

Article

Diversity of Native *Trichoderma* spp. Associated with *Theobroma cacao* and *Citrus sinensis* Crops in Peruvian Amazon: First Report for San Martín Region

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Abstract

Trichoderma species are recognized biocontrol agents and plant growth promoters with applications in sustainable agriculture. Despite the economic importance of cacao and orange crops in the San Martín region of the Peruvian Amazon, the diversity of native *Trichoderma* associated with these crops remains undocumented. This study provides the first report of *Trichoderma* diversity in rhizospheric soil and plant tissues of *Theobroma cacao* and *Citrus sinensis* in this region. Sampling was conducted in 18 sectors across five districts (195–785 m a.s.l.). Molecular identification was performed using the translation elongation factor 1-alpha (*tef1*) gene marker, with NCBI BLAST analysis against GenBank and phylogenetic analysis using the Neighbor-Joining method. From 59 initial isolates, 18 strains were successfully identified. Seven putative species (*T. koningiopsis*, *T. reesei*, *T. lentiforme*, *T. longibrachiatum*, *T. harzianum*, *T. spirale*, and *T. azevedoi*) were found associated with cacao, while four putative species (*T. erinaceum*, *T. parareesei*, *T. longibrachiatum*, and *T. virens*) were identified from orange crops. Sequence identity ranged from 94.38% to 100%. BLAST comparison initially returned equal identity scores between *T. harzianum* and *T. lentiforme* for five strains (T-3, T-11, T-14, T-15, and T-16); subsequent phylogenetic placement using a broader GenBank reference panel resolved all five within the *T. lentiforme* clade. Phylogenetic placement also refined two further identifications beyond their initial BLAST match: strain T6-SR-B grouped with *T. azevedoi* rather than *T. harzianum*, and strain T12-R-A grouped with the sister species *T. parareesei* rather than *T. reesei*. These findings establish a baseline for *Trichoderma* biodiversity in the region and identify potential candidates for biocontrol applications.

Keywords: biodiversity; biocontrol; cacao; molecular identification; orange; Peruvian Amazon; *tef1* gene



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1. Introduction

The cultivation of cacao, a fruit tree native to the Amazon basin of South America [1,2], is the main source of income for many producers and farmers, especially in Africa, Central America and South America [3]. Reports from 2018 show that cacao production reached a total of 4.6 million tons worldwide [3], benefiting 40–50 million people [4]. San Martín, a region and province of Peru, represents 38% of the total national production. Cacao cultivation depends on efficient microorganisms, making it highly mycotrophic in order to develop and grow [5].

Citrus sinensis L. (orange) is the most cultivated citrus fruit globally, accounting for over 40% of citrus exports. World orange production in 2021/22 exceeded approximately 48 million tonnes. In Peru, the main producing regions are Junín (55% of national production), followed by San Martín (11%), Lima (7%), Ica (6%), Puno (5%), and Cusco (5%) [6,7].

Given the importance of these crops in Peru and worldwide, agricultural production is increasing; however, phytosanitary problems arise, mainly caused by pathogenic fungi. Fungal phytopathogens are among the main problems in cacao and citrus plantations in tropical countries [8,9]. Plant pathogens represent a major threat to the agricultural industry, with up to 40% of yields from economically important crops lost annually due to plant pathogens and pests [10,11]. Losses associated with plant diseases impose a high economic burden, with an estimated annual loss of US\$220 billion [12].

Trichoderma species are recognized as effective biocontrol agents against numerous plant pathogens and as plant growth promoters [13]. They fulfill various functional roles in the plant–soil system [14], including biofertilization, bioprotection, and bioremediation. The use of microbial remediation techniques not only allows for effective cleaning and reduction in environmental contamination but also restores and reconstructs ecosystems, maintaining their stability [15,16]. With the growing development of high-throughput sequencing technology and molecular information technology [17], numerous new species of microbial strains have been discovered and selected.

