Syst. Biol. 68(4):555–572, 2019

DOI:10.1093/sysbio/syy082
Advance Access publication December 4, 2018

Golden Orbweavers Ignore Biological Rules: Phylogenomic and Comparative Analyses Unravel a Complex Evolution of Sexual Size Dimorphism

MATJAŽ KUNTNER[1,2,3,4,*], CHRIS A. HAMILTON[5], REN-CHUNG CHENG[2,6], MATJAŽ GREGORIČ[2], NIK LUPŠE[2,7], TJAŠA LOKOVŠEK[2], EMILY MORIARTY LEMMON[8], ALAN R. LEMMON[9], INGI AGNARSSON[3,10], JONATHAN A. CODDINGTON[3], AND JASON E. BOND[11]

[1]*Evolutionary Zoology Laboratory, Department of Organisms and Ecosystems Research, National Institute of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia;* [2]*Evolutionary Zoology Laboratory, Biological Institute ZRC SAZU, Novi trg 2, SI-1001 Ljubljana, Slovenia;* [3]*Department of Entomology, National Museum of Natural History, Smithsonian Institution, 10th and Constitution, NW, Washington, DC 20560-0105, USA;* [4]*Centre for Behavioural Ecology and Evolution, College of Life Sciences, Hubei University, 368 Youyi Road, Wuhan, Hubei 430062, China;* [5]*Department of Entomology, Plant Pathology, & Nematology, University of Idaho, 875 Perimeter Dr. MS 2329, Moscow, ID 83844-2329, USA;* [6]*Department of Life Sciences, National Chung Hsing University, No.145 Xingda Rd., South Dist., Taichung City 402, Taiwan;* [7]*Division of Animal Evolutionary Biology, Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague, Czech Republic;* [8]*Department of Biological Science, Florida State University, 319 Stadium Dr., Tallahassee, FL 32306-4295, USA;* [9]*Department of Scientific Computing, Florida State University, 400 Dirac Science Library, Tallahassee, FL 32306-4120, USA;* [10]*Department of Biology, University of Vermont, 316 Marsh Life Science Building, 109 Carrigan Drive, Burlington, VT 05405-0086, USA; and* [11]*Department of Entomology and Nematology, University of California Davis, 1 Shields Drive, Davis, CA 95616, USA*

[*]*Correspondence to be sent to: Evolutionary Zoology Laboratory, Department of Organisms and Ecosystems Research, National Institute of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia;*
E-mail: matjaz.kuntner@nib.si.

Received 05 July 2018; reviews returned 20 November 2018; accepted 26 November 2018
Associate Editor: Adrian Paterson

Abstract.—Instances of sexual size dimorphism (SSD) provide the context for rigorous tests of biological rules of size evolution, such as Cope's rule (phyletic size increase), Rensch's rule (allometric patterns of male and female size), as well as male and female body size optima. In certain spider groups, such as the golden orbweavers (Nephilidae), extreme female-biased SSD (eSSD, female:male body length ≥2) is the norm. Nephilid genera construct webs of exaggerated proportions, which can be aerial, arboricolous, or intermediate (hybrid). First, we established the backbone phylogeny of Nephilidae using 367 anchored hybrid enrichment markers, then combined these data with classical markers for a reference species-level phylogeny. Second, we used the phylogeny to test Cope and Rensch's rules, sex specific size optima, and the coevolution of web size, type, and features with female and male body size and their ratio, SSD. Male, but not female, size increases significantly over time, and refutes Cope's rule. Allometric analyses reject the converse, Rensch's rule. Male and female body sizes are uncorrelated. Female size evolution is random, but males evolve toward an optimum size (3.2–4.9 mm). Overall, female body size correlates positively with absolute web size. However, intermediate sized females build the largest webs (of the hybrid type), giant female *Nephila* and *Trichonephila* build smaller webs (of the aerial type), and the smallest females build the smallest webs (of the arboricolous type). We propose taxonomic changes based on the criteria of clade age, monophyly and exclusivity, classification information content, and diagnosability. Spider families, as currently defined, tend to be between 37 million years old and 98 million years old, and Nephilidae is estimated at 133 Ma (97–146), thus deserving family status. We, therefore, resurrect the family Nephilidae Simon 1894 that contains *Clitaetra* Simon 1889, the Cretaceous *Geratonephila* Poinar and Buckley (2012), *Herennia* Thorell 1877, *Indoetra* Kuntner 2006, new rank, *Nephila* Leach 1815, *Nephilengys* L. Koch 1872, *Nephilingis* Kuntner 2013, *Palaeonephila* Wunderlich 2004 from Tertiary Baltic amber, and *Trichonephila* Dahl 1911, new rank. We propose the new clade Orbipurae to contain Araneidae Clerck 1757, Phonognathidae Simon 1894, new rank, and Nephilidae. Nephilid female gigantism is a phylogenetically ancient phenotype (over 100 Ma), as is eSSD, though their magnitudes vary by lineage. [Body size evolution; female gigantism; web asymmetry; Cope's rule; Rensch's rule; Nephilidae; Phonognathidae; Orbipurae.]

Evolution of body size is often attributed to biological laws. Rensch postulated phyletic increase in body size, that is, size increase in evolutionary time, and referred to it as Cope's rule (Rensch 1948), now attributed to Depéret (Bokma et al. 2016). Authors disagree on whether the rule is generally valid or even biologically meaningful (Stanley 1973; Gould 1997), at what phylogenetic scale it may be applied (Heim and Knope 2015), and, importantly, how to test it (Hone and Benton 2005). Interpretations of this rule range from overall short-term fitness advantages of larger body size (Waller and Svensson 2017) to long-term size increases over geologic time (Hunt and Roy 2006).

Sexual size dimorphism (SSD) often seems to be correlated with extreme morphological, behavioral, and life history phenotypes in either sex. In female-biased size dimorphic organisms, SSD is defined as female-to-male body size ratio (or correlated body parts; Fairbairn 2007). Extreme, female-biased values of SSD (we term those SSD values that exceed 2.0 as eSSD) are rare in any animal group and eSSD provides a heuristic definition to identify extreme phenotypes (Scharff and Coddington 1997; Kuntner and Cheng 2016). In certain spider groups, eSSD is the norm, and values exceeding 5.0 are common (Hormiga et al. 2000). Such clades are the most extreme (eSSD) examples among all terrestrial animals (Kuntner and Elgar 2014), and thus figure prominently in studies of gendered body size evolution (Vollrath and Parker 1992; Coddington et al. 1997; Foellmer and Moya-Laraño 2007).

Prior studies have utilized nephilid and argiopine spider phylogenies to investigate patterns of sex-specific size evolution (Kuntner and Coddington 2009; Cheng and Kuntner 2014; Kuntner and Elgar 2014), but shared macroevolutionary patterns are scarce (Kuntner and Cheng 2016). For example, nephilid females and males were thought to grow larger phyletically, with the slope of female size evolution being steeper, thus eSSD was maintained (Kuntner and Elgar 2014). In argiopines (Araneidae), phyletic size change showed no net trend in either sex and eSSD declined over time (Cheng and Kuntner 2014). Nephilids, but not argiopines, seem to follow Cope's rule. These prior studies of reconstructed size evolution concluded that argiopine size, nephilid female size, and SSD drifted randomly in time (i.e., they appear to follow a model of Brownian motion). However, nephilid male size fit a single optimum, under an Ornstein–Uhlenbeck model (Kuntner and Cheng 2016). Male and female nephilid sizes evolved independently (Kuntner and Coddington 2009; Higgins et al. 2011; Kuntner and Elgar 2014), but in argiopines, male and female sizes were significantly correlated (Cheng and Kuntner 2014; Kuntner and Cheng 2016).

In many vertebrates, variation in male size contributes more to SSD than does variation in female size, but the opposite is true in the cases of female-biased SSD, as seen in numerous invertebrates (Abouheif and Fairbairn 1997). This observation is formulated in Rensch's rule that predicts positive allometry for male size versus female size in male-size dimorphic animals, either within species or clades, whereas the converse Rensch's rule predicts negative allometry in female-size dimorphic lineages (Abouheif and Fairbairn 1997; Fairbairn 1997; Blanckenhorn et al. 2007a). However, Rensch's rule has not been found to hold in spiders at any phylogenetic level or fauna (Foellmer and Moya-Laraño 2007; Cheng and Kuntner 2014). Specifically, argiopines were isometric and nephilid sizes were uncorrelated (Kuntner and Cheng 2016).

As the ratio of gendered sizes, SSD is probably not a single trait under selection. Therefore, SSD is best considered as an epiphenomenon of potentially complex, taxon-specific, and evolutionary changes in the size of each gender. The ratio is plausibly selected only if the direct interaction of males and females of different sizes affects fitness (Ramos et al. 2005; Lupše et al. 2016). Spider size variation in each gender can be caused by multiple proximate causes (Kuntner and Elgar 2014; Kuntner and Cheng 2016). Selection for larger, more fecund females, larger and stronger males, or for smaller, more agile males are all credible drivers of size evolution (Elgar 1991; Vollrath and Parker 1992; Head 1995; Higgins 2002; Moya-Laraño et al. 2002, 2009; Foellmer and Moya-Laraño 2007; Danielson-François et al. 2012; Cheng and Kuntner 2015). However, each factor may select for a different size, so that net selection on size could be equivocal (Kuntner and Elgar 2014).

