The *Tetragnatha kauaiensis* genome sheds light on the origins of genomic novelty in spiders

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Abstract

Spiders (Araneae) have a diverse spectrum of morphologies, behaviours and physiologies. Attempts to understand the genomic-basis of this diversity are often hindered by their large, heterozygous and AT-rich genomes with high repeat content resulting in highly fragmented, poor-quality assemblies. As a result, the key attributes of spider genomes , including gene family evolution, repeat content, and gene function, remain poorly understood. Here, we used Illumina and Dovetail Chicago technologies to sequence the genome of the long jawed spider *Tetragnatha kauaiensis*, producing an assembly distributed along 3,925 scaffolds with a N50 of ~2 Mb. Using comparative genomics tools, we explore genome evolution across available spider assemblies. Our findings suggest that the previously reported and vast genome size variation in spiders is linked to the different representation and number of transposable elements. Using statistical tools to uncover gene-family level evolution, we find expansions associated with the sensory perception of taste, immunity and metabolism. In addition, we report strikingly different histories of chemosensory, venom and silk gene families, with the first two evolving much earlier, affected by the ancestral whole genome duplication in Arachnopulmonata (~450 million years ago) and exhibiting higher numbers. Together, our findings reveal that spider genomes are highly variable and that genomic novelty may have been driven by the burst of an ancient whole genome duplication, followed by gene family and transposable element expansion.

Significance statement

Despite being one of the most charismatic animal lineages, progress on spider genome evolution lags due to the challenges in sequencing and assembling their genomes, which involve genome size and repeat content. Here, we sequence the genome of *Tetragnatha kauaiensis*, a spider endemic to Hawai'i, and compare it to other available spider genomes. We find variation in terms of repeats and transposable elements; expansions in gene-content associated with metabolism, sensory perception and immunity; and wide variation of chemosensory genes and venom genes.

Introduction

With nearly 50,000 described species ("World Spider Catalog," 2021), and dating back ~350 million years (Fernández et al. 2018), spiders (Chelicerata, Araneae) have conquered most terrestrial ecosystems, from the cold Arctic to arid deserts (Jackson and Cross 2011; Dimitrov et al. 2012; Garrison et al. 2016; Fernández et al. 2018). Spiders play a key role in terrestrial ecosystems regulating community dynamics as major arthropod predators (Herberstein and Wignall 2011; Wilder 2011), having evolved a diverse array of adaptive solutions, which include, a rich cocktail of venoms to neutralize prey (Binford 2001; King and Hardy 2013), a colour palette essential for camouflaging, mimicking and signaling (Oxford and Gillespie 1998; Croucher et al. 2013; Cotoras et al. 2016), and

the ability to produce silk for spinning webs and subduing prey (Vollrath 1999; Garb et al. 2010; Sanggaard et al. 2014).

Despite the advances in spider ecology, evolution and systematics, knowledge of spider genomes still lags relative to other taxa. Most of the available spider genomes are of poor quality, being highly fragmented (Garb et al. 2018) and lack a substantial part of the genome, with only three recent exceptions involving chromosome-resolved genomes (Escuer et al. 2021; Fan et al. 2021; Sheffer et al. 2021). Several factors contribute to the sparse availability of high-quality spider genome assemblies, including the lack of a model organism among spiders (sensu *Drosophila melanogaster* in flies and *Tribolium castaneum* in beetles) (Brewer et al. 2014), and the challenges associated with sequencing spider genomes, which are characterized by high AT-content, repeats, heterozygosity, and often large genome sizes (Garb et al. 2018). Focus on non-model organism genomes shows that increased taxon-sampling leads to an improved understanding of the diversity and function of molecular mechanisms across the tree of life (McGregor et al. 2008), as it overcomes the biases from the limited number of model taxa, and highlights the idiosyncrasies throughout the tree of life. Consequently, a better representation of spider genomes will certainly help understanding spider diversity and evolution (McGregor et al. 2008).

A systematic analysis of spider genomes has the potential to unveil the genomic foundation of spider evolution. For example, the detection of duplicate Hox clusters suggested an ancestral whole genome duplication in the common ancestor of modern spiders and scorpions (Arachnopulmonata; Schwager et al. 2007), and this evidence was later on confirmed by the first spider genomes (Clarke et al. 2015; Schwager et al. 2017; Leite et al. 2018). The implications of whole genome duplications may, however, be multifarious and complex (Ohno 1970). On one hand, genome duplication may act as a catalyst for molecular novelty. Under this framework, the retention of duplicated genes and other genetic components may act as ‘reservoirs of genetic variation’, through processes of gene neo- and sub-functionalization (Lynch and Force 2000), and be of use when organisms encounter novel selective pressures (Li et al. 2018; Nieto Feliner et al. 2020; Schmickl and Yant 2021). Considering the evidence for gene duplicates in spider genomes, including spidroins (silk genes) (Sanggaard et al. 2014; Clarke et al. 2015; Babb et al. 2017; Garb et al. 2018; Sheffer et al. 2021), venoms (Sanggaard et al. 2014; Gendreau et al. 2017; Haney et al. 2019), chemosensory (Vizueta et al. 2018; Vizueta et al. 2019; Vizueta, Escuer, et al. 2020) gene families may yield insights on phenotypic innovation and the adaptation to novel environments. On the other hand, since genome duplication leads to a significant re-organization of the genome, it may cause deregulation of gene-expression networks or unlock the epigenetic suppression of transposable elements, which may proliferate across the genome and result in decreased fitness for the organism – ‘the genomic shock hypothesis’ (McClintock 1984; Choi et al. 2020). In such a scenario, one expects to find variation in transposable element proliferation across genomes, and ultimately a substantial variation of genome size. The proliferation of transposable elements may thereby underlie genome size variation in spiders, which ranges

between 0.74 - 5.73 C values (0.7 Gb - 5.6 Gb) (Gregory and Shorthouse 2003)(http://www.genomesize.com/ checked in April 15th 2021; values for: *Habronattus borealis*, *Tetragnatha elongata*, respectively). Comparisons between different genome assemblies may yield important insights on the prevalence of gene duplications, neofunctionalization, and transposable element dynamics across different lineages.

Here, we report a genome assembly of the Hawaiian spider *Tetragnatha kauaiensis* and place it in the context of currently available spider genomes to assess signatures of genome evolution across spider lineages (Supplementary Table 1). To do so, we first explore the completeness and duplication rates across the spider assemblies. Considering the role of transposable elements in driving genome size variation, we also assess transposable element load in each genome. Third, we quantify the expansion and contraction of gene families (based on gene similarity metrics), and classify the function of these families using Gene Ontology (GO). Finally, we delve deeper into the identification and comparison of chemosensory, venom and spidroin (silk) genes, studying duplicates in a phylogenetic context. Focus on these three categories is grounded on their central role to the survival and fitness of spiders, and benefits from extensive research, including hand curated genes and databases.

Results

The *Tetragnatha kauaiensis* genome

The *T. kauaiensis* genome assembly has a size of ~1.08 gigabases (Gb), distributed along a total of 132,391 contigs, comprising 3,925 scaffolds. The largest scaffold is ca. 10,5 megabases (Mb), while the estimated scaffold-N50 for the assembly is ~2 Mb (Supplementary Table 2). The assembly has a GC content of ~33.3%, in line with the remaining spider genomes (lowest GC content *L. hesperus* with 28.59% and highest content is *S. mimosarum* with a GC content of 33.62; Supplementary Table 2). The assembly has no obvious contaminants or associated symbionts, as determined by Blobtools (Supplementary Figure 1). The majority of scaffolds have a similar GC composition, despite variations in coverage. From all 3,925 scaffolds, 2,774 were labelled as no-hits (comprising only a total of ~32.46 Mb of the assembly), and 889 labelled as Arthropods (~886 Mb).

Annotation of the *Tetragnatha kauaiensis* genome yielded 38,907 genes, comprising 213,695 exons and 171,423 introns (Supplementary Table 3). Together, all genes cover 290,369,064 bp (290 Mb) representing 26.7% of the genome with 41,209,078 bp (41 Mb, 3.8% of the genome) being coding sequences (cds). The mean gene length is 7,463 bp (Supplementary Table 3), the longest gene is 208,580 bp long (208 kb), and 89.7% of BUSCOs are retrieved as complete.

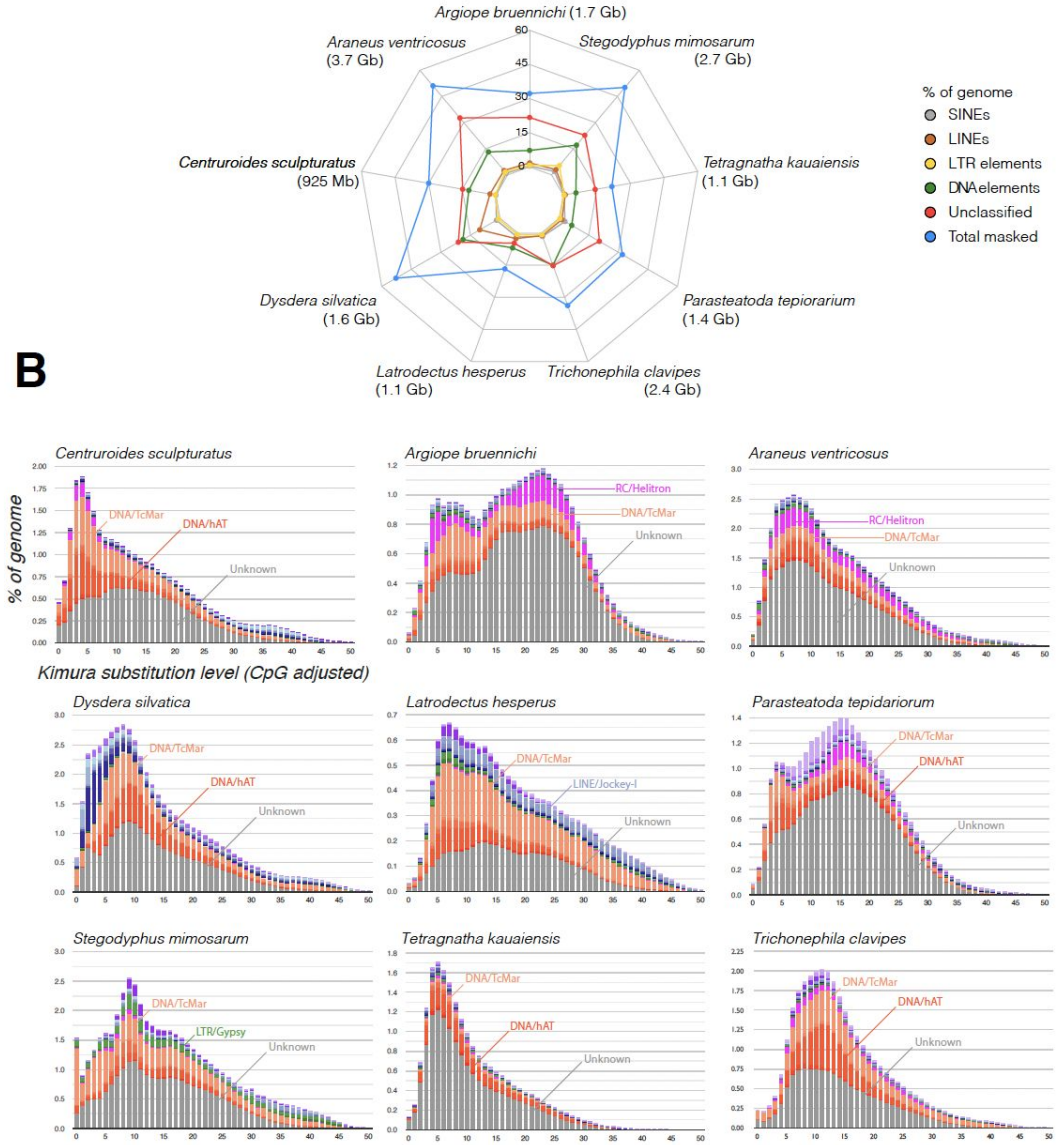

Figure 1: Transposable element (TE) and repeat characterization a) Web diagram showing the representation of TE and repeats in the assemblies. Assemblies and correspondent assembly sizes are represented on the edges of the web diagram. Different transposable element families or repeats are presented in different colours on the plot, and the total content masked by RepeatMasker is shown in blue. The numbers for each web-line indicate the % of the genome occupied by each transposable element, or the % masked. **b)** Repeat/transposable element landscape plots for the various assemblies. The three most represented transposable element categories are present for every genome (e.g. DNA/TcMar, DNA/hAT, and unknown for *T. kauaiensis*). Each plot shows the Kimura substitution level (x axis) and % of genome covered by repeats (y axis).

