

1 **Sex-based *de novo* transcriptome assemblies of the parasitoid wasp**

2 ***Encarsia suzannae*, a host of the manipulative heritable symbiont**

3 ***Cardinium hertigii***

4 **Running title:** Sex-based transcriptome assemblies of *Encarsia suzannae*

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## 23 Abstract

24 Minute parasitoid wasps in the genus *Encarsia* are commonly used as biological pest control  
25 agents of whiteflies and armored scale insects in greenhouses or in the field. They are also a key host of  
26 the bacterial endosymbiont *Cardinium hertigii* which can cause a suite of reproductive manipulation  
27 phenotypes, including parthenogenesis, feminization, and cytoplasmic incompatibility; the last being most  
28 thoroughly studied in *Encarsia suzannae*. Despite their biological and economic importance, there are  
29 currently no published *Encarsia* genomes and only one public transcriptome. In this study, we applied a  
30 mapping-and-removal approach to eliminate known contaminants from previously-obtained Illumina  
31 sequencing data. We generated *de novo* transcriptome assemblies for both female and male *E. suzannae*  
32 which contain 45,986 and 54,762 final coding sequences, respectively. Benchmarking Single-Copy  
33 Orthologs (BUSCO) results indicate both assemblies are highly complete. Preliminary analyses revealed  
34 the presence of homologs of sex-determination genes characterized in other insects and putative venom  
35 proteins. These transcriptomes will be valuable tools to better understand the biology of *Encarsia* wasps  
36 and their evolutionary relatives. Furthermore, the separate male and female assemblies will be particularly  
37 useful references for studies involving insects of only one sex.

38 **Key words:** *Encarsia*, parasitoid wasp, cytoplasmic incompatibility, transcriptome, *Cardinium*

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## 40 Background

41 *Encarsia suzannae* are minute parasitoid wasps within the order Hymenoptera and are of interest  
42 due to their unusual behavior and biology, their use in biological control of the important whitefly pest  
43 *Bemisia tabaci*, their relatedness to the widespread greenhouse biological control agent *Encarsia formosa*,  
44 and because they harbor a bacterial endosymbiont capable of host reproductive manipulation, *Cardinium*  
45 *hertigii*. *Cardinium*, in the bacterial phylum *Bacteroidota*, shows independent evolution of reproductive  
46 manipulation from the well-known alphaproteobacterial *Wolbachia* [1]. Like other Hymenoptera, *E.*  
47 *suzannae* are haplodiploid and reproduce via arrhenotoky (arrhenotokous parthenogenesis) in which

48 haploid males are produced via unfertilized eggs and females are derived from fertilized diploid eggs [2].  
49 Unusually, most *Encarsia* species, including *E. suzannae*, are also autoparasitoids, with females  
50 developing in and consuming the nymphs of the sweet potato whitefly, *B. tabaci*, while male wasps  
51 develop as hyperparasitoids, consuming the pupae of conspecific females or other aphelinid parasitoids of  
52 whiteflies. After consuming their host, both male and female *Encarsia* pupate in the whitefly cuticle and  
53 emerge as adults [3]. Many *Encarsia* species are effective parasites of whitefly species, which are  
54 widespread pests causing up to billions of dollars in crop losses yearly as they can directly damage plants  
55 by feeding and are able to transmit more than 200 different plant viruses to a multitude of plant species [4,  
56 5]. As a result, *Encarsia* species have been widely used as pest control agents to limit whitefly  
57 populations in field or greenhouse settings [6-8]. Their unusual autoparasitic biology [9], sex allocation  
58 behavior, and host selection have also been the focus of study in these intriguing wasps [10].

59         Like many insects, *Encarsia* may be infected with maternally-transmitted intracellular bacterial  
60 endosymbionts, such as *Wolbachia* and *Cardinium*, which influence their transmission by manipulating  
61 host reproduction [11] or oviposition behavior [12] to favor infected females. These manipulations  
62 include the induction of asexual reproduction via thelytokous parthenogenesis [13, 14], as well as a type  
63 of male reproductive sabotage called cytoplasmic incompatibility (CI) [15]. CI causes the offspring of  
64 infected males and uninfected females to die early in development, yet females infected with the same  
65 symbiont can successfully mate with infected or uninfected males. This sabotage proceeds via a two-step  
66 mechanism in which the symbiont alters male sperm with a fatal modification, then rescues infected  
67 offspring from this fatal modification when present in the fertilized egg. Together, the modification and  
68 rescue steps of CI grant infected females with a relative fitness advantage over uninfected females, which  
69 drives the symbiont to high frequencies in host populations [11]. The role of endosymbionts in arthropod  
70 biology, evolution, and speciation have been a subject of intense study [16-18]. Much of this research has  
71 focused on symbiont-induced CI given its potential role in insect speciation [19-21], its application in  
72 arthropod pest population control [22, 23], and its ability to drive desirable genetic traits through  
73 populations (e.g. resistance to arthropod-borne diseases) [24].

