A chelicerate Wnt gene expression atlas: novel insights into the complexity of arthropod Wnt-patterning

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Abstract
The Wnt genes represent a large family of secreted glycoprotein ligands that date back to early animal evolution. Multiple duplication events generated a set of 13 Wnt families of which 12 are preserved in protostomes. Embryonic Wnt expression patterns (Wnt-patterning) are complex, representing the plentitude of functions these genes play during development. Here, we comprehensively investigated the embryonic expression patterns of Wnt genes from three species of spiders covering both main groups of true spiders, Haplogynae and Entelegynae, a mygalomorph species (tarantula), as well as a distantly related chelicerate outgroup species, the harvestman *Phalangium opilio*. All spiders possess the same ten classes of Wnt genes, but retained partially different sets of duplicated Wnt genes after whole genome duplication, some of which representing impressive examples of sub- and neo-functionalization. The harvestman, however, possesses a more complete set of 11 Wnt genes but with no duplicates. Our comprehensive data-analysis suggests a high degree of complexity and evolutionary flexibility of Wnt-patterning likely providing a firm network of mutational protection. We discuss the new data on Wnt gene expression in terms of their potential function in segmentation, posterior elongation, and appendage development and critically review previous research on these topics. We conclude that earlier research may have suffered from the absence of comprehensive gene expression data leading to partial misconceptions about the roles of Wnt genes in development and evolution.

Keywords: Wnt, Mygalomorpha, Opiliones, Spiders, Appendage development, Arthropod evolution, Gene duplication

Introduction
Wnt genes are important for the regulation of many aspects of animal development (reviewed in [92]). They encode secreted glycoprotein ligands that bind to different families of transmembrane receptors such as Frizzled and LRP5/6 (reviewed in e.g., [51]). Binding of Wnt molecules to their dedicated receptors activates intracellular signaling cascades that regulate target gene transcription (reviewed in e.g., [69, 88, 78]).

The last common ancestor of arthropods possessed 12 Wnt genes. However, loss of Wnt genes is common among arthropods [21, 30, 39], which is most obvious in model insects like *Drosophila melanogaster* and *Triobium castaneum* that have only retained seven and nine Wnt genes, respectively (e.g., [39]). Other arthropods have retained representatives of most (e.g., the myriapods *Glomeris marginata* and *Strigamia maritima*, and the spider *Parasteatoda tepidariorum*) or all (the crustacean *Daphnia pulex*) of the 12 Wnt families found in arthropods [22, 39]. In spiders, however, some Wnt genes are represented by two paralogs, the result of a whole genome duplication (WGD) that took place in the lineage
leading to Arachnopulmonata (e.g., spiders, whip spiders, scorpions) [46, 81].

Research on chelicerates in general and spiders in particular has greatly expanded in the last two decades providing key insights into the genomics, development, evolution, and ecology of arthropods more broadly (e.g., [11, 15, 20, 27, 57, 64, 76, 85]. However, despite the increasing interest in both Wnt-signaling and chelicerate research, we still lack truly comprehensive data about the expression profiles of Wnt genes in any chelicerate species. This includes the current main model species Parasteatoda in which Wnt genes have been studied rather intensively. However, also these studies do neither cover all Wnt genes nor all aspects of embryonic expression [39, 58]. In general, data on Wnt gene expression from other spider and chelicerate species are scarce. Therefore, we further explored the expression of all Parasteatoda Wnt genes, including those that were not investigated in previous studies. In order to establish a basis for comparative studies, we also characterized the embryonic expression profiles of all known Wnt genes in two other spiders, the cellar spider Pholcus phalangioides and the tarantula Acanthoscurria geniculata representing the haplogyne clade of araneomorphs and the mygalomorph infraorder, respectively (Fig. 1). With respect to gene duplication, the analysis revealed partially different complements of Wnt genes in these different spider lineages. Furthermore, we discovered conserved as well as divergent expression patterns of spider Wnt genes with respect to those of the harvestman Phalangium opilio, which did not have an ancestral WGD (Fig. 1). Our data reveal some patterns of sub- and neo-functionalization of Wnt genes after duplication and retention in spiders. More importantly, however, our data strongly suggest that Wnt gene patterning is subject to a high degree of redundancy, combinatorial function and function-shuffling (i.e., the adoption of a function of a given Wnt gene by another Wnt gene, e.g., [56, 84]. In summary, this chelicerate Wnt gene atlas highlights the complexity and evolutionary flexibility of Wnt gene expression and function. This in mind, we suggest that gene expression analyses and functional studies targeting a single (or more) Wnt gene(s) have to be interpreted with care, especially with

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**Fig. 1** Research organisms and their embryos. **A** Chelicerate phylogeny. ‘True spiders’ (Haplogyne and Entelegyne) — separated by the morphology of their female mating apparatus, represented by Pholcus and Parasteatoda, respectively. Mygalomorpha is represented by Acanthoscurria expanding the study towards spiders sensu lato. True spiders possess a pair of book lungs on the second opisthosomal segment (O2), a pair of tracheal tubes on O3, and spinnerets on O4 and O5. In tarantulas, book lungs develop on both, O2 and O3, and the spinnerets on O4 are rudimentary. A whole genome duplication (WGD) in the lineage leading to Arachnopulmonata is indicated. The harvestman Phalangium represents a chelicerate outside Arachnopulmonata, and thus a species that has not undergone a WGD. In comparison to spiders, harvestmen only have one pair of tracheal tubules on their opisthosoma (O2), and do not possess book lungs and spinnerets. **B** Adult female of the common house spider Parasteatoda tepidariorum. **C** Adult female of the cellar spider Pholcus phalangioides holding a cocoon with her chelicerae. **D** Adult female of the tarantula Acanthoscurria geniculata. **E** Adult female of the harvestman Phalangium opilio on a house wall. **F** Size comparison of the embryos of the investigated chelicerate species. The embryos are stained with the nuclear dye SYBR-green.
respect to questions concerning the evolution of animals and their development.

Methods
Animal husbandry, embryo collection and in situ hybridization
Parasteatoda embryos were collected from the colony established in Uppsala, Sweden, and were treated as described in Prpic et al. [73] (Fig. 1A, B, F). Embryos of Pholcus were collected from wild-caught specimens in Munich and Lower Saxony, Germany, and were treated as described in Turetzek and Prpic [89] (Fig. 1A, C, F). Acanthoscurria embryos were collected from the established colony in Cologne, Germany, and were treated as described in Pechmann and Prpic [67] (Fig. 1A, D, F). Embryos of Phalangium were collected from wild-caught specimens in Uppsala, Sweden (Fig. 1A, E, F). Several males and females were kept together in large (40 L) plastic boxes. Clutches of eggs were deposited by the females into petri dishes with moistened peat moss. The embryonic chorion was dissolved in commercial bleach (Klorix) for 3–5 min followed by rinsing of the embryos in tap water. Embryos were then fixed in a 50% volume of 4% formaldehyde in phosphate buffered saline (PBS) and 50% volume heptane for 12–16 h at room temperature on a gently rocking platform. After fixation, embryos were transferred to 100% methanol and stored at −20 °C. Prior to in situ hybridization experiments, the vitelline membrane was removed with fine forceps. All in situ hybridizations were performed using a standardized protocol published in Janssen et al. [42]. We apply the staging system of Parasteatoda [60], as accurately as possible, to all here investigated species to simplify comparison of gene expression data. For further information on the different developmental stages, we refer to the original descriptions by Turetzek and Prpic [89] (Pholcus), Pechmann [68] (Acanthoscurria) and Juberthie [44] (Phalangium). In this study, we investigated all stages from the formation of the early germ band to dorsal closure, for Parasteatoda and Pholcus, we also investigated the earlier germ disc stage (stages 4 and 5). In the other species, this disc is unfortunately too fragile to survive the fixation and in situ hybridization procedures.

