Diversity, Host Ranges, and Potential Drivers of Speciation Among the Inquiline Enemies of Oak Gall Wasps (Hymenoptera: Cynipidae)

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Abstract

Animals that exploit living spaces of other animals (inquilines) may have specialized traits that adapt them to extended phenotypes of their ‘hosts’. These adaptations to host traits may incur fitness trade-offs that restrict the host range of an inquiline such that shifts to new hosts might trigger inquiline diversification. Speciation via host shifting has been studied in many animal parasites, but we know less about the role of host shifts in inquiline speciation. Synergus Hartig (Hymenoptera: Cynipidae: Synergini) is a speciose but taxonomically challenging genus of inquilines that feed inside galls induced by oak gall wasps (Hymenoptera: Cynipidae: Cynipini). Here, we report on a large collection of Synergus reared from galls of 33 oak gall wasp species in the upper Midwestern United States. We integrated DNA barcodes, morphology, ecology, and phenology to delimit putative species of Synergus and describe their host ranges. We find evidence of at least 23 Synergus species associated with the 33 gall wasp hosts. At least five previously described Synergus species are each complexes of two to five species, while three species fit no prior description. We find evidence that oak tree phylogeny and host gall morphology define axes of specialization for Synergus. The North American Synergus have experienced several transitions among gall hosts and tree habitats and their host use is correlated with reproductive isolation. It remains too early to tell whether shifts to new hosts initiate speciation events in Synergus inquilines of oak gall wasps, or if host shifts occur after reproductive isolation has already evolved.

Key words: Synergus, Cynipidae, ecological speciation, Quercus, gall-forming insects

Many plant-feeding insects are attacked by diverse and complex communities of natural enemies (Price 1980, Rodriguez and Hawkins 2000, Lewis et al. 2002). In general, these natural enemy communities are dominated by one or more trophic levels of parasitic wasps (parasitoids) that lay eggs into or onto their hosts, and whose larvae feed on the still-living host’s body. The particular ubiquity of parasitoids (Noyes 2012, Forbes et al. 2018) has led to much research into the mechanisms underlying their evolutionary success, with shifts by specialist parasitoids onto new host species frequently invoked as a potential driver of their speciation (e.g., Stireman et al. 2005, Forbes et al. 2009, Joyce et al. 2010, Hood et al. 2015, Kester et al. 2015, Hamerlinck et al. 2016). A related body of work has employed DNA sequence data to reveal cryptic host specialization among insects—including parasitoids—initially thought to be oligophagous (Smith et al. 2006, 2008, 2011; Condon et al. 2008, 2014; Desneux et al. 2009; Forbes et al. 2012).

While parasitoids continue to be rewarding systems for evolutionary biologists interested in the relationship between host use and reproductive isolation, another group of insect natural enemies promises equally interesting insights but is far less well studied in this context. Inquilinous insects (inquilines) exploit the homes and/or use resources produced by other insects (Roskam 1979). Unlike parasitoids, which directly parasitize the host insect, inquilines ‘attack’ the extended phenotype of their ‘host’ insect, and their effect can range from a benign commensalism to a malignant kleptoparasitism. Though inquilines are common and taxonomically diverse in the context of some habitats (Sanver and Hawkins 2000), few studies to date have examined life history characters or host ranges for individual inquiline species (Stone et al. 1995, 2017), and fewer still have asked how ecology might influence their evolution and diversity (though see: Abrahamson et al. 2003, Blair et al. 2005).

Defined by their strategy of exploiting a habitat created by another animal, inquilines are a common associate of insects that induce the development of galls—fleshy, highly structured growths of tissue—on plants (Sanver and Hawkins 2000). Larvae of gall-inducing insects benefit from the assured source of food that their gall provides and the protection from predators that concealment inside the gall
Synergus Hartig (Hymenoptera: Cynipidae: Synerginae) is a genus of inquiline wasps whose larvae feed on galls made on oak trees (Quercus L.; FAGALES: FAGACEAE) by many of the ~1,000 species of oak gall wasp (Hymenoptera: Cynipidae: Cynipini) (Pénzes et al. 2017). Most Synergus do not create their own primary galls (though see Abe et al. 2011, Ide et al. 2018). Instead, they induce secondary chambers in galls made by other cynipid wasps (Askew 1961, Pénzes et al. 2012). Some Synergus do not mortally injure the developing galgal wasp, while others sever the nutrient supply and can be lethal to other gall inhabitants (Csóka et al. 2005, Ács et al. 2010). Synergus are common across oak gall communities: often multiple species attack the same galler species (e.g., Melika 2006, Nazemi et al. 2008, Bird et al. 2013, Forbes et al. 2015, Weinersmith et al. 2020), though in many galls only one Synergus is known (Burks 1979) and in still others they may be replaced by other inquiline genera (e.g., Joseph et al. 2011).

