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The origins of the Psechridae: Web-building lycosoid spiders

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ABSTRACT

Psechrids are an enigmatic family of S.E. Asian spiders. This small family builds sheet webs and even orb webs, yet unlike other orb weavers, its putative relatives are largely cursorial lycosoids – a superfamily of approximately seven spider families related to wolf spiders. The orb web was invented at least twice: first in a very ancient event, and then second, within this clade of wolf-like spiders that reinvented this ability. Exactly how the spiders modified their silks, anatomy, and behaviors to accomplish this transition requires that we identify their precise evolutionary origins – yet, thus far, molecular phylogenies show poor support and considerable disagreement. Using phylogenomic methods based on whole body transcriptomes for psechrids and their putative relatives, we have recovered a well-supported phylogeny that places the Psechridae sister to the Ctenidae – a family of mostly cursorial habits but that, as with all psechrids, retains some cribellate species. Although this position reinforces the prevailing view that orb weaving in psechrids is largely a consequence of convergence, it is still possible that some components of this behavior are retained or resurrected in common with more distant true orb weaving ancestors.

1. Introduction

Spiders serve as evolutionary models for the study of biodiversity and adaptive radiations owing largely to the remarkable physiological adaptations and diverse behavioral repertoires that have produced what are among the most successful of terrestrial predators (Vollrath and Selden, 2007). Perhaps the most remarkable prey capturing adaptation in spiders, which is thought responsible for major adaptive radiations (Bond and Opell, 1998), is in the production and innovative use of silk, and most notably in its use in building orb webs. The orb web consists of a largely 2-dimensional, vertically aligned frame with radii connecting to a central hub, and an adhesive spiral designed to capture insects in flight. Because these webs must absorb the kinetic energy of flying insects, the silk used in the radii have evolved the essential properties of being both extremely tough and extremely stretchy (Sensenig et al., 2012). Moreover, building this intricate, symmetrical, and highly regular snare requires a complex series of stereotypical behaviors that are often phylogenetically conserved (Eberhard, 1982).

For the vast majority of orb weavers, the origin of this web design is ancient and probably singular, but in the case of *Fecenia*, a small genus in the family Psechridae, the orb web has evolved more recently and independently from within a large clade of mostly cursorial and ground sheet weaving spiders (Agnarsson et al., 2013; Blackledge et al., 2012a; Levi, 1982). This remarkable case of convergence (Fig. 1) offers an ideal opportunity to study how silk proteins, web-building behaviors, and anatomy coevolve to recreate this remarkable snare. However, concomitant with these studies is the need to estimate reliably the phylogeny of psechrids and their kin so that innovations can be understood in terms of homologies and analogies with respect to their antecedents, exaptations, atavisms, and the like.

Getting the phylogeny wrong can mislead us in how we infer the evolution of traits. For example, prior research argued that *Fecenia* silks could never fully match the properties of true orb weavers because, as recently as five years ago, it was assumed that true orb weavers originated separately (Fig. 2 Left), and that this group uniquely and exclusively acquired a key silk protein called major ampullate spidroin 2 (MaSp2) (Blackledge et al., 2012a; Blackledge et al., 2012b). MaSp2 is rich in proline residues that are thought to disrupt intermolecular bonds resulting in high extensibility and supercontraction (Gatesy et al., 2001; Hayashi and Lewis, 1998). However, more recent phylogenomic analyses (Bond et al., 2014; Fernandez et al., 2014; Garrison et al., 2016) provide strong support for an older origin for true orb weavers where RTA-clade spiders (i.e. a large clade of spiders having a

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Fig. 1. Photograph of the web of *Fecenia protensa* from Dong Phaya Yen Mountains, Thailand. The spider uses a leaf retreat, much like the araneid genus *Acusilas* (Murphy and Murphy, 1983). The outcome is a web with an uncanny resemblance to the classic orb webs of the "Orbiculariae" spiders.

retrolateral tibial apophysis on the male palp, which includes the Lycosoidea – labeled as "R" on Fig. 2) are likely descendants of orb weaving ancestors (Fig. 2 Right). Under this scenario, key innovations attributed to true orb weavers, such as the MaSp2 protein, could be retained in RTA-clade spiders such as *Fecenia*. If true, it would be a mistake to assume that all silk protein innovations and web-building behaviors that allowed for orb web convergence by *Fecenia* had to have evolved completely de novo: at least some of these could result from atavistic reemergence. It is therefore well worth establishing a solid phylogenetic framework between psechrids and other taxa so as not to be misled by incorrect phylogenetic assumptions.

