Ecology and Niche Evolution of Species in the Ground Beetle Genus *Nebria* (Carabidae: Nebriini)

By

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Dissertation Abstract

Understanding why species live where they do is a fundamental goal of macroecology and macroevolution. Examining these processes across multiple spatial scales and across time can provide unique insight into the macroecological and macroevolutionary processes which affect species distributions. Trait-based approaches provide a useful framework for testing which ecological processes most strongly influence local community composition, especially across environmental gradients where species diversity varies. Ecomorphological traits are a useful phenotype to understand niche evolution as they are easily measured and predictive of ecological niche. Habitat use is an important aspect of niche, particularly in abiotically challenging environments. In ecosystems with steep ecotones, the relationships between habitat use, ecological traits, and niche evolution can become clear, particularly when examining an adaptive radiation comprised of habitat specialists. Here, we integrate ecological and evolutionary methods to test the importance of environmental filtering, resource/habitat partitioning, habitat specialization, and range size evolution in shaping a mainly montane ground beetle species assemblage (Carabidae: Nebriini: *Nebria*). Species in this genus have a Holarctic distribution and occupy a diversity of montane habitats. Species in the genus *Nebria* occupy sequentially overlapping, but statistically discernable elevational bands along mountain streams, from 0-3000 m. above sea level. I sampled species in this diverse genus and found that habitat and microhabitat features are reliable predictors of species presence, and that more similar species are more likely to coexist. I found weak evidence for the partitioning of consumed resources (using stable isotope analysis), and that local community composition does not predict presence of individual species. I found evidence for convergence in morphotypes based on habitat specialization, and that ecomorphological traits, specifically pronotal and elytral features, are evolving slower than expected under Brownian motion evolution, indicating stabilizing selection. Range size is an important aspect of niche, and range size evolution is best explained by ecological speciation, in which ancestral range is divided among daughter taxa. This process is in contrast with dispersal-vicariance processes, in which diversification arises through genetic isolation across geographic barriers. Species tend to be closely related to their neighbors, and species diversity (turnover and nestedness) does not vary with climatic variables. Habitat specialization is evident, to the extent that ecological speciation best explains range size evolution, despite the sky-island distribution of these communities. Niche specialization is a known phenomenon in challenging environments, such as extreme latitudes, in caves, or in subterranean habitats. Previously, in zoological studies physiological traits were assumed to be critical to the survival of alpine species, yet thermal tolerance does not vary among species of *Nebria* despite great differences in elevational specialization across species. While patterns, such as Bergmann's Law, are known in vertebrate communities, few analogous patterns are known for arthropod communities across latitudinal and elevational gradients. Species inhabiting isolated archipelago-like habitat patches are often expected to diversify through genetic drift, rather than niche specialization or ecological selection. Nearctic species of *Nebria* suggest this assumption is invalid. Thus, studying species in this genus has illuminated patterns of ecomorphological trait ecology and evolution that have provided novel insight into why species in extreme environments live where they do, the processes which explain this phenotype-niche relationship, and the importance of ecological speciation in such a diverse, distributed community.

Dissertation Introduction

A major goal of both macroecology and macroevolution is to reliably predict species' habitat use, community assembly, and niche evolution (Austin, 2007; Liu et al., 2009; Weber et al., 2017). Environmental filtering shapes the fundamental niche for a given species (Grinnell, 1917), constraining the habitats and geographical range species can occupy. Secondarily, community composition can shape a species' realized niche through interactions such as competition for resources (Elton, 1946). As species interact with their environment, across generations, their niche changes as their traits evolve. Employing a trait-based approach to understanding these dynamics can facilitate the generalization of these trends across taxa. The relationships between functional traits and habitat use, community composition, and niche evolution are often used to test the alternative roles of species interactions (Nolte et al., 2017) and environmental filtering (Cadotte et al., 2015) in shaping community assembly across time and space. Functional traits are key phenotypic characteristics of a species that significantly influence performance, evolving in response to both species' interactions and niche utilization (McGill et al., 2006; Cadotte et al., 2015; D'Andrea & Ostling, 2016). For example, when multiple species possess similar functional traits, competitive interactions may limit similar species from occupying the same functional niche (Elton, 1946). As a result, the relationship between functional traits and environmental variation within communities and across evolutionary timescales can provide insight into the processes that predict and shape niche (Sanderson & Pimm, 2015; Gobbi et al., 2017; Nolte et al., 2017).

Adaptive radiations in abiotically challenging environments provide a useful context to study these processes and relationships. Adaptive radiations possess high rates of diversification and ecological specialization across taxa. The combination of recent, shared ancestry and ecological differentiation across taxa provides an opportunity to clearly test which evolutionary processes contributed significantly to niche development. In abiotically challenging environments, environmental filtering is often the primary process predicting species distributions, while species interactions are secondary. In montane and alpine habitats, for example, ecological gradients are steep and mountain geography often leads to isolated natural replicates, as is the case in the volcanic Cascades Range, U.S.A. (Graham et al., 2015). The ecology and evolution of adaptive radiations has been studied in a variety of communities including finches (Grant & Grant, 2002), cichlids (Sturmbauer, 1998), *Anolis* spp. lizards (Butler & King, 2004), and spiders (Gillespie et al., 2018), among other taxa. However, this study is one of the first to examine these processes in a temperate, montane environment with taxonomically simple communities. Riparian and alpine arthropod communities in the northern Cascades Range are notably enriched for species in the ground beetle genus *Nebria* (Carabidae: Nebriini). Species in this genus are some of the most abundant arthropod taxa in these habitats, and they are generalist carnivores. They are not significantly predated by other taxa (pers. obs.), and they hunt opportunistically. The northern Cascades Range contains ongoing sites of alpine arthropod research, providing a baseline of data to study species of *Nebria*’s habitat use and species distributions (Mann et al., 1980; Schoville et al., 2012; Slatyer & Schoville, 2016). Species of *Nebria* in snowfields, riparian habitats, and other sites have been studied for over 40 years (Kavanaugh, 1978; Mann et al., 1980; S. Schoville pers. comm.).

The goal of this dissertation research is to quantify the most important niche-determining factors that govern the distribution of alpine arthropods. Members of the Holarctic beetle genus *Nebria* (Carabidae: Nebriini) provide a unique opportunity to address this goal. This speciose genus (>400 species) has been observed to have up to 12 species on a single peak ranging in altitude from 0 – 3000 m above sea level (Slatyer & Schoville, 2016). It is rare to find a study system where many similar species occupy a variety of habitats across climatic and elevational gradients (Elton, 1946), even considering arthropods on tropical islands (Gillespie & Roderick, 2002). Species of *Nebria* have been observed in a variety of microhabitats including alpine snowfields and lower elevation riparian zones in montane habitats. Using these communities, we can experimentally approach the question: Which species traits and ecological/evolutionary processes influence niche in temperate montane habitats?

In my first chapter, I examine the importance of environmental filtering, resource consumption, and local community composition on habitat use. This is accomplished by conducting surveys of species of *Nebria* along elevational and ecological gradients, measuring select species functional traits, quantifying abundance, and measuring resource overlap among species of *Nebria*. I then test the predictive relationships between species traits, community composition, and habitat use. In my second chapter, I examine whether these morphological functional traits converge across alpine communities where local species diversity varies, to determine if ecomorphological traits consistently respond to environmental filtering in geographically isolated montane ecosystems. In my third chapter, I use maximum entropy niche modelling to estimate the range size of species of *Nebria*, then reconstruct ancestral range size using dispersal-extinction cladogenesis and dispersal-vicariance models to test the importance of ecological speciation and ancestral range size in predicting current range size and the degree to which species of *Nebria* are habitat specialists. I compare the explanatory power of these two models to assess which diversification processes best explain changes in range and habitat use.

Significance

The data obtained as a result of this project include images and measurements of functional traits, isotopic data, the distributions of species of *Nebria* in the Cascades Range and elsewhere, predictive models of the relationships between functional traits and habitat use, and microclimate data spanning the elevational range of mountains. This dissertation also contributes models of alpine species distributions, as well as the use of evolutionary analyses to inform conservation efforts. These data and results have been made publicly available to support conservation efforts in the National Parks Service. The insular nature of alpine habitats makes alpine species particularly vulnerable to climate change (Barnett et al., 2005; Spehn & Koerner, 2005). As anthropogenic climate change progresses, alpine habitats will experience warmer and more xeric conditions, shifting and possibly contracting the elevational distribution of these habitats. This will reduce the stability and resilience of these alpine communities as species chase their climatic niche, are challenged to accommodate their physiological limits at the edges of their range, and as they possibly encounter new competitive interactions in their shifting distributional range (Janzen, 1967; Barnett et al., 2005; Spehn & Koerner, 2005). Species adapted to relatively stable alpine conditions will be among the most vulnerable to these perturbances (Janzen, 1967; Spehn & Koerner, 2005; Chown, 2012). This study and others like it will inform management decisions and conservation efforts in alpine and montane habitats, as well as contribute to our general knowledge of species' ecology and niche evolution. Understanding how and why species live where they do will guide conservationists in prioritizing critical habitat, or critical regions with high diversity in habitat features.

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Table of Contents

Chapter 1: "Functional traits and habitat use: investigating community assembly in a mostly montane community (Carabidae: *Nebria* spp.)"

Authors: Jillian Schat, David H. Kavanaugh, Jacki Whisenant, Genevieve Anderegg, Huijun Xiao, and Sean D. Schoville.

Open Research Statement: Data used in this study are open access: https://datadryad.org/stash/share/Dd_j1rSQ0fkAfkNGwRWTF57Z3il_6CsUccDf6KKwLfg

Abstract:

The processes that influence community assembly, such as competition for resources and environmental filtering, are often scale-dependent and vary across ecotones. Trait-based ecology provides a useful framework for testing which ecological processes most strongly influence local community composition, especially across environmental gradients where species diversity varies. Where environmental filtering dominates, species distributions are expected to be defined by strong turnover along environmental gradients, with more similar species occupying more similar habitats. Where interspecific competition dominates, species are expected to diverge in relative abundance and resource utilization at sites, so species can co-occur. Here, we integrate measurements of functional traits, microhabitat usage, isotopic composition ($\delta^{15}N$ and $\delta^{13}C$), and abundance to test the importance of environmental filtering and resource/habitat partitioning in shaping a montane ground beetle species assemblage (Carabidae: Nebriini: *Nebria* spp.) in the isolated, volcanic peaks of the northern Cascades Range, U.S.A. Across species of *Nebria*, body size, pronotal shape, temperature preference, and isotopic enrichment varies across habitats, and habitat/microhabitat features were reliable predictors of species presence. Resource consumption among mid-elevation species on Mt. Rainier – the peak with the greatest species diversity – is highly overlapping. Species turnover and nestedness varied significantly across habitat gradients and peaks throughout this region and varied nearly significantly across sites, and across habitat types and sites, more similar species are more likely to coexist. These results suggest that environmental filtering is the primary process structuring this species assemblage, although we find detailed evidence for microhabitat niche partitioning among species of *Nebria* at the site-scale.

Introduction:

A major goal of ecology is to reliably and efficiently predict habitat use and the composition of natural communities (Austin, 2007; Liu et al., 2009). Abiotic factors, such as environmental filtering, and biotic processes, such as species interactions and niche partitioning, have been implicated as important sources of variation in species' distributions (Kraft et al., 2015). To examine the relative importance of these competing factors in a testable framework, ecologists are increasingly integrating tools that explain species distributions at multiple spatial and temporal scales (Garrick, 2011).

One important approach has been to use functional traits to disentangle the role of species interactions (Nolte et al., 2017) and the role of environmental filtering (Cadotte et al., 2015) in determining habitat use. Functional traits are key phenotypic characteristics of a species that significantly influence performance, evolving in response to both species' interactions and niche utilization (McGill et al., 2006; Cadotte et al., 2015; D'Andrea & Ostling, 2016). When multiple species possess similar functional traits, competitive interactions limit similar species from occupying the same functional niche (Elton, 1946). As a result, the relationship between functional traits and environmental variation within communities can provide insight into the processes that predict habitat use (Sanderson & Pimm, 2015; Gobbi et al., 2017; Nolte et al., 2017). By integrating multiple forms of morphological and physiological functional traits it becomes more apparent which ecological processes drive species distributions and community composition. In addition, trophic interactions play a key role in predicting local community composition, and stable isotope composition is a reliable way to integrate this feature (McNabb et al., 2001; Kamenova et al., 2018). Stable isotope composition (δ^{15}N and δ^{13}C) can be used to estimate resource overlap among species and across sites (Jackson et al., 2011). If competitive interactions over resources significantly impact species distributions, then isotope composition should be able to separate species into distinct ecological niche space.

Documenting variation in functional traits and resource consumption as a function of environmental factors would indicate that environmental filtering is a prominent ecological process influencing habitat use (but see Kraft et al., 2015). Alternatively, if interspecific competition were driving species distributions, then co-occurrence of functionally similar species would be unlikely, and co-occurrence of more functionally distinct species would be more likely (Brandl & Topp, 1985). Montane habitats are useful systems to employ this approach, as ecological gradients are steep and mountain geography often leads to isolated natural replicates, as is the case in the volcanic Cascades Range, U.S.A. (Graham et al., 2015). In montane ecosystems, there is a long-standing notion that elevational limits are primarily influenced by ecophysiological traits and habitat specialization (Janzen, 1967). Recent research has tended to support the importance of environmental filtering in montane habitats (Kohli et al., 2018). However, there is evidence that the relative importance of competition and environmental filtering vary in relation to elevation (Machac et al., 2011), perhaps in response to stress-resource gradients (Weiher & Keddy, 1995). Interestingly, in one large-scale synthesis of global avian communities, the relative importance of competition and environmental filtering varied in a site-specific manner (Montaño-Centellas et al., 2020). This suggests the relative importance of interspecific competition might increase with increasing species diversity and/or the degree of ecological overlap of species in each community. To resolve this, it will be helpful to examine species assemblages (groups of related species) with high ecological overlap, as this could provide a strong test of how important interspecific competition is in structuring montane communities.

To understand the extent to which environmental filtering and interspecific competition influence species distributions and local community composition, we examine variation in species presence, resource consumption, and ecomorphological and ecophysiological traits across sites and peaks with changing species composition and microhabitat characteristics (**Figure 1**). A montane species complex of the ground beetle genus *Nebria* (Carabidae: Nebriini) thrives in cold temperate environments, and their assemblages provide an excellent opportunity to assess the relative importance of environmental filtering and competitive interactions in shaping communities. Species of *Nebria* are found in riparian and talus habitats that are kept cold by alpine snowmelt. It is an abundant and speciose genus, with up to 12 species on a single peak in the northern Cascades Range (Slatyer & Schoville, 2016). Species of *Nebria* have been studied extensively in this volcanic mountain range due to their diversity, their habitat use variability (spanning a broad elevational range, 0 – 3000 m above sea level), and the accessibility of sites where they occur (Kavanaugh, 1978; Mann et al., 1980; Schoville et al., 2012; Slatyer & Schoville, 2016). It is rare to find so many closely related species in a single macrohabitat (Elton, 1946), even considering tropical islands (Gillespie & Roderick, 2002). In diverse species assemblages, functional traits should evolve rapidly if competition or ecological filtering are important, making them easily detectable. Functional traits are readily measured in *Nebria*, including morphological traits and physiological traits (Slatyer & Schoville, 2016; Weng et al. 2020), as is abundance and resource consumption through analysis of stable isotope composition. Most ground beetles, including species of *Nebria*, are opportunistic carnivores across larval and adult life stages, occasionally even consuming congeneric species (Raso et al., 2014). Using species of *Nebria* as a focal system, this study will examine how species' presence, microhabitat use, and species' functional traits vary across sites in relation to species composition and environmental variation.

Community assembly processes can be scale dependent, and so we will examine several spatial scales – site and peak – as well as habitat gradients that vary both within and among sites. Changes in community composition across scales can indicate which ecological processes most influence species distribution. When there is a high degree of species turnover across scales, ecological filtering and habitat partitioning tend to dominate (Baselga, 2010). Nestedness may instead describe species diversity across scales – a pattern in which some sites contain a more diverse species set, while other sites contain subsets of the overall species diversity. Where nestedness is predominant, competition and niche partitioning strongly influence local community composition (Baselga, 2010). To this end, this study will test: 1) whether habitat and microhabitat features vary across sites and elevations used by species of *Nebria*, 2) how functional traits and resource consumption vary across species of *Nebria*, and 3) how variation in traits and resource consumption relates to variation in habitat use and local community composition.

Methods:

Focal Taxa

The taxa included are: *Nebria acuta* Lindroth, *N. crassicornis* Van Dyke, *N. eschscholtzii* Ménétriés, *N. gebleri* Dejean, *N. kincaidi* Schwarz, *N. mannerheimii* Fischer von Waldheim, *N.*

meanyi Van Dyke, *N. metallica* Fischer von Waldheim, *N. paradisi* Darlington, *N. piperi* Van Dyke, *N. sahlbergii* Fischer von Waldheim, and *N. vandykei* Bänninger. Subspecific lineages within these taxa have recently been elevated to full species status (Kavanaugh et al., 2021). Newly elevated species within these groups are morphologically and ecologically similar, often superficially indistinguishable, yet occupy isolated, distinct volcanic peaks in the Cascades Range. Due to the high similarity among these closely related species, we refer to them as species groups using a single species name. It is known that some species groups track highly similar ecological niches through climate cycles (Schoville et al., 2012; Schat et al., 2022).

Microhabitat Surveys

The elevational distribution of 12 species of *Nebria* are known for Mt. Rainier National Park, Washington, U.S.A. from extensive surveys (Kavanaugh 1978; Slatyer & Schoville, 2016). Microhabitat surveys are time-intensive, and so were conducted only on Mt. Rainier as it is the peak with the highest species diversity and habitat heterogeneity, therefore habitat partitioning is expected to be most pronounced here. Each site was surveyed once, and all surveys were conducted with permission (MORA-2019-SCI-0017). Five sites along the Nisqually River were selected as a focal area for this study, as several had been previously surveyed and species of *Nebria* were known to occur there (Slatyer & Schoville, 2016). The Nisqually River runs along the south side of Mt. Rainier and maintains a large variety of habitat types over a gradual change of ~2000 m elevation. Each survey site was at least 1 km away from the next nearest site. For rivers on other slopes of Mt. Rainier, surveys were conducted at similar elevations as the Nisqually River sites for ease of comparison. The rivers on Mt. Rainier and elsewhere in the northern Cascades Range are supplied primarily by snowpack and glacial meltwater, and so stream flow fluctuates considerably during summer months. However, sites were only surveyed if it was clear that they had not been recently disturbed by flooding.

From June to August 2019, 21 sites were surveyed across eight tributaries covering all slopes of the mountain: Carbon River, North Puyallup River, Tahoma Creek, Nisqually River, Ohanapecosh River, Deer Creek, Fryingpan Creek, and White River. Species of *Nebria* are crepuscular, with most of their activity taking place in the first three hours after sundown (Mann et al., 1980). Surveys were conducted as soon as night fell and lasted up to three hours. Specimens were collected along 100 m length transects, parallel to the riverbed. Surveys were conducted by walking in parallel lines, normal to the 100 m axis of the transect, from the edge of the river to the edge of the forest habitat. Riparian habitats where species of *Nebria* are found largely lack vegetation and transition abruptly to forest. This abrupt change in habitat and substrate marks the edge of the riverbed, and species of *Nebria* are rarely found in heavily forested habitats. At sites with extremely wide riverbeds – rocky substrate extending over 100 m between the river and forest – specimens were collected along the steep embankment between the edge of the river and the consistently "flat" patch of rocky habitat. *Nebria crassicornis* can be found away from riparian habitats (D. Kavanaugh, pers. Comm.), but these surveys did not extend beyond the riparian zone. At each site, specimens were collected for functional trait analysis.

Sites on Mt. Rainier were surveyed for microhabitat information – substrate type, substrate temperature, distance of each beetle from the river's edge – as well as ambient temperature, relative humidity, and elevation. The levels of substrate type were categorized as: mostly sand, sand & small rocks, small rocks (<10 cm in diameter), medium rocks (10-50 cm in diameter), large rocks (>50 cm), vegetated riparian zone, and snow. An analysis of variance (ANOVA), followed by a Tukey Honest Signal Difference test, was used to determine which substrate types formed statistically discernable groups based on their typical elevation. A chi-squared test was used to determine any significant association between these substrate types and species presence. Ambient temperature and relative humidity were recorded at the beginning and end of each survey and averaged (arithmetic mean). Multiple regression was then used to determine whether microhabitat characteristics varied significantly across elevations and sites. Any variables correlating with Julian Day ($r > 0.500$) were not included in subsequent analysis as this indicates a strong seasonal effect of sampling. Multinomial logistic regression was used to assess which microhabitat characteristics best predicted species' presence. All statistical analysis was completed in R v 3.5.3 (https://www.r-project.org).

Functional Traits

Both ecophysiological and ecomorphological traits are known to vary along environmental gradients in carabid species (Thiele, 1977). Among key ecophysiological traits, thermal tolerance is often considered predictive of latitudinal and elevational distributions (Addo-Bediako et al., 2000; Amundrud & Srivastava, 2020). However, past research demonstrated that thermal tolerance breadth is highly conserved in species of *Nebria* at Mt. Rainier (Slatyer & Schoville, 2016), as CT_{min} (minimum critical thermal limit) and CT_{max} (maximum critical thermal limit) do not seem to vary substantially across species or populations across elevational gradients (CT_{max} is only ~2^{o}C higher in the low elevation species *N. eschscholtzii* and *N. mannerheimii*). These experiments were conducted on adults, and very little is known about the larval stages of species of *Nebria*, which overwinter under deep snowdrifts and are very difficult to rear in laboratory settings. It is possible that there is variation in thermal tolerance across species, but that this variation is only present in juveniles. A lack of variation across adults of different species reduces the likelihood that climatic extremes act as a key filtering mechanism for community composition, but it does not rule out the importance of temperature preference, which we measure here. Another ecophysiological variable, desiccation tolerance, appears to vary among some species (Slatyer & Schoville, 2016). Therefore, we conducted physiological assays of temperature preference and desiccation rate to evaluate the role of ecophysiological traits in habitat use. We also measured several ecomorphological traits that can be used to distinguish species of *Nebria* (Palestrini et al., 2012) and are considered important for habitat usage and locomotory performance in carabid beetles (Fountain-Jones et al., 2015). Based on previous morphological trait analyses, we measured aspects of the head, pronotum, and elytra, as well as body size, as these features readily distinguish most species of *Nebria* at Mt. Rainier (Kavanaugh, 1978).

Prior to assessing thermal preference, beetles were stored for at least two weeks with a moist cotton round and food (mealworms) under a 12-hour light cycle (~5 ℃). Specimens were placed on a table with lanes and a thermal gradient ranging from approximately 0˚C to 25˚C. The location of each beetle was recorded along the thermal table at time 0 and after 15, 25, 35, 45, 55, and 65 minutes. A thermal camera (FLIR ONE® Thermal Imager, 2nd generation) was used to determine the temperature at the location of each beetle after the assay was complete. Thermal preference assays were conducted on 153 specimens across 10 species: *N. acuta* (n = 12), *N. crassicornis* (n = 15), *N. eschscholtzii* (n = 16), *N. gebleri* (n = 7), *N. mannerheimii* (n = 19), *N. metallica* (n = 19), *N. paradisi* (n = 28), *N. piperi* (n = 27), *N. sahlbergii* (n = 7), and *N. vandykei* (n = 3). An ANOVA, followed by a Tukey Honest Signal Difference test assessed any statistical difference between species.

Prior to desiccation assays, specimens were kept alive in a refrigerator in small, individual plastic containers with a moist cotton round and starved for 48 hours at 5˚C. Emptying gastrointestinal tracts prior to measuring desiccation rate ensured that any mass lost during the assay was either due to cuticular water loss, or to general metabolic processes (*i.e.* respiration). During the assay, each specimen was placed in a small plastic vial that was closed with a breathable cloth. These vials were placed within a sealed 50 mL falcon tubes with 5 mL of Drierite™, which reduces relative humidity to < 5% (Slatyer and Schoville, 2016). Specimens' initial mass, as well as their mass after 8 hours, after 24 hours, and after 48 hours were recorded. Weight loss due to desiccation was recorded from 424 specimens across 10 species: *N. acuta* (n = 47), *N. crassicornis* (n = 51), *N. eschscholtzii* (n = 50), *N. gebleri* (n = 50), *N. mannerheimii* (n = 67), *N. metallica* (n = 19), *N. paradisi* (n = 10), *N. piperi* (n = 52), *N. sahlbergii* (n = 54), and *N. vandykei* (n = 24). An ANOVA, followed by a Tukey Honest Signal Difference test assessed any statistical difference between species.

For morphological analyses, specimens were collected during surveys on Mt. Rainier in 2019 and stored in ethyl acetate saturated fiber until morphometric analysis. Preservation of specimens in ethanol has been shown to alter stable isotope composition (Jesus et al., 2015). A total of 330 individuals were photographed using a Leica scope (Leica Camera AG, 2020) and measured using ImageJ (Schneider et al., 2012). This included 433 individuals across 10 species: *N. acuta* (n = 49), *N. crassicornis* (n = 47), *N. eschscholtzii* (n = 47), *N. gebleri* (n = 48), *N. kincaidi* (n = 20), *N. mannerheimii* (n = 49), *N. meanyi* (n = 3), *N. metallica* (n = 19), *N. paradisi* (n = 38), *N. piperi* (n = 45), *N. sahlbergii* (n = 42), and *N. vandykei* (n = 26). Morphological traits measured included: antennal scape length, elytral length (along elytral suture) and width (widest point, orthogonal to the length, the location of which varied slightly among individuals and species), minimum distance between eyes (dorsally), right metathoracic femoral and tibial length, ratio of the pronotal width at its widest point to its base – pronotum width ratio (an estimate of pronotum shape), and the dorsoventral thickness of each specimen at the widest thoracic cross section. A principal components analysis was used to assess which morphological traits contributed the most to among-species variation. These results were confirmed using an ANOVA.

Local Community Composition

To understand how species composition might influence trophic ecology, surveys were extended to sites throughout the northern Cascades Range in 2021. Sites were selected based on historical surveys to measure abundance where species of *Nebria* composition is known to vary. These sites include Royal Basin in Olympic National Park, and, in the North Cascades ecoregion (encompassing North Cascades National Park), Surprise Mountain, Monte Cristo Peak, Whitechuck Mountain, Table Mountain, Cascade Pass, and the Skagit River. All sites were visited in June-August 2021 and samples were collected with permission (MORA-2019-SCI-0017, NCCO-2021-SCI-0025, OLYM-2021-SCI-0046, and WA State Scientific Collection Permit # SCHAT 21-148). Each site was visited twice to quantify species density, assuming a closed population at each site. These survey methods allowed direct comparison to previous abundance surveys (Mann et al., 1980; Slatyer & Schoville, 2016), as well as occupancy surveys (Kavanaugh, 1978; Glesne et al. 2000). Abundance surveys are inherently dependent on assumptions of detectability. These species are easy to spot across habitats, but they are certainly more visible against snow or sand than among rocky substrates. Therefore, to incorporate detection probabilities, distance data was used from surveys on Mt. Rainier in 2019 to calculate a detection curve as distance from the river's edge increases across various substrates. Empirical cumulative distribution functions were estimated from histograms of individual abundance as distance from the stream increases. These detection probabilities were used to adjust the observed values recorded from abundance surveys in 2021 throughout the northern Cascades Range. Surveys conducted in 2019 on Rainier across a variety of substrates provide a good baseline for detectability under optimal conditions.

To understand whether the relative abundance of one species varies with the relative abundance of another species, the proportion of a population composed of one species when others were present was compared to the mean proportion of the population when others were absent. For example, when assessing how the relative abundance of *N. acuta* correlates with the relative abundance of *N. mannerheimii*, one would subtract the average proportion of the population – across sites – which is *N. acuta* when *N. mannerheimii* is absent from the average proportion of *N. acuta* across sites when *N. mannerheimii* is present. If for example, *N. acuta* was 0.75 of the population on average when *N. mannerheimii* was absent, and 0.25 of the population whenever *N. mannerheimii* was present, the resulting D_{ij} value would be negative (-0.50). This indicates that *N. acuta* is relatively more abundant at sites where *N. mannerheimii* is absent. A paired t-test was used to assess differences in abundance of co-existing species when other species were present or absent.