Just a few studies have used molecular data to address the evolution and host ranges of Synergus, and all of these have focused on the Palearctic region. A three-gene (COI, cyt b, and 28S) phylogeny of inquiline associated with oak gall wasps included 23 of the 30 described Western Palearctic Synergus species and generally supported the hypothesis that they are monophyletic relative to other gall-associated inquiline genera (Ács et al. 2010). Ács et al. (2010) also suggested that some species, including the apparent generalist Synergus umbraculus (Olivier), might be amalgams of several morphologically convergent lineages. This phylogeographic work did not directly address species host ranges at the host plant or gall wasp species level. Two subsequent studies (Bihari et al. 2011, Stone et al. 2017) addressed the population genetics of one of the lineages of S. umbraculus identified by Ács et al. (2010; lineage 19). Both studies found a strong geographic signal without any strong signal of host-associated genetic differentiation. However, all host gall wasps in these studies were in genus Andricus and all collections save for one were from oaks in the same taxonomic section (Quercus sect. Quercus) (Bihari et al. 2011, Stone et al. 2017).

Though much more remains to be addressed relative to the role of host in diversification of Palearctic Synergus, even less is known about Synergus in the Nearctic despite the latter being more species-rich and with a far greater diversity of potential gall hosts (Pénzes et al. 2012, 2018). Seventy-eight species of Synergus have been described in the Nearctic (Lobato-Vila et al. 2019), though the total number is predicted to be far higher (Lobato-Vila and Pujade-Villar 2017). Synergus diversity is hypothesized to positively correlate with the diversity of potential gall hosts and the Nearctic has a greater diversity of both oaks (~300 species, compared to just ~40 species in the Western Palearctic; Stone et al. 2002, Lobato-Vila and Pujade-Villar 2017, Hipp et al. 2018) and oak-associated cynipid gall wasps (~700 species, compared with 140 in the western Palearctic; Stone et al. 2002).

Our primary goal in the current study is to characterize patterns of diversity, host range, and ecology for a subset of the Nearctic Synergus, with an eye toward understanding how changes in host use might be tied to their diversification. We employ a regionally focused collection and Synergus-rearing effort from galls found growing on 15 species of oak trees, infer reproductive barriers using molecular barcodes, ecology, and morphology, and ask the following questions: 1) Do putative Synergus species that attack multiple different gall types harbor additional host- or habitat-associated genetic structure? 2) Along what axes (gall wasp species/gall morphology/tree species), if any, do Synergus specialize? We also use our limited molecular data to make a first inference regarding how Nearctic and Palearctic Synergus are related to one another.

**Methods**

**Collections**

We employed a collection approach largely focused on galls in the Midwestern United States (Fig. 1). We collected galls from August 2015 through October 2017. We recorded geographical location, date of collection, tree host, and the species of gall wasp (based on gall characteristics, Weld 1959). We stored each collection of galls in an individual cup in an incubator that mimicked seasonal variations in temperature, humidity, and light/dark cycle. Each day, we checked all cups in the incubator and placed any emergent insects into 95% ethanol, recording the date of emergence. We identified each insect to family or genus using a variety of taxonomic resources (Goulet and Huber 1993, Gibson et al. 1997, Wahl 2015). In total, we collected >24,000 galls, representing 103 gall wasp species (inferred using gall morphologies and tree host association based on Weld 1959).

![Fig. 1. Map of gall collections that yielded Synergus samples used in this study. For a list of samples, locations, and tree and gall associations, see Supp Table 2 (online only).](https://academic.oup.com/isd/article/4/6/3/5997471)
Identification of Putative Species

We first separated *Synergus* by gall association and then sorted individuals into morphotypes based on characters used by previous authors to distinguish species (e.g., color, mesopleural striation, antennal segmentation, patterns of syntergal punctures, etc.). Representatives from each morphotype were also identified to species—where possible—using keys by Gillette (1896), Lobato-Vila and Pujade-Villar (2017), and Lobato-Vila et al. (2019), or matched to described species based on morphological descriptions and records of host associations found in Fullaway (1911), McCracken and Egbert (1922), or Muesebeck et al. (1951). From each morphologically defined species, we chose individuals (usually females) to interrogate genetically. To allow for detection of possible cryptic diversity, we chose individuals that represented a diverse set of collection sites and from across each morphotype’s spectrum of eclosion dates. In cases where a *Synergus* morphotype emerged from the same gall wasp species but from different oak tree species, we also chose representatives from each tree habit. We photographed a single forewing and a profile of the body of each wasp used in genetic work (Supp Figs. S1–S46 [online only]).

