limb elements (Carroll, 1996; Parra-Olea and Wake, 2001). In this paper I will test whether the evolution of wormlike morphology in a salamander genus *Batrachoseps* (Plethodontidae) is correlated with the use of more seasonal, xeric, and less predictable habitat than other *Batrachoseps*. This relationship was first hypothesized by Maiorana (1976) as adaptive in the seasonally arid habitat occupied by *Batrachoseps* in western North America. Maiorana (1976) expected fossoriality to be advantageous to escape the adverse surface conditions of seasonally dry weather.

No extant terrestrial salamanders have lost their limb function but several species have highly reduced limbs and wormlike morphology. These species are contained in two sister tribes of lungless salamanders (Plethodontidae) the neotropical Bolitoglossini and the nearctic Batrachosepini. Among the twelve genera in Bolitoglossini wormlike morphology has evolved at least three times (Parra-Olea and Wake, 2001). Batrachosepini contains a single genus, *Batrachoseps*, in which species show variation in their degree of elongation and associated traits. Due to the interest in the origin of snakes many authors have investigated potential selective drivers of snakelike morphology in squamates (Evans, 2015). Much less attention as been given to what drives evolution of snakelike body forms in terrestrial salamanders (Parra-Olea and Wake, 2001).

The salamander genus *Batrachoseps* contains 22 described species that show variation in traits associated with the theme of snakelike or wormlike body evolution. Wormlike and more generalized forms do not differ significantly in overall length but wormlike forms are significantly narrower (Brame and Murray, 1968). Until relatively recently most wormlike forms were thought to represent a monophyletic group and the derived condition in *Batrachoseps* (Hendrickson, 1954; Brame and Murray, 1968; Maiorana, 1976). Until the late 1970s morphological differences in *Batrachoseps* were used by researchers to create phylogenetic hypotheses (Hendrickson, 1954, Yanev, 1978). When phylogenetic hypotheses for *Batrachoseps* based on DNA were studied they revealed that wormlike species are deeply divergent genetically and are mostly members of separate well-supported monophyletic groups within the genus (Figure 1, see also Yanev, 1978; Jockusch et al., 1998; Jockusch et al., 2001; Jockusch et al., 2014). Homoplasy is widespread in salamanders and description of cryptic species in *Batrachoseps* drew comparison with another North American plethodontid, *Plethodon glutinosus* which also displays morphological similarity among distinct lineages (Highton et al., 1989; Wake, 2009). Somewhat minimized amid these discoveries is the opportunity to understand the role of selection in driving convergent morphological evolution in *Batrachoseps*. Using the current phylogeny it is possible to conduct robust tests to assess whether environmental factors may drive convergence for wormlike morphology in *Batrachoseps*.

Both the sources of selection and developmental pathways that result in snakelike or wormlike morphology have received attention from researchers (Abdala et al., 2015; Martil et al., 2015). Wormlike morphology is achieved due to a lengthening of the body while maintaining similar body width or by narrowing the body while maintaining similar length (Brandley et al., 2008). This can be achieved by a lengthening of individual vertebra or an increase in the number of vertebra (Parra-Olea and Wake, 2001). In *Batrachoseps* the latter pattern is observed (Brame and Murray, 1968). While Jockusch (1997) provided evidence that fecundity selection drives an increase in number of trunk vertebrae, selection on the decrease in head and body diameter was not explained. Gans (1975) considered fossorial movement through crevices as, "The only mechanism that may plausibly have required a limit on the relative diameter of an animal…" Despite the strong language of Gans (1975) fossorial movement may not be the only factor that can result in evolution of a reduced diameter. Miniaturization may arise as a result of life history evolution due to progenic paedomorphosis from selection in unstable environments (Gould, 1977; Hanken and Wake, 1993). Unstable and unpredictable environments, where opportunities to increase fitness are uncertain due to high levels of density independent selection on juveniles, are expected to produce species which reach sexual maturity earlier in the developmental process (Gould, 1977; Hanken and Wake, 1993). This type of morphological evolution is a type of paedomorphic heterochrony which is considered important for the evolution of morphological novelty (Gould, 1977; Gould, 1988).

Wake (1966) identified paedomorphosis in several *Batrachoseps* based skeletal features, in particular fusion of the premaxilla. These paedomorphic species were all later assigned to subgenus *Batrachoseps* (Jockusch and Wake, 2002). Wake (1966) concluded that these species (sg. *Batrachoseps*) display a greater degree of paedomorphosis than sg. *Plethopsis*, which have paired premaxilla as adults. Based on this pattern the current working hypothesis is that the wormlike *Batrachoseps* are miniaturized via paedomorphosis and evolved elongated trunks secondarily as a result of fecundity selection (Wake, 1966; Roth et al., 1980; Jockusch, 1997). If a decrease in body diameter (miniaturization) is consequence of paedomorphosis due to selection on life history in unstable or unpredictable environments then selection on life history alone could result in wormlike morphology in *Batrachoseps*. This line of reasoning is consistent with the expectation of Maiorana (1976) that wormlike forms represent morphological adaptation to xeric, seasonal, and unpredictable environments than those utilized by ancestral forms with generalized morphology. Several authors have tied key life history activities in *Batrachoseps*, namely feeding and reproduction, to the precipitation events (Hendrickson, 1954; Cunningham, 1960; Maiorana, 1976, 1978). Paedomorphosis in *Batrachoseps* has thus far not been labeled as either neoteny or progenesis. Both neoteny and progenesis result in reproductively viable adults that retain some larval characters, but they achieve this end through different developmental pathways. Neoteny is the slowing of somatic development while the development of reproductive organs proceeds. Progenesis results in paedomorphosis by precocious development of the reproductive system in an individual with juvenile traits (Gould, 1977). Neoteny has been found in salamanders who occupy stable environments with low juvenile mortality (Sprules, 1974; 1974a).

Morphological diversity in *Batrachoseps* has been acknowledged by researchers since their earliest discovery (Cope, 1865). Descriptions of new species and the discovery of new populations over the past 40 years continues to deepen our knowledge of diversity in *Batrachoseps* (Brame and Murray, 1968; Wake, 1996; Wake et al., 2002). The basal split in *Batrachoseps* is between three relict species (see Figure 1 and Figure 2) in sg. *Plethopsis* and 19 species in sg. *Batrachoseps* (Jockusch et al., 2014). All wormlike species are contained in sg. *Batrachoseps*. Osteological and morphological differences between the two subgenera were first described by Stebbins and Lowe (1949), expanded upon by Wake (1966), and later discussed with more recent species descriptions (Marlow et al., 1979; Wake et al., 2002). Subgenus *Batrachoseps* appears to represent the derived condition based on several skeletal features most notably the premaxilla which remains fused throughout the life cycle while the premaxilla is paired in adult *Plethopsis* (Wake, 1966; Marlow et al., 1979; Wake et al., 2002). This feature indicates that sg. *Batrachoseps* is paedomorphic to a greater degree than sg. *Plethopsis* (Wake, 1966). This developmental shift appears to have facilitated evolution of the variable gross morphology observed in sg. *Batrachoseps*, where both wormlike and generalized forms are present (Figure 1 and Figure 3). Maiorana (1976) proposes wormlike morphology is an adaptation to environments that are both seasonally and unpredictably inhospitable for salamanders. Her comprehensive conceptual model considered both life history and physical demands on *Batrachoseps* over their lifetimes. A life history model that allowed for optimization by *Batrachoseps*, between the traditional r- and K- selection paradigms, via resource allocation to reproduction and storage was particularly timely (Maiorana, 1976; Gould, 1977; Stearns, 1980). The wormlike morphology in *Batrachoseps* studied by Maiorana (1976) was beneficial for effective storage of lipids in the large tail and for accessing sub-surface refugia during seasonal dry periods. Wormlike gross morphology facilitates escape from adverse surface conditions via cracks and burrows in the substrate (Maiorana, 1976; Maiorana, 1978). Selection for the ability to escape into sub-surface refugia should be stronger in dry, unpredictable, and highly seasonal environments (Maiorana, 1976). To illustrate her point she used data on the consistency, seasonality, and predictability of rainfall across most of the range of the wormlike *B. attenuatus*[1] and *B. major*. Her deduction regarding adaptation to a fossorial lifestyle is consistent with proposals for the evolution of elongation in lizards (Gans, 1975) and the evolution of miniaturization in interstitial invertebrates (Hanken and Wake, 1993; Rundell and Leander, 2010; Struck et al., 2015). Selection that favors a small diameter carries obvious advantages in moving through intersticies as it allows access to a wider range of crevices (Gans, 1975; Maiorana, 1978).

