



First record of Cynipidae in Brazil: A new species of gall wasp of the genus *Diastrophus* Hartig (Hymenoptera: Cynipidae: Diastrophini)

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Abstract

We describe *Diastrophus brasiliensis* Oliveira & Melo **sp. nov.**, a gall inducer on stems of *Rubus brasiliensis* Mart. and *R. sellowii* Cham. & Schlitdl. (Rosaceae). This represents the first record of the family Cynipidae in Brazil and the second species of *Diastrophus* Hartig from the Neotropical region. The new species differs from the remaining *Diastrophus* mainly by its entirely rugose mesoscutum. We also generated molecular data for the new taxon and conducted an exploratory phylogenetic analysis to investigate its placement within Diastrophini.

Key words: 28S, CO1, Cynipoidea, gall inquilines, Paraná

Introduction

The family Cynipidae is commonly known as gall wasps due to their ability to induce the development of modified plant tissues, called galls, in their host plants. These galls serve as the environment in which the wasp larvae develop (Stone *et al.* 2002). Not all Cynipidae species induce galls, some of them develop as inquilines in galls induced by other cynipid species (Sanver & Hawkins 2000; van Noort *et al.* 2007; Ronquist *et al.* 2015; Ronquist *et al.* 2018) and by other insect groups (Hearn *et al.* 2024; Tataroglu 2025). This family comprises approximately 1,400 described species, distributed across all continents except Antarctica, and exhibits its highest diversity in the Nearctic and western Palearctic regions (Nieves-Aldrey 1994; Melika 2006; Melika *et al.* 2021; Natasi *et al.* 2024).

Cynipidae has been traditionally divided into 13 extant tribes (Ronquist *et al.* 2015; Lobato-Vila *et al.* 2022). Recently, Blaimer *et al.* (2020), using a phylogenetic analysis of Ultraconserved Elements (UCEs), recovered Diplolepidini, Eschatocerini, Paraulacini and Pediastpidini outside the family, rendering Cynipidae non-monophyletic. Hearn *et al.* (2024), using protein-coding sequences from genome and transcriptome assemblies, corroborated most of the results from Blaimer *et al.* (2020), except for the placement of Eschatocerini. Currently, the family is divided into 10 tribes, namely: Aulacideini, Aylacini, Ceroptresini, Cynipini, Diastrophini, Eschatocerini, Phanacidini, Qwaqwaiini, Rhoophilini, and Synergini (Lobato-Vila *et al.* 2022; Hearn *et al.* 2024).

Prior to this study, the native fauna of Cynipidae in South America was restricted to eight species: three species of *Synergus* Hartig, one species of *Zapatella* Pujade-Villar & Melika, and one species of *Diastrophus* Hartig, all from Colombia, along with the endemic tribe Eschatocerini, represented by the single genus *Eschatocerus* Mayr with three species restricted to southern South America (Pujade-Villar *et al.* 2012; Nieves-Aldrey *et al.* 2013; Nieves-Aldrey & Blas 2015; Lobato-Vila *et al.* 2020). Currently, three non-native cynipid wasp species have been introduced to South America from Europe: *Phanacis hypochoeridis* (Kieffer) (Phanacidini; Argentina and Chile), *Plagiotrochus suberi* (Cynipini; Argentina), and *Timaspis cichorii* (Kieffer) (Phanacidini; Chile) (Pujade-Villar & Díaz 2001; Nieves-Aldrey & Grez 2007).

Among extant cynipid tribes, Diastrophini represents a monophyletic group (Ronquist *et al.* 2015; Blaimer *et al.* 2020; Hearn *et al.* 2024) composed by two genera of gall inducers, *Diastrophus* Hartig and *Xestophanes*

Förster, developing on Rosaceae (*Rubus* L., *Potentilla* L. and *Fragaria* L.); two genera of inquilines, *Periclistus* Förster, which attacks galls induced by *Diplolepis* Geoffroy (Cynipoidea, Diplolepididae), and *Synophromorpha* Ashmead, inquilines in galls induced by *Diastrophus* (Buffington *et al.* 2020); and the recently described genus *Xestophanopsis* Pujade-Villar & Wang, whose biology remains unknown (Pujade-Villar *et al.* 2019). Currently, the genus *Diastrophus* comprises 21 species (Schick *et al.* 2003; Melika & Klymenko 2005; Nieves-Aldrey *et al.* 2013; Wachi *et al.* 2013), including two species recently described from Taiwan (Davis *et al.* 2024). The genus occurs throughout the Palearctic region, with four described species; the Nearctic region, with 14 species; the Oriental region, with two species; and the Neotropical region, with only one species.

In this study, we describe a new species of *Diastrophus* from southern Brazil, reared from stem galls on native species of *Rubus*. We also generated molecular data for the new taxon and conducted an exploratory phylogenetic analysis to investigate its placement within Diastrophini. This represents the first record of the family Cynipidae in Brazil and the second known species of this genus in the Neotropical region.

Material and methods

Specimen sampling and morphological study

The specimens studied here are deposited in the Coleção Entomológica Pe. Jesus Santiago Moure, Universidade Federal do Paraná, Curitiba, Brazil (DZUP) and Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZSP). In the list of type material, original data from the labels are provided here as *ipsis litteris* transcriptions surrounded by double quotation marks (“”), with backslash (\) indicating different lines on the same label. Morphological terminology follows Huber & Sharkey (1993), Ronquist (1994), and Nieves-Aldrey (2001), with cuticular sculpture nomenclature based on Harris (1979). The following abbreviations are used: antennal flagellomeres (F1, F2, etc.), metasomal terga and sterna (T1–T7 and S1–S8, respectively), POL (postocellar distance; the distance between the inner margins of the posterior ocelli), and OOL (ocellar-ocular distance; the distance from the outer edge of a posterior ocellus to the inner margin of the compound eye). High-resolution images were captured using a Leica DMC4500 4.12.0 camera paired with a Leica M205 C stereomicroscope, with image stacking performed in Zerene Stacker (v1.04) and final editing in Adobe Photoshop.

