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Research

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Untangling host specialization in a "double dark taxa" system

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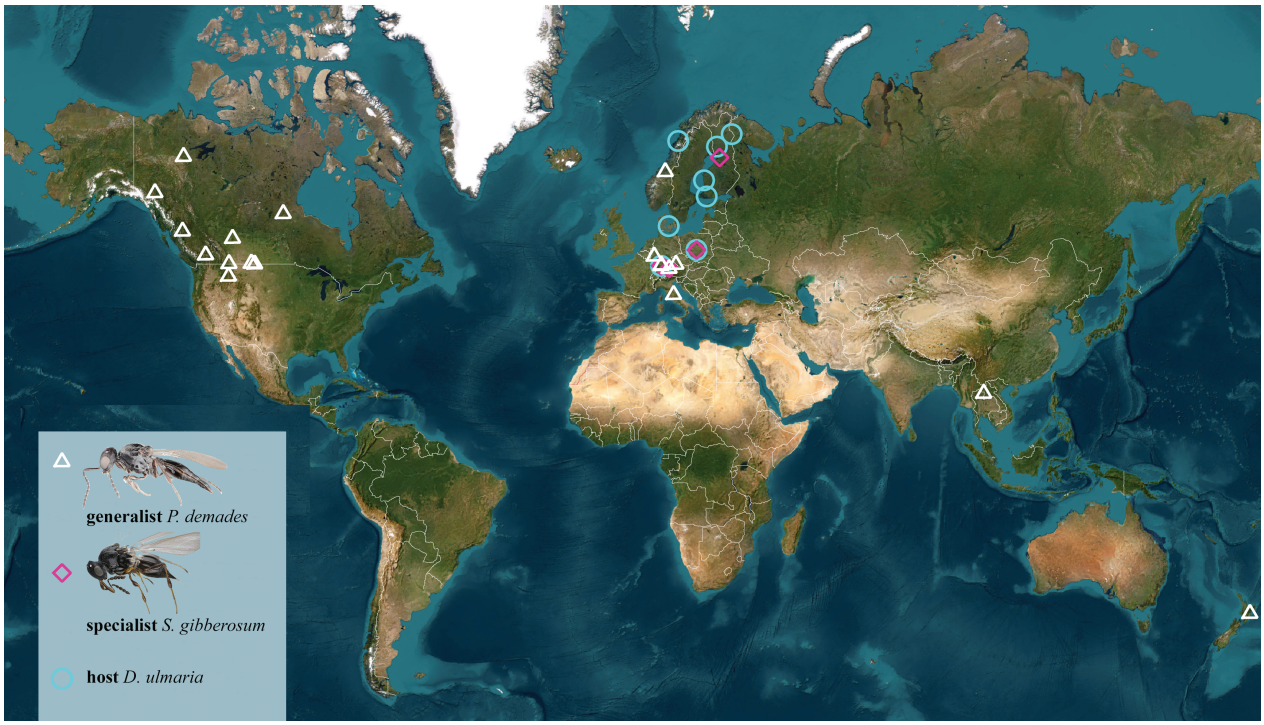
Platygastrine wasps (Hymenoptera: Platygastridae) are parasitoids of gall midges (Diptera: Cecidomyiidae). They and their hosts are exceptionally abundant and speciose, with great relevance to agriculture and biodiversity research. Both groups are also "dark taxa," whose species identification and ecological associations are obscured by a history of taxonomic confusion and neglect. Verified host records are few in number and limited in scope. In order to understand host specialization, more records are needed. However, rearing Cecidomyiidae is challenging, as many species require living host tissue to complete development. There is no universal rearing method for Cecidomyiidae and their parasitoids. The present work applies an exploratory approach to rearing gall midges, with the aim of obtaining accurate host associations and parasitoid identifications. We obtained 5 species of Platygastrinae from reared material, 3 of which are identified and diagnosed. *Platygaster demades* Walker (= *Platygaster marchali* Kieffer, **syn. nov.** = *Platygaster ornata* Kieffer, **syn. nov.**) is not host-specific, attacking Cecidomyiidae on Rosaceae worldwide, including *Filipendula ulmaria*. *Synopeas gibberosum* Buhl apparently specializes on *Dasineura ulmaria* (Bremi) on *F. ulmaria*. *Synopeas rhanis* (Walker) is known only from galls of *D. urticae* (Perris), but may attack other midge species on *Urtica dioica*. *Amblyaspis* sp. emerged from *Hartigiola annulipes* (Hartig) galls on *Fagus sylvatica*, and *Synopeas* sp. was associated with *Mycodiplosis* sp. on *Rubus* sp. Illustrations, DNA barcodes, and distributions are provided. We discuss challenges to understanding "double dark taxa" interactions, implications for biological control, and possible solutions for future research on these important but neglected systems.

Keywords: galls, host range, taxonomic impediment, Palearctic, parasitoid wasps.

Graphical Abstract



Introduction

Gall midges (Diptera: Cecidomyiidae) are evidently the most diverse group of flying insects, with worldwide estimates in excess of 1 million species (Hebert et al. 2016). It is therefore unsurprising that their parasitoids (Hymenoptera: Platygastridae) are similarly dominant. Srivathsan et al. (2023) ranked platygastrid abundance and diversity first among hymenopteran families, and fourth among all families of flying insects in global Malaise trap samples, although these rankings merit further investigation due to pervasive misidentifications in reference databases. Besides comprising a disproportionate share of terrestrial biodiversity, Cecidomyiidae and their parasitoids are economically important. Many herbivorous cecidomyiid species feed on food crops, forestry plants, or invasive weeds, while predatory species control aphids and other pests (Gagné and Jaschhof 2017). However, both Cecidomyiidae and Platygastrinae are "dark taxa," whose species identification and ecological associations are often obscured by a history of taxonomic confusion and neglect (Hausmann et al. 2020).

Although some Platygastridae exploit other hosts, the majority of species attack gall midges (Chen et al. 2021). The gall midge parasitoids, all classified in the subfamily Platygastrinae, include more than 1,800 described species (Awad et al. 2023b). The "superficial species impediment" (Meier et al. 2022) in Platygastrinae is exceptionally severe, and taxonomic progress is stymied by a few genera which are both remarkably species-rich and morphologically difficult to distinguish.

Chief among these is the genus *Platygaster* Latreille, 1809. With nearly 700 described species, it is the largest genus in the subfamily and even in the whole superfamily Platygastroidea (MBD 2023). *Platygaster* has no morphological synapomorphies and is always placed at the end of keys, being defined by a lack of distinguishing features. It also includes many species of interest to agriculture (Vlug 1995, Sampson et al. 2006, He and Wang 2015) and to biological invasions (Moore et al. 2023).

The genus *Synopeas* Förster, 1856, is the second-largest in Platygastrinae with nearly 400 described species (Awad et al. 2023b). *Synopeas* specimens are easily recognized by a fusion of the first and second metasomal tergites and the presence of a ventral pronotal pit (Fig. 4). However, as is the case with most Platygastrinae, species-level identification of *Synopeas* is difficult to impossible based on morphology alone. Recent studies integrating molecular, morphological, and ecological data have provided diagnostic improvements (Awad et al. 2021), but much work remains to resolve the taxonomic issues in the group.