Documenting native *Trichoderma* diversity associated with perennial crops is an emerging line of research in the Amazon basin, and previous work provides an important frame of reference for the present study. In Brazilian rubber trees (*Hevea brasiliensis*), Nascimento-Brito et al. [18] recovered a broad endophytic *Trichoderma* community, including four species new to science, underscoring how little of the genus' diversity in Amazonian perennial-crop systems has been formally described. Similarly, Gazis and Chaverri [19] surveyed fungal endophytes, including *Trichoderma*, in wild rubber trees in the Peruvian Amazon and found host- and tissue-associated patterns of colonization that had not previously been reported for the region. In cacao specifically, Chavez-Jalk et al. [20] demonstrated that endophytic *Trichoderma* strains isolated from Peruvian nurseries improved agronomic performance of *T. cacao* ecotypes, while in a comparable tropical smallholder-crop system, Mulatu et al. [21] characterized the diversity of *Trichoderma* in the rhizosphere of coffee (*Coffea arabica*) in Ethiopia and evaluated its potential for biocontrol of coffee wilt disease. Taken together, these studies show that native *Trichoderma* assemblages associated with tropical perennial crops tend to be regionally distinct and taxonomically under-sampled, and that closing this gap has direct applied value for biocontrol prospecting. Despite the recognized importance of *Trichoderma* in sustainable agriculture and the economic significance of cacao and orange crops in the San Martín region, no equivalent baseline exists for native *Trichoderma* species associated with these two crops in this specific area. This study aims to provide the first report of *Trichoderma* diversity associated with *T. cacao* and *C. sinensis* crops in the Peruvian Amazon, establishing a baseline for future taxonomic studies and identifying potential candidates for biocontrol applications.

2. Materials and Methods

2.1. Study Site

The San Martín region covers an area of 51,253.31 km² and has a predominantly sub-tropical and tropical climate. The geographic location and environmental conditions of the sampling sites presented elevations ranging from 195 to 785 m a.s.l., with a mean annual precipitation of 1111 mm and a mean annual temperature of 24.9 °C. The study site comprises 18 sectors where samples were taken from *Theobroma cacao* L. and *Citrus sinensis* L. crops. These sectors are distributed in the districts of Chazuta, Sauce, Cacat-

achi, San Martín, and Cabo Alberto Leveau in the San Martín province and region. The full geographic coordinates and environmental conditions of each sampling site are provided in Table S1 (Supplementary Materials); Figure 1 shows the spatial distribution of the sampling plots.

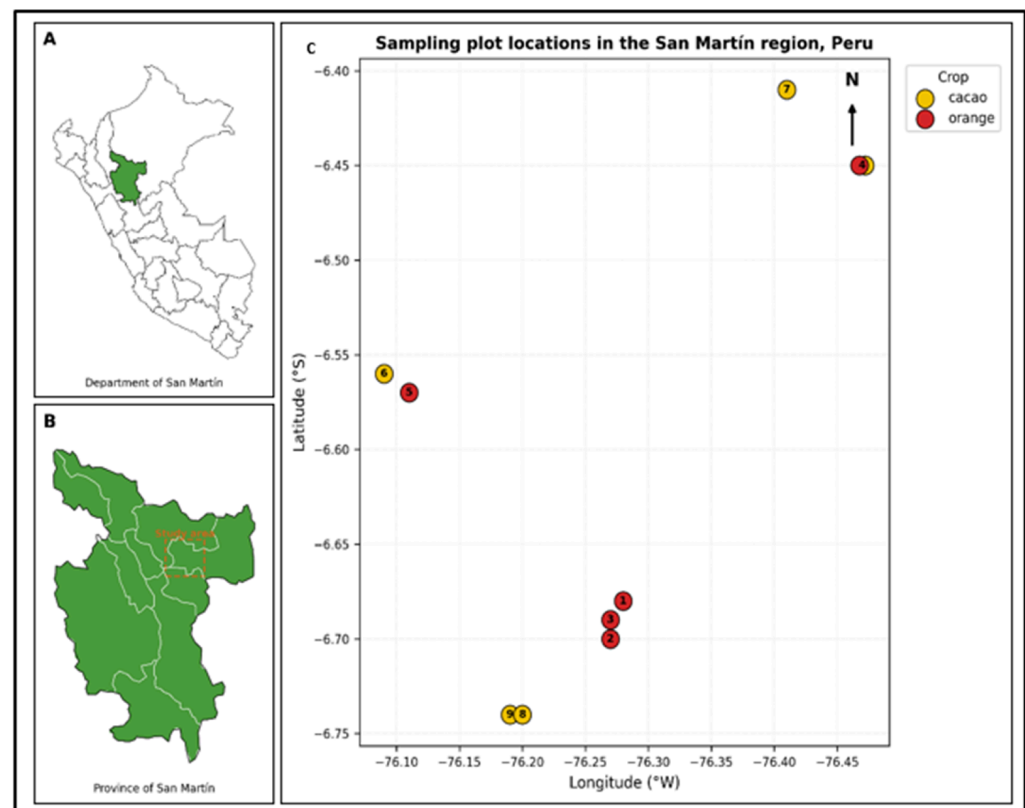


Figure 1. Map of the study area indicating the distribution of the plot sampling in the districts of San Martín, Peru. (A) Location of the San Martín department within Peru. (B) Location of the study area within the San Martín province. (C) Detailed distribution of the 18 sampling plots.

