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A new species of gall wasp *Diastrophus* Hartig, 1840 (Hymenoptera: Cynipidae) from Sichuan, China

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Abstract

We describe a new species of gall wasp associated with *Rubus lambertianus* Ser. in Sichuan, China: *Diastrophus emeiensis* Hu, Nieves-Aldrey, Zhang & Fang **sp. nov.** (Cynipidae: Diastrophini) based on morphological and molecular data. This is the first *Diastrophus* Hartig, 1840 species recorded from mainland China, and is recovered as the sister group to the rest of *Diastrophus* based on our phylogenetic analysis. The diagnosis, distribution, and biology of *D. emeiensis* is provided, along with a brief discussion of the evolutionary history of *Diastrophus*.

Key words: *Rubus*, Rosaceae, Diastrophini, Emei, bramble

Introduction

Gall wasps in the genus *Diastrophus* Hartig, 1840 (Hymenoptera: Cynipidae, Diastrophini) includes 22 known species, most of which are found in North America, but have also been recorded from Brazil, Colombia and East Asia (Davis *et al.* 2024; Nieves-Aldrey *et al.* 2013; Oliveira & Melo 2025; Ronquist *et al.* 2015; Schick *et al.* 2003; Wachi *et al.* 2013). *Diastrophus* species have been recorded as gall inducers on Rosaceae plants in the genera *Rubus* L., *Fragaria* L., and *Potentilla* L. A single host record for the species *Diastrophus smilacis* Ashmead, 1896 in the US was erroneously recorded on *Smilax* L. (Smilacaceae), but has since been corrected as the true gall inducer is *Aprostocetus smilax* Gates & Zhang, 2020 (Chalcidoidea: Eulophidae, Tetrastichinae) (Gates *et al.*

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2020). A second doubtful species, *Diastrophus hieraci* Melika & Klymenko, 2005, was more recently recorded as a gall inducer on *Hieracium* spp. (Asteraceae) from Ukraine, but this species seems to be both a host plant and cynipid misidentification with *Diastrophus mayri* Reinhard, 1876 on *Potentilla* (Nieves-Aldrey unpublished data). No *Diastrophus* species have previously been reported from mainland China, but three species have been found in neighboring regions: *D. japonicus* Wachi, Ide & Abe, 2013, *D. renai* Davis & Nastasi, 2024 and *D. wushei* Davis & Nastasi 2024. Only the biology of *D. japonicus* is known, which induces swollen stem galls on *Rubus palmatus* Thunb. (Wachi *et al.* 2013).

As part of our ongoing survey of Cynipoidea diversity in Sichuan, China (Fang *et al*. 2020a; 2020b; 2024; 2025), we discovered a new species of *Diastrophus* from *Rubus lambertianus* Ser. We used a combination of morphological and molecular data to confirm its identity as a new species and the first record of the genus from mainland China, and provide a formal taxonomic description.

Materials & Methods

Study materials

Galls were collected by Nieves-Aldrey & Fang in October–November 2018 in Emeishan and several sites close to Mt. Emei on *R. lambertianus* (Figs. 1A–C). Some galls were stored in rearing cages under normal lab conditions until emergence of adults. A subset of galls were dissected after collection to reveal the internal structure of larval chambers and location of the larvae (Figs. 1D–G). A total of three adult females were recovered.

Specimen preparation

We examined characters using a Leica M205C stereomicroscope illuminated by an incandescent gooseneck lighting system as well as LED illuminators. Measurements were taken using an optical micrometer. Images were taken with a Sony a6000 camera, a Cognysis Stackshot automated rail, and a series of microscope objectives (Nikon BE 4x infinity corrected, Mitutoyo Mplan apo 10x, Olympus UMplan fluor 10x) depending on magnification. 50 to 500 images were combined for each composite photograph using Helicon Focus (v 8.3.2). Final output images were edited in FastStone (v 7.7) image viewer, and scale bars added in ImageJ (v 1.54g). We edited images in Adobe Photoshop and prepared plates using Adobe Illustrator (Adobe Inc.). The type materials are deposited in Emeishan Biological Resources Research Station (EBRRS), Emeishan, Sichuan, China (curator Zhiqiang Fang).

Abbreviations and Terminology

We follow morphological terminology based on Hymenoptera Anatomy Ontology (HAO, Yoder *et al.* 2010) and Melika (2006) for Cynipidae-specific characters; as well as Harris (1979) for cuticular sculpture definitions. The following abbreviations are used: POL (posterior ocellar length), LOL (lateral ocellar length) OOL (ocular ocellar length), and Fn (flagellomere number, 1–12).