Based on her own extensive field work (Maiorana, 1974) and work by previous authors (Hendrickson, 1954; Cunningham, 1960) Maiorana (1976) identified rainfall amount and seasonal pattern as the most important environmental factors likely to influence life history and morphological evolution in *Batrachoseps*. The strong link between precipitation, feeding, and reproduction is well supported by observations of *Batrachoseps* specifically and amphibians in general (Burke, 1911; Maslin, 1939; Stebbins, 1949; Davis, 1952, Hendrickson, 1954; Cunningham, 1960). Central to Maiorana's (1976) hypothesis are three predictions about where wormlike morphology would evolve: 1) in environments with periodic food availability 2) in environments relatively xeric compared to generalized forms 3) in environments with unpredictable fluctuations in climate. In combination with the

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[1] *B. attenuatus* in Maiorana (1976) was actually an amalgam of *B. attenuatus*, *B. gavilanensis*, and *B. nigriventris* see Table 1

previous studies Maiorana (1976) gathered extensive field observations which informed her predictions (Maiorana, 1974). Unfortunately her study was constrained by the lack of an accurate phylogeny and limited data and analytical tools for assessing environmental variability across the range of *Batrachoseps*. A more recent effort to understand morphological evolution in *Batrachoseps* was conducted by Jockusch (1997), who was able to utilize an accurate genetic phylogeny while investigating intra- and interspecific variation in number of trunk vertebrae along a gradient of increasingly xeric conditions from coastal California to inland habitats (Jockusch, 1997). The results from Jockusch (1997) implicate fecundity selection as the driver of axial elongation due to increase in number of trunk vertebrae.

Analyses by Maiorana (1976) and Jockusch (1997) were limited in their use of environmental data. Both authors highlighted the importance of rainfall to the life history of amphibians. This is true for ephemeral pond breeding species as well as fully terrestrial direct-developing taxa such as *Batrachoseps*. Field data for two species of wormlike *Batrachoseps* each show that seasonal precipitation facilitates feeding, reproduction, and hydration (Hendrickson, 1954; Cunningham, 1960; Maiorana, 1974). In order to quantify the seasonality and predictability of rainfall across the range of *Batrachoseps* Maiorana (1976) used Colwell's indices (Colwell, 1974) to quantify the predictability and seasonality of precipitation in coastal habitats of California. Unfortunately her use of these data to conduct robust tests in an evolutionary context was limited by an erroneous phylogeny, lack of spatially discrete climate data, and lack of analytical methods for handling these data. In 1976 the complex phylogeny of *Batrachoseps* was only beginning to take shape and only eight species (seven described) were recognized (Table 1), see also Brame and Murray, 1968; Marlow et al., 1979). Intervening studies have uncovered more diversity in three ways: species which are deeply divergent genetically but similar morphologically; morphologically distinct species that were thought to represent intraspecific variation; and the discovery of new species. Maiorana's (1976) target species, *B. attenuatus*, is now understood to be four separate species from deeply divergent lineages (Table 1 and Figure 1). In addition to an improved understanding of the species tree for *Batrachoseps* there is now greater access to environmental variables through Geographic Information System (GIS) data across the range of *Batrachoseps*. These environmental variables are available as temporally and spatially explicit GIS datasets which allows for interspecific comparison of habitat based on species occurrence records (Vertnet, 2010). Precipitation data through time are available for nearly the entire range of *Batrachoseps* at a scale of 4 km^2 (Daly et al., 2008; PRISM, 2013). With the improved understanding of interspecific relationships and the availability of spatially explicit environmental variables it is now possible to put the predictions of Maiorana (1976) to a robust test in a phylogenetic context.

In this paper I will revisit the hypotheses of Maiorana (1976) and explicitly test whether wormlike species occupy habitat that is more seasonal, more xeric, and less predictable than other taxa in *Batrachoseps*. To facilitate comparison, each of the three central predictions by Maiorana (1976) will be tested using methodologies consistent with those used in her original paper. Like many plethodontid salamanders *Batrachoseps* are dormant when surface conditions, namely temperature and moisture, are not suitable for small amphibians with high surface-to-volume ratios (Hendrickson, 1954). However the most important life history activities, feeding and reproduction occur at the surface during and after precipitation events when surface conditions are suitable. In a way this seasonal activity pattern in *Batrachoseps* is comparable to that of amphibians that breed and feed in ephemeral ponds. Predictability and seasonality of precipitation events influence how consistently feeding and reproduction take place from year to year. The length of wet and dry seasons account for the length of time species have for feeding and reproduction, which impacts an individual's ability to acquire fat stores and find mates. In the highly seasonal climate of California the least hospitable environments for amphibians are those with relatively short and unpredictable wet seasons and long and predictable dry seasons (Maiorana, 1976). This combination results in selection due to short and unpredictable opportunities for surface activity which are crucial for feeding, reproduction, and hydration in *Batrachoseps* (Hendrickson, 1954; Cunningham, 1960; Maiorana, 1974).

Materials and methods

The goal of these analyses is to test whether the evolution of wormlike morphology in *Batrachoseps* is associated with environments that are more seasonal, more xeric, and less predictable. I focused my analysis on three hypothesized correlations from Maiorana (1976) between environment and wormlike morphology: a) periodic food availability b) a relatively xeric environment and c) unpredictable fluctuations in climate. One to three metrics were used to test each of these hypotheses. Colwell's indices (Colwell, 1974) are one way to measure predictability and periodicity of data. These metrics quantify the ability of time (seasonality = M) or state (constancy = C) to predict the probability of a given event (Colwell, 1974). When applied to precipitation data the events are rainfall events of a chosen magnitude (Maiorana, 1976). Time bins are delineated, month in this case, and a tally of rainfall events across the study period is compiled. Predictability (P) is the sum of the ability of time (M) and state (C) to predict the probability of an event. Colwell's M for monthly precipitation was used as a proxy for periodic food availability. How xeric an environment is was quantified using dry season length (the number of months with precipitation <6mm); dry season precipitation (mean precipitation in those months); and total annual precipitation. The predictability of climate fluctuations was quantified using Colwell's P (Colwell, 1974). These metrics were used to test for differences in the locations where the two subgenera sg. *Plethopsis* and sg. *Batrachoseps* have been found. Subgenus *Batrachoseps* has 19 species and contains both wormlike and non-wormlike taxa while sg. *Plethopsis* has 3 species which are all among the most morphologically robust forms (Figure 3). Analysis of variance (ANOVA) was used to compare subgenera. Secondly I tested whether wormlike morphology is correlated with the chosen climate variables across species within sg. *Batrachoseps*. This analysis is at the root of what Maiorana (1976) proposed in her original paper. Morphology was quantified as a single multivariate score (Jockusch et al., 2012). Phylogenetic independent contrasts (PIC) were used to account for the influence of heredity for both morphology and environment (Felsenstein, 1985).

Materials

Morphological species data for sg. *Batrachoseps* is from measurements by Martinez-Solano (Jockusch et al., 2012). I made morphological measurements for sg. *Plethopsis* (Figure 3). A single continuous variable, *S*, was created to quantify morphological shape independent of absolute size. *S* was created using 9 linear morphological measures from each of the 22 described species in *Batrachoseps* (Figure 1). To compare variation in shape, not just differences in size, residuals of the regression of each variable against standard length (SL) were analyzed. In salamanders SL is measured from the tip of the snout to the caudal end of the vent. Correlations between residuals were calculated, and the forelimb-length residual was excluded because of its high correlation ($R^2 = 0.90$) with the hind limb-length residual. Principal Component Analysis (PCA) of the covariance matrix of the remaining seven residuals, as implemented by the prcomp command in R (R, 2014), was used to capture the main axes of morphological variation. Linear measurements used were standard length, SL, which is the length from the tip of the snout to the posterior angle of the vent, head width, snout–gular fold length, axilla–groin length, chest width behind the forelimbs, forelimb length, hind limb length, maximum foot width and head depth behind the eyes (Jockusch et al., 2012).

Environmental data used in this analysis are monthly precipitation data from the conterminous United States. Spatial resolution for the data are from cells 4 km^2 in size. Data are from the PRISM dataset of Recent Years precipitation (Daly et al., 2008; PRISM, 2013). Temporal resolution for this dataset is monthly averages. The period of time used in the Recent Years dataset is from January 1981 to November 2013. This PRISM dataset is biologically appropriate since precipitation values represent rainfall or snowmelt, as opposed to snowfall, so precipitation values indicate the timing and duration of surface moisture from precipitation (Daly et al., 2008; PRISM, 2013). While this does not necessarily represent the conditions under which species of *Batrachoseps* evolved in the distant past it is a proxy for environmental conditions during the most recent interglacial cycle. It also has the benefit in allowing for the comparison of intra-annual variability. Nearly the entire distribution of *Batrachoseps* is contained within this dataset with the exception of a handful of *B. major* populations that occur in Baja California (Martinez-Solano, 2012).