Identification of Wnt genes
Reciprocal BLAST searches (tBLASTn) were performed against the embryonic transcriptomes of Pholcus [41], Phalangium [83] and Acanthoscurria [68], as well as the genome of Parasteatoda [81], using published arthropod and onychophoran Wnt protein sequences as baits. RNA isolation, library preparation and sequencing with Illumina HiSeq2000 for Pholcus was previously described [41]. The reads of the Pholcus transcriptome were de novo assembled after quality trimming and filtering with Trimmomatic [3] using Trinity (version r20140717, –seq-type fq –JM 240 G – run_as_paired –CPU 6 [19]. Retrieved protein sequences were aligned by applying T-Coffee with default parameters in MacVector v12.6.0 (Additional file 10). Phylogenetic analysis was performed as described in Panara et al. [66], using MrBayes [31]. Sequence identifiers of all identified sequences are listed in Additional file 11.

Gene cloning
Total RNA from Parasteatoda and Phalangium was isolated from a mix of embryonic stages using TRIzol (Invitrogen). For Phalangium, we isolated mRNA from total RNA using the Dynabeads mRNA Purification Kit (Invitrogen) followed by reverse transcription into cDNA (SuperScriptII first-strand synthesis system for RT-PCR, Invitrogen). For Pholcus and Acanthoscurria, RNA isolation and cDNA synthesis were carried out as previously described [90] (Pholcus), [68] (Acanthoscurria). Genes were amplified using RT-PCR with gene-specific primers (in most cases a second/nested PCR was performed using a second set of primers and the first PCR as template). For Pholcus, some Wnt genes were isolated using gene-specific primers in combination with degenerate primers, (Additional file 12). Gene fragments obtained were cloned into pCR-II or pCR2.1 (TA Cloning Kit Dual Promoter, Invitrogen) or Pjet1.2 (CloneJET PCR Cloning Kit), and sequenced using the commercial sequencing services offered by Macrogen or Eurofins Genomics.

Data documentation
Staining of embryos was either documented from whole mounts, in the form of flat-mounted parts of the embryos, or in the form of dissected appendages. For the dissection of appendages, we used fine tungsten needles recycled from burned-out old-fashioned light bulbs that were sharpened in the flame of a Bunsen burner.

Bright field microscopy and visualization of the nuclear dye SYBR-green were performed under a MZ-FLIII Leica dissection microscope using a Leica DC490 digital camera equipped with an external UV-light source. Whenever necessary and appropriate, linear adjustments were performed on color, contrast and brightness with the image-processing software Adobe Photoshop CC 2018.

Results
Wnt genes in spiders and a harvestman
We reanalyzed the Wnt gene repertoire of Parasteatoda and surveyed the repertoires of these genes in additional spiders, Pholcus and Acanthoscurria, as well as the harvestman Phalangium screening embryonic transcriptomes of all species and the genome of Parasteatoda.
Our phylogenetic analysis was similar to those by Harper et al. [21].

The common ancestor of chelicerates likely possessed a “complete” set of the 12 Wnt genes typical for protostomes, despite common lineage-specific losses within this subphylum (Figs. 2, 3; Additional file 1: Fig. S1) [21, 39]. Spiders appear to have lost their Wnt9 and Wnt10 orthologs, while these genes are retained in other chelicerates such as the harvestman Phalangium [21]. Two paralogs of Wnt1, Wnt7, and Wnt11 have been retained (after the WGD in Arachnopulmonata) but the second paralog of Wnt1 has been lost in most true spiders [21]. The lack of a second Wnt4 paralog in Pholcus and the presence of two paralogs of Wnt4 in Acanthoscurria, as well as some lineages of entelegyne spiders [21] suggest that loss of a second Wnt4 gene occurred independently in at least two lineages of spiders (towards Parasteatoda, and towards Pholcus) (Figs. 2, 3). The apparent loss of a second Wnt4 gene in Pholcus may be representative for Haplogynae as a whole as we could not identify a second copy in the published genome of another basally branching haplogyne spider, the recluse Loxosceles reclusa (data not shown). Please note that the lack/loss of a gene is difficult to prove, even in the era of full genome sequencing. Most genomes, although “sequenced” are not complete, or have not been assembled completely. The situation in spiders is even more complicated because of the many duplicated genes and often enlarged intronic regions. Many of the published spider genomes are thus far from having the complete set of genes. The usage of transcriptomic data (as used in our study), using a combination of sequencing methods as well as several rounds of
reannotations helps to improve these issues. That is why the *Parasteatoda* genome is still one of the best annotated genomes present.

**Wnt1**

In all investigated species, at least one paralog of *Wnt1* is expressed in a subset of cells in the pre-cheliceral region, along the ventral side of the appendages (including the opisthosomal limb buds that correspond to the breathing organs and the spinnerets), dorsally in the labrum (except for the harvestman), and in the posterior of the developing embryo (Figs. 4, 5; Additional files 2, 3, 5, 6, 7: Figures S2, S3, S5–7). The posterior expression is either corresponding to the hindgut primordium that is located posterior to the segment-addition zone (marked with SAZ), or the posterior part of the SAZ. While this expression appears early during development in other spiders suggesting a role as posterior patterning gene, in *Parasteatoda* this expression is restricted to later developmental stages indicating that it may indeed correspond to the hindgut rather than be involved in segment addition (Fig. 4B, C). Interestingly, there are two paralogs of *Wnt1* in *Acanthoscurria*. The second paralog, *Wnt1.2*, is exclusively expressed in the SAZ (Fig. 4M, N), while the other paralog, *Wnt1*, is expressed similar to the single *Wnt1* gene in the other species, but is lacking expression in the SAZ (Fig. 4I–L). This represents an impressive example of sub-functionalization after WGD. With the exception of *Acanthoscurria*, for all species studied dorsal stripes of expression appear in the opisthosoma late during embryogenesis (Figs. 4, 5). Only in *Parasteatoda*, there is a line of expression dorsal in the head and the limb-bearing segments (Fig. 4C, D).

In true spiders, *Wnt1* is expressed in the form of segment polarity gene (SPG)-like transverse stripes, but such stripes are restricted to some of the head segments [39] (Fig. 4F). In *Acanthoscurria*, there are no SPG-like stripes of expression (Fig. 4I–N). In *Phalangium*, however, SPG-like stripes are present early during development, and in all developing segments (including posteriorly added segments) (Fig. 5A–F). Note that expression of *Wnt1* in the developing books lungs of *Parasteatoda* is in the form of three separate domains as previously described for another entelegyne spider, *Cupiennius salei* [9] (Fig. 4C(inlay)). Expression patterns of spider and harvestman *Wnt1* genes are summarized in schematic Figs. 4O and 5G, respectively.