Molecular and Phylogenetic Analysis

Genomic DNA was destructively extracted from one individual of *Diastrophus emeiensis* sp. nov. We used PCR primers mlCOIintF (Leray *et al.* 2013) and HCO2198 (Folmer *et al.* 1994) to amplify of a 313 base pair sequence of the DNA barcode region of the mitochondrial cytochrome c oxidase subunit I (COI) gene following the protocol from Kaartinen *et al.* (2010). Amplicons were sequenced using Sanger chemistry by Tsingke Biotechnology, Chengdu, on an ABI3730XL capillary sequencer, and base calling was confirmed using Sequencher 5.4.6. (Gene Codes Corporation). We downloaded publicly available COI sequences of previously described *Diastrophus*

species and we chose *Periclistus brandtii* (Ratzeburg, 1831), *Synophromorpha sylvestris* (Osten Sacken, 1861), and *Xestophanes potentillae* (Retzius, 1783) as outgroups within the tribe Diastrophini (Nylander *et al.* 2004; Malm & Nyman, 2014). For detailed specimen information see Table 1. We reconstructed a phylogenetic tree using maximum likelihood in IQ-TREE v2.36 (Minh *et al.* 2020), with the best evolutionary model determined using ModelFinder (Kalyaanamoorthy *et al.* 2017). Node support was established using 1000 replicates of ultrafast bootstrap (Hoang *et al.* 2018). Pairwise distance was also calculated using MEGA11 (Tamura *et al.* 2021) using *p*-distance.

TABLE 1. List of species included in the phylogenetic analysis of *Diastrophus* based on mitochondrial COI. WP = western Palearctic, EP = eastern Palearctic, NE = Nearctic.

Species	GenBank	Host	Distribution	Reference
Diastrophus emeiensis	PV828417	*Rubus lambertianus*	EP	Present Study
Diastrophus mayri	DQ012639	*Potentilla* spp.	WP	Nylander *et al.* 2004
Diastrophus potentillae	AY368914	*Potentilla canadensis/simplex*	NE	Nylander *et al.* 2004
Diastrophus rubi	DQ012640	*Rubus* spp.	WP	Nylander *et al.* 2004
Diastrophus turgidus	AY368913	*Rubus strigosus*	NE	Nylander *et al.* 2004
Xestophanes potentillae	AY368912	*Potentilla* spp.	WP	Nylander *et al.* 2004
Periclistus brandtii	KF936633	Inquiline	WP	Malm & Nyman 2014
Synophromorpha sylvestris	AY368911	Inquiline	NE	Malm & Nyman 2014

Results

***Diastrophus emeiensis* Hu, Nieves-Aldrey, Zhang & Fang sp. nov.**
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(Figs. 2–5)

Type material. HOLOTYPE: F, **CHINA**: Sichuan, Emeishan, San Dao Gou Road, ex *Rubus lambertianus* (EM-Morpho-159), 29.594N, 103.379E, 765m a.s.l., gall collected 25.X.2018 (EM-2612), adult emerged 11.IV.2019 (Tube-010780), JL Nieves-Aldrey leg. Emeishan gall expedition 2018, deposited in EBRRS. PARATYPE: 1F, same as holotype, Tube-010779, deposited in EBRRS.

Etymology: Named after the region of Mt. Emei, Sichuan, where the species was collected.

Diagnosis:

Diastrophus emeiensis differs from the other three known East Asian *Diastrophus* species by the following characteristics:

1) *D. wushei*: Antennae 14-segmented (13-segmented in *D. wushei*); F1 as long as F2 (F1 longer than F2 in *D. wushei*) (Fig. 3D).

2) *D. renai*: Head and mesosoma are black (red brown in *D. renai*) (Figs. 3B,C); hind coxa dark brown (yellow in *D. renai*) (Figs. 3, 4B); parapsidal line present (absent in *D. renai*) (Fig. 3A); posterior margin of mesoscutellar fovea indistinct (distinct in *D. renai*) (Fig. 5A).

3) *D. japonicus*: Rs straight (slightly curved in *D. japonicus*) (Fig. 5C); F1 as long as F2 (F1 longer than F2 in *D. japonicus*) (Fig. 3D); ratio of POL: OOL: LOL = 2.5: 2.8: 1 (3:5:2 in *D. japonicus*) (Fig. 3B); pronotum smooth to coriaceous, sparsely setose (pubescent, strigate in *D. japonicus*); parapsidal lines apparent (rudimentary in *D. japonicus*) (Fig. 3A); galls are located asymmetrically to one side of the stem and leaf petiole (integral stem swelling for galls of *D. japonicus*) (Fig.1 A–C).