Spider webs are, in an ecological sense, extensions of a spider's phenotype and have evolved in diverse ways (Blackledge et al. 2011). Web characteristics can plausibly affect somatic trait evolution, but studies that statistically test links between body size and web characteristics across diverse species are rare, and outdated (see Eberhard's 1990 review). Nephilids are ideal models for addressing such evolutionary questions (Table 1). The golden orbweavers, genus *Nephila*, are conspicuous tropical spiders (Kuntner 2017). Massive, colorful females construct their characteristic orb web with a golden shine, and these webs are unusually large, often over a meter wide (Kuntner et al. 2008). Tiny males, over 10 times smaller, and 100 times lighter than corresponding conspecific females (Kuntner et al. 2012), are less striking (Fig. 1a), and, notoriously, are often cannibalized by females (Elgar 1991; Schneider and Elgar 2001). *Nephila* and related genera (Kuntner et al. 2013) are popular lab animals and their biology is well understood (Fig. 1b,c). Because of female body size and web gigantism, nephilids have become models to study extreme phenotypes, especially their traits such as tough silk, large webs, eSSD, and sexually conflicted behaviors (Kuntner et al. 2009, 2016; Blackledge et al. 2011; Kuntner and Elgar 2014). Additionally, *Trichonephila clavipes* (formerly *Nephila*; Table 1) is the first orb web spider with an annotated genome (Babb et al. 2017). By revealing an unprecedented diversity of silk genes and their complex expression, the study by Babb et al. suggested new directions of genetic and biomaterial research.

The evolution of these remarkable traits continues to puzzle biologists, but well-formulated hypotheses are short-lived if phylogenetic estimates continue to change. A robust and resolved species-level phylogeny is crucial to understanding the evolution of extreme phenotypes, and to settle nephilid taxonomy and classification. Published species-level phylogenies attempted precisely that; one based on morphological and behavioral phylogenetic data (Kuntner et al. 2008), and the other two (Su et al. 2011; Kuntner et al. 2013) on a few, commonly used mitochondrial and nuclear markers. Such data sets have well-understood limitations (Agnarsson et al. 2013). Phylogenomics can test these earlier hypotheses of phylogeny with much larger data sets, and perhaps provide a more reliable and accurate understanding of evolutionary history.

The use of molecular data in spider phylogenetics has a relatively short history, with the first large studies starting to appear at the turn of the millennium (reviewed in Agnarsson et al. 2013). Until recently, research was necessarily limited to the few markers that could be amplified across spiders (Agnarsson et al. 2013; Dimitrov et al. 2017; Wheeler et al. 2017), which typically did a poor job recovering older nodes. The phylogenomics era brought forth transcriptomics and targeted sequence capture to begin more rigorously addressing many questions regarding spider evolution (Bond et al. 2014; Fernández et al. 2014, 2018; Garrison et al. 2016; Hamilton et al. 2016a; Starrett et al. 2017; Kallal et al. 2018; Wood et al. 2018). Many formerly stable hypotheses have not survived this data revolution, including the monophyly of orbweavers,

TABLE 1. Currently valid contemporary genera and species of the family Nephilidae, including taxonomic changes proposed here

Genus	Species	Author	Comment
Clitaetra		Simon 1889	
	clathrata	Simon 1907	
	*episinoides**	Simon 1889	
	irenae	Kuntner 2006	
	perroti	Simon 1894	
	simoni	Benoit 1962	
Herennia		Thorell 1877	
	agnarssoni	Kuntner 2005	
	deelemanae	Kuntner 2005	
	etruscilla	Kuntner 2005	
	gagamba	Kuntner 2005	
	jernej	Kuntner 2005	
	milleri	Kuntner 2005	
	*multipuncta**	Doleschall 1859	
	oz	Kuntner 2005	
	papuana	Thorell 1881	
	sonja	Kuntner 2005	
	tone	Kuntner 2005	
Indoetra		Kuntner 2006	New rank
	*thisbe**	Simon 1903	New combination
Nephila		Leach 1815	
	constricta	Karsch 1879	
	*pilipes**	Fabricius 1793	
Nephilengys		L. Koch 1872	
	*malabarensis**	Walckenaer 1842	
	papuana	Thorell 1881	
Nephilingis		Kuntner 2013	
	borbonica	Vinson 1863	
	*cruentata**	Fabricius 1775	
	dodo	Kuntner & Agnarsson 2011	
	livida	Vinson 1863	
Trichonephila		Dahl 1911	New rank
	antipodiana	Walckenaer 1842	New combination
	clavata	L. Koch 1878	New combination
	*clavipes**	Linnaeus 1767	New combination
	edulis	Labillardière 1799	New combination
	fenestrata	Thorell 1859	New combination
	inaurata	Walckenaer 1842	New combination
	komaci	Kuntner & Coddington 2009	New combination
	plumipes	Latreille 1804	New combination
	senegalensis	Walckenaer 1842	New combination
	sexpunctata	Giebel 1867	New combination
	sumptuosa	Gerstäcker 1873	New combination
	turneri	Blackwall 1833	New combination

Note: The genus *Nephila* includes additional names that are thought to be synonyms of *Nephila* or *Trichonephila* species listed above. Type species indicated by asterisks.

relationships of primitive araneomorphs, patterns of spider diversification, and ages of major spider groups (Bond et al. 2014; Fernández et al. 2014; Garrison et al. 2016; Maddison et al. 2017). While relationships among spider families seem to be stabilizing, few species-level phylogenomic studies have been published (Hamilton et al. 2016b; Chamberland et al. 2018).

Here, we use anchored hybrid enrichment (AHE) methodology (Lemmon et al. 2012) to provide a well-corroborated species-level phylogeny and to estimate lineage ages. We test the reciprocal monophyly of Nephilidae *sensu* Kuntner (2006) and Kuntner et al. (2013) and its genera using 22 ingroup taxa, then use this constrained backbone phylogeny to place an additional nine nephilid species using the classical markers (Kuntner et al. 2013; total nephilid diversity ~37 spp.). We use this nephilid topology to test hypotheses on body and web form and size evolution, detailed

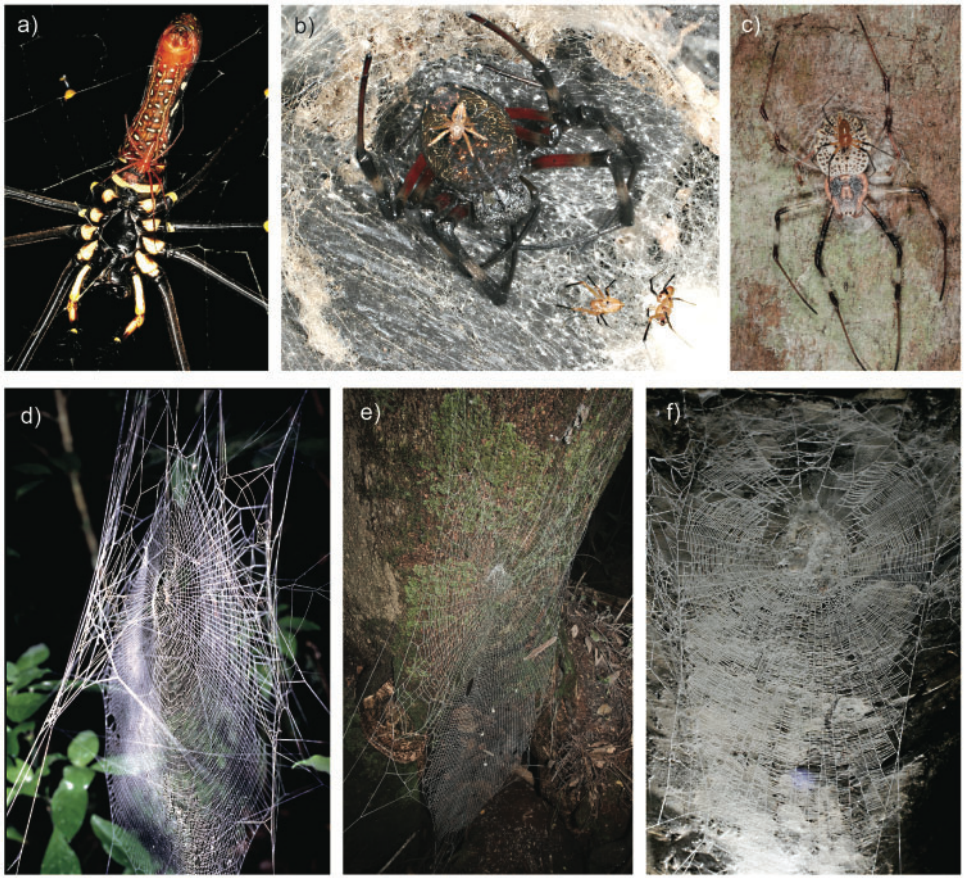

FIGURE 1. Representatives of nephilid genera, their sexual size dimorphism, and web types: (a) *Nephila pilipes* male on a female; (b) *Nephilingis cruentata* males accumulating around a female; (c) *Herennia multipuncta* male sitting on a female; (d) aerial orb web of *Trichonephila clavipes*; (e) hybrid web of *Nephilingis livida*; and (f) arboricolous ladder web of *Clitaetra episinoides*.

below. We also infer nephilid age using the 97–100 Ma monotypic Cretaceous Burmese amber *Geratonephila burmanica* Poinar 2012 as a constraint. The phylogeny represents the foundation to correct and refine the taxonomy of Nephilidae (Table 1). And finally, given the new phylogeny, we pose three broad questions: 1) Do male and female size evolve independently, do they evolve toward optima, and how does their evolution affect SSD?; 2) Do nephilids obey Cope's rule and the converse Rensch's rule?; 3) What is the relationship among spider body size and web size, web types, web architecture, and SSD?

MATERIALS AND METHODS

Phylogenomics

We employed the AHE targeted-sequencing approach for spiders (outlined in Hamilton et al. 2016a) to target 585 single copy orthologous loci from across the genome. These loci have been shown to possess sufficient variation for resolving both shallow and deep-scale evolutionary relationships throughout the Araneae. Hamilton et al. (2016a), Maddison et al. (2017), and Godwin et al. (2018) have used AHE to recover genus and species-level relationships within spider families, Theraphosidae, Salticidae, and Halonoproctidae/Ctenizidae.

