

One hundred and sixty years of taxonomic confusion resolved: *Belonocnema* (Hymenoptera: Cynipidae: Cynipini) gall wasps associated with live oaks in the USA

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Gall wasps (Hymenoptera: Cynipidae) in the genus *Belonocnema* induce galls on live oaks (*Quercus* series *Virentes*), forming multilocular root galls in the sexual generation and unilocular leaf galls in the asexual generation. Using morphological characters, host records, museum specimens, flight propensity and phylogenetic analysis of published cytochrome *c* oxidase subunit I (*COI*) and nuclear SNP data, we resolve the long-standing taxonomic confusion within *Belonocnema* and recognize three distinct species that are distributed throughout the southern and south-eastern USA: *B. fossoria* (rev. stat.), *B. kinseyi* (rev. stat.) and *B. treatae*, while *B. quercusvirens* is treated as *species inquirenda*. The presence of mitonuclear discordance results in the failure of a mitochondrial DNA barcode region to distinguish between *B. fossoria* and *B. treatae*, while recognizing *B. kinseyi*, despite the three species being clearly separated based on morphology and phylogenetic analysis of SNP data. We provide re-descriptions and an updated dichotomous key for both asexual and sexual generations of these widespread species. Finally, as *Belonocnema* has emerged as a model organism for ecological and evolutionary studies, we clarify the species examined in published studies to date.

ADDITIONAL KEYWORDS: DNA barcoding – galls – host plant associations – mitonuclear discordance – *Quercus* – *Virentes*.

INTRODUCTION

Gall wasps (Hymenoptera: Cynipidae) represent one of the largest radiations of specialized insect herbivores, with over 1400 known species worldwide and likely many more undescribed (Ronquist *et al.*, 2015). Over 70% of this diversity resides within the tribe Cynipini, which induce galls on oaks (*Quercus* L., Fagaceae). Oaks are the dominant woody plant in North America, in both biomass and species richness (Cavender-Bares, 2019). Like their hosts, the North American oak gall wasps are species-rich with around 700 species in ~30 genera (Buffington *et al.*, 2020; Nicholls *et al.*, 2018),

many of which are endemic. Cynipini often exhibit cyclical parthenogenesis (heterogony), whereby temporally segregated sexual and asexual generations alternate to complete the life cycle (Pujade-Villar *et al.*, 2001). The sexual and asexual generations typically develop in galls on different host-plant tissues, and both the morphology of the galler and the galls induced vary between generations (Stone *et al.*, 2002).

All known members of the North American genus *Belonocnema* (Mayr, 1881) are found in the south-eastern and southern USA and are host-specific to species of live oaks (*Quercus* section *Quercus* series *Virentes* Nixon). The *Virentes* includes four species distributed throughout the southern and south-eastern USA, and three species in Mexico, Central America and Cuba (Hipp *et al.*, 2018; Cavender-Bares, 2019). Here we resolve the long-standing taxonomic

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confusion surrounding those *Belonocnema* taxa that develop on the four live oak species that are distributed throughout southern and south-eastern USA: *Quercus fusiformis* Small, *Q. geminata* Small, *Q. minima* Small and *Q. virginiana* Miller.

The 160-year taxonomic history of *Belonocnema* is convoluted. This is due in part to: (1) the confusion arising from multiple names being given to the sexual and asexual generations, a problem that has commonly plagued Cynipini literature (Abe *et al.*, 2007); (2) naming of *Belonocnema* species on the basis of alternate generations reared from different, but closely related, host-plant species that themselves range from sympatric to parapatric to allopatric; and (3) the changing taxonomic status of host plants linked to galler descriptions (Muller, 1961).

Belonocnema belongs to the *Cynips*-group within Cynipini, which originated from the Nearctic and has since spread to the Palaearctic region, including many species that have brachypterous (short-winged) or apterous (wingless) forms (Liljeblad *et al.*, 2008). Sexual-generation *Belonocnema* oviposit on the undersides of newly unfurled leaves of live oak in the spring. The asexual generation then develops within detachable, smooth, pea-shaped, unilocular 'leaf galls' (Fig. 1C, E). Leaf galls are pigmented and vary from white to yellow to pink to red as they develop throughout the summer (Lund *et al.*, 1998: fig.1). As the asexual generation develops to the penultimate stage in late autumn, leaf galls lignify and turn brown. The asexual generation emerges from November through December (Driscove *et al.*, 2019) and oviposits into small rootlets sprouting clonal above-ground growth just below the soil surface. The sexual generation then develops within the irregularly shaped, multilocular galls, often in clusters, that form at the site of oviposition (Fig. 1A; Lund *et al.*, 1998: figs 3, 4). Asexual females are either androphores or gynophores; thus, individual root galls house either all-male or all-female offspring (Cryer, 2003). Developing root galls are also pigmented, but remain fibrous, turgid and not fully lignified through March–April, at which time the sexual generation emerges to repeat the life cycle (Lund *et al.*, 1998).

Osten Sacken first described spherical leaf galls found on 'live oak' collected from Georgia as *Cynips quercusvirens*, without reference to reared adults and without specification of which live oak species was the host, thereby initiating the long-standing taxonomic confusion (Osten Sacken, 1861). The genus *Belonocnema* was first established by Mayr in 1881, on the basis of sexual-generation adults of *Belonocnema treatae* (Mayr, 1881) (Fig. 2A–D, F) collected by the noted 19th-century naturalist Mary Treat, a correspondent of Charles Darwin. She collected the galls in Green Cove Spring, Florida, from root galls developing on

Q. virginiana (Mayr, 1881; Melika & Bechtold, 2001). However, due to a printing error, the genus was first *Belenocnema* [sic] before being subsequently corrected (Mayr, 1902; Melika & Bechtold, 2001). Ashmead (1881) then described the same sexual-generation adults as a new genus *Dryorhizoxenus* with the original designation of *Dryorhizoxenus floridanus* Ashmead, 1881 as type species. Ashmead subsequently synonymized *Belonocnema* with *Dryorhizoxenus* (Ashmead, 1885), but later recognized that Mayr's *Belonocnema* name had priority (Ashmead, 1886). Weld (1921) then described two asexual-generation species associated with leaf galls. *Belonocnema fossoria* Weld, 1921 (Figs 1D, 3A–D) was named based on adults reared from leaf galls collected from *Q. geminata* (Fig. 1C) and *Q. virginiana* from Clearwater, Florida. However, the host record from *Q. virginiana* was likely in error as subsequent exhaustive collections from the south-eastern USA have consistently associated *B. fossoria* with only *Q. geminata* (Driscove *et al.*, 2019; Hood *et al.*, 2019). *Belonocnema kinseyi* Weld, 1921 (Fig. 4A–D) was named on the basis of adults (Fig. 1F) reared from leaf galls (Fig. 1E) on live oak from Boerne, Texas, then known as *Q. virginiana* var. *fusiformis* (Small) Sargent, but now known as *Q. fusiformis* (Muller, 1961). Weld also treated Osten Sacken's leaf galls as a junior synonym of his *B. fossoria*, as he argued that '...the classification of the Cynipidae must be based upon the adults rather than upon their work' (Weld, 1921). However, this synonymy was reversed in the *Catalog of Hymenoptera in America north of Mexico* (Burks, 1979) and *B. fossoria* became a junior synonym of *B. quercusvirens* under *International code of zoological nomenclature* Article 1, section 3: 'Excluded from the provisions of the Code are names proposed...after 1930, for the work of extant animals'. More recently, Lund *et al.* (1998) demonstrated that asexual-generation *B. kinseyi* emerging from leaf galls on *Q. fusiformis* in Texas subsequently induced root galls from which the sexual generation, then known as *B. treatae*, emerged, thus synonymizing *B. kinseyi* as the asexual generation of *B. treatae*. The recent literature exploring the taxonomy and phylogenetic history of the Cynipini (Melika & Abrahamson, 2002; Liljeblad *et al.*, 2008) has continued to recognize only two species: *Belonocnema treatae*, where both asexual and sexual generations are known, ranging across the entire southern and south-eastern USA where the live oaks (*Q. fusiformis*, *Q. geminata* and *Q. virginiana*) occur (Burks, 1979; Lund *et al.*, 1998) and *B. quercusvirens*, which has a much more limited range of Georgia and Florida (*Q. geminata* and *Q. virginiana*), with only the asexual generation having been described (Burks, 1979). *Quercus minima* has also been recorded as a host plant, although the

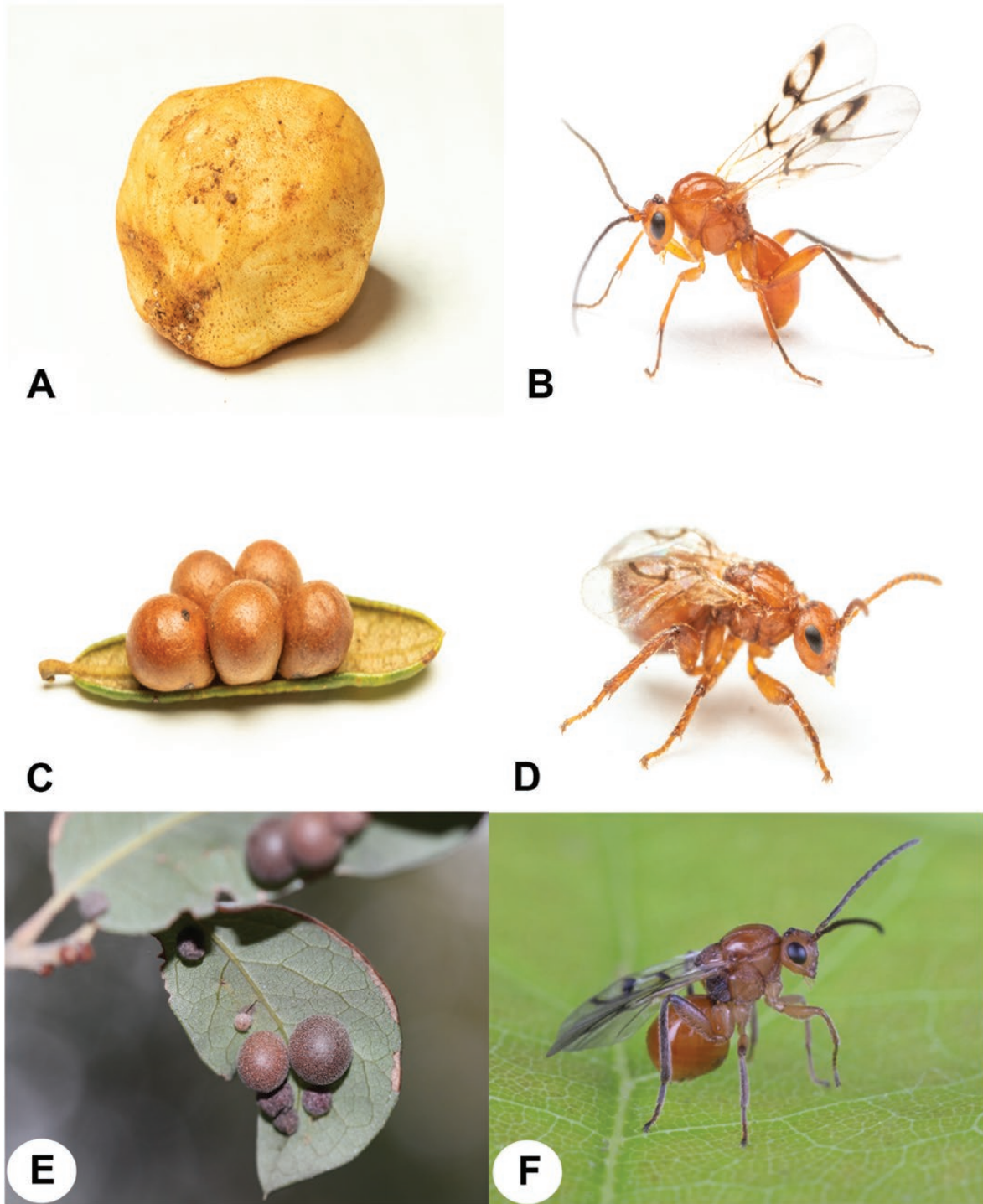


Figure 1. *Belonocnema fossoria* (A–D), *Belonocnema kinseyi* (E, F). A, sexual-generation root gall. B, adult female sexual generation. C, fully lignified asexual-generation leaf gall on *Q. geminata*. D, adult asexual generation. E, fully lignified asexual-generation leaf gall on *Q. fusiformis*. (Leaf galls produced by *B. kinseyi* on *Q. fusiformis* and *Q. virginiana* are indistinguishable from leaf galls produced by *B. treatae* on *Q. virginiana*.) F, adult asexual generation (by Jena Johnson).

