



Implications of a cheliceral axial duplication in *Tetragnatha versicolor* (Araneae: Tetragnathidae) for arachnid deutocerebral appendage development

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Abstract

The homology of the arachnid chelicera with respect to other head appendages in Panarthropoda has long been debated. Gene expression data and the re-interpretation of early transitional fossils have supported the homology of the deutocerebrum and its associated appendages, implying a homology between primary antennae (mandibulates), chelicerae (euchelicerates), and chelifores (sea spiders). Nevertheless, comparatively little is known about the mechanistic basis of proximo-distal (PD) axis induction in chelicerates, much less the basis for cheliceral fate specification. Here, we describe a new cheliceral teratology in the spider *Tetragnatha versicolor* Walckenaer, 1841, which consists on a duplication of the PD axis of the left chelicera associated with a terminal secondary schistomely on the fang of the lower axis. This duplication offers clues as to potential shared mechanisms of PD axis formation in the chelicera. We review the state of knowledge on PD axis induction mechanisms in arthropods and identify elements of gene regulatory networks that are key for future functional experiments of appendage development in non-insect model systems. Such investigations would allow a better understanding of PD axis induction of modified and poorly studied arthropod limbs (e.g., chelicerae, chelifores, and ovigers).

Keywords Axis duplication · Chelicerae · Deutocerebral appendage · Teratology · Araneoidea

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Introduction

The homology of the arachnid cheliceral segment (and sea spider cheliforal segment) with respect to other head segments in Panarthropoda was a historically contentious topic (Budd 2002; Maxmen et al. 2005; Brenneis et al. 2008). It was traditionally thought that the midbrain of mandibulates (the deutocerebrum, or the second part of the arthropod tripartite brain) was absent in Chelicerata, and therefore, its associated appendage was not present. This inference was based on the anatomy of the chelicerate brain, namely, (1) the comparable organization of the ganglion innervating the chelicerae to the one on the tritocerebral ganglion in crustaceans and insects (Weygoldt 1985); (2) the relative position of the cheliceral ganglion with respect to the stomodeum; and (3) the location of the post-esophageal commissure (Siewing 1963). This understanding was overturned by gene expression data from different chelicerate taxa, such as spiders (Damen et al. 1998), mites (Telford and Thomas 1998), and sea spiders (Jager et al. 2006). These works demonstrated that the cheliceral/cheliforal segment of Chelicerata represented the first appendage-bearing segment of the arthropod head that

lacks Hox gene expression, comparable to the first antennal segment of Mandibulata. This interpretation was additionally supported by the investigation of chelicerate neuroanatomy in horseshoe crabs (Mittmann and Scholtz 2003) and sea spiders (Brenneis et al. 2008).

This revised homology of deutocerebral appendages has prompted the re-interpretation of early transitional fossils (Chen et al. 2004; Aria and Gaines 2015). Structures putatively linking the antennae and the chelicerae as part of a single transformational series include the antenniform chelicerae of the Silurian synziphosurines *Dibasterium durgae* Briggs et al. 2012 and *Offacolus kingi* Orr et al. 2002 (Sutton et al. 2002; Briggs et al. 2012; Sharma et al. 2013), as well as the multi-flagellate and multi-chelate deutocerebral appendages of groups like leanchioliids (Megacheira, commonly known as the great-appendage group; Chen et al. 2004; Aria and Gaines 2015; Setton et al. 2017). Nevertheless, the wide anatomical and functional gulf between antenna and chelicera poses challenges from the perspective of developmental genetics and homologizing regions of the proximo-distal (PD) axis in deutocerebral appendages, by comparison to structures like walking legs (Bruce and Patel 2020). This is partly attributable to the evolutionary lability of spatiotemporal dynamics among the genes that regionalize the PD axis (leg gap genes) across phylogenetic scales, particularly for gnathal and sensory serial homologs of walking legs (Prpic et al. 2003; Angelini and Kaufman 2005). An additional complication is the retention of numerous orthologs of developmental patterning genes resulting from a shared whole-genome duplication in the common ancestor of Arachnospulmonata (Panscorpiones + Tetrapulmonata; Schwager et al. 2017; Ontano et al. 2021), resulting in the subdivision of expression and function of numerous leg gap gene paralogs in the spider models often prioritized as exemplars of Chelicerata in comparative developmental studies (Prpic and Damen 2004; Pechmann and Prpic 2009; Turetzek et al. 2016; Gainett and Sharma 2020; Nolan et al. 2020).