**Wnt2**

We identified a single *Wnt2* ortholog in all spider species, but not in the harvestman. In all spiders, *Wnt2* is expressed in a subset of cells in the pre-cheliceral region (Fig. 6; Additional file 3, 6, 7: Fig. S3B, S6B, S7B). Notably, this domain appears already during early germ band stages in *Parasteatoda* and covers a larger area of the brain in later stages compared to *Pholcus* and *Acanthoscurria*, the latter displaying the smallest brain expression domain (Fig. 6). In *Pholcus* and *Acanthoscurria*, *Wnt2* is expressed in the SAZ throughout development, but in *Parasteatoda*, there is no such posterior expression (Fig. 6). Similarly, in *Pholcus* and *Acanthoscurria* *Wnt2* is expressed along the ventral side of the prosomal appendages (except for the labrum), but in *Parasteatoda* expression is restricted to some dot-like domains along the ventral side of the appendages (Fig. 6; Additional files 3, 6, 7: Figs. S3, S6, S7). Expression of spider *Wnt2* genes is summarized in the schematic Fig. 6K.

**Wnt4**

In most spiders, there are two paralogs of *Wnt4* [21], in *Pholcus* and *Parasteatoda*, however, only one *Wnt4* is present (*Parasteatoda*) or has been identified in an embryonic transcriptome (*Pholcus*) (Fig. 3). Only in *Acanthoscurria*, we were able to identify two paralogs of *Wnt4*.

*Wnt4* exhibits quite diverse expression among spiders and between these animals and the harvestman (Fig. 7; Additional files 3, 5, 6, 7: Fig. S3, S5–S7). The only common features are the dot-like domains in the distal ectoderm of the legs and pedipalps of spiders, and the expression in the labrum (except for *Acanthoscurria*). In the harvestman, however, expression in the pedipalps and legs is different to the spiders and restricted to a distal portion of the limb mesoderm (cf. Additional files 3, 5, 6, 7: Fig. S3, S5–S7). Although expression in the legs and pedipalps of spiders is mainly restricted to ventral tissue, one of the two tarantula *Wnt4* genes (*Wnt4.2*) is expressed in dorsal (and rather proximal) domains (cf. panels C and D of Additional file 3: Figure S3). Patterns of presence and absence in the prosomal appendages of spiders differs between the investigated species (Additional files 3, 5, 6, 7: Figs. S3, S5–S7). In all species (except *Acanthoscurria*), there is a complex pattern of expression in the pre-cheliceral region (Fig. 7). In all species, *Wnt4* is expressed at the posterior pole of the developing embryo, although the signal in *Acanthoscurria* is very weak and thus may represent background (Fig. 7). In true spiders, expression in the posterior is clear, but only appears at relatively late developmental stages, while comparative expression appears very early during germ band formation in the harvestman (Fig. 7O). Only in the tarantula, one of the two *Wnt4* paralogs (*Wnt4.1*) is expressed in SPG-like stripes early during development (Fig. 7H), and in the harvestman a unique ventral expression appears during later stages in the opisthosoma (Fig. 7Q). Another unique expression is present for *Parasteatoda* *Wnt4*
Expression of Wnt1 genes in spiders. Expression of Wnt1 in Parasteatoda (A–D), Pholcus (E–H), and Acanthoscurria (I–L (Wnt1.1), M, N (Wnt1.2)). In all panels, except panel O, anterior is to the left. Ventral views, except panels C, D, F, and H (lateral views). Developmental stages are indicated. Filled circles (•) in panels C and D mark expression along the dorsal rim of the prosoma. Asterisks in panel E mark the center of the germ disc (the later posterior region of the germ band). Asterisks in panels D and H mark dorsal stripes of expression. The arrow in panel C points to the book lung that expresses Wnt1 in the form of three separate domains (cf. inlay in panel C). Panels indicated with an apostrophe (’’) represent SYBR-green stained embryos corresponding to the embryo shown in the panel without apostrophe. Expression patterns are summarized in panel O, anterior is up. Abbreviations: ch, chelicera; L, leg; lr, labrum; m, mouth; O, opisthosomal segment; pc, pre-cheliceral region; pp, pedipalp; saz, segment-addition zone; sp, spinneret.
forming a dorsal stripe separating the prosoma from the opisthosoma (Fig. 7C). The expression patterns of Wnt4 genes are too diverse to identify possible patterns of sub- or neo-functionalization in Acanthoscurria. Here, expression patterns of other chelicerate species that retained two paralogs could help to clarify an ancestral feature of Wnt4. Expression patterns of Wnt4 genes are summarized in the schematic Fig. 7S.

**Wnt5**

In Parasteatoda and Pholcus, expression of Wnt5 starts after germ band formation and shortly before the limb buds begin to grow out (Fig. 8A, E). The same pattern is seen in the early germ bands of Acanthoscurria and Phalangium, but we do not know if expression starts already earlier in these species (Fig. 8I, N). This expression most likely correlates with the limb primordia. Furthermore, in all species, Wnt5 is expressed in a large domain of the pre-cheliceral region and the ventral nervous system (Fig. 8; Additional files 3, 5, 7: Figs. S3, S5–S7). Wnt5 is also expressed in all appendages, including the opisthosomal limb buds, but not in the labrum (with the exception of dot-like domains late in Acanthoscurria and Pholcus) (Fig. 8; Additional files 3, 5, 7: Figs. S3, S5–S7). Interestingly, in all species, the limb expression resembles leg-gap gene like domains. In all species, Wnt5 is also expressed in the dorsum of the opisthosomal segments; likely, this expression is correlated with the development of the heart (arrowhead in Figs. 8D, G, H, M, O–Q, S) (cf. [37]). In the three spiders, but not in the harvestman, Wnt5 is also expressed in the stomodeum (Fig. 8C, F, J; Additional file 7: Figure S7D). Wnt5 expression is summarized in the schematic Fig. 8T.

**Wnt6**

In all species, Wnt6 is expressed along the ventral side of all appendages, including the opisthosomal limb buds (Fig. 9; Additional files 3, 5, 6, 7: Figs. S3, S5–7). In the labrum, Wnt6 is expressed dorsally but note that Phalangium Wnt6 is not expressed in the labrum at all (Fig. 9H, L; Additional files 5, 7: Figs. S5D, S7E). In Parasteatoda, expression starts when the limb buds begin to grow out (Fig. 9A). In Acanthoscurria, the earliest Wnt6 expression commences just before the formation of the limb buds.
in a SPG-like fashion (Fig. 9). In *Pholcus* and *Phalangium*, expression starts earlier and in SPG-like transverse stripes before the onset of limb bud development (Fig. 9E, N). The anterior-most stripe is correlated with later expression in the pre-cheliceral region. This expression was not observed in *Parasteatoda* or *Acanthoscurria* (Fig. 9). The early stripes later become restricted to expression in the developing appendages and thin...
Fig. 7  (See legend on previous page.)
stripes of expression ventral to the base of the appendages, most prominently seen in *Phalangium* where the germ band halves do not split unlike in spiders (Fig. 9R). In *Phalangium* and *Parasteatoda*, *Wnt6* is expressed in the SAZ, but while this expression is already present in early stages of the harvestman, expression in this spider appears later during germ band extension (Figs. 9A, N). The other two spiders do not express *Wnt6* posteriorly (Fig. 9), except for an early transient posterior domain in *Acanthoscurria* (Fig. 9E). In all spiders, *Wnt6* is also expressed dorsal to the base of the appendages, which is especially prominent in the opisthosoma (Fig. 9). This expression is likely correlated with the development of the heart and in *Acanthoscurria*, the developing heart tube itself expresses *Wnt6* (Fig. 9K, M). Additional expression of *Wnt6* was observed in the stomodaenum of the harvestman (Fig. 9S), and in the form of transverse segmental stripes in the ventral sulcus (the region between the split germ band halves) of the tarantula (Fig. 9L). Similar stripes of expression in the ventral sulcus have been reported for *nebrin* expression in spiders including *Parasteatoda*, suggesting that *Wnt6* may be involved in axonal guidance [48]. Expression of *Wnt6* is summarized in the schematic Fig. 9T.