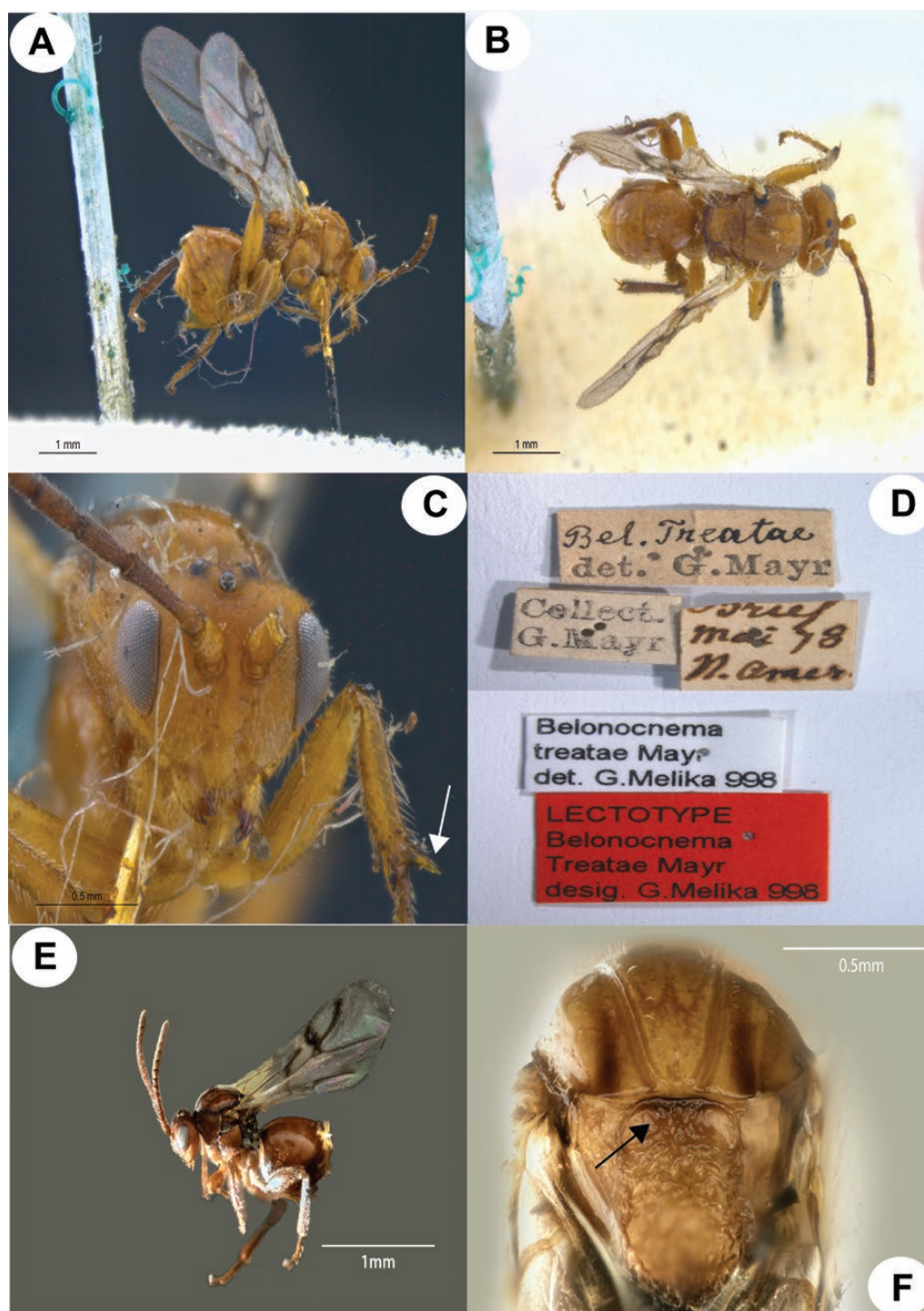


Figure 2. *Belonocnema treatae*. A, lateral habitus of sexual-generation female lectotype. B, dorsal habitus of sexual-generation female lectotype. C, frontal view of sexual-generation female lectotype, arrow pointing to tibial spur. D, label of lectotype specimen. E, lateral habitus of asexual generation female. F, dorsal view of sexual generation female, arrow pointing to scutellar fovea. (Photos A–D by Dominique Zimmermann, NHMW.)

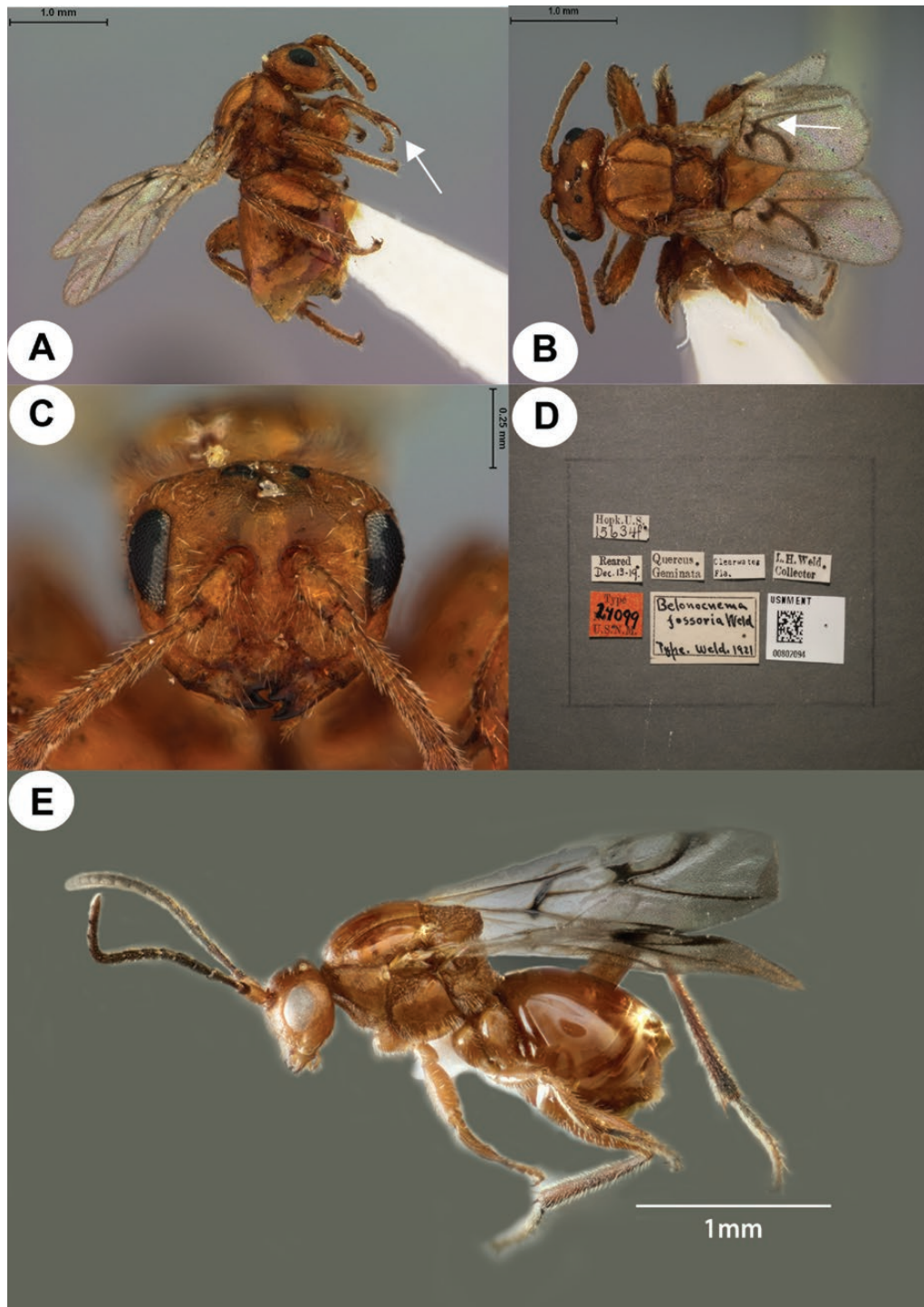


Figure 3. *Belonocnema fossoria*. A, lateral habitus of asexual-generation female syntype, arrow pointing to tibial spur. B, dorsal habitus of asexual-generation female syntype. C, frontal view of asexual-generation female syntype. D, label of syntype specimen. E, lateral habitus of sexual-generation female. (Photos A–D by Rachel Osborn, USNM: <http://n2t.net/ark:/65665/36d24dd5f-fe3b-4085-aecd-0568264b43a9>, Accessed 28 September 2020)

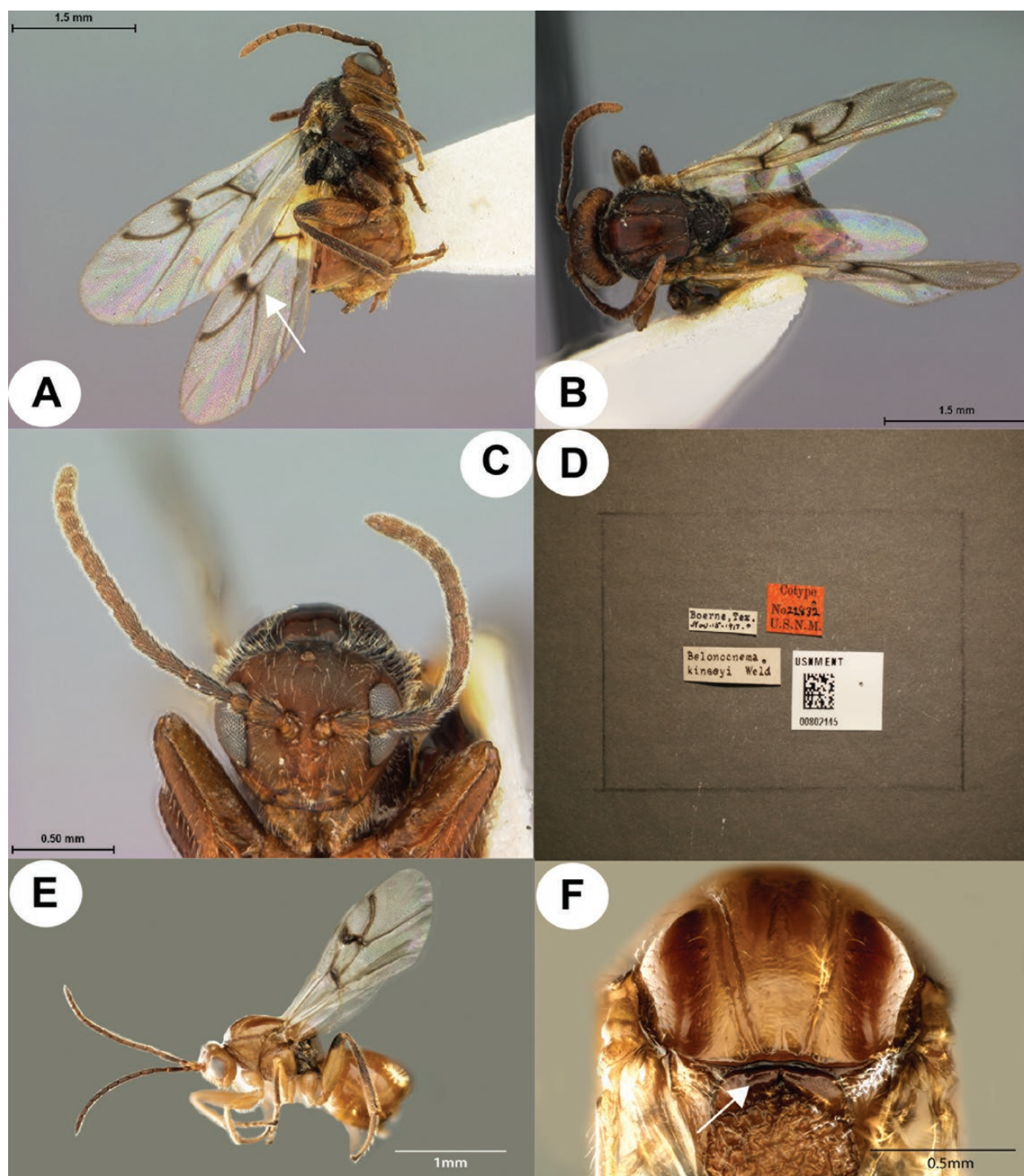


Figure 4. *Belonocnema kinseyi*. A, lateral habitus of asexual-generation female syntype, arrow pointing to areolet. B, dorsal habitus of asexual-generation female syntype. C, frontal view of asexual-generation female syntype. D, label of syntype specimen. E, lateral habitus of sexual-generation female. F, dorsal view of sexual-generation female, arrow pointing to scutellar fovea. (Photos A–D by Rachel Osborn, USNM: <http://n2t.net/ark:/65665/m3098f569a-1f76-44b1-8519-c06bf912057d>, Accessed 28 September 2020)

validity of this species is somewhat doubtful due to low genetic differentiation (Cavender-Bares *et al.*, 2015). The authors have never collected *Belonocnema* from specimens identified as *Q. minima*.