74 The *cEper1* strain of *Cardinium hertigii* is the causal agent of CI in *E. suzannae* [15]. This  
75 symbiosis between *cEper1* and *E. suzannae* is the best-studied instance of *Cardinium*-induced CI, and this  
76 strain of *Cardinium* has been well-characterized by genomic and transcriptomic data [1, 3]. However,  
77 sequence information on the host, *E. suzannae*, is extremely sparse: it currently lacks a sequenced genome  
78 and a transcriptomic profile, hampering the molecular identification of host-symbiont interactions. Here,  
79 we have generated separate *de novo* assembled transcriptomes for male and female *E. suzannae* using  
80 previously obtained RNA-seq data generated to characterize the *Cardinium hertigii* transcriptome [3]. To  
81 our knowledge, there is only one other publicly available *Encarsia* transcriptome: that of the widely used  
82 greenhouse whitefly biocontrol agent *Encarsia formosa*, which has been published as part of a  
83 phylogenetic characterization of Chalcidoidea parasitoid wasps [25, 26]. Based on differences in  
84 morphology and lifestyle between *E. suzannae* and *E. formosa*, as well as their phylogenetic relationship,  
85 the two species are distantly related within the diverse *Encarsia* genus [27-30]. This dataset will be a  
86 valuable asset in an ecologically important lineage of chalcidoid wasps (Aphelinidae) that is sorely  
87 lacking sequencing data, as well as provide the first molecular characterization of the host in the model  
88 *Cardinium* CI system.

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## 90 **Methods**

### 91 **Sample information and sequencing**

92 Transcriptome data was obtained as described by Mann et. al [3] which considered only  
93 *Cardinium* reads. Here, we focused on the host (non-*Cardinium*) reads from that dataset. In brief, the  
94 initial *E. suzannae* culture was obtained in 2006 in Weslaco, TX from whitefly (*B. tabaci*) hosts. Male  
95 and female wasps were reared separately in a laboratory culture as described previously [3]. For females,  
96 mated *E. suzannae* were introduced to cages bearing whitefly nymphs on cowpea (*Vigna unguiculata*)  
97 plants. For males, unmated *E. suzannae* were provided with *Eretmocerus* sp. nr. *emiratus* larvae or pupae  
98 developing within whitefly nymphs. Total RNA from 6 groups of 350-500 male or female 1- to 3-day old

99 *E. suzannae* wasps was extracted using the Trizol reagent (Invitrogen) followed by digestion of genomic  
100 DNA with the Turbo DNA-free kit (Ambion). The quality of extracted RNA was assessed with an Agilent  
101 2100 bioanalyzer (Agilent Technologies) and three libraries for each sex were generated with the  
102 NEBNext Ultra RNA library prep kit combined with rRNA depletion via the Ribo-Zero Magnetic Gold  
103 kit (Epicentre Biotechnologies). Samples were sequenced on an Illumina HiSeq2500 platform at the  
104 Vienna BioCenter Core Facilities (VBCF) NGS unit [31], producing a range of 127 to 162 million 50bp  
105 paired-end reads per sample [3].

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### 107 **Read preparation and assembly**

108 Raw read files were processed with BBDuk from the BBTools suite of software (v37.36) [32] to  
109 remove Illumina adapter sequences, trim and/or filter out whole reads with a quality score less than 15,  
110 and remove reads shorter than 36bp after trimming via the following options: “ref=adapters.fa ktrim=r  
111 ordered k=23 hdist=1 mink=11 tpe tbo maq=15 qtrim=rl trimq=15 minlen=36”. We utilized FastQC  
112 (v0.11.9) to visualize the sequence quality of each sample before and after trimming and to confirm the  
113 successful removal of adapter sequences [33]. Due to the complex biology of this species and its host  
114 insects, sequence contamination from a variety of organisms throughout the rearing system is inevitable,  
115 including *Cardinium cEper1*, the different insect hosts of male and female *E. suzannae*, and the  
116 endosymbionts of those insect hosts. Thus, we employed a mapping-and-removal approach to enrich for  
117 *E. suzannae* reads prior to assembly and limit the generation of contaminating transcripts. In this  
118 approach, BMap (from BBTools) was used to initially map quality-trimmed reads to the genomes of  
119 *Cardinium hertigii cEper1* and the endosymbionts of *Bemisia tabaci* MEAM1 (with which *E. suzannae*  
120 females and males have direct or indirect contact): *Hamiltonella defensa*, *Portiera aleyrodidarum*, and  
121 *Rickettsia* sp. MEAM1 [34, 35]. It was also determined that the *E. sp. nr. emiratus* hosts of *E. suzannae*  
122 males contain *Wolbachia* [36]; thus, the *Wolbachia* wPip genome was added and mapped to the male  
123 samples. Reads that did not map to any of these bacterial genomes with greater than 94% identity (to  
124 allow for a difference of 3 nucleotides between sequenced transcripts and reference endosymbiont

