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A phylogenomic perspective on the evolutionary history of the stonefly genus *Suwallia* (Plecoptera: Chloroperlidae) revealed by ultraconserved genomic elements

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Abstract

Evolutionary biologists have long sought to disentangle phylogenetic relationships among taxa spanning the tree of life, an increasingly important task as anthropogenic influences accelerate population declines and species extinctions, particularly in insects. Phylogenetic analyses are commonly used to identify unique evolutionary lineages, to clarify taxonomic designations of the focal taxa, and to inform conservation decisions. Advances in DNA sequencing techniques have increasingly facilitated the ability of researchers to apply genomic methods to phylogenetic analyses, even for non-model organisms. Stoneflies are non-model insects that are important bioindicators of the quality of freshwater habitats and landscape disturbance as they spend the immature stages of their life cycles in fresh water, and the adult stages in terrestrial environments. Phylogenetic relationships within the stonefly genus *Suwallia* (Insecta: Plecoptera: Chloroperlidae) are poorly understood, and have never been assessed using molecular data. We used DNA sequence data from genome-wide ultraconserved element loci to generate the first molecular phylogeny for the group and assess its monophyly. We found that Palearctic and Nearctic *Suwallia* do not form reciprocally monophyletic clades, and that a biogeographic history including dispersal, vicariance, and founder event speciation via jump dispersal best explains the geographic distribution of this group. Our results also strongly suggest that *Neaviperla forcipata*

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Author contribution

Conceptualization: Derek Houston, Jordan Satler, Kevin Alexander. Data curation: Derek Houston, Jordan Satler, Hannah Carroll. Formal analysis: Derek Houston, Jordan Satler, Hannah Carroll. Funding acquisition: Derek Houston, Kevin Alexander. Investigation: Derek Houston, Jordan Satler, Taylor Stack, Autumn Moya, Alissa Bevan. Methodology: Derek Houston, Jordan Satler, Taylor Stack, Hannah Carroll, Autumn Moya, Alissa Bevan. Project administration: Derek Houston, Kevin Alexander. Resources: Derek Houston, Jordan Satler, Kevin Alexander. Supervision: Derek Houston, Jordan Satler. Visualization: Derek Houston, Jordan Satler, Hannah Carroll. Writing - original draft: Derek Houston. Writing - review & editing: Derek Houston, Jordan Satler, Taylor Stack, Hannah Carroll, Autumn Moya, Alissa Bevan, Kevin Alexander.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

(Neave, 1929) is nested within *Suwallia*, and the concept of the genus *Suwallia* should be revised to include it. Thus, we formally propose a new taxonomic combination wherein *Neaviperla forcipata* (Neave, 1929) is reclassified as *Suwallia forcipata* (Neave, 1929). Moreover, some *Suwallia* species (e.g., *S. amoenacolens, S. kerzhneri, S. marginata, S. pallidula*, and *S. starki*) exhibit pronounced cryptic diversity that is worthy of further investigation. These findings provide a first glimpse into the evolutionary history of *Suwallia*, improve our understanding of stonefly diversity in the tribe Suwallini, and highlight areas where additional research is needed.

Keywords

UCEs; Phylogenetics; Biodiversity; Stoneflies; Aquatic insects

1. Introduction

Evolutionary biologists have long sought to disentangle phylogenetic relationships among a wide variety of organisms, and phylogenetic trees fundamentally inform our understanding of evolutionary processes. Gaining a better understanding of biodiversity is increasingly important as anthropogenic influences such as habitat fragmentation, climate change, exotic species introductions, overexploitation, and pollution contribute to population declines in biological populations and reduce biodiversity (Diamond, 1989; Isbell, 2010; Maxwell et al., 2016). Insects are particularly at risk, and have exhibited steep declines in recent decades (Thomas et al., 2004; Hallmann et al., 2017; Sánchez-Bayo and Wyckhuys, 2019), although these declines may be more pronounced in terrestrial than in freshwater insects (van Klink et al., 2020).

Stoneflies (Insecta: Plecoptera) belong to an ancient, circumglobal order of aquatic insects that originated in the Permian, and extant suborders are postulated to have evolved corresponding with the break-up of Pangaea (Stewart and Ricker, 1997; Zwick, 2000; Ding et al., 2019). Unsurprisingly, speciation events that have occurred more recently are also common within the group (Zwick, 2000). Order Plecoptera contains more than 3,700 species, with many more being described each year (Fochetti and de Figueroa, 2008; DeWalt and Ower, 2019). Rapid rates of stonefly species descriptions in China and South America highlight our general lack of knowledge of, as well as our need to better understand, the diversity of this group in the face of the looming biodiversity crisis. Indeed, many existing plecopteran phylogenies need additional molecular data to be more accurately reconstructed (Wang et al., 2018). Recent phylogenomic research has provided important insights into family-level relationships of North American plecopterans (South et al., 2021). However, inter- and intra-generic relationships among many stonefly taxa remain poorly understood.

The chloroperlid genus *Suwallia* is distributed across Nearctic and eastern Palearctic regions (Alexander and Stewart, 1999; Cary and Jacobi, 2008; Kondratieff et al., 2019; Fig. 1). *Suwallia* is postulated to be a monophyletic group based on distinctive morphological characteristics (e.g., morphology of genitalia, wing venation patterns, coloration; Surdick, 1985). There are currently 26 described *Suwallia* species (Alexander and Stewart, 1999; Chen and Du, 2015; Chen, 2019; DeWalt et al., 2020) (see Table 1). Another member

of the tribe Suwallini, *Neaviperla forcipata*, was synonymized with *Suwallia* due to low diagnostic characters across various life stages (Alexander and Stewart, 1999). It was later re-established as a valid genus (Baumann and Lee, 2014). Clearly, the phylogenetic relationships and taxonomic status of these genera warrant additional scrutiny. Molecular data from some members of the genus *Suwallia* have been included in higher-level phylogenetic studies of the Plecoptera (Terry, 2004; Wang et al., 2018; Ding et al., 2019; South et al., 2021), as representatives used in proof-of-concept studies using DNA barcoding primers (Hebert et al., 2003), and in comparative analyses examining speciation rates in high elevation areas in tropical vs. temperate ecosystems (Polato et al., 2018). However, a comprehensive phylogenetic analysis of the genus based on molecular data has yet to be conducted.

In this study, we used ultraconserved elements (UCEs) to infer phylogenetic relationships within the genus *Suwallia* and to assess the validity of the genus *Neaviperla* using museum specimens. Ultraconserved elements are genomic regions that are highly conserved across evolutionarily disparate taxa that are flanked by increasingly variable DNA sequences (with distance from the core region), and are a powerful tool for modern phylogenomic analyses (Bejerano et al., 2004; Siepel et al., 2005; Crawford et al., 2012; Faircloth et al., 2012, 2013, 2020; Smith et al., 2014; Faircloth, 2015; Wachi et al., 2018; Zhang et al., 2019). Moreover, this approach has successfully recovered UCEs from older museum specimens that were previously considered to be unusable in phylogenetic analyses because of their highly degraded DNA (Burrell et al., 2015; Blaimer et al., 2016; Jones and Good, 2016; McCormack et al., 2016; Ruane and Austin, 2017; Chen et al., 2018; Wood et al., 2018). We used UCE data generated from museum stonefly specimens to test the following hypotheses:

H1: *Suwallia* species distributed in the Nearctic and those with Palearctic distributions will form reciprocally monophyletic clades, and the historical biogeography of the group is best explained by vicariant events.

H2: Previously unrecognized cryptic genetic diversity, in the form of non-monophyly, will be recovered within *Suwallia*, particularly in species with broad geographic distributions.

H3: Phylogenetic analyses based on UCE sequences will place *Neaviperla forcipata* within *Suwallia*, rendering the genus paraphyletic.

2. Materials & methods

2.1. Sampling and DNA extraction

Ethanol-preserved *Suwallia* specimens housed in the Faunal Museum in the Department of Natural and Environmental Sciences at Western Colorado University were used in this study. Samples were collected between the years 1941–2018 (Table 1). All specimens were identified to species multiple times by coauthor K. Alexander, an expert on chloroperlid stoneflies. Four morphotypes were classified *a priori* as *Suwallia* species a, b, d, and e, representing either undescribed species, or populations displaying morphological variation within described species. When possible, we included multiple individuals from distinct geographic sampling localities (Fig. 1), but multiple samples were not available for all

species (Table 1). To reduce the negative consequences of destructive sampling, we digitally photographed each specimen (dorsal, ventral, and side views, against both white and black backgrounds) using a Leica S9i stereo macroscope prior to DNA extraction. These images have been made available in a Dryad repository (see Data Accessibility below).

Specimens were air-dried to let ethanol completely evaporate from the tissues, then we used a Qiagen DNeasy Blood and Tissue Kit to extract whole genomic DNA following the manufacturer's recommended protocol. At the onset of the DNA extraction procedure, we placed whole air-dried specimens into their respective lysis buffer/proteinase K solutions, then pierced each abdomen with a sterilized dissecting pin prior to the first incubation step of the protocol to facilitate lysis of internal organs. Following DNA extractions, we retrieved exoskeletons, rinsed them with ethanol, then placed them into labeled, ethanol filled vials for long-term storage as vouchers in case external morphology needed reassessment.