Belonocnema is emerging as a model system for studies of insect–plant relationships, speciation and multitrophic interactions (Table 1). Due to the confusing taxonomic history, and the hitherto unknown geographic and host-plant-related genomic structure recently uncovered by Schuler *et al.* (2018) and Driscove *et al.* (2019), most of these studies have been published under the names *B. treatae*, with wasps developing on *Q. virginiana* and *Q. geminata* in the south-eastern USA described as ‘host-associated’ populations. Table 1 updates the taxa examined in studies involving *Belonocnema*, based on the work presented herein.

An initial inspection of genetic differentiation within *B. treatae* as a function of host-plant association examined three *Q. geminata* and three *Q. virginiana* host-associated populations across a portion of the range of the host plant in the south-east. No evidence of host-plant-related differentiation or geographic structure was found, based on inspection of a concatenated mtDNA sequence made up of a 416-bp fragment of the cytochrome *b* (*Cytb*) gene and a 593-bp fragment of the *COI* gene (Egan *et al.*, 2012a). However, more robust sampling, involving 23 host-associated populations of *B. treatae* distributed across all three live oak species spanning the south-eastern USA from Florida to Texas, using a 633-bp *COI* sequence, showed evidence of two distinct geographic clades (Schuler *et al.*, 2018), with a western clade ranging from Texas to Mississippi and an eastern clade spanning from Mississippi to Florida. Most recently, the roles of host-plant association and geography in structuring genetic differentiation in *B. treatae* were evaluated by sampling 58 sites distributed across the primary host plants, *Q. fusiformis*, *Q. geminata* and *Q. virginiana*, throughout the entire known geographic range of *Belonocnema* across the southern and south-eastern USA. Based on genome-wide sampling of over 40 000 SNPs from over 1200 individuals, evidence of three deeply divergent genetic clusters (i.e. three putative species) was discovered (Driscove *et al.* 2019: fig. 2). Evidence of gene flow among clusters was restricted to admixture between two lineages at a single site. Individuals from this site in Gautier, Mississippi, are included as material examined in this study. Our goal herein is to clarify the taxonomy of *Belonocnema*, considering the recently published molecular evidence for three species, and to provide (re)descriptions of both generations, along with an updated taxonomic key based on morphological characters for the known species that occur in North America north of Mexico.

MATERIAL AND METHODS

MOLECULAR DATA

Discordance between mitochondrial and nuclear data has been observed in European oak gall wasps (Cook *et al.*, 2002; Rokas *et al.*, 2003; Nicholls *et al.*, 2012), thus it is not surprising that the analysis of *COI* and SNP data also produced conflicting results with respect to the structure of genetic variation within *Belonocnema*. To provide a phylogenetic context of lineage differentiation we re-analysed the *COI* data and subsamples of the genotype-by-sequencing data. The *COI* data from Schuler *et al.* (2018) represents $N = 96$ unique haplotypes found among 463 individuals collected from 23 populations across the range of the three host plants in Oklahoma, Texas, Mississippi, Alabama, Florida and Georgia. Maximum likelihood analysis was performed using IQ-TREE v.2.0.5 (Minh *et al.*, 2020), using ModelFinder for each codon position (Kalyanamoorthy *et al.*, 2017). The HKY+F+I model for codon positions 1 and 2 and the TPM2+F+G4 model for codon position 3 were selected by ModelFinder. Ultrafast Bootstrap (Hoang *et al.*, 2017) and Shimodaira–Hasegawa approximate likelihood ratio test (Guindon *et al.*, 2010) were used as support values. We subsampled the SNP data generated using genotyping-by-sequencing from Driscove *et al.* (2019) by randomly selecting one wasp from each of the 58 population (See Supporting Information, Table S1) as representative, and reprocessing the BAM files in the original study using STACKS v.2.5.3 (Catchen *et al.*, 2013). We randomly selected one SNP per contig to minimize linkage disequilibrium. The phylogenetic analysis of SNP data was identical to that of the *COI* data, with the exception that it was conducted without any partitioning. The data matrix consisted of 49 053 SNPs, analysed using the K3P+ASC model selected using ModelFinder. The output trees were visualized in R v.4.0 (R Core Team, 2020) using the packages ggtree v.2.2.0 and treeio v.1.12.0 (Wang *et al.*, 2020; Yu *et al.*, 2017). The locality of specimens sampled for both the *COI*- and SNP-based phylogenetic analyses are shown in Figure 5.

MORPHOLOGICAL EXAMINATION

To characterize and compare the morphology of the three putative species identified by the analysis of Driscove *et al.* (2019), we inspected a wide range of material ($N = 269$) sampled from across the geographic ranges of all three major host plants (Fig. 5). Specimens were mostly collected by the authors, along with additional members of the respective labs.

Table 1. Updated taxonomic names for studies using *Belonocnema* species

Publication	Taxon designation	Generation studied	Revised Taxon	Host plant	Location	Focus
Lund <i>et al.</i> , 1998	<i>B. treatae</i>	AS/Sex	<i>B. kinseyi</i>	<i>Qf</i>	TX	Lifecycle closure
Pujade-Villar <i>et al.</i> , 2001	<i>B. treatae</i>	AS/Sex	<i>B. kinseyi</i>	N/A	N/A	Lifecycle diversity
Melika & Abrahamson, 2002	<i>B. quercusvirens</i>	AS/Sex	<i>B. fossoria</i>	N/A	N/A	Taxonomy
Price <i>et al.</i> , 2004	<i>B. quercusvirens</i>	AS	<i>B. fossoria</i>	<i>Qg</i>	FL	Restoration ecology
	<i>B. treatae</i>		<i>B. treatae</i>	<i>Qm</i>		
Egan & Ott, 2007	<i>B. treatae</i>	AS	<i>B. kinseyi</i>	<i>Qf</i>	TX	Local adaptation
Liljeblad <i>et al.</i> , 2008	<i>B. treatae</i>	AS/Sex	<i>B. kinseyi</i>	N/A	N/A	Phylogeny
Hood & Ott, 2010	<i>B. treatae</i>	AS/Sex	<i>B. kinseyi</i>	<i>Qf</i>	TX	Developmental plasticity
Hood & Ott, 2011	<i>B. treatae</i>	AS/Sex	<i>B. kinseyi</i>	<i>Qf</i>	TX	Life history evolution
Egan <i>et al.</i> , 2011	<i>B. treatae</i>	AS	<i>B. kinseyi</i>	<i>Qf</i>	TX	Natural selection gall size
Egan <i>et al.</i> , 2012a	<i>B. treatae</i>	Sex	<i>B. treatae</i>	<i>Qv</i>	FL	Reproductive isolation:
			<i>B. fossoria</i>	<i>Qg</i>	FL	Mate choice
Egan <i>et al.</i> , 2012b	<i>B. treatae</i>	Sex	<i>B. treatae</i>	<i>Qv</i>	FL	Reproductive isolation:
			<i>B. fossoria</i>	<i>Qg</i>	FL	Habitat isolation
Egan <i>et al.</i> , 2013	<i>B. treatae</i>	AS/Sex	<i>B. treatae</i>	<i>Qv</i>	FL	Host plant associated
		AS/Sex	<i>B. fossoria</i>	<i>Qg</i>	FL	differentiation
Ott & Egan, 2014	<i>B. treatae</i>	AS	<i>B. kinseyi</i>	<i>Qf</i>	TX	Natural selection gall size
Gokhman <i>et al.</i> , 2015	<i>B. treatae</i>	AS/Sex	<i>B. kinseyi</i>	<i>Qf</i>	TX	Cyclical deuterothy/karyotype
Forbes <i>et al.</i> , 2016	<i>B. treatae</i>	AS/Sex	<i>B. kinseyi</i>	<i>Qf</i>	TX	Insect natural enemies
		AS/Sex	<i>B. kinseyi</i>	<i>Qv</i>		
			<i>B. treatae</i> x			
			<i>B. kinseyi</i> hybrid			
Reynolds <i>et al.</i> , 2016	<i>B. treatae</i>	AS	<i>B. kinseyi</i>	<i>Qf</i>	TX	Natural selection gall size
Hood & Ott, 2017	<i>B. treatae</i>	AS/Sex	<i>B. kinseyi</i>	<i>Qf</i>	TX	Life history evolution
Zhang <i>et al.</i> , 2017	<i>B. treatae</i>	AS/Sex	<i>B. kinseyi</i>	<i>Qf</i>	TX	Host plant associated
		AS/Sex	<i>B. treatae</i>	<i>Qv</i>	FL	differentiation: immigrant
		AS/Sex	<i>B. fossoria</i>	<i>Qg</i>	FL	inviability
Schuler <i>et al.</i> , 2018	<i>B. treatae</i>	AS/Sex	<i>B. kinseyi</i>	<i>Qf</i>	Range wide	<i>Wolbachia</i> infection dynamics
		AS/Sex	<i>B. treatae</i>	<i>Qv</i>	Range wide	
		AS/Sex	<i>B. fossoria</i>	<i>Qg</i>	Range wide	
Driscoll <i>et al.</i> , 2019	<i>B. treatae</i>	AS	<i>B. kinseyi</i>	<i>Qf</i>	Range wide	Host plant associated
		AS	<i>B. treatae</i>	<i>Qv</i>	Range wide	genomic differentiation
		AS	<i>B. fossoria</i>	<i>Qg</i>	Range wide	
Hood <i>et al.</i> , 2019	<i>B. treatae</i>	AS/Sex	<i>B. treatae</i>	<i>Qv</i>	FL	Reproductive isolation:
		AS/Sex	<i>B. fossoria</i>	<i>Qg</i>	FL	host plant phenology
Zhang <i>et al.</i> , 2019	<i>B. treatae</i>	AS/Sex	<i>B. treatae</i>	<i>Qv</i>	FL, AL	Cascading reproductive isolation
		AS/Sex	<i>B. fossoria</i>	<i>Qg</i>	FL, AL	

Table 1. Continued

Publication	Taxon designation	Generation studied	Revised Taxon	Host plant	Location	Focus
Cronin <i>et al.</i> , 2020	<i>B. quercusvirens</i>	AS	<i>B. fossoria</i>	<i>Qg</i>	FL	Restoration ecology
Genome (PRJNA623416)	<i>B. treatatae</i>	Sex	<i>B. kinseyi</i>	<i>Qv</i>	TX	Genome
Zhang <i>et al.</i> , 2020	<i>B. treatatae</i>	AS/Sex	<i>B. treatatae</i>	<i>Qv</i>	FL	Reproductive isolation
Zhang <i>et al.</i> , 2021	<i>B. treatatae</i>	AS/Sex	<i>B. fossoria</i>	<i>Qg</i>		asymmetric immigrant fitness
			<i>B. treatatae</i> x	<i>Qv</i>	FL	Hybrid fitness
			<i>B. fossoria</i> hybrid	<i>Qg</i>		

Additional materials and type specimens from the Smithsonian National Museum of Natural History (NMNH), Museum of Natural History of Vienna (photo only, NHMW), Florida State Collection of Arthropods (FSCA), University of Central Florida Collection (UCFC) and University of Texas at Austin Insect Collection (UTIC) were also examined. Individuals from Gautier, Mississippi, where genetic admixture occurs between eastern and western *Belonocnema*, are assigned as two different species based on morphological characters.

