STATE WILDLIFE GRANT PROJECT - FINAL REPORT

Project T20 – 2

Systematics of Species of Special Concern in the Genus *Orconectes* from the Ozark Highlands. Case Studies from Species Pairs: *Orconectes neglectus neglectus, Orconectes neglectus chaenodactylus* and *Orconectes macrus, Orconectes nana*

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Introduction

The Ozark Highlands physiographic province represents an island of upland habitat in Arkansas, Kansas, Missouri and Oklahoma. The Ozark Highlands are approximately bordered to the north by the Missouri River, to the east by the Mississippi River and to the south by the floodplain of the Arkansas River. Aquatic fauna from this province are unique and phylogenetically related to taxa either in adjacent drainages from the same province(e.g. Switzer and Wood 2002) or in other isolated portions of the Interior Highlands, i.e. Eastern Highlands, Ouachita Highlands, (Wiley and Mayden 1985; Mayden 1988; Strange and Burr 1997). The total Interior Highlands island habitats are remnants of a once continuous upland region that was altered by repeated advances and retreats of glacial ice from the north (Mayden 1988), with the maximum southern extent of glacial movement roughly traced by the Missouri and Ohio Rivers. The aquatic vertebrate fauna of the region has arguably been studied much more extensively than the invertebrate fauna, despite the fact that these invertebrate communities make up a significant component of the biodiversity from streams in the region.

One of the most familiar components of the invertebrate fauna of streams in eastern North America are the crayfishes. The Ozark Highlands is home to many endemic species of crayfish, and although the distribution of each of these endemics varies, they often overlap in at least a portion of their respective ranges. Two species (or subspecies) pairs endemic to the region with partially overlapping distributions are *Orconectes nana* and *Orconectes macrus* (Figure 1), and *Orconectes neglectus neglectus* and *Orconectes neglectus chaenodactylus* (Figure 2).

Orconectes macrus was formerly recognized as a subspecies of *O. nana* and both taxa are endemic to streams on the western edge of the Ozark Highlands (Williams 1952). *Orconectes nana* is restricted to the Illinois River basin and *O. macrus* is found in tributaries to the Neosho River north of the Illinois River. Both stream systems are tributaries of the Arkansas River. In addition to the core range of this species pair, one allopatrically distributed population of *O. nana* (previously recognized as *O. macrus*) is found in Prairie Creek in the White River basin. *Orconectes neglectus neglectus* is found syntopically with both *O. nana* and *O. macrus* throughout tributaries of the Arkansas River. The other subspecies, *O. n. chaenodactylus*, as currently recognized, is restricted to the North Fork White River (Williams 1952). Intergrades have been hypothesized between the two subspecies throughout the remainder of the White River (Williams 1952). In addition, *O. n. neglectus* is known from isolated regions of Oklahoma (Blue River), Kansas (Kansas River, Republican River) and Nebraska (Republican River). This disjunct distribution, though not identical, is also noted in several highland fishes (e.g. *Etheostoma cragini*, *Phoxinus erythrogaster*).

In an effort to better understand the inter-relationships of the sister species, or subspecies pairs, as well as define the distribution and species limits of these taxa from a historical (evolutionary) perspective, a molecular phylogenetic approach was utilized. The generated data were used to test hypotheses concerning species boundaries and distributions in both pairs, evaluate the subspecies status in *O. neglectus*, determine whether full species status is warranted in *O. macrus* and infer biogeographic histories for these crayfishes from the southern half of the Ozark Highlands.

Materials and Methods

Ninety-one individuals of the *Orconectes nana* and *O. macrus* complex were sampled (Table 1, Figure 3) and 208 individuals were sampled from recognized subspecies of *Orconectes neglectus* (Table 2, Figure 4). In addition, four species of genus *Cambarus* and two species of genus *Procambarus* were included as well as five other species of genus *Orconectes* outside of the focal taxa to reconstruct a phylogenetic hypothesis for both the *O. nana* and *O. macrus* species pair and the *O. neglectus* subspecies pair. *Procambarus acutus* was recovered from the dataset of Crandall and Fitzpatrick (1996), available from the lead author's website, and was used as the outgroup for all analyses. All other samples were collected from 2000 to 2006 by hand, seine, or dipnet. All phylogenetic and population genetic analyses were carried out separately for the two groups under investigation and for clarity will be presented in sections by species and subspecies for ease in both the Results and Discussion.

Total genomic DNA was isolated using the QIAGEN DNeasy extraction kit as described by the manufacturer for animal tissues. Modifications to the protocol included an overnight tissue digestion and a final elution volume of 200 µl in ddH20. Polymerase Chain Reaction (PCR) was used to amplify approximately 650 base pairs (bp) of the Cytochrome Oxidase I (COI) locus from the mitochondrial genome. Primers used for amplification were H-COI 5'- TAAACTTCAGGGTGACCAAAAAATCA-3' and L-COI 5'- GGTCAACAAATCATAAAGATATTG-3'. 50 µl PCR reactions consisted of 1-4 µl of total genomic DNA, 0.4 µM of each primer, 1 unit of Taq DNA polymerase, 5 µl of 10X DNA buffer, 2mM MgCl2, 0.8 mM dNTP's, and ddH2O to volume. PCR protocols were as follows: an initial denaturation at 94 °C for 4 minutes, 45 cycles of 94 °C for 1 minute, 50 °C for 1 minute, 72 °C for 1 minute, and a final extension of 72 °C for 4 minutes.

PCR amplified fragments were cleaned-up using the QIAGEN MinElute PCR purification kit. The cleaned products were then used in cycle sequencing reactions with Applied Biosystems BigDye terminated cycle sequencing kits. Thermal cycling for cycle sequencing was performed with PCR amplification primers: initial denaturing step at 96° C for 1 minute, 45 cycles of 96° C for 30 seconds, 50° C for 15 seconds and 60° C for 4 minutes. Cycle sequencing reactions were completed by Macrogen, Inc. (Seoul, South Korea).

Sequence data were analyzed by eye for base calling using 4Peaks (Griekspoor and Groothius). Complete edited sequences were aligned using CLUSTAL X (Thompson et al., 1997) and checked by eye. Aligned sequences were analyzed in either PAUP*4.10b (Swofford 2003) or PHYLIP (Felsenstein, 2005) for Maximum Parsimony (MP) phylogeny reconstruction and in Mr.Bayes v.3.04b (Huelsenbeck and Ronquist, 2001) for Bayesian phylogeny reconstruction.

Maximum parsimony searches conducted in PAUP* v4.10b (Swofford 2003) were with default parsimony search settings. A heuristic search was performed with 10 replicates of random sequence addition while holding 1 tree at each step. Searches that recovered multiple trees were consolidated by both strict and majority-rule consensus trees to estimate support for reconstruction of recovered nodes. Branch swapping was performed with TBR and the steepest descent option was in effect. Bootstrapping with 10,000 pseudo-replications was implemented using a full heuristic search holding one tree at each step (Felsenstein, 1985). Parsimony searches in PHYLIP were completed with DNAPARS. Recovered trees were imported into PAUP* 4.10b for subsequent consensus reconstruction and bootstrap analyses as described above.

Bayesian phylogenetic inference was performed with Mr.Bayes v. 3.04b (Huelsenbeck and Ronquist, 2001). Two independent runs, each consisting of five million generations, were completed implementing the MCMCMC search algorithm, and MrModelTest (Nylander 2004) was used to select the best evolutionary parameters for the data as partitioned by codon position (i.e. 1st, 2nd, 3rd codon). The recovered log likelihood scores at each sampling interval (1000 generations) were plotted against the generation number to establish when stationarity was reached. Those trees that were part of the burn-in (i.e. pre-stationarity) were removed and all of the remaining trees from each independent run were compiled into a single tree file and the resultant posterior probability scores (PPS) were used to infer support for the nodes in a 50% majority rule consensus phylogenetic hypothesis.

Given the low levels of divergence, i.e. zero to few nucleotide synapomorphies, sometimes observed in intraspecific (or closely related inter-specific) taxa, several standard intraspecific population genetic and phylogeographic methodologies were utilized for investigating the interrelationships of individuals from clades of species in *Orconectes* under investigation here. Statistical Parsimony, as implemented by TCS (Templeton et al. 1992) was used to reconstruct a network hypothesis for both the *O. nana* and *O. macrus* group and the *O. neglectus* subspecies based on the 95% connection limit. A haplotype numbering scheme was developed for each individual based on recovered networks. In general the root haplotypes were the first numbered haplotype of the network and all other haplotypes in each network were subsequently numbered. Separate numbering schemes were used for *O. nana* and *O. macrus* and *O. neglectus*, but haplotypes were sequential across networks in each project.

Pairwise nucleotide divergence tables for network comparisons were completed for recovered root nodes of each group. Average pairwise genetic distances using Kimura 2-parameter estimates for within and between group averages were calculated using MEGA 3.0 (Kumar et al., 2004). Pairwise Φ_{ST} estimates for haploid (mitochondrial) loci (analogous to F_{ST} estimates for diploids) for the a priori grouping scheme were also computed in ARLEQUIN (Schneider et al., 2000). DNAsp (Rozas and Rozas, 1999) was used to estimate current (θ_π) effective population size (N_e) and LAMARC (Kuhner et al., 2005) was used for historical estimates of N_e (θ_w) (Buhay and Crandall 2005). Essentially, current estimates are based on pairwise differences and historical estimates are based on segregating sites (polymorphism) within a priori defined groups.

Results

Orconectes nana and *Orconectes macrus*

642 nucleotides of CO-I sequence data were generated for 91 individuals from the *O. nana* and *O. macrus* species pair. 442 nucleotide characters were constant, 45 characters were variable but uninformative for parsimony reconstruction, and the remaining 164 characters were parsimony informative. Parsimony searches recovered 108 most parsimonious trees at 579 steps each. The backbone (i.e. terminal individuals

trimmed and showing only nodes supporting recovered groupings) of the strict consensus hypothesis is shown in Figure 5. Bootstrap and Posterior Probability support respectively are shown above the nodes in each figure depicting hypothesized inter-relationships. The maximum parsimony hypothesis shows the majority of individuals (n=86) of *O. nana* and *O. macrus* sampled are monophyletic and sister taxa. Each taxon is further subdivided into two clades (A & B, Figure 6 and 7 for *O. nana* and *O. macrus* respectively). The remaining 5 individuals (n=4 *O. nana* and n=1 *O. macrus*) that are recovered outside the larger *nana*/*macrus* clade are most likely due to either interspecific hybridization events with sympatrically and syntopically occurring individuals, or due to retained ancestral polymorphisms. These two processes are difficult to tease apart (Funk and Omland 2003). However, when these data are analyzed in the larger CO-I phylogeny (Taylor and Knouft 2006) the four individuals field identified as *O. nana* are recovered in a clade with *O. meeki* and the individual *O. macrus* is recovered in a clade sister to *O. marchandi* also containing *O. hylas*, *O. peruncus*, *O. quadruncus* (a species group allopatrically distributed), *O. ozarkae*, and *O. punctimanus*.

The two independent Bayesian searches each resulted in a total of 5001 sampled trees. Burn-in was complete by 50,000 generations (i.e. 50 sampled trees) and the first 100 trees (100,000 generations) were removed, leaving 4900 trees for reconstructing the majority-rule consensus. Posterior probability scores are the percentage of trees each node is recovered in after burn-in trees are removed. The posterior probabilities are shown on the recovered maximum parsimony hypothesis shown in Figures 5, 6, and 7. The recovered topologies were very similar to the MP hypothesis.

Reconstructed TCS networks of hypothesized inter-relationships among haplotypes, although phenetic, were completed for the *O. nana* and *O. macrus* data set. 10 nucleotide differences was the maximum number allowed for inter-connection of any two haplotypes into a network with 95% confidence (Figure 8 &9). That is to say that in each of the presented networks all haplotypes that are interconnected are 10 or fewer nucleotides differentiated (less than 1.5% divergent). Conversely, the two networks (Figure 8 & 9) are separated by 10 or more nucleotides (greater than 1.5% divergent). In fact comparing the hypothesized root haplotypes (noted by the square surrounding the haplotype number, and frequency of observation (in parentheses)) the two networks are separated by 53 nucleotides (8.3% divergence). In addition the differentiation between the two clades recovered in both *O. nana* and *O. macrus* are maximally 2.0% (13 nucleotides) divergent.

Average pairwise inter- and intraclade divergence values are given above the diagonal and along the diagonal respectively (Table 3). *Orconectes nana* and *O. macrus* are approximately 1.5% divergent within their respective clades. Interclade divergence, i.e. between species, is 9.2 to 9.7%. *Orconectes macrus* (clade A) shows an average of 1.4% divergence within the clade indicating a substantial amount of nucleotide sequence variation within this recovered natural group. Intraspecific Φ_{ST} values (0=complete mixing, 1=complete subdivision) are given below the diagonal in Table 3, and the values range from 0.686 between clades of *O. macrus* to 0.818 between clades *O. nana*. Between *O. nana* and *O. macrus* the values range from 0.95 to 0.97. All pairwise comparisons showed significant isolation with $p<0.0001$ in all cases. Effective population size estimates (Table 4) indicate that in all but one case (*O. macrus* clade B) historical effective population sizes were higher than current estimates. In *O. macrus* clade B the sample size is very low, and the result may be an artifact.

Orconectes neglectus neglectus and *Orconectes neglectus chaenodactylus*

642 nucleotides of CO-I sequence data were generated for 208 individuals of the *Orconectes neglectus* subspecies pair. 402 nucleotide characters were constant, 57 characters were variable but uninformative for parsimony, and the remaining 183 characters were parsimony informative. Parsimony searches recovered 244 equally parsimonious trees each 788 steps. The backbone phylogeny (i.e. terminal individuals trimmed and showing only nodes supporting recovered groupings) of the strict consensus hypothesis is shown in Figure 10. Bootstrap and Posterior Probability support respectively are shown above the nodes in each figure depicting hypothesized inter-relationships. 205 of the 208 *O. neglectus* individuals that data were generated for in this study are recovered in a polytomy along with one individual each of *O. ozarkae*, *O. punctimanus*, and *O. marchandi*. Two individuals, one of each *O. neglectus* subspecies, were included from the data set of Taylor and Knouft (2006). In the primary recovered clade of subspecies of *O. neglectus* (Figures 10, 11, 12, 13 14, and 15) there are 9 clades from throughout the range of *O. neglectus*: Clades A-1, A-2, A-3, A-4, B-1, B-2, B-3, C-1 and C-2.

Clade A *O. neglectus* (Figures 11 and 12) contains 94 individuals distributed throughout most of the White River basin (Figure 16). Clade A is subdivided into 4 clades of *O. neglectus* that are each geographically restricted to portions of the basin

(Figure 17; except for A-5 which is one individual of *O. punctimanus* and is found in Spring River). Clade A-1 contains 61 individuals that include all sampled members of *O. neglectus chaenodactylus* from North Fork White River, as well as sampled locations downstream of the confluence of North Fork and mainstem White River (Figure 16). Pairwise nucleotide differences among root haplotypes for each network (explained above in *O. nana* and *O. macrus*) are given in Table 5, each of the 5 clades are differentiated by 10 or more nucleotides and are not connected into one large network (Figure 18). 57 pairwise nucleotide differences (~9.0% sequence divergence) separate clade A-1 from clade A-2. Clade A-2 (n=4 individuals) is the most geographically restricted clade based on sampling employed in this study and is completely contained in Jimmie Creek (36.33702N, -92.67578W; Figure 17), a tributary to lower Bull Shoals Reservior. Geographically, between clades A-1/A-2 and A-3/A-4 sits Little North Fork White River in Missouri from which no samples are included or possessed. Clade A-3 (n=6 individuals) is 42 nucleotides (6.5%) divergent from clade A-2 and 55 nucleotides (8.6%) divergent from clade A-1. Clade A-3 occurs in the generally east-flowing region of the White River (Figure 17) and its tributaries below the dam on Table Rock Lake down through upper portions of Bull Shoals reservoir. Clade A-4 (n=22 individuals) is 40 (6.2%), 43 (6.7%), and 45 (7.0%) nucleotides (percent) divergent from clades A-1, A-2, and A-3 respectively. Representatives of clade A-4 are distributed throughout tributaries of Table Rock Lake upstream along the White River through tributaries occurring on the eastern edge of Beaver Lake. Haplotypes of *O. neglectus* occurring in the very upper White River are for the most part unknown, as we were unable to obtain any samples from multiple collection sites (see discussion of clade B-1 below) throughout the range, though sampling efforts were attempted. The individual *O. punctimanus* representing the basal member of clade A (A-5) is from Spring River and when analyzed in a larger *Orconectes* CO-I data set comes out in a clade with *O. nais* and *O. deanae*; all three are recovered basally to the clade described as A in this report.

Clade B *O. neglectus* (Figures 13 and 14) is recovered and subdivided into B-1, B-2 and B-3. B-3 is a default classification as B-2 is distinct and derived within B-3. All three clades and constituent individuals recovered in clades comprising B are all reconstructed into one network (Figure 19), and nucleotide or percent divergences are not given, though each clade represents a distinct and isolated portion of the network hypothesis (Figure 17). Clade B-1 (n=44 individuals) is restricted, based on these data, to the Illinois River drainage and to two sampled sites in the upper White River (36.04929N, -94.16243W; a creek tributary in Fayetteville, AR on the western edge of the White River drainage, and 36.15561N, -93.73473W; Withrow Springs on the eastern edge (i.e a west flowing tributary) of upper Beaver Lake). Clade B-2 (n= 27 individuals) is restricted to the upper Neosho River tributaries including Shoal Creek and Sugar Creek in Missouri and Little Sugar Creek and tributaries of Elk River in Arkansas (Figure 17). In addition, two individuals from Prairie Creek, in the White River, occur with sampled members recovered in the upper Neosho clade. Clade B-3 (n=15 individuals) is restricted to Spavinaw Creek and Flint Creek (Figure 17).

Clade C *O. neglectus* (Figure 15) is subdivided into two clades: C-1 and C-2. Clade C-1 (n=20 individuals) is 16 nucleotides (2.5%) divergent from C-2. Clade C-1 is geographically restricted to Crooked Creek, an east flowing tributary of White River above the mouth of North Fork White River (Figure 17). Interestingly all 4 individuals sequenced from Long Creek (Boone County, AR; 36.34715N, -93.28105W) are recovered in clade C-1 even though this is a tributary of Table Rock Lake in the White River. Also, 5 individuals sequenced from Knob Creek (Izard, Co., AR; 36.08175N, -91.98671W) contain haplotypes recovered in network C-1 and the other 5 individuals are recovered in clade A-1. One individual containing a haplotype found in network C-1 is also found in a tributary to Big Creek (36.31957N, -92.38549W; North Fork drainage). Clade C-2 is restricted to Buffalo River (Figure 17).

Two other individuals: *O. neglectus* 606 and 528 are recovered in clades outside of the main clades of *O. neglectus*. Orconectes neglectus 606 is recovered in a clade with *O. eupunctus*, a taxon restricted to the Spring River, while individual 606 is found in tributaries of the Neosho Basin on the opposite side of the taxon's range. *Orconectes neglectus* 528 is found in the Illinois drainage where *O. nana* occurs sympatrically and this individual is most likely the result of a hybridization event among these two species with a third species *O. meeki* (data not shown).