We obtained AHE sequence data for 22 nephilids (representing 59% of the known 37 total species; see Table 1), and for 11 outgroups. High-quality genomic DNA (≥1 μg) for all specimens was extracted from leg tissue stored in ≥95% ethanol at -80°C, using an optimized protocol on the MagMAX Express magnetic particle processor robot (Vidergar et al. 2014). DNA concentration was evaluated through agarose gel electrophoresis and spectrophotometry using a NanoDrop ND-1000. Not all ingroup nephilids were sequenced due to library prep or sequencing failure, or lack of specimens for sufficient quality tissues or DNA—as some taxa are rare in molecular collections.

AHE data, including library preparation, enrichment, and sequencing, were generated at the Center for Anchored Phylogenomics at Florida State University (www.anchoredphylogeny.com) following Lemmon et al. (2012), Prum et al. (2015), and Hamilton et al. (2016a). Up to 500 ng of each DNA sample was sonicated to a fragment size of approximately 300–800 bp using a Covaris E220 ultrasonicator. Indexed

libraries were then prepared following Meyer and Kircher (2010), but with modifications for automation on a Beckman-Coulter Biomek FXp liquid-handling robot (see Hamilton et al. 2016a for details). Size-selection was performed after blunt-end repair using SPRI select beads (Beckman-Coulter Inc.; 0.9x ratio of bead to sample volume). Indexed samples were pooled at equal quantities (16 samples per pool), and then each pool was enriched using the AHE Spider Probe kit v1 (Hamilton et al. 2016a) and a modified v2 (C. A. Hamilton et al, unpublished), which refines the previous v1 capture probes to capture the same loci but yield greater enrichment within araneomorph spiders. After enrichment, the two reactions were pooled in equal quantities and sequenced on one PE150 Illumina HiSeq 2500 lane (35.2 Gb) at Florida State University Translational Science Laboratory in the College of Medicine. Prior to assembly, overlapping paired reads were merged following Rokyta et al. (2012). For each read pair, the probability of obtaining the observed number of matches by chance was evaluated for each possible degree of overlap. The overlap with the lowest probability was chosen if the *P*-value was less than 10^{-10}, a stringent threshold that helps avoid chance matches in repetitive regions (Rokyta et al. 2012). Read pairs failing to merge were utilized but left unmerged during the assembly. Subsequent bioinformatic pipelines (data processing, sequence assembly, quality control, orthology search, and alignment) follow Hamilton et al. (2016a), with contigs derived from fewer than 20 reads being removed before orthology assessment. Alignments were performed in MAFFT v. 7 (Katoh and Standley 2013) with gaps treated as missing characters.

We defined two AHE data sets, based on "strict" and "loose" trimming/masking thresholds ("strict": goodSites = 14, propSame = 0.5, missingAllowed = 5; "loose": goodSites = 16, propSame = 0.6, missingAllowed = 11) in order to evaluate matrix occupancy on phylogeny estimation. The "strict" data set comprised 206 loci (42,396 bp total sites; 13,338 informative sites) with 9% missing data, while the "loose" consisted of 367 loci (89,212 bp total sites; 27,129 informative sites) with 22% missing data, each with the same 34 taxa. We partitioned the data by locus and concatenated, with the resulting supermatrix analyzed using maximum likelihood in IQ-TREE v1.4.2 (Nguyen et al. 2015) using the –m TEST command and 1000 rapid bootstraps. IQ-TREE analyses allow for a much larger suite of evolutionary models to be tested for and applied per locus/partition. RogueNaRok (Aberer et al. 2013) was used to investigate the presence of rogue taxa and whether they were influencing any part of the inference or topology. In addition to analyzing the supermatrix data, we employed ASTRAL v4.10.12 (Mirarab and Warnow 2015), a genome-scale coalescent-based species tree estimation, on both "loose" and "strict" individual gene trees inferred using IQ-TREE, and employing the same parameters as the supermatrix (above). We evaluated the AHE topologies for topological congruence/disagreement and clade support, selecting a representative AHE topology to be used as the backbone constraint.

Lastly, we added nine nephilid species that lacked AHE data (explained above), but for which three loci (*cox1*, 16S rRNA, and ND1) were available (Kuntner et al. 2013). Using the AHE topology as a constraint, we placed *Herennia papuana* and *H. tone*, *Nephilingis borbonica* and *N. livida*, *Trichonephila inaurata*, *T. senegalensis*, and *T. clavata*, *Clitaetra episinoides* and *Indoetra thisbe*. This chimaeric data set was created by merging the "strict" data set with a legacy data set of three standard loci from previous Nephilidae phylogeny inference (*cox1*, 16S rRNA, and ND1; Kuntner et al. 2013), where all tips included these three loci. A simplified backbone phylogeny was created ("strict" and "loose" inferences produced identical topologies), by stripping branch lengths and simplifying names. This tree was then used as a constraint for the chimaeric inference. The total data set comprised 209 loci (44,579 bp) for 45 taxa. The combined "constraint" analysis placed a total of 31 nephilid species representing 84% of nephilid species diversity (Table 1) within a reference, species-level phylogeny, and was inferred using IQ-TREE with the same parameters as above. All consensus sequences, alignments, tree files, and scripts are available on Dryad at http://dx.doi.org/10.5061/dryad.h07s9d1.

Divergence Estimation

Lineage divergence times were estimated using the RelTime maximum likelihood method explicitly proposed for dating nodes from large phylogenomic data sets (Tamura et al. 2012). In order to evaluate consistency, divergence times were estimated for both the 367 loci ("loose") supermatrix, to include more putatively informative sites for dating, and the 206 loci ("strict") data set. The "constraint" data set was not evaluated due to increases in missing data for those taxa that were added. Each ML best tree was used as the reference topology for each respective analysis; topologies were identical between "strict" and "loose". Local clocks were used for each lineage, with no clock rates merged. The HKY substitution model was employed with gamma distributed rates among sites (and five discrete gamma categories). Partial deletion was used, with a cutoff of 50% missing data at a site.

Due to the paucity of available or informative fossils, we explored a suite of different program parameters and fossil calibration min/max boundaries (see Discussion section for more detail). Two nodes were calibrated. To set a minimum age on the *Trichonephila* clade, we reevaluated fossils from the Dominican amber described as *Nephila*: *N. dommeli* Wunderlich 1982, *N. breviembolus* Wunderlich 1986, *N. furca* Wunderlich 1986, *N. tenuis* Wunderlich 1986, and *N. longembolus* Wunderlich 1986. Judging from their male palpal anatomical details as featured in Wunderlich (1986), these are indeed nephilids (Kuntner et al. 2013), although precise genus placement is unclear for the first three listed species.

On the other hand, *N. tenuis* and *N. longembolus* fit well the *Trichonephila* morphological diagnosis, their embolic conductor length and curvature closely resembling the African *T. senegalensis* and *T. sumptuosa*, respectively (Kuntner 2005). The estimated ages for the Dominican amber can therefore calibrate the *Trichonephila* clade as defined in this paper. Because we cannot assume when a lineage might have split or gone extinct, we set a hard minimum of 16 Ma (the age of the fossils), and a softer maximum age of 23 Ma on the *Trichonephila* clade. This boundary was an attempt to account for date flexibility, aging the fossils slightly older than the rock where they were recovered.

Kuntner et al. (2013) used the age of *Palaeonephila* Wunderlich 2004 from Baltic amber (Wunderlich 2004a) as a minimum bound for the stem group of nephilids. They assessed at least some of the four described species of *Palaeonephila* as potentially true nephilids: "Morphological evidence placing the nephilid fossils described from Baltic amber (Wunderlich 2004) is inconclusive, except in the case of *Palaeonephila*, which exhibits a striated cheliceral condyle (boss), as do all extant nephilids…". Here, we evaluate a much older fossil to date early nephilid divergences. *Geratonephila burmanica* Poinar 2012, from early Cretaceous Burmese amber, is thought to be an ancestral nephilid (Poinar and Buckley 2012). The strongest nephilid synapomorphy is striae on the cheliceral boss (Kuntner et al. 2008), but this feature is not mentioned or visible in the *Geratonephila* description—cheliceral striae are rarely visible in any amber specimen (but, see *Palaeonephila* above). Striae are difficult to see even in extant male nephilids, and, in any case, our request to examine the type was ignored. The palpal morphology resembles a nephilid because the embolic conductor fully encloses the embolus, the cymbium is cup shaped, the paracymbium has an apophysis, and the bulb lacks the tegular apophyses typical of araneids and phonognathids. In fact, the *G. burmanica* palp closely resembles *C. episinoides* from the Comoros (compare Poinar and Buckley's 2012 figure 3 with Kuntner (2006) figure 13). Both have globular cymbia mislabeled as the tegulum by Poinar and Buckley (P&B), similar paracymbia (not labeled by P&B), tegula (mislabeled as subtegulum by P&B), embolic bases (not labeled by P&B), and embolic conductors (not labeled by P&B). The *Geratonephila* tegulum and the embolic conductor seem to be rotated out of their usual position. *Geratonephila* could be an early offshoot of *Clitaetra*, but since we were unable to verify the type specimen, we conservatively treat it as a stem nephilid.

To set an age for the Cretaceous *Geratonephila*, a hard minimum boundary of 97 Ma was set corresponding to the minimum age of the Burmese amber as suggested by geological evidence (Shi et al. 2012; Poinar 2018; see Discussion section), as well as a softer maximum boundary at 146 Ma, the beginning of the Cretaceous. If *G. burmanica* was treated as a stem *Clitaetra* at 146–97 Ma, the dated splits would be vastly older than those in published phylogenomic analyses (Bond et al. 2014; Garrison et al. 2016). Our more conservative dating scheme placing *Geratonephila* at the stem of the Nephilidae appears to be a more justifiable placement.

Body Size

We measured the total body length, carapace length and width, and first leg patella + tibia length for a total of 480 males and females of the 28 nephilid species that are known from both sexes (males and females are known for 76% of nephilid species, all measured here; Supplementary Table S1 available on Dryad). We calculated SSD as the ratio of female to male values for the above measurements (Supplementary Table S1 available on Dryad), then translated SSD to the sexual dimorphism index (SDI) as SSD - 1 (Cheng and Kuntner 2014; Kuntner and Cheng 2016) for the use in comparative analyses.

