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Scientific Note

A close relationship: *Euwallacea fornicatus* (Eichhoff, 1868) haplotype H22 (Coleoptera: Curculionidae: Scolytinae) and *Ricinus communis* L. (Euphorbiaceae) in Brazil

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Abstract. *Euwallacea fornicatus* (Eichhoff, 1868) (Coleoptera: Curculionidae: Scolytinae), the polyphagous shot hole borer (PSHB), is native to Asia and it has been recorded in Argentina (2023), Brazil (2024), and recently in Uruguay and Türkiye (2025), among other regions of the world. We document, for the first time, *E. fornicatus* on castor bean, *Ricinus communis* L. (Euphorbiaceae), in Brazil, which also represents the first record on this host for South America. A molecular phylogenetic analysis of PSHB from castor bean across Brazil, based on mitochondrial gene *cytochrome c oxidase subunit I* (COI) sequences, revealed a single haplotype (H22) in South America. We recorded PSHB on castor bean in 28 municipalities from five Brazilian states. Our results highlight the importance of including *R. communis* in PSHB monitoring programs.

Keywords: Ambrosia beetle, Breeding host, Castor bean, Polyphagous shot hole borer.

Euwallacea fornicatus (Eichhoff, 1868) (Coleoptera: Curculionidae: Scolytinae) is native to Asia and is now established in various regions of the world (Gomez & Johnson 2019; Gallego et al. 2025). In South America, the pest has been reported in Argentina, Uruguay (Ceriani-Nakamurakare et al. 2023; 2025), and Brazil (Covre et al. 2024).

The *E. fornicatus* complex comprises four species, namely *E. fornicatus* (polyphagous shot hole borer – PSHB), *Euwallacea perbrevis* (Schedl, 1951) (tea shot hole borer a – TSHBa), *Euwallacea fornicatior* (Eggers, 1923) (tea shot hole borer b – TSHBb), and *Euwallacea kuroshio* Gomez & Hulcr, 2018 (Kuroshio shot hole borer – KSHB) (Smith et al. 2019). Within PSHB, a genetically divergent COI haplotype (termed haplotype 22 or H22) has been identified in China (Taiwan) (see Stouthamer et al. 2017; Liu et al. 2022; Wang et al. 2022).

The *E. fornicatus* complex is associated with approximately 600 plant species, 110 of which are considered reproductive hosts, including several economically important crops (Mendel et al. 2017; Acer et al. 2025). Among these, *Ricinus communis* L. (Euphorbiaceae) has been recognized as a reproductive host for the PSHB (King 1941; Gadd & Loos 1947; Eskalen et al. 2012; Mendel et al. 2012; Li et al. 2016). This plant is widespread and frequently regarded as a weed in urban and agricultural settings (WFO 2025). However, castor bean is also cultivated for its oil, which serves multiple purposes (Milani et al. 2011).

Reports of *E. fornicatus* in South America indicate low or no genetic variability, suggesting a single invasion event (Ceriani-Nakamurakare et al. 2023; 2025; Covre et al. 2024). While several tree species have been documented as hosts of *E. fornicatus* in the region, we report, for the first time, the presence of *E. fornicatus* on *R. communis* in Brazil.

The sampling of *E. fornicatus* specimens was conducted exclusively from castor bean (*R. communis*) across 46 municipalities in 10 Brazilian states from April 2023 to September 2024. Records encompassed *in situ* observations of stem damaged by the pest and manual collection of specimens (S1, Fig. 1; S2, Tab. 1).

The specimens found in *R. communis* were identified as *E. fornicatus* using the keys of Gomez et al. (2018) and Smith et al. (2019). Voucher specimens (ESALQENT001897–ESALQENT001918) were deposited in the "Luiz de Queiroz" Entomology Museum (MELQ-ESALQ/USP), Piracicaba, São Paulo, Brazil.

To identify *E. fornicatus* haplotypes from castor bean, the first third of the *cytochrome c oxidase subunit I* (COI) gene was amplified using PCR with the specific primer pair COI_1455b and COI_r750 (Smith & Cognato 2014). Initially, DNA was extracted from the entire body of *E. fornicatus* using the CTAB method (Doyle & Doyle 1987) from specimens collected in four Brazilian states: Paraná (n = 3), Rio Grande do Sul (n = 5), Santa Catarina (n = 5), and São Paulo (n = 3).

PCR reactions were performed in a total volume of 25 µL with Invitrogen™ reagents, containing 12.5 µL ultrapure water, 2.5 µL buffer (10×Mg^{2+}), 2 µL of $MgCl_2$ (50 mM), 0.8 µL of dNTPs (10 µM), 2 µL of each primer (5 nM), 3 µL of DNA (40 ng), and 0.2 µL of Taq platinum DNA Polymerase (1 U). Primary denaturation lasted 3 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 54 °C, and 2 min at 72 °C, with a final polymerization for 10 min at 72 °C.