Species occurrence records were taken from museum specimen records and uploaded from Herpnet.org (now Vertnet.org). Additional records were downloaded from the University of California Santa Barbara vertebrate collection. All records were reviewed for taxonomic mistakes. These were mostly the result of museum records not reflecting taxonomic changes due to recent genetic studies (Yanev, 1978; Jockusch et al., 1998; Jockusch et al., 2000; Jockusch and Wake, 2002; Jockusch et al., 2014). Records were also checked for obvious outliers and those records removed. This included an introduced population of *B. major* in the California Central Valley (Martínez-Solano et al., 2012). To minimize the influence of multiple records for a single locality the original data were collapsed by the locality description as it appeared in the record and then by decimal coordinates latitude and longitude as they appeared in the record. This was accomplished in ArcMap 10.2 using the Dissolve tool. This resulted in 3711 valid records with monthly precipitation data across 396 months from 1981-2013.

From these 3711 records extreme outliers were identified using the Tukey method (Thompson, 2008) for the following climate variables: annual precipitation, precipitation during the dry season (< 6 mm/ month), length of the dry season, Colwell's predictability index (P), Colwell's constancy index (C), and Colwell's seasonality index (M) (Colwell, 1974). Records which qualified as extreme outliers, > 3 interquartile ranges from the upper or lower quartiles in each species, were removed. After outliers were removed 3519 records remained. In order for the seasonality and predictability records to better reflect actual impact on surface activity only cells where annual rainfall was in 25th percentile and below for each species were chosen for further analysis. This reflects the expectation that climate is less of a selection pressure on life history in these wetter environments. The result of this was most clearly seen in the wide-ranging *B. attenuatus* as most occurrences from the relatively mesic coastal forests of northern California were removed. Statistical analysis was performed on these 25% most xeric environments occupied by each species as this is most likely to represent the environments were selection due to precipitation patterns is the strongest. In total 932 records were used in morphology-precipitation correlation analyses.

Methods

Monthly rainfall values from January 1981 to November 2013 were sampled from the PRISM Recent Years dataset in ArcMap 10.1 using specimen records (Herpnet.org, 2012) and the Extract Multi Values to Points tool in the Spatial Analyst extension (ESRI, 2012). These data were then used to create a data table used to quantify how xeric, periodic, and predictable precipitation was for each locality. Precipitation data with a cell size of 4 km^2 were used (Daly et al., 2008; PRISM, 2013). In order to quantify the periodic nature of precipitation regimes across the range of *Batrachoseps* I followed the example of Maiorana (1976) and used Colwell's indices (Colwell, 1974). These indices use three metrics to describe environmental predictability: Contingency (M), Constancy (C), and Predictability (P). Contingency (M) describes the seasonality or periodic nature of the environmental variable of interest.

Correlations between wormlike morphology and the three core predictions from Maiorana (1976) were tested using the following metrics. Colwell's M was used as a proxy for periodic food availability. Based on past observation *Batrachoseps* feed at the surface and precipitation is heavily relied upon for surface activity (Hendrickson, 1954; Cunningham, 1960; Maiorana, 1974; 1976). Since only the most xeric habitats were used for each species, annual precipitation from the 25% quantile and below, the seasonality metric M should represent movement in and out of times where the habitat is suitable for surface activity. Three metrics were used to measure how xeric habitat is, annual precipitation, length of dry season, and average monthly precipitation in the dry season. These three metrics are informative of the ways a habitat can be harsh due to lack of precipitation. Annual precipitation is often used in ecological studies as a general measure of the mesic nature of a habitat (Soberon and Nakamura, 2009). Maiorana (1976) suggested that the length of the dry season as well as how consistently dry it is represent important aspects of selection in highly seasonal environments. Unlike Maiorana (1976) who presupposed a winter wet season of December – February, I used data from each cell to calculate dry season values. In some cases the wet and dry seasons are not aligned with the wet winter/dry summer paradigm (Table 2). Using individual cells allows for a more accurate account of the timing of surface activity for each species. Colwell's measure of predictability, P, is an obvious choice for quantifying unpredictable fluctuations in climate. To analyze the data from *Batrachoseps* the hydrostats package (Bond, 2015) was used in the statistics program r (R, 2014). Monthly rainfall was broken into 10 logarithmically increasing classes following the method of Maiorana (1976): 0.0 cm, 0.25 cm, 0.50 cm, 1.0 cm, 2.0 cm, 4.0 cm, 8.0 cm, 16.0 cm, 32.0 cm, >32.0 cm. Length and precipitation of the dry season was quantified using an average monthly threshold of < 0.6 cm (Maiorana, 1976). Dry season was the maximum consecutive months when mean rainfall in each month is <0.6 cm. This was measured on a cell-by-cell basis. Analyses focused on the indices of annual precipitation, the length of the dry season, and the amount of precipitation in the dry season. These factors are considered relevant to the life history of *Batrachoseps* since they control the predictability, timing, and length of time species have for surface activity which is when feeding and reproduction take place (Hendrickson, 1954; Cunningham, 1960; Maiorana, 1976).

Statistical methods

Comparisons between sg. *Plethopsis* and sg. *Batrachoseps* were performed using analysis of variance tests (ANOVA). Because this is inherently a phylogenetic test of monophyletic groups more specialized phylogenetic methods were not used. Phylogenetic independent contrasts (PIC) were used for tests within sg. *Batrachoseps*. To account for phylogenetic signal in morphological and climatic variables I used a current species tree (Jockusch et al., 2014) and performed phylogenetic independent contrasts in r using the ape package (Felsenstein, 1985; Paradis et al., 2014; r, 2014). A single species, *B. aridus* is excluded from PIC analyses since the most accurate species tree (E. L. Jockusch, pers. comm.) is based on nuclear genes and material for the Federally Endangered Species *B. aridus* is not available (Jockusch et al., 2014). The undescribed sp. nov. Arguello was also excluded pending formal description (Jockusch et al., 2014).

Five measurements from each variable of interest were taken. These five measurements are inferred to represent environments where selection on *Batrachoseps* is increasingly more intense. For annual precipitation, dry season precipitation, and annual predictability (P) PIC correlations were measured for the mean, the 25% quantile, the 10% quantile, the 5% quantile, and the 1% quantile. For dry season length and annual seasonality (M) PIC correlations were measured for the mean, the 75% quantile, the 90% quantile, the 95% quantile, and the 99% quantile.

Results

Morphology

Morphological scores appear to be effective at quantifying the differences described by previous authors (Stebbins and Lowe, 1949; Maiorana, 1976; Stebbins, 2003). Within *Batrachoseps* the distribution of Shape (S) scores is not significantly different than normal based on a Wilks-Shapiro test (W = 0.96, Prob < W =0.51). Wormlike species described by Maiorana (1976) as “derived” have the smallest shape scores and uniquely fall within lowest 25% of all scores. All monophyletic groups with more than one species show interspecific morphological variation in shape. Three of four monophyletic groups within sg. *Batrachoseps* contain at least one wormlike species. The *diabolicus* group is the exception as its most elongate member has a shape score of 2.20.

Subgenus *Batrachoseps* vs. subgenus *Plethopsis*

Food availability is more periodic in habitat were subgenus *Batrachoseps* is found compared with sg. *Plethopsis*. Analysis of variance (ANOVA) results reveal a significant difference in seasonality (Colwell’s M) between habitats occupied by sg. *Batrachoseps* compared to habitat occupied by sg. *Plethopsis*. Across the five measurements considered the strongest difference was found using mean values of the seasonality metric M, $R^2 = 0.31$, p-value = 0.007. Scores from other quantiles hovered around 0.2 but remained significant at < 0.05 level (Table 3).

Three measures were used to quantify how xeric an environment is, Length of dry season (months), monthly precipitation during dry season (millimeters), and total annual precipitation (millimeters). Length of dry season showed a slight interaction with subgenus in mean values, $R^2 = 0.14$, p-value = 0.09. The relationship was far weaker and insignificant in all other tests, never higher than 0.004 (0.78) (see Table 3). Scores were also weak and insignificant for annual precipitation, $R^2 = -0.2$, p-value = 0.45 for mean values was the most significant (Table 3). ANOVA for dry season precipitation did show a significant difference between the subgenera (Table 3 and Figure 4). Subgenus *Batrachoseps* occupy environments with significantly less dry season precipitation. This relationship was consistent across all measurements with R^2 falling between 0.36-0.39 with p-values ≤ 0.004. Interestingly the most northerly member of *Plethopsis*, *B. wrighti* does not have a dry season where monthly precipitation falls below 6 mm.