As a result, comparative functional investigations of chelicerate segmentation and chelicerate fate specification have been grounded in the harvestman *Phalangium opilio* Linnaeus, 1758, which does not share the whole genome duplication exhibited by arachnospulmonates. It was shown in this model system that the chelicerae (like their mandibulate counterpart, the deutocerebral antennae) share the requirement of *homothorax* (*hth*) in the absence of Hox input for specification of deutocerebral appendage identity (Ronco et al. 2008; Sharma et al. 2015), further supporting the homology of these appendage types. This is consistent with expression dynamics of *hth* and *Dll* across panarthropods, with non-antagonistic expression domains between *Dll* and the proximal patterning genes *hth/extradenticle* (*exd*) common across deutocerebral appendages (Dong et al. 2002; Prpic and Damen 2004; Pechmann

and Prpic 2009; Janssen et al. 2014; Sharma et al. 2012, 2015).

Beyond the role of *hth* in specifying chelicerate identity, as well as a separate role for the medial PD axis gene *dachshund* (*dac*) in patterning the proximal segment in groups with a plesiomorphic three-segmented chelicera (Sharma et al. 2012, 2013; Nolan et al. 2020), little is known about chelicerate fate specification, the genetic basis for the patterning of the chela, and more broadly, the functional dynamics of PD axis patterning in Chelicerata. A recent investigation of the distal antennal selector *spineless* (*ss*) revealed that the antennal expression domain of this transcription factor is restricted to mandibulates; *ss* does not appear to play any role in the patterning of the distal chelicera (Setton et al. 2017). This data point suggests that a role for *ss* in distal antennal fate specification was acquired at the base of Mandibulata and not earlier; the identity of chelicerate selector genes remains unknown.

In the absence of developmental genetic data, one clue to how structures are formed can be gleaned from the investigation of naturally occurring teratologies. In contrast to insects, the number of documented naturally occurring malformations in arachnids is rather limited. As it relates to chelicerate patterning, an ideal study taxon is the long-jawed orb-weaving spiders. *Tetragnatha* Menge, 1866 (Tetragnathidae) are characterized by large chelicerae with a diverse arrangement of associated teeth, and sometimes additional cusps on the respective fangs. This degree of variation had made the tetragnathid chelicerae taxonomically relevant, like in any other spider taxa (Castanheira et al. 2019). Moreover, the structural complexity of tetragnathid chelicerae provides reliable landmarks for the identification of homologous structures. Here, we describe a new chelicerate malformation phenotype on *Tetragnatha versicolor* Walckenaer, 1841 and review its implications for chelicerate PD axis patterning.

Material and methods

The collection information of the examined specimen is “USA: Pinecrest, CALIF. Tuolumne Co. VII-6-1947 1♀ *Tetragnatha versicolor* (det. D. Cotoras 2020).” It is deposited at the California Academy of Sciences under accession number CASENT 9081591.

The specimen was originally in a vial with more than 20 other specimens (male, female, and juveniles) under the identification of *Tetragnatha laboriosa* Hentz, 1850. Our identification was corroborated using Levi (1981), Álvarez-Padilla et al. (2009), and Álvarez-Padilla and Hormiga (2011). Structure names follow Castanheira et al. (2019).