It also differs from the eastern Palearctic species *Diastrophus rubi* (Bouché): Antennae 14-segmented (13-segmented in *D. rubi*); F1 as long as F2 (F1 1.3× F2 in *D. rubi*) (Fig. 3D); Mesoscutellar foveae posterior margins indistinct (well-delimited in *D. rubi*) (Fig. 5A); head is 2.7× as broad as long (2.1–2.2× in *D. rubi*); scutellum is 1.3× as long as wide (rounded to wider than long in *D. rubi*); and galls are located asymmetrically to one side of the stem and leaf petiole (integral stem swelling for galls of *D. rubi*) (Fig.1 A–C).

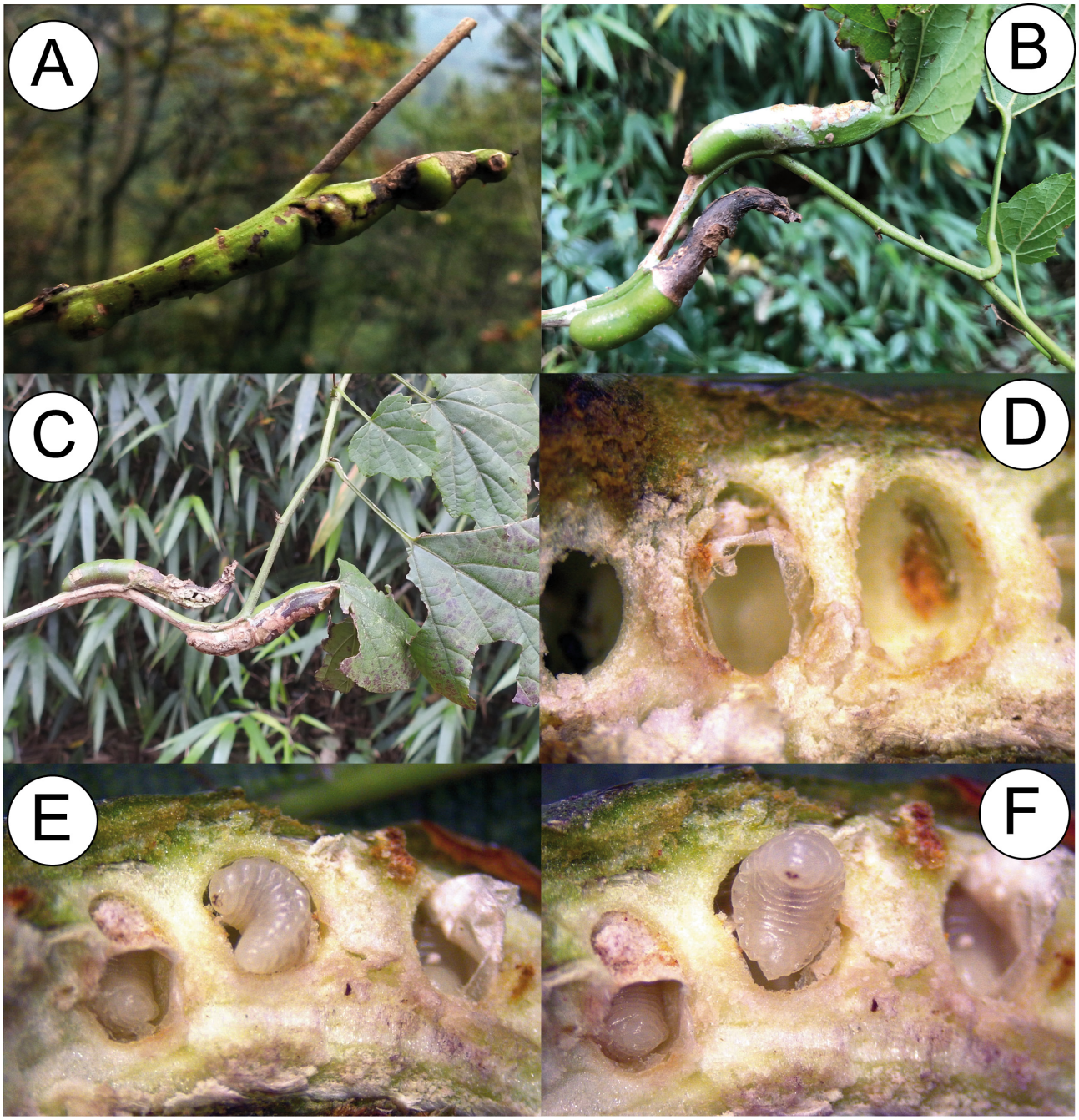

FIGURE 1. A–C. Galls of *Diastrophus emeiensis*. **D.** Larval chamber of *D. emeiensis*. **E.** Larva of a second cynipid species, probably an inquiline *Synophromorpha* sp. in lateral view. **F.** Same larva in ventral view.