Unpredictability of the environment was quantified using Colwell's P for monthly rainfall. No significant difference was found in the environmental predictability of habitat occupied by sg. *Plethopsis* and sg. *Batrachoseps*. R^2 values ranged from $R^2 = 0.02$ (p-value = 0.53) to $R^2 = 0.0003$ (0.94) when comparing the 5% quantile (Table 3).

Periodic availability of food (Colwell's M)

Results from PIC on periodic food availability (Colwell's M) and morphology (Shape) show a modest trend at the mean which becomes increasingly strong and significant at the extremes (Table 4). This trend is in the level expected if wormlike species are adapted to more seasonal environments. At the 75% quantile of M the Pearson correlation = -0.55 (p-value = 0.02). These results strengthen to -0.68 (p-value = 0.003) in comparing the 90%

quantile values of M, to -0.69 (p-value = 0.002) at the 95% quantile values of M, and to -0.8 (p-value = 0.0001) at the 99% quantile values of M. (Figure 5).

Dry season and precipitation

Dry season precipitation has a significant positive relationship with morphology within the 10% most extreme environments but not at the mean or the 25% quantile (Table 4). Using 10% quantile values of dry season precipitation in PIC analysis yielded a Pearson correlation of 0.49 (p-value = 0.05) (Table 4). At the 5% and 1% quantiles the correlation is stronger, Pearson correlation = 0.69 (p-value = 0.002) at 5% and 0.71 (p-value = 0.001) at 1% (Table 4 and Figure 6). Neither annual precipitation nor length of dry season showed a significant correlation with morphology for any of the measurements used. For both variables the correlations were weak and non-significant. Phylogenetic independent contrasts of annual precipitation mean values had a Pearson correlation of -0.2 (p-value = 0.45) with morphology. This relationship shifted to 0.14 (0.6) in comparing the 1% quantile values (Table 4). Dry season length showed a similar pattern (Table 4) with mean values yielding a Pearson correlation of -0.07 (p-value = 0.8) and the correlation of morphology with 99% quantile values at 0.09 (p-value = 0.75).

Unpredictable environment (P annual)

Colwell’s P is not significantly correlated with morphology for any of the quantiles considered here (Table 4). Significance never rises above 0.58 and the Pearson correlation coefficients are all weak and range from -0.14 to 0.045.

Predictions made by Maiorana (1976) regarding the influence of a xeric and unpredictable environment on evolution in *Batrachoseps* do not appear to be broadly valid. However the relationship between seasonality and morphology in *Batrachoseps* is significant for both the split between the two subgenera and the degree of elongation within sg. *Batrachoseps*. Seasonality (M) and dry season precipitation are significantly different between sg. *Plethopsis* and sg. *Batrachoseps* (Table 3 and 4). The strength of this difference shrinks at the higher quantiles of M while the difference in dry season precipitation remains constant. This is due to M being the result of differences in both dry season and wet season precipitation. Perhaps most compelling in light of the original investigations of Maiorana (1976) is the strong correlation between degree of elongation within sg. *Batrachoseps* seasonal environments. Using the 90% quantile values in PIC correlation tests resulted in a strong negative correlation of -0.68 with a p-value of 0.003 (Table 4). Using 99% quantile values boosts this relationship to -0.8 with a p-value = 0.0001 (Table 4 and Figure 5).

Discussion

Members of subgenus *Batrachoseps* occupy more seasonal environments than those of subgenus *Plethopsis*. This result links the greater degree of paedomorphosis in sg. *Batrachoseps* (Wake, 1966) with more seasonal and hence less stable environments. Two results illustrate the difference in environmental stability between these two groups. Colwell's metric for seasonality (M) and dry season precipitation both differ significantly between the two subgenera (Table 3 and 4). Subgenus *Batrachoseps* inhabits more seasonal habitats that have more xeric dry seasons. This positive relationship between degree of paedodomorphosis and seasonality suggests that paedomorphosis in *Batrachoseps* is the result of progenesis (Gould, 1977). Classic models of paedomorphosis in salamanders involve species with aquatic larval stages where metamorphosis is facultative (Gould, 1977, sources therein). Even within a single species the proportion of paedomorphic individuals can vary from population to population (Gould 1977 and sources therein). Differences in the stability of the aquatic environment along with the productivity of the adjacent terrestrial environment are good predictors of the proportion of paedomorphic individuals in a population (Snyder, 1956; Sprules, 1974: Sprules, 1974a). In stable aquatic environments with few predators the proportion of paedomorphic individuals is higher and paedomorphosis is due to neoteny. In relating this paradigm to the fully terrestrial *Batrachoseps*, the pond where breeding and feeding can take place is replaced conceptually by a wet season with enough precipitation to facilitate feeding and breeding behaviors. If paedomorphic species of *Batrachoseps* were neotenic paedomorphs they would be associated with stable mesic habitat. As the opposite is observed I conclude that paedomorphosis in *Batrachoseps* is the result of progenic heterochrony.

Progenesis appears to reach its most extreme in the wormlike taxa of sg. *Batrachoseps*. In these species many features remain miniaturized, most obviously the limbs, while resources go toward growing the body cavity via increase in the number of axial vertebrae (Roth et al., 1980; Jockusch, 1997). This appears consistent with the idea of r-selection where the optimal strategy is to maximize reproduction in a limited amount of time. One of the compelling things about Maiorana's (1976) study was its timely critique of the r- and K-selection paradigm as they relate to *Batrachoseps*. This critique was taken up by various authors around the same time (Gould, 1977; Stearns, 1980). Maiorana (1976) postulated that individual *Batrachoseps* are able to adopt r- or K- selection strategies depending on environmental conditions via resource allocation. It may be possible that local optimization of life history strategy could be associated with interspecific morphological disparity and the speciation process itself through assortative mating. Clutch sizes, egg sizes, and time to hatching reported by Jockusch and Mahoney (1997) appear consistent with a greater degree of r-selection in wormlike *Batrachoseps* compared to *Plethopsis* as well as the ability to vary clutch size in response to local conditions. Mean clutch sizes for two wormlike taxa, *B. attenuatus* (6.8 – 13.4) and *B. gregarius* (7.3 – 16.3; listed as *B. nigriventris* in pub.) were larger and gestation times shorter compared with mean clutch size in *B. wrighti* (5.1, sd= 2.0). *Batrachoseps wrighti* is in sg. *Plethopsis* and occupies the most stable and wet habitat of all *Batrachoseps* (Table 2). Analysis of general seasonality trends and patterns during the years Jockusch and Mahoney (1997) collected eggs might further reinforce the link between these patterns and seasonal rainfall. Jockusch (1997) showed that number of trunk vertebrae is sexually dimorphic in several wormlike species, with females having more trunk vertebrae, and that this dimorphism is more pronounced among inland populations. She tied this observation to the general trend of inland habitat being more xeric (Jockusch, 1997). Differences in seasonality were not considered. Although it receives no formal treatment here the data indicate that increasing inland dryness manifests in a particular way that is consistent with the conclusions presented here, namely that inland populations are more xeric because they receive less dry season precipitation. These xeric and constant dry seasons likely put selection pressure on a population by increasing dry season mortality independent of population density (Maiorana, 1976). Maximizing reproduction over the shortest amount of time appears to be under more intense selective pressure in the inland environments sampled by Jockusch (1997). The current hypothesis for evolution of wormlike morphology in *Batrachoseps* is that these are miniaturized species which secondarily evolved axial elongation (Roth et al., 1980; Hanken and Wake, 1993). If instead *Batrachoseps* are viewed as progenic the mechanism for explaining morphological diversity is simplified as largely the result of a single selective mechanism: r-selection drives progenesis and results in miniaturization of certain features in wormlike species. For this reason the standard size measure used for salamanders, snout-to-vent length (SVL) is stymies effective comparisons in *Batrachoseps*. When just SVL is compared robust and wormlike forms can look remarkably similar (Brame and Murray, 1968). While length may be similar the rest of the animal is much smaller in wormlike forms.