125 genomes) were retained. These reads were then subsequently mapped to the *B. tabaci* MEAM1 genome  
126 with a more stringent 97% identity threshold using BBMap to avoid mapping *E. suzannae* reads from  
127 genes highly conserved in both *Encarsia* and *Bemisia* (see Table 1 for mapping and removal details).  
128 Again, only unmapped reads were retained for assembly, as these final reads are expected to be mainly  
129 attributed to *E. suzannae*.

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151 **Table 1: Pre-assembly contaminant read mapping and removal of *Encarsia suzannae* transcriptome**  
 152 **sequencing data**

Organism	Reason for removal	Proportion of trimmed reads mapped	GenBank accession no.
<i>Cardinium hertigii</i> cEper1	CI-causing secondary <i>E. suzannae</i> endosymbiont	<u>Female</u> : 1.183 % <u>Male</u> : 0.991 %	GCA_000304455.1
<i>Portiera aleyrodidarum</i> MEAM1	Primary endosymbiont of <i>B. tabaci</i>	<u>Female</u> : 0.043 % <u>Male</u> : 0.035 %	GCA_002285875.1
<i>Rickettsia</i> sp. MEAM1	Secondary endosymbiont of <i>B. tabaci</i>	<u>Female</u> : 0.058 % <u>Male</u> : 0.065 %	GCA_002285905.1
<i>Hamiltonella defensa</i> MEAM1	Secondary endosymbiont of <i>B. tabaci</i>	<u>Female</u> : 0.040 % <u>Male</u> : 0.037 %	GCA_002285855.1
<i>Bemisia tabaci</i> MEAM1	Parasitized by female <i>E. suzannae</i> offspring and <i>E. sp. nr. emiratus</i>	<u>Female</u> : 5.343 % <u>Male</u> : 5.289 %	GCA_001854935.1
<i>Wolbachia pipientis</i> wPip (male only)	Secondary endosymbiont of <i>E. sp. nr. emiratus</i> which is parasitized by male <i>E. suzannae</i> offspring	<u>Female</u> : N/A <u>Male</u> : 0.050 %	GCA_000073005.1

153 List of organisms from which reads were removed prior to assembly with Trinity. Quality-controlled  
 154 reads were mapped to the genomes of listed organisms and reads that mapped to any of the references  
 155 were removed.

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160 We assembled separate transcriptomes for male and female *E. suzannae* whole adult wasps with  
161 the remaining unmapped reads using Trinity v2.6.6 with default settings [37]. Transcript abundance was  
162 then estimated for each with kallisto using the “align\_and\_estimate\_abundance.pl” command bundled  
163 with Trinity [38]. Transcripts with an estimated abundance below 0.5 transcripts per million (TPM) were  
164 removed from both assemblies as these may be lowly expressed isoforms of other transcripts, poorly  
165 assembled or chimeric transcripts, or are simply contaminants and not from *Encarsia* [39, 40]. Next,  
166 TransDecoder v5.5.0 [41] was used to predict coding sequences within the remaining transcripts in each  
167 assembly and translate those coding sequences into predicted protein sequences with a minimum amino  
168 acid length of 67. Similar protein-coding sequences were then clustered using CD-HIT v4.6.8 [42, 43]  
169 with a threshold of 95% amino acid identity, and the longest protein isoform was assigned as the  
170 representative sequence for that cluster. The final assemblies are presented as the nucleotide sequence of  
171 the representative protein for each cluster. For a comprehensive list of the number of reads or transcripts  
172 at each step in the pipeline, see Table 2.

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186 **Table 2: *E. suzannae* transcriptome read and transcript statistics**

	<i>E. suzannae</i> female	<i>E. suzannae</i> male	<i>E. formosa</i>
<b>Total number of reads</b>	439,763,386	449,368,298	14,341,314
<b>Reads after trimming and mapping</b>	401,213,202	412,945,938	N/A
<b>Initial transcripts</b>	146,798	211,544	48,232
<b>Final transcripts</b>	122,465	136,359	47,852*
<b>Coding sequences</b>	45,986*	54,762*	27,161
<b>Average length of final sequences (bp)</b>	697.74	692.03	772.51
<b>Assembly N50</b>	1,275	1,200	1,237
<b>Average % GC</b>	44.94	44.88	37.5
<b>% Annotated</b>	58.27	65.34	0
<b>Assembly software</b>	Trinity v2.6.6	Trinity 2.6.6	SOAPdenovo-Trans-31kmer v1.01
<b>Reference</b>	This study	This study	[25]

187 Assembly and annotation statistics at each step in the pipeline for the *E. suzannae* transcriptomes

188 published here as compared to the previously-published *E. formosa* transcriptome assembly.