We follow Liljeblad & Ronquist (1998), Melika (2006) and Buffington *et al.* (2020) for terminology on Cynipidae morphological structures and abbreviations for fore wing venation, and Harris (1979) for patterns of cuticular sculpture. The following measurements and abbreviations were used: F1–Fn, the first and the following flagellomeres; POL (post-ocellar distance), the distance between the inner margins of posterior ocelli; OOL (ocellar–ocular distance), the distance from the outer margin of lateral ocellus to the inner margin of compound eye; LOL (lateral–ocular distance), the distance between lateral and frontal ocellus; transfacial line, distance between inner margins of compound eyes measured across the toruli; width of radial cell, measured as the distance between the upper margin of the fore wing and the radial sector (Rs) vein. Images of specimens were captured using a Canon 7D Mark II with a Mitutoyo M Plan Apo 10× objective mounted onto the Canon EF Telephoto 70–200 mm zoom lens, and the Canon MT–24EX Macro Twin Lite Flash (Tokyo, Japan) with custom-made diffusers to minimize hot spots. Image series were merged into a single in-focus, composite image with the program Zerene Stacker v.1.04. Post-imaging processing was completed with the editing tools in Photoshop CC, and plates were generated using Illustrator CC. Voucher specimens are deposited at NMNH (USNMENT 01735336–523), FSCA (FSCA 00094816–43), UCFC (UCFC0577291–307), UTIC (UTIC 265051–57) and the research collection of the Ott lab (Texas State University, San Marcos, USA) and Egan lab (Rice University, Houston, USA).

FLIGHT PROPENSITY

Because of the marked differences in both wing size and conformity among adult asexual-generation *Belonocnema* associated with *Q. geminata* leaf galls (Figs 1D, 3A, small-crumpled wings) and *Belonocnema* associated with both *Q. virginiana* (Fig. 2E) and *Q. fusiformis* (Fig. 4A), (large–non-crumpled wings), we tested for flight ability of asexual *Belonocnema* associated with each host plant. Flight propensity was tested by aspirating newly enclosed one-day old adults from *Q. geminata* ($N = 4$ sites; 135

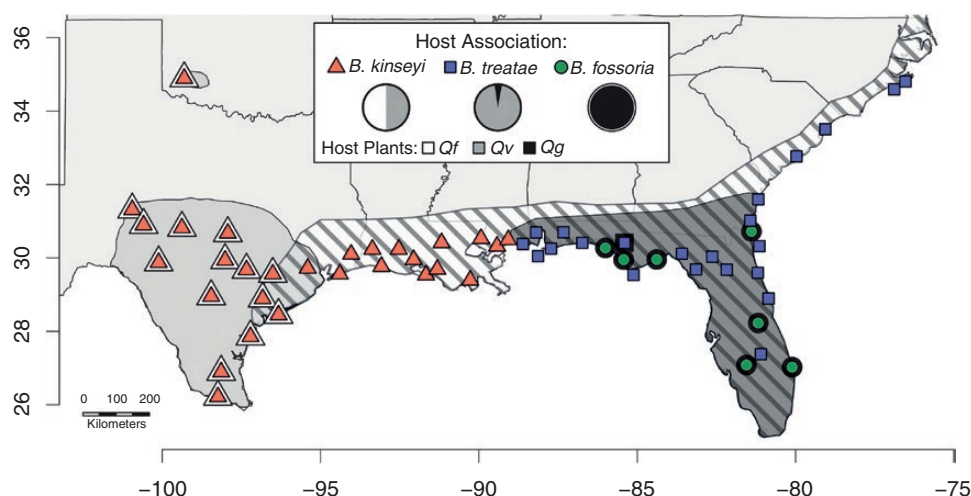


Figure 5. Geographic distribution of *Belonocnema* species across the geographic ranges of their host plants *Quercus fusiformis* (light grey), *Q. virginiana* (diagonal pattern) and *Q. geminata* (dark grey) within the southern and south-eastern United States along with a summary of host-plant associations for each *Belonocnema* species. The proportions of each species using each host plant are based on the 58 sites and 1219 individuals from Driscove *et al.* (2019). Site symbol and inner colour denote *Belonocnema* species, while the outline colour indicates host-plant species sampled at each site; Qf = white; Qg = black; and Qv = no outline. Plant ranges redrawn from Cavender-Bares *et al.* (2015).

wasps), *Q. virginiana* ($N = 12$ sites; 123 wasps) and *Q. fusiformis* ($N = 4$ sites; 53 wasps) individually into an empty standard fruit-fly vial, which was placed opening upwards on the lab bench and then covered by an inverted 1-L clear plastic bottle to form a closed chamber. We then monitored behaviour at room temperature for 10-min trials and noted whether gall wasps reached the wall of the larger chamber by flying or by crawling only.

RESULTS

Maximum likelihood analysis of *COI* variation suggests two strongly supported clades (Fig. 6). The first clade includes samples now identified as *B. kinseyi* from Oklahoma, Texas and Mississippi found on *Q. fusiformis* and *Q. virginiana*; while the second clade includes samples now identified as *B. treatae* and *B. fossoria* from Alabama, Georgia, Florida and Mississippi found on *Q. virginiana* and *Q. geminata*, respectively. In contrast, the maximum likelihood tree based on SNP data supports three distinct lineages (Fig. 7), with samples identified as *B. fossoria* found exclusively in Florida and attacking *Q. geminata* as distinct from *B. treatae* (same locality and host info as *COI* data). The *Belonocnema kinseyi* clade was also recovered by analysis of the SNP data for the same localities and hosts, including additional samples from Louisiana. *Belonocnema treatae* was recovered from Alabama, Georgia, Florida, Mississippi, North Carolina and South Carolina, almost exclusively from

Q. virginiana, except for the sample collected from *Q. geminata* in Parker, Florida.

The results of the morphological examination confirmed previous, as well as novel, diagnostic characters between each of the three *Belonocnema* lineages. This includes lineage-specific variation in tibial spurs, scutellar foveae, wing Rs veins and wing areolets. In addition, reduced wing structure in the asexual generation of *Belonocnema* associated with *Q. geminata* was observed. This morphological variation is now included in a new dichotomous key for the asexual and sexual generations of the three *Belonocnema* species within the taxonomic revision (see below).

The results of the flight test demonstrated that asexual-generation *Belonocnema* with intact and fully developed wings associated with both *Q. fusiformis* and *Q. virginiana* are capable of flight (macropterous), while *Belonocnema* with reduced wings associated with *Q. geminata* do not fly (brachypterous; see Table 2 for flight test results).

TAXONOMIC REVISION

GENUS *BELONOCNEMA* (MAYR, 1881)

Type species: *Belonocnema treatae* (Mayr, 1881).

Diagnosis: *Belonocnema* can be distinguished from all other Cynipini genera by the combination of the curved spine on the anterior side of the fore tibia (Fig. 3A), and the strongly angulate Rs with narrow black

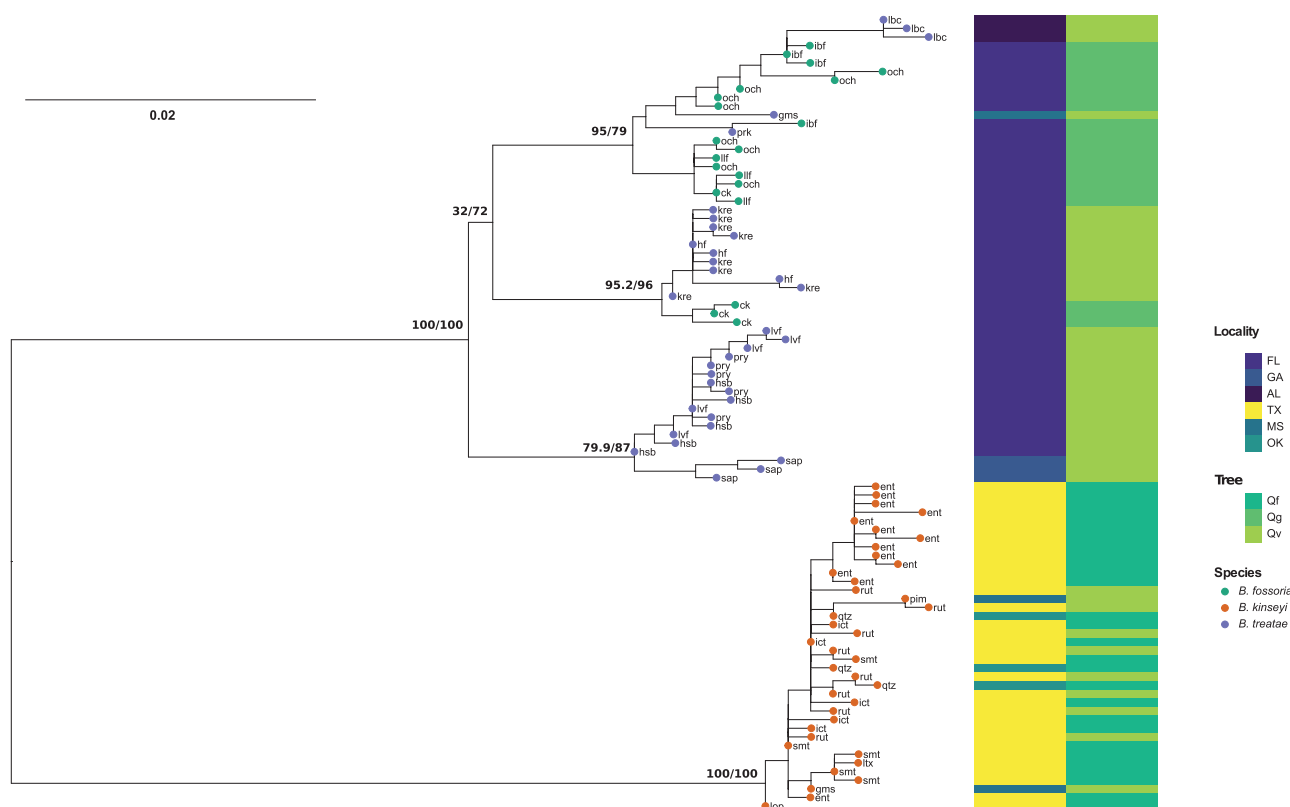


Figure 6. Maximum likelihood tree for *Belonocnema* of the southern and south-eastern USA based on sampling of 23 populations and COI data collected by Schuler *et al.* (2018). Numbers at nodes represent ultrafast bootstrap and SH-aLRT. (See Supporting Information, Table S1 for corresponding site abbreviations.)

stripes, which form a short radial cell in the fore wing (Fig. 4A; Melika & Abrahamson, 2002). The genus is restricted to live oaks (*Quercus* section *Quercus* series *Virentes*). Sexual-generation males have 15 antennal segments with F1 excavated, while the sexual females and asexual females have 14 and 13 antennal segments, respectively. The sexual females are larger than asexual-generation conspecifics (Hood & Ott, 2017: fig. 2A) based on measurement of the length of the right tibia length, often used as a proxy for body size, (e.g., the average tibial length of sexual females of *B. kinseyi* = 1.46 ± 0.007 mm vs. that of asexual females = 1.05 ± 0.004 mm; Hood & Ott, 2017).