Other large platygastrine genera include *Leptacis* Förster, 1856 (nearly 300 described species); *Inostemma* Haliday, 1833 (109 species); and *Amblyaspis* Förster, 1856 (88 species) (MBD 2023). *Inostemma* was redescribed, diagnosed, and keyed by Masner and Huggert (1989), while *Leptacis* and *Amblyaspis* were diagnosed by Awad et al. (2023a) and keyed by Awad et al. (2023b). Serious revisionary work has never been attempted for any of these genera and all remain in a state of taxonomic chaos, especially in the Palearctic region. Diagnosable species groups are needed to break up these genera into manageable parts.

While studying parasitoids of the soybean gall midge *Resseliella maxima* Gagné, Melotto et al. (2023) defined a distinctive species group of *Synopeas*. The *Synopeas rhanis* group is characterized by the elevation of the mesoscutum relative to the mesoscutellum in lateral view (Fig. 4a). This unique characteristic is visible even in poor illustrations and noted even in vague descriptions, allowing for its detection among the superficial work that constitutes the majority of previous *Synopeas* research.

Melotto et al. (2023) listed 26 described species in the *S. rhanis* group, including representatives from every continent except Antarctica. Several species were described from reared material, meaning that their ecological associations are well-supported. The recently discovered *Synopeas maximum* Awad and Talamas, 2023 is a parasitoid of the soybean gall midge in Minnesota and nearby

states. Both *S. maximum* and its host likely originated on a native North American legume before moving to soybean (Melotto et al. 2023). *Synopeas cynipsiphilum* (Ashmead, 1887) was reared from an oak gall in Florida. In Italy, *S. oleae* Buhl and Viggiani, 2008 was reared from *Lasioptera berlesiana* Paoli, which lives in the tunnels of the olive fruit fly, *Bactrocera oleae* (Rossi). The almond bud gall midge, *Odinadiplosis amygdali* (Anagnostopoulos), is the host of *Synopeas talhouki* Vlug, 1976, in Lebanon.

There are published ecological associations for other species in the *S. rhanis* group. However, due to the difficulty of identification, the accuracy of these historical European records is questionable. Stefani (1900) listed *S. prospectum* Förster, 1861, originally described from the Swiss Alps, as a parasitoid of *Asphondylia punica* Marchal, 1897, on *Atriplex halimus* in Sicily. The namesake of the species group, *S. rhanis* (Walker, 1835) has been associated with *Dasineura ulmaria* (Bremi) on meadowsweet, *Dasineura urticae* (Perris) on nettle, and even the aphid predator *Aphidoletes aphidomyza* (Rondani) (Vlug 1995).

Host associations in the *rhanis* group, as well as the rest of *Synopeas* and Platygastrinae in general, remain poorly understood. Although there are a handful of published host records, these tend to focus on agricultural crops and most do not provide information on host specialization or generalization. In order to understand the biodiversity and ecology of this important group, more host records are needed, and rearing parasitoids directly from hosts remains one of the best methods to obtain accurate host data (eg Baine et al. 2023, Bruun et al. 2024). However, rearing gall midges can be challenging. As many species require living host tissue to complete development, timing of collection and maintenance of proper environmental conditions are critical to success. Furthermore, in temperate zones, many species require temperature changes to simulate overwintering. There is no universal rearing method for Cecidomyiidae and their parasitoids, although it is possible to develop reliable systems for individual species.

Additionally, the taxonomic confusion surrounding the identification of Platygastrinae means that records in the literature, even recent literature, may not be entirely reliable. Multiple lines of evidence are required to accurately identify Platygastrinae to species, including morphological comparison to type material as well as any available ecological, geographic, and molecular data. Unfortunately, the integrative approach has only recently been adopted for Platygastrinae, and superficial descriptions and unverifiable identifications continue to appear in the literature.

To obtain accurate host associations and parasitoid identifications, the present work applies integrative taxonomic methods to platygastrine specimens reared from cecidomyiid larvae in southwestern Germany. Three parasitoid species are identified, illustrated, and diagnosed using modern techniques, with additional observations on the local gall midge fauna and their parasitoids. These contributions represent an updated standard for parasitoid identifications and propose further avenues for the advancement of ecological and taxonomic understanding of "double dark taxa" systems.

Materials and Methods

Specimen Collection

Gall collection took place between July and mid-October 2021. Collection sites were in the vicinity of Stuttgart, southwest Germany, in woodlands and meadows near the towns (postal codes in brackets) of Plieningen (70599), Musberg (70771), Stetten (70771), and Ehningen (71139). Sites were visited once every 2 wks from July to September. In October, only sites with high numbers of galls were visited.

Gall midge identification was based on host plant identity (Schauer and Caspari 1990, Spohn et al. 2015) and gall morphology (Bellmann et al. 2018, Ellis 2020). Cecidomyiid larvae were collected from 9 plant species representing 8 families: *Achillea millefolium* L. (Asteraceae); *Dactylis glomerata* L. (Poaceae); *Daucus carota* L. (Apiaceae); *Fagus sylvatica* L. (Fagaceae); *Filipendula ulmaria* (L.) Maxim., *Rubus* L. sp. (Rosaceae); *Sambucus nigra* L. (Adoxaceae); *Tilia cordata* Mill. (Malvaceae); and *Urtica dioica* L. (Urticaceae). Eleven putative species of Cecidomyiidae were collected, 1 per host plant species except for *F. sylvatica* and *F. ulmaria*, which each hosted 2 gall morphotypes (Table 1).

Insect Rearing

Due to the high variability of midge galls and lack of established rearing protocols, we attempted a variety of methods to obtain gall midges and their parasitoids. All galls were kept at room temperature with a natural daylight cycle.

Galls were kept in Falcon tubes, ventilated plastic rearing boxes, or Petri dishes. We tried adding locally collected garden soil, baked at 50°C for 24 h, but this did not yield good results. Sterile cotton pads (diameter 6 cm, thickness 0.5 cm) in Petri dishes, dampened with tap water, provided a substrate for whole galls or for individual larvae. To obtain larvae without plant material, leaves were dissected with a razor blade and forceps (for *U. dioica* and *F. sylvatica*) or a whole branch of the plant was put in a vase inside a rearing box until the larvae absconded (for *F. ulmaria*).

Two strategies were successful. Keeping whole galls in a container with no water or soil was better suited to harder galls (*D. carota*, *F. sylvatica*, and *T. cordata*). Keeping individual larvae on damp cotton pads worked best for softer plant tissue galls (*A. millefolium*, *F. ulmaria*, and *U. dioica*). The latter strategy also worked for the midges from *Rubus*, which did not emerge from galls but were apparently free-living, likely feeding upon a rust fungus. Keeping soft plant tissue galls intact was not effective, as the galls either dried out or began to grow mold.

Reared midges and wasps were collected soon after eclosion and transferred to 100% ethanol for later analyses. Platygastrinae of each host were keyed morphologically to genus level (Awad et al. 2023b). Species were identified by comparison to type material (Talamas 2022, 2023, 2024). Reared specimens are deposited in the State Museum of Natural History Stuttgart (SMNS) and detailed specimen data are provided in Supplementary File 1.