Genome characterization and evolution

The analyzed assemblies vary widely in size. *A. ventricosus* has the largest assembly with 3.6 Gb (Supplementary Table 2), while *T. kauaiensis* has the smallest assembly with 1,085,571,486 bp (1.1 Gb). In between these extremes, we find the genomes of *S. mimosarum* (2.7 Gb), *T. clavipes* (2.4 Gb), *A. bruennichi* (1.7 Gb), *D. silvatica* (1.4 Gb), *P. tepidariorum* (1.5 Gb) and *L. hesperus* (1.1 Gb).

Considering the 3-fold variation in genome size and the evidence for ancient whole genome duplications in Chelicerata (Shingate et al. 2020) and Arachnida (Schwager et al. 2017; Harper et al. 2020), and the suggestion that there has been a large-scale (whole genome or chromosomal) duplication event within spiders (Clarke et al. 2015), we explored the possibility of whole genome duplication private to spider genomes by interrogating the number of homologs in the Hox genes clusters. Using Hox genes 1-5, and based on a threshold of 95% identity, we find no evidence for an additional ancestral whole genome duplication in the studied spider genomes. We found zero, one or two homologs for Hox 1 (Supplementary Table 4). For Hox 2, we found two homologs in all genomes, with the exception of *A. ventricosus,* where we only find a single homolog (Supplementary Table 4). For Hox 3, there was only one homolog in all genomes, with the exception of *P. tepidariorum* (2 candidates) and *T. clavipes* (no candidate). For Hox 4, we found two homologous genes in *T. kauaiensis*, *P. tepidariorum*, *L. hesperus* and *S. mimosarum*, one in *T. clavipes* and another in *D. sylvatica*. *A. ventricosus*, however, had four homologs for the Hox4 gene. Finally, for Hox 5, we identified one homolog in all genomes, with the exception of *A. ventricosus* and *P. tepidariorum* where we found two homologous genes. This suggests that, with the exception of the outlier with four copies (*Araneus* Hox4), Hox genes are present in 1 or 2 copies.

Transposable element variation

We find variation in repeat content and tempo of repeat accumulation across the spider assemblies (Figure 1; Supplementary Table 5). For example, 10.3% of the *D. silvatica* genome is composed of LINEs, whereas all other studied spiders had at most 3% LINEs (Figure 1A). *S. mimosarum* had 5.40% of its genome covered by LTR elements, while *A. ventricosus*, which is the second LTR-element most rich genome, had only 1.60% (Figure 1). Interspersed repeats varied between 52.84% in *D. silvatica* and 16.53% in *L. hesperus* (Supplementary Table 5). Unclassified repeats ranged between 32.64% (*A. ventricosus*), and 4.71% *L. hesperus* (Supplementary Table 5). Overall, Repeatmasker identified between 16.71% -52.84% of total repeat content (Figure 1 A; Supplementary Table 5). The correlation coefficient (R) between genome size and the % of masked genome is R=0.65, and the correlation coefficient (R) between total length of the masked genome and genome size is R=0.962. Finally, we find variability in the accumulation of transposable elements through time, as represented by the shape of the transposable element/repeat landscape plot curves (Figure B). For instance, the *A. bruennichi* and *P. tepidariorum* assemblies show two peaks in transposable element accumulation, whereas all the others display a single peak. *S. mimosarum*, however, has a recent burst in Tc1/mariner (DNA/TcMar) transposable elements (Figure 1B). Despite the differences in the accumulation of transposable element/repeats through time, we note that the

Tc1/mariner group (DNA/TcMar) is present as one of the top three most represented transposable elements in all the assemblies, and and hAT transposons (DNA/hAT) are also among the three-dominant categories in 6 assemblies. There is, however, variation across assemblies, as shown by the high numbers of Helitrons (RC/Helitron) in two of the Araneidae assemblies (*A. bruennichi* and *A. ventricosus*), Gypsy (LTRGypsy) in S. *mimosarum*, and Jockey (LINE/Jockey-l) in *L. hesperus*.

The analysis of genome completeness, as assessed by BUSCO scores, suggests that spider assemblies are considerably fragmented and missing substantial parts of the genome (Supplementary Table 6). For instance, the *D. silvatica*, *L. hesperus* and *T. clavipes* genomes have only, respectively, 66%, 38.6% and 52% complete BUSCOs (Arachnid odb10). Completeness in the remaining genomes ranged between 80-99%. Duplicated BUSCOs ranged between 30.5% (*P. tepidariorum*) and 3.2% (*S. mimosarum*). Notably, the two biggest genomes, *A. ventricosus* (3.6Gb) and *S. mimosarum* (2.7Gb) have 18.4% and 3.2% duplicated BUSCOs (Supplementary Table 6, Arachnid dataset odb10). The percentage of complete single-copy, duplicated, fragmented, and missing BUSCOs is concordant between the Arthropod and Arachnid sets (Supplementary Table 6).

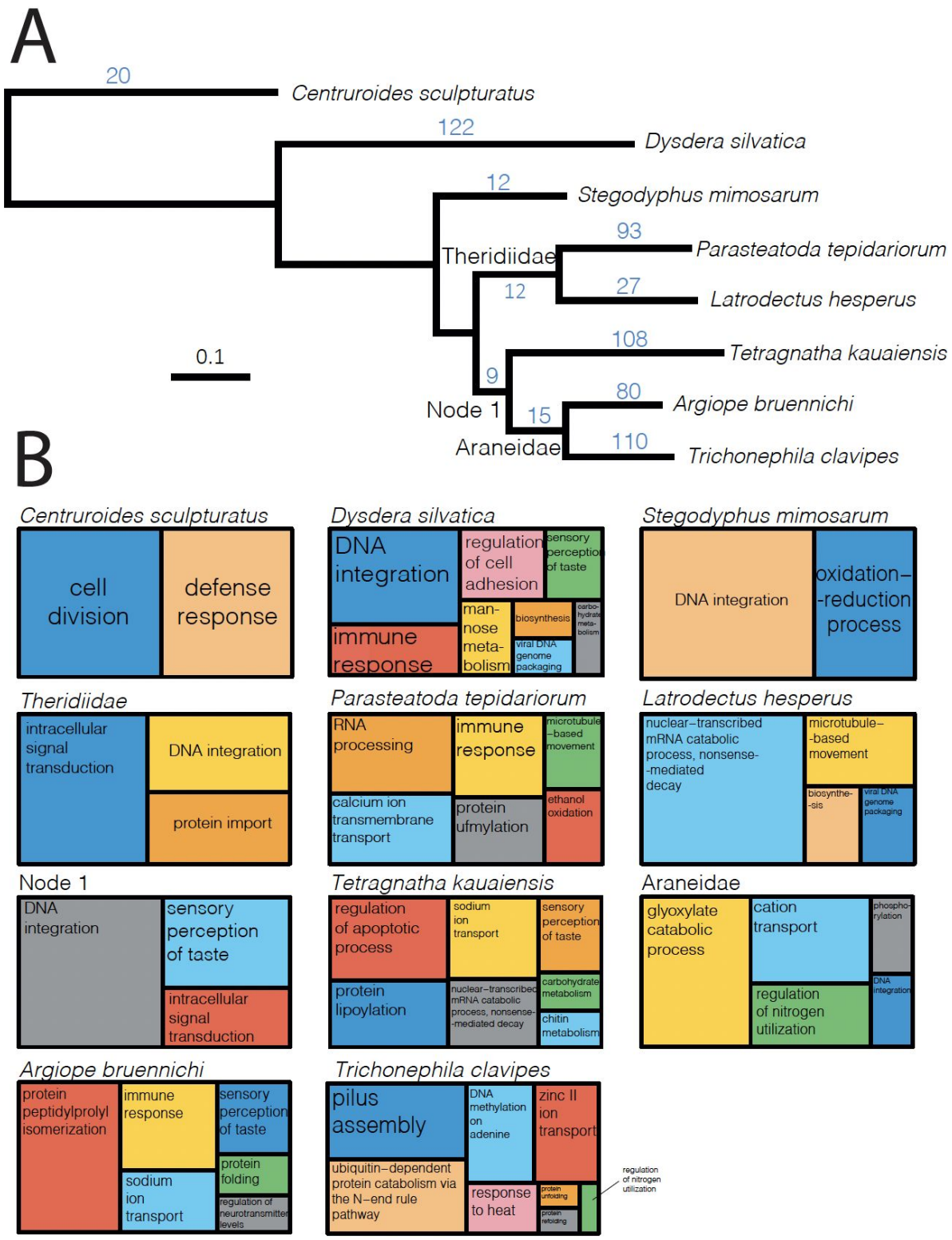

Figure 2. Gene family expansion A) Tree topology obtained for single-copy orthologs. Numbers in blue indicate significantly expanded gene families as determined by CAFE. B) Treemap representation of Gene Ontology Biological Function Annotation of the significantly expanded gene

families as retrieved by REVIGO. Branches/Nodes with significant expansions, including Araneidae, Theridiidae, and Node 1 are represented together with the different genomes.

Gene-family evolution

Since studying gene family evolution requires a phylogenetic backbone, we used the tree obtained from OrthoFinder based on 286 single-copy orthologs (orthologs are genes in different species that evolved from a common ancestral gene; Figure 2A). The tree topology has *T. kauaiensis* (Tetragnathidae) as sister lineage to the clade comprising the two members of Araneidae (*A. bruennichi* and *T. clavipes*). The clade encompassing all the aforementioned is sister to the Theridiidae (*L. hesperus* and *P. tepidariorum*). In turn, *S. mimosarium* (Eresidae) is the sister to Araneoidea (represented here by Tetragnathidae, Araneidae and Theridiidae). *D. silvatica* (Dysderidae) is the sister to the clade comprising all the aforementioned spiders (Figure 2A). This topology is in agreement with recent and comprehensive phylogenomic analyses of spiders (Fernández et al. 2018).