The amplification was confirmed using an agarose gel, and the PCR product was purified with Exonuclease I (EXO I) and thermosensitive alkaline phosphatase (FastAP). Sequencing was performed using the Sanger method. The sequences were reviewed and edited with BioEdit, deposited in the National Center for Biotechnology Information (NCBI) [PQ580203–PQ580218].

To characterize the haplotype of *E. fornicatus* specimens from South America, 57 sequences were retrieved from NCBI GenBank. Among these, 21 sequences correspond to H21-H42, with *E. kuroshio* (haplotype H21) serving as an outgroup (Stouthamer et al. 2017). Additionally, 23 sequences correspond to H43-48, and H52 (Wang et al. 2022); haplotypes H68-H70 (Smith et al. 2019); H66 and H67 (Gomez et al. 2018); H53 (Mitchell & Maddox 2010); as well as H73, H77, and H80 (Liao et al. 2023). Sequences from Brazil (Covre et al. 2024), Argentina, Uruguay (Ceriani-Nakamurakare et al. 2023; 2025, Spain (Gallego et al. 2025), and Türkiye (Acer et al. 2025) were included, with accession numbers (OR773079-81, OR016051, PQ667063, PQ849270, and PX488929), respectively. Notably, all Brazilian sequences here produced share the same genotype; thus, only one (PQ580203) was used for molecular inferences. The sequences were aligned using Muscle software.

Terminal positions in the alignment, that were not covered by all sequences in the dataset, were trimmed, resulting in a 563-bp alignment (except for PX488929, which is only 394 base pairs long with 392 base pairs aligned) used for analyses in the MEGA 11 program (Tamura et al. 2021). The genetic relationship between the produced sequences and the *E. fornicatus* complex was also represented in a maximum likelihood phylogenetic tree inferred using IQ-TREE v2.3.2 software (Minh et al. 2020). The best-fit model, as selected by the Modelfinder software, was HKY+F+I+R2 (Kalyaanamoorthy et al. 2017). Maximum likelihood based on IQ-TREE with 10,000 ultrafast bootstraps tests (Hoang et al. 2018) (Fig. 1).

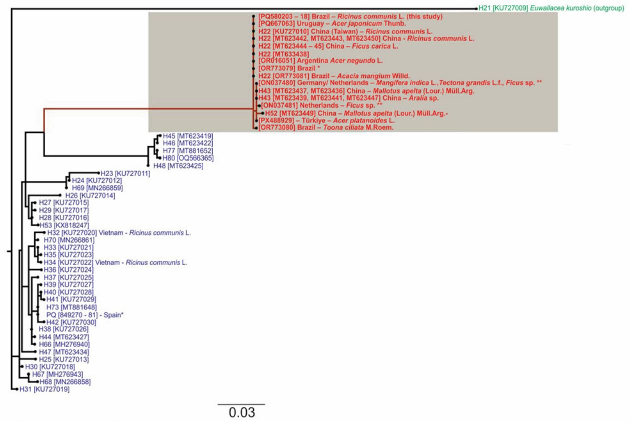

Figure 1. Maximum likelihood phylogenetic tree based on partial mitochondrial COI gene sequences (563 bp), illustrating the relationships among individuals from South America with previously published haplotypes; The haplotype H21 (*Euwallacea kuroshio*) was used as the outgroup. Known host plants are listed after each country record. *Unknown host, specimens captured traps; **Unclear host identity.

The characterization of stem damage (holes/galleries) by *E. fornicatus* (Fig. 2A-F) was conducted on four *R. communis* plants, approximately 3 years old, in Piracicaba, São Paulo, Brazil (22°45'13.38"S; 47°35'9.94"W, 607 m a.s.l.). The stems of the four plants were cut into ten 30 cm segments taken from the collar region to a height of 3 m. For each segment, we measured: (1) stem diameter, calculated from the circumference at the midpoint; (2) the total number of holes; (3) stem development (unlignified or lignified); and (4) plant height at the cut point.

To evaluate whether attack intensity (number of holes in the stem) varied with plant height, development stage (tender or woody), and stem diameter, a Generalized Linear Mixed Model (GLMM) was used, assuming a Poisson distribution (Fig. 4A-B, S3, Fig. 2) and treating plant as a random variable. Model significance was assessed using the Type III Wald Chi-square test (α = 5%), and pairwise comparisons were made using the Tukey test with Bonferroni adjustment. Analyses were performed in R version 4.2.3 (R Core Team 2024), utilizing *ggplot2* (Wickham et al. 2016), *emmeans* (Lenth et al. 2019), *multcomp* (Hothorn et al. 2016), and *lme4* (Bates et al. 2024) packages.