While superfamily relationships deep within the spider tree have greatly improved though phylogenomics, well-supported phylogenies of lycosoid spiders continue to elude us. Five recent papers that address these relationships using traditional molecular markers (Agnarsson et al., 2013; Bayer and Schonhofer, 2013; Blackledge et al., 2012a; Moradmand et al., 2014; Wheeler et al., 2016), each arrive at a different set of relationships (Fig. 3), and all but one fail to support monophyly of the Psechridae. Consistent with some older morphological studies (Davila, 2003; Griswold, 1993), these results tend to group psechrids closer to true wolf spiders (Lycosidae) rather than tropical wolf spiders (Ctenidae). In contrast, other studies that include morphological data group psechrids with the ctenids (Polotow et al., 2015; Ramírez, 2014). The placement of psechrids within the Lycosoidea remains recalcitrant and enigmatic.

To help resolve the origins of the Psechridae, we used published transcriptomes from a select group of spiders and arachnid outgroups to build a set of spider-specific core orthologs, and then used this core as the basis for assembling orthologous groups (OGs) with the transcriptomes of lycosoid species. We performed multiple analyses of different sets of data, each assembled under more or less stringent criteria.

2. Methods

2.1. Taxon sampling

We downloaded eleven spider transcriptomes from the NCBI Sequence Read Archive (SRA), including from all ten available RTA-clade species. To this, we added ten additional transcriptomes: *Ctenus corniger* (Ctenidae), *Hippasa holmerae* (Lycosidae), *Sosippus placidus* (Ly-

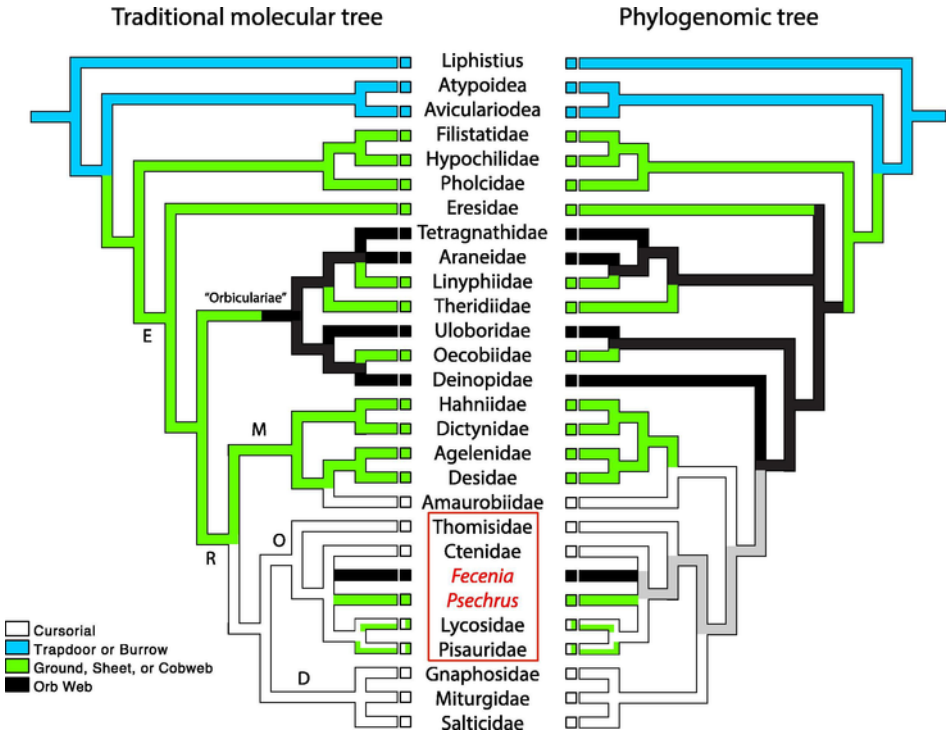

Fig. 2. Evolution of web use illustrated on a traditional molecular phylogeny tree of spiders compared with a phylogenomic tree of spiders. Left side: traditional molecular tree (modified from Blackledge et al. 2012a), with orb-weaving originating in the Orbiculariae completely independently of *Fecenia*. Right side: phylogenomic tree (modified from Garrison et al. 2016), showing that *Fecenia* likely descends from orb-weaving ancestors. Web type character optimization modified from Garrison et al. (2016). Clade labels: D, Dionycha; E, Entelegynae; M, marronoid clade; O, oval calamistrum clade; R, RTA-clade. Lycosoid taxa, the focus of this study, are circumscribed in a red box. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