For 96 samples extracted before 2017, we used a DNeasy Blood and Tissue kit (Qiagen, Germantown, MD) to destructively extract DNA from specimens. For another 55 *Synergus* extracted after 2017, we used a CTAB/PCI approach following the methods from Chen et al. (2010). The mtCOI region was amplified using the primers LepR1 5’ TAAACTTCTGAGATGTCAAAAATTCA 3’ and LepF1 5’ ATCCAAGACTATAAGGATTTGG 3’ (Smith et al. 2008). We cleaned DNA using EXOI and SAP before Sanger sequencing both the forward and reverse sequence on an ABI 3730 in the University of Iowa’s Carver Center for Genomics. We edited and aligned sequences in Geneious v.8.1.9 (Biomatters, Inc., San Diego, CA) and determined the model with the best fit to our sequences using AIC in jModelTest2 (Darriba et al. 2012), which chose a GTR+I+Γ model. We constructed phylogenetic trees using a Bayesian approach (MrBayes v3.2, Ronquist et al. 2012), performing two independent searches with four chains run for 3,000,000 generations, sampling every 1,000 generations. Log-likelihood plots of the MCMC iterations were visualized to confirm convergence and select a standard 25% burn-in. We also constructed a phylogenetic tree using a maximum likelihood approach with RAxML v8.2, using a GTR+Γ model with 1,000 bootstrap pseudoreplicates (Stamatakis 2014). Sequences are available on GenBank: accession numbers MT124785-MT124935 (see Supp Table 2 [online only]). We also combined our mtCOI sequences with a previously published data set of Palearctic *Synergus* mtCOI sequences from Ács et al. (2010) and generated Bayesian and maximum likelihood trees following the same methods as above.

We generated molecular species hypotheses using two different but complementary approaches: Automatic Barcode Gap Discovery (ABGD; Puillandre et al. 2012) and PTP (Poisson Tree Processes model; Zhang et al. 2013). ABGD uses a sequence alignment to infer a confidence interval for genetic divergence within species. Ideally, a clear difference emerges between interspecific and intraspecific comparisons, defining a ‘barcode gap’ for the sample set. ABGD was run with the default prior range (0.001, 0.1), a Jukes–Cantor model of distance, and minimum slope increase of 1. PTP (Zhang et al. 2013) is a coalescence-based approach that requires a rooted phylogenetic tree as its input. We used the updated version bPTP, which uses branch lengths as proxies for substitutions and provides posterior Bayesian support values to each node describing the likelihood that descendants of that node belong to the same species. We used the online server (http://species.h-its.org/) and used our Bayesian tree of *Synergus* samples (Supp Fig. 47 [online only]) as the input tree. We ran 100,000 MCMC generations with a 10% burn-in. We confirmed convergence by visualizing the likelihood trace plots of the iterations.

Results

Summary of Collections and Species Hypotheses

We reared 12,272 individual natural enemies from 88 different gall types. Of these natural enemies, 1,645 were *Synergus* wasps reared from galls of 33 gall wasp species (Supp Tables 3 and 4 [online only]). Among these, we identified 14 *Synergus* morphospecies, 11 of which corresponded closely to previously described taxa (see Synthesis below for details). Although relative support values differed for Bayesian and maximum likelihood mtCOI trees, topologies were the same. The Bayesian tree of 148 individual *Synergus* (Fig. 2; Supp Fig. 47 [online only]) demonstrates extensive phylogenetic structure corresponding to host gall wasp and oak section association.

The synthesis of our data with the Palearctic *Synergus* sequences previously published by Ács et al. (2010) produced Bayesian and maximum likelihood trees with the same topology: the Palearctic *Synergus* formed a clade within the Nearctic *Synergus* (Fig. 3; Supp Figs. 48 and 49 [online only]).

For ABGD, there was no clear barcode gap and thus we investigated two different partitions—one more liberal (more splitting) and one more conservative (more clumping). The liberal partition suggested 36 groups, whereas the conservative partition suggested 21 groups (Fig. 2; Supp Fig. 50 [online only]). The highest support solution for bPTP suggested 31 groups (Fig. 2; Supp Fig. 51 [online only]).