Whole genomic DNA quality was visually assessed via gel electrophoresis in 1.5X agarose gels stained with GelRed nucleic acid dye. We then quantified DNA concentrations using a Qubit™ 1X dsDNA HS Assay Kit. Following quantification, we concentrated samples with low concentrations by drying them in a vacuum centrifuge, then rehydrated them in 120 μL of AE buffer, after which we quantified concentrations a second time using the same Qubit™ kit.

2.2. UCE sequencing

DNA quality varied among samples, so we employed three different approaches to ensure that DNA fragments were within the appropriate size range (300–600 bp) for Illumina sequencing: 1) We sheared samples with high molecular weight DNA using a Covaris ME220 Focused-Ultrasonicator (with the following settings, depending on apparent DNA quality: D70, PP50, DF10, CB1000, T 20°C for the highest quality samples; D63, PP50, DF10, CB1000, T 20°C for slightly degraded samples; D40, PP25, DF10, CB1000, T20°C for more degraded samples that still had bright bands of high molecular weight). After sonication, we performed a double size selection by first removing large fragments (greater than 800 bp) using a 0.6X SPRI bead cleanup, then increasing the SPRI bead concentration in the sample to 1.5X to remove small fragments (<150 bp) (Bronner et al., 2009). 2) For genomic samples with bright bands within the appropriate size range, and only faint bands of high molecular weight DNA, we bypassed sonication and only used a 0.6X-1.5X SPRI bead double cleanup. 3) Highly degraded samples were included 'as is' to avoid shearing already degraded DNA to unusable small fragments, but the smallest (i.e., unusable) fragments were removed using a 1.5X SPRI bead cleanup. After shearing and size selection, we again visually assessed size distributions following gel electrophoresis as described above.

DNA libraries were prepared in half reactions using KAPA Hyperprep Kits (KAPA Biosystems) following the manufacturer's protocol that included detailed instructions for end repair and A-tailing, ligating adaptors, cleaning samples post-ligation, amplifying libraries, and cleaning the libraries post-amplification. Glenn et al. (2019) provide a comprehensive overview of this procedure.

Currently, there are no published bait kits designed for target enrichment of UCEs in Plecoptera, but the protocol is robust, only requiring an ~80% match between baits and the targeted UCE to be effective (Faircloth, 2015, 2017; Branstetter et al., 2017). A recent bioinformatic analysis (Bossert and Danforth, 2018) predicted that baits originally designed for hymenopterans (Branstetter et al., 2017) should also capture ~300 loci in plecopterans. It was also suggested that baits developed for hemipterans (Faircloth, 2017) could capture loci in plecopterans (Brant Faircloth, personal communication with DDH). Hence, we opted to use both the Hymenoptera 2.5Kv2 bait design (Branstetter et al., 2017) and the Hemiptera 2.7Kv1 bait design (Faircloth, 2017) to capture UCE loci *in situ*. Both the hymenopteran and hemipteran bait designs were commercially synthesized as RNA target capture arrays (myBaits, MYcroarray, Arbor Biosciences).

To capture targeted UCEs, we pooled libraries in equimolar amounts and conducted hybridization capture in 1/8th reactions. We captured UCEs in separate reactions for each pool of eight samples using probe sets Hymenoptera 2.5Kv2 and Hemiptera 2.7Kv1, following the steps outlined in the myBaits protocol 4.0.1. We subsequently pooled the captured hymenopteran and hemipteran targets, assessed DNA size, quantity and quality using an Agilent 2100 Bioanalyzer, then conducted one last size selection step using BluePippin before sending the pooled sample to GENEWIZ (South Plainfield, NJ) for Illumina sequencing. We sequenced libraries in parallel in a single lane of an Illumina HiSeq 4000 with 150-cycle paired end reads.

2.3. Phylogenetic inference

We analyzed UCE data following the PHYLUCE v1.6.7 pipeline (Faircloth, 2015). In brief, we trimmed raw sequence reads using illumiprocessor v2.0.9 (https://github.com/faircloth-lab/illumiprocessor) a wrapper around trimmomatic v0.39 (Bolger et al., 2014), assembled and aligned contigs using SPAdes v3.12.0 (Bankevich et al., 2012; Nurk et al., 2013), matched UCEs to the hymenopteran and hemipteran probe sets, extracted UCE loci and aligned them using MAFFT v7.407 (Katoh and Standley, 2013), trimmed matrix edges missing sequence data for more than 35% of the taxa, and removed ambiguously aligned internal sites using Gblocks v0.91b (Castresana, 2000). Because the baits used were not specifically developed for plecopterans, the number of UCEs captured was expected to be lower than a typical data set with taxon-specific baits. To strike a balance between minimizing missing data and maximizing sampled loci, we retained UCEs containing sequences for at least 50% of the individuals. A visual representation of presence/absence of UCE data is displayed in Fig. 2.

For phylogenetic analysis, we first estimated a concatenated maximum likelihood (ML) phylogeny in IQ-TREE v1.6.12 (Nguyen et al., 2015). We partitioned the dataset by UCE locus, using a GTR + γ model per partition, and generated nodal support values through 1000 repetitions of the ultrafast bootstrap approximation (Hoang et al., 2018). We also used two coalescent-based species tree approaches for phylogeny estimation. First, we estimated a species tree with ASTRAL-III v5.7.3 (Zhang et al., 2017). As this method uses gene trees as input data, we estimated ML gene trees for each locus with IQ-TREE, using ModelFinder (Kalyaanamoorthy et al., 2017) to estimate a model of best substitution with BIC, and

generating nodal support values as described above. Following recommendations by Zhang et al. (2017), poorly supported nodes (bootstrap values < 10) were collapsed in each ML gene tree. Nodal support values were generated for the ASTRAL species tree with local posterior probabilities (Sayyari and Mirarab, 2016). Next, we estimated a species tree with SVDQuartets (Chifman and Kubatko, 2015) as implemented in PAUP* v4.0a166 (Swofford, 2003). SVDQuartets uses site patterns in the nucleotide data to estimate a species tree under the multispecies coalescent model. We evaluated all quartets and conducted standard bootstrapping to generate nodal support values. For both species tree approaches, the *a priori* designation of individuals to species was informed by a combination of taxonomy and monophyly in the ML concatenated tree. For samples where species designations were unclear, we treated them as their own operational taxonomic unit (OTU).

To visualize the presence and absence of UCEs across taxa, we created a list of all UCEs in our 50% data matrix. We concatenated the nexus files into a data frame containing all individuals and coded them for the presence (1) or absence (0) of each UCE. We then color-coded the cells marked ‘present’ in each individual to match the colors for the corresponding genus in Fig. 3. The visualization was created using the color2D.matplot function in the R package plotrix v.3.7–8 (Lemon, 2006) in R v.4.0.2 (R Core Team, 2020).

2.4. Biogeographic history

To infer the biogeographic history of this group of stoneflies and identify an appropriate biogeographic model for the data, we conducted a biogeographic analysis using the R package BioGeoBEARS v.1.1.2 (Matzke, 2013) in R v.4.0.2 (R Core Team, 2020). We designated each species by its contemporary geographic range, defined very broadly as occurring in the Nearctic or the Palearctic. We calculated geographic centroids for all Nearctic and all Palearctic samples, respectively, and measured the Haversine distance between centroids using the R package geosphere v.1.5–10 (Hijmans et al., 2019). That distance (8,129 km) was then used in the matrix included in the BioGeoBEARS distance file. We used the phylogeny estimated from IQ-TREE as input data. First, we pruned tips from the phylogeny using the R package ape v.5.4.1 (Paradis and Schliep, 2019), resulting in a tree with one representative per species (or monophyletic group, for the species exhibiting cryptic lineage diversity). We then used the chronos function in ape applying the correlated rate model (Sanderson, 2002) to convert the pruned tree to an ultrametric chronogram. We then used the chronogram to test the following biogeographic models: DEC, a dispersal-extinction-cladogenesis biogeographic model (Ree and Smith, 2008; Matzke, 2014; Massana et al., 2015); DIVALIKE, a maximum likelihood-based biogeographic model similar to DIVA (Ronquist, 1997); BAYAREALIKE, a maximum likelihood-based biogeographic model similar to Bay Area (Landis et al., 2013); and all of these models with an additional parameter (+J) that accounts for founder event speciation via jump dispersal (i.e., DEC+J, DIVALIKE+J, and BAYAREALIKE+J). Note that the DEC+J model has been critiqued for not adequately modelling founder event speciation (Ree and Sanmartín, 2018).