**Wnt7**

All spiders investigated here possess two *Wnt7* paralogs (Fig. 3). In true spiders, one *Wnt7* gene (*Wnt7.1*) is expressed in the posterior SAZ region (Fig. 10A, B, D, H, I). While this is the only expression of *Wnt7.1* observed in *Pholcus*, *Parasteatoda* *Wnt7.1* is also expressed in the developing limb buds including the opisthosomal buds, and in part of the brain and the ventral nervous system (Fig. 10B–D). In the limbs, this expression is predominantly present along the ventral side, but a dot of expression is also visible proximally and dorsal (Additional file 7: Fig. S7F). In the tarantula, *Wnt7.1* expression is restricted to late embryonic stages and mainly in the ventral ectoderm of the appendages, except for the labrum that does not express *Wnt7.1* (Fig. 11A, B; Additional file 4: Figure S4A).

In all spiders, *Wnt7.2* is expressed in the appendages (Figs. 10, 11; Additional files 4, 6, 7: Figs. S4B, S6F, S7G). In *Parasteatoda*, *Wnt7.2* is expressed in the form of several dot-like domains along the dorsum of the labrum, the pedipalps, the legs and the opisthosomal limb buds, but ventral in the chelicerae (Additional file 7: Figure S7G). In addition, there is a dot-like expression ventrally and close to the tip of the legs. In *Pholcus*, however, expression in chelicerae, pedipalps, legs, and opisthosomal appendages is restricted to the dorsal-proximal region (Additional file 6: Figure S6F). In *Acanthoscurria*, expression in the chelicerae is ventral, as described for *Parasteatoda*, and expression in the pedipalps and legs is restricted to a dorsal-proximal patch as described for *Pholcus* (Additional file 4: Figure S4B). Additionally, *Wnt7.2* is expressed in four dominant large domains in the pre-cheliceral region of *Parasteatoda* (Fig. 10E; Additional file 7: Figure S7G). Similar expression is present in *Pholcus* and *Acanthoscurria* albeit in smaller domains (Figs. 10N, 11C). In the spiders *Parasteatoda* and *Pholcus*, *Wnt7.1* and *Wnt7.2*, respectively, are also expressed in the developing ventral nervous system (Fig. 10D, M). In the harvestman *Phalangium*, the single copy of *Wnt7* is only expressed in the dorsal-proximal region of the pedipalps and the legs (but not the labrum or the chelicerae) (Fig. 11F–H; Additional file 5: Figure S5E). Expression of *Wnt7* genes is summarized in schematic Figs. 10O, 11J.

**Wnt8**

In all investigated spiders, *Wnt8* is expressed in the ventral ectoderm of the chelicerae, the pedipalps, the legs and the opisthosomal limb buds (Additional files 4, 6, 7: Figs. S4C, S6G, S7H) but only in *Parasteatoda* expression is also present dorsally in the labrum (Additional file 7: Fig. S7H). In *Pholcus* and *Acanthoscurria* (but not *Parasteatoda*), *Wnt8* is expressed in the stomodaemum (Fig. 12F(inlay), J). In all spiders, expression starts early during embryogenesis in the form of transverse segmental stripes that are reminiscent of SPG expression (Fig. 12; Additional file 8: Figure S8). In *Parasteatoda*, expression starts already during the germ disc stage as a central patch and a ring close to the rim of the disc (Fig. 12A). The latter transforms into expression in the pre-cheliceral region, which is also present in the other spiders. The central patch of expression in *Parasteatoda*, however, that later represents expression in the SAZ, is not present in *Pholcus*. Indeed, the earlier reported strong expression of *Wnt8* in the SAZ of *Parasteatoda* [58] is neither present in the entelegyne spider *Pholcus* nor the tarantula *Acanthoscurria*. Like a typical SPG, in all spiders *Wnt8* is expressed in the form of transverse stripes in all newly forming posterior segments (Fig. 12C–F, K; Additional file 8: Fig. 8D). In *Phalangium*, *Wnt8* expression

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**Fig. 8** Expression of Wnt5 genes. Expression of Wnt5 in *Parasteatoda* (A–D), *Pholcus* (E–H), *Acanthoscurria* (I–M) and *Phalangium* (N–S). In all panels, anterior is to the left. Panels A, B, E, G, N, O, and Q show lateral views. Panels C, D, H, I, L, P, R, and S show ventral views. Panel M represents a dorsal view. Panel A represents SYBR-green staining of the embryo shown in panels A. Developmental stages are indicated. Asterisks in panel A mark faint stripes of expression. In all panels, arrows and arrowheads point to expression in the ventral nervous system and the heart, respectively. Expression patterns are summarized in panel T, anterior is up. Abbreviations as in Fig. 4.
Fig. 8 (See legend on previous page.)
As the appendages develop, expression is restricted to Wnt9 and Wnt10. We did not identify any orthologs of Wnt9 and Wnt10 in the spider species studied here. In Phalangium, however, we found representatives of both subfamilies (Fig. 3). Wnt9 is first expressed in a SPG-like pattern as transverse segmental stripes covering the region where the limbs will form and the most ventral tissue of the embryo (Fig. 13A). These early stripes correspond to a domain in the anterior head, the chelicerae-bearing segment, the pedipalpal segment and the first leg-bearing segments. As the appendages develop, expression is restricted to a central segment along the ventral side of the chelicerae, the pedipalps and the legs (and their endites), but in the labrum Wnt9 is dorsally expressed (Fig. 13B–D; Additional file 5: Fig. S5F). Later during development, expression appears in the stomodaemum (Fig. 13C).

Expression of Wnt10 also starts early during development and in the form of transverse stripes; note however, that these stripes are not continuous (cf. expression of Wnt9). Instead, expression in the ventral region of the embryo is missing (Fig. 13F–I). We assume that these stripes are correlated with the primordia of the appendages. The most anterior expression domains are located in the pre-cheliceral region. Later during development, expression is observed centrally along the ventral side of the appendages (including the endites) (Fig. 13G–I; Additional file 5: Figure S5G). Unlike Wnt9, Wnt10 is not expressed in the labrum. Expression in the posterior pole of the embryo is comparable to that of Wnt9, but no stripes were observed in the opisthosomal segments (Fig. 13H, I). Late during embryogenesis, expression of Wnt10 appears in the stomodaemum (Additional file 5: Figure S5G). Expression of Wnt9 and Wnt10 is summarized in Fig. 13E, J, respectively.

