

**Section 6 (Texas Traditional) Report Review**

Attachment to letter dated **OCT 10 2008**

TPWD signature date on report 12/8/07

**Project Title:** Phylogeography of cave crickets (*Ceuthophilus* spp.) in Central Texas: A keystone taxon for the conservation and management of federally listed endangered cave arthropods

**Final or Interim Report?** Final

**Grant #:** E-60

**Reviewer Station:** Austin ESFO

**Lead station was contacted and concurs with the following comments:**

Yes    No    Not applicable (reviewer is from lead station)

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**Interim Report (check one):**

- is acceptable as is
- is acceptable as is, but comments below need to be addressed in the next report
- needs revision (see comments below)

**Final Report (check one):**

- is acceptable as is
- is acceptable, but needs minor revision (see comments below)
- needs major revision (see comments below)

**Comments:**

FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. E - 60

Endangered and Threatened Species Conservation

**Phylogeography of cave crickets (*Ceuthophilus* spp.)  
in central Texas: A keystone taxon for the conservation and management of federally  
endangered cave arthropods**

Prepared by:

Steve Taylor



Carter Smith  
Executive Director

Mike Berger  
Division Director, Wildlife

5 December 2007

FINAL REPORT

STATE: Texas GRANT NUMBER: E - 60

GRANT TITLE: Phylogeography of cave crickets (*Ceuthophilus* spp.) in central Texas: A keystone taxon for the conservation and management of federally endangered cave arthropods

REPORTING PERIOD: 10/01/04 to 12/1/07

OBJECTIVE(S):

To examine broad spatial patterns of genetic relatedness and population structure among cave-inhabiting *Ceuthophilus* populations of the Edwards Plateau and Balcones Escarpment in central Texas in relation to: 1) the known distribution and biology of the species, 2) the geological context in which they occur, and 3) the genetic relatedness of other cavernicoles presently being studied by others (e.g., Hedin & Paquin), and 4) the management and regulatory issues related to terrestrial karst invertebrates in central Texas.

Summary Of Progress:

Please see Attachment A (.pdf file).

Significant Deviation:

None

Location: Several Central Texas Counties

Cost: Not available at time of this report

Prepared by: Craig Farquhar

Date: 5 December 2007

Approved by:   
C. Craig Farquhar

Date: 8 Dec 07

# Phylogeography of cave crickets (*Ceuthophilus* spp.) in central Texas: A keystone taxon for the conservation and management of federally listed endangered cave arthropods

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**30 November 2007**

**Illinois Natural History Survey Technical Report 2007 (58)**

prepared for:

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Cover: Female (left) and male  
*Ceuthophilus (Geotettix)* sp. mating in  
Fern Cave, Val Verde County, Texas.  
Photo by Jean K. Krejca, 18 April 2004.

## Abstract

*Ceuthophilus* spp. (Orthoptera: Rhaphidophoridae) in two subgenera (*Ceuthophilus* and *Geotettix*) were examined phylogenetically, morphologically, and biogeographically in order to improve our understanding of these species that carry nutrients into caves and are thus closely tied to rare and endangered troglobites in central Texas. Crickets were collected from 43 caves across central Texas, and outgroup taxa were collected in west Texas, Kentucky, Missouri, New Mexico, and Mexico. We analyzed 1263 base pairs of mitochondrial DNA from the COI and ND5 genes and complemented this data with morphometric analyses of 19 morphological characters. Phylogenetic trees from the molecular analysis allowed us to select representatives from 25 clades for morphological work.

The molecular data show relatively high levels of genetic variation and phylogeographic structure. This variation is higher than that predicted by the currently accepted species level taxonomy of *Ceuthophilus*. These genetically divergent lineages uncovered by our DNA sequence data may be cryptic species. Members of the subgenus *Ceuthophilus* are known to commonly forage outside of caves leading us to predict that they might also be better at dispersing than members of the subgenus *Geotettix*, which are not known to leave their caves. However, contrary to our expectations, haplotype phylograms of the two subgenera indicate that the subgenus *Ceuthophilus* has deeper genetic structure than the subgenus *Geotettix*. Therefore, *Ceuthophilus* populations have been isolated for a longer period than *Geotettix* populations. Another unexpected result is that multiple haplotypes from genetically divergent groups are found in the same cave on multiple occasions, another indicator of cryptic species.

The morphological dataset included 19 discrete characters and 31 continuous characters that were used to create a morphology-based tree and plotted in a principal component analysis. The morphological and genetic trees are similar only on a rough level in that the two subgenera are generally distinct (though *Geotettix* is monophyletic in the genetic analysis and one morphological analysis and paraphyletic in another morphological analysis). Morphological characters are also divergent within monophyletic clades and convergent among clades, also pointing to probable undescribed species.

As *Ceuthophilus* spp. are a key component in central Texas karst invertebrate conservation, identifying and describing any new species is a high conservation priority. Different species may have different life history patterns, foraging behaviors, and use of caves.

## Introduction

Understanding the role that cave crickets play in cave communities is important for the management and protection of endangered karst invertebrates in central Texas. Cave crickets are key species (Taylor et al. 2005, Lavoie et al. 2007) in cave communities because 1) they often occur in large numbers, and 2) they are one of the primary modes of transporting energy from the surface environment into the caves. Effective conservation and management of Texas' federally listed endangered karst invertebrates (USFWS 1988, 1993, 2000) requires understanding the direct and indirect dependence of cave communities on the biology of *Ceuthophilus* cave cricket taxa. However, our knowledge of the occurrence and distribution of Texas' cave cricket taxa remains in its infancy. Here we seek to expand our understanding of the spatial distribution of evolutionary lineages (both described and undescribed species) of central Texas *Ceuthophilus* species. These data can be utilized in future studies to better define the biology (food sources, annual life cycle, numbers of eggs produced, energy flow) of both the crickets and the endangered karst invertebrates.

The genus *Ceuthophilus* currently includes 89 valid species and geographical races in North America. The generic taxonomy and morphology of *Ceuthophilus* was thoroughly revised by Hubbell (1936) who studied 71 primary types and over 17,000 specimens (Strohecker 1936). He divided the genus into three subgenera: *Ceuthophilus (Hemiudeopsylla)* with eight species restricted to southwestern North America; *Ceuthophilus (Geotettix)* including 20 species mostly distributed in the Great Basin and Great Plains regions; and the nominotypical *Ceuthophilus (Ceuthophilus)* that includes 62 species which comprise the majority of species inhabiting forested areas of North America. Furthermore, Hubbell (1936) accommodated all species in species groups, and in turn accommodated those in series, in an attempt to express his understanding of relationships ("close alliance") within the genus. Since Hubbell's revision, the genus *Ceuthophilus* has received little attention, although various researchers recognize a number of undescribed species. For example, unpublished observations by Reddell, corroborated by Krejca and Taylor, suggested that *C. (G.) cunicularis* represents more than one species. Many *Ceuthophilus* taxa cannot be easily distinguished, and the taxonomy of the genus *Ceuthophilus* is in need of revision because the species descriptions of Hubbell's (1936) monograph refer to differing sets of characters, confounding what should be fairly straightforward species identifications (Cohn 2000).

The present study is an assessment of the molecular and morphological variation of species of cavernicolous *Ceuthophilus* species occurring in Texas and Mexico. We examine broad spatial patterns of genetic relatedness and population structure among cave-inhabiting *Ceuthophilus* of the Edwards Plateau and Balcones Escarpment in central Texas in relation to the:

- 1) known distribution and biology of the species,
- 2) geological context in which they occur,
- 3) genetic relatedness of other cavernicoles
- 4) management and regulatory issues related to terrestrial karst invertebrates in central Texas.

Species definitions by Hubbell (1936) and hypotheses of relationships generated based on morphology are compared to a phylogenetic hypothesis generated based on DNA-sequence data. The primary species of interest are cave crickets typical of central Texas caves. They belong to the undescribed *Ceuthophilus (C.)* "species B", *Ceuthophilus (G.) cunicularis* which is thought to

represent a complex of two or more taxa, not all of which have been described, and the widely spread and often abundant *Ceuthophilus (C.) secretus*.

### Cave cricket biology

*Ceuthophilus (Ceuthophilus) secretus* (Figure 1) and other, undescribed, *Ceuthophilus (Ceuthophilus)* species in central Texas forage at night on the surface, returning to the cave to roost during the day. Their feces, dead bodies, and eggs (laid in the caves) constitute a significant portion of the available energy in the cave environment. Crickets which emerge from central Texas caves to forage at night are currently recognized as *C. (C.) secretus* and *Ceuthophilus (Ceuthophilus)* “species B” – the latter is an undescribed taxon, with unclear affinities to *C. (C.) secretus*. Recently, Taylor *et al.* (2003, 2004, 2005) demonstrated that *C. (C.) secretus* commonly forages more than 100 meters from cave entrances. A third species, *Ceuthophilus (Geotettix) cunicularis*, is also reported from many of the endangered species caves, though it rarely, if ever, leaves caves to forage.



Figure 1. *Ceuthophilus (Ceuthophilus) secretus* from Fort Hood, Texas (Bell and Coryell counties).

## Relationships between *Ceuthophilus* and federally listed endangered cave invertebrates

The life histories of endangered cave arthropods and the various (likely greater than three) species of *Ceuthophilus* are tightly interconnected. The most obvious relationship is the predator/prey relationship between the endangered *Rhadine* beetles (Mitchell 1971) and one or more of the cricket taxa. Cave-adapted *Rhadine* beetles (Figure 2) are known to prey upon eggs of *Ceuthophilus* species. Adult female *Ceuthophilus* spp. insert their ovipositors deeply in the substrate and deposit a single, fairly large egg. Based on more detailed studies of Rhabdiphoridae crickets elsewhere (Kane and Poulson 1976, Hubbell and Norton 1978, Griffith 1990, Lavoie et al. 2007) it is likely that the crickets make a series of false oviposition holes to limit beetle depredation. One of us (JKK) has observed a female *Ceuthophilus* repeatedly inserting her ovipositor in the substrate, and studies on eastern and midwestern North American *Hadenoecus* species demonstrate an obligate relationship between the cave cricket species and their beetle predators (e.g., Poulson et al. 1995, Helf et al. 1996, Studier 1996, Lavoie et al. 2007). These beetle/cricket species pairs are unique in different geographic areas (e.g., the karst of central Kentucky versus the Appalachians). In central Texas, three species of *Rhadine* are federally listed as endangered (*Rhadine persephone* in Travis and Williamson counties, plus *Rhadine exilis* and *Rhadine infernalis* in Bexar County), and it is probable that a significant portion of their food comes in the form of cave cricket eggs.



Figure 2. *Rhadine reyesi* (Coleoptera: Carabidae) with a *Ceuthophilus* sp. egg in a Coryell County, Texas cave. Two large holes in the clay substrate (upper left and lower right) are thought to be oviposition holes of *Ceuthophilus* sp. which may have been enlarged by *Rhadine reyesi* searching for cricket eggs. Photo by Jean Krejca and Steve Taylor.

Relationships between other federally listed endangered cave arthropods and cave crickets are less direct but clearly important. In Bell and Coryell counties (the northeastern limit of our study area), Taylor et al. (2003) documented similar spatial distributions of *Cicurina* spiders, cave-adapted springtails, and *C. (C.) secretus*. It appears that the springtails feed on the guano of roosting cave crickets (and/or the fungi and bacteria which are breaking down the cricket guano) and the predatory *Cicurina* spiders are, in turn, feeding on the springtails. Cokendolpher (personal communication 2002) successfully maintained *Cicurina* spp. in the laboratory on a diet of cave springtails, corroborating portions of the above relationships. In addition, one of us (JRR) observed both pseudoscorpions (*Tartarocreagris* spp.) and troglobitic *Cicurina* spp. spiders feeding on *Ceuthophilus* spp. nymphs.

The relationship between other endangered cave arthropods (e.g., *Batrissodes* spp.) and the cave crickets in central Texas is less clear, but cave crickets, and their feces and eggs, provide one of the most important sources of energy driving the cave ecosystem (excluding a few caves dominated by colonial bats or others which regularly flood) (Taylor et al. 2003, 2004, 2007). Poulson (1992) reviewed in detail the better-studied terrestrial cave communities of Mammoth Cave, Kentucky, and

discussed demonstrable links between the raphidophorid *Hadenoecus subterraneus* and various cave-adapted invertebrates.

*Ceuthophilus* biology and distribution in Bell and Coryell counties has been shown to differ between the more vagile *C. (C.) secretus* and the seemingly cave-limited crickets presently referred to as *C. (G.) cunicularis* (Taylor *et al.* 2003). *Ceuthophilus (C.) secretus* leave these caves at night to forage during the warmer months, sometimes traveling more than 100 m from a cave entrance, and occasionally moving between caves (Taylor *et al.* 2004). In another similarly mobile cave cricket species, *Ceuthophilus (Ceuthophilus) gracilipes*, Cockley *et al.* (1977) found relatively low levels of genetic variation within and between populations. At the other extreme, *C. (G.) cunicularis* was never observed leaving caves during studies by Taylor *et al.* (2003, 2005, 2007) and was only once been observed exiting a cave during extensive monitoring at Camp Bullis in Bexar County (Veni and Associates 2006). Within caves, *C. (C.) secretus* are typically found on bedrock ceilings whereas *C. (G.) cunicularis* are typically found on the walls and floors (Taylor *et al.* 2003). Finally, carbon and nitrogen stable isotope data (Taylor *et al.* 2004, 2007) suggest that *C. (C.) secretus* and *C. (G.) cunicularis* forage on different foods and may even occupy differing trophic levels. Collectively, these differences in ecology and mobility may be reflected in levels of population genetic structure, as has been found in other cavernicolous arthropods (Caccone 1985, Caccone and Sbordoni 1987).

### Endangered Species Management Implications

The management of the federally listed endangered terrestrial cave invertebrates requires an understanding of the phylogenetic relationships and population genetic structure of the cave crickets upon which they depend. Different cricket species, whether named or merely identified as genetically unique entities, may well have differing life history strategies that have implications for the federally listed endangered terrestrial troglobites in central Texas. The foraging range of these crickets is a crucial piece of information for effective management of cave and karst ecosystems. For example, land managers may wish to control access to, or control red imported fire ant (RIFA, *Solenopsis invicta*) populations in all or part of the foraging range of the crickets to help maintain natural surface and cave communities. The obvious differences in microhabitat utilization by *C. (C.) secretus* and *C. (G.) cunicularis* documented in Bell and Coryell counties (Taylor *et al.* 2003) may not be applicable to populations in Travis/Williamson or Bexar counties if, as it is strongly suspected, the *C. (G.) cunicularis* in Bell and Coryell counties is actually an undescribed species. Making management recommendations with regard to cave crickets within the range of the endangered species caves depend on knowing what cricket taxa are present, because the biology of unstudied taxa may differ from related taxa in different areas. Also, patterns of genetic variation in cave crickets may be useful for identifying areas of unique biological diversity.

## **Materials and Methods**

We collected mitochondrial DNA (mtDNA) sequence data to compare patterns of genetic structure between the three cricket species across their geographic ranges. Mitochondrial sequences have been useful for comparing levels of population genetic structure in other cavernicoles (Caccone and Sbordoni 2001). In total we sequenced mtDNA from 309 individual cave crickets.

## Sampling locations and collection methods

The geographic area encompassed by this study focus primarily on counties within the Balcones Escarpment and Edwards Plateau (Figure 3). The federally listed endangered cave invertebrates are confined to the Balcones Escarpment within Bexar, Travis, and Williamson counties, with a few localities in eastern Burnet County. Cave crickets in the subgenera *Geotettix* and *Ceuthophilus* are codistributed across central Texas. We sampled multiple individuals from each subgenus at each cave where possible, with *Ceuthophilus* specimens from 43 caves distributed across 20 Texas counties and two caves in Mexico. We also obtained specimens from caves in Kentucky (*Hadenoeus subterraneus*), Missouri (*Ceuthophilus* [*Ceuthophilus*] *gracilipes*, *Ceuthophilus* [*Ceuthophilus*] *williamsoni*), and New Mexico (*Ceuthophilus* [*Ceuthophilus*] *longipes*, *Ceuthophilus* [*Geotettix*] *carlsbadensis*, and *Ceuthophilus* [*Ceuthophilus*] *conicaudus*) to serve as outgroup taxa (Figure 3), for a total of 50 sampling sites (Table 1). These sample sites were selected to maximize our understanding of potential barriers to gene flow. We suspect that major geological discontinuities (Figure 4), rivers (Figure 5) or ecoregional differences (Figure 6) could serve as barriers to gene flow,

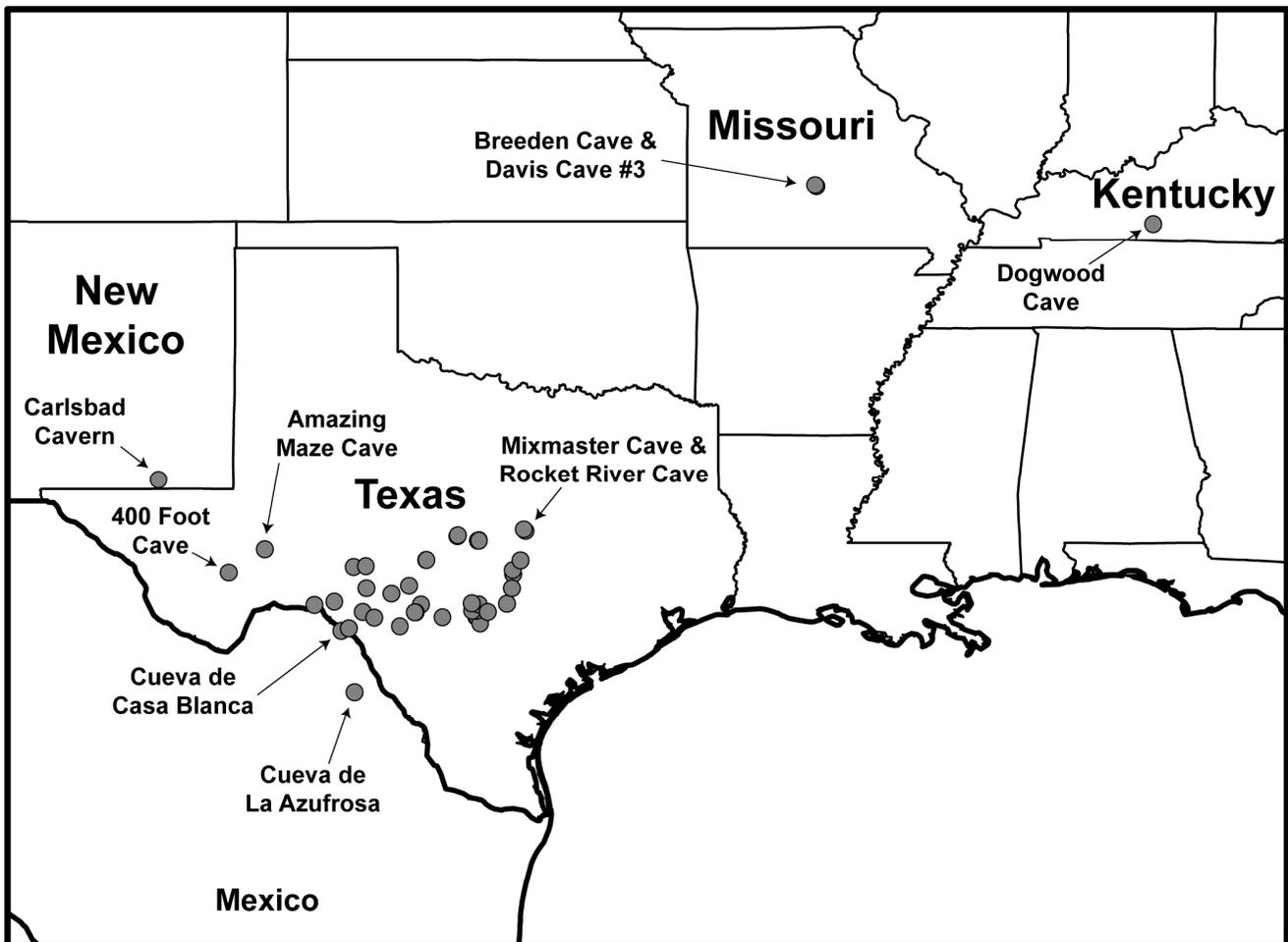


Figure 3. Distribution of all sites from which Rhaphidophoridae (Orthoptera) were sampled for this study, including outgroup taxa.