Results

The specimen was identified as *T. versicolor*, contrary to the original label indicating “*T. laboriosa*.” DNA sequencing of other samples from the vial showed that it had a mix of *T. laboriosa* and *T. versicolor*. The studied specimen was not sequenced, so its identification was based on morphology. In particular, the recognized traits which distinguished the female *T. versicolor* (Fig. 1a) from *T. laboriosa* were as follows: (1) in *T. versicolor*, lateral eyes are closer together than median eyes, whereas in *T. laboriosa*, lateral eyes are usually as far apart as the median eyes; (2) in *T. versicolor*, there is an outer cusp of the fang (OC), which is absent in *T. laboriosa*; (3) in *T. versicolor*, there is a guide tooth on the upper row (Gu) followed by a diastema and then a series of teeth decreasing in size (U), whereas in *T. laboriosa*, the most distal tooth (corresponding by position with Gu) is not as differentiated from the others; (4) in *T. versicolor*, the guide tooth on the lower row (Gl) is closer to the other lower teeth (L), whereas in *T. laboriosa*, there is a larger diastema to the remaining teeth; and, finally, (5) *T. versicolor* has proportionally more elongated chelicerae than *T. laboriosa* (Levi 1981). In addition, the specimen has dusky markings in the abdomen, which is a character mentioned for *T. versicolor* in a revision of the group for North America (Levi 1981).

The specimen presents a duplication of the PD axis on the left chelicera associated with a terminal secondary schistomely on the fang of the lower axis (Figs. 1b and 2). Each axis has the upper and lower rows of cheliceral teeth and one fang. The upper axis has a relative configuration similar to the wild type, which includes the orientation of the fang. By contrast, the fang of the lower axis, which presents the terminal secondary schistomely, is pointing in the opposite direction (outwards). The outer cusp (OC) is present in both fangs. The guide tooth of the upper row (Gu) is present in the upper

Fig. 1 Female chelicerae, internal view: **a** right chelicera (normal phenotype), **b** left chelicera (malformed phenotype). The upper axis presents the normal phenotype, while the lower axis has a fang with a terminal secondary schistomely. Note the presence of two proximal extra teeth between the upper and lower rows of teeth on the lower axis. eu, extra teeth; Gl, guide tooth on the lower row; Gu, guide tooth on the upper row; L, remaining teeth from the lower row; OC, outer cusp of the fang; U, remaining teeth from the upper row

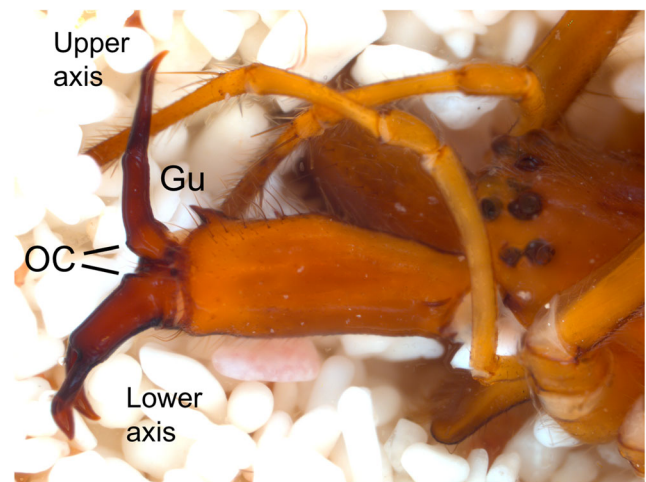
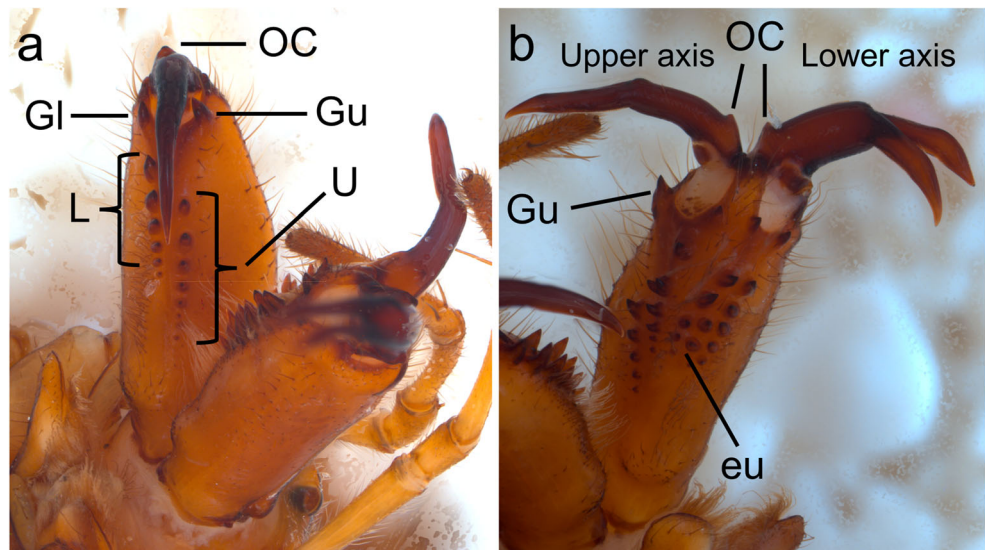