The first specimens were discovered among samples from Malaise traps placed in the municipality of São José dos Pinhais, Paraná, kindly provided by Dr. Alexandre Domahovski. One female was found in a catch of a trap placed in October, 2021, while two additional males were found later among stored samples from October, 2016. After the discovery of these three specimens, and knowing that *D. colombianus* Nieves-Aldrey was reared from *Rubus* (see Nieves-Aldrey *et al.* 2013), we decided to inspect other sites in the region previously known to harbor populations of native *Rubus*. We focused on plants growing on roadsides near the coordinates 25.5122°S, 49.0033°W, in Piraquara, Paraná, which was visited for the first time in May 25th, 2024. On this occasion, we collected a few *Rubus* stems showing signs of gall formation, in addition to having emergence holes. Additional galled stems were collected in the same site on October 17th, 2024. In this visit, we took photographs of the attacked plants and stems under field conditions. The plant branches were kept in plastic containers in the laboratory for emergence of the insects. The species of *Rubus* were identified using the key from Carpanezzi *et al.* (2019), while the larvae of *Diastrophus* were identified morphologically based on diagnostic characteristics described by Nieves-Aldrey *et al.* (2005).

DNA sequencing

One larva and three adults (two females and one male) of the new species were used for DNA extraction. The specimens were subjected to non-destructive DNA extraction using the Solid-Phase Reversible Immobilization (SPRI) technique (Hultman *et al.* 1989) with Sera-MagTM SpeedBeads Carboxylate-Modified Magnetic Particles (CytivaTM). We selected two molecular markers for analysis: the mitochondrial cytochrome oxidase I (COI) gene and the large subunit ribosomal RNA (28S) gene. For COI amplification, we used the primers dgLCO1490 (5'-GGTCAACAAATCATAAAGAYATYGG-3') and dgHCO2198 (5'-TAAACTTCAGGGTGACCAARAAYCA-3') (Meyer, 2003). For 28S amplification, we employed the primers For28Vesp (5'-AGAGAGAGTTCAAGAGTACGTG-3') and Rev28SVesp (5'-GGAACCAGCTACTAGATGG-3') (Hines *et al.* 2007). Polymerase chain reaction (PCR) was performed with PlatinumTM Taq DNA Polymerase (Invitrogen/Thermo Fisher Scientific) following the manufacturer's protocol. The amplification program included an initial denaturation step at 94°C for 2 minutes,

followed by 35 cycles of 94°C for 40 seconds, 50°C (CO1) or 53°C (28S) for 45 seconds, and 72°C for 1 minute, with a final extension step at 72°C for 7 minutes. PCR products were verified by 1% agarose gel electrophoresis, purified via SPRI, and sequenced using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™) under standard protocols.

Additional sequences for other species of *Diastrophus* and for the outgroup were obtained from GenBank (www.ncbi.nlm.nih.gov/genbank) and BOLD Systems (<https://v3.boldsystems.org/>) (Table 1). For outgroup comparisons, we used available sequences from other Diastrophini (*Periclistus*, *Synophromorpha*, and *Xestophanes*), from the Ceroptresini (*Ceroptres* Hartig), Phanacidini (*Phanacis* Förster) and the Aulacideini (*Antistrophus* Walsh, and *Aulacidea* Ashmead). Choice of outgroup and tree rooting were based on the phylogenetic results of Hearn *et al.* (2024).

TABLE 1. Terminal taxa used in the phylogenetic analysis for this study, with their corresponding database accession numbers. For *D. brasiliensis*, AG refers to adult vouchers and L to the larval voucher.

Terminal	CO1	28S	Database
<i>Antistrophus silphii</i>	AY368917.1	AY368943.1	GenBank
<i>Aulacidea freesei</i>	DQ012627.1	DQ012585.1	GenBank
<i>Ceroptres cerri</i>	AY368910.1	AY368935.1	GenBank
<i>Ceroptres clavicornis</i>	EF486872.1	EF487120.1	GenBank
<i>Diastrophus brasiliensis</i> sp. nov. (AG86)	PV590576	PV590598	GenBank
<i>Diastrophus brasiliensis</i> sp. nov. (AG87)	PV590577	PV590599	GenBank
<i>Diastrophus brasiliensis</i> sp. nov. (AG88)	PV590578	-	GenBank
<i>Diastrophus brasiliensis</i> sp. nov. (L3)	PV590575	-	GenBank
<i>Diastrophus kincaidii</i>	SSWLA4873-13	-	BOLD Systems
<i>Diastrophus mayri</i>	DQ012639.1	-	GenBank
<i>Diastrophus potentillae</i> (a)	OPPFM1850-17	-	BOLD Systems
<i>Diastrophus potentillae</i> (b)	KT708154.1	-	GenBank
<i>Diastrophus potentillae</i> (c)	AY368914.1	AY368940.1	GenBank
<i>Diastrophus potentillae</i> (d)	OPPFM2907-17	-	BOLD Systems
<i>Diastrophus potentillae</i> (e)	OPPFM2963-17	-	BOLD Systems
<i>Diastrophus rubi</i> (a)	DQ012640.1	DQ012598.1	GenBank
<i>Diastrophus rubi</i> (b)	DTNHM7858-23	-	BOLD Systems
<i>Diastrophus rubi</i> (c)	DTNHM7859-23	-	BOLD Systems
<i>Diastrophus</i> sp. 1	KR880473.1	-	GenBank
<i>Diastrophus</i> sp. 2	OPPFM2651-17	-	BOLD Systems
<i>Diastrophus</i> sp. 3	OPPFC602-17	-	GenBank
<i>Diastrophus</i> sp. 4	KR792067.1	-	GenBank
<i>Diastrophus</i> sp. 5	KR802854.1	-	GenBank
<i>Diastrophus</i> sp. 6 (a)	KR405594.1	-	GenBank
<i>Diastrophus</i> sp. 6 (b)	KR895356.1	-	GenBank
<i>Diastrophus</i> sp. 7	OPPDC596-17	-	BOLD Systems
<i>Diastrophus</i> sp. 9	KR408439.1	-	GenBank
<i>Diastrophus</i> sp. 10	SSEIA3236-13	-	BOLD Systems
<i>Diastrophus</i> sp. 8	CNPEP1220-14	-	BOLD Systems
<i>Diastrophus turgidus</i>	AY368913.1	AY368939.1	GenBank
<i>Periclistus arefactus</i>	HQ968038.1	-	GenBank
<i>Periclistus brandtii</i>	AF395181.1	AF395152.1	GenBank
<i>Periclistus orientalis</i>	MN633410.1	MN633411.1	GenBank

.....continued on the next page

TABLE 1. (Continued)

