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TO:
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Using a combination of mostly genetic data and analyses, complemented with examination of genital morphology for adult female specimens, we identified **26 eyeless *Cicurina* specimens** from Travis and Hays counties as follows:

Cave	Date collected	Collector	Number of specimens	Agency	Voucher #	Identification
FK-32	8/17/20	T Clark	2 imm	Travis Co.	TCNR 1029A, B	***C. buwata* Chamberlin & Ivie, 1940**
FK-2	12/12/19	J Krejca	1 imm	Zara	ZARA_ 12832	***C. buwata* Chamberlin & Ivie, 1940**
FK-2	12/17/19	J Owen	1 imm	Zara	ZARA_ 12835	***C. buwata* Chamberlin & Ivie, 1940**
FK-2	1/19/20	J Watson	1 imm	Zara	ZARA_ 12982	***C. buwata* Chamberlin & Ivie, 1940**
Adobe Springs	5/17/21	T Clark	2 imm	Travis Co.	TCNR 1029A, B	***C. buwata* Chamberlin & Ivie, 1940**
Cortana Cave	8/31/18	M Sanders	1 imm	City of Austin	SDSU_ G3846B	***C. buwata* Chamberlin & Ivie, 1940**
Collaboration Cave	7/16/18	D Thompson	1 imm	City of Austin	SDSU_ G3840	***C. buwata* Chamberlin & Ivie, 1940**
Rolling Rock Cave	11/14/18	M Sanders	1 imm	City of Austin	SDSU_ G3838	***C. buwata* Chamberlin & Ivie, 1940**
Jester Estates Cave	11/19/19	M Sanders	2 imm	City of Austin	SDSU_ G3837A, B	***Cicurina travisae* (Gertsch 1992)**

Cortana Cave	8/31/18	M Sanders	1 adult F	City of Austin	SDSU_ G3846A	***Cicurina travisae* (Gertsch 1992)**
Perry Park Cave	8/18/20	C Strickland	1 imm	City of Austin	SDSU_ G3836	***Cicurina travisae* (Gertsch 1992)**
Pond Party Pit	8/28/18	D Thompson	1 adult F	City of Austin	SDSU_ G3842	***Cicurina travisae* (Gertsch 1992)**
Hammett's Cave	9/10/19	T Clark	1 imm, 1 adult F	Travis Co.	TCNR 1021A (imm), B (F)	***Cicurina nov. sp.***
Pennie's Cave	11/15/18	P Sprouse	1 adult M	Zara	ZARA_ 10244	***Cicurina bandida* Gertsch 1992**
Pipeline Cave	8/21/18	M Sanders	2 adult F, 1 adult M	City of Austin	SDSU_ G3849A-C	***Cicurina bandida* Gertsch 1992**
Wyoaka Cave	7/23/19	C Strickland	2 imm	City of Austin	SDSU_ G3847A, B	***Cicurina bandida* Gertsch 1992**
Y2 Cave	3/14/20	C Strickland	1 imm	City of Austin	SDSU_ G3839	***Cicurina bandida* Gertsch 1992**
Millennium Cave	1/30/18	J Scalise	1 imm	City of Austin	SDSU_ G3841	***Cicurina bandida* Gertsch 1992**
Hoskin's Cave	3/22/19	C Strickland	1 imm	City of Austin	SDSU_ G3848B	***Cicurina bandida* Gertsch 1992**

Methodological Details

We used phylogenetic analysis of nuclear ultra-conserved elements (UCEs; see ultraconserved.org/) to identify *Cicurina* specimens. Working with UCE data, it is possible to capture hundreds of orthologous genetic regions ("loci") from a set of specimens using cost-effective, scalable lab techniques (Faircloth et al 2012). At shallow taxonomic levels (e.g., within species, between closely-related species), most phylogenetic information is coming from variable regions that flank the core UCE; recent publications have shown that UCE flanking regions carry enough phylogenetic information to resolve shallow evolutionary divergences with high confidence (e.g., Starrett et al 2017).

Genomic DNA was extracted from spider leg tissues using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). All extractions were quantified using a Qubit Fluorometer (Life Technologies, Inc.) and quality was assessed via gel electrophoresis on a 1% agarose gel. Up to 500 ng of genomic DNA was then shipped to Arbor Biosciences, where UCEs were captured

using the Spider UCE probeset (Kulkarni et al. 2020). Captured libraries were sequenced using NovaSeq S4 (PE150) technology.