From a total of 608 significant gene family expansions in all branches, 572 occurred in terminal branches (Figure 2B). There were 451 significant expansions, and 157 significant contractions, of which 124 occurred in terminal branches (Supplementary Figures 1-4).

GO annotations of the significantly expanded gene families which were characterized under 'biological process' were organized by REVIGO and are represented in Figure 2B. Broadly, we find expansions associated with feeding metabolism and sensory perception, mannose metabolism in the genome of *D. silvatica* and chitin metabolism in *T. kauaiensis* (Figure 2B). Expansions in carbohydrate metabolism are found in *D. silvatica* and *T. kauaiensis*, while Araneidae has glyoxylate catabolic process expanded (Figure 2B). Expansions in sensory perception of taste are found in *D. silvatica*, *T. kauaiensis*, *A. bruennichi*, and in Node 1 (Figure 2B). Immune response is found in the genomes of *D. silvatica*, *P. tepidariorum* and *A. bruennichi*, while sodium ion transport is found in *T. kauaiensis* and *A. bruennichi* (Figure 2B).

When considering significant expansions in all GO categories (i.e. biological process, molecular function and cellular component), we find expansions associated with taste (including sensory perception of taste in Node 1, *A. bruennichi*, and *D. silvatica*; detection of chemical stimulus involved in sensory perception of taste in *A. bruennichi* and Node 1; molecular function taste receptor activity; is found in *A. bruennichi* and *T. kauaiensis;* Supplementary Table 7). We also find evidence for expansions related to various metabolic processes, including carbohydrate metabolic process , and mannose metabolic process in *D. silvatica*, while protein catabolic process, 3,4-dihydroxybenzoate catabolic process, fatty acid catabolic process, pyruvate metabolic process, glucose metabolic process, protein metabolic process, lipid catabolic process, lipid metabolic process, and fatty acid metabolic process are found in *T. clavipes*. The *P. tepidoriorum* genome includes expansions in peptidoglycan catabolic process and lipid metabolic process, while that of *T. kauaiensis* includes expansions in chitin

metabolic process, carbohydrate metabolic process. Theridiidae includes expansions in lipid metabolic process, while Araneidae includes changes in taurine catabolic process. Finally, catalytic activity, is expanded in the genomes of *D. silvatica*, *L. hesperus*, *T. clavipes*, *T. kauaiensis*. Other notable expansions include the regulation of neurotransmitter levels, structural constituent of eye lens in *A. bruennichi*, defence response and toxin activity in *C. sculpturatus*, and response to heat in *T. clavipes*. The biological process for 'sodium channel activity' is found expanded in *A. bruennichi*, *T. clavipes* and *P. tepidariorum*, while the molecular function for 'sodium channel activity' is found in *A. bruennichi* and *T. kauaiensis* . Proteolysis (i.e. breakdown of proteins) , the break down of process is expanded in *A. bruennichi*, *C. sculpturatus*, *D. silvatica*, *L. hesperus*, *P. tepidariorum*, *S. mimosarum*, *T. kauaiensis* and Theridiidae.

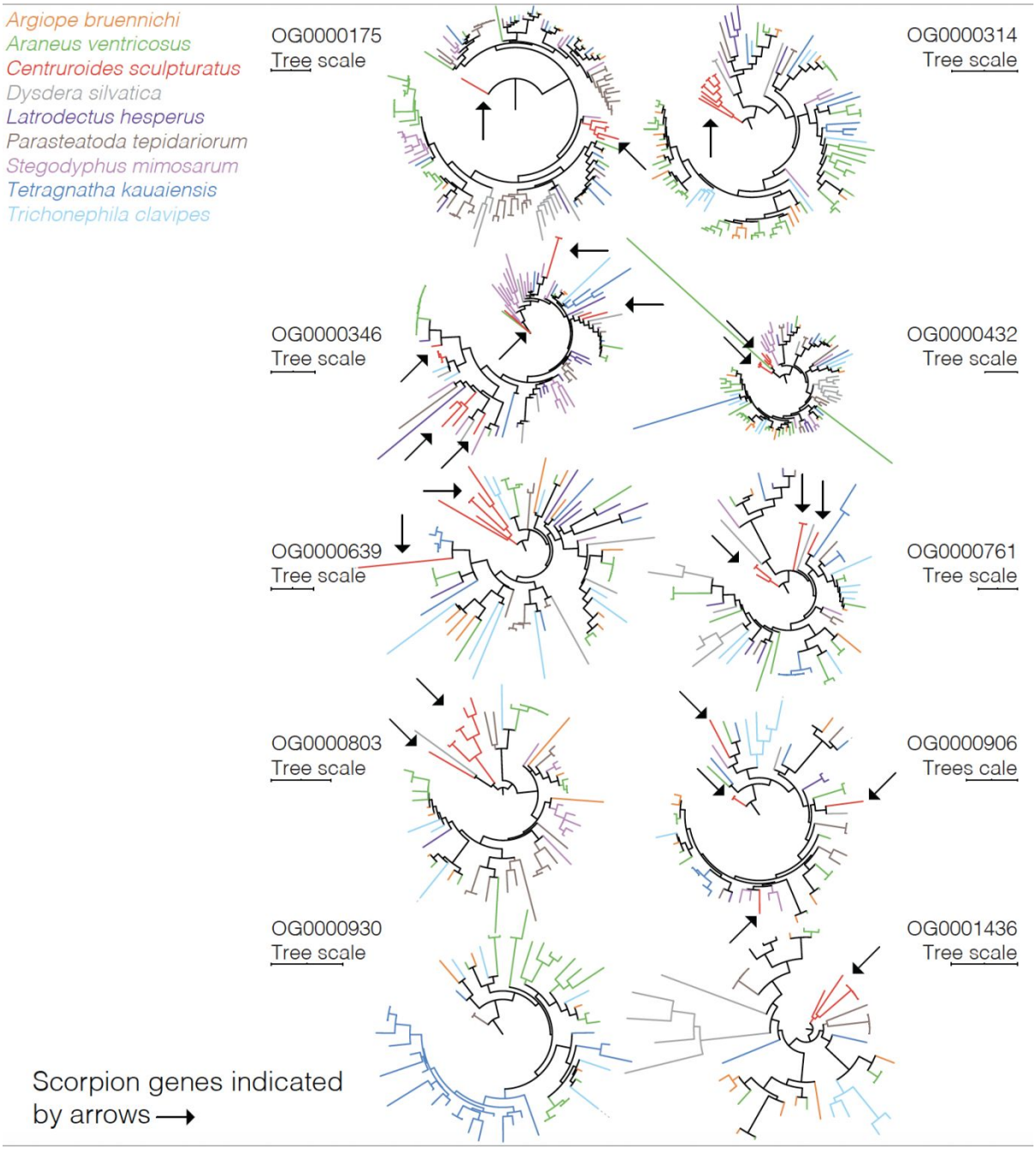

Figure 3. Venom gene phylogenies. Phylogenies for the 10 largest orthogroups of identified venom genes. For each tree we indicate the Orthogroup ID and tree scale. Different colours correspond to different species, as displayed in the legend. Arrows highlight scorpion toxin genes, and show that most orthogroups in were already present in before the split between scorpions and spiders.

Venom gene-family variation

The combination of BLAST and TOXIFY identified a total of 559 toxins in the studied genomes (Supplementary Table 8), included as part of 189 orthogroups. The orthogroups with most genes are displayed on Figure 3 and include OG0000175 (135 genes, Astacin-like metalloproteases as determined by NCBI-blast), OG0000314 (105 genes, Neprilysins or endothelin-converting proteins),

OG0000346 (99 genes, uncharacterized proteins), OG0000432 (86 genes, Techylectin), OG0000639 (68 genes, various toxin-types) , OG0000761 (61 genes, Zonadhesins, various various toxin-types), OG0000803 (59 genes, Astacin-like metalloproteases), OG0000916 (54 genes, Papilins, Kunitz-type serine protease inhibitor) OG0000930 (54 genes, Astacin-like metalloproteases), OG0001436 (41 genes, uncharacterized proteins). The two most toxin-rich assemblies were the *A. bruennichi* and *P. tepidariorum* where 154 and 200 toxins were identified, respectively. The scorpion genome, *C. sculpturatus*, yielded 31 toxins, whereas *D. silvatica* and *L. hesperus* yielded 13 and 16 toxins, respectively (Supplementary Table 8).

Phylogenetic analyses of the orthogroups show that most venom families were present before the split between scorpions and spiders (Figure 3). Different spider genomes include species-specific expansions (i.e. groups of 5 or more genes from a single genome that cluster as a monophyletic clade), and many of these have relatively large branch lengths. Specifically, we find evidence for various expansions in *P. tepidariorum* (4 expansions, one with 7 genes, another with 12, one with 7 and one with 9 genes), one expansion in *A. ventricosus* (one expansion with 11 closely related genes), one in *D. silvatica* (one expansion in 6 genes) and one in *C. sculpturatus* (5 genes expanded) in OG0000175 (Figure 3). In OG0000314, we found an expansion private to the three Araneidae genomes, including *A. bruennichi*, *A. ventricosus* and *T. clavipes*), various expansions exclusive to the *A. ventricosus* genome, and one expansion specific to the scorpion genome (9 genes). In OG000346, we found various expansions on the *S. mimosarum* (9 genes), *P. tepidariorum* (5 genes), *A. ventricosus* (8 genes) genomes. In OG000432 we found genome-specific expansions in *D. silvatica* (8 genes; Figure 3). In OG0000639, we found an expansion in *C. sculpturatus* (5 genes), and in OG0000803 there are two 5-gene expansions, one in *C. sculpturatus*, another in *A. ventricosus*. OG0000930 is only present in *T. kauaiensis* (1 expansion with 20 genes), *A. ventricosus*, *A. bruennichi*, *T. clavipes* and *P. tepidariorum*. OG0001436 is expanded in *C. sculpturatus* (5 genes).

