

1 **Widespread retention of ohnologs in key developmental gene families following whole genome**
2 **duplication in arachnoplumonates**

3 Amber Harper¹, Luis Baudouin Gonzalez¹, Anna Schönauer¹, Michael Seiter², Michaela Holzem^{1,3},
4 Saad Arif^{1,4}, Alistair P. McGregor^{1,4*}, Lauren Sumner-Rooney^{5,*}

5 ¹Department of Biological and Medical Sciences, Faculty of Health and Life Sciences, Oxford Brookes
6 University, Oxford, OX3 0BP, United Kingdom.

7 ²Department of Evolutionary Biology, Unit Integrative Zoology, University of Vienna, Althanstrasse 14,
8 1090 Vienna, Austria.

9 ³Division of Signalling and Functional Genomics, German Cancer Research Centre (DKFZ),
10 Heidelberg, Germany and Department of Cell and Molecular Biology, Medical Faculty Mannheim,
11 Heidelberg University, Heidelberg, Germany.

12 ⁴Centre for Functional Genomics, Oxford Brookes University, Oxford, OX3 0BP, United Kingdom.

13 ⁵Oxford University Museum of Natural History, University of Oxford, Oxford OX1 3PW, United Kingdom.

14 *Corresponding authors: amcgregor@brookes.ac.uk (APM) and lauren.sumner-rooney@oum.ox.ac.uk
15 (LSR).

16 **Abstract**

17 Whole genome duplications (WGD) have occurred multiple times in the evolution of animals, including
18 in the lineages leading to vertebrates, teleosts, horseshoe crabs and arachnoplumonates. These
19 dramatic genomic events initially produce a wealth of new genetic material, which is generally followed
20 by extensive gene loss. It appears that developmental genes such as homeobox genes, signalling
21 pathway components and microRNAs, however, tend to be more frequently retained in duplicate
22 following WGD (ohnologs). These not only provide the best evidence for the occurrence of WGD, but
23 an opportunity to study its evolutionary implications. Although these genes are relatively well studied in
24 the context of vertebrate WGD, genomic and transcriptomic data for independent comparison in other
25 groups are scarce, with patchy sampling of only two of the five extant arachnoplumionate orders. To
26 improve our knowledge of developmental gene repertoires, and their evolution since the

27 arachnoplumonate WGD, we sequenced embryonic transcriptomes from two additional spider species
28 and two whip spider species and surveyed them for three important gene families: Hox, Wnt and
29 frizzled. We report extensive retention of ohnologs in all four species, further supporting the
30 arachnoplumonate WGD hypothesis. Thanks to improved sampling we were able to identify patterns of
31 likely ohnolog retention and loss within spiders, including apparent differences between major clades.
32 The two amblypygid species have larger ohnolog repertoires of these genes than both spiders and
33 scorpions; including the first reported duplicated *Wnt1/wg*, the first *Wnt10* recovered in an arachnid,
34 and broad retention of frizzled genes. These insights shed light on the evolution of the enigmatic whip
35 spiders, highlight the importance of the comparative approach within lineages, and provide substantial
36 new transcriptomic data for future study.

37 **Introduction**

38 The duplication of genetic material is widely accepted to be an important contributor to the evolution of
39 morphological and physiological innovations (Ohno 1970; Zhang 2003). The most dramatic example of
40 this is whole genome duplication (WGD), when gene copy numbers are doubled and retained paralogs
41 or ohnologs can then share ancestral functions (subfunctionalisation) and/or evolve new roles
42 (neofunctionalization) (Ohno 1970; Force et al. 1999; Lynch and Conery 2000). The occurrence of two
43 rounds (2R) of WGD in the early evolution of vertebrates has long been associated with their taxonomic
44 and morphological diversity (e.g. Ohno 1970; Holland et al. 1994; Dehal and Boore 2005; Holland
45 2013a), and a subsequent 3R in teleosts is linked to their success as the most diverse vertebrate group
46 (e.g. Meyer and Schartl 1999; Glasauer and Neuhauss 2014). However, this remains controversial and
47 difficult to test (Donoghue and Purnell 2005) and in several animal lineages there is not a clear
48 association between WGD and diversification (Mark Welch et al. 2008; Flot et al. 2013; Havlak et al.
49 2014; Kenny et al. 2016; Nong et al. 2020). Along with vertebrates, chelicerates also appear to be
50 hotspots of WGD, with up to three rounds reported in horseshoe crabs (Kenny et al. 2016; Nong et al.
51 2020), one in the ancestor of arachnoplumonates (spiders, scorpions, and their allies) (Schwager et al.
52 2017), and potentially two further rounds within the spider clade Synspermiata (Král et al. 2019).
53 Chelicerates demonstrate a highly variable body plan, occupy a wide range of habitats and ecological
54 niches, and have evolved a variety of biologically important innovations such as venoms and silks
55 (Schwager et al. 2015). They therefore offer an excellent opportunity for comparison with vertebrates

56 concerning the implications of WGD for morphological and taxonomic diversity, and genome evolution
57 in its wake.

58 Over the past 20 years, the house spider *Parasteatoda tepidariorum* has emerged as a model species
59 to study the impacts of WGD on arachnid evolution and development. Genomic and functional
60 developmental studies have found retained ohnologs of many important genes, with evidence for neo-
61 and subfunctionalisation in many of these compared to single-copy orthologs in arachnids lacking WGD
62 (Janssen et al. 2015; Leite et al. 2016, 2018; Turetzek et al. 2016, 2017; Schwager et al. 2017;
63 Baudouin-Gonzalez et al. 2020). Work on the scorpions *Centruroides sculpturatus* and *Mesobuthus*
64 *martensii* has consistently complemented findings in *P. tepidariorum*, with genomic studies recovering
65 many ohnologs in common with spiders (Di et al. 2015; Sharma et al. 2015; Leite et al. 2018). Although
66 high quality genome assemblies are required for the analysis of synteny between gene duplicates, these
67 remain relatively scarce in arachnids. Work on the *P. tepidariorum*, *Ce. sculpturatus* and *Me. martensii*
68 genomes has been complemented by targeted studies of individual gene families and transcriptomic
69 surveys (e.g. Schwager et al. 2007; Sharma et al. 2012; Leite et al. 2018; Gainett and Sharma 2020).
70 Combined with phylogenetic analyses, the identification of large-scale gene duplications using
71 transcriptomics can provide evidence of WGD events and their timing in the history of arachnid
72 evolution. Although transcriptomes can yield variant sequences of individual genes, from different
73 alleles or individuals in mixed samples, these are generally easy to filter out from truly duplicated loci
74 owing to substantial sequence divergence in the latter. They also offer the double-edged sword of
75 capturing gene expression, rather than presence in the genome; pseudogenised or silenced duplicates
76 are not detected, but neither are functional genes if they are not expressed at the sampled timepoint or
77 tissue. Such studies have produced strong additional evidence for an ancestral WGD, with patterns of
78 duplication coinciding with our expectations for arachnoplumonate ohnologs (Clarke et al. 2014, 2015;
79 Sharma et al. 2015; Turetzek et al. 2017; Leite et al. 2018; Gainett and Sharma 2020; Gainett et al.
80 2020).

81 Comparison of WGD events among arachnoplumonates, horseshoe crabs and vertebrates indicates
82 that despite extensive gene loss following duplication events, certain gene families are commonly
83 retained following duplication (Holland et al. 1994; Schwager et al. 2007, 2017; Kuraku and Meyer 2009;
84 Di et al. 2015; Sharma et al. 2015; Kenny et al. 2016; Leite et al. 2016, 2018). These typically include

85 genes from the conserved developmental 'toolkit' of transcription factors (TFs), cell signalling ligands
86 and receptors, and microRNAs (Erwin 2009). Among these, several have stood out as focal points in
87 the study of gene and genome duplications. The Hox gene group of homeobox genes regulate the
88 identity of the body plan along the antero-posterior axis of all bilaterian animals (McGinnis and Krumlauf
89 1992; Abzhanov et al. 1999; Carroll et al. 2005; Pearson et al. 2005; Hueber and Lohmann 2008;
90 Holland 2013b). Four clusters of these key developmental genes were retained after 1R and 2R in
91 vertebrates (Holland et al. 1994; Meyer and Scharf 1999; Kuraku and Meyer 2009; Pascual-Anaya et
92 al. 2013), and the arachnoplumonate WGD is evident in the almost universal retention of Hox gene
93 duplicates in sequenced genomes, with two ohnologs of all ten arthropod Hox genes in the scorpion *M.*
94 *martensii* (Di et al. 2015; Leite et al. 2018), all except *Hox3* being represented by two copies in *Ce.*
95 *sculpturatus* (Leite et al. 2018), and all except *fushi tarazu* (*ftz*) in *P. tepidariorum* (Schwager et al.
96 2017). Systematic studies of Hox gene expression patterns in the latter demonstrated that all nine pairs
97 of Hox paralogs exhibit signs of sub- or neofunctionalization (Schwager et al. 2017). This high level of
98 retention and functional divergence lends strong support to the importance of Hox gene duplication in
99 the evolution of the arachnoplumonate body plan, and further consolidates the position of this family as
100 a key indicator of WGD.

101 In addition to TFs, the ligands and receptors of some signalling pathways of the developmental toolkit
102 (e.g. Hedgehog, Wnt, TGF- β , NHR) also demonstrate higher copy numbers in vertebrates and other
103 groups subject to WGD, including arachnoplumonates (Holland et al. 1994; Meyer and Scharf 1999;
104 Shimeld 1999; Pires-daSilva and Sommer 2003; Cho et al. 2010; Janssen et al. 2010, 2015; Hogvall et
105 al. 2014). The Wnt signal transduction pathway plays many important roles during arthropod
106 development, including segmentation and patterning of the nervous system, eyes and gut (Erwin 2009;
107 Murat et al. 2010). In the canonical pathway, Wnt ligands bind to transmembrane receptors, such
108 Frizzled, to trigger translocation of β -catenin to the nucleus and mediate regulation of gene expression
109 (Cadigan and Nusse 1997; Hamilton et al. 2001; Logan and Nusse 2004; van Amerongen and Nusse
110 2009). There are thirteen subfamilies of Wnt genes found in bilaterians, as well as multiple receptor
111 families and downstream components. In contrast to the extensive retention of Hox ohnologs following
112 WGD, Wnt duplicates in *P. tepidariorum* appear to be restricted to *Wnt7* and *Wnt11*, with the remaining
113 eight subfamilies represented by single genes (Janssen et al. 2010). However, these are the only
114 reported Wnt gene duplications in arthropods despite several recent surveys (Bolognesi et al. 2008;

115 Murat et al. 2010; Hayden and Arthur 2013; Meng et al. 2013; Hogvall et al. 2014; Janssen and Posnien
116 2014; Holzem et al. 2019), and beyond *P. tepidariorum* no other arachnoplumonates have been
117 systematically searched. Similarly, duplications within the four *frizzled* gene subfamilies appear to be
118 restricted to arachnoplumonates among arthropods, wherein only *fz4* is duplicated in both *P.*
119 *tepidariorum* and *M. martensii* (Janssen et al. 2015).

120 Several Wnt families have also been retained after the 1R and 2R events in vertebrates, for example
121 there are two copies each of *Wnt2*, *Wnt3*, *Wnt5*, *Wnt7*, *Wnt8*, *Wnt9*, and *Wnt10* in humans (Miller 2001;
122 Janssen et al. 2010). However, no subfamilies are represented by three or four copies in humans and
123 so there is some consistency with arachnoplumonates in that the Wnts may be more conservative
124 markers of WGD, to be used in combination with Hox and other homeobox genes.

125 The extensive and consistent retention of key developmental genes like Hox genes apparent in *P.*
126 *tepidariorum* and *Ce. sculpturatus*, and Wnt genes in *P. tepidariorum*, strongly support the occurrence
127 of an ancestral WGD in arachnoplumonates. However, data are only available for a handful of species
128 so far, resulting in very patchy taxonomic sampling. For example, only *P. tepidariorum* and *Pholcus*
129 *phalangoides* have been comprehensively surveyed for homeobox genes among spiders (Leite et al.
130 2018), omitting the large and derived retrolateral tibial apophysis (RTA) clade, which includes jumping
131 spiders, crab spiders and other free hunters, and the systematic identification of Wnt genes has been
132 restricted to only *P. tepidariorum*. Spiders and scorpions are by far the most speciose of the
133 arachnoplumonates, and there may be additional diversity in their repertoires of these important
134 developmental gene families of which we are not yet aware.

135 In addition, and perhaps more urgently, only two of the five arachnoplumonate lineages have dominated
136 the field thus far; sufficient genomic information for comparison is lacking beyond spiders and scorpions.
137 Also represented in Arachnoplumonata are the amblypygids (whip spiders), relatively understudied and
138 enigmatic animals comprising around 190 extant species. They exhibit highly derived morphology of
139 the pedipalps, which are adapted to form raptorial appendages, and of the first pair of walking legs,
140 which are antenniform and can comprise more than 100 segments (Weygoldt 2009). Despite the
141 scarcity of transcriptomic or genomic data for amblypygids (whip spiders) (Garb et al. 2018; though see
142 Gainett and Sharma 2020; and Gainett et al. 2020 for recent advances), their widely accepted position
143 within Arachnoplumonata implies that they were also subject to an ancestral WGD. A recent survey of

144 the *Phrynus marginemaculatus* transcriptome supported this in the recovery of multiple duplicate Hox
145 and leg gap genes (Gainett and Sharma 2020). Particularly given the derived nature of their
146 appendages, this group could shed substantial light on genomic and morphological evolution following
147 WGD.

148 To better understand the genomic consequences of WGD in a greater diversity of arachnoplumbrate
149 lineages, we sequenced *de novo* embryonic transcriptomes from two spiders belonging to the derived
150 RTA clade and two amblypygids. We surveyed Hox, Wnt and frizzled genes in these species and
151 existing genomic and transcriptomic resources for as examples for comparison with other arachnids,
152 both with and without an ancestral WGD, improving sampling at both the order and sub-order levels.

153 **Materials and methods**

154 *Embryo collection, fixation and staging*

155 Embryos of mixed ages were collected from captive females of the amblypygids *Charinus acosta*
156 (Charinidae; parthenogenetic, collected at one day, one month and two months after the appearance
157 of egg sacs) and *Euphrynichus bacillifer* (Neoamblypygi: Phrynichidae; mated, collected at
158 approximately 30% of development), the wolf spider *Pardosa amentata* (collected in Oxford, UK) and
159 mixed stage embryos of the jumping spider *Marpissa muscosa* (kindly provided by Philip Steinhoff and
160 Gabriele Uhl) and stored in RNAlater.