Fig. 2 Female left chelicera, external view (malformed phenotype). OC, outer cusp of the fang; Gu, guide tooth on the upper row. The upper axis presents the normal phenotype, while the lower axis has a fang with a terminal secondary schistomely

axis, but absent in the lower one. Instead, there is a tooth which does not have the characteristic size difference associated with the rest of the proximal teeth. In the most proximal position in between the upper and lower rows of teeth of the lower axis in the left chelicera, there are two extra teeth (eu) (Fig. 1b).

Discussion

This study represents the first reported teratology in a *Tetragnatha* spider and, to our knowledge, the first described naturally occurring cheliceral axial duplication in an adult specimen. The fact that it occurs in the presence of adult structures allows for a better interpretation of the observed duplication. Previous experimental studies of thermal alternation

during embryonic development of *Eratigena atrica* (C. L. Koch, 1843) (Agelenidae) resulted in a small portion of chelicerae anomalies (Napiórkowska et al. 2016a). Among those anomalies were bifurcated chelicerae with two fangs: polymely, consisting of an additional chelicera; polymely with simultaneous heterosymely, consisting of three fused chelicerae with their respective fangs on the same side of the body (see Figs. 5–7 on Jacuński et al. 2004), and other deformities with atypical chelicerae shapes that were difficult to interpret (Napiórkowska et al. 2010; Jacuński et al. 2004). Regardless of the similarity with some of those phenotypes, it is difficult to associate what is observed in *T. versicolor* with the same cause.

The fact that the malformation presented here occurs in only one of the chelicerae is congruent with previous experimental evidence that shows independence between chelicerae in the expression of teratological phenotypes (Jacuński et al. 2004). As the duplication occurs on the teeth rows and their respective fang, both structures might correspond to a single developmental module under the same regulatory mechanism, probably associated with PD differentiation.

Given that *Tetragnatha* spiders capture their prey with a web and not by ambush predation, it appears possible that an individual with a suboptimal cheliceral structure might be able to survive in the wild. The anatomical position of the upper axis appears to be functional, as it is similar to the right chelicera. Therefore, considering that this was an adult specimen, it is possible that this malformation did not strongly affect its capacity to survive. A different situation is the one regarding reproduction, because a key step in the *Tetragnatha* copulation process is the cheliceral lock between male and female. This immobilization of the chelicerae prevents the female from eating the male during mating (Simkovic and Andrade 2019). In all *Tetragnatha* species, the males even have dorsal apophyses, which may facilitate the lock (Castanheira et al. 2019). We speculate that this double-fang condition might have prevented any successful copulation.