Raw sequence reads were processed with the PHYLUCE pipeline (Faircloth 2016). Trimmomatic v0.39 (Bolger et al. 2014) was used to trim adapters and low quality base calls using the following commands: PE ILLUMINACLIP:$adaptersfasta:2:30:10:2:keepBothReads LEADING:5 TRAILING:15 SLIDINGWINDOW:4:15 MINLEN:40. SPADES v3.15.2 (Prjibelski et al. 2020) was then used for assembling clean reads, using the commands: spades.py --sc --careful --cov-cutoff auto. Contigs were matched to probes using default minimum coverage and minimum identity values. UCE loci were aligned with MAFFT (Katoh & Standley 2013) and trimmed with GBLOCKS (Castresana 2000; Talavera & Castresana 2007) implemented in the PHYLUCE pipeline.

Using individual UCE loci alignments (n=731) as separate partitions, optimal models were selected using PartitionFinder2 (Lanfear et al. 2012) in IQ-TREE 2 (Nguyen et al. 2015). IQ-TREE 2 was also used to reconstruct concatenated maximum likelihood trees.

A species tree was also estimated under a multispecies coalescent model using ASTRAL version 5.7.8 (Mirarab et al. 2014; Mirarab and Warnow 2015; Rabiee et al. 2019). Input gene trees (n=1816) were estimated using IQ-TREE 2, and treated as unrooted. Internal branch lengths were estimated in coalescent units, with branch support measured as posterior probability values (a function of number of loci and quartet frequencies, Sayyari and Mirarab 2016).

Results:

We generated new UCE data for 26 specimens. To place these data into a broader phylogenetic context, we included additional UCE data from prior studies in the Hedin lab; all prior specimens had been previously identified using a combination of morphology and genetic placement. Phylogenies were rooted using data for *Cicurina cicur* (type species for the genus) from Germany.

Phylogenomic analyses of 731 UCE loci (IQTREE) and 1816 UCE loci (ASTRAL) result in robust recovery (support values > 95) of the following eyeless *Cicurina* groups relevant to Travis and Hays counties (FIGS 1-3): an "**ME Clade_NE**", which includes species with ME ("mostly elongate") spermathecae (following the Hedin *et al* (2018) naming convention), 2) within this clade are species from **north of the Colorado River** (sister species *C. travisae* and *C. buwata*, and a more northern *C. browni* group), and species from **south of the Colorado River** (*C. nov sp*, *C. bandida* sister to *C. cf. ubicki* and *C. platypus*). We draw three primary conclusions from our phylogenomic analyses:

1) The described species *C. travisae*, *C. buwata*, and *C. bandida* all form well-supported groupings (support values > 95) genetic, and each of these genetic groupings is anchored by adult specimens with diagnostic morphologies. Based on high support for inclusion within genetic clades, we were **able to provide the *C. bandida, C. travisae, and C. buwata* identifications for the specimens in the Table above.**

2) We discovered an apparent case of sympatry at Cortana Cave (FIGS 1-3). SDSU_G3846B is an immature specimen that falls within the *C. buwata* group. SDSU_G3846A is an adult female specimen that falls within the *C. travisae* group; unfortunately, the entire abdomen of this

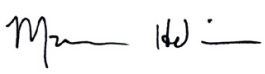
female specimen was inadvertently destroyed during the DNA extraction process, so we cannot confirm the morphological identify of this particular specimen. This case of potential sympatry might be similar to the situation at Gallifer Cave (geographically close to Cortana Cave), where Gertsch (1992) had identified specimens as *C. buwata*, but Hedin (2005) found different specimens genitally allied with *C. travisae.* **Additional sampling will be needed at Cortana Cave to confirm this potential case of sympatry**, otherwise rare in eyeless *Cicurina* from Texas (see Paquin and Dupérré 2009; Hedin et al 2018).

3) Two specimens from Hammett Cave are found further west than other **known** (described) *Cicurina* in this region (FIG 3), based on maps published in Paquin and Dupérré (2009, figs. 131-132). Importantly, we note that many blind, immature *Cicurina* have been recorded from caves of western Travis county (Paquin and Dupérré 2009, fig. 7), but because none of these prior specimens were adult, they have never been attributed to described species.