Redescription: Female antenna 13-segmented (asexual generation) (Fig. 4C) and 14-segmented (sexual generation) (Fig. 4E), male antenna 15-segmented and filiform. Head weakly sculptured, almost smooth or alutaceous to finely coriaceous. Occiput without distinct and sharp occipital carina. Ventral area of genae without vertical carinae. Malar space much shorter than height of compound eye and malar sulcus present. Lower face without striae radiating from sides of clypeus. Mesoscutum smooth and shiny. Notauli complete (Fig. 3B), anterior parallel and parapsidal lines absent (Figs 2F,

4F). Scutellar foveae present (Figs 2F, 4F). Mesopleuron smooth and shiny. Propodeal carinae sharply curved. Second metasomal tergum large and medially setose. Radial cell of the forewings open, partially infumated, Rs strongly angulate, with or without areolet (Figs 3B, 4A). Fore tibia prolonged on the anterior side into a curved spine (Figs 2C, 3A), with or without middle tibia curved spine. Tarsi with swollen base, not toothed. Body colour varies from light yellow to orange to reddish brown.

BELONOCNEMA FOSSORIA, WELD, 1921, REV. STAT.

(Figs 1A–D, 3)

Belonocnema quercusvirens, Burks, 1979.

Material examined: Asexual generation – Syntype 1F ‘USA: FL, Clearwater, Reared Dec-13–19, *Quercus geminata*, L.H.Weld Collector, Hopk. U.S. 15634f, Type 24099, USNMMENT 00802094’; Paratype 9F same locality as Syntype USNMMENT 00893032, 00893095, 00893115, 00893123, 00893218; 4F ‘USA: SC, Charleston Co. S. Car ’43, *Q. virginiana*, 1160a, USNM’; 2F ‘USA: FL, Archbold Biol. Stn., 27.1846, -81.3521, 19/X/2016, Ott Lab, *Q. geminata*’; 5F ‘USA:

KEY TO THE ASEXUAL AND SEXUAL GENERATIONS OF THE THREE *BELONOCNEMA* SPECIES OF THE SOUTHERN AND SOUTH-EASTERN USA:

1. Antennae with 14 (females) or 15 (males, F1 excavated) segments.....2 (sexual generation)
- 1'. Antennae with 13 segments 4 (asexual generation)
2. Spur on the anterior side of fore tibia longer than basitarsus and tibial spurs (Fig. 3A). Middle tibia with a smaller spur. Found on *Q. geminata* in Georgia and Florida.....*B. fossoria*
- 2'. Spur on the anterior side of fore tibia shorter than basitarsus, approximately the same length as tibial spurs (Fig. 2C). Middle tibia without a spur. Found on *Q. fusiformis* or *Q. virginiana*..... 3
3. Scutellar foveae deeply excavated, delimited on all sides, separated narrowly by a carina (Fig. 4F). Rs vein thickened but not infumated (Fig. 4E). Found west of Gautier, Mississippi (30.3858° N, 88.6117° W), associated with *Q. fusiformis* and *Q. virginiana**B. kinseyi*
- 3'. Scutellar foveae shallow, weakly delimited posteriorly, separated broadly by a ridge (Fig. 2F). Rs vein thickened and infumated (Fig. 2E). Found east of Gautier, Mississippi, associated predominantly with *Q. virginiana* and rarely with *Q. geminata* *B. treatae*
4. Spur on the anterior side of fore tibia longer than basitarsus and tibial spurs (Fig. 3A). Middle tibia with a smaller spur. Fore wing small and curved, areolet absent (Fig. 3A). Found on *Q. geminata* in Georgia and Florida*B. fossoria*
- 4'. Spur on the anterior side of fore tibia shorter than basitarsus, approximately the same length as tibial spurs. Middle tibia without a spur. Fore wing not curved, areolet present (could be small and indistinct) (Fig. 4A). Found on *Q. fusiformis* or *Q. virginiana* 5
5. Reddish brown in colour, areolet in fore wing small and indistinct (Fig. 4A). Found west of Gautier, Mississippi, associated with *Q. fusiformis* and *Q. virginiana*.....*B. kinseyi*
- 5'. Yellowish brown in colour, areolet in fore wing large and distinct (Fig. 2E). Found east of Gautier, Mississippi, associated predominantly on *Q. virginiana* and rarely with *Q. geminata* *B. treatae*

FL, Dickinson State Pk., 27.0261, -80.1090, 18/X/2016, Ott Lab, *Q. geminata*'; 5F 'USA: FL, Oceanside Village, 29.9542, -85.4277, 30/X/2016, Ott Lab, *Q. geminata*'.

Sexual Generation – 7F3M 'USA: FL, Archbold Biol. Stn., 27.1846, -81.3521, IV/2018, Egan Lab, *Q. geminata*'; 5F5M 'USA: FL, Dickinson State Pk., 27.0261, -80.1090, III/2017, Egan Lab, *Q. geminata*'; 5F5M 'USA: FL, Lake Lizzie, 28.2277, -81.1800, III/2018, Egan Lab, *Q. geminata*'; 4M 'USA: FL, Volusia Co. Daytona Beach, IV-6-1998, Urban beachside UV light trap, C Yorke, S. Fullerton, UCFC 0017693, 0017694, 0017695, 0017696'; 1F2M 'USA: FL, Brevard Co. Malabar, Malabar Rd. 30 Mar – 25 May 2000, P.J. Russell, Z. Prusak, S.M. Fullerton, UCFC 0079161, 0100100, 0101364'; 2F3M 'USA: FL, Brevard Co. Titusville, SR 405, 21 Feb–15 May 2001, Z. Prusak, P.J. Russell, S.M. Fullerton, UCFC 0078923, 0079318, 0093022, 0093669, 0103143'; 2F2M 'USA: FL, Orange Co. Rk. Spr. Rn. St. Res. IV-13–1995, S.M. Fullerton, UCFC 0201995, 0202004, 0202411, 0202415'; 5F5M 'USA: FL, Orange Co. UCF 28°36'37"N 81°12'01"W LLP Flatwds, M. Carey, S.L. Kelly, S.M. Fullerton, III-28–2008, UCFC 0463902, 0463926, 0463936, 0463954, 0463957, 0464351, 0464355, 0464384, 0464390, 0464524. 8F4M 'USA: FL, Orange/Osceola Co. Walt Disney World, 24 Mar–28 Apr 1998, Z. Prusak, S. Fullerton, UCFC 0017132, 0017146, 0017173, 0017230, 0017232, 0017601, 0017602, 0017603, 0017604, 0017608, 0017787, 0017793'; 2M

'USA: FL, Sarasota Co. MCC- Venice Campus, III-31-1997, K.J. Maharay, S.M. Fullerton, UCFC 0018361, 0018363'; 2F1M 'USA: FL, Seminole Co. Econ. Wild. Area IV-8-2000, T. Smith, UCFC 0054591, 0054607, 0060190'; 2F1M 'USA: FL, Seminole Co. Lower Wekiva River St. Preserve, 28-IV-2001, P.J. Russell, S.M. Fullerton, UCFC 0108938, 0109646, 0109778'; 3F 'USA: FL, Seminole Co. Oveido, rural yard, Malaise trap, IV-7-1994, S.M. Fullerton, UCFC 0202173, 0202175, 0202190'; 4F6M 'USA: FL, Seminole Co. Oveido, rural yard, UV light, 28°39'25"N 81°10'44"W, S.M. Fullerton, III-28–IV-12-2009, UCFC 0446980, 0446991, 0446992, 0446993, 0446994, 0446996, 0448649, 0448658, 0448659, 0448660'.

Diagnosis: *Belonocnema fossoria* can be distinguished from the other two known species by the spur on the anterior side of fore tibia longer than basitarsus and tibial spurs (Fig. 3A). The asexual generation has small, non-functional wings, lack of areolet in the front wing, and the middle tibia with an additional spur.

Description

Asexual female (Figs 1D, 3A–C): Body length 2.5–3.5 mm ($N = 12$). Reddish brown; tip of mandibles, wing veins dark brown (Fig. 3A). Head finely coriaceous with sparse white setae; slightly rounded in dorsal view; 2.3×

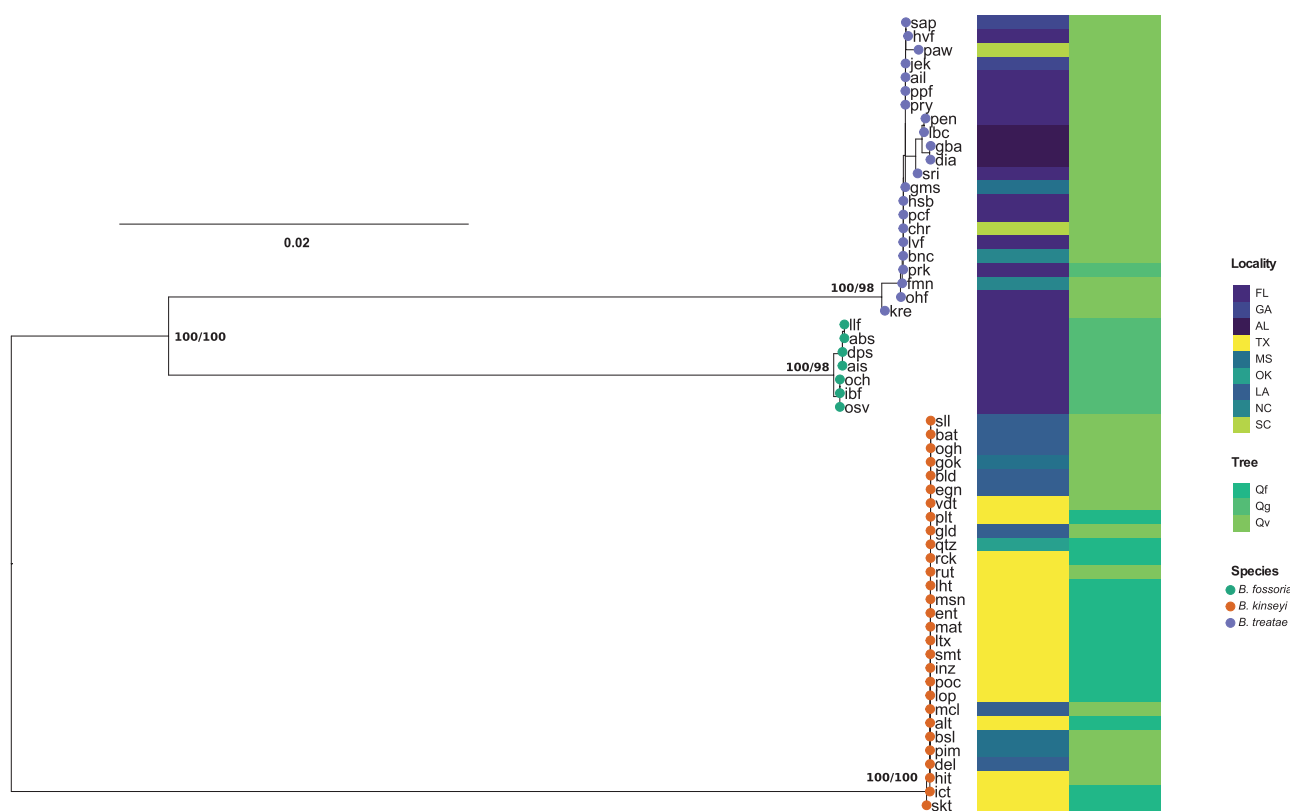


Figure 7. Maximum likelihood tree for *Belonocnema* of the southern and south-eastern USA based on sampling and SNP data collected by Driscove *et al.* (2019), using 1 individual sampled randomly from each of $N = 58$ populations. Numbers at nodes represent ultrafast bootstrap and SH-aLRT. (See Supporting Information, Table S1 for corresponding site abbreviations.)

as broad as long in dorsal view; 1.2× as broad as long in frontal view; slightly broader than mesosoma. Gena alutaceous, not broadened behind eye in dorsal view; 1.2× as broad as cross diameter of eye. Malar space alutaceous, without striae radiating from clypeus; eye 2.1× higher than length of malar space. Inner margins of eyes parallel. OOL 1.6× longer than POL; OOL 2.3× longer than LOL; ocelli ovate, all equal in size. Transfacial distance 1.8× longer than height of eye and 1.5× longer than height of lower face (Fig. 3C); diameter of antennal torulus 2.3× longer than distance between them, distance between torulus and eye margin 1.4× longer than diameter of torulus. Lower face finely coriaceous, with white setae, without striae radiating from clypeus, median area not elevated. Clypeus trapezoid, flat, broader than high, with deep anterior tentorial pits, distinct epistomal sulcus and clypeopleurostomal line. Frons finely coriaceous, glabrous; vertex, interocellar area, occiput is finely coriaceous. Postgena coriaceous, glabrous. Antenna 13 segmented, longer than head + mesosoma; F1 shorter than the length of scape + pedicel, 1.7× longer than F2 (Fig. 3C).

