

Drosophila are hosts to the first described parasitoid wasp of adult flies

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Parasitoid wasps are exceptionally diverse and use specialized adaptations capable of manipulating the physiology and behaviour of host organisms¹. In more than two centuries since the first records of *Drosophila*-parasitizing wasps, nearly 200 described and provisional parasitoid species of drosophilids have been identified². These include endoparasitoids and ectoparasitoids, as well as species attacking larval and pupal hosts³. Despite a deep history of research attention and remarkable biodiversity, a wasp species that attacks and develops inside the adult stage of a fly host has not been described previously. Here we report the discovery of a wasp species that infects the adult stage of fruit flies in the genus *Drosophila*, including one of the most deeply studied model organisms in biology, *Drosophila melanogaster*. Notably, this wasp can be easily collected from backyard fly baits and has a broad geographic distribution throughout the eastern USA. We document its life history and unique host interactions, including egg-laying into and larval emergence from adult flies, and provide protocols to raise wasps from wild-caught host flies. Our results emphasize the need for ongoing research investment in insect biodiversity and systematics. As parasitoid research continues to uncover unusual biology and supports fundamental mechanistic insights into immunity⁴, metabolism⁵, ecology⁶, evolution^{7–9} and behaviour^{10–12}, we anticipate that this wasp's association with the laboratory model organism, *D. melanogaster*, will provide new research opportunities across the life sciences.

Evolutionary conflict between parasites and hosts is a driving force behind some of the most specialized adaptations observed across the tree of life^{13–15}. Among the insects, such adaptations are used by the parasitoid wasps, which lay their eggs and develop in or on a live host. In this paper we describe a wasp species that parasitizes the adult stage of *Drosophila* flies—a parasitoid–host relationship that has not been described previously. Research on the natural parasites of the model fruit fly *Drosophila* has been ongoing since the early nineteenth century. In more than two centuries since the first records of *Drosophila*-parasitizing wasps (for example, *Tanycarpa bicolor* (Nees 1814)), nearly 200 described and provisional species of parasitoids of drosophilids have been identified². These include figitid and braconid wasps, which lay their eggs within fly larvae, and pteromalid, diapriid and encyrtid wasps, which attack host pupae³. Despite this deep history and rich phylogenetic diversity, a wasp species that attacks and develops inside the adult stage of *Drosophila* or any other fly has not been reported previously.

Parasitoid wasps of adult insects

Parasitoids of adults (imagobionts) are known to attack other orders of holometabolous insects. Members of some genera of the braconid wasp subfamily Euphorinae attack coleopteran, hymenopteran and neuropteran adults^{16–19}. Other euphorines use larval coleopterans,

lepidopterans and raphidiopterans²⁰ as well as nymph and adult stages of several paurometabolous insects including psocopterans, orthopterans and hemipterans^{16,17}. Outside Euphorinae, imagobiosis is rare²¹. This may be due to increased mobility of potential hosts, thickness of the chitinous exoskeleton and development of defensive responses not available to all juvenile stages (for example, kicking appendages, powerful mandibles and potent chemicals)²¹. Even so, imagobionts have thrived in this unique niche, as evidenced by the effectiveness with which they parasitize and manipulate host biology and the peculiar mechanisms sometimes involved. For example, an RNA virus and potentially other venom components of the euphorine *Dinocampus coccinellae* paralyse adult spotted lady beetles to elicit a cocoon-guarding behaviour following larval emergence from the host body¹¹.

The subfamily Euphorinae is also characterized by repeated host shifts across major insect orders. It is among the most successful subfamilies in the family Braconidae with regard to species diversity and host range—the subfamily is composed of 54 unique genera with a host range spanning 8 insect orders, before this study^{16,17,19,20}. Despite these elements of striking diversity, frequent host shifts and remarkable solutions to the unique challenges of imagobiosis, many aspects of euphorine wasp biology remain understudied, perhaps owing in part to challenges in laboratory rearing and missing host records for many species collected as adults.

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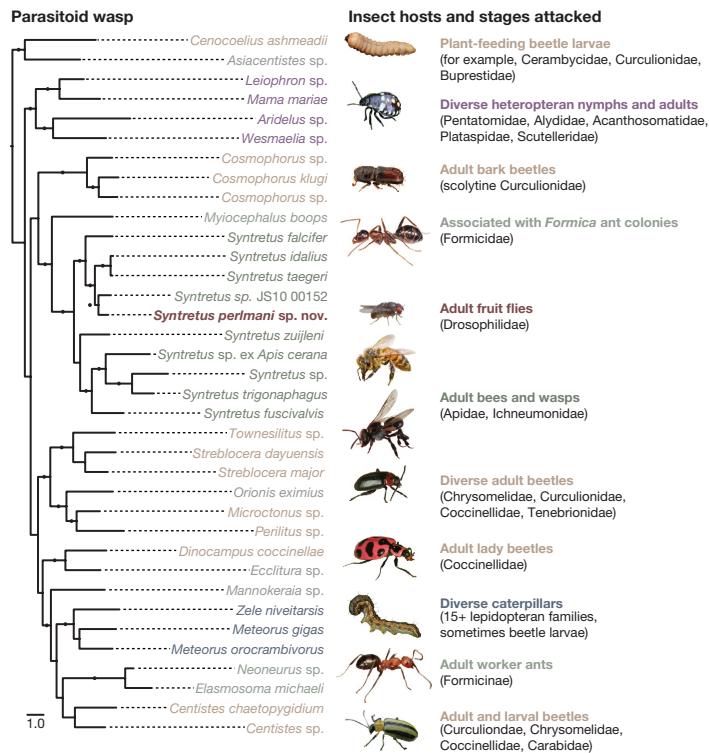


Fig. 1 | Adult *Drosophila* are parasitized by a previously undescribed species in the euphorine genus *Syntretus*. A phylogram constructed from concatenated nuclear and mitochondrial gene sequences of select euphorine wasps (left), with images and descriptions of the host species (right). Nuclear loci are genes encoding carbamoylphosphate synthase domain protein (*CAD*), 18S rRNA and 28S rRNA. The mitochondrial locus is *COI*. Members of the sister taxa *Cenocoelius* and *Asiacentistes* are included as outgroups. The gene sequences of *S. perlmani* group with high support inside the genus *Syntretus*. Branches with Shimodaira–Hasegawa-like maximum likelihood support values ≥ 0.9 are labelled with a circle. Taxon labels are coloured by host order. GenBank sequence accession numbers are listed in the Supplementary Data. Host insect photographs are adapted with permission as follows (top to bottom, excluding the fruit fly): cerambycid larva, Gilles San Martin under a CC BY 2.0 licence; *Nezara viridula*, Bugwood.org, Robert and Lesley Ingram under a CC BY 3.0 licence; *Pityogenes chalcographus*, Gilles San Martin under a CC BY 2.0 licence; *Formica* sp., Bugwood.org, Joseph Berger under a CC BY 3.0 licence; *Apis mellifera*, Bugwood.org, David Cappaert under a CC BY 3.0 licence; *T. carbonaria*, Alison Bockoven; *Dionycha triangularis*, Bugwood.org, Joseph Berger under a CC BY 3.0 licence; *Coleomegilla maculata*, Bugwood.org, Whitney Cranshaw under a CC BY 3.0 licence; *Helicoverpa armigera*, Bugwood.org, Gyorgy Csoska under a CC BY 3.0 licence; *Formica obscuriventris*, Gary Alpert; *Acalymma vittatum*, Bugwood.org, G.J. Holmes under a CC BY 3.0 licence.

Here we report a previously undescribed euphorine parasitoid wasp belonging to the genus *Syntretus* in the hymenopteran-parasitizing tribe Syntretini. Although *Syntretus* species have long been known to occur in North America^{22,23}, their hosts have remained undescribed. In the Old World, the hosts of just four syntretines have been described^{24–27}. As these are all hymenopteran hosts, it was widely assumed that Hymenoptera is the sole host order for this tribe; however, the most recent revision for European *Syntretus* emphasized the paucity of biological information regarding species in this genus²⁸. The wasp species naturally infects the adult stage of several divergent host species in the genus *Drosophila* across the eastern USA, including *Drosophila affinis* and *D. melanogaster*, an unexpected host order record both for the genus *Syntretus* and the subfamily Euphorinae as a whole. We show that flies infected with this wasp are easily collected from fruit fly baits in both rural and suburban backyards in Mississippi, Alabama and North Carolina. We also present protocols to rear adult wasps from wild-caught

host flies, and we document their life history and host interactions with photographs and video. Overall, this discovery creates new avenues of research with both euphorines and *Drosophila* and presents unexpected new biology and ecology in a highly studied model organism.