Miniaturization has occurred several times within the Plethodontidae (Hanken and Wake, 1993; Wake, 2009). In Bolitoglossini, the sister tribe to all *Batrachoseps* (Vieites et al., 2011) the smallest of all salamanders, the fully terrestrial genus *Thorius*, display vastly different morphology and life history traits compared to *Batrachoseps* (Wake and Lynch, 1976; Wake, 1987; Rovito et al., 2013). While the most elongate *Batrachoseps* have relatively large clutch sizes (Jockusch and Mahoney, 1997) this pattern is reversed in *Thorius*. All *Thorius* appear to have smaller clutch sizes than wormlike *Batrachoseps*, rarely >10 (J. Hanken, pers. comm.) but larger species of *Thorius* have more eggs than more minute species. This pattern is consistent with degree of miniaturization in *Batrachoseps* being the result of progenesis while degree of miniaturization in *Thorius* is the result of neoteny. Disparity in the environmental stability between the neotropical *Thorius* and the nearctic *Batrachoseps* is also consistent with neotenic paedomorphosis in *Thorius* and progenesis in *Batrachoseps* (Gould, 1977). This proposed mechanistic difference between these two miniaturized species may also explain the absence of the fifth digit from the pes in

Batrachoseps while this digit is retained in *Thorius* and all other neotropical bolitoglossines (Wake, 1966; Wake, 2012). Absence of the fifth digit from the pes of the progenic *Eurycea quadridigitata*, a salamander in the same subfamily as *Thorius* and *Batrachoseps*, is consistent with this hypothesis (Bonnet et al., 2014).

Maiorana (1976) conceived of wormlike *Batrachoseps* as species under intense selection to seek refuge from dry surface conditions for months at a time. Her conclusion was based on results that indicated wormlike morphology is key accessing sub-surface refuges with suitable microclimate (Maiorana, 1978). Results presented here indicate that instead of being associated strictly with wormlike morphology the shift toward more seasonal habitat occurred at the basal split between the two extant subgenera in *Batrachoseps*. This split is clearly associated with the presence of wormlike forms since the wormlike *B. attenuatus* is the outgroup to all other sg. *Batrachoseps*. Origin of sg. *Batrachoseps* and its paedomorphic development also appears to have facilitated increased morphopotentiality (Liem, 1973) within this group compared with sg. *Plethopsis* (Figure 3). Gould (1988) pointed out that the evolution of progenesis, "…gains its macroevolutionary power by presenting to future selective contexts a rapidly generating, unusual mosaic of juvenile and adult characters…". While "rapidly generating" may seem out of place in a discussion of *Batrachoseps*, which have extremely low metabolic rates (Feder, 1976), the "mosaic of juvenilized and adult characters" observed in sg. *Batrachoseps* may be the key to their relative evolutionary success compared with sg. *Plethopsis*. The hypothesis for progenic development presented here as well as the conclusions of Jockusch (1997) both emphasize female fecundity via the increase in trunk size. From first principles an increase in trunk size can be achieved by increasing length or width. As only slender forms are observed selection on decreased body diameter from semi-fossorial habits of *Batrachoseps* likely plays a role in the evolution of wormlike morphology (Gans, 1975; Maiorana's (1976). The degree to which differences in substrate covary with the climatic variables used in this inquiry is unclear although a connection between precipitation seasonality and the associated plant community is plausible.

Despite the strong correlation between morphology and climatic seasonality other factors are also likely contribute to the complex array of selective pressures faced by individuals of each species. Diversity of forms in sg. *Batrachoseps* compared with sg. *Plethopsis* may be the result of selection for interstitial locomotion to reach sub-surface refuges in environments with different types of substrate. This would be an extension of Maiorana's (1976) logic. Selection on *Batrachoseps* to seek refuge during dry periods has strong support from studies of *Batrachoseps* and other salamanders (Hendrickson, 1954; Cunningham, 1960; Maiorana, 1974; Mathis, 1990). The conclusion that wormlike morphology is always the most advantageous way to reach those refugia has much less support. Locomotor differences across morphotypes within salamanders in the family Plethodontidae were treated by Edwards (1976) but movement through interstitial space was not analyzed. Two robust forms in sg. *Batrachoseps* occur at high elevations and are often associated with rocky substrate or talus slopes (Brame and Murray, 1968; Wake, 1996; Hansen et al., 2005; Hansen and Wake, 2005). These robust phenotypes may be the result of selection for locomotion though saxicolous habitat where longer limbs may be advantageous for moving along and around rock faces and facets to reach microclimate refugia during dry surface conditions. Tests of locomotor performance across and through different substrates are lacking.

Two known synapomorphies within sg. *Batrachoseps* deserve further investigation in light of the findings presented here. Both have the potential to influence how species cope with highly seasonal environments. Accessing refuges from dry surface conditions likely involves selection on gross morphology but surviving for several months without additional moisture and likely without much additional oxygen would appear to involve intense selection on physiology (refs in Hillman et al book). A pair of physiology studies on species in sg. *Batrachoseps* found the ability to regulate osmolarity of the blood as a way to limit water loss (Licht et al., 1975; Jones and Hillman, 1978). This trait is likely ubiquitous in sg. *Batrachoseps* osmoregulation through urea retention occurs in both the basal *B. attenuatus* and members of the *pacificus* group (Figure 1). This ability to slow water loss would clearly be advantageous during seasonal dry periods. It is unknown whether this trait is present in sg. *Plethopsis*.

A second trait that is reported only to occur in sg. *Batrachoseps* is the ability to enucleate red blood cells (Mueller et al., 2008). The presence of a large number of enucleate red blood cells in *Batrachoseps* has been known for some time but a clear explanation for what drives this trait continues to elude researchers (Emmel, 1923; Villalobos et al., 1988; Mueller et al., 2008). Explanations to date have focused on the role of miniaturization and genome size in driving observed patterns. No clear pattern has yet emerged as neither miniaturized morphology, genome size, or nucleus size are particularly strong predictors of the percent of enucleate blood cells present in a specimen (Villalobos et al., 1988; Mueller et al., 2008). One of the difficulties is the large amount of variance in percent of enucleate red blood cells observed even within members of a single species from one population (Mueller et al., 2008). Physiological hypotheses have been discussed but as yet have not been tested.

One limitation of the current analysis is that it only accounts for moisture inputs due to rain and snow. Other sources of moisture may create suitable microclimates and allow local persistence of populations of *Batrachoseps*. The inability to account for these moisture inputs has the potential to invalidate findings presented here if wormlike taxa, and sg. *Batrachoseps* in general, are strongly associated with moisture inputs aside from precipitation. In fact the opposite appears to be true. Several robust taxa and the two southern members of sg. *Plethopsis* are often associated with moisture inputs aside from precipitation. Springs are thought to be necessary for the persistence of desert taxa *B. campi* (*Plethopsis*) and *B. aridus* (*Batrachoseps*). Each is closely associated with perennial springs and anomalous wet canyons in an otherwise xeric desert landscape (Hansen and Wake, 2005a; Hansen and Wake, 2005b). Near surface groundwater has also been observed in several sites of rock or talus habitat where *B. stebbinsi* is found (author pers. comm.). Early descriptions of *B. relictus* call attention to its use of surface water and *B. robustus* (*Plethopsis*) is commonly found under cover along the margins of seasonal streams (Hansen and Wake, 2005c). Perhaps most interesting is the example of *B. pacificus* which is both robust in proportion and large in size and appears to be a classic case of island gigantism (Case, 1982; Stebbins, 2003). Precipitation measures used in this study measured average dry season length as four months long from June-September. When monthly fog data are considered (Baguskus and Rastogi, unpub. data) this dry season was effectively eliminated at sample sites as moisture input for all months was >6mm. If *B. pacificus* occupies sites that lack a dry season it shares this trait only with the species found in wet northern forests, *B.*

wrighti (*Plethopsis*). These observations support the conclusion that species in highly seasonal environments are often dependent on rainfall for surface activity while robust forms from less seasonal habitats generally benefit from water input aside from precipitation.

Subgenus *Plethopsis* is a group that has fascinated researchers since description of *B. wrighti* as, "A remarkable new salamander from Oregon" over 75 years ago (Bishop, 1937). Ranges for these three species are highly disjunct and they each occupy very different types of habitat. *Batrachoseps wrighti* is found in the wet coniferous forest and western foothills of the Cascade Range, *B. robustus* in the arid and high elevation forest on the Kern Plateau and Scodie Mountains of the southern Sierra Nevada of California, and *B. campi* in the wet margins of springs and seeps in the high desert. Data presented in this study find that relatively low seasonality is the common thread in all of these habitats (Table 2 and Figure 4). Despite the much lower total annual precipitation for *B. campi* and *B. robustus* the seasonality of their habitat is low compared to habitat occupied by sg. *Batrachoseps* (Table 2). The implication is that surface or near-surface activity may be possible for much of the year for all three species in sg. *Plethopsis*. Conditions are marginal for *B. campi* and *B. robustus* compared to *B. wrighti* which may explain the more robust morphology and lower surface to volume ratio of these two species compared with *B. wrighti*. Despite the wide separation of their ranges these three species appear to have diverged in only the last 3 million years, more recent than much of the divergence in sg. *Batrachoseps*. More puzzling still is that *B. campi* is more closely related to *B. wrighti*, 820 km away, that it is to *B. robustus* whose range is less than 50 km away. Formal treatment of the process that led to this disjunct distribution over such short time scales is pending and tectonic activity along with Pleistocene glacial cycles appear to be the most likely influences (D. Wilson, pers.

comm.; sierra glaciation sources). Vicariant speciation due to tectonic activity has also been implicated for species of *Batrachoseps* on the California coast (Wake, 2006; Martinez-Solano et al., 2012).