161 *Transcriptomics*

162 Total RNA was extracted from mixed aged embryos, pooled by species, of *C. acosta*, *E. bacillifer*, *Pa.*
163 *amentata* and *M. muscosa* using QIAzol according to the manufacturer's instructions (QIAzol Lysis
164 Reagent, Qiagen). Illumina libraries were constructed using a TruSeq RNA sample preparation kit
165 (including polyA selection) and sequenced using the Illumina NovaSeq platform (100bp PE) by
166 Edinburgh Genomics. Quality of raw reads was assessed using FastQC v0.11.9 (Andrews 2010).
167 Erroneous k-mers were corrected using rCorrector (default settings, Song and Florea 2015) and
168 unfixable read pairs (from low-expression homolog pairs or containing too many errors) were discarded
169 using a custom Python script (available at
170 [https://github.com/harvardinformatics/TranscriptomeAssemblyTools/blob/master/FilterUncorrectable](https://github.com/harvardinformatics/TranscriptomeAssemblyTools/blob/master/FilterUncorrectablePEfastq.py)
171 [PEfastq.py](https://github.com/harvardinformatics/TranscriptomeAssemblyTools/blob/master/FilterUncorrectablePEfastq.py) courtesy of Adam Freeman). Adapter sequences were identified and removed and low

172 quality ends (phred score cut-off = 5) trimmed using TrimGalore! v0.6.5 (available at
173 <https://github.com/FelixKrueger/TrimGalore>). *De novo* transcriptome assembly was performed using
174 only properly paired reads with Trinity v2.10.0 (Haas et al. 2013) using default settings. Transcriptome
175 completeness was evaluated on the longest isoform per gene using BUSCO v4.0.2 (Seppey et al. 2019)
176 along with the arachnid database (arachnida_odb10 created on 2019-11-20; 10 species, 2934
177 BUSCOs) and the arthropod database (arthropoda_odb10 created on 2019-11-20; 90 species, 1013
178 BUSCOs).

179 *Identification of gene candidates*

180 To identify Wnt, frizzled and Hox gene candidates in *C. acosta*, *E. bacillifer*, *Pa. amentata* and *M.*
181 *muscosa*, we performed TBLASTN (version 2.6.0+) searches of the assembled embryonic
182 transcriptome, using Wnt and frizzled protein sequences previously identified in *P. tepidariorum*
183 (Janssen et al. 2010, 2015) and homeodomain protein sequences of the *Drosophila melanogaster* Hox
184 genes from HomeoDB2 (Zhong and Holland 2011). Existing Wnt and Frizzled protein predictions were
185 collected for *Ce. sculpturatus* from NCBI (PRJNA422877, Supplementary Data Tables 1-3). Predicted
186 protein sequences were obtained using the Translate ExpASy online tool
187 (<https://web.expasy.org/translate/>) and the standard genetic code.

188 *Phylogenetic analysis*

189 Transcript identity was confirmed by reciprocal BLAST and the construction of maximum likelihood
190 trees. Amino acid sequences of Hox, Wnt and frizzled family genes of known identity from selected
191 arthropods (*Bicyclus anynana*, *Bombyx mori*, *Daphnia pulex*, *Drosophila melanogaster*, *Parage*
192 *aegeria*, *Strigamia maritima*, and *Tribolium castaneum*) and an onychophoran (*Euperipatoides*
193 *kanangrensis*) were retrieved from NCBI (Accession numbers: Supplementary Tables 1-3). Alignments
194 of full protein sequences were performed in Clustal Omega using default parameters (Goujon et al.
195 2010; Sievers et al. 2011). Maximum likelihood trees were generated from whole-sequence alignments
196 to assign genes to families and study the relationship between candidate duplicates. Phylogenetic
197 analyses were performed in IQ-Tree (v2.0.3, Nguyen et al. 2015) using ModelFinder to identify optimal
198 substitution models (VT+F+R10 for Hox, LG+R8 for *Wnt*, JTT+5 for *fz*; Kalyaanamoorthy et al. 2017)
199 and 1000 bootstrap replicates. Trees were visualised in FigTree v.1.4.4
200 (<http://tree.bio.ed.ac.uk/software/figtree/>).

201 *Identification of duplicate genes*

202 Where more than one sequence was identified as a potential candidate for a single gene, several factors
203 were examined to eliminate the possibility that they were isoforms, individual variants, or fragments of
204 the same gene. Nucleotide and protein alignments (Supplementary Data Files 1-4) were inspected and
205 if sequences did not overlap, the shorter one was discarded. If sequences showed very high similarity
206 from alignments, or potential duplicates resolved as short-branch sister pairs in phylogenetic analysis,
207 as expected from isoforms or individual variants, the shorter sequence was discarded. Candidate
208 duplicates that passed these tests were also BLASTed back against source transcriptomes to confirm
209 their origin.

210 **Results and Discussion**

211 *Transcriptome assemblies*

212 To further study the outcomes of WGD in the ancestor of arachnoplumonates we carried out RNA-Seq
213 on embryos of two further spider species, *Pa. amentata* and *M. muscosa*, and two species of
214 amblypygids, *C. acosta* and *E. bacillifer*.

215 RNA-Seq for the four species produced between 222,479,664 and 272,844,971 raw reads, reduced to
216 211,848,357 and 260,853,757 after processing. Trinity assembled between 184,142 and 316,021
217 transcripts in up to 542,344 isoforms (Table 1). Contig N50 ranged from 592 bp in *M. muscosa* to 978
218 bp in *E. bacillifer*, and from 1461 bp (*M. muscosa*) to 2671 bp (*E. bacillifer*) in the most highly expressed
219 genes (representing 90% of total normalised expression) (Table 1).

220 Transcriptomes were found to be between 83.7% (*C. acosta*) and 89.4% (*E. bacillifer*) complete
221 according to BUSCO scores compared to the arthropod database, with between 3.5% and 9.5%
222 duplicated BUSCOs. Compared to the arachnid databases, transcriptomes were 82%-90.1% complete
223 for single-copy BUSCOs and contained between 5.3%-12.9% duplicated BUSCOs (Table 1).

224 To explore the extent of duplication in these arachnoplumonates we then surveyed the copy number of
225 Hox, Wnt and Frizzled genes in their transcriptomes in comparison to other arachnids. It is important to
226 note that the absence of genes recovered from transcriptomes does not eliminate the possibility that
227 they are present in the genome, as the transcriptomes will only capture genes expressed at the relevant
228 point in development. Mixed-stage embryonic samples may yield more transcripts for the same reason.

229 *Duplication of Hox genes in spiders and amblypygids*

230 The Hox gene repertoires for the two spiders are largely consistent with previous surveys of the *P.*
231 *tepidariorum* genome (Figure 1), which has two copies of all genes except for *ftz*. There are three
232 exceptions: the recovery of a single copy of *Deformed (Dfd)* in *M. muscosa*, the apparent absence of
233 *ftz* in *Pa. amentata*, and the presence of one, rather than two, copies of *Hox3* in both species (Figure
234 1). Perhaps more comparable is previous analysis of the embryonic transcriptome of the synspermiatan
235 spider *Ph. phalangioides*, which detected single transcripts of *labial (lab)*, *ftz* and *Ultrabithorax (Ubx)*,
236 but two copies of the remaining Hox genes (*proboscipedia*, *Hox3*, *Dfd*, *Sex combs reduced*,
237 *Antennapedia*, *Abdominal-A*, *And abdominal-B*; Leite et al. 2018). From these combined data it seems
238 likely that having a single copy of *ftz* is common across all spiders, but both copies are retained in all
239 other arachnoplumonates studied to date (Figures 1-2). This is consistent with the loss of one copy of
240 *ftz* in the common ancestor of all spiders, following WGD. The absence of *Hox3* duplicates in *M.*
241 *muscosa* and *Pa. amentata* could indicate a lineage-specific loss in the RTA clade, which unites
242 salticids, lycosids and their allies. Indeed, only one copy of *Hox3* has been recovered in *Cupiennius*
243 *salei*, a ctenid also belonging to the RTA clade (Schwager et al. 2007). Other apparent losses, of *Dfd-*
244 *A* in *M. muscosa* and *Ubx* and *lab-A* in *Ph. phalangioides*, may be specific to Salticidae and
245 Synspermiata/Pholcidae, respectively, if they are absent from the genome.

246 Both amblypygids exhibit extensive duplication of Hox genes, in line with expectations following the
247 arachnoplumonate WGD (Figures 1-2). *Charinus acosta* appears to have two copies of all surveyed
248 Hox genes except for *pb*. We recovered single copies of *pb*, *Scr* and *Ubx* in *E. bacillifer*, but two copies
249 of *lab*, *zen*, *Dfd*, *ftz*, *Antp*, *abdA* and *abdB*. The absence of a second copy of *pb* in both *C. acosta* and
250 *E. bacillifer*, which are relatively distantly related within Amblypygi, suggests a loss in the common
251 ancestor of all amblypygids. This is also supported by a recent survey of *Phrynus marginemaculatus*,
252 which recovered a single copy of *pb* but duplicates of all other Hox genes (Gainett and Sharma 2020).
253 Embryos of *C. acosta* were collected at multiple stages of development, supporting the hypothesis that
254 this may be a true loss, rather than absence of expression at a particular developmental stage.
255 However, the apparent additional absence of *Scr* and *Ubx* duplicates in *E. bacillifer* could equivocally
256 indicate lineage-specific losses or absence of expression at a single timepoint.

257 The duplication of Hox genes is consistent among the three arachnoplumonate orders studied to date,
258 and specific repertoires appear to be fairly conserved at the order level (this study; Schwager et al.
259 2007, 2017; Cao et al. 2013; Di et al. 2015; Leite et al. 2018). Given that this is the level at which overall
260 body plans are conserved, this is perhaps not surprising. The potential loss of a *Hox3* duplicate in the
261 spider RTA clade (in *M. muscosa* and *Pa. amentata*, and *Cu. salei*, Schwager et al. 2007) is an unusual
262 example of infraorder variation in Hox repertoires. Although initial analyses found that the expression
263 patterns of the two *Hox3* ohnologs overlapped in *P. tepidariorum* (Schwager et al. 2017), both duplicates
264 were still expressed. As other intraorder losses of Hox genes were only observed in single species from
265 embryonic transcriptomes, it would be premature to conclude that they are genuinely absent from the
266 genome.

267 Thanks to the relatively conserved expression patterns of Hox genes along the anterior-posterior axis
268 of chelicerates, we can begin to make tentative inferences about the possible macroevolutionary
269 implications of duplication and loss. For example, an anticipated duplicate of *pb* has been lost in both
270 amblypygids but persists in spiders and scorpions. In spiders, both *pb* paralogs are expressed in the
271 pedipalp and leg-bearing segments, separated temporally (Schwager et al. 2017). Given the highly
272 derived nature of the raptorial pedipalps and the antenniform first pair of walking legs in amblypygids,
273 it is perhaps surprising that this duplicate was not retained. However, this might indicate that other Hox
274 genes expressed in the anterior prosomal segments (e.g. *lab*, *zen*, or *Dfd*) may contribute to these
275 morphological innovations. A good candidate for future study might be *lab*: a single ortholog is
276 expressed in both the pedipalps and the first walking leg in the harvestman *Phalangium opilio* (Sharma
277 et al. 2012), and expression patterns and experimental manipulation provide evidence for functional
278 divergence between the two *lab* paralogs, also expressed in the pedipalps and first walking legs, in *P.*
279 *tepidariorum* (Pechmann et al. 2015; Schomburg et al. 2020).

280 *Wnt gene repertoires exhibit both between- and within-lineage diversity*

281 Consistent with previous studies of *P. tepidariorum*, we found representatives of ten *Wnt* subfamilies in
282 *M. muscosa* and *Pa. amentata* transcriptomes, with all three spiders lacking *Wnt3*, *Wnt9*, and *Wnt10*.
283 The absence of *Wnt3* (in both amblypygids and both spiders) is consistent with all other protostomes
284 (Janssen et al. 2010; Murat et al. 2010; Hogvall et al. 2014), but the absence of *Wnt9* and *Wnt10* in
285 spiders indicates losses in the spider ancestor. We did not recover duplicates of *Wnt2*, *Wnt8-10*, *Wnt16*

286 or *WntA* in any arachnoplumonate lineage. This suggests loss shortly after WGD in the common
287 ancestor of all arachnoplumonates.

288 Both *M. muscosa* and *Pa. amentata* expressed two copies of *Wnt7* and *Wnt11*, in line with *P.*
289 *tepidariorum*, as well as a second copy of *Wnt4* that is absent in *P. tepidariorum*.

290 Representation of the Wnt subfamilies in our amblypygid transcriptomes is higher than any other
291 arachnid studied to date, including those with high-quality genome assemblies (Janssen et al. 2010;
292 Hogvall et al. 2014; Holzem et al. 2019). We recovered transcripts from twelve out of thirteen
293 subfamilies in *C. acosta* (missing *Wnt3*) and eleven in *E. bacillifer* (missing *Wnt3* and *Wnt9*) (Figure 3).
294 Two copies of *Wnt1/wg*, *Wnt4* and *Wnt7* were recovered for both species, with an additional duplicate
295 of *Wnt6* in *C. acosta*. Unlike all other arachnoplumonates surveyed, we only identified a single *Wnt11*
296 gene in amblypygids, suggesting a lineage-specific loss following WGD. Most of the duplicate Wnt
297 genes identified in our analysis appear to be likely ohnologs; conclusive confirmation of this requires
298 synteny analysis of fully sequenced genomes, but the relationships between paralogs resolved by
299 phylogenetic analysis generally do not support more recent tandem duplications. Duplicates of *Wnt7*
300 were previously identified in *P. tepidariorum* (Janssen et al. 2010), and are recovered in this study from
301 all four transcriptomes and existing sequence data from *Ce. sculpturatus*. These paralogs did not
302 resolve as sister pairs in phylogenetic analyses and sequence similarity between paralogs was low (61-
303 73%, Supplementary Data File 2). *Wnt7* ortholog groups formed two well-supported clades within
304 spiders and amblypygids, suggesting retention of *Wnt7* ohnologs in these groups following the
305 arachnoplumonate WGD (Figure 4). The two *Wnt7* sequences from *Ce. sculpturatus* also demonstrated
306 low similarity (66%, Supplementary Data File 2) but did not resolve in separate clades and may indicate
307 a lineage-specific duplication. *Wnt11* duplicates were recovered from transcriptomes of *M. muscosa*
308 and *Pa. amentata*, and from the published genome of *Ce. sculpturatus*. These formed two separate and
309 well-supported clades (78% and 95%, Figure 4), also including genomic sequences from *P.*
310 *tepidariorum* duplicates. Sequence similarity between paralogs was very low (40-50%, Supplementary
311 Data File 2); combined with their phylogenetic placement, we conclude that this reflects the likely
312 retention of ohnologs following WGD. Only the amblypygids appear not to have retained two copies of
313 *Wnt11* following ancestral duplication. Paired *Wnt4* paralogs detected in *M. muscosa* and *Pa. amentata*
314 form well supported clades with duplicates recovered in the amblypygids (bootstrap \geq 96%; Figure 4)

315 and show substantial sequence divergence within species (56-65% similarity, Supplementary Table 2),
316 indicating that they are again likely to represent retained ohnologs following the arachnoplumonate
317 WGD, despite being lost in the lineage to *P. tepidariorum*.