A wasp living inside adult fruit flies

While monitoring nematode infections in wild fruit flies in March 2023, we found a parasitoid wasp larva inside the abdomen of an adult male *D. affinis*. Following amplification and sequencing of the mitochondrial cytochrome oxidase subunit I gene locus (*COI*), we (L.D.M., T.C.A. and M.J.B.) identified this larva as a euphorine braconid wasp, which are known for their unusual strategy of ovipositing and developing within adult insects. Subsequently, specimens were examined and confirmed by S.R.S. as a previously undescribed species of *Syntretus* on the basis of morphology and the relevant taxonomic literature^{16,23,28,29}. Nuclear and mitochondrial DNA sequences also support assignment of this species to the euphorine genus *Syntretus* (Fig. 1), some of which are known to use adult hymenopterans as hosts^{17,29}.

Four described syntretine species have well-documented host records: *Syntretus xanthocephalus* parasitizes the ichneumonid parasitoid wasp *Phaeogenes invisor*²⁴, *Syntretus splendidus* parasitizes *Bombus* species²⁵, *Syntretus trigonaphagus* parasitizes the meliponine (stingless) bee *Tetragonula carbonaria*²⁷, and *Syntretromorpha szaboi* parasitizes the Asiatic honeybee *Apis cerana*²⁶. As *COI* sequences are not available for any of these species, we also sequenced the *COI* locus of *S. trigonaphagus* and mapped short sequence reads from a parasitized *A. cerana* (Supplementary Data) to tie *COI* sequence data to host records in the tribe (Fig. 1). The sequences of these two bee-parasitizing species group together within the genus, whereas *S. perlmani* is allied with other species for which hosts have not been identified. We note that without visual inspection of the *A. cerana* parasite, the species identity cannot be confirmed, and we treat it as *Syntretus* sp. ex *A. cerana*. These relationships are recovered from nuclear and mitochondrial loci individually (Extended Data Fig. 1a,b) and from the concatenated sequence data (Fig. 1). As shown previously, the monophyly of tribes within Euphorinae are strongly supported, although the deeper nodes relating to intertribal relationships are not fully resolved¹⁹. However, both the previous and present analyses agree on the placement of the ant-parasitizing members of the tribe Myiocephalini (*Myiocephalus boops*) as sister to Syntretini, suggesting that the use of hymenopteran hosts is ancestral in Syntretini (that is, the direction of this host shift was from Hymenoptera to Diptera).

We also retrieved all *COI* sequences attributed to *Syntretus* from the Barcode of Life Data System (BOLD) and GenBank, regardless of association with a described species or host record, to estimate sequence divergence between the previously undescribed wasp and existing records (a phylogram built from these data is available as Extended Data Fig. 1c; database queried 31 July 2023). The most closely related *COI* sequence on the basis of patristic distance (branch length) was collected in Arizona, USA in 2010 (BOLD sequence ID 10BBHYM-0318). Shared sequence identity is 92.7% with this entry across 552 bases. On the basis of nucleotide divergence from available records, distinguishing morphological traits (described below) and association with *Drosophila* hosts, we propose that the wasp is a previously undescribed species in the genus *Syntretus* and we assign the name *Syntretus perlmani* (see the ‘Etymology’ section in the species description).

Infection frequency and life history

To estimate the infection frequency of *S. perlmani*, we collected and screened more than 6,000 male *D. affinis* from Mississippi, Alabama and North Carolina, USA, between April 2023 and February 2024 (Supplementary Data). Infection in male *D. affinis* can be visually confirmed without dissection between days 7 and 18 post oviposition, at which

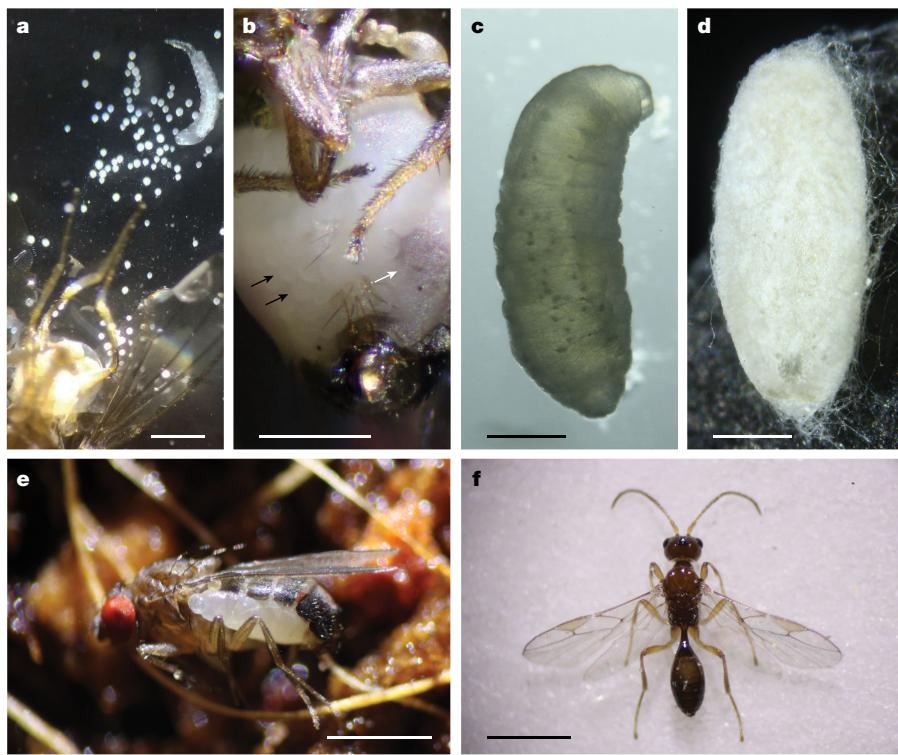


Fig. 2 | Life stages of *S. perlmani*. **a,b**, The development of wasp larvae inside host flies (**a**) is accompanied by growth of wasp teratocytes (**b**, black arrows), which can be seen through the host abdominal cuticle and obstruct the view of the testes (**b**, white arrow). **c**, The second and following larval instars lack a head capsule and tail spike, and the final instar grows to nearly the length of the host fly (see Supplementary Video 2). **d**, Pupal development takes place within

a white silken cocoon as is typical of euphorine wasps. **e**, Larval emergence is always from the abdomen and has been observed to occur between the second and third tergites (dorsolaterally) or laterally through a tear in the abdominal cuticle. **f**, The adult wasp (male shown) is small, yellowish brown and approximately 1.5 mm in length. Scale bars, 0.5 mm (**a–d**) and 1 mm (**e,f**).

point the wasp larva and teratocytes grow large enough to swell the abdomen and obstruct the view of the host's brightly pigmented testes (Fig. 2b). Teratocytes are specialized cells that originate from the embryonic membrane of some braconid parasitoids. They persist throughout larval development, exhibiting massive growth and decreasing number over time³⁰. Just under 1% of males were infected during the entire collection interval, although this varied through the year from 0.5% to 3% as did host abundance (Extended Data Fig. 2). Wild-caught females were also visually inspected but were not reliably scored for infection in this way; however, we dissected 477 female *D. affinis* and found just one infection. Most of the dissected females were collected in October when the infection rate in male flies was 1.6% (12 of 730).

We reared *S. perlmani* from wild-caught males to observe behaviour and development in the laboratory. Following oviposition, larval development within the host takes 18.2 ± 1.4 days ($n = 24$; Supplementary Data) at 21°C (Fig. 3a,b). For the entire duration until the time of larval emergence, host flies remain active within housing vials. Consistent with typical syntretine development, two distinct larval forms are observed; the early instars have a prominent head, mandibles and a spiked tail (Fig. 2a), and late instars adopt a grub-like (hymenopteriform) form with no distinct head or tail²⁵ (Fig. 2c).

Late into larval development, teratocyte numbers are reduced, possibly consumed by the larva³¹, and the larva can be observed moving within the host abdomen (Supplementary Video 1). Mature larvae chew through the host cuticle, usually dorsolaterally between abdominal tergites two and three, or laterally through the abdominal cuticle (Fig. 2e and Supplementary Video 2). The emerged larva is active (Supplementary Video 3) and may explore a substrate for hours before selecting a pupation site. As reported for other *Syntretus* spp., larvae prefer to burrow into a loose substrate and cocoon beneath the surface.