Table 1. Names and county-level locations for sites sampled for this study.

Country	State	County	Caves
Mexico	Coahuila		Cueva de La Azufrosa, Cueva de Casa Blanca
USA		Barren	Dogwood Cave
		Pulaski	Breeden Cave, Davis Cave #3
		Eddy	Carlsbad Cavern (& outside of cave)
		Bandera	near Haby Salamander Cave
			MARS Shaft, Poor Boy Baculum Cave, Robber
		Bexar	Baron Cave, Tall Tales Cave
			400 Foot Cave
		Brewster	Camp Bullis Bat Cave, Camp Bullis Cave No. 1,
		Comal	Preserve Cave, Temple of Doom
			Mixmaster Cave, Rocket River Cave
		Coryell	Deep Cave, Devils Sinkhole, Punkin Cave, Writing
			on the Rocks Cave
		Hays	Ezell's Cave
		Kendall	Dead Man's Cave
		Kerr	Schroeder Bat Cave
		Kinney	Kickapoo Cavern
		Mason	Behren's Grotto, Porcupine Pit, Swift Cave
		Medina	Ground Hog Cave
		Pecos	Amazing Maze Cave
		Real	All the Wonders and Joys Cave, Little Dry Frio
San Saba	(a cave without any name), Cicurina Cave, Lemons		
	Ranch Cave, Puberty Pit, Rattlesnake Drop, Turtle		
	Shell Cave		
Sutton	Caverns of Sonora, IH-10 Cave,		
Travis	Lamm Cave, Lost Oasis Cave, Testudo Tube		
Uvalde	Finley Bat Cave		
Val Verde	Big Tree Cave, (cave near Del Rio), Fern Cave		
Williamson	Temples of Thor		

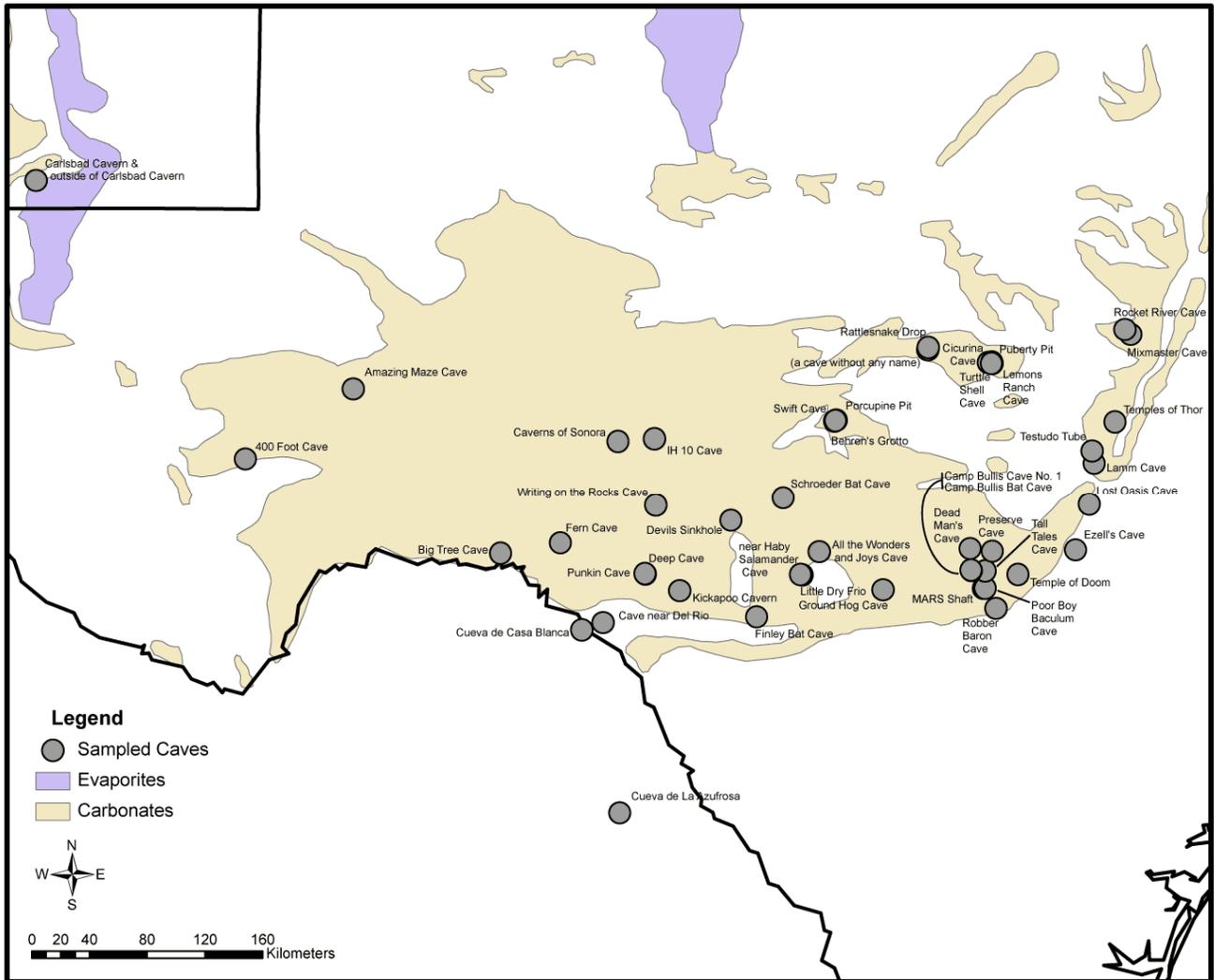


Figure 4. Distribution of sites in Texas, New Mexico, and Mexico from which *Ceuthophilus* spp. (Orthoptera: Rhabdophoridae) were obtained for this study, overlaid on the distribution of karst (not including Mexico). Karst map modified after Veni and Duchene (2001).

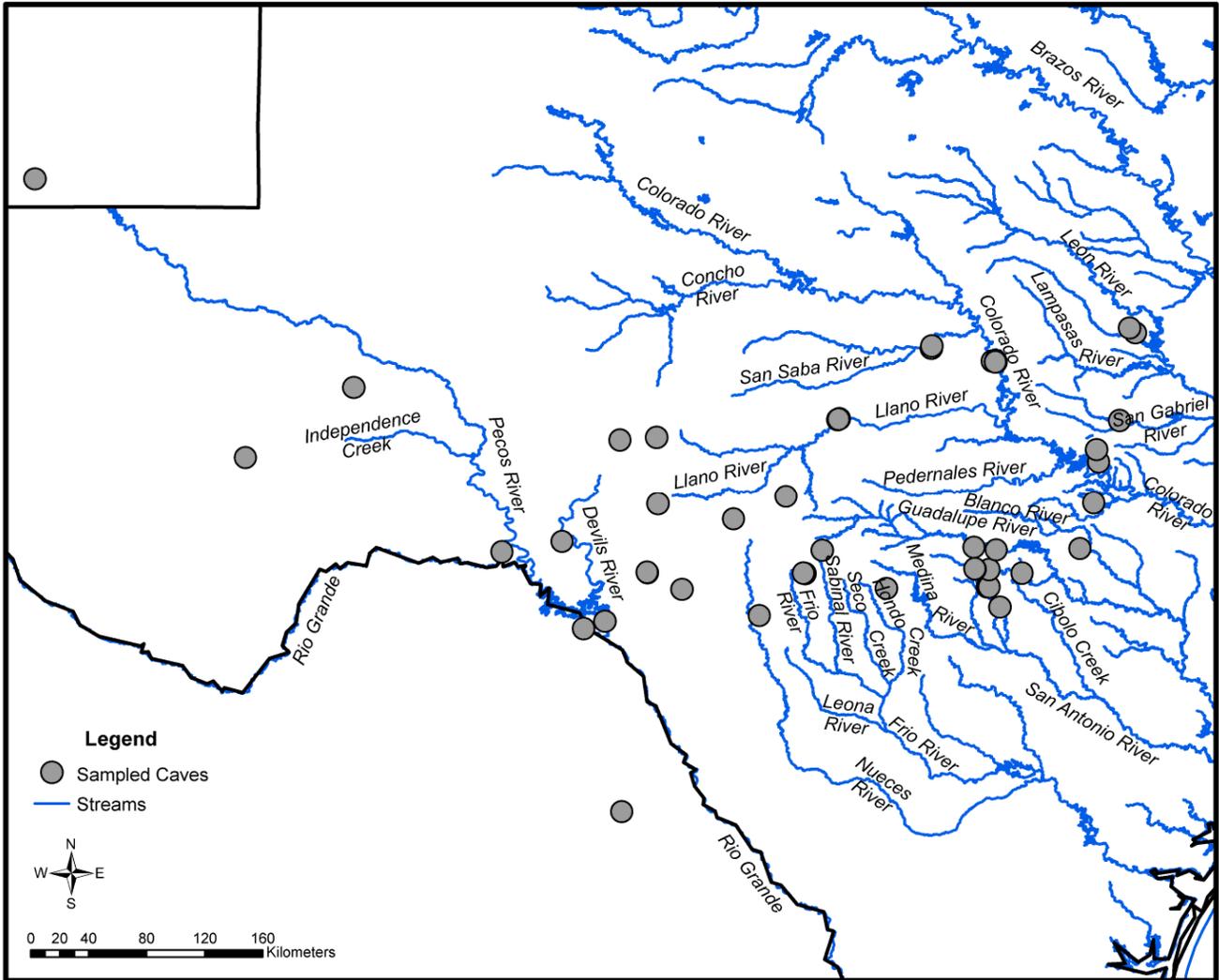


Figure 5. Distribution of sites in Texas, New Mexico, and Mexico from which *Ceuthophilus* spp. (Orthoptera: Rhaphidophoridae) were obtained for this study, overlaid on the major streams and rivers of Texas.

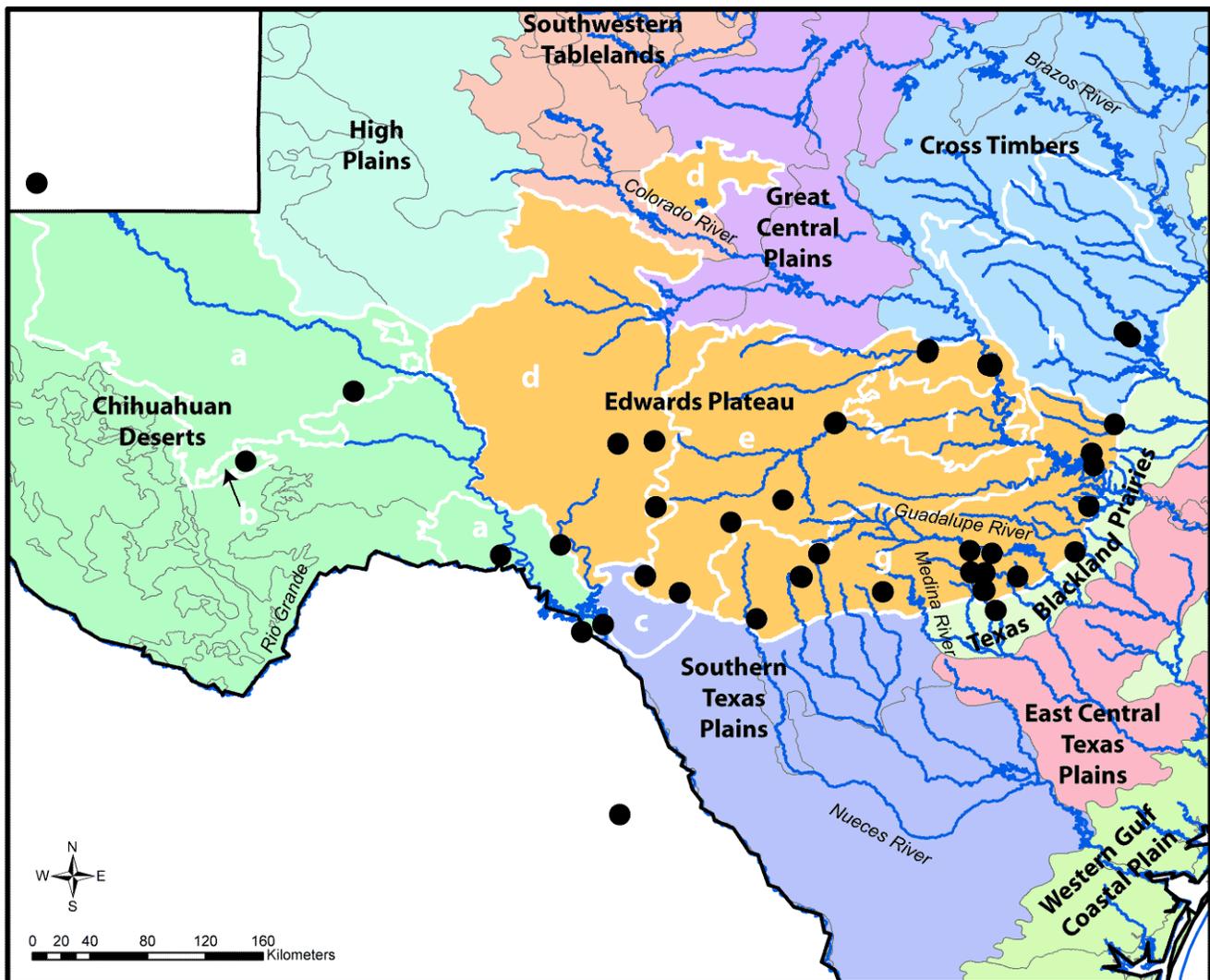


Figure 6. Distribution of study sites in Texas, New Mexico, and Mexico from which *Ceuthophilus* spp. (Orthoptera: Rhaphidophoridae) were obtained for this study, overlaid on US EPA Level III and IV Ecoregions of Texas (Griffith *et al.* 2004). Level III Ecoregions indicated by colors and bold black text, with selected Level IV regions outlined in white, with white text. Chihuahuan Deserts: a. Chihuahuan Basins and Playas, b. Chihuahuan Montane Woodlands; Southern Texas Plains: c. Semiarid Edwards Bajada; Edwards Plateau: d. Semiarid Edwards Plateau, e. Edwards Plateau Woodland, f. Llano Uplift, g. Balcones Canyonlands; Cross Timbers: h. Limestone Cut Plain.

especially in the largely cave-limited *C. (G.) cunicularis*. Because *C. (C.) secretus* is able to travel over land for significant distances (more than 100 meters over short periods of time [Taylor *et al.* 2003, 2004, 2005]) and can move between karst features (Taylor *et al.* 2004), higher levels of gene flow may be evident in this species.

### Collecting methods used and tissue sampling

Specimens were captured by hand and placed in 95% ethanol to preserve DNA. An effort was made to collect several adult individuals of both sexes and both subgenera at each site. In the lab, a single leg was removed from each specimen for use in DNA extraction. Selected specimens were used for morphological analysis in this study, and these, along with the remaining portion of each specimen will be deposited in the insect collection of the Illinois Natural History Survey as vouchers available for future analyses. Unlike the federally list endangered karst invertebrates, the cave crickets usually occur in sufficiently large numbers (sometimes thousands of individuals in a single cave) that collecting 3-5 individuals from a single cave on a one-time basis will have an inconsequential effect on the population size.