Wnt11

In Parasteatoda and Pholcus, Wnt11 is represented by two paralogs (Fig. 3). However, in both species, expression of Wnt11.1 was not detected in any of the investigated embryonic stages (cf. [39]). In Acanthoscurria and Phalangium only one copy of Wnt11 was found. In Parasteatoda and Phalangium, expression of Wnt11.2 and Wnt11, respectively, appears early during embryogenesis in the SAZ (Fig. 14A, B, I), but in Pholcus and Acanthoscurria, there is no such posterior expression (Fig. 14F, G, I). In the appendages of all investigated animals (including the opisthosomal buds), expression was observed in the ventral ectoderm, except for the labrum where expression is dorsal (the labrum of the tarantula and the harvestman do not express Wnt11) (Fig. 14; Additional files 4, 5, 6, 7: Figs. S4D, S5H, S6H, S7I). Expression in the chelicerae of the harvestman is internal, likely mesodermal (Additional file 5: Fig. S5H). Expression of Wnt11 is summarized in Fig. 14M.

Wnt16

In all investigated species, Wnt16 is expressed in a SPG-like pattern in the form of transverse segmental stripes (Fig. 15; Additional file 9: Fig. S9). In Pholcus, Acanthoscurria and Phalangium, these stripes appear early during development (cf. Fig. 15E, I, N, O with Additional file 9: Figure S9B), while in Parasteatoda the expression starts later coinciding with limb bud formation (Fig. 15A). In spiders, there is no (or only weak) expression in the posterior SAZ, but in the harvestman, Wnt16 is dominantly expressed in the SAZ (Fig. 15O, Q). In all species, Wnt16 is also expressed in the pre-cheliceral region and the stomodaemum (Fig. 15A, B, E–G, J, L, P, R, S; Additional files 5, 7: Figs. S5I, S7J). In Acanthoscurria, Wnt16 is expressed on the dorsal side of the labrum and two thin longitudinal stripes of expression run on either side of the stomodaemum (Fig. 15L). Common to all analyzed species, expression in the appendages is restricted to the ventral side including the ventral sector of the endites (if present); in the labrum, expression is always dorsal (Fig. 15; Additional files 4, 5, 6, 7: Figs. S4E, S5I, S6I, S7J). In all spiders, Wnt16 is expressed in the form of short stripes (or patches) dorsal to the opisthosomal limb buds (Fig. 15D, H, M). Comparable expression is also present in the opisthosomal and the leg-bearing segments in Phalangium (Fig. 15S). Expression of Wnt16 is summarized in Fig. 15T.

WntA

In all species, WntA is expressed in the SAZ (Fig. 16). In all species, except Parasteatoda, expression is present...
Fig. 9 (See legend on previous page.)
in the pre-cheliceral region (Fig. 16E, F, K, O, S), and the ventral nervous system along either side of the midline (Fig. 16E–H, L, M, R). Expression in the developing appendages is diverse. In Phalangium, expression of WntA in chelicerae, pedipalps and legs is exclusively mesodermal.

| Pt-Wnt7.1 | E st10.2 | Pt-Wnt7.2 | F st10.2 | L4 |
|-----------|----------|-----------|----------|----|
| A st8.1  | B st11   | C st12    | D st12   |    |
|           |          | pc        |          |    |
|           |          |           | L1       |    |

### Fig. 10
Expression of Wnt7 genes in true spiders. Expression of Wnt7 in Parasteatoda (A–D (Wnt7.1), E–G (Wnt7.2)), and Pholcus (H, I (Wnt7.1), J–N (Wnt7.2)). In all panels (except panel N), anterior is to the left. Panels A, B, H, I, K and M show lateral views. Panels C, D, F, G, J and L ventral views. Panels E and N show anterior views; in panel N anterior is up. The inlay in panel B shows the SAZ of a slightly older embryo. Panels indicated with an apostrophe represent SYBR-green staining of the embryos in corresponding panels. Developmental stages are indicated. In all panels, arrows mark expression dorsal to the base of the limbs. The arrowhead in panel M points to expression in the ventral nervous system. Expression patterns are summarized in panel O, anterior is up. Abbreviations as in Fig. 4.
In Parasteatoda and Pholcus WntA is expressed in one or several patches in the dorsal ectoderm of the legs, pedipalps and the chelicerae (Additional files 6, 7: Figs. S6J, S7K). Additionally, WntA is expressed in the mesoderm of these appendages in Pholcus (Additional file 6: Figure S6J). In Acanthoscurria expression in the limbs is weak, but still dorsal and distal ectodermal expression domains as well as expression in the mesoderm are present in at least the pedipalps and the legs (Additional file 4: Figure S4F). Only in Parasteatoda, WntA expression was also observed in the dorsal tissue of the labrum (Fig. 16C; Additional file 7: Figure S7K). Expression of WntA is summarized in Fig. 16T.

**Discussion**

**Is Wnt1 (wingless) a bona fide segment-polarity gene in spiders?**

In *Drosophila melanogaster*, the transcription factor encoding gene engrailed (*en*) and the signaling molecule encoding gene **Wnt1** (*wingless* (*wg*)) demarcate the parasegmental boundary with *wg* being expressed anterior to this boundary, and *en* being expressed posterior to this boundary (e.g., [17, 26, 63]). Subsequent research in other arthropods and closely related groups like tardi-grades and onychophorans revealed that the expression domains of these genes are highly conserved (e.g., [12, 13, 33, 65, 74]).

A deviation from this apparent conservation, however, has previously been suggested for the spider Parasteatoda where Wnt1 is not expressed in the form of a SPG-like pattern or the SAZ [39]. Indeed, already Damen [9] realized that expression of Wnt1 in the spider *Cupiennius salei* is dissimilar from its expression in other arthropods, and is indeed lacking in cells anterior to *en* in the ventral region of the developing embryo. He suggested that another Wnt gene, Wnt5, could perhaps partially substitute the function of Wnt1 in the ventral tissue, while Wnt1 would still play its “regular” role as SPG in dorsal tissue [9]. Although this appears to be an interesting idea, a closer look at the expression of Wnt5 in *Cupiennius* and other chelicerates reveals a likely role in the patterning of the ventral nervous system, rather than a role as a SPG (Fig. 8). Although Wnt5 is expressed relatively early during embryogenesis in
arthropods, and the initial expression in the early germ band is in the form of transverse stripes, these stripes soon transform into patch-like domains in the ventral nervous system, and the domains in posteriorly added segments never develop into SPG-like stripes [9, 39], this study). Consequently, Wnt5 likely does not act
in combination with Wnt1 during spider segmentation. Both papers, Damen [9] and Janssen et al. [39] also suggested that a second Wnt1 gene could exist in spiders that could pattern the ventral tissue. It was therefore exciting to discover two Wnt1 paralogs in the spider Acanthoscurria, but neither of the Wnt1 genes in this species is expressed like a SPG (Fig. 4). In Pholcus, Wnt1 is also not expressed like a typical SPG,
but instead (as with *Parasteatoda*) is only detected in the form of transverse stripes in a subset of the anterior segments, and no such stripes appear in the newly forming posterior segments (Fig. 4). It appears thus that at least in spiders, *Wnt1* does not function as a *bona fide* SPG. In the harvestman, however, *Wnt1* is expressed in the form of a typical SPG, and hence it is likely that in this group of arachnids, the ancestral function of *Wnt1* has been retained (Fig. 5). It would be interesting to analyze the expression of *Wnt1* genes...
in other Arachnopulmonata, especially whip spiders which also appear to have retained two copies of this gene after the ancestral WGD [21] to better understand the evolution of this gene in chelicerates.