DNA Analysis

DNA extraction was performed with the Qiagen DNeasy Blood & Tissue Extraction Kit following an updated protocol based on Cruaud et al. (2019). We extracted DNA from all platygastrine specimens and from the putative hosts of identifiable platygastrine species. COI barcodes were obtained through PCR using the primer combination COI_PF2/HCO2198 (Folmer et al. 1994, Kaartinen et al. 2010) for wasps and LCO1495/HCO2198 (Folmer et al 1994) for Cecidomyiidae. PCR was performed in a 25-µl reaction with 10-µl DNA template; sequences were obtained through bidirectional Sanger sequencing. Assembling and sequence proofing was done in Geneious Prime. Sequences are available on GenBank (PP824835–41, PP824781–802).

DNA barcodes were obtained from 22 platygastrine specimens and 7 cecidomyiid specimens reared from *F. sylvatica*, *F. ulmaria*,

Table 1. Gall collection and insect emergence

Plant family	Plant species	Putative midge species	Collection site(s)	Collection date (successfully reared)	Time to adult eclosion	Substrate	Adult midges	Platygastrinae	Other parasitoids
Asteraceae	*Achillea millefolium* L.	*Rhopalomyia millefolii* (Löw, 1850)	Stetten	11 Sep 2021	7 to 8 d	Cotton	2	—	—
Poaceae	*Dactylis glomerata* L.	*Mayetiola dactylidis* Kieffer, 1896	Musberg, Stetten	—	—	—	—	—	—
Apiaceae	*Daucus carota* L.	*Kiefferia pericarpiicola* (Bremi, 1847)	Ehningen	6 to 20 Sep 2021	2 to 6 d	Dry	—	—	37 Eulophidae 1 Torymidae
Fagaceae	*Fagus sylvatica* L.	*Hartigiola annulipes* (Hartig, 1839)	Ehningen, Musberg	27 Sep 2021	4 wk	Dry	—	3 *Amblyaspis*	—
		Mikiola fagi (Hartig, 1839)					—	—	1 Eulophidae
Rosaceae	*Filipendula ulmaria* (L.) Maxim.	*Dasineura ulmaria* (Bremi, 1847)	Musberg, Plieningen, Stetten	14 Sep to 10 Oct 2021	5 d to 6 wk	Cotton, soil	195	23 *Synopeas gibberosum*	1 Eulophidae
		Dasineura pustulans (Rübsaamen, 1889)			4 wk		—	1 *Platygaster demades*	—
	Rubus L. sp.	*Mycodiplosis* sp. (Winnertz, 1853)	Musberg		2 to 4 wk	Cotton	11	1 *Synopeas*	
Adoxaceae	*Sambucus nigra* L.	*Placochela nigripes* (Löw, 1877)	Musberg	—	—	—	—	—	—
Malvaceae	*Tilia cordata* Mill.	*Didymomyia tiliacea* (Bremi, 1847)	Ehningen, Musberg	13 Sep 2021	15 d	Dry	—	—	1 Eulophidae
Urticaceae	*Urtica dioica* L.	*Dasineura urticae* (Perris, 1840)	Ehningen, Musberg, Plieningen	13 to 27 Sep 2021	3 to 4 wk	Cotton	5	1 *Synopeas rhanis*	8 Torymidae

Rubus sp., and *U. dioica*. Additional sequences were obtained from the German Barcode of Life project, BOLD Systems, and GenBank.

The GBIF Sequence ID (GBIF 2020), BLAST on GenBank (Camacho et al 2009, Benson et al 2012), and BOLD BLAST (Ratnasingham and Hebert 2007) tools were employed for recovery of sequences with >95% identity with our data. These were pulled and included in the species delimitation analyses, specimen metadata was also recovered and included in the distribution (BOLD:ACC4428, BOLD:ADV9551, BOLD:ADI4543, BOLD:ADI5201, BOLD:AAG7995, BOLD:AEB6878, BOLD:ADN1775, BOLD:ADZ7300, BOLD:ADV6097). Species delimitation was performed with ASAP (Puillandre et al. 2021) and Species Identifier (Meier et al. 2006).

The distribution map was created with QGIS using the ArcGIS template (QGIS 2024).

Morphology

Morphological terms follow Hymenoptera Anatomy Ontology (Yoder et al. 2010). Additional species-level characters follow Awad et al. (2021) and Melotto et al. (2023). Microphotography was performed using a focus-stacking system with 10× and 20× Mitutoyo objective lenses. Image stacks were rendered in Helicon Focus. Post-processing (addition of scale bars, removal of stacking artifacts, and color balance adjustment) was conducted in Adobe Photoshop. Full morphological treatments are provided in Supplementary File 1.

Results

Rearing

Seven plant species produced adult midges, adult parasitoids, or both (Table 1). No adult insects were obtained from *D. glomerata* or *S. nigra*. Dry containers led to successful rearings from *D. carota, F. sylvatica,* and *T. cordata*. Damp cotton pads led to successful rearings from the other plant species. The only successful rearings on soil were from *F. ulmaria*.

The only plants which yielded both adult Cecidomyiidae and Platygastrinae were *F. ulmaria*, *Rubus* sp., and *U. dioica* (Fig. 1). *Achillea millefolium* yielded only Cecidomyiidae, while *F. sylvatica* produced only Platygastrinae. Other parasitoids belonging to the Chalcidoidea emerged from *D. carota, F. sylvatica, F. ulmaria,* and *T. cordata*.