### Sample sizes and localities to be samples

We collected 1263 base pairs (bp) of mitochondrial DNA (mtDNA) sequence data (COI and ND5 regions) from 122 individual cave crickets in the subgenus *Geotettix*, including *C. (G.) cunicularis*, *Ceuthophilus (Geotettix) polingi*, and 179 individual cave crickets in the subgenus *Ceuthophilus* including *C. (C.) secretus*, *C. (C.) conicaudus*, and the undescribed taxon *C. (C.)* “species B.” We also collected mtDNA sequence data from the following outgroup taxa to root phylogenetic trees in some analyses: *H. subterraneus* ( $n = 1$ ), *C. (C.) gracilipes* ( $n = 1$ ), *C. (C.) longipes* ( $n = 2$ ), *C. (G.) carlsbadensis* ( $n = 3$ ), and *C. (C.) williamsoni* ( $n = 1$ ).

### Extraction, PCR, and DNA sequencing

We extracted whole genomic DNA from a single leg of each specimen using a Dneasy extraction kit following the kit protocol for animal tissues (Qiagen, Valencia, California). For each specimen we sequenced 1263 bp of mitochondrial DNA (mtDNA) including 850 bp of Cytochrome Oxidase I (COI) and 413 bp of nicotinamide adenine dinucleotide dehydrogenase subunit 5 (ND5). We amplified an 850 bp fragment of COI using the primers C1-J-1718 (Simon *et al.* 1994) and H7005 (Hafner *et al.* 1994) and sequenced this fragment using the C1-7-1718, H7005, and two of three internal primers designed specifically for this study: CeuthCOIL (5'-GATCCTGCTGGTGGAGGAGATCC-3'), and either CeuthCOIH (5'-GAATTGGATCTCCTCCACCAGCAGG-3') or CeuthCOIHcunn (5'-GAATTGGATCTCCTCCTCCTGCGYGG-3'). We amplified and sequenced a 413 bp fragment of ND5 using the primers F7081 and R7495 (Yoshizawa 2004). For COI PCR amplifications we used the following thermal cycling profile: 94° C for 2 min, 35 cycles of 94° C for 30s, 46° C for 30s, 72° C for 1 min, and 72° C for 7 min. Whereas for ND5 PCR amplifications we used the following thermal cycling profile: 94° C for 2 min, 35 cycles of 94° C for 30s, 42° C for 30s, 65° C for 30s, and 65° C for 7 min. We verified all PCR products on a 1% agarose gel and purified these PCR products using a QIAquick PCR Purification kit (Qiagen, Valencia, California).

Cycle sequencing reactions were performed at the University of Illinois DNA sequencing facility using an ABI Big Dye kit (Applied Biosystems, Foster City, California), the above-listed

primers, and approximately 75 ng of purified PCR product. We ran purified sequencing reaction products on an ABI 3730 capillary electrophoresis system (Applied Biosystems) and used Sequencher (ver. 4.5, GeneCodes Co., Ann Arbor, Michigan) to reconcile double-stranded sequences and to align sequences for analysis.

### Phylogenetic and population genetic analyses

We used MacClade (version 4.05; Maddison and Maddison 2002) to collapse the entire dataset down to unique sequences (haplotypes) for phylogenetic analysis. We reconstructed separate phylogenetic trees for the subgenera *Geotettix* and *Ceuthophilus* using Neighbor-Joining analysis as implemented in the computer programs PAUP\* (ver. 4.0beta10; Swofford 2001) and used the outgroups listed above to root the topologies. We also calculated uncorrected pairwise sequence divergence (p-distance) using PAUP\* (Swofford 2001) between taxa in these two subgeneric clades to compare the relative levels of divergence within each of the subgenera.

A reduced taxon dataset was used to perform a Bayesian phylogenetic analysis as implemented in the computer program MrBayes (ver. 3.1.1; Ronquist and Huelsenbeck 2003). For the Bayesian analysis we analyzed the COI and ND5 mtDNA sequences for forty-seven terminal taxa using a mixed-model approach by partitioning each gene by codon position (total of 6 partitions). The analysis was conducted with two independent runs of 5,000,000 generations each with 4 mcmc chains. A majority-rule consensus with average branch lengths and posterior probabilities was constructed based on 10,854 distinct trees after a 1,000,000 generation burn-in (trees sampled prior to the analysis reaching stationarity) was discarded (Leaché and Reeder 2002).

To assess whether there was an influence of geography on the phylogenetic results, the resulting Bayesian tree for the *Ceuthophilus* (*Geotettix*) subgenus was divided into clades containing one to four of the previously defined genetic lineages. This tree was then plotted onto a map using geographical coordinates of the specimens (terminal taxa) with the module Cartographer of Mesquite (Maddison & Maddison 2006a, 2006b). We also compared this tree topology to the morphological data and used it to identify matching pairs of males and females for morphological analysis.

### Taxon sampling for morphological study

For purposes of reference throughout this report, we named monophyletic lineages, or clades, of crickets using letters that were arbitrarily assigned based on the molecular phylogeny. These letter designations do not necessarily imply species breaks, nor do they relate to terminology used by other authors and researchers for undescribed species (e.g., *Ceuthophilus* “species B”). The *Ceuthophilus* included in this study are divided into two main groups, corresponding to their respective subgenera: (1) monophyletic lineages A-J, *C. (C.) gracilipes*, *C. (C.) conicaudus*, and *C. (C.) secretus* (belonging to nominative subgenus *Ceuthophilus*); and (2) monophyletic lineages K-T, *C. (G.) carlsbadensis*, *C. (G.) cunicularis*, and *C. (G.) polingi* (all belonging to subgenus *Geotettix*). Morphological characters were studied from adult male and female specimens from each lineage (see specimen list in Table 1). The morphology of lineage I was not studied because only immature specimens were available, and lineage Q was further divided into Q and Q2. Voucher numbers and geographical origins are listed in Table 2.

Table 2. Voucher numbers and Texas county of origin (except for Mexico [MX] state, New Mexico [NM] and Missouri [MO] counties) of adult *Ceuthophilus* specimens for each molecular lineage which were studied for the morphological analysis.

Subgenus	Molecular Lineage (Clade)	Male	Female
<i>C. (Ceuthophilus)</i> spp.	A	-	448: Sutton
	B	-	310: Travis
	C	083: Comal	140: Brewster
	D	444: Medina	443: Medina
	E	459: Val Verde	460: Val Verde
	E	180: Mason	
	F	-	318: Williamson
	G	-	215: Bexar
	H	-	452: Uvalde
	J	-	139: Brewster
	<i>C. (C.) conicaudus</i>	258: Eddy (NM)	259: Eddy (NM)
	<i>C. (C.) gracilipes</i>	009: Pulaski (MO)	
	<i>C. (C.) longipes</i>	-	253: Eddy
	<i>C. (C.) secretus</i>	229: Travis	228: Travis
<i>C. (Geotettix)</i> spp.	K	038: Edwards	043: Edwards
	L	078: Val Verde	336: Edwards
	L	079: Val Verde	
	M	240: Bexar	219: Bexar
	N	323: Williamson	324: Williamson
	O	463: Val Verde	461: Val Verde
	P	-	425: Bexar
	Q	446: Medina	
	Q2	116: Kerr	114: Kerr
	R	454: Uvalde	
	S	138: Brewster	143: Brewster
	T	366: Coahuila (MX)	385: Coahuila (MX)
	<i>C. (G.) carlsbadensis</i>	257: Eddy (NM)	256: Eddy (NM)
	<i>C. (G.) cunicularis</i> (Q)	220: Bexar	241: Bexar
<i>C. (G.) polingi</i> (R)	148: Mason	153: Mason	
<i>C. (G.) polingi</i> (R)	290: Coahuila (MX)		

Morphological discrete character sampling

Our morphological data matrix for 25 terminal taxa, including eight male terminalia characters and 11 female external characters (Table 3, Appendix 1). These discrete characters consisted of those related to structural shape and serially homologous structures, such as spurs and denticulations. We plotted histograms of the variation observed (bin size = 10 when range was <50 and 15 when range >50) in those characters involving counts (characters 15-19), and states were delimited according to consistent breaks in the continuity of the count classes. Some character states are illustrated (Figure 7) and character and state descriptions are listed in Appendix 1. Analysis of the count data was not weighed according to the relative magnitude of the states. For *C. (C.) longipes* and seven molecular lineages, no males were available and those related characters were scored as missing data (“?”) in the matrix.

Table 3. Data matrix of discrete morphological characters of Texas *Ceuthophilus*. List of character and states are presented in Appendix 1 and illustrations of these characters in Figure 4.

Clade	Characters																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A	?	?	?	?	?	?	?	?	0	0	1	0	0	0	1	1	1	1	2
B	?	?	?	?	?	?	?	?	1	0	0	0	0	0	1	1	1	0	3
C	1	1	1	0	0	0	-	0	0	0	0	0	1	0	1	1	1	0	2
D	1	1	0	1	0	0	-	1	1	0	1	1	0	1	1	1	1	2	3
E	1	1	0	1	0	0	-	1	1	0	1	1	0	1	1	1	1	3	4
F	?	?	?	?	?	?	?	?	1	0	0	0	0	0	1	1	1	0	2
<i>C. (C.) secretus</i>	1	1	0	1	0	0	-	1	0	0	1	1	0	1	1	1	1	3	4
G	?	?	?	?	?	?	?	?	1	0	0	0	1	0	1	1	1	0	2
H	?	?	?	?	?	?	?	?	1	0	0	0	1	0	1	1	1	1	2
<i>C. (C.) conicaudus</i>	1	1	0	0	0	0	-	0	0	0	1	2	0	0	1	1	1	1	2
J	?	?	?	?	?	?	?	?	0	0	1	0	0	0	1	1	1	0	4
<i>C. (G.) carlsbadensis</i>	0	0	0	0	2	0	-	0	0	1	1	0	0	0	1	2	2	5	1
K	0	0	0	0	1	1	1	0	0	1	1	0	0	0	1	1	2	3	1
L	0	0	0	0	1	1	0	0	0	1	1	0	1	0	1	1	1	3	1
M	0	0	0	0	1	1	1	0	0	1	1	0	1	0	1	1	1	1	1
N	0	0	0	0	1	1	0	0	0	1	1	0	1	0	2	2	1	1	1
O	0	0	0	0	1	1	0	0	0	1	1	0	0	0	4	3	4	4	1
P	?	?	?	?	?	?	?	?	0	1	1	0	1	0	1	1	1	1	1
<i>C. (G.) cunicularis</i> (Q)	0	0	0	0	1	1	0	0	0	1	1	0	1	0	1	1	1	1	2
Q2	0	0	0	0	1	1	0	0	0	1	1	0	0	0	2	2	3	4	1
<i>C. (G.) polingi</i> (R)	0	0	0	0	1	0	-	0	0	1	1	0	1	0	1	1	1	1	0
S	0	0	0	0	0	1	1	0	0	1	1	0	1	0	1	1	1	1	2
T	0	0	1	0	1	1	0	0	0	1	1	0	0	0	3	2	2	3	0
<i>C. (C.) longipes</i>	?	?	?	?	?	?	?	?	0	0	0	0	0	0	0	0	0	0	3
<i>C. (C.) gracilipes</i>	1	1	1	0	0	0	-	0	?	?	?	0	?	?	?	?	?	?	?

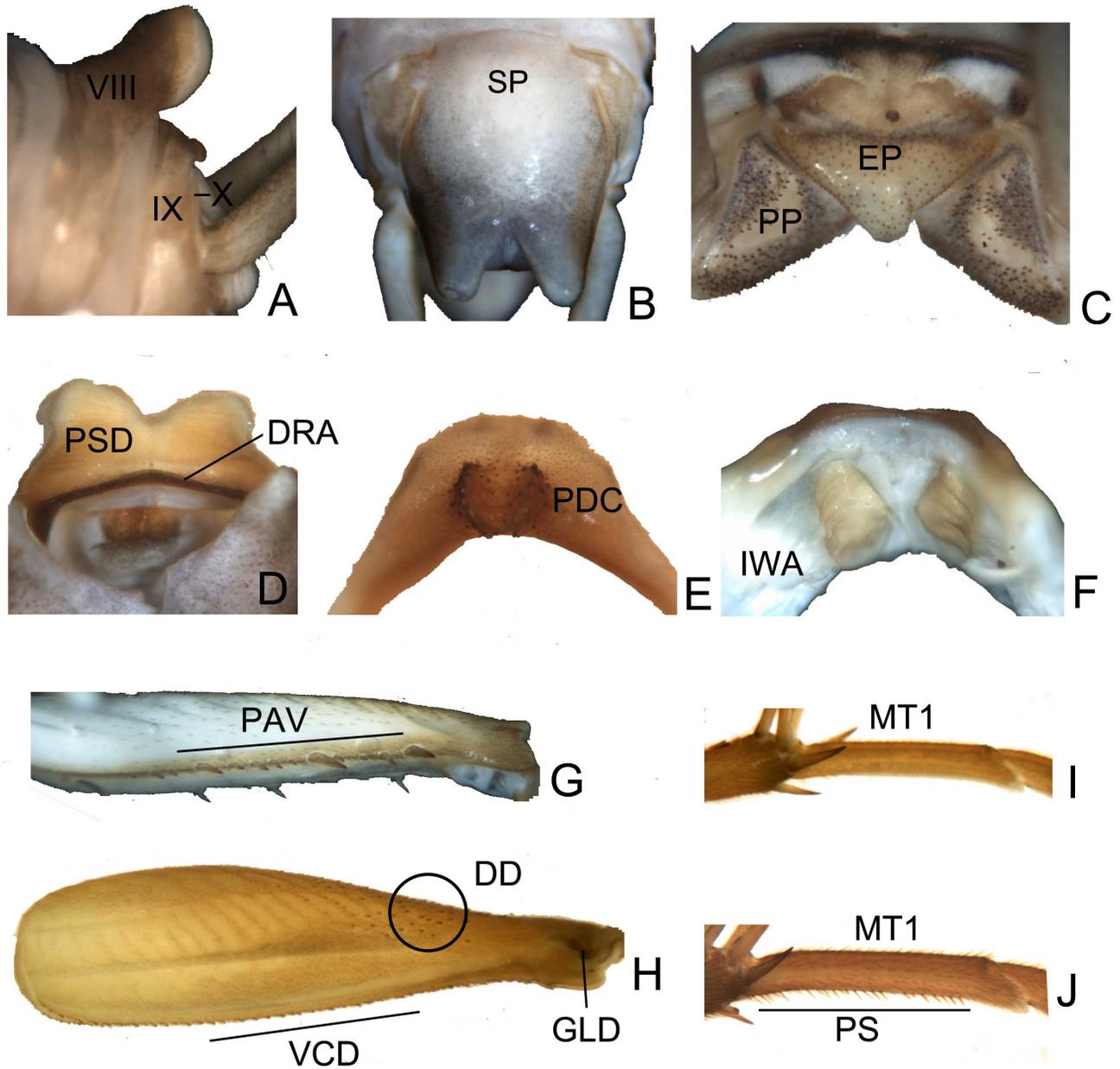


Figure 7. Morphological structures used for defining discrete characters for the phylogenetic analysis of *Ceuthophilus* species. A-F: male terminalia; A: terminal abdominal segments, lateral view, tergites labeled VIII-X; B: subgenital plates (SP), ventral view; C: epiproct (EP) and paraprocts (PP), caudal view; D: pseudosternite dorsum (PSD) with thickened dorsal rim of arch (DRA), dorsocaudal view; E: pseudosternite dorsum with pair of parallel crests (PDC); F: internal wall or arch (IWA) with pair of thickened folds, caudal view; G-J: female appendages; G: profemur with anteroventral row of setae (PAV), anterior view; H: metafemur with dorsal denticulations (DD), genicular lobe denticle (GLD), and ventral carina denticulations (VLD), anterior view; I: metatarsomere I (MT1) without plantar setae, lateral view; J: metatarsomere I (MT1) with row of plantar setae (PS), lateral view.

## Morphological continuous character sampling

Continuous data for female structural characters were measured based on photographs taken of the specimens. Thirty-one measurements of the external morphology were taken for each specimen. Most of these measurements were previously used by Hubbell (1936) in species descriptions, including six diagnostic ones, which he called “primary dimensions.” Some of these measurements were posteriorly discarded because they appeared correlated ( $R^2 > 0.90$ ) with another (e.g. length of PD vs. AD calcar, length of protarsomere I vs. metatarsomere I, length of metafemur vs. metatibia, interocular distance vs. infraocular distance, etc.). The 17 remaining measurements were used in a principal components analysis (PCA) run in Systat 10.0 (SPSS, Inc.). Representative measurements (Figure 8) were reduced to two factors, which explained  $>85\%$  of the total variation. Three separate analyses were run, one including all the lineages studied (Table 2), and two others including only representatives of each of the two main clades. PCA scores for each individual cricket were plotted in 2-dimensional graphs to allow a graphical visualization of the lineages studied based on their measurements.

Finally, an average linkage cluster was produced using pairwise Euclidean distances between the component scores of each taxon. This cluster analysis was compared with the molecular phylogenetic hypotheses.

## Phylogenetic analysis of morphological data

The independent measurements were divided by the pronotum length of each specimen, to correct for variations in body size – the pronotal length is considered a more accurate measure of body size than overall length (Hubbell 1936), resulting 16 ratios (Table 4, Appendix 2). These ratios were then used to reconstruct a phylogenetic hypothesis in Tree analysis using New Technology (TNT, Goloboff 1999), which allows the combined parsimony analysis of continuous and discrete characters. Exact tree searches were conducted using implicit enumeration and the most parsimonious trees condensed into a strict consensus tree. Parsimony bootstrap values were also calculated.