Mesosoma longer than high in lateral view.

Propleuron alutaceous, with few setae. Mesoscutum smooth, glabrous between notauli, alutaceous lateral to notaulus; longer than broad (width measured across base of tegulae); notauli complete, deeply impressed for full length; median mesoscutal line distinct; anterior parallel lines and parapsidal lines absent (Fig. 3B); Mesoscutellum only slightly longer than broad, slightly broader posteriorly; shorter than mesoscutum, uniformly rugose, overhanging metanotum; scutellar foveae present but shallow and indistinct. Mesopleural triangle large, sparsely setose; Mesopleuron smooth, glabrous, with a few white setae along ventral and anterior margins. Lateral propodeal carinae distinct, central propodeal area glabrous, with rugae; lateral propodeal area alutaceous, with dense white setae; nucha short, coriaceous. Legs short and stout; tibia setose on anterior edge; fore tibia prolonged on the anterior side into a curved spine (Fig. 3A), longer than basitarsus and the tibial spur; tarsal claws simple with a slight ridge but never a full tooth. Middle tibia also with curved spine but smaller than basitarsus and tibial spurs; middle and hind tibia with two spurs. Tarsi

Table 2. Flight test for *Belonocnema* asexual generation

Taxon	Host Plant	*Site	N	% Fly
<i>B. kinseyi</i>	<i>Q. fusiformis</i>	qtz	11	27
		ict	3	66
		lop	21	76
		ent	18	94
		Total	53	72
<i>B. treatae</i>	<i>Q. virginiana</i>	hit	23	87
		pim	13	69
		gld	35	100
		bsl	12	100
		gok	1	100
		sri	1	100
		pry	14	80
		hsb	2	100
		ais	3	100
		kre	1	0
		fmn	17	94
		bnc	1	100
		Total	123	90
<i>B. fossoria</i>	<i>Q. geminata</i>	ibf	1	0
		prk	11	0
		osv	36	0
		och	17	0
		ais	14	0
		llf	56	0
		Total	135	0

N = number tested; * see [Supporting Information, Table S1](#) for corresponding site abbreviations.

covered in setae. Fore wing hyaline, shorter than body and often curved upwards, margin with dense cilia; Radial cell 2× as long as wide; 2r and Rs infumated, Rs curved upwards and thickened at apex; radial cell open; areolet absent; Rs + medial vein (M) reaching to M; cubitus-anal crossvein (cu-a) absent; first cubitus vein (cu1) broken ([Fig. 3B](#)). Metasoma slightly longer than head + mesosoma, as long as high in lateral view, smooth and glabrous; second metasomal tergite occupies setose medially; all subsequent tergites without setae, smooth, glossy; ventral spine of the hypopygium short, prominent part as long as broad in ventral view, with white setae extending beyond the apex of spine ([Fig. 3A](#)).

Sexual female ([Figs 1B, 3E](#)): Body length 3.5–4.5 mm ($N = 16$). Reddish brown; pedicel, flagellomeres, vertex, tip of mandibles, tarsal claws, wing veins dark brown ([Fig. 3E](#)). Head finely coriaceous with sparse white setae; slightly rounded in dorsal view; 1.8× as broad as long in dorsal view; 1.3× as broad as long in frontal view; slightly broader than mesosoma. Gena alutaceous, not broadened behind eye in dorsal view; 0.7× as broad as cross diameter

of eye. Malar space alutaceous, without striae radiating from clypeus; eye 2.1× higher than length of malar space. Inner margins of eyes parallel. OOL 1.6× longer than POL; OOL 1.4× longer than LOL; ocelli ovate, all equal in size. Transfacial distance 1.6× longer than height of eye and 1.3× longer than height of lower face; diameter of antennal torulus 2× longer than distance between them, distance between torulus and eye margin 1.3× longer than diameter of torulus. Lower face finely coriaceous, with white setae, without striae radiating from clypeus, median area not elevated. Clypeus trapezoid, flat, broader than high, with deep anterior tentorial pits, distinct epistomal sulcus and clypeo-pleurostomal line. Frons finely coriaceous, glabrous; vertex, interocellar area, occiput is finely coriaceous. Postgena coriaceous, glabrous. Antenna 14 segmented, longer than head + mesosoma; F1 shorter than the length of scape + pedicel, 1.3× longer than F2 ([Fig. 3E](#)). Mesosoma longer than high in lateral view. Propleuron alutaceous, with few setae. Mesoscutum smooth, glabrous between notauli, alutaceous lateral to notaulus; longer than broad (width measured across base of tegulae); notauli complete, deeply impressed for full length; median mesoscutal line distinct; anterior parallel lines and parapsidal lines absent; mesoscutellum only slightly longer than broad, slightly broader posteriorly; shorter than mesoscutum, uniformly rugose, overhanging metanotum; scutellar foveae present. Mesopleuron smooth, glabrous, with a few white setae along ventral and anterior margins; mesopleural triangle setose. Lateral propodeal carinae bent outwards, central propodeal area glabrous, with rugae; lateral propodeal area alutaceous, with dense white setae; nucha short, coriaceous. Legs short and stout; tibia setose on anterior edge; fore tibia prolonged on the anterior side into a curved spine, longer than basitarsus and the tibial spur; tarsal claws simple with a slight ridge but never a full tooth. Middle tibia also with curved spine but smaller than basitarsus and tibial spurs; middle and hind tibia with two spurs. Tarsi covered in setae ([Fig. 3E](#)). Fore wing hyaline, shorter than body and often curved upwards, margin with dense cilia; radial cell 2× as long as wide; 2r and Rs infumated, Rs curved upwards and thickened at apex; radial cell open; areolet present; Rs + M reaching to M; cu-a absent; cu1 broken ([Fig. 3E](#)). Metasoma slightly longer than head + mesosoma, as long as high in lateral view, smooth and glabrous; second metasomal tergite occupies setose medially; all subsequent tergites without setae, smooth, glossy; ventral spine of the hypopygium short, prominent part 1.5× as long as broad in ventral view, with white setae extending beyond the apex of spine ([Fig. 3E](#)).

Male: Body length 3.2–4.1 mm ($N = 15$). Colour and sculptures like the sexual female, Antenna 15 segmented; F1 is curved, excavated, and incised medially. Metasoma smaller than head + mesosoma.

Gall: Smooth, single-chambered pea galls (5.88–6.45 mm in diameter) on the ventral side of leaves for the asexual generation (Fig. 1C); irregular shaped, multilocular galls often in clusters on the small rootlets for the sexual generation (Fig. 1A; Egan *et al.*, 2013). Host plant: *Quercus geminata*.

Distribution: Georgia, Florida (USA).

Biology: The small, often bent wings of the asexual generation (Figs 1D, 3A) appear to be non-functional (Table 2), and the large tibial spur and short, stout legs are likely fossorial adaptations that allows *B. fossoria* to reach the rootlets of *Q. geminata* in sandy soil where these species co-occur. The sexual generation emerges from early March to mid-April, corresponding with the timing of leaf flush of their host *Q. geminata* (Hood *et al.*, 2019).

Remarks: Osten Sacken's (1861) original description of *C. quercusvirens* was based on the asexual galls alone collected on 'live oak' in Georgia, the wasp itself was described by Weld (1921) as *B. fossoria*. As both *B. treatae* and *B. fossoria* can be found in the region and both induce similar galls, it is difficult to know which species Osten Sacken collected and named. However, names described before 1930, and which were applied only to the product of an animal, are valid names for the organism itself under ICZN Article 1, section 3. Therefore, we here propose the name *B. quercusvirens* as *species inquirenda*.

BELONOCNEMA KINSEYI, WELD, 1921, REV. STAT.

(Figs 1E, F, 4)

Belonocnema treatae, Lund *et al.*, 1998, female, male, asexual, sexual generation, gall.

Material examined: Asexual – Syntype 1F 'USA: TX, Boerne, Nov.-15–1917, Cotype 22832, USNM 00802145'; 46F 'same locality as Syntype, Nov.-15–Dec.1-1917, USNM'; 5F 'USA: LA: Golden Meadow, 29.3939, -90.2729, 22/X/2016, Ott Lab, *Q. virginiana*'; 5F 'USA: LA, Oak Grove Hwy, 29.7668, -92.9750, 21/X/2016, Ott Lab, *Q. virginiana*'; 2F 'USA: MS, Gautier, 30.3803, -88.6104, 28/X/2016, Ott Lab, *Q. virginiana*'; 5F 'USA: MS, Picayune, 30.5271, -89.6813, 30/X/2016, Ott Lab, *Q. virginiana*'; 5F 'USA: OK, Quartz Mountain, 34.8901, -99.3011, 17/X/2018, Ott Lab, *Q. fusiformis*'; 5F 'USA: TX, Encino, 26.8942, -98.1352, 13/IX/2015, Ott Lab, *Q. fusiformis*'; 5F 'USA: TX, High Island, 29.5612, -94.3918, 17/X/2016, Ott Lab, *Q. virginiana*'; 5F 'USA: TX, Live Oak Park, 27.8544, -97.2105, 1/XI/2015, Ott Lab, *Q. fusiformis*';

5F 'USA: TX, Luling, 29.6739, -97.6350, 5/XI/2016, Ott Lab, *Q. fusiformis*'; 5F 'USA: TX, Pleasanton, 28.9523, -98.4509, 23/X/2016, Ott Lab, *Q. fusiformis*'; 5F 'USA: TX, Rocksprings, 29.8751, -100.1086, 13/XI/2016, Ott Lab, *Q. fusiformis*'.

Sexual generation – 4F 6M 'USA: TX, Rice University, 28.7174, -95.4023, III/2018, Egan Lab, *Q. virginiana*'; 2F UTIC 200066, 200067 'USA, TX, Travis Co: Austin nr Austin Mem. Park Cemetery, 30.3281, -97.7543, 210 m 14.III.2016, A.L.Wild, UV Light, 20–2300 h'; 6F 6M 'USA: LA, Golden Meadow, 29.3939, -90.2729, III/2018, Egan Lab, *Q. virginiana*'; 5F 5M 'USA: MS, Picayune, 30.5271, -89.6813, III/2016, Ott Lab, *Q. virginiana*'; 5F 5M 'USA: OK, Quartz Mountain, 34.8901, -99.3011, III/2016, Ott Lab, *Q. fusiformis*'; 4F 5M 'USA: TX, Encino, 26.8942, -98.1352, III/2015, Ott Lab, *Q. fusiformis*'; 5F 4M 'USA: TX, Live Oak Park, 27.8544, -97.2105, III/2015, Ott Lab, *Q. fusiformis*'; 4F 4M 'USA: TX, San Marcos, 29.9373, -98.0099, 11/XI/2016, Ott Lab, *Q. fusiformis*'.

Diagnosis: *Belonocnema kinseyi* can be distinguished from *B. fossoria* by the spur on the anterior side of fore tibia shorter than basitarsus and tibial spurs in both generations. It can also be separated from *B. treatae* in the sexual generation by the deeply delimited scutellar foveae separated narrowly by a carina, and the reddish brown colour along with an indistinctive areolet in the asexual generation.