318 We have identified two copies of *Wnt1/wg* in both the amblypygid transcriptomes and in the previously
319 published genome of the scorpion *Ce. sculpturatus*. To the best of our knowledge this is the first time a
320 duplication of *Wnt1/wg* has been reported in any animal surveyed to date. Since this is highly unusual
321 it requires critical interpretation. We can eliminate the possibility of individual variation in *C. acosta*, as
322 embryos are produced by parthenogenesis and are therefore clones, and in *Ce. sculpturatus*, as the
323 sequences were recovered from a single individual's published genome (Supplementary Table 2).
324 Sequence similarity between paralogs was low (72-76%, Supplementary Data File 2), even compared
325 to similarity between *Wnt1* orthologs at the order level (e.g. 91% between *M. muscosa* and *Pa.*
326 *amentata*), reducing the likelihood that we are detecting allelic variation within individuals. We also
327 inspected nucleotide alignments and found lower paralog sequence similarity than evident from amino
328 acid sequences (65-69%, Supplementary Data File 3), indicating synonymous evolution. Although
329 synteny analysis is required for conclusive confirmation, our phylogenetic analysis indicates that the
330 amblypygid duplicates are likely to be ohnologs retained from the arachnoplumonate WGD, as they
331 form separate, well-supported clades with other arachnid *Wnt1*s (bootstrap values $\geq 79\%$; Figure 4).
332 The resolution of the *Ce. sculpturatus Wnt1* paralogs had lower support and their relationship is
333 therefore more ambiguous. The current placement of *Cs-Wnt1-2* as sister to *Cs-Wnt1-1+(Ca-Wnt1-*
334 *1+Eb-Wnt1-1)* lends support to a lineage-specific duplication, but support for this topology is middling
335 (75%, Figure 4), and it is noteworthy that the two *Ce. sculpturatus* sequences are recovered from
336 different genomic scaffolds (see Supplementary Table 2 for accession numbers).

337 The presence of *Wnt10* in both amblypygids is also intriguing because it is absent from all other
338 arachnids surveyed so far. These sequences were placed within an arthropod *Wnt10* clade with high
339 bootstrap support (100%; Figure 4), according to our phylogenetic analysis. Whether this indicates
340 multiple losses of *Wnt10* in all other arachnid lineages, the recovery of a lost *Wnt10* in amblypygids, or
341 the co-option of another gene, is unclear. Insights from other groups, such as harvestmen, will shed
342 further light on this in future.

343 In contrast to the widespread retention of Hox duplicates, these new data indicate that the retention of
344 duplicate Wnt genes is less common and restricted to certain subfamilies. Our understanding of specific
345 Wnt functions among arthropods is more limited than that of Hox genes, but *Wnt* expression patterns
346 in *P. tepidariorum* are available for a tentative comparison. For example, the two *Wnt7* paralogs show
347 clear functional divergence in *P. tepidariorum*, with *Wnt7-1* expressed in the segment addition zone
348 (SAZ) and *Wnt7-2* at the base of the appendages with some signal in the head lobes (Janssen et al.
349 2010). Whether this separation is consistent in all arachnopulmonates remains to be determined, but
350 levels of sequence divergence between newly identified ohnologs are similar to that of *P. tepidariorum*
351 (62%).

352 Conversely, previous attempts to characterise the expression patterns of *Wnt11* paralogs in *P.*
353 *tepidariorum* only detected expression of *Wnt11-2* (Janssen et al. 2010). Given the retention of *Wnt11-*
354 *1* in both spiders and scorpions, and the considerable divergence between paralogous sequences,
355 *Wnt11* could be a good candidate for sub- or neofunctionalization, but the role of *Wnt11-1* remains
356 unknown. Similarly, *Wnt4* expression in *P. tepidariorum* is restricted to a few cells at the posterior edge
357 of the germ band, towards the end of embryogenesis (Janssen et al. 2010). These authors noted that
358 this was in stark contrast to *Platynereis dumerilii*, where *Wnt4* is expressed in segments, the ventral
359 midline and the SAZ (*Wnt4* is absent in insects, preventing a closer phylogenetic comparison). If *Wnt4*
360 has an ancestrally complex role, as suggested by *Pl. dumerilii*, the very restricted expression of *Wnt4*
361 in *P. tepidariorum* could be the result of subfunctionalisation followed by loss of one paralog, which is
362 apparently retained in RTA-clade spiders and amblypygids (Figures 3-4). The expression patterns of
363 *Wnt4-2* in these groups will help to clarify this in future. Alternatively, as insects have lost *Wnt4* entirely,
364 there may simply be reduced *Wnt4* functionality across arthropods. However, this hypothesis stands at
365 odds with not only the retention of both ohnologs in two large clades, but their detectable expression
366 during development as evidenced by RNA-Seq.

367 The discovery of duplicate *Wnt1/wg* is particularly exciting: duplicates of this Wnt gene have not yet
368 been detected in any other metazoans, even following multiple rounds of WGD in vertebrates and
369 teleosts (see <https://web.stanford.edu/group/nusselab/cgi-bin/wnt/vertebrate>). Horseshoe crabs, which
370 have also undergone multiple WGD and retain multiple Hox clusters (Kenny et al. 2016; Nong et al.
371 2020; Shingate et al. 2020), have not yet been systematically surveyed for Wnt genes. Embryonic

372 transcriptomes so far have only recovered one copy of *Wnt1* (Chen et al. 2016), but this should be a
373 priority for future study. In arthropods *Wnt1/wg* performs a wide variety of roles, including in segment
374 polarisation and in appendage and nervous system development (Murat et al. 2010) and has an
375 accordingly complex expression pattern in *P. tepidariorum*, appearing in the L1 and L2 segments, limb
376 buds, and dorsal O2 and O3 segments (Janssen et al. 2010). In theory, therefore, there is ample
377 potential for subfunctionalisation. Functional analysis of *Wnt1/wg* duplicates in amblypygids and
378 scorpions will no doubt prove extremely interesting in the future.

379 *Frizzled duplicates are retained in amblypygids, but not in spiders*

380 The transcriptomes of the spiders *M. muscosa* and *Pa. amentata* contained orthologs of *fz1*, *fz2* and
381 *fz4*, but *fz3* was absent (Figures 5-6). The same subfamilies are represented in the *P. tepidariorum*
382 genome, but a single copy of *fz3* was identified in *Ph. phalangioides* (Janssen et al. 2015); thus,
383 entelegyne spiders may universally lack *fz3* but it was likely present in the ancestor of all spiders.
384 Analysis of the *M. muscosa* transcriptome also returned a second copy of *fz2*, which is not shared by
385 any other arachnid to date. These two paralogs form a well-supported clade (95%, Figure 6), indicating
386 that this is the result of a lineage-specific tandem duplication followed by rapid sequence divergence in
387 *fz2-2* (sequence similarity 53%, Supplementary Data File 4). Although two copies of *fz4* were identified
388 in *P. tepidariorum* (Janssen et al. 2015), we only detected single copies in transcriptomes from *M.*
389 *muscosa* and *Pa. amentata*, and only one was recovered from an embryonic transcriptome of *Ph.*
390 *phalangioides* (Janssen et al. 2015). However, Janssen et al. (2015) demonstrated that the expression
391 of the two *fz4* paralogs in *P. tepidariorum* is separated temporally. Therefore, we might not expect to
392 detect both transcripts in embryos of similar stages, and an additional copy may be present in the
393 genome.

394 Both amblypygid species have a large repertoire of *frizzled* genes compared to other arachnids,
395 expressing all four orthology groups with two copies each of *fz1*, *fz3* and *fz4* (Figures 5-6). Duplicates
396 of *fz1* and *fz3* appear to be unique to amblypygids. The *fz1* duplicates could be ohnologs retained from
397 the arachnopulmonate WGD, as they form separate clades with the *fz1* genes of other
398 arachnopulmonates and exhibit reasonable sequence divergence (support values $\geq 98\%$, paralog
399 sequence similarity 76-77%; Figure 6). The origin of the *fz3* duplication is less clear; although the four
400 amblypygid sequences form two separate clades, they are separated only by the placement of *Cs-fz3*,

401 which has low support (35%; Figure 6). Therefore we cannot yet confirm the timing of the duplication.
402 Two copies of *fz4* are also found in the genomes of *P. tepidariorum* and the scorpions *Me. martensii*
403 (Janssen et al. 2015) and *Ce. sculpturatus*. Sequences from *P. tepidariorum* and *Ce. sculpturatus*
404 resolve in separate clades with other arachnopulmonate sequences (*fz4-1*; support value 62%; Figure
405 6) or together with *S. maritima* (*fz4-2*, support value 99%; Figure 6). They also demonstrate
406 considerable sequence divergence (44% similarity in *P. tepidariorum* and 57% in *Ce. sculpturatus*;
407 Supplementary Data File 4). We propose that these duplicates are probably retained from the ancestral
408 WGD. The position of duplicates in the amblypygids, however, is less well resolved. Paralogs do not
409 form within-species clades (Figure 6) and have fairly low sequence similarity (56-59%, Supplementary
410 Data File 4), nor do all four sequences form an amblypygid clade. The four *fz4* genes were all placed
411 within the same clade as *Pt-fz4-1* and *Cs-fz4-1*, and pairs of orthologs diverge sequentially (Figure 6).
412 This conflicts with an origin in WGD. Ortholog pairs (e.g. *Ca-fz4-1* and *Eb-fz4-1*) returned 100%
413 bootstrap support, but deeper relationships were more ambiguous (support values $\geq 51\%$). As a result,
414 we cannot conclusively identify their origin but we hypothesise that these *fz4* duplicates reflect a
415 lineage-specific duplication in either the ancestor of amblypygids or that of Pedipalpi (the larger clade
416 to which amblypygids belong).

417 Previous studies of spiders, scorpions, and ticks indicated that *frizzled* repertoires in these groups are
418 restricted to three or four copies, often with incomplete representation of the four orthology groups.
419 Analysis of the new transcriptomes for the spiders *M. muscosa* and *Pa. amentata* is consistent with this
420 pattern, albeit with a unique duplication of *fz2* in the jumping spider. We also recovered a single copy
421 of *fz2* in *Ce. sculpturatus*, which was missing from previous work on *Me. martensii* (Janssen et al. 2015).
422 The absence of *fz2* in the latter could result from a lineage-specific loss or an issue with genome
423 assembly. In contrast, all four *frizzled* subfamilies were recovered in both amblypygid species, with
424 three of these present in duplicate. Based on our data, it appears that the *frizzled* repertoire of
425 amblypygids is around twice the size of all other arachnids and may have followed a very different
426 evolutionary trajectory to spiders and scorpions following WGD. The expanded repertoire of frizzled
427 genes in amblypygids is intriguing since they have also retained most Wnts and indeed several Wnt
428 subfamilies are duplicated, and therefore it is possible that some specialised ligand-receptor
429 interactions have evolved compared to other arthropods (Wu and Nusse 2002). However, although
430 frizzled genes encode key receptors for Wnt ligands, they have other Wnt-independent functions, so

431 the expansion of the frizzled gene repertoire could be related to the evolution of alternative signalling
432 roles (Janssen et al. 2015; Yu et al. 2020).

433 *Conclusions: arachnoplumonate genome evolution in the wake of WGD*

434 Our new transcriptome data and phylogenetic analyses provide the most comprehensive survey of Hox,
435 Wnt and frizzled genes in arachnids to date, and substantially improve the density and breadth of
436 taxonomic sampling for key developmental genes in Arachnoplumonata. We have identified intraorder
437 variation at the level of major clades in spiders, which could help us better understand their
438 morphological evolution. In new data for a third arachnoplumonate lineage, the amblypygids, we find
439 additional evidence supporting an ancestral WGD and are better able to reconstruct the chronology of
440 gene duplications and losses in spiders and scorpions. These transcriptomic resources are among the
441 very first available for amblypygids and will aid future investigations of this fascinating group. We also
442 find evidence of consistent evolutionary trajectories in Hox and Wnt gene repertoires across three of
443 the five arachnoplumonate orders, with inter-order variation in the retention of specific paralogs.

444 By improving taxonomic coverage within the spider lineage we are better able to polarise some
445 loss/duplication events and identify potential new trends within the spiders, particularly illustrating
446 separations between synspermiatan and entelegyne spiders, and between the derived RTA clade and
447 other spiders. Despite being unable to ultimately conclude that some missing transcripts reflect genuine
448 genomic losses, it appears that the evolution of these developmental genes in spiders is more
449 complicated than we thought. It may be that these gene repertoires are genuinely more variable within
450 spiders than they are in amblypygids or scorpions; spiders are by far the most taxonomically diverse
451 arachnoplumonate order, and the apparent diversity of repertoires may simply reflect this. Conversely,
452 the higher apparent intraorder diversity of gene repertoires may be an artefact of increased sampling in
453 spiders (up to four or five species for specific gene families) compared to the one or two available
454 resources for scorpions and amblypygids; we may detect more diversity within these groups with
455 increased sampling. Nonetheless, we see two notable trends within spiders, outlined below.

456 First, we see several characters that appear to unite the RTA clade, which contains almost half of all
457 extant spider species (World Spider Catalog 2019), having diversified rapidly following its divergence
458 from the orb weavers (Garrison et al. 2016; Fernández et al. 2018; Shao and Li 2018). *M. muscosa* and
459 *Pa. amentata* both exhibit the apparent loss of *Hox3* and *fz4* paralogs and the retention of a *Wnt4*

460 duplicate, in contrast to *P. tepidariorum* and *Ph. phalangioides*. Although frizzleds and Wnts have not
461 been surveyed in *Cu. salei*, also a member of the RTA clade, previous studies of Hox genes have so
462 far only recovered a single copy of *Hox3* (Schwager et al. 2007). The identification of genetic trends
463 potentially uniting this group is exciting, even if the macroevolutionary implications are unclear: as
464 described above, the possible functions of a *Wnt4* paralog are elusive in the context of very specific
465 *Wnt4* expression in *P. tepidariorum*. Members of the RTA clade are very derived compared to other
466 araneomorph spiders, both morphologically (e.g. male pedipalp morphology and sophisticated eyes)
467 and ecologically (most are wandering hunters), and their rapid diversification would align with clade-
468 specific genetic divergence (Garrison et al. 2016; Fernández et al. 2018; Shao and Li 2018).