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Figure 8 (on following page). Morphological measurements taken from *Ceuthophilus* species. A: head and thorax, lateral view; PL: pronotum length; DPL: distal segment of maxillary palpus; B: face, frontal view; EA: eye area; IOL: interocular distance; C: face, lateral view; FD: fastigium distance to mesal margin of antennal fossa; D: profemur, anterior view; PFL: profemur length; E: profemur distal end, anterior view; PAV: distalmost setae of the anteroventral row length; F: mesofemur, dorsal view; GSL: posterior genicular spur length; G: metafemur, anterior view; MFW: femur largest width; MFL: femur length; H: tibial apex and metatarsus, anterior view; SDL: tibial subdistal spur length; ADC: tibial anteodorsal calcar length; MTL: tarsomere I length; I: metatarsus apex, anterior view; TCL: tarsal claw length; J: cercus, lateral view; CL: cercus length; K: ovipositor, lateral view; VVL: ventral valve length; VVA: ventral valve armed.

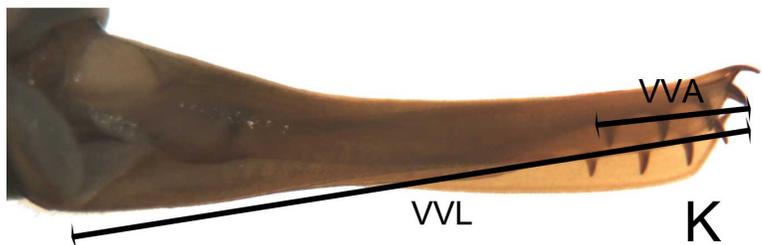
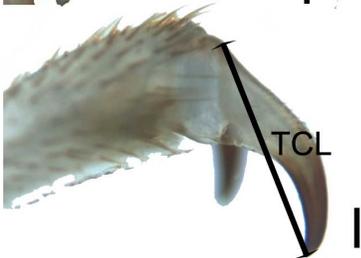
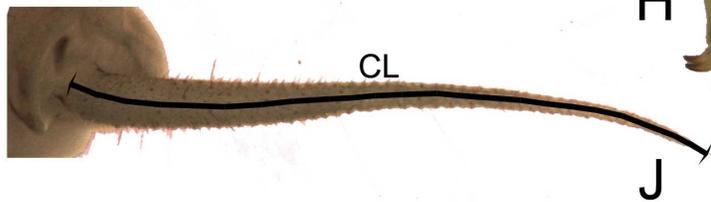
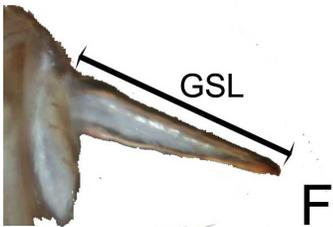
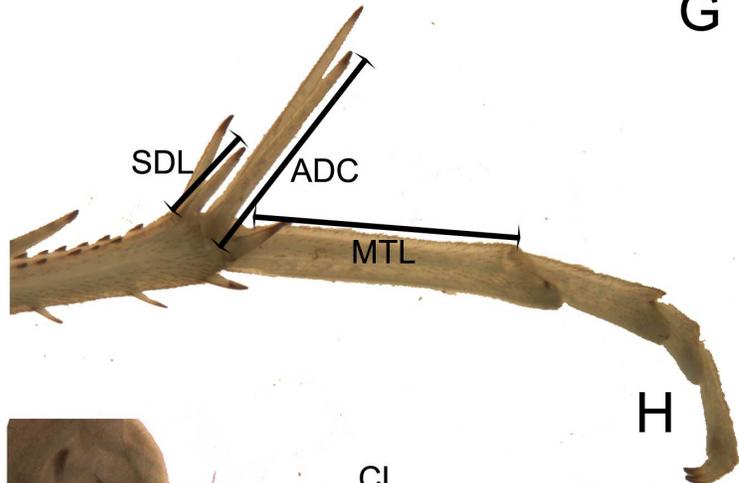
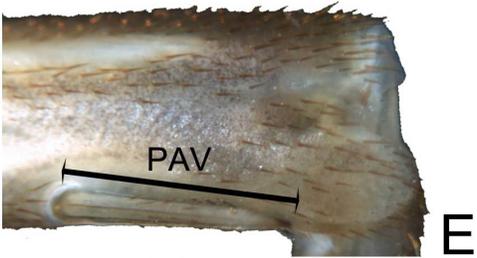
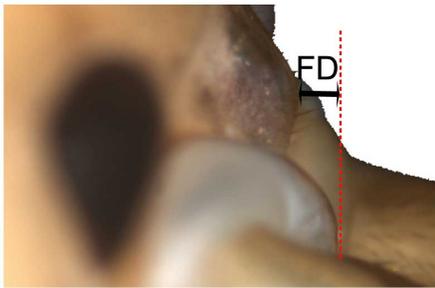
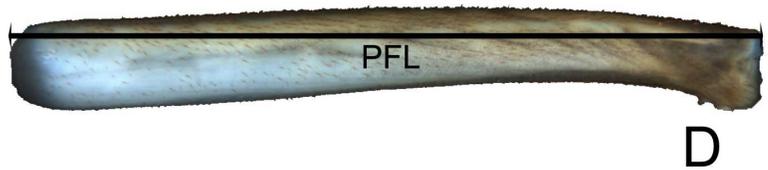
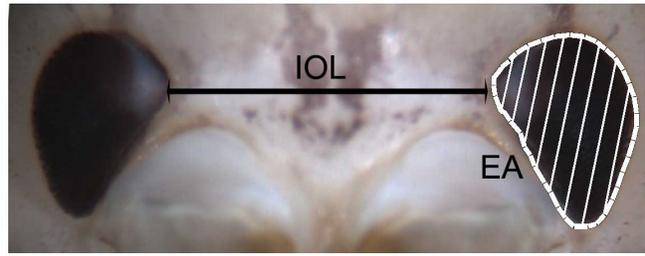
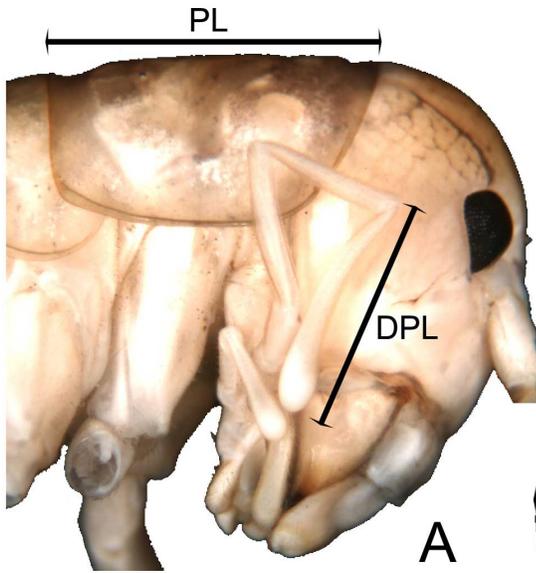


Table 4. Data matrix of continuous characters of female *Ceuthophilus*. Corresponding number for each ratio is listed in Appendix 2 and illustrations of measurements are presented in Figure 5.

<b>Taxon/Ratio</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>
A	0.18	0.45	0.02	0.65	1.56	0.21	0.19	2.99	0.87	0.24	0.52	0.64	0.17	1.27	2.25	0.27
B	0.14	0.34	0.04	0.69	1.77	0.20	0.17	3.27	0.76	0.28	0.49	0.71	0.16	1.13	2.19	0.31
C	0.15	0.37	0.03	0.69	1.67	0.18	0.15	3.00	0.72	0.22	0.56	0.70	0.17	1.17	2.50	0.40
D	0.21	0.32	0.03	0.67	1.63	0.18	0.19	3.19	0.77	0.21	0.52	0.67	0.14	1.23	2.19	0.40
E	0.18	0.37	0.02	0.62	1.62	0.18	0.19	3.16	0.74	0.23	0.55	0.63	0.11	1.20	2.28	0.42
F	0.17	0.35	0.03	0.64	1.83	0.18	0.16	3.27	0.73	0.25	0.60	0.74	0.16	1.27	2.48	0.25
<i>C. (C.) secretus</i>	0.17	0.33	0.05	0.62	1.56	0.22	0.20	3.24	0.84	0.24	0.64	0.66	0.12	1.09	2.15	0.34
G	0.18	0.35	0.04	0.56	1.67	0.16	0.20	3.48	0.84	0.23	0.57	0.76	0.20	1.23	2.12	0.32
H	0.14	0.40	0.02	0.78	1.90	0.21	0.22	3.39	0.77	0.24	0.59	0.85	0.17	1.44	2.27	0.37
<i>C. (C.) conicaudus</i>	0.14	0.39	0.02	0.63	1.44	0.23	0.17	3.10	0.94	0.21	0.50	0.60	0.16	1.24	1.85	0.34
J	0.14	0.34	0.05	0.44	1.80	0.10	0.12	3.02	0.61	0.14	0.27	0.64	0.17	0.86	3.04	0.46
<i>C. (G.) carlsbadensis</i>	0.10	0.36	0.03	0.50	1.44	0.09	0.09	2.61	0.58	0.20	0.31	0.52	0.19	1.03	1.39	0.37
K	0.09	0.33	0.02	0.55	1.42	0.12	0.11	2.35	0.63	0.23	0.35	0.50	0.16	1.07	1.24	0.26
L	0.08	0.34	0.01	0.50	1.32	0.10	0.12	2.40	0.59	0.20	0.31	0.53	0.20	0.98	1.22	0.31
M	0.07	0.34	0.02	0.60	1.34	0.12	0.10	2.22	0.61	0.19	0.33	0.54	0.17	1.18	1.26	0.32
N	0.06	0.34	0.04	0.48	1.32	0.12	0.12	2.17	0.59	0.17	0.36	0.51	0.18	1.02	1.14	0.26
O	0.09	0.36	0.03	0.53	1.60	0.09	0.14	2.63	0.60	0.26	0.38	0.55	0.21	1.19	1.11	0.29
P	0.08	0.19	0.02	0.57	1.41	0.14	0.09	2.49	0.66	0.17	0.28	0.55	0.18	1.09	1.28	0.33
<i>C. (G.) cunicularis</i>	0.07	0.35	0.02	0.53	1.33	0.12	0.12	2.22	0.64	0.14	0.33	0.53	0.16	0.98	1.32	0.34
Q2	0.08	0.34	0.02	0.52	1.47	0.16	0.14	2.34	0.62	0.28	0.40	0.50	0.17	1.20	1.13	0.25
<i>C. (G.) polingi</i>	0.08	0.29	0.02	0.48	1.27	0.14	0.12	2.30	0.67	0.21	0.36	0.51	0.16	0.97	1.18	0.34
S	0.11	0.54	0.05	0.50	2.40	0.19	0.17	3.95	0.95	0.28	0.48	0.89	0.25	1.50	1.96	0.57
T	0.07	0.38	0.03	0.58	1.55	0.14	0.09	2.59	0.63	0.22	0.32	0.49	0.21	1.23	1.16	0.28
<i>C. (C.) longipes</i>	0.13	0.41	0.02	0.72	2.09	-	0.14	3.67	0.64	0.23	0.40	0.87	0.21	0.90	2.83	0.50

## Results and Discussion

### Sequence Attributes

The aligned matrix of 1263 bp of mtDNA sequence for 306 individuals (five outgroup, 301 ingroup) provided a total of 420 variable characters, of which 293 were potentially parsimony informative. Among the 301 *Ceuthophilus* ingroup taxa sequenced for this project, many share identical mtDNA sequences and hence the dataset was reduced to 182 unique haplotypes for phylogenetic analysis. These 182 unique haplotypes were divided among two subgenera, *Geotettix* and *Ceuthophilus*. Fifty-nine unique haplotypes were found in members of the subgenus *Geotettix* and 123 unique haplotypes were found among members of the subgenus *Ceuthophilus*.

Among ingroup taxa in the subgenus *Geotettix*, uncorrected sequence divergence ranged from 0.0 to 2.3% (mean = 1.1%). Whereas for the subgenus *Ceuthophilus*, uncorrected sequence divergence are higher and ranged from 0.0 to 9.3% (mean = 2.7%). These ranges and means are concordant with the pattern seen in the phylograms (Figures 9, 10) of these subgenera.

### Phylogenetic Analyses and Patterns of Genetic and Phylogenetic Diversity

Neighbor-joining analyses of each subgenus produced well-supported phylogenies (Figures 11, 12). The monophyly of both the *Geotettix* and *Ceuthophilus* subgeneric clades were well supported by bootstrapping (88% for the *Ceuthophilus* subgeneric clade, 100% for the *Geotettix* subgeneric clade). The Bayesian mixed-model analysis of the reduced taxon dataset (47 terminal taxa) also produced a strongly supported phylogenetic hypothesis (Figure 13) with all except six nodes supported by  $\geq 90\%$  posterior probability. As expected, the subgenera *Geotettix* and *Ceuthophilus* are reciprocally monophyletic in this analysis. Several salient findings emerged from our analysis, which are discussed below.

First, the neighbor-joining haplotype phylogram for the subgenus *Ceuthophilus* shows deeper internal branches leading to clades than does the haplotype phylogram for the subgenus *Geotettix* (Figures 9, 10). Branch lengths in the Bayesian tree produced from the reduced dataset (Figure 13) also show deeper (older) genetic structure in the subgenus *Ceuthophilus* than that found in the codistributed subgenus *Geotettix*.

At least some crickets in the subgenus *Ceuthophilus* are known to be more vagile than those in the subgenus *Geotettix* (Taylor et al. 2003, 2004, 2005). Taylor et al. (2003, 2004, 2005) found that *Ceuthophilus* (*C.*) *secretus* individuals traveled more than 100 m from their cave entrance to forage at night, whereas *C. (G.) cunicularis* were never observed leaving their caves. Similarly, Veni and Associates (2006) observed only one instance of *C. (G.) cunicularis* exiting a cave during their extensive monitoring at Camp Bullis (Bexar County, Texas). Hence, we predicted that patterns of genetic structure for these two subgenera would differ with the cave-limited subgenus *Geotettix* showing higher levels of genetic structure than those in the subgenus *Ceuthophilus*. Instead, the genetic data differ from our prediction, with the subgenus *Ceuthophilus* having higher levels of genetic variation (uncorrected p-distance 0.0 - 9.3%) than found in *Geotettix* (uncorrected p-distance 0.0-2.3%). This pattern is also borne out in the phylogenetic analyses, which show that across the same geographic area, the subgenus *Ceuthophilus* has longer internal branches, and hence longer times since diversification than the subgenus *Geotettix*. One possible interpretation of this pattern is that subgenus