Resource Consumption – Stable Isotope Composition

To quantify variation in resource consumption within and among species of *Nebria*, we examined isotope composition across the northern Cascades Range. During surveys in 2021, aquatic and terrestrial plant samples were collected from each site to act as a site-specific baseline for trophic position. All beetles collected for stable isotope analysis were sampled between July and August 2021 and immediately placed in a vial containing ethyl acetate-saturated fibers. Specimens across 12 species were analyzed including: *N. acuta* (n = 179), *N. crassicornis* (n = 5), *N. eschscholtzii* (n = 3), *N. kincaidi* (n = 6), *N. mannerheimii* (n = 47), *N. meanyi* (n = 34), *N. metallica* (n = 14), *N. paradisi* (n = 5), *N. piperi* (n = 35), *N. sahlbergii* (n = 10), and *N. vandykei* (n = 5). Plant samples were placed individually by site in moisture-permeable vials surrounded by a desiccant (Drierite©, $CaSO_4$). Elements of an adult insect exoskeleton often reflect the integration of larval and adult feeding, though this depends on the age of the specimen (Henden & Guédot, 2021). The left elytron was removed from each beetle and placed into a tin capsule prior to being shipped to the UC Davis Stable Isotope Facility for bulk stable isotope analysis (https://stableisotopefacility.ucdavis.edu/). Plant material was immediately placed in a desiccation chamber, identical in design to the chambers used in desiccation tolerance assays. Before placement in the tin capsules, plant material was pulverized and homogenized. Plant samples contained leaves, stems, bark, and flowers from riparian or aquatic vegetation.

MANOVAs were used to assess differences in δ^{15}N and δ^{13}C enrichment in riparian vs. aquatic plants across sites, elevations, and peaks. Δ^{15}N and δ^{13}C values for each beetle were standardized by site using plant enrichment (by subtracting plant enrichment from beetle enrichment), and a MANOVA assessed variation in the isotopic enrichment of species of *Nebria* with respect to δ^{15}N and δ^{13}C across site, elevation, and peak. Random Forest tests were used to assess which predictor contributed to the most variation in isotope composition. Increases in node purity were used to assess variable importance. These values indicate the relative number of times that a variable was used to classify a datum, and so an estimation of how informative that variable is. Standard ellipse area overlap was used to assess overlap of isotopic enrichment among co-occurring species on Mt. Rainier. Mount Rainier hosts the greatest species diversity in the northern Cascades Range, and so if resource partitioning is prominent, it will be most detectable there.

Habitat Use and Community Assembly

To assess whether variation in traits and resource consumption explains variation in habitat use, habitat features were regressed on species' traits and isotopic enrichment. To accommodate discrepancies in sample sizes for each of these variables, each variable was subset by species and resampled. The variable by which each species was least represented was used to create a baseline sample size to be collected from every other variable. If n observations were made for species x in their limiting variable, all variables were randomly sampled to achieve $n-1$ observations for species x across all variables. This was repeated 100 times for each species for each variable, and each iteration was evaluated using a generalized linear model. Based on a ratio of deviance to null deviance for each of these models, an average R^2 value was calculated from these 100 models to evaluate the relationships between functional traits, resource consumption, and habitat use. This R^2 value represents the proportional improvement of a model that includes the predictors over a model which includes only the intercept. The significance of this R^2 value was assessed against a distribution of R^2 values from 1000 null models. These null models were created from regressions using 1000 randomized trait and habitat matrices.

To assess changes in species diversity across sites, peaks, and habitat types, nestedness (β_{Nes}) (Baselga, 2010) and turnover Simpson's dissimilarity index (β_{Sim}) were calculated for each of these scales. B_{Nes} is equivalent to the difference between Sørensen's dissimilarity index and Simpson's dissimilarity index (Baselga, 2010). These values were compared to distributions of 1000 null β_{Nes} or β_{Sim} values, created by randomizing presence and absence of species across sites, habitat types, and peaks – while maintaining the total number of each species observed at each spatial scale, as well as the number of total observations at each spatial scale. Substrate type was used as a proxy for habitat type because substrate type varies across elevations, correlates with other habitat features, and provides a categorical variable by which habitat varieties can be delineated.

To assess whether similar species are more likely to co-occur, a correlation matrix of species traits was examined for statistical association with co-occurrence matrices of species across sites, habitat types, or peaks, using abundance data. The correlation matrix of species traits represents the similarity of species based on their functional traits. The co-occurrence matrices possess correlation values between species indicating the likelihood of coexistence between species at each spatial scale. A positive correlation between the trait matrix and a co-occurrence matrix would indicate that species are likely to coexist when their traits are more similar to one another, indicating strong environmental filtering. A negative correlation would imply species are more likely to coexist when their traits are more dissimilar to one another, indicating strong niche partitioning. No correlation would indicate that the species traits measured do not significantly relate to local community composition at that spatial scale.

Results:

Survey Data

Local species composition varies across elevations on Mt. Rainier (ANOVA: $F_{9,1232} = 61.61$, $p < 0.001$). A Tukey Honest Signal Difference test showed that there are statistically discernable, sequentially overlapping elevational ranges across species (**Figure 2**). *Nebria eschscholtzii* is a low elevation species, while *N. mannerheimii, N. piperi, N. metallica,* and *N. sahlbergii* are all low- to mid-elevation species. *Nebria acuta* occurs from low to high elevation, and *N. crassicornis* overlaps in elevation with *N. gebleri* in a mid-high elevation range. *Nebria* *paradisi* and *N. vandykei* occupy only high elevational ranges. *Nebria kincaidi* and *N. meanyi* were not found on Mt. Rainier in these surveys. *Nebria kincaidi* typically occurs at high elevations, and *N. meanyi* typically occurs at intermediate elevations.

Microhabitat Surveys

Substrate types vary across elevation (ANOVA: $F_{6,1235} = 73.19$, $p < 0.001$). However, based on a Tukey HSD test, "mostly sand", "sand and rocks", and "small rocks" all form one statistical group based on elevation (**Appendix 1 Table 1**). This substrate group is subsequently referred to as "gravel". A chi-squared test showed a significant association between the remaining five substrate types and species presence ($X^2_{40} = 934$, $p = 4 \cdot 10^{-170}$) (**Figure 3**). *Nebria crassicornis, N. eschscholtzii,* and *N. metallica* were all typically found on gravel. *Nebria sahlbergii* was associated with medium rocks, while *N. acuta* and *N. piperi* were associated with medium and large rocks. *Nebria gebleri* was most associated with vegetation-covered boulders in riparian areas but has been found alongside large rivers and small streams as well. *Nebria kincaidi* was most associated with large rocks and snow. *Nebria paradisi* and *N. vandykei* are associated with snow. This approximates the sequentially overlapping elevational range of these species (**Figure 2**).

Microhabitat features which correlate strongly with Julian Day (Pearson's $r = 0.500$) were disregarded from further analysis, as they could be merely a function of seasonality. Only relative humidity was strongly correlated with Julian Day (Pearson's $r = 0.800$). Based on regression of habitat features on elevation and location, ambient temperature, substrate type, substrate temperature, and distance of the beetle from the river's edge vary across elevations and across sites (adjusted $R^2 = 0.509$, $p < 0.001$). A multinomial logistic regression assessed whether these habitat features can predict species presence. Based on a likelihood ratio test, the model which included these features was more likely to accurately predict species presence than a model without these features ($X^2_{50,10} = 539$, $p < 0.001$). Some coefficients from these analyses had standard errors overlapping with zero. Based on coefficient values with standard errors not overlapping with zero (**Appendix 1 Table 2**), *N. crassicornis* and *N. eschscholtzii* tend to be found on substrates with smaller grain size and warmer temperature, and *N. eschscholtzii* is more likely to be found further from the river's edge. *Nebria gebleri* is found in habitats with larger grain sizes, colder substrate temperatures, and closer to the river. *Nebria kincaidi* and *N. paradisi* are both associated with colder substrate temperatures and greater distance from the river. *Nebria paradisi* is associated with high-elevation substrates. *Nebria mannerheimi* is associated with warmer substrates and is typically relatively further from the river, while *N. metallica* has opposite trends and prefers warmer ambient temperatures. *Nebria piperi* and *N. sahlbergii* are associated with colder ambient temperatures and being closer to the river, and *N. sahlbergii* prefers small grain-sized substrate. Lastly, *N. vandykei* is associated with colder ambient temperatures and is typically found further from the river's edge.

Functional Traits

There was a significant difference in temperature preference between species ($F_{9,1061} = 41.6$, $p < 0.001$). Except for *N. gebleri* and *N. metallica*, these preferences generally reflect the elevational distribution of these species (**Appendix 1 Table 3**). *Nebria metallica* – a low elevation specialist – overlapped in temperature preference with *N. paradisi* and *N. vandykei* – two high elevation specialists. *Nebria gebleri* and *N. eschscholtzii* – mid-high and low elevation specialists, respectively – also overlapped in temperature preference. Barring these exceptions, higher elevation species seem to prefer relatively colder temperatures, and lower elevation species seem to prefer warmer temperatures.

Proportional weight loss due to desiccation (weight loss/initial weight) was not normally distributed within *N. eschscholtzii*, *N. mannerheimii*, *N. piperi*, and *N. vandykei*. Each of these species contained a small set of individuals who lost a high proportion of weight, but otherwise the variation in distribution of their desiccation rate was unimodal and similar among species. There was a significant difference in desiccation tolerance among species ($F_{9,414} = 8.204$, $p = 2.99e-11$). However, these results were driven exclusively by *N. gebleri* (**Appendix 1 Table 4**). Because variation in this ecophysiological trait was driven by one species, it was left out of subsequent analyses.

Species can be distinguished in morphospace (**Figure 4)**. A plot of cumulative variance showed inflection after PC2, and so PC1 and PC2 were included in further analysis. These two main eigenvectors explain 88.6% of the morphological variation (PC1 = 77.1% and PC2 = 11.5% of the variance). All morphological characters, except for pronotal width ratio and antennal scape length, were highly correlated and loaded on PC1. PC1 was used as a proxy for "body size" in subsequent analyses. PC2 is almost entirely explained by pronotal width ratio and 97.2% of the variation in pronotal width ratio is loaded on to PC2. Based on ANOVAs, both body size ($F_{11,421} = 182.6$, $p < 0.001$) and pronotal shape ($F_{11,421} = 92.55$, $p < 0.001$) vary among species. While PC3 is almost entirely explained by antennal scape length, PC3 was not considered a significant source of morphological variation in further tests.

Local Community Composition

Density distribution curves across substrate types were very similar(**Appendix 1 Figure 1**). At 10 meters distance from the river, detection probabilities reached over 90% regardless of substrate type. *Nebria gebleri* was left out of this analysis as it did not occur at any of the sites surveyed for relative abundance. Among alpine specialists, *N. vandykei* and *N. paradisi*, the presence of species *j* does not appear to negatively impact species *i*'s abundance (**Figure 5**). The low elevation specialists, *N. eschscholtzii* and *N. metallica*, also did not seem to negatively impact one another's abundance. *Nebria kincaidi* and *N. meanyi*, species found at mid-high elevations, seemed to seldom coexist if at all. Interestingly, *N. acuta*, the species with the broadest elevational range, seemed most impacted by the presence of other species, except among low elevation specialists. Among pairs of species which co-occur, there was no difference in abundance of species whether others were present or absent ($t_{42} = -0.370$, $p = 0.693$).

Resource Consumption – Stable Isotope Composition

Based on a MANOVA for plant isotopic data, $\delta^{13}C$ enrichment does not vary between aquatic or riparian plants ($F_{1,18} = 1.226$, $p = 0.283$), across sites ($F_{9,18} = 2.31$, $p = 0.0624$), across elevations ($F_{2,18} = 1.289$, $p = 0.300$), nor across peaks ($F_{8,18} = 1.53$, $p = 0.215$). Aquatic plants' $\delta^{13}C$ enrichment ranged in composition from $-32.75\,{}^{0}/_{00}$ to $-18.85\,{}^{0}/_{00}$. Terrestrial plants' $\delta^{13}C$ enrichment ranged from $-32.47\,{}^{0}/_{00}$ to $-18.21\,{}^{0}/_{00}$. Similarly, plant $\delta^{15}N$ enrichment does not vary by plant type ($F_{1,18} = 2.02$, $p = 0.172$), across sites ($F_{9,18} = 1.22$, $p = 0.343$), across elevation ($F_{2,18} = 0.649$, $p = 0.534$), nor peaks ($F_{8,18} = 1.99$, $p = 0.107$). Terrestrial plant samples from Mt. Baker (high elevation) and from Mt. Monte Cristo became contaminated with mold, and so aquatic plant samples were used to calibrate isotope enrichment of beetles from these sites. Survey sites where species of *Nebria* were sampled for stable isotope analysis were at low (as close to sea level as possible), mid (intermediate), and high (above tree line) elevations for each peak. Based on MANOVAs, δ^{13}C enrichment varies among species of *Nebria* ($F_{10,316} = 60.5$, $p < 0.001$), sites ($F_{6,316} = 110$, $p < 0.001$), elevational bands ($F_{2,316} = 63.5$, $p < 0.001$), and peaks ($F_{8,316} = 310$, $p < 0.001$). Similarly, δ^{15}N enrichment varies among species of *Nebria* ($F_{10,316} = 33.5$, $p < 0.001$), sites ($F_{6,316} = 4.55$, $p < 0.001$), elevational bands ($F_{2,316} = 60.7$, $p < 0.001$), and peaks ($F_{8,316} = 32.5$, $p < 0.001$). Based on Random Forest tests, site was the most important variable in predicting variation in δ^{13}C and δ^{15}N (increase in node purity by 1178 and 160.3, respectively). These values indicate the relative number of times that a variable was used to classify a datum, and so an estimation of how informative that variable is. Peak (469.7), followed by species identity (57.65), and lastly, elevation (22.25) also contained information about variation in δ^{13}C. Elevation (84.52), followed by peak (27.81), and lastly species identity (20.84) also contained information about variation in δ^{15}N.

Standard ellipse overlap was calculated for mid-elevation species on Mt. Rainier. Mount Rainier hosts the highest species diversity in the northern Cascades Range, as well as the greatest habitat heterogeneity. This focal site was chosen due to the high overlap in species of *Nebria* distributions at mid-elevations. *Nebria acuta*, *N. mannerheimii, N. piperi,* and *N. sahlbergii* were found at this focal site. *Nebria crassicornis* was present at this site, but only two specimens were observed. Based on 100 draws from a Bayesian estimate of posterior ellipse overlap, *Nebria acuta* overlaps with *N. sahlbergii* by 69.3%, *N. piperi* by 71.0%, and *N. mannerheimii* by 69.7%. *Nebria sahlbergii* overlaps with *N. piperi* by 60.3% and *N. mannerheimii* by 59.6%. *Nebria piperi* and *N. mannerheimii* overlap by 64.8% (**Figure 6**).

Habitat Use and Community Assembly

Habitat features were regressed on species' traits and isotopic enrichment. Ambient temperature, substrate type, substrate temperature, and distance of each beetle from the river's edge varied across sites where species of *Nebria* were found and reliably predicted their presence. Distance from the river's edge was not normally distributed, and so was log-transformed, after adding $1 \cdot 10^{-11}$ to zeros. Body size, pronotal width ratio, temperature preference, nitrogen enrichment, and carbon enrichment varied significantly across species of *Nebria*. Carbon enrichment was not normally distributed, even following log-transformation, and so was excluded from this analysis. A generalized linear model was used to test the ability of functional traits to explain variation in habitats and microhabitats. Species' traits and nitrogen enrichment explain a significant portion of the variation in habitats and microhabitats used by species of *Nebria*. (ambient temperature: $R^2 = 0.118$, $p = 0.001$; substrate temperature: $R^2 = 0.188$, $p = 0$; substrate type: $R^2 = 0.145$, $p = 0$) except for distance of the beetle from river's edge ($R^2 = 0.0286$, $p = 0.353$) (**Appendix 1 Figure 2**). Smaller beetles with squarer pronota, who prefer warmer temperatures and have a more enriched diet with respect to nitrogen, prefer warmer ambient temperatures, surface temperatures, and substrates typically found at lower elevations.

Species turnover and nestedness were examined in relation to spatial scale (site and peak), as well as habitat gradient (as determined by substrate variation). Species turnover was significant across habitat types ($\beta_{Sim} = 0.269$, $p = 0$) and nearly significant across peaks ($\beta_{Sim} = 0.525$, $p = 0.064$) as was species' nestedness (habitat: $\beta_{Nes} = 0.299$, $p = 0$; peak: $\beta_{Nes} = 0.189$, $p = 0.064$) (**Appendix 1 Figure 3**). Neither species turnover nor nestedness varied significantly across sites ($\beta_{Sim} = 0.798$, $p = 0.121$; $\beta_{Nes} = 0.0894$, $p = 0.121$). Across peaks, species turnover explains more variation in species composition. Species turnover and species nestedness were similarly important along habitat gradients.

Across habitat types, species who share more similarities are more likely to coexist (Pearson's r = 0.721, p = $3.05 \bullet 10^{-14}$), indicating strong environmental filtering. Across sites, there is a nearly significant relationship between species coexistence and species similarity (Pearson's r = 0.198, p = 0.0771). Across peaks, there is no relationship between coexistence and similarity (Pearson's r = 0.0465, p = 0.680).

Discussion:

In communities with many closely related species, competitive interactions are often predicted to drive species distributions more than physiological constraints due to high niche overlap (Elton, 1946; Niemelä, 1993). However, a competing hypothesis is that species distributions in regions with pronounced ecotones might be more strongly structured by axes of environmental variation (Hutchinson, 1959). In environments with extreme conditions, species often partition their habitats into microhabitats, where conditions are locally stable (Gereben, 1995; Scheffers et al., 2014). If environmental filtering were the dominant ecological process dictating species ranges, we would expect to observe a direct relationship between species' traits, particularly ecophysiological traits (Janzen, 1967), and habitat and microhabitat features, as well as a high degree of turnover across ecotones (Baselga, 2010).

In the northern Cascades Range, we found that the presence of species of *Nebria* does vary across ecotones and can be predicted by a combination of ecophysiological and ecomorphological traits. Species composition varies significantly across peaks and habitat types (*i.e.* substrate variation within sites and across elevations). Across habitat types, nestedness and turnover explain similar amounts of variation in species composition. Across peaks, turnover explains much more variation in species across peaks than nestedness, suggesting that dispersal across peaks is low. Simultaneously, temperature preference and ecomorphological traits varied in relation to substrate microhabitat and elevation. If competitive interactions for resources were a primary factor in shaping communities, differences in relative abundance and resource utilization should have varied more strongly among species within sites as an outcome of niche competition (Brandl & Topp, 1985). Instead, our results show that resource consumption varies more across locations than among species, and diet enrichment overlaps greatly among coexisting species. Across sites, local species abundance is not strongly influenced by species composition. However, while our results imply that environmental filtering is the dominant process for this community, we did observe that species differ in microhabitat usage at sites, functional traits did not converge at the site scale, and species do vary in their resource consumption.

Functional trait variation across ecological gradients

It is surprising that physiological constraints don't directly predict habitat use in this montane beetle community (Slatyer and Schoville, 2016), as physiological tolerance typically influences species presence in other alpine communities (Block et al., 2003; Pallarés et al., 2019; Schat, et al., 2022). The observed variation in the temperature tolerance of species of (Slatyer & Schoville, 2016) and desiccation tolerance is relatively minor and is indicated in only one or two species. Temperature tolerance and desiccation tolerance are generally regarded as defining features of insect communities' distributions (King & Sinclair, 2015). However, temperature preference appears to differ significantly among species of *Nebria* in our study system and is associated with their elevational range. This indicates that species of *Nebria* diverge in behavior as they diverge in microhabitat use, a potential mechanism for microhabitat partitioning. Indeed, a similar result has been shown to be important in other species of *Nebria* from the European Alps (Gereben, 1995). Nestedness does explain some of the diversity in these communities across habitat types, and habitat partitioning is an often-proposed mechanism for such patterns (Gering et al., 2003; Baselga, 2010; da Silva et al., 2018). One caveat is that studies of ground beetle distributions have found that environmental filtering can be secondary to other processes, such as priority effects (Gobbi et al., 2007). Unfortunately, our data do not provide tests for the importance of priority effects or historical contingency. However, two observations can be made. First, annual variation in snowmelt frequently disrupts downstream habitats, yet species distributions have remained relatively constant for more than 50 years (Kavanaugh, 1979; Erwin et al., 1979; Mann & Edwards, 1980; Slatyer & Schoville, 2016). This suggests rather stable responses to existing environmental gradients, even when relative abundance might be impacted by stochastic events over time. Secondly, over evolutionary timescales, allopatric populations of these species have responded to climate cycles by tracking their preferred habitats in different mountains (Schoville, et al., 2012; Schat et al., 2022), and therefore current species' distributions don't strongly indicate priority effects or historical contingency.

As species of *Nebria* follow their respective ecological niches over time, morphological variation appears to be a good predictor of where species occur. Morphological features such as pronotal shape and body size could greatly impact how these geophilic insects navigate substrate texture (Weiser & Kaspari, 2006; Talarico et al., 2007; Jachertz et al., 2019). These flightless insects forage, hide, reproduce, and oviposit in terrestrially bound communities, so their ability to rapidly traverse a spectrum of grain sizes present in riparian habitats along an elevational gradient is important. Substrate type does vary significantly with elevation, as expected with the erosion and weathering from precipitation and glacial processes (Shuster et al., 2011). Substrate variation has been known to drive community assembly in ground beetles (Gobbi et al., 2007; Jachertz et al. 2019) and habitat partitioning at the local scale (Komlyk & Brygadyrenko, 2020). The consistent relationship between morphological features and environment has encouraged the use of ground beetles as indicator species in a variety of habitat types (Koivula et al., 2002; Schirmel et al., 2015; Nolte et al., 2017). Thus, our results are consistent with other studies on ground beetles indicating that morphological traits directly influence how individuals interact with their environment – such as foraging regime (Talarico et al., 2007).

Resource consumption across elevational gradients

Within a trophic guild, isotopic enrichment can be used as a reliable proxy for resource consumption and competitive hierarchy (Jackson et al., 2011). While there were significant differences in $\delta^{13}C$ and $\delta^{15}N$ enrichment across species of *Nebria*, enrichment also varied along elevational and latitudinal (peak) gradients and varied most strongly by site. If competition for resources were driving species distributions, it is expected that $\delta^{13}C$ and $\delta^{15}N$ enrichment would vary most strongly among species within sites (Zalewski et al., 2014; Parent et al., 2014). Instead, even species in relatively high-diversity environments, such as on Mt. Rainier, can overlap isotopically by as much as 71%. It is notable, however, that this analysis only includes bulk isotope composition. While average enrichment among species was high, this is not necessarily indicative that dietary composition strongly overlaps. Isotopic enrichment is the result of nutrient integration across multiple feeding events. For example, an individual with a mid-enrichment diet and an individual with a highly variable diet may have similar overall enrichment levels due to averaging across time.

Interestingly, while enrichment was stratified by elevation across species of *Nebria*, there was no relationship between elevation and stable isotope enrichment in plants in these riparian habitats. One would expect isotopic enrichment in δ^{13}C and δ^{15}N to vary across elevations in plants, as plant communities are much more diverse downstream (Zah et al., 2001). Based on the δ^{13}C enrichment of plant samples collected, these communities' food webs seem to be terrestrially sourced with continual allochthonous input across elevations (Zah et al., 2001; Cloern et al., 2022). This can be further examined by assessing δ^{2}H and δ^{18}O enrichment in plants and beetles, as these isotopes vary among flowing water (steam-originated sources) and pre-precipitation water sources, such as lakes, which tend to irrigate terrestrial ecosystems (Mondal et al., 2023). What could explain the discrepancy between plant and beetle isotope enrichment is a shift in diet across elevations. Carnivorous alpine arthropod communities are often supported by aeolian deposition (wind-blown organic matter), a mercurial influx of resources that vary seasonally and primarily include smaller herbivorous insects, as well as algae-feeding Collembola in snowfields (Edwards, 1987), both low-enrichment resource. Downstream riparian habitats may support a greater diversity of inputs available to species of *Nebria*, including other ground beetle species. Compound-specific analysis would illuminate whether this apparent enrichment is due to an enriched diet, or an enriched baseline in the food web of downstream communities. While environmental filtering appears to be the primary factor in predicting habitat use among species of *Nebria*, our results do not rule out a secondary role for local habitat partitioning.

Community dynamics under climate change

At lower altitudes on more northern peaks in the Cascades Range, such as Mt. Baker, similar habitats are found that occur at higher altitudes on more southern peaks, such as Mt.

Rainier. Elevational ranges are more compressed on northern peaks, shortening habitat ecotones. This shortening effectively reduces the available riparian habitat to species of *Nebria* and reduces the ability of species to coexist by partitioning habitats into microhabitats. Across more southern peaks, ecotones are extended over a larger range, especially on larger mountains. Across peaks, mid-elevation habitats are likely to have the highest overlap of species' ranges and possess an intermediate climate. While many species can occupy this intermediate elevational range, such as *N. acuta*, *N. mannerheimii*, *N. piperi*, and *N. sahlbergii*, there is only one peak in the northern Cascades where all the species in this study have been recorded to co-occur – Mt. Rainier. Mount Rainier is one of the tallest peaks in the Cascades Range and possesses the greatest range in habitat types across elevations. It is likely that this environmental variation and extended ecotone permits more extensive habitat partitioning among otherwise ecologically similar species. At the same time, more southern peaks, such as those found in the Sierra Nevada Range, are drier, and moisture is often a limiting resource of insects (King & Sinclair, 2015). As climate change progresses, communities occupying peaks with relatively shorter ecotones will be among the most vulnerable. Our limited evidence for interspecific competition suggests species are most vulnerable to changes in abiotic conditions, rather than biotic interactions. In the triage of conservation, it may be most prudent to prioritize management, including preservation of habitat, at peaks where many species can coexist, and greater habitat heterogeneity is present.

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Author Contributions:

JS and SS designed and wrote the primary draft of the study. DK provided conceptual guidance. JS, JW, GW, and HX collected the data. JS conducted all statistical analyses. JS and SS contributed to the writing of the manuscript.

Conflict of Interest Statement:

The authors do not have any conflicts of interest to declare.

<u>Figures</u>

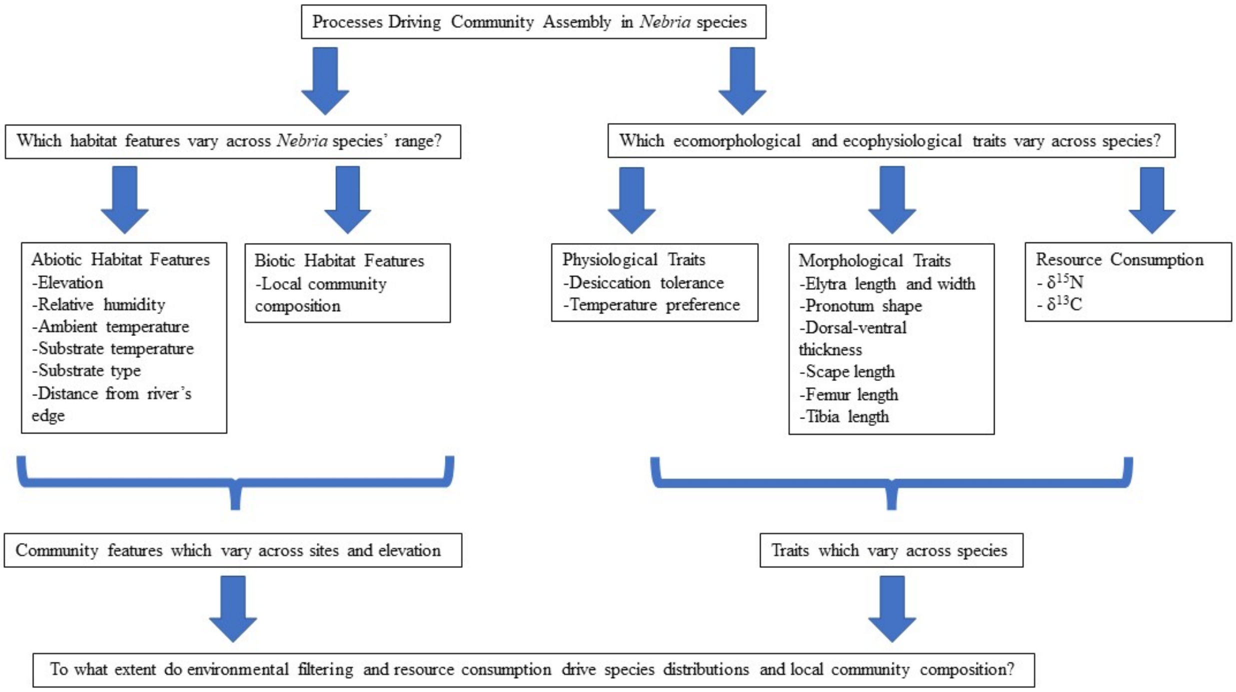


Figure 1 Conceptual diagram relating community composition to functional traits and habitat and community features.