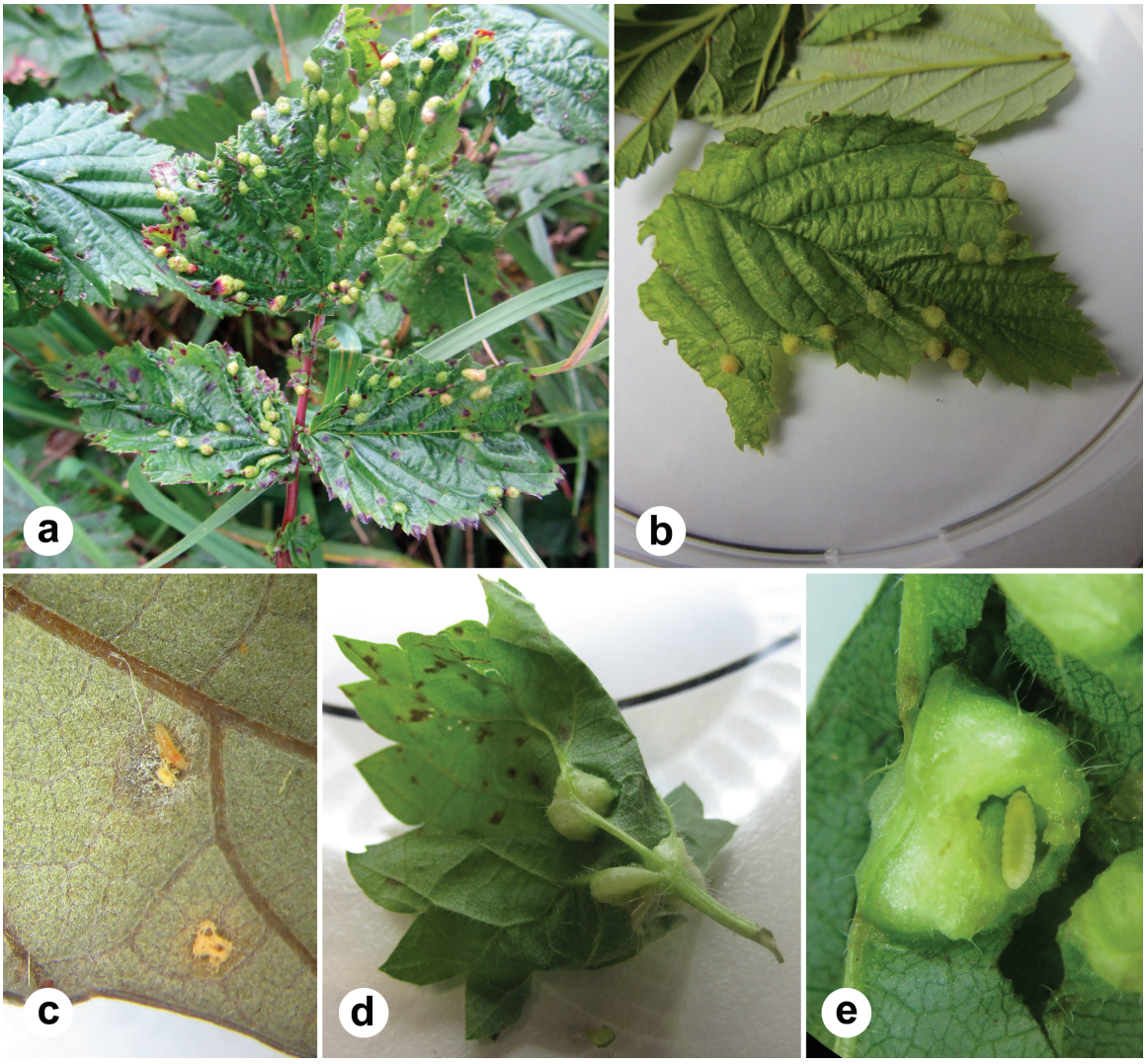


Fig. 1. Galls and larvae producing both Platygastrinae and Cecidomyiidae. (a and b) *Dasineura ulmaria* galls on *Filipendula ulmaria*. (c) *Mycodiplosis* sp. on leaf fungus of *Rubus* sp. (d and e) *Dasineura urticae* galls on *Urtica dioica*.

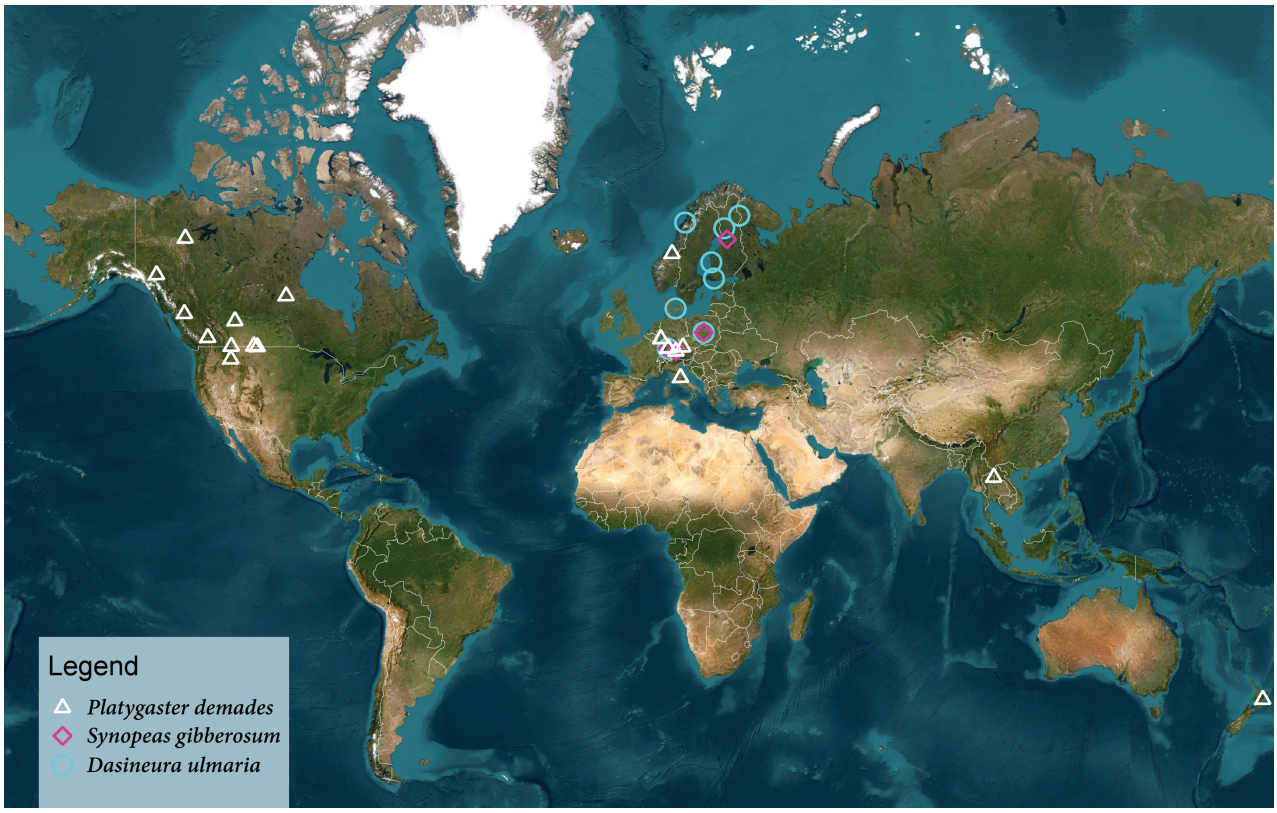


Fig. 2. Map of Cecidomyiidae and Platygastrinae species reared from *Filipendula ulmaria*.

Most of the Platygastrinae belonged to the genus *Synopeas*. *Filipendula ulmaria* and *U. dioica* produced members of the *S. rhanis* group. The only *Synopeas* not in the *rhanis* group was associated with fungus-feeding cecidomyiid larvae on *Rubus* sp.

DNA Barcoding

COI barcodes indicate the presence of 5 species of Platygastrinae: 1 *Amblyaspis*, 1 *Platygaster*, and 3 *Synopeas*. One platygastrine species emerged from each plant species except for *F. ulmaria*, which yielded both *Platygaster* and *Synopeas* (Fig. 2). All species matched other Palearctic sequences in BOLD/Genbank. One *Synopeas* species also retrieved matches from Canada, and the *Platygaster* species also matched sequences identified as *P. demades* from North America and New Zealand.

Although the galls on *U. dioica* were apparently induced by *D. urticae*, sequences from the reared Cecidomyiidae did not match reliably identified records of *D. urticae* in BOLD, indicating the presence of an inquiline, predator, or fungivore. The *Mycodiplosis* on *Rubus* matched other sequences identified as *Mycodiplosis* from Germany and China. The species of Cecidomyiidae which emerged from *F. ulmaria* matched other sequences identified as the gall inducer *D. ulmaria* in BOLD (Fig. 2).