Description. Female. Body length 2.5 mm (n = 2).

***Coloration*.** Antennae dark brown. Head black and mesosoma black; tegula brown. Metasoma dark brown (Fig. 2–4). Wings hyaline, infuscated in large areas around R1, 2r, Cu1 (Fig. 5C). All coxae, femora and tarsal claws are dark brown; tibiae and tarsi slightly lighter (Fig. 2).

FIGURE 2. Holotype lateral habitus of *Diastrophus emeiensis*.

Antennae. 0.75× as long as body; 14-segmented. F1 as long as F2 (Fig. 3D). Placodeal sensilla present on F2–F12.

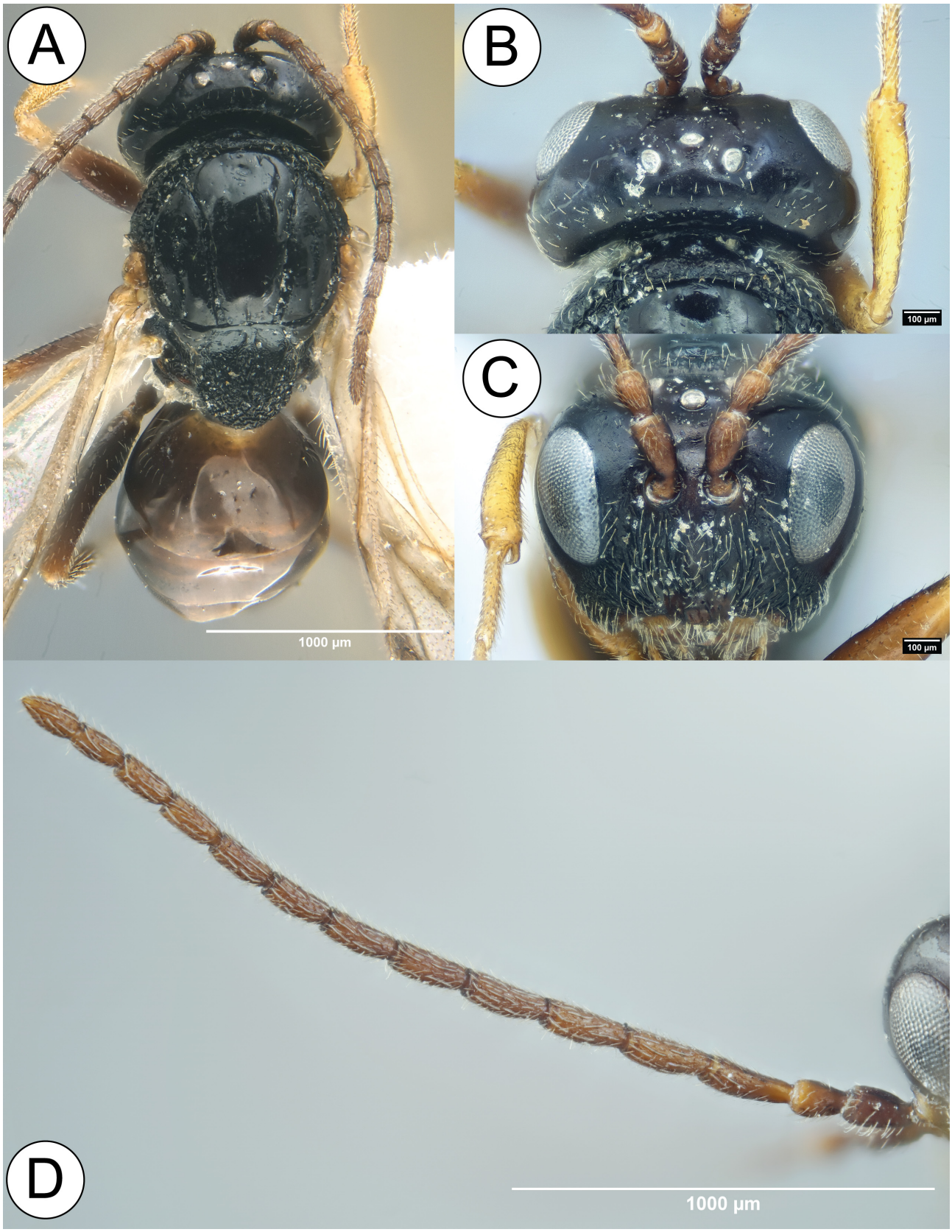

FIGURE 3. Holotype of *Diastrophus emeiensis*. **A)** Dorsal habitus. **B)** Dorsal head. **C)** Frontal head. **D)** Lateral antenna.