3. Results

3.1. Sampling and DNA extraction

We extracted DNA from 66 individual stoneflies representing 25 species from 5 genera (Table 1), including 20 of 26 *Suwallia* species, 4 unidentified *Suwallia* species (*S.* sp. a, *S.* sp. b, *S.* sp. d, and *S.* sp. e, designated as such because they may represent putative cryptic species, or they may represent slight morphological variants of described *Suwallia* species; K. Alexander *personal observation*), *N. forcipata*, and outgroups from the chloroperlid genera *Alloperla, Plumiperla, Sweltsa*, and *Triznaka* (Fig. 1; Table 1).

3.2. UCE sequencing

Illumina sequencing of 64 *Suwallia* samples produced a total of 407,352,395 total reads from a single lane (Appendix A). Raw sequences have been deposited in the NCBI Sequence Read Archive (BioProject PRJNA667287). The number of reads sequenced varied among samples from 1,062,265 (*S.* sp. a [Su50]) to 22,887,098 (*T. signata* [Su63]) (Fig. 2; Appendix A). In total, we captured and sequenced 1412 UCE loci (1241 using hemipteran baits, 171 using hymenopteran baits) with a length of 248,705 bp and a total of 23,361 informative sites. Of those loci, 296 UCEs comprised the 50% sampling matrix (272 hemipteran, 24 hymenopteran). The number of UCEs per sample ranged broadly (Appendix A), from 87 (*S. amoenacolens* [Su09]) to 826 (*S.* sp. e [Su05]). Missing data in the 50% sampling matrix can be visualized in Fig. 2.

3.3. Phylogenetic inference

A lineage tree generated by IQ-TREE using the concatenated UCE loci (Appendix B) shows several species as non-monophyletic (e.g., *S. amoenacolens, S. kerzhneri, S. marginata, S. pallidula, S. starki*, and *N. forcipata*). This phylogeny also reveals that *N. forcipata* renders *Suwallia* paraphyletic. These results are consistent with the results of our coalescent-based species tree reconstructions (see below). Notably, *S.* sp. a does not appear to be monophyletic, *S.* sp. b is nested within a clade that contains *S. lineosa* and one *S. pallidula* individual (Su16), *S.* sp. d is indistinguishable from *S. salish*, and *S.* sp. e is fully nested within *S. nipponica*.

Our estimated species tree for the genus *Suwallia* is depicted in Fig. 3. Species tree estimates were consistent between the two coalescent-based approaches. The only differences between species trees generated by Astral (Fig. 3) and SVDQuartets (Appendix C) were the placement of *S. sierra*, the position of a *S. marginata* and *S. starki* clade, and the relationships between *S. lineosa, S.* sp. b, and *S. pallidula* (Su16). In the Astral tree, *S. lineosa* was sister to *S.* sp. b (with *S. pallidula* sister to *S. lineosa/S.* sp. b), but *S.* sp. b was sister to *S. pallidula* (Su16) in the SVDQuartets species tree (with S. lineosa sister to *S.* sp. b/*S. pallidula*). Moreover, the placement of *S. starki* (Su27, Su28, Su29, Su30) and *S. marginata* (Su14, Su15) differs slightly between Astral and SVDQuartets species trees (Fig. 3; Appendix C) because the node that places them as sister to *S. autumna, S. talalajensis* and *S. wardi* is not well supported in the Astral tree.

Notably, in our species trees (Fig. 3; Appendix C), two *N. forcipata* samples (Su31, Su37) form a well-supported clade that is nested well within *Suwallia*, and is sister to a clade containing *S. dubia, S.* species b, *S. lineosa*, and one of the *S. pallidula* samples (Su16). The other *N. forcipata* individual (Su65) is in a different clade containing six *Suwallia* samples, *S. decolorata* (Su49), two *S.* sp. a individuals (Su50, Su52), one *S. pallidula* individual (Su20), *S. sachalina* (Su48), and two of the presumed outgroup taxa, *Plumiperla diversa* (Su64) and *Triznaka signata* (Su63). Moreover, one *S. amoenacolens* individual (Su10) is in a clade with *a priori* defined outgroups *Alloperla fraterna* (Su60) and *Sweltsa oregenensis* (Su61, Su62).

Suwallia that are distributed in the Palearctic and Nearctic do not form reciprocally monophyletic groups (Fig. 3; Appendix D). Similarly, some *a priori* designated species do not form monophyletic groups. The five *S. pallidula* samples included herein are in five different positions in the tree (Fig. 3). Similarly, the *S. marginata* sample from Virginia (Su13) differs in phylogenetic placement from *S. marginata* samples from Canada (Su14, Su15), which are sister to four *S. starki* samples (Su27, Su28, Su29, Su30). However, two other *S. starki* (Su26, Su66) individuals are sister to *S. pallidula* (Su17), and are more closely related to *S. salish* (Su34, Su35), *S.* species d (Su55, Su56, Su57), *S. thoracica* (Su33), *S. amoenacolens* (Su09, Su11, Su12), and *S. sublimis* (Su41) than to the aforementioned *S. starki* individuals (Fig. 3; Appendix C). *Suwallia kerzhneri* samples (Su32 and Su58) also exhibit vast phylogenetic distance. Similarly, some of the unidentified species do not form monophyletic groups. For example, *S.* sp. a does not form a monophyletic group; instead, all three individuals extend from nodes of a clade that includes *S. decolorata* (Su49), *S. kerzhneri* (Su32), *S. pallidula* (Su20), *S. sachalina* (Su48), *N. forcipata* (Su65), *P. diversa* (Su64), and *T. signata* (Su63) (Fig. 3). *Suwallia* species b forms a well-supported monophyletic group that is closely related to *S. lineosa* and one *S. pallidula* individual (Su16), but these relationships differed in the SVDQuartets and Astral trees (Fig. 3, Appendix C). *Suwallia* species d individuals are non-monophyletic, with individuals that are closely related to *S. salish, S. starki* (Su26, Su66), and one *S. pallidula* individual (Su17) (Fig. 3). *Suwallia* species e is fully nested within *S. nipponica* (Fig. 3), although, *S.* species e and *S. nipponica* were sister taxa in the concatenated lineage tree generated by IQ-TREE (Appendix B).

3.4. Biogeographic history

Palearctic and Nearctic *Suwallia* do not form reciprocally monophyletic clades (Fig. 3; Appendix D). BioGeoBEARS results show that DIVALIKE+J has the best likelihood score (Table 2), making it the most appropriate model for these data, especially considering that DEC+J does not model jump dispersal well (Ree and Sanmartín, 2018), even though the likelihood scores of both models were similar. The DIVALIKE+J biogeographic model accounts for anagenetic dispersal (i.e., range expansion), vicariance, and founder event speciation via jump dispersal (Matzke, 2014). There appear to have been at least four jump dispersal events from the Nearctic to the Palearctic (*S. talalajensis, S. thoracica, S. sachalina*, and a *S. kerzhneri/jezoensis/teleckojensis* ancestor followed by speciation), and at least one dispersal event from the Palearctic to the Nearctic (a common ancestor to *T. signata, S.*

sachalina, N. forcipata [Su65], *P. diversa*, and *S. pallidula* [Su20]) followed by speciation (see Fig. 3; Appendix D).

4. Discussion

Herein, we used genome-wide sequence data to present the first molecular phylogeny for the chloroperlid stonefly genus *Suwallia* (Fig. 3). This phylogeny contains representatives from 20 of the 26 described species (~77%), and allows us to make some inferences about the group's taxonomy and evolutionary history. These results refute our first hypothesis after revealing that Palearctic and Nearctic *Suwallia* species did not form reciprocally monophyletic clades. Rather, some clades that primarily comprise species distributed in the Palearctic or Nearctic include lineages from the opposite hemisphere (Fig. 3; Appendix D), with our biogeographic modeling approach suggesting a complex history including dispersal, vicariance, and jump dispersal. This suggests that multiple, potentially bidirectional dispersal events across the Bering Land Bridge have occurred. Unfortunately, fossil data that could be used for molecular clock calibrations for molecular dating in this group are lacking. Only two chloroperlid stoneflies, †*Dipsoperla kunikanensis* and †*Dipsoperla serpentis*, are known from the fossil record (Sinitshenkova, 1987, 1990; Nicholson et al., 2015; DeWalt et al., 2020), both occurring in the Palearctic, but neither are members of the Suwallini, and thus could not be used to calibrate this phylogeny given our limited sampling of chloroperlid stoneflies. Adult stoneflies are winged and capable of flight, but tend to be poor dispersers that mostly disperse within stream corridors and exhibit little overland dispersal among separate drainage basins (Nebeker and Gaufin, 1968; Schultheis et al., 2002; Kauwe et al., 2004; Petersen et al., 2004; Macneale et al., 2005; Elbrecht et al., 2014; Sproul et al., 2014). However, periodic dispersal events across freshwater drainage systems on the Bering Land Bridge would be plausible. In fact, long-distance dispersal has been shown to be important in the biogeography of some stoneflies (Kauwe et al., 2004; Nelson, 2008; DeWalt and South, 2015; Kondratieff et al., 2019). The BioGeoBEARS results suggest that rare jump dispersal events resulting in new genetically isolated lineages (Matzke, 2014) have been important in *Suwallia*'s biogeographic history. Stoneflies belong to an ancient insect order that is known to be approximately 300 million years old (at least) based on fossil evidence from Pennsylvanian strata (Béthoux et al., 2011). Stoneflies subsequently diversified following the break-up of the supercontinent of Pangaea, but the evolution of most extant lineages was probably much more recent, with the earliest fossils representing extant families dating to the Jurassic Period (Zwick, 2000). While Pleistocene events have influenced stonefly diversification through vicariance in some areas (McCulloch et al., 2010), it has been postulated that montane forest-dwelling insects, including chloroperlids, would not have had access to the Bering Land Bridge during the Pleistocene (Zwick, 2009), but it is also hypothesized that *trans*-Beringian dispersal has been relatively common in stoneflies (Stewart and Ricker, 1997). If so, dispersal between Palearctic and Nearctic regions may have been during earlier episodes of low sea levels that exposed the land bridge. Hence, additional fossil discoveries or better understanding of mutation rates would be necessary for molecular clock calibrations to estimate the timing of diversification within this group.