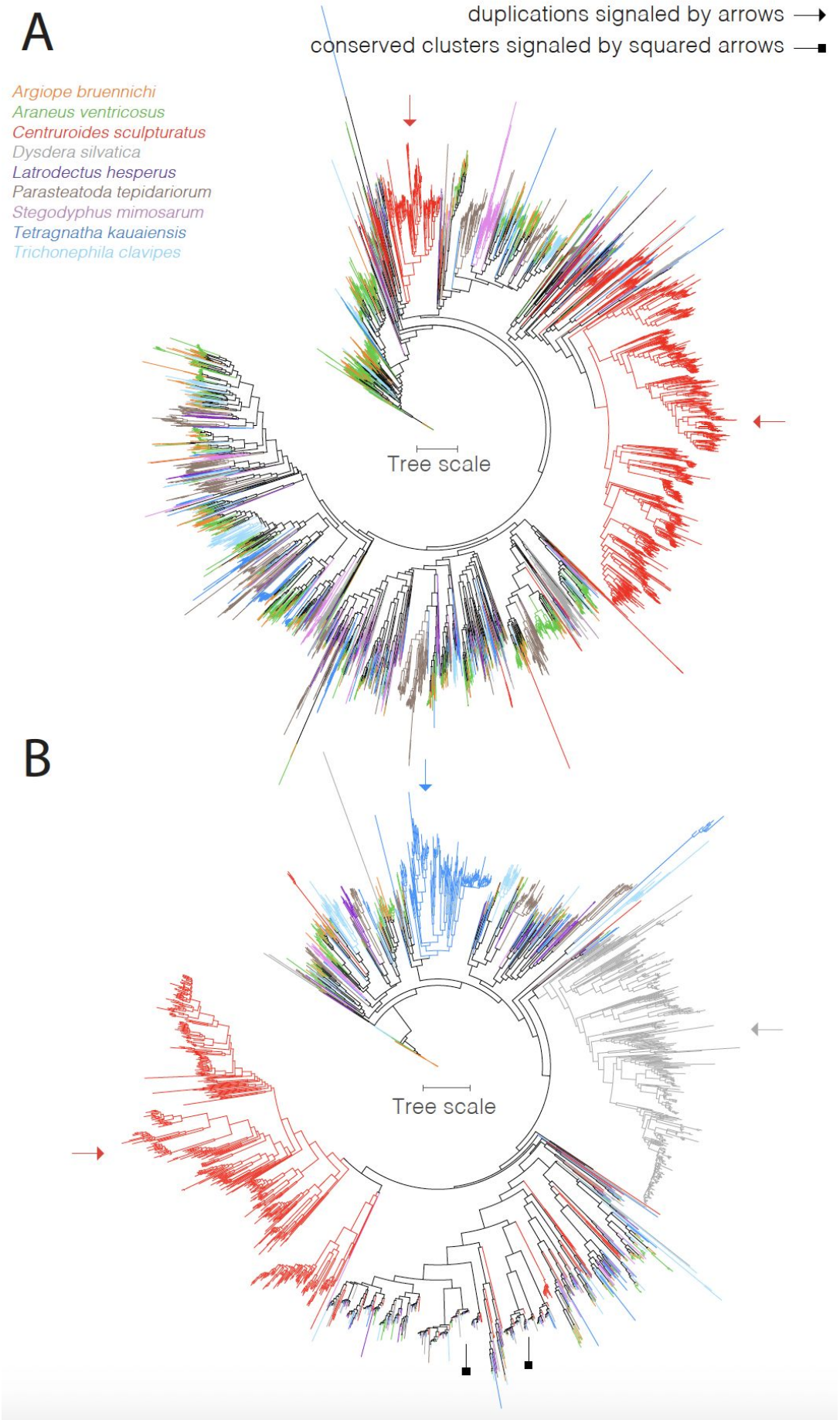

Figure 4. **Gustatory and Ionotropic reception phylogenies** a) Gustatory receptor phylogeny. The phylogeny has 5,595 genes and includes every GR identified in the assemblies herein studied. b) Ionotropic receptor phylogeny. The phylogeny has 1,932 genes and includes every IR identified in the assemblies herein studied. Arrows indicate major duplications private to specific genomes, whereas squared arrows highlight potentially conserved IR genes (small branch length and small duplicates).

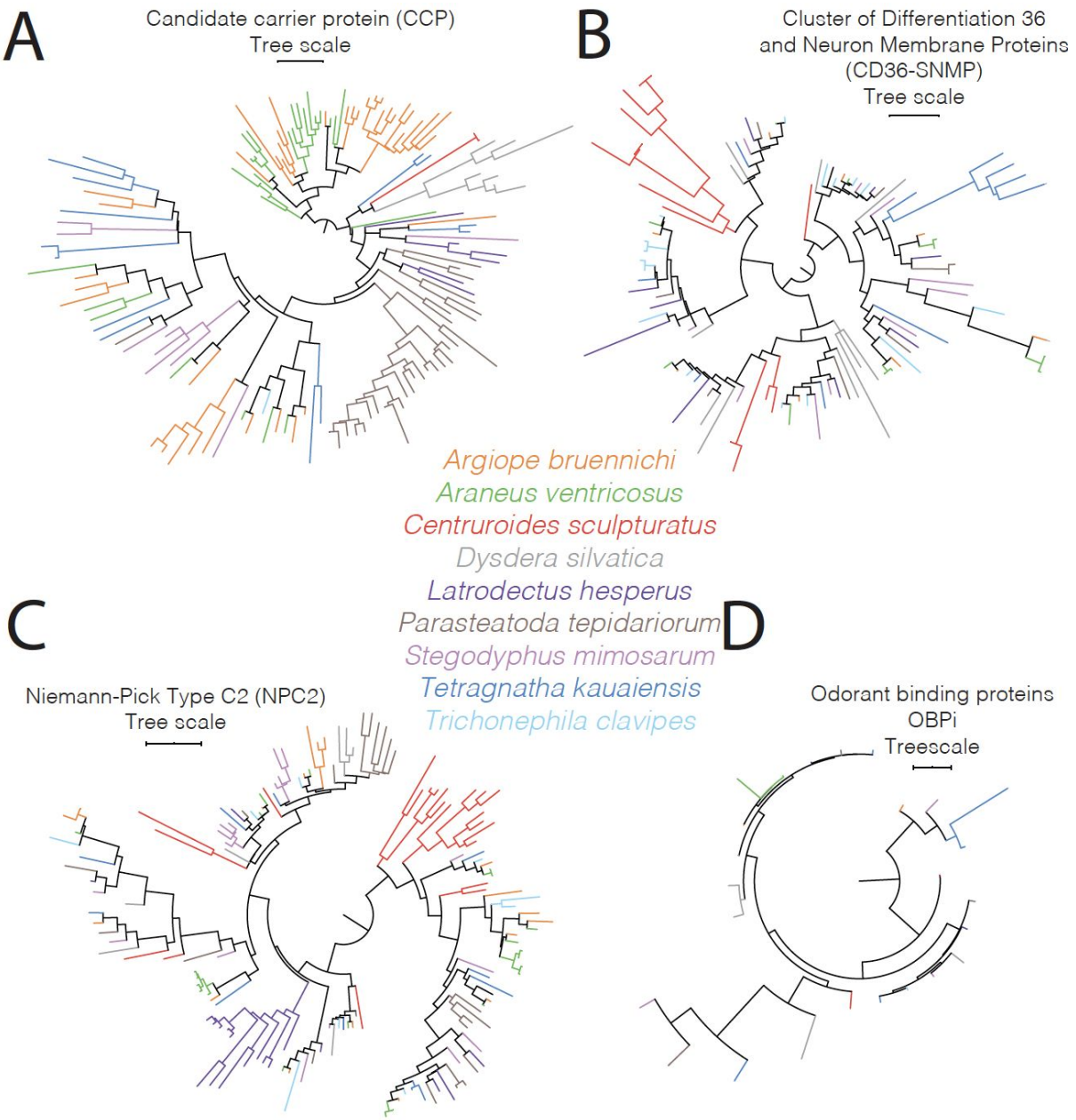

Figure 5. Phylogeny of other chemosensory genes. a) candidate carrier protein (CCP) phylogeny; b) cluster of differentiation 36 and neuron membrane proteins (CD36-SNMP) phylogeny; c) Niemann-Pick type C2 (NPC2), phylogeny; d) Odorant binding proteins (OBP-like) phylogeny.

Chemosensory gene-family variation

We identified a total of 5,595 candidate gustatory receptors (GRs), 1,934 candidate ionotropic receptors (IRs), 25 candidate Odorant binding proteins (OBP-like), 147 candidate Niemann-Pick type C2 (NPC2), 137 candidate carrier protein (CCP), and 998 candidate cluster of differentiation 36 and neuron membrane proteins (CD36-SNMP; Supplementary Table 9; Figures 4-5). GRs exhibited a large interspecific variation (Figure 4), ranging between 1,436 GRs in *A. ventricosus* and 84 in *L. hesperus*. *C. sculpturatus*, the outgroup, had 1,648 GRs (Supplementary Table 9). The *D. silvatica* genome has the most IR/iGluR genes with 443 genes (Supplementary Table 9; Figure 4). We detected a total of 25 OBP-like genes, with 5 being present in *T. kauaiensis*, 4 in *D. silvatica* and in *S. mimosarum*, 3 in *P. tepidariorum* and all remaining genomes having only 1 or 2 OBP-like genes (Supplementary Table 9; Figure 5). From the 147 identified NPC2, *D. silvatica* had the least NPC2-genes (7 genes) and *A. ventricosus* the most (23). *A. bruennichi* had the most CCP, with 41 genes, while *C. sculpturatus* and *T. clavipes* had only 1 CCP (Supplementary Table 9; Figure 5). Finally, we identified at least 8 and at most 16 CD36-SNMP genes. *T. clavipes*, and *C. sculpturatus* had the most CD36-SNMP genes with 16 and 14, respectively, while *P. tepidariorum* and *A. bruennichi* had the least with 8 (Supplementary Table 9).

Analysis of phylogenetic patterns suggests that the chemosensory portfolio is driven by a highly dynamic diversification process. For instance, within GRs there are two genome-specific expansions of genes in the scorpion, one including 1,237 genes and another 235 genes (Figure 4). A similar pattern is observed in the IRs where we find two genome-specific expansions private to the scorpion genome (88 genes, and 382 genes; Figure 4), a large genome-specific gene group with 392 genes in *D. silvatica*, and another in the *Tetragnatha* genome including 139 genes. In CCPs, we found expansions in *A. bruennichi* (5 genes and 13 genes), *P. tepidariorum* (21 genes), *A. ventricosus* (8 genes) and *D. silvatica* (6 genes; Figure 5A). In CD36-SNMP we found expansions in the scorpion, (9 genes), and in *T. kauaiensis* (5 genes; Figure 5B). In NPC2, we found expansions in *L. hesperus* (14 genes), *P.* tepidariorum (6 genes), and *C. sculpturatus* (14 genes; Figure 5C), while in CD36-SNMP (Figure 5D) we found expansions in the *T. kauaiensis* (5 genes), and *C. sculpturatus* (9 genes) genomes.