469 Second, although data are only available for a single representative of the plesiomorphic clade
470 Synspermiata, *Ph. phalangioides*, these suggest lineage-specific losses of *lab* and *Ubx* paralogs and
471 the only example of *fz3* found in spiders so far. The presence of *fz3* is consistent with other
472 arachnoplumonate groups and suggests that it was present in the spider ancestor and only lost in the
473 more derived entelegyne lineages (as seen in *P. tepidariorum*, *M. muscosa* and *Pa. amentata*). If *lab*
474 and *Ubx* duplicates are indeed absent from the genome of *Ph. phalangioides*, this unusual loss of Hox
475 genes represents an interesting divergence between these two major groups. Synspermiatan spiders
476 are separated from other spiders by their relatively simpler genitalia and the absence of a cribellum,
477 which was putatively present in ancestral spiders but lost in Synspermiata (Michalik and Ramírez 2014).
478 Although they are unlikely to be directly responsible, the divergence in gene repertoires we see between
479 *Ph. phalangioides* and the other spider lineages might provide a starting point for understanding these
480 important morphological differences.

481 The amblypygids emerge as a key group of interest for studying the impacts of WGD owing to their high
482 levels of ohnolog retention. Our transcriptomes, from representatives of two major clades, provide new
483 evidence supporting a WGD in the ancestor of arachnoplumonates and demonstrating widespread
484 retention of ohnologs in three major families of developmental genes (consistent with the retention of
485 many duplicated regulators of eye development in other species; Gainett et al. 2020). In all three gene
486 families we studied, repertoires were largest in the amblypygid species. This was particularly the case
487 in *C. acosta*, which belongs to the less speciose and more plesiomorphic infraorder Charinidae within
488 living Amblypygi. Although this study represents just two amblypygid species and three gene families,

489 this appears to contradict widespread predictions of diversification with the duplication of important
490 developmental genes such as Hox (e.g. Van De Peer et al. 2009). Of particular interest are the
491 amblypygid Wnt gene repertoires. We have identified from their transcriptomes, and from the published
492 genome of *Ce. sculpturatus*, the first reported duplicates of *Wnt1/wg* in any animal, as well as the first
493 reported *Wnt10* in any arachnid. Future functional studies of these genes and their expression during
494 development will be critical to understanding the evolutionary impacts of these unusual components of
495 amblypygid gene repertoires. Amblypygids also represent a potential model group for studying the
496 evolution of arthropod body plans, owing to the unusual and derived morphology of the pedipalps and
497 especially the first walking legs. Thanks to a substantial existing body of work on anterior-posterior
498 patterning, segmentation and appendage development in spiders and other arachnids, we may have a
499 chance to crack the genetic underpinnings of these dramatic evolutionary innovations (Pechmann et al.
500 2009; Sharma et al. 2012, 2014; Turetzek et al. 2016, 2017; Schwager et al. 2017; Baudouin-Gonzalez
501 et al. 2020; Schomburg et al. 2020).

502 Finally, our analysis of existing genomic data for *Ce. sculpturatus* has recovered several Wnt and
503 Frizzled gene duplications, similar to spiders and amblypygids. However, in contrast to those groups,
504 our phylogenies have sometimes supported within-lineage duplication in *Ce. sculpturatus*, as opposed
505 to the retention of ohnologs following WGD, even when these are observed in spiders and amblypygids.
506 This was the case for *Wnt1/wg*, *Wnt6*, *Wnt7*, and potentially *fz2* (Figures 4,6). However, levels of
507 sequence similarity in these cases were comparable for *Ce. sculpturatus* paralogs and amblypygid and
508 spider ohnologs, when we might expect within-lineage duplicates to show higher similarity. The
509 resolution of the paralogous sequences in our phylogenetic analyses could be confounded by the early-
510 branching position of scorpions within Arachnoplumonata, which means paralogs would be expected
511 to appear towards the bottom of ortholog clades and are more vulnerable to movement.

512 Overall, our new data provide further evidence of an ancestral arachnoplumonate WGD, identify
513 evolutionary patterns within gene families following WGD, reveal new diversity in spider gene
514 repertoires, better contextualise existing data from spiders and scorpions, and broaden the phylogenetic
515 scope of available data for future researchers. However, other arachnid groups, both with and without
516 ancestral WGD, require further study. Thelyphonids (vinegaroons or whip scorpions) and schizomids
517 form a clade with amblypygids (Pedipalpi) and should also have been subject to the arachnoplumonate

518 WGD. Future work on these groups will shed light on the unusual patterns of gene retention we find in
519 both major clades of amblypygids. Pseudoscorpions especially require urgent attention; due to their
520 uncertain phylogenetic placement and lack of genomic data, we don't currently know whether they have
521 also been subject to the arachnoplumbrate WGD. Finally, to better contextualise the genomic changes
522 that occur following the arachnoplumbrate WGD, we require further data on arachnids without WGD,
523 namely harvestmen and ticks. The ability to compare rates of sequence divergence, within-lineage gene
524 duplication, and, eventually, functional properties of developmental genes in these groups will provide
525 critical comparative data for arachnoplumbrates.

526

527 **Acknowledgements**

528 This work was supported by the John Fell Fund, University of Oxford (award 0005632 to LSR), a NERC
529 grant (NE/T006854/1) to LSR and APM, and a Leverhulme Trust grant (RPG-2016-234) to APM. AH
530 was funded by a BBSRC DTP studentship. The authors are very grateful to Philip Steinhoff and Gabriele
531 Uhl (University of Greifswald) for providing embryos of *M. muscosa* and to Simon Ellis (University of
532 Oxford) for IT support.

533

534 **References**

- 535 Abzhanov, A., A. Popadic, and T. C. Kaufman. 1999. Chelicerate Hox genes and the homology of
536 arthropod segments. *Evol. Dev.* 1:77–89.
- 537 Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data.
- 538 Baudouin-Gonzalez, L., A. Schoenauer, A. Harper, G. Blakeley, M. Seiter, S. Arif, L. Sumner-Rooney,
539 S. Russell, P. P. Sharma, and P. Alistair. 2020. The evolution of Sox gene repertoires and
540 regulation of segmentation in arachnids. *BioRxiv*, doi:
541 <https://doi.org/10.1101/2020.06.04.133389>.
- 542 Beermann, A., R. Pruhs, R. Lutz, and R. Schroder. 2011. A context-dependent combination of wnt
543 receptors controls axis elongation and leg development in a short germ insect. *Development*
544 138:2793–805.
- 545 Bolognesi, R., A. Beermann, L. Farzana, N. Wittkopp, R. Lutz, G. Balavoine, S. J. Brown, and R.
546 Schröder. 2008. *Tribolium* Wnts: Evidence for a larger repertoire in insects with overlapping
547 expression patterns that suggest multiple redundant functions in embryogenesis. *Dev. Genes*
548 *Evol.* 218:193–202.
- 549 Brown, S. J., J. P. Fellers, T. D. Shippey, E. A. Richardson, M. Maxwell, J. J. Stuart, and R. E. Denell.
550 2002. Sequence of the *Tribolium castaneum* homeotic complex: The region corresponding to the
551 *Drosophila melanogaster* Antennapedia complex. *Genetics* 160:1067–1074.
- 552 Cadigan, K. M., and R. Nusse. 1997. Wnt signaling: A common theme in animal development. *Genes*
553 *Dev.* 11:3286–3305.
- 554 Cao, Z., Y. Yu, Y. Wu, P. Hao, Z. Di, Y. He, Z. Chen, W. Yang, Z. Shen, X. He, J. Sheng, X. Xu, B.
555 Pan, J. Feng, X. Yang, W. Hong, W. Zhao, Z. Li, K. Huang, T. Li, Y. Kong, H. Liu, D. Jiang, B.
556 Zhang, J. Hu, Y. Hu, B. Wang, J. Dai, B. Yuan, Y. Feng, W. Huang, X. Xing, G. Zhao, X. Li, Y.
557 Li, and W. Li. 2013. The genome of *Mesobuthus martensii* reveals a unique adaptation model of
558 arthropods. *Nat. Commun.* 4. Nature Publishing Group.
- 559 Carroll, S., J. Grenier, and S. Weatherbee. 2005. From DNA to diversity. Blackwell Publishing Ltd,
560 London.
- 561 Chen, M., C. Wang, W. Wang, G. Ji, B. Hu, M. Du, G. Liu, Z. Li, W. Wang, X. Lin, W. Zheng, and J.
562 Chen. 2016. De novo assembly and characterization of early embryonic transcriptome of the
563 horseshoe crab *Tachypleus tridentatus*. *PLoS One* 11:1–18.
- 564 Chipman, A. D., D. E. K. Ferrier, C. Brena, J. Qu, D. S. T. Hughes, R. Schröder, M. Torres-Oliva, N.
565 Znassi, H. Jiang, F. C. Almeida, C. R. Alonso, Z. Apostolou, P. Aqrabi, W. Arthur, J. C. J. Barna,
566 K. P. Blankenburg, D. Brites, S. Capella-Gutiérrez, M. Coyle, P. K. Dearden, L. Du Pasquier, E.
567 J. Duncan, D. Ebert, C. Eibner, G. Erikson, P. D. Evans, C. G. Extavour, L. Francisco, T.
568 Gabaldón, W. J. Gillis, E. A. Goodwin-Horn, J. E. Green, S. Griffiths-Jones, C. J. P.
569 Grimmelikhuijzen, S. Gubbala, R. Guigó, Y. Han, F. Hauser, P. Havlak, L. Hayden, S. Helbing,
570 M. Holder, J. H. L. Hui, J. P. Hunn, V. S. Hunnekuhl, L. R. Jackson, M. Javaid, S. N. Jhangiani,
571 F. M. Jiggins, T. E. Jones, T. S. Kaiser, D. Kalra, N. J. Kenny, V. Korchina, C. L. Kovar, F. B.
572 Kraus, F. Lapraz, S. L. Lee, J. Lv, C. Mandapat, G. Manning, M. Mariotti, R. Mata, T. Mathew, T.
573 Neumann, I. Newsham, D. N. Ngo, M. Ninova, G. Okwuonu, F. Onger, W. J. Palmer, S. Patil, P.
574 Patraquim, C. Pham, L. L. Pu, N. H. Putman, C. Rabouille, O. M. Ramos, A. C. Rhodes, H. E.
575 Robertson, H. M. Robertson, M. Ronshaugen, J. Rozas, N. Saada, A. Sánchez-Gracia, S. E.
576 Scherer, A. M. Schurko, K. W. Siggins, D. N. Simmons, A. Stief, E. Stolle, M. J. Telford, K.
577 Tessmar-Raible, R. Thornton, M. van der Zee, A. von Haeseler, J. M. Williams, J. H. Willis, Y.
578 Wu, X. Zou, D. Lawson, D. M. Muzny, K. C. Worley, R. A. Gibbs, M. Akam, and S. Richards.
579 2014. The first myriapod genome sequence reveals conservative arthropod gene content and
580 genome organisation in the centipede *Strigamia maritima*. *PLoS Biol.* 12.
- 581 Cho, S. J., Y. Vallès, V. C. Giani, E. C. Seaver, and D. A. Weisblat. 2010. Evolutionary dynamics of
582 the *wnt* gene family: A lophotrochozoan perspective. *Mol. Biol. Evol.* 27:1645–1658.

- 583 Clarke, T. H., J. E. Garb, C. Y. Hayashi, P. Arensburger, and N. A. Ayoub. 2015. Spider
584 Transcriptomes Identify Ancient Large-Scale Gene Duplication Event Potentially Important in
585 Silk Gland Evolution. *Genome Biol. Evol.* 7:1856–1870.
- 586 Clarke, T. H., J. E. Garb, C. Y. Hayashi, R. A. Haney, A. K. Lancaster, S. Corbett, and N. A. Ayoub.
587 2014. Multi-tissue transcriptomics of the black widow spider reveals expansions, co-options, and
588 functional processes of the silk gland gene toolkit. *BMC Genomics* 15:1–17.
- 589 Dehal, P., and J. L. Boore. 2005. Two rounds of whole genome duplication in the ancestral vertebrate.
590 *PLoS Biol.* 3.
- 591 Di, Z., Y. Yu, Y. Wu, P. Hao, Y. He, H. Zhao, Y. Li, G. Zhao, X. Li, W. Li, and Z. Cao. 2015. Genome-
592 wide analysis of homeobox genes from *Mesobuthus martensii* reveals Hox gene duplication in
593 scorpions. *Insect Biochem. Mol. Biol.* 61:25–33. Elsevier Ltd.
- 594 Ding, X., J. Liu, L. Zheng, J. Song, N. Li, H. Hu, X. Tong, and F. Dai. 2019. Genome-wide
595 identification and expression profiling of Wnt family genes in the silkworm, *Bombyx mori*. *Int. J.*
596 *Mol. Sci.* 20:1–13.
- 597 Donoghue, P. C. J., and M. A. Purnell. 2005. Genome duplication, extinction and vertebrate evolution.
598 *Trends Ecol. Evol.* 20:312–319.
- 599 Erwin, D. H. 2009. Early origin of the bilaterian developmental toolkit. *Philos. Trans. R. Soc. B Biol.*
600 *Sci.* 364:2253–2261.
- 601 Ferguson, L., F. Marlétaz, J. M. Carter, W. R. Taylor, M. Gibbs, C. J. Breuker, and P. W. H. Holland.
602 2014. Ancient expansion of the Hox cluster in Lepidoptera generated four homeobox genes
603 implicated in extra-embryonic tissue formation. *PLoS Genet.* 10.
- 604 Fernández, R., R. J. Kallal, D. Dimitrov, J. A. Ballesteros, M. A. Arnedo, G. Giribet, and G. Hormiga.
605 2018. Phylogenomics, Diversification Dynamics, and Comparative Transcriptomics across the
606 Spider Tree of Life. *Curr. Biol.* 28:1489-1497.e5.
- 607 Flot, J. F., B. Hespeels, X. Li, B. Noel, I. Arkhipova, E. G. J. Danchin, A. Hejnol, B. Henrissat, R.
608 Koszul, J. M. Aury, V. Barbe, R. M. Barthélémy, J. Bast, G. A. Bazykin, O. Chabrol, A. Couloux,
609 M. Da Rocha, C. Da Silva, E. Gladyshev, P. Gouret, O. Hallatschek, B. Hecox-Lea, K. Labadie,
610 B. Lejeune, O. Piskurek, J. Poulain, F. Rodriguez, J. F. Ryan, O. A. Vakhrusheva, E. Wajnberg,
611 B. Wirth, I. Yushenova, M. Kellis, A. S. Kondrashov, D. B. M. Welch, P. Pontarotti, J.
612 Weissenbach, P. Wincker, O. Jaillon, and K. Van Doninck. 2013. Genomic evidence for ameiotic
613 evolution in the bdelloid rotifer *Adineta vaga*. *Nature* 500:453–457.
- 614 Force, A., M. Lynch, F. B. Pickett, A. Amores, Y. L. Yan, and J. Postlethwait. 1999. Preservation of
615 duplicate genes by complementary, degenerative mutations. *Genetics* 151:1531–1545.
- 616 Gainett, G., J. A. Ballesteros, C. R. Kanzler, J. T. Zehms, J. M. Zern, S. Aharon, E. Gavish-Regev,
617 and P. P. Sharma. 2020. How spiders make their eyes: Systemic paralogy and function of retinal
618 determination network homologs in arachnids. *BioRxiv* 1–13.
- 619 Gainett, G., and P. P. Sharma. 2020. New genomic resources and toolkits for developmental study of
620 whip spiders (Amblypygi) provide insights into arachnid genome evolution and antenniform leg
621 patterning. *BioRxiv* 1–39.
- 622 Garb, J. E., P. P. Sharma, and N. A. Ayoub. 2018. Recent progress and prospects for advancing
623 arachnid genomics. *Curr. Opin. Insect Sci.* 25:51–57. Elsevier Inc.
- 624 Garrison, N. L., J. Rodriguez, I. Agnarsson, J. A. Coddington, C. E. Griswold, C. A. Hamilton, M.
625 Hedin, K. M. Kocot, J. M. Ledford, and J. E. Bond. 2016. Spider phylogenomics: untangling the
626 Spider Tree of Life. *PeerJ* 4.
- 627 Glasauer, S. M. K., and S. C. F. Neuhauss. 2014. Whole-genome duplication in teleost fishes and its
628 evolutionary consequences. *Mol. Genet. Genomics* 289:1045–1060.