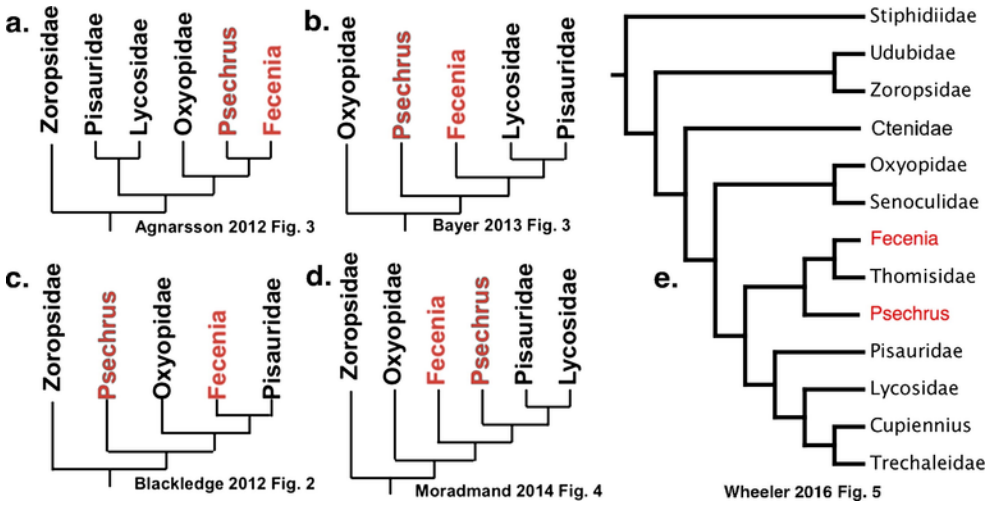

Fig. 3. Relationships among lycosoid spiders based on traditional molecular markers (Agnarsson et al., 2013; Bayer and Schonhofer, 2013; Blackledge et al., 2012a; Moradmand et al., 2014; Wheeler et al., 2016). Tree (c), but without *Fecenia*, approximates results from mitochondrial ribosomes (Fang et al., 2000). Tree (e) was inferred using traditional molecular markers constrained by a skeletal tree that was based on transcriptomic data (Wheeler et al., 2016). Psechrids are highlighted in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cosidae), *Odo patricius* (Xenoctenidae), *Oxyopes* sp. (Oxyopidae), *Nilus albocinctus* (Pisauridae), *Sphedanus quadrimaculatus* (Pisauridae), *Fecenia protensa* (Psechridae), *Psechrus singaporensis* (Psechridae), *Thomisus spectabilis* (Thomisidae). These taxa were selected to target the Psechridae and putative related families, especially Ctenidae, Lycosidae, and Pisauridae. The remaining lycosoid family, Trechaleidae, is not represented in our analysis due to difficulties in obtaining fresh tissues. Recent analysis using seven traditional molecular markers give substantial support for the Trechaleidae grouping with *Cupiennius* as sister group to the Lycosidae (Albo et al., 2017). We predicted that the Psechridae would be positioned well outside of the pisaurid-lycosid clade, so did not estimate its omission to be problematic. See Table 1 for a complete list of the tissue sources for the transcriptomes.

2.2. Extraction and sequencing

Whole body RNA was extracted from single adult female spiders using the TRIzol™ Reagent protocol (Simms et al., 1993). Commercial services company AIT Biotech (now NovogeneAIT) prepared cDNA libraries from the RNA extractions and the Genome Institute of Singapore performed paired-end sequencing on the Illumina HiSeq 4000 platform.

2.3. Analysis pipeline

A core ortholog approach was employed for putative ortholog selection by closely following the methods described in Garrison et al. (2016), but with just some minor modifications.

Table 1
Transcriptomes used in this study. In bold are new transcriptomes contributed by this study. Under SRA Run Accession, citation are abbreviated as: B, (Bond et al., 2014); Fe, (Fernandez et al., 2014); Fr, (French et al., 2014); G, (Garrison et al., 2016); M, (Meng et al., 2015).