Synthesis

While any one of morphological differences, mtCOI sequence divergence, and patterns of differing host association in sympatry would not alone be sufficiently strong evidence of species boundaries, agreement between them builds a much stronger argument. We use concordance between these three factors to define a final count of putative species in our collections (Fig. 2). Among the original 14 morphologically defined species, we find a revised total of 23–27 species. In particular, at least five previously described *Synergus* species in our collections are each composed of at least two or more taxa, each with a more limited range of host galls than morphological species definitions initially implied. What follows is an account of each morphological ‘species’ alongside a summary of its revised status in light of these new results. Species are named below in the order in which they appear in our mtCOI tree, from top to bottom (Fig. 2). When a species was reared from >1 different gall types, we discuss apparent morphological or location-based similarities among these gall hosts. We also plot collection and emergence dates of all *Synergus* specimens to offer a sense of their phenology as it relates to host use (Fig. 4).

*Synergus laeviventris* (Osten-Sacken) (clades 1–3) (wing and lateral habitus images: Supp Figs. S1–S6 [online only]) is a complex of at least three species, differing from one another in their host gall use. One species (clade 1) attacks the bud galls of *Andricus pisciformis* Beutenmüller and *Andricus quercusfusconodosus* (Basset) on white oak (*Quercus alba* L.) and swamp white oak (*Quercus bicolor* Willd.)—both in oak section *Quercus*. A second species (clade 2) uses *Disholcaspis quercusglobulus* (Fitch) bullet galls on white oak (section *Quercus*), and a third (clade 3) attacks several different oak species.
oak apple galls (Andricus Hartig and Amphibolips Reinhard) found on red oak (Quercus rubra L.) and pin oak (Quercus palustris Münchh.)—both section Lobatae. Previous rearing records (Gillette 1896) associate S. laeviventris with the oak apple gall Amphibolips quercusspongifica Osten-Sacken, as well as from three bullet galls: D. quercusglobulus, Disholcaspis rubens (Gillette), and Atrusca quercuscentricola (Osten-Sacken). Clades 1 and 3 are unusual among our collections in that we found evidence of two or three temporally distinct generations, each attacking different gall species (Fig. 5).

Synergus unk. #1 (clade 4) (Supp Figs. S7 and S8 [online only]) was reared from an unknown twig gall on a bur oak (Quercus macrocarpa Michx.; section Virentes). This Synergus ran closest to Synergus batatoides Ashmead in the Gillette (1896) key, but its color and sculpturing did not match that description. Synergus batatoides are associated with Callirhytis quercusbatatoides (Ashmead) stem galls in southern live oaks (Quercus virginiana Mill.; section Virentes). Since we do not have specimens of S. batatoides for comparison, it is not clear whether this is just a morphological variant of a species previously described only from the southern United States.
or a different species. This species was also notable as one of the earliest-emerging Synergus in our collections (Fig. 4).

Synergus confereae Ashmead (clade 5) (Supp Figs. S9 and S10 [online only]) was reared from Callirhytis quercusventricosa and previously from the same host by Ashmead (1885). We found no additional host or genetic structure within this species. Our samples were taken from single oaks (Quercus imbricaria; section Lobatae) in both Iowa City, IA, and St. Louis, MO.

Synergus lignicola (Osten-Sacken) (clade 6) (Supp Figs. S11 and S12 [online only]) was reared from Callirhytis quercusgennmaria (Ashmead) on red oaks (section Lobatae) from Traverse City, MI. These wasps closely match the description of Synergus davisi (Beutenmüller 1907) and were reared from the same host gall from which S. davisi was previously described, but a recent revision of some Nearctic Synergus (Lobato-Vila et al. 2019) synonymized S. davisi with S. lignicola. Our female S. lignicola specimens have an incomplete division in their final antennal segments, such that they can appear to have either 13 or 14 segments.

Synergus nr. lignicola (clade 7) (Supp Figs. S11 and S12 [online only]) were reared from Callirhytis quercuspunctata (Bassett) collected on pin oaks in St. Louis, MO. This twig gall is the same host from which S. lignicola was previously reared (another known host is the morphologically similar Callirhytis cornigera (Osten-Sacken)) (Gillette 1896), but it differs in having 14 (instead of 13) female antennal segments, and a slight incision in the posterior-dorsal edge of its syntergite. At the more conservative partition, ABGD did not separate this species from S. lignicola (clade 6), though they were distinguished by both the BPT method and the more liberal ABGD partition. The difference in morphology and host galls suggests that they are two different species, though a lack of a sympatric collection here allows for the possibility that differences are due to geographic isolation rather than reproductive isolating barriers.