The terminal secondary schistomely of the fang on the lower axis in the malformation presented here is a phenotype comparable to those reported in other taxa and appendages, such as the terminal bifurcations described in myriapod legs (Vega-Román and Hugo-Ruiz 2015) and antennal bifurcations in *Xyletineurus bombycinus* (Erichson, 1847) (Coleoptera: Ptinidae) (Honour and Lüer 2020). No spiders are known to have bifurcated fangs, but several species in the family Tetragnathidae possess short protrusions along the fangs, such as the two inner cusps (IC) on the female of *Tetragnatha argentinensis* Mello-Leitão (1931), or the cheliceral fang outgrowth (CFO) on several species of the genus *Glenognatha* Simon, 1887 (Cabra-García and Brescovit 2016; Castanheira et al. 2019). Those protruding structures might be formed by some kind of main axis duplication or “ramification” at a smaller scale, than what is presented here.

To test this idea, more detailed studies on the determinants of fang morphology are required.

Mechanisms of appendage axis duplication in Arthropoda

Teratologies are fascinating phenomena from the perspective of developmental biology, in that they provoke questions of mechanism and process that led to those anomalies. Within chelicerates, intriguing teratologies have spanned the number and position of eyes (reviewed by Jimenez and Llinas 2002), appendages (Juberthie 1968; Jacuński et al. 2002a, b; Scholtz and Brenneis 2016; Napiórkowska and Templin 2017; Di et al. 2018), reproductive organs (Izquierdo 2021), and bicephaly (Mikulska and Jacuński 1970; Templin et al. 2009; Napiórkowska et al. 2016b). The last of these teratologies has received the most attention from the standpoint of embryological and genetic investigations, as antero-posterior axis duplication of this type is thought to be associated with the incidence of an axis organizer. Indeed, seminal experiments by Holm (1952) showed that a specific cell population of the spider embryo, called the cumulus, acts as the axis organizer. Grafts of this region from one embryo to another are able to induce a secondary axis in the recipient. A counterpart of this experiment in horseshoe crab embryos suggested that the cumulus may be an ancient and phylogenetically widespread organizer (Itow et al. 1991). Detailed work on the cell dynamics and genetics of this region of the embryo in the past 20 years has linked secretion of Decapentaplegic (Dpp) from the cumulus with axis-patterning activity in multiple spider species (Akiyama-Oda and Oda 2003; Oda et al. 2020; Pechmann 2020). Besides grafts of the cumulus, other experimental approaches that can drive antero-posterior axis duplication in chelicerates include fluctuations of humidity (Buczek 2000) and temperature (Jacuński 1984; Juberthie 1962; Jacuński et al. 2002a, b; Napiórkowska et al. 2016a, b; Napiórkowska and Templin 2018), chemical induction (Itow and Sekiguchi 1979), and laser ablation (Oda et al. 2020).

By comparison to the dynamics of the cumulus and antero-posterior axis patterning, little is known about the mechanisms of limb PD axis duplication in chelicerates. Putative causes of this phenotype can be divided into two groups: (1) an external teratogen and (2) a malformation during development, molt, or regeneration caused by an aberrant developmental process. The possibility of an external teratogen is unlikely, as it would require one with very localized action and without a widespread environmental effect, as no other individuals with the same phenotype were found in a vial corresponding to a 1-day collection event with >20 samples in total. Without natural history data on the embryogenesis and postembryonic development of this specimen, we are unable to evaluate the possibility of mechanical damage driving an axis bifurcation during