Supplementary Video 4 captures an emerged larva spinning silk. The fully constructed cocoon is white and appears opaque (Fig. 2d) until development is nearly complete and a darkly pigmented adult becomes visible. At 21°C , the adult wasp (Fig. 2f) emerges from the cocoon after 23.2 ± 1.4 days ($n = 22$; Supplementary Data).

Female wasps fed on honey water (1:1) begin ovipositing into adult flies in the first 24 h of emergence from the cocoon. To oviposit, euphorines bend the metasoma (abdomen) ventrally and anteriorly below the mesosoma (thorax) and head (so the ovipositor tip is directed forwards in front of the face), and then step forwards while extending the ovipositor into the host. Observed host reactions to the attack range from no response (Supplementary Video 5) to rapid and sometimes successful retreat (Supplementary Video 6). In one observed encounter, the host fly appeared to recognize an oviposition attempt and engage the wasp with aggressive defensive behaviours (Supplementary Video 7). In this case, the parasitoid approached to oviposit only from the side and back, repeatedly retreating when confronted head-on.

We attempted to establish a permanent culture of *S. perlmani* by allowing female wasps reared from wild-caught hosts to parasitize flies in the laboratory. Oviposition success from five female wasps in the laboratory ranged from 48% to 63% (Extended Data Fig. 3 and Supplementary Data). A larva emerged from 8–60% of parasitized hosts, cocoons from 8–30%, and adult wasps from 0–25%. The wide range of reproductive success across female wasps may be explained in part by modifications to the rearing approach with each attempt. For example, we exposed the first female wasp almost exclusively to male hosts ($n = 124$) to mirror the apparent host sex bias in the wild but recovered no adult wasps from these exposures. For the second female wasp, 17% of the hosts we offered (of $n = 82$) were female, and we reared five adults from these and three from the male hosts. For the last 3 female wasps, we offered 89–100% female hosts ($n = 87, 59$ and 19)

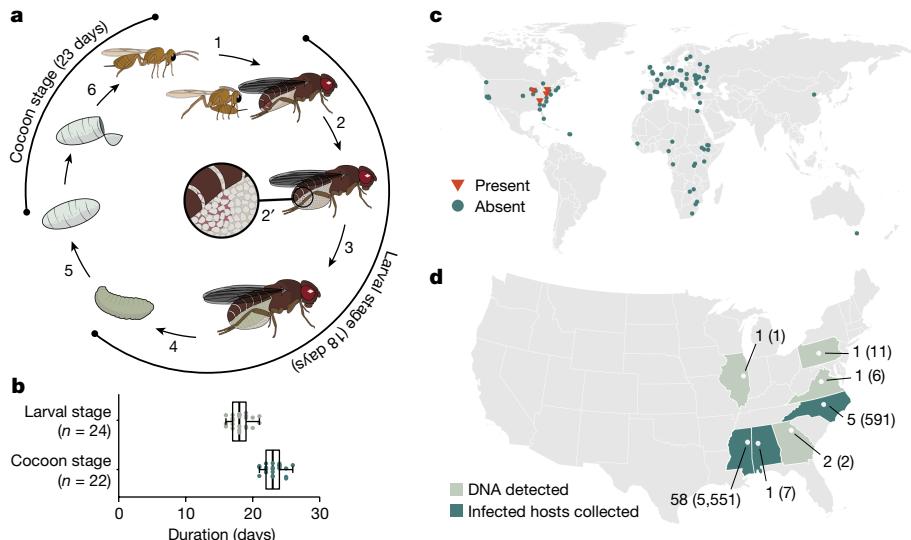


Fig. 3 | Life cycle, oviposition outcomes and geographic distribution of *S. perlmani*. **a**, The life cycle of *S. perlmani*, including oviposition (1), early larval development (2), appearance of teratocytes (2'), late larval development and reduction in teratocyte number (3), larval emergence (4), cocoon formation and metamorphosis (5) and adult emergence (6). **b**, Duration of larval and cocoon stages for $n = 24$ and $n = 22$ wasps, respectively. Data are observations collected while rearing wasps from wild-caught and laboratory-infected flies. Centre line, median; box limits, upper and lower quartiles; whiskers, $1.5 \times$ quartile range. **c**, Global map showing the sampling locations of wild-caught *D. melanogaster* pools from which reads were sequenced³².

Green points indicate collection sites where *S. perlmani* mitochondrial DNA reads were absent and red triangles indicate collection sites where *S. perlmani* mitochondrial DNA reads were present. **d**, Map of the contiguous USA showing the locations where *S. perlmani* have been identified through field collections of *D. affinis* (dark green) or through in silico surveys of host DNA data (light green). Labels beside each state indicate the number of *S. perlmani*-infected *D. affinis* males caught and the total number collected in parentheses, or the number of DNA read sets positive for *S. perlmani* DNA reads and the total number analysed.

and reared 16 adult wasps, all from female hosts (Extended Data Fig. 3 and Supplementary Data). By contrast, all of the infected wild flies we identified from visual screens and attempted to rear wasps from were male ($n = 28$) and adults completed development in 39% of these cases (Supplementary Data). For additional effects of host sex on wasp development in the laboratory, see Extended Data Fig. 4.

Host range and distribution

To explore the possibility that *S. perlmani* may use *D. melanogaster* as a natural host and to examine its broader geographic distribution, we used an in silico approach. We downloaded DNA-sequencing data generated from wild-caught *D. melanogaster* collected primarily from North America, Europe and Africa³² ($n = 234$; Fig. 3c). We sequenced and assembled the mitochondrial genome of *S. perlmani* and used it to map reads from *D. melanogaster* datasets with $\geq 99\%$ nucleotide identity. *S. perlmani* reads are present in data from four states in the USA—Georgia, Virginia, Pennsylvania and Illinois (Fig. 3c,d and Supplementary Data). No sequence evidence for *S. perlmani* infection was found in *D. melanogaster* data collected from any site outside the USA. To confirm that *S. perlmani* can complete its life cycle in *D. melanogaster*, we exposed male and female flies to adult wasps in the laboratory. We observed *S. perlmani* attacking male *D. melanogaster* (Supplementary Video 5), but we failed to rear adults from them ($n = 11$ confirmed infections). However, from female *D. melanogaster*, we reared nine adult wasps ($n = 44$ confirmed infections). This rate of embryo-to-adult success (20.5%) is comparable to results from female *D. affinis* hosts in the laboratory, and suggests, in combination with the sequence mapping results, that *D. melanogaster* is used as a host by *S. perlmani* in the wild.

D. affinis and *D. melanogaster* are distantly related species in the *Drosophila* subgenus *Sophophora*, estimated to have shared a common ancestor 17–31 million years before present³³. The ability of *S. perlmani* to parasitize both species despite divergence between innate immune effectors³⁴ raises the possibility that *S. perlmani* may be capable of

parasitizing many species of *Drosophila*. To test its ability to parasitize additional hosts, we exposed female *Drosophila acutilabella* (subgenus *Drosophila*) to *S. perlmani* in the laboratory. Adult wasps were reared from 14 parasitized hosts ($n = 67$ confirmed infections), an overall embryo-to-adult success rate of 20.9%. However, we also found evidence for resistance to *S. perlmani* in a member of the subgenus *Drosophila*; in 15 confirmed infections of *Drosophila immigrans*, we found encapsulated wasp eggs in every case (Extended Data Fig. 5), but no teratocytes or wasp larvae. Altogether, these results indicate that *S. perlmani* may have a broad potential host range among *Drosophila* species, limited in part by species-specific immune interactions.

Taxonomic classification and description

Family Braconidae Latreille, 1829
 Subfamily Euphorinae Foerster, 1862
 Tribe Syntretini Shaw, 1985
 Genus *Syntretus* Foerster, 1862
 Subgenus *Syntretus* Foerster, 1862
Syntretus perlmani sp. nov. Shaw & Ballinger, 2024

Etymology. This species is named after Steve Perlman (University of Victoria, British Columbia, Canada), in recognition of his contributions to research and mentorship in the field of *Drosophila*–parasite interactions.

Type material. Holotype: female (deposited in the University of Wyoming Insect Museum, UWIM), USA, Mississippi, Oktibbeha County, Starkville; Global Positioning System (GPS): 33.494303, -88.753891; locality: residence backyard, pine and cedar forest; L.D.M. collector; reared from infected host adult male *D. affinis*; collection date of infected fly: 12 July 2023; parasitoid larval emergence date: 21 July 2023; adult wasp emergence date, 13 August 2023; date of death: 24 August 2023; tube label: Buttercup.