Synergus oneratus (Harris) (clades 8–11; Supp Figs. S15–S22 [online only]) may contain as many as five species among the samples in our collection, each defined by COI sequence similarity, host association, and morphological variation. These Synergus showed some variation in phenology, but generally emergence was either within a few months of gall collection or after more than a year (Fig. 4). One species (clades 8a and 8b) was reared from Acraspis Mayr galls and other leaf galls on various white oaks (section Quercus). This species was split into two additional groups by the ‘liberal’ ABGD partition but since both groups contained Synergus from the same Acraspis pezomachoides Osten-Sacken collection in Peducah, KY, this split does not appear to be supported by ecology or geography. A second species (clade 9) was reared from Andricus biconius Weld and Andricus robustus Weld in post oaks (Quercus stellata Wångenh.; section Quercus), and a third species (clade 10) was reared from Andricus quercusurobilianus (Osten-Sacken) in bur, white, and swamp white oaks (section Quercus). A fourth species (clade 11) was reared from a variety of stem and leaf galls on oaks in section Quercus. Previous taxonomic work has already suggested that S. oneratus species contains two geographic varieties differing in the amount of black on the mesonotum: S. oneratus oneratus (some yellow) in the Midwest and S. oneratus coloradensis (entirely black) in the west (Gillette 1896). The discovery of S. oneratus oneratus in California (Fullaway 1911, McCracken and Egbert 1922) indicated a more complex distribution. Here, clades 10 and 11 (Supp Figs. S19 and S21 [online only]) appear to better match the description of S. oneratus oneratus, while clade 8 (Supp Figs. S15 and S17 [online only]) is closer to S. oneratus coloradensis. No females of clade 9 were available for this comparison.

Synergus unk. #2 (clade 12) (Supp Fig. S23 [online only]) was represented by a single male reared from a Pholox nigra (Gillette) gall on leaves of bur oak in Konza, KS (clade 12). Because many keys do not address males, the failure to attach a name to this species does not mean that it is undescribed.

Synergus villosus Gillette (clades 13 and 14) (Supp Figs. S24 and S25 [online only]) includes two distinct species. One species (clade 13) was reared from Callirhytis lanata (Gillette) and Dryocosmus imbricarcae Ashmead, a fuzzy leaf gall and a stem bullet gall, respectively, both on red oak (section Lobatae) in Tiffin, IA. A second species (clade 14) was reared from Acraspis villosa Gillette, a fuzzy leaf gall, on bur oak (section Quercus) in Urbana, IL. Some caution should be used in definitively separating clades 13 and 14, as collections were from different states and differences could be due to geography. The original description of S. villosus (Gillette 1896) was from A. villosa galls collected in Iowa and the morphology of these wasps exactly matches our Illinois wasp, while the samples from red oak showed them to have entirely yellow mesonota, which differs from the bur oak collection and the original S. villosus description (thorax entirely black). Wasps from both clades emerged 9–11 mo after gall collection (Fig. 4).

Synergus magnus Gillette (clade 15) (Supp Fig. S26 [online only]) was reared from Amphisbopis quercusugulans (Osten-Sacken) leaf galls and from unidentified acorn galls on black oaks (Quercus velutina Lam.) and red oaks, respectively (both section Lobatae). This species was previously reared from an Amphisbopis cookii Gillette gall (Gillette 1896). All S. magnus emerged within 1–2 mo of the gall collection data (Fig. 4).

Synergus erinacei Gillette (clades 16 and 17) (Supp Figs. S27–S29 [online only]) contains either one or two different species. One putative species (clade 16) was reared from Acraspis erinacei (Beutenmüller) and A. pezomachoides leaf galls on white oaks (section Quercus). The second (clade 17) was reared from Acraspis macrocarpa Bassett on bur oaks (section Quercus). The two S. erinacei clades are also separated by geography, so a definitive conclusion regarding species status requires additional work.

Synergus walshii Gillette (clades 18 and 19) (Supp Figs. S30–S33 [online only]) contains two putative species. One (clade 18) was
reared from galls of two Phyllothes Ashmead species on bur oak and swamp white oak (section Quercus), the other (clade 19) from various Andricus leaf galls on bur, white, and post oaks (section Quercus). Previous collections of S. walshii (Gillette 1896) were from Andricus quercusflocci (Walsh) galls, one of the clade 19 hosts.

Synergus punctatus Gillette (clades 20, 22–25) (Supp Figs. S34, S35, and S37–S41 [online only]) may contain up to five distinct species. The first species (clade 20) was reared from leaf galls of Andricus nigricens Gillette on swamp white oak (section Quercus). A second species (clade 22) was reared from Acraspis and Philox Fitch leaf galls on white, bur, and chinquapin oaks (Quercus muehlenbergii Engelm.) (section Quercus) and females had some color variation, with thorax and abdomen color ranging from black to a chestnut-brown. Two additional putative species—both with a mix of black- and brown-bodied individuals—were reared from galls of A. robustus (clade 23) and A. erinacei (clade 24) galls on post and white oaks, respectively. We are more tentative about definitively splitting clades 23 and 24 due to conflation of genetic and host differences with geographic distance.