Could another Wnt gene substitute for Wnt1-function during segmentation in spiders? Our analysis shows that several Wnt genes are indeed expressed in a pattern that is similar to the expression of Wnt1 in other arthropods (summarized in Fig. 17). Besides the expression in the form of transverse segmental stripes anterior to en (i.e., in about the middle of the segment), another important factor is the temporal appearance of expression: a substitute for Wnt1 should be expressed early during segment formation.

In Pholcus, Acanthoscurria and even in Phalangium (except for Wnt8), Wnt6, Wnt8, and Wnt16 are expressed like SPGs during segment formation in both the anterior segments that form from the early blastoderm and the germ disc, and the posterior segments that are added from the posterior SAZ (Figs 9, 12, 15, 17; Additional files 8, 9: Figs. 58, 59). In Parasteatoda, however, Wnt6 is not expressed in a SPG-like fashion (Fig. 9), and Wnt8 has been extensively studied, and it has been found that it is an important factor for the establishment of the SAZ and thus posterior elongation [58]. Although expressed in the germ disc (from which the anterior segments are formed) there are no obvious anterior SPG-like phenotypes in Wnt8 knock-down embryos [58]. However, a SPG-like function could be masked by the function of yet another Wnt gene such as Wnt16. If Wnt8 substitutes partially for Wnt1, then this function may have evolved in the lineage leading to spiders (or any lineage within Arachnopulmonata), because in the outgroup, the harvestman Phalangium, Wnt8 is not expressed in a SPG-like pattern. In arthropods outside Chelicerata, Wnt8 genes are either missing, or their expression (and function) is quite diverse [4, 5, 14, 16, 22, 30, 39]. This suggests that Wnt8 has flexibility to assume different functions during evolution, and this may speak for Wnt8 as a potential (at least partial) substitute for Wnt1 in spiders (Fig. 17).

The most likely candidate for substituting for Wnt1 function in spider segmentation, however, appears to be Wnt16. In all chelicerate species, Wnt16 is expressed in a typical SPG-like pattern both during anterior and posterior segment formation (Fig. 15; Additional file 9: Fig. S9). In other arthropods, and even in an onychophoran, Wnt16 is also expressed in a SPG-like pattern suggesting a conserved role in segmentation [8, 22, 28, 39]. Wnt16 has thus far not been in the focus of scientific studies, and this may be correlated to the fact that holometabolic insects, the most intensively studied arthropod species (cf. data on Drosophila (reviewed in Murat et al. [62], have lost Wnt16 (e.g., [39], [21]). In this context, it would be interesting to investigate the expression of Wnt16 in insects that have retained this gene, and to perform Wnt16 knock-down studies in spiders.

Wnt-signaling is likely involved in posterior elongation, but Wnt8 is not a conserved factor in this network

Wnt8 is one of the few arthropod Wnt genes for which functional data exist outside Drosophila. In the spider Parasteatoda and the beetle Tribolium castaneum, RNAi-mediated knockdown of Wnt8 results in truncated embryos. This has been interpreted as evidence that Wnt8 represents a conserved component of an ancestral posterior gene regulatory network in arthropods [5, 58, 79], or even in animals in general (e.g., [47, 49], reviewed in [59]). In many arthropods, however, Wnt8 has been lost (e.g., [21]). In such species, another Wnt gene must regulate posterior segment addition, as exemplified for the cockroach Periplaneta americana, where knockdown of Wnt1 causes posterior truncation [6]. This is not unexpected because Wnt-patterning likely includes a high degree of redundancy and combinatorial gene function as suggested by the similar expression patterns of multiple different Wnt genes in any given species (e.g., [4, 8, 22, 39]), and as shown for Wnt1 and Wnt8 in Tribolium [5].

In the spiders we studied here, Wnt8 is not expressed at the posterior pole of the embryo with the exception of Parasteatoda (Figs. 12, 17). The most parsimonious explanation is thus that the role of Wnt8 in Parasteatoda represents an apomorphy for this spider species, or possibly Entelegynae as a whole, but not for spiders or chelicerates in general; note that Wnt8 is not expressed in the SAZ in the harvestman Phalangium either. Similarly, the posterior expression of Wnt8 in Tribolium may represent a synapomorphy of Tribolium or beetles in general because Wnt8 is missing or not expressed in the SAZ of other arthropods such as myriapods and crustaceans and

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(See figure on next page.)

Fig. 15 Expression of Wnt16 genes. Expression of of Wnt16 in Parasteatoda (A-D), Pholcus (E-H), Acanthoscurria (I-M) and Phalangium (N-S). In all panels, anterior is to the left. Panels A, D, E, F, and S represent lateral views. Other panels represent ventral views, except panel M (dorsal view). The inlay in panel M shows the saz of the the same embryo (ventral view). The inlay in H shows a lateral view on the tail and the saz. Panel F represents SYBR-green staining of the embryo shown in L. Arrow and arrowheads in panel D point to expression along the dorsal rim of the prosoma and dorsally in the opisthosoma, respectively. Developmental stages are indicated. Arrowheads in panels H, M, and S point to dorsal expression in the opisthosoma. Asterisks in panels J and K mark expression ventral to the base of the limbs. Expression patterns are summarized in panel T, anterior is up. Abbreviations as in Fig. 4.
Fig. 15 (See legend on previous page.)
other insects (e.g., [10, 39]). This finding further strengthens the view that Wnt genes can be co-opted into existing gene regulatory networks to work in combination with or even replace the function of another Wnt gene.

**Wnt-signaling in anterior–posterior axis elongation**

In all previously investigated species that develop via posterior elongation, which is the vast majority of all arthropods, and also the vast majority of animals in general, at least one Wnt ligand is always expressed posteriorly in the developing embryo, and loss of one or more Wnt genes causes truncation of the main body axis (reviewed in [53, 93]). Equally, knocking-down the function of Wnt-signaling by targeting key-components of Wnt pathways, or inducing over-activity of Wnt-signaling, lead to posteriorly truncated embryos or disturbances in the posterior patterning network (e.g., [2, 23, 75, 82]).