Terminal	COI	28S	Database
<i>Periclistus piceus</i>	MK813816.1	-	GenBank
<i>Periclistus pirata</i>	DQ012649.1	DQ012606.1	GenBank
<i>Periclistus</i> sp.	MF905093.1	-	GenBank
<i>Phanacis centaureae</i>	AY368927.1	AY368953.1	GenBank
<i>Synophromorpha</i> sp.	HQ929306.1	-	GenBank
<i>Synophromorpha sylvestris</i>	AY368911.1	AY368937.1	GenBank
<i>Xestophanes potentillae</i>	AY368912.1	AY368938.1	GenBank

Phylogenetic analysis

For molecular data processing, forward and reverse reads were first aligned using BioEdit (Hall 1999) to generate consensus sequences. Sequence alignment for each marker was performed in MAFFT v6 (Katoh *et al.* 2019) using default parameters, followed by manual inspection and correction of any detected errors in BioEdit. The aligned sequences from both markers were then concatenated using SequenceMatrix (Vaidya *et al.* 2011). Maximum likelihood analysis was conducted using a partitioned approach by gene, with the best-fit substitution models (K3Pu+F+I+G4 for COI and TIM3e+I for 28S) selected through ModelFinder (Kalyaanamoorthy *et al.* 2017). Phylogenetic reconstruction was performed in IQ-TREE (Nguyen *et al.* 2015) using ultrafast bootstrap approximation with 1000 replicates and an automatic stopping criterion, with the final tree visualized in FigTree v1.3.1 (Rambaut 2007).

Results

Molecular phylogenetic results

The maximum likelihood analysis produced a well-supported phylogeny (bootstrap ≥ 90 for all major clades), confirming the monophyly of Diastrophini and all genera within it (Fig. 1). The topology showed: (1) *Periclistus* as sister to the remaining Diastrophini (BS = 100), (2) a strongly supported *Xestophanes* + *Synophromorpha* clade (BS = 100) sister to *Diastrophus* (BS = 100), and (3) *D. rubi* as sister to the remaining species of *Diastrophus* (BS = 100). The newly described species, *D. brasiliensis*, came out nested deeply within *Diastrophus*, in a clade containing other named species, as *D. mayri* and *D. turgidus*. The branch leading to *D. brasiliensis* is distinctly long, indicating an isolated position within the genus.

Taxonomy

Diastrophus Hartig, 1840

Diagnosis. Mesoscutum smooth and weakly sculptured in most species, except for *D. brasiliensis* **sp. nov.** and *D. colombianus* which exhibits a variable degree of rugulosity (see below). Notauli complete and well-impressed. Mesopleuron almost entirely smooth and shining, medially with some fine longitudinal striae. Fore wing with marginal cell open. Metasoma never with syntergite. (Diagnosis modified from Melika 2006 and Nastasi *et al.* 2024).



FIGURE 1. Phylogenetic relationships based on CO1 and 28S markers, obtained through maximum likelihood analysis. Branch support is given by bootstrap.

***Diastrophus brasiliensis* sp. nov.**

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Figures 2–4

Diagnosis. *Diastrophus brasiliensis* sp. nov. can be easily distinguished from all other species of *Diastrophus* by the following combination of features: surface of mesonotum strongly rugulose to rugose (mostly smooth and shiny in other species); dorsal axillar surface distinctly rugose (varying in other species from shiny to rugose); and mesopleuron medially with fine longitudinal striae (varying in other species from shiny, coriaceous, to striated). In addition, the new species can be distinguished from *D. colombianus*, the only other South American species, by its mostly rugulose mesoscutum (rugulosity in *D. colombianus* is restricted to the area between the parapsidal signa and the posterior half of the notauli) and its broadly and finely striated lateral mesopleuron (in *D. colombianus*, the mesopleuron is mostly smooth and the striation is restricted to a narrow medial band).

Description. Holotype female. **Measurements** (in mm). Approximate body length 2.8. Forewing length, including tegula, 3.8. Head length 0.8. Head width 0.9; POL 0.14; OOL 0.16. **Color.** Integument predominantly black. Mandible dark brown, teeth black distally. Tegula and antenna dark reddish brown. Coxae, trochanters, basal half of femora, and last tarsomeres brown; apical half of femora, tibiae and tarsomeres 1–4 reddish brown. Wing membrane mostly hyaline, with some infuscated regions, veins dark brown to black, 1st abscissa of Rs (Median vein) and 2r-rs (2r) darker than remaining veins. **Pilosity.** Mostly pale and short. Antennae with scattered setae, each as long as half the width of F1. Head with very scattered setae. Anterior margin of clypeus and base of mandible with denser, longer setae. Pronotum moderately setose, especially on its lateral surface. Mesoscutum and scutellum very sparsely setose. Lateral mesopleuron glabrous, except for subalar triangle; ventral mesopleural area with scattered setae. Ventral metapleural area and lateral portion of propodeum densely setose. Legs densely setose, except for a glabrous stripe on the dorsal regions of the coxae, trochanters, and femora. Wing membrane homogeneously setose; setae about as long as half the width of Rs+M. Dorso-lateral area of T2 with a curved row of sparse, short setae. Posterior region of T6 and T7 with sparse, short setae. Hypopygial spine ventrally with a row of sparse, short setae