Several *Suwallia* species do appear to exhibit cryptic genetic diversity, supporting our second hypothesis. For example, individuals identified as *S. pallidula, S. starki, S. marginata, S.* sp. a, *S. kerzhneri*, and *S. amoenacolens* are not monophyletic (within described species), but appear in multiple places in the phylogeny. Whereas some of these phylogenetic placements may be attributable to missing data, this result requires further investigation. *Suwallia* species typically occur at high elevations, so broadly distributed taxa may represent evolutionarily divergent groups that are currently considered a single species. For example, *S. pallidula* is distributed in the Rocky Mountains, Sierra Nevada, and Cascade Range in North America, as well as some mountains in the Basin and Range Province (Alexander and Stewart, 1999; Baumann et al., 2017). The *S. pallidula* samples included herein were collected from New Mexico, Colorado, Oregon, and Montana (Table 1), and do not form a monophyletic clade (Fig. 3). Rather, all five *S. pallidula* samples we included are more closely related to other *Suwallia* species than to the other *S. pallidula* samples (Fig. 3). Similarly, *S. starki*, which occupies the largest geographic range of all North American *Suwallia* species (Alexander and Stewart, 1999; Baumann et al., 2017), occupies two phylogenetically disparate clades (Fig. 3). Indeed, many of the cryptic lineages are from populations that are separated by broad geographic distances (see Fig. 1). Given that stoneflies tend to be poor dispersers, and many of these populations occupy sky islands that are surrounded by uninhabitable terrain at low elevations, many of these populations may have been on independent evolutionary trajectories for much longer than previously recognized. Increased geographic sampling is necessary to better understand species limits and species distributions in these stoneflies.

The monospecific chloroperlid *N. forcipata* renders *Suwallia* paraphyletic, supporting our third hypothesis. Therefore, we reject the hypothesis that *Neaviperla* is a valid genus, as postulated by Baumann and Lee (2014), and propose a new classification following the classification previously published by Alexander and Stewart (1999), with *N. forcipata* reclassified as *Suwallia forcipata*. It is unclear why one *Neaviperla* (hereafter *S. forcipata*) sample (Su65) did not cluster with the other two conspecifics (Su31 and Su37). This could be due to sample misidentification, an artifact of missing data, unrecognized cryptic diversity within the species, or a hybridization event. As *Suwallia forcipata* has a unique morphology, we do not think that a misidentification is likely as these identifications were confirmed multiple times throughout this study for all included taxa. Missing data may be playing a role. However, of the three *S. forcipata* samples included, all three were represented in 37 UCE loci in the final data set, and they were not monophyletic in any of those 37 gene trees (Appendix E). Broader sampling efforts are necessary to evaluate cryptic species diversity and possible intergeneric hybridization. Regardless, all three *Suwallia forcipata* samples exhibit phylogenetic affinities for other *Suwallia* species rather than standing as a unique monospecific genus, and therefore we have reclassified *Neaviperla forcipata* (Neave, 1929) as *Suwallia forcipata* (Neave, 1929).

Finally, this research demonstrates that UCE baits designed for specific taxa can be useful in other groups. In our dataset, overwhelmingly more UCEs were captured using hemipteran baits (Faircloth, 2017) than by hymenopteran baits (Branstetter et al., 2017), but the total loci captured were only a fraction (~10%) of the number that is possible with UCE sequencing. While the hymenopteran and hemipteran baits were undoubtedly

useful, this highlights a need for plecopteran specific baits for stonefly phylogenetics using UCEs, which could be developed effectively using the workflow described by Faircloth (2017) and publicly available stonefly genomes (e.g., *Amphinemura sulcicollis* [Macdonald et al., 2016], *Isoperla grammatica* [Macdonald et al., 2016], *Lednia tumana* [Hotaling et al., 2019]). As previously mentioned, missing data resulting from our use of UCE baits developed for other taxonomic groups may have influenced phylogenetic relationships among some samples (Brown and Thomson, 2017; Smith et al., 2020), particularly those with the lowest numbers of UCEs sequenced, but theory states that maximum-likelihood models can properly accommodate missing data (Yang and Rannala, 2012), and studies have shown that phylogenetic position can still be accurate for taxa with extensive missing data if enough characters are included in the analyses (Wiens and Morrill, 2011; Roure et al., 2013; Streicher et al., 2016; Molloy and Warnow, 2018). For example, two *S. amoenacolens* samples (Su09, Su10) did not form a monophyletic clade with the other two (Su11, Su12) that were monophyletic (Fig. 3), but one of those samples (Su09) had the fewest UCE loci sequenced (87 total; 50 included in the 50% data matrix) (Fig. 2; Appendix A), casting some doubt on its phylogenetic position herein if the missing data were non-randomly distributed. Similarly, several samples (*S. pallidula* [Su20], *S. kerzhneri* [Su32], *S. sachalina* [Su48], *S. decolorata* [Su49], *S.* sp. a [Su50-52], and *N. forcipata* [Su65]) grouped with the outgroup taxa *P. diversa* and *T. signata* (Fig. 3), yet all had large numbers of missing UCE loci (Fig. 2; Appendix A). We anticipate that some of these relationships would shift with a more complete data matrix. Nevertheless, these data still provide important insights into the evolution of the group.

5. Conclusions

In conclusion, based on sequence data from hundreds of genome-wide UCE loci, we have reconstructed the first molecular phylogeny for the chloroperlid stonefly genus *Suwallia*, and we make the taxonomic revision to include the monospecific genus *Neaviperla* within *Suwallia*. We have also demonstrated that Palearctic and Nearctic stoneflies within the genus do not form reciprocally monophyletic groups, and that a biogeographic history of dispersal, vicariance, and jump dispersal has been important in shaping the distribution of these chloroperlid stoneflies. It is well understood that comprehensive sampling is fundamental to obtaining accurate estimations of phylogenies (Heath et al., 2008). Indeed, additional taxonomic and geographic sampling (including broader sampling of chloroperlid stoneflies) coupled with stonefly specific UCE baits would greatly enhance our understanding of the evolution of this group of stoneflies, particularly in evaluating apparent cryptic genetic diversity within several *Suwallia* species.

The new taxonomic combination is:

Suwallia forcipata (Neave, 1929), new combination

Alloperla forcipata Neave, 1929:160. Holotype ♂ (Canadian National Insect Collection), Lake Edith, Jasper National Park, Canada

Alloperla (Neaviperla) forcipata: Ricker, 1943:142

Neaviperla forcipata: Illies, 1966:448

Suwallia forcipata: Alexander & Stewart, 1999:202.

Suwallia forcipata: Stewart & Stark, 2002:280

Suwallia forcipata: Stark, 2012

Suwallia forcipata: Stewart and Stark in Merritt et al., 2008

Suwallia forcipata: DeWalt et al., 2014

Neaviperla forcipata: Baumann and Lee, 2014.

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Data Accessibility

DNA sequence data are publicly archived in the NCBI Short Reads Archive (BioProject PRJNA667287). All data sets, trees, scripts, and R code used in analyses are publicly available via Dryad (https://doi.org/10.5061/dryad.pc866t1ms) and GitHub (https://github.com/ddhouston/suwalliaphylogenomics2020).

Appendix A

Sample ID numbers, species designations, number of raw reads, number of UCEs sequenced for each sample, and number of loci included in the 50% matrix used for phylogenetic analyses. SRA accession numbers are also provided, all archived in BioProject PRJNA667287.