Is there an “ancestral” posterior Wnt factor? In *Tribolium*, double knockdown of *Wnt1 + Wnt8* causes more severe effects than the mere knockdown of either of these two genes alone suggesting that they may work together [5]. In another species, the cockroach *Periplaneta*, knockdown of *Wnt1* also results in truncated embryos, further suggesting that *Wnt1* may be an ancestral factor of posterior elongation, at least in insects [6]. Data from the cricket *Gryllus bimaculatus* and the true bug *Oncopeltus fasciatus*, however, show that knockdown of *Wnt1* has no effect on posterior elongation, although disruption of the complete canonical Wnt pathway causes truncation suggesting that *Wnt1* may act in combination with other Wnt factors [1, 61], reviewed in [93]. Interestingly, however, *Wnt1* cannot be involved in posterior elongation in *Parasteatoda* because it is not expressed in the posterior of the embryo [39] (Fig. 4). However, *Wnt1* shows posterior embryonic expression in most arthropod species and in outgroups such as onychophorans and priapulids (e.g., [12, 54]) (Fig. 17). *Wnt1* is thus likely a conserved factor of posterior elongation, and the situation in the model spider *Parasteatoda* likely presents a derived feature.

To further investigate the possibility that other Wnt genes may be involved in posterior elongation we summarized the findings from arthropods, an onychophoran and a priapulid, all for which comprehensive expression data of the complete complement of Wnt genes are available [4, 8, 22, 28, 29, 36, 39, 40]. Several Wnt genes are typically expressed in the posterior embryo, but often their distribution is little conserved among different species including arthropods. These genes could, however, still contribute to posterior elongation and segment addition, either alone or in concert with other Wnt genes (Fig. 17). However, the summary of posteriorly expressed Wnt genes reveals two other Wnt genes beyond *Wnt1* that are expressed in the posterior of developing embryos of most species. *Wnt6* is expressed posteriorly in the priapulid and all arthropods except *Acanthoscurria* (Fig. 17) [29]. Like many Wnt genes, *Wnt6* is highly under-investigated and so expression data are relatively scarce and the function of this gene has not been studied in many species. Interestingly, however, *Wnt1* and *Wnt6* appear to be ancient paralogs as revealed by phylogenetic analyses (e.g., [7, 10, 21, 29, 39], this study) and their conserved synteny in at least insects and crustaceans (data on Wnt gene synteny in other arthropods are not available), a lophotrochozoan species, the owl limpet *Lottia* [10, 39], and some chordates [84]. In addition, *Wnt1* and *Wnt6* have overlapping expression patterns in many species (e.g., [4, 35, 39], this study). It is therefore possible that *Wnt6* may have had an ancestral role in posterior elongation like *Wnt1*. To test this further the function of *Wnt6* should be assessed via gene knockdown in species where this technique is established and where *Wnt6* is expressed in the posterior of the embryo, including the beetle *Tribolium* [4].

Another Wnt gene with posterior expression in all investigated arthropod species, and even the onychophoran (albeit weakly) and the priapulid, is *WntA* (Figs. 16, 17) [29]. In *Tribolium*, knockdown of *WntA* does not cause any phenotype, neither on its own nor in combination with *Wnt1* and/or *Wnt8* [5]. Although *WntA* is thus likely not involved in posterior segmentation in *Tribolium*, this does not exclude the possibility that it is in other arthropods. In order to answer this question conclusively, further research is required including functional studies.

**Wnt genes in arthropod appendage development**

In *Drosophila*, Wnt-signaling is an important regulator of limb development. In the developing limb discs, Wnt1 (*wg*) is expressed in the ventral sector of the disc, and loss of its function causes dorsalization of the limbs. In the dorsal sector of the discs, *decapentaplegic* (*dpp*) and its downstream target gene *optomotor-blind* (*omb*) are expressed (reviewed in [70]). In all hitherto investigated arthropods, the expression of *Wnt1* and *omb*...
Fig. 16 (See legend on previous page.)
during limb development are highly conserved suggesting that their function is conserved as well (e.g., [38, 71, 72], this study). In Tribolium, functional studies revealed conserved function of Wnt1 in ventral limb development [18]. A functional study in a hemimetabolous insect, the true bug Oncopeltus, however, suggested that this function may be restricted to holometabolous insects [1]. Other functional data on the possible function of Wnt1 in limb development are not available, and it is therefore unclear if the situation in Oncopeltus is conserved in other arthropods, or if it represents an exception. In any case, a reoccurring feature of Wnt genes is their expression along the ventral side of outgrowing appendages (Fig. 17). Expression of Wnt genes in the dorsal of appendages, however, is much rarer and never in the same striking continuous patterns as displayed for the ventral side (except for the labrum that likely rotated by 180° during evolution and therefore expresses Wnt genes predominantly along its dorsal side [45]. In onychophorans, however, a closely related group of animals, Wnt genes are expressed in the tips of the growing appendages [12, 28]. Thus, the ventral appendage-patterning by the Wnt genes might represent a conserved feature restricted to arthropods. Either way, the fact that multiple Wnts are expressed along the ventral side of the developing appendages in all investigated arthropod species strongly suggest that they have a function in ventral limb development, either individually or in combination. Therefore, functional studies targeting a single Wnt gene, as performed in Oncopeltus [1], could easily overlook the involvement of Wnt-patterning in ventral vs dorsal appendage development. To circumvent problems caused by redundant function(s) of multiple Wnts in studying arthropod limb development, known downstream targets of Wnt, such as the T-box encoding transcription factor H15/midline, could instead be addressed by means of e.g., RNAi-mediated knockdown [38, 71, 86, 87]. Another transcription factor that is expressed along the ventral sector of all appendages in all arthropods and even an onychophoran is the forkhead-box encoding gene FoxB. This gene appears to act upstream of Wnt-signaling and may thus provide yet another alternative to study the role(s) of Wnt-signaling in appendage development [24].

**Insight into the complexity of arthropod Wnt-patterning: a potpourri of functional redundancy, combinatorial function, function-shuffling, and neo- and sub-functionalization**

Wnt-patterning, the interaction of the multiple Wnt ligands with the plentitude of their potential receptors,
is highly complex (e.g., [25, 32, 50]). We can assume that many (if not the most) Wnts possess very similar biochemical features, such as their receptor-binding sites (e.g., [34, 77]). As a consequence, Wnts are in many cases able to interact with more than one type of receptor, and multiple Wnts can likely interact (albeit with different stringency) with the same receptor [43]. As a result, a given Wnt can be co-opted relatively easily into a GRN replacing another Wnt (e.g., [55]. For the same reasons, different Wnts can act redundantly, as long as they share the same regulatory elements and are thus co-expressed. Co-expression also allows Wnt genes to function combinatorial (e.g., [4, 5, 39, 91]). The control of a given developmental feature or genetic interaction can thus be under control of a set of Wnt genes, possibly in a dose-dependent manner, (reviewed in e.g., [94]), rather than a single Wnt. In summary, this provides a complex network of mutational protection, and thus the loss of one of these redundant/complementary Wnt factors (caused by either depletion of the gene, or regulatory changes) may not alter the development of the organism very much. Indeed, it has been shown that function-shuffling occurs regularly in Wnt genes, often associated with gene loss [52, 56, 84]. The latter, however, is not mandatory, especially when the gene is part of multiple GRNs. In spiders, we frequently observe Wnt gene expression domain losses and gains, such as the dominant posterior expression of Wnt8 in Parasteatoda, but in no other spider, or the loss of the segment-polarity like pattern of wg/Wnt1 in spiders, although this pattern is conserved in the harvestman and arthropods in general (both cases discussed above). Gain of an expression pattern on the other hand is for example represented by the expression of Wnt2 in the SAZ and the ventral surface of the appendages in basally branching spiders (possibly followed by a loss in entelegyne spiders) (summarized in Fig. 17). Although function-shuffling is not necessarily accompanied by gene loss, it could explain the loss of Wnt9 and Wnt10 class genes in spiders (Fig. 3). Function-shuffling could also explain why Wnt genes are often expressed in similar patterns, e.g., along the ventral side of the appendages, a feature that cannot easily be explained by ancestry. The reconstruction of the ancestral patterns of Wnt genes is also likely impeded by function-shuffling (associated with the acquisition of shared expression patterns). The reoccurring expression of Wnts in the SAZ (likely associated with posterior elongation) and the regionalization of the brain could represent ancestral features of Wnt gene function because the central nervous system and posterior elongation are ancestral features of most animals. Reoccurring expression along the ventral side of the appendages and the segment-polarity like patterns, however, likely are conserved features of (pan)arthropods and thus must have evolved in the lineage leading to this group of animals, long after the establishment of the protostomian Wnt complement (e.g., [39]).