on each side. **Sculpturing.** Face with strong, irregular carinae radiating from clypeus. Upper paraocular area with somewhat longitudinal carinae reaching antennal socket. Lower paraocular area with somewhat curved carinae near mandibles and lateral margin of clypeus. Clypeus with vertical striae. Gena, frons, and vertex coriaceous, very sparsely and shallowly punctate. Pronotum strongly rugose, especially on pronotal plate and dorsolateral areas; lateral area with well-developed longitudinal rugae. Mesoscutum with strong rugose sculpture, except for coriaceous area between parapsidal line and tegula. Scutellum strongly rugose, except for shiny scutellar foveae. Dorsal axillar surface rugose. Mesopleuron with longitudinal striae medially, striation broader near pronotum and becoming narrower toward metapleuron, giving the striated region a triangular form; dorsal and ventral areas of mesopleuron smooth; subalar triangle rugose. Metapleuron and propodeum rugose. Micropunctures present on posterior margins of both tergum and sternum of metasomal segments 4–7. **Structure.** Head in dorsal view 2.5x wider than long, slightly broader than mesosoma; rounded in frontal view, 1.25x wider than long. Gena in lateral view expanded, 0.5x as wide as compound eye. Clypeus subquadrate, about as wide as long; ventral margin weakly projecting over mandibles. Epistomal sulcus visible and slightly curved upwards. Malar space 0.6x as long as compound eye length. Antennal sockets situated slightly above midlength of compound eye. Right mandible with three teeth; left with two teeth. Antenna with 13 flagellomeres, all with visible placodeal sensilla; pedicel 1.3x as long; F1–F12 longer than wide, F1 about 3.5x as long as its maximum width, F2–F4 about 0.9x as long as F1, remaining flagellomeres gradually decreasing in length; F13 subtriangular, subequal in length to F12. Pronotal plate distinct. Notauli complete, distinct and deeply impressed. Parapsidal line distinct and deeply impressed. Scutellar foveae oval, 0.26x as long as scutellum; foveal septum triangular, about 0.5x as wide as scutellum at its widest region posteriorly. Forewing with radial cell 3x longer than wide; R1 and Rs not reaching anterior wing margin; areolet absent. Basitarsi as long as combined lengths of tarsomeres 2 and 3. T2 about one-third of metasoma length.

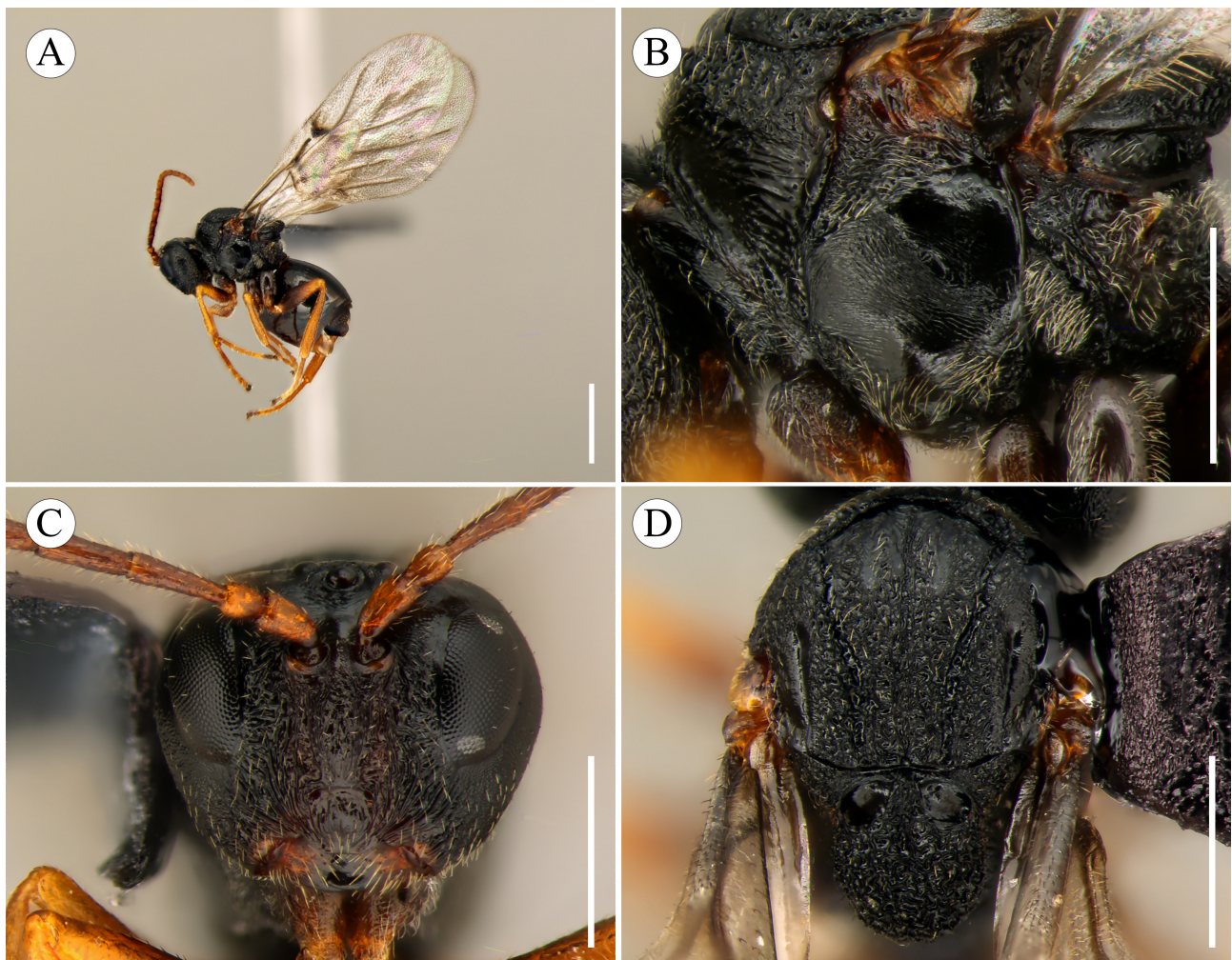


FIGURE 2. *Diastrophus brasiliensis* sp. nov. (holotype, female). **A**, habitus, lateral view; **B**, mesosoma, lateral view; **C**, head, frontal view; **D**, mesosoma, dorsal view. Scale bars: A, 1mm; B–D, 0.5mm.