189 A \* indicates the number and type of final sequences in the public version of each assembly.

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## 196 **Quality control and data validation**

197           Along with our mapping-and-removal approach to limit contamination while enriching for  
198 *Encarsia* reads prior to assembly, we also utilized additional methods to improve the quality of our  
199 assemblies. First, to comply with NCBI's Transcriptome Shotgun Assembly (TSA) database  
200 requirements, we removed all coding sequences below 200bp. Furthermore, a blastn of remaining  
201 sequences against NCBI's vector database was conducted to identify contaminating sequences and  
202 synthetic RNA spike-in controls, and hits with 100% nucleotide identity to vector sequences were  
203 removed from each assembly [44]. Prior to submission, any remaining coding sequences flagged by  
204 NCBI's contamination check as sequencing vectors or contaminants were also removed. In total, 71 and  
205 109 contaminating sequences were removed from the female and male assemblies, respectively.

206           The final assemblies were then assessed for completeness using BUSCO v5.3.2 in protein mode  
207 against the hymenoptera\_odb10 reference lineage (v2020-08-05) [45, 46]. The female and male  
208 assemblies were found to possess, respectively, 82.1% and 82.6% of 5,991 complete orthologs identified  
209 as single-copy and nearly universal within the order Hymenoptera (present in >90% of species tested).  
210 This indicates a high level of completeness for both *E. suzannae* transcriptomes, although with varying  
211 degrees of duplication (shown in Table 3).

212           One issue which we are unable to rectify with the currently available sequencing data is the  
213 presence of *E. sp. nr. emiratus* transcripts within the male *E. suzannae* assembly. As mentioned above,  
214 haploid male *E. suzannae* eggs are laid into *Eretmocerus* pupae, and since this host does not have a  
215 sequenced genome (in contrast to *B. tabaci*), we could not apply the same mapping-and-removal approach  
216 to *E. sp. nr. emiratus*. This may be at least partly responsible for the elevated number of total sequences  
217 and duplicated BUSCOs in the *E. suzannae* male assembly compared to the female assembly (see Tables  
218 2 and 3), but there are likely other contributing factors. Due to the relatedness of *Encarsia* and  
219 *Eretmocerus*, we are unable to differentiate sequences originating from those organisms at the read or  
220 assembled transcript level without a reference genome for either. However, we are confident that the  
221 abundance of *Eretmocerus* transcripts in the male assembly is low and many may have been removed

222 from the assembly during the transcript abundance filtering step. This is evidenced by the very low  
223 *Eretmocerus* biomass present in/on fully emerged adult *E. suzannae* (larval *Encarsia suzannae* void their  
224 gut prior to pupation [47]), and using the average abundance of *B. tabaci* reads in either assembly as a  
225 proxy for *E. sp. nr. emiratus* reads suggests an abundance of around 5% for *Eretmocerus* (Table 1).

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248 **Table 3: Prediction of *E. suzannae* transcriptome assembly completeness using BUSCO**

<b>BUSCO results</b>	<b>Male <i>E. suzannae</i></b>		<b>Female <i>E. suzannae</i></b>	
	<b>BUSCOs present</b>	<b>Percent of total</b>	<b>BUSCOs present</b>	<b>Percent of total</b>
Complete BUSCOs	4953	82.6 %	4915	82.1 %
Complete single-copy BUSCOs	3591	59.9 %	4492	75.0 %
Complete duplicated BUSCOs	1362	22.7 %	423	7.1 %
Fragmented BUSCOs	279	4.7 %	280	4.7 %
Missing BUSCOs	759	12.7 %	796	13.2 %
Total BUSCO groups searched	5991	100 %	5991	100 %

249 Assessment of assembly completeness using BUSCO v5.3.2 to search assembled proteins against a  
250 database of proteins identified as Hymenopteran BUSCOs. All BUSCO groups searched were determined  
251 to be present in single copy in >90% of Hymenopteran species tested; therefore, a high number of  
252 complete single-copy BUSCOs indicates a comprehensive and non-redundant assembly [45].

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264 **Annotation**

265 The male and female *E. suzannae* assemblies are available as unannotated coding sequences at  
266 NCBI's TSA database under the accession numbers GJLB00000000 and GJLI00000000, respectively.