- 629 Goujon, M., H. McWilliam, W. Li, F. Valentin, S. Squizzato, J. Paern, and R. Lopez. 2010. A new
630 bioinformatics analysis tools framework at EMBL-EBI. *Nucleic Acids Res.* 38:695–699.
- 631 Haas, B. J., A. Papanicolaou, M. Yassour, M. Grabherr, P. D. Blood, J. Bowden, M. B. Couger, D.
632 Eccles, B. Li, M. Lieber, M. D. MacManes, M. Ott, J. Orvis, N. Pochet, F. Strozzi, N. Weeks, R.
633 Westerman, T. William, C. N. Dewey, R. Henschel, R. D. LeDuc, N. Friedman, and A. Regev.
634 2013. De novo transcript sequence reconstruction from RNA-Seq: reference generation and
635 analysis with Trinity.
- 636 Hamilton, F. S., G. N. Wheeler, and S. Hoppler. 2001. Difference in XTcf-3 dependency accounts for
637 change in response to β -catenin-mediated Wnt signalling in *Xenopus* blastula. *Development*
638 128:2063–2073.
- 639 Havlak, P., N. H. Putnam, J.-X. Yue, H. J. Brockmann, C. W. Nossa, K. Y. Vincent, and J. Lv. 2014.
640 Joint assembly and genetic mapping of the Atlantic horseshoe crab genome reveals ancient
641 whole genome duplication. *Gigascience* 3:1–21.
- 642 Hayden, L., and W. Arthur. 2013. Expression patterns of Wnt genes in the venom claws of
643 centipedes. *Evol. Dev.* 15:365–372.
- 644 Hogvall, M., A. Schönauer, G. E. Budd, A. P. McGregor, N. Posnien, and R. Janssen. 2014. Analysis
645 of the Wnt gene repertoire in an onychophoran provides new insights into the evolution of
646 segmentation. *Evodevo* 5:1–15.
- 647 Holland, L. Z. 2013a. Evolution of new characters after whole genome duplications: Insights from
648 amphioxus. *Semin. Cell Dev. Biol.* 24:101–109. Elsevier Ltd.
- 649 Holland, P. W. H. 2013b. Evolution of homeobox genes. *Wiley Interdiscip. Rev. Dev. Biol.* 2:31–45.
- 650 Holland, P. W. H., J. Garcia-Fernandez, N. A. Williams, and A. Sidow. 1994. Gene duplications and
651 the origins of vertebrate development. *Development* 120:125–133.
- 652 Holzem, M., N. Braak, O. Brattström, A. P. McGregor, and C. J. Breuker. 2019. Wnt gene expression
653 during early embryogenesis in the nymphalid butterfly *Bicyclus anynana*. *Front. Ecol. Evol.* 7.
- 654 Hueber, S. D., and I. Lohmann. 2008. Shaping segments: Hox gene function in the genomic age.
655 *BioEssays* 30:965–979.
- 656 Janssen, R., M. Le Gouar, M. Pechmann, F. Poulin, R. Bolognesi, E. E. Schwager, C. Hopfen, J. K.
657 Colbourne, G. E. Budd, S. J. Brown, N. M. Prpic, C. Kosiol, M. Vervoort, W. G. Damen, G.
658 Balavoine, and A. P. McGregor. 2010. Conservation, loss, and redeployment of Wnt ligands in
659 protostomes: Implications for understanding the evolution of segment formation. *BMC Evol. Biol.*
660 10:1–21.
- 661 Janssen, R., and N. Posnien. 2014. Identification and embryonic expression of *Wnt2*, *Wnt4*, *Wnt5* and
662 *Wnt9* in the millipede *Glomeris marginata* (Myriapoda: Diplopoda). *Gene Expr. Patterns* 14:55–
663 61.
- 664 Janssen, R., A. Schönauer, M. Weber, N. Turetzek, M. Hogvall, G. E. Goss, N. H. Patel, A. P.
665 McGregor, and M. Hilbrant. 2015. The evolution and expression of panarthropod frizzled genes.
666 *Front. Ecol. Evol.* 3:1–14.
- 667 Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. Von Haeseler, and L. S. Jermiin. 2017.
668 ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14:587–
669 589.
- 670 Kenny, N. J., K. W. Chan, W. Nong, Z. Qu, I. Maeso, H. Y. Yip, T. F. Chan, H. S. Kwan, P. W. H. H.
671 Holland, K. H. Chu, and J. H. L. L. Hui. 2016. Ancestral whole-genome duplication in the marine
672 chelicerate horseshoe crabs. *Heredity (Edinb)*. 116:190–199.
- 673 Král, J., M. Forman, T. Kořínková, A. C. Reyes Lerma, C. R. Haddad, J. Musilová, M. Řezáč, I. M.
674 Ávila Herrera, S. Thakur, A. S. Dippenaar-Schoeman, F. Marec, L. Horová, and P. Bureš. 2019.

- 675 Insights into the karyotype and genome evolution of haplogyne spiders indicate a polyploid
676 origin of lineage with holokinetic chromosomes. *Sci. Rep.*
- 677 Kuraku, S., and A. Meyer. 2009. The evolution and maintenance of Hox gene clusters in vertebrates
678 and the teleost-specific genome duplication. *Int. J. Dev. Biol.* 53:765–773.
- 679 Leite, D. J., L. Baudouin-Gonzalez, S. Iwasaki-Yokozawa, J. Lozano-Fernandez, N. Turetzek, Y.
680 Akiyama-Oda, N. M. Prpic, D. Pisani, H. Oda, P. P. Sharma, and A. P. McGregor. 2018.
681 Homeobox gene duplication and divergence in arachnids. *Mol. Biol. Evol.* 35:2240–2253.
- 682 Leite, D. J., M. Ninova, M. Hilbrant, S. Arif, S. Griffiths-Jones, M. Ronshaugen, and A. P. McGregor.
683 2016. Pervasive microRNA duplication in chelicerates: Insights from the embryonic microRNA
684 repertoire of the spider *Parasteatoda tepidariorum*. *Genome Biol. Evol.* 8:2133–2144.
- 685 Logan, C. Y., and R. Nusse. 2004. The Wnt signaling pathway in development and disease. *Annu.*
686 *Rev. Cell Dev. Biol.* 20:781–810.
- 687 Lynch, M., and J. S. Conery. 2000. The evolutionary fate and consequences of duplicate genes.
688 *Science* (80-.). 290:1151–1155.
- 689 Mark Welch, D. B., J. L. Mark Welch, and M. Meselson. 2008. Evidence for degenerate tetraploidy in
690 bdelloid rotifers. *Proc. Natl. Acad. Sci. U. S. A.* 105:5145–5149.
- 691 McGinnis, W., and R. Krumlauf. 1992. Homeobox genes and axial patterning. *Cell* 68:283–302.
- 692 Meng, F., I. Braasch, J. B. Phillips, X. Lin, T. Titus, C. Zhang, and J. H. Postlethwait. 2013. Evolution
693 of the eye transcriptome under constant darkness in *Sinocyclocheilus* cavefish. *Mol. Biol. Evol.*
694 30:1527–1543.
- 695 Meyer, A., and M. Scharf. 1999. Gene and genome duplications in vertebrates: The one-to-four (-to-
696 eight in fish) rule and the evolution of novel gene functions. *Curr. Opin. Cell Biol.* 11:699–704.
- 697 Michalik, P., and M. J. Ramírez. 2014. Evolutionary morphology of the male reproductive system,
698 spermatozoa and seminal fluid of spiders (Araneae, Arachnida) - Current knowledge and future
699 directions. *Arthropod Struct. Dev.* 43:291–322.
- 700 Miller, J. R. 2001. Protein family review The Wnts. *Gene* 1–15.
- 701 Murat, S., C. Hopfen, and A. P. McGregor. 2010. The function and evolution of Wnt genes in
702 arthropods. *Arthropod Struct. Dev.* 39:446–452. Elsevier Ltd.
- 703 Nguyen, L. T., H. A. Schmidt, A. Von Haeseler, and B. Q. Minh. 2015. IQ-TREE: A fast and effective
704 stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* 32:268–
705 274.
- 706 Nong, W., Z. Qu, Y. Li, T. Barton-Owe, A. Y. P. Wong, H. Y. Yip, H. T. Lee, S. Narayana, T. Baril, T.
707 Swale, J. Cao, T. F. Chan, H. S. Kwan, N. S. Ming, G. Panagiotou, P.-Y. Qian, J.-W. Qui, K. Y.
708 Yip, N. Ismail, S. Pati, A. John, S. S. Tobe, W. G. Bendena, S. G. Cheung, A. Hayward, and J.
709 H. L. Hui. 2020. Horseshoe crab genomes reveal the evolutionary fates of genes and
710 microRNAs after three rounds (3R) of whole genome duplication. *BioRxiv* 1–29.
- 711 Ohno, S. 1970. *Evolution by Gene Duplication*. Springer, New York.
- 712 Pascual-Anaya, J., S. D’Aniello, S. Kuratani, and J. Garcia-Fernández. 2013. Evolution of Hox gene
713 clusters in deuterostomes Hox content in invertebrate deuterostomes.
- 714 Pearson, J. C., D. Lemons, and W. McGinnis. 2005. Modulating Hox gene functions during animal
715 body patterning. *Nat. Rev. Genet.* 6:893–904.
- 716 Pechmann, M., A. P. McGregor, E. E. Schwager, N. M. Feitosa, and W. G. M. Damen. 2009. Dynamic
717 gene expression is required for anterior regionalization in a spider. *Proc. Natl. Acad. Sci. U. S.*
718 *A.* 106:1468–1472.

- 719 Pechmann, M., E. E. Schwager, N. Turetzek, and N. M. Prpic. 2015. Regressive evolution of the
720 arthropod tritocerebral segment linked to functional divergence of the Hox gene *labial*. *Proc. R.*
721 *Soc. B Biol. Sci.* 282.
- 722 Pires-daSilva, A., and R. J. Sommer. 2003. The evolution of signalling pathways in animal
723 development. *Nat. Rev. Genet.* 4:39–49.
- 724 Schomburg, C., N. Turetzek, and N. M. Prpic. 2020. Candidate gene screen for potential interaction
725 partners and regulatory targets of the Hox gene *labial* in the spider *Parasteatoda tepidariorum*.
726 *Dev. Genes Evol.* 230:105–120. *Development Genes and Evolution.*
- 727 Schwager, E. E., A. Schönauer, D. J. Leite, P. P. Sharma, and A. P. McGregor. 2015. Chelicerata.
728 Pp. 99–139 in A. Wanninger, ed. *Evolutionary Developmental Biology of Invertebrates 3:*
729 *Ecdysozoa I: Non-Tetraconata.* Springer-Verlag, Wien.
- 730 Schwager, E. E., M. Schoppmeier, M. Pechmann, and W. G. M. Damen. 2007. Duplicated Hox genes
731 in the spider *Cupiennius salei*. *Front. Zool.* 4.
- 732 Schwager, E. E., P. P. Sharma, T. Clarke, D. J. Leite, T. Wierschin, A. D. Buffry, H. Chao, H. Dinh, H.
733 Doddapaneni, S. Dugan, S. L. Lee, I. Maeso, S. C. Murali, D. M. Muzny, R. Nunes, C. Wolff, K.
734 C. Worley, G. Bucher, R. A. Gibbs, J. Coddington, H. Oda, S. Richards, and A. P. McGregor.
735 2017. The house spider genome reveals an ancient whole-genome duplication during arachnid
736 evolution. *BMC Biol.* 15:1–27. *BMC Biology.*
- 737 Seppey, M., M. Manni, and E. M. Zdobnov. 2019. BUSCO: Assessing Genome Assembly and
738 Annotation Completeness. P. in M. Kollmar, ed. *Gene Prediction. Methods in Molecular Biology,*
739 *Vol 1962.* Humana, New York, NY.
- 740 Shao, L., and S. Li. 2018. Early Cretaceous greenhouse pumped higher taxa diversification in spiders.
741 *Mol. Phylogenet. Evol.* 127:146–155. Elsevier.
- 742 Sharma, P. P., M. A. Santiago, E. González-Santillán, L. Monod, and W. C. Wheeler. 2015. Evidence
743 of duplicated Hox genes in the most recent common ancestor of extant scorpions. *Evol. Dev.*
744 17:347–355.
- 745 Sharma, P. P., E. E. Schwager, C. G. Extavour, and G. Giribet. 2012. Hox gene expression in the
746 harvestman *Phalangium opilio* reveals divergent patterning of the chelicerate opisthosoma. *Evol.*
747 *Dev.* 14:450–463.
- 748 Sharma, P. P., E. E. Schwager, C. G. Extavour, and W. C. Wheeler. 2014. Hox gene duplications
749 correlate with posterior heteronomy in scorpions. *Proc. R. Soc. B Biol. Sci.* 281.
- 750 Shimeld, S. M. 1999. Gene function, gene networks and the fate of duplicated genes. *Semin. Cell*
751 *Dev. Biol.* 10:549–553.
- 752 Shingate, P., V. Ravi, A. Prasad, B. H. Tay, K. M. Garg, B. Chattopadhyay, L. M. Yap, F. E. Rheindt,
753 and B. Venkatesh. 2020. Chromosome-level assembly of the horseshoe crab genome provides
754 insights into its genome evolution. *Nat. Commun.* 11. Springer US.
- 755 Sievers, F., A. Wilm, D. Dineen, T. J. Gibson, K. Karplus, W. Li, R. Lopez, H. McWilliam, M. Remmert,
756 J. Söding, J. D. Thompson, and D. G. Higgins. 2011. Fast, scalable generation of high-quality
757 protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* 7.
- 758 Song, L., and L. Florea. 2015. Rcorrector: Efficient and accurate error correction for Illumina RNA-seq
759 reads. *GigaScience* 4:1–8. *GigaScience.*
- 760 Stauber, M., H. Jäckle, and U. Schmidt-Ott. 1999. The anterior determinant bicoid of *Drosophila* is a
761 derived Hox class 3 gene. *Proc. Natl. Acad. Sci. U. S. A.* 96:3786–3789.
- 762 Turetzek, N., S. Khadjeh, C. Schomburg, and N. M. Prpic. 2017. Rapid diversification of homothorax
763 expression patterns after gene duplication in spiders. *BMC Evol. Biol.* 17:1–12. *BMC*
764 *Evolutionary Biology.*