The Hammett Cave specimens included here form a highly distinctive genetic grouping, separated by long branches from described clades/ species in this region (FIGS 1-3). Finally, one of these specimens is an adult female (#TCNR 1021B). We have examined and imaged this important specimen (FIG 4), but here do not make details comparisons with other regional taxa. Overall, the combined data (geographic, genetic, morphological) **support specimens from Hammett Cave as an undescribed species. We hypothesize that this undescribed species is likely more widespread in western Travis county** (see Paquin and Dupérré 2009, fig. 7)**. More collecting (and UCE data collection) from this region will be important.**

Please contact me with any questions or concerns (mhedin@sdsu.edu).

Sincerely,



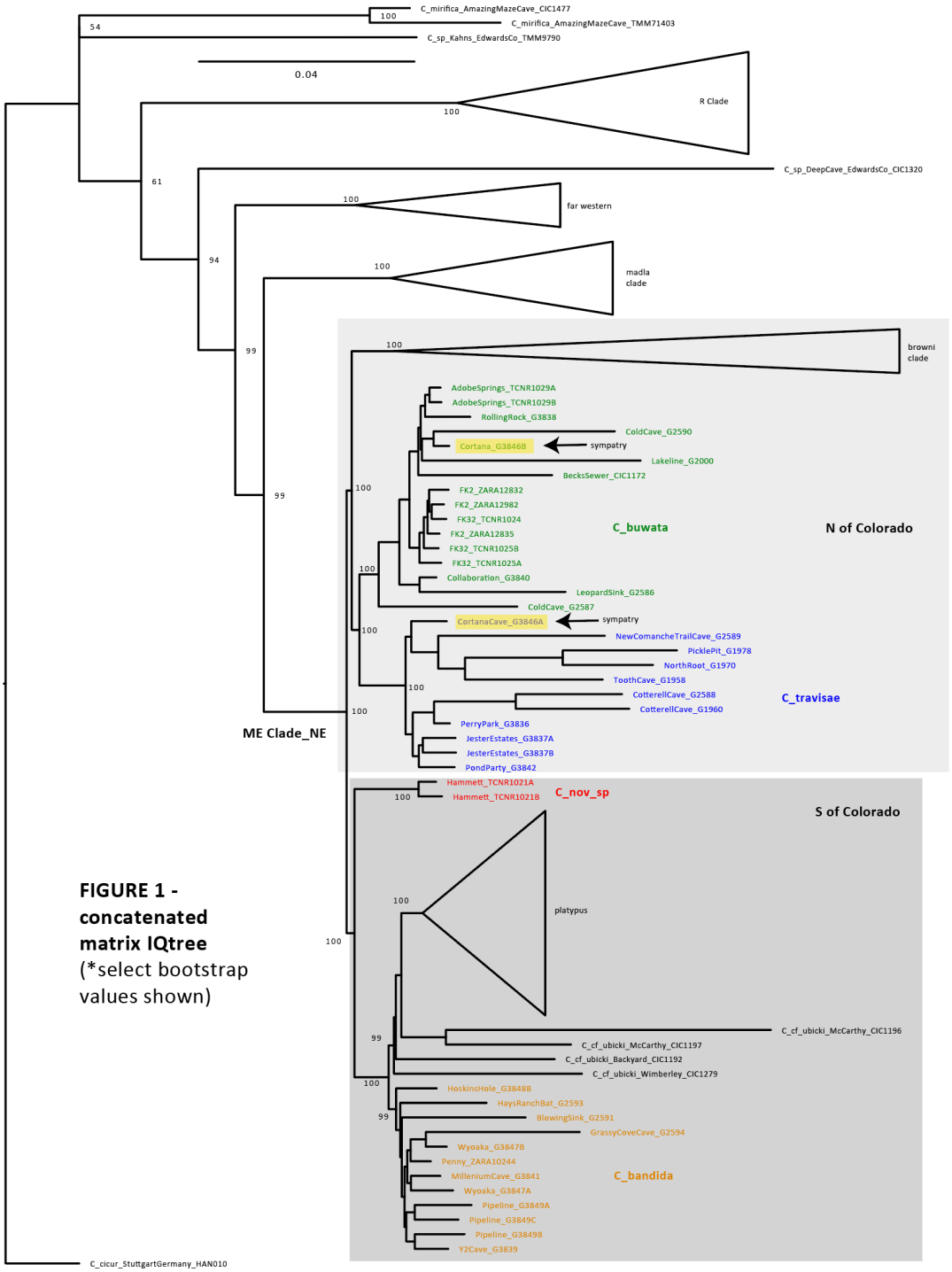


FIGURE 1 - concatenated matrix IQtree (*select bootstrap values shown)