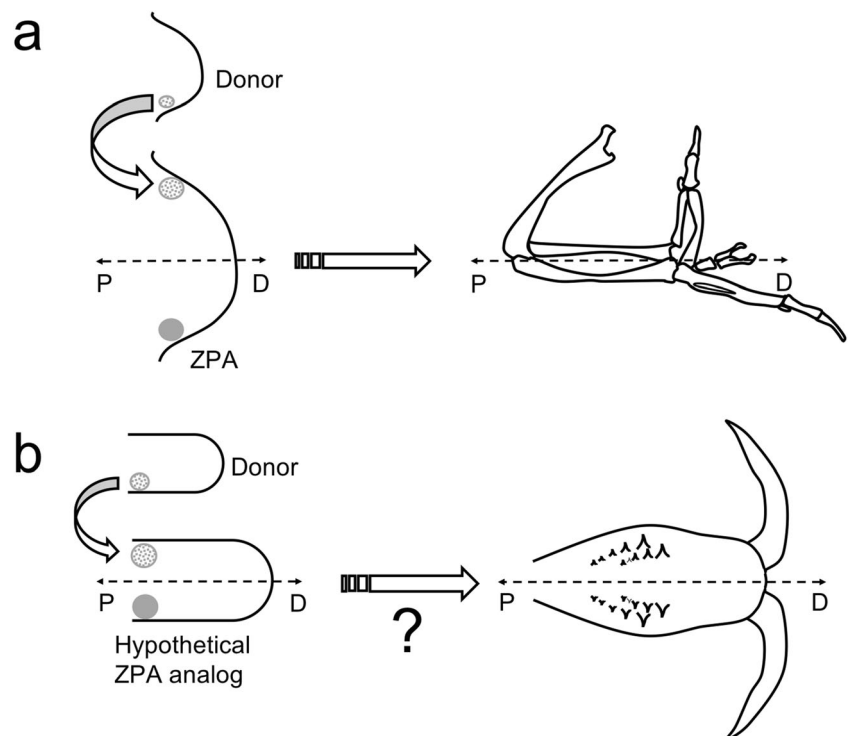
development. Along the same line, a malformation due to a failed regeneration process appears to be unlikely, as the chelicerae in *Tetragnatha* are not known to regenerate.

Heterosymely (fusions of adjacent appendages) is known to be linked to changes in temperature in multiple arachnid orders (Juberthie 1968; Jacuński et al. 2002a, b; Napiórkowska and Templin 2017), but temperature and humidity changes are not causally linked to PD axis duplication. A recent report on an ectopic leg in the sea spider *Pycnogonum litorale* (Strøm, 1762) offered some clues as to causes of such duplications; in this case, the specimen belonged to a captive-bred colony and was known to have been damaged at the site of the ectopic leg's induction in its first juvenile stage (Scholtz and Brenneis 2016). It was inferred that this anomaly was consistent with the boundary model (Meinhardt 1986), wherein appendages develop at the boundaries of two different cell populations (anterior and posterior); mechanical damage causes displacement of a third group of cells, causing juxtaposition of morphogens that confer anterior, posterior, and ventral fate (the requirements of PD axis induction) during regeneration. The result of this displacement is the induction of a new appendage axis (Scholtz and Brenneis 2016). The boundary model has garnered broad support from developmental genetic data, principally through functional studies of *dpp*, *wingless* (*wg/Wnt-1*), and their downstream targets *Sp6-9*, *Distal-less* (*Dll*), and *homothorax* (*hth*), in PD axis induction (reviewed by Estella et al. 2012). In this regard, *dpp* and *wg* are thought to act in a manner comparable to Sonic hedgehog (Shh) signaling in the posterior

limb bud of vertebrates. In a classic experiment, it was shown that ectopic expression of Shh in the anterior limb bud of a developing chick induces distal duplication of the PD axis; modulating the timing and intensity of expression alters the completeness of the duplication, with higher levels of Shh ectopic expression inducing greater differentiation of the ectopic PD axis (Yang et al. 1997). This experiment is understood to reflect the result of experimental grafts of the Zone of Polarizing Activity (ZPA, an Shh-expression center) to the anterior limb bud, which also results in distal axis bifurcation (Saunders and Gasseling 1968; Tickle 2017). Comparable dynamics have demonstrably evolved in early axis patterning of insects and cephalopod limbs as well (Tarazona et al. 2019). A similar PD determination mechanism could be occurring on the early cheliceral limb bud with the presence of a hypothetical ZPA analog (Fig. 3). If this model were accurate, it is predicted that graft experiments or implantation of Dpp- and Wg-soaked beads could result in the cheliceral PD axis duplication, which would replicate the phenotype described here.