Head. In dorsal view 2.7× as broad as long; in anterior view 1.4× as tall as wide; equal to mesosoma in dorsal view. Ratio of POL: OOL: LOL = 2.5: 2.8: 1 (Fig. 3B). Compound eye height to malar space ratio 2×; Frontal face with radiating striae, reaching toruli. Gena moderately expanded behind the compound eye. Face with sparse short setae (Fig. 3C). Toruli situated at mid-height of compound eye. Supraclypeal area raised, narrowing ventrally, with coriaceous sculpture, setose. Clypeus rectangular, 2× as long as wide, with trapezoidal raised area; ventral margin strongly projecting over mandibles. Gena in frontal view curved throughout, coriaceous. Vertex and upper face smooth with setiferous punctures (Fig. 3B). Upper occiput smooth.

Mesosoma*.* Pronotum smooth to coriaceous, sparsely setose. Pronotal plate rugose, with scattered setae (Fig. 3A). Mesoscutum 0.8× as long as wide in dorsal view; mostly glabrous and smooth, with sparse setose on the lateral lobes. Parapsidal lines apparent. Anterior parallel lines indistinct. Medial mesoscutal impression present as a notch. Notauli complete (Fig. 3A). Scutellum rounded, 1.3× as long as wide in dorsal view. Mesoscutellar disc rugose; with short setae. Circumscutellar carina absent. Mesoscutellar foveae elliptical, with posterior margins somewhat indistinct, medially separated by a wide, weak carina; base of foveae coriaceous (Fig. 5A). Dorsal axilla smooth. Mesopleuron mostly smooth, with striate to rugulose sculpture on the anterior edge (Fig. 4A). Lateral propodeal area with dense setae. Medial propodeal area delimited by carinae, with vertical rugae (Fig. 5B). Nucha with irregular, longitudinal sulci.

Fore Wing. 1.2× as long as body. Radial cell open, 3.3× as long as wide. Veins R1 and Rs not reaching wing margin. Rs straight. Rs+M not reaching M anteriorly. Areolet present. Apical margin of wing with a fringe of setae. Basal cell with scattered setae (Fig. 5C).

Legs. Tarsal claw strongly lobed, measuring 1/2 of length of apical tooth. Hind coxa setose along the posterior edge, and with one row of setae laterally (Fig. 4B).

Metasoma. Shorter than combined length of head and mesosoma. Gt_2 with a single row of setae laterally, glabrous dorsally; Gt_7 with sparse setae. Hypopygial spine short, not extending beyond the hypopygium, with setae along its entire length (Fig. 4B).

Males. Unknown.

Distribution. Mt. Emei, Sichuan, China.

Biology. The new species induces a multilocular stem and petiole gall on *Rubus lambertianus* (Figs. 1A–C). The gall is located asymmetrically to one side of the stem and leaf petiole, rather than being integral to these structures, and its development often causes death of the associated leaf. The gall is irregularly sausage-shaped, usually with a rounded base distinct from the stem, and tapering into an affected petiole. The gall is green (sometimes with reddish tints) like the stem, and is smooth and shiny. Each gall extends along the stem and petiole for c. 70mm, with a maximum width of 8 mm. Dissection revealed an internal structure showing two types of larval chambers and larvae: the *Diastrophus* gall inducer larval chamber is regularly ovoid and its internal wall is covered by a fine membranous envelope (Fig. 1D). The second type of larval chambers has a less regular form which lacks the membranous lining. They are occupied by larvae that show stronger mandibles (three teeth) than *Diastrophus,* which probably represent an undescribed inquiline *Synophromorpha* Ashmead, 1903 species (Figs. 1E, F).

Variation. The depression on the anterior edge of mesopleuron is less prominent on the paratype.