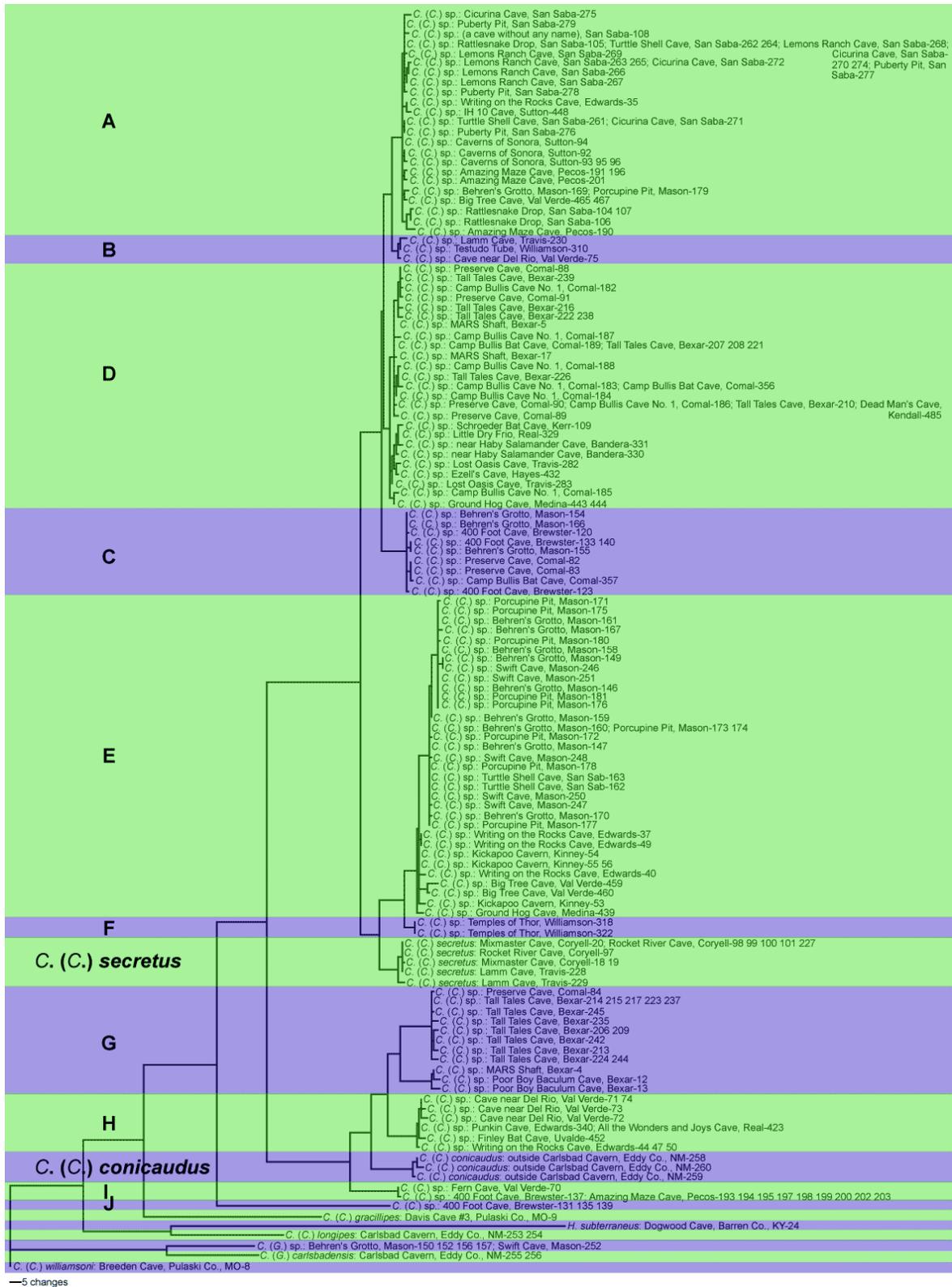
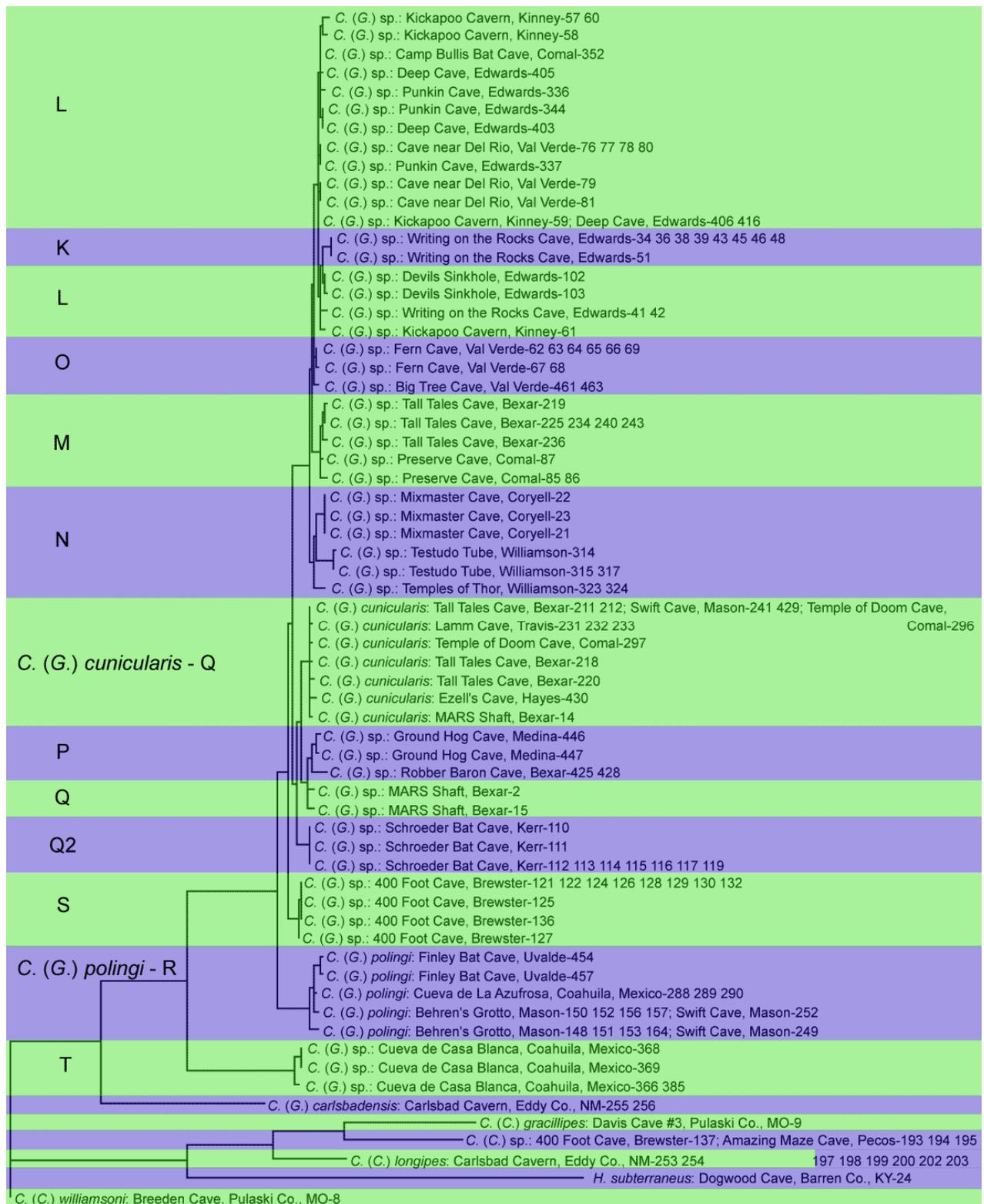


Figure 9. *Ceuthophilus* (*Ceuthophilus*) spp. neighbor-joining tree, constructed from uncorrected p-distances, showing the genealogical relationships of haplotypes. Branch lengths are proportional to uncorrected p-distance as indicated by the scale bar. Samples are given as: *Site Name, County-Specimen Numbers*. Bold letters on left correspond to letters used in text.



– 5 changes

Figure 10. *Ceuthophilus* (*Geotettix*) spp. neighbor-joining tree, constructed from uncorrected p-distances, showing the genealogical relationships of haplotypes. Branch lengths are proportional to uncorrected p-distance as indicated by the scale bar. Samples are given as: *Site Name, County-Specimen Numbers*. Bold letters on left correspond to letters used in text.

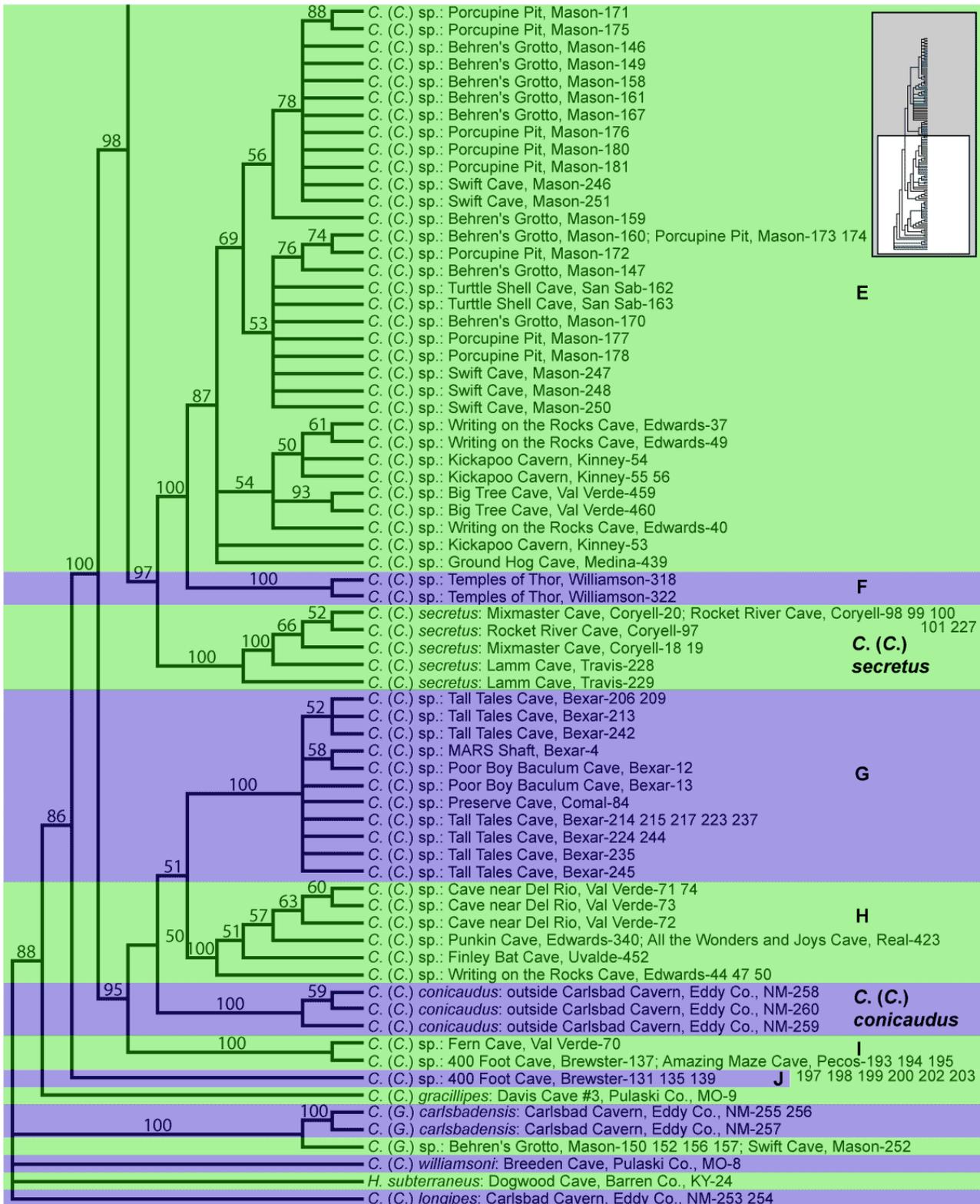


Figure 11. *Ceuthophilus* (*Ceuthophilus*) spp. haplotype neighbor-joining bootstrap consensus tree, constructed from uncorrected p-distances. Numbers above branches are support from 1000 bootstrap replicates (only values >50% are shown). Samples are given as: *Site Name, County-Specimen Numbers*. Bold letters on right correspond to letters used in text. Inset shows portion of tree displayed on this page (in white), remaining tree in inset (in gray) shown on following page as a continuation of this figure. (This figure continued on following page.)

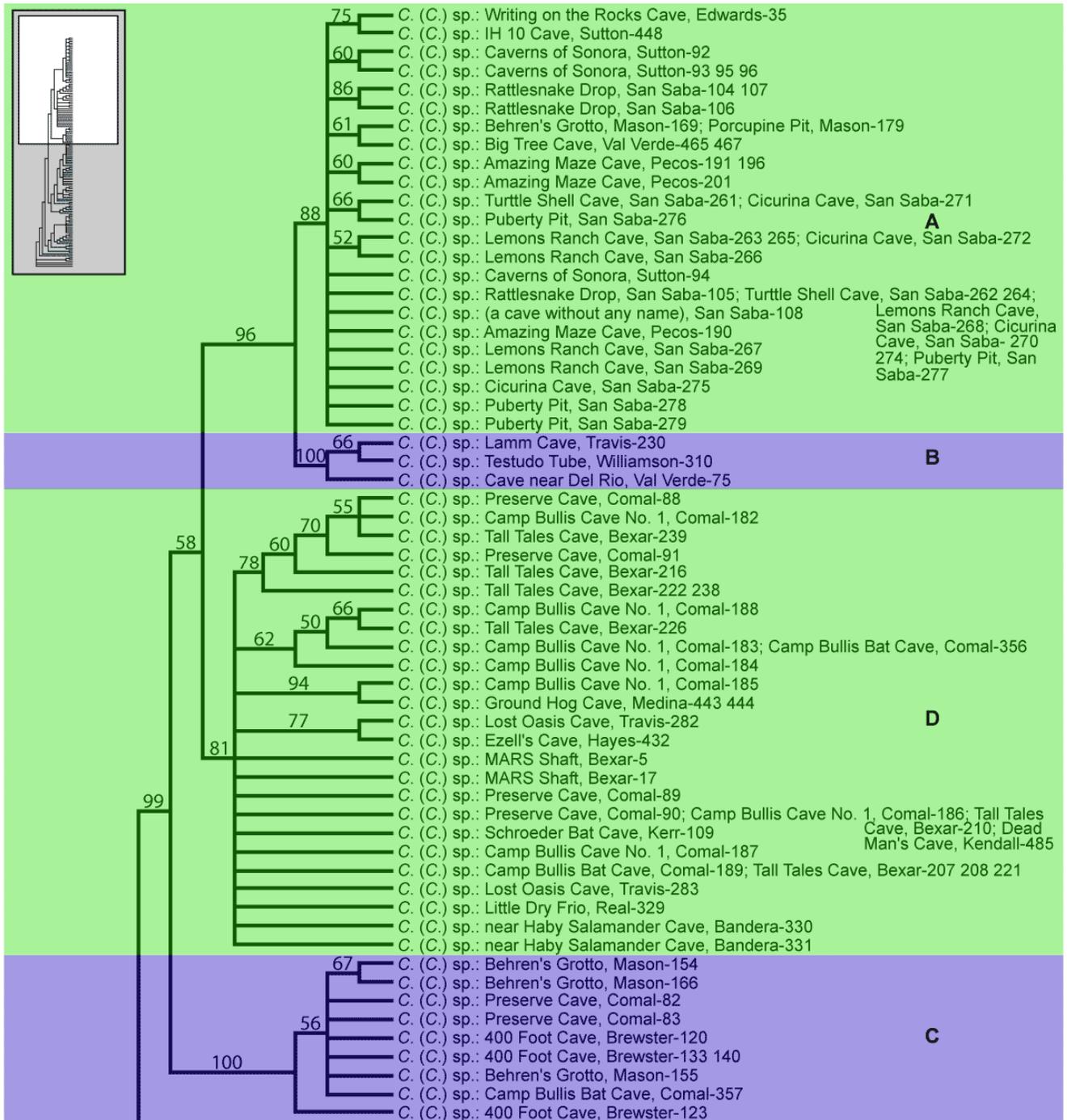


Figure 11 (continued). *Ceuthophilus* (*Ceuthophilus*) spp. haplotype neighbor-joining bootstrap consensus tree, constructed from uncorrected p-distances. Numbers above branches are support from 1000 bootstrap replicates (only values >50% are shown). Samples are given as: *Site Name, County-Specimen Numbers*. Bold letters on right correspond to letters used in text. Inset shows portion of tree displayed on this page (in white), remaining tree in inset (in gray) shown on preceding page as the first part of this figure.

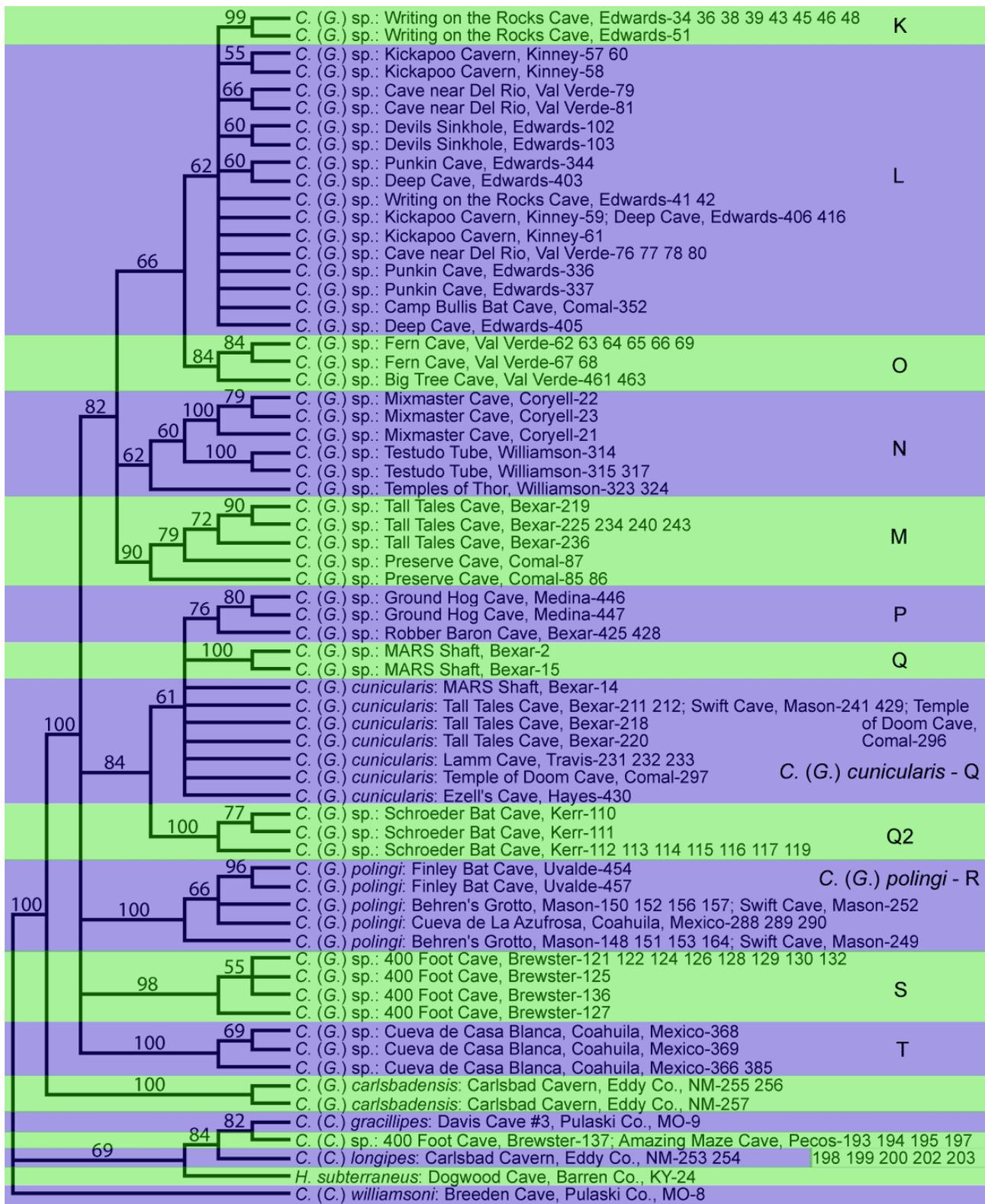


Figure 12. *Ceuthophilus (Geotettix)* spp. haplotype neighbor-joining bootstrap consensus tree, constructed from uncorrected p-distances. Numbers above branches are support from 1000 bootstrap replicates (only values >50% are shown). Samples are given as: *Site Name, County-Specimen Numbers*. Bold letters on right correspond to letters used in text.