Synergus unk. #3 (clade 21) (Supp Fig. S36 [online only]) was reared from Disholcaspis Dalla Torre & Kieffer bullet galls on branches of white, swamp white, and post oaks (section Quercus) and differed from S. punctatus by having females with mostly chestnut-brown heads, and <1/3 of the posterior edge of their syntergite covered with punctures.

Synergus campanula Osten-Sacken (clades 25–27) (Supp Figs. S42–S46 [online only]) contains between two and three species. One species (clade 25) was collected from clustered midrib leaf galls of Andricus dimorphus (Beutenmüller) on dwarf chinquapin oak (Quercus prinoides Willd.; section Quercus). The other species (or two) (clades 26 and 27) were reared from a mix of leaf and stem galls from white, swamp white, and post oaks (section Quercus). One potential difference between wasps in clades 26 and 27 is their emergence timing, with clade 26 wasps all emerging 10–12 mo after gall collection, while clade 27 wasps had two emergence peaks: one within the first few months after galls were collected and the other the following year (Fig. 4).
Discussion

Nearctic Synergus Diversity and Taxonomy

One immediate lesson from this study is that the Nearctic Synergus harbor both cryptic and undiscovered species. For 5 of 11 previously named species in our collections, we find evidence of additional genetic, morphological, phenological (Fig. 4), and/or ecological structure indicative of >1 species. Three other putative species apparently fit no previous description. Because this was a geographically limited survey, it thus seems very likely that many more undiscovered species exist in North America, both as members of morphologically cryptic assemblages, and as undescribed species. Indeed, when recent authors have focused on the Nearctic, they have consistently added new species (e.g., Lobato-Vila and Pujade-Villar 2017; Lobato-Villa et al. 2018, 2019). The incompleteness of the record is perhaps unsurprising as most taxonomic and phylogenetic work has emphasized the Synergus of the western Palearctic (Penzes et al. 2012) while the most comprehensive list of Nearctic Synergus species is now >40 yr old (Burks 1979).

A taxonomic revision of the Nearctic Synergus that incorporates molecular, morphological, and ecological data appears warranted, and, though such a revision is beyond the scope of this paper, it might be informed by some of our findings. Morphological characters historically used to sort Synergus into taxonomically relevant groups do not necessarily reflect characters shared due to common evolutionary histories. The Palearctic Synergus were initially divided into two major morphological groups (Mayr 1872): Section I, which are univoltine and usually not lethal to their associated galler, and Section II, which tend to be both bivoltine and lethal (Wiebes-Rijks 1979). The two sections also differed in patterns of microscopic punctures on their metasomal tergites. These sections were more recently revealed to be paraphyletic (Ács et al. 2010). One of the potential reasons for the disconnect between morphological and molecular data is that Synergus can be morphologically cryptic as well as exhibit variance in morphology within species (Wiebes-Rijks 1979).

One morphological character previously suggested as useful for organizing the Nearctic fauna seems immediately relevant given these new results. Gillette (1896) split the genus into three ‘natural’ groups based on whether females of each species have 13, 14, or 15 segments in their flagella. Among our collections, we found only 14- and 15-segmented females (with *S. lignicola* being indeterminately 14-segmented), but all 15-segmented females (clades 12–15) grouped together on the mtCOI tree (Fig. 2; Supp Figs. 47 and 52 [online only]), suggesting a common ancestry among these samples. While we acknowledge caveats about inferring too much from a single short mitochondrial sequence (Funk and Omland 2003; and more on this below), this may be an early indication that antennal segment number is an informative
character. To assist future efforts in this regard, we include supplemental profile and forewing pictures of wasps from each clade (Supp Figs. S1–S46 [online only]) and have deposited examples of most clades into the collection of the University of Iowa Museum of Natural History.

Host Ranges and Axes of Specialization

Our primary motivation for this study was to evaluate the extent to which inquilines specialize on aspects of their host environments, which then would stand as an initial, indirect assessment of how changes in host use might result in lineage divergence. Evidence from our study suggests that Nearctic Synergus specialize on aspects of host plant identity, gall morphology, and/or gall phenology. One obvious axis of specialization was host tree section. Though we made large collections of galls from several oak species in both section Quercus (white oaks) and section Lobatae (red oaks), we found no evidence of a Synergus species that was reared from both sections. This pattern conforms to previous studies, which have suggested Synergus may often be specific to closely related oaks species (Pujade-Villar et al. 2003). Other Cynipidae, including the gall wasp hosts of these Synergus, also show fidelity to oaks with similar chemistry (which generally means more closely related oaks; Abrahamson et al. 1998, 2003).