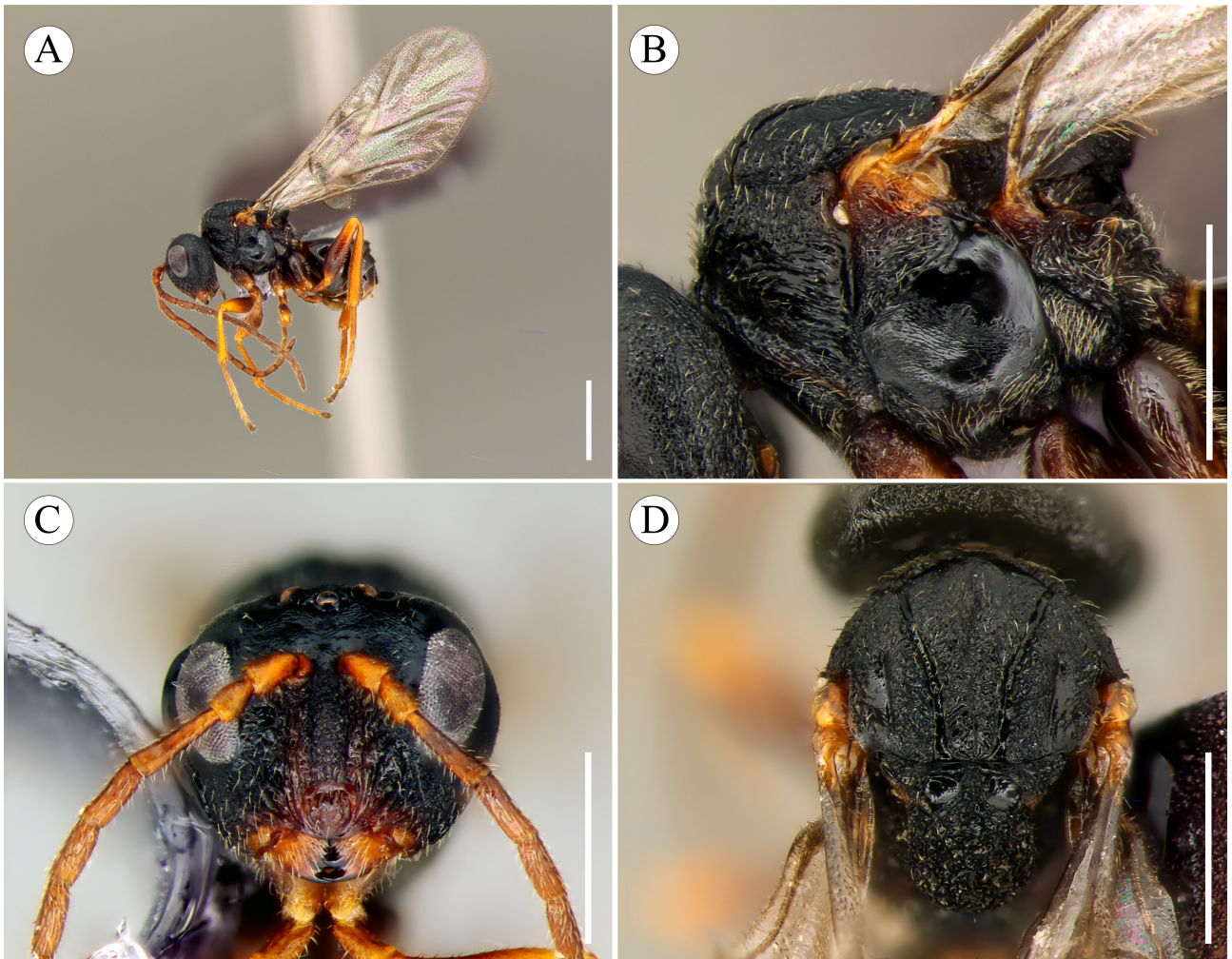


FIGURE 3. *Diastrophus brasiliensis* sp. nov. (paratype, male). **A**, habitus, lateral view; **B**, mesosoma, lateral view; **C**, head, frontal view; **D**, mesosoma, dorsal view. Scale bars: A, 1mm; B–D, 0.5mm.

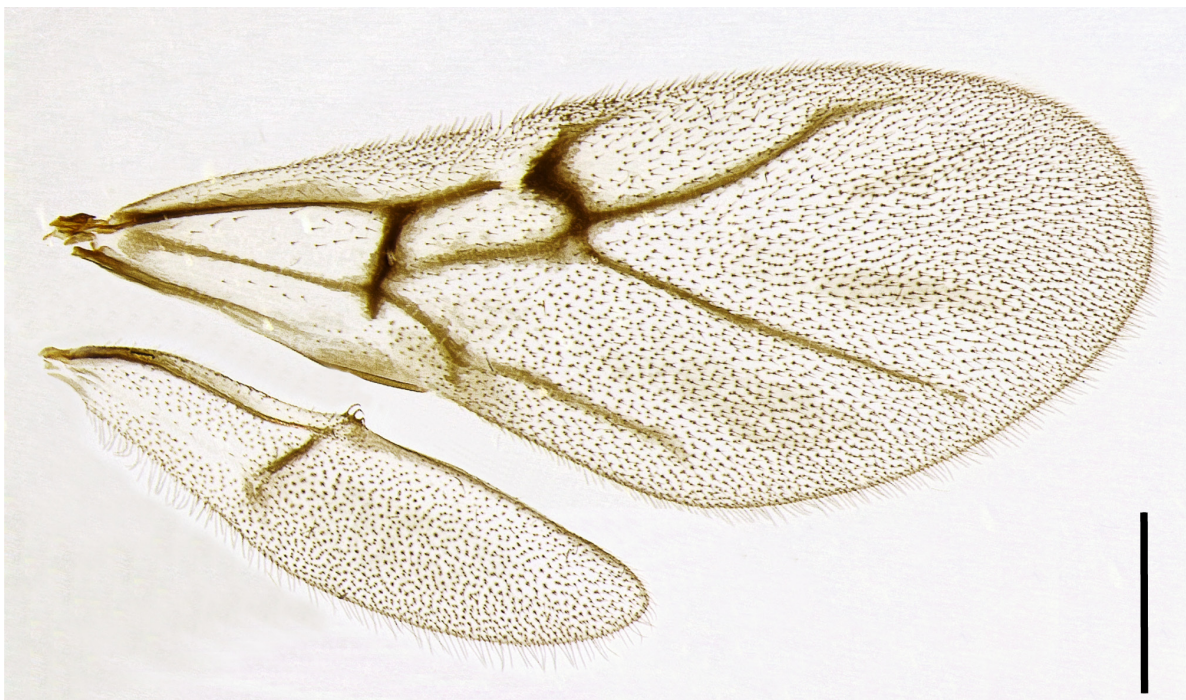


FIGURE 4. Fore- and hindwing of *Diastrophus brasiliensis* sp. nov. Scale bar: 0.5mm.