Molecular Data. The COI fragment of *D. emeiensis* was 313 bp in length after trimming, and is deposited in GenBank with accession number PV828417. The K3Pu+F+G4 model was used to construct the phylogeny in Fig. 6 after it was identified as the best fit to the data by Modelfinder. *Diastrophus* was recovered as monophyletic and sister to *Xestophanes*, and *D. emeiensis* was recovered as a distinct lineage that is sister to the rest of *Diastrophus*, which includes *D. mayri*, *D. potentillae* Bassett, 1864, *D. rubi*, and *D. turgidus* Bassett, 1870 (Fig. 6). The pairwise distance of *D. emeiensis* from other *Diastrophus* species is 8.7–12.2% (Table 2).

FIGURE 4. Holotype of *Diastrophus emeiensis*. **A)** Lateral mesosoma. **B)** Lateral metasoma.

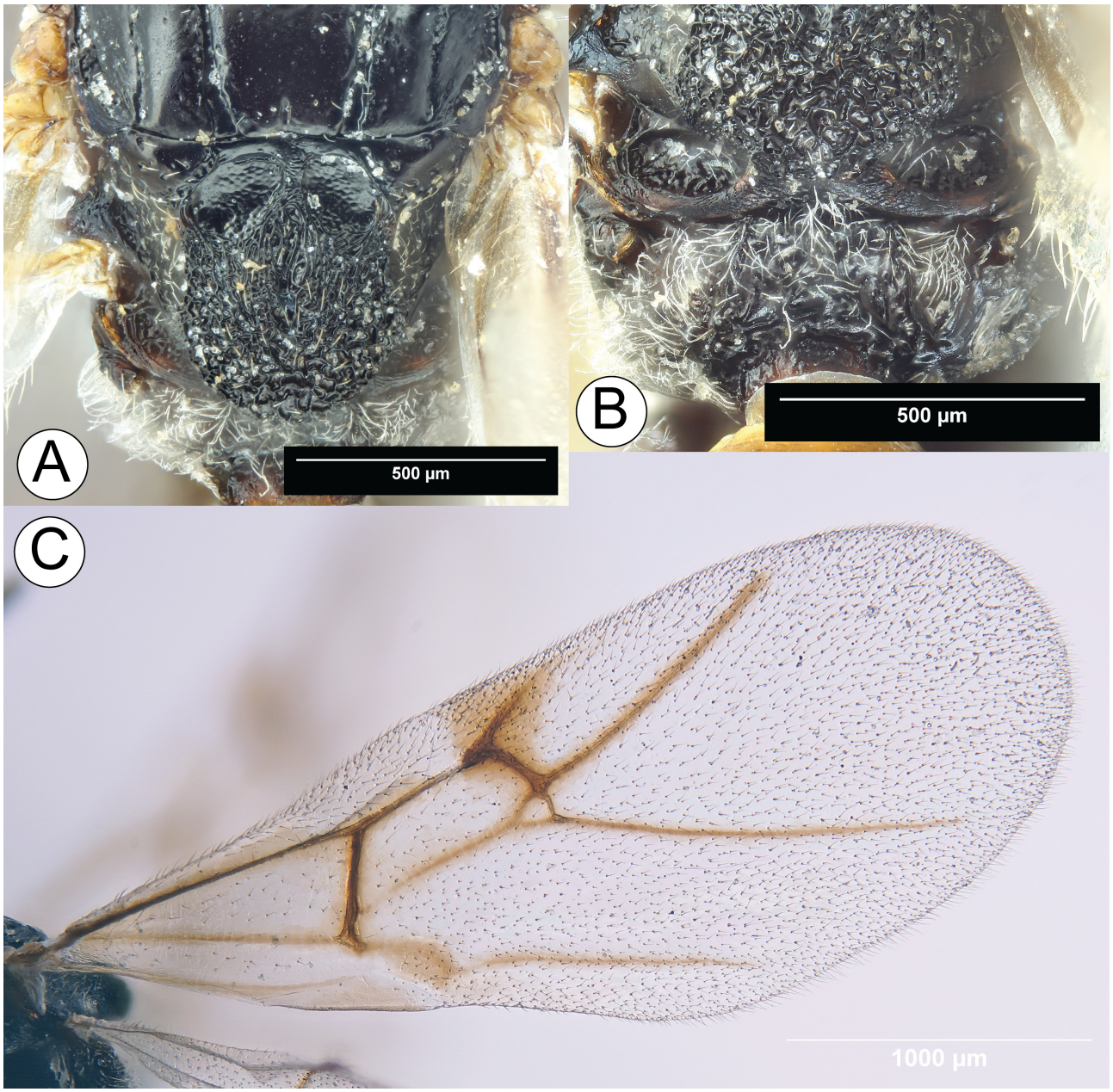

FIGURE 5. Holotype of *Diastrophus emeiensis*. **A)** Dorsal scutellum. **B)** Dorsal propodeum. **C)** Fore wing.