- 765 Turetzek, N., M. Pechmann, C. Schomburg, J. Schneider, and N. M. Prpic. 2016. Neofunctionalization
766 of a duplicate dachshund gene underlies the evolution of a novel leg segment in arachnids. *Mol.*
767 *Biol. Evol.* 33:109–121.
- 768 van Amerongen, R., and R. Nusse. 2009. Towards an integrated view of Wnt signaling in
769 development. *Development* 136:3205–3214.
- 770 Van De Peer, Y., S. Maere, and A. Meyer. 2009. The evolutionary significance of ancient genome
771 duplications. *Nat. Rev. Genet.* 10:725–732. Nature Publishing Group.
- 772 Weygoldt, P. 2009. Evolutionary morphology of whip spiders: towards a phylogenetic system
773 (Chelicerata: Arachnida: Amblypygi)*. *J. Zool. Syst. Evol. Res.* 34:185–202.
- 774 Wu, C. H., and R. Nusse. 2002. Ligand receptor interactions in the Wnt signaling pathway in
775 *Drosophila*. *J. Biol. Chem.* 277:41762–41769.
- 776 Yu, J. J. ., A. Maugarny-Cales, S. Pelletier, C. Alexandre, Y. Bellaiche, J.-P. Vincent, and I. J.
777 McGough. 2020. Frizzled-dependent Planar Cell Polarity without Wnt Ligands. *bioRxiv*
778 2020.05.23.108977.
- 779 Zhang, J. 2003. Evolution by gene duplication: An update. *Trends Ecol. Evol.* 18:292–298.
- 780 Zhong, Y. F., and P. W. H. Holland. 2011. HomeoDB2: Functional expansion of a comparative
781 homeobox gene database for evolutionary developmental biology. *Evol. Dev.* 13:567–568.
- 782 2019. World Spider Catalog. Natural History Museum Bern.
- 783

Table 1. Assembly metrics for transcriptomes of *Charinus acosta*, *Euphrynichus bacillifer*, *Marpissa muscosa* and *Pardosa amentata*.

* Based on longest Isoform per gene

** Same as contig N50 but based on top most highly expressed genes that represent 90% of the total normalized expression data

*** The number of genes for which Ex90N50 is calculated

¹ 10 species, n=2934 BUSCOs; C=Complete [D=Duplicated], F=Fragmented, M=Missing.

² 90 species, n=1013 BUSCOs; C=Complete [D=Duplicated], F=Fragmented, M=Missing.

Species	Raw Reads	Processed Reads	#Trinity Genes	#Trinity Isoforms	Contig N50 (bases)*	Ex90N50 (bases)**	#Ex90N50 genes***	Arachnid Scores (C[D],F,M) ¹	BUSCO Scores (C[D],F,M) ²
<i>C. acosta</i>	272,844,971	260,853,757	237,678	334,267	896	2406	31,012	94.9%[12.9%],1.0%,4.1%	93.2%[9.5%],1.5%,5.3%
<i>E. bacillifer</i>	249,938,618	239,034,000	184,142	285,861	978	2671	22,647	93.8%[7.1%],1.5%,4.7%	92.9%[3.5%],0.7%,6.4%
<i>Pa. amentata</i>	266,764,548	256,911,378	316,021	542,344	652	1758	38,423	95.4%[5.3%],0.9%,3.7%	92.9%[4.5%],1.2%,5.9%
<i>M. muscosa</i>	222,479,664	211,848,357	276,943	473,878	592	1461	46,196	94.7%[6.1%],1.3%,4.0%	90.8%[3.9%],1.9%,7.3%

Figures

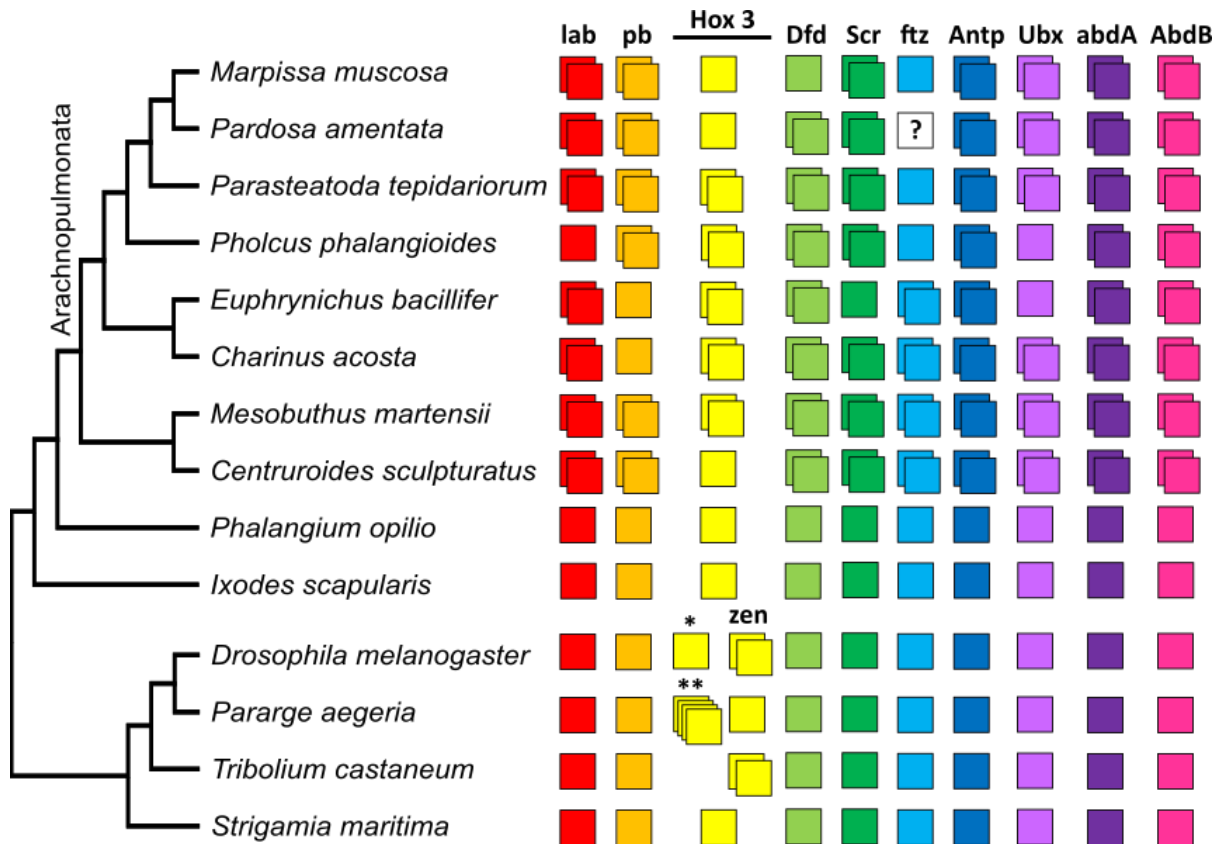


Figure 1. Repertoires of Hox genes in arachnids and other selected arthropods. Hox genes are represented by coloured boxes with duplicated Hox genes indicated by overlapping boxes. *ftz* was not found in the *Pa. amentata* transcriptome. Figure includes Hox repertoires previously surveyed in the arachnids *P. tepidariorum* (Schwager et al. 2017; Leite et al. 2018), *Ce. sculpturatus* (Schwager et al. 2017), *Me. martensii* (Di et al. 2015), *Pha. opilio* (Sharma et al. 2012), *I. scapularis* (all genomes) and *Ph. phalangioides* (embryonic transcriptome; Leite et al. 2018), the myriapod *S. maritima* (Chipman et al. 2014) and the insects *D. melanogaster*, *T. castaneum* (Zhong and Holland 2011) and *Par. aegeria* (Ferguson et al. 2014). The insect *Hox3* homolog *zen* has undergone independent tandem duplications in *T. castaneum* to yield *zen* and *zen2*; in cyclorrhaphan flies to yield *zen* and *bicoid* (*) (Stauber et al. 1999); and in the genus *Drosophila* to yield *zen2* (Brown et al. 2002). The nymphalid butterfly *P. aegeria* is representative of most species of ditrysian Lepidoptera which possess four distinct *Hox3* genes termed Special homeobox genes (*ShxA*, *ShxB*, *ShxC* and *ShxD*) (**) and the canonical *zen* gene (Ferguson et al. 2014).

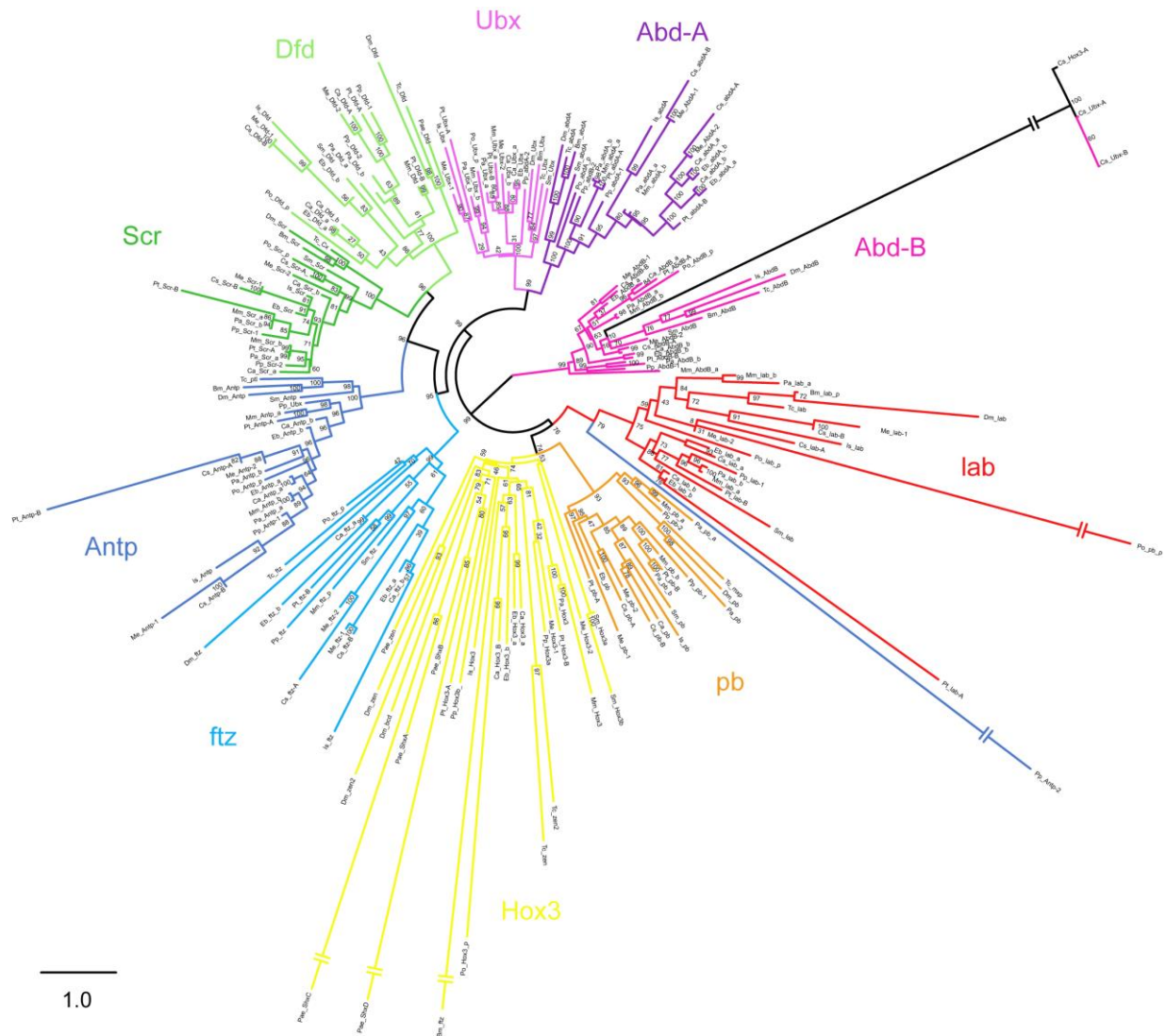


Figure 2 Maximum likelihood phylogeny of Hox amino acid sequences. The Hox genes are shown as different colours (after Figure 1). Panarthropods included: *M. muscosa* (Mm), *Pa. amentata* (Pa), *P. tepidarium* (Pt), *Ph. phalangioides* (Pp), *E. bacillifer* (Eb), *C. acosta* (Ca), *Me. martensii* (Me), *Ce. sculpturatus* (Cs), *Pha. opilio* (Po), *I. scapularis* (Is), *D. melanogaster* (Dm), *Par. aegeria* (Pae), *T. castaneum* (Tc), and *S. maritima* (Sm). Node labels indicate ultrafast bootstrap support values. See Supplementary Table 1 for accession numbers, Supplementary Data File 1 for amino acid sequence alignments.