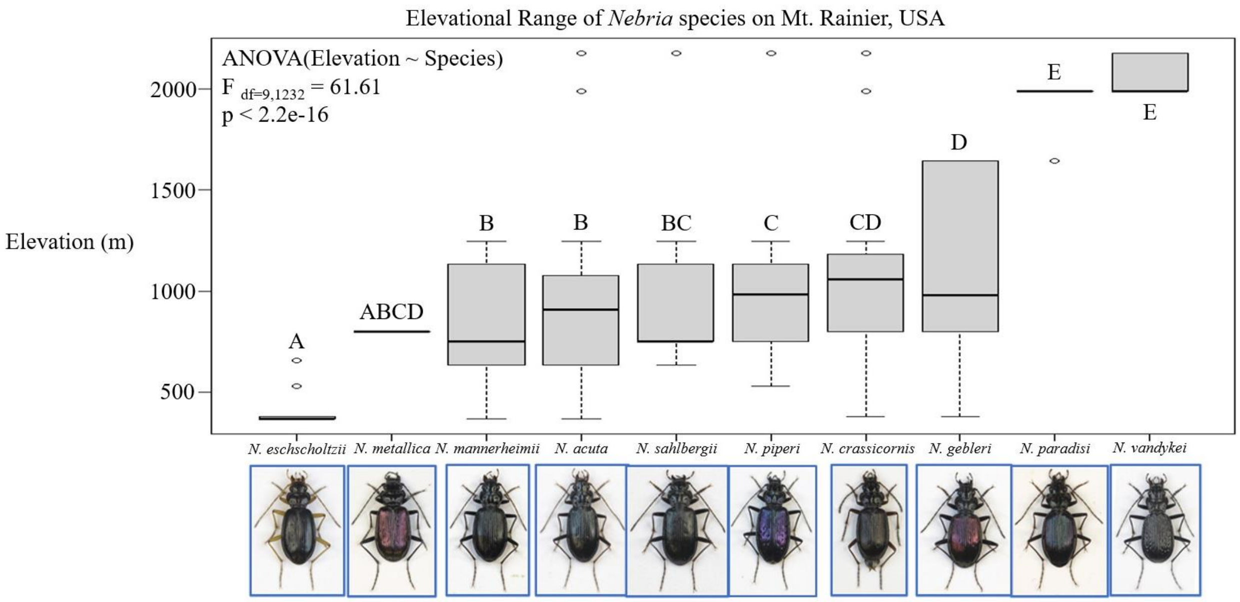


Figure 2 Species of *Nebria* sampled in this study and their elevational ranges on Mt. Rainier. Letters above box plots indicate statistically grouped elevational ranges based on an ANOVA followed by a Tukey HSD test (PC: Roman Dudko, 2014).

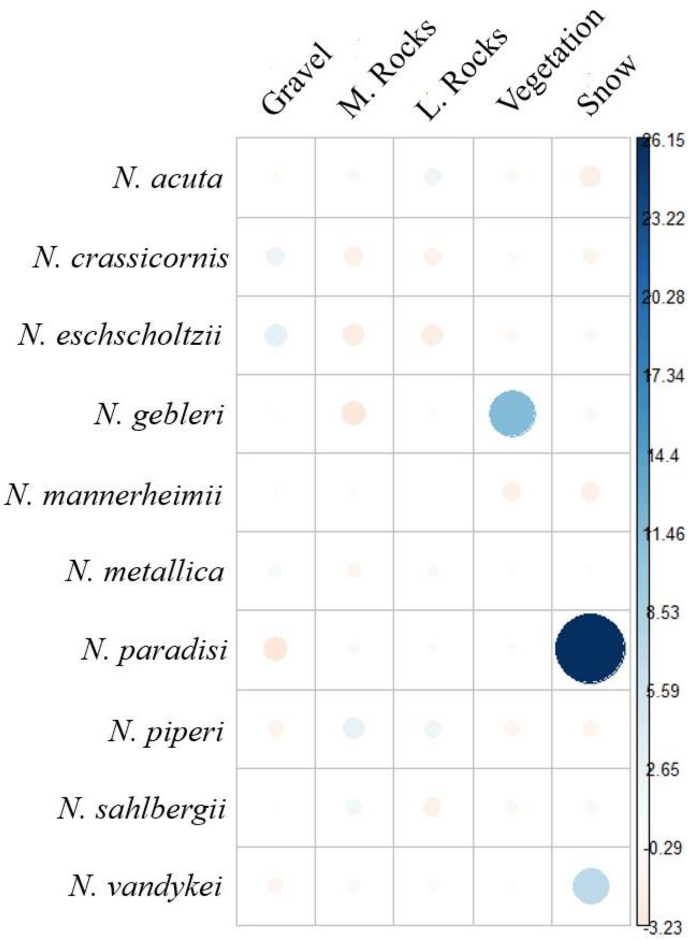


Figure 3 Residuals from a chi-squared analysis of substrate and species presence. Darkening bluer hues indicate that the species is more likely to be found on that type of substrate. Darkening redder hues indicate that the species is unlikely to be found on that type of substrate.

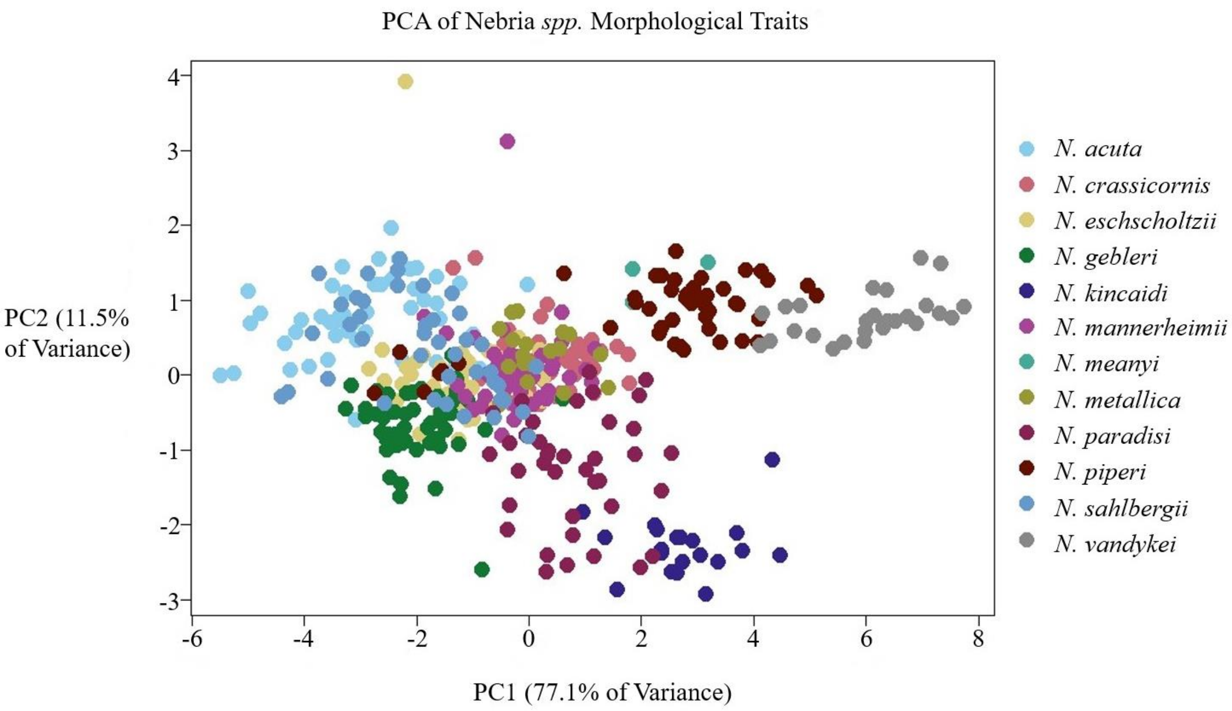
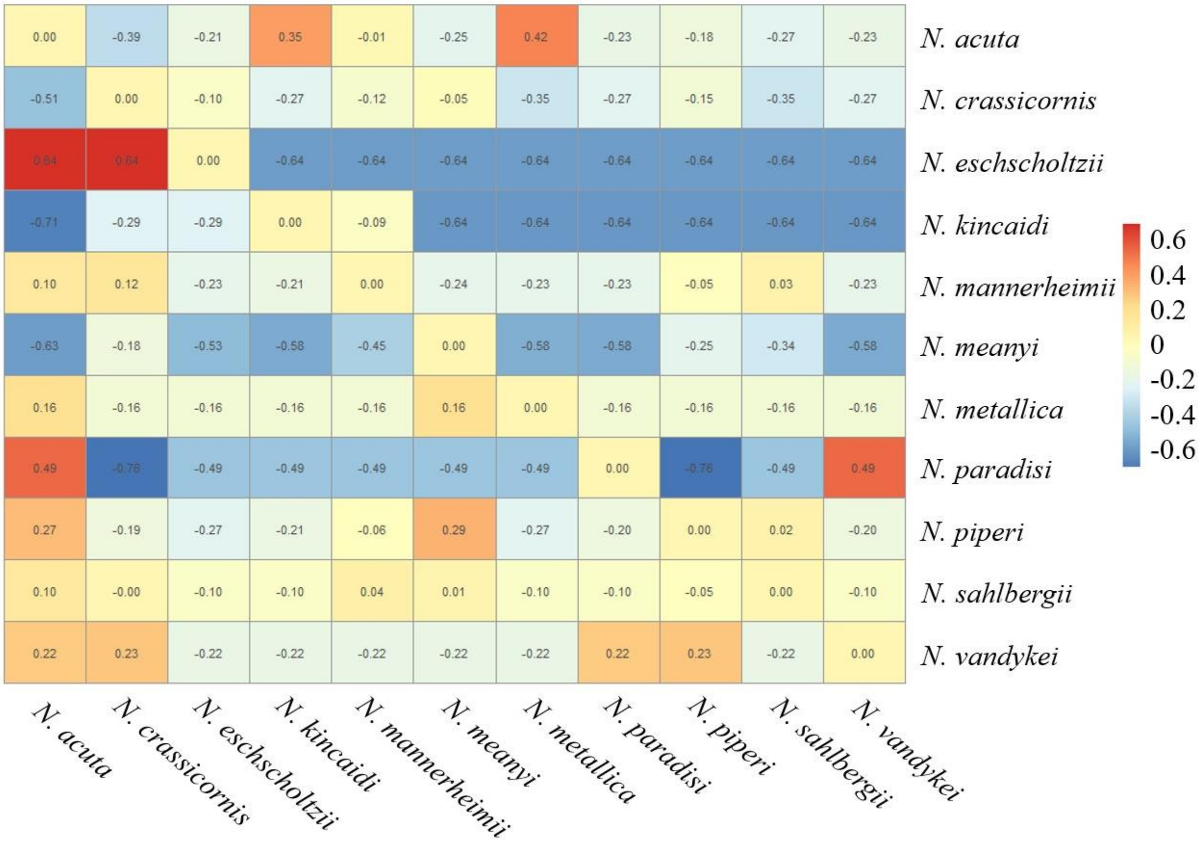


Figure 4 Principal components analysis of morphological features of species of *Nebria*. Morphological traits measured included: antennal scape length, elytral length (along elytral suture) and width (widest point, orthogonal to the length, the location of which varied slightly among individuals and species), minimum distance between eyes (dorsally), right metathoracic femoral and tibial length, ratio of the pronotal width at its widest point to its base – pronotum width ratio (an estimate of pronotum shape), and the dorsoventral thickness of each specimen at the widest thoracic cross section.



	N. acuta	*N. crassicornis*	*N. eschscholtzii*	*N. kincaidi*	*N. mannerheimii*	*N. meanyi*	*N. metallica*	*N. paradisi*	*N. piperi*	*N. sahlbergii*	*N. vandykei*
N. acuta	0.00	-0.39	-0.21	0.35	-0.01	-0.25	0.42	-0.23	-0.18	-0.27	-0.23
N. crassicornis	-0.51	0.00	-0.10	-0.27	-0.12	-0.05	-0.35	-0.27	-0.15	-0.35	-0.27
N. eschscholtzii	0.64	0.64	0.00	-0.64	-0.64	-0.64	-0.64	-0.64	-0.64	-0.64	-0.64
N. kincaidi	-0.71	-0.29	-0.29	0.00	-0.09	-0.64	-0.64	-0.64	-0.64	-0.64	-0.64
N. mannerheimii	0.10	0.12	-0.23	-0.21	0.00	-0.24	-0.23	-0.23	-0.05	0.03	-0.23
N. meanyi	-0.63	-0.18	-0.53	-0.58	-0.45	0.00	-0.58	-0.58	-0.25	-0.34	-0.58
N. metallica	0.16	-0.16	-0.16	-0.16	-0.16	0.16	0.00	-0.16	-0.16	-0.16	-0.16
N. paradisi	0.49	-0.76	-0.49	-0.49	-0.49	-0.49	-0.49	0.00	-0.76	-0.49	0.49
N. piperi	0.27	-0.19	-0.27	-0.21	-0.06	0.29	-0.27	-0.20	0.00	0.02	-0.20
N. sahlbergii	0.10	-0.00	-0.10	-0.10	0.04	0.01	-0.10	-0.10	-0.05	0.00	-0.10
N. vandykei	0.22	0.23	-0.22	-0.22	-0.22	-0.22	-0.22	0.22	0.23	-0.22	0.00

Figure 5 Heatmap depicting the difference in species' (*i*) relative abundances when other species (*j*) are present or absent. Redder hues and more positive values mean that species *i* is more abundant when species *j* is present than when species *j* is absent. Bluer hues and more negative values mean that species *i* is more abundant when species *j* is absent than when species *j* is present.

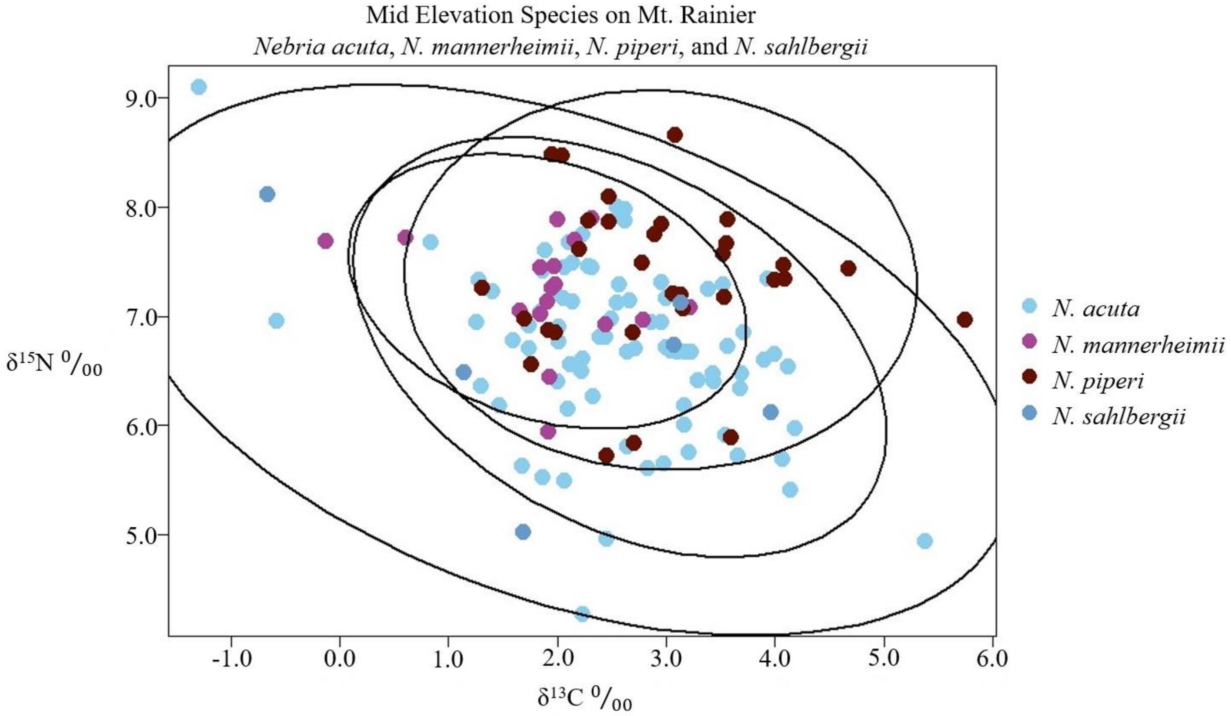


Figure 6 Isotopic overlap (δ^{13}C and δ^{15}N) of mid-elevation species on Mt. Rainier, plotted with standard ellipses from maximum likelihood estimates of isotopic variation within sites. Species include *N. acuta*, *N. sahlbergii*, *N. piperi*, and *N. mannerheimii*. *Nebria crassicornis* was found at this site, but excluded from this analysis as only two individuals were observed at this site. Each ellipse is one species.

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Chapter 2: "Ecomorphological convergence following niche shifts in montane ground beetles (Carabidae: *Nebria*)"

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Open Research Statement: Morphometric data, specimen photographs, and R code used in this study are open access: https://doi.org/10.5061/dryad.m63xsj486.

Abstract

A major goal of macroevolution is to uncover generalizable patterns of phenotype-niche relationships. Ecomorphological traits provide for a useful phenotypeas they are easily measured and predictive of ecological niche. Habitat use is an important aspect of niche; a species' abiotic requirements are foundational to that species' fundamental niche and remain an important aspect of the realized niche for many species. Searching for generalizable patterns between ecomorphological traits and habitat use within adaptive radiations in abiotically challenging environments can be fruitful. High diversification and high ecological specialization unfolding in the context of strong environmental filtering provide opportunit to understand the role of evolutionary processes such as parallel and convergent evolution in forming observed trait-habitat use patterns. Monatne species complexes of the Holarctic ground beetle genus *Nebria* (Carabidae: Nebriini) provide such an opportunity. In these communities, environmental filtering is the primary factor predicting species presence. Their ecomorphological traits predict habitat use, and species of *Nebria* are habitat specialists. These species occupy sequentially overlapping, but statistically discernable elevational bands along streams, from 0-3000 m. above sea level. Using their molecular phylogeny, habitat use, and morphometrics, this study aimed to understand the role of parallel and convergent evolution in ecomorphological trait evolution. Ecomorphological traits measured in this study included: pronotal ratio (widest point divided by the base), pronotal shape (Fourier decomposition), elytral length, elytral ratio (length divided by width), elytral shape (Fourier decomposition), and antennal scape length. Each of these traits are evolving slower than expected under Brownian motion evolution. Species of *Nebria* cluster in multivariate morphospace according to relatedness, but habitat use and relatedness together are the best predictors of morphological variation. In addition, there is evidence for convergence upon a “riparian” morphotype based on morphological similarity, and additional evidence for “riparian” and “alpine” morphotypes when phylogenetic distance is considered. In communities with strong environmental filtering, similar species are more likely to coexist. In some communities, rapid diversification followed by slowed ecomorphological evolution indicates that convergent evolution plays a significant role in shaping community composition and niche acquisition. A combination of ancestral constraint and convergence explains trait-habitat use patterns in this community. Microhabitat features can be an important aspect of the distribution of species of *Nebria* and should be examined closely in future studies.

Introduction

A major goal in macroevolution is to discover generalizable patterns of phenotypic evolution and niche evolution (Weber et al., 2017). Examining the evolutionary processes that underlie adaptive radiations provides critical insight into the rules shaping community formation, through phenomena such as convergent evolution. Convergent evolution often arises in species radiations as a result of the interaction between traits and environment, where different species evolve similar traits in response to resource availability and/or habitat-based performance (terHost et al., 2010; Muschick et al., 2012). One common pattern is for physical form to evolve predictably to environmental conditions (Vidal-Garcia & Keogh, 2015). The similarity in physical forms can also arise due to parallel evolution, which occurs when selection on shared ancestral variation leads to the same phenotype (Losos, 2011). While these processes can be difficult to distinguish in a recent species radiation, if the relationship between functional traits (D’Andrea & Ostling, 2016) and niche occupancy (Elton, 1946) can be explained by patterned, repeated development of traits across a phylogeny, as opposed to recent shared ancestry, convergent evolution must be occurring (Pigot et al., 2020). When such patterns are found, macroecologists can begin to disentangle the relative importance of ecological processes such as succession, competition, and environmental filtering to community assembly.

Numerous studies have documented how ecological opportunity drives adaptive radiations, for example in finches (Grant & Grant, 2002), cichlids (Sturmbauer, 1998), *Anolis* spp. lizards (Butler & King, 2004), and spiders (Gillespie et al., 2018), among other taxa. Separately, other studies have looked for repeated ecological trait patterns in nature, such as size ratios among closely competing beetles (Hutchinson, 1959; Brandl & Topp, 1985) or other functional morphological traits (Stayton, 2006; Pigot et al., 2020). By mapping functional morphological traits and habitat usage onto phylogenetic trees, one can test the relationship between traits and niche while controlling for evolutionary history. Here we examined this phenomenon in the Holarctic ground beetle lineage *Nebria* (Coleoptera: Carabidae), which appears to represent a relatively recent species radiation (Slatyer & Schoville, 2016; Kavanaugh et al., 2021). Species of *Nebria* occur in riparian habitats across a wide range in elevation (0 – 3000 m. above sea level), where they occupy sequentially overlapping, but statistically discernable elevational ranges and have close associations with microhabitat type (Kavanaugh, 1979). In these communities, environmental filtering appears to be the dominant ecological process influencing species distributions (J. Schat, unpublished data). The microhabitats used by species of *Nebria* vary in composition from small sandy riverbeds, pebble-bound streams, rocky rivers, boulder-entrenched rivers, to talus snowfields. These habitats occur in isolated mountains across the Northern Hemisphere, where independent lineages of these species have evolved due to geological barriers (Kavanaugh et al., 2011). While adaptive radiations have been studied in many different environments, few examine cold temperate communities where species richness is typically low. *Nebria* is an interesting example of a taxon that has high species richness in these habitats, forming species assemblages of co-occurring species at local scales.

Body size and pronotal width ratio are both ecomorphological traits that can reliably predict habitat type of the 12 species of *Nebria* present on Mt. Rainier, U.S.A in the Cascades Range (J. Schat, unpublished data), as well as *Nebria* species in Russia (pers. comm. R.Y. dudko). In many species, ecomorphological traits evolve as a result of niche expansion or contraction (D’Andrea & Ostling, 2016), making these features ideal characteristics for testing hypotheses of repeated patterns in niche acquisition. Pronotal and elytral shapes represent morphological traits related to habitat usage and locomotion in carabid species, and are known to correlate with environmental gradients (Thiele, 1977). By comparing variation in these ecomorphological traits across a phylogeny, the relative rates of ecomorphological divergence can be estimated. For comparison, antennal scape shape will be used to compare the rate and pattern of morphological trait evolution, as it is not expected to vary in relation to habitat type, yet frequently diverges among closely related species (Weng et al, 2020). Here, we will examine whether morphological functional traits converge within riparian or alpine communities where constituent species of *Nebria* vary, to determine if ecomorphological traits consistently respond to habitat selection in geographically isolated montane ecosystems. Specifically, we hypothesize that species will show evidence of convergence in ecomorphological traits in relation to habitat specialization (*i.e.*, riparian versus alpine habitats). Under a null model of Brownian motion, trait evolution follows a random walk that is constrained by recent ancestry, with more closely related species being more similar and variation increasing linearly with evolutionary distance (Pagel, 1997; Ives & Zhu, 2006; Ives, 2020). Repeated evolution of similar forms in similar environments, across distantly related species in a phylogeny would provide strong evidence for convergent evolution (Losos, 2011), due to directional selection pressures.

Methods

Focal Taxa

Species of *Nebria* have a Holarctic distribution and occupy primarily talus snowfield and riparian habitats in montane ecosystems (Kavanaugh, 1978; Schoville et al., 2012; Kavanaugh et al., 2021; Chapter 1). This study focuses on species of *Nebria* within their Nearctic range due to the accessibility of specimens for morphometric analysis, but it includes Palearctic species of *Nebria* that are closely related to our focal taxa in order to represent the phylogenetic clades as completely as possible (**Figure 1**, figure made using the R packages mapview (Appelhans et al., 2022), sf (Pebesma et al., 2018, 2023), and tidyverse (Wickham et al., 2019)). Specimens that were photographed for morphometric analysis are housed within the California Academy of Sciences, U.S.A. Across 79 species, an average of 17 specimens per species were used in this study (mean = 17.4, sd = 4.00, see sampling details in **Appendix 1 Table 1**).

Morphometrics

Photographs of species of *Nebria* were taken using a Leica camera mounted to a dissecting scope (Leica Camera AG, 2020). Raw images of the pronotum and elytra were processed to generate silhouettes for downstream analysis, using the software Inkscape (Inkscape 2020). Antennal scape length, elytral length (along elytral suture), ratio of the elytral length to the elytral width (elytral width ratio), and ratio of the pronotum at the widest point to the base (pronotal width ratio) were measured on each specimen using ImageJ (Schneider et al., 2012) (**Figure 2**). These features are known to vary across species of *Nebria* in the northern Cascades Range and predict habitat usage (J. Schat, unpublished data).

All statistical analyses in this study were performed using R v4.2.0.1 (R Core Team, 2021). A multivariate analysis of variance (MANOVA) was used to assess whether these traits vary across the species of *Nebria* analyzed in this study. The Student's T-test was used to assess whether these traits vary between sexes. Fourier decomposition was used to extract shape features from silhouettes (**Figure 2**) of elytra and pronota using the R package Momocs (Bonhomme et al., 2014), while the tibble package was used to organize data tables (Wickham, 2023).

Habitat Use

Locality data from specimen labels were used to estimate habitat and local climate conditions. When available, GPS coordinates were used to directly extract local climatic information. Otherwise, approximate locations were determined based on specimen labels. Google Earth (Google Maps 2021) was used to approximate the substrate type, elevation, and coordinates (when not directly stated on labels) where beetles were collected. The online database WorldClim (Fick & Hijmans, 2017) was used to estimate mean annual ground temperature (˚C), maximum annual ground temperature (˚C), minimum annual ground temperature (˚C), mean annual solar radiation (kJ/m^2/day), mean annual vapor pressure (kPa), annual precipitation volume (mm), and mean annual wind speed (m/s) for each site. Estimates for each of these variables are accurate to the nearest 30 arcseconds (~1 km^2). Habitat variables were examined for cross-correlation and were only included in further analysis if they were not strongly correlated with one another (if the absolute value of Pearson's $r < 0.75$). Species of *Nebria* respond to shifts in climate and utilize microhabitats to reduce microclimate variation across their ontological development. For example, larvae and adults retreat under snowdrifts or rocks during the daytime, whereas they hunt for Collembola and other arthropod prey at night (Slatyer & Schoville, 2016; Mann et al., 1980; Edwards, 1987).

The tree line in montane ecosystems represents a stark demarcation of alpine habitat. In systems where environmental filtering is dominant, such contrasting, close-proximity habitats can be a useful setting in which to test for ecomorphological convergence based on habitat type. Körner & Paulsen (2004) found that alpine habitats in temperate zones typically have a seasonal (growing season) mean ground temperature of 7-8˚C, while subarctic and boreal alpine habitats have a seasonal mean ground temperature of 6-7˚C. This thermal threshold corresponds to the tree line in these habitats, with alpine habitats found above the tree line. While the specimens used in this study could be categorized as temperate, boreal, or subarctic based on their locality data, many species analyzed in this study occupy ranges that span multiple biomes. We tested for convergence in "alpine" (above the tree line) and "riparian" (below the tree line) morphotypes using thresholds of mean ground temperature (6˚C or colder, as well as 7˚C or colder) to define alpine habitats. Convergence under each of these thermal conditions was assessed separately.

Phylogenetic Relatedness

A multi-locus molecular phylogeny by Kavanaugh et al. (2021) was used to identify the role of phylogenetic relatedness in trait variation (**Figure 3**). This phylogeny was based on maximum likelihood estimates of relatedness using a concatenated dataset that included the nuclear ribosomal gene 28S, nuclear protein coding genes CAD2, PEPCK, Topo, and wg, and the mitochondrial genes 16S-ND1, COI BC, and COI PJ (Kavanaugh et al., 2021). Nodes with less than 90% bootstrap support were collapsed. While the full phylogeny is rooted with numerous outgroups and other *Nebria* taxa, we pruned the tree to contain only our focal taxa. The assignment of species to riparian and alpine habitats suggests these designations are not clade-specific, but rather repeatedly occur throughout the phylogeny of *Nebria*.

Statistical Analysis of Morphometrics, Habitat Use, and Phylogenetic Relatedness

K means cluster analysis was used to discern if species cluster in morphospace. Linearly measured traits (elytral length, elytral ratio, pronotal ratio, and antennal scape length), elytral shape, and pronotal shape were each analyzed separately. The number of clusters was determined based on minimizing the within-group sum of squared distance from k centroids.

Next, in a separate analysis, regression was used to assess the ability of habitat use and phylogenetic relatedness to predict morphological variation in species of *Nebria*. Habitat features and relatedness were assessed separately in two different models, and together in the same model. Based on a power analysis using the R package simmr (Green & Macleod, 2015), a sample size of approximately fifteen individuals per species is needed to detect an effect size of 0.05 with a power of 80% and $\alpha = 0.05$ in a generalized mixed model. This sample size was achieved in nearly all species. In models that included relatedness as a predictor, the phylogeny of species of *Nebria* was converted to a correlation matrix of relatedness among species, assuming Brownian motion as a model for the rate of evolution. In models that included habitat features, latitude and elevation were included as fixed effects. These models used a correlation matrix of linearly measured traits (antennal scape, elytral length, elytral ratio, pronotal ratio), or a correlation of pronotal shape features, or a correlation matrix of elytral shape features as response variables. Likelihood ratio tests were used to compare models which included relatedness and/or habitat characteristics as predictors.