Web Types and Size

Nephilids spin three major types of webs. *Nephila* and *Trichonephila* spin large (often ≥100 cm diameter), completely aerial orb webs (Fig. 1d) (Kuntner et al. 2008; Kuntner 2017). The nephilid aerial web differs from typical orb architecture (e.g., Araneidae and Tetragnathidae) in details of radii, frames, and spirals, but especially in its asymmetrically placed hub. In contrast, the arboricolous ladder web of *Herennia* and *Clitaetra* (Fig. 1f) is least similar to orbs. These ladder-shaped webs are spun only on tree trunks. They have parallel (rather than converging) side frames attached to the trunk, relatively horizontal, parallel "spirals," and hubs that attach to the substrate (Kuntner 2005, 2006). The third architecture, typical of *Nephilengys* and *Nephilingis*, is intermediate between the first two, neither fully aerial nor substrate bound ("hybrid"; Fig. 1e). The upper frames and hubs attach to substrate (e.g., trees or house roofs), but their aerial capture areas are rich in radii and spirals (Kuntner 2007; Kuntner and Agnarsson 2011; Kuntner et al. 2013). Hybrid webs vary in size but can be extremely large: *Nephilingis livida* webs are up to 151 cm high, easily surpassing *Nephila* or *Trichonephila* aerial webs (up to 116 cm), and *Herennia* ladders (up to 100 cm). The heavy female spiders hide in substrate retreats during the day.

In the field, we measured three web parameters: the a) horizontal and b) vertical diameters, as well as the c) distance from the hub to the top edge (Kuntner et al. 2010) for 18 species (representing 47% species diversity; Supplementary Table S2 available on Dryad). We calculated web area (WA) using the formula WA = $(a/2)*(b/2)*\pi$ (Blackledge and Gillespie 2002; Gregorič et al. 2011), the hub displacement (HD) index using the formula HD index = $(b-c)/b$ (Kuntner et al. 2008), and the ladder index (LI) using the formula LI = b/a (Peters 1937; Kuntner et al. 2008). Larger values of HD index and LI imply more asymmetric webs.

Comparative Analyses

Using the reference topology, pruned to include only the ingroup Nephilidae, we analyzed data on body size, WA, and type in a comparative framework. We used maximum likelihood to estimate ancestral state reconstructions of body size and SSD using the 'fastAnc' function in the R package 'phytools' (Revell 2012). To evaluate alternative optimizations, we also inferred ancestral body size and SSD under squared change parsimony in Mesquite v.3.04 (Maddison and Maddison 2015). We used phylogenetically independent contrasts (PICs) in Mesquite to calculate correlations in continuous data. To test Cope's rule, we used a linear regression model that regressed ancestral sizes against cladogenetic events. To test for Rensch's rule, we performed an allometric analysis (Model II regression analysis) using the function "lmodel2" in the R package "lmodel2" (Legendre 2014) with the PICs of $\log_{10}$-transformed body sizes. We ran major-axis regression of male body size on female body size with 10,000 simulations. To test optimum size evolution, we fitted three evolutionary models (Brownian motion vs. single optimum Ornstein–Uhlenbeck vs. Brownian motion with a directional trend) on nephilid size data using the function "fitContinuous" in the R package "geiger" (Harmon et al. 2008). We selected the best fit model using a likelihood ratio test. To examine the relationships between female web characteristics and the size of both sexes and web types, we employed Bayesian analyses of generalized linear mixed models, with phylogeny as a random factor, via the function "MCMCglmm" in the R package "MCMCglmm" (Hadfield 2010). We treated WA, HD index, and LI as dependent variables, and female size and male size, as well as web type, as independent variables. If regression revealed significant differences between web types, additional multiple comparisons of dependent variables among the three web types were calculated. Finally, we tested if body size and SSD differed among web types via "MCMCglmm" analyses. Supplementary Appendix S1 available on Dryad provides the R code for all above analyses.

Results

Phylogeny

AHE phylogenomics inferred robust support for the relationships of 22 nephilid species (Fig. 2a). All analyses on the concatenated data (Supplementary Fig. S1 available on Dryad) ("strict" or "loose"), as well as species trees (Supplementary Fig. S2 available on Dryad), agree on nephilid monophyly, generally with robust bootstrap support throughout. Additionally, no taxa were discovered to be influencing the phylogenetic inference. *Nephilengys*, *Herennia*, and *Nephilingis* are monophyletic and confined to well-defined biogeographical regions: *Nephilengys* + *Herennia* is Australasian, *Nephilingis* is Afrotropical. *Clitaetra* is also monophyletic (but only represented by two Afrotropical species). *Trichonephila* species (Table 1) were all formerly in classical *Nephila*, but our phylogenetic results unequivocally establish this classical *Nephila* as diphyletic (Fig. 2a). These genera, as well as the topological position of the true *Nephila* (i.e., sister to all other Nephilidae), are fully supported in all analyses, the next distal clade separating *Clitaetra* from the remaining nephilids is strongly supported in the concatenated analyses (Fig. 2a; Supplementary Fig. S1 available on Dryad), but relatively weakly in ASTRAL analyses (Supplementary Fig. S2 available on Dryad).

The AHE data (Supplementary Figs. S1–S3 available on Dryad) supports the monophyly of three major araneoid clades, nephilids (NEP), araneids (ARA; represented here by *Araneus, Cyclosa, Verrucosa*, and *Caerostris*), and phonognathids (PHO; represented by *Phonognatha, Deliochus*, and *Leviellus*). These clades are consistently well supported, and, if combined, could be considered as Araneidae *s.l.* (Dimitrov et al. 2017). However, the sister relationships among the three vary, with most concatenated data recovering NEP + ARA (Supplementary Figs. S1 and S3 available on Dryad) and most ASTRAL analyses recovering NEP + PHO (Supplementary Fig. S2 available on Dryad), but always with low support. No analyses support ARA + PHO.

Finally, the maximum likelihood analyses of the "chimaeric data set", created by merging the "strict" data set with a legacy data set of three standard loci from previous Nephilidae phylogeny inference (*cox1*, 16S rRNA, and ND1; Kuntner et al. 2013), resulted in a well-resolved tree with strong support for placement of the additional taxa, our reference species-level phylogeny (Supplementary Fig. S4 available on Dryad). Because the topology does not diverge from our AHE data, we converted this topology to an undated, relative rates ultrametric tree (Fig. 3) for comparative analyses.

Fossil Evidence and Divergence Time Estimation

Time-calibrated analysis using *Geratonephila* places the origin of nephilids well into the Cretaceous, estimated at 133 Ma. The confidence intervals of this estimation *sensu* Tamura et al. (2018) span 97–146 Ma (Fig. 2b). *Herennia* is found to have originated around 26 Ma (13–42), *Nephilengys* around 15 Ma (5–27), *Nephilingis* around 26 Ma (10–45), *Trichonephila* around 60 Ma (36–88), *Clitaetra* around 51 Ma (25–81), and *Nephila* around 25 Ma (10–45). These estimated ages are older than prior analyses (Kuntner et al. 2013), a common trend in the age of phylogenomics (Garrison et al. 2016; Fernández et al. 2018). The origins of Araneidae and Phonognathidae are both estimated at around 140 Ma, although with large confidence intervals—a theoretical issue investigated by Tamura et al. (2018) (Fig. 2b), and low taxonomic sampling.

Taxonomy

Our primary rationales for taxonomic decisions are monophyly and estimated node age (Fig. 2b). Ages of