Another feature observed for Wnt genes is the retention of both copies after duplication that adds yet another level of complexity. As we see in spiders, duplicated genes always display quite different expression pattern, suggesting that these genes have not been incorporated into the redundancy-based mutational protection network that the complexity of Wnt gene expression most likely provides. Instead, if retained, one copy of a given Wnt gene must have required new functions and thus expression patterns (neo-functionalization) (e.g., Wnt4 and Wnt7, Figs. 7, 10 and 11). Compared to other genes, most copies of Wnt genes disappeared after duplication (cf. with duplicated and almost fully retained Hox gene clusters in spiders (e.g., [80, 81] or the multitude of duplicated homeodomain genes [46]). This further strengthens the idea that the interaction of Wnt genes is dose-dependent and may be disturbed by the presence and transcription of a new duplicate. Cases of sub-functionalization, i.e., the subdivision of function and thus expression are rather rare in duplicated Wnt genes. One impressive example, however, is represented by the expression of the two Wnt1 ohnologs in the tarantula Acanthoscurria (Fig. 4).

The fact that many Wnt genes are expressed in similar patterns demands comprehensive studies including all genes that share a given expression pattern in order to investigate the function of “Wnt” in a given developmental or evolutionary context. As this study shows and tries to highlight, these Wnt genes may not necessarily be paralogs, but may represent members of other classes of Wnt genes. As the expression of Wnt genes appears to change frequently during the course of evolution, possibly as a result of function-shuffling or the general exchange of regulatory elements, developmental studies concerning the function of a given Wnt gene should rather address Wnt gene patterning as a whole (the complement of Wnt genes with identical/similar expression). Future evolutionary studies, comparing of gene expression and their function among a variety of more or less related animals, however, should include a sufficient number of species along the phylogenetic tree to reveal possibly changing expression patterns (and potential function). The latter is of the uttermost importance in order to draw any relevant conclusion from such data in terms of evolutionary processes. Essential to both kinds of studies is the comprehensive knowledge about Wnt gene expression in any given research organism, a task this paper aims to contribute to.
Supplementary Information

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Additional file 1: Fig. S1 The complements of arthropod and onychophoran Wnt genes. Full species names that are not listed in the legend of Fig. 1: Acaryosphasoph pium (Hexapoda: Homoptera), Anopheles gambiae (Hexapoda: Diptera),Apis mellifera (Hexapoda: Hymenoptera), Daphnia pulex (“Crustacea”: Branchiopoda), Drosophila melanogaster (Hexapoda: Diptera), Eupenipatioides kanagakensis (“Onychophora”), Glomeris marginata (Myriapoda: Diplopoda), Stigmamia mantima (Myriapoda: Chilopoda), Thamnocephalus platyurus (“Crustacea”: Branchiopoda), Tribolium castaneum (Hexapoda: Coleoptera). Abbreviations: e, expression has been studied, but no specific signal has been reported; E, expression has been studied; F, functional studies have been performed.

Additional file 2: Fig. S2 Early expression of Phalangium Wnt1. A. Posterior view, anterior to the left. B. Dorsal view, anterior to the left. C and D, posterior views, anterior to the left. Developmental stages are indicated. The asterisks mark the posterior of the embryo proper. Abbreviations: df, dorsal field; saz, segmentation-addition zone.

Additional file 3: Fig. S3 Expression in the appendages of Acanthoscurria. Abbreviations: l(l), lateral view; p (posterior view), ch, chelicera; en, endite; L, leg; pp, pedipalp. Appendage-type and orientation are the same for all Wnt genes, as indicated for Wnt1.

Additional file 4: Fig. S4 Expression in the appendages of Acanthoscurria (continued). Abbreviations: l(l), lateral view; p (posterior view); ch, chelicera; en, endite; L, leg; pp, pedipalp. Appendage-type and orientation are the same for all Wnt genes, as indicated for Wnt1.

Additional file 5: Fig. S5 Expression in the appendages of Phalangium. All panels show anterior views. Appendage-type and orientation are the same for all Wnt genes, as indicated for Wnt1. The asterisk marks the tip of the chelicerae that often attract unspecific staining at late developmental stages. Abbreviations: ch, chelicera; en, endite; L, leg; lr, labrum; m, mouth; pc, pre-cheliceral region; pp, pedipalp.

Additional file 6: Fig. S6 Expression in the appendages of Pholcus. All appendages are shown from ventral, except last panel in I (posterior view of a leg). Appendage-type and orientation are the same for all Wnt genes, as indicated for Wnt1. Abbreviations: ch, chelicera; L, leg; pp, pedipalp.

Additional file 7: Fig. S7 Expression in the appendages of Parasteatoda. Labrum and chelicerae are shown from anterior, pedipalps and legs are shown from ventral. Appendage-type and orientation are the same for all Wnt genes, as indicated for Wnt1. Arrows point to expression in the labrum. Asterisks mark expression at the dorsal rim of the head. Abbreviations: ch, chelicera; en, endite; L, leg; m, mouth; pc, pre-cheliceral region; pp, pedipalp.

Additional file 8: Fig. S8. Early expression of Parasteatoda and Acanthoscurria Wnt11. In all panels, anterior is to the left, ventral views (except panels A and C (lateral views)). Developmental stages are indicated. Panels marked with an apostrophe represent SYBR-green images of the embryo shown in the regular panels. Abbreviations as in Fig. 4.

Additional file 9: Fig. S9. Early expression of Parasteatoda Wnt16. In all panels, anterior is to the left, ventral views (except panel B (lateral view)). Inlay picture in A represents SYBR-green image of the embryo shown in the regular panel. Developmental stages are indicated. Abbreviations as in Fig. 4.

Additional file 10. Wnt gene alignment.

Additional file 11. Accession Numbers.

Additional file 12. Primers.

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Authors’ contributions

RI designed the project, performed most of the experiments and documented the data. MP provided all Acanthoscurria related material, NT provided all Pholcus related material and screened spider genomes for the presence and absence of Wnt genes. All authors discussed the results. RJ wrote the first draft of the manuscript. All authors contributed to the final version of the manuscript. All authors read and approved the final version of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article (and its additional information files).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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