Linking life history attributes to environmental variables continues to be a challenge for researchers modeling species distributions. It may be possible to use Colwell's indices to link life history evolution to predict species distributions and boundaries (Colwell, 1974). As a practical application, the use of Colwell's indicies may have the potential to inform spatial modeling efforts (Colwell, 1974; Maiorana, 1976). Species distribution models often use locality data (Vertnet, 2015) along with climate and other habitat variables to predict distributions of a species' ecology (Phillips and Dudik, 2008). This study shows that standard precipitation measures such as annual and seasonal totals proved less informative for differentiating between species than seasonality, Colwell's M. Colwell's indices have the advantage of relating to the timing of important life history events for an organism if they are properly calibrated. In this study the calibration was based on in several years of data collected by Maiorana (1974) as well as other researchers of *Batrachoseps*. Multi-year field studies that allow realistic calibration of Colwell's indices or other environmental metrics appear to be falling out of favor, particularly in graduate Ph.D. research. Spatial environmental data and computational tools are now widely available. Precipitation data at relatively fine temporal and spatial scales are available for much of North America from the PRISM dataset and computational programs available to analyze this data freely available with the hydrostats package in r (R, 2014; Bond, 2015). Detailed life history studies are needed if habitat and distribution models are to prove meaningful for future generations.

Tables

Table 1: A summary of the history of species descriptions in genus *Batrachoseps*. *B. attenuatus* and *B. relictus* as recognized by Maiorana (1976) contain 11 species. Maiorana (1976) describes wormlike forms as “derived”. Wormlike forms in the current paper are species whose morphology score is in the 25th percentile and below (≤ 2.1) see text for details.

	Species (category) Maiorana, 1976	Species (morphology score) Jockusch et al., 2014
1.	*B. attenuatus* (derived)	*B. attenuatus* (0.69) *B. gavilanensis* (1.50) *B. gregarius* (0.25) *B. nigriventris* (1.02)
2.	*B. pacificus* (ancestral)	*B. pacificus* (4.59)
3.	*B. major* (derived)	*B. major* (1.88)
4.	*B. wrightorum* (ancestral)	*B. wrighti* (4.89)
5.	*B. relictus* (ancestral)	*B. altasierrae* (2.31) *B. diabolicus* (2.20) *B. kawia* (2.50) *B. luciae* (2.68) *B. minor* (2.99) *B. incognitus* (2.98) *B. relictus* (2.93)
6.	*B. simatus* (ancestral)	*B. simatus* (2.49)
7.	*B. stebbinsi* (ancestral)	*B. stebbinsi* (5.32)
8.	*B. aridus* (ancestral)	*B. aridus* (4.50)
18.	Undiscovered/undescribed	*B. campi* (7.70)
19.	Undiscovered/undescribed	*B. bramei* (3.89)
20.	Undiscovered/undescribed	*B. gabrieli* (5.05)
21.	Undiscovered/undescribed	*B. regius* (3.06)
22.	Undiscovered/undescribed	*B. robustus* (6.62)

Table 2: Mean monthly precipitation averages in millimeters ordered by shape. Note the differences in length and timing of dry seasons (shaded boxes). Two metrics that reflect seasonality are also included the 90% quantile value for seasonality (M) and the 10% quantile value for dry season precipitation (Dry PPT). Subgenus *Plethopsis* indicated by (*P*). Shading indicates the dry season for each species, where average monthly ppt< 6mm.

Species	Shape	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	M (Q90%)	Dry PPT (Q10%)
B. campi (*P*)	7.7	24.4	23.3	15	6	**3.9**	**3.1**	**5.1**	6.3	4.5	5.5	13.4	21.7	0.12	3.4
B. robustus (*P*)	6.62	71.8	54	50.3	13.6	6.3	**3.3**	9.1	9.6	7.7	20.3	33.2	47.3	0.21	3.5
B. stebbinsi	5.32	50.2	48.3	44.1	14.7	6.9	**1.7**	**2.7**	**5.6**	4.1	12.2	30.1	43.1	0.2	2.3
B. gabrieli	5.05	150	172	122	49.6	20	**5.7**	**1.7**	**4.1**	8.7	40.2	60.8	111	0.195	2.6
B. wrighti (*P*)	4.89	224	176	189	152	112	81.3	24.5	22.3	60.2	133	255	256	0.2	NA
B. pacificus	4.59	63.2	72.7	48.8	21.5	6.6	**1.1**	**0.5**	**0.9**	**4.4**	13.1	25.4	44.3	0.191	1.3
B. aridus	4.5	47.3	47.5	21.6	**5.1**	**0.8**	**0.9**	14.2	17.9	13.6	7.3	12.5	34.4	0.13	2.3
B. bramei	3.89	75.9	74.2	59.9	21.3	9.4	**2.3**	**4.7**	7.4	5.5	12.5	38.7	68.7	0.2	3.4
B. regius	3.06	117	112	104	54.4	25.1	8.9	**1.9**	**0.6**	10.6	37	61	110	0.21	1.2
B. minor	2.99	123	115	97.9	35.8	11.8	**2.4**	**0.2**	**0.7**	8	30.7	48.8	102	0.24	1.0
B. incognitus	2.98	100	110	89.9	32.9	10.3	**3**	**0.2**	**0.8**	6.4	22.7	41.6	90.2	0.23	1.3
B. relictus	2.93	47.4	48.9	61.5	36.2	9.2	**3.7**	**0.3**	**1.7**	**4.1**	16.6	28.5	48.8	0.22	2.4
B. luciae	2.68	100	94.9	81.4	37.5	13.5	**5**	**0.4**	**1**	**4.4**	25.3	51.9	81.5	0.25	2.1
B. kawia	2.5	119	117	111	56.1	24.9	8.3	**1.7**	**0.8**	10.3	33.9	68.8	101	0.22	1.1
B. simatus	2.49	47.4	48.9	61.5	36.2	9.2	**3.7**	**0.3**	**1.7**	**4.1**	16.6	28.5	48.8	0.22	2.4
B. altasierrae	2.31	133	131	117	60	26.9	8.6	**3**	**3.4**	7.6	29	77.6	116	0.23	2.4
B. diabolicus	2.2	141	128	122	59.4	27.7	10	**1.2**	**1**	9.4	43.6	79.5	124	0.23	0.8
B. major	1.88	58.5	66.4	51.5	19.2	**5.4**	**1.9**	**0.9**	**1.1**	**3.6**	13.8	24.7	48.1	0.21	1.9
B. gavilanensis	1.5	67.6	65.3	61	22	9	**2.3**	**0.3**	**0.8**	**4.9**	17.4	31.2	57	0.24	1.6
B. nigriventris	1.02	72.2	77.6	63.1	24.2	8.4	**1.9**	**0.6**	**1.1**	**3.8**	17.5	30.4	57.5	0.23	1.2
B. attenuatus	0.69	100	96.4	80.5	35.7	16.7	**5**	**0.2**	**1**	**5**	26.9	62.4	99.7	0.25	1.8
B. gregarius	0.25	94.2	89.7	88.1	44.4	19.3	6.7	**1.5**	**3.3**	6.2	24.7	55.7	82	0.22	0.9

Table 3: R^2 statistics for analysis of variance (ANOVA) tests comparing seasonality (M), dry season length, predictability (P), dry season precipitation, and annual precipitation for sg. *Batrachoseps* and sg. *Plethopsis* (shown as R^2 (p-value)).