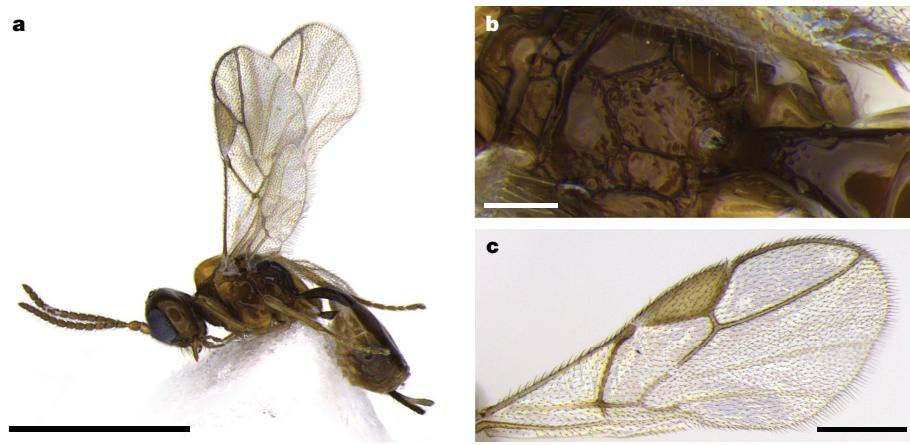


Fig. 4 | Morphology of *S. perlmani*. **a**, Holotype lateral habitus. **b**, Holotype propodeum. **c**, Holotype forewing venation. Scale bars, 1 mm (a), 100 µm (b) and 400 µm (c).

Paratype. One female, same data as holotype except collection date of infected fly: 5 April 2023; (parasitoid) larval emergence date: 15 April 2023; adult (wasp) emergence date: 7 May 2023; date of death: 12 June 2023; tube label: Dorothy (UWIM). One female, same data as holotype except collection date of infected fly: 28 May 2023; (parasitoid) larval emergence date: 9 June 2023; adult (wasp) emergence date: 3 July 2023; date of death: 8 July 2023; tube label: Betsy Bobbin (UWIM). One female, same data as holotype except collection date of infected fly: 13 July 2023; (parasitoid) larval emergence date: 24 July 2023; adult (wasp) emergence date: 18 August 2023; date of death: 22 August 2023; tube label: Leeloo (UWIM). One female, same data as holotype except collection date of infected fly: 14 July 2023; (parasitoid) larval emergence date: 23 July 2023; adult (wasp) emergence date: 16 August 2023; date of death: 4 September 2023; tube label: Barbie (UWIM). One male, same data as holotype except collection date of infected fly: 13 June 2023; (parasitoid) larval emergence date: 22 June 2023; adult (wasp) emergence date: 16 July 2023; date of death: 25 July 2023; tube label: Javier Bardem (UWIM). One male, same data as holotype except collection date of infected fly: 14 July 2023; (parasitoid) larval emergence date: 24 July 2023; adult (wasp) emergence date: 16 August 2023; date of death: 29 August 2023 (preserved alive); tube label: Keith (UWIM). One female, North Carolina, Wake County, Apex; GPS: 35.741349, -78.834234, locality: watermelon bait in residential neighbourhood; M.J.B. collector; reared from infected host adult male *D. affinis*, collection date of infected fly: 13 June 2023; (parasitoid) larval emergence date: 22 June 2023; adult (wasp) emergence date: 18 July 2023; date of death: 29 July 2023; tube label: Alice (UWIM). One male, same data as preceding female except no collection data; laboratory-reared from mother (Alice); host: female adult *D. affinis*; oviposition date: 21 July 2023; adult (wasp) emergence date: 29 July 2023; date of death: 6 September 2023 (preserved alive); note: 13 flagellomeres; tube label: Male offspring of Alice (UWIM).

Description of the holotype. Female, length of forewing 1.98 mm, length of body 1.45 mm.

The details of the head are as follows. Antenna with 14 antennomeres (scape, pedicel and 12 flagellomeres), flagellomeres moderately bristly setose (Fig. 4a and Extended Data Fig. 6c; antennomere measurements and ratios in the Supplementary Discussion); mandible long and slender, 3.3 times as long as basal width; length of maxillary palpus (248.1 µm) 0.7 times height of head (348.5 µm); face smooth and shining, moderately setose, with setae arising from distinct punctures (Extended Data Fig. 6c,d); face shape roughly isosceles trapezoidal, narrowest above, where eyes converge most closely, widest along lower margin near clypeus; face greatest width 2.2 times wider than face height medially; clypeus smooth (except setae and scattered

punctures), slightly convex, lower clypeal margin even with lower level of eyes; clypeal setae long, especially ventrally, longest setae more than 2 times as long as facial setae (Fig. 4a; Extended Data Fig. 6c); frons smooth and moderately setose laterally, more glabrous medially, and without a median groove or carina, rather flat in front of anterior ocellus; gena, temple and vertex mostly smooth and shining (especially as compared to frons and face), only a few scattered small setae ventrally on gena; ocelli small, lateral ocellus width just slightly greater than basal flagellomere (F1) width; eye shape ovoid, narrowest dorsally, broader ventrally, slightly protruding anteriorly but not extending beyond face; eye in lateral view 1.6 times taller than greatest width; greatest eye width 1.1 times gena width; temples parallel-sided behind eyes; length of malar equal to basal width of mandible; occipital carina well-developed laterally, weak dorso-medially; hypostomal carina not visible.

The mesosoma is shown in Fig. 4a,b. Antescutal depression narrow; mesoscutum smooth (including notaular area anteriorly), glabrous; notaular absent; scutellar disc slightly convex, entirely smooth and glabrous (devoid of setae); scutellar sulcus with three cross-carinae delimiting two large submedial foveae; metapleuron mostly smooth, except weakly rugulose basally; propodeum anteriorly with a short median carina (about equal in length to median carina of scutellar sulcus), posteriorly gradually declivous and with a large pentagonal areola, laterally with rugosity and costulae; propodeal spiracle circular, slightly broader than width of carinae delimiting pentagonal areola (Fig. 4b).

The forewing is shown in Fig. 4c. Pterostigma 2.9 times longer than wide; vein r short, emerging from pterostigma distinctly beyond middle; vein $1-R1$ 1.14 times longer than pterostigma length; vein RS long, only slightly curved, ending near apex of wing, 1.43 times longer than pterostigma length, and 9.88 times longer than vein r length; marginal cell maximum length times longer than maximum width; cross-vein $1-cu-a$ nearly even with base of vein $1-M$; wing membrane moderately densely and evenly setose over the entire wing, basal and subbasal cells as densely setose as other cells.

The details of the hindwing are as follows. Vein $1-SC + R$ is tubular and sclerotized; vein $1r-m$ not separated from vein $2-SC + R$ (Extended Data Fig. 6a,b).

The legs are shown in Fig. 4a and Extended Data Fig. 6a). Hind femur 4.38 times longer than maximum width; hind tibia 11.25 times longer than maximum width; hind basitarsus 6.0 times longer than maximum width; fore femur 3.33 times longer than maximum width; tarsi with short setosity ventrally; tarsal claws small, slender and strongly curved.

The details of the metasoma are as follows. First tergite (T1) narrow and slender basally, gradually wider posteriorly, basal width 0.2 times length of T1, apical width 0.65 times T1 length; first tergite smooth basally and apically, medially with faint rugulose sculpture, laterally

with margin weakly carinate, giving the appearance of a distinct edge separating the dorsal surface of T1 and its lateral sides (Fig. 4a); laterope absent (Fig. 4a); ovipositor sheath long and slender, slightly widened apically, slightly narrower medially, and with scattered long setae over entire length (Fig. 4a), visible portion of ovipositor sheath 11.6 times longer than median width (see details on variation below); ovipositor (in holotype) not visible except tip, appearing straight.

The colour is shown in (Fig. 4a,b and Extended Data Fig. 6). Yellowish brown; antenna (except for two basal segments) dark brown; first tergite, apical half of metasoma dorsally and ovipositor sheath darkened; apical half of hind tibia and tarsus slightly darker than remainder of legs; palpi, tegulae, coxae, trochanters, second and anterior half of third metasomal segment whitish; veins yellowish brown, but veins C + SC + R, and r of forewing and pterostigma brown; wing membrane subhyaline; both valves of ovipositor brown, but upper valve slightly darker.