Species	Family	Specimen ID	SRA Run Accession	Specimen Origin
Anahita punctulata	Ctenidae	AUMS11932	SRR3144072, G	Auburn, AL, USA
Ctenus corniger	**Ctenidae**	**WHP01407**	**SRR6360557**	**Singapore**
Cupiennius salei	"Ctenidae"	SAMN02189690	SRR880446, Fr	Lab reared
Dolomedes triton	Pisauridae	AUMS11906	SRR3144094, G	Opelika, AL, USA
Fecenia protensa	**Psechridae**	**WHP01462**	**SRR6360558**	**Singapore**
Habronattus signatus	Salticidae	HED004	SRR1514888, B	Ocotillo, CA, USA
Hibana sp.	Anyphaenidae	AUMS11902	SRR3144074, G	Auburn, AL, USA
Hippasa holmerae sundaica	**Lycosidae**	**WHP01460**	**SRR6360559**	**Singapore**
Homalonychus theologus	Homalonychidae	AUMS11917	SRR3144075, G	Imperial Co, CA, USA
Misumenoides formosipes	Thomisidae	AUMS6454	SRR3144080, G	Opelika, AL, USA
Nilus albocinctus	**Pisauridae**	**WHP01408**	**SRR6360560**	**Singapore**
Odo patricius	**Xenoctenidae**	**WHP01500**	**SRR6360553**	**Iquique, Chile**
Oxyopes sp.	**Oxyopidae**	**DQC01489**	**SRR6360554**	**Singapore**
Pardosa pseudoannulata	Lycosidae	SAMN03384319	SRR1833279, M	Nanjing, China
Peucetia longipalpis	Oxyopidae	AUMS5740	SRR1514898, B	Opelika, AL, USA
Pisaurina mira	Pisauridae	MCZ:IZ:19677	SRR1365651, Fe	University Park, MD
Psechrus singaporensis	**Psechridae**	**WHP01461**	**SRR6360555**	**Singapore**
Schizocosa rovneri	Lycosidae	AUMS5122	SRR1514894, B	Oxford, MS, USA
Sergiolus capulatus	Gnaphosidae	AUMS5674	SRR1514903, B	Opelika, AL, USA
Sosippus placidus	**Lycosidae**	**WHP01475**	**SRR6360556**	**Placid Lakes, FL, USA**
Sphedanus quadrimaculatus	**Pisauridae**	**DQC01465**	**SRR6360561**	**Singapore**
Thomisus spectabilis	**Thomisidae**	**WHP01453**	**SRR6360562**	**Singapore**

All transcriptomes were assembled using Trinity (Grabherr et al., 2011). Proteins were predicted from each transcriptome using TransDecoder (Haas et al., 2013). An all-versus-all BLASTP approach (Altschul et al., 1990), with an e-value cut-off of 10^{-5}, was used to derive a core ortholog set from the transcripts of *Damon variegatus*, *Acanthoscurria geniculata*, *Dolomedes triton*, *Ero leonina*, *Hypochilus pococki*, *Leucauge venusta*, *Liphistius malayanus*, *Megahexura fulva*, *Neoscona arabesca*, *Stegodyphus mimosarum*, and *Uloborus sp.* – all available from SRA. Markov clustering, with an inflation parameter 2.1, was performed using OrthoMCL (Li et al., 2003). For the resulting set of putatively orthologous group (OGs), sequences shorter than 100 amino acids in length were discarded. Candidate OGs were each aligned using MAFFT (Katoh et al., 2005) with automatic alignment and a maxiterate value of 1000. Each alignment was screened for evidence of paralogy using FastTree 2 (Price et al., 2010) and PhyloTreePruner (Kocot et al., 2013). FastTree 2 used "slow" and "gamma" options; PhyloTreePruner used a node collapse parameter of 0.95. OGs were discarded should they be found in fewer than 10 of the 11 taxa and not found in the taxon with greatest number of identified OGs, *Megahexura fulva*. With the remaining alignments, profile hidden Markov models (pHMMs) were built using hmmbuild as implemented in HMMER (Eddy, 2011).