Convergence and Trait Evolution

Traits undergoing directional selection will likely experience faster rates of change than expected by random chance (Clegg et al., 2002), especially in the early stages of this process. Traits undergoing stabilizing selection will likely experience slower rates of change than expected by random chance (Wojcieszek & Simmons, 2012, but see Lemos et al., 2001). The function search.shift in the R package RRphylo (Castiglione et al., 2019) was used to search for shifts in the evolutionary rate of ecomorphological traits across species lineages. It uses the results from a phylogenetic ridge regression (using the function RRphylo in the same package), which estimates the ability of the phylogeny to explain current phenotypes. The search.shift function then estimates clade-specific evolutionary rates which explain this relationship. In this study, the minimum clade size was set to one branch. We also tested differential rates of ecomorphological traits between alpine and riparian species. Using the “sparse” argument for status.type, instead of “clade”, both “alpine” and, separately “riparian” was tested for differential rate of evolution from the rest of the tree. More than half the species are alpine, and so testing for both specializations as the “state” of interest may yield different results.

Morphological convergence towards alpine and riparian morphotypes were assessed among species of *Nebria* assuming an alpine mean ground temperature of 6˚C, and separately, 7˚C. To assess convergence upon alpine or riparian morphotypes, two methods were used. First, several metrics of convergence were calculated (C1-4) based on the methods described in Stayton (2015). C1 tests for evidence of convergence by calculating: $1\text{-}D_{tip}/D_{max}$. Here, D_{tip} represents the greatest phenotypic distance between species, and D_{max} represents the greatest phylogenetic distance between species. Values for C1 range between 0-1, with a larger value indicating greater convergence. C2 estimates the proportion of phenotypic distance "closed" by convergent evolution and is calculated by: D_{tip}/D_{max}. C3 indicates the proportion of morphological change within a lineage that can be explained by convergence and is calculated by: $C2/L_{tot.lineage}$. The value $L_{tot.lineage}$ is the total morphological change in that lineage from their ancestral state. Lastly, C4 indicates the proportion of morphological change within a clade that can be explained by convergence and is calculated by: $C2/L_{tot.clade}$. The value $L_{tot.clade}$ is the total morphological change in that clade from the ancestral state (Stayton 2015). These calculations were performed using the function calcConv in the R package convevol (Brightly & Stayton 2023). The significance of these values was assessed using permutation tests.

Second, species of *Nebria* were plotted in multivariate morphospace. The angle between pairs of species were used to approximate the similarity between species, with a smaller angle indicating greater similarity (Sansalone et al., 2020). Using the search.conv function in the R package RRphylo (Castiglione et al., 2019), convergence upon alpine or riparian morphotypes was assessed. If angles between species that occupy the same habitat type are smaller than the average angle among all species (if alpine species cluster with other alpine species in morphospace, for instance), then species may be converging on a habitat-specific morphotype. Additionally, if no convergence is occurring, the angle between species should increase with time since divergence (Sansalone et al., 2020). The angle between species of the same habitat category, and angle between species per unit time was used to quantify convergence. The significance of these findings was assessed using permutation tests.

Results

Morphometrics

We first assessed whether *Nebria* species could be distinguished based on our measured morphological trait data. Based on MANOVA tests, elytral ratio ($F_{78,1295} = 13.21$, $p < 0.001$), pronotal ratio ($F_{78,1295} = 41.39$, $p < 0.001$), antennal scape length ($F_{78,1295} = 135.9$, $p < 0.001$), and elytral length ($F_{78,1295} = 132.1$, $p < 0.001$) all vary significantly among species of *Nebria*. Only elytral length varies by sex ($t_{1371} = 2.96$, $p = 3 \bullet 10^{-3}$). While statistically discernable, the distributions of elytral length of males and females are highly overlapping (**Appendix 1 Figure 1**), and so males and females were grouped together by species in further analyses. All linearly measured morphological traits are approximately normally distributed across species of *Nebria* (**Appendix 1 Figure 2**).

Morphometrics, Habitat Use, and Phylogenetic Relatedness

We next examined whether habitat features and phylogenetic relatedness account for variation in morphological traits among species of *Nebria*. Habitat characteristics where species of *Nebria* were collected are approximately normally distributed across species (**Appendix 1 Figure 3**). Based on the within-group sum of squared distance from centroids, elytral shape features formed four clusters in morphospace, pronotal shape features formed three clusters, and linearly measured traits together formed three clusters. MANOVAs showed that none of these clusters correspond to variation in habitat use (**Appendix 2 Figures 4-6**), including variation in elevation, latitude, longitude, solar radiation, vapor pressure, mean precipitation, wind speed, substrate type, nor mean, maximum, or minimum ground temperature. However, after converting the phylogeny to a correlation matrix, analyses of variance (ANOVA) showed that relatedness explains the clustering of species in multivariate morphospace (elytral shape features: $F_{3,6237} = 85.4$, $p < 0.001$; pronotal shape features: $F_{2,6238} = 363$, $p < 0.001$; linearly measured traits: $F_{2,6238} = 102$, $p < 0.001$) (**Figure 4**). Tukey HSD tests showed that all clusters were statistically discernable from one another in elytral shape, pronotal shape, and the morphospace of linearly measured traits.

Linear regression was used to assess how much variation in morphological traits could be explained by phylogenetic relatedness and habitat use. Models that included both relatedness and habitat use explained a small, but significant portion of variation in morphological traits (linearly measured traits: adjusted $R^2 = 0.0546$, $p < 0.001$; pronotal shape: adjusted $R^2 = 0.121$, $p < 0.001$; elytral shape: adjusted $R^2 = 0.0738$, $p < 0.001$). Based on likelihood ratio tests, models that included both relatedness and habitat use outperformed models that only included relatedness (linear traits: $X_6^2 = 135$, $p < 0.001$; pronotal shape: $X_6^2 = 203$, $p < 0.001$; elytral shape: $X_6^2 = 379$, $p < 0.001$) or habitat use (linear traits: $X_5^2 = 57.3$, $p < 0.001$; pronotal shape: $X_5^2 = 323$, $p < 0.001$; elytral shape: $X_5^2 = 262$, $p < 0.001$) as predictors.

Trait Evolution and Tests for Convergence

We finally assessed whether morphological traits showed differential rates of evolution and evidence for convergence upon habitat type. Following a Bonferroni correction for multiple comparisons, we found that the evolutionary rate of all ecomorphological traits, as well as antennal scape length, decreases across species lineages (**Figure 3**). Elytral length evolution slowed at nodes 95 (rate Δ: $-4.95 \cdot 10^{-3}$, $p = 0.02$), 111 (rate Δ: $5.27 \cdot 10^{-3}$, $p = 0.01$), and 123 (rate Δ: $7.43 \cdot 10^{-3}$, $p = 0.01$). Elytral ratio evolution slowed at nodes 104 (rate Δ: $3.88 \cdot 10^{-4}$, $p = 0.01$) and 130 (rate Δ: $3.79 \cdot 10^{-4}$, $p = 0.01$). Pronotal ratio evolution slowed at nodes 111 (rate Δ: $6.80 \cdot 10^{-4}$, $p = 0.01$) and 130 (rate Δ: $8.40 \cdot 10^{-4}$, $p = 0.01$). Antennal scape length evolution slowed at nodes 94 (rate Δ: $4.19 \cdot 10^{-2}$, $p = 0.03$), 111 (rate Δ: $4.51 \cdot 10^{-2}$, $p = 0.01$), and 123 (rate Δ: $6.45 \cdot 10^{-2}$, $p = 0.01$). Pronotal shape evolution slowed at nodes 107 (rate Δ: $3.44 \cdot 10^{-4}$, $p = 0.01$) and 130 (rate Δ: $3.90 \cdot 10^{-4}$, $p = 0.01$). Elytral shape evolution slowed at nodes 106 (rate Δ: $2.36 \cdot 10^{-4}$, $p = 0.01$) and 130 (rate Δ: $2.67 \cdot 10^{-4}$, $p = 0.01$). Nodes 81, 93, and 123 correspond to species complexes *Boreonebria*, *Reductonebria*, and *Catonebria*, respectively. Node 123, the species complex *Catonebria*, is the location of shifts in elytral length and antennal length rates. The subgenus *Catonebria* diverges at node 130, corresponding to shifts evolutionary rate of elytral ratio, pronotal ratio, pronotal shape, and elytral shape. This species complex includes some of the largest species of *Nebria* known. Within the *Reductonebria* species complex, there are two clades – the subgenus *Reductonebria*, which diverges at node 94, and the subgenus *Erwinebria*, which diverges at node 104. With the divergence of *Reductonebria*, antennal scape length evolution slows, and with the divergence of the subgenus *Erwinebria*, elytral ratio evolution slows. The subgenus *Reductonebria* is dominated by riparian species (90.9% of species in this clade) whereas the subgenus *Erwinebria* is dominated by alpine species (80% of species in this clade). These shifts in ecomorphological trait evolution at these nodes may correspond to shifts in habitat use. In addition, analyses were performed with alpine as the “state”, and separately with riparian as “state”. In either of these cases, there is no difference in evolutionary rate of any ecomorphological traits between alpine and riparian species.

Morphological convergence for alpine and riparian morphotypes was assessed among species of *Nebria*, assuming an alpine mean ground temperature of 6˚C, and separately, 7˚C. Elytral shape appears to be converging among riparian species. This is consistent whether using a 6˚C (C1 = 0.268, p = 0.009; C2 = 0.000423, p = 0.012; C3 = 0.111, p = 0.004) or 7˚C (C1 = 0.256, p = 0.025; C2 = 0.000409, p = 0.027; C3 = 0.108, p = 0.015) threshold of mean ground temperature to categorize habitat type (**Table 1**). Pronotal shape also appears to be converging among riparian species, relative to other species in their clade (6˚C: C4 = 0.000521, p = 0.0003; 7˚C: C4 = 0.000496, p = 0.008) (**Table 1**). Linearly measured morphological traits appear to be converging among alpine species, relative to other species in their clade (6˚C: C4 = 0.00578, p = 0.055; 7˚C: C4 = 0.00546, p = 0.05) (**Table 1**).

Based on linear measurements of morphology (antennal scape length, elytral length, elytral ratio, and pronotal ratio), riparian species cluster together in morphospace (6˚C: θ = 2.49, p = 0.058; 7˚C: θ = 2.55, p = 0.072) (**Table 2**). They do not cluster in morphospace based on pronotal shape (6˚C: θ = 2.28, p = 0.456; 7˚C: θ = 2.31, p = 0.532) or elytral shape (6˚C: θ = 1.56, p = 0.380; 7˚C: θ = 1.60, p = 0.498). Alpine species do not cluster together in multivariate morphospace whether analyzing linearly measured traits (6˚C: θ = 3.25, p = 0.955; 7˚C: θ = 3.23, p = 0.945), pronotal shape (6˚C: θ = 2.30, p = 0.470; 7˚C: θ = 2.28, p = 0.423), or elytral shape (6˚C: θ = 1.57, p = 0.258; 7˚C: θ = 1.57, p = 0.230) (**Table 2**).

Discussion

Convergent Evolution of Morphospace among Species of Nebria

The similarity of ecomorphological traits among riparian species suggests that there is convergence upon morphotypes. Evidence is much stronger for the riparian morphotype, and includes linearly measured traits, elytral length, elytral ratio, pronotal width ratio, and scape length. When accounting for phylogenetic distance between species, elytral shape appears to be undergoing convergence in riparian species, as is pronotal shape when compared to non-riparian species in the clade. When accounting for phylogenetic distance between species, only linearly measured traits appear to be converging among the alpine morphotype. A small, but significant proportion of the variation in ecomorphological traits is best predicted by relatedness and habitat use together, but the clusters that species form in multivariate morphospace are only predicted by relatedness. This apparent discrepancy may be explained by the way habitat features are included in these analyses. The habitat features used in this study measure habitat states to the nearest ~1km^2, whereas species of *Nebria* tend to specialize on habitat and microhabitat features (J. Schat unpublished data). A linear combination of course-grain habitat features may not be enough to explain morphospace partitioning, and finer scale analyses of microhabitat features could provide more explanatory power.

All ecomorphological traits and antennal scape length are evolving slower than expected under Brownian motion evolution. This suggests that these traits are undergoing stabilizing selection. Elytral length is often used as a proxy for body size (Sukhodolskaya, 2013; Luzyanin et al., 2021), which along with pronotal ratio and elytral length, have been shown to be important predictors of habitat use (Schat et al., 2022). The evolutionary rates of all measured traits shift several times throughout the evolutionary history of species used in this study. Some of these shifts appear to coincide with changes in habitat specialization, while others coincide with divergence of the specific clades. Within adaptive radiations, early rapid diversification, followed by decreases in trait evolution can indicate convergence upon ecological morphotypes (Sansalone et al., 2020).

Habitat Use in Montane Communities

Montane communities possess ecotones spanning elevations and latitudes, and species of *Nebria* are known to specialize in niches defined by ecotones in these habitats (Grinnell, 1917; Kavanaugh, p35-57; Gobbi et al., 2007; Schoville et al., 2012; Schoville & Slatyer, 2016; Schat et al., 2022). The convergence observed in elytral shape and pronotal shape suggests that the gestalt of these morphotypes may be consistent with riparian or alpine specialization, such as having ovoid (alpine) or angular (riparian) elytra. Within riparian or alpine communities, further habitat specialization at the microhabitat scale may explain the rate of evolution of body size (elytral length) and body proportions (elytral ratio and pronotal ratio). Morphological traits, specifically body size and pronotal width ratio, have been shown to predict habitat and microhabitat use in northern Cascades Range communities (J. Schat unpublished data, Schat et al., 2022), and the scaling of morphological traits is known to vary with elevation (Sukhodolskaya & Ananina, 2015). The consistency of these relationships in these communities, regardless of local community composition, suggests that environmental filtering dictates species persistence. Environmental filtering is known to be a dominant factor in predicting species diversity in alpine and montane habitats (Janzen, 1967; Machac et al., 2011), including in ground beetles (Gobbi et al., 2007). The convergent processes indicated in this study support similar findings as other studies focusing on temperate, montane communities (Weng et al., 2020; Schoville et al., 2023).

In communities with strong environmental filtering, phenotypic similarity among coexisting species is expected (Cavender-Bares et al., 2006). In communities with many closely related species, this similarity can be due to relatedness. However, in clades with high diversification rates, similarity may be the result of convergent evolution. This appears to be the case in these communities. Convergence upon morphotypes related to niche has been seen in other ground beetle communities (Ingerson-Mahar, 2014; Maddison et al., 2019; Baulechner et al., 2020). Morphology is a known predictor of habitat use (Madison & Maruyama, 2019), resource consumption (Konuma & Chiba, 2007), and extinction risk (Nolte et al., 2019) among ground beetles. Elytral shape, pronotal shape, and antennal scape length are known to vary across species within ground beetle communities and predict habitat use (Ribera et al., 1999). The consistent relationships between habitat use, species diversity, and functional diversity among ground beetles is what has made them such reliable bioindicators of changes in habitat (Koivula et al., 2002) and ecosystem diversity (Pakeman & Stockan, 2014).

Glacial Cycles and Habitat Partitioning

As habitat specialists, species of *Nebria* respond to climatic cycles by chasing their climatic niche across elevations (Schoville et al., 2012). In some Palearctic communities of these species, the age of the glacial retreat has been known to impact species habitat occupancy (Gobbi et al., 2007). While some species of *Nebria* are still fully winged, many are brachypterous or flightless (Kavanaugh, 1985), thus long-distance dispersal is limited. During glacial maxima, alpine habitats shift to lower elevations, and during glacial minima, the elevational range of riparian habitats lengthens and expands up mountain slopes. Throughout glacial cycles, shifting habitats can allow species to more readily mix among adjacent mountain ranges, facilitating dispersal and possibly hybridization (Schoville et al., 2012). As glaciers retreat and riparian habitats expand upwards, microhabitat partitioning may act as an environmental filter on body proportions and body size as ecologically important traits under selection (Brehm et al., 2019; Mena et al., 2020). As species move upwards to chase their climatic niche, selection upon allometric scaling may play an increasingly important role in environmental filtering and habitat partitioning. This may explain why the riparian morphotype is so stable despite habitat variability in riparian zones. As species' ranges expand or move upwards, they may face novel selective pressures influencing body proportion. Allometric scaling has been shown to change as body size increases with elevation (but see Egset et al., 2012 and Shelomi et al., 2012). This is consistent with finer scale (spatially) studies of these communities across elevations and habitat types in the northern Cascades Range (J. Schat unpublished data).

In contrast, the lack of absolute similarity among alpine species may be due to weaker environmental filtering on morphotype. Microhabitat features, rather than general habitat characteristics, are predictive of invertebrate habitat occupancy (Sinclair et al., 2001; Gobbi et al., 2021). Despite limited evidence for alpine convergence in this study, in previous work we have shown that alpine specialists exhibit niche conservatism in morphological shape when they become isolated across mountain ranges (Schat et al., 2022). This most likely arises as a form of stabilizing selection, although parallel evolution could be explored through more careful phylogenomic analysis.

Conclusions and future directions

Within the radiation of species of *Nebria*, morphological variation is best predicted by a combination of habitat use and ancestral constraint. There is evidence for convergence among species of *Nebria* with respect to alpine and riparian habitat specialization, but k means clusters suggest phylogenetic relatedness drives an important part of the pattern in morphospace. It is not surprising that ancestral constraint predicts some of the morphological variation among closely related species. However, the importance of habitat use in predicting morphological variation, the morphological similarity of ecotypes, and the consistency of stabilizing selection upon these ecomorphological traits indicates that convergent processes significantly impact phenotypic variation. The methods used in this study employ comparative approaches, designed to test whether traits are predictably associated with their function while controlling for phylogenetic history. In future studies, optimality approaches, such as is discussed in Hansen (1997) and demonstrated in studies such as Bravo et al. (2014), may further illuminate the relationship between convergence and stabilizing selection in this system. In addition, more effort should be placed in examining convergence at the microhabitat scale. Microhabitats are subsets of habitats, which maintain more stable climatic conditions on short time scales (Scheffers et al., 2014). While the Worldclim database contains a rich resource of habitat data across global scales, environmental data is only accurate to the nearest ~1km^2. Sampling habitat variability within these regions would provide insight into the potential habitat variation where species of *Nebria* are found. During periods of rapid climate change, these pockets of stability may become important aspects of environmental filtering. Ground beetles are useful indicators of significant habitat change (Messer, 2009; Schirmel et al., 2015). Understanding how they respond to environmental changes is key to improving conservation outcomes over long time scales.

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Tables and Figures

Table 1 Extent of convergence on a riparian and alpine morphotypes across species of *Nebria* based on C1-4 metrics as described in Stayton (2015). Statistical significance ($p < 0.05$) was calculated from permutation tests and is denoted by *. Values with $p < 0.10$ are indicated by ^. Riparian habitats are those which have a mean ground temperature of greater than 6˚C – 7˚C. Alpine habitats are those which have a mean ground temperature of 6˚C – 7˚C or less.

Riparian Morphotype (>6˚C)	C1	C2	C3	C4
Linearly Measured Traits	0.375	0.615	0.168	0.00168
Pronotal Shape	0.165	0.000534	0.0675	**0.000521***
Elytral Shape	**0.268***	**0.000423***	**0.111***	0.000710
Riparian Morphotype (>7˚C)	C1	C2	C3	C4
Linearly Measured Traits	0.371	0.576	0.167	0.00174
Pronotal Shape	0.167	0.000547	0.0692	**0.000496***
Elytral Shape	**0.256***	**0.000409***	**0.108***	0.000835
Alpine Morphotype (<=6˚C)	C1	C2	C3	C4
Linearly Measured Traits	0.316	0.604	0.167	**0.00578***
Pronotal Shape	0.173	0.000566	0.0672	0.0000971
Elytral Shape	0.175	0.000261	0.0720	0.00146
Alpine Morphotype (<=7˚C)	C1	C2	C3	C4
Linearly Measured Traits	0.321	0.620	0.169	**0.00546***
Pronotal Shape	0.174	0.000563	0.169	0.00546
Elytral Shape	0.176	**0.176***	0.0719	0.00140

Table 2 Angular distance (θ) between riparian and alpine species in multivariate morphospace. Statistically significant values ($p < 0.05$) are indicated by *. Values with $p < 0.10$ are indicated by ^. Riparian habitats are those which have a mean ground temperature of greater than 6˚C – 7˚C. Alpine habitats are those which have a mean ground temperature of 6˚C – 7˚C or less.

Riparian Morphotype (>6˚C)	θ	θ Adjusted by Time
Linearly Measured Traits	**2.49***	77.3
Pronotal Shape	2.28	64.5
Elytral Shape	1.56	58.9
Riparian Morphotype (>7˚C)	θ	θ Adjusted by Time
Linearly Measured Traits	**2.55**^	80.4
Pronotal Shape	2.31	66.6
Elytral Shape	1.60	62.3
Alpine Morphotype (<=6˚C)	θ	θ Adjusted by Time
Linearly Measured Traits	3.25	66.0
Pronotal Shape	2.30	52.2
Elytral Shape	1.57	40.1
Alpine Morphotype (<=7˚C)	θ	θ Adjusted by Time
Linearly Measured Traits	3.23	66.3
Pronotal Shape	2.28	51.9
Elytral Shape	1.57	40.1

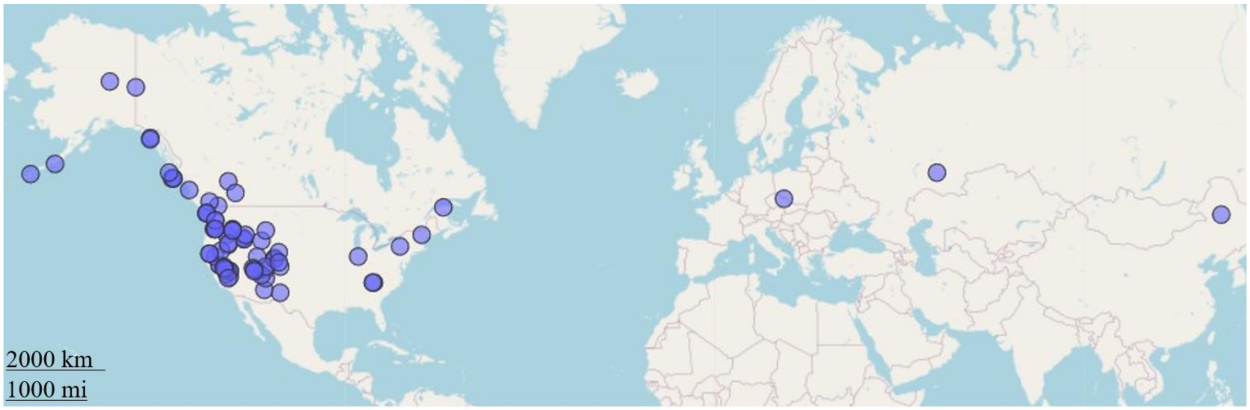

Figure 1 Map of locations were specimens of species of *Nebria* used in this study were collected.

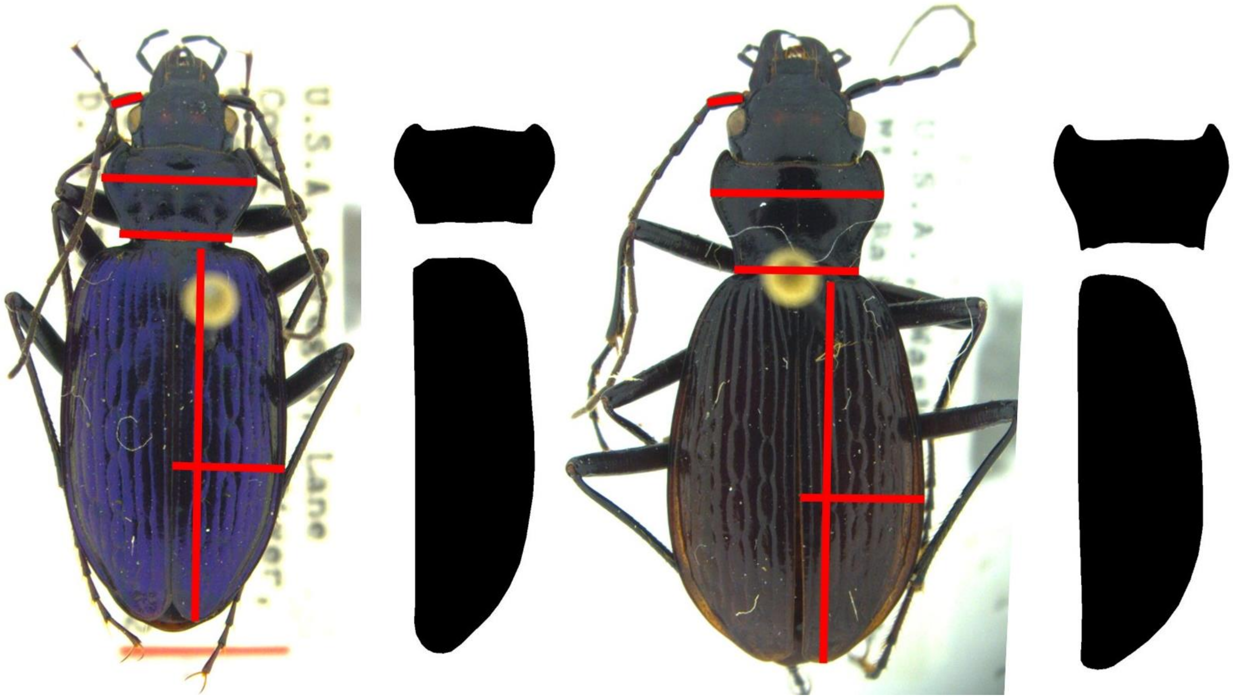

Figure 2 Specimens of *Nebria piperi* (left), a riparian species, and *N. vandykei* (right), an alpine species (PC: Jillian Schat, 2022). Images are not to scale. Red lines indicate where measurements were taken to assess linearly measured morphological traits, including elytral length, elytral ratio (length divided by width), pronotal width ratio (widest distance divided by the base), and antennal scape length. Silhouettes are examples of pronotal and elytral shape silhouettes created in Inkscape.

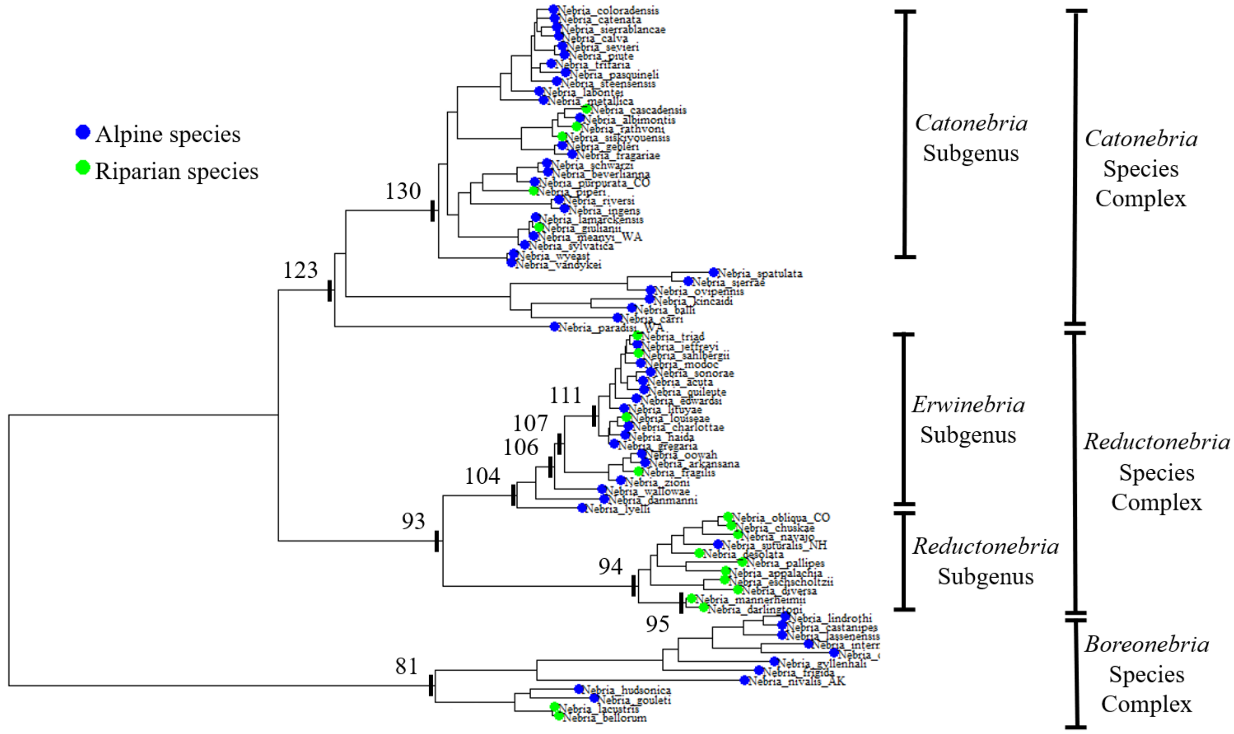

Figure 3 Phylogeny of species of *Nebria* used in this study based on the multilocus Maximum Likelihood phylogeny found in Kavanaugh et al. (2021). Colors indicate whether species were collected in alpine or riparian habitats based on a threshold mean ground temperature of 6˚C. Bars indicate nodes at which the evolutionary rate of one or more traits changed. Nodes 81, 93, and 123 correspond to the species complexes *Boreonebria*, *Reductonebria*, and *Catonebria*, respectively. Nodes 130, 104, and 94 correspond to the subgenera *Catonebria*, *Erwinebria*, and *Reductonebria*, respectively. Elytral length evolution slowed at nodes 95, 111, and 123. Elytral ratio evolution slowed at nodes 104 and 130. Pronotal ratio evolution slowed at nodes 111 and 130. Antennal scape length evolution slowed at nodes 94, 111, and 123. Pronotal shape evolution slowed at nodes 107 and 130. Elytral shape evolution slowed at nodes 106 and 130.