Details of variation are as follows. Antenna with 12 or 13 flagellomeres. On the basis of other material reared in Mississippi (not included in the type series), it seems that flagellomere number may be correlated with the size and sex of the host fly (Extended Data Fig. 4). Wasps that develop in male host flies are slightly smaller, and wasps reared from these have 12 flagellomeres (most of the type series show this character state). Wasps that develop from female host flies are slightly larger, and the resulting wasps have 13 flagellomeres.

The appearance of the ovipositor varies depending on the degree to which it is exerted at the time the specimen is preserved. In the holotype the ovipositor is in its resting position, appearing straight, partly drawn inside the body, resting within the ovipositor sheaths, with the ovipositor tip just barely visible. In other specimens, the ovipositor is extended farther out of the body, appears thicker basally, thinner apically, and is slightly curved. In specimens with the ovipositor extended, the hypopygium (last sternite) is bent ventrally, and the ovipositor sheathes are folded dorsally, being partly concealed basally by the apex of the metasoma but extending dorsally well above the metasoma dorsum. In specimens with the ovipositor fully visible and extended, the ovipositor is clearly much longer than the hind tibia length.

Distribution. USA, Mississippi and North Carolina. Collected in May–August. However, screenings of global DNA datasets performed in this study suggest a broader distribution across the eastern USA.

Differential diagnosis. *Syntretus perlmani* sp. nov is a tiny species that is distinct from other known *Syntretus* species by virtue of its small size (body length about 1.5 mm) and utilization of adult *Drosophila* flies as hosts. The antenna is shorter than the head and mesosoma combined, and there are 12–13 flagellomeres. Often the antenna has only 12 flagellomeres (the lowest number recorded for any North American *Syntretus* species).

Of the North American species, *S. perlmani* is most similar to *Syntretus brevicornis* Muesebeck, which also has a small body size and short antenna. Of the two species, *S. perlmani* is the smaller, with a body length of about 1.5 mm, as compared to 2.2 mm for *S. brevicornis*. As far as we are aware, *S. perlmani* has the smallest body length of any known *Syntretus* species, worldwide. The hind femur of *S. perlmani* is longer and narrower than in *S. brevicornis* (hind femur about 4.4 times longer than wide in *S. perlmani*, as compared with 4.0 times longer than wide in *S. brevicornis*). The ovipositor is longer in *S. perlmani* with the ovipositor being as long as the hind tibia and basal three hind tarsomeres combined, whereas the ovipositor is about equal to the hind tibia length in *S. brevicornis*. The colour patterns of the two species are distinctly different, especially that of females. In the holotype of *S. perlmani* the pronotum laterally is dark brown contrasting with the mesonotum, which is yellow or light yellowish brown. In the holotype of *S. brevicornis* this pattern is reversed, with the pronotum laterally being light yellowish brown and the mesonotum being dark brown. For additional taxonomic details, see the Supplementary Discussion.

Discussion

In this study, we report the discovery of a parasitoid wasp that uses adult dipterans as hosts by attacking and infecting the adult stage of *Drosophila* flies. Parasitism of the adult stage of insects is an unusual life strategy among wasps but it is commonplace in the subfamily Euphorinae^{16,17,35–37}. Although Euphorinae is among the most diverse of the braconid wasp subfamilies, many features of these wasps remain understudied, including the origins and advantages of adult parasitism. Fossil evidence establishes a minimum age of >30 million years for euphorines^{38,39}, and phylogenetic evidence in congruence with host records indicates that the earliest euphorines were probably parasitoids of larval hosts, possibly chrysomelid beetles^{16,17,40}. Although it has been considered that the rarity of adult parasitism may be due to a number of obstacles²¹, use of adult hosts may also offer unique advantages, including reduced competition for hosts⁴¹ and reduced potency of the host cellular immune response^{42,43}.

We anticipate that continuing work will improve the culturability of *S. perlmani*. Parasitoid success reached around 25% of laboratory-infected (Extended Data Fig. 3) and 39% of wild-infected hosts. We identified differential developmental outcomes for wasps that develop within female hosts, including increased survival, body size and antenna length (Extended Data Fig. 4). Yet, we observe that the frequency of *Syntretus* infections in field collections is much higher in male *D. affinis* compared to *D. affinis* females. Observed infection rates may be biased by the ease of detecting infected males, but our direct dissections also indicated a very low rate of infection in wild females. Sex-based differences in wasp detection and response have been identified in *Drosophila*; for example, female flies alter egg-laying and sexual behaviour in environments where larva-parasitizing wasps are present^{12,44}. We might speculate that similarly elevated sensitivity to wasp presence by females could result in behavioural responses that reduce successful attacks in the field (for example, increased vigilance, activity or retreat).

Molecular phylogenetics and morphological assessment of *S. perlmani* agree that the previously undescribed euphorine belongs to the genus *Syntretus*. Until now, all syntretine host records have been other hymenopterans^{24–27}; therefore, two possibilities can explain the use of *Drosophila* hosts: either the host range of *S. perlmani* spans several orders of holometabolous insects as is the case for some pupal parasitoids (for example, members of the pteromalid genus *Trichomalopsis*⁴⁵), or a shift from hymenopterans to dipterans underlies its current use of *Drosophila* hosts. Although there are no described cases in which a euphorine wasp uses hosts from more than one order, several order-spanning host shifts have been mapped to the phylogeny of Euphorinae, including two independent origins of hymenopteran parasitism from a probable coleopteran-parasitizing ancestor¹⁹. Within host families, however, some euphorines are highly successful generalists, using more than 50 different host species in the example of *D. coccinellae* and its coccinellid hosts⁴⁶. Other euphorine host records are limited to few or even a single host species²⁷, mirroring patterns of generalist and specialist infection strategies observed across parasitoid wasps, including those of *Drosophila*⁴⁷.

Our results do not yet resolve a primary host preference or geographic range of *S. perlmani*. By combining field collections with *in silico* screens, we identified two species of *Drosophila* flies that host *S. perlmani* in the eastern USA. The absence of *S. perlmani* from sequence data collected in Africa, within the ancestral range of *D. melanogaster*⁴⁸, suggests that this species has not historically been the primary host of *S. perlmani*. The low infection frequency of *D. affinis* in the field, and the extensive geography and host species diversity unrepresented by available sequencing data available at present, highlight the possibility that *S. perlmani* is present across a broader range and infects a greater diversity of drosophilids than demonstrated here. Drosophilidae is a diverse, cosmopolitan family containing more than 4,700 described

species⁴⁹, and our laboratory infections of 2 additional drosophilids in the subgenus *Drosophila*, *D. acutilabella* and *D. immigrans*, suggest that future studies of host range are likely to uncover additional hosts and examples of resistance throughout the genus.

Next we consider how diverse the dipteran-parasitizing syntretines might be. Members of the tribe with hymenopteran host records have a broad distribution, having been collected in the UK, India and Australia^{24–27}. Additionally, dissections of adult *Bombus* spp. have revealed parasitoid larvae, probably *Syntretus* spp., in the eastern USA, the UK and Sweden²⁶. Diverse adult syntretines with no host records have been found from Korea⁵⁰ to Costa Rica (S.R.S., unpublished data) and more than 150 COI sequences from the USA and Canada have been collected and deposited in BOLD with no host records (Extended Data Fig. 2). Although many more hymenopteran-parasitizing species are probably among these, we hope knowledge that some of this unstudied species diversity may have evolved in association with adult dipterans will motivate a broader approach to collecting and monitoring wild flies for identification of previously undescribed parasitoids.

Underlying each parasitoid infection are battles fought on behavioural and physiological fronts, including conserved immune pathways and genetic processes^{4–7}. By studying how hosts avoid and defend against parasitoids and how wasps overcome these adaptations, research in insect-parasitoid models is advancing understanding of the behaviour^{10–12}, physiology^{4,5} and evolution^{7–9,51} of biological systems. For example, experiments with *Drosophila* and parasitoids have demonstrated the evolvability of enhanced immunity-based resistance as well as fitness costs associated with evolved resistance⁵¹. Work on *Drosophila*-parasitoid systems has also advanced understanding of the genetic mechanisms underlying reproductive biology⁵² and mating behaviour¹² in response to parasitoid pressure. We expect that the unique lifestyle of *S. perlmani* and its evolutionary divergence from previously known parasitoids of *Drosophila* will support new avenues to extend research in these areas. For example, a euphorine parasitoid of adult *Drosophila* may be useful to study: functional specialization of innate immunity across *Drosophila* development; cues and behaviours mediating natural enemy sensing, defence and avoidance; host searching and discrimination by parasitoids; and functional evolution of parasitoid effectors, including venom and teratocyte proteins. Combined with the genetic tractability of *D. melanogaster*, this parasitoid provides new molecular and behavioural interfaces at which the mechanisms underpinning parasite–host interactions can be investigated.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-024-07919-7>.