Orthology was inferred with predefined sets of orthologs using HaMStR v.13.2.3 (Ebersberger et al., 2009). Translations of all transcripts were matched to the spider-specific pHMMs that we had generated. The reference set for reciprocal best hit scoring came from *Acanthoscurria geniculata*'s OGs. Orthologs were pooled and filtered. Sequences shorter than 75 amino acids were discarded. OGs sampled for fewer than 11 taxa were then discarded. Each OG was then aligned using MAFFT with "auto", "localpair" and "maxiterate 1000" parameters. Trimming using ALISCORE (Misof and Misof, 2009) and ALICUT (Kück, 2009) removed ambiguous regions. A consensus sequence was derived for each alignment with infoalign (Rice et al., 2000). Sequences were deleted if they differed by more than an infoalign *change* value of 75 with respect to the consensus. Sequences were deleted if they had more than nine gaps on either side of a region with 20 or fewer mistranslated amino acids. Alignment columns were deleted if they had fewer than four non-gap characters. Following these edits, another round of alignment deletion applied to those shorter than 75 amino acids in length. Finally, sequences that did not overlap with all other sequences in the alignment by at least 20 amino acids were deleted, and OGs sampled for fewer than 12 taxa were discarded.

Five sets of OGs were assembled using a range of criteria. Matrix 1 consisted of the full set OGs. From this set, OGs were sorted based on the amount of gene occupancy, and OGs with the same degree of gene occupancy were then sorted by gene length. The first half of this list was discarded such that matrix 2 consisted of those in the larger half. Again, based on the sorted OGs, matrix 3 consisted matrix 2 reduced by half. Matrices 4 and 5 were the outcome of optimizing matrix 1 using the programs BaCoCa (Kück and Struck, 2014) and MARE (Meyer et al., 2011) respectively. BaCoCa retained 50% of the full matrix on the basis of the most phylogenetically informative sites. MARE assessed the matrix by partition, providing a measure of tree-likeness for each gene and optimizing the matrix for information content with an alpha value of 5. Concatenation of OGs was done with FASconCAT (Kück and Meusemann, 2010).

All analyses were performed on amino acid sequences. Separate maximum likelihood searches were conducted for each matrix. The optimal tree for each matrix was arrived at using the program ExaML 3.0.1 (Kozlov et al., 2015). For each dataset, models of amino acid substitution were selected using the AUTO command in ExaML, which can automatically determine the best-scoring protein substitution model for each partition via a test procedure that uses BIC (Bayesian Information Criterion). The gamma parameter was estimated using a model of rate heterogeneity with four discrete rates. RAxML v. 8.2.10 (Stamatakis, 2014) produced the parsimony trees used to initiate searches. ExaML produced the bootstrap consensus results based on 300 replicates per dataset. For bootstrapping, we reused the gamma parameters and the best-scoring protein substitution models from the prior optimizations. Finally, we used RAxML v. 8.2.10 to generate unrooted gene trees for each gene in Matrix 1 in order to serve as inputs for the coalescent-based species tree analysis in ASTRAL (Mirarab et al., 2014).

3. Results

SRA accession numbers and provenance metadata for all specimens are presented in Table 1. Summary statistics for ten novel spider transcriptomes, nine of which belong to the superfamily Lycosoidea, are presented in Table 2. The core spider-specific ortholog set, derived from ten spiders and one amblypygid, resulted in 4446 spider-specific pHMMs. This figure is smaller than the 4934 OGs obtained by Garrison et al. (2016) owing to our criteria being slightly more stringent.

The five matrices generated from the spider core set of orthologs range in size from 646 to 2581 OGs. The number of OGs, total amino acid length, and percent missing are summarized in Table 3. These numbers are smaller than those obtained by Garrison et al. (2016), again owing to our more stringent criteria, but each dataset is longer in length due to our narrower taxonomic scope.

All analyses show strong support for the Dionycha (two-clawed spiders, indicated as "D" on Fig. 2), and the Lycosoidea *sensu* Polotow et al. (2015), i.e. including Ctenidae, Lycosidae, Oxyopidae, Pisauridae, Psechridae, and Thomisidae (see Fig. 4). Unlike Garrison et al. (2016), the Pisauridae is monophyletic (although the inclusion of *Dolomedes* is fully supported only on the largest dataset) and the Oxyopidae falls outside of the Lycosidae-Pisauridae clade. Unlike Wheeler et al. (2016), the Psechridae is monophyletic and is sister to the Ctenidae.

In a separate set of analyses, we included the "ctenid" *Cupiennius salei* (SRR880446, (French et al., 2014)) and found that it grouped with the Lycosidae, far from the other ctenids (Fig. S1) – a polyphyly that is

Table 2
Assembly data for sequenced transcriptomes, including number of reads, contigs, average length, and number of TransDecoder predicted proteins. SPID is the number of OGs matched by the spider-specific set of pHMMs.