Less specialization was evident at finer levels of plant taxonomic organization. For example, Synergus species reared from galls on white oak (Q. alba) were also often reared from galls on swamp white oak (Q. bicolor), bur oak (Q. macrocarpa), or post oak (Q. stellata)—all North American members of section Quercus, the white oaks. Specialization on oak section rather than oak species may explain why prior assessments of host plant specialization in the Palearctic Synergus—where most samples have been from the same oak section—have not resolved strong genetic structure associated with host plant (e.g., Bihari et al. 2011, Stone et al. 2017). Given that Synergus in this study specialize on either red and white oaks, it will be interesting in the future to include Synergus associated from oaks in section Virentes (the live oaks), another North American group which overlaps geographically with the southern ranges of some of the oaks in this study.

While Synergus did not appear to specialize at the oak species level (below the section level), they did specialize on galls of different

**Fig. 6.** Photos of galls collected in this study from which Synergus emerged. Gall photos are grouped based on similar morphology and location found on tree. Numbers following the species name of the gall indicate the Synergus clades (see Fig. 2) that were reared from that host.
morphology. Though our haphazard collections give us an incomplete picture of all hosts attacked by each species, most species tended to favor galls that shared similar characteristics (Figs. 2 and 6). Most strikingly, even when we reared Synergus from different gall hosts across the course of the year, each generation nevertheless conserved elements of gall morphology. The clade 1 S. laeviventris wasps, for example, emerged in late May from small bud galls of Andricus pisiformis collected in early April, and then adults emerged again in the fall from a second small bud gall (A. quercusfrondosus) collected in August and September (Fig. 5). Adults of this species may then oviposit into pre-winter bud galls of A. pisiformis or perhaps attack a third overwintering host that we did not collect in this study.

Similarly, we captured three discrete generations of S. laeviventris clade 3 wasps, each attacking oak apple galls containing a central cell surrounded by radiating fibers, but each made by different galler species (Fig. 5).

Conservation of host gall morphology suggests that Synergus specialization conforms to the ‘Enemy Hypothesis’ (Bailey et al. 2009). That is: galls with similar qualities will exclude certain natural enemies and therefore similar galls will tend to be attacked by the same or similar species (Ward et al. 2019). Brookfield (1972) similarly suggested that inquilines and parasitoids of galls depend more on gall morphology than other factors such as gall wasp species or tree host (note, however, that this last is belied by our data). Because the evolutionary relationships among the Nearctic gall wasps themselves are not yet known, it is not possible to distinguish gall morphology from gall wasp phylogeny. A phylogeny of North American gall wasps will be helpful in this respect.

Phenology has also been previously considered as an important axis of adaptation and specialization for Synergus because some developing galls may offer only a limited ‘window of opportunity’ during which an inquiline might successfully oviposit (Penzes et al. 2012). The S. laeviventris clades 1 and 3 wasps described above appear to fit this pattern, with each of their respective two or three generations attacking different galls, presumably during some window of early gall development (Fig. 5). In contrast to this pattern, the long (July–October) emergence schedule of S. punctatus clade 24 wasps from A. erinacei galls (Fig. 7) suggests that these Synergus may
undergo multiple generations per year and are capable of attacking *A. erinacei* galls for much of the time that these galls occur on leaves (though an alternative interpretation here is that *S. punctatus* is univoltine and that harvesting galls from leaves somehow induces *Synergus* emergence). Some individual *S. punctatus* also undergo a period of winter diapause that ends shortly before the next generation of *A. erinacei* appears on leaves again, further highlighting their phenological tracking of this host (Fig. 7).

Characterizing *Synergus* host ranges and their relative level of specialization has important connotations for conservation and pest control. Oak gall wasps have been transported into non-native ranges (Walker et al. 2002, Prior and Hellmann 2010), with some classified as invasive due to their impact on trees (Buffington et al. 2016, Davis 2017). *Synergus*, because they are often the most abundant enemy of some gall species, may be particularly important in the control of alien galls (Schonrogge et al. 1996, 2000), and their host breadth could inform likelihood of colonization. For instance, knowledge about host gall characters might allow for biocontrol efforts that employ native *Synergus* rather than introducing non-native parasites or inquilines (though the latter can also be successful: Moriya et al. 1989).