The two independent Bayesian searches each resulted in 5001 trees. Burn-in was complete by 200,000 generations (i.e. 200 sampled trees) and the first 250 trees (250,000 generations) were removed, leaving 4,750 trees for reconstructing the majority-rule consensus. Posterior probability scores are the percentage of trees in which each node is recovered after burn-in trees are removed. The posterior probabilities are shown on the recovered maximum parsimony hypothesis (shown in Figures 10, 11, 12, 13, 14, and 15). The recovered topology was very similar to the MP hypothesis and is not presented.

Reconstructed TCS networks of hypothesized inter-relationships among haplotypes, although phenetic, were completed for the *O. neglectus* subspecies data set

(Figure 18, 19, and 20). 10 nucleotide differences was the maximum number allowed for inter-connection of any two haplotypes into a network with 95% confidence. That is to say that in each of the presented networks all haplotypes that are interconnected are 10 or fewer nucleotides differentiated (less than 1.5% divergent). Thus, the networks presented in Figures 18, 19, and 20 are separated by ten or more nucleotides (greater than 1.5% divergent). Pairwise comparisons among networks based on the number of pairwise differences among hypothesized root haplotypes are given in Table 2. Root haplotypes are designated by the square surrounding the haplotype number, and frequency of observation (in parentheses) in Figures 18, 19, and 20.

Average pairwise inter- and intraclade divergence values are given above the diagonal and along the diagonal respectively (Table 6). *Orconectes neglectus* ranges from 0.0 to 2.9% divergent within clades, with the highest value (2.9%) observed in clade A-3 indicating that there is substantial variation between the recovered sub-clades. All other intra-clade comparisons show 0.6% or less divergence. Average interclade divergence, i.e. between recovered clades, ranged from a low of 2.2% (A-1 compared to A-2) to 12% (A-3 compared to C-1). Intraspecific Φ_{ST} values (0=complete mixing, 1=complete subdivision) are given below the diagonal in Table 3, and the values range from a low of 0.58 to a high of 1.0. All but six pairwise comparisons showed significant isolation and $p<0.005$ in all cases where pair-wise significance was recovered. Effective population size estimates (Table 7) indicate that in all but one case (*O. neglectus* clade A-3) the historical effective population size was higher than current estimates. The one case (clade A-3) that shows higher effective population size estimates for the current population than the historical estimates is also the clade that has 2.9% intraclade divergence. Thus the high level of intra-clade divergence may translate to higher pairwise differences than polymorphic sites for this group.

Discussion and Conclusions

General

Identical haplotypes are sometimes found in individuals distributed across widespread geographic areas, that is to say that maximal endemicity cannot be demonstrated or hypothesized for a species based on a given set of data (in this case mitochondrial DNA sequence data), or that lineages, and the associated polymorphisms within the lineage as a whole, once occurred across widespread areas. However, in many circumstances this is not the case. That is to say that recovered lineages are deeply divergent and each lineage is highly restricted geographically. Confined lineages (maximal endemics) can arise via at least two different mechanisms that, in the end, are very difficult to distinguish between: mutations arising in a lineage after genetic drift has fixed the entire species (or lineage) for one common haplotype across the entire species' (lineage) range, or genetic drift fixing subdivided groups for different ancestral polymorphic haplotypes.

Orconectes nana and *Orconectes macrus*

Orconectes nana and *O. macrus* were described by Williams 1952, and were first recognized as subspecies: *O. nana nana* and *O. nana macrus*. The two subspecific entities were subsequently elevated to full species status. Williams (1952) placed the two taxa into the Hylas species group, allopatrically distributed from remaining members of this group. Fitzpatrick (1987) restructured genus *Orconectes* with a subgeneric classification system and placed both species into subgenus *Procericambarus*, and more specifically into the Spinosus group separating them from other species of the Hylas group. In the group classifications of subgenus *Procericambarus* (Fitzpatrick Jr. 1987) *O. quadruncus* was separated into its own group (Quadruncus group) apart from *O. hylas* and *O. peruncus* (both members of the Hylas group) though phylogenetically this is a tightly recovered species group (Taylor and Knouft 2006), and thus the criterion used for groups may be phenetic and not the result of common ancestry. The distinctness of *O. nana* and *O. macrus* morphologically is largely based on variation of gonopod length. The etymology of *O. nana* comes from its small size while *O. macrus* gained its name from the larger gonopods when compared to *O. nana* (Williams 1952).

The differences in gonopod length (and presumably in annulus ventralis position) could lead to reproductive isolation in cases where the two allopatric species may come into secondary contact. The sampling provided by this study indicates that these two species have not been in contact with one another for an extensive period of time (as indicated by the deep divergence between recognized species). Additionally, these data indicate that there is sub-division within each of the recognized species (i.e. clades A and B in both species, 1.4% and 1.5% divergence in *O. nana* and *O. macrus* respectively) and that the subdivision within each clade is geographically structured, as is the divergence between each of these species (~9.5% divergence).

Orconectes nana, as recognized by the mitochondrial lineage data recovered in this study, is primarily confined to the Illinois River drainage (clade A), though there is a disjunt population located in Prairie Creek (White River) and the six individuals analyzed from that location are recovered in the clade with all *O. nana* sampled from the Illinois River. A previous stream connection between the Neosho River and the White River has been hypothesized based on faunal distribution patterns in fishes (Branson 1967) and data presented here from *O. nana* support this hypothesized former connection. Additionally, the population in Prairie Creek had previously been identified as *O. macrus*, but the data presented here prompted a re-examination of those individuals identified as *O. macrus* and based on re-examined gonopod length, in conjunction with these data, resulted in the change of identification. Thus, these molecular data were able to pinpoint a mis-identification and demonstrate how both morphology and molecules can work in conjunction to better understand faunal distribution patterns. The second clade of *O. nana* (clade B) is restricted to Flint Creek, a tributary to the Illinois River. Despite the distinct lineage represented by clade B, it may be premature to elevate this lineage to full species status as the remaining downstream portions of both Flint Creek and Illinois River have yet to be sampled and analyzed.

Orconectes macrus, as recognized by the mitochondrial lineage data recovered in this study is found as far south as Spavinaw Creek and its' tributary Beaty Creek. It is also found in sampled tributaries of Little Sugar Creek and Elk River in Arksansas. The range of *O. macrus* extends much further north than was sampled for this study.

Orconectes macrus can be found throughout the Spring River and its tributaries, exclusive of North Fork Spring River, as well as portions of Shoal Creek in Missouri (Pflieger 1996). In addition *O. macrus* is known from Spring River in Cherokee County, Kansas (Ghedotti 1998). Two clades of *O. macrus* (A and B) are recovered in these analyses. Clade B *O. macrus* contains only five individuals that were sampled from Butler Creek, a locality that lies between Honey Creek and Gordon Hollow, all are tributaries to the Elk River. Since much of the range of this taxon has yet to be sampled this result may be an artifact of the sampling employed here, and may not necessarily indicate genetic subdivision within the tributaries of the Elk River. Though it is possible with more data and denser sampling that the subdivision noted will continue to be supported.

Orconectes neglectus neglectus and *Orconectes neglectus chaenodactylus*

Orconectes neglectus neglectus was described by (Faxon 1885), and *O. n. chaenodactylus* was described by Williams 1952. *Orconectes n. chaenodactylus* was restricted to North Fork White River by Williams (1952) and he also noted intergrades between the two subspecies in portions of headwaters of North Fork in Missouri and in tributaries downstream of the mouth of North Fork in Arkansas. *Orconectes n. neglectus* is hypothesized to occur throughout the rest of the White River basin including all tributaries exclusive of North Fork. Additionally, the nominal subspecies is found throughout the same portions of Neosho River where *O. nana* and *O. macrus* are distributed (see above), as well as in four disjunct portions in Kansas, Nebraska and

Oklahoma. Interestingly, the localities in Oklahoma have been either omitted from hypotheses of the historical distribution (Williams 1954) or interpreted as an introduction outside of the native range (Taylor et al., 2004). The latter is not improbable, as *O. neglectus neglectus* has become established in New York (Daniels et al., 2001) and on the western slope of the Rocky Mountains. The western Rocky Mountain population was formerly recognized as *O. transfuga* (Fitzpatrick 1966). Interstingly, *O. transfuga* Fitzpatrick, has been synonymized with *O. neglectus neglectus*, but before this it was assigned to the Spinosus group (Fitzpatrick 1987), while the two subspecies of *O. neglectus* (with which *O. transfuga* was synonymized with) were assigned to the Rusticus Group by Williams (1954). Fitzpatrick (1987) re-assigned both subspecies to the Forceps Group in subgenus *Procericambarus*, again indicating that taxonomy for some of this subgenus may not represent the natural genealogical history of the group, and indeed molecular sequence data (Crandall and Fitzpatrick Jr. 1996) and allozymes (Fetzner Jr. 1996) have borne this out. The disjunct localities described above have no samples that are included in any of the analyses and for the most part will be left out of the discussion presented here. The areas of co-distribution (Neosho River basin) occupied by *O. neglectus* and the *O. nana* and *O. macrus* species pair allow a test of the hypotheses presented for historical subdivisions for *O. nana* and *O. macrus*.

The allopatric distribution of the two recognized subspecies may have allowed sufficient time for reproductive isolation to occur. Thus upon secondary contact these subspecies (i.e. hypothesized lineages) would retain integrity, despite possibility for hybridization (historically hypothesized as intergrades). To this end, sampling employed for this study indicates that there are three deeply divergent lineages, one more than the hypothesized number of subspecies. And, within each of these three lineages there ranges from a minimum of 2 to a maximum of 4, isolated, less deeply divergent, and independently evolving lineages. Based on the data presented here for the nominal subspecies, *O. n. neglectus*, there are three lineages, in *O. n. chaenodactylus*, there are four lineages, and in a third undescribed taxon there are two lineages. Given the highly divergent haplotypes recovered in the taxon currently recognized as *Orconectes neglectus* along with previous recognition of subspecies in the taxon it is without much difficulty that we propose elevation to full species status for both subspecies of *O. neglectus*, and note that there is at minimum a third, as of yet unrecognized, species. The remainder of this discussion will refer to these species as *O. neglectus*, *O. chaenodactylus*, and the third species will be referenced as *O.* sp. cf. *neglectus*.

The type locality of *O. neglectus* is Mill Creek in Wabaunsee County, Kansas (Faxon 1885). This is a tributary to the Kansas River, and currently has no connection to the Ozark Highlands province, however the Ancestral Plains Stream (Metcalf 1966) is proposed to have connected these areas historically, and indeed is hypothesized as the explanation for the distribution of this species (Williams 1954) and others (Kreiser et al., 2001). Based on this information and until samples from the type locality of *O. neglectus* are analyzed we confine this taxon to the western edge of the Ozark Highlands, i.e. tributaries of the Arkansas River. In addition to this geographically isolated portion of the Ozark Highlands, and as defined by these mtDNA data, *O. neglectus* is also found in two tributaries to the upper White River, specifically on the western edge (east-flowing) tributaries of the White River. The recovery of a single individual in an eastern (west-flowing) tributary of the Upper White River is intriguing and may be explained by the retention of ancestral polymorphism, hybridization, or possibly by a recent introduction (i.e. ‘bait-bucket’). If this is a retained ancestral polymorphism then it represents a large pool of variability present across the entire range of this species pre-isolation of the lineages in the Arkansas and White River tributaries as the haplotypes at this location are 52 nucleotides differentiated. Additionally, it is indicated that effective population sizes were quite large historically (at the time of ancestral subdivision), and that effective population sizes continue to be large to the present day, thus making it more likely that the population could maintain large amounts of diversity (i.e. genetic drift as a mechanism would be slow to sort to fixation) in the location. Of the 7 individuals sampled from the stream reach only one individual had this haplotype. However, it should be noted that the recovered pattern for this occurrence would look no different, in a phylogenetic or population genetic context, if the individual were the result of a recent introduction. In fact, a recent introduction of *O. chaenodactylus* ((Magoulick and DiStefano 2007), noted as *O. neglectus* in their publication) into the Spring River basin is an example of a cross-drainage introduction that is presumably by bait-bucket. In addition to significant subdivision between this geographic locality (Arkansas River tributaries) and the remainder of the *O. neglectus* species group range, there is significant subdivision within *O. neglectus*, as recognized here, indicating that each of the three sub-units (depicted as smaller blue polygons circumscribed by dark borders in Figure 17) are not maintaining maternal gene flow among any of these sub-units, and indeed warrant further investigation and potential elevation to full species.

The historical distribution of *O. neglectus*, as recognized taxonomically, was not confined to the western portion of the Ozark Highlands as it is here, but instead occurred in the range described above as well as throughout the remainder of the White River drainage to the exclusion of North Fork White River where *Orconectes chaenodactylus* occurs. However, throughout the remainder of the White River region there are two widespread mitochondrial lineages that encompass even further sub-divided lineages. *Orconectes* sp. cf. *neglectus* is confined to three localities in this area: Buffalo River and Crooked Creek (both are east flowing tributaries to the mainstem White River), and a north flowing direct tributary to White River. These localities are not interconnected currently but form a ‘pocket’ in the central portion of the White River indicating historically there was an interconnection among these regions (Figure 17). The lineage is further subdivided into two groups as shown in Figure 17, and there is significant isolation between the two sub-groups indicating that there is no maternal gene flow between localities. In addition to this confined area in the central White River basin, the lineage has representatives in two other localities, and in both locations unusual color patterns were noted. For example in the collection of CBD 06-48, both species (as indicated by color morphs) were collected together, and while only one of each color variant (species) was sequenced they each separated into their respective clades indicating that some pre- or post-mating isolation mechanism is keeping the taxa distinct in this locality. In the second locality, CBD 06-08, ten individuals were sequenced and 50% are recovered in the *O.* sp. cf. *neglectus* clade and 50% are recovered in the *O. chaenodactylus* clade again indicating that the lineages are intact and maintaining their independence. This is in the area hypothesized by Williams (1954) to contain intergrades, and as mentioned above the fact that both haplotypes are recovered in this location may indicate that the species have come into contact with one another after accumulation of pre-mating or post-mating isolation mechanisms. In addition there is, as mentioned above, significant differentiation among the localities indicating the need for future work to determine if more than one species inhabits the area.

Orconectes chaenodactylus, as recognized by the mtDNA lineage recovered in North Fork, occupies the remainder of the White River basin, exclusive of the range of *O.* sp. cf. *neglectus*. Like other recognized species of the *O. neglectus* complex (see above) there are several (n=4) significantly isolated maternal lineages that occur throughout the White River basin that, with further investigation, may indicate that additional species need be recognized from this clade. Ranges for each of the localities of the clades comprising *O. chaenodactylus* are discussed above, but it is evident that isolation events have taken place historically that have subdivided members of *O. chaenodactylus* throughout White River as shown in Figure 17.

Fine scale geographic variation was recovered throughout the range of the *O. neglectus* species group. The mtDNA locus used here was able to shed light upon historical events and present day parameters of populations. Species comprising this group are highly variable in color pattern and at least slightly variable in body shape (CBD, personal observation) and these mtDNA data provide a robust hypothesis and a framework for further testing subdivision of these species with alternative data sets (e.g. nuclear sequence data and/or morphological data). These data suggest periods of inter-connection between drainage basins and isolation within drainages. Combined, these aspects point to a long and complex geologic history for the southern Ozark Highlands province.

Management Implications

Based on results from these studies of *Orconectes* species pairs from the Ozark Highlands it is clear that the level of biodiversity is much higher than currently recognized taxonomically. In addition, the results also point to intra-drainage complexity in relationships within species groups. Specifically, there is a significant amount of subdivision throughout the White River that warrants further investigation in *Orconectes chaenodactylus* and *O*. sp. cf. *neglectus*. These data also indicate that significant subdivision occurs between populations of *O. neglectus* and *O. nana* and potentially *O. macrus* in tributaries of Neosho River. Taken as a whole, these data strongly indicate that anthropogenic movement of these taxa, or any subdivided lineages within the examined geographic areas, via bait-bucket introduction, aquarists, or otherwise, across drainage reaches would be detrimental to the complex history of these species, as well as impact their evolutionary future, as distinct units recovered here could be compromised by inter-lineage secondary contact, which without reinforcement of pre- or post-mating isolation mechanisms could result in 'hybridization', and thus swamp out millions of years of independent evolutionary history that has accrued within these isolated lineages. All efforts should be made to reduce any such movement, thus maintaining the natural condition for this significant component of biodiversity of the Ozark Highlands.

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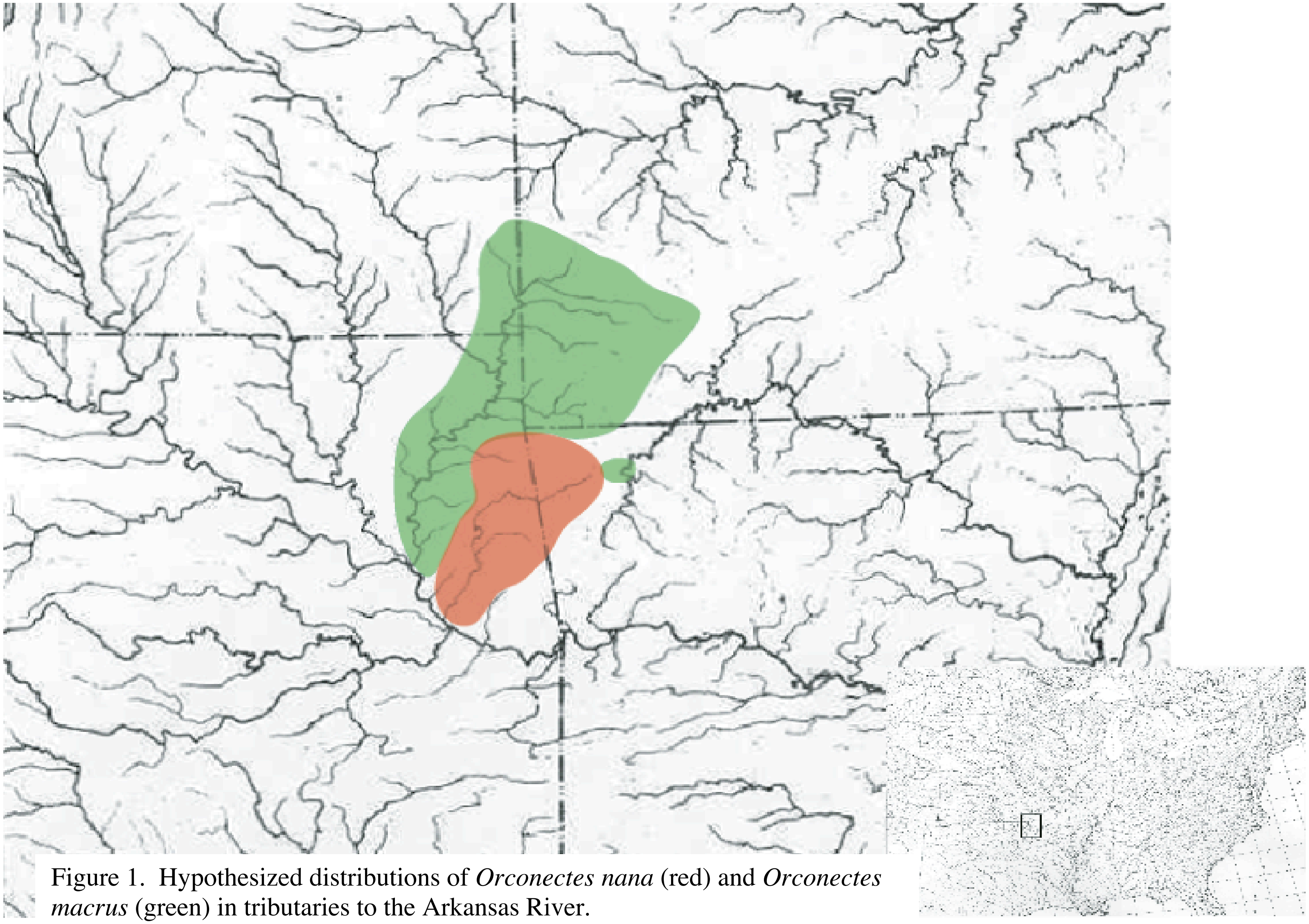


Figure 1. Hypothesized distributions of *Orconectes nana* (red) and *Orconectes macrus* (green) in tributaries to the Arkansas River.