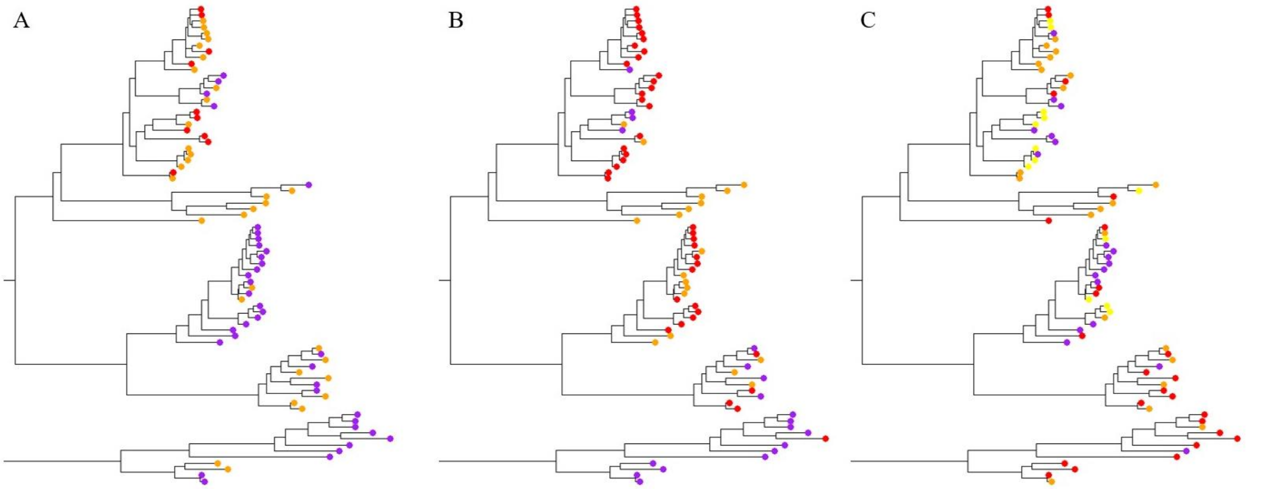

Figure 4 Phylogeny of species of *Nebria* used in this study based on the multilocus Maximum Likelihood phylogeny found in Kavanaugh et al. (2021). Images of the phylogenies were trimmed for visualization, but the topology is unchanged. K-means clustering was used to assess how species cluster in morphospace. Based on the within-group sum of squared distance from centroids, linearly measured traits together formed three clusters (panel A), pronotal shape features formed three clusters (panel B), and elytral shape features formed four clusters in morphospace (panel C). Different colors represent different clusters.

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Chapter 3: "Range size evolution in an adaptive radiation of montane ground beetles (Carabidae: *Nebria*)"

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Open Research Statement: Morphometric data, specimen collection locations, and R code used in this study are open access: https://orcid.org/0000-0002-7253-1607.

Abstract

Range size is an important aspect of niche, and range size evolution can be used to understand how clades diversify. Understanding why species live where they do is a fundamental question in macroecology, and understanding how they acquired the niches they possess across generations is a fundamental aspect of macroevolutionary biology. By studying the relationship between range size evolution, ecomorphological trait variation, and diversification, the processes influencing niche evolution and speciation can become clear. In communities wherein ecological speciation is the dominant process shaping species niches and ranges, it is expected that closely related species would share nearby ranges, and ancestral ranges would be divided among daughter taxa. This process is referred to as dispersal-extinction cladogenesis. In communities wherein dispersal-vicariance cladogenesis dominates, neighboring species would be more distantly related and not share recent ancestral ranges. These opposing processes were examined using the Nearctic distribution of species in the the subgenus *Nebria Catonebria*. Species in this subgenus are habitat specialists and occupy a wide range of habitats, from sea level to alpine talus and snowfields. Despite the sky island distribution of these communities, ecological speciation explains range size evolution better than dispersal-vicariance models. In a community where environmental filtering

predicts community composition and habitat specialization is evident, niche specialization plays a dominant role in lineage diversification.

Introduction

A species' range size can be indicative of its habitat use, potential resource consumption, and distribution across a landscape. A populations' range size is impacted by species interactions (Tingley et al., 2014; Afkhami et al., 2014) and environmental change, such as climate change (Pozsgai et al., 2015; Pureswaran et al., 2018). As such, the evolution of range size can be used to investigate the processes that determine why species live where they do. Within a monophyletic group, ancestral range size, range size of extant taxa, and relatedness among taxa can indicate which evolutionary processes explain range size evolution (Lamm & Redelings, 2009). For example, local adaptation and ecological speciation may play a prominent role in diversification, or alternatively, geographical isolation and genetic drift may be more important to clade diversification (Rundle & Nosil, 2005). In scenarios where ecological speciation dominates, ancestral range sizes are compartmentalized into distinct or nested ranges occupied by daughter taxa (Ree et al., 2005). Conversely, when genetic isolation among subpopulations is more important, ancestral ranges are often large and cross geological barriers, allowing for spatial separation and gradual cladogenesis. This might commonly occur in ecosystems with prominently partitioned habitats, such as island systems or mountain ranges (Losos & Ricklefs, 2009). These alternative range evolution hypotheses, known as the dispersal-extinction cladogenesis hypothesis (Matzke, 2014) and the dispersal-vicariance hypothesis (Ronquist, 1997), respectively, propose opposing mechanisms for diversification and range size evolution.

Range size increase likely precedes diversification, and ecological speciation is more easily detected than sympatric speciation. Range size studies are often in island systems with species who rely heavily on long-distance transportation for survival/reproduction, such as birds (Purushotham & Robin, 2016), palm trees and other highly dispersive plants (Price & Wagner, 2004; Bacon et al., 2012), and small winged arthropods or those capable of ballooning (Hiller et al., 2019). Species that have a montane "sky island" distribution are less frequently studied but may provide interesting test cases for systems in which strong environmental variation influences divergence dynamics and dispersal abilities of taxa may be more limited. Range size can be correlated with degree of niche specialization, with specialists occupying smaller geographic ranges (Slatyer et al., 2013), but the underlying relationship is more contingent on the spatial distribution of niches, such as habitat types, across a landscape or seascape (Grinnell, 1947; Sexton et al., 2017). Indeed, the fundamental niche of a given species is most often predicted by abiotic constraints and habitat use, and overestimating range size is inevitable when these constraints are not accounted for, particularly in communities with sky island (or aquatic island) distributions. It is the goal of this study to test which mechanism best explains range size evolution in an adaptive radiation of a montane species complex of ground beetles in the genus *Nebria* (Carabidae: Nebriini), which are habitat specialists that occupy a multitude of range sizes and vary in dispersal ability (Kavanaugh, 1979; Slayter & Schoville, 2016; Gobbi et al., 2007). This is a diverse genus with approximately 500 species that occur mostly in montane habitats (Kavanaugh et al., 2021), where some are alpine specialists with atrophied wings and limited dispersal abilities, some are low-elevation specialists with fully developed wings in more interconnected habitats, and some are mid-elevation specialists whose ranges overlap with low and high elevation species to varying degrees (Kavanaugh, 1985). Species of *Nebria* are known to chase their climatic niche during the progression of glacial cycles

(Schoville et al., 2012; Gobbi et al., 2021), and so their ranges fluctuate with changing environmental conditions. Communities of species of *Nebria* are primarily influenced by environmental filtering, and their habitat use is predicted by ecomorphological traits (Schat et al., 2022). Because montane ecosystems have steep ecotones, species of *Nebria*'s habitat use is primarily predicted by abiotic habitat features that stratify across elevation and latitude (Gobbi et al., 2021; Chapter 1). For species in this genus, ecomorphological traits such body length and pronotal shape (Chapter 2) are predictive of habitat use. Understanding the relationship between range size evolution and functional trait evolution can indicate the importance of ecological speciation in diversification.

Here we focus on species of *Nebria* with a Nearctic distribution, as we have comprehensive data on their geographical ranges and a recently published molecular phylogeny that is comprehensive for Nearctic species (Kavanaugh et al., 2021). We first estimate range size for each species using maximum entropy niche models (Phillips et al., 2006). The distributions and community composition of species of *Nebria* are primarily predicted by habitat and microhabitat features (Chapter 1; Schat et al., 2022; Slatyer & Schoville, 2016; Panza & Gobbi, 2022), and maximum entropy niche models provide a habitat-focused approach to modelling habitat suitability. Maxent uses no prior assumptions about species distributions and penalizes complex models to prevent over-fitting. This approach requires no prior information about species' absences and so has become an increasingly popular approach to modelling species distributions (Urbani et al., 2015; Brygadyrenko et al., 2021; Busch et al., 2021). We then characterize patterns of endemism, phylogenetic evenness, and ecomorphological evenness of species of *Nebria* within this region. Species diversity – nestedness (Baselga, 2012) – and species turnover (Leprieur et al., 2012) across their regional distribution, degree of relatedness within local communities, and evenness of ecomorphological diversity across habitats may lend insight into the processes contributing to cladistic diversification. Next, we analyze the evolutionary processes that influence range size using two approaches. Range size evolution is first modeled using dispersal-extinction cladogenesis. Dispersal-extinction cladogenesis is an approach which models range evolution through ecological speciation (Ree & Smith, 2008). It assumes no relationship between range size and diversification and assumes that ancestral range size is split among daughter taxa (Ree & Smith, 2008). Second, we fit a model of dispseral-vicariance cladogenesis. Dispersal-vicariance cladogenesis models assume allopatric speciation is the primary mechanism of diversification, with species becoming genetically isolated from one another following geographic separation (Ronquist, 1997). As habitat specialists with sky island distributions, these communities present an exciting opportunity to test these two hypotheses. We will compare these two models to test the relative importance of ecological vs. allopatric speciation in explaining the diversification of these lineages in the montane element of their Nearctic range.

Methods

Focal taxa

A total of 24 species were included in this study, including members of the subgenera *Catonebria* and *Boreonebria* (Kavanaugh et al., 2021). The mean sample size per species was 56, with a standard deviation of 79 (**Table 1**). While occurrence data for additional species of *Nebria* were available, species were only included in this study if at least five occurrences were recorded. *Nebria gebleri* had the most observations, with 297 occurrences recorded.

Species occurrence data represent records from field surveys and museum collections, which were first compiled by Kavanaugh (1979) and have been continually updated with new collection data (Kavanaugh, pers. comm.). Species occurrences data were screened for duplicate occurrences and these were removed before downstream analyses.

A multi-locus molecular phylogeny by Kavanaugh et al. (2021) was used to identify the role of phylogenetic relatedness with respect to species distributions and range size evolution. This phylogeny was based on maximum likelihood estimates of relatedness using a concatenated dataset that included the nuclear ribosomal gene 28S, nuclear protein coding genes CAD2, PEPCK, Topo, and wg, and the mitochondrial genes 16S-ND1, COI BC, and COI PJ (Kavanaugh et al., 2021). Nodes with less than 90% bootstrap support were collapsed. While the full phylogeny is rooted with numerous outgroups and other *Nebria* taxa, we pruned the tree to contain only our focal taxa.

Species of Nebria distributions

The Bioclim database (Fick & Hijmans, 2017) was used to estimate the climatic features where species of *Nebria* occur, derived from monthly temperature and rainfall values measured over the period of 1970-2000. This database includes 19 environmental features with estimates accurate to the nearest ~1km^2. A principal components analysis was used to assess which bioclimatic variables (BIO1-19) vary most across the distributions of species of *Nebria* and which variables are highly correlated with one another where species of *Nebria* are found. The subset of these variables contributing significantly and orthogonal with one another in PC space were used to create maximum entropy niche models. Using environmental features where species of *Nebria* were observed, maximum entropy niche models were created to estimate the range sizes of species of *Nebria* (Phillips et al., 2006). Maxent models rely only on “presence” data and do not require “presence/absence” observations. This modelling approach uses the principle of maximum entropy to estimate the suitability of habitats based on occurrence data and environmental factors where individuals are found.

The R packages raster (Hijmans, 2023), dismo (Hijmans et al., 2023), dplyr (Wickham et al., 2023), and rJava (Urbanek, 2021) were used to create raster files of predicted ranges of species of *Nebria*. Rasters of Bioclim variables were cropped to a range of 32˚N to 80˚N (datum = WGS84). No known species of *Nebria* have been observed beyond these latitudes. For each species, a buffer of 100 km was created around species occurrences, these polygons were united into one area, and this area was used to sample background training data for the niche model. For each species, 100*n observations background points were sampled from this region. Species' ranges were then predicted using the native R function "predict". To test the predictive power of these models, for each species, 75% of observations were used as training data, while 25% of observations were reserved for testing these models using the native R function "evaluate". The area under the curve (AUC) statistic was used to assess the predictive power of these models.

To estimate range sizes of species of *Nebria*, species distribution models were used to generate rasters, wherein each cell of the raster possessed a suitability estimate for each species, ranging from 0-100%. These rasters were cropped to contain areas within 100 km of recorded species' occurrences. Within this range, a suitability threshold of ≥70.0% was used to select cells where species were likely to be found. This value is close to the mean value of habitat suitability within 100 km of species occurrences. Within 100 km of species' occurrences, the mean suitability of each cell was 70.9%. The sum of cells with a suitability of at least 70.0% was used as an approximation of the range size for each species.

Ecomorphological variation and species diversity

Photographs of species of *Nebria* were taken using a Leica camera mounted to a dissecting scope (Leica Camera AG, 2020). Specimens from which these images were taken are housed at the California Academy of Sciences, U.S.A. Antennal scape length, elytral length (along elytral suture), ratio of the elytral length to the elytral width (elytral width ratio), and ratio of the pronotum at the widest point to the base (pronotal width ratio) were measured on each specimen using ImageJ (Schneider et al., 2012). These features are known to vary across species of *Nebria* in the northern Cascades Range and predict habitat usage (Chapter 1). Combining morphometric data, the molecular phylogeny, and the range sizes of species of *Nebria*, the R package epm (Pascal et al., 2022) was used to measure endemism, phylogenetic evenness, and ecomorphological evenness across the Nearctic region. In addition, two metrics of β diversity – nestedness (Baselga, 2012) and turnover (Leprieur et al., 2012), were also measured. Across the distribution of all species of *Nebria*, the R package epm was used to sample 10,000 grid cells where at least one species of *Nebria* was observed (based on range size estimates). Species predicted to occur at these locations were used to estimate variation in morphology between species in that sampled location and their nearest neighbor. To estimate phylogenetic evenness, this same sampling method was employed, and variance between nearest neighbor was calculated using patristic distances – the sum of the branch length between taxa. Endemism was calculated using species richness inversely weighted by range size. Measures of β were calculated using the same sample of grid cells.

Evolution of range size

Two hypotheses that could explain the evolution of range size among species are the dispersal-extinction cladogenesis hypothesis (Ree & Smith, 2008) (hereby after referred to as

DEC) and the dispersal-vicariance hypothesis (Ronquist, 1997) (hereby after referred to as DIVA). These range size evolution models were analyzed using the R package BioGeoBEARS (Matzke, 2013), which estimates how taxa occupy areas within a region across time, based on their current distributions and relatedness, to estimate how species may have acquired the ranges they currently possess. BioGeoBEARS lists possible "states" that species may occupy, and increasing state space dramatically increases computational time. The number of states available is based on the number of areas in a region and the maximum number of areas any species may occupy. Some studies have shown that allowing large maximum range sizes can lead to an overestimation of ancestral range size, especially since models such as these do not incorporate how easily taxa move across a landscape (Kodandaramaiah, 2010; Kodandaramaiah, 2010). To avoid this problem and reduce computational time, the Nearctic distributions of species of *Nebria* were divided into 8 areas (**Figure 1**), with *Nebria gebleri* and *N. piperi* occupying the maximum number of areas (n = 5). We focused our analysis on the 24 species of *Nebria* in the subgenus *Catonebria.* Species in this subgenus share an evolutionary history, ecological environment, and altogether, occupy a restricted range size (approximately 130˚W to 100˚W, and 35˚N to 65˚N). Two Nearctic species . – *N. C. catenata* and *N. C. metallica*, had to be excluded from this study because their range size exceeds the focal state space, and as such, their range evolution dynamics could be affected by processes unique to them and distinct from species whose ranges are contained in the focal region.

The DEC approach recreates ancestral state using maximum likelihood estimates and assumes that the ancestral range is immediately divided among the daughter taxa (though not necessarily symmetrically), coinciding with the diversification of these clades. This model assumes prominent ecological speciation from a continuous ancestral population, and it assumes no relationship between range size and speciation. Conversely, DIVA assumes that diversification occurs through primarily allopatric speciation – in which a population expands its range, subpopulations become geographically isolated from one another, followed by eventual genetic isolation. DIVA does not address ecological exclusion between taxa. Loglikelihood was used to compare DEC and DIVA models of range size evolution and current species distributions. BioGeoBEARS possesses the option to include a "jump" parameter in each of these models – a parameter which would estimate how likely species are to suddenly occupy a region unconnected to their current range. While these "+J" models have provided more effective models of many taxa (Matzke, 2022), species of *Nebria* are highly geophilic, and many species are brachypterous or flightless (Kavanaugh, 1985). As such, this variable would be an unrealistic addition to these models.

Results

Distributions of species of Nebria

A principal components analysis was used to assess which bioclimatic variables vary most where the focal species of *Nebria* occur, inclusive of all 41 species in the *Boreonebria* and *Catonebria* subgenera, including Palearctic species in this group. Based on this analysis, the Bioclim variables used to create maximum entropy niche models included: mean temperature of the warmest month (BIO10), mean temperature of the coldest quarter (BIO11), annual precipitation (BIO12), precipitation of the wettest month (BIO13), precipitation of the wettest quarter (BIO16), and precipitation of the warmest quarter (BIO18) (**Figure 2**). These habitat features are uncorrelated (Pearson's $r < 0.75$) with one another and vary greatly across these species distributions in their Holarctic range. Despite the initially wide-ranging geographic span upon which this principal component analysis was based, these environmental features resulted in models that accurately predicted occurrences of species of *Nebria* in their Nearctic range (**Table 2**). On average, AUC for all species models was 0.922 with a standard deviation of 0.0880. A threshold of 70% suitability was used to estimate range size of species of *Nebria*. The sum of cells above this value was used to estimate range size (**Table 1**, **Figure 3** depicts an example of this process, with a suitability map of *Nebria vandykei* across its estimated range, accompanied by a truncated map, showing only regions of 70% suitability and higher, **Appendix 3 Figure 1** depicts the estimated ranges of all taxa included in this study).

Ecomorphological variation and species diversity

Using a random sample of 10,000 locations where species of *Nebria* are found, phylogenetic evenness, endemism, morphological evenness, species nestedness, and species turnover were estimated. Endemism was low (min: $8.43 \cdot 10^{-6}$; max: $5.24 \cdot 10^{-3}$), indicating that species ranges overlap significantly with one another in this region. Phylogenetic relatedness, the variation of patristic distance between nearest neighbors, was also low (min: 0; max: $1.28 \cdot 10^{-3}$), indicating that most species are closely related to their neighbors. Morphological variance between nearest neighbors ranged from 0 to 2.62 and does not appear to covary with endemism or patristic distance between nearest neighbors (**Appendix 3 Figure 2**). Estimates of nestedness and turnover varied across their range, with nestedness ranging from 0 to 1, and species turnover ranging from 0 to 0.619 (**Figure 4**). Locations with high turnover and high nestedness were geographically interspersed with locations of low turnover and low nestedness. Species diversity (nestedness and turnover) was estimated where species of *Nebria* occur. The locations within the highest 5% and lowest 5% of each β diversity metric were sampled for climatic data. The mean climatic environment at these locations was compared to the general climatic environment where species of *Nebria* occur. However, the locations with the highest or lowest 5% of estimates for nestedness or turnover do not differ climatically from other locations within the ranges of species of *Nebria* (**Appendix 3 Table 1**).

Evolution of range size

We compared the DEC (**Figure 5**) and DIVA (**Figure 6**) models of range size evolution and diversification of species within *N. Catonebria* across their Nearctic range using maximum likelihood methods. To limit the state space, the geographic range of these species was divided into eight areas (**Figure 1**). In these models, species were permitted to occupy a maximum of five areas in total; this was the largest number of areas currently occupied by a species – *Nebria gebleri* and *N. piperi*. Using eight areas and a maximum of five areas per species, a state space of 219 possible ranges was generated. In the DEC and DIVA models of range size evolution, species dispersal was limited to areas adjacent to the area (or areas) they already occupied. Both models produced similar estimates of dispersal and low estimates of extinction rates per unit of time (ML tree is not scaled in years) (DEC: dispersal = 5, extinction = $3 \cdot 10^{-7}$; DIVA: dispersal = 5, extinction = $1 \cdot 10^{-12}$), but the DEC model better explained species diversification and range size evolution (DEC: loglikelihood = -124; DIVA: loglikelihood = -133).

Discussion

The study of range size evolution can provide insight into the relative importance of different drivers of species diversification, including the relative frequency of ecological and allopatric speciation during cladogenesis (Rundle & Nosil, 2005). Species like *Nebria* that have a largely montane “sky island” distribution have experienced dynamic environmental variation that may strengthen the relative importance of ecological speciation (Grossenbacher et al., 2014). Indeed, our results suggest that DEC models favoring ecological speciation provide a stronger fit to our data for Nearctic *N. Catonebria*. Habitat specialization has been shown to be a consistent feature of species of *Nebria* and a reliable predictor of local community composition (Chapter 1; Gereben, 1995).

Maxent species models illustrate the importance of habitat features in predicting species presence. Overall, AUC values were high for distribution models, illustrating high predictive accuracy based solely on habitat use. Ecomorphological traits have been shown to predict habitat use among species of *Nebria* (Chapter 1, Palestrini et al., 2012) and habitat use and range size among carabid beetles in general (Gutierrez & Menendez, 1997; Brandl & Topp, 1985; Homburg et al., 2012). Ecomorphological similarity between neighboring species varies but does not appear to vary with relatedness or endemism. Phylogenetic similarity (patristic distance) and endemism were low, indicating high relatedness among neighboring species and high range overlap. High rates of turnover across some sites may indicate strong environmental filtering (Baselga, 2010), and high degrees of nestedness across other sites may indicate niche partitioning (Baselga, 2010) – potentially an early stage of ecological speciation (Keller & Seehausen, 2011; Mammola et al., 2018). Habitat specialization is a prominent feature of these communities, and in this study, has been shown to be the primary process behind cladistic diversification. Ecological speciation is facilitated by niche specialization, in the case of species of *N. Catonebria*, habitat specialization. This is supported by the close relatedness between neighboring species. If diversification through dispersal and vicariance best explained species distributions, one would anticipate that neighboring species would be more distantly related to one another. Instead, short branch length distances between taxa suggests a recent, shared evolutionary past. While sympatric speciation is difficult to detect, ecological speciation can be inferred when range size evolution is best explained by a sympatric ancestral range and recent shared ancestry among neighboring species.

Ecological vs. allopatric speciation

Both allopatric (Faille et al., 2015) and ecological speciation (Goldberg et al., 2014; Delić et al., 2020) have been observed in carabid beetles. Ecological opportunity is a recognized mechanism behind adaptive radiations in montane ecosystems (McCulloch et al., 2021), and is a process that has been observed other abiotically extreme communities of insects, such as cave-dwelling carabids (Deharveng & Bedos, 2018). While sky island distributions inherently provide geographic isolation among species in different regions of the “archipelago”, the harsh conditions of extreme abiotic environments may be more important factors in diversification. In ecosystems where environmental filtering is a primary factor predicting species presence (Chapter 1), and habitat specialization is evident (Chapter 2), ecological niche evolution is an intuitive driver of speciation. However, we note that previous phylogeographic studies of *N. Catonebria* taxa show patterns of both ecological speciation (Schoville et al., 2012) and allopatric speciation (Schat et al., 2022) in different taxa, indicating both processes act to increase diversity through time.

Niche evolution

Range size can be a predictor of extinction risk (Nolte et al., 2019), and the ability for carabids to disperse to new habitats increases resilience to environmental change (Kotze & O’Hara, 2003) and reduces extinction risk. Species of *Nebria* are, on the whole, flightless (Kavanaugh, 1985), and their dispersal ability is greatly affected by climate. Species of *Nebria* are known to shift across elevations as glacial cycles progress (Schoville et al., 2012, Homburg et al., 2012; Gobbi et al., 2021). The DEC model of species of *N. Catonebria* range evolution indicates that many species shared overlapping ranges across multiple, wide-ranging areas approximately 25,000 years ago. During this time, the most recent glacial maxima began receding, approximately 20,000 years ago, and montane ecotones extended upwards in elevation (Kaufman et al., 2003). Subsequently, species of *N. Catonebria* across their Nearctic range began to occupy fewer areas and overlap with fewer species. It seems likely that as glaciers receded and the climate warmed, species of *N. Catonebria* became more isolated from one another as they chased their climatic niche up the mountains.

Conservation implications and future directions

Carabid beetles have been used as bioindicators due to their sensitivity to environmental changes in a variety of habitat types, including subterranean (Schuldt & Assmann, 2011), farmland (Scott & Anderson, 2003), and grassland and forest ecosystems (Rainio & Niemelä, 2003). Species of *Nebria* are one of many groups that will likely face range contraction as anthropogenic climate change continues. Montane insects (McCain & Garfinkel), alpine mammals (Rubidge et al., 2012), and alpine plants (Zu et al., 2021) will experience range contraction with the progression of anthropogenic climate change. As extinction rates have increased globally (Ceballos et al., 2017; Schachat & Labandeira, 2021), it has become increasingly important to understand the processes behind diversification. Functionally and phylogenetically diverse communities are often more resilient (Kühsel & Blüthgen, 2015), especially in abiotically challenging environments, even if individual taxa turnover (Filazzola et al., 2021). The responses by carabids to environmental changes can be predicted by their habitat use and ecomorphological traits (Qiu et al., 2023). Understanding which processes influence range size evolution and cladistic diversification can inform conservationists how to protect habitats. Where habitat continuity is important, such as in cases of ecological speciation, large extended preserves may be more beneficial for vulnerable species. Where allopatric speciation is most important, increased patchiness of habitat may be adequate to maintain diverse populations. In future studies, we suggest approaching these questions using finer-scale landscape partitioning and climate change projections to predict range evolution under a changing climate. While the Bioclim database contains a rich resource of habitat data across global scales, environmental data is only accurate to the nearest ~1km^2. Sampling habitat variability within these regions would provide insight into the potential habitat variation where species of *Nebria* are found.

Tables and Figures

Table 1 Species of *Nebria* included in this study, the number of occurrences recorded for each species, and estimated range size of each species (km^2).

Species	Sample Size	Range Size Estimate
N. albimontis Kavanaugh	6	0.0469 km^2
N. balli Kavanaugh	15	0.105 km^2
N. carri Kavanaugh	14	0.208 km^2
N. cascadensis Kavanaugh	50	0.366 km^2
N. coloradensis Van Dyke	17	0.125 km^2
N. gebleri Dejean	233	3.10 km^2
N. ingens Horn	28	0.0865 km^2
N. kincaidi Kavanaugh	28	0.624 km^2
N. labontei Kavanaugh	5	0.0467 km^2
N. meanyi Van Dyke	30	1.38 km^2
N. ovipennis LeConte	66	0.218 km^2
N. paradisi Darlington	25	0.175 km^2
N. pasquineli Kavanaugh	47	0.201 km^2
N. piperi Van Dyke	59	1.48 km^2
N. purpurata LeConte	43	0.323 km^2
N. rathvoni LeConte	63	0.320 km^2
N. riversi Van Dyke	9	0.0550 km^2
N. schwarzi Van Dyke	17	0.215 km^2
N. sevieri Kavanaugh	12	0.133 km^2
N. sierrae Kavanaugh	53	0.160 km^2
N. spatulata Van Dyke	13	0.0599 km^2
N. sylvatica Kavanaugh	10	0.0855 km^2
N. trifaria LeConte	44	0.716 km^2
N. vandykei Bänninger	7	0.175 km^2

Table 2 Relative contribution of each Bioclimatic variable to each species' maxent niche model and AUC of each maxent model. BIO10 = mean temperature of the warmest quarter, BIO11 = mean temperature of the coldest quarter, BIO12 = annual precipitation, BIO13 = precipitation of wettest month, BIO16 = precipitation of wettest quarter, and BIO18 = precipitation of warmest quarter.