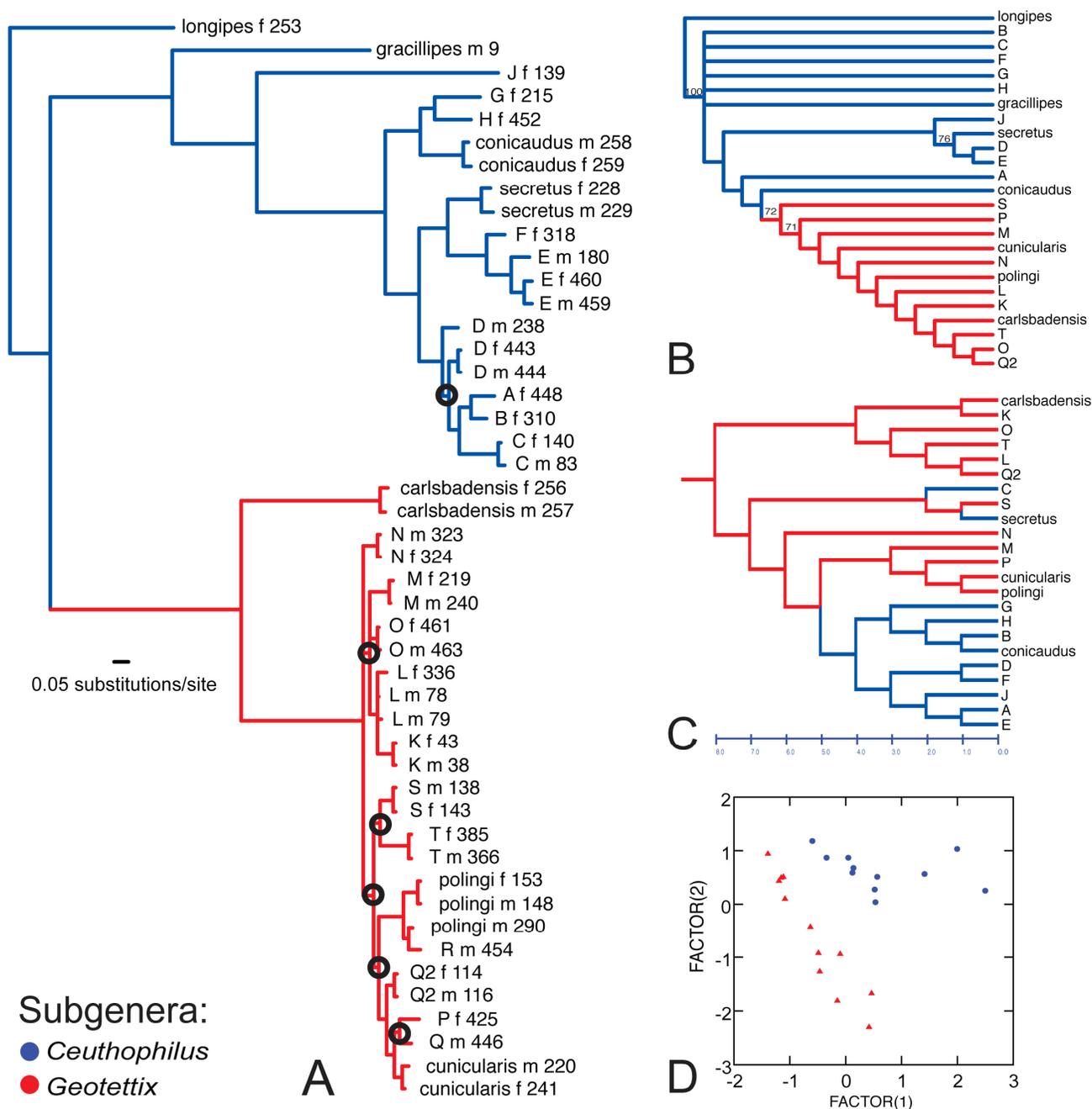


Figure 13. Phylogenetic, cluster, and principal component analyses (PCA) of *Ceuthophilus* species color-coded by subgenus. A: mixed-model Bayesian analysis of combined COI and ND5 sequences (1263 bp), all clades with posterior probabilities >0.9, except those marked with a circle; B: single lineage cluster using Euclidean distances among principal component scores reduced from 17 measurements of females, numbers above branches refer to bootstrap percentages; C: strict consensus of nine most parsimonious trees ( $L=72.810$ ) resulting from the analysis of 19 discrete and 16 continuous characters from males and females; D: 2-dimensional plots of PCA factors reduced from female measurement data of all *Ceuthophilus* specimens studied (>85% of explained variation) grouped by subgenus.

*Ceuthophilus* taxa have been isolated in particular caves or sets of caves for a longer period of time than the codistributed subgenus *Geotettix* taxa.

Second, in the neighbor-joining haplotype tree for the subgenus *Ceuthophilus*, different individual crickets from the same geographic areas (cave localities) fall out in multiple divergent haplotype groups (Figures 9, 11). For example, individual crickets from three distinct (divergent) haplotype groups are found in Behrens Grotto, Mason County (A, C, E), and Writing on the Rocks Cave, Edwards County (A, E, H). Haplotypes from two genetically distinct (divergent) groups are found in Porcupine Pit, Mason County (A, E), Tall Tales Cave, Bexar County (D, G), and Lamm Cave, Travis County, Texas (*C. [C.] secretus*, B). For the subgenus *Geotettix*, fewer caves harbor multiple divergent haplotype lineages (Figures 10, 12). In the case of MARS Shaft, Bexar County (*C. (G.) cunicularis*, Q) and Writing on the Rocks Cave, Edwards County (K, L), the co-occurring haplotype clades are not very divergent and likely do not represent unique species. Even so, Swift Cave, Mason County contains two described species in the subgenus *Geotettix* (*C. polingi*, *C. cunicularis*), and Tall Tales Cave, Bexar County contains two distinctly divergent haplotype clades (M, *C. cunicularis*). Thus, with notably fewer exceptions than in the *Ceuthophilus* subgenus, individual *Geotettix* from the same cave typically fall out into the same haplotype clade within the subgeneric haplotype tree. When both subgenera are considered together, however, there are a surprising number of caves at which more than two haplotype clades co-occur (Figure 15). Some of the caves with only one clade represented are undersampled, with additional clades present, but not represented in our samples.

Previous work has suggested that most caves held two *Ceuthophilus* cricket species, one from the subgenus *Ceuthophilus* and the other from the subgenus *Geotettix*. However, within both subgenera, undescribed species likely exist. Our phylogenetic data confirms this hypothesis and these data are consistent with the species level taxonomy of these cricket subgenera being higher than current taxonomy indicates. Both subgeneric phylogenetic trees show multiple divergent lineages that have likely been on their own evolutionary trajectories for a considerable period of time. Furthermore, the phylogenetic analysis of haplotypes identifies several instances in which individual crickets from the subgenus *Ceuthophilus* collected from the same locality fall out in two

or three different haplotype clades, which suggests that individual caves hold more than the traditionally hypothesized two species. For example, Behrens Grotto, Mason County, has one genetic lineage of *Geotettix* (*C. (G.) polingi*) and three lineages of the subgenus *Ceuthophilus* (A, C, E). Whether these genetic lineages are actually species will require further study, but certainly this pattern

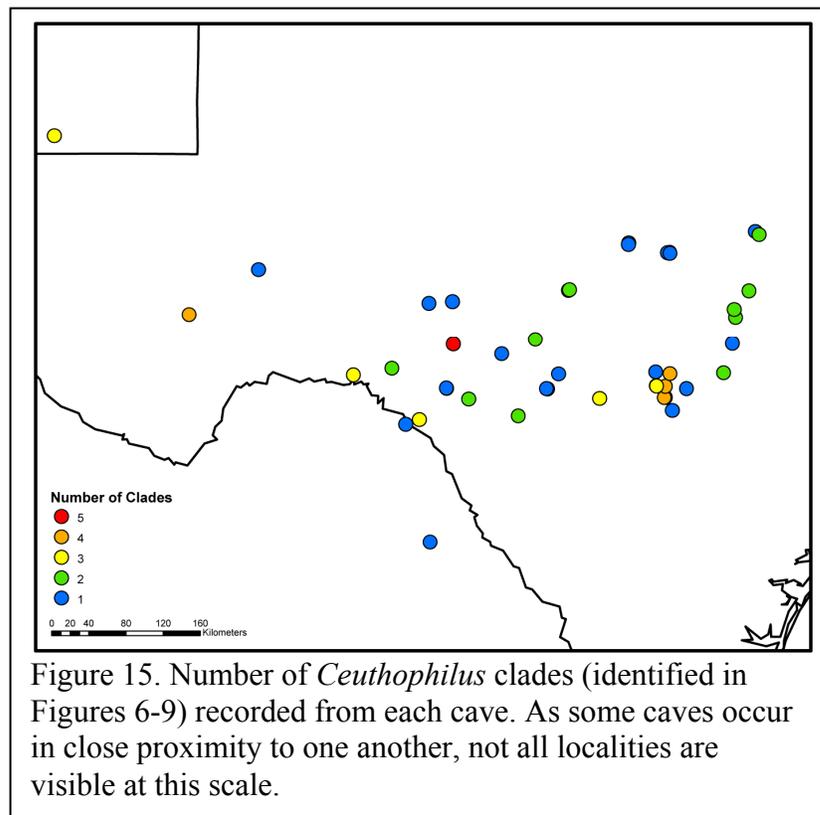


Figure 15. Number of *Ceuthophilus* clades (identified in Figures 6-9) recorded from each cave. As some caves occur in close proximity to one another, not all localities are visible at this scale.

of genetic diversity also suggests, in general, that the genus *Ceuthophilus* may hold more diversity than that described by Hubbell (1936).

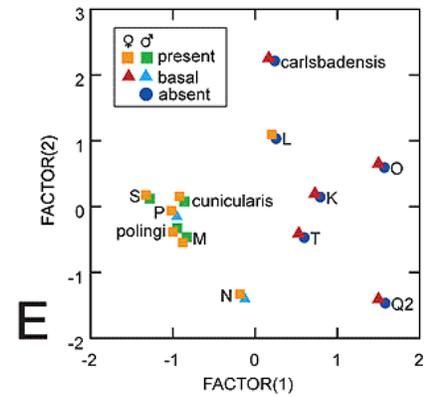
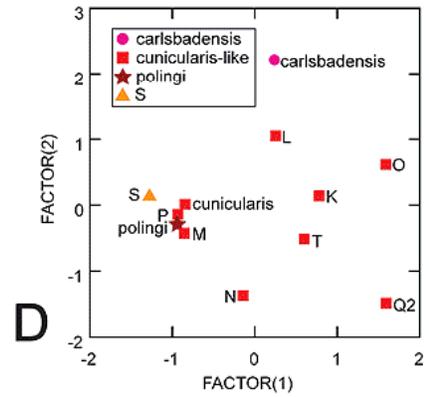
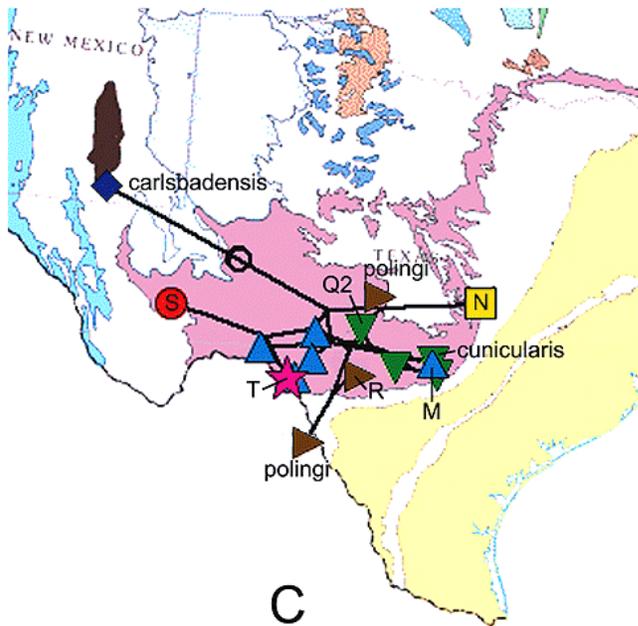
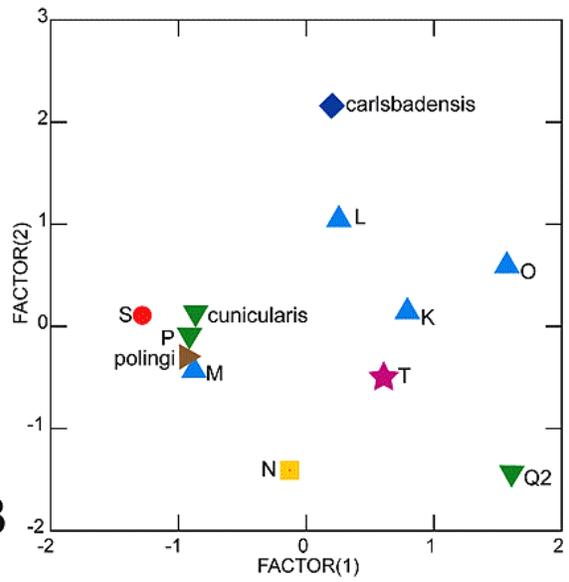
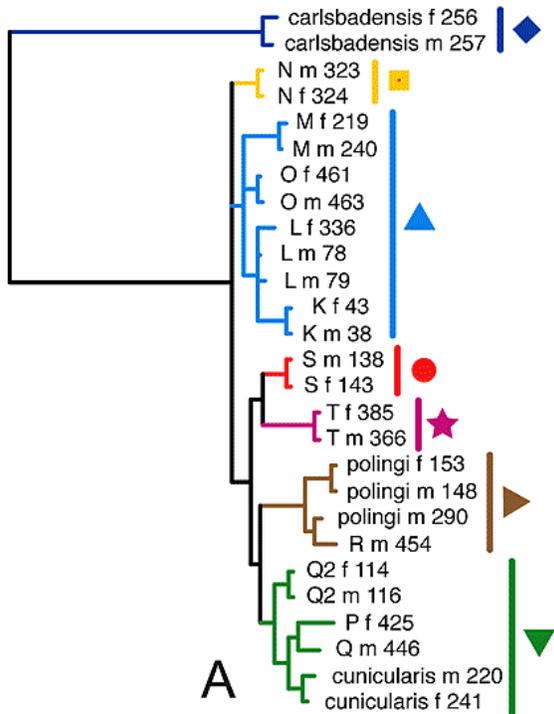
Lastly, our visual analysis of the phylogenetic tree mapped on geography, performed using Mesquite's Cartographer module (Maddison and Maddison 2006a, 2006b), suggests that geographically proximate individuals share more similar mtDNA sequences than do geographically distant specimens (Figure 16). Hence geographic distance may be related to patterns of genetic structure in these two cricket subgenera. For example, two biogeographic patterns stand out in the genetic analysis: 1) a major discontinuity just north of the Colorado River, a probable barrier to distribution, and 2) a discontinuity in far west Texas where karst rocks are not exposed and the surface is extremely arid.

In the subgenus *Ceuthophilus*, the discontinuity to the north of the Colorado River is evident when examining the relationship of clades *C. (C.) secretus*, F and E (Figures 9, 17). The first two clades occur only north of the discontinuity, in northern Travis, Williamson and Coryell counties, and the sister group to F, clade E, occurs only south of the discontinuity. However, clade B (Figures 9, 17) is inconsistent because it has representatives that cluster together from both sides (in particular, Lamm Cave). This inconsistency could arise from a history of multiple founders crossing the barrier at different times, causing one population (or a putative species) from Lamm Cave to cluster with others north of the divide and another to cluster with those that otherwise occur south of the divide. Another explanation is that clade B is all that remains of an older lineage that was widespread prior to the development of the Colorado River as a formidable barrier to distribution. A third possibility is that the historic path of the Colorado River swung as far north as Lamm Cave such that at some times in history that area was south, or very close to the Colorado River.

For *Geotettix* subgenus, clade N is entirely north of the Colorado River, while all the other clades, with the exception of one population, occur south of the Colorado River (Figures 10, 18). Again that exception is Lamm Cave in Travis County. The same possibilities exist for this inconsistency, and both subgenera deserve a higher density of sampling in the area to help clarify their evolutionary history. Other cave taxa show a similar pattern of species break at the Colorado River. The aquatic asellid isopod *Lirceolus* spp. occurs only south of the Colorado River, and the aquatic cirolanid isopod *Cirolanides texensis* occurs only south of the Colorado River with one exception, Longhorn Caverns (Krejca 2005). The troglobitic harvestman genus *Chinquipellobunus* occurs only south of the Colorado River, and the troglobitic harvestman *Texella reddelli* occurs only south while closely related *Texella* congeners occur north of this divide. Undoubtedly a more thorough search of

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Figure 16 (on following page). Phylogenetic and principal component analyses (PCA) of the subgenus *Ceuthophilus* (*Geotettix*). Genetic lineages are grouped based on major clades, which were color-coded and designated by a different symbol. A: cropped Bayesian analysis of COI+ND5 sequences; B: 2-dimensional plots of PCA factors reduced from female measurement data of specimens studied by genetic groups following part A of this figure; C: plot of phylogenetic tree over map of south-central USA using the information of geographical coordinates of terminal taxa, grouped following part A of this figure; D: 2-dimensional plots of PCA factors reduced from female measurement data of specimens studied grouped by pseudosternite shape; E: 2-dimensional plots of PCA factors reduced from female measurement data of specimens studied grouped by aspect of setae row on plantar surface of metatarsomere I of females and males. Geological/aquifer map in C adapted from Miller (1998).