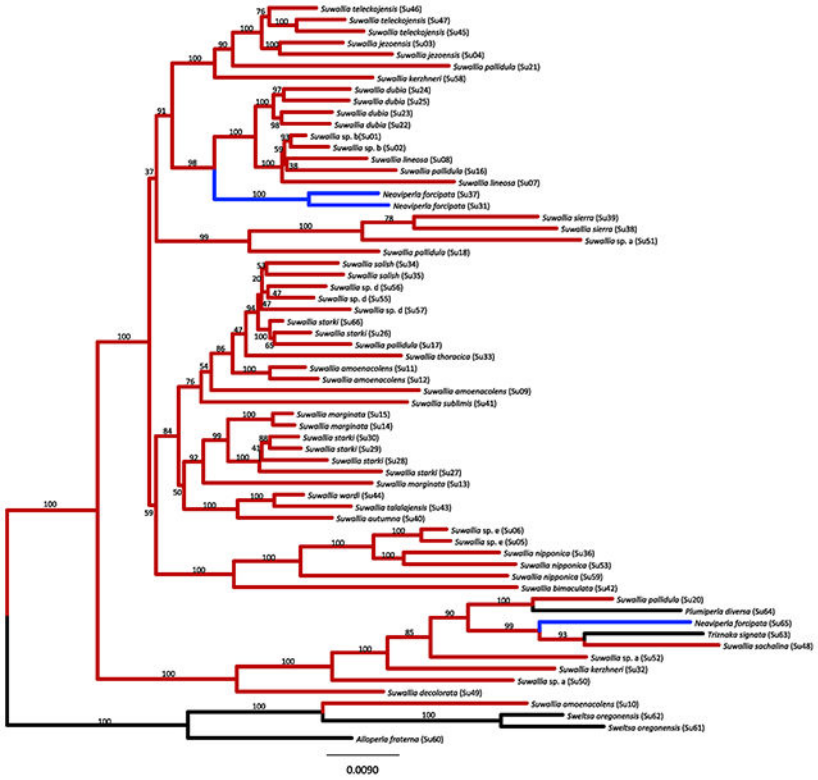
Sample ID	Species	# Raw Reads	# UCE loci (total)	# UCE loci (50% matrix)	SRA Accession #
Su01	*S.* sp. b	9,621,103	817	243	SAMN16364912
Su02	*S.* sp. b	5,460,742	661	250	SAMN16364913
Su03	*S. jezoensis*	17,405,980	385	200	SAMN16364914
Su04	*S. jezoensis*	5,066,132	376	172	SAMN16364915
Su05	*S.* sp. e	5,270,887	826	234	SAMN16364916

Sample ID	Species	# Raw Reads	# UCE loci (total)	# UCE loci (50% matrix)	SRA Accession #
Su06	*S.* sp. e	4,633,904	795	251	SAMN16364917
Su07	*S. lineosa*	5,769,877	173	104	SAMN16364918
Su08	*S. lineosa*	18,585,408	310	175	SAMN16364919
Su09	*S. amoenacolens*	3,471,908	87	50	SAMN16364920
Su10	*S. amoenacolens*	3,986,371	529	168	SAMN16364921
Su11	*S. amoenacolens*	5,173,006	791	248	SAMN16364922
Su12	*S. amoenacolens*	5,571,647	613	226	SAMN16364923
Su13	*S. marginata*	3,198,631	330	165	SAMN16364924
Su14	*S. marginata*	5,524,143	670	279	SAMN16364925
Su15	*S. marginata*	5,870,195	711	269	SAMN16364926
Su16	*S. pallidula*	6,194,234	324	168	SAMN16364927
Su17	*S. pallidula*	6,500,823	418	225	SAMN16364928
Su18	*S. pallidula*	3,262,714	265	138	SAMN16364929
Su20	*S. pallidula*	3,045,210	505	201	SAMN16364930
Su21	*S. pallidula*	3,809,120	149	85	SAMN16364931
Su22	*S. dubia*	5,118,206	570	242	SAMN16364932
Su23	*S. dubia*	4,848,552	521	244	SAMN16364933
Su24	*S. dubia*	2,707,569	343	206	SAMN16364934
Su25	*S. dubia*	3,719,401	344	184	SAMN16364935
Su26	*S. starki*	8,349,147	583	251	SAMN16364936
Su27	*S. starki*	6,594,121	316	155	SAMN16364937
Su28	*S. starki*	3,068,870	386	199	SAMN16364938
Su29	*S. starki*	3,095,095	568	253	SAMN16364939
Su30	*S. starki*	4,242,406	619	253	SAMN16364940
Su31	*N. forcipata*	3,857,093	218	136	SAMN16364941
Su32	*S. kerzhneri*	3,579,753	228	126	SAMN16364942
Su33	*S. thoracica*	4,437,823	167	94	SAMN16364943
Su34	*S. salish*	11,132,286	287	168	SAMN16364944
Su35	*S. salish*	11,574,416	290	166	SAMN16364945
Su36	*S. nipponica*	10,633,259	431	204	SAMN16364946
Su37	*N. forcipata*	4,944,469	556	204	SAMN16364947
Su38	*S. sierra*	6,284,883	333	160	SAMN16364948
Su39	*S. sierra*	14,493,519	512	192	SAMN16364949
Su40	*S. autumna*	1,621,942	303	180	SAMN16364950
Su41	*S. sublimis*	5,278,587	219	108	SAMN16364951
Su42	*S. bimaculata*	4,732,589	287	135	SAMN16364952
Su43	*S. talalajensis*	8,383,318	361	178	SAMN16364953
Su44	*S. wardi*	21,251,072	766	217	SAMN16364954
Su45	*S. teleckojensis*	4,140,311	451	191	SAMN16364955
Su46	*S. teleckojensis*	5,118,397	625	246	SAMN16364956
Su47	*S. teleckojensis*	4,091,373	409	196	SAMN16364957

Sample ID	Species	# Raw Reads	# UCE loci (total)	# UCE loci (50% matrix)	SRA Accession #
Su48	*S. sachalina*	6,371,823	200	101	SAMN16364958
Su49	*S. decolorata*	3,363,545	305	159	SAMN16364959
Su50	*S.* sp. a	1,062,265	135	85	SAMN16364960
Su51	*S.* sp. a	1,308,734	232	140	SAMN16364961
Su52	*S.* sp. a	1,365,260	131	68	SAMN16364962
Su53	*S. nipponica*	4,523,713	392	179	SAMN16364963
Su55	*S.* sp. d	3,861,324	534	240	SAMN16364964
Su56	*S.* sp. d	6,065,241	480	240	SAMN16364965
Su57	*S.* sp. d	2,387,687	327	191	SAMN16364966
Su58	*S. kerzhneri*	1,955,395	442	194	SAMN16364967
Su59	*S. nipponica*	8,753,119	242	138	SAMN16364968
Su60	*A. fraterna*	2,150,629	433	176	SAMN16364969
Su61	*S. oregonensis*	5,589,427	492	163	SAMN16364970
Su62	*S. oregonensis*	5,106,527	560	176	SAMN16364971
Su63	*T. signata*	22,887,098	469	181	SAMN16364972
Su64	*P. diversa*	18,530,268	658	184	SAMN16364973
Su65	*N. forcipata*	6,932,370	196	115	SAMN16364974
Su66	*S. starki*	10,417,478	751	264	SAMN16364975
Total		407,352,395			

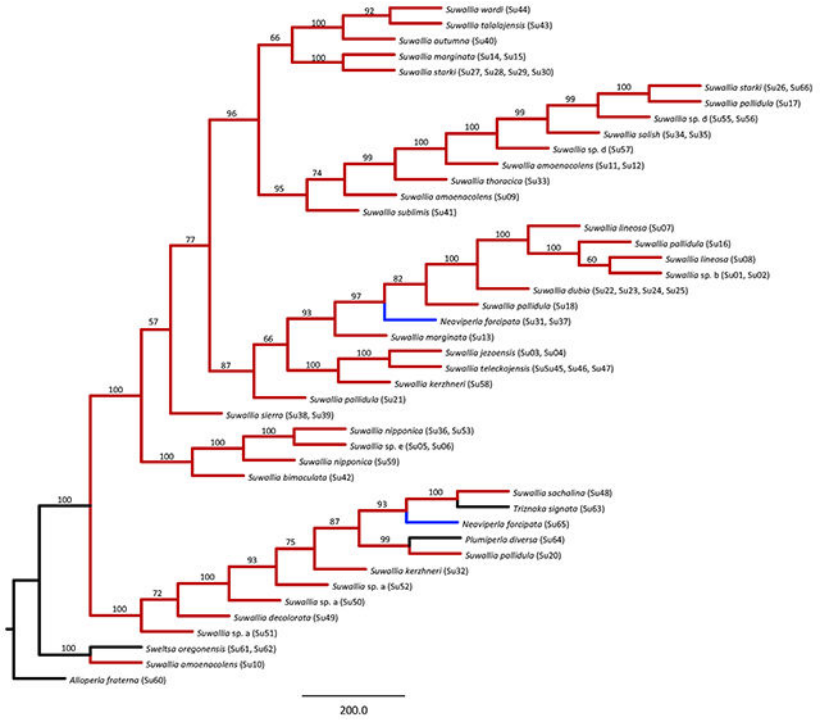
Appendix B

Lineage tree generated by concatenated UCE sequences using IQ-TREE. Genera are color coded as follows: *Suwallia* (maroon), *Neaviperla* (blue), and *a priori* defined outgroups (black).



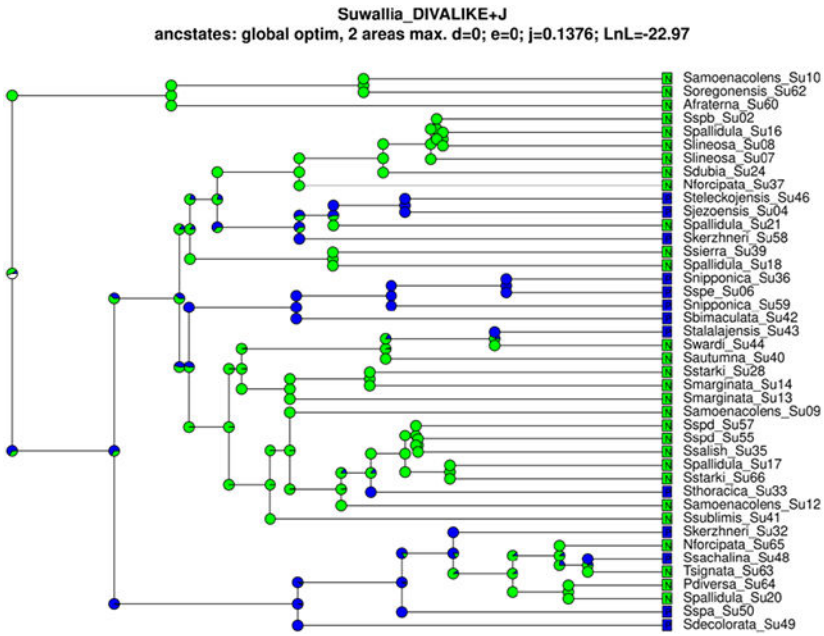
Appendix C

Coalescent-based species tree produced by our SVDQuartets analysis. Branches are colored by *a priori* species designations: *Suwallia* (maroon), *Neaviperla* (blue), and outgroups *Alloperla, Plumiperla, Triznaka*, and *Sweltsa* (black).



Appendix D

Results of BioGeoBEARS analysis, for which the model DIVALIKE+J was most appropriate for these data. Contemporary Nearctic distributions are designated at the tips of the phylogeny as green squares containing the letter N, and contemporary Palearctic distributions are designated as blue squares containing the letter P. The most probable ancestral distributions are designated using circles filled with the same colors, but nodes with more uncertainty are labelled with pie charts that display the probabilities for all possible ancestral ranges (including a probable Nearctic/Palearctic ancestral distribution at the basal node, colored in white).



Appendix E

The total number of gene trees (GT) shared among individuals with the same *a priori* species designations, along with the proportion of those gene trees for which that species is monophyletic (PM). Only the species with N > 1 are included herein (monophyly could not be tested for species where N = 1), and species that were missing for a locus were not counted for that gene tree. Individual gene trees are from the IQ-TREE analysis. Columns where N = 2 through N = 6 represent different thresholds for the number of individuals for a species in order to be tested. Dashed lines mark cells where the threshold for n is higher than the number of individuals included in our sampling (e.g., we included four *S. amoenacolens* individuals overall, so monophyly where N = 5 and N = 6 is not possible to test for that species). Bolded values represent the maximum available threshold for a particular species. Interpretation is as follows for three examples: Example 1 - *S. amoenacolens*. There were 21 gene trees where all four *S. amoenacolens* samples amplified and sequenced, and of those, none had *S. amoenacolens* forming a monophyletic group. There were 137 gene trees where three *S. amoenacolens* individuals amplified and sequenced for those loci (but not the same three individuals for each gene), and of those, 2% had *S. amoenacolens* forming a monophyletic group, and those were likely the gene trees that did not include the most divergent *S. amoenacolens* individual (Su10), that grouped with *S. oregonensis* and *A. fraterna* in our species tree. Example 2 - *N. forcipata*. There were 37 gene trees that included all three *Neaviperla* samples. Of those, none had the species as a monophyletic group. However, 19% of the 155 gene trees that included two *Neaviperla* samples had *Neaviperla* forming a monophyletic group, and in those cases, the two samples included were the two that claded together in the species tree (Su31 and Su37). Example 3 - *S. nipponica*. There were 63 gene trees that included all three *S. nipponica* samples, and *S. nipponica* formed a monophyletic group in 14% of those. This is because *S. nipponica* and *S.* sp. e were

considered as separate species *a priori*, yet *S.* sp. e is nested within *S. nipponica* in our results (and therefore is not a separate species).

	Tests of Monophyly for Individual Gene Trees									
	N = 2		N = 3		N = 4		N = 5		N = 6	
	GT	PM	GT	PM	GT	PM	GT	PM	GT	PM
S. amoenacolens	246	0.14	137	0.02	**21**	**0.00**	–	–	–	–
S. dubia	269	0.23	213	0.23	**102**	**0.20**	–	–	–	–
S. jezoensis	123	**0.16**	–	–	–	–	–	–	–	–
S. kerzhneri	88	**0.00**	–	–	–	–	–	–	–	–
S. lineosa	70	0.06	–	–	–	–	–	–	–	–
S. marginata	277	0.34	**144**	**0.15**	–	–	–	–	–	–
S. nipponica	187	0.30	**63**	**0.14**	–	–	–	–	–	–
S. pallidula	259	0.00	191	0.00	66	0.00	**9**	**0.00**	–	–
S. salish	105	**0.10**	–	–	–	–	–	–	–	–
S. sierra	108	**0.38**	–	–	–	–	–	–	–	–
S. starki	294	0.00	287	0.00	259	0.00	181	0.01	**59**	**0.02**
S. teleckojensis	232	0.22	**114**	**0.17**	–	–	–	–	–	–
S. sp. a	80	0.03	**25**	**0.00**	–	–	–	–	–	–
S. sp. b	**214**	**0.28**	–	–	–	–	–	–	–	–
S. sp. d	250	0.04	**132**	**0.02**	–	–	–	–	–	–
S. sp. e	**212**	**0.67**	–	–	–	–	–	–	–	–
N. forcipata	155	0.19	**37**	**0.00**	–	–	–	–	–	–
S. oregonensis	**115**	**0.65**	–	–	–	–	–	–	–	–

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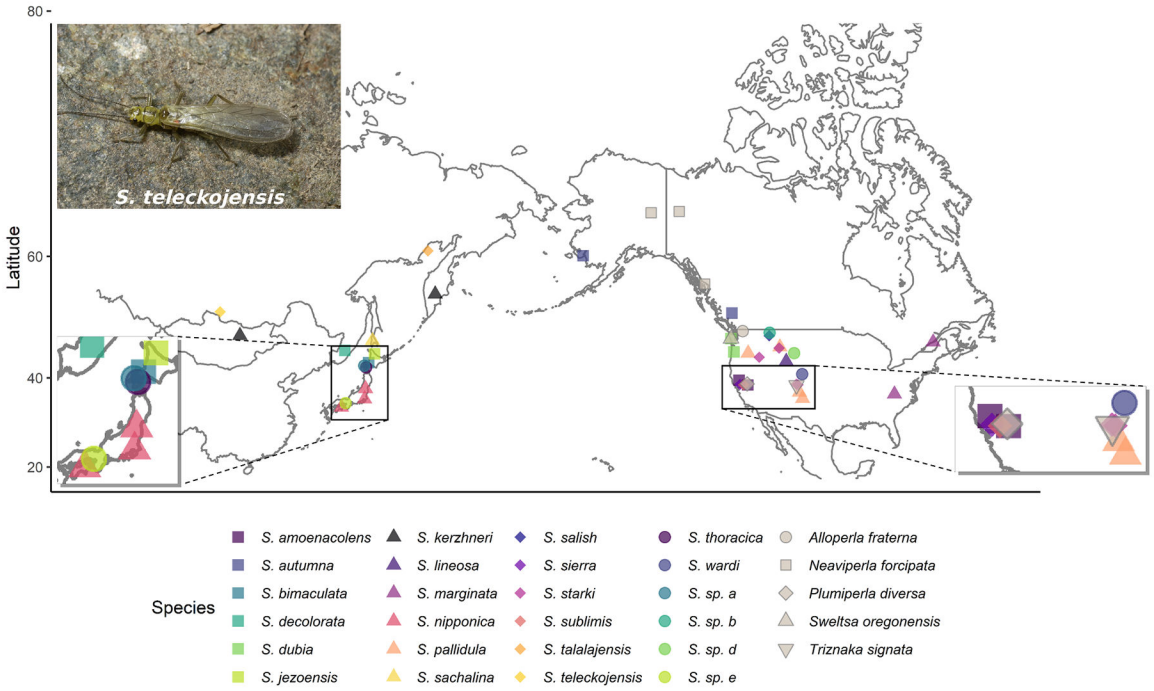

Fig. 1.