267 Here, we also provide annotation information for both assemblies from multiple sources.

268 The final clustered proteins were annotated through the eggNOG-mapper v2 web-based pipeline  
269 using default settings to assign taxonomy to sequences and generate an annotation report with Gene  
270 Ontology (GO) terms, Pfam domains, KEGG pathway info, and other relevant information [48, 49]. Final  
271 proteins were also subjected to a search using DIAMOND with the "--very-sensitive" option [50] against  
272 NCBI's non-redundant (nr) protein database (release 242.0) and a blastp search [51, 52] against a targeted  
273 database of well-annotated insect predicted proteomes consisting of *Nasonia vitripennis* Nvit\_psr\_1\_1  
274 (Genbank accession: GCA\_009193385.2), *Trichogramma pretiosum* Tpre\_2\_0 (Genbank accession:  
275 GCA\_000599845.3), and *Bemisia tabaci* MEAM1 (Genbank accession: GCA\_001854935.1) using an e-  
276 value cutoff of  $10^{-5}$ . Although not closely related to *Encarsia*, *Bemisia* was included in the targeted insect  
277 database as its thorough annotation and presence as an outgroup may be useful in annotating proteins  
278 retained in *Encarsia* that *Nasonia* or *Trichogramma* may have lost. This database was also found to  
279 generate fewer hits labeled as "hypothetical" or "uncharacterized" when compared to a search against the  
280 nr protein database. The annotation results from each reference for both assemblies were pooled into a  
281 single Microsoft Excel spreadsheet (Additional File 1) and we have also provided an additional .fasta file  
282 for each assembly containing the final nucleotide sequences with sequence headers containing annotations  
283 from the blastp against the targeted insect database for ease of use (female: Additional file 2; male:  
284 Additional file 3).

285 Approximately 58% and 65% of female and male assembled proteins were annotated by one of  
286 the listed methods, with the characterization against NCBI's nr database annotating the greatest number  
287 of proteins (26,155 female and 35,073 male), followed closely by the targeted insect database (24,478  
288 female and 33,353 male). Some transcripts of note that were annotated in both the male and female  
289 assemblies are putative homologs to an array of insect sex-determination genes characterized in

290 *Drosophila*. Homologs included *sex lethal (sxl)*, the master regulator of the *Drosophila* sex-determination  
291 cascade, and some genes it regulates, including *transformer (tra)*, *doublesex (dsx)*, and *fruitless (fru)*. *Sex*  
292 *lethal* controls the splicing of *tra* which itself is involved in the sex-specific splicing of *dsx* and *fru* [53].  
293 In *Drosophila*, splicing by *tra* results in either male isoforms of *dsx* and *fru* or a female isoform of *dsx*  
294 and a truncated and untranslated female *fru* isoform. The different *dsx* isoforms are crucial for male and  
295 female somatic sexual development while *fru* appears to be key in the generation of male courtship  
296 behavior in *Drosophila* [54, 55]. We also searched the assemblies for homologs of *wasp overruler of*  
297 *masculinization (wom)* [56], which was identified in *N. vitripennis* as the instructor of sex determination  
298 via the activation of *tra* expression and autoregulation which, in turn, results in female development, but  
299 found none. However, we cannot rule out the presence of *wom* in *E. suzannae* as this gene in *N.*  
300 *vitripennis* is mainly transcribed in diploid (female) embryos prior to 7 hours post oviposition and is not  
301 expressed in adults, which we sampled for our transcriptome assemblies. We also did not find homologs  
302 of *complementary sex determiner (csd)*, the instructor of sex determination in *Apis mellifera*.

303 Sex determination in the Chalcidoidea has been a matter of some speculation [57], but the  
304 presence of these transcripts provides insight into the nature of sex determination and development in *E.*  
305 *suzannae* and lays the foundation for understanding how the mechanisms of sexual development in  
306 *Encarsia* may interface with reproductive manipulation by *Cardinium*. Particularly applicable are cases of  
307 symbiont-induced parthenogenesis, in which unfertilized eggs are diploidized by the endosymbiont and  
308 biological females are produced [13, 58].

309 Furthermore, the identification of many transcripts harboring coding sequences annotated as  
310 putative venom proteins in both male and female *E. suzannae* transcriptomes is notable as these are  
311 believed to be important mechanisms used by female parasitoid wasps to enhance the survivability of  
312 their offspring. Venom proteins are diverse and are predicted to have a variety of impacts on the host  
313 undergoing parasitism, including immune system suppression, developmental arrest, lipid accumulation,  
314 apoptosis, and more [59]. In the case of *E. suzannae*, parasitism causes the whitefly host to undergo  
315 developmental arrest during a late nymphal stage. As arrest occurs regardless of wasp larva survival, it is

316 possible that it is induced by venom injected into the whitefly during oviposition [15]. The presence of  
317 predicted proteins annotated as venom proteins in the male *E. suzannae* assembly is intriguing since only  
318 female wasps host feed and lay eggs into their host while adult males would seemingly have no need to  
319 express venom genes. It is unclear whether these putative proteins are actually venom genes expressed in  
320 male *E. suzannae* or if they were annotated as such due to the presence of domains similar to those found  
321 in venom proteins. Regardless, detecting putative venom proteins in *E. suzannae* provides more insight  
322 into how these wasps effectively parasitize their hosts; however, it should be noted that reliable  
323 identification of venom proteins will require additional experimental verification.