Species	BIO10	BIO11	BIO12	BIO13	BIO16	BIO18	AUC
N. albimontis	70.1	0.1	0	29.9	0	0	0.988
N. balli	11	39.3	2.1	20.6	2.6	24.3	0.946
N. carri	90.3	0	9.6	0	0.1	0.1	0.951
N. cascadensis	28.6	29.7	0.2	0.4	32.6	8.6	0.918
N. coloradensis	86.6	6.9	0.5	0.3	3.4	2.3	0.929
N. gebleri	18.4	19.1	35.5	1	2.7	23.3	0.730
N. ingens	11.5	82	0.8	0	0	5.7	0.987
N. kincaidi	12.3	53.2	0.1	9.1	8.2	17.1	0.743
N. labontei	0	100	0	0	0	0	0.998
N. meanyi	26.9	16.8	4.2	10	30.3	11.7	0.953
N. ovipennis	63	11.5	11.8	0	0	13.7	0.975
N. paradisi	56.9	14.1	0	14	9.1	6	0.979
N. pasquineli	61.6	14.3	7.3	2.4	13	1.4	0.903
N. piperi	54.5	17.8	1.3	13.8	0.9	11.6	0.857
N. purpurata	74.6	2.5	12.9	0.2	8.2	1.6	0.921
N. rathvoni	0.8	50.1	0.3	37.8	0.8	10.2	0.928
N. riversi	86.5	0	0	0	13.4	0.1	0.983
N. schwarzi	12	1.2	5.4	81	0	0.4	0.975
N. sevieri	79.1	0.1	7.6	0	4.6	8.5	0.964
N. sierrae	77.4	4.4	1.2	0.7	0	16.8	0.985
N. spatulata	0	82.2	7.8	3	5.4	1.5	0.983
N. sylvatica	0	96.5	0	0	1.1	2.3	0.691
N. trifaria	2.8	0.8	84.9	7.5	0.3	3.7	0.843
N. vandykei	31	22.6	0.4	7.3	4.9	33.9	1.00
Mean(sd)	70.1(33.1)	27.7(32.4)	8.08(18.1)	9.96(18.2)	5.9(8.88)	8.53(9.13)	0.922(0.0880)

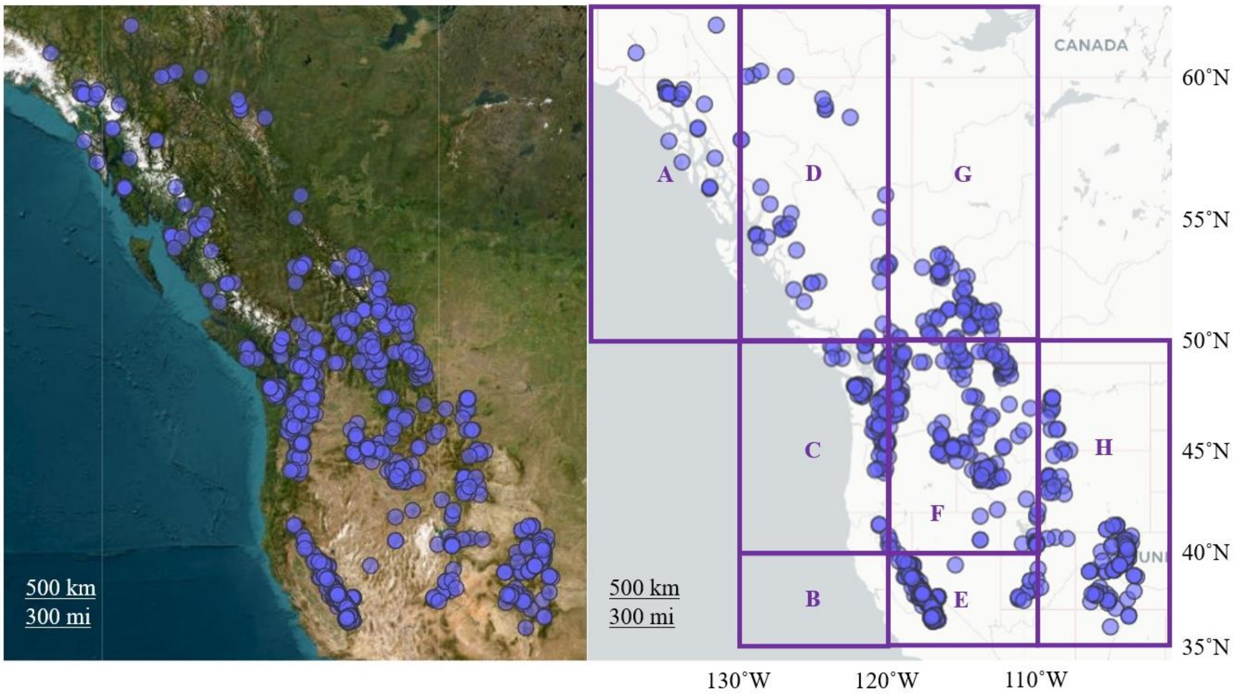

Figure 1 Occurrences of 24 species of *Nebria Catonebria* in their Nearctic range. The left panel contains a satellite image of the region in which these species were observed, and the right panel contains the same observations, but layered over an ordinal grid (WGS84). Areas labeled A-H were used in DEC and DIVA models.

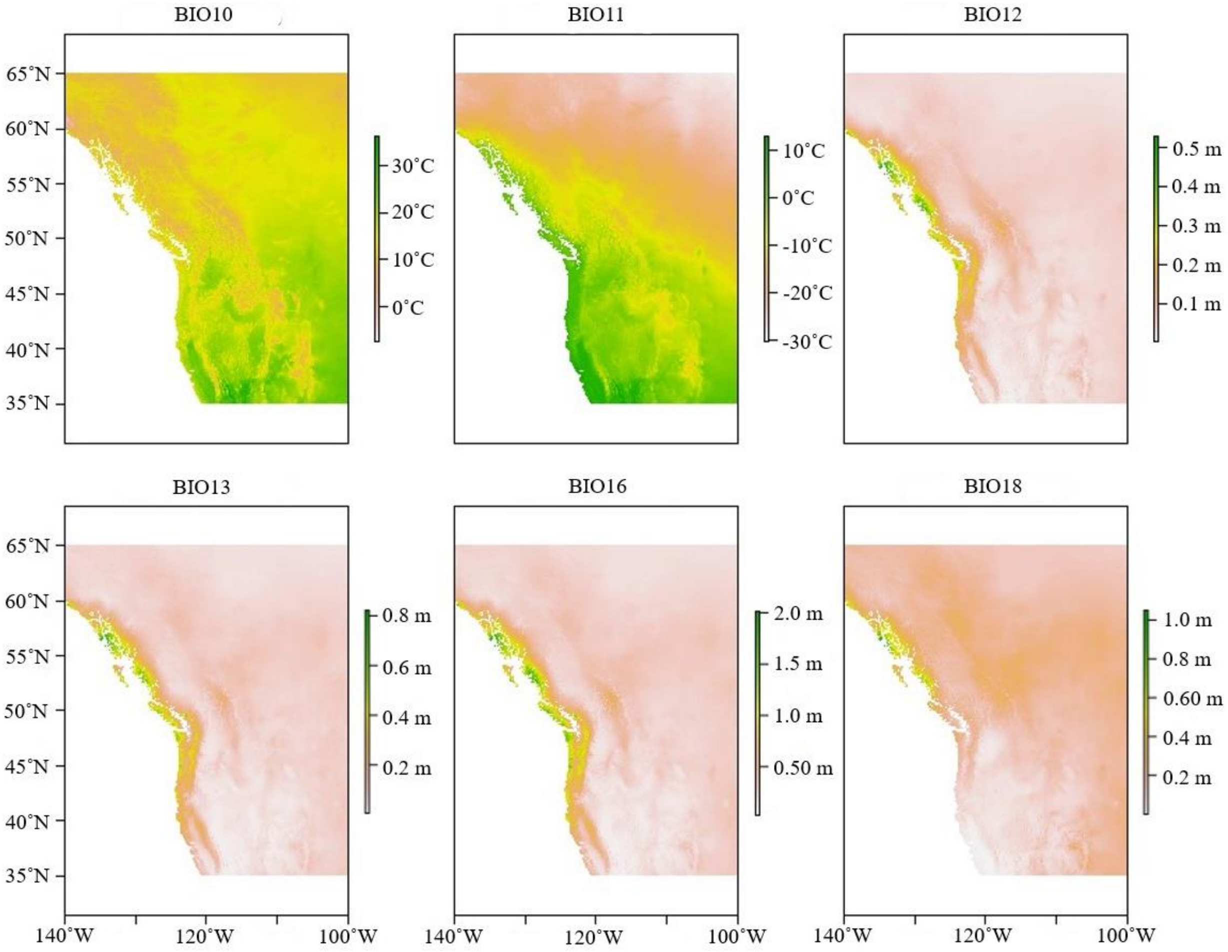

Figure 2 Variation in habitat features where species of Nearctic *N. Catonebria* occur. BIO10 = mean temperature of the warmest quarter (˚C), BIO11 = mean temperature of the coldest quarter (˚C), BIO12 = annual precipitation (m), BIO13 = precipitation of wettest month (m), BIO16 = precipitation of wettest quarter (m), and BIO18 = precipitation of warmest quarter (m).

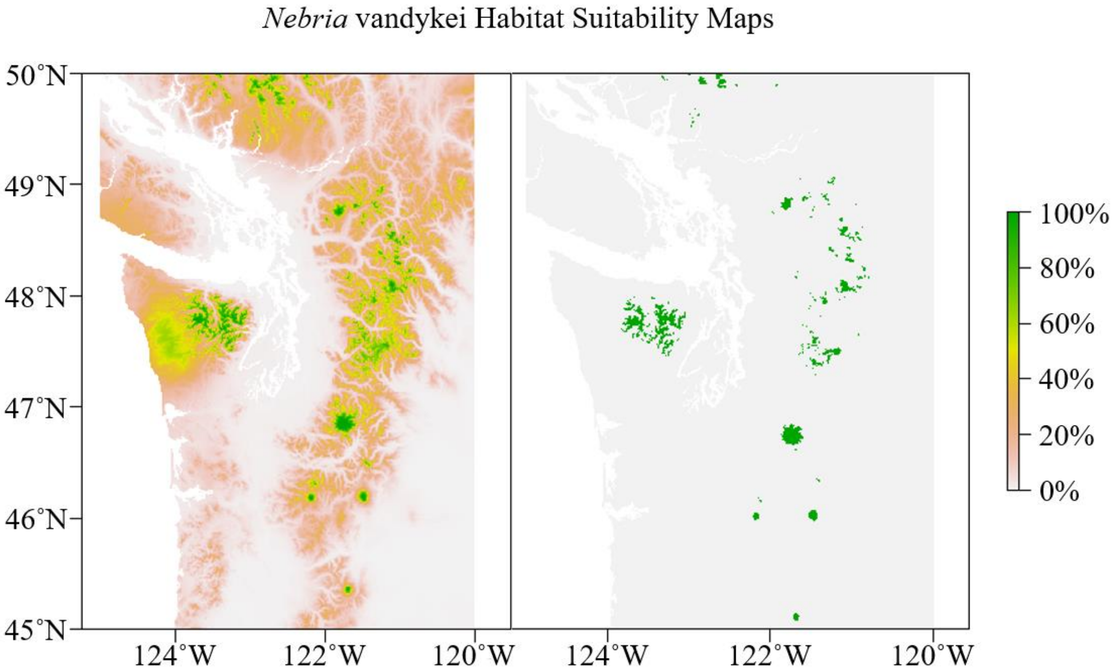

Figure 3 Habitat suitability maps for *Nebria vandykei* based on their maximum entropy niche model. The left panel shows habitat suitability variation within 100 km of observed occurrences of *N. vandykei* on a scale of 0-100% suitability. The right panel shows regions of which are considered at least 70% suitable for *N. vandykei*; these pixels were summed to estimate the range size of *N. vandykei*.

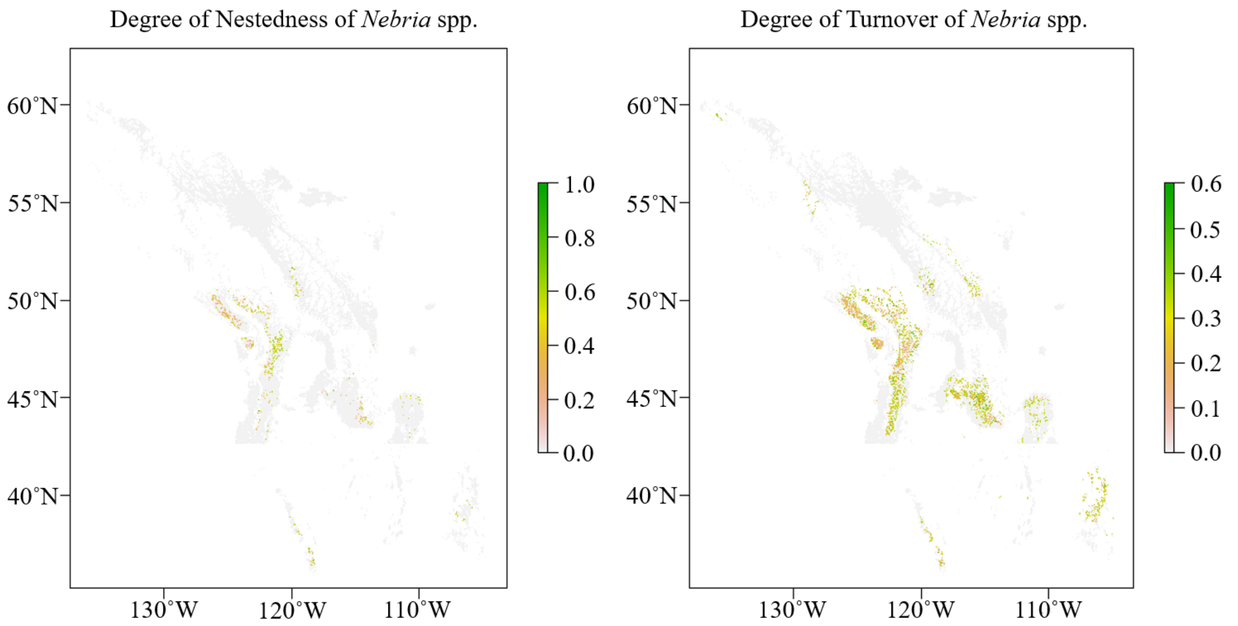

Figure 4 Estimates of species nestedness and turnover across sites where species of *N. Catonebria* occur. The left panel is a heatmap estimating nestedness, and the right panel is a heatmap estimating species turnover across the range of species of *N. Catonebria*.

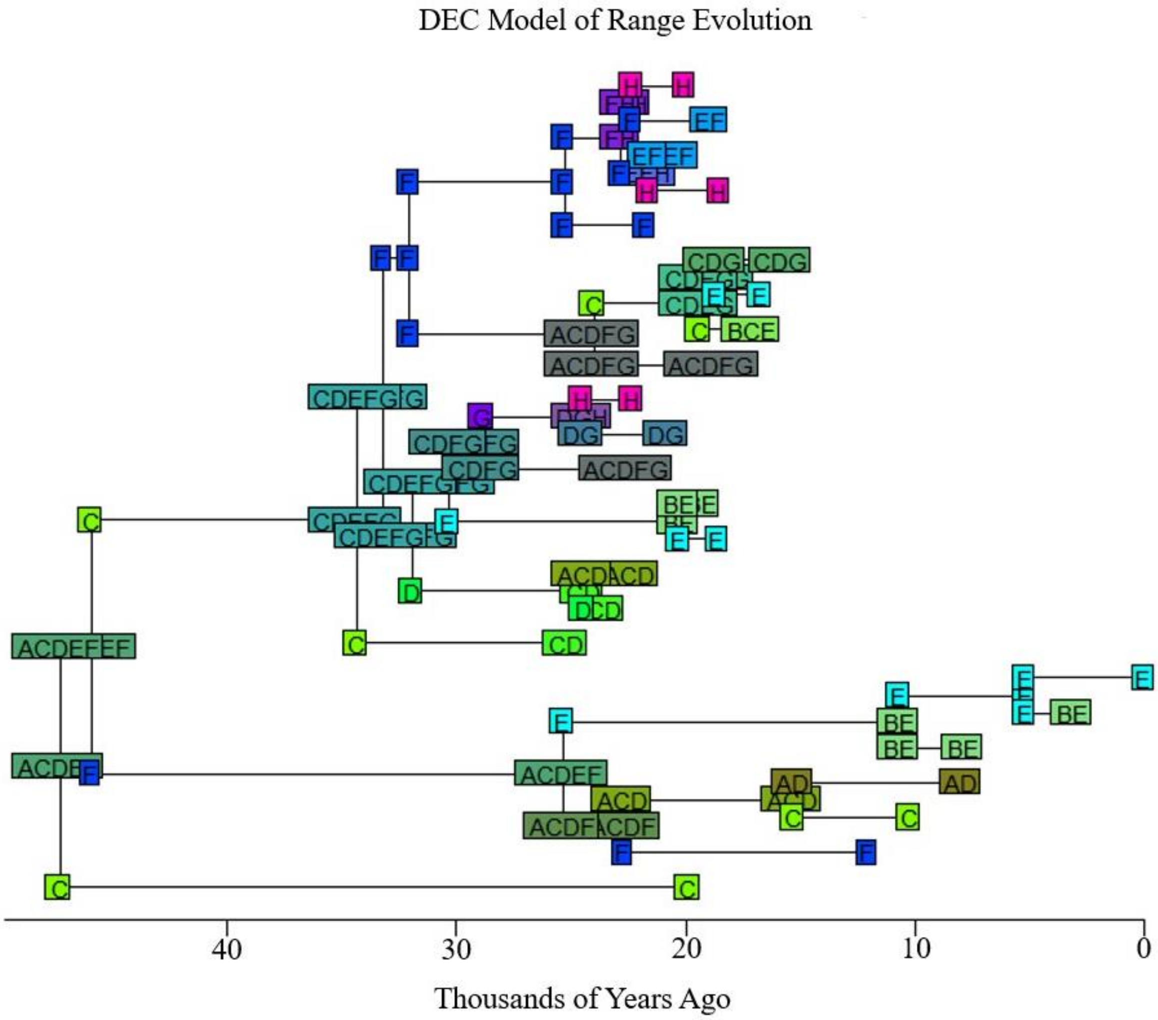

Figure 5 DEC model of ancestral range reconstruction using maximum likelihood (loglikelihood = -124). Letters indicate areas in the geographic range of species of *N. Catonebria* (see Figure 1). Ancestral states with multiple letters indicate that species occupied all areas listed.

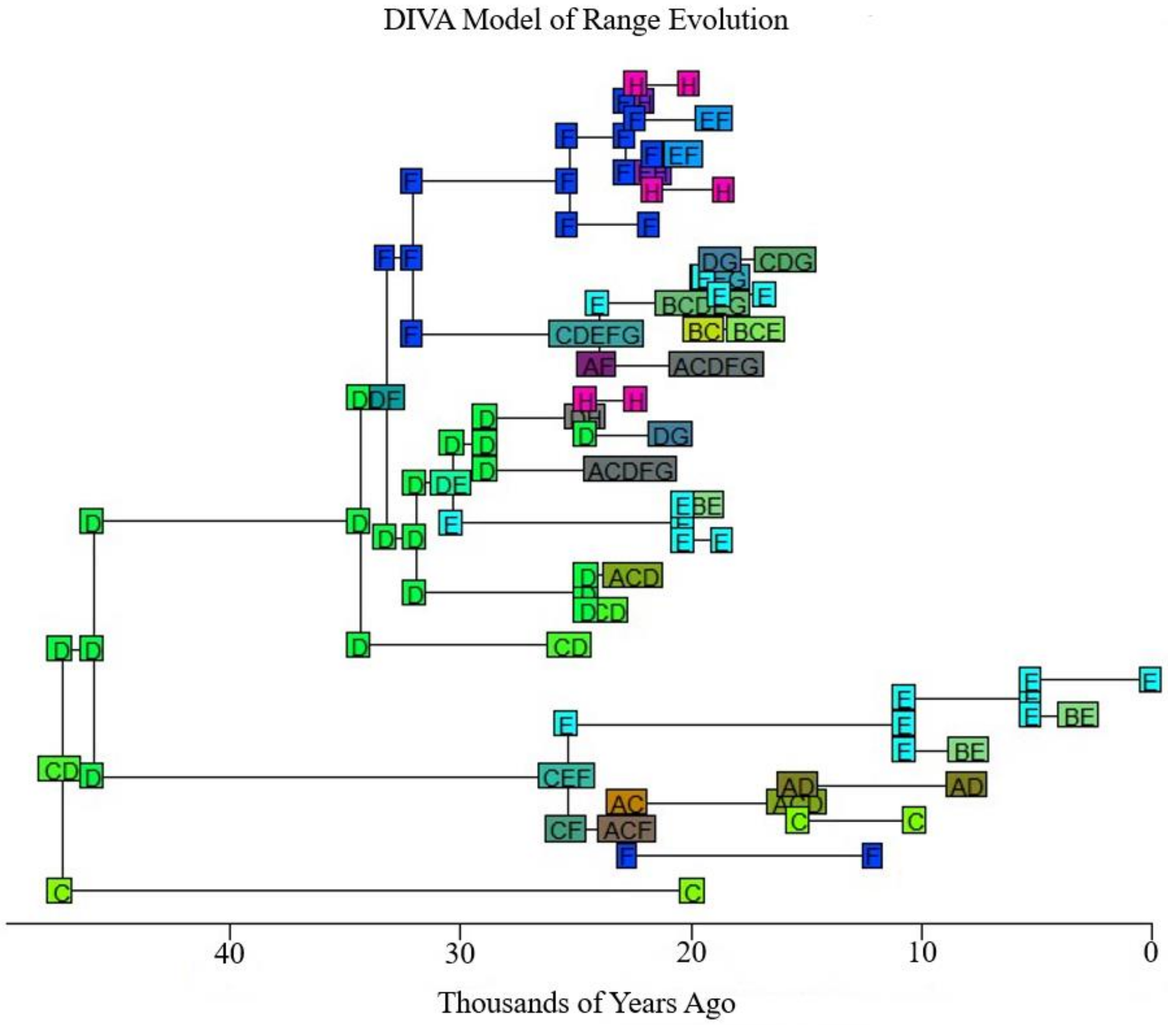

Figure 6 Figure 3 DIVA model of ancestral range reconstruction using maximum likelihood (loglikelihood = -132). Letters indicate areas in the geographic range of species of *N. Catonebria* (see Figure 1). Ancestral states with multiple letters indicate that species occupied all areas listed.

Table 1 β diversity (nestedness and turnover) was estimated where species of *Nebria* occur. The locations within the highest 5% for estimates for both β diversity metrics, and the locations within the lowest 5% for both β diversity metrics was sampled for climatic data. The mean climatic environment (below) at these locations was compared to the general climatic environment where species occur. There is no climatic difference between locations with relatively high β diversity and the general regions where species of *Nebria* occur. There was no climatic difference between locations with relatively low β diversity and the general region where species of *Nebria* occur. BIO10 = mean temperature of the warmest quarter (˚C), BIO11 = mean temperature of the coldest quarter (˚C), BIO12 = annual precipitation (m), BIO13 = precipitation of wettest month (m), BIO16 = precipitation of wettest quarter (m), and BIO18 = precipitation of warmest quarter (m).

	BIO10	BIO11	BIO12	BIO13	BIO16	BIO18
Locations with Highest 5% Nestedness	12.1	-3.78	1300	201	557	159
Locations with Lowest 5% Nestedness	11.4	-11.1	708	94.1	250	178
Locations with Highest 5% Turnover	12.8	-3.94	1080	159	448	135
Locations with Lowest 5% Turnover	11.4	-11.2	702	93.0	247	179

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Conclusions

The studies of ecological and evolutionary biology provide complementary approaches to discovering why species live where they do. Each of these fields propose scale-dependent hypotheses and methods for analyzing the processes that influence community composition, niche evolution, and habitat use. Individual creatures interact with their environment and vie for resources with the aim of increasing fitness. Populations wax and wane with climate cycles, speciation, and extinction. As a species responds to its environment, its niche shifts to increase their probability of survival and reproduction. Niche evolution is shaped by many axes, such as habitat use, patterns of resource consumption, and interspecific interactions, resulting in a multidimensional hyper-space which defines the environment for each species. Despite our theoretical understanding of this phenomenon, we still lack an understanding of the relative importance of different niche axes in different species, or how they vary across space, time, and environment.

In chapter 1, I investigated the ecological processes that explain species habitat use and community composition, where I found strong evidence for the importance of environmental filtering and weak evidence for the importance of resource consumption or community composition. While the latter two factors are important in other ecosystems, montane communities are often stratified across elevations due to environmental filtering. I observed this pattern in species of *Nebria* communities. Further, the stratification of habitat use was predicted by ecomorphological functional traits, not physiological tolerances. For geophilic, crepuscular, insects, the ability to traverse various substrates to hunt and mate and to seek refuge during the day is critical to their survival and appears to be relatively more important in determining species range limits than other factors. While this study provides evidence for the importance of environmental filtering, it is limited. This study includes only ecological surveys of adults and not the larval ecology of species of *Nebria*. While larvae are morphologically indistinguishable (D. Kavanaugh pers. comm.), they may occupy different functional roles than their adult counterparts. Species of *Nebria* overwinter as larvae, and the extent to which they interact with one another and with adults is unclear. Understanding their diet, behavior, and habitat use will provide useful information about how ontogeny affects niche. In addition, including more methods which more directly examine species interactions would provide clarity as to the role of competition in habitat use. While resource consumption, relative abundance, and local community composition appear primarily affected by abiotic factors, without a more direct approach, such as behavioral assays, it is difficult to truly dismiss the role of species interactions. Species of *Nebria* are the most abundant arthropods in the habitats where they occur, but there are some other species that share these environments. Wolf spiders, stoneflies, and *Bembidion* spp. have been observed where species of *Nebria* thrive. While somewhat rare, it is possible that one or several of these species have a significant impact on species of *Nebria* behavior and ecology. Field removal or transplant experiments may shed light on this possibility.

In chapter 2, I tested whether these trait-habitat use relationships evolve repeatedly in species of *Nebria*. Parallel and convergent evolution are not mutually exclusive, but they indicate different eco-evolutionary processes. Evidence I found strongly supports convergent evolution of morphotypes based on habitat specialization. Alpine and riparian species have, separately, converged on distinct habitat-specific morphotypes, and much of the variation in morphotype can be predicted with habitat use alone. However, when including relatedness, the explanatory power of models increases. While environmental filtering seems to have provided a selective pressure for habitat-specific morphotypes, relatedness did remain an important factor in explaining morphological variation. In an adaptive radiation of closely related species, a combination of relatedness and habitat specialization explains morphotype convergence. The pronotal shape, elytral shape, and linear measurements of these traits in species of *Nebria* indicate that convergence is shaping the evolution of morphology. In addition to these traits, leg length, body proportions (allometric scaling), and other features, such as body mass, may also be under ecological selection. In Chapter 1, body size was used as a proxy for several traits, including leg length and distance between the eyes. Body size is significantly related to habitat use, so examining these traits may provide further information about how species are adapting to their environment across generations. Additionally, examining the genetics of these traits could provide additional insight into how plastic these ecomorphological traits are. Given the importance of ecomorphological traits in predicting habitat use, it would be useful to understand how these traits have evolved at times of climatic change, such as with glacial fluctuations. Understanding how flexible and genetically diverse these traits may be indicative of how predictive these traits will be in the future as the climate continues to change.

Lastly, in chapter 3, I examined the relationship between ancestral range size and modern range size. Range size can be predictive of habitat specialization, and species of *Nebria* are known habitat and microhabitat specialists. To understand the evolutionary processes behind range size and its relationship with diversification, I used maximum entropy niche models to estimate range size. With these ranges, I estimated ecomorphological evenness across locations in species distributions, how closely related neighboring species are, how endemic species appear to be, and species diversity across sites. Further, I compared the predictive power of two models – dispersal-extinction cladogenesis and dispersal-vicariance cladogenesis to assess the importance of ecological speciation in this adaptive radiation of ground beetles. I found that morphology does not appear to covary with endemism or relatedness between neighbors, and I found that endemism across sites is quite low. Neighboring species tend to be closely related, and species diversity is not related to local climatic features. Within their Nearctic range, ecological speciation explains range size evolution better than dispersal-vicariance analysis. Community composition is predicted by habitat features (Chapter 1), and species are habitat specialists – to the extent that I found evidence for convergence upon habitat-specific morphotypes. In future studies, including metrics of habitat specialization would provide useful information about the role of ecological niche specialization in diversification. This study found evidence for the importance of ecological speciation but does not address whether the strategy of the habitat specialist or habitat generalist tends to precede diversification. In ecological speciation, ancestral range is split between daughter taxa, implying increasing specialization of one or both taxa. However, examining this further by including ancestral state reconstruction of niche breadth or niche position would increase certainty in this area.