Taxonomy

Amblyaspis sp.
(Fig. 3)

Host associations. *Hartigiola annulipes* galls on *F. sylvatica*.

Geographic distribution. Palearctic.

Remarks. The genus *Amblyaspis* is in a state of taxonomic disarray, particularly in the Palearctic. Identification of these specimens is further complicated by the fact that they are all male, while female specimens are generally the standard for description in Platygastroidea. The lack of females could possibly indicate a suboptimal host (Godfray 1994), but it is not possible to draw confident conclusions from so few specimens. The male antenna is somewhat remarkable, with a highly enlarged sex segment (Fig. 3a) and deeply excavated scape (Fig. 3b).

Platygaster demades Walker, 1835
(Fig. 4)

> *Platygaster marchali* Kieffer, 1906; **new synonym**
> *Platygaster ornata* Kieffer, 1906; **new synonym**

Diagnosis (female). *Platygaster demades* may be recognized by the following combination of characters: clava nonabrupt, narrow; frons with diagonal striae/rugulae (Fig. 4a); occiput transversely striate; lateral pronotum almost entirely sculptured; mesopleuron smooth; notauli present; mesoscutellum rounded; T3 and T4 transverse; T5 with longitudinally rugulose sculpture (Fig. 4c and d), slightly wider than long to longer than wide.

Host associations. *Dasineura ulmaria* and *D. pustulans* galls on *F. ulmaria*; *Dasineura mali* on *Malus* spp.; *Dasineura pyri* on *Pyrus* spp.; possibly others (see remarks).

Geographic distribution. Palearctic; Canada; United States; New Zealand.

Remarks. *Platygaster demades* is a biological control agent. It was deliberately introduced to New Zealand to control the pear

Fig. 3. Male *Amblyaspis* sp. (SMNS_Hym_Pla_001714) reared from *Hartigiola annulipes* galls on *Fagus sylvatica*. (a) Lateral habitus, with male sex segment indicated. (b) Dorsal habitus. Scale bar = 0.2 mm.

leaf-curling midge *D. pyri* and is also a major parasitoid of the apple leaf-curling midge *D. mali* in New Zealand and Canada (Miller 1926, He and Wang 2015, Cossentine et al. 2020). Records with molecular data are all associated with Rosaceae (*Filipendula*, *Malus*, and *Pyrus*). Shaw (1969) provided a record from *Wachtliella ericina* (Löw) on *Erica carnea* (Ericaceae). Tondini et al. (2023) identified *P. demades* from *D. oleae* (Angelini) on olive (Oleaceae). Bruun et al. (2024) reported it from galls on *Urtica* (Urticaceae) and *Quercus* (Fagaceae) as well as *Rubus* (Rosaceae). It is entirely possible that some or all of these records are accurate. However, the parasitoid identifications should be validated by examination of voucher specimens.

The type specimens of *Platygaster ornata* and *P. marchali* are lost (Notton 2010), but there is no evidence that these differ from *P. demades* or from one another. Both species were reared from *F. ulmaria* in eastern France, very close to western Germany, and the original descriptions are indistinguishable from the observed morphology of *P. demades*. Marchal (1906) mentions minor differences in development, which are consistent with experimentally confirmed life history variation (He et al. 2010, He and Wang 2015). The

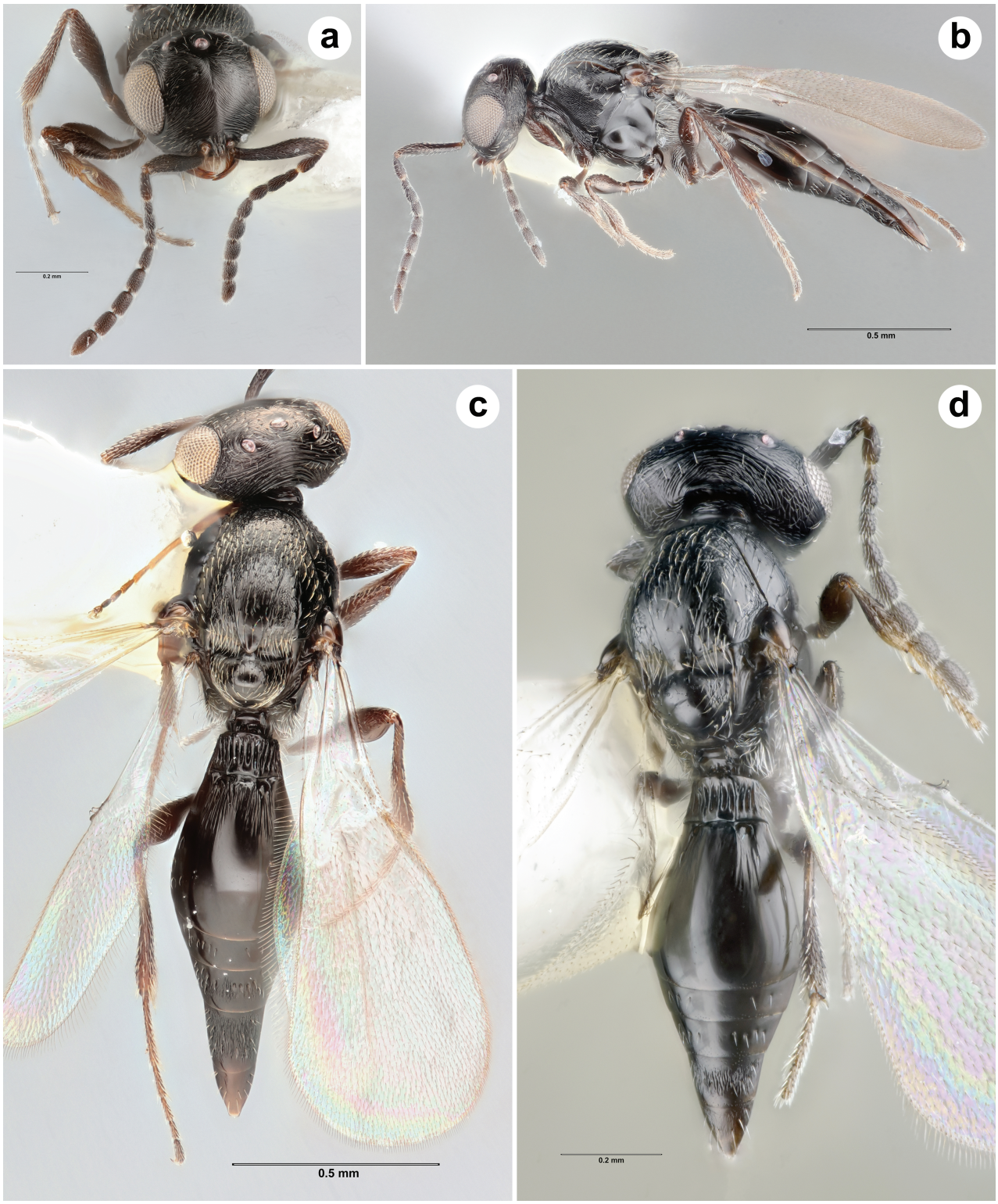


Fig. 4. *Platygaster demades* females. (a–c). SMNS_Hym_Pla_000525. (d) SMNS_Hym_Pla_001707, reared from Cecidomyiidae on *Filipendula ulmaria*. Scale bar = 0.2 mm (a, d); 0.5 mm (b, c).

species concepts of Buhl (2006) are not based on type material and their scientific foundation is uncertain. Our data demonstrate intraspecific variation in characters traditionally used to separate species, such as the sculpture of metasomal tergites 2 and 4 and the shape of metasomal tergite 5 (Fig. 4c and d). In general, it seems that larger specimens (Fig. 4c) have more extensive sculpturing and a more elongate metasomal tergite 5 than do smaller specimens (Fig. 4d).