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### 325 **Transcriptome comparisons**

326 As stated above, the only other currently publicly available transcriptome of an *Encarsia* species  
327 belongs to *E. formosa* [26]; thus, limited comparisons can be made within this genus. An overview of all  
328 currently known *Encarsia* transcriptomes is shown in Table 2. Compared to the *E. formosa* transcriptome  
329 assembly, the male and female *E. suzannae* assemblies were generated from more initial reads and  
330 produced more transcripts pre-filtering, meaning they could be subject to more stringent transcript  
331 filtering than the *E. formosa* assembly. While the *E. formosa* assembly underwent limited post-assembly  
332 contaminant filtering, the *E. suzannae* assemblies utilized additional measures to 1) limit potential  
333 nonsense, low-abundance, and redundant transcripts through post-assembly filtering and processing, and  
334 2) eliminate as many contaminants as possible prior to assembly via mapping-and-removal. Furthermore,  
335 the publicly available *E. formosa* assembly consists of full-length mRNA transcripts instead of coding  
336 sequences as seen in the *E. suzannae* assemblies [25]. After running TransDecoder on the *E. formosa*  
337 transcripts, only 27,161 coding sequences were predicted using a minimum length of 50 amino acids.  
338 This indicates that the female (45,986) and male (54,762) *E. suzannae* assemblies contain twice or nearly  
339 twice as many coding sequences compared to the *E. formosa* assembly, even though the *E. formosa*  
340 coding sequences were predicted with a shorter minimum protein size than *E. suzannae*.

341 Finally, OrthoVenn2 was used to determine orthologous groups between the predicted proteins in  
342 both *E. suzannae* assemblies presented in this paper and the *E. formosa* assembly published elsewhere  
343 [26, 60]. Using default settings and an e-value cutoff of  $1e^{-5}$ , 8,816 orthologs were found to be shared  
344 across all three transcriptomes, and a total of 22,015 orthologous groups were shared between male and  
345 female *E. suzannae* out of a total of 23,265 and 23,346 clusters, respectively (see Figure 1), indicating a  
346 high degree of similarity between the different sex assemblies but also showing the presence of over one  
347 thousand sex-specific protein clusters. It is also striking that female and male *E. suzannae* transcriptomes  
348 are equally similar to the *E. formosa* transcriptome despite the fact that *E. formosa* exists as an asexual  
349 species consisting of nearly all females (due to the presence of parthenogenesis-inducing *Wolbachia*) and  
350 its transcriptome therefore only reflects female individuals [61].

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## 352 **Conclusion and re-use potential**

353 We are confident that our assemblies are among the purest possible transcriptome representations  
354 of *E. suzannae* using the currently available data and assembly and filtering tools (for a list of all software  
355 and their versions utilized in this study, see Table 4). This study is also one of the first to present sex-  
356 specific transcriptome assemblies of a single insect species. In an organism such as *E. suzannae*, where  
357 males and females develop within different hosts, are impacted differently by endosymbiotic bacteria, and  
358 exhibit distinct behaviors, it is highly valuable to have available a reference database for both sexes to  
359 ensure more accurate studies when wasps of only one sex are used. Furthermore, these assemblies greatly  
360 expand our host knowledge of the *Cardinium* *cEper1* CI system and pave the way for future studies  
361 exploring how this endosymbiont interacts with its *E. suzannae* host in causing CI. We also believe that  
362 these data will be a valuable tool to other researchers as a reference when studying the diverse members  
363 of the ecologically important genus *Encarsia* and other chalcidoid parasitic wasps, many of which have  
364 interesting biology and potential as pest biological control agents.