Through this work, I have found strong evidence for the importance of environmental filtering and habitat use. These processes have been shown to shape the ecology and evolution of species of *Nebria*, more so than resource consumption or community composition. In future work, I propose examining the importance of these factors further by incorporating microhabitat use. Chapter 1 surveys microhabitat use through detailed ecological surveys, but given the geographic scope encompassed by Chapters 2 and 3, microhabitat variation is not an axis of niche that could be considered. Species of *Nebria* seek refuge during the day under rocks, sand, and other substrate, and have been shown to be closely associated with microhabitat features, such as substrate type and substrate temperature. Microhabitats are climatically stable subsets of larger habitats and could serve as important refugia for species adjusting to climate change. Montane habitats in the northwestern United States and southwestern Canada, where much of these data were collected, are expected to become hotter and drier as climate change progresses, with increasingly irregular precipitation. For geophilic species with limited dispersal capability, rapid changes in climate could result in large declines in species' populations, and the beginnings of this have already been observed. Microclimates could provide important refugia, providing valuable resources of time and space, perhaps for long enough to allow populations to adapt. There is currently debate as to how alpine species have historically responded to glacial cycles, and examining the role of microhabitat could shed light on this topic. While there is strong support for some species chasing their thermal niche, when possible, others species may be bound to their current location due to locally adapted traits. Ecological traps may create difficulties for species attempting to chase their thermal niche if other traits are more functional in their current habitat. If sufficient microhabitats exist, low-elevation species may seek refuge in these microniches, negating the need to shift their range. Microhabitats are a largely unexplored feature of above-ground habitats and could provide important refugia to species seeking climate refuge. I look forward to pursuing avenues of research such as these in my career.

Appendix 1: Supplementary Material for Chapter 1

Table 1 Tukey honest significance test of elevational differences between substrate types. Only statistically indiscernible substrate types based on the elevation at which they typically occur are included ($p > 0.05$): mostly sand, sand and small rocks, and small rocks. All other substrates are statistically indiscernible from one another. Upper and lower bounds are calculated from a 95% confidence interval.

Substrate 1 Elevation – Substrate 2 Elevation	Difference	Lower Bound	Upper Bound	p value
Sand and Rocks – Mostly Sand	8.49	-71.3	88.3	0.999
Small Rocks – Mostly Sand	-45.5	-127	36.0	0.651
Small Rocks – Sand and Rocks	-54.0	-125	16.6	0.266

Table 2 Coefficients from a multinomial logistic regression analyzing trends in habitat use among species of *Nebria* found on Mt. Rainier. All species except for *N. meanyi* are included in this analysis. *Nebria acuta* was used as the reference species. Standard errors are indicated in parentheses.

Species	Intercept	Ambient Temp.	Substrate Type	Surface Temp.	Dist. from River
N. crassicornis	1.02(0.682)	-0.181(0.0377)	-0.595(0.171)	0.102(0.0363)	0.0251(0.0274)
N. eschscholtzii	-4.03(1.38)	-0.0145(0.0696)	-1.64(0.555)	0.292(0.0556)	0.129(0.0236)
N. gebleri	2.55(0.821)	-0.0470(0.0496)	-0.317(0.178)	-0.349(0.0533)	-0.0663(0.0641)
N. kincaidi	5.71(13.3)	-1.02(1.84)	-0.676(1.16)	-1.71(0.725)	0.499(0.479)
N. mannerheimii	-1.52(0.566)	-0.0304(0.0307)	-0.0991(0.110)	0.124(0.0284)	0.0394(0.0215)
N. metallica	1.83(15.4)	0.340(0.259)	-6.74(14.8)	-0.223(0.205)	-3.86(1.84)
N. paradisi	-3.04(2.34)	-0.133(0.193)	0.976(0.468)	-0.296(0.170)	0.209(0.0718)
N. piperi	0.604(0.656)	-0.0840(0.0361)	-0.0263(0.129)	-0.0214(0.0342)	-0.231(0.0637)
N. sahlbergii	3.39(0.822)	-0.289(0.0491)	-0.684(0.220)	-0.00443 (0.0483)	-0.162(0.0780)
N. vanydkei	3.11(2.21)	-0.751(0.243)	-0.464(0.500)	-0.0915(0.137)	0.235(0.122)

Table 3 Differences in temperature preference among species of *Nebria*. Lower and upper bounds are calculated from a 95% confidence interval of the difference between species' preferences. Only significant ($p < 0.05$) results are included.

Nebria species 1 – *Nebria* species 2	Difference	Lower Bound	Upper Bound	p value
N. gebleri – N. acuta	3.38	1.23	5.53	0.0000323
N. metallica – N. acuta	-2.92	-4.59	-1.25	0.0000016
N. paradisi – N. acuta	-3.69	-5.25	-2.13	~0
N. gebleri – N. crassicornis	2.60	0.533	4.67	0.00286
N. metallica – N. crassicornis	-3.70	-5.26	-2.14	~0
N. paradisi – N. crassicornis	-4.47	-5.91	-3.02	~0
N. vandykei – N. crassicornis	-3.46	-6.32	-0.600	0.00517
N. metallica – N. eschscholtzii	-4.77	-6.31	-3.24	~0
N. paradisi – N. eschscholtzii	-5.54	-6.96	-4.12	~0
N. vandykei – N. eschscholtzii	-4.53	-7.38	-1.69	0.0000225
N. mannerheimi – N. gebleri	-2.05	-4.05	-0.054	0.0384
N. metallica – N. gebleri	-6.30	-8.30	-4.30	~0
N. paradisi – N. gebleri	-7.07	-8.98	-5.16	~0
N. piperi – N. gebleri	-2.28	-4.20	-0.365	0.00653
N. sahlbergii – N. gebleri	-3.06	-5.48	-0.641	0.00260
N. vandykei – N. gebleri	-6.06	-9.19	-2.94	~0
N. metallica – N. mannerheimi	-4.25	-5.72	-2.78	~0
N. paradisi – N. mannerheimi	-5.02	-6.36	-3.67	~0
N. vandykei – N. mannerheimi	-4.01	-6.82	-1.20	0.000288
N. piperi – N. metallica	4.01	2.66	5.37	~0
N. sahlbergii – N. metallica	3.24	1.24	5.24	0.0000144
N. piperi – N. paradisi	4.79	3.57	6.01	~0
N. sahlbergii – N. paradisi	4.01	2.10	5.92	~0
N. vandykei – N. piperi	-3.78	-6.53	-1.03	0.000613

Table 4 Proportional weight loss differences due to desiccation between species of *Nebria*. Upper and lower bounds are calculated from a 95% confidence interval of the difference between species. Only significant ($p < 0.005$) results are included.

Nebria species 1 – *Nebria* species 2	Difference	Lower Bound	Upper Bound	p value
N. gebleri – N. acuta	0.151	0.0651	0.238	0.0000043
N. gebleri – N. crassicornis	0.117	0.0321	0.201	0.000849
N. gebleri – N. eschscholtzii	0.109	0.0245	0.194	0.00256
N. mannerheimi – N. gebleri	-0.155	-0.234	-0.0754	0.0000002
N. metallica – N. gebleri	-0.244	-0.362	-0.126	<2e-16
N. piperi – N. gebleri	-0.116	-0.200	-0.0323	0.000801
N. sahlbergii – N. gebleri	-0.119	-0.202	-0.0353	0.000481
N. vandykei – N. gebleri	-0.220	-0.329	-0.111	<2e-16

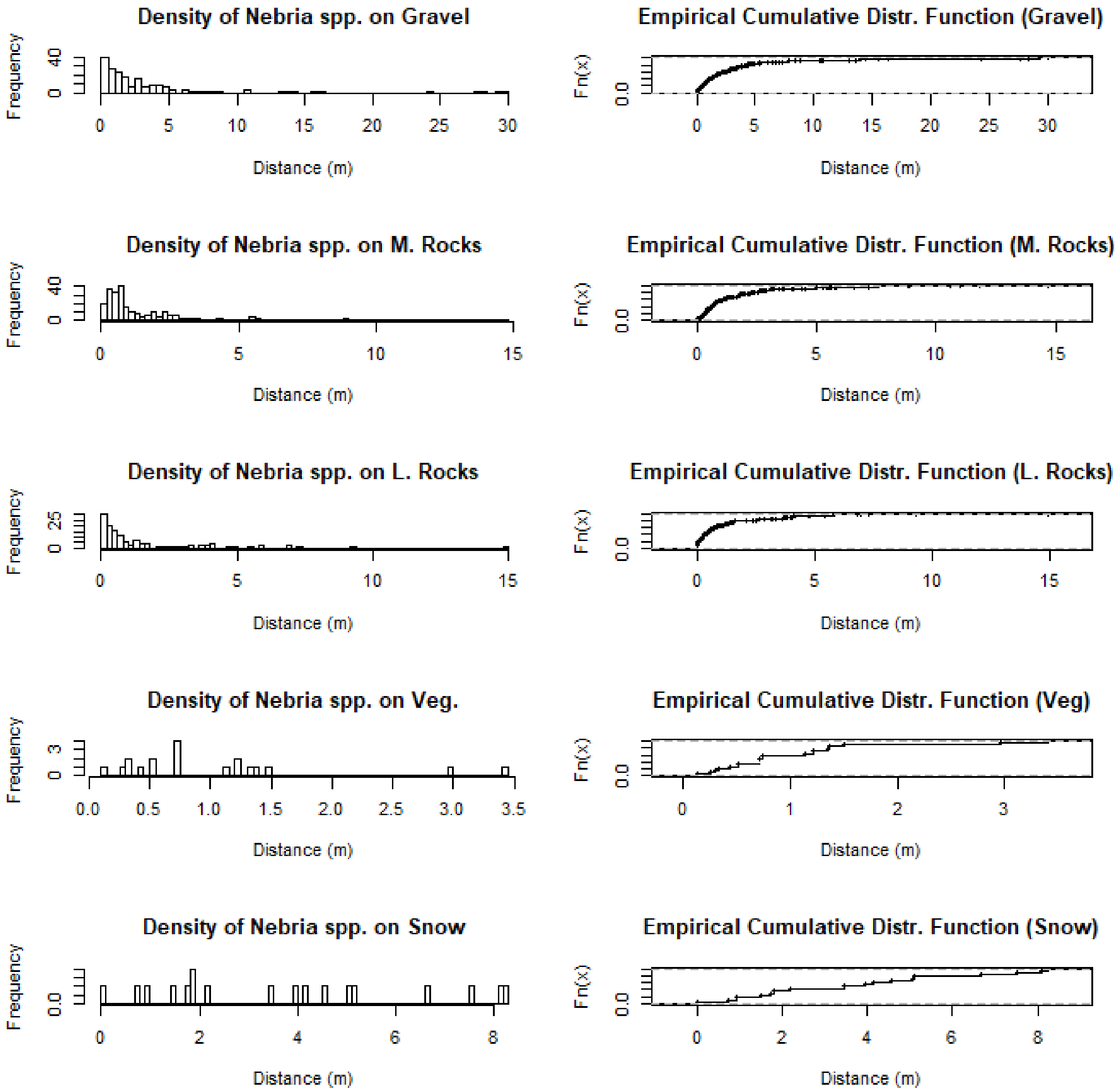
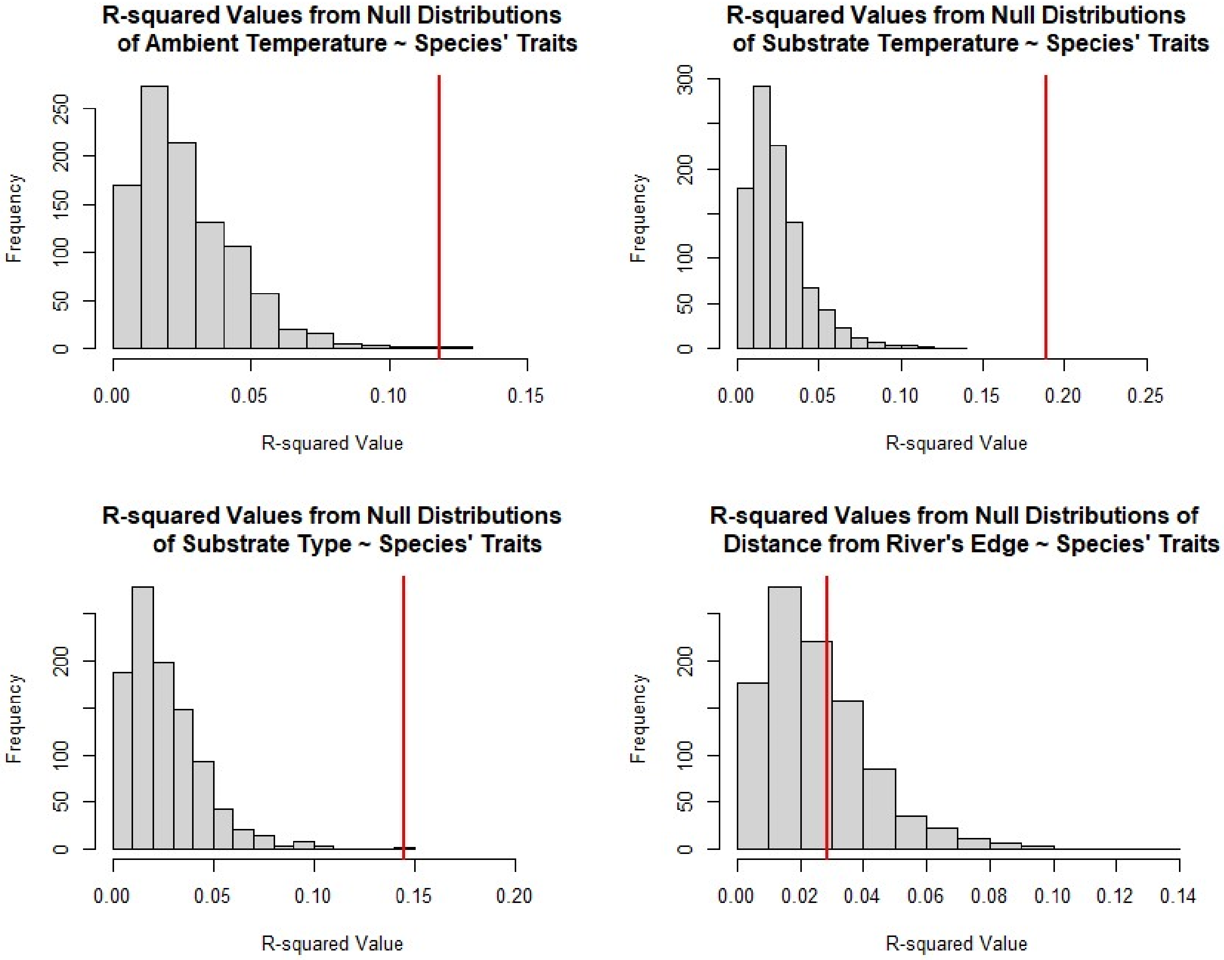


Figure 1 The panels on the left show the density of species of *Nebria* individuals moving away from the stream edge on different habitat substrates. The curves on the right show the cumulative distribution function of these histograms.


R-squared Values from Null Distributions of Ambient Temperature ~ Species' Traits

R-squared Values from Null Distributions of Substrate Temperature ~ Species' Traits

R-squared Values from Null Distributions of Substrate Type ~ Species' Traits

R-squared Values from Null Distributions of Distance from River's Edge ~ Species' Traits

Figure 2 Histograms indicating null distributions of adjusted R^2 values from regression models of habitat characteristics and traits. These null models were made by randomizing trait values and habitat features across individuals. Red lines indicate the R^2 values calculated using the observed survey data (ambient temperature: $R^2 = 0.118$, $p = 0.001$; substrate temperature: $R^2 = 0.188$, $p = 0$; substrate type: $R^2 = 0.145$, $p = 0$; distance from river's edge: $R^2 = 0.0286$, $p = 0.353$).

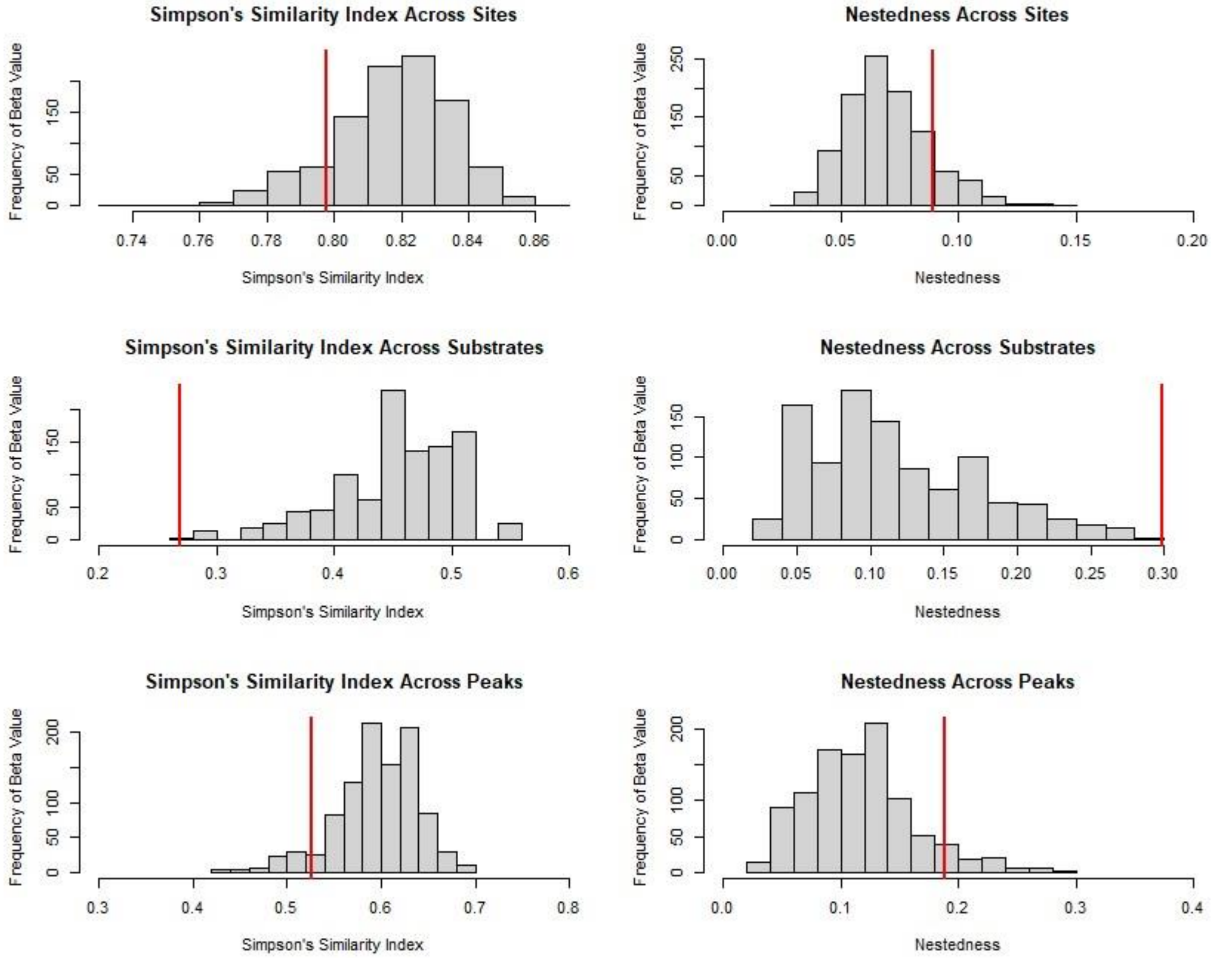


Figure 3 Histograms indicating null distributions of β_{Sim} (left) and β_{Nes} (right) values across sites (top), substrates (middle), and peaks (bottom). Null β values were computed from 1000 randomized matrices of species compositions across locations, maintaining the total number of occurrences for each species. Red lines indicate the β values calculated using the observed survey data (β_{Sim}: site = 0.798, p = 0.121; substrate = 0.269, p = 0; peak = 0.525, p = 0.064; β_{Nes}: site = 0.0894, p = 0.121; substrate = 0.299, p = 0; peak = 0.189, p = 0.064).

Appendix 2: Supplementary Material for Chapter 2

Table 1 Sample sizes of species of *Nebria* analyzed in this study.

Species	Sample Size
Nebria acuta Lindroth	20
Nebria albimontis Kavanaugh	13
Nebria appalachia Darlington	17
Nebria arkansana Casey	20
Nebria balli Kavanaugh	19
Nebria bellorum Kavanaugh	10
Nebria beverlianna Kavanaugh	19
Nebria calva Kavanaugh	20
Nebria carri Kavanaugh	20
Nebria cascadensis Kavanaugh	20
Nebria castanipes Kirby	10
Nebria catenata Casey	20
Nebria charlottae Lindroth	20
Nebria chuskae Kavanaugh	10
Nebria coloradensis Van Dyke	12
Nebria crassicornis Van Dyke	9
Nebria danmanni Kavanaugh	18
Nebria darlingtoni Kavanaugh	19
Nebria desolata Kavanaugh	18
Nebria diversa LeConte	20
Nebria edwardsi Kavanaugh	20
Nebria eschscholtzii Ménétriés	20
Nebria fragariae Kavanaugh	20
Nebria fragilis Casey	20
Nebria frigida Sahlberg	19
Nebria gebleri Dejean	20
Nebria giulianii Kavanaugh	17
Nebria gouleti Kavanaugh	19
Nebria gregaria Fischer von Waldeim	16
Nebria gyllenhali Schönherr	20
Nebria haida Kavanaugh	20
Nebria hudsonica LeConte	20
Nebria ingens Horn	19
Nebria intermedia Van Dyke	10
Nebria jeffreyi Kavanaugh	16
Nebria kincaidi Schwarz	19
Nebria labontei Kavanaugh	15
Nebria lacustris Casey	10

Nebria lamarckensis Kavanaugh	19
Nebria lassenensis Kavanaugh	10
Nebria lindrothi Kavanaugh	10
Nebria lituyae Kavanaugh	20
Nebria louiseae Kavanaugh	20
Nebria lyelli Van Dyke	20
Nebria mannerheimii Fischer von Waldheim	20
Nebria meanyi Van Dyke	18
Nebria metallica Fischer von Waldheim	17
Nebria modoc Kavanaugh	13
Nebria navajo Kavanaugh	18
Nebria nivalis Paykull	9
Nebria obliqua LeConte	8
Nebria oowah Kavanaugh	20
Nebria ovipennis LeConte	20
Nebria pallipes Say	20
Nebria paradisi Darlington	19
Nebria pasquineli Kavanaugh	20
Nebria piperi Van Dyke	19
Nebria piute Erwin & Ball	19
Nebria purpurata LeConte	20
Nebria quileute Kavanaugh	19
Nebria rathvoni LeConte	20
Nebria riversi Van Dyke	5
Nebria sahlbergii Fischer von Waldheim	20
Nebria schwarzi Van Dyke	20
Nebria sevieri Kavanaugh	20
Nebria sierrablancae Kavanaugh	18
Nebria sierrae Kavanaugh	19
Nebria siskiyouensis Kavanaugh	20
Nebria sonorae Kavanaugh	13
Nebria spatulata Van Dyke	20
Nebria steensensis Kavanaugh	20
Nebria suturalis LeConte	20
Nebria sylvatica Kavanaugh	20
Nebria triad Kavanaugh	19
Nebria trifaria LeConte	20
Nebria vandykei Bänninger	20
Nebria wallowae Kavanaugh	19
Nebria wyeast Kavanaugh	20
Nebria zioni Van Dyke	8

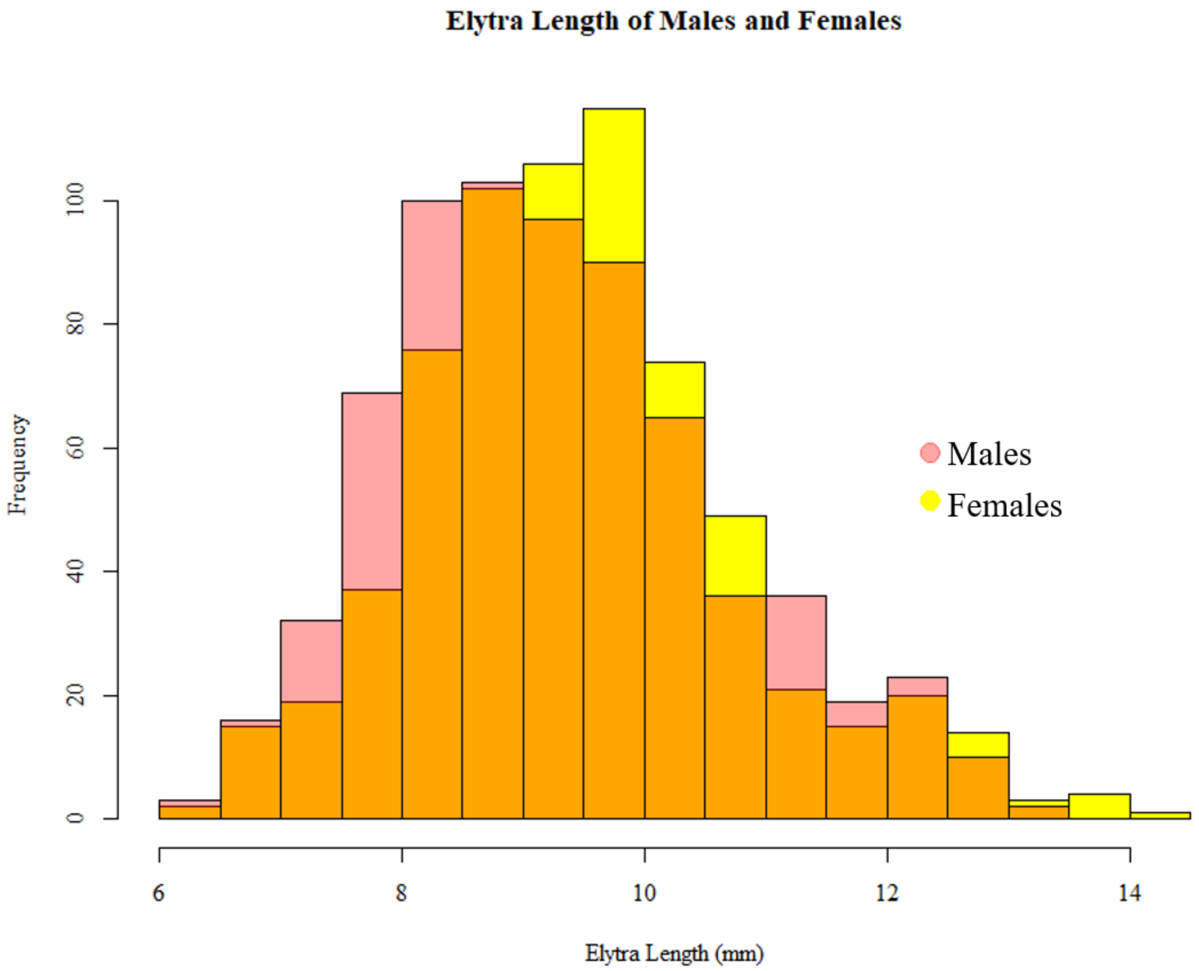


Figure 1 Elytral length of males and females across species of *Nebria*. Males are indicated by light red, and females are indicated by yellow. While elytral length is statistically discernable between males and females, there is high overlap in the distribution of these values across sexes.