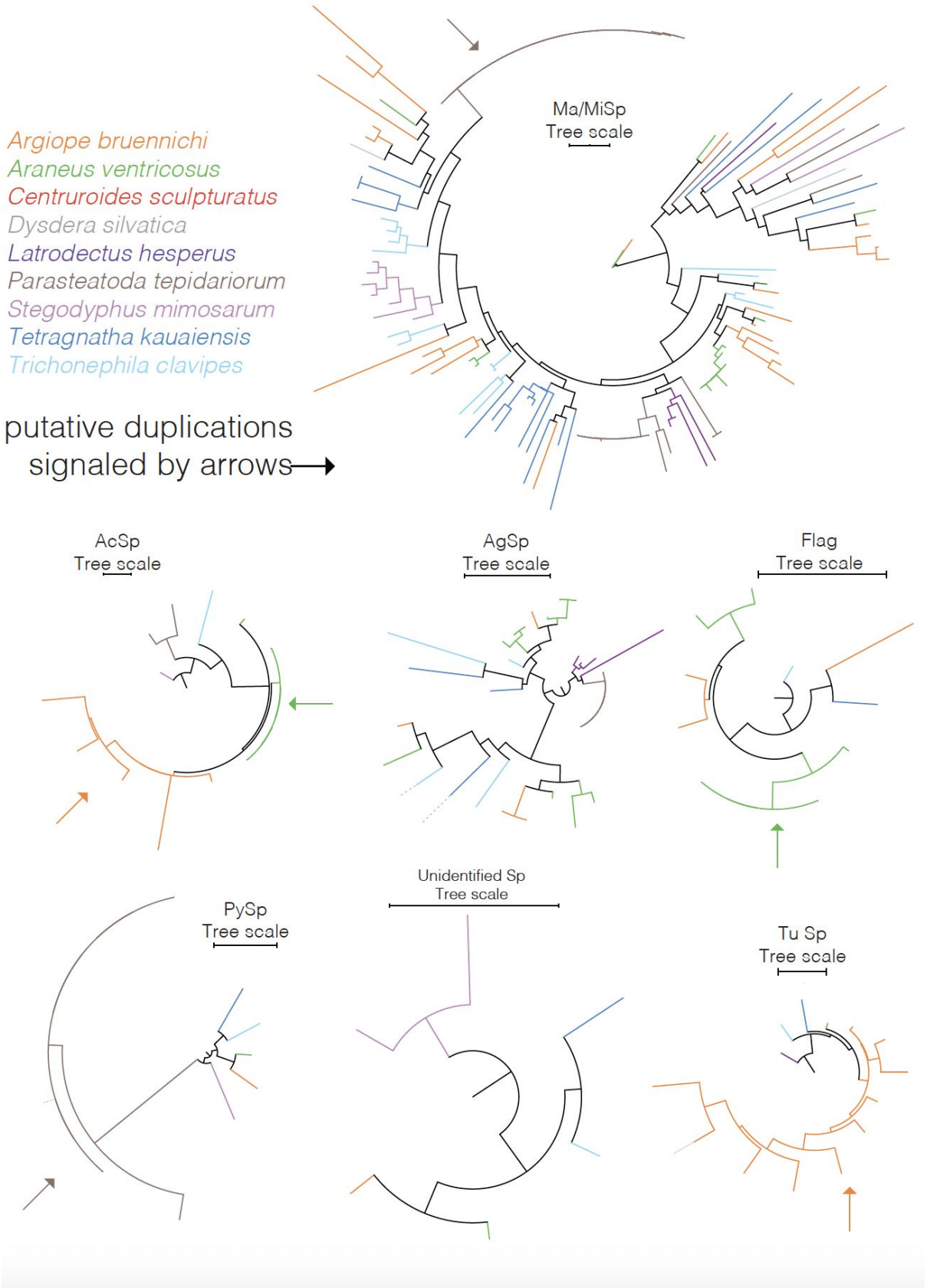

Figure 6. Silk genes (spidroins) phylogeny These including Major and Minor Ampullate spidroins (Ma/MiSp), Aciniform spidroins (AcSp), Aggregate spridroins (AgSp), Flagelliform spridroins (Flag),

Pyriform spridroins (PySp), an unidentified spridroins group present in the *T. clavipes* genome and the Tubuliform spidroins (TuSp).

Silk gene-family

We identified a total of 24 putative spridroins in the genome of *T. kauaiensis* (Supplementary Table 9). After querying these to the NCBI protein database, we identified 1 Flagelliform spridroin (Flag), 4 Aggregate spridroins (AgSp), 8 Major Ampullate spridroins (MaSp), 3 Minor Ampullate spridroins (MiSp), 1 Tubuliform spridroins (TuSp), 1 Pyriform spridroin (PySp) and 1 Aciniform spridroin (AcSp). There was one spidroin for which NCBI did not yield any results, and 4 where the database retrieved more than a single gland as a top-hit (Supplementary Table 9). Alignments are provided in the supplementary.

Phylogenetic patterns of spidroin shows several genome-specific expansions of the Ma/Mi spridroins, including two separate expansions in the *P. tepidariorum* genome (25 genes and 10 genes; Supplementary Table 10; Figure 6), a single expansion in *S. mimosarum* including 7 genes, another in *A. ventricosus* including 8 genes, and another in *T. kauaiensis* including 7 genes. In the remaining spridroins, we find genome-specific expansions in AgSp and PySp in *P. tepidariorum*, with 9 and 6 genes, respectively. In AcSp there are two smaller lineage-specific clades in *A. bruennichi* and *A. ventricosus*. There is a genome-specific expansion in *A. bruennichi* for the TuSp gland, with 7 genes (Supplementary Table 10; Figure 6).

Discussion

In this study, we report the sequence assembly of the *Tetragnatha kauaiensis* genome, and explore genome evolution across the available spider assemblies. To do so, we controlled for the quality of the assemblies, by focusing on contiguity and completeness (i.e. how complete a genome is from a gene content perspective based on the presence of universal single copy genes), finding that many of these assemblies are highly fragmented and incomplete. We find a wide variation in gene content, repeat content, and genome size in the surveyed spider genomes, which indicates a highly dynamic pattern of genome evolution. While the low quality of some assemblies did not hamper comparative analyses of the surveyed spider genomes, results should be interpreted with caution. By surveying all repeats and transposable elements (hereafter 'the repeatome') and studying Hox gene duplications, we find that the observed genome size differences are likely driven by the expansion of the repeatome. We also find significant gene-family expansions associated with sensory perception of taste, immunity and metabolism, which may underlie the diverse biology of spiders. We confirm previous work showing that venoms and chemosensory genes are present in high numbers across the assemblies, and discuss the role of a putative ancient whole genome duplication in generating the diversity we observe in spiders.

Repeat content underlie genome size variation in spiders

Previous evidence from flow cytometry, Feulgen image analysis densitometry, and genome assembly sizes have found wide variation in genome size in spiders (Gregory and Shorthouse 2003; Sanggaard et al. 2014; Král et al. 2019). For instance, Gregory and Shorthouse (2003) assembled a large dataset comprising 115 species from 19 different families of spiders, finding that spider genomes vary between 5.73 - 0.79 C (~7 Gb for the jumping spider *Habronattus borealis* – ~724 Mb for the long-jawed orbweaver *Tetragnatha elongata*). They also reported a wide variation within relatively closely related species. For instance, genome size in the Salticidae family ranged between 1.73 – 5.73 C (between *Habronattus borealis* and the peppered jumping spider *Pelegrina galathea*). Our results are in line with this evidence, since we found variation in genome size among spider assemblies (in our dataset the largest genome was *A. ventricosus* with 3.6 Gb, and the smallest was *T. kauaiensis* with 1.08 Gb). We also report variation between relatively closely related species (i.e. within the Araneidae family, where we included three assemblies, genome sizes ranged between 3.6 Gb and 1.7 Gb). Similar to previous reports, we do not find a clear phylogenetic pattern of genome size variation across the spider tree of life (Gregory and Shorthouse 2003).

Genome size may increase through whole genome duplication, where the whole genome doubles itself, or through small scale duplication of genetic elements which may include duplication of genes or transposable elements. Recent evidence, using flow cytometry, has revealed a whole genome duplication in caponiid spiders (Král et al. 2019), which indicates the potential of further whole genome duplications in spiders, other than the duplication ~450 million years ago (Schwager et al. 2007; Schwager et al. 2017). While we have no caponiids in our dataset, we found no evidence of recent whole genome duplication specific to spiders on the analyzed assemblies. This evidence comes from several sources. First, there is a low % of double copy BUSCO genes – a set of highly curated genes, single copy genes. The scorpion assembly has a duplicate BUSCO score of 26 %, whereas spider genomes range between 26% - 0.8 %, in *P. tepidariorum* and *L. hesperus* (note that *L. hesperus* assembly has many missing BUSCOs, which is indicative of a poor assembly quality). Second, analysis of Hox genes shows that these genes are mostly present in 2 copies, with a single exception of four Hox4 in *A. ventricosus*. The four copies of Hox4 in *A. ventricosus* could be an artefact due to the similarity between Hox genes, and we were not able to obtain candidates for Hox1 using the 95% cut-off threshold. The BUSCO-pattern together with that from the Hox genes are in line with the evidence for a ancestral whole genome duplication in Arachnopulmonata. Third, an important finding of our work is that variation in genome size of spiders is largely driven by the duplication of genetic elements, and specifically, the repeatome (transposable elements and repeats). Indeed, we find a R=0.95 correlation between the 'length of the masked repeats' and the 'genome size' – a strong indication of the role of the repeatome in underlying genome size changes (Figure 1). Expansions of the repeatome are generally constrained in animal lineages since bigger genomes translate to higher

cell-economy costs through the increase of cell size. In addition to this, proliferation of transposable elements may interfere with gene expression when these selfish elements jump in front of a gene promoter (Choi and Lee 2020). Considering the strikingly different representation of the repeatome that we find here, including the variation in transposable element accumulation through time, we speculate that transposable elements may have had a role in the regulation and variation of gene expression across spiders, likely underlying some of the observed morphological and physiological diversity.

By conducting a *de novo* annotation of repeats and using the same version and library of repeats for every genome, we guaranteed a standardization of the repeat identification, thereby removing potential biases due to the use of different databases and pipelines. Variation in some elements, both in terms of classes and extent along the genome, was substantial. For instance, Long Interspersed Nuclear Elements (LINESs) occupy less than >2% in every assembly, but occupy 10.3% of the *D. silvatica* assembly. This may suggest mechanisms to purge LINEs from some clades, or an expansion specific to *D. silvatica* (and possibly closely related species). Furthermore, DNA elements had a three-fold variation, ranging between 5.59 % (*T. kauaiensis*) and 18.82 % (*D. silvatica*). Despite the overall variation in numbers and accumulation of the repeatome through time, there was a clear dominance of DNA/TcMar and DNA/hAT elements (both DNA elements) across the assembly when considering the top three most represented categories (Figure 1B), suggesting these elements are the most prolific and present across spiders, and potentially scorpions (keep in mind we have single scorpion genome in our analyze using the same version and library of repeats for every genomes). Future studies on spider genome assemblies should put transposable element variation in the context of the spider phylogeny, and should benefit from an increased sampling of spider genomes. The differential presence of repeats and transposable elements may indicate that mechanisms to eliminate these elements such as nonhomologous end joining, or illegitimate recombination may be active in these genomes (Choi et al. 2020). A phylogenetic framework together with ancestral character reconstructions, focusing on transposable element data, will certainly elucidate the patterns of activation and deactivation of certain transposable element classes, and how changes in transposable element proliferation may be linked to particular events in the evolution of spiders. For instance, a caponiid genome, where a more recent genome duplication was detected (Král et al. 2019), may help understand the impacts of whole genome duplication and transposable element proliferation in spiders. This would allow testing the ‘genomic shock’ hypothesis after genome duplication in spiders. Finally, the variation in the repeatome is in line with those of the remaining arthropods, where variation in transposable elements load was deemed as an important predictor for genome size (Wu and Lu 2019; Gilbert et al. 2021).