Description

Asexual female (Figs 1F, 4A–C): Body length 2.6–3.3 mm ($N = 22$). Reddish brown; tip of mandibles, mesosoma (except for mesoscutum), wing veins, anterior third of first gastral tergite, anterior edge of fore, meso, and metacoxae, and distal edge of hind femora black (Fig. 4A). Head finely coriaceous with sparse white setae; slightly rounded in dorsal view; 2.1× as broad as long in dorsal view; 1.4× as broad as long in frontal view; slightly broader than mesosoma. Gena alutaceous, not broadened behind eye in dorsal view; equally broad as cross diameter of eye. Malar space alutaceous, without striae radiating from clypeus; eye 2.3× higher than length of malar space. Inner margins of eyes parallel. OOL 1.1× longer than POL; OOL 2.2× longer than LOL; ocelli ovate, all equal in size. Transfacial distance 1.4× longer than height of eye and 1.3× longer than height of lower face (Fig. 4C); diameter of antennal torulus 2× longer than distance between them, distance between torulus and eye margin 2× longer than diameter of torulus. Lower face finely coriaceous, with white setae, without striae radiating from clypeus, median area not elevated. Clypeus trapezoid, flat, broader than high, with deep anterior tentorial pits, distinct epistomal sulcus and

clypeo-pleurostomal line. Frons finely coriaceous, glabrous; vertex, interocellar area, occiput is finely coriaceous. Postgena coriaceous, glabrous. Antenna 13 segmented, longer than head + mesosoma; F1 shorter than the length of scape + pedicel, 1.6× longer than F2 (Fig. 4A). Mesosoma longer than high in lateral view. Propleuron alutaceous, with few setae. Mesoscutum smooth, glabrous between notauli, alutaceous lateral to notaulus; longer than broad; notauli complete, deeply impressed for full length; median mesoscutal line distinct; anterior parallel lines and parapsidal lines absent (Fig. 4B); mesoscutellum only slightly longer than broad, slightly narrower posteriorly; shorter than mesoscutum, uniformly rugose, overhanging metanotum; scutellar foveae present. Mesopleural triangle covered with dense white setae, mesopleuron smooth, glabrous, with a few white setae along ventral and anterior margins. Lateral propodeal carinae distinct, bent outwards; central propodeal area rugose; lateral propodeal area alutaceous, with dense white setae; nucha short, coriaceous. Tibia setose on anterior edge; fore tibia prolonged on the anterior side into a curved spine, much shorter than tibial spur and basitarsus; tarsal claws simple with a slight ridge but never a full tooth. Middle and hind tibia with two spurs (Fig. 4A). Fore wing hyaline, longer than body, margin with dense cilia; radial cell 2× as long as wide; 2r infumated, Rs curved upwards and thickened at apex; radial cell open; areolet small and indistinct; Rs + M reaching to M; cu-a absent; cu1 broken (Fig. 4A). Metasoma shorter than head + mesosoma, 1.2× longer than high in lateral view, smooth and glabrous; second metasomal tergite setose medially; all subsequent tergites without setae, smooth, glossy; ventral spine of the hypopygium short, prominent part 1.3× as long as broad in ventral view, with white setae extending beyond the apex of spine (Fig. 4A).

Sexual female (Fig. 4E, F): Body length 3.5–4.0 mm ($N = 15$). Yellowish brown; scape, flagellomeres, tip of mandibles, propodeum, wing veins, distal edge of hind femora, hind tibia, and tarsi dark brown (Fig. 4E). Head finely coriaceous with sparse white setae; slightly rounded in dorsal view; 2.4× as broad as long in dorsal view; 1.2× as broad as long in frontal view; slightly broader than mesosoma. Gena alutaceous, not broadened behind eye in dorsal view; equally broad as cross diameter of eye. Malar space alutaceous, without striae radiating from clypeus; eye 2.3× higher than length of malar space. Inner margins of eyes parallel. OOL 1.1× longer than POL; OOL 2.2× longer than LOL; ocelli ovate, all equal in size. Transfacial distance 1.8× longer than height of eye and 1.7× longer than height of lower face; diameter of antennal torulus 2.3× longer than distance between them, distance between torulus

and eye margin 1.6× longer than diameter of torulus. Lower face finely coriaceous, with white setae, without striae radiating from clypeus, median area not elevated. Clypeus trapezoid, flat, broader than high, with deep anterior tentorial pits, distinct epistomal sulcus and clypeo-pleurostomal line. Frons finely coriaceous, glabrous; vertex, interocellar area, occiput is finely coriaceous. Postgena coriaceous, glabrous. Antenna 14 segmented, longer than head + mesosoma; F1 shorter than the length of scape + pedicel, 1.6× longer than F2 (Fig. 4E). Mesosoma longer than high in lateral view. Propleuron alutaceous, with few setae. Mesoscutum smooth, glabrous between notauli, alutaceous lateral to notaulus; longer than broad; notauli complete, deeply impressed for full length; median mesoscutal line distinct; anterior parallel lines and parapsidal lines absent; mesoscutellum only slightly longer than broad, slightly narrower posteriorly; shorter than mesoscutum, uniformly rugose, overhanging metanotum; scutellar foveae deeply excavated, fully delimited on all sides, separated narrowly by carina (Fig. 4H). Mesopleuron smooth, glabrous, with a few white setae along ventral and anterior margins. Lateral propodeal carinae distinct, straight; central propodeal area punctate; lateral propodeal area alutaceous, with dense white setae; nucha short, coriaceous (Fig. 4F). Tibia setose on anterior edge; fore tibia prolonged on the anterior side into a curved spine, much shorter than tibial spur and basitarsus; tarsal claws simple with a slight ridge but never a full tooth. Middle and hind tibia with two spurs (Fig. 4E). Fore wing hyaline, longer than body, margin with dense cilia; radial cell 2.3× as long as wide; 2r infumated, Rs curved upwards and thickened at apex; radial cell open; areolet small and indistinct; Rs + M reaching to M; cu-a absent; cu1 broken (Fig. 4E). Metasoma shorter than head + mesosoma, 1.2× longer than high in lateral view, smooth and glabrous; second metasomal tergite setose medially; all subsequent tergites without setae, smooth, glossy. Ventral spine of the hypopygium short, prominent part 1.5× as long as broad in ventral view, with white setae extending beyond the apex of spine (Fig. 4E).

Male: Body length 2.6–3.9 mm ($N = 16$). Colour darker than sexual female, scape, pronotum, mesoscutum and mesopleuron, metasoma dark brown, sculptures like the sexual female. Antenna 15 segmented; F1 is curved, excavated, and incised medially. Metasoma smaller than head + mesosoma; see Lund *et al.* (1998: fig. 7) for comparison of lateral habitus of sexual generation male and female.

Gall: Smooth, unilocular pea-like galls on the ventral side of leaves for the asexual generation (Fig. 1E; Lund *et al.* 1998: figs 1, 2), irregular shaped, multilocular

clusters of galls on the small rootlets for the sexual generation (Lund *et al.* 1998: figs 3, 4).

Host plant: *Quercus fusiformis* (Oklahoma, Texas, Mexico) and *Q. virginiana* (east Texas to Florida, north to North Carolina).

Distribution: Louisiana, Mississippi, Oklahoma, Texas.

Biology: The asexual generation has long, straight wings and is capable of flight (Table 2). Given the distribution of its host *Q. fusiformis* (Cavender-Bares *et al.*, 2015), this species likely also occurs in northern Mexico.

Remarks: *Belonocnema kinseyi* was previously synonymized by Lund *et al.* (1998) based on experimental rearing that linked the asexual and sexual generations developing on *Q. fusiformis* in Texas. Given the molecular and morphological evidence showing that populations west of the Gulf of Mexico represent a distinct species, the synonymy by Lund *et al.* (1998) is, therefore, rejected and *B. kinseyi* 17.15 is restored as a valid species. The genome of this species, *B. kinseyi*, has been sequenced and annotated (NCBI SRA: PRJNA623416) originally under the name *B. treatae*.

BELONOCNEMA TREATAE (MAYR, 1881)

(FIG. 2)

Dryorhizoxenus floridanus Ashmead, 1881, female, male, sexual generation, gall.

Belenocnema [sic!] *treatae* Mayr, 1881, female, sexual generation.

Material examined: Asexual generation – 12F ‘USA: FL: Jacksonville Type 2813, USNM, *Dryorhizoxenus floridanus*’; 6F ‘USA: FL: E. Florida, USNM’; 1F ‘USA: FL: LaBelle, IV-20–21, USNM’; 1F ‘USA: FL: Tampa 14-4, USNM’; 1F ‘USA: FL: Manatee Co., 111–26, R.F. Tinker, USNM’; 5F ‘USA: AL, Dauphin Island, 30.2504, -88.1325, 29/X/2016, Ott Lab, *Q. virginiana*’; 2F ‘USA: FL, Archbold Biol. Stn., 27.1846, -81.3521, 28/X/2016, Ott Lab, *Q. virginiana*’; 5F ‘USA: FL, Kissimmee River, 27.3780, -81.0968, 18/X/2016, Ott Lab, *Q. virginiana*’; 5F ‘USA: FL, Perry, 30.1161, -83.5895, 14/X/2015, Ott Lab, *Q. virginiana*’; 5F ‘USA: GA, Jekyll Island, 31.0174, -81.4297, 28/X/2016, Ott Lab, *Q. virginiana*’; 3F ‘USA: MS, Gautier, 30.3803, -88.6104, 28/X/2016, Ott Lab, *Q. virginiana*’; 5F ‘USA: NC, Fort Macon, 34.6951, -76.6862, 30/X/2016, Ott Lab, *Q. virginiana*’; 5F ‘USA: SC, Charleston, 32.7688, -79.9734, 30/X/2016, Ott Lab, *Q. virginiana*’.

Sexual generation – 1F Lectotype (photo only) ‘Brief mai 78 N. Amer., Collect G. Mayr, Bel. Treatae det. G. Mayr, LECTOTYPE *Belonocnema treatae* Mayr desig. G. Melika 998’ NHMW. 5F 6M ‘USA: AL, Gulf Shores, 30.2558, -87.7205, 31/X/2016, Ott Lab, *Q. virginiana*’; 5F 5M ‘USA: FL, Kissimmee River, 27.3780, -81.0968, III/2016, Ott Lab, *Q. virginiana*’; 10M ‘USA: FL, Okeechobee, 27.2434, -80.8276, III/2017, Ott Lab, *Q. virginiana*’; 5F 5M ‘USA: FL, Perry, 30.1161, -83.5895, III/2016, Ott Lab, *Q. virginiana*’.

Diagnosis: *Belonocnema treatae* can be distinguished from *B. fossoria* by the spur on the anterior side of fore tibia shorter than basitarsus and tibial spurs in both generations. It can also be separated from *B. kinseyi* in the sexual generation by the weakly delimited scutellar foveae separated broadly by a ridge and the yellowish brown colour along with a distinctive areolet in the asexual generation.