TABLE 2. Pairwise COI *p*-distance between specimens included in this study.

	Taxa	1	2	3	4	5	6	**7**	**8**
1	PV828417|*Diastrophus emeiensis*								
2	DQ012639|*Diastrophus mayri*	0.0865							
3	AY368914|*Diastrophus potentillae*	0.1218	0.0970						
4	DQ012640|*Diastrophus rubi*	0.0897	0.0970	0.1002					
5	AY368913|*Diastrophus turgidus*	0.0929	0.0892	0.1064	0.0876				
6	AY368912|*Xestophanes potentillae*	0.0994	0.1049	0.1158	0.0845	0.0782			
7	KF936633|*Periclistus brandtii*	0.1624	0.1608	0.1508	0.1407	0.1642	0.1256		
8	AY368911|*Synophromorpha sylvestris*	0.1154	0.1174	0.1095	0.0923	0.1127	0.0642	0.1189	

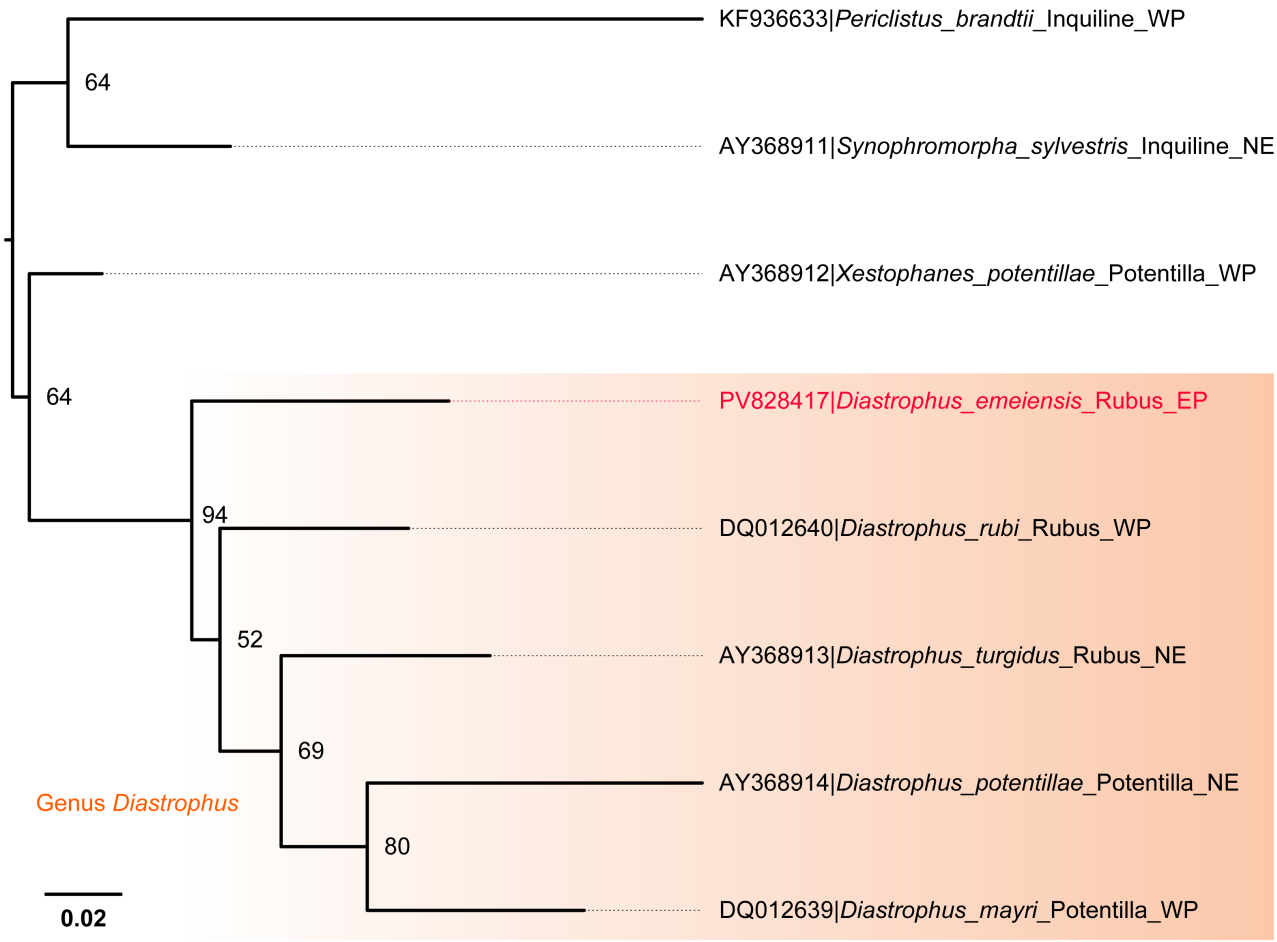

FIGURE 6. Maximum likelihood phylogram of *Diastrophus* mitochondrial DNA COI, along with host data and distribution (WP: western Palearctic; EP: eastern Palearctic; NE: Nearctic). Numbers at nodes indicate support values estimated in ultrafast bootstrap.

Discussion

Relative to cynipids on Fagaceae, the biology, diversity and distributions of cynipids on other hosts is rather poorly known. This is especially true for mainland East Asia, where even the Fagaceae-associated cynipids are massively understudied (Abe *et al.* 2007; Fang *et al.* 2024). A lack of biological and phylogenetic information for eastern Palearctic Cynipidae, including *Diastrophus*, has impeded our understanding of global phylogenetic relationships and biogeographical patterns (Abe *et al.* 2007; Davis *et al.* 2024; Fang *et al.* 2024; Pang *et al.* 2020; Wachi *et al.* 2013).

Diastrophus emeiensis is the first species of *Diastrophus* recorded in mainland China, and the second bramble (*Rubus*)-associated gall wasp with host plant information and gall morphology (Fig. 1) in East Asia (Davis *et al.* 2024; Wachi *et al.* 2013). *Rubus lambertianus* is found widely across East and Southeast Asia (POWO, 2025), thus the range of *D. emeiensis* could potentially extend far beyond Sichuan. The structure and location of the gall of *D. emeiensis* are as we would expect given known species in the genus (Schick *et al.* 2003).