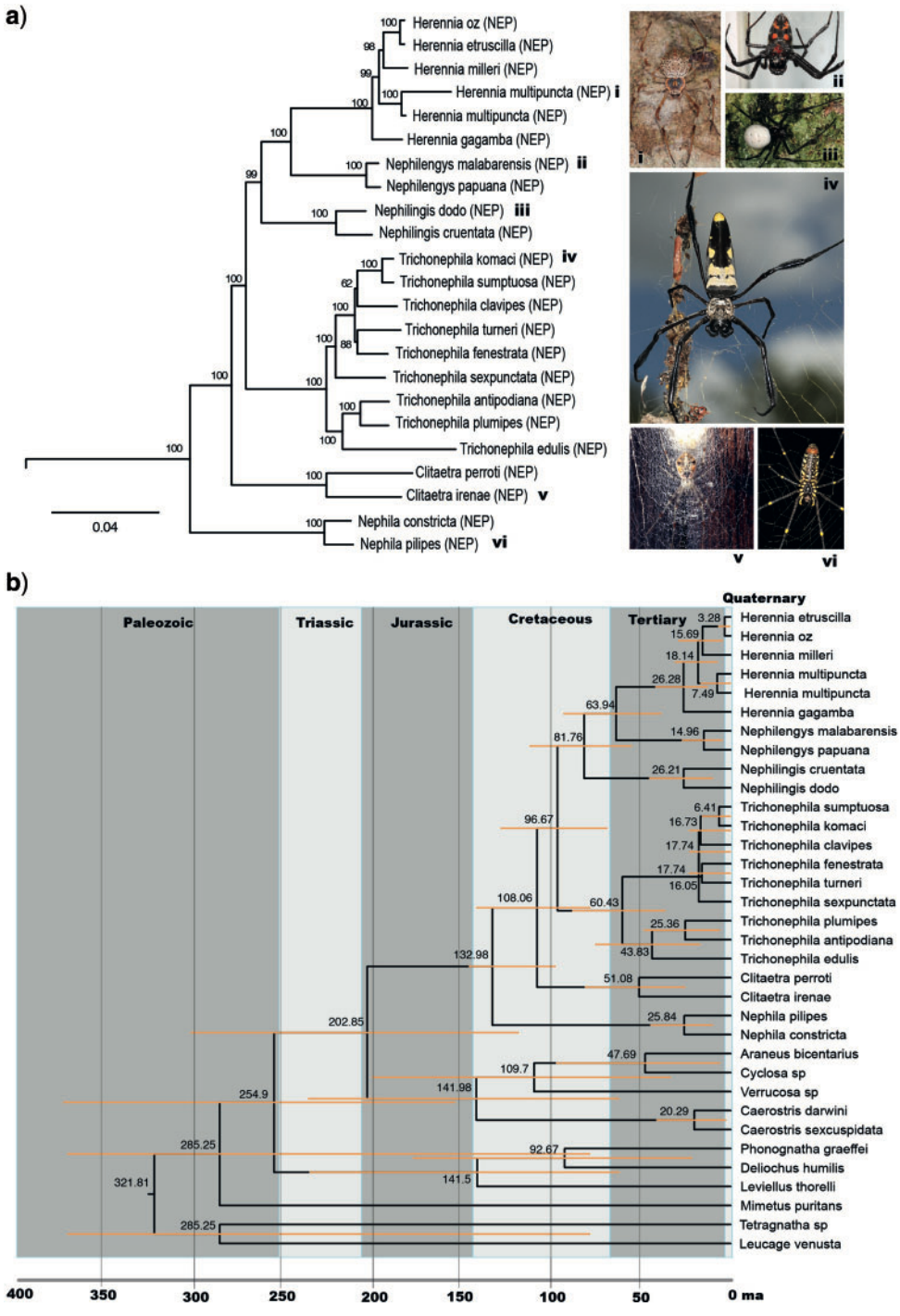

FIGURE 2. AHE resolves the relationships of 22 nephilid species. a) Maximum likelihood inference of concatenated data from 367 loci ("loose" data set); This tree shows the ingroup, with field photographs of typical representatives; for entire and other topologies, see Supplementary Figures S1–S3 (available on Dryad). b) Chronogram on the same taxon and AHE sample with labeled node ages in million years and their confidence intervals. NEP = Nephilidae.

all genera are comparable (Fig. 3, orange vertical bar). Table 1 summarizes the taxonomic changes listed below. Because classical *Nephila* is diphyletic, (Fig. 2a), *Nephila* Leach 1815 includes only its type, the Australasian *N. pilipes*, and the African *N. constricta*. We assign the remaining 12 species to the circumtropical *Trichonephila* Dahl 1911, new rank (formerly a *Nephila* subgenus; Dahl 1911). As its type species, we designate *Aranea clavipes* Linnaeus 1767. We elevate *Indoetra* Kuntner 2006, new rank, to genus (formerly a subgenus of *Clitaetra*; Kuntner 2006), and designate as its type species *I. thisbe* new combination from Sri Lanka. *Clitaetra* now

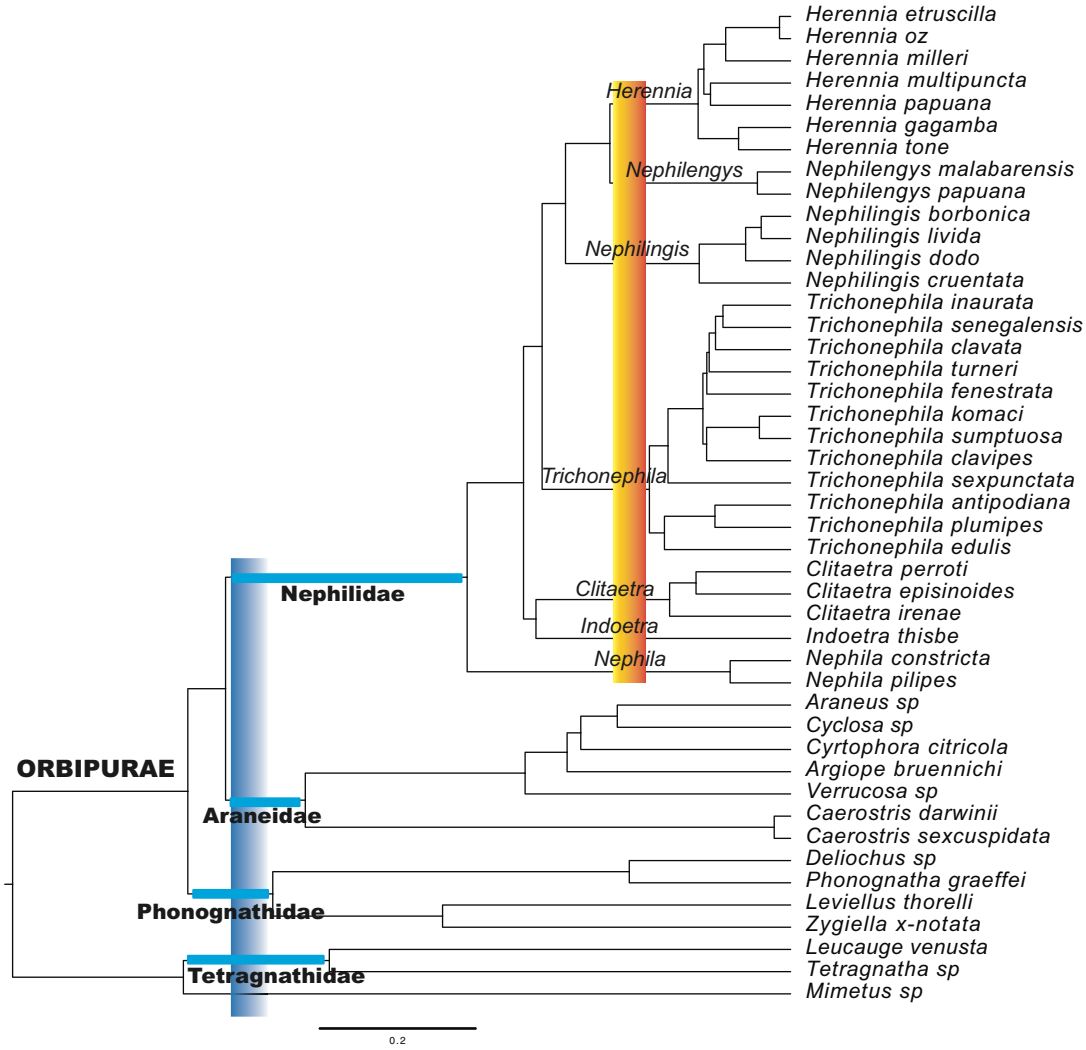

FIGURE 3. Phylogeny of Nephilidae with taxonomic implications. Ultrametricized tree is from a constrained, species-level phylogeny (see Supplementary Fig. S4 available on Dryad). The clade age taxonomic criterion aligns the families Nephilidae, Araneidae, Phonognathidae, and Tetragnathidae (older, blue vertical bar on the left), as well as the seven nephilid genera (more recent, orange vertical bar on the right).

includes the African and Western Indian Ocean island fauna.

For the same reasons, we formally recognize Nephilidae as a family (Fig. 3, blue vertical bar), and argue against its proposed synonymy with Araneidae by Dimitrov et al. (2017; see also Discussion section). Kuntner 2006 elevated Nephilidae Simon 1894 to family rank and defined it phylogenetically as the least inclusive clade containing *Clitaetra*, *Herennia*, *Nephila*, and *Nephilengys*. That definition also includes *Trichonephila*, *Nephilingis*, and *Indoetra*. A striated cheliceral boss, an unreversed synapomorphy, diagnoses Nephilidae (Kuntner 2006). The family Araneidae Clerck 1757 is defined as the least inclusive clade containing *Araneus*, *Argiope*, *Caerostris*, *Cyclosa*, *Cyrtophora*, and *Verrucosa*. The family Phonognathidae Simon 1894 new rank is defined as the least inclusive clade containing *Deliochus*, *Leviellus*, *Phonognatha*, and *Zygiella*. Previously treated as Zygiellidae Wunderlich 2004, or as Zygiellinae (Hormiga and Griswold 2014; Gregorič et al. 2015; Dimitrov et al. 2017; Kallal and Hormiga 2018; Kallal et al. 2018), the family group name Phonognathidae (Simon 1894 as Phonognatheae) has precedence. Wunderlich 2004b based Zygiellidae on Zygielleae Simon 1929, with type genus *Zygiella* F.O.P. Cambridge 1902, when Zygiellidae did not contain *Phonognatha*. Because *Phonognatha* is now part of this clade, as are *Deliochus* and *Artifex* (Kallal and Hormiga 2018), the oldest family group name prevails (International Code of Zoological Nomenclature, Article 23.3). Phonognathidae Simon 1894 thus includes the genera Artifex Kallal and Hormiga, 2018, Deliochus Simon 1894, Leviellus Wunderlich 2004, Phonognatha Simon 1894, and Zygiella F. O. Pickard-Cambridge 1902. Note that Parazygiella Wunderlich 2004 and Stroemiellus Wunderlich 2004 are synonyms of Zygiella, and Leviellus, respectively (Gregorič et al. 2015).

If nephilids (and phonognathids) are included in Araneidae *s.l.* as proposed by works referenced

above, that family becomes extraordinarily old and morphologically complex compared with other spider families (see Orbipurae, Fig. 3). If Nephilidae, Araneidae, and Phonognathidae are recognized as families (Fig. 3, blue horizontal and vertical lines), their ages, morphology, and phylogenetic distinctiveness are comparable to other spider families (Garrison et al. 2016; Fernández et al. 2018). Additionally, Araneidae, Nephilidae, and Phonognathidae are reciprocally monophyletic. We propose the rankless name Orbipurae (from orb + pure or classic, a Latinized feminine plural) for this clade, defined as the least inclusive clade containing *Nephila pilipes* (Fabricius 1793), *Araneus angulatus* (Clerck 1757), and *Phonognatha graeffei* (Keyserling 1865).