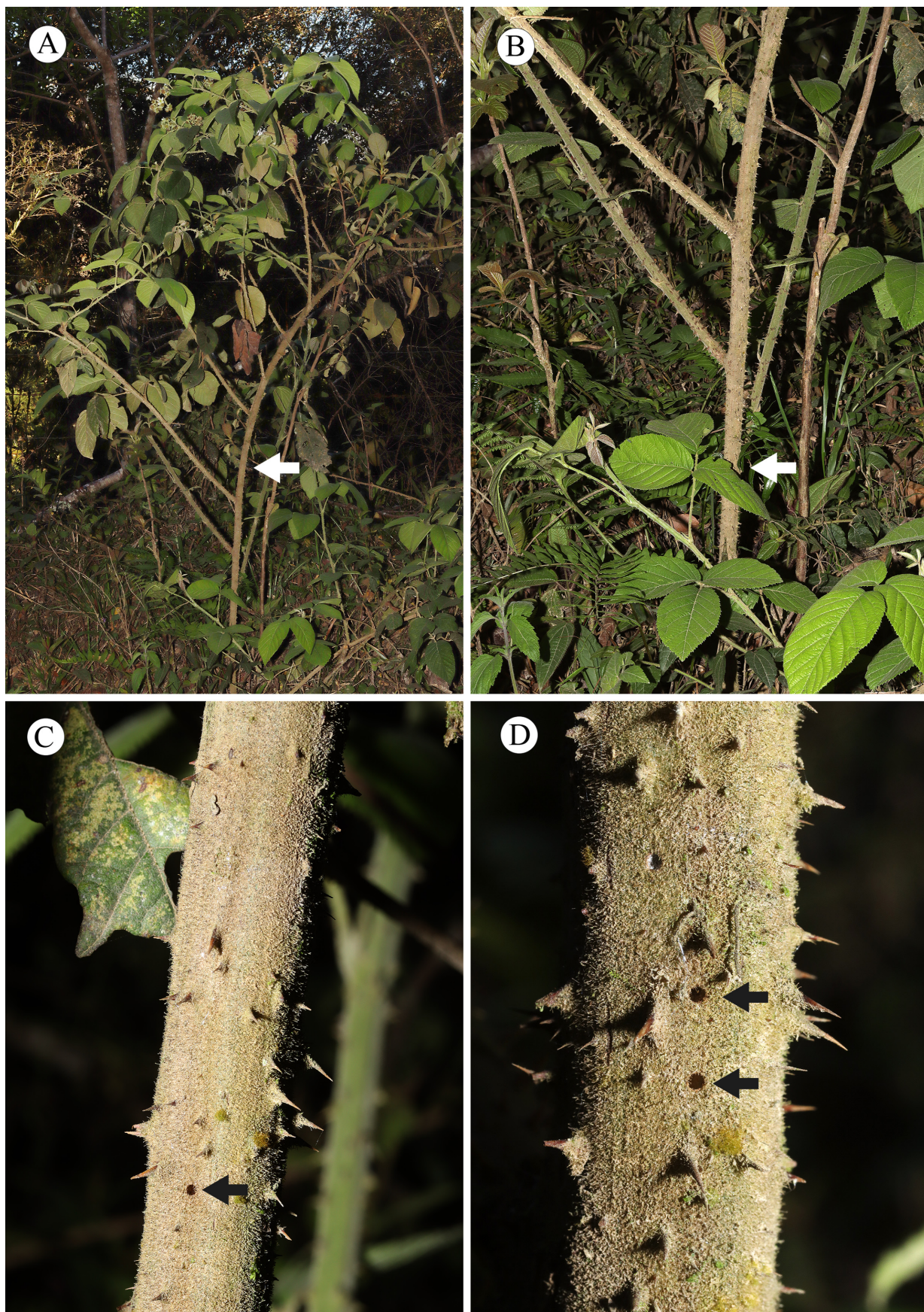


FIGURE 5. *Rubus brasiliensis* Mart. (Rosaceae). **A–B**, Overall habitus; **C–D**, Close-up view of a galled stem. White setae point to the galled stems; black setae indicate the emergence holes.