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Methods

Fly collections and parasitoid screening

Drosophila were collected at banana, watermelon and mushroom baits by net and aspirator. GPS locations of collection sites are included in the 'Type material' section. Flies were examined at the time of collection for displaced or obstructed testes and teratocytes and re-examined periodically for 1 week post-collection. If no evidence of infection was visible after 1 week in the laboratory, flies were recorded as uninfected. Infected flies were monitored in vials at low density until teratocyte abundance decreased and the larva became visible directly beneath the abdominal cuticle. At this stage, they were transferred to a dish for larval emergence and pupation. Dish recommendations and other rearing instructions are available in the Supplementary Methods.

Sequencing and phylogenetic analysis

DNA was extracted from the *S. perlmani* larva using PrepMan Ultra (Applied Biosystems). The *COI* locus of *S. perlmani* was amplified by PCR using the primer set LCO1490 and HCO2198⁵³ and sequenced at Sequetech (California). The thermal cycling programme was: 95 °C for 2 min followed by 40 cycles of 95 °C for 30 s, 48 °C for 30 s, 72 °C for 1 min. *S. trigonaphagus* larvae dissected from *T. carbonaria* were a gift from R. Gloag. They were collected in Southeast Queensland, Australia in 2018 and stored in 100% ethanol at -80 °C. DNA was extracted using the phenol–chloroform method and *COI* sequences were amplified as described for *S. perlmani*. *CAD*, 18S rRNA and 28S rRNA sequences were amplified using the primer sets described in ref. 19 and the same thermal cycling programme as used for *COI* amplification. Products were Sanger sequenced by Azenta Life Sciences.

Nucleotide sequences of euphorines and outgroups were retrieved from GenBank (accession numbers available in the Supplementary Data). For all phylogenies, sequences were aligned using MAFFT 7.49 (ref. 54) and phylogenies were built using the Jukes–Cantor model in FastTree 2.1.11 (ref. 55).

S. perlmani infections in the laboratory

All adult wasps and *D. affinis* flies were maintained in insect rearing chambers held at 21 °C with 16 h light and 8 h darkness. *D. melanogaster* flies were maintained at 25 °C before exposures, and 21 °C during exposures and wasp development. Flies were exposed to single wasp females in groups of 5–10 in polystyrene *Drosophila* vials containing banana or glucose agar and 50 µl of honey water on the vial closure. Exposures were carried out in 24-h intervals. After each interval, female wasps were transferred by aspirator (to minimize CO₂ exposure) to a new vial containing unexposed flies.

S. perlmani mitochondrial genome sequencing

DNA was extracted using the phenol–chloroform method from 13 larvae of *S. perlmani* collected in Starkville, MS or reared from mothers collected in Starkville. An Oxford Nanopore DNA library was prepared using the Ligation Sequencing Kit V14 (SQK-LSK114) and 6.11 gigabases (Gb) were sequenced on a MinION flow cell (FLO-MIN114). Reads 1 kb and longer were retained using seqtk⁵⁶ and assembled with Flye 2.9.1 (ref. 57). The assembly did not close the mitochondrial genome, despite high coverage by long reads, but it contains extensive repeat elements flanking the AT-rich control region that were not fully resolved. Similar repeat elements are increasingly recognized in long-read mitochondrial genomes of other insect taxa⁵⁸.

In silico detection of *S. perlmani* infections

Sequence Read Archive data generated from wild-caught *Bombus* spp., *Apis* spp. and *D. melanogaster* were downloaded and mapped to the

S. perlmani mitochondrial genome using BBMap⁵⁹. All *D. melanogaster* reads used were generated by the *Drosophila* Evolution over Space and Time project³². Accession numbers are listed in the Supplementary Data, along with identity thresholds and other parameters used for each mapping analysis. Data points were plotted with the rnaturrearthdata R package⁶⁰. As the mitochondrial genome assembly contains unresolved repeats, we included a single contiguous region of the assembly as the reference sequence for read mapping. This subset included all mitochondrial tRNAs, rRNAs and proteins as well as the intergenic sequences between them (14,866 nucleotides) but excluded the repeats and control region.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Data generated during this research are included in the Supplementary Information. Nucleotide sequences are available on GenBank under accession numbers PP583658, PP477065, PP885366–PP885369, PP894828 and PP894829. The previously undescribed species is registered in ZooBank (<http://zoobank.org>; LSID urn:lsid:zoobank.org:act:42350E17-2D77-4B57-8DB5-018D2A90E351). Source data are provided with this paper.

Code availability

Code used to execute bioinformatics analyses using existing software can be accessed at <https://github.com/mooreld>.

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Author contributions M.J.B. made the primary observation and supervised research. L.D.M., T.C.A. and M.J.B. collected flies. L.D.M. and M.J.B. carried out infections and organism care. L.D.M. and M.J.B. designed rearing procedures. T.C.A. and M.J.B. carried out genome sequencing. L.D.M. and M.J.B. carried out bioinformatics analyses. M.J.B. recorded videos. M.J.B. and S.R.S. captured photographs. S.R.S. and M.J.B. described the species. All authors prepared figures and tables. L.D.M., S.R.S. and M.J.B. wrote the paper.

Competing interests The authors declare no competing interests.

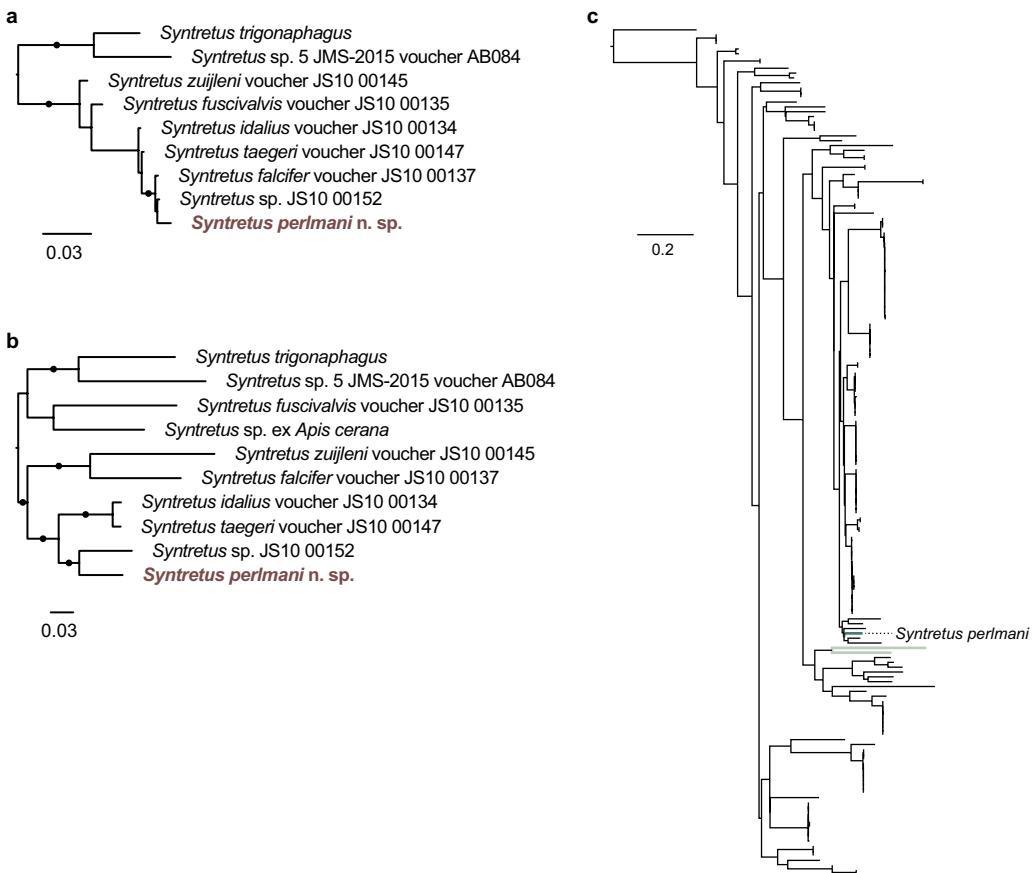
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-024-07919-7>.