Synopeas gibberosum Buhl, 1997
(Fig. 5)

Diagnosis. Synopeas gibberosum can be separated from other species of *Synopeas* by the following combination of characters: scutoscutellar sulcus deep, causing mesoscutum to be elevated relative to mesoscutellum; hyperoccipital carina present between lateral

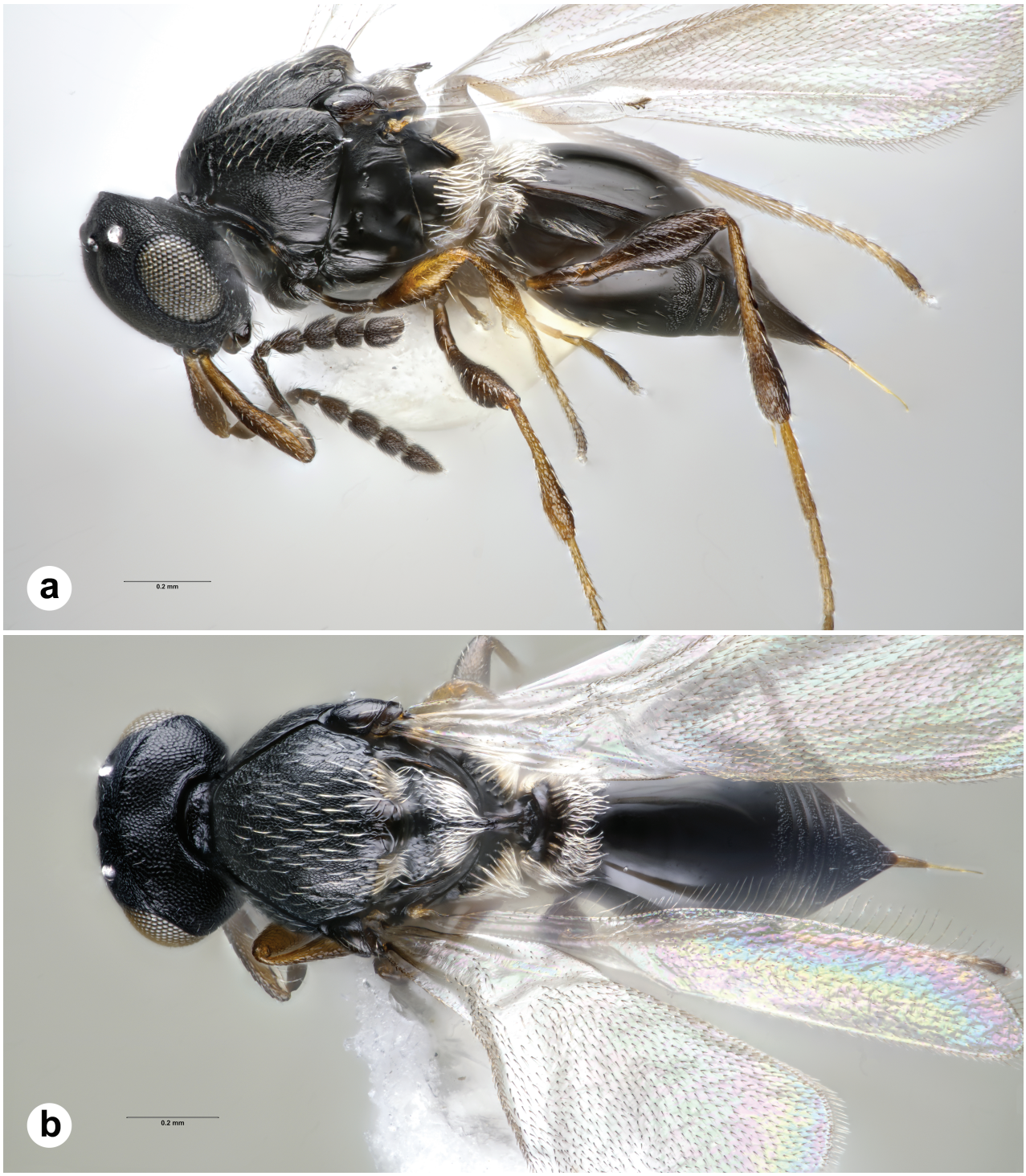


Fig. 5. *Synopeas gibberosum* female (SMNS_Hym_Pla_001695) reared from *Dasineura ulmaria* galls on *Filipendula ulmaria*. (a) Lateral habitus. (b) Dorsal habitus. Scale bar = 0.2 mm.

ocelli, sharp, fine, and laterally weakened; mesoscutellar spine well-developed, originating from the dorsal apex of the mesoscutellum and pointing posteriorly; metasomal sternite 2 in both sexes with microsculpture at posterior margin and in posterolateral corners; female metasomal sternite 6 and tergite 6 entirely sculptured, triangular, equilateral.

Host associations. *Dasineura ulmaria* galls on *F. ulmaria*.

Geographic distribution. Europe.

Remarks. Our host association data are consistent with those of Bruun et al. (2024) and suggest that the specimens identified as *S. rhanis* by Marchal (1906) likely belonged instead to *S. gibberosum*.

Synopeas rhanis (Walker, 1835)
(Fig. 6)