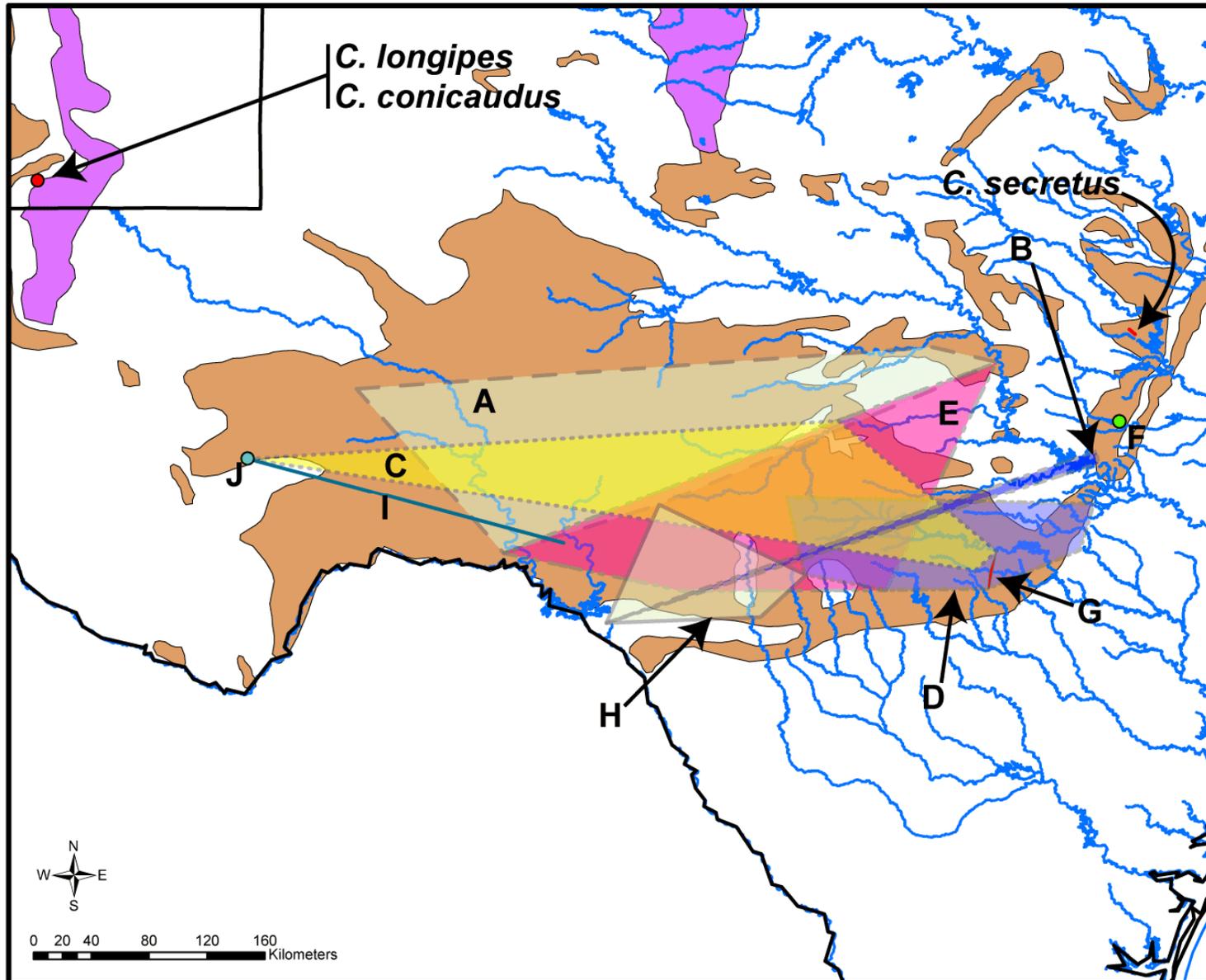


Figure 17. Distribution of clades sampled for *Ceuthophilus* (*Ceuthophilus*) spp. Clades recorded from a single site are indicated by small circles (*C. [C.] longipes*, *C. [C.] conicaudus*, F, J); clades recorded from two sites are indicated by lines (*C. [C.] secretus*, I); clades recorded from three or more sites are indicated by minimum convex polygons (A, B, C, D, E, G, H).

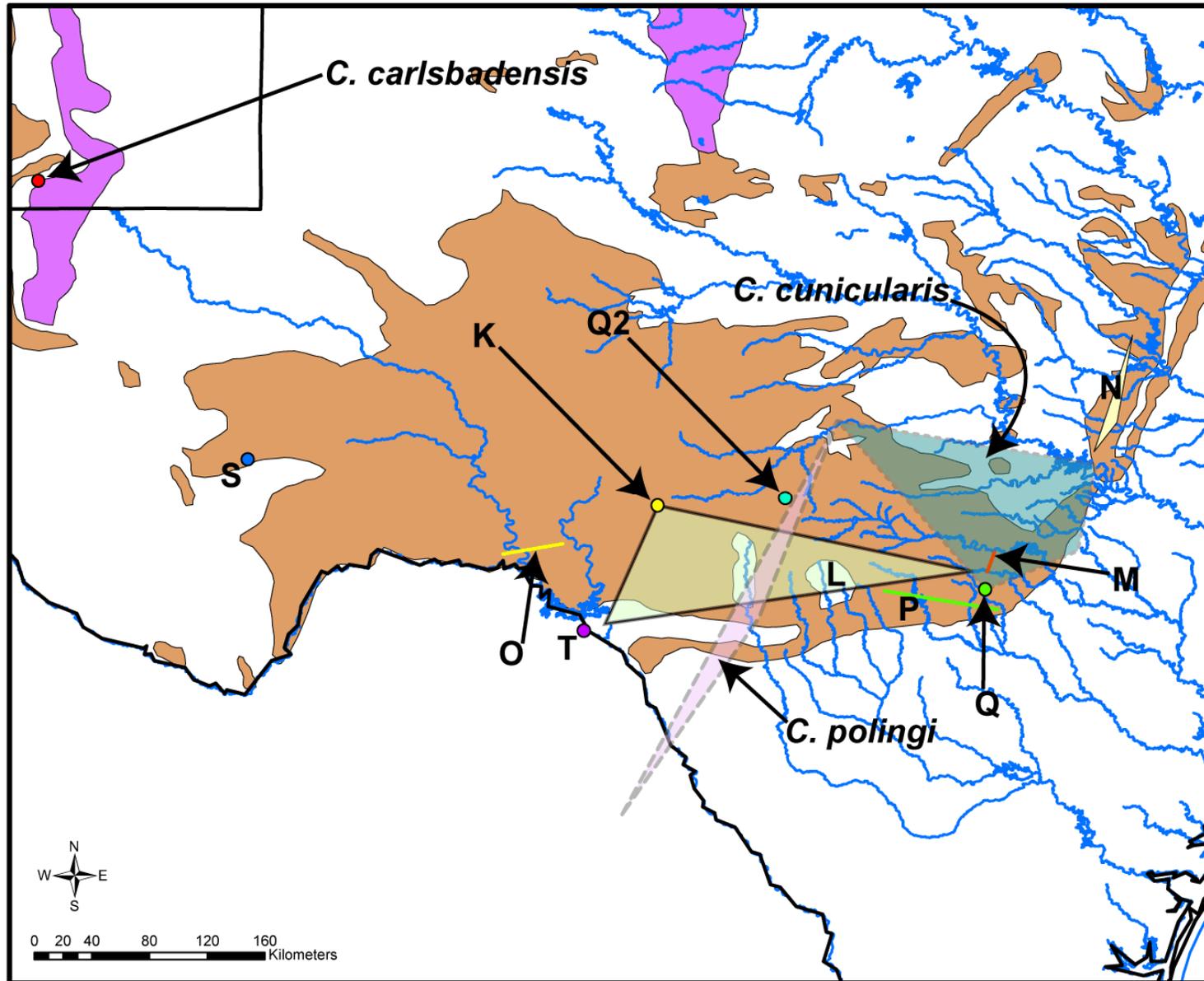


Figure 18. Distribution of clades sampled for *Ceuthophilus (Geotettix)* spp. Clades recorded from a single site are indicated by small circles (*C. [G.] carlsbadensis*, K, S, T, Q, Q2); clades recorded from two sites are indicated by lines (M, O, P); clades recorded from three or more sites are indicated by minimum convex polygons (*C. [G.] cunicularis*, *C. [G.] polingi*, L, N).

the distribution of cave taxa would reveal more examples of the pattern. A phylogenetic analysis of approximately 18 species of troglobitic *Cicurina* spiders across central Texas did not yield any examples of populations that occurred on both sides of the Colorado River, though this hypothesis was not explicitly tested or discussed (Paquin and Hedin 2004). In *Eurycea* salamanders, the Colorado River represents the most basal node in the phylogenetic tree, dividing the northern group from all other lineages (Chippindale et al. 2000). In *Plethodon* salamanders, phylogenetic analyses showed a major phylogenetic break several kilometers north of the Colorado River, and hypothesized that these populations immediately north of the river clustered with southern populations because they occurred on terrace deposits that were located south of the river until the Pleistocene (Baird et al. 2006). Our data do not perfectly fit this model because our populations north of the river are not on terrace deposits.

Regarding the discontinuity of populations in west Texas and Mexico, both subgenera show similar but not totally consistent patterns. For the subgenus *Ceuthophilus*, a deep branch separates one population from 400 Foot Cave (Clade J) from the rest of Texas and some of New Mexico. This partially correlates with the Pecos River as a barrier, except that Amazing Maze Cave population clusters with others from east of the Pecos (Figure 17). Possibly in the farther upstream reaches of the Pecos, near Amazing Maze Cave, the River more frequently runs dry and does not pose a significant barrier to migration. As in the example at Lamm Cave, above, 400 Foot Cave also has a population that clusters with Clade C, probably a result of multiple invasions, historic widespread species, and changes in past climactic conditions that may have taken down present-day barriers.

In the subgenus *Geotettix*, there are deep branches separating isolated species in New Mexico and Mexico [*C. (G.) carlsbadensis* and Clade T] corresponding to the desert in far west Texas and the Rio Grande (Figure 18). As in the previous discussion, however, there are some inconsistencies with the Rio Grande as a barrier because one of the two Mexico populations examined, *C. polingi* in Cueva de La Azufrosa, clusters with those from Uvalde and Mason counties. Molecular phylogenetic analysis of aquifer isopods in west Texas and northern Mexico also showed deep splits at the Pecos and Rio Grande rivers (Krejca 2005), and distribution breaks of aquifer asellid isopod species (Lewis 2001) occur at the non-cavernous region between the Carlsbad Cavern, New Mexico area and west Texas (white space in west Texas, Figure 17).

Our analysis shows that cave cricket genetic distance is dictated by geographic distance, but discussion herein demonstrates that major barriers present in other cave taxa are also shared by crickets. The common occurrence sympatric lineages at the same caves complicates our analysis, and in some areas, such as those immediately adjacent to large barriers (Colorado, Pecos, Rio Grande rivers), more sampling will be needed to clarify these relationships. Additionally, further post-hoc hypothesis testing specific to the presumed barriers, such as with a parametric bootstrap analysis (similar to those done in Krejca 2005) and Mantel tests of genetic distances versus great circle geographic distances, could improve our understanding of the role those barriers play. Such analyses will be incorporated into our study prior to final publication in a peer-reviewed journal.

### Morphological species

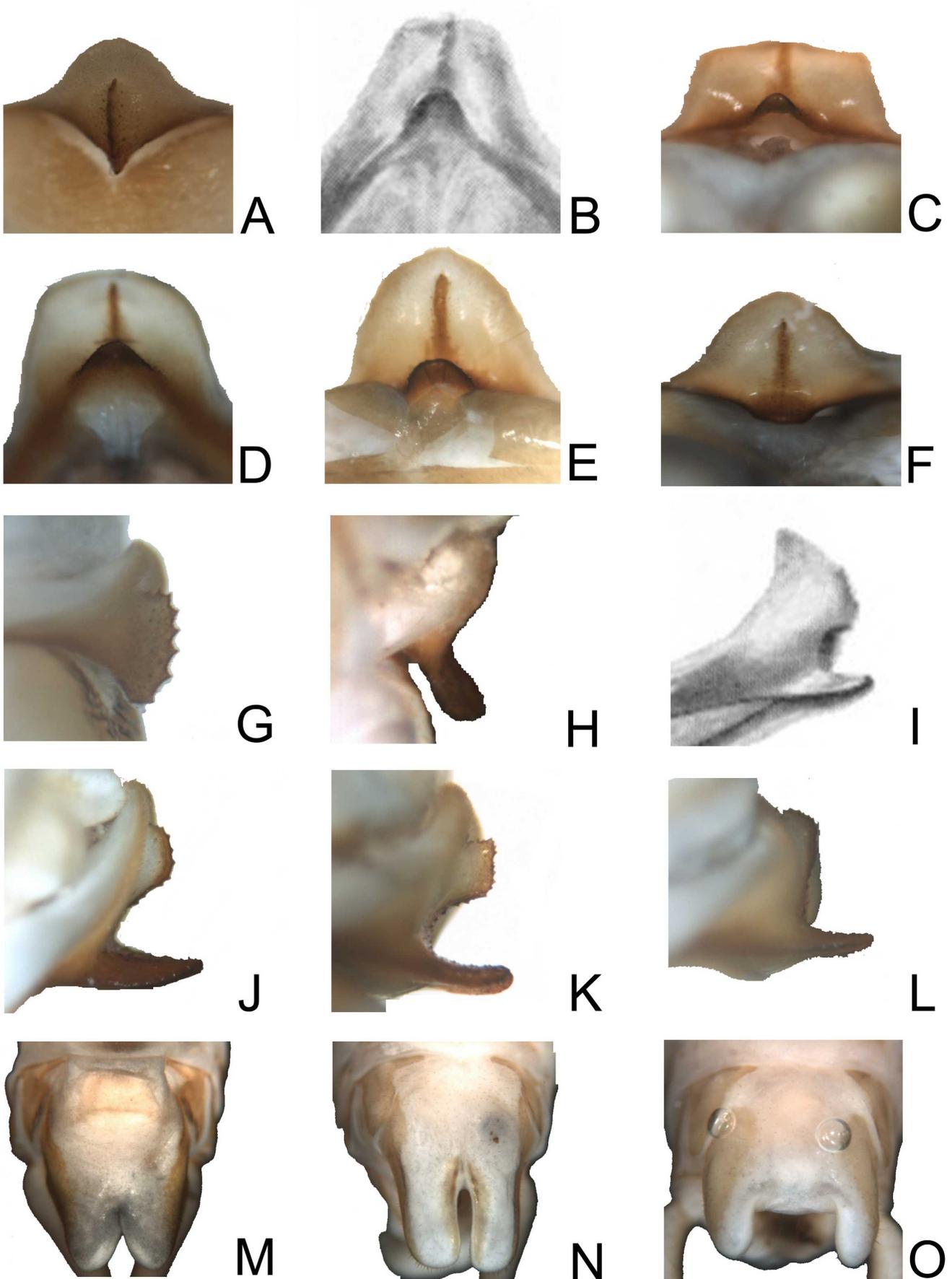
Morphological species concepts were mostly based on the shape of the sclerotized pseudosternite, which Hubbell (1936) used extensively in his taxonomic revision of the genus *Ceuthophilus* and considered that “The modifications of this structure are of the utmost taxonomic value in the study of the genus.” Based on the study of the pseudosternite morphology, the identity of *C. (C.) secretus* and *C. (G.) carlsbadensis* was confirmed. One male specimen previously identified as

*C. (C.) conicaudus* (#258) keys to this species using Hubbell (1936), but the arch dorsum of the pseudosternite in the specimen is a bit broader and less projected than in the specimen figured by Hubbell (1936) and has two ventral subdistal spurs of metatibiae. More than 95% of *C. (C.) conicaudus* specimens (n>80) studied by Hubbell had a single subdistal spur, which he considered a relatively constant character and used it as a main differentiating character of *C. (C.) conicaudus* and *C. (C.) secretus*, 98% of the latter having two subdistal spurs. However, this male specimen and sympatric female (#259) lack the main reliable diagnostic characters of *C. (C.) secretus*: (1) pseudosternite arch internally with two sclerotized tumid lobes (Figure 19F); (2) paraprocts with short and stout spines (Figure 19C); and female ovipositor with six apical teeth. The specimens were collected in Carlsbad Cavern, Eddy County, New Mexico, and specimens of *C. (C.) conicaudus* from caves in Dark Canyon, just north of Carlsbad Caverns National Park and 20.1 km southwest of the city of Carlsbad were studied by Hubbell (1936) when he described the species as new. In addition, several other researchers studied crickets in southeastern New Mexico, and these, too, refer to *C. (C.) conicaudus* (Barr and Reddell [1967], Campbell [1976], Cokendolpher [2001], Cokendolpher and Polyak [1996, 2004], Northup and Kuper [1987], and Northup *et al.* [1993]; see Lavoie *et al.* [2007] for a review of literature). Therefore, this lineage is herein referred to as *C. (C.) conicaudus*, despite the incongruencies.

Other morphological species belonging to the subgenus *Ceuthophilus* (*Geotettix*) were herein identified as *C. (G.) cunicularis*, and *C. (G.) polingi*, with clade S also seeming markedly unique (see discussion below). Male specimen #220 keys unequivocally to *C. (G.) cunicularis*, a species described from Texas (Bexar, Hays, and Travis counties). Hubbell (1936) relates this species to *C. (G.) polingi* and *Ceuthophilus* (*Geotettix*) *umbratilis*, but distinguishes it because of the male terminalia. Although all three species mentioned share a similar median longitudinal crest (Figure 19A-F), only *C. (G.) cunicularis* has a median triangular projection on the margin of dorsal rim, recurvate in lateral view (Figure 19G, I-L). Interestingly, this basic pseudosternite shape is also found in almost all genetic lineages of *Ceuthophilus* (*Geotettix*) (Figure 16D). However, males of all these other lineages do not key to *C. (G.) cunicularis* basically because the plantar surface of their metatarsomere I does not have a complete row of setae along most of its length (Figure 7I, 16E). The only exception is lineage M, which does have a complete row of setae (Figure 7J), but the projection on the margin of dorsal rim is much broader and less triangular than that described for *C. (G.) cunicularis*. Furthermore, although all these lineages do share the same basic plan, there is quite a bit of intergrading variation of some key characteristics in the male pseudosternite; such as, in the shape and size of the dorsal rim process, the degree to which this process is reflected upward, and the height and shape of the dorsal crest. This variation is further made difficult to quantify due to subjective artifacts as the positioning of the sclerite. Additionally, the male specimen from Comal County has the profemur anteroventral carina (Figure 7G: PAV) with 2-3 spurs and mesofemur anteroventral carina with five spurs, consistent with the original description of *C. (G.) cunicularis*. Therefore, only these specimens from Comal County are henceforth referred to as *C. (G.) cunicularis*, while other members of the Q lineage (including Q2) are not.