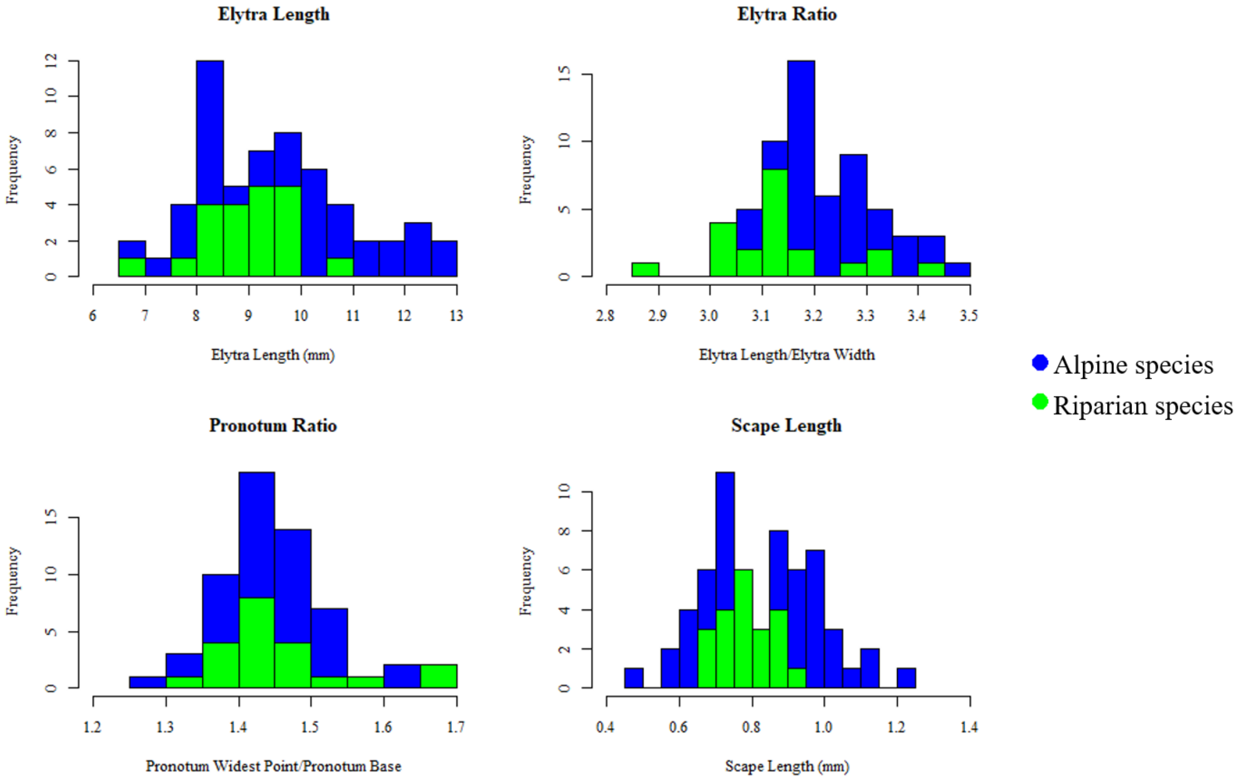


Figure 2 Linearly measured morphological traits of species of *Nebria*. Colors indicate whether species were collected in alpine or riparian habitats based on a threshold mean ground temperature of 6˚C.

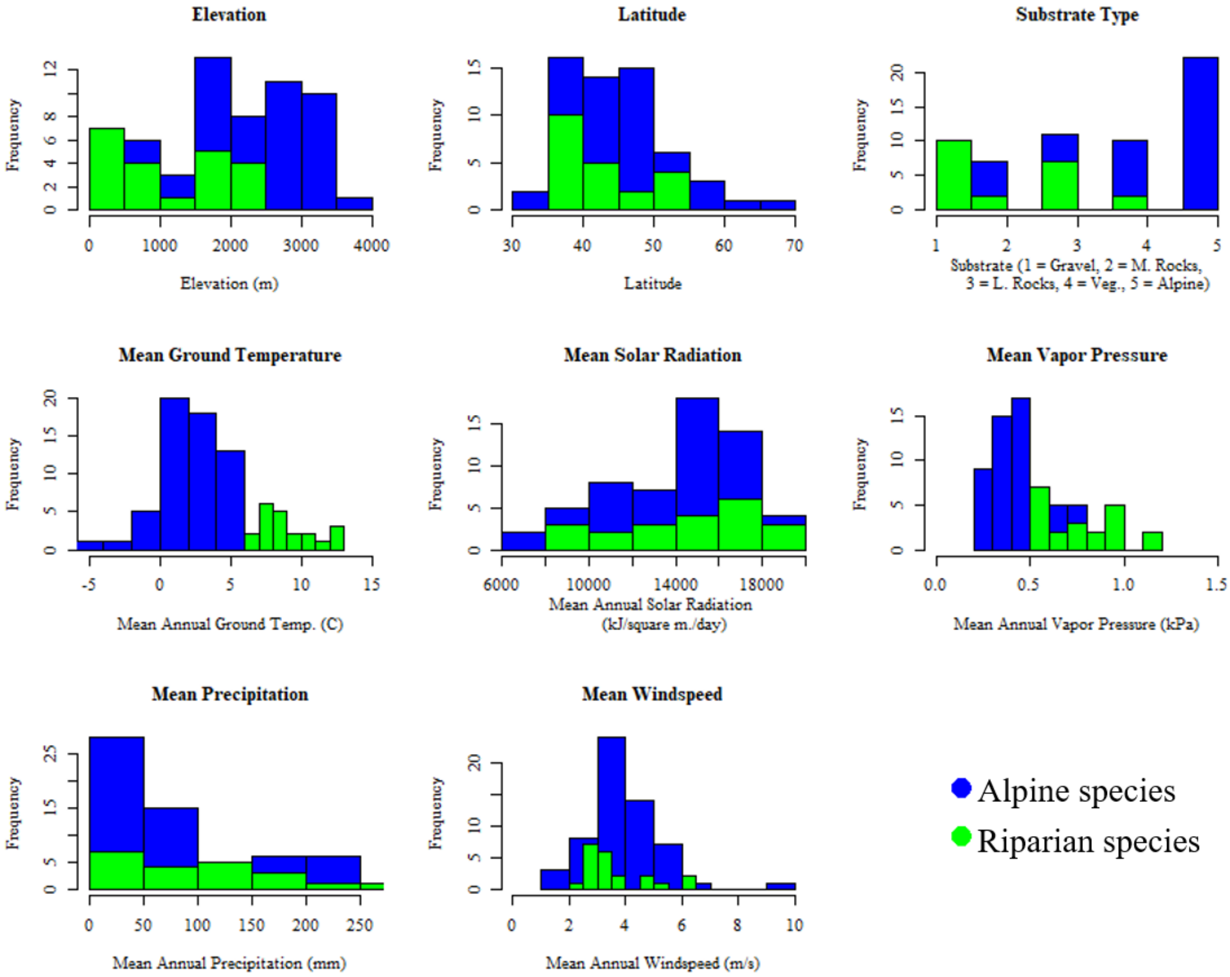


Figure 3 Habitat characteristics where species *Nebria*. were collected using the Worldclim database (Fick & Hijmans, 2017). Colors indicate whether species were collected in alpine or riparian habitats based on a threshold mean ground temperature of 6˚C.

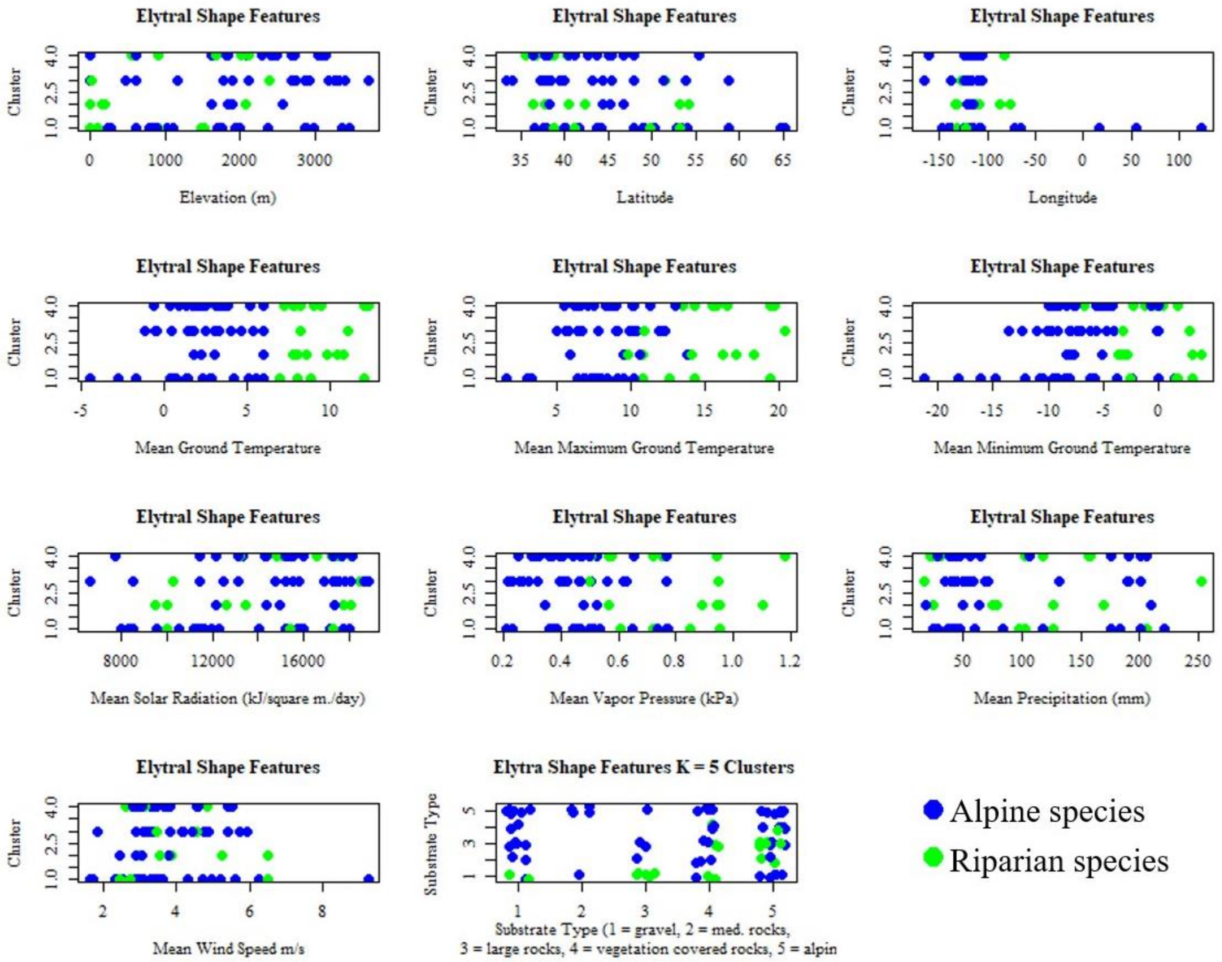


Figure 4 Elytral shape features formed k = 4 clusters in morphospace. These clusters do not appear to correspond to variation in habitat use. Colors indicate whether species were collected in alpine or riparian habitats based on a threshold mean ground temperature of 6˚C. For comparison with substrate type, elytral shape features were coerced to five clusters, corresponding to the categories of substrate type used in this study.

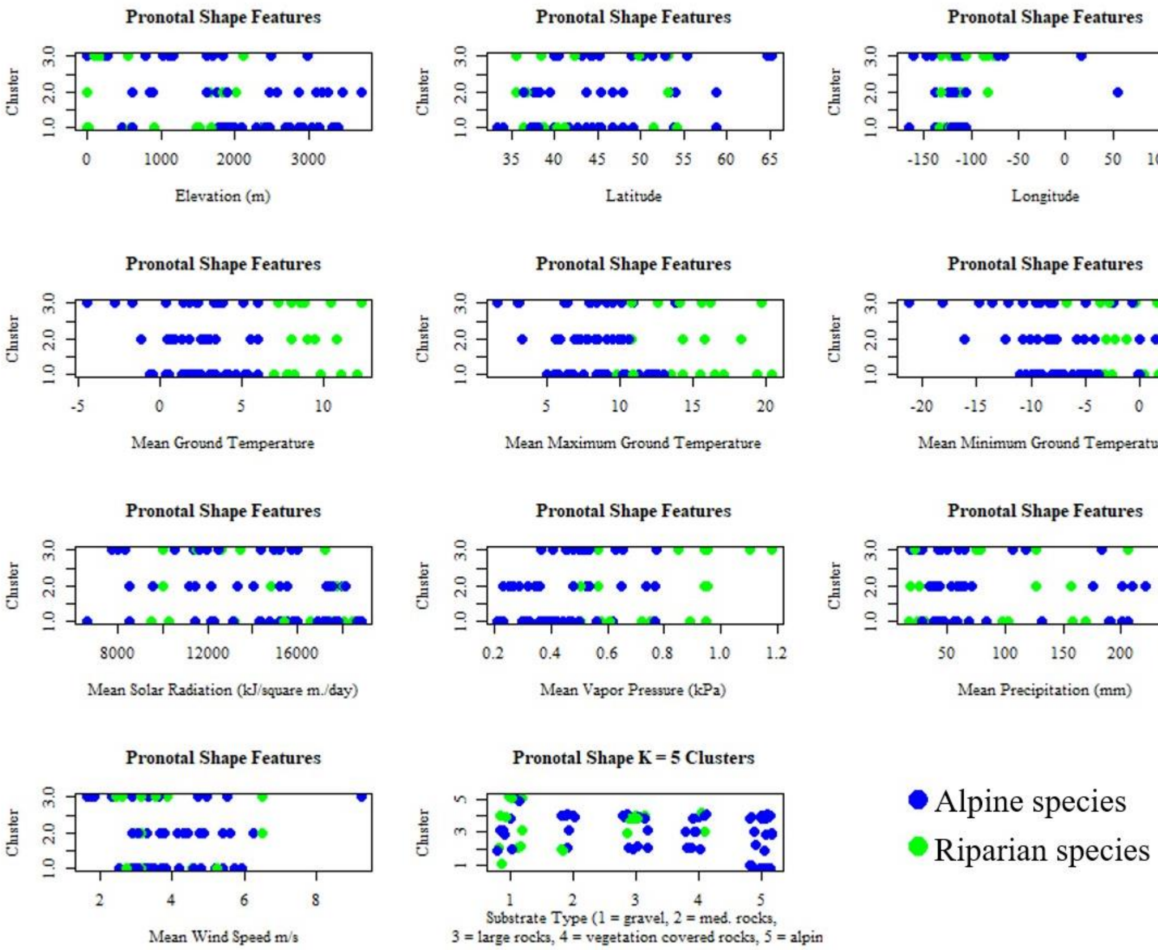


Figure 5 Pronotal shape features formed k = 3 clusters in morphospace. These clusters do not appear to correspond to variation in habitat use. Colors indicate whether species were collected in alpine or riparian habitats based on a threshold mean ground temperature of 6˚C. For comparison with substrate type, pronotal shape features were coerced to five clusters, corresponding to the categories of substrate type used in this study.

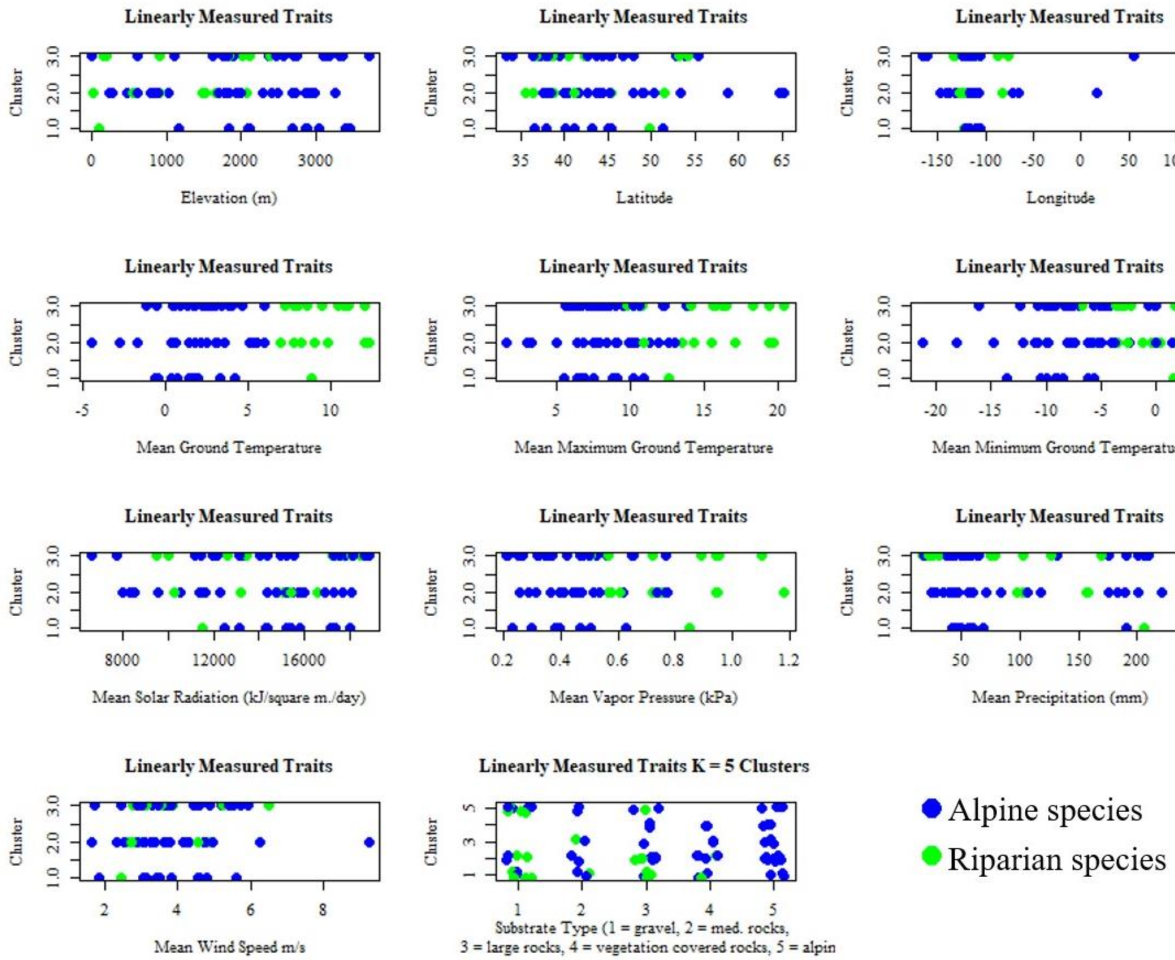


Figure 6 Linearly measured traits formed k = 3 clusters in morphospace. These clusters do not appear to correspond to variation in habitat use. Colors indicate whether species were collected in alpine or riparian habitats based on a threshold mean ground temperature of 6˚C. For comparison with substrate type, linearly measured traits were coerced to five clusters, corresponding to the categories of substrate type used in this study.

Appendix 3: Supplementary Material for Chapter 3

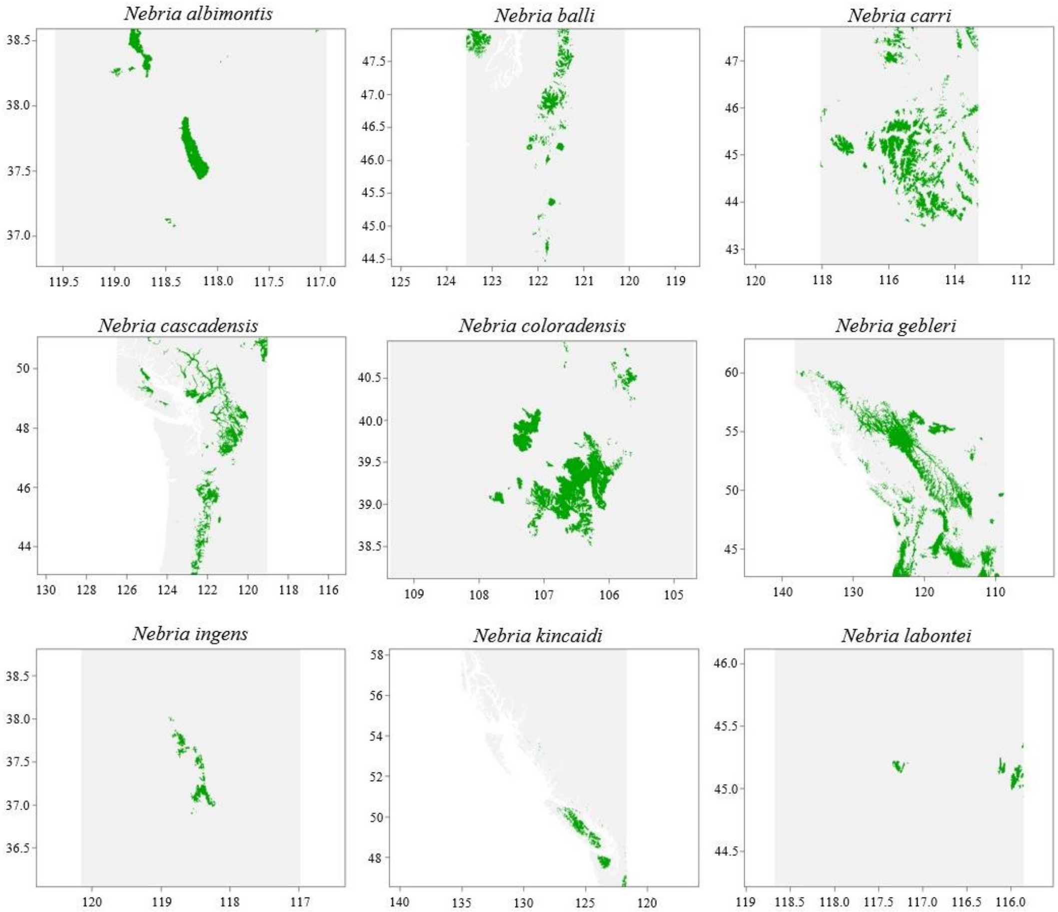


Figure 1 Range maps of species of *Nebria*. based on suitability thresholds of 70.0% or higher shown in green, based on maxent niche models of species distributions (continued on the next page).

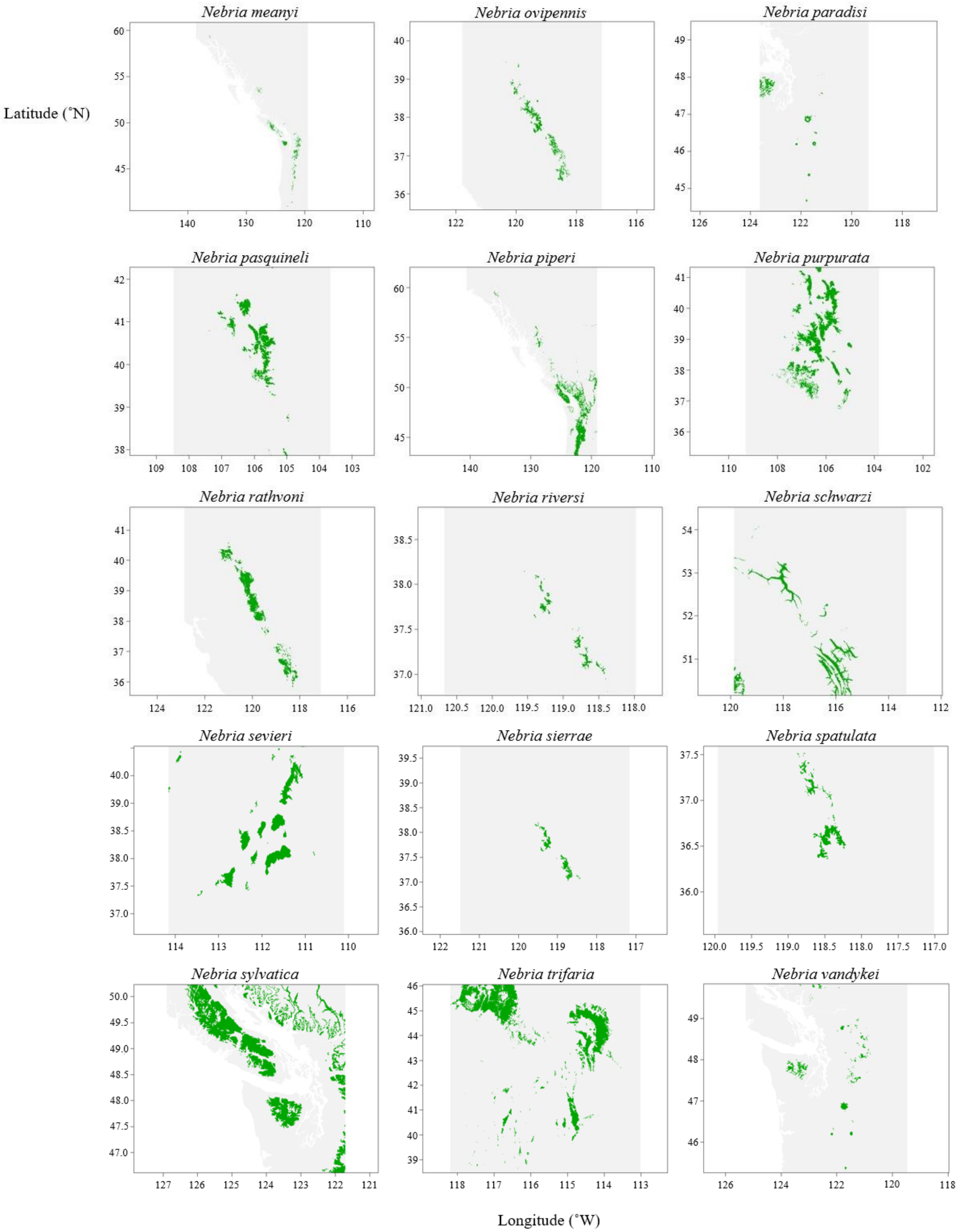


Figure 1 Cont. Range maps of species of *Nebria*. based on suitability thresholds of 70.0% or higher shown in green, based on maxent niche models of species distributions.

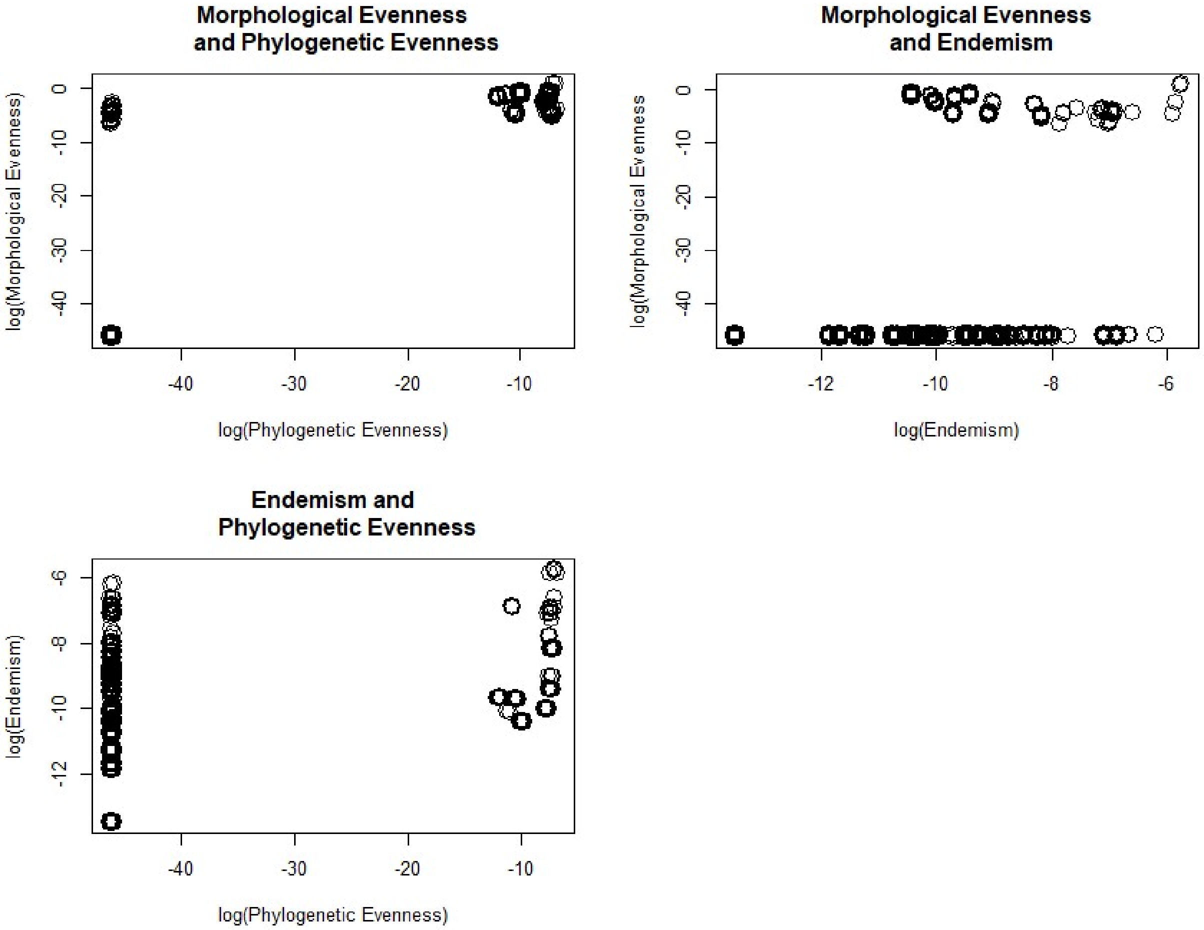


Figure 2 Scatterplots of log-scaled morphological variation, phylogenetic relatedness, and endemism trends where Nearctic species of *N. Catonebria*. are found. Phylogenetic relatedness is estimated by measuring the patristic distance between species pairs (the total phylogenetic branch length between them). Endemism is calculated using species richness inversely weighted by range size.