All specimens collected from castor bean plants in Brazil (S1, Fig. 1; S2, Tab. 1) were identified as *E. fornicatus*, haplotype 22 (H22). The PSHB haplotype from this study clustered with sequences from Argentina (Ceriani-Nakamurakare et al. 2023), Brazil (Covre et al. 2024) China (Wang et al. 2022; Liao et al. 2023), Germany, Netherlands (Schuler et al. 2023), and Spain (Gallego et al. 2025), Uruguay (Ceriani-Nakamurakare et al. 2025), and Türkiye (Acer et al. 2025) (Fig. 1; red clade) (Mitchell & Maddox 2010; Stouthamer et al. 2017; Gomez et al. 2018; Smith et al. 2019; Wang et al. 2022; Liao et al. 2023; Ceriani-Nakamurakare et al. 2023; 2025; Covre et al. 2024; Acer et al. 2025). Molecular analyses confirm that, to date, haplotype H22 is the only one identified in South America (Ceriani-Nakamurakare et al. 2023; 2025; Covre et al. 2024).

Figure 2 A-F. *Euwallacea fornicatus* (Eichhoff, 1868) (Coleoptera: Curculionidae: Scolytinae) damage: A) *R. communis*. B) Castor bean stem with damage caused by *E. fornicatus*. C) Stems of infested plants. D) Sawdust (frass tubes or noodles) formed by the PSHB activity (red arrow). (E-F) Gallery and holes caused by PSHB.

Beyond *R. communis* (Stouthamer et al. 2017; Liu et al. 2022), H22 has also been recorded on *Camellia sinensis* (L.) Kuntze (Theaceae) in Taiwan (China) (Liao et al. 2023), *Ficus carica* L. (Moraceae), *R. communis*, *Sindora glabra* Merr. ex de Wit (Fabaceae) in China (Wang et al. 2022), *Acacia mangium* Willd. (Fabaceae), *Khaya grandifoliola* C.DC., and *Toona ciliata* M.Roem. (Meliaceae) in Brazil (Covre et al. 2024), *Acer negundo* L. in Argentina, *A. japonicum* Thunb. (Sapindaceae) in Uruguay (Ceriani-Nakamurakare et al. 2025), and *Acer platanoides* L. (Sapindaceae) in Türkiye (Acer et al. 2025). Indicating, therefore, that H22 is a highly polyphagous pest.

Along with H22, haplotypes H32 and H34 have been identified colonizing castor bean plants in Vietnam (Stouthamer et al. 2017) (Fig. 1; blue clade). It is important to note that, so far, H22 has not been recorded attacking avocado plants, *Persea americana* Mill. (Lauraceae). Therefore, the *E. fornicatus* records on avocado trees in Brazil (Covre et al. 2024) should be verified at the haplotype level.

The phylogenetic tree reveals two distinct clusters within PSHB, suggesting the presence of variants (red and blue clusters) (Fig. 1). Our findings support the questions raised by Stouthamer et al. (2017) and further questioned by Liao et al. (2023) regarding the classification of H22. Additionally, Wang et al. (2022) found that H22 is present in China and shows high genetic similarity with haplotypes H43 and H52, suggesting it may represent a separate variant species (Fig. 1). Therefore, a thorough review of the *E. fornicatus* complex based on regional genotyping is recommended.

Our survey across 46 Brazilian municipalities detected PSHB in 28 municipalities within five states (S2, Tab. 1). Our results support the widespread occurrence of PSHB previously reported in Brazil (Covre et al. 2024). In dissected castor bean plants, we found eggs, larvae, pupae, and adults (males and females) (Fig. 3A-H). We observed a higher proportion of females compared to males in the galleries, which aligns with previous studies (Stouthamer et al. 2017). The number of holes varied across the sampled plants. The four sampled plants showed variable numbers of holes: Plant 1 (n = 977 holes), Plant 2 (n = 30 holes), Plant 3 (n = 335 holes), and Plant 4 (n = 572 holes). The distribution of holes was mainly concentrated in the basal third of the plant.