Functional data on PD axis specification and induction remain limited in Chelicerata. Investigations of Dpp signaling have prioritized early embryogenesis in spiders (Akiyama-Oda and Oda 2003, 2006); surprisingly, there are no functional data in chelicerates with respect to the role of *dpp* in appendage fate specification or appendage dorsoventral axis patterning. Similarly, no functional data exist for arthropod *wg* homologs outside of derived insects; *Wnt8*, the only Wnt pathway member associated with a phenotype in an arachnid, has only been implicated in the patterning of opisthosomal

Fig. 3 Hypothesized mechanistic similarities in PD axis duplication between chick forelimb and spider chelicera. **a** Summary representation of the classic ZPA graft experiment which results in mirror-image distal duplication of the adult limb. **b** A hypothesized developmental mechanism to replicate the phenotype of cheliceral PD axis duplication observed in this study. As in the chicken graft experiment, this scenario postulates the presence of an analog of the ZPA in the proximal region of the early developing cheliceral limb bud. Note the chelicera diagram is rotated 90° to the right with respect to the position in Fig. 1b



segments (McGregor et al. 2008). Recently, it was shown that knockdown of the Wnt co-receptor *arrow* in the spider *Parasteatoda tepidariorum* (C. L. Koch, 1841) (Theridiidae) results in disrupted segmentation of the entire germ band and loss of appendages (Setton and Sharma 2018, 2021); while demonstrative of broader conservation of the Wnt pathway, this data point does not narrow down the functions of specific Wnts in chelicerate appendage patterning. Additional candidates that are associated with a distal bifurcation of the antennal PD axis in *Drosophila melanogaster* Meigen, 1830 include *Dip3* (extra antenna; duplication is achieved by splitting of the antennal field or by homeotic transformation of the eye disc; Duonga et al. 2008), *hth* (ectopic expression causes a duplication on the antennal PD axis; Yao et al. 1999), *obake* (misexpression induces a mirror image duplication and sometimes a triplication of the antenna; Dworkin et al. 2001), *CG17836/Xrp1* (antennal duplication by the formation of an additional PD axis by the *CG17836/Xrp1*-dependent ectopic induction of *wg*, *dpp*, and *hedgehog* (*hh*) in eye-antennal disc; Tsurui-Nishimura et al. 2013), and *eyK* (eyeless strain; in addition to the eye size reduction, the loss-of-function phenotype includes mirror image duplications of the antennae, either separated or fused at the base; Sang and Burnet 1963). Functional experiments in those genes which replicate the here described phenotype could help to shed light on the developmental mechanisms of the PD determination of the chelicerae.

Future experimental directions

Mutants and teratologies are useful tools for investigations of developmental mechanisms. Given the established homology of antennae and chelicerae, we present several potential mechanisms to account for the chelicerate axis duplication described in *T. versicolor*, as targets for future investigations. The establishment of large-bodied spiders as new models amenable to a broader swath of experimental approaches (Setton et al. 2019; Pechmann 2020) offers the hope of a refined understanding of PD axis duplications, as observed in the tetragnathid described herein. As first steps to establishing mechanisms of axis induction in any chelicerate, future investigations must adapt bead implantation techniques in limb buds of mygalomorph embryos to test the functions of Dpp, Wg, and Hedgehog in appendage fate specification, with the prediction that artificial co-induction of Dpp and Wg signals in the dorsal compartment of the chelicerate limb bud can drive distal duplication of the PD axis. In tandem with these approaches, laser ablation experiments should be performed at later stages of development (comparable to experiments by Oda et al. 2020), in order to assess whether mechanical disruption of boundaries between morphogen gradients can be causally linked to distal duplication of the PD axis (Scholtz and Brenneis 2016). A better understanding of chelicerate axis patterning could shed

light on the various adaptations of the chelicerae for different modes of feeding and reproduction, as exemplified by Tetragnathidae.

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Availability of data and material The studied specimen is deposited at the California Academy of Sciences under accession number CASENT 9081591.

Code availability (software application or custom code) Not applicable

Author contribution DDC conceived the study; found, identified, and photographed the specimen; analyzed the data; and wrote the paper. PdSC and PPS analyzed the data and wrote the paper.

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Declarations

Conflict of interest The authors declare no competing interests.

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