Species	Reads	Contigs	Av. Length	TransDecoder	SPID
Ctenus corniger	34113263	240527	573.3	85565	3616
Fecenia protensa	20672432	178423	580.9	65179	3630
Hippasa holmerae	35258023	241214	620.4	87465	3656
Nilus albocinctus	14143482	130776	604.0	56263	3593
Odo patricius	11673148	121635	591.8	47922	3509
Oxyopes sp.	20199221	165775	536.2	69799	3627
Psechrus singaporensis	19392535	181029	578.5	71193	3619
Sosippus placidus	13465604	143295	597.3	63618	3588
Sphedanus quadrimaculatus	16746904	180516	575.9	69045	3647
Thomisus spectabilis	17500046	125786	577.0	56516	3581

Table 3
Summary of phylogenomic analyses for the five concatenated datasets and the ASTRAL analysis. #OGs: number of orthologous groups; #AAs: sum of amino acids in final concatenated alignment.

Analysis	#OGs	#AAs	% Missing	Log likelihood
Matrix 1: All genes	2581	822,142	18.77%	−7,796,144.01
Matrix 2: 1st reduce	1291	523,525	12.47%	−4,887,882.43
Matrix 3: 2nd reduce	646	314,446	9.92%	−2,768,450.39
Matrix 4: BaCoCa	1290	474,416	10.61%	−4,591,021.35
Matrix 5: MARE	1387	480,201	12.04%	−3,965,515.72
ASTRAL Analysis	2581	–	–	–

in keeping with other morphological and molecular results, e.g. (Albo et al., 2017; Polotow et al., 2015; Wheeler et al., 2016). However, given that this transcriptome was extracted from a small portion of leg hypodermis, as opposed to a whole body, the narrower, limited scope of expressed genes has a destabilizing effect on the rest of the tree, so we decided to exclude it from our main reported analysis (Fig. 4). It could be that deep sequencing from very specific tissue types introduces paralogs with conflicting histories.

Unlike Garrison et al. (2016), the oxyopids and thomisids are together monophyletic; however this relationship is only supported by the concatenated datasets and not the ASTRAL analysis, which is also the case for hybrid enrichment analysis (Maddison et al., 2017). Additionally, this clade collapses with the inclusion of the *Cupiennius* transcriptome from leg hypodermis (Fig. S1). The close affinity of xenoctenids, miturgids, and salticids, the former represented by *Odo patricius*, is consistent with hybrid enrichment results with miturgids represented by *Zora spinimana* (Maddison et al., 2017).

4. Discussion

Prior phylogenetic efforts using the usual set of mitochondrial and nuclear genes have failed to recover consistent patterns of relationship among lycosoid spiders, particularly with respect to psechrids (Fig. 3). In most cases they don't confirm psechrid monophyly (Bayer and Schonhofer, 2013; Blackledge et al., 2012a; Moradmand et al., 2014; Wheeler et al., 2016), often reporting close affinities with oxyopids (Fig. 3a, c) and thomisids (Fig. 3e). Our phylogenomic results (Fig. 4) are consistent with prior results using transcriptomics (Bond et al., 2014; Fernandez et al., 2014; Garrison et al., 2016) and anchored hybrid enrichment (Maddison et al., 2017). We improve on Garrison et al. (2016), in that we recover monophyletic support for pisaurids (though the inclusion of *Dolomedes* is not fully supported) and we find that oxyopids do not group with ctenids. Our results provide independent confirmation to the findings of Agnarsson et al. (2013) and Maddison et al. (2017) that miturgids are sister group to salticids.

That psechrids are confirmed as monophyletic lends credence to the hypothesis that the cone web of juvenile *Fecenia* is derived from a sheet web, akin to what *Psechrus* weaves. This hypothesis proposes that the sequence in web evolution starts with a sheet web without a leaf or detritus retreat evolving into a cone web with a detritus retreat at its apex, and then flattening the cone into a disk with a vertical reorientation and the use of a leaf retreat instead of detritus (Robinson and Lubin, 1979). The monophyly of psechrids also cements confidence in morphological synapomorphies, e.g. both genera having relatively long legs I and II, with the tibia of legs I being more than three times the width of the carapace; having dense cymbial scapulae; having a nearly straight posterior eye row; and having claw tufts with three claws (Griswold, 1993).

The close affinity of psechrids and ctenids has never before been suggested by molecular data alone (e.g. Bayer and Schonhofer, 2013; Wheeler et al., 2016), but is supported by some analyses that include morphological data (e.g. Polotow et al., 2015). In Polotow et al. (2015) this relationship is supported by a number of synapomorphies, including having more than three retromarginal cheliceral teeth, a subtegulum lobe, a condyle at base of embolus that interlocks with the subtegular retromargin, a fixed embolus base with a sclerotized attachment to the tegulum, four pairs of ventral spines on metatarsus I, and five or more pairs of ventral spines on tibia I. This affinity is strengthened by the fact that unlike lycosids and pisaurids, some ctenids (e.g. *Acanthoctenus*) share with psechrids a cribellum and oval calamistrum.

Female ctenids, lycosids, and pisaurids, being largely cursorial, are typically obliged to carry around their egg sacs: lycosids, trechaleids,

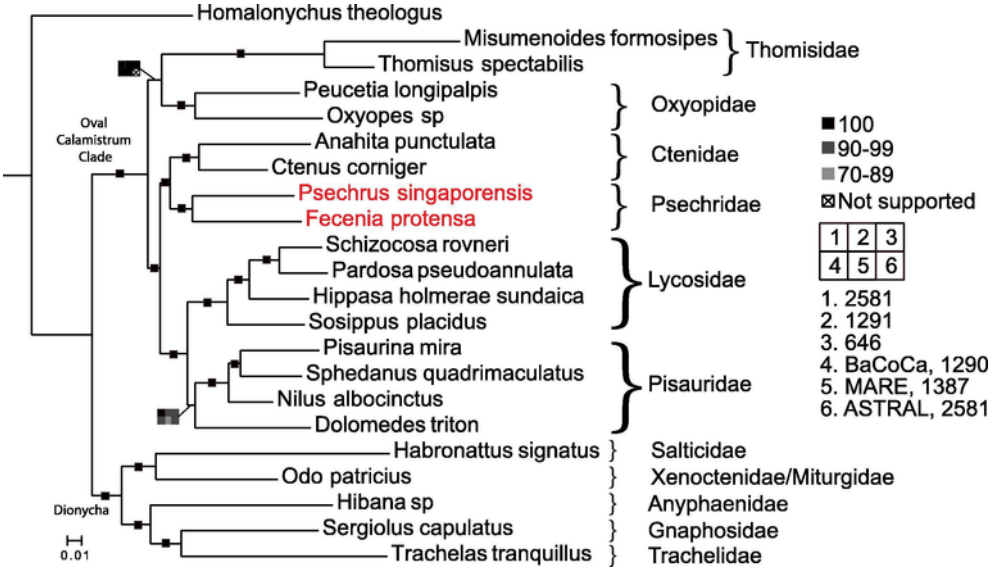

Fig. 4. Summary result for the relationships among the Lycosoidea using the ExaML analysis of dataset 1. Box plots indicate node-specific bootstrap value ranges for each analysis of matrices 1–5, plus the ASTRAL analysis. Nodes with a single solid block indicates bootstrap values of 100% for all analyses.

and *Cupiennius* attached to their spinnerets; other ctenids and pisaurids in their chelicerae. That *Psechrus* carries its egg sacs in its chelicerae, despite living in sheet webs and no longer being cursorial, suggests an affinity with pisaurids and ctenids (Agnarsson et al., 2013; Levi, 1982). Our prior analysis that included *Cupiennius salei* placed this species separate from other ctenids and instead sister to lycosids, thereby uniting taxa that have lost the habit of carrying egg sacs in their chelicerae and instead attach them to their spinnerets. Naturally, *Fecenia* has also lost the egg sac carrying habit, as this would be physically awkward in the context of navigating around a mostly vertical orb web: it prefers to attach it to the inside of the leaf retreat as a flattened disk near the entrance (Robinson and Lubin, 1979). Thus, it would seem that cheliceral egg sac carrying first evolved at the base of the clade uniting ctenids, psechrids, lycosids, and pisaurids, and then later was lost in *Fecenia* and some ctenids (e.g. *Enoploctenus*, *Acanthoctenus*), and further evolved into spinneret-carrying in the case of the *Cupiennius*-trechaleid-lycosid clade.