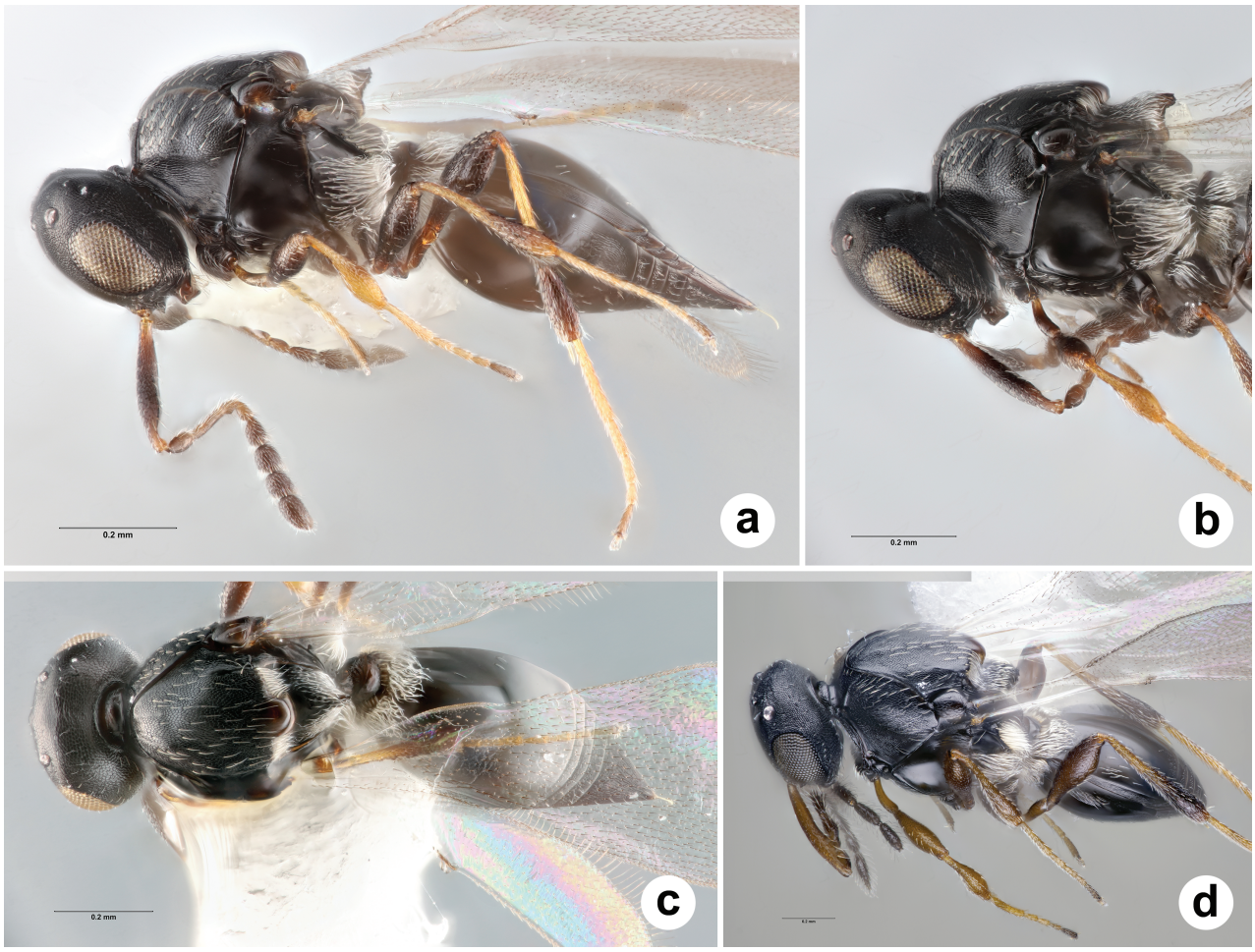


Fig. 6. *Synopeas rhanis*. (a) Female, lateral habitus, SMNS_Hym_Pla_000785. (b) Female, lateral habitus, SMNS_Hym_Pla_000780. (c) Dorsal habitus, SMNS_Hym_Pla_000785. (d) Male, SMNS_Hym_Pla_001692, reared from *Dasineura urticae* galls on *Urtica dioica*. Scale bar = 0.2 mm.

Diagnosis. *Synopeas rhanis* can be separated from other species of *Synopeas* by the following combination of characters: scutoscutellar sulcus deep, causing mesoscutum to be elevated relative to mesoscutellum; hyperoccipital carina absent; mesoscutellar spine short to extremely reduced, originating from the dorsal apex of the mesoscutellum; metasomal sternite 2 in both sexes with microsculpture at posterior margin and in posterolateral corners; female metasomal sternite 6 and tergite 6 entirely sculptured, triangular, equilateral to slightly longer than wide.

Host associations. *Dasineura urticae* galls on *U. dioica*.

Geographic distribution. Europe.

Remarks. Historical confusion has likely led to numerous misdiagnoses of species in the *rhanis* group, which are morphologically quite similar, especially when examined at low magnification. The mesoscutellar spine exhibits some intraspecific variation (Fig. 6a, b, and d), further complicating diagnosis. Vlug (1995) records 3 different gall midge hosts from early- to mid-20th-century literature, including *D. urticae*, *D. ulmaria*, and the aphid predator *A. aphidomyza*. Our observations and those of Vlug (1985) and Bruun et al. (2024) only support an association with galls of *D. urticae*, although hosts may include other cecidomyiid species on *U. dioica*.

Synopeas sp.
(Fig. 7)

Host associations. Collected with *Mycodiplosis* sp., on rust fungus on *Rubus* sp.

Geographic distribution. Germany, Canada.

Remarks. This species exemplifies the "double dark taxa" problem. Although it is somewhat morphologically distinctive (Fig. 7), we cannot be certain of its identity or whether it has already been described. Similarly, the host could not be identified confidently to species. *Mycodiplosis* is a cosmopolitan genus of fungivores, which has not been revised since the dissertation of Holz (1970). Both *Synopeas* and *Mycodiplosis* are in dire need of integrative taxonomic revision for the Holarctic region, but progress requires further development of specialist expertise, institutional support, and research funding.

Discussion

Accurate diagnostics are fundamental to understanding patterns of host specialization (Rosen and DeBach 1973). In order to be useful, host and parasitoid species concepts must be universal, that is, shared by all researchers in all fields, and they must be consistent throughout time. Taxonomic confusion obscures a great deal of otherwise valuable host association data in literature and museum collections, especially in the Palearctic. Although resolving this confusion is particularly complex in dark taxa, it is absolutely critical to ensure the universality and continuity of species concepts.

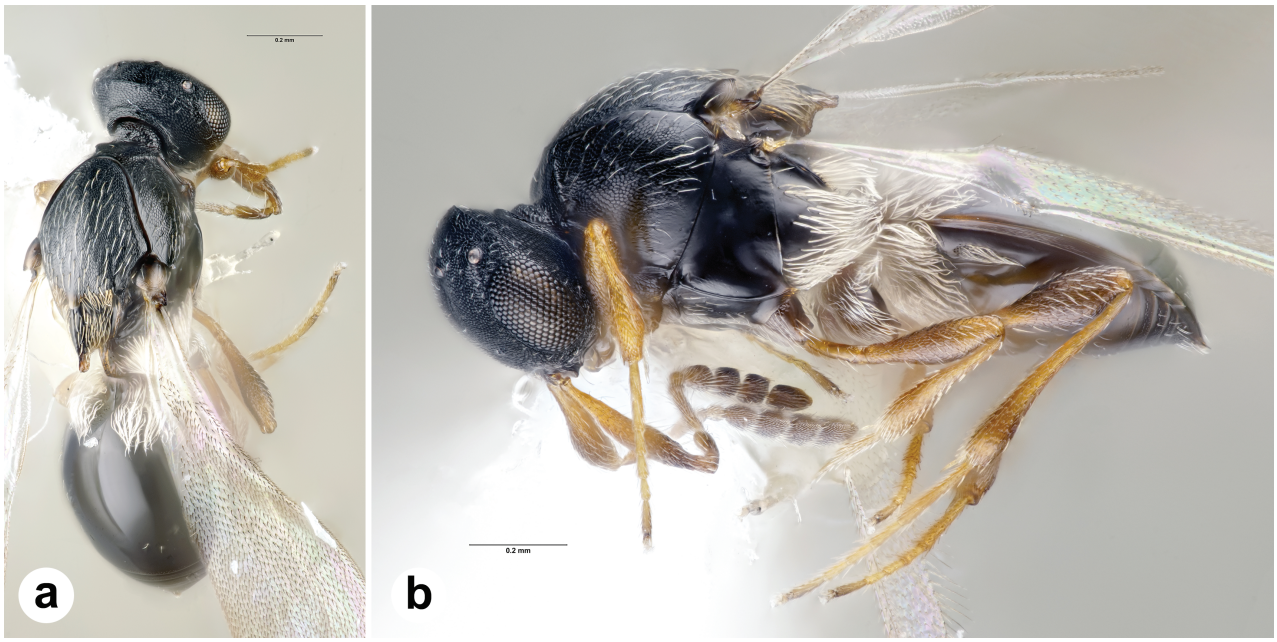


Fig. 7. Female *Synopeas* sp. (SMNS_Hym_Pla_001684) associated with *Mycodiplosis* sp. on *Rubus* sp. Scale bar = 0.2 mm.