Body Size, SSD, and Web Evolution

Supplementary Table S1 (available on Dryad) provides average sizes of male and female body parts for 28 species (n = 480 specimens). The average nephilid total female body length is 20.4 mm (range 3.5–36.1) and male body length is 4.2 mm (2.5–7.1). The average SSD is 5.0 (1.4–11.4).

Supplementary Table S2 (available on Dryad) summarizes female web features for 18 of the 28 species in Supplementary Table S1 (available on Dryad). A parsimony reconstruction of web types (Fig. 4a) suggests that the aerial web is ancestral in nephilids, and thus homologous in *Nephila* and *Trichonephila*. *Herennia*, *Clitaetra*, and *Indoetra* have arboricolous webs. *Clitaetra* and *Indoetra* webs are planar with space between the tree surface and the web, but *Herennia* webs are convex, following tree trunk curvature, with a hub cup attached to the tree. Interestingly, these two arboricolous web types evolved independently (Fig. 4a). Their natural history on tree trunks is similar, but convergent. Hybrid webs spun by *Nephilengys* and *Nephilingis* evolved once and are homologous (Fig. 4a). Below we address the three broad questions posed in the Introduction.

(i) *Do male and female size evolve independently, do they evolve toward optima, and how does their evolution affect SSD?* —PIC analyses suggest that male and female sizes are independent, both when considering total body length (r^2 = 0.0008, two-tailed P = 0.89), as well as carapace length (r^2 = 0.016, two-tailed P = 0.52). Brownian motion is the best fit model for nephilid female size and SSD evolution, and Ornstein–Uhlenbeck model is the best fit model for male size (Table 2). This suggests that male size evolution, but not female, is driven toward an optimal body size.

Maximum likelihood optimizations reveal complex patterns of size evolution in nephilids (Fig. 4a,b) and the alternative, squared change parsimony optimizations likewise reveal numerous increases and decreases in female and male sizes (Supplementary Fig. S6a,b available on Dryad). All terminals and deeper phylogenetic nodes are eSSD except three island *Clitaetra* and *Indoetra* species that independently evolved moderate ratios on Madagascar, Comoros, and Sri Lanka (Fig. 4c and Supplementary Fig. S6c available on Dryad). The inferred nephilid root eSSD is 5.1, which is maintained or increased in *Nephila*, and notably in the tropical *Trichonephila*, *Nephilingis cruentata* (Africa), *N. livida* (Madagascar), and *Nephilengys malabarensis* (SE Asia). Conversely, the Australian *T. edulis* and *T. plumipes* occupy more temperate areas, and independently evolved smaller eSSD.

(ii) *Do nephilids obey Cope's rule and the converse Rensch's rule?*—Ancestral size reconstructions by cladogenetic events (Supplementary Fig. S5 available on Dryad) reveal that female size does not significantly increase (although it trends upward), while male size significantly increases and SSD stagnates, and a linear regression model analysis confirms this (Supplementary Appendix S1 and Supplementary Fig. S5 available on Dryad). These patterns reject Cope's rule.

Model II regression analysis on PIC data detects no relation between male and female size (Supplementary Appendix S1 available on Dryad, Ma slope = 0.287 (-0.307 to 1.153), P = 0.170; see Discussion section). Male and female size show no negative allometry, as would be predicted by converse Rensch's rule.

(iii) *What is the relationship among spider body size and web size, web types, web architecture, and SSD?*—Overall, the MCMCglmm analyses reveal that species with larger females spin larger webs (Supplementary Appendix S1 available on Dryad). Female body size significantly differs between web types: species with the largest females spin aerial webs, intermediate sized females spin hybrid webs, and the smallest females spin arboricolous webs (Supplementary Appendix S1 available on Dryad). However, contrary to expectation, MCMCglmm analyses reveal that hybrid webs occupy a larger space than aerial webs (Supplementary Appendix S1 available on Dryad). Reconstruction of WA (Fig. 5) also shows that *Nephilingis* hybrid webs are largest, that *Nephila* and *Trichonephila* aerial webs are smaller, and that arboricolous webs are smallest, particularly in *Clitaetra*.

MCMCglmm analyses suggest that female body size does not affect the ladder index (Supplementary Appendix S1 available on Dryad). Instead, LI in arboricolous webs is significantly higher than in hybrid or aerial webs (Supplementary Appendix S1 available on Dryad); thus, web type affects its "ladderness". Finally, HD and spider body size or web type are unrelated (Supplementary Appendix S1 available on Dryad).

Comparative results furthermore suggest that SSD in arboricolous species is significantly lower than SSD in aerial species, and that SSD in hybrid species does not significantly differ from others (Supplementary Appendix S1 available on Dryad). The convergent evolution of arboricolous webs seems to correspond with a decline in SSD.

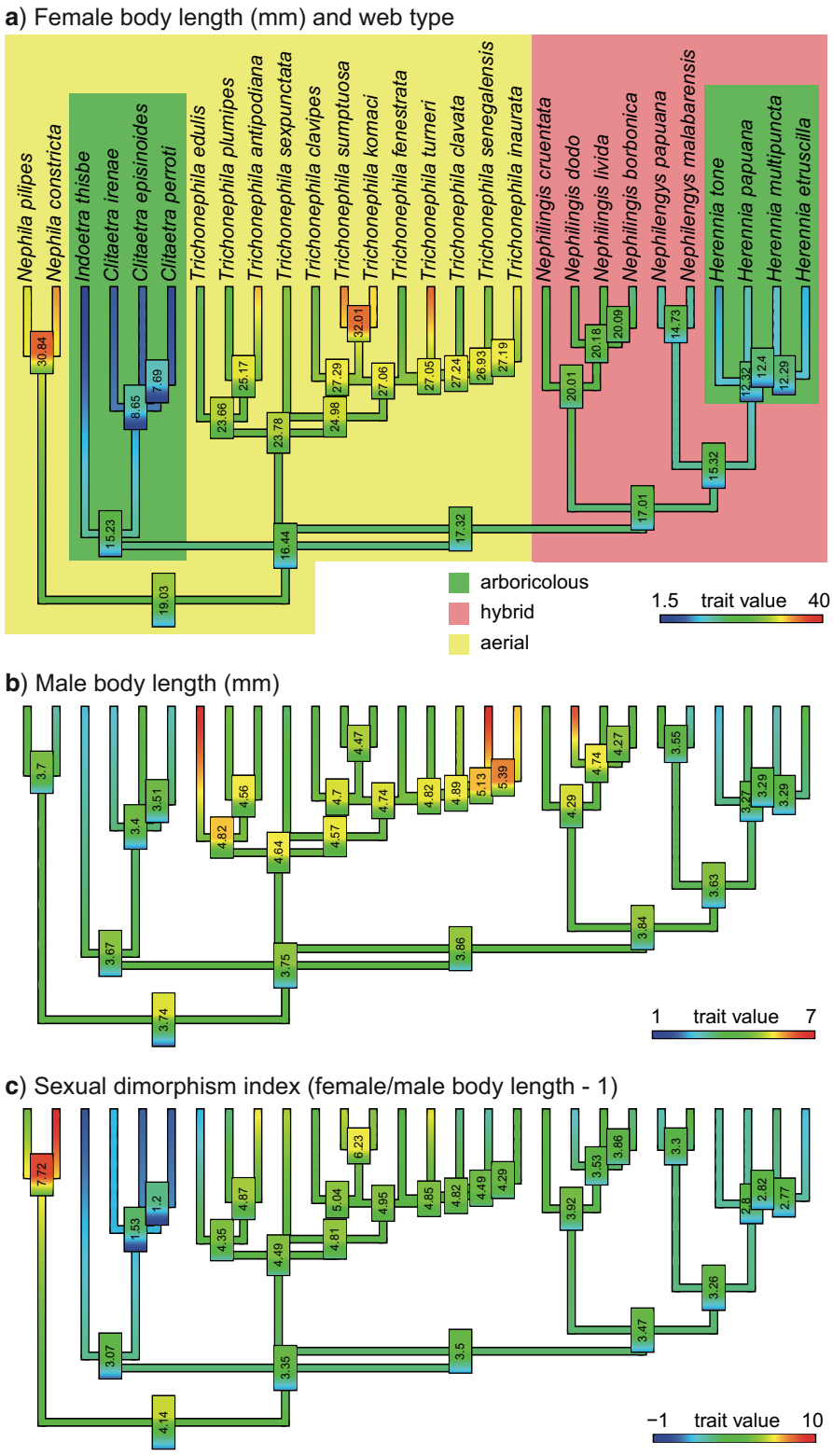

FIGURE 4. The evolution of body size and SSD in nephilid spiders. a) Female body length, as well as male body length (b), and SSD (c) show numerous increases and decreases. An ultrametric tree was pruned to contain the 28 taxa with known size variation in both sexes. Sexual dimorphism index is calculated as female/male body length - 1. Size and index values were optimized on the tree using maximum likelihood (for an alternative optimization using square change parsimony, see Supplementary Fig. S6 available on Dryad). Clades are color coded by web type in (a).

TABLE 2. Evolutionary model fitting for nephilid body size and sexual size dimorphism

Model	BM	OU	Trend
FBL			
lnL	−91.672	−91.672	−91.370
AIC	187.344	189.344	188.740
P-value		1.000	0.437
MBL			
lnL	−46.935	−44.474	−45.394
AIC	97.869	94.948	96.789
P-value		**0.027**	0.079
SDI			
lnL	−61.291	−60.976	−61.028
AIC	126.581	127.953	128.055
P-value		0.428	0.468

Note: BM = Brownian motion model; OU = single-optimum Ornstein–Uhlenbeck model; Trend = Brownian motion model with a directional trend; FBL = female body length; MBL = male body length; SDI = sexual dimorphism index. *P*-values are from the likelihood ratio test as compared with BM.
The significant *P*-value is bolded.

DISCUSSION

We provide a robust new phylogeny of Nephilidae, a model arachnid group for genomic, ecological, biomaterial, and evolutionary research that enables numerous new evolutionary tests to establish patterns in size, web type, and web feature evolution. This phylogenetic foundation also offers an objective rationale for family and genus-level taxonomy. Our comparative analyses build upon newly acquired data sets that contain measured body sizes of all nephilid species known from both sexes, as well as field-obtained web size data for nearly half the species diversity.