Variable	Mean	75% quantile	90% quantile	95% quantile	99% quantile
Seasonality (M)	**0.31 (0.007)**	**0.2 (0.04)**	**0.19 (0.046)**	**0.197 (0.039)**	**0.20 (0.036)**
Dry season length (months)	0.14 (0.09)	0.004 (0.78)	0.0005 (0.92)	0.001 (0.88)	0.001 (0.88)
	Mean	25% quantile	10% quantile	5% quantile	1% quantile
Predictability (P)	0.02 (0.53)	0.0008 (0.9)	0.0001 (0.96)	0.0003 (0.94)	0.002 (0.84)
Dry season precipitation	**0.37 (0.004)**	**0.39 (0.002)**	**0.36 (0.004)**	**0.38 (0.003)**	**0.36 (0.004)**
Annual precipitation	0.07 (0.22)	0.08 (0.2)	0.06 (0.3)	0.07 (0.24)	0.07 (0.23)

Table 4: Pearson correlation values from PIC of shape and seasonality (M), dry season length (months), predictability (P), dry season precipitation, and annual precipitation. Shown as Pearson Correlation (p-value).

Variable	Mean	75% quantile	90% quantile	95% quantile	99% quantile
Seasonality (M)	-0.4 (0.11)	**-0.55** **(0.02)**	**-0.68** **(0.003)**	**-0.69** **(0.002)**	**-0.8** **(0.0001)**
Dry season length (months)	-0.07 (0.8)	0.3 (0.24)	0.16 (0.54)	0.09 (0.75)	0.09 (0.75)
	Mean	25% quantile	10% quantile	5% quantile	1% quantile
Predictability (P)	-0.13 (0.6)	-0.14 (0.58)	-0.11 (0.67)	-0.09 (0.74)	0.045 (0.86)
Dry season precipitation	0.26 (0.32)	0.39 (0.12)	**0.49** **(0.05)**	**0.69** **(0.002)**	**0.71** **(0.001)**
Annual precipitation	-0.2 (0.45)	-0.08 (0.76)	0.02 (0.94)	0.06 (0.82)	0.14 (0.6)

Figures

Figure 1: Most recent published phylogeny based on 5 nuclear genes (Jockusch et al., 2014). Numbers in parentheses are shape scores. High scores indicate robust species with generalized morphology. As numbers get smaller the degree of elongation increases. Species with scores at or below the 25th percentile (≤ 2.1) are categorized as wormlike in this study; see text for details. These correspond to the "derived" designation in Maiorana (1976).

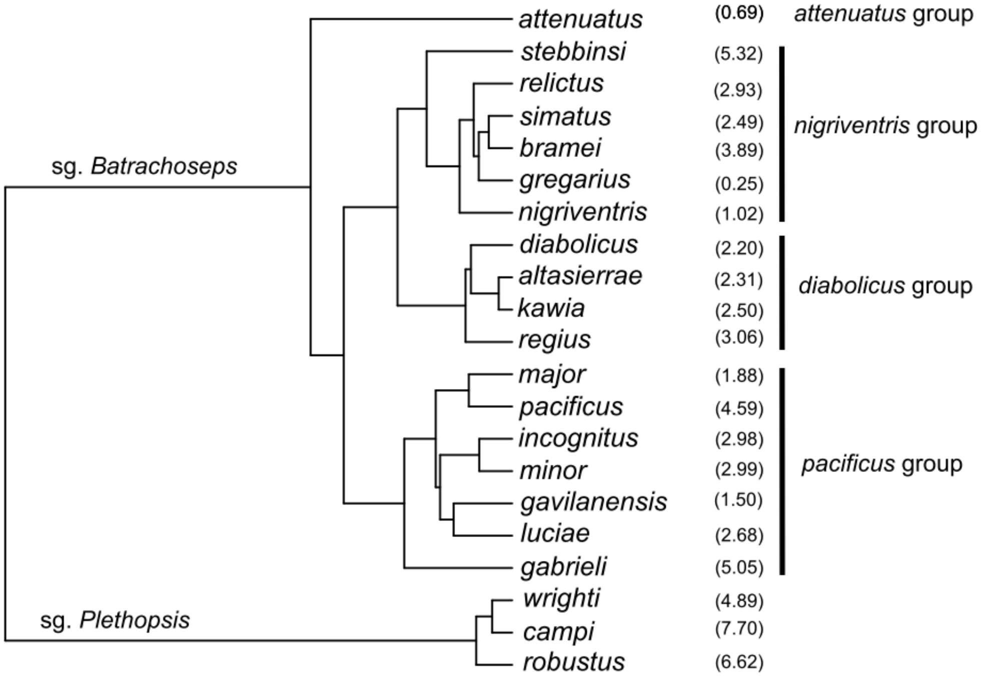


Figure 2: Map showing range of sg. *Plethopsis* (black dots) which contains three relict species and sg. *Batrachoseps* (yellow dots) which contains 19 described species and is distributed relatively continuously.

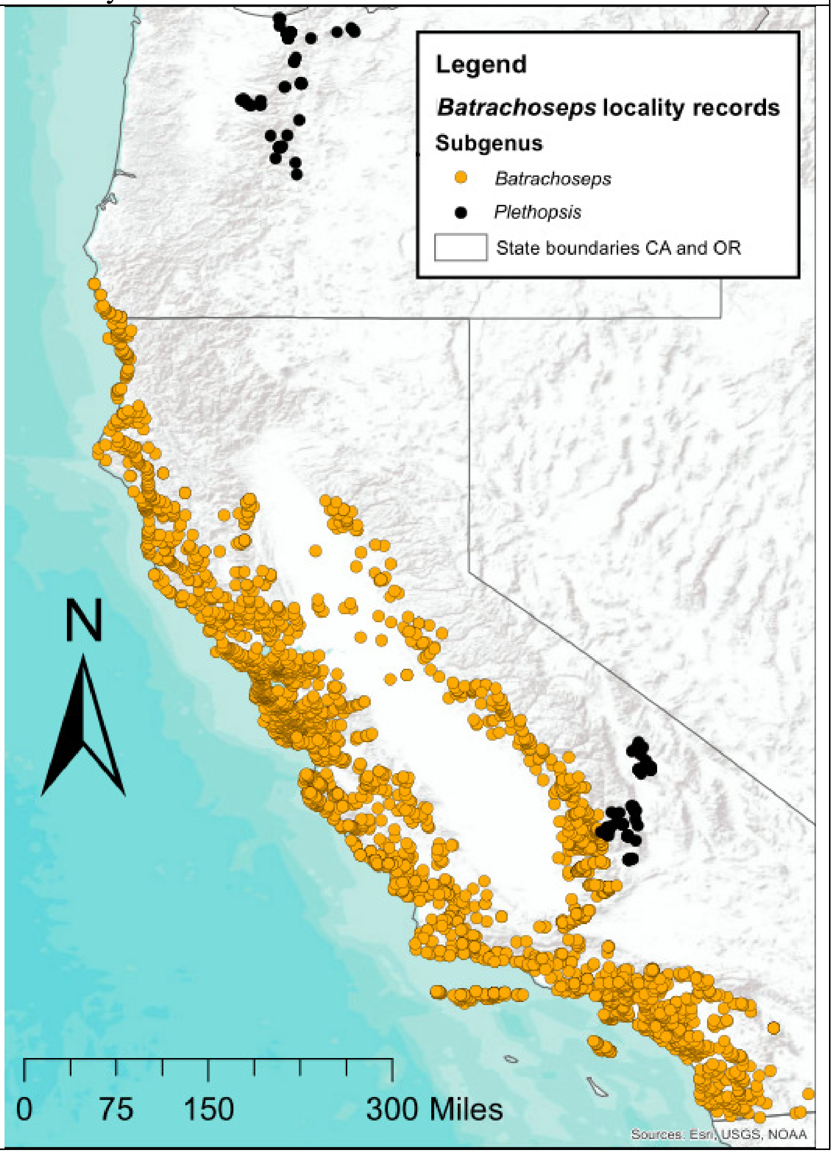


Figure 3: Shape variation for all 22 described species of Batrachoseps. Shape value shown as normal density distribution based on mean and standard deviation. Images above curves demonstrate how scores relate to morphological differences (from Stebbins, 2003). Dashed lines indicate that shape score does not include the tail, defined as posterior to the vent. Asterisk under apex of curve signifies three members of sg. *Plethopsis*.