FIGURE 2 - ASTRAL tree
(*local posterior probability support values shown)

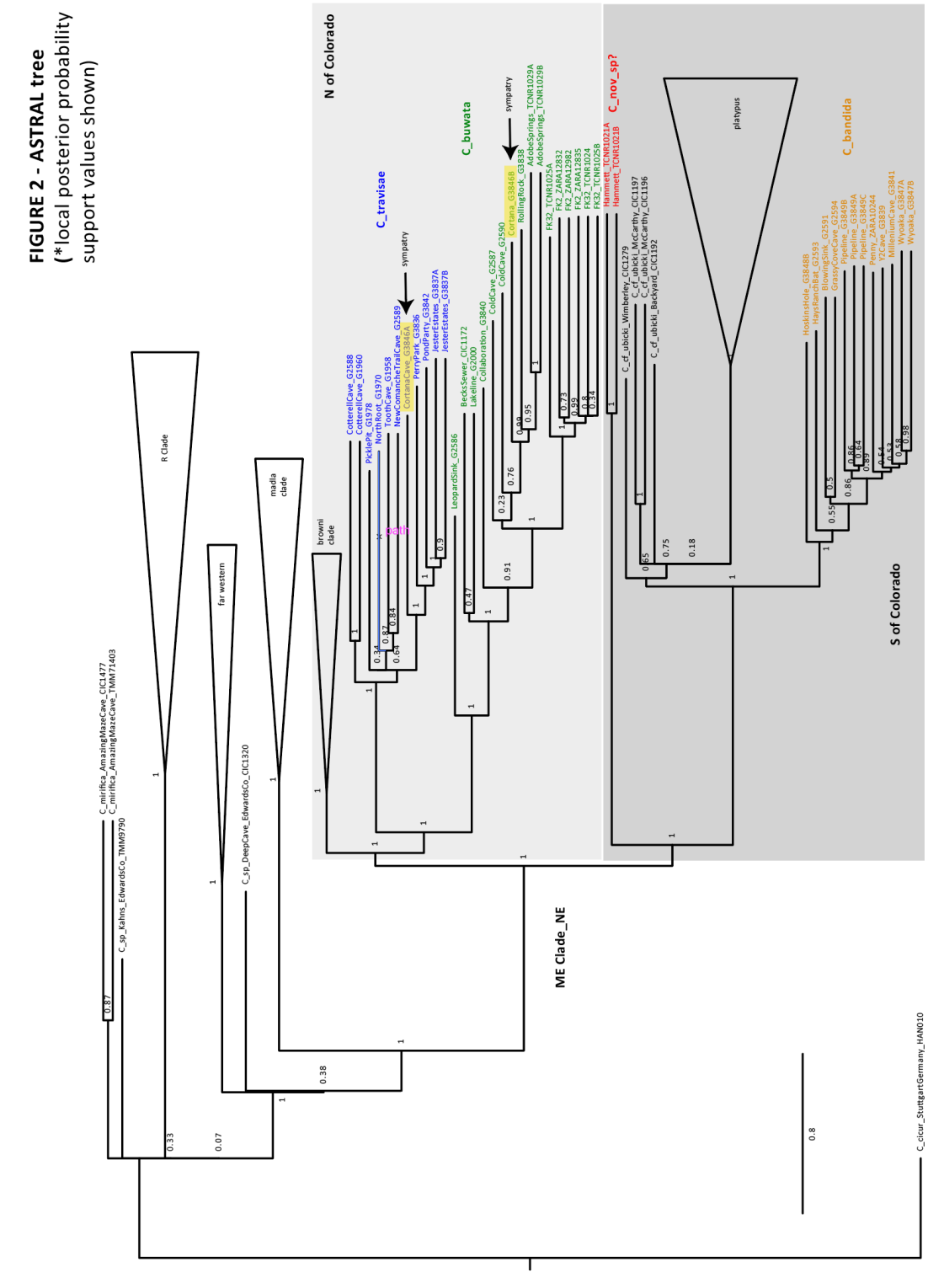
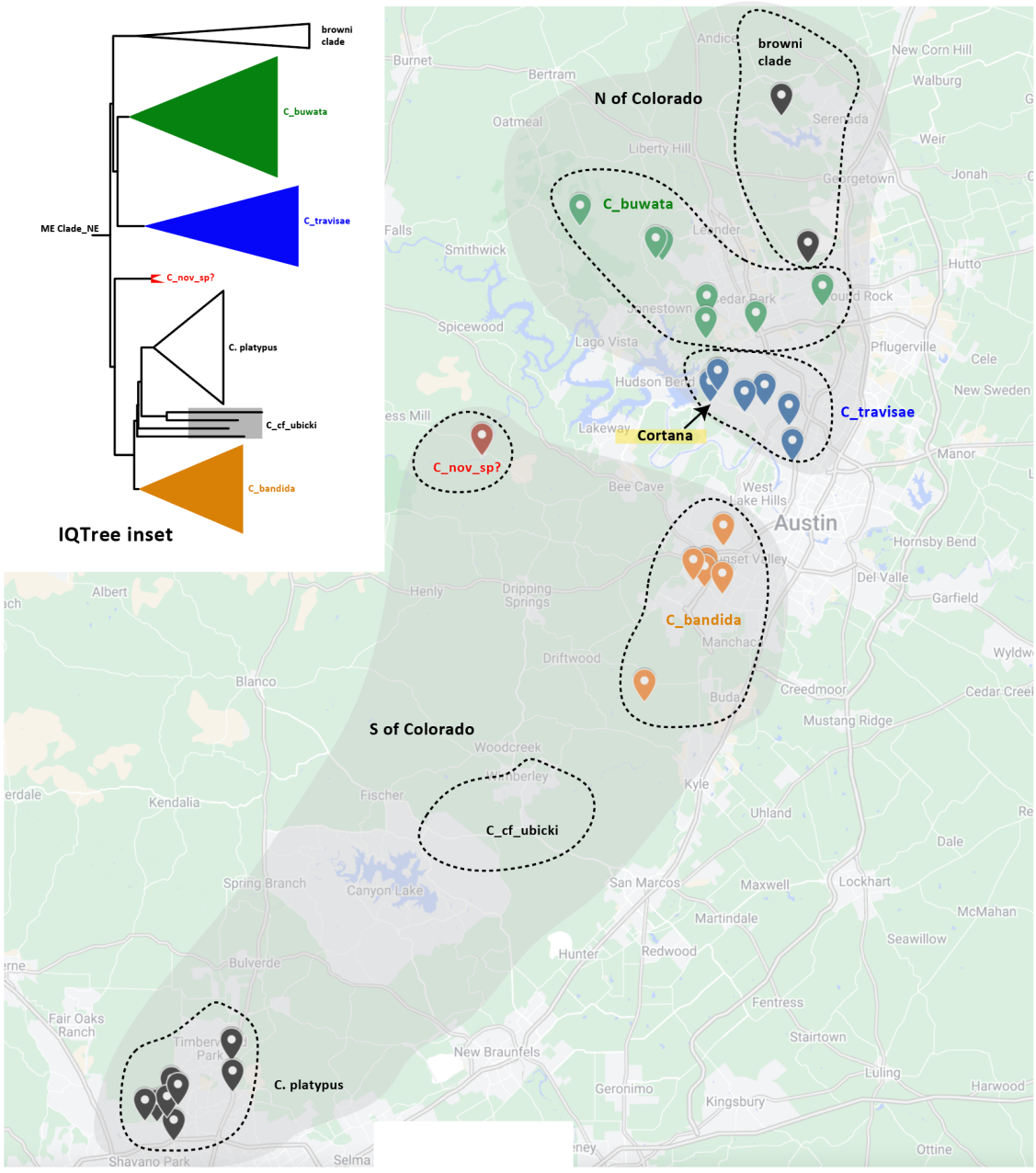


FIGURE 3 - Map of Species Distributions



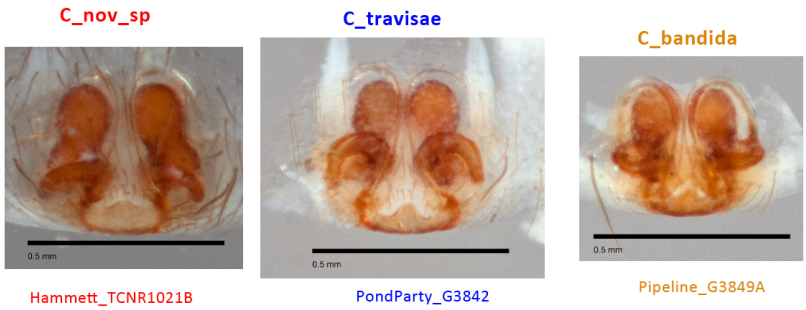


FIGURE 4 - female spermathecal morphology (*all views ventral)

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