Implications for *Synergus* evolution

One leading explanation for why parasitoid wasps are so diverse is that adaptations to new insect hosts may lead to host-associated differentiation and speciation (Stireman et al. 2005; Feder and Forbes 2010, Kaiser et al. 2013, König et al. 2015). When a parasitoid adopts a new host, trade-offs associated with adaptation to the new host can result in reproductive isolation from individuals attacking the ancestral host (Hood et al. 2015). We know surprisingly little about whether the same ecological differences can drive lineage divergence in insect inquilines: one exception is the tumbling flower beetle *Mordellistena convicta* (Coleoptera: Mordellidae), which feeds on the galls of two lineages of *Eurosota solidaginis* gall flies (Diptera: Tephritidae) on goldenrods (*Solidaginis*; Compositae). Life history and molecular work has shown that both *E. solidaginis* and *M. convicta* have formed host-associated lineages on two different species of goldenrod, suggesting that speciation has ‘cascaded’ across trophic levels (gall former to inquiline) (Abrahamson et al. 2003, Eubanks et al. 2003, Rhodes et al. 2012). This beetle system offers a compelling hint that ecological changes might lead to lineage divergence in inquilines, but missing is a broader study of ecological and host use patterns within an entire inquiline clade.

What do we learn about the role of host shifts in *Synergus* evolution? Though not a comprehensive phylogenetic history of these species, our mtCOI tree of Nearctic *Synergus* (Fig. 2; Supp Figs 47 and 50 [online only]) nevertheless suggests many historical shifts between host tree sections (*Quercus* to *Lobatae*), between gall wasp species, and between gall morphologies. This is not a definitive signature that host shifts drive speciation in *Synergus*, but the broad correlation between ecological changes and the evolution of reproductive isolation suggests this as a leading hypothesis. Transitions among host wasps, galls, and tree sections all represent changes that—to differing extents—require the evolution of novel behaviors, life histories, and strategies for circumventing host defenses. The accumulation of such divergent characters in the context of different habitats has been shown to directly (Craig et al. 1993, Forbes et al. 2005, Fujiyama et al. 2013, Doellman et al. 2019, Hood et al. 2019) and indirectly (Nosil and Hohenlohe 2012) lead to the evolution of reproductive isolation in many different insect systems.

An alternative to the above hypothesis is that ecological divergence among *Synergus* occurs after reproductive isolation evolves between lineages. For instance, perhaps *Synergus* speciate in the absence of divergent ecological selection (e.g., Nosil and Flaxman 2011), and then later shift into new hosts or habitats upon secondary contact because partitioning of environments facilitates coexistence of ecologically similar sympatric species (Dufour et al. 2017). One advantage of our renewed exploration of the Nearctic *Synergus* is that it suggests taxa for further study that may allow the disentanglement of these competing hypotheses. For instance, clades where individuals with little or no mtCOI differentiation have been reared from galls of different morphologies (e.g., clades 3, 9, 27) could be interrogated further. If experimental tests revealed significant host-associated reproductive isolation and/or population genetic analyses suggested evidence of host-associated genetic differentiation, these might support a hypothesis that difference in host use is contributing to divergence. Similarly, as new samples of *Synergus* from across a wider geographic area are added to this data set, an increasing role for geographic isolation might emerge between sister species.

Notes on the Relationship Between Nearctic and Palearctic *Synergus*

This first genetic analysis of a large number of Nearctic *Synergus* allows for a preliminary assessment of their relationships to the Palearctic *Synergus*. Our summary tree that includes the Ács et al. (2010) Palearctic *Synergus* (Fig. 3) suggests at the very least that these are two different sets of fauna, with no species apparently spanning both North American and Eurasia. Further, this combined mtCOI tree suggests that the Nearctic *Synergus* are not a monophyletic group. Because this is based on a single gene, we are hesitant to make strong conclusions about the geographic origins, but if the Nearctic *Synergus* are not monophyletic (as others have previously suggested; Bozsó et al. 2014), it suggests that the history of movement of these inquilines between continents bears close attention. Similarly, as additional data are collected, it will be interesting to study in greater detail the uncertain phylogenetic relationship between *Synergus* and its putative sister genus, *Saphonecrus* Dalla Torre and Kieffer (Bozsó et al. 2014). Future multilocus analyses promise to reveal additional insights into these histories.

Supplementary Data

Supplementary data are available at *Insect Systematics and Diversity* online.

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Author Contributions

A.K.G.W. and A.A.F. conceived of the study. All authors collected and analyzed data and wrote the manuscript.
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