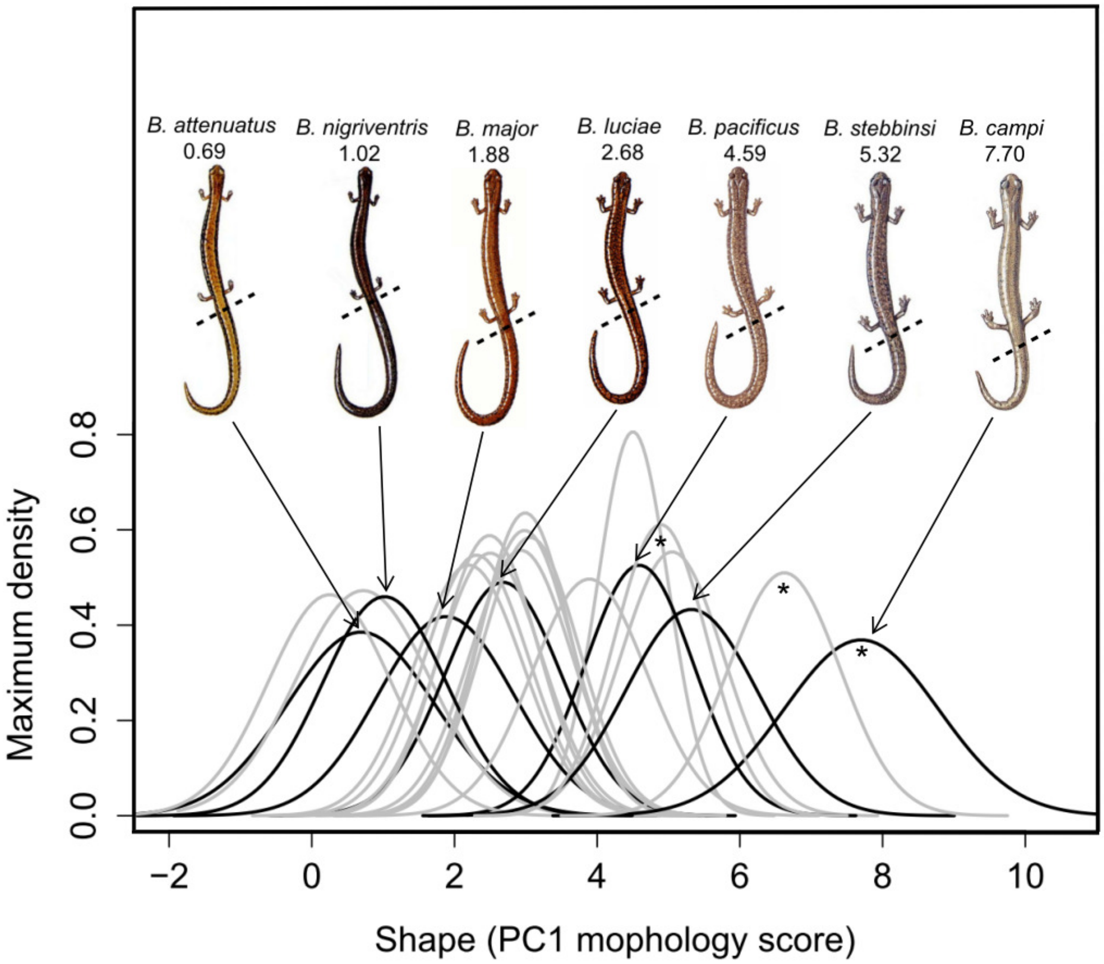


Figure 4: Box plots show ANOVA results for mean values of annual seasonality (Colwell's M) and dry season precipitation (mm). The sg. *Batrachoseps* is found in habitats that are significantly more seasonal ($R^2 = 0.31$, p-value = 0.007), and have significantly less dry season rainfall ($R^2 = 0.37$, p-value = 0.004). One species in sg. *Plethopsis*, *B. wrighti*, is found in the wet forests of central Oregon and does not have a dry season where monthly precipitation falls below 6 mm. See text for details.

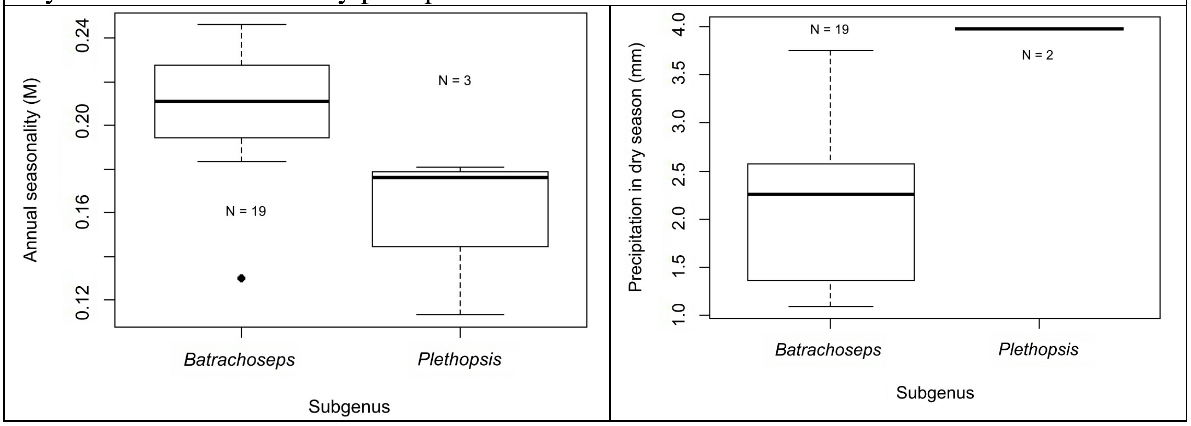


Figure 5: Correlation of phylogenetic independent contrasts for morphology (Shape) and annual seasonality of precipitation (M). PIC scores for the most extreme 10% and 1% values are shown here which relate to the 90% and 99% quantiles for each species.

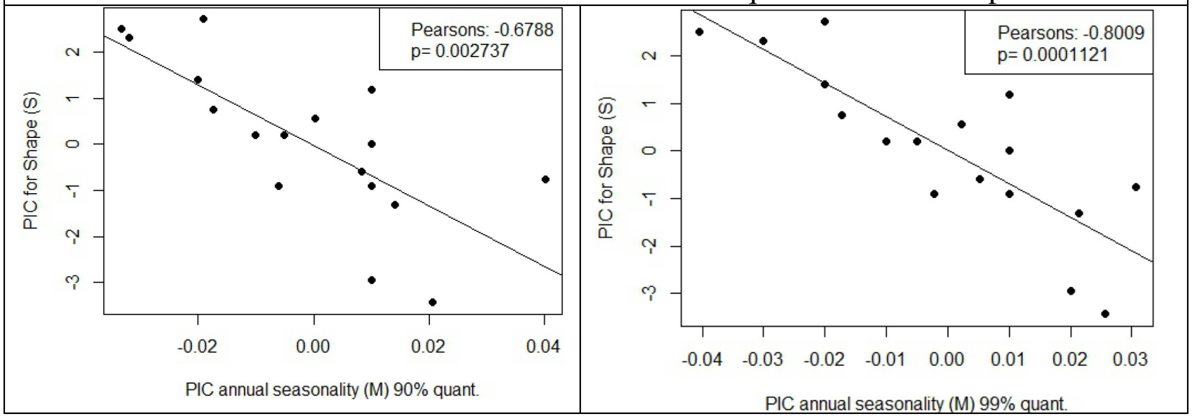
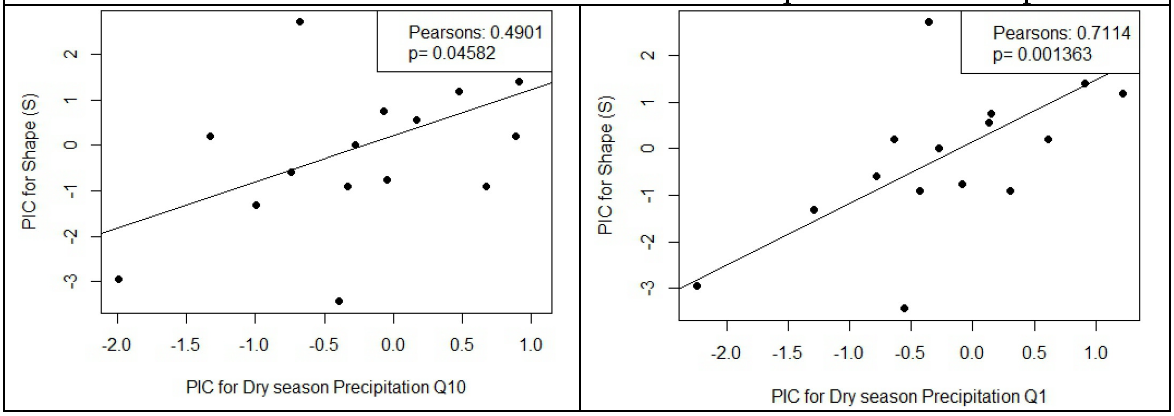


Figure 6: Correlation of phylogenetic independent contrasts for morphology (Shape) and annual dry season precipitation (mm/month). PIC scores for the most extreme 10% and 1% values are shown here which relate to the 10% and 1% quantiles for each species.



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