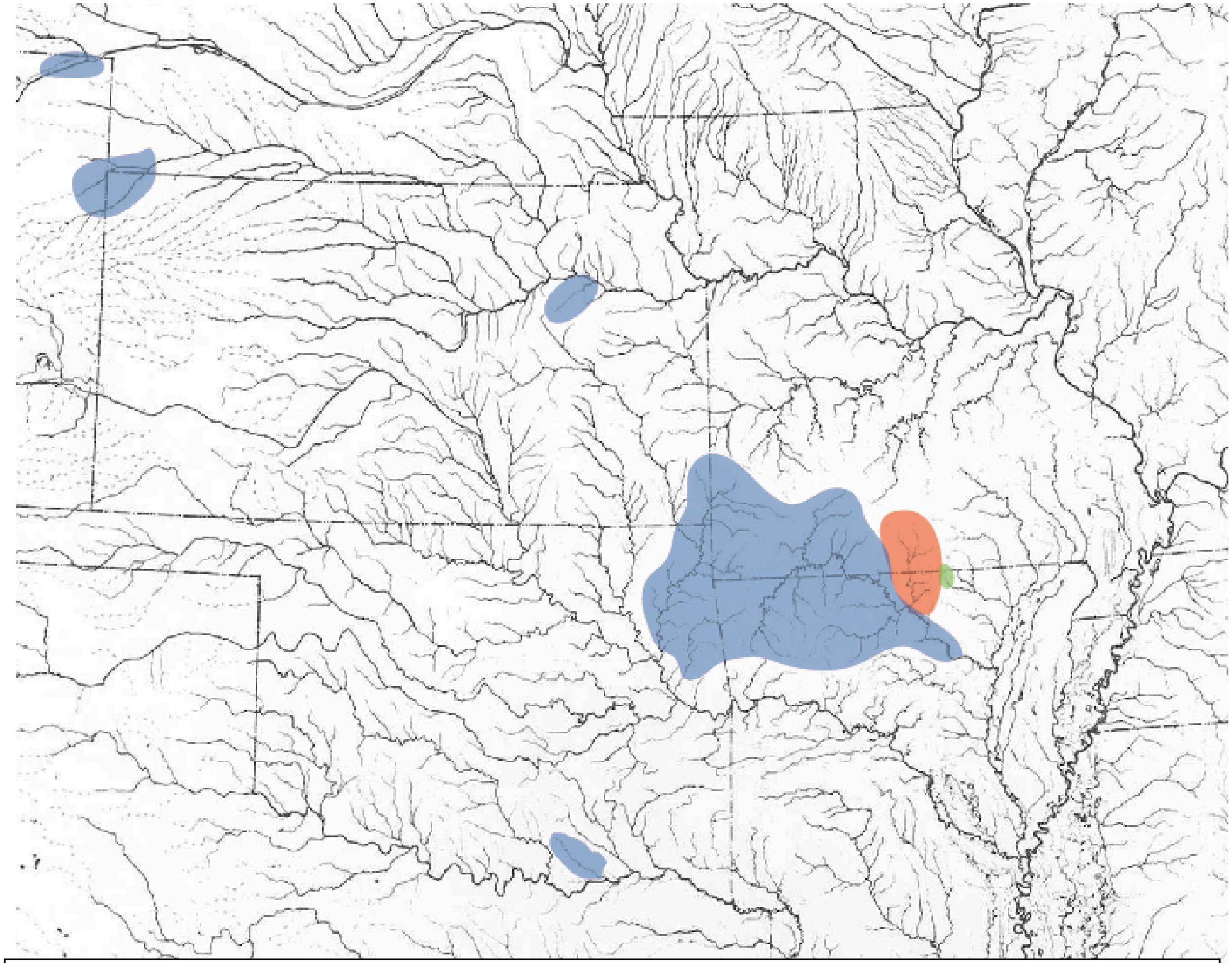


Figure 2. Map showing hypothesized distribution for both subspecies of *Orconectes neglectus*. *Orconectes neglectus neglectus* is shown in blue and *O. n. chaenodactylus* is shown in red. The area in green represents a recent introduction outside the native range.

Table 1. Individuals sampled and their species identification for the interspecific investigation in *Orconectes nana* and *Orconectes macrus*. Cytochrome Oxidase I (CO-I) haplotypes are given along with the frequency of observation. Asterisks indicate species field identifications that were overturned in phylogenetic recovery. Field numbers correspond to first author's field collection information.

Species ID (Lab Number)	Sample Location	Drainage	CO-I Haplotype (Total Observations)	Longitude	Latitude	State:County	Field Number
Orconectes nana (478)	Elk Horn Spring Branch	Illinois	12 (2)	-94.31139	36.06019	AR: Washington	CBD 06-22
O. nana (479)	Elk Horn Spring Branch	Illinois	9 (1)	-94.31139	36.06019	AR: Washington	CBD 06-22
O. nana (480)	Elk Horn Spring Branch	Illinois	12 (2)	-94.31139	36.06019	AR: Washington	CBD 06-22
O. nana (481)	Elk Horn Spring Branch	Illinois	1 (7)	-94.31139	36.06019	AR: Washington	CBD 06-22
O. nana (482)	Elk Horn Spring Branch	Illinois	5 (1)	-94.31139	36.06019	AR: Washington	CBD 06-22
O. nana (488)	Hamestring Creek	Illinois	2 (2)	-94.28716	36.0951	AR: Washington	CBD 06-23
O. nana (489)	Hamestring Creek	Illinois	1 (7)	-94.28716	36.0951	AR: Washington	CBD 06-23
O. nana (490)	Hamestring Creek	Illinois	2 (2)	-94.28716	36.0951	AR: Washington	CBD 06-23
O. nana (503)	Tributary of Illinois River	Illinois	6 (1)	-94.49955	36.15478	AR: Benton	CBD 06-24
O. nana (504)	Tributary of Illinois River	Illinois	1 (7)	-94.49955	36.15478	AR: Benton	CBD 06-24
O. nana (505)	Tributary of Illinois River	Illinois	41 (2)	-94.49955	36.15478	AR: Benton	CBD 06-24
O. nana (506)	Tributary of Illinois River	Illinois	1 (7)	-94.49955	36.15478	AR: Benton	CBD 06-24
O. nana (507)	Tributary of Illinois River	Illinois	1 (7)	-94.49955	36.15478	AR: Benton	CBD 06-24
O. nana (508)	Tributary of Illinois River	Illinois	41 (2)	-94.49955	36.15478	AR: Benton	CBD 06-24
O. nana (509)	Sager Creek	Illinois	17 (2)	-94.52324	36.19098	AR: Benton	CBD 06-25
O. nana (510)	Sager Creek	Illinois	16 (1)	-94.52324	36.19098	AR: Benton	CBD 06-25
O. nana (511)	Sager Creek	Illinois	17 (2)	-94.52324	36.19098	AR: Benton	CBD 06-25
O. nana (512)	Sager Creek	Illinois	15 (1)	-94.52324	36.19098	AR: Benton	CBD 06-25
O. nana (513)	Sager Creek	Illinois	13 (10)	-94.52324	36.19098	AR: Benton	CBD 06-25
O. nana (519)	Spring Fed Creek along Cornhoff Rd.	Illinois	1 (7)	-94.30472	36.15227	AR: Washington	CBD 06-26
O. nana (520)	Spring Fed Creek along Cornhoff Rd.	Illinois	4 (1)	-94.30472	36.15227	AR: Washington	CBD 06-26
O. nana (521)	Spring Fed Creek along Cornhoff Rd.	Illinois	7 (4)	-94.30472	36.15227	AR: Washington	CBD 06-26

O. nana (522)	Spring Fed Creek along Cornhoff Rd.	Illinois	8 (1)	-94.30472	36.15227	AR: Washington	CBD 06-26
O. nana (523)	Spring Fed Creek along Cornhoff Rd.	Illinois	7 (4)	-94.30472	36.15227	AR: Washington	CBD 06-26
O. nana (529)	Little Osage Creek	Illinois	1 (7)	-94.2714	36.2537	AR: Benton	CBD 06-27
O. nana (530)	Little Osage Creek	Illinois	10 (6)	-94.2714	36.2537	AR: Benton	CBD 06-27
O. nana (531)	Little Osage Creek	Illinois	10 (6)	-94.2714	36.2537	AR: Benton	CBD 06-27
O. nana (532)	Little Osage Creek	Illinois	3 (1)	-94.2714	36.2537	AR: Benton	CBD 06-27
O. nana (533)	Little Osage Creek	Illinois	10 (6)	-94.2714	36.2537	AR: Benton	CBD 06-27
O. nana (534)	Little Osage Creek	Illinois	1 (7)	-94.2714	36.2537	AR: Benton	CBD 06-27
O. nana (535)	Little Osage Creek	Illinois	10 (6)	-94.2714	36.2537	AR: Benton	CBD 06-27
O. nana (586)*	Spavinaw Creek	Neosho	33 (1)	-94.41488	36.39635	AR: Benton	CBD 06-32
O. nana (587)*	Spavinaw Creek	Neosho	30 (7)	-94.41488	36.39635	AR: Benton	CBD 06-32
O. nana (588)*	Spavinaw Creek	Neosho	30 (7)	-94.41488	36.39635	AR: Benton	CBD 06-32
O. nana (589)*	Spavinaw Creek	Neosho	30 (7)	-94.41488	36.39635	AR: Benton	CBD 06-32
O. nana (590)*	Spavinaw Creek	Neosho	30 (7)	-94.41488	36.39635	AR: Benton	CBD 06-32
O. nana (617)*	Beaty Creek	Neosho	26 (1)	-94.60036	36.41686	AR: Benton	CBD 06-35
O. nana (618)*	Beaty Creek	Neosho	27 (1)	-94.60036	36.41686	AR: Benton	CBD 06-35
O. nana (619)*	Beaty Creek	Neosho	29 (1)	-94.60036	36.41686	AR: Benton	CBD 06-35
O. nana (621)*	Beaty Creek	Neosho	28 (1)	-94.60036	36.41686	AR: Benton	CBD 06-35
O. nana (627)*	Spavinaw Creek	Neosho	31 (1)	-94.58675	36.34262	AR: Benton	CBD 06-36
O. nana (628)*	Spavinaw Creek	Neosho	30 (7)	-94.58675	36.34262	AR: Benton	CBD 06-36
O. nana (629)*	Spavinaw Creek	Neosho	30 (7)	-94.58675	36.34262	AR: Benton	CBD 06-36
O. nana (630)*	Spavinaw Creek	Neosho	32 (1)	-94.58675	36.34262	AR: Benton	CBD 06-36
O. nana (631)*	Spavinaw Creek	Neosho	30 (7)	-94.58675	36.34262	AR: Benton	CBD 06-36
O. nana (637)	Flint Creek	Illinois	14 (1)	-94.48721	36.24226	AR: Benton	CBD 06-37
O. nana (638)	Flint Creek	Illinois	13 (10)	-94.48721	36.24226	AR: Benton	CBD 06-37

O. nana (639)	Flint Creek	Illinois	13 (10)	-94.48721	36.24226	AR: Benton	CBD 06-37
O. nana (640)	Flint Creek	Illinois	13 (10)	-94.48721	36.24226	AR: Benton	CBD 06-37
O. nana (641)	Flint Creek	Illinois	13 (10)	-94.48721	36.24226	AR: Benton	CBD 06-37
O. nana (642)**	Flint Creek	Illinois	40 (2)	-94.48721	36.24226	AR: Benton	CBD 06-37
O. nana (643)**	Flint Creek	Illinois	40 (2)	-94.48721	36.24226	AR: Benton	CBD 06-37
O. nana (649)	Tributary of East Flint Creek	Illinois	13 (10)	-94.42128	36.26191	AR: Benton	CBD 06-38
O. nana (650)	Tributary of East Flint Creek	Illinois	13 (10)	-94.42128	36.26191	AR: Benton	CBD 06-38
O. nana (651)	Tributary of East Flint Creek	Illinois	13 (10)	-94.42128	36.26191	AR: Benton	CBD 06-38
O. nana (652)	Tributary of East Flint Creek	Illinois	13 (10)	-94.42128	36.26191	AR: Benton	CBD 06-38
O. nana (653)	Tributary of East Flint Creek	Illinois	13 (10)	-94.42128	36.26191	AR: Benton	CBD 06-38
Orconectes macrus (541)*	Prairie Creek at Atalanta Lake	White River	11 (2)	-94.1032	36.3343	AR: Benton	CBD 06-28
O. macrus 542*	Prairie Creek at Atalanta Lake	White River	11 (2)	-94.1032	36.3343	AR: Benton	CBD 06-28
O. macrus 543*	Prairie Creek at Atalanta Lake	White River	7 (4)	-94.1032	36.3343	AR: Benton	CBD 06-28
O. macrus 544*	Prairie Creek at Atalanta Lake	White River	7 (4)	-94.1032	36.3343	AR: Benton	CBD 06-28
O. macrus 545*	Prairie Creek at Atalanta Lake	White River	10 (6)	-94.1032	36.3343	AR: Benton	CBD 06-28
O. macrus 546*	Prairie Creek at Atalanta Lake	White River	10 (6)	-94.1032	36.3343	AR: Benton	CBD 06-28
O. macrus 552	Spanker Creek	Elk / Spring / Neosho	18 (9)	-94.21	36.43025	AR: Benton	CBD 06-29
O. macrus 553	Spanker Creek	Elk / Spring / Neosho	18 (9)	-94.21	36.43025	AR: Benton	CBD 06-29
O. macrus 554	Spanker Creek	Elk / Spring / Neosho	18 (9)	-94.21	36.43025	AR: Benton	CBD 06-29
O. macrus 555	Spanker Creek	Elk / Spring / Neosho	25 (1)	-94.21	36.43025	AR: Benton	CBD 06-29
O. macrus 556	Spanker Creek	Elk / Spring / Neosho	19 (1)	-94.21	36.43025	AR: Benton	CBD 06-29

O. macrus 557	Spanker Creek	Elk / Spring / Neosho	23 (1)	-94.21	36.43025	AR: Benton	CBD 06-29
O. macrus 558	Spanker Creek	Elk / Spring / Neosho	24 (2)	-94.21	36.43025	AR: Benton	CBD 06-29
O. macrus 565	Little Sugar Creek	Elk / Spring / Neosho	20 (1)	-94.25027	36.47566	AR: Benton	CBD 06-30
O. macrus 566	Little Sugar Creek	Elk / Spring / Neosho	18 (9)	-94.25027	36.47566	AR: Benton	CBD 06-30
O. macrus 567	Little Sugar Creek	Elk / Spring / Neosho	18 (9)	-94.25027	36.47566	AR: Benton	CBD 06-30
O. macrus 568	Little Sugar Creek	Elk / Spring / Neosho	24 (2)	-94.25027	36.47566	AR: Benton	CBD 06-30
O. macrus 569	Little Sugar Creek	Elk / Spring / Neosho	21 (1)	-94.25027	36.47566	AR: Benton	CBD 06-30
O. macrus 570	Little Sugar Creek	Elk / Spring / Neosho	18 (9)	-94.25027	36.47566	AR: Benton	CBD 06-30
O. macrus 576	Gordon Hollow	Elk / Spring / Neosho	22 (2)	-94.29852	36.4884	AR: Benton	CBD 06-31
O. macrus 577	Gordon Hollow	Elk / Spring / Neosho	18 (9)	-94.29852	36.4884	AR: Benton	CBD 06-31
O. macrus 578	Gordon Hollow	Elk / Spring / Neosho	18 (9)	-94.29852	36.4884	AR: Benton	CBD 06-31
O. macrus 579	Gordon Hollow	Elk / Spring / Neosho	18 (9)	-94.29852	36.4884	AR: Benton	CBD 06-31
O. macrus 580	Gordon Hollow	Elk / Spring / Neosho	22 (2)	-94.29852	36.4884	AR: Benton	CBD 06-31
O. macrus 597	Butler Creek	Elk / Spring / Neosho	37 (2)	-94.45797	36.48433	AR: Benton	CBD 06-33
O. macrus 598	Butler Creek	Elk / Spring / Neosho	37 (2)	-94.45797	36.48433	AR: Benton	CBD 06-33
O. macrus 599	Butler Creek	Elk / Spring /	38 (3)	-94.45797	36.48433	AR: Benton	CBD 06-33

		Neosho					
O. macrus 600	Butler Creek	Elk / Spring / Neosho	38 (3)	-94.45797	36.48433	AR: Benton	CBD 06-33
O. macrus 601	Butler Creek	Elk / Spring / Neosho	38 (3)	-94.45797	36.48433	AR: Benton	CBD 06-33
O. macrus 607	Honey Creek	Neosho	39 (1)	-94.56296	36.48066	AR: Benton	CBD 06-34
O. macrus 608	Honey Creek	Neosho	35 (1)	-94.56296	36.48066	AR: Benton	CBD 06-34
O. macrus 609	Honey Creek	Neosho	34 (2)	-94.56296	36.48066	AR: Benton	CBD 06-34
O. macrus 610	Honey Creek	Neosho	36 (1)	-94.56296	36.48066	AR: Benton	CBD 06-34
O. macrus 611	Honey Creek	Neosho	34 (2)	-94.56296	36.48066	AR: Benton	CBD 06-34

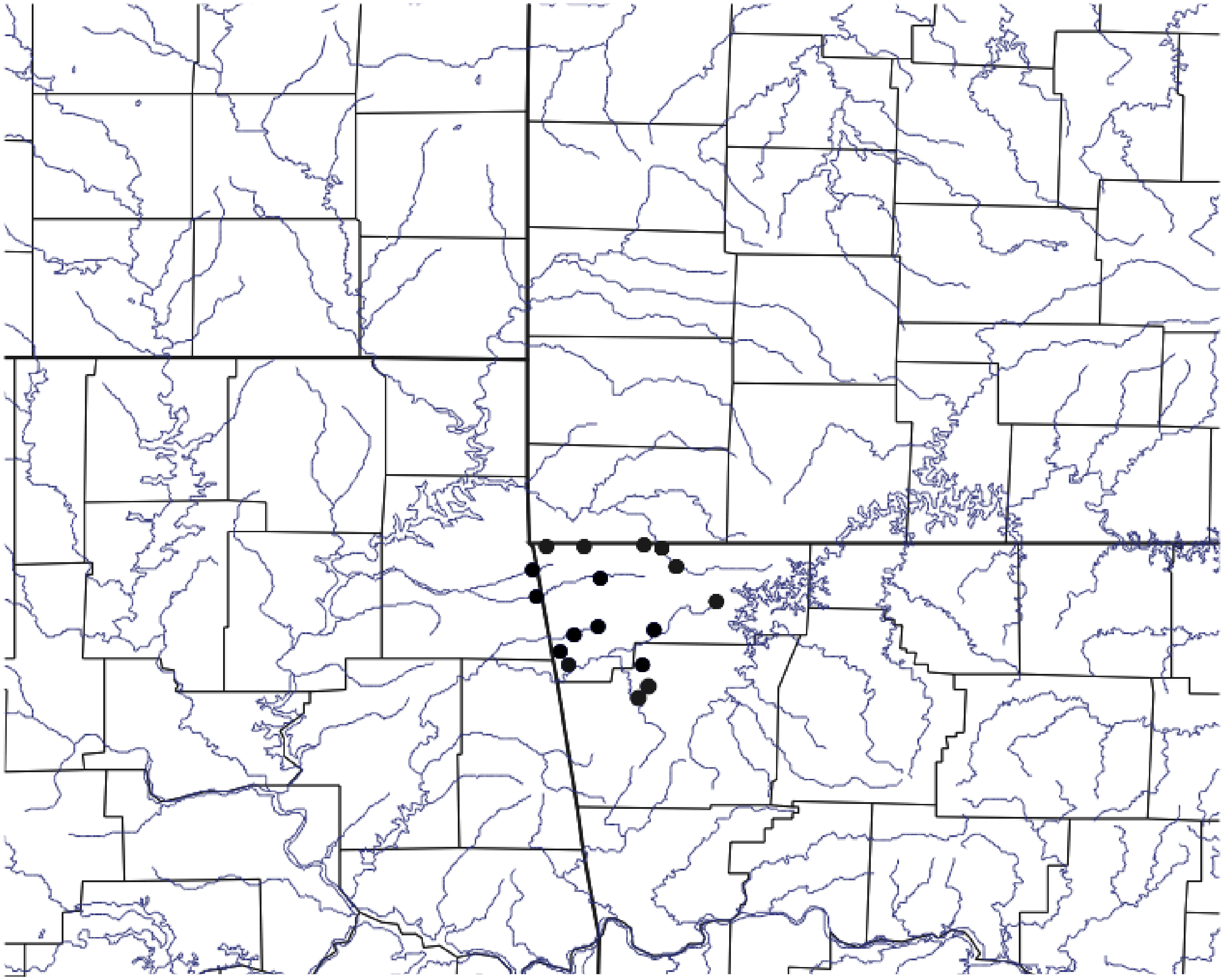


Figure 3. Sampling localities of *O. nana* and *O. macrus* species pair employed in this study.

Table 2. Individuals sampled and species identification for the interspecific investigation in *Orconectes neglectus neglectus* and *Orconectes neglectus chaenodactylus*. Cytochrome Oxidase I (CO-I) haplotypes are given along with the frequency of observation (in parentheses). Field numbers correspond to first author's field collection information or to INHS Catalog Number.

Species ID (Lab Number)	Sample Location	Drainage	CO-I Haplotype (Total Observations)	Longitude	Latitude	State:County	Field Number
Onegneg	Lick Creek	White	66(1)			MO:Ozark	INHS 8887
Onegcha	Indian Creek	White	36(8)			MO:Stone	INHS 8789
76_1	Roark Creek	White	84(1)	-93.185575	36.683317	MO:Taney	
77_1	Roark Creek	White	85(1)	-93.185575	36.683317	MO:Taney	
82_1	Roark Creek	White	86(1)	-93.185575	36.683317	MO:Taney	
95_1	North Fork White River	White	50(1)	-92.16525	36.84428	MO:Douglas	
99_1	Rippee Creek at County Rd. 320	White	40(1)	-92.544903	36.848014	MO:Douglas	
100_1	Rippee Creek	White	36(8)	-92.481502	36.864892	MO:Douglas	
105_1	Hudson Creek		23(1)	-94.012335	36.882184	MO:Barry	
106_1	Big Sugar Creek	Neosho	24(1)	-94.3513	36.6093	MO:McDonald	
108_1	Roaring River	White	71(1)	-93.781563	36.554519	MO:Barry	
109_1	James River	White	72(2)	-93.128291	37.192195	MO:Greene	
112_1	James River	White	72(2)	-92.921553	37.264898	MO:Webster	
Oneg100A	Crooked Creek	White	53(8)	-93.0472	36.2438	AR:Boone	CBD 05-20
Oneg100B	Crooked Creek	White	53(8)	-93.0472	36.2438	AR:Boone	CBD 05-20
Oneg101A	Little Buffalo River	White	79(1)	-93.1837	36.001	AR: Newton	CBD 05-16
Oneg101B	Little Buffalo River	White	80(1)	-93.1837	36.001	AR: Newton	CBD 05-16
O. neglectus 340	Bear Creek	White	78(1)	-93.07526	36.4498	AR:Boone	CBD05-21
O. neglectus 341	Bear Creek	White	76(1)	-93.07526	36.4498	AR:Boone	CBD05-21
O. neglectus 342	Bear Creek	White	77(1)	-93.07526	36.4498	AR:Boone	CBD05-21
O. neglectus chaenodactylus 354	Sylamore Creek	White	46(3)	-92.21116	35.99472	AR:Stone	CBD 06-06
O. neglectus chaenodactylus 355	Sylamore Creek	White	46(3)	-92.21116	35.99472	AR:Stone	CBD 06-06

O. neglectus chaenodactylus 356	Sylamore Creek	White	44(1)	-92.21116	35.99472	AR:Stone	CBD 06-06
O. neglectus chaenodactylus 357	Sylamore Creek	White	46(3)	-92.21116	35.99472	AR:Stone	CBD 06-06
O. neglectus chaenodactylus 358	Sylamore Creek	White	43(1)	-92.21116	35.99472	AR:Stone	CBD 06-06
O. neglectus chaenodactylus 360	Sugarloaf Creek	White	39(10)	-92.15289	36.07009	AR:Stone	CBD 06-07
O. neglectus chaenodactylus 361	Sugarloaf Creek	White	39(10)	-92.15289	36.07009	AR:Stone	CBD 06-07
O. neglectus chaenodactylus 362	Sugarloaf Creek	White	39(10)	-92.15289	36.07009	AR:Stone	CBD 06-07
O. neglectus chaenodactylus 363	Sugarloaf Creek	White	39(10)	-92.15289	36.07009	AR:Stone	CBD 06-07
O. neglectus chaenodactylus 364	Sugarloaf Creek	White	39(10)	-92.15289	36.07009	AR:Stone	CBD 06-07
O. neglectus chaenodactylus 365	Sugarloaf Creek	White	39(10)	-92.15289	36.07009	AR:Stone	CBD 06-07
O. neglectus chaenodactylus 366	Sugarloaf Creek	White	39(10)	-92.15289	36.07009	AR:Stone	CBD 06-07
O. neglectus chaenodactylus 367	Sugarloaf Creek	White	39(10)	-92.15289	36.07009	AR:Stone	CBD 06-07
O. neglectus chaenodactylus 368	Sugarloaf Creek	White	39(10)	-92.15289	36.07009	AR:Stone	CBD 06-07
O. neglectus chaenodactylus 369	Sugarloaf Creek	White	39(10)	-92.15289	36.07009	AR:Stone	CBD 06-07
O. neglectus 370	Knob Creek	White	45(5)	-91.98671	36.08175	AR:Izard	CBD 06-08
O. neglectus 371	Knob Creek	White	45(5)	-91.98671	36.08175	AR:Izard	CBD 06-08
O. neglectus 372	Knob Creek	White	45(5)	-91.98671	36.08175	AR:Izard	CBD 06-08
O. neglectus 373	Knob Creek	White	54(1)	-91.98671	36.08175	AR:Izard	CBD 06-08
O. neglectus 374	Knob Creek	White	53(8)	-91.98671	36.08175	AR:Izard	CBD 06-08
O. neglectus 375	Knob Creek	White	64(1)	-91.98671	36.08175	AR:Izard	CBD 06-08

O. neglectus 376	Knob Creek	White	56(1)	-91.98671	36.08175	AR:Izard	CBD 06-08
O. neglectus 377	Knob Creek	White	45(5)	-91.98671	36.08175	AR:Izard	CBD 06-08
O. neglectus 378	Knob Creek	White	53(8)	-91.98671	36.08175	AR:Izard	CBD 06-08
O. neglectus 379	Knob Creek	White	45(5)	-91.98671	36.08175	AR:Izard	CBD 06-08
O. neglectus chaenodactylus 380	Big Creek	White	52(1)	-91.9772	36.39643	AR:Fulton	CBD 06-09
O. neglectus chaenodactylus 381	Big Creek	White	47(11)	-91.9772	36.39643	AR:Fulton	CBD 06-09
O. neglectus chaenodactylus 382	Big Creek	White	38(10)	-91.9772	36.39643	AR:Fulton	CBD 06-09
O. neglectus chaenodactylus 383	Big Creek	White	38(10)	-91.9772	36.39643	AR:Fulton	CBD 06-09
O. neglectus chaenodactylus 384	Big Creek	White	47(11)	-91.9772	36.39643	AR:Fulton	CBD 06-09
O. neglectus chaenodactylus 385	Big Creek	White	38(10)	-91.9772	36.39643	AR:Fulton	CBD 06-09
O. neglectus chaenodactylus 386	Big Creek	White	38(10)	-91.9772	36.39643	AR:Fulton	CBD 06-09
O. neglectus chaenodactylus 387	Big Creek	White	51(3)	-91.9772	36.39643	AR:Fulton	CBD 06-09
O. neglectus chaenodactylus 388	Big Creek	White	51(3)	-91.9772	36.39643	AR:Fulton	CBD 06-09
O. neglectus chaenodactylus 389	Big Creek	White	51(3)	-91.9772	36.39643	AR:Fulton	CBD 06-09
O. neglectus chaenodactylus 390	Un-named Tributary of Bennetts River	White	36(8)	-92.10587	36.42821	AR:Fulton	CBD 06-10
O. neglectus chaenodactylus 392	Un-named Tributary of Bennetts River	White	48(2)	-92.10587	36.42821	AR:Fulton	CBD 06-10
O. neglectus chaenodactylus 393	Un-named Tributary of Bennetts River	White	48(2)	-92.10587	36.42821	AR:Fulton	CBD 06-10
O. neglectus chaenodactylus 394	Un-named Tributary of Bennetts River	White	36(8)	-92.10587	36.42821	AR:Fulton	CBD 06-10

O. neglectus chaenodactylus 395	Un-named Tributary of Bennetts River	White	36(8)	-92.10587	36.42821	AR:Fulton	CBD 06-10
O. neglectus chaenodactylus 396	Un-named Tributary of Bennetts River	White	42(1)	-92.10587	36.42821	AR:Fulton	CBD 06-10
O. neglectus chaenodactylus 397	Un-named Tributary of Bennetts River	White	41(1)	-92.10587	36.42821	AR:Fulton	CBD 06-10
O. neglectus chaenodactylus 398	Un-named Tributary of Bennetts River	White	47(11)	-92.10587	36.42821	AR:Fulton	CBD 06-10
O. neglectus chaenodactylus 399	Un-named Tributary of Bennetts River	White	38(10)	-92.10587	36.42821	AR:Fulton	CBD 06-10
O. neglectus 400	South Fork Spring River	White	38(10)	-91.8632	36.457	AR:Fulton	CBD 06-11
O. neglectus 401	South Fork Spring River	White	38(10)	-91.8632	36.457	AR:Fulton	CBD 06-11
O. neglectus 402	South Fork Spring River	White	38(10)	-91.8632	36.457	AR:Fulton	CBD 06-11
O. neglectus 403	South Fork Spring River	White	38(10)	-91.8632	36.457	AR:Fulton	CBD 06-11
O. neglectus 404	South Fork Spring River	White	47(11)	-91.8632	36.457	AR:Fulton	CBD 06-11
O. neglectus 405	South Fork Spring River	White	47(11)	-91.8632	36.457	AR:Fulton	CBD 06-11
O. neglectus 406	South Fork Spring River	White	38(10)	-91.8632	36.457	AR:Fulton	CBD 06-11
O. neglectus 461	King's River	White	70(4)	-93.59427	36.14374	AR:Madison	CBD 06-16
O. neglectus 462	King's River	White	70(4)	-93.59427	36.14374	AR:Madison	CBD 06-16
O. neglectus 463	King's River	White	74(1)	-93.59427	36.14374	AR:Madison	CBD 06-16
O. neglectus 464	King's River	White	75(1)	-93.59427	36.14374	AR:Madison	CBD 06-16
O. neglectus 465	King's River	White	70(4)	-93.59427	36.14374	AR:Madison	CBD 06-16
O. neglectus 466	King's River	White	70(4)	-93.59427	36.14374	AR:Madison	CBD 06-16
O. neglectus 467	War Eagle Creek	White	68(2)	-93.69495	36.12206	AR:Madison	CBD 06-17
O. neglectus 468	War Eagle Creek	White	68(2)	-93.69495	36.12206	AR:Madison	CBD 06-17
O. neglectus 469	Withrow Springs	White	69(2)	-93.73473	36.15561	AR:Madison	CBD 06-19
O. neglectus 470	Withrow Springs	White	69(2)	-93.73473	36.15561	AR:Madison	CBD 06-19
O. neglectus 471	Withrow Springs	White	1(25)	-93.73473	36.15561	AR:Madison	CBD 06-19

O. neglectus 473	Trib to Town Branch at Hwy 16 Fayetteville, AR	White	1(25)	-94.16243	36.04929	AR:Washington	CBD 06-21
O. neglectus 474	Trib to Town Branch at Hwy 16 Fayetteville, AR	White	1(25)	-94.16243	36.04929	AR:Washington	CBD 06-21
O. neglectus 475	Trib to Town Branch at Hwy 16 Fayetteville, AR	White	1(25)	-94.16243	36.04929	AR:Washington	CBD 06-21
O. neglectus 476	Trib to Town Branch at Hwy 16 Fayetteville, AR	White	1(25)	-94.16243	36.04929	AR:Washington	CBD 06-21
O. neglectus 477	Trib to Town Branch at Hwy 16 Fayetteville, AR	White	1(25)	-94.16243	36.04929	AR:Washington	CBD 06-21
O. neglectus 483	Elk Horn Spring Branch	Illinois	8(1)	-94.31139	36.06019	AR:Washington	CBD 06-22
O. neglectus 484	Elk Horn Spring Branch	Illinois	10(2)	-94.31139	36.06019	AR:Washington	CBD 06-22
O. neglectus 485	Elk Horn Spring Branch	Illinois	10(2)	-94.31139	36.06019	AR:Washington	CBD 06-22
O. neglectus 486	Elk Horn Spring Branch	Illinois	4(2)	-94.31139	36.06019	AR:Washington	CBD 06-22
O. neglectus 487	Elk Horn Spring Branch	Illinois	4(2)	-94.31139	36.06019	AR:Washington	CBD 06-22
O. neglectus 493	Hamestring Creek	Illinois	7(1)	-94.28716	36.0951	AR:Washington	CBD 06-23
O. neglectus 494	Hamestring Creek	Illinois	1(25)	-94.28716	36.0951	AR:Washington	CBD 06-23
O. neglectus 495	Hamestring Creek	Illinois	11(1)	-94.28716	36.0951	AR:Washington	CBD 06-23
O. neglectus 497	Hamestring Creek	Illinois	2(1)	-94.28716	36.0951	AR:Washington	CBD 06-23
O. neglectus 498	Tributary of Illinois River	Illinois	1(25)	-94.49955	36.15478	AR:Benton	CBD 06-24
O. neglectus 499	Tributary of Illinois River	Illinois	1(25)	-94.49955	36.15478	AR:Benton	CBD 06-24
O. neglectus 500	Tributary of Illinois River	Illinois	1(25)	-94.49955	36.15478	AR:Benton	CBD 06-24
O. neglectus 501	Tributary of Illinois River	Illinois	1(25)	-94.49955	36.15478	AR:Benton	CBD 06-24
O. neglectus 502	Tributary of Illinois River	Illinois	1(25)	-94.49955	36.15478	AR:Benton	CBD 06-24
O. neglectus 514	Sager Creek	Illinois	14(1)	-94.52324	36.19098	AR:Benton	CBD 06-25
O. neglectus 515	Sager Creek	Illinois	1(25)	-94.52324	36.19098	AR:Benton	CBD 06-25
O. neglectus 516	Sager Creek	Illinois	9(1)	-94.52324	36.19098	AR:Benton	CBD 06-25
O. neglectus 517	Sager Creek	Illinois	12(1)	-94.52324	36.19098	AR:Benton	CBD 06-25

O. neglectus 518	Sager Creek	Illinois	1(25)	-94.52324	36.19098	AR:Benton	CBD 06-25
O. neglectus 524	Spring Fed Creek at Cornhoff Road	Illinois	1(25)	-94.30472	36.15227	AR:Washington	CBD 06-26
O. neglectus 525	Spring Fed Creek at Cornhoff Road	Illinois	1(25)	-94.30472	36.15227	AR:Washington	CBD 06-26
O. neglectus 526	Spring Fed Creek at Cornhoff Road	Illinois	1(25)	-94.30472	36.15227	AR:Washington	CBD 06-26
O. neglectus 527	Spring Fed Creek at Cornhoff Road	Illinois	1(25)	-94.30472	36.15227	AR:Washington	CBD 06-26
O. neglectus 528	Spring Fed Creek at Cornhoff Road	Illinois	88(1)	-94.30472	36.15227	AR:Washington	CBD 06-26
O. neglectus 536	Little Osage Creek	Illinois	1(25)	-94.2714	36.2537	AR:Benton	CBD 06-27
O. neglectus 537	Little Osage Creek	Illinois	6(1)	-94.2714	36.2537	AR:Benton	CBD 06-27
O. neglectus 538	Little Osage Creek	Illinois	1(25)	-94.2714	36.2537	AR:Benton	CBD 06-27
O. neglectus 539	Little Osage Creek	Illinois	1(25)	-94.2714	36.2537	AR:Benton	CBD 06-27
O. neglectus 540	Little Osage Creek	Illinois	1(25)	-94.2714	36.2537	AR:Benton	CBD 06-27
O. neglectus 547	Prairie Creek	White	33(2)	-94.1032	36.3343	AR:Benton	CBD 06-28
O. neglectus 548	Prairie Creek	White	27(1)	-94.1032	36.3343	AR:Benton	CBD 06-28
O. neglectus 559	Spanker Creek	Elk/Spring/Neosho	34(12)	-94.21	36.43025	AR:Benton	CBD 06-29
O. neglectus 560	Spanker Creek	Elk/Spring/Neosho	34(12)	-94.21	36.43025	AR:Benton	CBD 06-29
O. neglectus 561	Spanker Creek	Elk/Spring/Neosho	34(12)	-94.21	36.43025	AR:Benton	CBD 06-29
O. neglectus 562	Spanker Creek	Elk/Spring/Neosho	26(2)	-94.21	36.43025	AR:Benton	CBD 06-29
O. neglectus 563	Spanker Creek	Elk/Spring/Neosho	32(1)	-94.21	36.43025	AR:Benton	CBD 06-29
O. neglectus 571	Little Sugar Creek	Elk/Spring/Neosho	34(12)	-94.25027	36.47566	AR:Benton	CBD 06-30
O. neglectus 572	Little Sugar Creek	Elk/Spring/Neosho	34(12)	-94.25027	36.47566	AR:Benton	CBD 06-30
O. neglectus 573	Little Sugar Creek	Elk/Spring/Neosho	34(12)	-94.25027	36.47566	AR:Benton	CBD 06-30
O. neglectus 574	Little Sugar Creek	Elk/Spring/Neosho	34(12)	-94.25027	36.47566	AR:Benton	CBD 06-30
O. neglectus 575	Little Sugar Creek	Elk/Spring/Neosho	34(12)	-94.25027	36.47566	AR:Benton	CBD 06-30