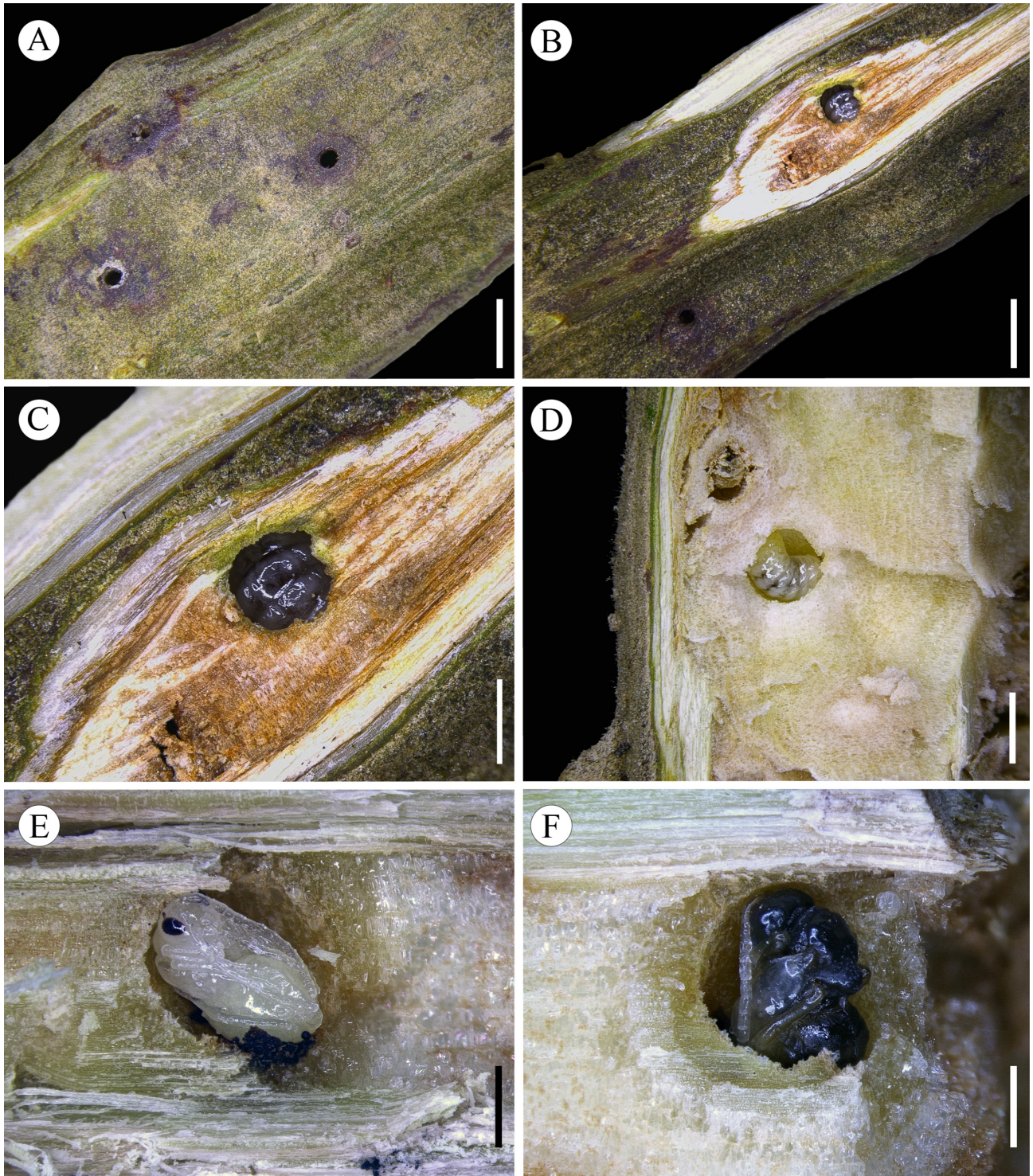


FIGURE 6. Galls of *Diastrophus brasiliensis* **sp. nov.** in two *Rubus* species, showing different developmental stages. **A**, external view of gall and emergence holes in *R. sellowii* Cham. & Schltdl.; **B–D**, partially dissected gall chamber containing larvae in *R. sellowii*; **E**, white pupae within gall chamber, with visible feces, in *R. brasiliensis*; **F**, pigmented pupae inside gall chamber in *R. brasiliensis*. Scale bars: A, B and D, 2 mm; C, E and F, 1 mm.

Male. As in female except as follows: body size tending to be smaller; F2–F4 about 0.8x as long as F1; metasoma more compact.

Etymology. The specific name is based on the country where the type series was collected.

Type material. Holotype ♀, “Brasil, PR, Piraquara, \25.5122°S 49.0033°W, \965m, G.A.R. Melo” “Nascido em 19.x.2024\ de galha em ramo de \ *Rubus* coletada em \ 17.x.2024”. Paratypes: 3 ♀ and 3 ♂, same data as holotype; 1 ♀, same data except “Nascido em 16.x.2024...”; 1 ♀ and 2 ♂, same data except “Nascido em 17.x.2024...”; 5 ♀, same data except “Nascido em 21.x.2024...”; 5 ♀ and 6 ♂, same data except “Nascido em 25.x.2024...”; 3 ♀ and 7 ♂, same data except “Nascido em 26.x.2024...”; 7 ♀ and 8 ♂, same data except “Nascido em 28.x.2024...”; 12 ♀ and 2 ♂, same data except “Nascido em 29.x.2024...”; 7 ♀ and 4 ♂, same data except “Nascido em 1.xi.2024...”; 6 ♀ and 6 ♂, same data except “Nascido em 4.xi.2024...”; 7 ♀ and 1 ♂, same data except “Nascido em 6.xi.2024...”; 4 ♀ and 1 ♂, same data except “Nascido em 9.xi.2024...”; 4 ♀ and 2 ♂, same data except “Nascido em 11.xi.2024...”; 2 ♂ “Brasil, PR, S.J.Pinhais, \25.605°S 49.193°W, \880m, 1–30.x.2016, \A. C. Domahovski”; 1 ♀, same data except “1–30.x.2019”. The holotype and 100 paratypes are deposited in the DZUP collection; 6 ♀ and 4 ♂ paratypes will be sent to the MZSP collection.

Variation. In some females, the last flagellomere is fused with the preceding one, resulting in antennae with only 12 flagellomeres. Sometimes, this is observed in only one of the antennae. This fusion can also be partial and the constriction between the last two flagellomeres being perceptible on one side only. In males, the length of the 13th flagellomere can be variable, with most of them having a short F13, as in most females, while in some the F13 is slightly longer than F12. In a single male, we observed a partial constriction on its long F13, giving the impression of antennae with 14 flagellomeres.

Biology. Two species of *Rubus*, *R. brasiliensis* Mart. and *R. sellowii* Cham. & Schlitdl., were confirmed as host plants for *D. brasiliensis* sp. nov. The galls were generally cryptic (Fig. 5), with less pronounced external hypertrophy of the plant tissues. However, galls on *R. sellowii* (Fig. 6A–D) exhibited more pronounced hypertrophy compared to those on *R. brasiliensis*.

A single galled branch of *R. sellowii* was obtained on May 25th. Upon its partial dissection (Fig. 6A–D), carried out three days later, we obtained a few larvae that were identified as belonging to *Diastrophus* based on their asymmetrical mandibles (see Nieves-Aldrey *et al.* 2005). Identification of the larvae was corroborated by the CO1 sequence obtained from one of them (L3 in Fig. 1). Three females (May 29th, June 20th, 2024) and one male (June 6th, 2024) of Torymidae, plus one female and one male (June 18th, 2024) of Eurytomidae emerged from this branch, but no adult *Diastrophus*.

During a second visit to the sampling site, we obtained additional galled branches from *R. brasiliensis* (Fig. 5). The branches already had many emergence holes and several adult *Diastrophus* emerged in the subsequent weeks (see Type Material). Dissection of one branch, on the collecting day, revealed both white and pigmented pupae of *Diastrophus* within the gall chambers (Fig. 6E–F). In addition to the *Diastrophus* adults, the following insects emerged from the galled branches of *R. brasiliensis*: 9 females of Eulophidae; 6 females and 2 males of Eurytomidae; 9 females of Ichneumonidae (Orthocentrinae); 2 females of Pteromalidae; 1 male of Torymidae; and 4 females and 4 males of Sciaridae (Diptera). These other Hymenoptera are likely inquiline of the *Diastrophus* galls or their larval parasitoids, except for the Orthocentrinae ichneumon wasps, which are known to attack Sciaroidea (see Gauld 2006: 480).

Discussion

At first glance, the new species of *Diastrophus* from Brazil differs markedly from the rest of the genus as it has an entirely rugose mesoscutum (Fig. 2D, 3D). In contrast, other species of *Diastrophus* have a polished mesoscutum, a feature that has been used as diagnostic for the genus (Nieves-Aldrey 1994; Nastasi *et al.* 2024). Currently, only *D. colombianus* exhibits a partly rugose mesoscutum, with the area between the parapsidal signa and the posterior half of the notauli showing some rugosity (Nieves-Aldrey *et al.* 2013). This indicates that only the South American species of *Diastrophus* exhibit some degree of rugosity on the mesoscutum.

Our phylogenetic analyses corroborate the relationships between Diastrophini genera as recovered in previous studies (Blaimer *et al.* 2020; Pang *et al.* 2020). While these relationships are generally well resolved, the placement of the recently described genus *Xestophanopsis* (see Pujade-Villar *et al.* 2019) remains uncertain. Within *Diastrophus*, phylogenetic relationships are not well resolved, especially due to the limited molecular data available and the high number of unidentified terminals. Previous morphological studies by Shick *et al.* (2003), Aldrey *et al.* (2013) and Wachi *et al.* (2013) have proposed phylogenetic hypotheses for the genus, but their low consistency indices point

to high levels of homoplasy in the morphological character dataset. Therefore, until comprehensive morphological and/or molecular analyses of all *Diastrophus* species are conducted, the relationships within the genus will remain uncertain.

Although *D. brasiliensis* **sp. nov.** is clearly nested within the genus, it is not closely related to any of the other lineages included in our analyses. Its unusually long branch (Fig. 1) points to an ancient divergence from other clades. We suspect that *D. brasiliensis* **sp. nov.** will be shown to be closely related to *D. colombianus*, based not only on their shared Neotropical distribution (Table 2) but also on the similarities of their biology, including gall morphology, as well as possession of rugose sculpture on the mesoscutum.

Prior to this study, there were no confirmed records of Cynipidae in Brazil, although older literature suggested their presence in the country, particularly by classifying *Myrtopsen* Rübsaamen (Thrasorinae, Figitidae) within this family. However, the occurrence of this wasp family in Brazil was suggested by Melo *et al.* (2012) when referring to *Eschatocerus* Mayr, a cynipid genus known from Argentina and Uruguay, given that one of the host plants of *E. acaciae* Mayr, *Vachellia caven* (Molina) Seigler & Ebinger (Fabaceae; previously placed in *Acacia*), occurs in a small area of Rio Grande do Sul, in southern Brazil.

TABLE 2. Known species of *Diastrophus*, with indication of their main geographic distribution and host plants. Modified from Schick *et al.* (2003). WN: Western Nearctic; EN: Eastern Nearctic; WP: Western Palearctic; EP: Eastern Palearctic; OR: Oriental; NT: Neotropical.

Species	Distribution	Host Plant	Reference
<i>D. austrior</i> Kinsey, 1922	WN	<i>Rubus</i>	Burks (1979)
<i>D. bassetti</i> Beutenmüller, 1892	EN	<i>Rubus</i>	Burks (1979)
<i>D. brasiliensis</i> sp. nov.	NT	<i>Rubus</i>	Present study
<i>D. colombianus</i> Nieves-Aldrey, 2013	NT	<i>Rubus</i>	Nieves-Aldrey <i>et al.</i> (2013)
<i>D. cuscutoeformis</i> Osten Sacken, 1863	EN	<i>Rubus</i>	Burks (1979)
<i>D. fragariae</i> Beutenmüller, 1915	EN	<i>Fragaria</i>	Burks (1979)
<i>D. fusiformans</i> Ashmead, 1890	EN, WN	<i>Potentilla</i>	Burks (1979)
<i>D. hieracii</i> Melika & Klymenko, 2005	EP	Unknown*	Nastasi <i>et al.</i> (2025)
<i>D. japonicus</i> Wachi, Ide & Abe, 2013	EP	<i>Rubus</i>	Wachi <i>et al.</i> (2013)
<i>D. kincaidii</i> Gillette, 1893	WN	<i>Rubus</i>	Burks (1979)
<i>D. mayri</i> Reinhard, 1877	WP	<i>Potentilla</i>	Nieves-Aldrey (1994)
<i>D. minimus</i> Bassett, 1900	EN	<i>Potentilla</i>	Burks (1979)
<i>D. nebulosus</i> (Osten Sacken, 1861)	EN	<i>Rubus</i>	Burks (1979)
<i>D. niger</i> Bassett, 1900	EN	<i>Potentilla</i>	Burks (1979)
<i>D. potentillae</i> Bassett, 1864	EN	<i>Potentilla</i>	Weld (1952)
<i>D. radicum</i> Bassett, 1870	EN, WN	<i>Rubus</i>	Burks (1979)
<i>D. renai</i> Davis & Nastasi, 2024	OR	Unknown	Davis <i>et al.</i> (2024)
<i>D. rubi</i> (Bouché, 1834)	WP	<i>Rubus</i>	Nieves-Aldrey (1994)
<i>D. smilacis</i> Ashmead, 1896	EN	Unknown**	Gates <i>et al.</i> (2020)
<i>D. tumefactus</i> Kinsey, 1920	EN	<i>Potentilla</i>	Burks (1979)
<i>D. turgidus</i> Bassett, 1870	EN	<i>Rubus</i>	Burks (1979)
<i>D. wushei</i> Davis & Nastasi, 2024	OR	Unknown	Davis <i>et al.</i> (2024)

*Melika & Klymenko (2005) originally indicated *Hieracium* (Asteraceae) as the host plant (see Nastasi *et al.* 2025);

**Previously thought to induce galls on *Smilax*, but this association was erroneous (Gates *et al.* 2020).

We expect that additional species of *Diastrophus* will likely be found in the Neotropical region, especially along the Andes, taking into account that South America is home to many other native species of *Rubus*. Considering that galls made by the Neotropical *Diastrophus* are somewhat inconspicuous, that the period of adult emergence is quite short, and that these wasps are very rarely collected in Malaise traps, discovery of new species should most likely occur through targeted surveys. Indeed, we recently collected in Serra da Bocaina, northeastern São Paulo, some

Rubus stems bearing galls, from which no insects emerged, but upon dissection revealed a malformed, crumpled dead female of *Diastrophus* morphologically distinct from the species herein described. Furthermore, these surveys may also reveal the presence of *Synophromorpha* Ashmead, an inquiline diastrophine genus associated with galls made by *Diastrophus*, whose distribution in the New World includes Mexico and the United States (Nastasi *et al.* 2024).

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