Evolutionary Implications

Male and female body sizes evolve independently in Nephilidae, apparently a rare case in animals, and a result that confirms some of the differential equilibrium model predictions of body size evolution (Blanckenhorn 2005). The differential equilibrium model recognizes opposing selection pressures on males and females that, when summed, could push the size of each gender in different directions. Additionally, nephilid gender body sizes evolve independently from one another, but female size is non-directional (Brownian motion), whereas male size is driven toward an optimum (Ornstein–Uhlenbeck model). As suggested by the trendline in Supplementary Fig. S5 (available on Dryad), this optimal male body size may lie between 3.2 mm and 4.9 mm.

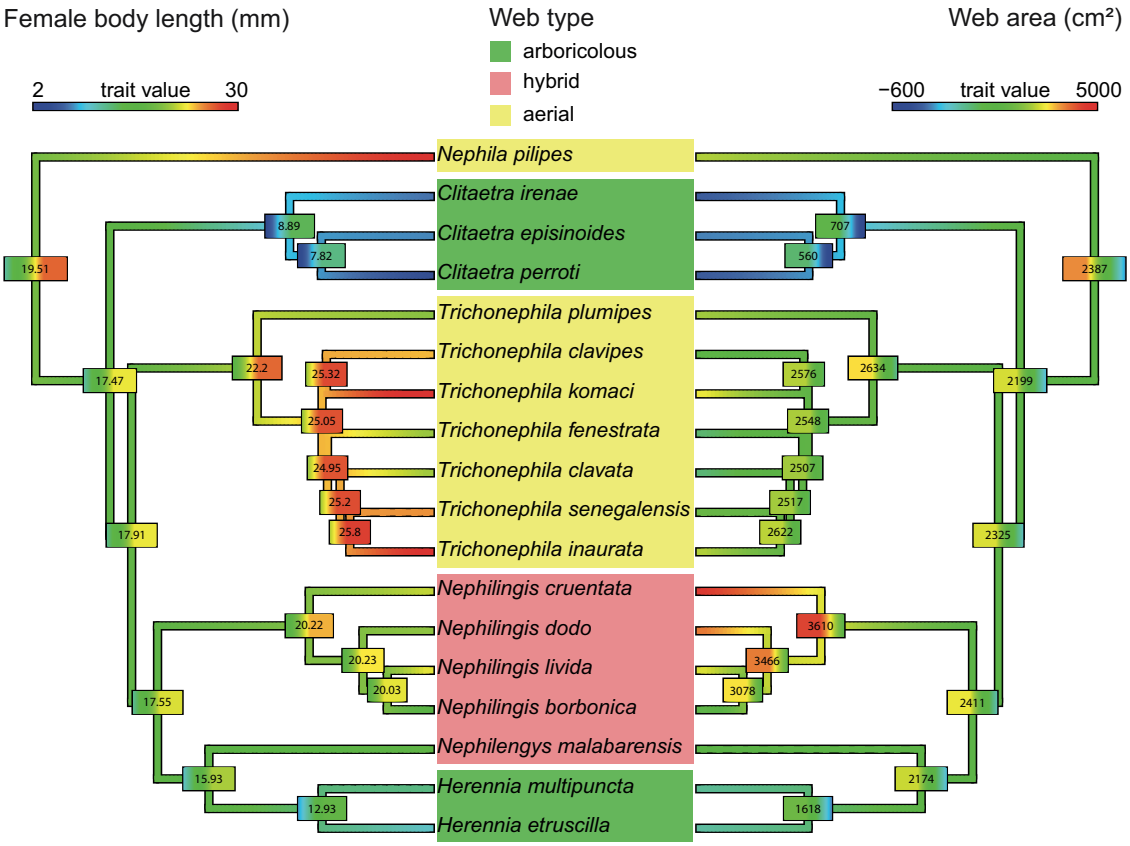

FIGURE 5. The evolution of female body size and web area optimized using maximum likelihood. Web types per terminal species are colored as in Figure 4.

As one would expect, female body and web size are strongly and positively correlated. However, even though the largest females spin aerial orb webs, the largest hybrid-type webs are spun by relatively smaller females. The smallest females spin arboricolous, ladder webs. Evolution from aerial toward hybrid webs, as well as the convergent origin of arboricolous webs (once from aerial and once from hybrid webs) have paralleled declines in female body size. Note that since adult males do not spin webs, male web size was not investigated.

Our analyses show that SSD in arboricolous species is smaller than in aerial species, while SSD in hybrid species is not significantly different from either the aerial or the arboricolous clades. In both cases where arboricolous webs evolve, the web site or architecture may constrain gender sizes so that SSD is less. On the other hand, aerial and hybrid webs may facilitate large female body sizes, as they provide less space constraint (Harmer and Herberstein 2009), or enable the capture of larger prey, or easier prey manipulation, or perhaps escape from common substrate dwelling predators. Whatever the reason, the evolutionary result is greater SSD in species on aerial and hybrid webs compared with those on arboricolous webs. Finally, female body size does not affect the web ladder index. Instead, it is the ecology of an arboricolous web that requires a higher ladder index than in hybrid or aerial webs.

Cope's and Rensch's Rules

Several approaches to detect phyletic trends in size yield diverse results. Assessing phyletic size trends in extant and fossil species (Moen 2006) is a strong test. Conventional statistics across ancestor-descendant pairs (Solow and Wang 2008) or model fitting (Monroe and Bokma 2010) are others. Phyletic size increase is supported in the fossil record, but not in extant mammals (Alroy 1998; Monroe and Bokma 2010). Cope's rule could be a long-term accumulation of responses to short-term ecological variables. For example, extinct ostracods grew larger as the climate cooled (Hunt and Roy 2006).

Unlike the temperature-related ostracod example, nephilid male phyletic size increase is likely due to gendered fitness advantages of large size at the individual level (Kingsolver and Pfennig 2004).

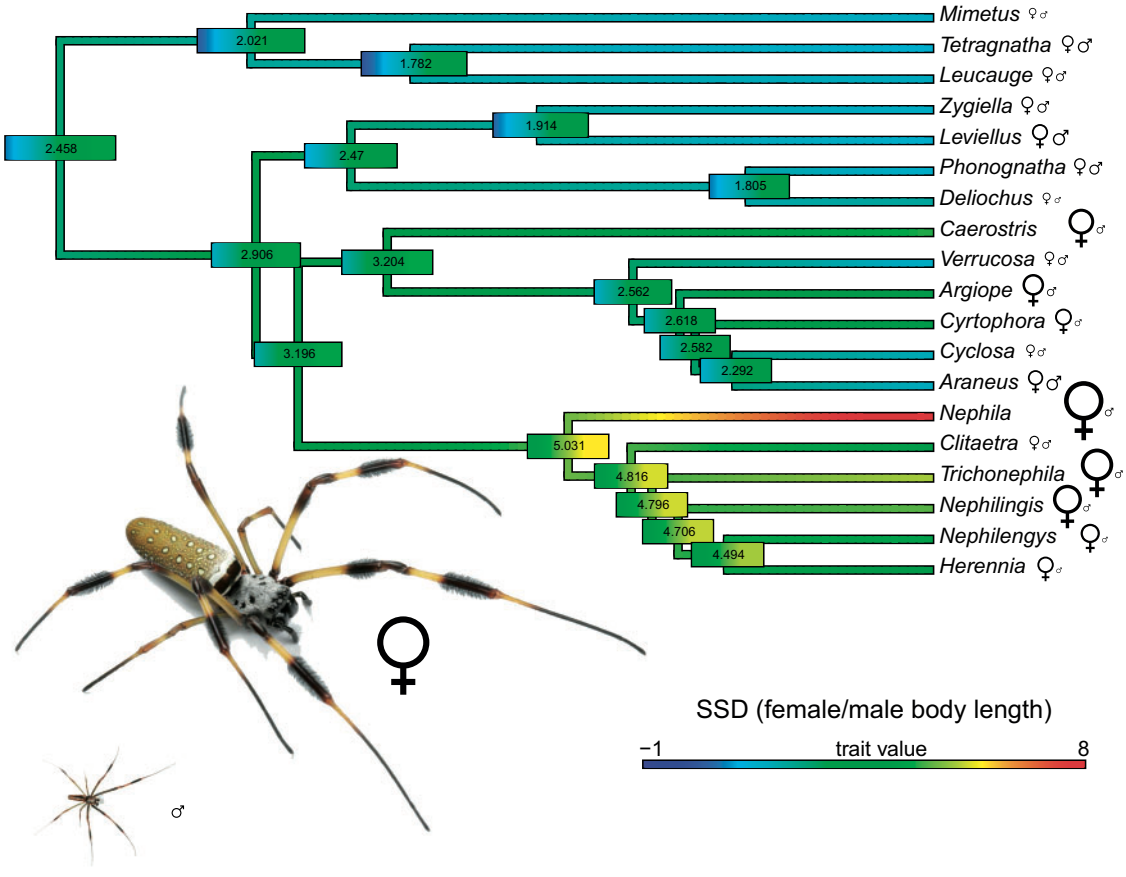

FIGURE 6. The evolution of SSD (as simple female to male body size ratio) on a genus level phylogeny. The genus level topology is from AHE analyses, the SSD ratios are optimizations for each nephilid genus (Supplementary Fig. S6c available on Dryad) or exemplar SSD data for outgroups (Supplementary Table S3 available on Dryad). The reconstruction is maximum likelihood (for parsimony alternative, see Supplementary Fig. S7 available on Dryad). Inset image shows *Trichonephila clavipes* female and male. The sizes of female and male symbols for all terminals correspond to relative total body lengths (Supplementary Table S3 available on Dryad), thus provide visuals of observed SSD.