Gene duplicates

Observed patterns in the explored gene families, namely venoms and chemosensory, suggest a central role in the evolution of spiders (Figure 3-5). The presence of most gene families in the scorpion genome and in spider genomes suggests an ancestral status (Vizueta, Escuer, et al. 2020), while variation in gene numbers and their branch lengths along the phylogeny is an indication of divergence, and thereby indirect evidence of the acquisition of novel gene functions (i.e. neofunctionalization). Gene duplicates generally experience relaxation of purifying selection or gene dose compensation and, if one of the copies does not get sub- or neofunctionalized through time, it will be lost. Indeed, we manually curated chemosensory genes, finding a low ratio of pseudogenes (Supplementary Table 9). There are large genome-specific duplications detected in *C. sculpturatus*, *T. kauaiensis* and *D. sylvatica* in the two largest chemosensory families (Figure 4 A, B). This is an indicator of the importance of gustatory (GRs) and ionotropic receptors (IRs) in *T. kauaiensis* and *D. silvatica*, and we speculate it may be associated with the colonization of islands (*T. kauaiensis* is part of a Hawaiian radiation of spiders, and *D. silvatica* is part of a Macaronesian radiation) where environmental conditions can be very different (disharmonic biotas, open ecological niches) (Vizueta et al. 2019). We note that, unfortunately, the taxonomic range (i.e. 1 single genome for Tetragnathidae and 1 single for Dysderidae) does not allow dissecting whether these changes are shared by other members of the families, whether they are private to the species in question (*D. silvatica*, *T. kauaiensis*) or even to the adaptive radiation (in Hawai'i and Macaronesia). Similarly, since we only included a single scorpion assembly, we cannot comment on whether the expansions observed in *C. sculpturatus* are specific to all scorpions, or just the *C. sculpturatus* genome.

Despite the aforementioned evidence, not every gene family is present in very high numbers. For example, we only detected 25 OBP-like genes in all genomes, and the small number of genes together with the short branch lengths confirms that the OBP-like are a relatively conserved family of genes in arachnids (Vizueta et al. 2017). In addition to the OBP-like, we also find few silk genes, with very short branch lengths (notice *P. tepidariorum* in PySp and Ma/MiSp, *A. ventricosus* in Flag and AcSp), which may be indicative of very recent duplications in silk genes (Garb et al. 2007; Clarke et al. 2014; Clarke et al. 2015). These results are in line with those of Clarke et al. (2015) who used transcriptomics to suggest that a large-scale duplication occurred early in the divergence of spiders, and that multiple independent duplication events in silk genes have likely taken place afterwards. Our results, however, have to be interpreted with caution since silk genes are composed of sequences (of often hundreds) of repeated aminoacids (Clarke et al. 2015), being therefore hard to reconstruct in entirely in the gene annotation process, and being typically fragmented onto separate fragments. Considering the fragmentation of most assemblies, it is possible that some duplicates consist of gene fragments.

Significant expansion of metabolism, immunity and sensory perception gene families

Using a statistical approach to detect expansion of gene families, we find that most expansions are in terminal branches. As a direct comparison, recent analyses on 76 insect assemblies were able to identify 147 expanded gene families, comprising 9,601 genes, in the branch corresponding to insects ('the Last-Insect-Common-Ancestor'; Thomas et al. 2020), thereby providing evidence for 'ancient expansions' particular to insects. Thomas et al (2020), however, included 10 times more genomes than we did, and some of the spider genomes in our dataset lack substantial data, as indicated by the BUSCO scores (Supplementary Table 6). Thus, it is possible that spiders have their own set of 'ancient expansions', which we were not able to detect due to the limitations of our dataset. It is also possible that the inclusion of fragmented assemblies (*D. silvatica* and *L. hesperus*) leads to an inflation of expanded gene families on closely related assemblies (e.g. *P. tepidariorum*). We expect that the addition of more highly completed spider genomes will help to further our understanding of the evolutionary history of gene families in spiders.

Despite the challenges in the dataset, we find notable evidence for various gene families expansions in spiders. Specifically, using gene ontology annotations (GO) we find that gene families associated with various metabolic functions, sensory perception of taste, and immune functions are expanded. This pattern is similar to the pattern found in arthropods whichi includes expansions of metabolic genes (Thomas et al. 2020). These independent pieces of evidence suggest that gene duplications associated with metabolism, immunity and sensory functions may have been instrumental to the evolution of arthropods in general, but also spiders specifically. We speculate that these expansions may contribute to the success, in terms of number of species and adaptation to different environments in spiders. As chromosome resolved assemblies become cheaper and technically less challenging, revising the role of gene expansions and gene contractions will certainly yield important insights towards the understanding of genome evolution of spiders.

Conclusion

We have sequenced the *Tetragnatha kauaiensis* genome, and explored patterns of genome evolution across various genome assemblies. Comparative genomics analyses including *T. kauaiensis*, 1 scorpion (outgroup), and 7 additional spiders assemblies suggest that variation of transposable elements and repeat content are associated with the wide variation of spider genome sizes. We also found many duplications in chemosensory and venom genes, consistent with the evidence that the evolution of toxins and the ability to perceive the environment are ancestral attributes of spider evolution. Our results suggest that the evolutionary history of spiders is characterized by gene-family expansions associated with sensory perception of taste, metabolism and immune responses, and by multiple gene duplication events. While we uncovered interesting patterns of genome evolution, we acknowledge the limitations of this work due to the lack of high-quality genomes. We hope that, however, this work catalyzes enthusiasm in the spider research community to produce and analyse more high-quality genomes.

Methods

Tetragnatha kauaiensis - Genome sequencing, assembly, annotation and quality verification

We sequenced the genome of a single individual of *T. kauaiensis* using a paired-end and a non-size selected mate-pair library on a lane of Illumina HiSeq4000 (individual ID AJR402, collected 31/May/2013 by AJ Rominger in Kaua'i, at 22.1412, -159.6206). Using these libraries we built a base assembly using ALLPATHS-LG with default parameters in addition to 'HALOIDIFY = True' (Gnerre et al. 2011). We then sequenced an additional individual using the Dovetail Chicago method (AJR443, collected 03/June/2013 by AJ Rominger in Kaua'i, at 22.1469, -159.6638), which was used to scaffold the initial assembly using the HiRise software (Koch 2016; Putnam et al. 2016).

The quality of the assembly was first assessed using BUSCO v3.0.2 arthropoda db v9 (Simão et al. 2015), which searches for highly conserved genes in the assembly. Then we used the Assemblathon 2 script (https://github.com/ucdavis-bioinformatics/assemblathon2-analysis) (Bradnam et al. 2013), which assesses scaffold and contig statistics, to evaluate the quality of the assembly. Annotation of repeats was carried out by identifying and building a database of repeats along the genome using RepeatModeler followed by masking them using RepeatMasker (Tarailo-Graovac and Chen 2009). We explored the draft assembly for contaminants, including gut-microbiota and wet-lab contaminants using Blobtools (Koutsovoulos et al., 2016; Laetsch et al., 2017; Dominik R Laetsch and Blaxter, 2017)(Supplementary Figure 1).

To determine protein-coding genes and their locations along the genome, we used BRAKERv1 (Hoff et al. 2019). We used whole-body *T. kauaiensis* transcriptome reads previously generated by (Yim et al. 2014) (SRR1313313, SRR1427109). Raw transcriptomic reads were cleaned using Trimmomatic (Bolger et al. 2014) and aligned to the generated genome using STAR (Dobin et al. 2013). The resulting binary alignment map (BAM) file was provided to BRAKERv1 as RNA-based evidence. The final annotation was assessed by BUSCOv4.0.1 (Seppey et al. 2019), using the Arthropoda10 (1,013 genes) and Arachnida10 (2,943 genes) gene sets.

Genomes used for comparative genomics

We searched the I5K and NCBI databases and the literature for published and available spider genomes (data consulted on 23rd October 2019). In total, we downloaded nine spider genomes (Supplementary Table 1), their general feature format (gff3), and predicted protein files (faa; Supplementary Table 1). From the available genomes, we selected those with a contig-N50 above 8,000 bp in order to avoid genomes that were highly fragmented. This included the genomes of *Stegodyphus mimosarum* (Sanggaard et al. 2014), *Latrodectus hesperus* (BCM-HGSC website), *Parasteatoda tepidariorum* (Gendreau et al. 2017), *Trichonephila clavipes* (Babb et al. 2017), *Dysdera silvatica (Sánchez-Herrero et al. 2019)*, *Araneus ventricosus* (Kono et al. 2019) and *Argiope bruennichi* (Sheffer et al. 2021). Additionally, we downloaded the genome of the bark scorpion *Centruroides sculpturatus* (Schwager et al. 2017) as an outgroup.

Characterization of spider genomes

We characterized spider genomes based on the (i) continuity and completeness of the assemblies, (ii) assembly size, (iii) repeat-content, and (iv) broad genomic features. Specifically, (i) the continuity of each genome serves as a proxy of the overall quality of an assembly, and it affects the detection of genes, repeat sequences and transposable elements (Peona et al. 2018). We characterized the contiguity of the assemblies using the Assemblathon 2 script, as described above for *T. kauaiensis*, retrieving contig-N50, scaffold-N50, total number of contigs, total number of scaffolds, maximum scaffold size, assembly size and GC content. (ii) The 'completeness' of the assemblies, is generally defined as an overview of the genes which may be missing, fragmented, duplicated or present in a single copy in an assembly. To assess the completeness of the genomes, we used BUSCO v4.0.1 as outlined above for *Tetragnatha kauaiensis* (the Arthropoda10 set including 1013 genes; and the Arachnida10 set including 2,943 genes). (iii) To assess repeat content, we used Repeat-Modeler v2.0.1 and Repeat-Masker-v4.1.0. Repeat content in the genome includes simple repeats (typically 1-5 base pairs, e.g. AAA, TTTTT), tandem repeats (100-200 base pairs), segmental duplications (10,000 - 300,000 base pairs), and interspersed repeats (SINES, which are non-functional copies of RNA genes that were reintegrated into the genome; DNA transposons; LINES, which are non-retrovirus retrotransposons). We ran RepeatModeler and RepeatMasker for each genome to screen and annotate DNA sequences *de novo*, thereby annotating and masking repeats. We retrieved repeat-statistics including % of the genome covered by different repeats and transposable element landscape plots. Finally, (iv) we assessed broad genomic features including, among others, the number of genes, coding sequences, introns, gene length using Another Gff Analysis Toolkit v0.4.0 (AGAT available at https://github.com/NBISweden/AGAT/; agat_sp_functional_statistics.pl, and agat_sp_statistics.pl). The association between total genome size, and % of masked sequences and total length of masked genome was assessed with a correlation using the cor() function in R.

Spider genome evolution

Previous work suggests that the whole genome duplication in the common ancestor of scorpions and spiders can be linked to the diversification of spiders (Schwager et al. 2007; Schwager et al. 2017). To better understand the presence of whole genome duplication in the studied lineages, we used two complementary approaches. We first analyzed repeat content variation in the available spider genomes (as described above), since differences in repeat content may translate to differences in genome size. Second, we downloaded the Hox genes 1-5 from the *P. tepidariorum* genome, and searched for these in the remaining spider genomes using BLAST (Altschul et al. 1990). Hox gene-copies are prime candidates for detecting whole genome duplications since they are functionally constrained (Leite et al. 2018). For example, a 1:4 ortholog ratio is maintained between the *Drosophila melanogaster* genome and vertebrate genomes, indicating the two whole genome

duplications which occurred in the lineage of modern vertebrates (Hakes et al. 2007; Schwager et al. 2017).

Spider gene-family evolution

Another component of genome evolution is gene-family expansion and reduction, or the gain and loss of gene-copies. Focusing on the predicted-proteins resulting from the annotations of the spider genomes, we first cleaned and filtered sequences using Kinfin's filter_fastas_before_clustering.py (Laetsch and Blaxter 2017) removing sequences shorter than 30 amino acids. We then removed all isoforms of a given gene, keeping only the longest isoform using in-house scripts. For this analysis, we removed the genome of *A. ventricosus* since it has twice the number of genes compared to the other spider genomes, and this biases the analysis. Cleaned and isoform-free prediction-proteins were then analyzed using Computational Analysis of Family Evolution (CAFE v 4.2.1) (De Bie et al. 2006). Briefly, we first determined gene-similarity (based on BLAST- e-values) in the dataset using an all-by-all blast approach. We then applied a Markov Cluster algorithm (MCL; mcxload, mcl mcxdump) (Enright et al. 2002), and parsed the output using the mcl2rawcafe.py script. These clusters (gene-families) are then integrated in a phylogenetic-backbone, which was retrieved from OrthoFinder's single-copy orthologs (Emms and Kelly 2015). This tree was then converted to an ultrametric format with r8s (Sanderson 2003), using the divergence time of 175 million years between Tetragnathidae (*T. kauaiensis*) and Araneidae (*A. bruennichi*) as a calibration point (Fernández et al. 2018). We used Dendroscope's Graphical User Interface (GUI) to visualize trees and remove bootstrap support (Huson and Scornavacca 2012). Using the main pipeline of CAFE, we estimated the birth-death parameter lambda ($\lambda = 0.0021$) for the dataset and obtained information on gene-family under significant evolution.

Genes belonging to gene-families that have undergone significant changes, that is, fast evolving families, were annotated using Gene Ontology terms (GO:terms) using the command-line version of Interproscan v5.34-73.0 (Ashburner et al. 2000). GO term annotations for genes belonging to expanded or reduced gene families were summarized and plotted as a treemap using R (Team and Others 2013) with REVIGO's treemap script (Supek et al. 2011) .

Silk, chemosensory and venom gene variation

To investigate venom gene evolution, we downloaded all toxin sequences available in the Arachnoserver v3.0 (Pineda et al. 2018), and used these as a database to query proteins from the spider and scorpion genomes with BLAST. Hits with e-values below 1e-10 were considered as candidate venom-genes. However, since venom proteins are potentially highly divergent and typically short, BLAST searches may result in a high proportion of false positives. To address this issue, we ran TOXIFY on the candidates, a pipeline specifically designed to identify toxins using deep learning algorithms (Cole and Brewer 2019). TOXIFY generates a prediction score between 0 and 1 where the

higher the score, the more likely a molecule is to be a venom, and we selected values above 0.75 as a criterion here. After TOXIFY, we kept a list of 589 putative venom genes across the assemblies. We then used OrthoFinder, obtaining an orthogroup-assignment for each of these 589 venom genes, finding that they group in 189 orthogroups. From these 189 groups, we selected the 10 biggest (in terms of gene number), identified the toxin-group using NCBI nr protein database, and aligned the genes within orthogroups using mafft v7.455 (Katoh and Standley 2013). These alignments were then used to obtain a maximum likelihood (ML) phylogenetic tree with bootstrap estimate (automatic determination of the substitution model) using IQ-Tree v1.6.12 (Nguyen et al. 2015; Chernomor et al. 2016; Kalyaanamoorthy et al. 2017; Hoang et al. 2018). The resulting phylogeny was plotted, formatted, coloured and labelled using the iTOL web server (Letunic and Bork 2019).

Considering the recent evidence on the wide variation in chemosensory gene-family size in Chelicerates (Vizueta et al. 2017; Vizueta et al. 2018), we searched the available genomes for Gustatory Receptors (GRs), Ionotropic Receptors (IRs), Niemann-Pick Type C2 (NPC2), Odorant binding proteins (OBP-like), Candidate carrier protein (CCP), Cluster of Differentiation 36 and Neuron Membrane Proteins (CD36-SNMP). To do so, we used BITACORA v1.2 (Vizueta, Escuer, et al. 2020; Vizueta, Sánchez-Gracia, et al. 2020), using its GeMoMa algorithm (Keilwagen et al. 2019), benefiting from a curated chemosensory database used in Vizueta et al (2018). To ensure the quality of the annotations, we performed a round of manual curation of the results, guaranteeing that (i) only a single isoform was selected and (ii) that putative annotation artefacts including small fragments, chimeric annotations or identical proteins by misassembly of duplicated contigs were removed. Finally, curated gene members were classified as pseudogenes (i.e. sequences with in-frame stop codons), partial or putatively complete functional proteins. The identified GRs, IRs, NPC2, OBP-like, CCP and CD36-SNMP were aligned using mafft, and a tree was generated and plotted using IQ-Tree and iTOL as described above.

We next identified spidroins (silk genes). To do so, we used a combination of BLAST searches using N-domains published with the *T. clavipes* genome, and the NCBI accession numbers for N-terminals and C-terminals from Vienneau-Hathaway et al. (2017). We extracted hits with an e-value below 1e-10 and candidate silk genes were then queried in NCBI nr database search (blastp) to classify the gland to which they belong based on NCBI's top hit. After labelling the gland, we did an orthogroup assignment using OrthoFinder as described above, and built a phylogeny for the silks in each gland, using the same method as described above for venom genes.

Data availability statement

The raw data is available through ENA (https://www.ebi.ac.uk/ena/browser/home), ID: PRJEB48087. The assembly and annotation is available through DRYAD (https://doi.org/10.5061/dryad.b2rbnzsgr).

Acknowledgements

JC is immensely grateful to Torsten H. Struck, for the freedom to pursue his interests, and for mentorship and stewardship (this is NHM genomics contribution X). JC is extremely grateful to Mark Blaxter for receiving him in his laboratory and opening the assembly, annotation and comparative genomics world – 'lang may yer lum reek'. We thank Andy J. Rominger for the field collection of specimens. JC thanks Lewis Steven, Andrea Martínez Martínez, and Dom Laetsch for their time, patience and expertise on gene and repeat annotation. A note of appreciation to Samuel Abalde, for advice on venom identification and classification. JC is thankful to Nina Sokolov, Wagner Menezes, Lisa Carroll, Katherine Magoulick, Aahan Agrawaland, Leke Hutchins, and Nik Susič, Leif Egil Loe, for support, friendship and advice ('em cada esquina, um amigo'). This paper was possible due to a Peder Sæther grant which funded JC to visit and stay with RG. A NORBIS travel internationalization grant guaranteed funding for JC to be trained by Mark Blaxter in Edinburgh. RF acknowledges support from the Ministerio de Economía y Competitividad and the Ministerio de Ciencia of Spain (RyC2017-22492 and PID2019-108824GA-I00). JR and JV are supported by the Ministerio de Economía y Competitividad and the Ministerio de Ciencia of Spain (CGL2016-75255 and PID2019-103947GB). We are indebted to three anonymous reviewers for their insightful and detailed comments which have made this work considerably more coherent and stronger.

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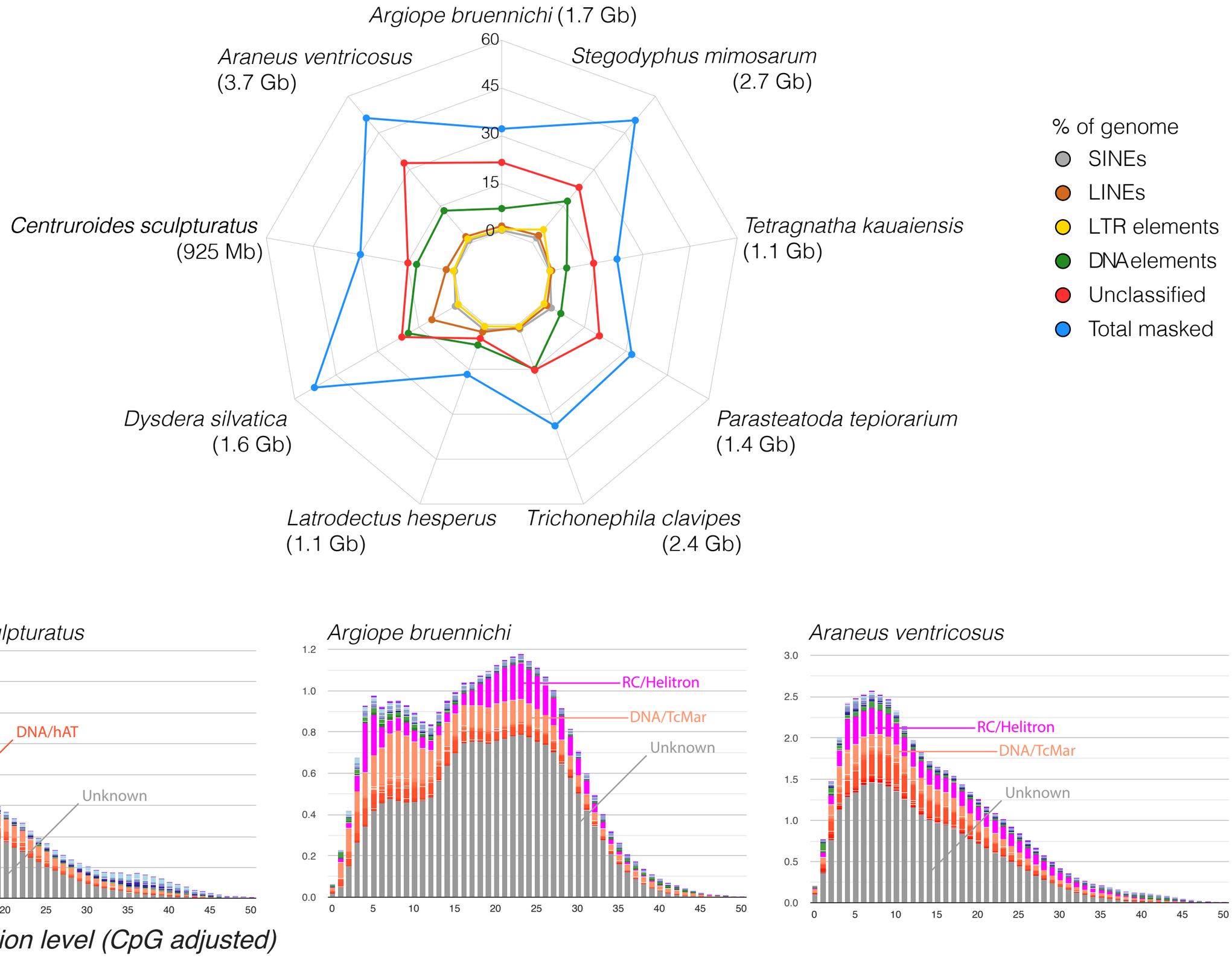