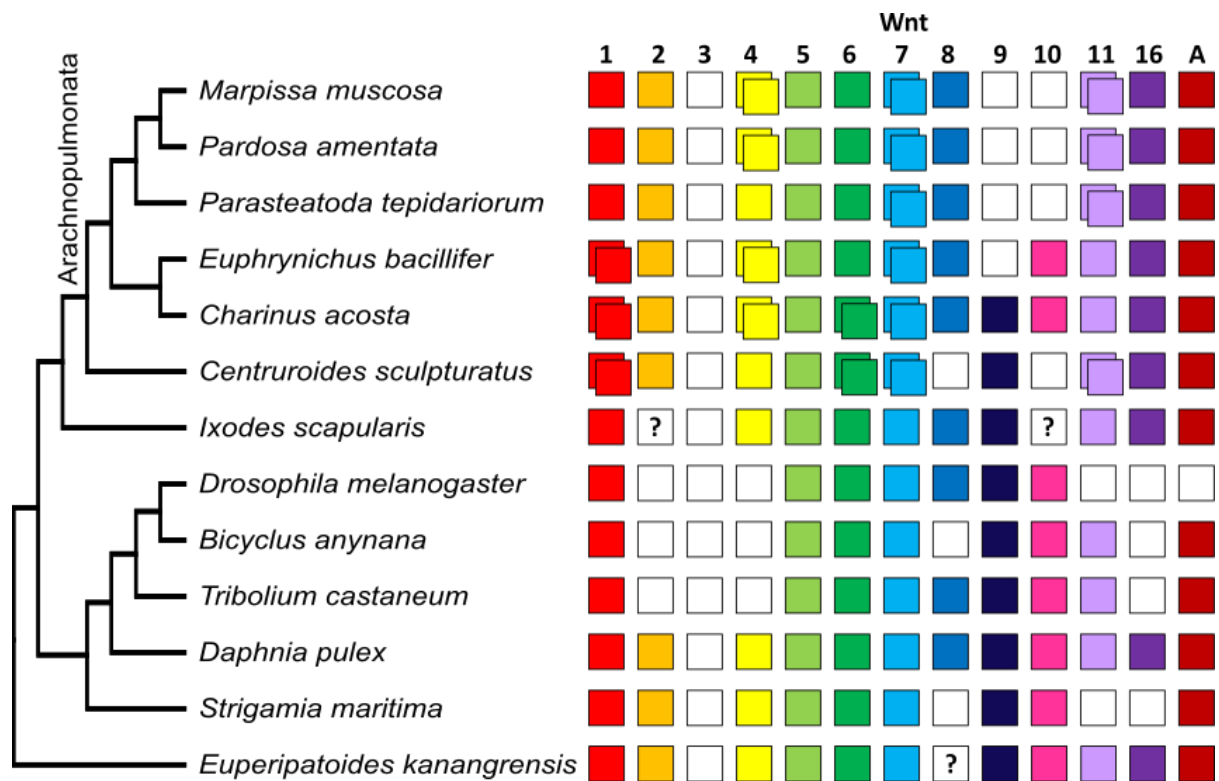


Figure 3 Repertoires of Wnt subfamilies in arthropods and an onychophoran. The Wnt subfamilies (1-11, 16 and A) are represented by coloured boxes with duplicated genes represented by overlapping boxes and putatively lost subfamilies indicated by white boxes. Question marks indicate subfamilies that have not been found but were probably not detected due to the lack of an available genomes or genome assembly quality. Figure includes Wnt repertoires recovered in this study and previously surveyed in the arachnids *P. tepidariorum* and *I. scapularis* (Janssen et al. 2010); the insects *D. melanogaster*, *T. castaneum* (Bolognesi et al. 2008) and *B. anynana* (Ding et al. 2019; Holzem et al. 2019); the crustacean *Da. pulex* (Janssen et al. 2010); the myriapod *S. maritima* (Chipman et al. 2014); and the onychophoran *Eu. kanangrensis* (Hogvall et al. 2014).

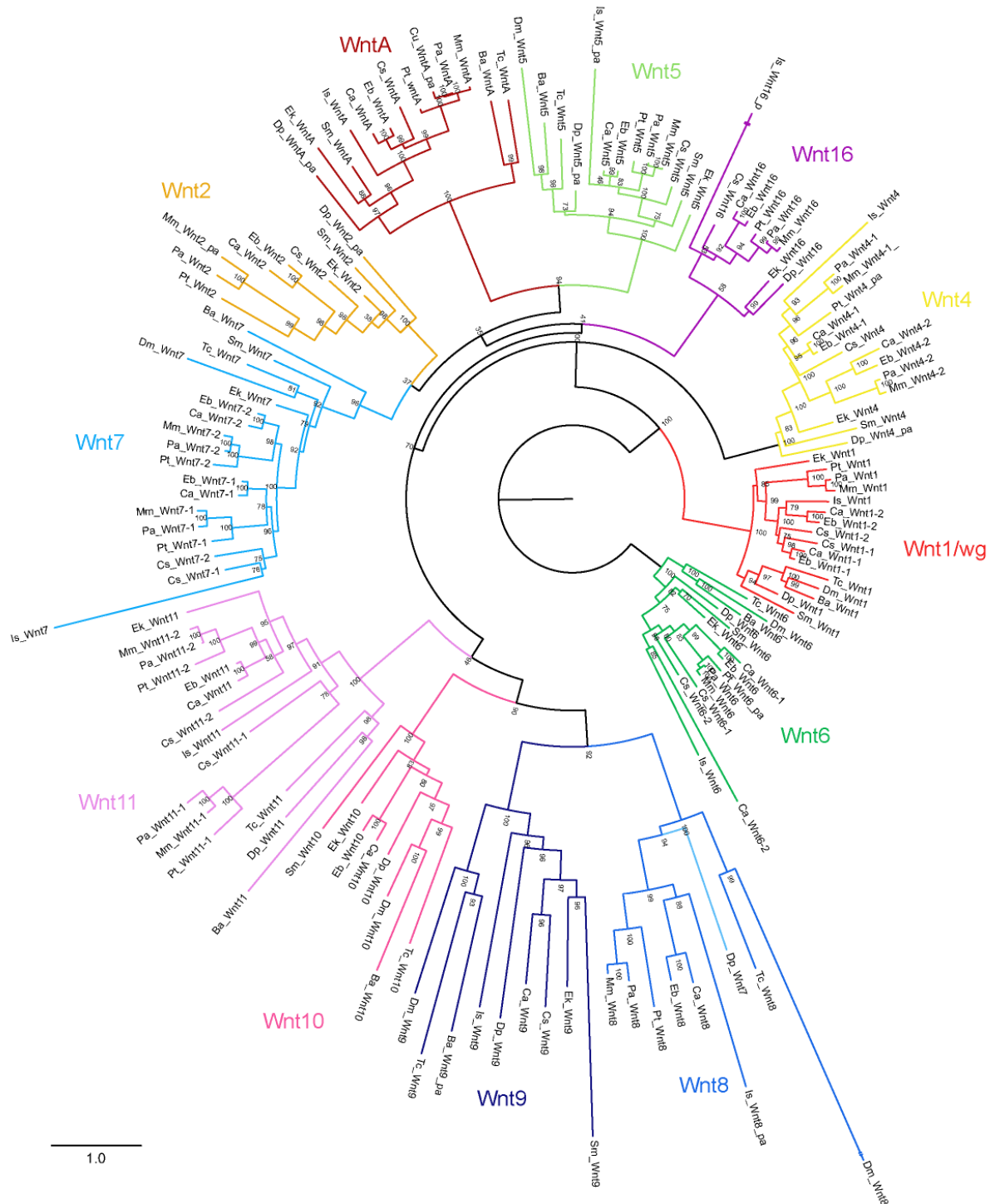


Figure 4. Maximum likelihood phylogeny of Wnt amino acid sequences. The 12 Wnt subfamilies are shown as different colours (after Figure 3). Panarthropods included: *P. tepidarium* (Pt), *Cu. salei* (Cu), *Pa. amentata* (Pa), *M. muscosa* (Mm), *C. acosta* (Ca), *E. bacillifer* (Eb), *Ce. sculpturatus* (Cs), *I. scapularis* (Is), *D. melanogaster* (Dm), *B. anynana* (Ba), *T. castaneum* (Tc), *Da. pulex* (Dp), *S. maritima* (Sm), and *Eu. kanangrensis* (Ek). Node labels indicate ultrafast bootstrap support values. See Supplementary Table 2 for accession numbers, Supplementary Data File 2 for amino acid sequence alignments, and Supplementary Data File 3 for nucleotide sequence alignments of *Wnt1/wg* duplicates in *C. acosta*, *Ce. sculpturatus* and *E. bacillifer*.

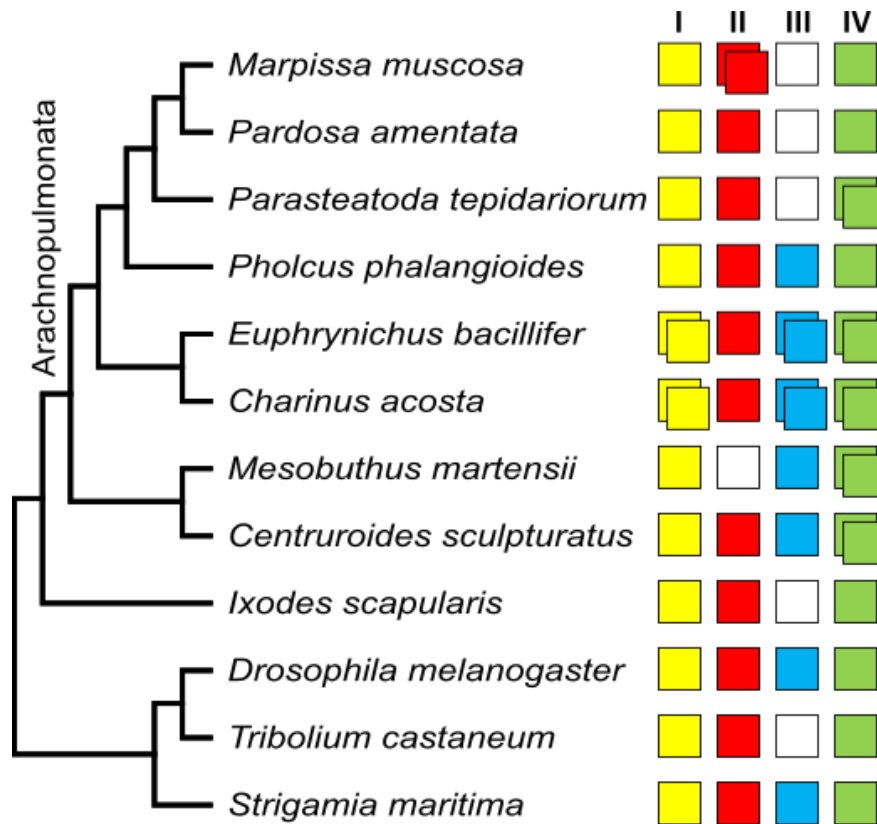


Figure 5. Repertoire of *frizzled* genes in arachnids and other selected arthropods. The four *frizzled* orthology groups (FzI, FzII, FzIII, and FzIV) are represented by coloured boxes, with duplicated genes represented by overlapping boxes and gene loss represented by a white box. Figure includes *frizzled* repertoires previously surveyed in the arachnids *P. tepidariorum*, *Ce. sculpturatus*, *Me. martensii*, *I. scapularis* (all genomes), and *Ph. phalangioides* (embryonic transcriptome; Janssen et al. 2015); the myriapod *S. maritima* (Janssen et al. 2015); and the insects *D. melanogaster* and *T. castaneum* (Beermann et al. 2011).

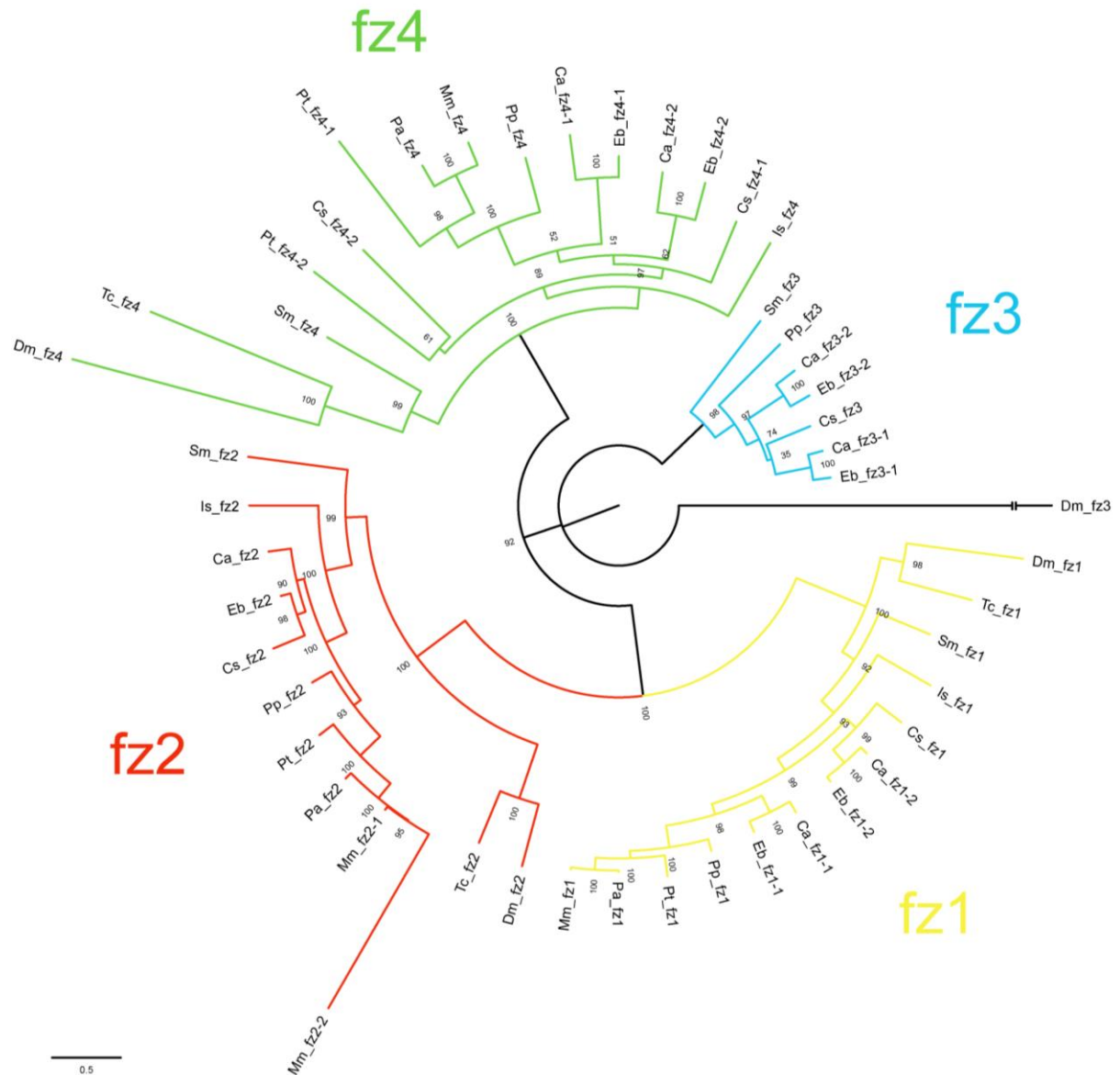


Figure 6 Maximum likelihood phylogeny of Frizzled proteins. The *frizzled* genes are shown as different colours (after Figure 5). Panarthropods included: *M. muscosa* (Mm), *Pa. amentata* (Pa), *P. tepidariorum* (Pt), *Ph. phalangioides* (Pp), *E. bacillifer* (Eb), *C. acosta* (Ca), *Me. martensii* (Me), *I. scapularis* (Is), *D. melanogaster* (Dm), *T. castaneum* (Tc), and *S. maritima* (Sm). Node labels indicate ultrafast bootstrap support values. See Supplementary Table 3 for accession numbers and Supplementary Data File 4 for alignments.