Correspondence and requests for materials should be addressed to Logan D. Moore or Matthew J. Ballinger.

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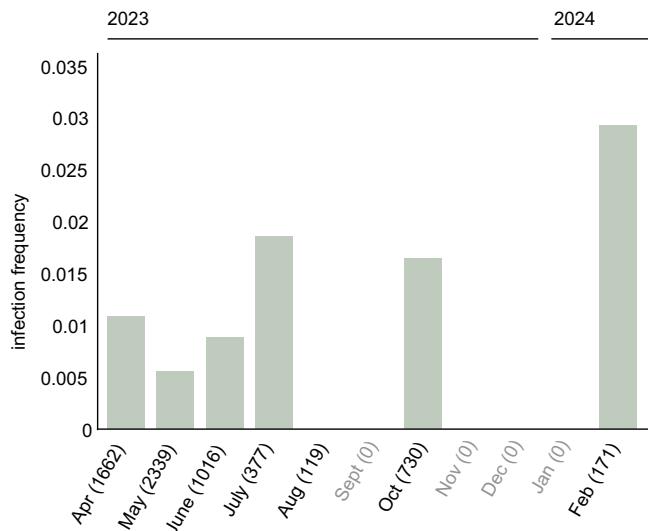
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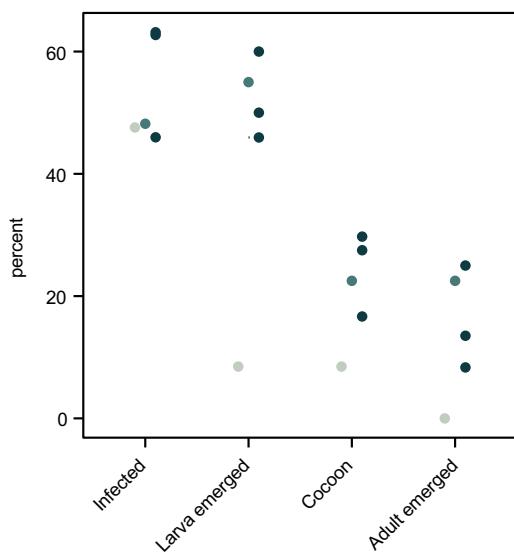
Extended Data Fig. 1 | Phylogenetic relationships in the genus *Syntretus*.

(a) and (b) Phylogenograms including members of the genus *Syntretus* constructed using concatenated (a) nuclear and (b) mitochondrial sequences. Nuclear loci are carbamoylphosphate synthase domain protein (*CAD*), *18S rRNA*, and *28S rRNA*. The mitochondrial locus is *COI*. Branches with SH-like support ≥ 0.9 are labeled with a circle. (c) A phylogram of the mitochondrial cytochrome oxidase subunit I (*COI*) locus of wasps attributed to the genus *Syntretus* showing the

abundance and diversity of syntretine sequences in online databases. Included are all publicly available sequence entries on GenBank and the Barcode of Life Data System (BOLD). Species for which host records are known are shown as bold branches; those in light green are *Syntretus trigonaphagus* and the syntretine parasitoid of *Apis cerana*. The tree is rooted on the *COI* sequence of *Myiocephalus boops*.

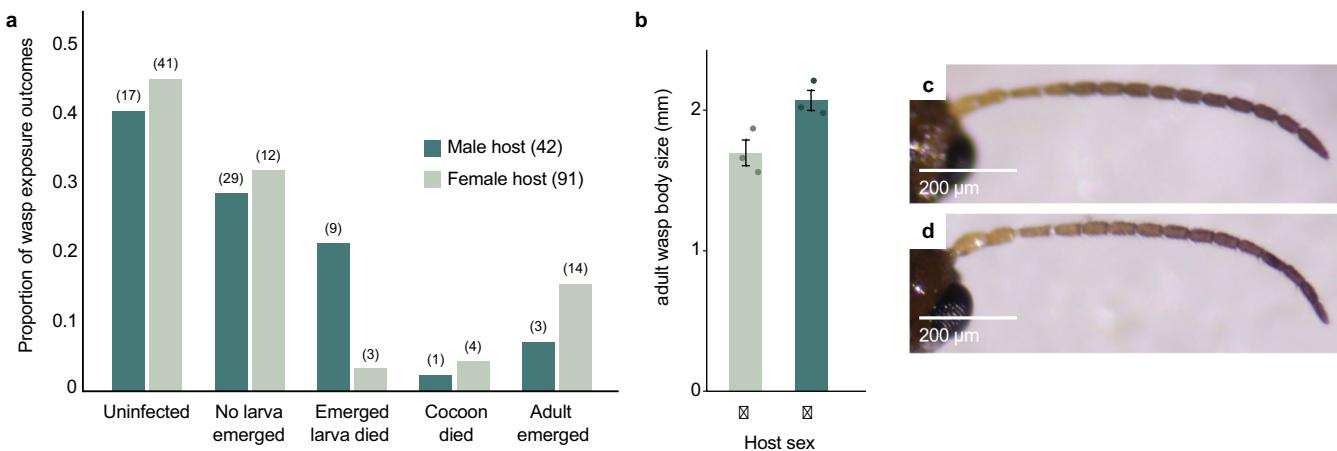


Extended Data Fig. 2 | Infection frequency of *Syntretus perlmani*. Infection frequency of *S. perlmani* in wild-caught *D. affinis* males in Starkville, MS from April 2023 to February 2024. Fly abundance decreased into the late summer with increased temperature and reduced rainfall. Baits were placed in September, but visitation was low. Baits were not placed in November or December. One collection in late January yielded three males, one of which was infected, and is combined with the February data. Sample size (n) is shown in parentheses.



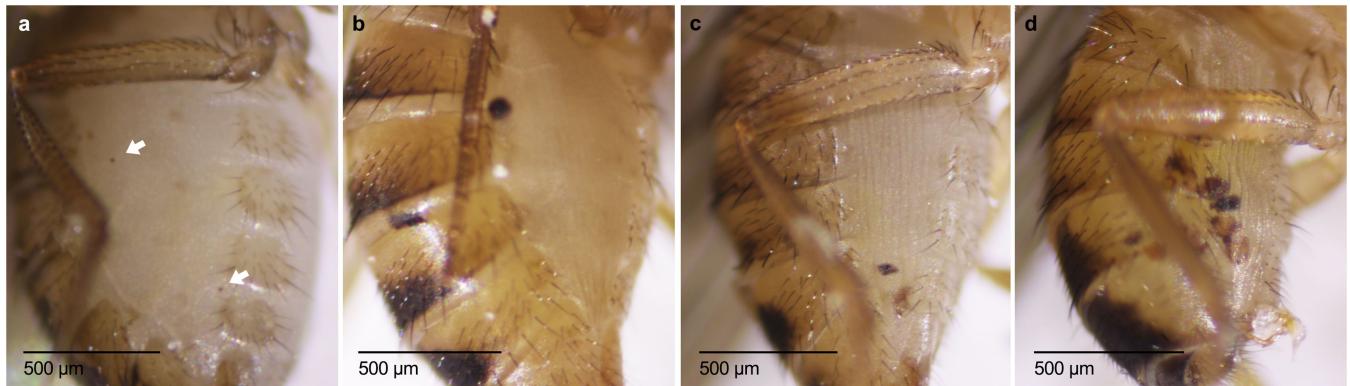
Extended Data Fig. 3 | Parasitism success of *Syntretus perlmani* in laboratory exposures.

Percent success is shown categorized by outcome or stage achieved following exposure. Data is shown for all *D. affinis* and *D. melanogaster* exposures, including both host sexes. For larvae emerged, cocoons, and adults emerged, values calculated are percent of confirmed infections. Points are shaded by the position of the female wasp in the time of exposures, where the lightest color points correspond to data from the first trials, and the darkest points to the most recent. Results are shown for $n = 5$ female wasps.


Extended Data Fig. 4 | Effects of host sex on *Syntretus perlmani* development.