O. neglectus 581	Gordon Hollow	Elk/Spring/Neosho	31(1)	-94.29852	36.4884	AR:Benton	CBD 06-31
O. neglectus 582	Gordon Hollow	Elk/Spring/Neosho	30(1)	-94.29852	36.4884	AR:Benton	CBD 06-31
O. neglectus 583	Gordon Hollow	Elk/Spring/Neosho	34(12)	-94.29852	36.4884	AR:Benton	CBD 06-31
O. neglectus 584	Gordon Hollow	Elk/Spring/Neosho	34(12)	-94.29852	36.4884	AR:Benton	CBD 06-31
O. neglectus 592	Spavinaw Creek	Neosho	20(1)	-94.41488	36.39635	AR:Benton	CBD 06-32
O. neglectus 593	Spavinaw Creek	Neosho	21(4)	-94.41488	36.39635	AR:Benton	CBD 06-32
O. neglectus 594	Spavinaw Creek	Neosho	17(3)	-94.41488	36.39635	AR:Benton	CBD 06-32
O. neglectus 595	Spavinaw Creek	Neosho	21(4)	-94.41488	36.39635	AR:Benton	CBD 06-32
O. neglectus 596	Spavinaw Creek	Neosho	22(1)	-94.41488	36.39635	AR:Benton	CBD 06-32
O. neglectus 602	Butler Creek	Elk/Spring/Neosho	34(12)	-94.45797	36.48433	AR:Benton	CBD 06-33
O. neglectus 603	Butler Creek	Elk/Spring/Neosho	35(1)	-94.45797	36.48433	AR:Benton	CBD 06-33
O. neglectus 604	Butler Creek	Elk/Spring/Neosho	33(2)	-94.45797	36.48433	AR:Benton	CBD 06-33
O. neglectus 605	Butler Creek	Elk/Spring/Neosho	34(12)	-94.45797	36.48433	AR:Benton	CBD 06-33
O. neglectus 606	Butler Creek	Elk/Spring/Neosho	87(1)	-94.45797	36.48433	AR:Benton	CBD 06-33
O. neglectus 612	Honey Creek	Neosho	26(2)	-94.56296	36.48066	AR:Benton	CBD 06-34
O. neglectus 613	Honey Creek	Neosho	25(2)	-94.56296	36.48066	AR:Benton	CBD 06-34
O. neglectus 614	Honey Creek	Neosho	25(2)	-94.56296	36.48066	AR:Benton	CBD 06-34
O. neglectus 615	Honey Creek	Neosho	28(1)	-94.56296	36.48066	AR:Benton	CBD 06-34
O. neglectus 616	Honey Creek	Neosho	29(1)	-94.56296	36.48066	AR:Benton	CBD 06-34
O. neglectus 622	Beaty Creek	Neosho	15(2)	-94.60036	36.41686	AR:Benton	CBD 06-35
O. neglectus 623	Beaty Creek	Neosho	16(2)	-94.60036	36.41686	AR:Benton	CBD 06-35
O. neglectus 624	Beaty Creek	Neosho	15(2)	-94.60036	36.41686	AR:Benton	CBD 06-35
O. neglectus 625	Beaty Creek	Neosho	19(1)	-94.60036	36.41686	AR:Benton	CBD 06-35
O. neglectus 626	Beaty Creek	Neosho	18(1)	-94.60036	36.41686	AR:Benton	CBD 06-35
O. neglectus 632	Spavinaw Creek	Neosho	21(4)	-94.58675	36.34262	AR:Benton	CBD 06-36

O. neglectus 633	Spavinaw Creek	Neosho	17(3)	-94.58675	36.34262	AR:Benton	CBD 06-36
O. neglectus 634	Spavinaw Creek	Neosho	16(2)	-94.58675	36.34262	AR:Benton	CBD 06-36
O. neglectus 635	Spavinaw Creek	Neosho	21(4)	-94.58675	36.34262	AR:Benton	CBD 06-36
O. neglectus 636	Spavinaw Creek	Neosho	17(3)	-94.58675	36.34262	AR:Benton	CBD 06-36
O. neglectus 644	Flint Creek	Illinois / Neosho	5(2)	-94.48721	36.24226	AR:Benton	CBD 06-37
O. neglectus 645	Flint Creek	Illinois / Neosho	13(4)	-94.48721	36.24226	AR:Benton	CBD 06-37
O. neglectus 646	Flint Creek	Illinois / Neosho	1(25)	-94.48721	36.24226	AR:Benton	CBD 06-37
O. neglectus 647	Flint Creek	Illinois / Neosho	13(4)	-94.48721	36.24226	AR:Benton	CBD 06-37
O. neglectus 648	Flint Creek	Illinois / Neosho	13(4)	-94.48721	36.24226	AR:Benton	CBD 06-37
O. neglectus 654	Tributary of East Flint Creek	Illinois / Neosho	5(2)	-94.42128	36.26191	AR:Benton	CBD 06-38
O. neglectus 655	Tributary of East Flint Creek	Illinois / Neosho	13(4)	-94.42128	36.26191	AR:Benton	CBD 06-38
O. neglectus 656	Tributary of East Flint Creek	Illinois / Neosho	1(25)	-94.42128	36.26191	AR:Benton	CBD 06-38
O. neglectus 657	Tributary of East Flint Creek	Illinois / Neosho	3(1)	-94.42128	36.26191	AR:Benton	CBD 06-38
O. neglectus 658	Tributary of East Flint Creek	Illinois / Neosho	1(25)	-94.42128	36.26191	AR:Benton	CBD 06-38
O. neglectus 659	Drainage Ditch in Eureka Springs, AR	White	65(6)	-93.73313	36.41038	AR:Carroll	CBD 06-39
O. neglectus 660	Drainage Ditch in Eureka Springs, AR	White	65(6)	-93.73313	36.41038	AR:Carroll	CBD 06-39
O. neglectus 661	Drainage Ditch in Eureka Springs, AR	White	73(1)	-93.73313	36.41038	AR:Carroll	CBD 06-39
O. neglectus 662	Drainage Ditch in Eureka Springs, AR	White	65(6)	-93.73313	36.41038	AR:Carroll	CBD 06-39
O. neglectus 663	Osage Creek at King's River	White	65(6)	-93.63763	36.39406	AR:Carroll	CBD 06-40
O. neglectus 664	Osage Creek at King's River	White	67(1)	-93.63763	36.39406	AR:Carroll	CBD 06-40
O. neglectus 665	Osage Creek at King's River	White	65(6)	-93.63763	36.39406	AR:Carroll	CBD 06-40
O. neglectus 666	Osage Creek at King's River	White	65(6)	-93.63763	36.39406	AR:Carroll	CBD 06-40
O. neglectus 667	Long Creek	White	62(2)	-93.28105	36.34715	AR:Boone	CBD 06-41

O. neglectus 668	Long Creek	White	61(1)	-93.28105	36.34715	AR:Boone	CBD 06-41
O. neglectus 669	Long Creek	White	62(2)	-93.28105	36.34715	AR:Boone	CBD 06-41
O. neglectus 670	Long Creek	White	59(1)	-93.28105	36.34715	AR:Boone	CBD 06-41
O. neglectus 675	Mill Creek	Buffalo / White	81(4)	-92.81523	36.02946	AR:Searcy	CBD 06-42
O. neglectus 676	Mill Creek	Buffalo / White	81(4)	-92.81523	36.02946	AR:Searcy	CBD 06-42
O. neglectus 677	Mill Creek	Buffalo / White	81(4)	-92.81523	36.02946	AR:Searcy	CBD 06-42
O. neglectus 678	Mill Creek	Buffalo / White	81(4)	-92.81523	36.02946	AR:Searcy	CBD 06-42
O. neglectus 683	Crooked Creek	Crooked Creek / White	58(1)	-92.67945	36.22259	AR:Marion	CBD 06-43
O. neglectus 684	Crooked Creek	Crooked Creek / White	55(1)	-92.67945	36.22259	AR:Marion	CBD 06-43
O. neglectus 685	Crooked Creek	Crooked Creek / White	53(8)	-92.67945	36.22259	AR:Marion	CBD 06-43
O. neglectus 686	Crooked Creek	Crooked Creek / White	60(1)	-92.67945	36.22259	AR:Marion	CBD 06-43
O. neglectus 691	Jimmie Creek	White	82(3)	-92.67578	36.33702	AR:Marion	CBD 06-44
O. neglectus 692	Jimmie Creek	White	82(3)	-92.67578	36.33702	AR:Marion	CBD 06-44
O. neglectus 693	Jimmie Creek	White	82(3)	-92.67578	36.33702	AR:Marion	CBD 06-44
O. neglectus 694	Jimmie Creek	White	83(1)	-92.67578	36.33702	AR:Marion	CBD 06-44
O. neglectus 699	Big Spring	White	53(8)	-92.54337	36.26638	AR:Baxter	CBD 06-45
O. neglectus 700	Big Spring	White	53(8)	-92.54337	36.26638	AR:Baxter	CBD 06-45
O. neglectus 701	Big Spring	White	63(1)	-92.54337	36.26638	AR:Baxter	CBD 06-45
O. neglectus 702	Big Spring	White	57(1)	-92.54337	36.26638	AR:Baxter	CBD 06-45
O. neglectus chaenodactylus 707	Tributary of East Pigeon Creek	White	47(11)	-92.36272	36.46901	AR:Baxter	CBD 06-46
O. neglectus chaenodactylus 708	Tributary of East Pigeon Creek	White	47(11)	-92.36272	36.46901	AR:Baxter	CBD 06-46
O. neglectus chaenodactylus 709	Tributary of East Pigeon Creek	White	47(11)	-92.36272	36.46901	AR:Baxter	CBD 06-46

O. neglectus chaenodactylus 710	Tributary of East Pigeon Creek	White	47(11)	-92.36272	36.46901	AR:Baxter	CBD 06-46
O. neglectus chaenodactylus 715	Pigeon Creek	White	49(1)	-92.37711	36.42172	AR:Baxter	CBD 06-47
O. neglectus chaenodactylus 716	Pigeon Creek	White	36(8)	-92.37711	36.42172	AR:Baxter	CBD 06-47
O. neglectus chaenodactylus 717	Pigeon Creek	White	36(8)	-92.37711	36.42172	AR:Baxter	CBD 06-47
O. neglectus chaenodactylus 718	Pigeon Creek	White	36(8)	-92.37711	36.42172	AR:Baxter	CBD 06-47
O. neglectus 724	Tributary of Big Creek	White	47(11)	-92.38549	36.31957	AR:Baxter	CBD 06-48
O. neglectus 725	Tributary of Big Creek	White	53(8)	-92.38549	36.31957	AR:Baxter	CBD 06-48
O. neglectus 726	Tributary of Big Creek	White	47(11)	-92.38549	36.31957	AR:Baxter	CBD 06-48
O. neglectus chaenodactylus 728	Tributary of Big Creek	White	37(1)	-92.38549	36.31957	AR:Baxter	CBD 06-48

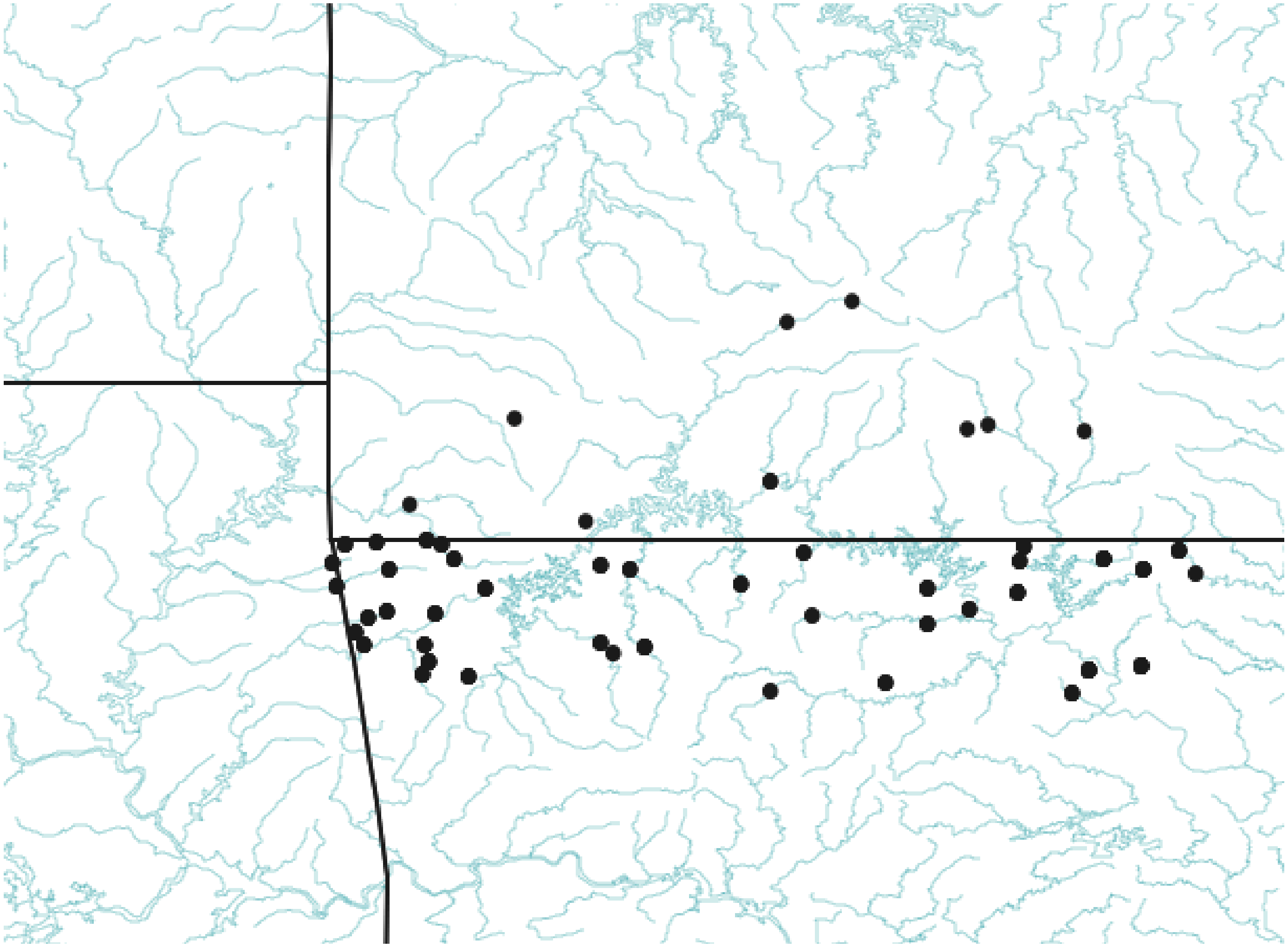
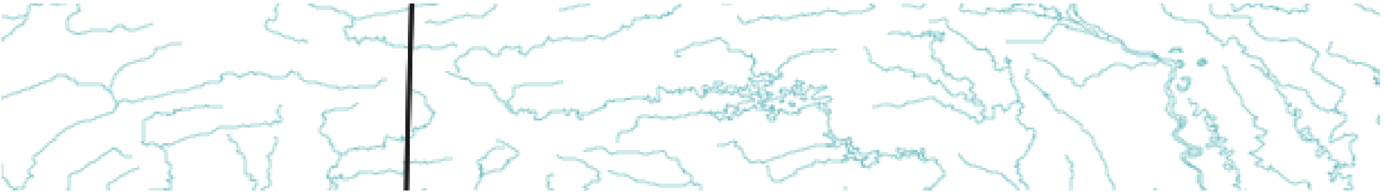


Figure 4. Map showing localities sampled for both subspecies of *Orconectes neglectus* used in the current study.



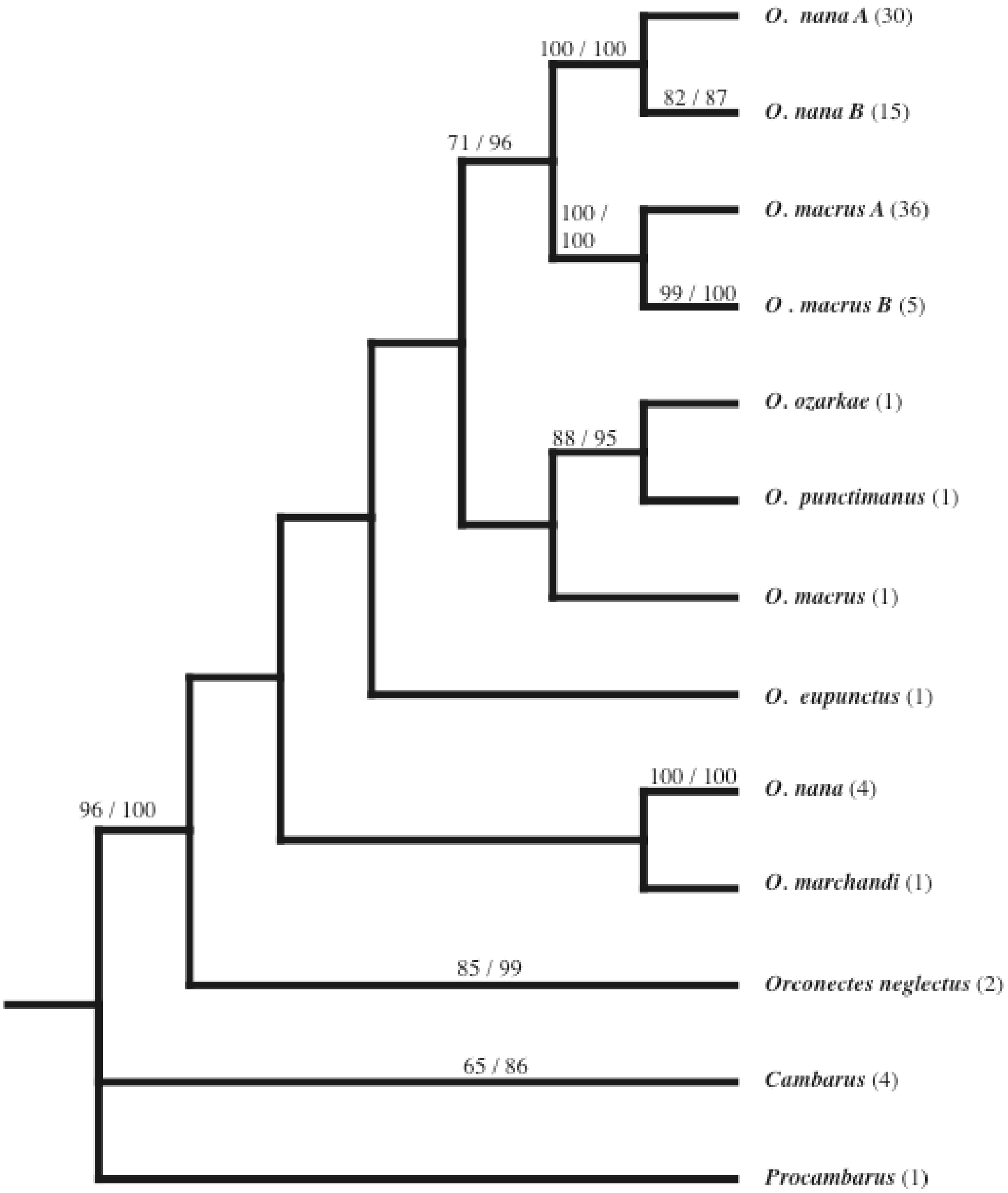


Figure 5. Backbone maximum parsimony phylogenetic hypothesis depicting interrelationships of *Orconectes nana* and *O. macrus*. Outgroup taxa were *Cambarus* and *Procambarus* and other ingroup taxa (*Orconectes*) were also used. Numbers above branches indicate bootstrap support (Maximum Parsimony) and Posterior Probability Scores (Bayesian) respectively. Numbers in parentheses after each taxon name indicate total number of individuals, or species, included.