Map showing locations where museum samples were collected. Sampling localities that are in close proximity in Japan and in western North America are expanded for clarity. Inset: *Suwallia teleckojensis* (Photo Credit: C. Riley Nelson, used with permission) from the Ulastai River, Mongolia (Judson and Nelson, 2012). *Suwallia* species are represented by assorted shapes and colors (see key). Genera other than *Suwallia* are represented by gray shapes.

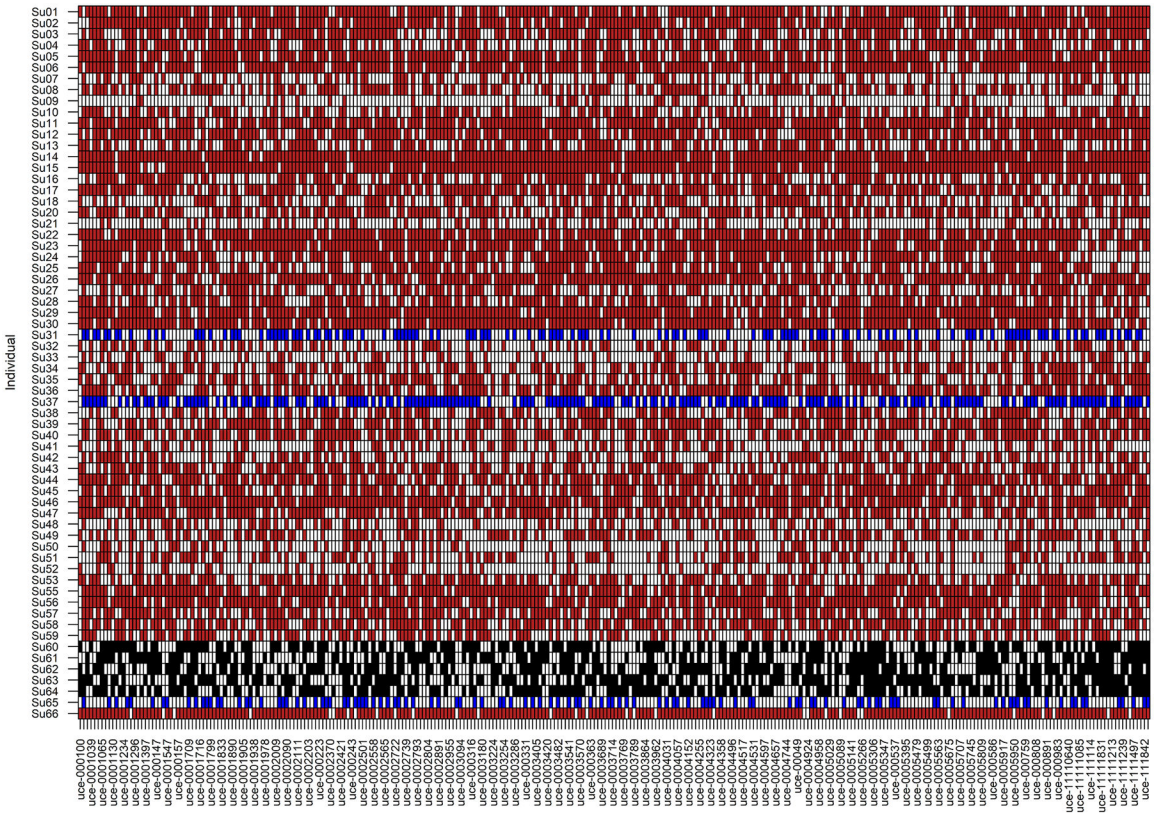

Fig. 2.

Data matrix showing UCE presence/absence data by specimen. Filled squares represent UCEs that were captured, sequenced, and included in our analyses, whereas white squares represent missing UCEs. Individual stoneflies are listed on the y-axis according to sample numbers provided in Table 1, and UCEs are listed on the x-axis. UCEs with a prefix of 000 in the number are from the hemipteran bait set, whereas those with a prefix of 111 are from the hymenopteran bait set. Colors represent the same scheme as depicted in Fig. 3: *Suwallia* (maroon), *Neaviperla* (blue), outgroup taxa (black). Note that Su19 and Su54 are not included in this panel, because one library failed (Su54; *S. autumna*), and the other (Su19; *S. pallidula*) was excluded because we were limited to 64 samples due to budgetary constraints, and we already had five other samples representing that species, including another representative from the same population. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

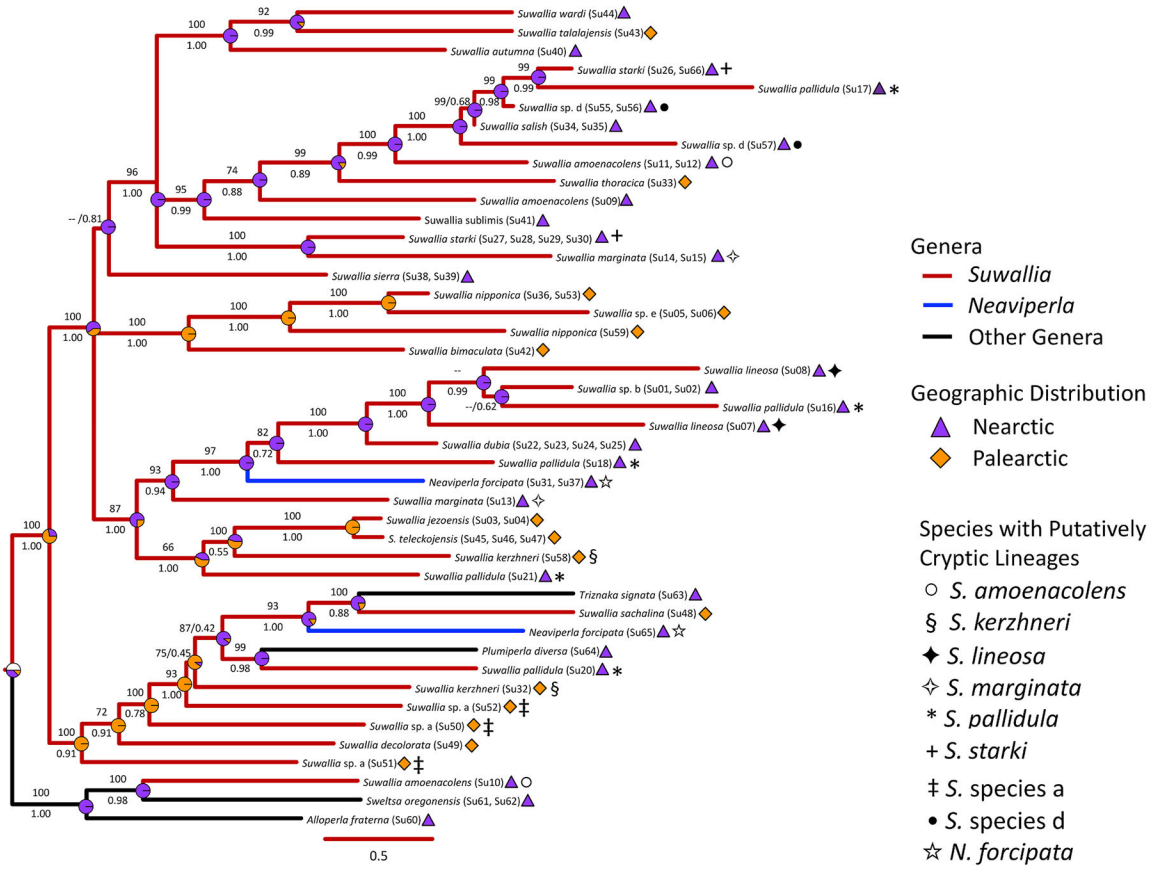

Fig. 3.

Species tree generated by ASTRAL-III for the chloroperlid stonefly genus *Suwallia* based on DNA sequence data from 296 UCE loci. Sample numbers are provided in parentheses following species names, and in cases where all samples representing a species formed a monophyletic group, all sample numbers are included. Support values above nodes are from SVDQuartets, and support values below nodes are local posterior probabilities from Astral. The only differences between species trees generated by each analysis were the placement of *S. sierra*, the relationships between *S. lineosa, S.* sp. b, and *S. pallidula* (Su16), wherein *S. lineosa* was sister to *S.* sp. b (instead of *S.* sp. b and *S. pallidula* [Su16] being sister taxa in the SVDQuartets species tree], and the placement of the *S. marginata* (Su14, Su15) and *S. starki* (Su27, Su28, Su29, Su30) clade (see Supplementary Files in GitHub). Branches are color coded by genus, and contemporary biogeographic distributions are denoted by purple triangles (Nearctic) and orange diamonds (Palearctic) at the tips. The most probable ancestral distributions are designated using circles filled with the same colors, but nodes with more uncertainty are labelled with pie charts that display the probabilities for all possible ancestral ranges (including a probable Nearctic/Palearctic ancestral distribution at the basal node, colored in white). Species that are not monophyletic, currently containing individuals that are separated by large phylogenetic distances and thus exhibiting putative cryptic genetic diversity, are marked using assorted symbols. (For interpretation of the

references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Comprehensive list of the currently recognized *Suwallia* species, four unidentified/putatively cryptic species (designated as such because they may represent cryptic species, or they may represent morphological variants of described *Suwallia* species), and other closely related chloroperlid genera (including the disputed genus *Neaviperla*) included as outgroup taxa. Samples of *S. asiatica, S. jihuae, S. shepardi, S. shimzui, S. tsudai, and S. wolongshana* were not available in the museum collection and therefore were not included in the study.