A growing collection of literature has attempted, but so far failed, to converge on general proximate causes, likely because male fitness benefits are governed by a mixture of natural and sexual selection (Kuntner and Elgar 2014). Our finding that female body size increase is not significant at the relatively long, over 100 million year, evolutionary timespan in nephilids, rejects Cope's rule. Female gigantism in this clade is obviously an ancient trait (Fig. 6).

Rensch's rule predicts that SSD increases with body size in male-biased, and decreases in female-biased SSD species (Abouheif and Fairbairn 1997; Blanckenhorn et al. 2007a,b). If female body size changes contribute more to SSD, common in female-biased SSD, then plotting male versus female size, using independent contrasts, should be hypoallometric (Abouheif and Fairbairn 1997; Fairbairn 2005; Foellmer and Moya-Laraño 2007). This pattern, termed converse Rensch's rule, should occur in spiders. However, while Abouheif and Fairbairn (1997) corroborated Rensch's rule in most male-biased SSD animal taxa, it largely failed in invertebrates (Blanckenhorn et al. 2007a,b). Organisms with female-biased SSD rarely follow the converse Rensch's rule (Webb and Freckleton 2007). In spiders, Foellmer and Moya-Laraño (2007) rejected the converse Rensch's rule at interspecific levels, except when analyzing raw (phylogenetically uncorrected) species data. Cheng and Kuntner (2014) rejected the converse Rensch's rule after analyzing both raw and phylogenetically corrected data. Nephilid uncorrected data supported the converse Rensch's rule, but not phylogenetically corrected data (Kuntner and Cheng 2016). Herein, our results corroborate that neither Rensch's rule, nor its converse, significantly explain SSD in spiders.

Taxonomy

Our phylogeny has aided the resolution of past taxonomic and classification controversies by recognizing seven contemporary genera (*Clitaetra*, *Herennia*, *Indoetra*, *Nephila*, *Nephilengys*, *Nephilingis*, and *Trichonephila*) within the family Nephilidae (Table 1), which also contains the fossil genera not placed phylogenetically (the Cretaceous *Geratonephila*, and the Tertiary *Palaeonephila*). Wheeler et al. (2017) and Dimitrov et al. (2017) treated Nephilinae as a subfamily of Araneidae with no explanation and based on poorly resolved phylogenies using a limited sampling of genes generally considered to be inappropriate for deep evolutionary inference. We argue that phylogenetic topological results, as well as lineage age, suggest, objectively, that Araneidae, Nephilidae, and Phonognathidae are comparably composed and phylogenetically positioned with respect to other spider families, and should be maintained as independent entities.

Phylogenomic, time-calibrated trees for spider families suggest a range of 37–98 Ma (Garrison et al. 2016; Fernández et al. 2018). If Nephilidae is a subfamily of Araneidae, our analysis suggests a family age ca. 200 Ma. If such a criterion is applied more broadly across spiders, it would require lumping of diverse spider groups into singular families. Garrison et al.'s chronogram (Fig. 4) at 100 Ma depth would unite not only *Nephila* (Nephilidae) and Araneidae, but also roughly 26 other taxonomically distinct spider families would disappear. Their exclusion from arachnological taxonomic use (WSC 2018) is clearly a poor option given their monophyly, age, and evolutionary complexity.

Taxonomic names serve the important purpose of increasing the information content of a classification (Hennig's 1965). If Nephilidae, Phonognathidae, and Araneidae are monophyletic lineages, they certainly deserve names. Treating them as subfamilies within an aberrantly ancient (and huge) family does not accomplish this goal, however their familial status is consistent with arachnological practice and makes it easier for workers to track future taxonomic changes (WSC 2018). The advent of well-supported, dated phylogenies can help resolve differing opinions about taxonomic rank by allowing Hennig's (1965) criterion of rank to be tied to lineage age ("…if the absolute rank of categories was linked to their time of origin, just as in geology the sequence of strata in different continents is made comparable by its correlation with specific periods of the earth's history…" p. 115). Though it is unlikely that taxonomic rank will ever be free of subjective opinion, rough norms can be established to guide the application of ranks to evolutionarily distinct monophyletic groups.

In sum, comparable family ages, monophyly, exclusivity, information content, morphological diagnosability, and prevailing community practices all support family rank for Nephilidae and Phonognathidae. Moreover, since recent analyses agree with the here monophyletic Araneidae, Nephilidae, and Phonognathidae (Dimitrov et al. 2017), proposing the rank-less name Orbipurae for this clade begins to restore some measure of cladistic hierarchy within the vast and diverse superfamily Araneoidea, and should therefore stimulate comparative work.

The Placement of **Geratonephila**, *and the Origin of Nephilidae*

The fossil lineage *Geratonephila* has two possible phylogenetic placements. After evaluating a conservative fossil calibration as a stem nephilid, we find the outcomes of the dating analysis to be realistic and consistent with previous knowledge. Alternatively, a less conservative placement would place *Geratonephila* as the lineage leading to extant *Clitaetra* species from Africa, Madagascar, and Comoros. Interestingly, the size of the described male *G. burmanica* (3.1 mm) agrees with the phylogenetic reconstruction of the male ancestor of *Clitaetra* (3.4 mm).

Burmese amber is estimated to be between 97 million years old and 110 million years old (Poinar and Buckley

2012; Shi et al. 2012), corresponding to our hard minimum age boundary at 97 Ma. Cruickshank and Ko (2003) attribute the deformation of the Hukawng Basin, the locality of this amber, to the collision of India with Asia, and its subduction beneath the Burma plate. They note that while the amber from these layers is certainly Cretaceous, a more precise age is yet to be determined. This ambiguity led us to establish a soft boundary at 146 Ma, the beginning of the Cretaceous. Although Shi et al. (2012) hypothesize the age of 98.79 ± 0.62 as a maximum age for Burmese amber fossils, more recent estimates suggest this amber age to be at least 100, and of Gondwanan origin (Poinar 2018). Burmese amber has also been found to contain fossil evidence for a more ancient origin of bees (Poinar and Danforth 2006). The origin of Nephilidae thus seems to date to Cretaceous (contra Kuntner et al. 2013), and the family's origin may be Gondwanan after all (Kuntner 2006).

Poinar and Buckley (2012) speculated that *Geratonephila* was a social spider, based only on the co-occurrence of a male and juvenile spider in the same amber inclusion. Insofar as no extant or fossil nephilid shows social behavior, it seems conservative to reject communal behavior in *Geratonephila* (see also Penney 2014). In *Clitaetra*, the first postmolt instar juveniles reside in the webs of their mothers, as do a number of other spider lineages (likely an ancestral state). And perhaps most importantly, male nephilids are known to inhabit female webs for long periods of time as they mate-guard females, waiting for them to mature and become reproductively viable (Kuntner et al. 2009). Together, these facts explain the male and juvenile *G. burmanica* in the same amber inclusion, as well as our decision to use *Geratonephila* as a stem nephilid for dating.

Conclusions

Biological rules concerning body sizes do not appear to apply to nephilid spiders. Cope's rule predicting phyletic size increase in both sexes, is at best naïve and refuted in this case. SSD complicates the interpretation of Cope's rule in lineages where gender sizes evolve independently because the rule applies to both sexes, not just one. Although SSD in nephilids has been proposed to be due primarily to female gigantism, female nephilid size does not significantly increase at macroevolutionary time scales. Even sexually dimorphic gigantism (Kuntner and Elgar 2014), in which both sexes increase in size, but females increase faster, is refuted by our results that only corroborate overall male size increase. The new allometric analyses of log body size also refute Rensch's rule, as previously suggested (Kuntner and Cheng 2016).

The emerging picture of the interplay between nephilid female and male body sizes, eSSD, as well as the interactions of these variables with web architecture and features, has become more complex. This complexity may be due to the considerable increase in taxon sampling, data density, and phylogenetic accuracy. Patterns that twenty years ago seemed clear based on sparse taxonomic sampling and even sparser quantitative data, now seem much more clade and biology-specific, frustrating both new and old efforts to generalize.

Despite the sometimes conflicting trends seen within Nephilidae, the clade stands as the most extreme example of female-biased SSD among terrestrial animals, as far as we know. Over the years, a large amount of nephilid data has been accumulated on associated phenomena such as fecundity or reduced predation pressure selection for larger females, male-male competition for larger males, or mortality selection for smaller males due to mate searching and avoiding cannibalism, selection for sperm or scramble competition, sexual conflict, genital mutilation or emasculation, and gravity (reviewed in Kuntner and Elgar 2014). However, none of these data can be reliably analyzed in a comparative framework without a stable phylogeny. Comparative perspectives are most powerful when combined with direct experimental data, but here, too, experimental design often depends on phylogenies. Our phylogeny bridges this gap for future comparative studies on this clade of spiders.

Supplementary Material

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.h07s9d1.

Funding

This work was supported by Slovenian Research Agency [grants J1-6729 and P1-0236 to M.K., and grant BI-US/17-18-011 to M.K. and I.A.], and in part from the US State Department through a Fulbright visiting scholar award, and the ZRC director's fund, both to M.K. Additional support came from the National Science Foundation [DEB-0841610 to J.E.B.; Doctoral Dissertation Improvement Grant—DEB-1311494 to C.A.H.]; Auburn University Department of Biological Sciences and College of Sciences and Mathematics (J.E.B.); an Auburn University Cellular and Molecular Biosciences Peaks of Excellence Research Fellowship (C.A.H.).

Acknowledgments

We thank Michelle Kortyna, Alyssa Bigelow, Kirby Birch, and Sean Holland for assisting with molecular data collection and bioinformatics analysis, Charles Haddad, and Daiqin Li for field assistance, Simona Kralj-Fišer, Klemen Čandek, Shakira Quiñones-Lebron, Eva Turk, Jutta Schneider, Fritz Vollrath, and George Poinar for helpful discussions, as well as Michael Rix and two anonymous reviewers for thorough, valuable feedback.

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