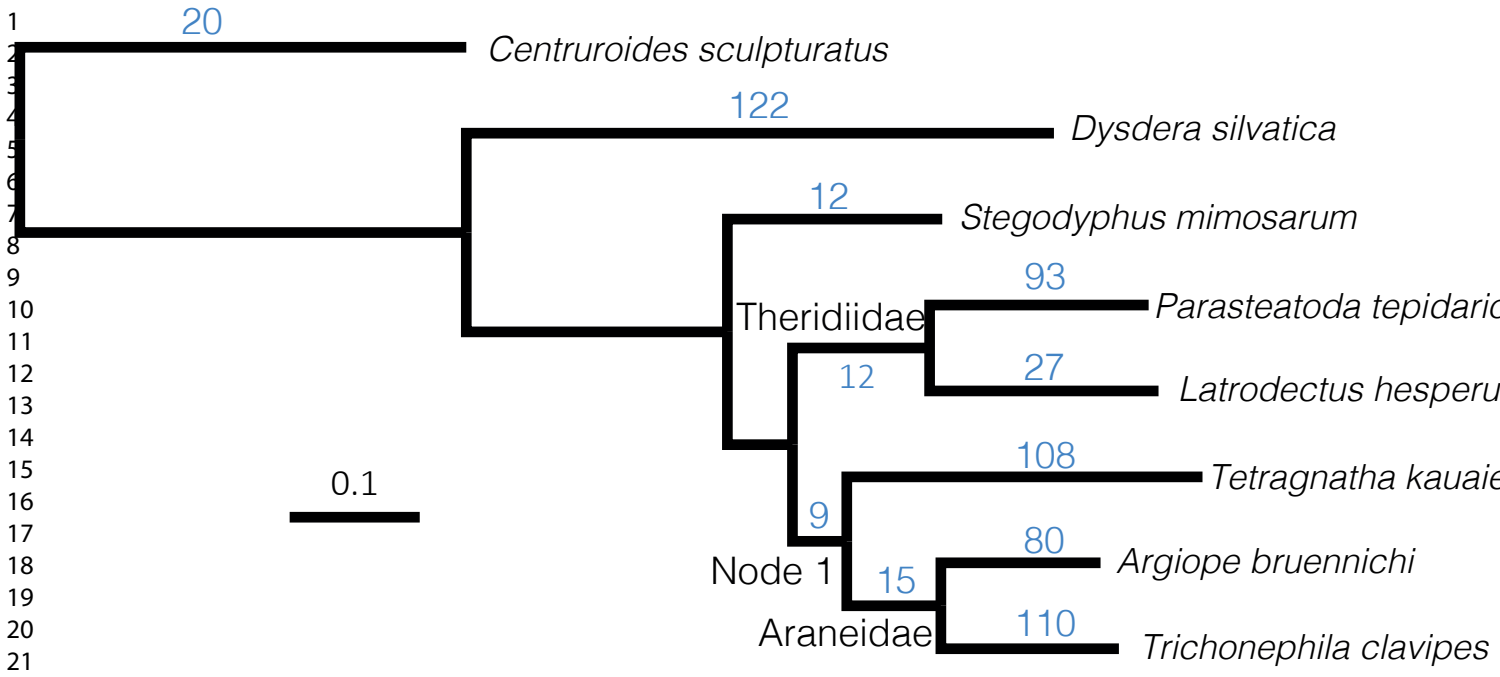

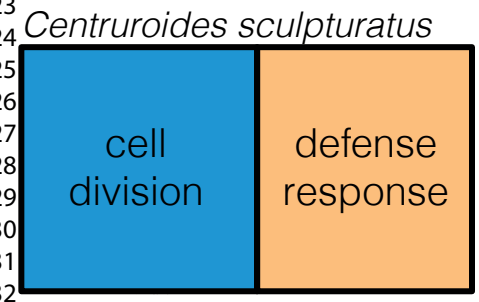

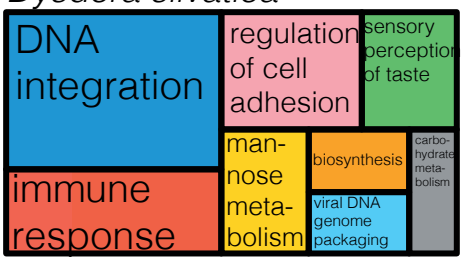

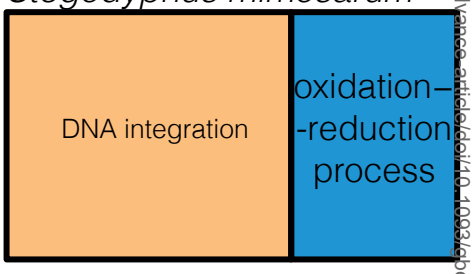

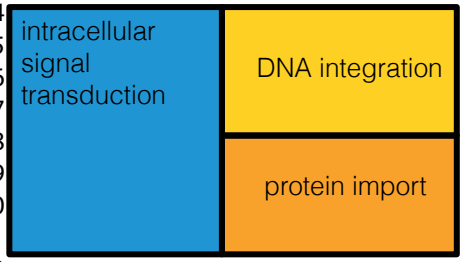

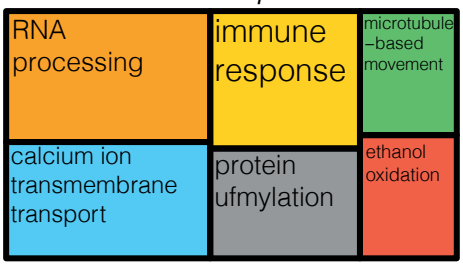

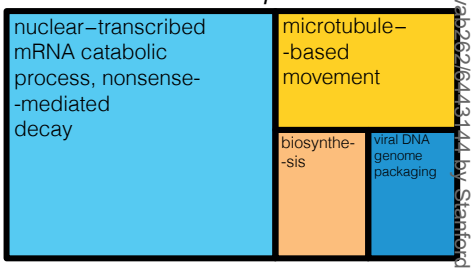

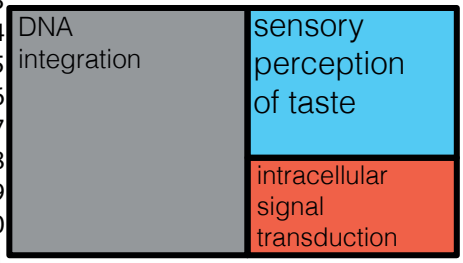

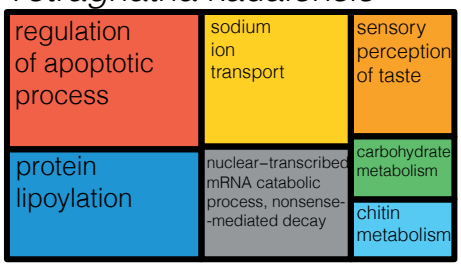

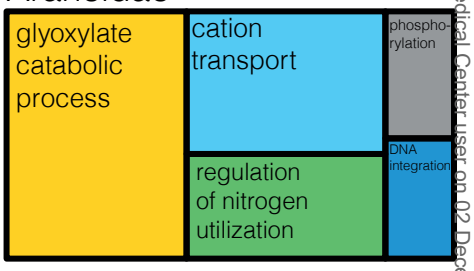

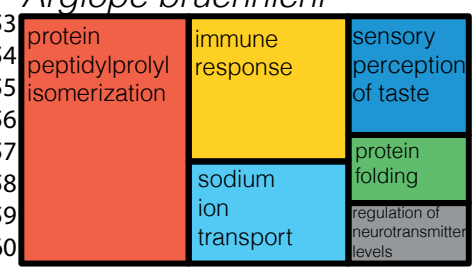

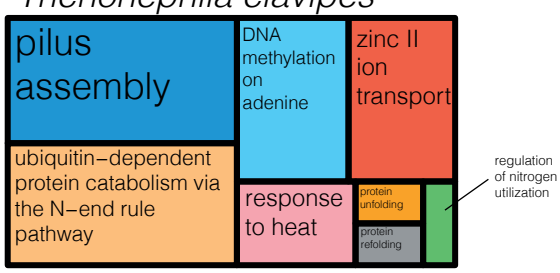

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Araneus ventricosus
Centruroides sculpturatus
Dysdera silvatica
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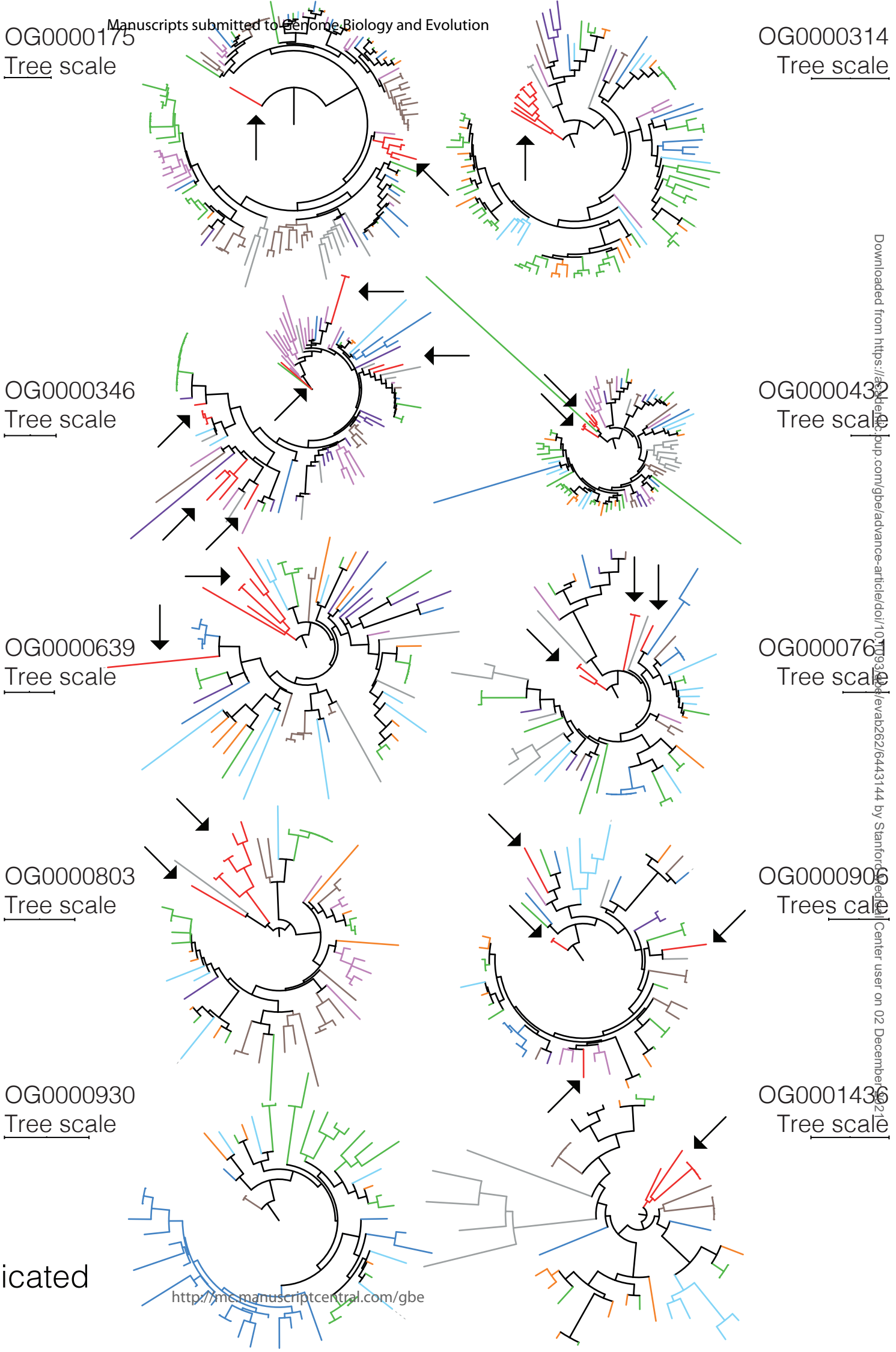

Scorpion genes indicated by arrows ⟶