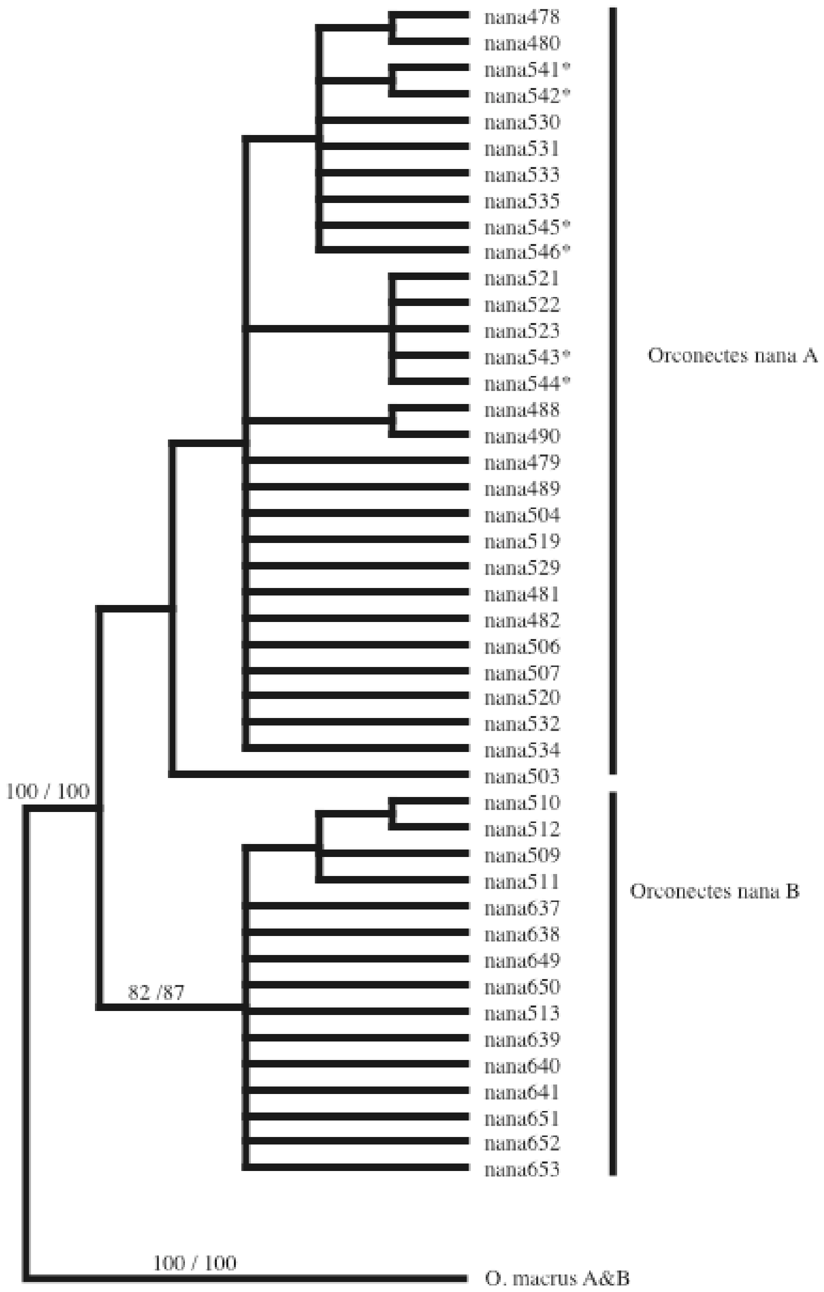


Figure 6. Reconstructed Maximum Parsimony hypothesis for *Orconectes nana*. Clades A and B are expanded from Figure 3. Bootstrapping and Posterior Probability scores support *O. nana* as monophyletic in 100% of the reconstructions. Asterisks after six individuals recovered in clade A were formerly recognized as *O. macrus*. The samples represent the only known allopatric population of this species pair and are confined the headwaters of the White River basin.

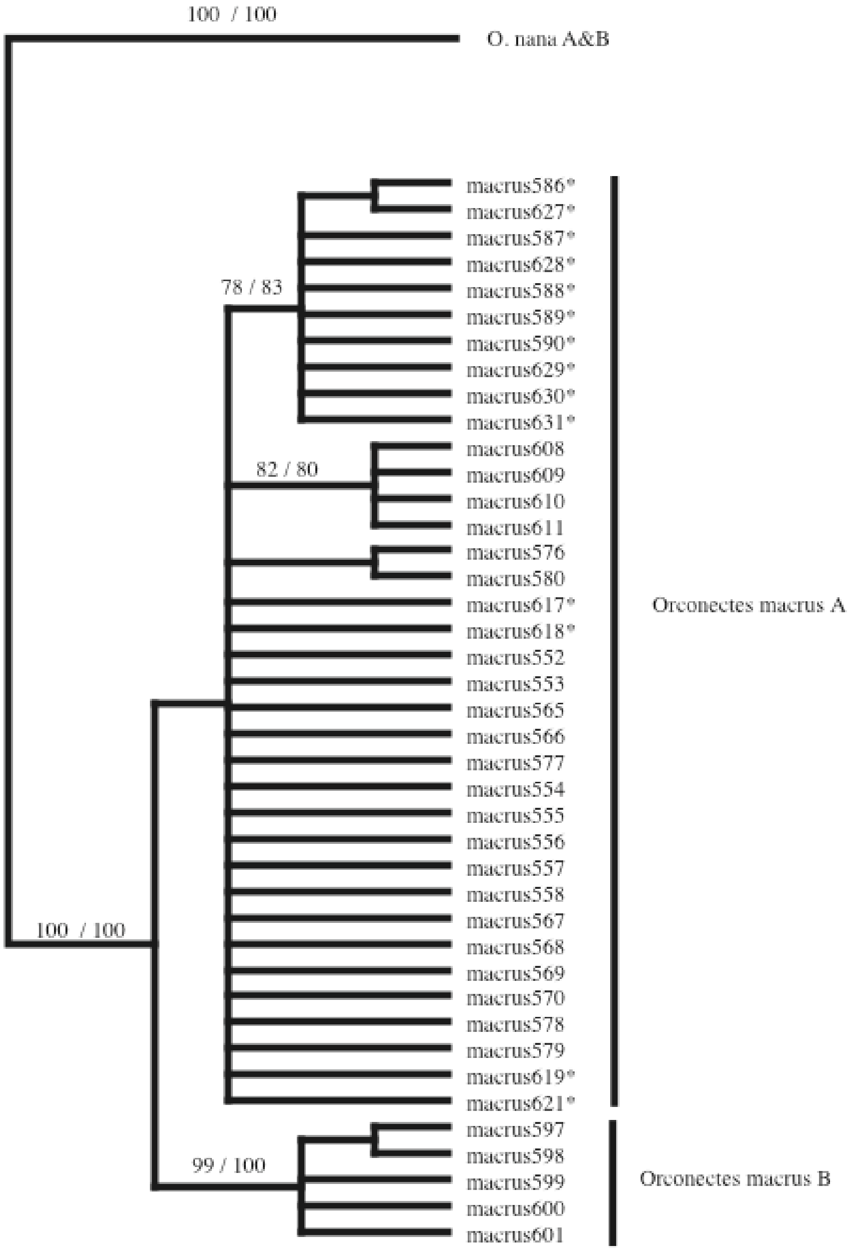


Figure 7. Reconstructed Maximum Parsimony hypothesis for *Orconectes macrus*. Clades A and B are expanded from Figure 3. Bootstrapping and Posterior Probability scores support *O. macrus* as monophyletic in 100% of the reconstructions. Asterisks after fourteen individuals recovered in clade A are recognized as *O. nana* based on hypothesized range of this taxon. Ten individuals (586-590, 627-631) form a distinct clade within clade A. These individuals are from two collections in Spavinaw Creek. The other four individuals in clade A with asterisks (617-619, 621) are from Beaty Creek, a tributary to Spavinaw Creek. Clade B contains individuals collected from Butler Creek a tributary to Elk River. Likewise individuals 608-611 were also collected from Honey Creek a tributary to Elk River, but further downstream.

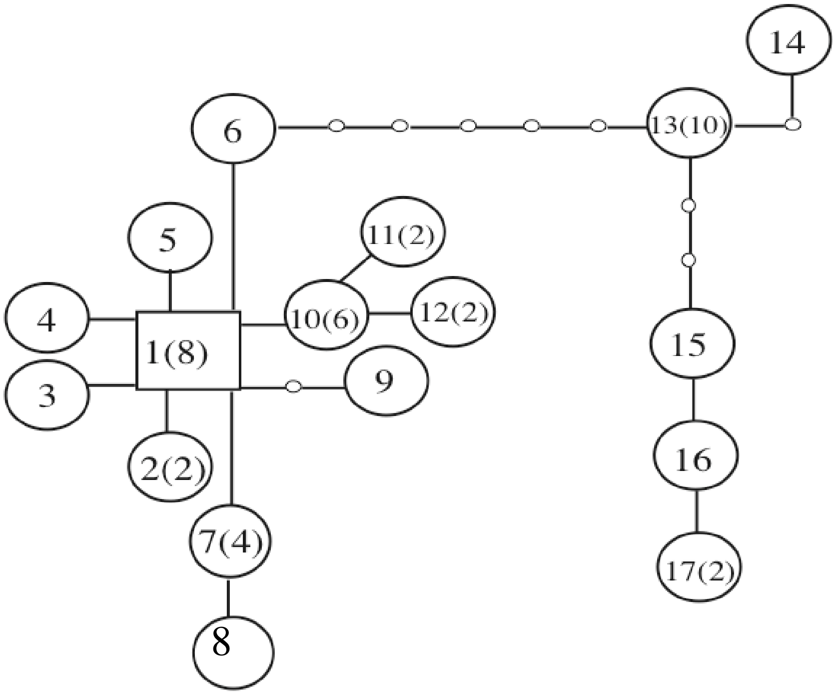


Figure 8. Population network representation of *O. nana*: clades A and B in Figure 6. Squares represent hypothesized ancestral haplotypes in each network, and circles are more derived haplotypes. Numbers inside squares and circles are haplotype number (and frequency of observation). See Table 1 for haplotypes correspondence to geographic locality and individual. Clade designations are as in Figures 5 and 6.

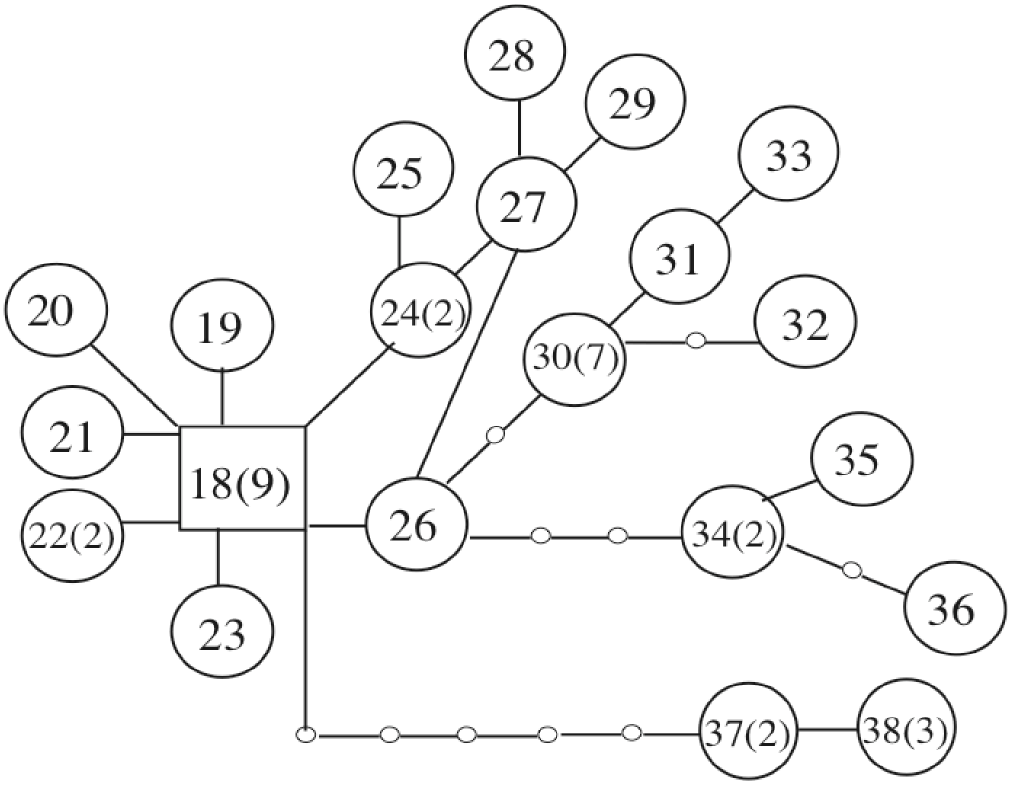


Figure 9. Population network representation of *O. macrus*: clades A and B in Figure 7. Squares represent hypothesized ancestral haplotypes in each network, and circles are more derived haplotypes. Numbers inside squares and circles are haplotype number (and frequency of observation). See Table 1 for haplotypes correspondence to geographic locality and individual. Clade designations are as in Figures 5 and 7.

Table 3. Average pairwise interclade divergence (above the diagonal) and intraclade divergence (along the diagonal) for recovered clades of *Orconectes nana* and *O. macrus*. Below the diagonal are inter- and intraspecific Φ_{ST} values. An asterisk indicates a p-value of less than 0.0001.

	O. nana A	*O. nana* B	*O. macrus* A	*O. macrus* B
O. nana A	0.002	0.015	0.093	0.092
O. nana B	0.818*	0.003	0.094	0.097
O. macrus A	0.956*	0.951*	0.014	0.014
O. macrus B	0.973*	0.976*	0.686*	0.000

Table 4. Sample size and current and historical estimates of effective population size for Orconectes nana and *O. macrus* based on recovered clades. In all but one case (*O. macrus* B) current estimates of effective population sizes are smaller than historical estimates. In *O. macrus* B the sample size of the recovered clade is small (N=5).

	N	Historical		Current	
		Theta	Ne	Theta	Ne
O. nana A	30	0.007028	159727.27	0.00250	56818.18
O. nana B	15	0.002986	67863.64	0.00256	58181.82
O. macrus A	36	0.027813	632113.64	0.00493	112045.45
O. macrus B	5	0.000739	16795.45	0.00094	21363.64

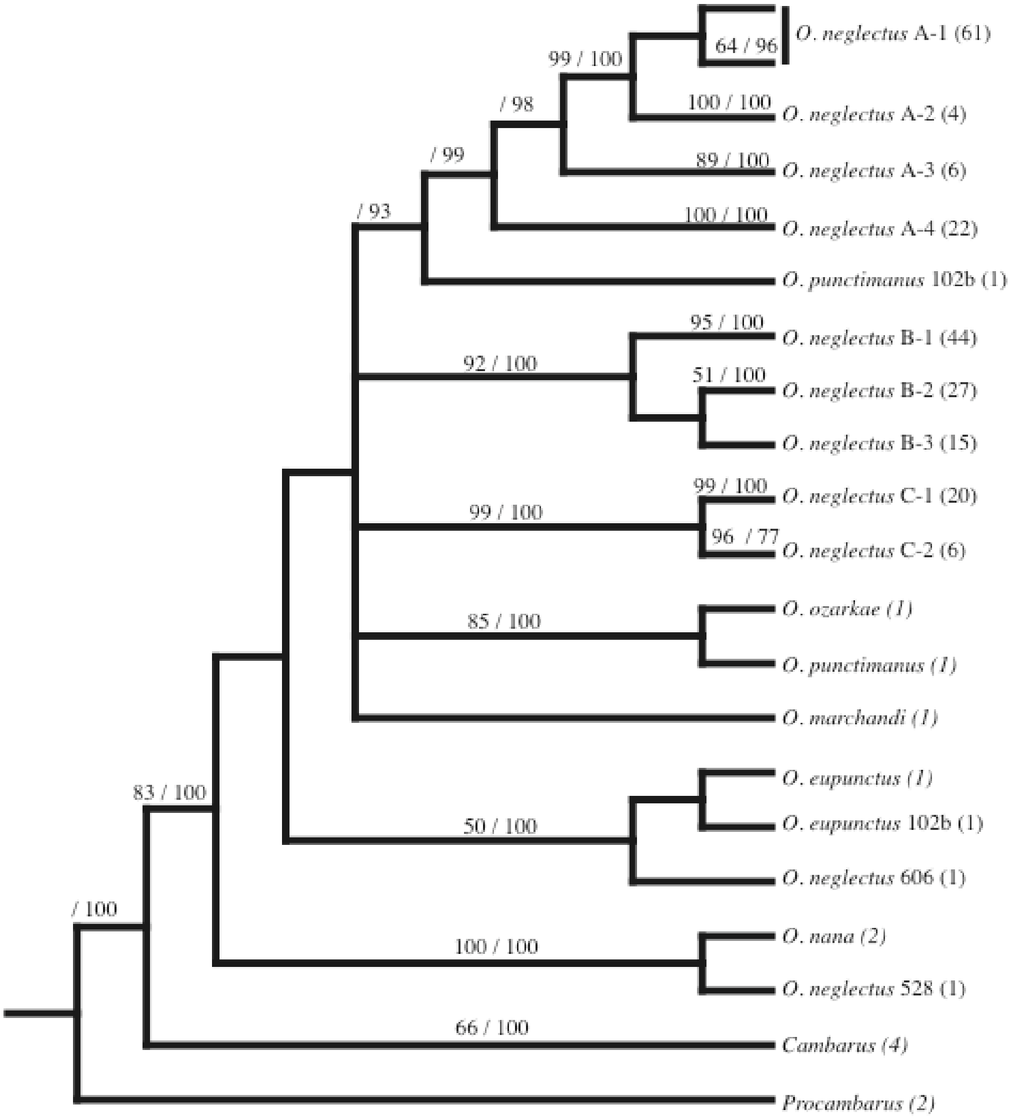


Figure10. Backbone phylogenetic hypothesis based on parsimony reconstruction showing recovered inter-relationships of *O. neglectus*. *Orconectes neglectus* is recovered in three clades (A, B, and C). In addition, each of these three clades are subdivided into numerical sub-clades for ease of discussion. Boostrap support and posterior probability scores, respectively, are given above the nodes. Number of individuals recovered in each clade are given in parentheses, i.e. 61 individuals are recovered in clade A-1.