Species	Sample ID	Locality	Lat/Long	Distribution	Date	N	Sex
S. amoenacolens Alexander & Stewart, 1999	Su09, Su10	Wolf Creek, CA	38.55, −119.71	USA	1995	2	♂, ♂
	Su11, Su12	Stony Creek, CA	39.51, −121.97	USA	1997	2	♂, ♂
S. asiatica Zhiltzova and Levanidova, 1978	n/a	n/a	n/a	Siberia	n/a	0	n/a
S. autumna Hoppe, 1938	Su40	Chilko River, BC	51.71, −123.86	Canada	1941	1	♀
	Su54[a]	Deering, Seward Peninsula, AK	60.07, −162.71	USA	1992	1	♂
S. bimaculata Okamoto, 1912	Su42	Sapporo Shi, Hokkaido, Japan	42.96, 141.25	Japan	1995	1	♀
S. decolorata Zhiltzova and Levanidova, 1978	Su49	Primorsk Territory	45.27, 135.01	Siberia	1975	1	♀
S. dubia Frison, 1935	Su22, Su23	Salt Creek, OR	44.99, −123.35	USA	1995	2	♂, ♂
	Su24, Su25	Quinault River, OR	47.39, −124.09	USA	1996	2	♂, ♂
S. jezoensis Kohno, 1953	Su03, Su04	Horonai-gawa, Hokkaido	44.61, 142.78	Japan	1996	2	♀, ♀
S. jihuae Chen, 2019	n/a	n/a	n/a	China	n/a	0	n/a
S. kerzhneri Zhiltzova and Zwick, 1971	Su32	Milkowo, Kamchatka	54.69, 158.62	Siberia	1968	1	♂
	Su58	Tuul R., Ulunbatar	47.94, 107.59	Mongolia	1992	1	♀
S. lineosa Banks, 1918	Su07, Su08	Bow Mountains, WY	43.21, −109.68	USA	1978	2	♀, ♀
S. marginata Banks, 1897	Su13	Fox Creek, VA	36.68, −81.40	USA	1978	1	♂
	Su14, Su15	Gaudreault River, Quebec	46.78, −71.27	Canada	1958	2	♀, ♂
S. nipponica Okamoto, 1912	Su36	Isobe, Japan	35.63, 140.16	Japan	1995	1	♀
	Su53	Mt. Tsurugi, Shikoku	33.85, 134.09	Japan	1993	1	♂
	Su59	Fukushima	37.79, 140.39	Japan	1996	1	♂
S. pallidula Banks, 1904	Su16	N. Fork John Day River, OR	44.75, −119.63	USA	1995	1	♀
	Su17	Porvenir Creek, NM	35.71, −105.42	USA	1996	1	♂
	Su18, Su19[b]	Massey Gulch, CO	37.06, −106.24	USA	1990	2	♂, ♂
	Su20, Su21	Gallatin River, MT	45.89, −111.32	USA	1947	1	♂, ♂
S. sachalina Zhiltzova, 1978	Su48	Ljutoga River	47.03, 142.21	Siberia	1973	1	♀
S. salish Alexander & Stewart, 1999	Su34, Su35	Flathead Lake, MT	47.92, −114.12	USA	1989	2	Nymph, Nymph

Species	Sample ID	Locality	Lat/Long	Distribution	Date	N	Sex
S. shepardi Alexander & Stewart, 1999	n/a	n/a	n/a	USA	n/a	0	n/a
S. shimizui Alexander & Stewart, 1999	n/a	n/a	n/a	Japan	n/a	0	n/a
S. sierra Baumann & Bottorff, 1997	Su38, Su39	Sacramento, CA	38.69, −121.75	USA	1986	2	♂, ♀
S. starki Alexander & Stewart, 1999	Su26, Su27	Quartz Creek, CO	38.61, −106.60	USA	1993	2	♂, ♀
	Su28	Madison River, MT	45.71, −111.53	USA	1995	1	♂
	Su29, Su30	Payette River, ID	43.99, −116.72	USA	1995	2	♂, ♀
	Su66	Mill Creek, CO	38.70, −107.05	USA	2018	1	♂
S. sublimis Alexander & Stewart, 1999	Su41	North Cosumnes River, CA	38.58, −120.78	USA	1989	1	♂
S. talalajensis Zhiltzova, 1976	Su43	Magadan Province	60.73, 156.70	Siberia	1977	1	♀
S. teleckojensis Šámal, 1939	Su45, Su46, Su47	Rio Archan, Siberia	51.93, 102.43	Siberia	1997	3	♂, ♀, ♂
S. thoracica Okamoto, 1912	Su33	Ojironai-gawa, Hokkaido	42.06, 140.57	Japan	1998	1	♂
S. tsudai Kawai, 1967	n/a	n/a	n/a	Japan	n/a	0	n/a
S. wardi Kondratieff & Kirchner, 1991	Su44	Ben Dalatour Scout Ranch, CO	40.74, −105.51	USA	1990	1	♂
S. wolongshana Du & Chen, 2015	n/a	n/a	n/a	China	n/a	0	n/a
S. sp. a (unidentified species)	Su50, Su51, Su52	Kenichi-gawa, Hokkaido	42.39, 140.05	Japan	1995	3	♀, ♀, ♀
S. sp. b (unidentified species)	Su01, Su02	Bear Autumn Creek, MT	48.45, −114.00	USA	1994	2	♀, ♀
S. sp. d (unidentified species)	Su55, Su56, Su57	North Tongue River, WY	44.76, −107.62	USA	1994	3	♂, ♂, ♂
S. sp. e (unidentified species)	Su05, Su06	Shinhotaka, Homsho	34.75, 135.20	Japan	1990	2	♀, ♂
Alloperla fraterna Frison, 1935	Su60	Happy Creek, WA	48.71, −121.04	USA	1996	1	♂
Neaviperla forcipata Neave, 1929	Su37	Burroughs River, AK	56.18, −130.95	USA	1992	1	Nymph
	Su31	Wolf Creek, AK	65.22, −144.97	USA	1992	1	♂
	Su65	Blackstone River, Yukon Territory	65.37, −137.63	Canada	1993	1	♂
Plumiperla diversa Frison, 1935	Su64	West Fork Carson River, CA	38.78, −119.88	USA	1996	1	♀
Sweltsa oregonensis Frison, 1935	Su61, Su62	Quinault River, WA	47.39, −124.09	USA	1996	2	♀, ♀
Triznaka signata Banks, 1895	Su63	Gunnison River, CO	38.51, −107.01	USA	2002	1	♂
Total						66	

[a]DNA library preparation for this *S. autumna* sample failed, presumably because of low DNA quality/quantity (0.09 ng/μl starting concentration, and no visible DNA in an agarose gel following electrophoresis).

[b]Excluded from myBAITS soak and subsequent UCE capture because of the 64-sample limit of our kit, *S. pallidula* as a species was represented by five other samples, and the sampling locality (Massey Gulch, CO) was already represented by another representative *S. pallidula* library (Su18).

Table 2

Results from BioGeoBEARS analysis. Each biogeographic model is displayed along with its log-likelihood score, the number of parameters included in each model, parameter values for dispersal (d), extinction (e), and founder (j), Akaike Information Criterion scores corrected for small sample sizes (AICc), and weighted Akaike Information Criterion scores (AICc_wt). Whereas the three models that account for jump dispersal (+J) all had similar likelihood scores, we selected the model with the best score that accurately models founder event speciation. Because DEC+J is a poor model of founder event speciation (Ree and Sanmartín, 2018), the most appropriate model for these data is DIVALIKE+J, displayed in bold font.

Model	LnL	# parameters	d	e	j	AICc	AICc_wt
DEC	−38.12660	2	0.21252	1.00×10^{-12}	0	80.56	3.49×10^{-7}
DEC+J	−23.12080	3	1.00×10^{-12}	1.00×10^{-12}	0.13773	52.87	0.36
DIVALIKE	−41.76024	2	0.39798	1.00×10^{-12}	0	87.83	9.22×10^{-9}
DIVALIKE+J	**−22.97417**	**3**	**1.00×10^{-12}**	**1.00×10^{-12}**	**0.13761**	**52.58**	**0.42**
BAYAREALIKE	−54.46515	2	0.22377	0.75839	0	113.24	2.80×10^{-14}
BAYAREALIKE+J	−23.58673	3	1.00×10^{-12}	1.00×10^{-7}	0.13080	53.81	0.23