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Figure 19 (on following page). Male genitalia structures of *Ceuthophilus* (*Geotettix*) species. A-F, pseudosternite arch dorsum, dorsocaudal view; A: *C. (G.) polingi*; B: *C. (G.) cunicularis*, modified from Hubbell (1936); C: *C. (G.) cunicularis*; D: lineage T; E: lineage Q2; F: lineage K; G-L: pseudosternite arch dorsum, lateral view; G: *C. (G.) polingi*; H: lineage S; I: *C. (G.) cunicularis*, modified from Hubbell (1936); J: lineage Q2; K: lineage L; L: lineage K; M-O: subgenital plates, ventral view; M: lineage L; N: *C. (G.) polingi*; O: lineage S.



A number of the divergent clades in the mitochondrial haplotypes trees may represent undescribed and/or cryptic species. For example, molecular lineage S may be morphologically and geographically distinct enough for it to be considered a new species within the subgenus *Geotettix*. Lineage S is separated geographically and geologically from other *Ceuthophilus* (*Geotettix*). We recorded this species only from 400 Foot Cave, outside the Edwards Plateau in the Glass Mountains of Brewster County, Texas. Other specimens collected in this cave are also very interesting and fall in lineages I and J of *Ceuthophilus* (*Ceuthophilus*), but unfortunately no adult specimens were collected of lineage I and only 2 adult females were collected from lineage J. Although, not highly genetically (Figure 13A) or morphometrically (Figure 13B) divergent from other *Ceuthophilus* (*Geotettix*) studied herein, the male genital characters of lineage S are quite distinct. Lineage S has very distinct subgenital plates with apicolateral projections very widely separated (Figure 19O), a pseudosternite arch with a dorsum lacking the median longitudinal crest, and a dorsal rim with a very broad subquadrate median process which is not distinctly upturned like other *cunicularis*-like pseudosternites (Figure 19H). Finally, it is worth noting that Cokendolpher and Polyak (1996) indicate that *C. (G.) carlsbadensis* is known from Brewster, Culberson, and Jeff Davis counties in Texas – it is possible that some of the collections to which they refer are attributable to our lineage S. More material from this area should be subject to morphological analysis to assess the range of structural variation before the taxonomic status of lineage S is finally determined.

Finally, some specimens belonging to the genetic lineage R were identified as *C. polingi* based on the distinctive pseudosternite morphology (Figure 19G, N). *Ceuthophilus (G.) polingi* was described based on two males from Davis County, Texas. This species is therefore herein recorded for the first time from Mason and Uvalde counties, Texas and Coahuila, Mexico. Strangely, a very large male specimen (#454) grouping with the remaining *C. (G.) polingi* (Figure 16A) from approximately the middle of this range (Uvalde County, Texas, see Figure 16C), not only has a *cunicularis*-like pseudosternite, but also lacks the complete row of setae along the plantar surface of the metatarsomere I (Figure 7I) diagnostic of *C. (G.) polingi*.

#### Congruence of DNA-based phylogeny and morphological data

The Bayesian phylogenetic analysis is based on DNA-sequences (Figure 13A), parsimony analysis of combined continuous and discrete morphological characters (Figure 13B), and cluster analysis based on distances of the principal component scores (Figure 13C). Overall, there is almost no congruence in the relationships suggested by each methodology and dataset. The only striking similarity is that both the molecular and combined morphology datasets generally support the two subgenera as distinct. They are monophyletic sister groups in the molecular analysis (Figure 13A), and one morphology tree shows *Geotettix* as a monophyletic lineage of *Ceuthophilus* (Figure 13B), while the other configures *Geotettix* as paraphyletic and *Ceuthophilus* as polyphyletic (Figure 13C). Although, the PCA score cluster resulted in a non-congruent hypothesis with the other datasets (Figure 13C), the 2-dimensional plots were successful in separating members of the two different subgenera (Figure 13D) and, considering the variability of some key characters (for example, the variation of characteristics in the male pseudosternite, discussed earlier), may be generally more useful when comparing morphology to genetics.

In the *Ceuthophilus* (*Ceuthophilus*) lineage, the male genitalia of relatively fewer specimens were studied because of the lesser availability of adult forms. Although the clades recovered by the Bayesian analysis had good statistical support (based on posterior probabilities), the three defined multi-lineage groups (Figure 20A, red circle, blue square, and green diamond) were not supported by the parsimony analysis of morphological characters (Figure 13B) and were not distinctly grouped in

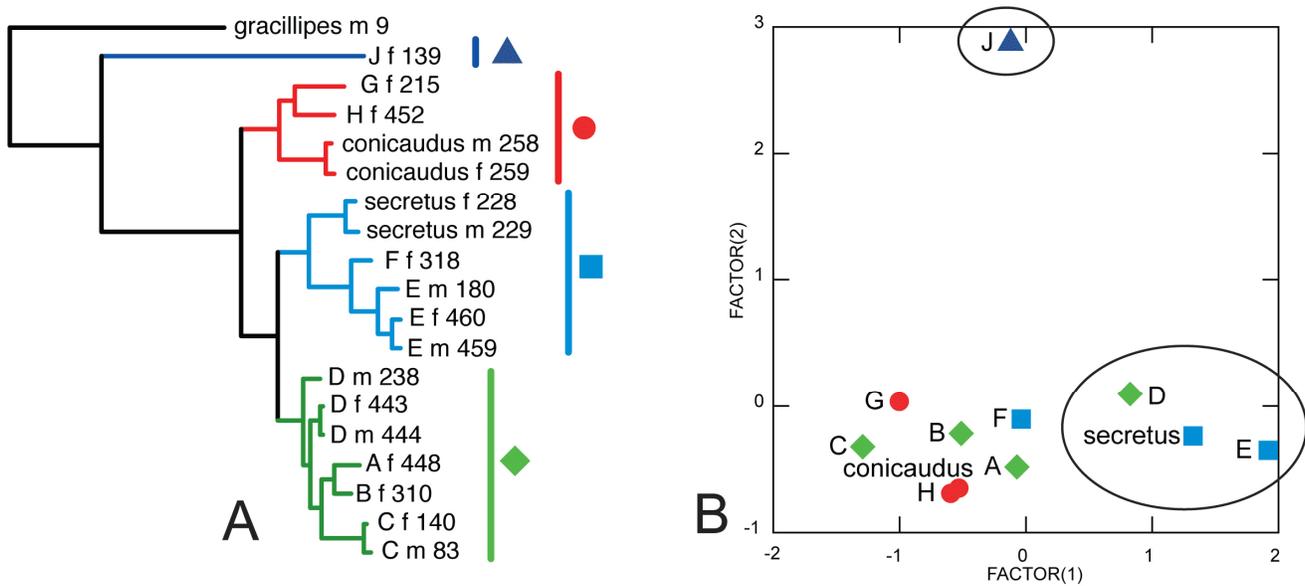


Figure 20. Phylogenetic and principal component analyses (PCA) of the subgenus *Ceuthophilus* (*Ceuthophilus*). Genetic lineages are grouped based on major clades, which were color-coded and designated by a different symbol. A: cropped Bayesian analysis of COI+ND5 sequences; B: 2-dimensional plots of PCA factors reduced from female measurement data of specimens studied by genetic groups following part A of this figure. Two distinctly morphometric groups are circled.

the 2-dimensional PCA plots (Figure 20B). Only one of the multi-lineage groups (*C. (C.) conicaudus* + G + H) (Figure 20A) was somewhat similar to the strict consensus of nine most parsimonious trees (Figure 13C). However, the PCA analysis shows a large separation of lineage J from other members of the subgenus, as does the DNA analysis (Figure 20). Furthermore, the morphometric data based on females supports the grouping of *C. (C.) secretus* + lineages D + E (Figure 20B), which was not recovered with the DNA analysis (Figure 20A). Both males and females of these three lineages have the distinguishing characters abovementioned for *C. (C.) secretus*. The DNA analysis also supports the grouping of lineage F within the *C. (C.) secretus* lineage. However, males of lineage F were not available for study, and although geographically consistent with *C. (C.) secretus* distribution, the female studied bore five teeth in the ventral valve instead of the characteristic six teeth. Aberrant females of *C. (C.) secretus* with only five teeth were previously studied by Hubbell (1936). The lack of information on the male genitalia and the unusual female genitalia of lineage F probably caused it to not group with *C. (C.) secretus* + D + E in the morphological analysis (Figure 13B).

Members of *Ceuthophilus* (*Geotettix*) are differentiated morphologically from other *Ceuthophilus* subgenera by its usually smaller size and more compact shape (shorter legs), ventral carina of the metafemur with several more denticulations, and a distinctive putative synapomorphy of males having the abdominal tergite VIII strongly produced, usually concealing the little-produced tergite IX, with its apex usually tumid and round (Figure 7A) (Hubbell 1936). Another synapomorphy recovered by this study is the presence of a denticle on the anterior genicular lobe of the metafemur in all studied *Ceuthophilus* (*Geotettix*) members. All studied specimens fall into what Hubbell (1936) defined as the *silvestris* group of species. Except for *C. (G.) carlsbadensis*, the phylogenetic history

based on DNA sequences shows very small divergences between members of this closely related group, which contributed to low statistical branch support of some multi-lineage groups (Figure 13A). As abovementioned, most studied males of this group have a *cunicularis*-like pseudosternite, with the exception of lineage S and *C. (G.) polingi* which appear to be confidently delimited morphological species. However, both of these lineages do not show strikingly longer branch lengths than other lineages within this clade. Whether both species are indeed genetically separated from the other *cunicularis*-like lineages is impossible to say with such a small taxon sampling in this analysis and lacking statistical support for the relevant multi-lineage clades in the phylogeny.

Finally, it is interesting to note a couple of minor incongruencies between the resulting DNA-sequence topology (Figure 16A) and morphometric analysis (Figure 16B) of *Ceuthophilus (Geotettix)*. A male specimen from Bexar County included in lineage M (#240) and sympatric female (#219), based on DNA sequences, appear to be more related to lineages K, L, and O (blue triangle group). However, the female is much more similar morphometrically to the group including *C. (G.) cunicularis*, P and Q (upside-down green triangle), both males and females have a complete row of plantar setae on metatarsomere I like *C. (G.) cunicularis* (Figure 16E), and their distribution seems more congruent with the green group (Figure 16C). Conversely, the male specimen (#116) belonging to Q2 and positioned in the upside-down green triangle seems more similar to members of the group containing lineages K, L, and O (blue triangle group). Although this specimen from Kerr County is not distinctly dissimilar in genitalic characters from males of the Q lineage, the sympatric female (#114) is morphometrically very distinct from most other *Ceuthophilus (Geotettix)* studied (Figure 16B). The metafemur armature is very well-developed similar to the female specimen from lineage O. Similar to the blue group, males lack the complete row of and females have a few basal setae on the plantar surface of metatarsomere I (Figure 16E).

Concerning the characters of presence and extent of the ventral row of setae on the plantar surface of metatarsomere I (Figure 7I, J), Hubbell (1936) considered the presence of these setae (*Type B* armature) a “retention by the adult of a condition universal among early instars, but which in most species is lost in some pre-adult stadium.” He further diagnosed the *silvestris*-group based on this *Type B* tarsal armature, with the exception of *C. (G.) carlsbadensis*, which normally has tarsus *Type A* (Hubbell 1936). Although he did not mention this character or specify the sexual dimorphism in the species descriptions of this group, it is used in both male and female taxonomic keys to species of *Ceuthophilus*. It is reported here as many additional lineages of the *silvestris*-group, which share a similar pseudosternite shape with *C. (G.) cunicularis*, but like *C. (G.) carlsbadensis*, their males lack setae on the plantar surface, and in all cases (except in lineage L) the females do have basal setae (Figure 16E). Clearly, the morphological character set used by Hubble (1936) (and in the present study) is inadequate to explain species-level differences among lineages. A search for additional characters, including the use of characters the male phallus (not examined in this study) should be undertaken, and future taxonomic decisions within the genus *Ceuthophilus* also should take genetic data into consideration.

## Conclusions

Both *Ceuthophilus* subgenera show considerable geographic and phylogenetic structure, as well as significant morphological variation. The longer branch lengths and morphological variation found in the subgenus *Ceuthophilus* suggests less genetic communication among populations in this subgenus relative to the subgenus *Geotettix*, a finding that is surprising and contrary to our expectation, because members of the subgenus *Ceuthophilus* commonly forage outside of caves in central Texas. In

addition, some caves have multiple mitochondrial lineages in them that possibly represent cryptic species, implicating that diversity is higher than previously expected. We identified 22 different clades (*C. [Ceuthophilus]* spp. [10 clades]: A, B, C, D, E, F, G, H, I, J, C. [*C. secretus*]; and *C. [Geotettix]* spp. [12 clades]: K, L, M, N, O, P, Q, Q2, R, S, *C. [G.] cunicularis*, *C. [G.] polingi*) in Texas caves which belong to the genus *Ceuthophilus* and are on independent evolutionary trajectories. Some of these clades may represent undescribed or cryptic species, and thus suggests that biotic diversity in *Ceuthophilus* is higher than current species level taxonomy indicates. These data point towards a need for additional conservation attention in a variety of Texas cave systems, and additional research, including additional genetic and traditional taxonomic work, are needed to make educated conservation decisions regarding the diverse fauna found in these cave systems.

## Acknowledgements

Many people helped make this report possible. In addition to the innumerable landowners and land managers, both private and government (including Texas Parks and Wildlife, The Nature Conservancy, The Texas Cave Management Association, U.S. Fish & Wildlife, Fort Hood, Camp Bullis, Fort Leonard Wood, Caverns of Sonora, etc.) and the cavers and other who created maps of caves that facilitated our visits, or helped out in phone calls or conversations in finding caves and helping us gain access, the following individuals made special efforts to help us acquire the samples needed for this study: Peter Sprouse, Kristopher Megahan, N. Parker, Rob Meyers, Peter Sprouse, A. Gluesenkamp, Vivian Loftin, Cindy A. Lee, Justin Shaw, Alan King, Jim Kennedy, M. Sanders, K. O'Connor, J. Chenowith, Alan King, Wayne Peplinski, Don Evan Selby, Allan Cobb, C. Phillips, C. Pekins, Bill Sawyer, Michael Cunningham, Michael E. Slay, Kurt L. Helf. Many of the above individuals participated in multiple trips and spent long hours driving between sites, crawling through caves, and fending off rattlesnakes. We thank Don Selby, who spent time trying to find *Ceuthophilus secretus* in the crawlspaces of homes in Dallas (the type locality). JoAnn Jacoby reviewed an earlier draft of this report. We apologize to those we've undoubtedly overlooked

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## Appendix 1. Morphological discrete characters and states.

### Male terminalia:

1. Abdominal tergite VIII: (0) well produced farther than tergite IX (Figure 4A); (1) not produced farther than state IX
2. Subgenital plates: (0) fused basally and apicolateral portions produced (Figure 4B); (1) divided basally by sulcus and apicolateral portions not distinctly produced
3. Epiproct shape: (0) triangular (Figure 4C); (1) subquadrate
4. Paraprocts sculpturing: (0) without stout spines; (1) with short and stout spines (Figure 4C)
5. Pseudosternite dorsum: (0) without longitudinal crests (Figure 4D); (1) with median crest; (2) with pair of parallel crests (Figure 4E)
6. Pseudosternite dorsal rim of arch: (0) without processes (Figure 4D); (1) with median process
7. Pseudosternite median process of dorsal rim of arch: (0) triangular; (1) broad, quadrangular
8. Internal wall of arch: (0) without folds; (1) with pair of tumid folds (Figure 4F)

### Female thorax and appendages:

9. Pronotal recumbent setae: (0) absent; (1) present
10. Metafemoral anterior genicular lobe denticle: (0) absent; (1) present (Figure 4H)
11. Metafemoral posterior genicular lobe denticle: (0) absent; (1) present
12. Metatibial subdistal ventral spurs: (0) 2:1, (1) 2:1:1; (2) 1:1
13. Metatarsomere I chaetotaxy: (0) absent or few basally (Figure 4I); (1) single row throughout most tarsomere (Figure 4J)

### Female ovipositor:

14. Ovipositor ventral valve number of teeth: (0) 5 (Figure 5K); (1) 6

### Female spur and denticulation counts:

15. Profemoral AV carina spurs (Figure 4G: PAV): (0) absent; (1) 1-7; (2) 9; (3) 11; (4) 23
16. Mesofemoral ventral carinae spurs: (0) absent; (1) 1-12; (2) 17-24; (3) 56
17. Metafemoral ventral carinae denticulations (Figure 4H: VCD): (0) absent; (1) 1-120; (2) 141-200; (3) 242; (4) 497
18. Metafemoral dorsal denticulations (Figure 4G: DD): (0) absent; (1) 1-25; (2) 38; (3) 60-93; (4) 103-110; (5) 129
19. Metatibial basal AD carina denticulations (before basal spur): (0) 7; (1) 9-10; (2) 12-14; (3) 16-17; (4) 19-21

Appendix 2. List of morphometric characters used to calculate the continuous ratios.

1. Eye area (Figure 5B)
2. Interocular distance (Figure 5B)
3. Fastigium distance to mesal margin of antennal fossa (Figure 5C)
4. Maxillary palp distal segment length (Figure 5A)
5. Profemur length (Figure 5D)
6. Profemur AV1 spur length (Figure 5E)
7. Mesofemur posterior genicular spur length (Figure 5F)
8. Metafemur length (Figure 5G)
9. Metafemur width (Figure 5G)
10. Metatibia dorsal subdistal spur length (Figure 5H)
11. Metatibia AD calcar length (Figure 5H)
12. Metatarsomere I length (Figure 5H)
13. Metarsal claw length (Figure 5I)
14. Cercus length (Figure 5J)
15. Ovipositor ventral valve length (Figure 5K)
16. Ovipositor ventral valve armed region length (Figure 5K)