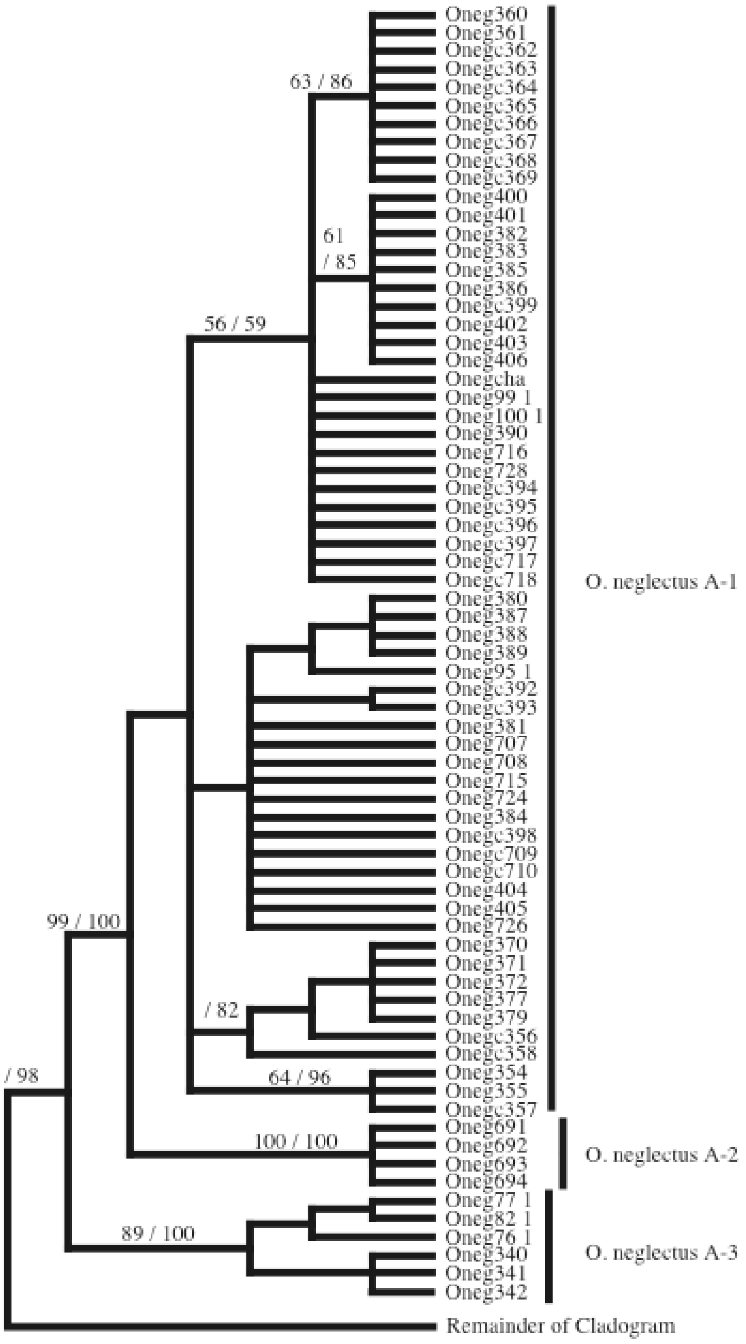


Figure 11. Reconstructed maximum parsimony phylogenetic hypothesis showing inter-relationships of individuals recovered in clades A-1, A-2, and A-3. Bootstrap and posterior probability scores, respectively, are given above branches. Several distinct clades are recovered within clade A-1. The remainder of the cladogram as in Figure 10.

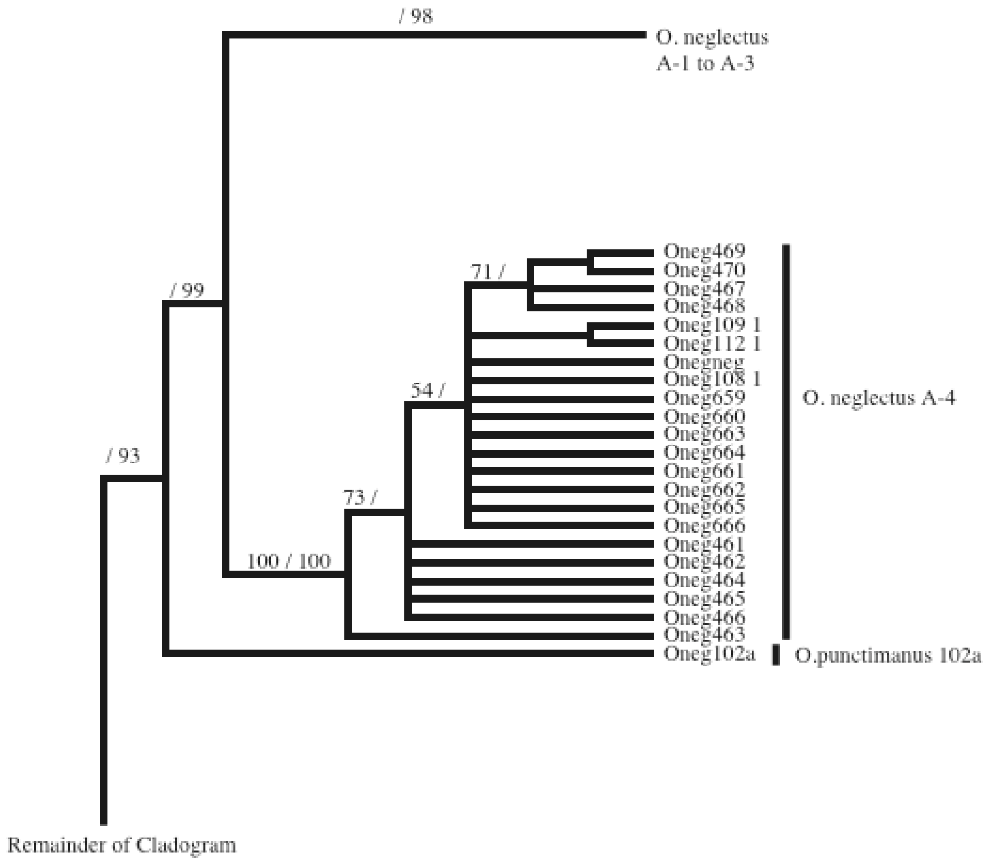


Figure 12. Reconstructed maximum parsimony phylogenetic hypothesis showing inter-relationships of individuals recovered in clades A-4 and that *O. punctimanus* from Spring River appears to be basal to all White River *O. neglectus* sampled. Bootstrap and posterior probability scores, respectively, are given above branches. The remainder of the cladogram as in Figure 10.

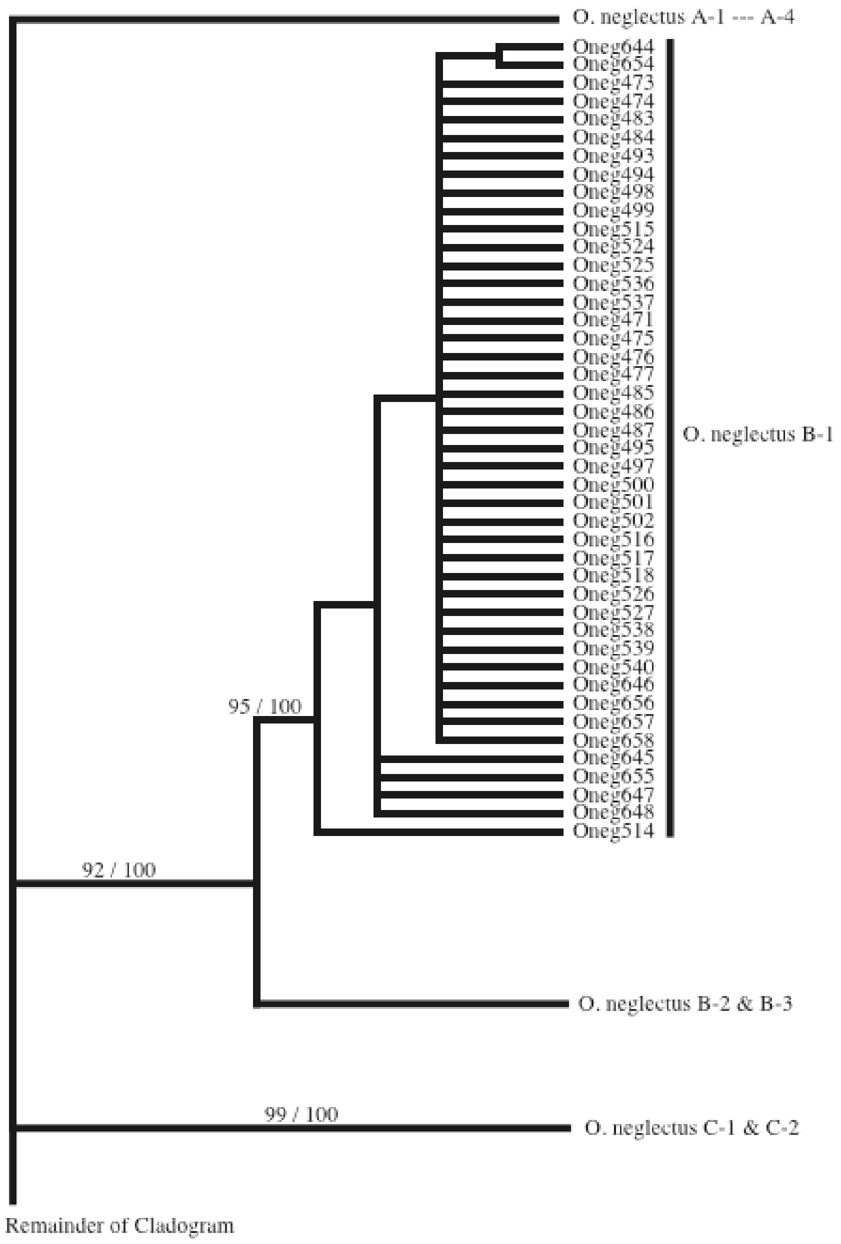


Figure 13. Reconstructed maximum parsimony phylogenetic hypothesis showing inter-relationships of individuals recovered in clades B-1. Bootstrap and posterior probability scores, respectively, are given above branches. The remainder of the cladogram as in Figure 10.

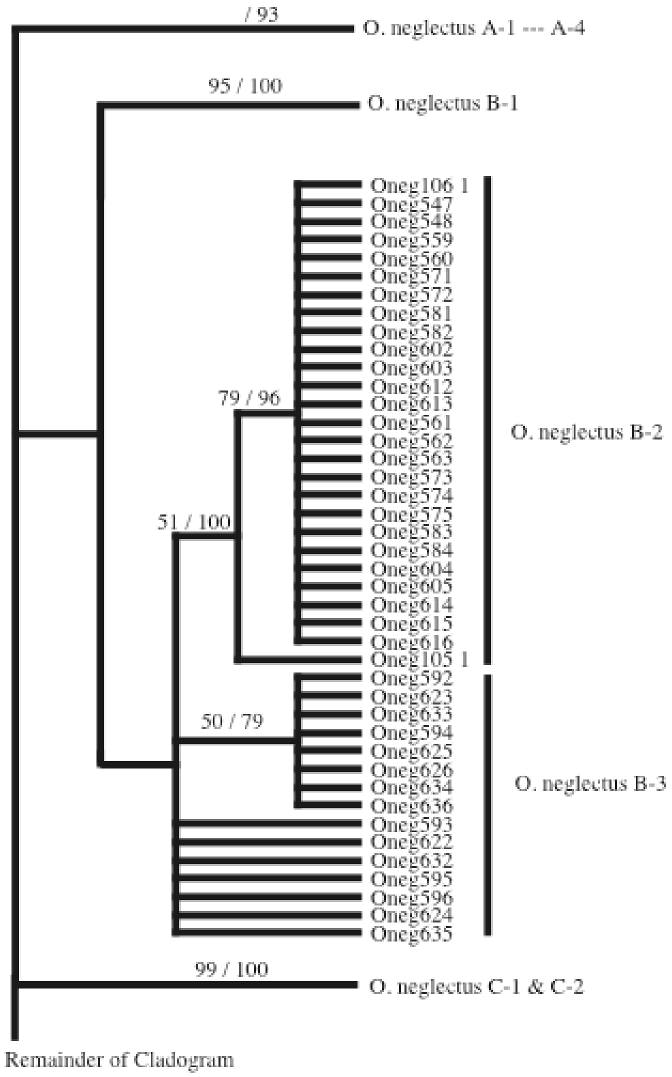


Figure 14. Reconstructed maximum parsimony phylogenetic hypothesis showing interrelationships of individuals recovered in clades B-2 and B-3. Bootstrap and posterior probability scores, respectively, are given above branches. The remainder of the cladogram as in Figure 10.

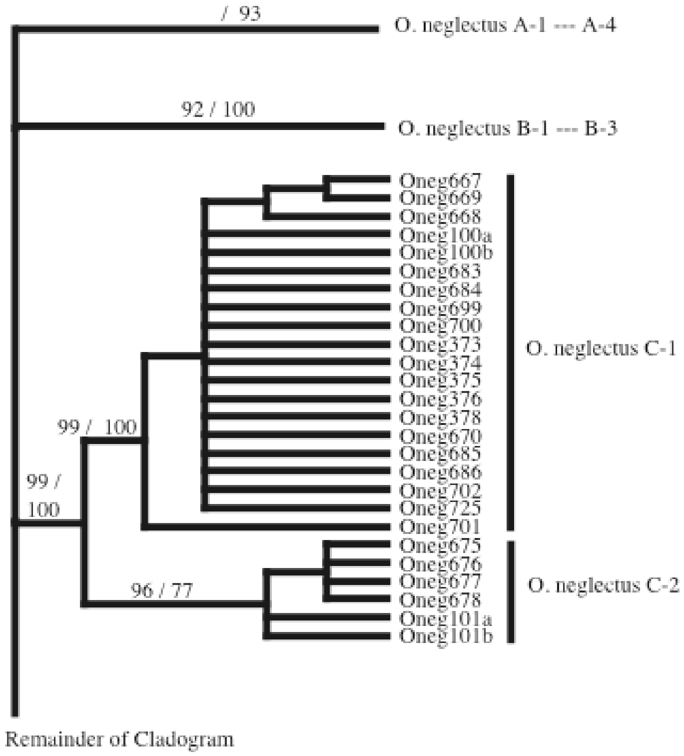


Figure 15. Reconstructed maximum parsimony phylogenetic hypothesis showing inter-relationships of individuals recovered in clades C-1 and C-2. Bootstrap and posterior probability scores, respectively, are given above branches. The remainder of the cladogram as in Figure 10.

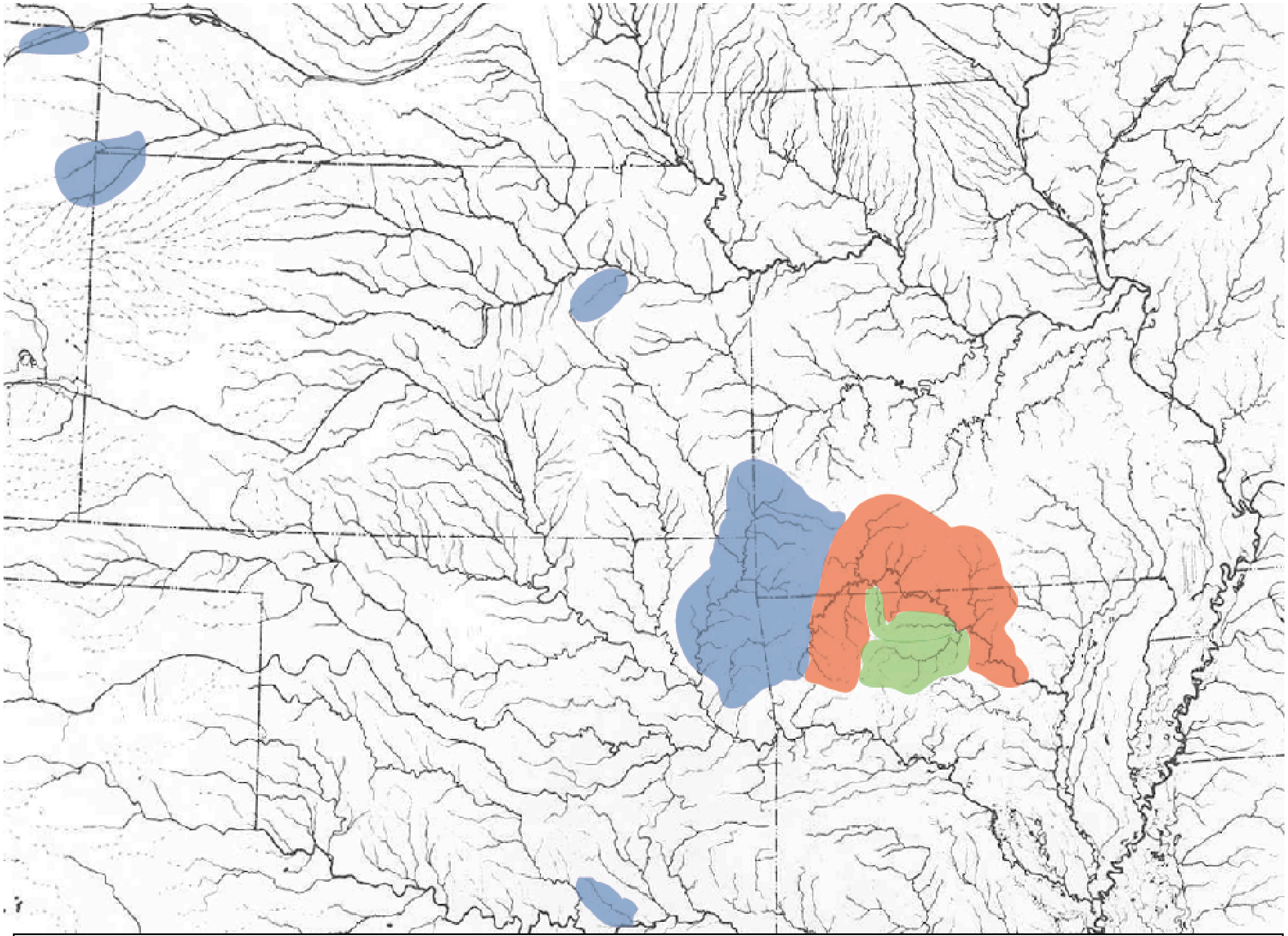


Figure 16. Map showing phylogenetic interpretation of mtDNA lineage distributions as revealed in this study. The blue distribution (except for disjunct portions) represents the distribution of *O. neglectus neglectus*. The distribution in red shows the clade inclusive of *O. neglectus chaenodactylus* (formerly confined to the North Fork White River) and how the clade is distributed throughout the White River. The green represents Clade C, an area of unrecognized lineage diversity in *O. neglectus*.