East Asia is the center of diversity for the *Rubus* subgenera *Batothamnus* (Focke) E.H.I. Krause and *Malachobatus* (Focke) Fritsch (Huang *et al.* 2023). The two East Asian *Diastrophus* species with known hosts are each associated with one of these subgenera: *D. japonicus* on *R. palmatus* (*Batothamnus*), and *D. emeiensis* on *R. lambertianus* (*Malachobatus*) (Wachi *et al.* 2013; Huang *et al.* 2023). China alone harbors more than 208 *Rubus* species, many of which are endemic (Wang & Wang 2019). Given this remarkable diversity, it is likely that numerous *Diastrophus* species associated with these hosts remain undiscovered in China and across East Asia. *Diastrophus* galls are the focal resource for associated *Synophromorpha* inquiline cynipids (putatively identified as

the larvae in our type 2 larval chambers) and a suite of associated parasitoids (Csóka *et al.* 2005; Gordinier 2003; Askew *et al.* 2006). In the Western Palearctic this includes species in at least four chalcidoid families: Eupelmidae (*Eupelmus* Dalman), Eurytomidae (*Eurytoma* Illiger), Ormyridae (*Ormyrus* Westwood) Torymidae (*Glyphomerus* Förster*, Torymus* Dalman) (Askew *et al.* 2006). It is thus likely that in addition to further *Diastrophus* species, many species in these associated guilds also remain to be discovered in East Asia.

Our phylogeny has recovered *D. emeiensis* as the sister group to the rest of *Diastrophus*, which includes members found in both the Nearctic and western Palearctic (Fig. 6). In terms of host plant, the *Rubus*-attacking lineages were recovered as early branching and in a step-like fashion, while the two species inducing galls on *Potentilla* are recovered as monophyletic (*D. mayri* and *D. potentillae*). This hints at a complex host use and biogeographic history within *Diastrophus*/Diastrophini, although this should be interpreted with caution as it is based on a single short mitochondrial DNA fragment. Wider taxon sampling (and particularly of eastern Palearctic species) using a much larger suite of unlinked genetic markers is necessary to explore these evolutionary patterns.

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Author Contributions

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Data analysis: WQH, JLNA, KM, CTT, YMZ
Funding acquisition: ZQF, GNS, KSM
Resource allocation: ZQF, YZ, KSM, GNS, YMZ
Writing first draft: WQH, YMZ
Editing: all authors

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