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369 **Table 4: Software and version specifications**

Software	Usage	Version	Reference(s)
BBTools	BBDuk for read trimming; BBMap for read mapping	37.36	[32]
FastQC	Visualization of sequence quality	0.11.9	[33]
SAMtools	.bam file manipulation	1.10	[62]
Trinity	<i>De novo</i> transcriptome assembly	2.6.6	[37]
kallisto	Transcript abundance estimation	0.46.2	[38]
TransDecoder	Prediction of coding sequences	5.5.0	[41]
CD-HIT	Clustering similar protein sequences	4.6.8	[42, 43]
BUSCO	Assessing assembly completeness	5.3.2	[45]
eggNOG-mapper v2	Annotation of assembled proteins	2.1.6	[48, 49]
Blast+	Annotation of assembled proteins	2.11.0	[51]
Diamond	Annotation of assembled proteins	2.0.4	[50]
OrthoVenn2	Orthologous protein group clustering and visualization	N/A	[60]

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### 379 **Availability of supporting data**

380 *E. suzannae* female and male raw read data and unannotated assemblies were submitted to NCBI's  
381 Sequence Read Archive (SRA) and Transcriptome Shotgun Assembly (TSA) databases under the  
382 BioProjects PRJNA737477 for male *E. suzannae* and PRJNA737478 for female *E. suzannae*. Detailed  
383 annotation information from multiple sources is provided in Additional file 1. Annotated female and male  
384 assemblies are available in FASTA format in Additional files 2 and 3, respectively. All raw sequencing  
385 data and the final assemblies from this study are publicly available.

386

### 387 **Figure legends**

388 **Figure 1: Orthologous groups between *E. formosa* females and male and female *E. suzannae***  
389 **transcriptomes.** The above figure shows an OrthoVenn2 diagram of orthologous groups between *E.*  
390 *formosa* females and male and female *E. suzannae* (e-value =  $1e^{-5}$ ) [60]. TransDecoder using a minimum  
391 amino acid length of 50 was run on the *E. formosa* assemblies to obtain coding sequences and the  
392 resulting peptide sequence output (27,161 sequences) was tested against the predicted proteins from the  
393 male and female *E. suzannae* transcriptomes. The topmost Venn diagram depicts the number of shared  
394 orthologous protein clusters between the three transcriptomes. The middle bar graph depicts the total  
395 number of orthologous clusters present for each transcriptome, and the bottom graph shows (left to right)  
396 the number of clusters that were shared by all three transcriptomes, by any two transcriptomes, or were  
397 unique to only one of the three assemblies.

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### 399 **Additional files**

400 **Additional file 1:** Annotation results for female and male *E. suzannae* from different annotation methods

401 **Additional file 2:** FASTA format file containing the female *E. suzannae* transcriptome as coding  
402 sequences with annotations in the sequence headers

403 **Additional file 3:** FASTA format file containing the male *E. suzannae* transcriptome as coding sequences  
404 with annotations in the sequence headers

405

## 406 **Abbreviations**

407 BUSCO: Benchmarking Universal Single-Copy Orthologs

408 CI: Cytoplasmic incompatibility

409 GO: Gene Ontology

410 KEGG: Kyoto Encyclopedia of Genes and Genomes

411 NCBI: National Center for Biotechnology Information

412 SRA: Sequence Read Archive

413 TPM: Transcripts per million

414 TSA: Transcriptome Shotgun Assembly

415 VBCF: Vienna BioCenter Core Facilities

416

## 417 **Competing interests**

418 The authors declare no competing interests.

419

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423

## 424 **Authors' contributions**

425 SSE and MSH conceived the experiments and provided supervision. DLS and SSE developed the analysis  
426 pipeline. ES, CS, and SEK performed experiments. DLS analyzed and visualized the data and wrote the  
427 draft manuscript. All authors wrote and edited the manuscript. SSE and MSH obtained funding.