Supplementary Data and Tables

Supplementary Data File 1 Alignment of full Hox protein sequences, Phylip format.

Supplementary Data File 2 Alignment of full Wnt protein sequences, Phylip format.

Supplementary Data File 3 Alignment of *Wnt1* duplicate nucleotide sequences in *Charinus acosta*, *Euphrynichus bacillifer* and *Centruroides sculpturatus*, Phylip format.

Supplementary Data File 4 Alignment of full Frizzled protein sequences, Phylip format.

Supplementary Table 1 Protein accession numbers for Hox gene sequences used in this study.

Species	Hox genes	Protein accession number
<i>Centruroides sculpturatus</i> (Cs)		SupFile1 (Leite et al. 2018) Predicted protein sequences were obtained using the Translate ExPASy online tool (https://web.expasy.org/translate/).
<i>Mesobuthus martensii</i> (Mm)		Supplementary file 2: Classification of scorpion homeobox genes. (Di et al. 2015)
<i>Parasteatoda tepidariorum</i> (Pt)		SupFile1 (Leite et al. 2018) Predicted protein sequences were obtained using the Translate ExPASy online tool (https://web.expasy.org/translate/).
	Ubx-B	XP_021004342.1
	abd-A	XP_015921999.1
<i>Pholcus phalangoides</i> (Pp)		SupFile1 (Leite et al. 2018) Predicted protein sequences were obtained using the Translate ExPASy online tool (https://web.expasy.org/translate/).
<i>Ixodes scapularis</i> (Is)		SupFile1 (Leite et al. 2018)
<i>Phalangium opilio</i> (Po)	lab	CCH51000.1
	pb	CCH51001.1
	Hox3	CCH51002.1
	Dfd	CCH51003.1
	Scr	CCH51004.1
	ftz	CCH51005.1
	Antp	CCH51006.1
	Ubx	CCH51007.1
	abdA	CCH51008.1
	AbdB	CCH51009.1
<i>Bombyx mori</i> (Bm)	lab	BAC99310.1
	Scr	NP_001037339.1
	ftz	NP_001037528.2
	Antp	NP_001037319.1
	Ubx	NP_001107632.1
	abdA	NP_001166808.1
	AbdB	NP_001139700.1
<i>Drosophila melanogaster</i> (Dm)	pb	NP_996163.1
	lab	NP_001246953.1
	bcd	NP_731111.1
	zen	NP_476793.1
	zen2	NP_476794.1
	Dfd	NP_477201.1
	Scr	NP_001368995.1

	ftz	NP_477498.1
	Antp	NP_996175.1
	Ubx	NP_996219.1
	abdA	NP_001247145.1
	AbdB	NP_001303474.1
<i>Pararge aegeria</i> (Pae)	pb	AIB07898.1
	zen	AIB07903.1
	Dfd	AIB07904.1
	ShxA	AIB07900.1
	ShxB	AIB07899.1
	ShxC	AIB07901.1
	ShxD	AIB07902.1
<i>Strigamia maritima</i> (Sm)		Table S30. Details of the manually annotated genes of <i>S. maritima</i>. (Chipman et al. 2014)
<i>Tribolium castaneum</i> (Tc)	mvp/pb	NP_001107807.1
	lab	NP_001107762.1
	zen	NP_001036813.1
	zen2	NP_001038090.1
	Dfd	NP_001034510.1
	Cx/Scr	NP_001034523.1
	ftz	NP_001034539.1
	ptl/Antp	NP_001034505.1
	Ubx	NP_001034497.1
	abdA	NP_001034518.1
	AbdB	NP_001034519.1

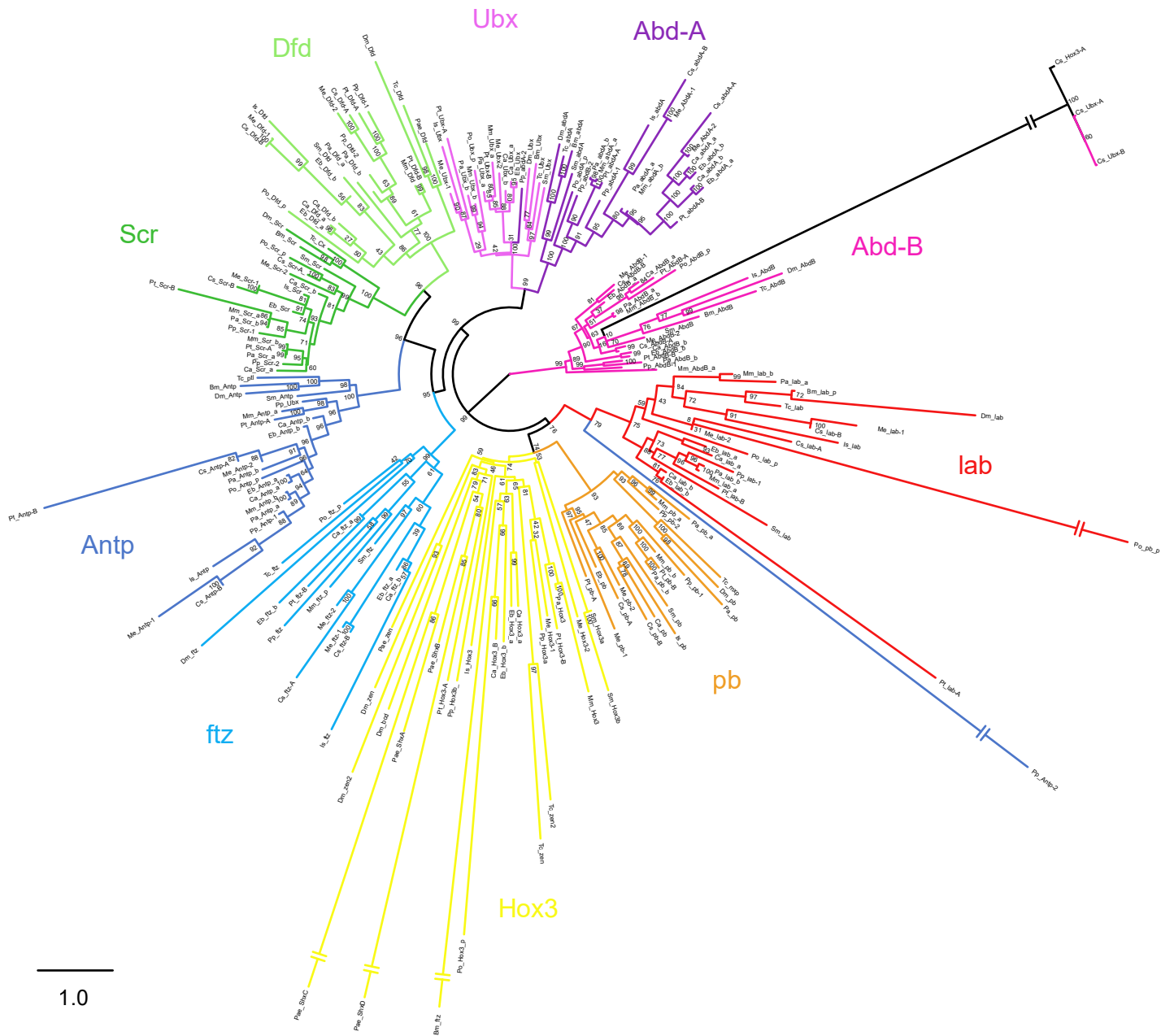
Supplementary Table 2. Protein accession numbers for all Wnt sequences used in this study. Note that the protein accession number could not be found for *Ixodes scapularis* Wnt8, the partial sequence from Janssen et al. (2010) was used in the Maximum likelihood tree of Wnt amino acid sequences (*).

Species	Wnt subfamily	Protein accession number
<i>Centruroides sculpturatus</i> (Cs)	Wnt1-1	XP_023219341.1
	Wnt1-2	XP_023228816.1
	Wnt2	XP_023224164.1
	Wnt4	XP_023222233.1
	Wnt5	XP_023215183.1
	Wnt6-1	XP_023219342.1
	Wnt6-2	XP_023228802.1
	Wnt7-1	XP_023215187.1
	Wnt7-2	XP_023220709.1
	Wnt9	XP_023228817.1
	Wnt11-1	XP_023240364.1
	Wnt11-2	XP_023228131.1
	Wnt16	XP_023224171.1
	WntA	XP_023218523.1
<i>Cupiennius salei</i> (Cu)	WntA (partial)	ADR79167.1
<i>Parasteatoda tepidariorum</i> (Pt)	Wnt1	XP_015906154.1
	Wnt2	NP_001310740.1
	Wnt4 (partial)	ADR79163.1
	Wnt5	NP_001310745.1
	Wnt6 (partial)	ADR79164.1
	Wnt7_1	NP_001310739.1
	Wnt7_2	NP_001310746.1
	Wnt8	ACH88002.1
	Wnt11_1	XP_015920223.1
	Wnt11_2	XP_015916686.1
	Wnt16	NP_001310769.1
<i>Ixodes scapularis</i> (Is)	Wnt1	XP_002407192.2
	Wnt4	XP_002436043.2
	Wnt5 (partial)	EEC11679.1
	Wnt6	EEC06108.1
	Wnt7	EEC17948.1
	Wnt8 (partial)	*
	Wnt9	EEC10449.1
	Wnt11	XP_002434188.1
	Wnt16 (partial)	EEC05882.1
	WntA	EEC02958.1
<i>Bicyclus anynana</i> (Ba)	Wnt1	XP_023955185.1
	Wnt5	XP_023937318.1
	Wnt6	XP_023955186.1

	Wnt7	XP_023935071.1
	Wnt9	XP_023953537.1
	Wnt10	XP_023955187.1
	Wnt11	XP_023934504.1
	WntA	XP_023937297.1
<i>Daphnia pulex</i> (Dp)	Wnt1	EFX86386.1
	Wnt2 (partial)	EFX87200.1
	Wnt4 (partial)	EFX72339.1
	Wnt5 (partial)	EFX66479.1
	Wnt6	EFX86167.1
	Wnt7	EFX66449.1
	Wnt8	EFX83364.1
	Wnt9	EFX86385.1
	Wnt10	EFX86388.1
	Wnt11	EFX77586.1
	Wnt16	EFX82994.1
	WntA (partial)	EFX69968.1
	<i>Drosophila melanogaster</i> (Dm)	Wnt1
Wnt5		NP_476924.1
Wnt6		NP_609108.3
Wnt7		NP_476810.1
Wnt8/D		NP_650272.1
Wnt9		NP_476972.2
Wnt10		NP_609109.3
<i>Euperipatoides kanangrensis</i> (Ek)	Wnt1 (partial)	ABY60732.1
	Wnt2	CDI40099.1
	Wnt4	CDI40100.1
	Wnt5	CDI40101.1
	Wnt6	CDI40102.1
	Wnt7	CDI40103.1
	Wnt9	CDI40104.1
	Wnt10	CDI40105.1
	Wnt11	CDI40106.1
	Wnt16	CDI40107.1
	WntA	CDI40108.1
<i>Tribolium castaneum</i> (Tc)	Wnt1	NP_001107822.1
	Wnt5	XP_974684.1
	Wnt6	NP_001164137.1
	Wnt7	XP_008196351.1
	Wnt8/D	XP_971439.1
	Wnt9	XP_015835609.1
	Wnt10	XP_015835532.1
	Wnt11	XP_015835988.1
	WntA	KYB26594.1

Supplementary Table 3. Protein accession numbers for Frizzled sequences used in this study.

Species	Frizzled genes	Protein accession number
<i>Centruroides sculpturatus</i> (Cs)	fz1	XP_023229313.1
	fz2	XP_023229226.1
	fz3	XP_023221477.1
	fz4-1	XP_023220057.1
	fz4-2	XP_023233147.1
<i>Parasteatoda tepidariorum</i> (Pt)	fz1	XP_015922960.1
	fz2	XP_015922948.1
	fz4-1	XP_015911110.1
	fz4-2	XP_015910102.1
<i>Ixodes scapularis</i> (Is)	fz1	EEC05379.1
	fz2	EEC03613.1
	fz4	XP_002402968.1
<i>Strigamia maritima</i> (Sm)	fz1 (SMAR014833)	Table S30. Details of the manually annotated genes of <i>S. maritima</i>. (Chipman et al. 2014)
	fz2 (SMAR012389)	
	fz3 (SMAR007293)	
	fz4 (SMAR009650)	
<i>Drosophila melanogaster</i> (Dm)	fz1	CAA38458.1
	fz2	AAC47273.1
	fz3	ABW09320.1
	fz4	NP_511068.2
<i>Tribolium castaneum</i> (Tc)	fz1	EFA04653.1
	fz2	EFA01325.1
	fz4	EFA09255.1



1.0