Although ctenids, lycosids, and pisaurids are generally considered cursorial hunters, it is not unusual for some species to use irregular ground sheets, such as sheet webs or funnel webs – e.g. *Sosippus*, *Hippasa*, and *Sphedanus* in our phylogeny. The use of ground sheets is particularly common in the marronoid clade (Wheeler et al., 2016), which is sister to the oval calamistrum clade and the dionychans (indicated on Fig. 2 as "M," "O," and "D" respectively). Moving back in time, we eventually reach cribellate orb weaving (i.e. using woolly microfibers to achieve stickiness) as an ancestral condition (Garrison et al., 2016). This pattern of web use across the phylogeny of araneomorph spiders raises the possibility that strict dependence on silk for prey capture is an ancestral condition, and that it is subsequently lost many times within RTA clade spiders. Could lycosoid spiders that are likewise dependent on webs – particularly those that use cribellate silk to trap prey, such as psechrids – have inherited this trait from deep ancestry, or are these reinventions of web use? The density of our taxon sampling is far too sparse to trace these characters with confidence, but the fact that psechrids have distal adhesive setae in the form of claw tufts, despite being completely web-dependent, suggests that they had cursorial ancestors that only later reverted to web building (Wolff et al., 2013).

Prior research concluded that because the MaSp2 spidroin had evolved in the orb-weaving "Orbiculariae" clade, completely separate from the RTA-clade (see Fig. 2 Left), it followed that *Fecenia*'s capture silk could never achieve the advanced mechanical performance of other orb weavers (Blackledge et al., 2012a). This reasoning, however, was based on an incorrect phylogeny as revealed by recent phylogenomic work (Bond et al., 2014; Fernandez et al., 2014; Garrison et al., 2016). Furthermore, that MaSp2 has been reported in the pisaurid *Euprosthenops australis* (Rising et al., 2007), suggests that it's quite possible for RTA-clade spiders, such as *Fecenia*, to have inherited MaSp2 from orb-weaving ancestors. However, it is premature to determine whether *Fecenia* specifically expresses MaSp2 without a detailed reevaluation of spidroin gene families, nomenclature, and orthology assignment. Recent work reports that many more MaSp gene copies are expressed in spiders than previously described, with the eresid *Stegodyphus mimosarum* having 11 MaSp genes (Sanggaard et al., 2014) and the araneid *Nephila clavipes* having 8 MaSp genes (Babb et al., 2017). Future research should investigate whether silk proteins, behaviors, and other innovations in true orb weavers (that were the antecedents of lycosoids) later facilitated the evolution of orb weaving in *Fecenia*. For example, it remains to be seen whether details of psechrid web-building behavior show signatures of reinvention or whether a subset of these behaviors may have been resurrected as atavisms.

5. Conclusion

Phylogenomic analysis of lycosoid transcriptomes results in a well-supported tree that is wholly different from all prior analyses based on traditional molecular markers, but similar to the most recent analyses based mostly on morphological data (Polotow et al., 2015). The Psechridae emerge as monophyletic and sister to the Ctenidae (minus *Cupiennius*). The Lycosidae and Pisauridae are each monophyletic sister groups. The Thomisidae and Oxyopidae are lycosoids that arise outside of the Ctenidae, Psechridae, Lycosidae, and Pisauridae clade – the latter clade characterized by females that carry their egg sacs. The claw tufts of Psechrids and the retention of egg sac carrying in *Psechrus* suggests that this family evolved from cursorial ancestors. Although the evolution of orb web prey capture by *Fecenia* is, therefore, largely *de novo*, since they have distant ancestors that once built orb webs it is still possible that they have retained or resurrected adaptations in common with true orb weavers.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.03.035.

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Glossary

Calamistrum : a row of specialized leg bristles on metatarsus of the hind legs used to comb out cribellate silk.
Cribellum : a silk-spinning organ that produces an ultra-fine wool to confer stickiness.
Lycosoidea : a superfamily of about seven araneomorph spider families, including wolf spiders, that are traditionally circumscribed by having grate-shaped tapeta in their secondary eyes.
RTA-Clade : A clade of descendants of araneomorph spiders that evolved a retrolateral tibial apophysis on the male pedipalp.

Appendix A. Supplementary material

Supplementary Fig. S1 — Summary result for the relationships among the Lycosoidea using the ExaML analysis of dataset 1, including *Cupiennius salei*. The inclusion of *C. salei*, which is from a deeply sequenced transcriptome of leg hypodermis (SRA accession SRR880446, (French et al., 2014)), appears to have destabilized the tree as compared with Fig. 4: each clade within the lycosids are no longer fully supported, and monophyly for each of psechrids and pisaurids is weakened. Additionally, oxyopids and thomisids no longer form a separate clade. Box plots indicate node-specific bootstrap value ranges for each analysis of matrices 1-5, plus the ASTRAL analysis. Nodes with a single solid block indicates bootstrap values of 100% for all analyses.