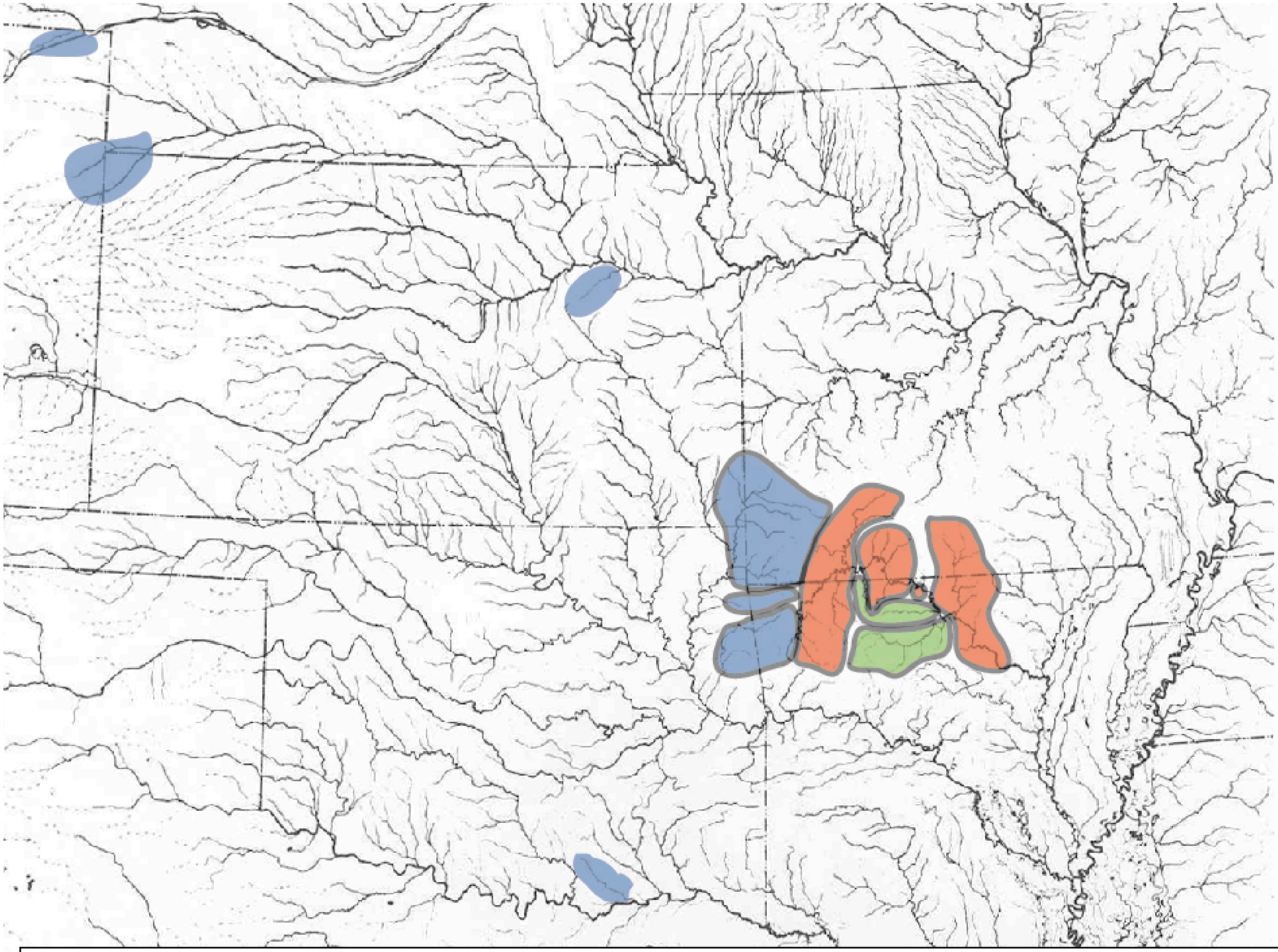


Figure 17. Map showing geographically restricted lineages (sub-clades) of A-1--A-4 (red), B-1—B-3 (blue), and C-1—C-2 (green). All geographically restricted sub-clades are bounded by dark grey lines.

Table 5. Absolute number of pairwise nucleotide differences between all hypothesized root node sequences (denoted as squares in Figures 10-15). A minimum of 40 nucleotides (6.2% sequence divergence) separate any two root sequences found among clades A, B, or C. Pairwise comparisons are not possible with B-2 and B-3 as they are all interconnected into one haplotype network with the root sequence found in clade B-1.

	A-1	A-2	A-3	A-4	B-1	B-2	B-3	C-1	C-2
A-1	----								
A-2	57	----							
A-3	55	42	----						
A-4	40	43	45	----					
B-1	46	49	54	52	----				
B-2	n/a	n/a	n/a	n/a	n/a	----			
B-3	n/a	n/a	n/a	n/a	n/a	n/a	----		
C-1	59	65	45	59	46	n/a	n/a	----	
C-2	41	56	62	56	40	n/a	n/a	16	----

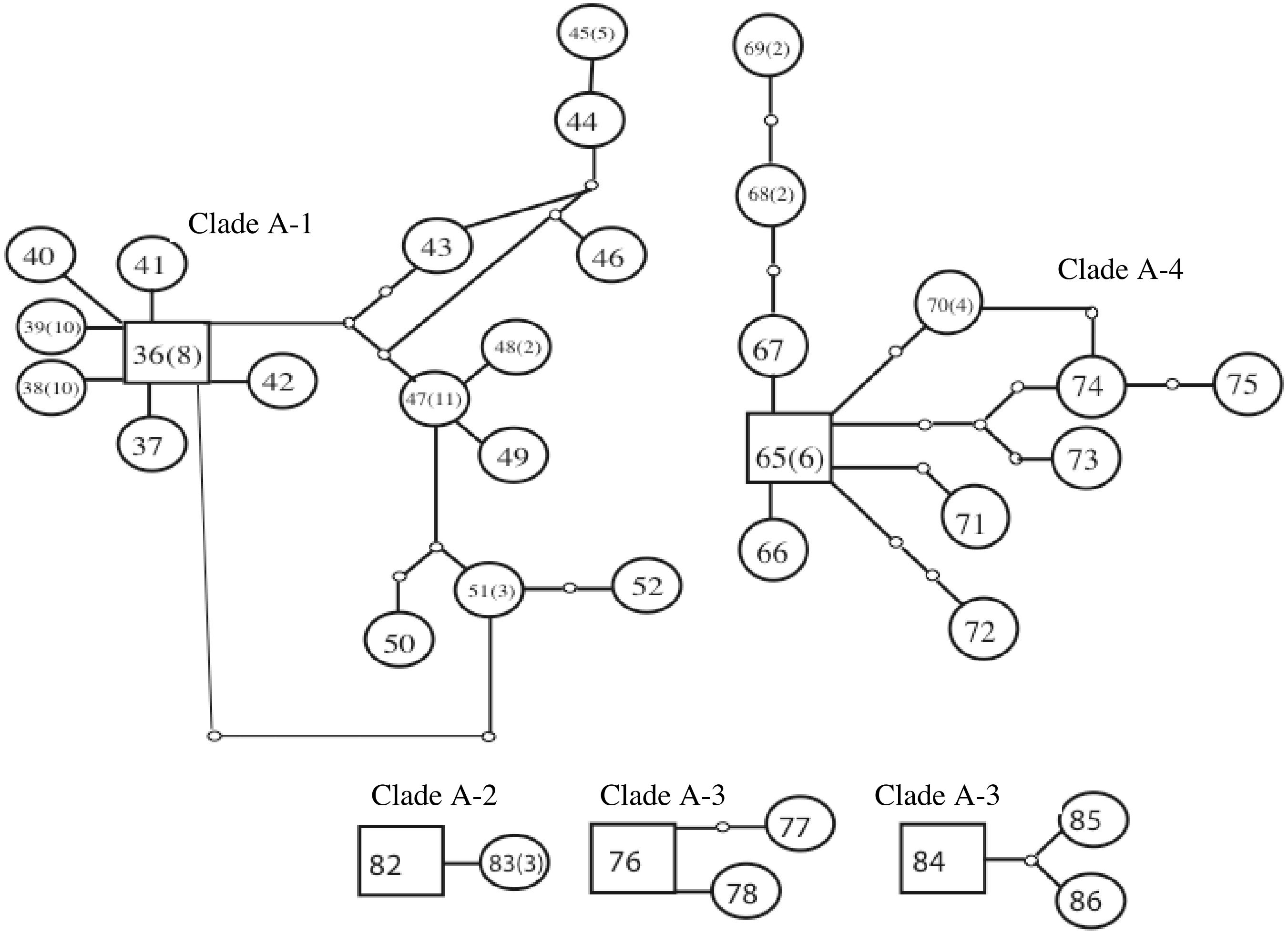


Figure 18. Population network representation of *O. neglectus chaenodactylus* as well as representatives of the nominal subspecies from throughout the remainder of the White River basin: clades A-1 to A-4 in Figure 10. Squares represent hypothesized ancestral haplotypes in each network, and circles are more derived haplotypes. Numbers inside squares and circles are haplotype number (and frequency of observation). See Table 2 for haplotypes correspondence to geographic locality and individual. Clade designations are as in Figures 11-12.

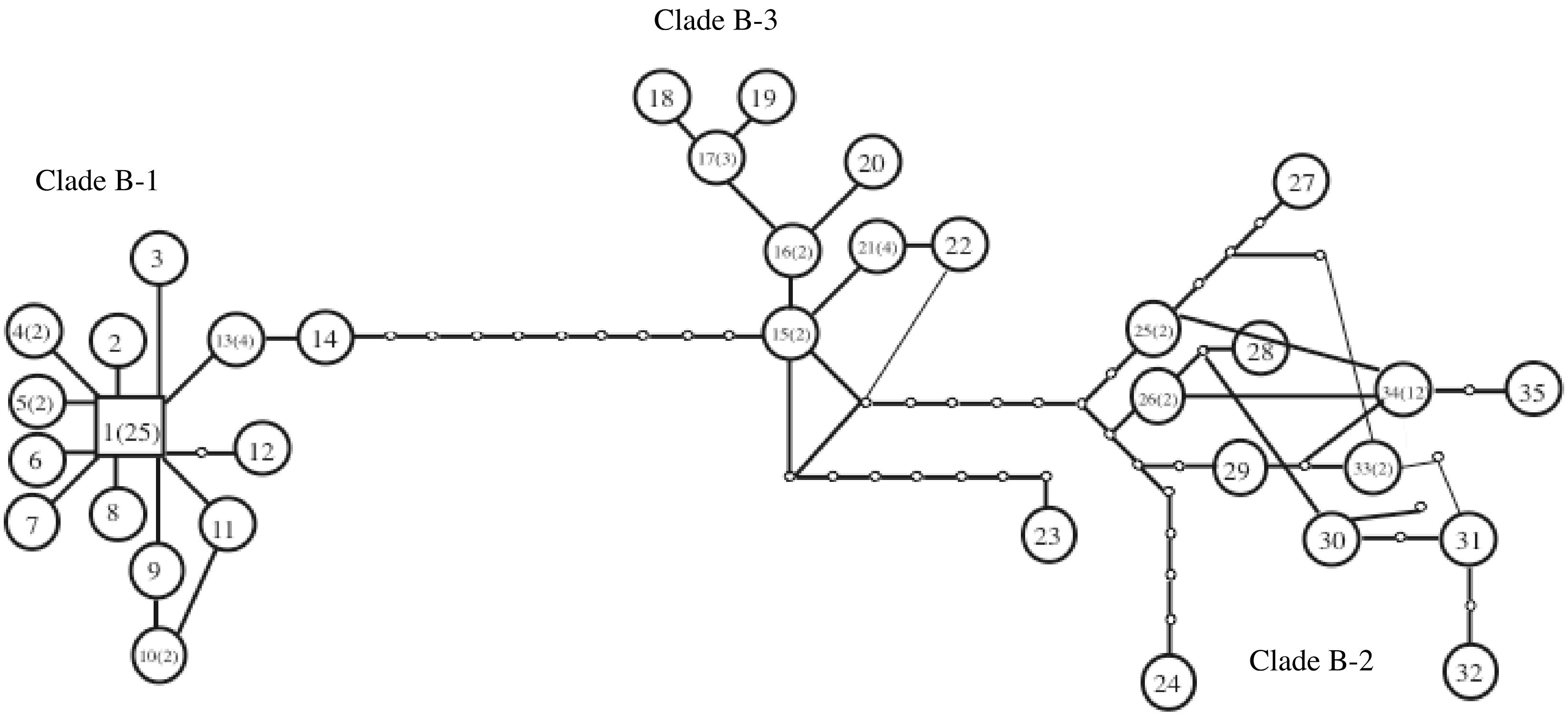
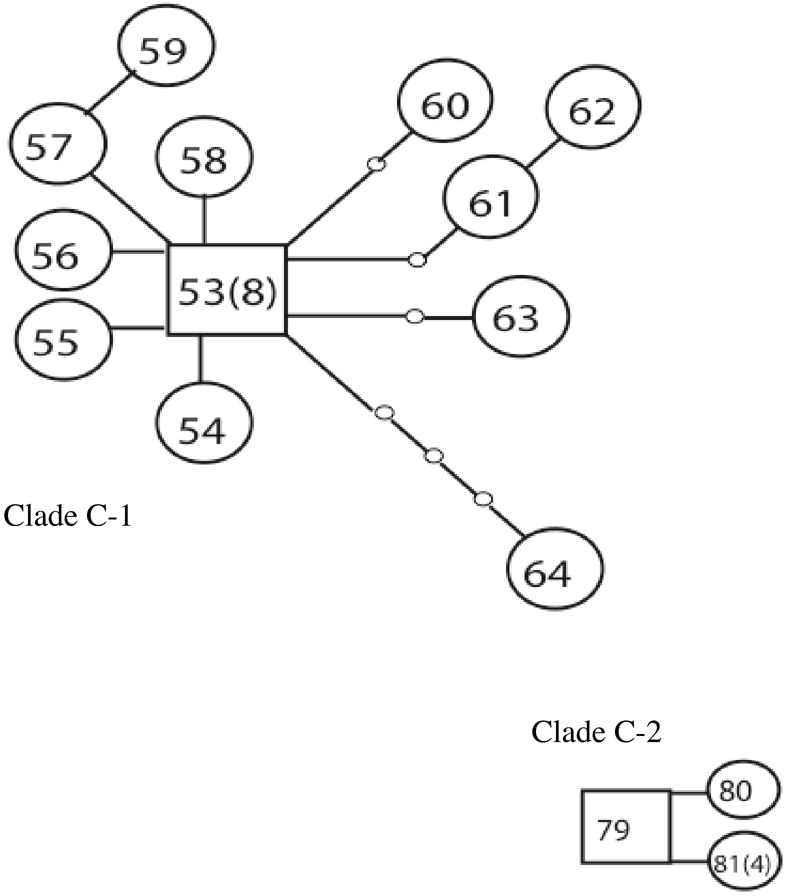


Figure 19. Population network representation of *O. neglectus neglectus*: clades B-1 to B-3 in Figure 10. Squares represent hypothesized ancestral haplotypes in each network, and circles are more derived haplotypes. Numbers inside both squares and circles are haplotype number (and frequency of observation). See Table 2 for haplotypes correspondence to geographic locality and individual. Clade designations are as in Figures 13-14.



Clade C-1

Clade C-2

Figure 20. Population network representation of unrecognized lineage diversity in the *O. neglectus* species complex: clades C-1 to C-2 in Figure 10. Squares represent hypothesized ancestral haplotypes in each network, and circles are more derived haplotypes. Numbers inside both squares and circles are haplotype number (and frequency of observation). See Table 2 for haplotypes correspondence to geographic locality and individual. Clade designations are as in Figure 15.

Table 6. Pairwise clade estimates of Φ_{ST} (below diagonal, * indicate statistically significant differentiation at $p<0.05$), average intra-clade divergence (along diagonal) and pairwise average inter-clade divergence estimates (above diagonal). Φ_{ST} estimates are analogous to F_{ST} estimates for haploid data with values ranging from 0 to 1, with 0 indicating complete panmixia and 1 indicating complete isolation. Nearly all pairwise comparisons are significant (the only ones that are not are between A-5 (a single individual) and other clades (with multiple individuals). The lowest value (outside of single individual comprsing A-5) is 0.73 that indicates a very high degree of isolation. Intraclade divergence estimates are generally low except in clade A-3 which has 2.9% divergence among the six individuals comprising the clade. Interclade divergence estimates range from 2.2% to 12.0%.

	A-1	A-2	A-3	A-4	B-1	B-2	B-3	C-1	C-2
O. neglectus A-1	0.006	0.022	0.062	0.057	0.074	0.088	0.079	0.098	0.085
O. neglectus A-2	0.78*	0.000	0.07	0.061	0.077	0.089	0.08	0.106	0.091
O. neglectus A-3	0.86*	0.73*	0.029	0.076	0.099	0.096	0.091	0.12	0.105
O. neglectus A-4	0.91*	0.91*	0.84*	0.005	0.079	0.092	0.08	0.095	0.084
O. neglectus B-1	0.95*	0.98*	0.95*	0.96*	0.002	0.029	0.015	0.07	0.062
O. neglectus B-2	0.94*	0.96*	0.91*	0.94*	0.91*	0.004	0.015	0.078	0.062
O. neglectus B-3	0.94*	0.98*	0.89*	0.94*	0.90*	0.79*	0.002	0.072	0.056
O. neglectus C-1	0.95*	0.97*	0.92*	0.95*	0.97*	0.95*	0.96*	0.003	0.028
O. neglectus C-2	0.94*	1.0*	0.84*	0.94*	0.98*	0.95*	0.98*	0.90*	0.000

Table 7. Current and historical estimates of θ used in N_e estimation. Effective population sizes have decreased substantially in current versus historical estimations. In the single exception to the decrease of effective population size, clade A-3, there is a high level of divergence (2.9%) within the recovered clade, whereas in all other clades the level of divergence are much lower. Increased amounts of sequence diversity within the clade may be contributing to this spurious result.

		Current		Historical	
	N	$\theta\pi$	N_e	θw	N_e
O. neglectus A-1	61	0.00573	130227.3	0.009835	223522.7
O. neglectus A-2	4	0.00000	n/a	0.000873	19840.9
O. neglectus A-3	6	0.03159	717954.5	0.018294	415772.7
O. neglectus A-4	22	0.00491	111590.9	0.01651	375227.3
O. neglectus B-1	44	0.00152	34545.5	0.007753	176204.5
O. neglectus B-2	27	0.00349	79318.2	0.018327	416522.7
O. neglectus B-3	15	0.00168	38181.8	0.004369	99295.5
O. neglectus C-1	20	0.00295	67045.5	0.012951	294340.9
O. neglectus C-2	6	0.00136	30909.1	0.001456	33090.9