Once the organisms have been identified, many challenges remain to understanding host specialization. It is relatively straightforward to show that a parasitoid species is a generalist, if it has been reared from multiple host species. However, it is much more difficult to establish that a given parasitoid species is a specialist. Just because a parasitoid has only ever been found on a given host does not necessarily mean that it never attacks any other species. Biological control has some well-established protocols for host range testing (Sands and Van Driesche 2003), which generally require significant financial investment and are thus limited to natural enemies of the most economically important pest species. These protocols require reliable rearing methods to maintain captive colonies of target and nontarget organisms.

The rearing methods presented here are preliminary and exploratory. Although the use of cotton pads to rear absconded cecidomyiid larvae is novel and yielded good results for galls on *F. ulmaria*, we suspect that soil-based methods may ultimately prove more effective and less labor-intensive for a wider range of taxa. Our choice of local garden topsoil and improvised quarantine-era sterilization technique were unsuccessful, but that does not mean that soil itself is a poor substrate. Other researchers have achieved reliable success with soil- and peat-based methods (Gagné and Moser 2013; Hans Henrik Bruun, pers. comm.; Charley Eiseman, pers. comm.).

Gall phenology plays a major role in the development of successful rearing protocols. Ideally, galls should be collected as close as possible to the date of pupation, to maximize the amount of healthy living plant tissue consumed by the larvae. We began collecting galls in July (summer), but we only obtained adult insects from galls collected in September and October (autumn). It should be noted that the eclosion dates in our study may not reflect natural phenology. It is possible that the shock of gall removal led to premature development for both midges and wasps, and we did not simulate overwintering, which probably influences the natural life cycle. However, we have captured adults of *P. demades*, *S. rhanis*, and the unidentified *Synopeas* species from the wild in October, so for those species at least, the laboratory emergence dates (Supplementary File 1) are plausibly close to natural.

Future rearing projects can offer valuable insights in 2 ways: the broad approach, surveying many species in a given region (eg Bruun et al. 2024); or the deep approach, carefully observing a single gall species and all of its associates throughout the entire life cycle (eg Baine et al. 2023). Whether the project takes a broad or a deep approach, future publications should clearly justify the basis of any parasitoid identifications, with underlying data accessible to other researchers for independent verification. Ideally, identifications should be accompanied by high-quality, full-body photographs and DNA barcodes. This combination of morphological, ecological, and molecular data will help to avoid misidentifications. Misidentifications can lead to false host associations, which only serve to exacerbate the "double dark taxa" problem.

Beyond rearing, molecular methods can potentially reveal host associations, especially as technology continues to develop and reference databases become more complete. It may be possible to detect parasitoid DNA from gall midge host remains, as has been done with hemipteran eggs (Gariepy et al. 2014, Bohacsova et al. 2016). Advances in sensitivity and filtering techniques may even enable the reliable detection of host DNA from adult parasitoids. In tightly coevolved systems, shared evolutionary history could possibly be revealed by comparative genomics. For example, lepidopteran genomes often bear evidence of polydnaviruses, which are used by ichneumonoid parasitoid wasps to suppress host immune response (Heisserer et al. 2023). The recent discovery of symbiotic viruses in some Platygastrinae (Guinet et al. 2024) could enable similar studies in Cecidomyiidae.

Within these double dark taxa systems, many ecological factors remain to be explored. Temperature could affect parasitoid phenology and performance with respect to different host species (Thierry et al. 2021, Pardikes et al. 2022). The effects of multispecies interactions are also not well understood. Within a single species of Cecidomyiidae, multiple species of parasitoids can occur, raising questions of competition and niche partitioning. Additionally, our galls on *U. dioica* yielded different cecidomyiid sequences than similar galls from Norway and Germany, which probably indicate the presence of an inquiline, predator, or fungivore. Whether

"specialist" parasitoids can attack multiple cecidomyiid host species on the same plant, and how this may influence host range expansion, have yet to be determined.

Our study indicates that the classical biological control agent *P. demades* is not host-specific. Multiple lines of evidence support its ability to parasitize multiple species of *Dasineura* on rosaceous plants, with additional literature suggesting that the host range may be even broader. Although much work has been done on the dynamics of *P. demades* in agricultural systems, nontarget effects in its introduced ranges are unknown. We were unable to find records of prerelease host range testing prior to its introduction to New Zealand in 1925 (Miller 1926) and to Canada in 1981 under the name *P. marchali* (Cossentine et al. 2020). The latter synonymy suggests that the introduction of *P. demades* to Canada was intentional and not accidental as previously thought (Mason et al. 2017). It would be interesting to examine the host associations of adventive *P. demades* in natural ecosystems. We would expect greater nontarget effects in Canada, as the native flora and fauna are more similar to those found in the Palearctic.

Untangling host specialization is of immediate relevance to biological control, but developing our knowledge of parasitoid ecological associations has great potential to inform other areas of study. For example, Forbes et al. (2018) demonstrated that the world's total hymenopteran diversity can theoretically be calculated from known parasitoid-to-host ratios. However, the current estimate of 883,000 to 1.15 million species is based on only 4 case studies, and further data are needed to improve accuracy and precision. We also have very little knowledge of how coevolution works in parasitoid–host systems. Most coevolutionary research involves herbivore–plant interactions, which may operate in different ways than parasitoid–host relationships (Medina et al. 2022). Finally, host specificity information is critical to insect conservation. Many natural enemies are likely to be in danger of extinction (Shaw and Hochberg 2001), but understanding their roles in the ecosystem allows us to determine which parasitoid species are most at risk (Abe et al. 2023), as well as which may threaten native species through anthropogenic introduction.

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Author contributions

Jessica Awad (Conceptualization [lead], Data curation [equal], Formal analysis [equal], Funding acquisition [supporting], Investigation [equal], Methodology [equal], Project administration [supporting], Supervision [equal], Validation [equal], Visualization [equal], Writing—original draft [lead], Writing—review & editing [equal]), Ronja Reinisch (Data curation [equal], Investigation [equal], Methodology [equal], Visualization [equal], Writing—original draft [supporting]), Marina Moser (Validation [equal], Visualization [equal], Writing—review & editing [equal]), Cristina Vasilita (Data curation [equal], Formal analysis [equal], Investigation [equal], Project administration [lead], Visualization [equal], Writing—original draft [supporting], Writing—review & editing [equal]), and Lars Krogmann (Funding acquisition [lead], Supervision [equal], Writing—review & editing [equal])

Supplementary material

Supplementary material is available at *Annals of the Entomological Society of America* online.

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