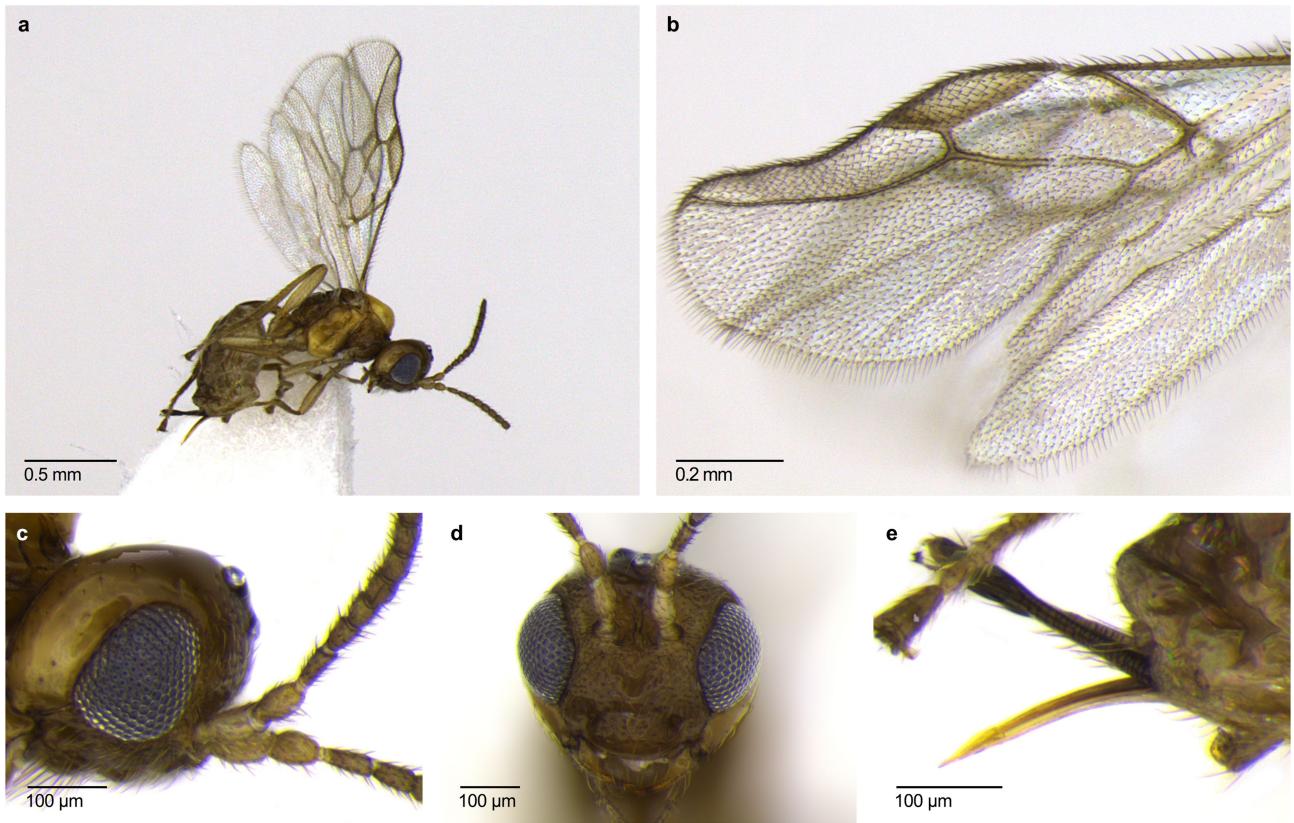
(a) Exposure outcomes after male and female *D. affinis* were exposed to female *S. perlmani* wasps. Data excludes rearing attempts using coconut husk, as no adults were reared using this substrate. (b) For a cohort of 6 male offspring of an unmated female wasp (“Alice”), adult body size is shown. Wasps that developed in a male host ($n = 3$) were significantly smaller than those that

developed in female hosts ($n = 3$; Welch’s t-test, $t_{(3,77)} = 3.23, p = 0.0349$, 95% CI [0.044-0.702]; two-sided). Error bars display standard error of the mean. Additionally, from the same sample set, wasps that developed in (c) male hosts had antennae with fewer flagellomeres (12) than (d) wasps that developed in female hosts (13).



Extended Data Fig. 5 | Melanotic encapsulation of *Syntretus perlmani* eggs in a resistant host species. Photographs of the lateral or ventral abdomen are shown for a permissive host species, *Drosophila acutilabella* (a), and a resistant host species, *Drosophila immigrans* (b-d; representative images from one

cohort of 15 infections). Arrows in (a) indicate small melanization spots associated with wasp oviposition. For both species, photographs were taken nine days post-oviposition by the same female wasp. The host pictured is female in (a-c) and male in (d).



Extended Data Fig. 6 | Additional morphology of *Syntretus perlmani*. (a) paratype lateral habitus, (b) paratype forewing and hindwing venation, (c) paratype head and antennomeres in profile, (d) paratype head, scape, and pedicel, (e) paratype ovipositor.

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	No custom software was generated for data collection. Code used to execute bioinformatics analyses using existing software packages can be accessed at https://github.com/mooreld .
Data analysis	No custom software was generated for data analysis. Code used to execute bioinformatics analyses using existing software packages can be accessed at https://github.com/mooreld .

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Data generated during this research are included within the accompanying supplement. Nucleotide sequences are available on GenBank under accession numbers

PP583658, PP477065, PP885366-PP885369, PP894828, and PP894829. A ZooBank record for the new species is available at [urn:lsid:zoobank.org:act:42350E17-2D77-4B57-8DB5-018D2A90E351](https://doi.zoobank.org/act:42350E17-2D77-4B57-8DB5-018D2A90E351).

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Reporting on race, ethnicity, or other socially relevant groupings	<input checked="" type="checkbox"/> Not applicable
Population characteristics	<input checked="" type="checkbox"/> Not applicable
Recruitment	<input checked="" type="checkbox"/> Not applicable
Ethics oversight	<input checked="" type="checkbox"/> Not applicable

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

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Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	The purpose of this project was to characterize the life history and range of a new wasp species that infects the adult stage of <i>Drosophila</i> hosts
Research sample	Research samples included wild male <i>Drosophila affinis</i> flies collected in rural and suburban areas. Other research samples included <i>Drosophila melanogaster</i> SRA data collected from GenBank following publication (Kapun et al., 2021) and <i>Apis cerana</i> SRA data collected from NCBI.
Sampling strategy	No sample size calculation was performed. We collected wild flies with the goal of estimating parasitoid wasp infection frequency and obtaining wasp larvae that could be reared to adulthood.
Data collection	Collection date, collection location, collection size, sex ratio of collection, host species, infected vs uninfected, date of wasp larval emergence, date of wasp adult emergence, date of wasp and host death, and infection burden on host. This data was collected by Logan D. Moore and Matthew J. Ballinger. Physical measurements of taxonomically relevant wasp characters were made by Scott R. Shaw.
Timing and spatial scale	Collection began 4/1/2023 and ended 2/10/2024. Samples were not organized into cohorts. Combining field collections in the southeastern USA with previously published SRA data sets, these data were collected at a global scale.
Data exclusions	<input checked="" type="checkbox"/> Not applicable
Reproducibility	<input checked="" type="checkbox"/> Not applicable
Randomization	<input checked="" type="checkbox"/> Not applicable
Blinding	<input checked="" type="checkbox"/> Not applicable

Did the study involve field work? Yes No

Field work, collection and transport

Field conditions	Mushroom, banana, tomato, and watermelon baits were placed in rural and suburban areas
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Location	33.456223, -88.787990; 33.461498, -88.787447; 33.4620642, -88.8054311; 33.956884, -83.435422; 33.230307, -87.528653; 38.776490, -92.235600; 35.497373, -82.524143; 35.741349, -78.834234; 33.456622, -88.841271; 33.494303, -88.753891; 33.491857, -88.753086; 33.424903, -88.759574; 33.34771, -88.733309; 33.538783, -88.633011
Access & import/export	All field samples were collected on public property or on private property with permission of the land owners.
Disturbance	Not applicable

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

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n/a	Involved in the study	n/a	Involved in the study
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Animals and other research organisms

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Laboratory animals	Drosophila affinis, Drosophila melanogaster, Drosophila acutilabella, Drosophila immigrans, and Syntretus perlmani
Wild animals	Vinegar flies (D. affinis, D. acutilabella, D. immigrans) were collected using mushroom, banana, tomato, and watermelon baits and were aspirated into polystyrene vials for transportation. Flies were euthanized at -20°C overnight or in 100% ethanol.
Reporting on sex	Not applicable
Field-collected samples	D. affinis, D. acutilabella, D. immigrans, and S. perlmani were maintained at 21°C with 16 hours light and 8 hours darkness in a Percival chamber. D. melanogaster was maintained at 25°C prior to infection and 21°C following infection.
Ethics oversight	No ethical approval or guidance is required for research involving non-endangered invertebrates

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable