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Uruguayan populations of *Gonipterus* Schoenherr, 1833 (Coleoptera, Curculionidae) belong to two species

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Abstract. The eucalypt snout beetle genus *Gonipterus* Schoenherr 1833 (Coleoptera, Curculionidae) has been present in Uruguay since the 1930s, although the identity of the species remained uncertain. We confirm the presence of at least two species, *G. platensis* (Marelli, 1926) and *G. pulverulentus* Lea, 1897, using both morphological and mitochondrial COI analyses. Both species occur in commercial *Eucalyptus* plantation throughout the country. This represents the first molecular evidence of their coexistence in Uruguay, providing key information for future management and biological control programs in eucalypt forestry.

Keywords. COI, *Eucalyptus*, forestry, *Gonipterus gibberus, G. platensis*, *G. pulverulentus*, *G. scutellatus*

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Introduction

Correct identification of insect pests is essential for accurate phytosanitary reporting, designing monitoring programs, and implementing biological control strategies. Misidentification can lead to ineffective management decisions and unnecessary economic losses, particularly in forestry systems where cryptic species complexes are common (Mapondera et al. 2012; Nanini et al. 2022). Furthermore, precise species delimitation is critical to ensure that biological control programs—especially those based on parasitoids—are resourcing to the correct host-specific natural enemies (Hajek 2004). Molecular tools such as mitochondrial COI sequencing have proven to be reliable complements to morphological identification in distinguishing closely related weevil species (Hall 1999; Simon et al. 2006).

The genus *Gonipterus* Schoenherr, 1833 (Curculionidae, Gonipterinae) was first reported in Uruguay in 1937 from urban *Eucalyptus* L'Hér. trees, and the species was initially misidentified as *Gonipterus gibberus* Boisduval 1835 (Trujillo 1963). It was assumed at that time that the insect species had dispersed from Argentina, where it had been previously reported as *Dacnirotatus platensis* Marelli, 1926, protonym of *Gonipterus platensis* (Marelli, 1926). Given the insect spread from the urban trees in the capital to planted eucalypts inrural areas, a biological control program with the parasitoid *Anaphes nitens* (Girault, 1928) (Hymenoptera, Myrmaridae) was rapidly deployed, leading to a first shipment of the biological control agent from South Africa in 1941 and later from Argentina in 1950 (Trujillo 1963; Porcile 1996). The taxonomic status of the *Eucalyptus* snout beetle in Uruguay, Argentina, and southern Brazil kept changing in the following decades, but generally accepting the occurrence of a single species (*Gonipterus scutellatus* Gyllenhal, 1833) or two species (*G. scutellatus* and *G. gibberus*) were thought, based on morphological descriptions of the adults and larvae(Rosado-Neto and Marques 1996).

Recent studies have shown that the so-called *G. scutellatus* species complex comprises several closely related and morphologically similar taxa historically confused under the name *G. scutellatus*. As treated by Mapondera et al. (2012), the complex includes *G. scutellatus* sensu stricto, *G. platensis*, *G. pulverulentus* Lea, 1897, *G. balteatus* Pascoe, 1870, and several undescribed species. Molecular phylogenetic analyses demonstrated that what was once regarded as a single cosmopolitan species actually represents multiple distinct taxa with different geographic distributions and invasion histories (Mapondera et al. 2012; Nanini et al. 2022). These findings have major implications for forestry pest management, as each species differs in host preference, climatic tolerance, and interaction with

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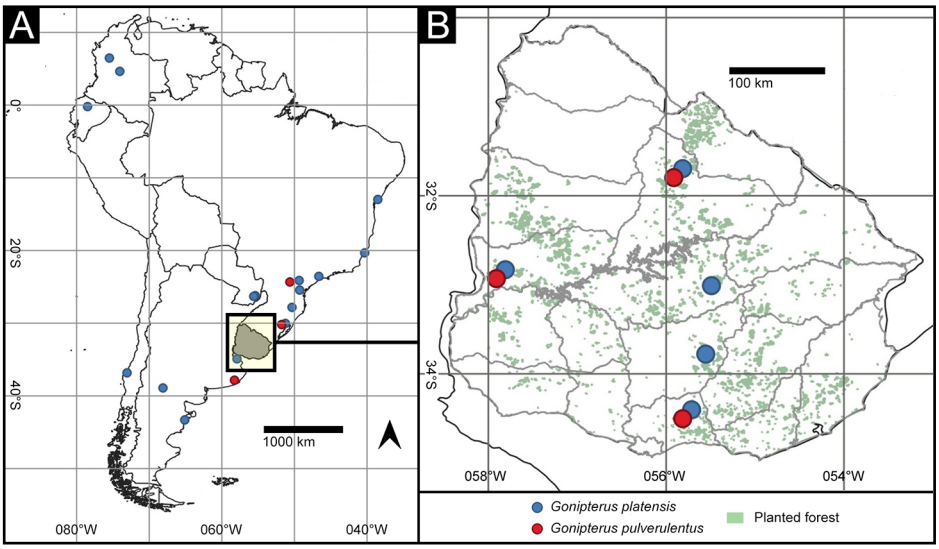

Figure 1. Distribution map of *Gonipterus* species. **A.** South America showing confirmed localities based on morphological and molecular identification; *G. platensis* exhibits a broad range extending from Argentina to Ecuador, whereas *G. pulverulentus* is restricted to Argentina, Uruguay, and southern Brazil. **B.** Specimen collection sites in Uruguay.

biological control agents such egg parasitoids (Schröder et al. 2019; Barten et al. 2023). Understanding this hidden diversity is therefore essential for developing precise monitoring and control strategies in affected eucalypt-growing regions.

The objective of this study was to determine the specific identity of the *Gonipterus* populations present in Uruguay by combining diagnostic morphological characters of adults and larvae with mitochondrial COI sequence data to assess whether they correspond to a single or multiple species.

Methods

Insect collections and morphological identification. Specimens were manually collected from *Eucalyptus* tree plantations in five departments (Canelones, Durazno, Florida, Río Negro, and Tacuarembó) covering the eucalypt forestry regions (Figure 1), from the 2022–2025 crop season. A distribution map was created using QGIS v. 3.44 (QGIS Development Team 2025). Voucher specimens were deposited in the Forestry Entomological Collection of the Instituto Nacional de Investigación Agropecuaria (INIA) in Tacuarembó, Uruguay. Larval and adult specimens were identified by morphological traits following Rosado-Neto and Marques (1996) and Mapondera et al. (2012). The diagnostic characters evaluated in adults included body colouration, rostrum pigmentation, distribution and colouration of scales on the pronotum, humeral region and elytra, presence or absence of an oblique-transverse median band of whitish scales on the elytra, development of basal elytral tubercles, and general dorsal habitus. In larvae, the evaluated traits included body colouration, abdominal pigmentation pattern, and presence or absence of longitudinal, dark abdominal stripes. These characters were used to distinguish *G. platensis* from *G. pulverulentus*. Male and female genitalia were also examined as diagnostic characters. Adult abdomens were removed with fine forceps, and genitalia were dissected. Genital structures were cleared in hot 10% potassium hydroxide (KOH), following the general procedure described by Rosado-Neto and Marques (1996), then rinsed in distilled water and examined using an Olympus SZ61 binocular stereomicroscope and a Nikon E100 microscope.

For males, the evaluated genital traits included the shape and curvature of the median lobe, the structure of the internal sac armature, number and arrangement of sclerites and dorsal processes, and the length, thickness and curvature of the gastral spicule. For females, diagnostic traits included the shape of the eighth sternite, curvature of the lateral margins, shape and divergence of the ventral-spicule arms, shape of the basal projection of the coxites, and position of the spermathecal duct.

Molecular identification. Larval individuals were preserved in ethanol 70% at 20 °C for DNA extraction. Selected individual larvae were selected and macerated in a mortar with liquid nitrogen. The DNeasy Plant Mini Kit (QIAGEN) extraction protocol was then executed. The DNA was eluted in 50 microliters of RNAse-treated TE buffer and analyzed using a nanodrop and gel electrophoresis.

The COI fragment was amplified via polymerase chain reaction (PCR) from the total DNA extracted from each specimen. The following primers described by Simon et al. (2006) were used: C1J2183 (Forward) (5′-CAACATTTATTTTGATTTTTTGG-3′) and TL2N3014 (Reverse) (5′-TCCATTGCACTAATCTGCCATATTA-3′). The PCR reactions were carried out following Nanini et al. (2022). A 2% agarose gel was used

to verify the PCR results by loading 5 µl of the amplified product. PCR product samples were submitted to Macrogen's sequencing service (Seoul, Korea) for forward and reverse sequencing. The obtained sequences were aligned and processed to generate a consensus sequence using BIOEDIT software (Hall et al. 1999). Specimen consensus sequences were deposited on GenBank (accession numbers PX501556, PX50155, PX501558, PX501559, PX501560, PX501561, PX501562, PX501563, PX501564, PX501565, PX501566, PX501567).

Phylogenetic analysis. Sequences reported herein were compared through phylogenetic analysis with sequences corresponding to the 34 haplotypes of the genus *Gonipterus* reported by Nanini et al. (2022). DNA sequences were aligned using the ClustalW algorithm (Thompson et al. 1994) implemented in MEGA11 software (Tamura et al. 2021). Phylogenetic relationships were inferred using the maximum-likelihood method under the Kimura 2-parameter (K2P) model, following Kimura (1980). Although other studies of the *G. scutellatus* species complex have employed more parameter-rich models, the K2P model yielded well-supported topologies consistent with previously published phylogenies of the complex (Mapondera et al. 2012; Nanini et al. 2022), confirming the reliability of the species assignments. The tree with the highest log-likelihood is shown. Bootstrap analysis was conducted with 1000 replicates to assess the statistical support for each node (Felsenstein 1985). All phylogenetic analyses were performed using MEGA 11 (Tamura et al. 2021).

Results

Order Coleoptera
Family Curculionidae

***Gonipterus platensis* (Marelli, 1926)**

New records. URUGUAY — Florida • Casupá; 33°46′43″S, 055°33′13″W; 136 m alt.; 18.VIII.2022; A. García-Feijó leg.; *Eucalyptus* sp. stand; GenBank PX501556; INIA CCGpla001♂ dry preservation, CCGpla002♀ dry preservation; CCGpla003♂ wet preservation, CCGpla004♀ wet preservation — Durazno • Carmen; 32°49′51″S, 057°48′30″W; 120 m alt.; 2.IX.2022; A. García-Feijó leg.; *Eucalyptus* sp. stand; GenBank PX501557; INIA CCGpla005♂ dry preservation, CCGpla006♀ dry preservation; CCGpla007♂ wet preservation, CCGpla008♀ wet preservation — Tacuarembó • Tacuarembó; 31°41′46″S, 055°48′48″W; 140m alt.; 20/VIII/2024; M. Suarez leg.; *Eucalyptus* sp. stand; GenBank PX501558, PX501559; INIA CCGpla009♂ dry preservation, CCGpla010♀ dry preservation; CCGpla011♂ wet preservation, CCGpla012♀ wet preservation — Canelones • San Ramón; 34°24′14″S, 055°42′47″W; 90 m alt.; 22/XII/2024; M. Suarez leg.; *Eucalyptus* sp. stand; GenBank PX501564, PX501565; INIA CCGpla013♂ dry preservation, CCGpla014♀ dry preservation; CCGpla015♂ wet preservation, CCGpla016♀ wet preservation — Río Negro • Young; 32°49′51″S, 057°48′30″W; 73 m alt.; 14/VII/2025; M. Suarez leg.; *Eucalyptus* sp. stand; GenBank PX501566, PX501567; INIA CCGpla017♂ dry preservation, CCGpla018♀ dry preservation; CCGpla019♂ wet preservation, CCGpla020♀ wet preservation. Figure 1.

***Gonipterus pulverulentus* Lea, 1897**

New records. URUGUAY — Tacuarembó • Tacuarembó; 31°41′46″S, 055°48′48″W; 140 m alt.; 20/VIII/2024; M. Suarez leg.; *Eucalyptus* sp. stand; GenBank PX501560, PX501561; INIA CCGpul001♂ dry preservation, CCGpul002♀ dry preservation; CCGpul003♂ wet preservation, CCGpul004♀ wet preservation. — Canelones • San Ramón; 34°24′14″S, 055°42′47″W; 90 m alt.; 22/XII/2024; M. Suarez leg.; *Eucalyptus* sp. stand; GenBank PX501562; INIA CCGpul005♂ dry preservation, CCGpul006♀ dry preservation; CCGpul007♂ wet preservation, CCGpul008♀ wet preservation — Río Negro • Young; 32°49′51″S, 057°48′30″W; 73 m alt.; 14/VII/2025; M. Suarez leg.; *Eucalyptus* sp. stand; GenBank PX501563; INIA CCGpul009♂ dry preservation, CCGpul010♀ dry preservation; CCGpul011♂ wet preservation, CCGpul012♀ wet preservation. Figure 1.

Morphological identification.

Adults—Adults of *G. platensis* and *G. pulverulentus* are externally similar but can be distinguished by their colour pattern, rostrum pigmentation, and the distribution of scales on the prothorax and elytra. In *G. pulverulentus*, the body is light brown, the rostrum is uniformly black, and the elytra bear a conspicuous, obliquely transverse, median band of whitish scales; dark scales are absent from the pronotum and basal elytral tubercles (Figure 2C). In contrast, *G. platensis* is darker brown to reddish brown, with the rostrum laterally black and medially reddish. The pronotum, humeral region, and basal elytral tubercles are densely covered with dark scales, while the elytra lack the whitish transverse band characteristic of *G. pulverulentus* (Figure 2A).

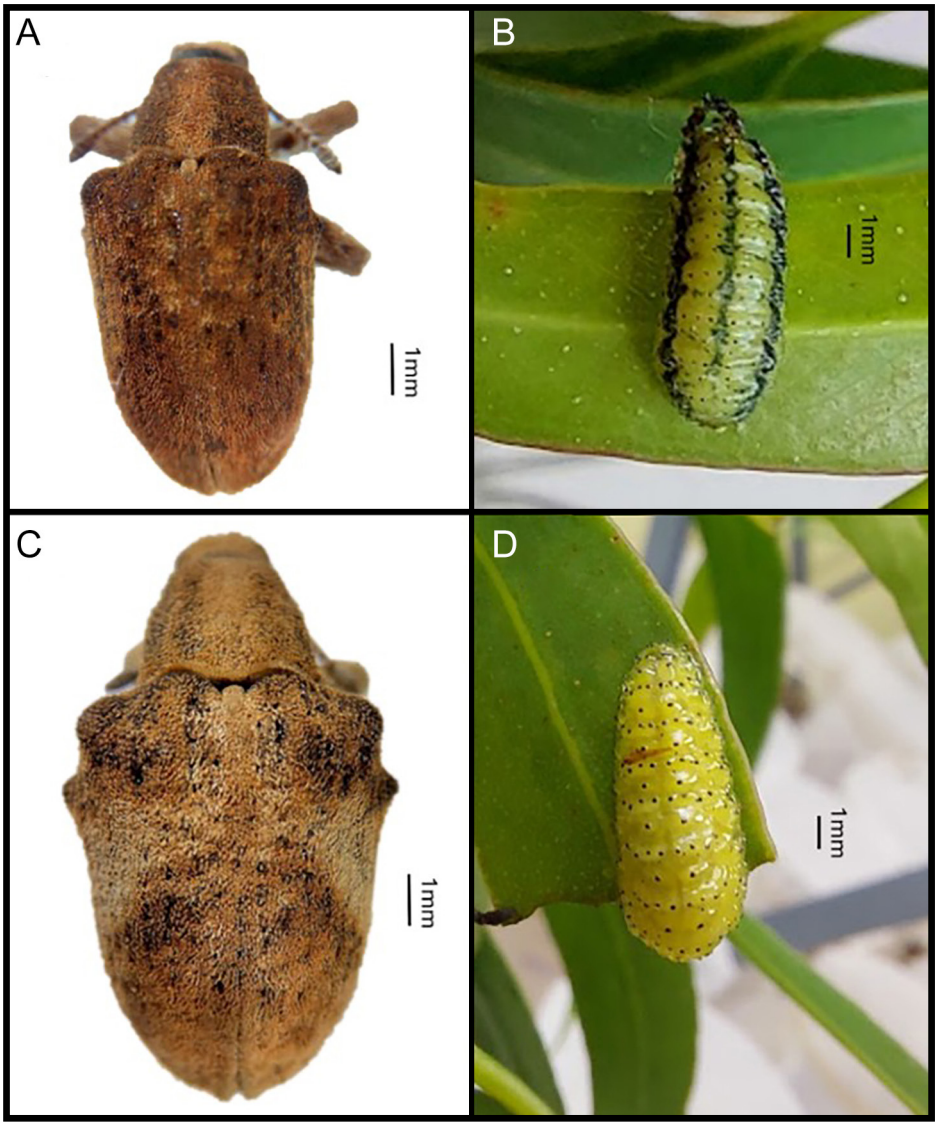

Figure 2. Dorsal views of *Gonipterus* species. **A.** Adult *G. platensis*. **B.** Larval *G. platensis*. **C.** Adult *G. pulverulentus*. **D.** Larval *G. pulverulentus.*

Larvae—Larvae of *G. pulverulentus* are light yellowish to cream and lack longitudinal dark stripes on the abdomen (Figure 2D). In *G. platensis*, the larvae exhibit three greenish to black longitudinal stripes, two lateral stripes, and one median dorsal stripe, less distinct in pupae (Figure 2B). Both species share an overall similar external morphology, but the genital structures provide reliable distinguishing characters (Rosado-Neto and Marques 1996).

Male genitalia—The median lobe in both species is elongated and slightly curved. *Gonipterus pulverulentus* shows a more complex armature of the internal sac, with three distinct sclerites and multiple dorsal processes (one on the right and two on the left; Figure 3A–C), whereas *G. platensis* bears only two lateral processes (one per side) that are shorter and simpler (Figure 3D–F). The gastral spicule of *G. pulverulentus* is very long, slender, and slightly curved, while in *G. platensis* it is thicker, shorter, and straight. Thus, the internal sac structure and spicule proportions constitute the main male diagnostic traits.

Female genitalia—The eighth sternite of *G. pulverulentus* has lateral margins recurved outward and ventral-spicule arms curved and divergent (Figure 3G), contrasting with *G. platensis*, where both margins and spicule arms are straight (Figure 3H). The basal projection of each coxite is long and straight in *G. pulverulentus* but short, broad, and slightly curved in *G. platensis*. The spermathecal duct attaches farther from the vagina in *G. pulverulentus* but closer in *G. platensis.*

In summary, *G. pulverulentus* can be recognized by its elongated gastral spicule, more intricate trilobed internal sac armature, recurved female sternite margins, and curved ventral-spicule arms; and *G. platensis* by its stout spicule, simpler armature with two lateral processes, straight sternite margins, and straight spicule arms.

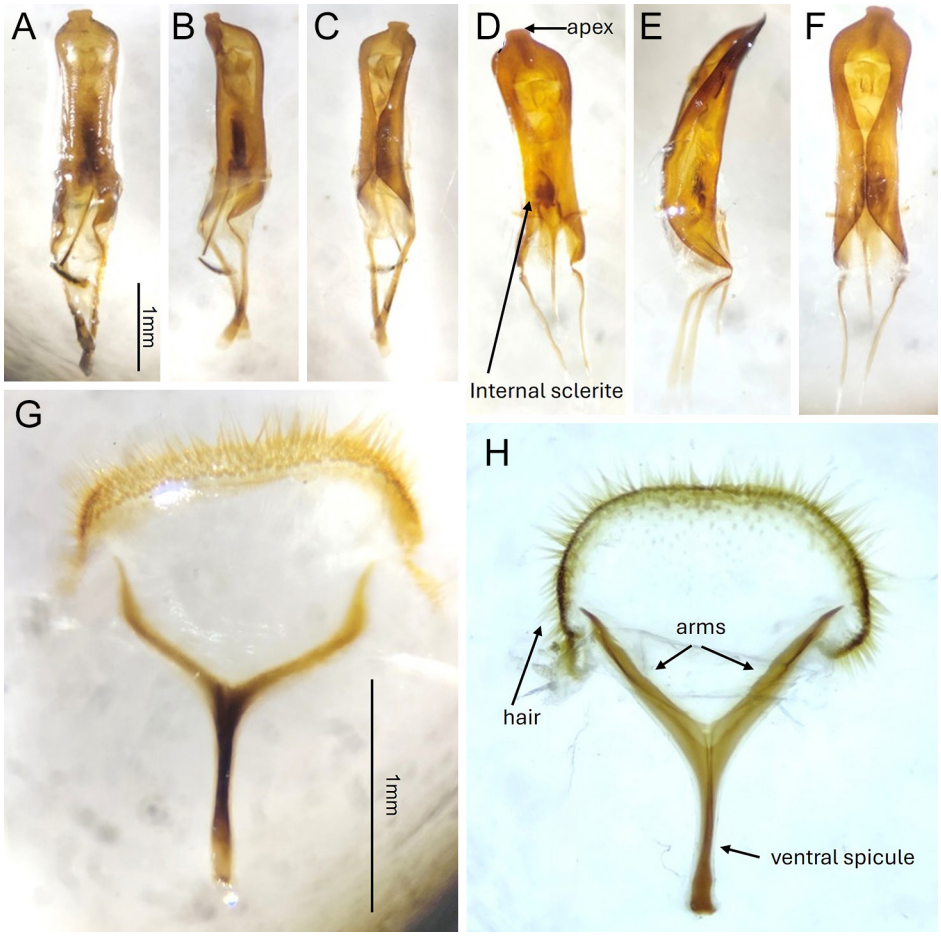

Figure 3. Genitalia of *Gonipterus* species. **A–C.** Ventral, lateral, and dorsal views of the aedeagus of *G. pulverulentus*. **D–F.** Ventral, lateral, and dorsal views of the aedeagus of *G. platensis*. **G.** Genitalia of female *G. pulverulentus*. **H.** Genitalia of female *G. platensis*.

Molecular identification. The maximum-likelihood phylogenetic analysis of the COI fragment (Figure 4) resolved all Uruguayan specimens into two well-supported clades corresponding to *G. platensis* and *G. pulverulentus*. Samples 1–17 from Uruguay clustered unambiguously with their respective reference haplotypes from the dataset of Nanini et al. (2022), with no Uruguayan specimen showing affinity to any other species of the *G. scutellatus* complex. The G. *platensis* Uruguayan sequences grouped with haplotypes previously reported from Argentina, southern Brazil, and other invaded regions, whereas the *G. pulverulentus* Uruguayan sequences were closely related to haplotypes known from Argentina and southern Brazil exclusively. Bootstrap support values at the nodes defining the *G. pulverulentus* and *G. platensis* clades were 81 and 65, respectively, confirming the reliability of the species assignments. No haplotype sharing was observed between the two species, and intraspecific genetic distances within each Uruguayan group were low, consistent with the reduced mitochondrial diversity previously described for both species in South America (Nanini et al. 2022).

Discussion

Our data confirm the presence of two species of the *Gonipterus scutellatus* complex in Uruguay, *Gonipterus platensis* and *G. pulverulentus*, co-occurring in commercial *Eucalyptus* plantations. Among them, *G. platensis* is the most widespread species of the complex, established in New Zealand, Europe, Africa, and the Americas (Mapondera et al. 2012). In South America, it has been recorded from Argentina (Marelli 1926), Chile (Mapondera et al. 2012), Brazil (Nanini et al. 2022), Ecuador (Crespo-Pérez et al. 2023), Colombia (Gamarra and Marin 2024), Paraguay (Benítez et al. 2025), and Uruguay (this study).

In contrast, *G. pulverulentus* shows a more restricted distribution, reported from Argentina and southern Brazil (Mapondera et al. 2012; Riquelme et al. 2018; Garcia et al. 2019) and confirmed in this study for Uruguay. The close relationship of Uruguayan *G. pulverulentus* sequences with haplotypes from Argentina and southern Brazil, and their absence from more northern latitudes, further supports the notion that this species has a more limited dispersal capacity and is associated with cooler climatic conditions (Nanini et al. 2022). The low intraspecific genetic distances observed in both species

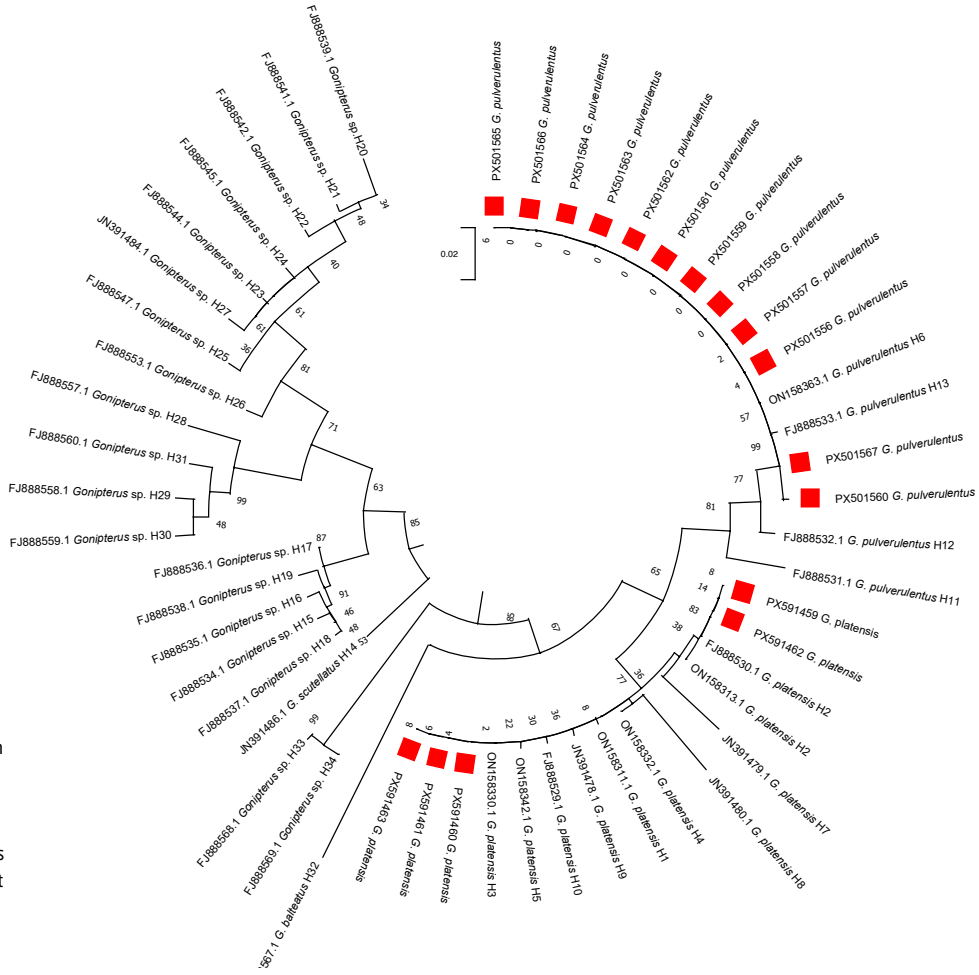

Figure 4. Phylogenetic tree of COI sequences showing the relationships between Uruguayan samples of *Gonipterus* species (samples 1–17) and other haplotypes reported globally (H1–H35, followed by the GenBank accession number and country of origin). Uruguayan samples are highlighted in red. The results confirm that the Uruguayan samples cluster with *G. platensis* and *G. pulverulentus.*

reinforce the pattern of reduced mitochondrial diversity typical of invasive populations established from a small number of founding individuals (Nanini et al. 2022).

Gonipterus populations in South America currently occupy a broad latitudinal gradient from approximately 43°S (Chubut, Argentina) to 6°N (Antioquia, Colombia), highlighting their capacity for climatic adaptation and regional spread. The phylogenetic placement of Uruguayan specimens within the *G. platensis* and *G. pulverulentus* clades (Figure 4) is consistent with a shared invasion history across the Southern Cone, likely derived from a single introduction event subsequently expanded through human-mediated dispersal associated with *Eucalyptus* seedling and timber transport (Nanini et al. 2022). These results resolve earlier misidentifications as *G. gibberus* and provide the first validated national record of *G. pulverulentus*.

The confirmation of two species of the *G. scutellatus* complex in Uruguay has important implications for forest pest management. Both species occur across different regions of the country with no apparent distribution pattern (Fig. 1B). In their native range in Tasmania, both species occur sympatrically and are strongly associated with *Eucalyptus ovata* Labill., occasionally utilizing other members of section *Maidenaria* L.D.Pryor & L.A.S.Johnson ex Brooker, such as *E. globulus* Labill. and *E. viminalis* Labill., while experimental studies show that *G. platensis* can develop on several *Eucalyptus* species in invaded areas (Cordero and Santolamazza 2000; Garcia et al. 2019). In Uruguay, however, commercial plantations are dominated by other *Eucalyptus* species, suggesting that both weevils persist mainly on secondary hosts, a shift that may affect fecundity, voltinism, damage patterns, and tritrophic interactions with parasitoids (Garcia et al. 2019; Valente et al. 2019). This distinction is particularly relevant for biological control with *Anaphes nitens*, the main classical agent released against the *Gonipterus* complex (Lanfranco and Dungey 2001). Because historical control programs in Uruguay assumed the presence of a single species (Trujillo 1963; Porcile 1996; Morey and Porcile 2002), the confirmation of two species may help explain variable field performance of *A. nitens*, since biological control outcomes in the *Gonipterus scutellatus* complex are likely influenced by the specific associations among eucalypt hosts, weevil species, and egg parasitoids (Garcia et al. 2019).Further studies should assess the

distribution of both *Gonipterus* species across the country, quantify parasitism rates of *A. nitens* on each species, and evaluate additional parasitoids reported in the native range, particularly *Anaphes tasmaniae* Huber & Prinsloo, 1990 and *Anaphes inexpectatus* Huber & Prinsloo, 1990, to better tailor integrated pest management strategies in the region.

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Additional information

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

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Author contributions

Conceptualization: GMC, NGP. Data curation: NGP, DTD. Formal analysis: DTD, NGP, GMC. Funding acquisition: NGP, DTD, GMC. Investigation: GMC, DTD, NGP. Methodology: DTD, NGP, GMC. Resources: GMC, NGP, DTD. Visualization: NGP, DTD. Writing – original draft: GMC, NGP, DTD. Writing – review and editing: GMC, NGP, DTD.

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Data availability

All data that support the findings of this study are available in the main text.

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