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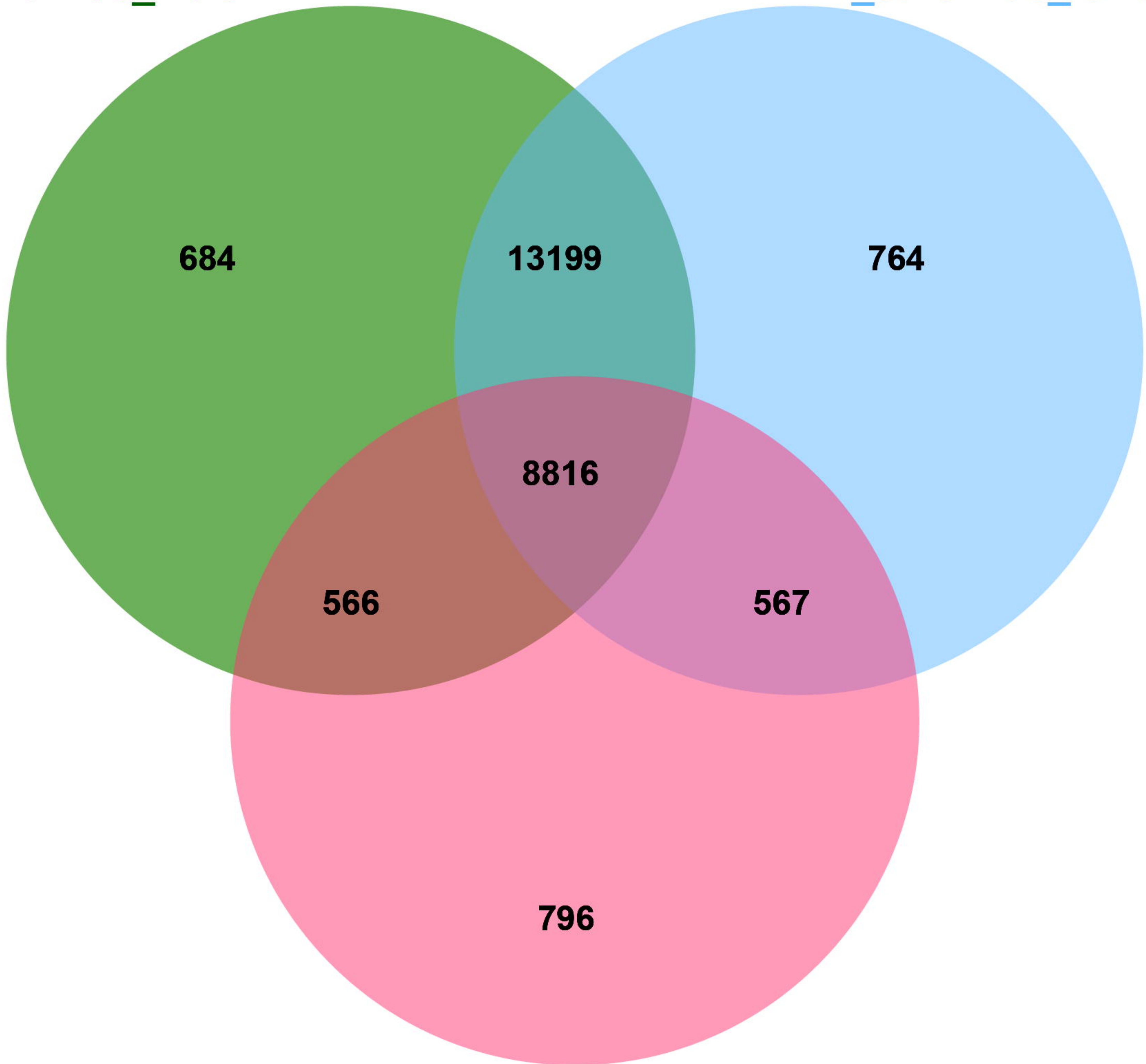
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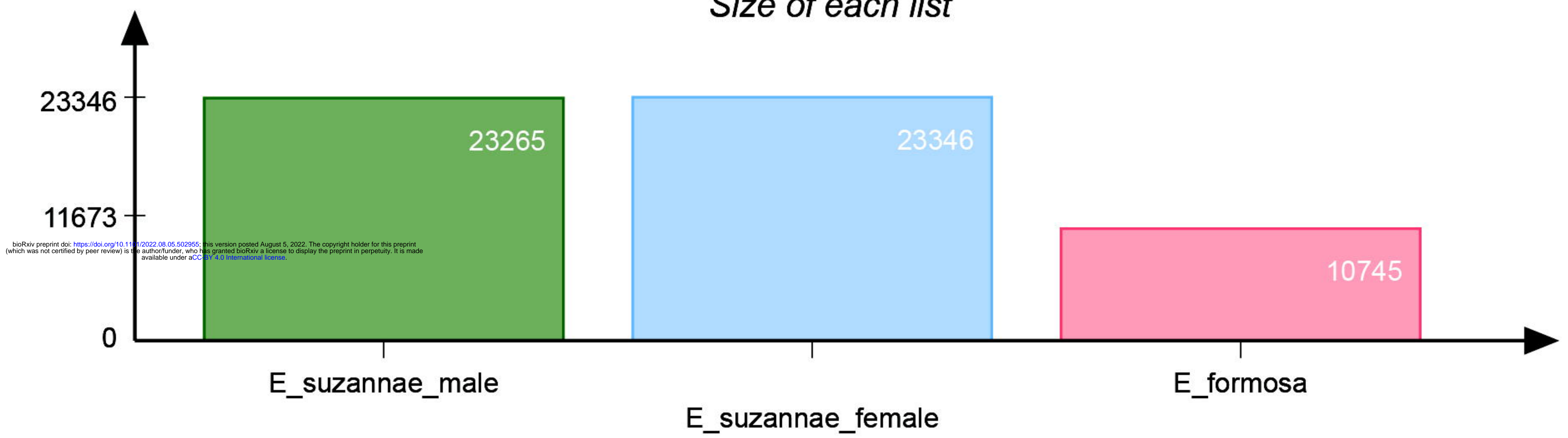
E\_suzannae\_male

E\_suzannae\_female



E\_formosa

Size of each list



Number of elements: specific (1) or shared by 2, 3, ... lists