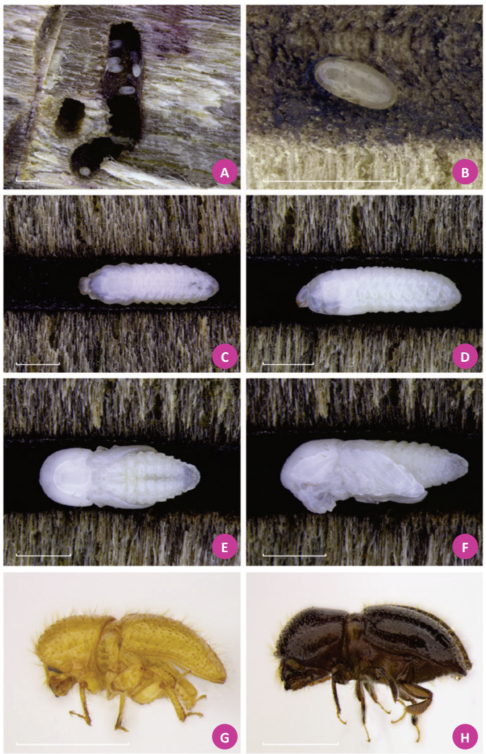

Figure 3 A-H. *E. fornicatus.* (A-B) Eggs. (C-D) Larva. (E-F) Pupa (dorsal and lateral views). (G-H) Male and female. (Scale = 1 mm).

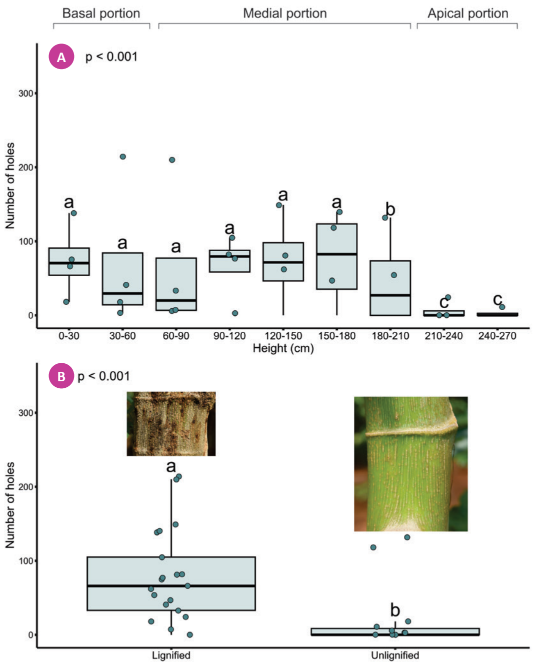

Figure 4 A-B. Influence of plant characteristics on damage caused by *Euwallacea fornicatus* (Eichhoff, 1868) (Coleoptera: Curculionidae: Scolytinae) to castor bean plants: A) Effect of plant height on the number of holes. B) Effect of stem developmental stage (unlignifed and lignified) on the number of holes. Different letters above bars mean significant difference.

The intensity of the PSHB attack on castor beans was significantly related to factors such as distance from the ground ($\chi^2 = 285.55$, df = 8, $p < 0.001$), stem diameter ($\chi^2 = 394.84$, df = 1, $p < 0.001$), and the developmental stage of the stem (unlignified or lignified) ($\chi^2 = 70.89$, df = 1, $p < 0.001$). Our results indicate that *E. fornicatus* H22 prefers mature castor bean plants with larger diameters (Fig. 4A-B; S3, Fig. 2), consistent with earlier reports (King 1941; Gadd & Loos 1947; Mendel et al. 2017).

Our findings highlight the importance of including *R. communis* in PSHB monitoring programs in Brazil. This underscores the need for coordinated efforts to prevent and control this invasive pest. H22 was observed colonizing fig trees (*Ficus carica*) (Wang et al. 2022) (Fig. 1), a crop for which Brazil is a leading producer (Oliveira et al. 2024). Thus, ongoing surveillance of this crop is crucial for early detection and effective pest management. Moreover, *E. fornicatus*, with its high dispersal capacity, presents an increasing threat to regional plant health and should be a priority in agroforestry monitoring efforts in Brazil.

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Authors' Contributions

NSS: Methodology, Visualization, Writing – original draft; LSD: Investigation, Writing – original draft; ASA: Investigation, Visualization, Writing – review & editing; SSN: Investigation, Writing – review & editing; FH: Investigation, Writing – original draft, Writing – review & editing; MSLA: Investigation, Resources; HLR: Investigation, Writing – review & editing; RAZ: Investigation, Writing – review & editing; MS: Investigation, Visualization, Writing – review & editing.

Conflict of Interest Statement

The authors declare no competing interests.

Ethical Approval

Not applicable.

Supplementary Material

Supplementary data for this article be accessed at doi: https://doi.org/10.17632/nh7b4w7fb9.1

Data Availability

The datasets generated during this study are available from the corresponding author upon reasonable request.

Generative AI Statement

The authors declare that no generative artificial intelligence tools were used in the preparation of this manuscript.

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