Description

Asexual female (Fig. 2E): Body length 2.8–3.2 mm ($N = 35$). Yellowish brown; tip of mandibles, scutellum, propodeum, mesopleural triangle, metapleuron, scutellum, hind tibia dark brown (Fig. 2E). Head finely coriaceous with sparse white setae; slightly rounded in dorsal view; 2× as broad as long in dorsal view; 1.3× as broad as long in frontal view; slightly broader than mesosoma. Gena alutaceous; not broadened behind eye in dorsal view; 1.1× broader than the cross diameter of eye. Malar space alutaceous, without striae radiating from clypeus; eye 2.2× higher than length of malar space. Inner margins of eyes parallel. OOL 1.2× longer than POL; OOL 2.4× longer than LOL; ocelli ovate, all equal in size. Transfacial distance 1.7× longer than height of eye and 1.8× longer than height of lower face; diameter of antennal torulus 1.3× longer than distance between them, distance between torulus and eye margin 3.3× longer than diameter of torulus. Lower face finely coriaceous, with white setae, without striae radiating from clypeus, median area not elevated. Clypeus trapezoid, flat, broader than high, with deep anterior tentorial pits, distinct epistomal sulcus and clypeo-pleurostomal line. Frons finely coriaceous, glabrous; vertex, interocellar area, occiput is finely coriaceous. Postgena coriaceous, glabrous. Antenna 13 segmented, longer than head + mesosoma; F1 shorter than the length of scape + pedicel, 1.6× longer than F2 (Fig. 2E). Mesosoma longer than high in lateral view. Propleuron evenly setose. Mesoscutum smooth, glabrous between notauli, alutaceous lateral to notaulus; longer than broad; notauli complete, deeply impressed for full length; median mesoscutal line distinct; anterior parallel lines and parapsidal lines absent; mesoscutellum only slightly longer

than broad, slightly narrower posteriorly; shorter than mesoscutum, uniformly rugose, overhanging metanotum; scutellar foveae present. Mesopleural triangle covered with dense white setae, mesopleuron smooth, glabrous, with a few white setae along ventral and anterior margins. Lateral propodeal carinae distinctly raised and bent outwards, central propodeal area rugose; lateral propodeal area alutaceous, with dense white setae; nucha short, coriaceous. Tibia setose on anterior edge. Fore tibia prolonged on the anterior side into a curved spine, much shorter than tibial spur and basitarsus; tarsal claws simple with a slight ridge but never a full tooth. Middle and hind tibia with two spurs. Fore wing hyaline, longer than body, margin with dense cilia. Radial cell $2.5\times$ as long as wide; $2r$ infumated, Rs curved upwards and thickened at apex; radial cell open; areolet large and distinct; $Rs + M$ reaching to M ; $cu-a$ absent; $cu1$ broken (Fig. 2E). Metasoma shorter than head + mesosoma, $1.1\times$ longer than high in lateral view, smooth and glabrous. Second metasomal tergite setose medially; all subsequent tergites without setae, smooth, glossy. Ventral spine of the hypopygium short, prominent part $1.2\times$ as long as broad in ventral view, with white setae extending beyond the apex of spine (Fig. 2E).

Sexual female (Fig. 2A–C, F): Body length 3.5–4.0 mm ($N = 15$). Yellowish brown; flagellomeres, tip of mandibles, metascutellum, propodeum, wing veins, middle and hind tibia, and tarsi dark brown (Fig. 2A). Head finely coriaceous with sparse white setae; slightly rounded in dorsal view; $1.8\times$ as broad as long in dorsal view; $1.2\times$ as broad as long in frontal view; slightly broader than mesosoma. Gena alutaceous, not broadened behind eye in dorsal view; $1.3\times$ broader than cross diameter of eye. Malar space alutaceous, without striae radiating from clypeus; eye $3\times$ higher than length of malar space. Inner margins of eyes parallel. OOL $1.3\times$ longer than POL; OOL $3.5\times$ longer than LOL; ocelli ovate, all equal in size. Transfacial distance $1.4\times$ longer than height of eye and $1.4\times$ longer than height of lower face (Fig. 2A); diameter of antennal torulus $2\times$ longer than distance between them, distance between torulus and eye margin equal to diameter of torulus. Lower face finely coriaceous, with white setae, without striae radiating from clypeus, median area not elevated. Clypeus trapezoid, flat, broader than high, with deep anterior tentorial pits, distinct epistomal sulcus and clypeo-pleurostomal line. Frons finely coriaceous, glabrous; vertex, interocellar area, occiput is finely coriaceous. Postgena coriaceous, glabrous. Antenna 14 segmented, longer than head + mesosoma; $F1$ shorter than the length of scape + pedicel, $1.4\times$ longer than $F2$ (Fig. 2C). Mesosoma longer than high in lateral view. Propleuron alutaceous, with few setae. Mesoscutum

smooth, glabrous between notauli, alutaceous lateral to notaulus; longer than broad; notauli complete, deeply impressed for full length (Fig. 2B); median mesoscutal line distinct; anterior parallel lines and parapsidal lines absent. Mesoscutellum only slightly longer than broad, slightly narrower posteriorly; shorter than mesoscutum, uniformly rugose, overhanging metanotum (Fig. 2B); scutellar foveae shallow, weakly delimited posteriorly, separated widely by a ridge (Fig. 2F). Mesopleuron smooth, glabrous, with a few white setae along ventral and anterior margins. Lateral propodeal carinae distinct, curved; central propodeal area rugose; lateral propodeal area alutaceous, with dense white setae; nucha short, coriaceous. Tibia setose on anterior edge. Fore tibia prolonged on the anterior side into a curved spine, much shorter than tibial spur and basitarsus (Fig. 2C); tarsal claws simple with a slight ridge but never a full tooth. Middle and hind tibia with two spurs. Fore wing hyaline, longer than body, margin with dense cilia. Radial cell $2.3\times$ as long as wide; $2r$ infumated, Rs curved upwards and thickened at apex; radial cell open; areolet small and indistinct; $Rs + M$ reaching to M ; $cu-a$ absent; $cu1$ broken (Fig. 2A). Metasoma shorter than head + mesosoma, $1.2\times$ longer than high in lateral view, smooth and glabrous; second metasomal tergite setose medially; all subsequent tergites without setae, smooth, glossy. Ventral spine of the hypopygium short, prominent part $1.3\times$ as long as broad in ventral view, with white setae extending beyond the apex of spine (Fig. 2A).

Male: 3.7 mm ($N = 26$). Colour and sculptures like the sexual female, antenna 15 segmented; $F1$ is curved, excavated and incised medially. Metasoma smaller than head + mesosoma.

Gall: Smooth, pea-like galls (4.92–5.50 mm) on the ventral side of leaves for the asexual generation, irregular shaped, multilocular galls often in clusters on the small rootlets for the sexual generation (Egan *et al.*, 2013). The leaf galls produced by *B. treatae* on *Q. virginiana* are indistinguishable from the leaf gall produced by *B. kinseyi* on both *Q. fusiformis* (Fig. 1E) and *Q. virginiana*.

Host plant: *Quercus virginiana* and rarely on *Q. geminata*.

Distribution: Alabama, Florida, Georgia, Mississippi, North Carolina, South Carolina.

Biology: The asexual generation has long, straight wings and is capable of flight (Table 2). Sexual-generation adults emerge from mid-March to end of April, corresponding with the timing of leaf flush of their main host *Q. virginiana* (Hood *et al.*, 2019).

Remarks: Few specimens of this species have been collected from *Q. geminata*, which is unsurprising given the much later leaf flushing time of *Q. geminata* (Hood *et al.*, 2019). While the lectotype of *B. treatae* designated from Gustav Mayr's collection at NHMW by Melika & Bechtold (2001) was not examined physically, the high-quality image of the habitus clearly shows the diagnostic characters of *B. treatae* (small fore tibial spine shorter than tibial spur, broadly separated scutellar foveae).

DISCUSSION

The accurate identification of gall wasp species along with their host-plant associations and knowledge of their geographic ranges are vital to studies of their ecology and evolutionary histories. In this study we have established that the genus *Belonocnema* distributed throughout the south-eastern and southern USA consists of three species that are clearly diagnosed on the basis of phylogenetic analysis of SNP data and patterns of morphological character variation. In conjunction with extensive sampling of *Belonocnema* across the geographic range of the three major host plants, our results have allowed us to map, in some detail, the geographic range of all three species and to identify regions of sympatry, parapatry and allopatry, as illustrated in Figure 5. In contrast, the popular DNA-barcoding approach, using a region of the mitochondrial DNA *COI* sequence, failed to resolve the three species. The discordance between mitochondrial and nuclear markers has been previously documented in European oak gall wasps (Cook *et al.*, 2002; Nicholls *et al.*, 2012; Rokas *et al.*, 2003). Our study provides the first example of this phenomenon in North America, where *COI* was unable to delimit *B. fossoria* from *B. treatae*, despite strong morphological and nuclear DNA evidence, perhaps obscured by mtDNA linkage with the endosymbiont and reproductive manipulator *Wolbachia* Hertig & Wolbach, 1924 (Schuler *et al.*, 2018). Given, the deep divergence and virtual absence of gene flow between these two lineages (Driscove *et al.*, 2019), it is of interest that *B. fossoria* and *B. treatae* are capable of mating and producing viable offspring (Hood *et al.*, 2019; Zhang *et al.*, 2019, 2021). However, temporal separation imposed by differences in the phenology of leaf flush between *Q. virginiana* and *Q. geminata*, habitat selection and mate preference, limit the opportunity for gene flow between the two gall former species, despite sympatry (Hood *et al.*, 2019; Zhang *et al.*, 2019). Additionally, asymmetrical immigrant inviability and reduced fecundity act to limit gene flow (Zhang *et al.*, 2020).

The characteristic elongated tibial spurs on the front legs of *Belonocnema* is likely an adaptation of

their fossorial life-style to reach the oak rootlets in the asexual generation. In addition, our flight test shows that the brachypterous asexual generation of *B. fossoria* cannot fly (Table 2), while the asexual generation of the other two *Belonocnema* species are capable of flight. This loss of flight in conjunction with the long tibial spurs in *B. fossoria* are both likely the result of adaptation to their environment as they specialize on *Q. geminata*, which is found on xeric, sandy soils (Cavender-Bares *et al.*, 2015). The loss of flight is common among oak gall wasps within the members of the *Cynips*-group, and is likely the result of convergent evolution, as this trait has evolved multiple times within Cynipini (Liljeblad *et al.*, 2008).

We were able to morphologically assign the five specimens from Gautier, MS, to either *B. kinseyi* or *B. treatae*, despite demonstrated genetic admixture of this population (Driscove *et al.*, 2019). Future studies focusing on the species boundaries between these two *Belonocnema* lineages should further sample this hybrid zone to determine the geographic extent of admixture, examine evidence for asymmetrical gene flow and to determine whether hybrids indeed have intermediate morphological and phenological traits compared to other populations. Furthermore, the role of the endosymbiotic bacteria *Wolbachia* in maintaining species boundaries in this region of overlap requires further work, as eastern and western *Belonocnema* harbour different strains that may play a role in reproductive isolation between lineages (Schuler *et al.*, 2018).

CONCLUSION

We have resolved the long-standing confusion about the taxonomic status, host-plant associations and geographic ranges of the three known species within the Cynipid genus *Belonocnema*. Our analyses clearly delineate three species on the basis of patterns of genetic variation, phylogenetic distinctions and morphological character inspection of both the asexual and sexual generations of the respective species. *Belonocnema* has been the focus of study for over 20 years and is increasingly employed in studies of the biology, ecology and evolution of gall former species; our results clarify the species status of the populations that have been, and continue to be, studied. Moreover, our 20 years of study (Table 1) highlights the challenge of gathering the detailed natural history, biogeographic, behavioural, ecological, genetic and genomic data to thoroughly address the hypothesis of species. While the scope of this paper focuses exclusively on the genus *Belonocnema* distributed throughout the southern and south-eastern USA, where *Belonocnema* has been exhaustively sampled from the three live oak species,

the diversity of this genus likely extends across other members of *Virentes* as the series includes seven named species of which three are found outside of the USA (Cavender-Bares *et al.* 2015). For example, as of yet unidentified *Belonocnema* asexual generation leaf galls have recently been observed from Veracruz, Mexico and Honduras on *Q. oleoides* Schlechtendal & Chamisso, and in Baja California, Mexico, on the narrowly distributed *Q. brandegeei* Goldman, 1916 (Egan *et al.*, unpublished data). Whether *Q. sagraeana* Nuttall, 1842, which is patchily distributed in western Cuba, is also host for *Belonocnema* is currently unknown. Additional sampling of regions where these members of *Virentes* occur may likely add to the known diversity of this genus and will facilitate comprehensive studies of *Belonocnema* taxonomy, differentiation in response to host plant associations and biogeographic and phylogenetic history.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. Collection site information and abbreviated site names (originally published in [Driscove *et al.*, 2019](#), as supplemental table S1).