2.2. Rhizospheric Soil Sampling

Eighteen rhizospheric soil samples were collected from *T. cacao* and *C. sinensis* crops distributed across five districts of the San Martín region. The sampling procedure was systematic and non-probabilistic, as recommended by Otzen and Manterola [22]. Sampling was conducted in a zigzag pattern, and plant tissue samples were collected using pruning scissors, selecting plants based on four criteria according to Harman et al. [13]. Five subsamples of 150 g of soil each were collected from each cultivation plot, 25–30 steps apart, at a depth of 0–20 cm, following the methodology of Cheng et al. [23]. The five subsamples obtained from each plot were homogenized into a representative sample of 150 g. Samples (plant material and soil) were collected in polypropylene plastic bags and then in Kraft paper bags to prevent UV damage, and were labeled for proper identification.

2.3. *Trichoderma* Isolation and Purification

Vegetative tissue (stems and leaves) was considered for the isolation of *Trichoderma* endophytes. In the microbiology laboratory, endophyte isolation was performed on potato dextrose agar (PDA) medium, following the protocol employed by Márquez-Dávila et al. [24]. For isolating *Trichoderma* spp. strains, 5 g of rhizospheric soil were placed in sterile flasks with 95 mL of sterile distilled water and shaken at 120 rpm for 30 min, followed by serial dilutions. From the 10^{-5} dilution, 1 mL was extracted and distributed on Petri dishes with PDA medium (Becton, Dickinson and Company (BD), Franklin Lakes,

NJ, USA) plus 0.5 g of oxytetracycline. Plates were incubated at 25 °C in darkness for 48–72 h until colony growth was observed. Subsequently, plates were exposed to artificial light to stimulate fruiting body development.

Emerging colonies were subcultured and purified individually. *Trichoderma* isolates were grown on Petri dishes with Difco™ PDA medium (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 25 °C for 5 days [13]. A total of 59 strains of *Trichoderma* spp. were isolated. From these, 19 strains were selected for molecular identification through visual and morphological evaluation of colony characteristics—growth pattern, pigmentation, and colony margin/shape—grouping isolates that shared a consistent morphotype and retaining one representative strain per recognizably distinct morphotype at each sampling site, in order to capture the morphological variability observed without submitting redundant isolates for sequencing. These 19 strains were sent to the BIOTECOOP laboratory (Lima, Peru) for molecular identification. Of these, 18 strains were successfully identified; one sample (T-4) was excluded due to contamination during processing.

2.4. DNA Extraction, PCR and Sequencing

The 19 strains were cultured in potato dextrose (PD) liquid medium. Each flask was inoculated with four 5 mm agar discs containing mycelium from each *Trichoderma* strain (three flasks per strain), then incubated with shaking at 150 rpm for 15 days at laboratory room temperature of 25 °C (± 2). After incubation, mycelium was separated from the culture medium by filtering through sterile cotton within a laminar flow cabinet, collected in sterile Petri dishes, and sent to a specialized laboratory. DNA extraction was performed at the BIOTECOOP laboratory using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions.

PCR was performed by amplification of the translation elongation factor 1-alpha (*tef1*) gene using primers EF1 (5'-ATGGGTAAGGARGACAAGAC-3') and EF2 (5'-GGARGTACCAGTSATCATGTT-3') [25]. Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA) was used for PCR product synthesis. The PCR product was sequenced by capillary electrophoresis at Macrogen Inc. (Chile), and identification was performed by comparison with the NCBI GenBank database. The 18 *tef1* gene sequences generated in this study were deposited in GenBank (NCBI) under accession numbers PZ233892–PZ233909, with a hold until the published release date. The correspondence between each strain code, its putative species assignment, and its individual GenBank accession number is provided in Table S2 (Supplementary Materials).

2.5. Phylogenetic Analysis

Sequences were analyzed using BLAST (<https://blast.ncbi.nlm.nih.gov>, accessed on 26 August 2026) (Basic Local Alignment Search Tool) on the NCBI platform, and sequences with similarity percentages above 98% were selected for downstream analysis. Multiple sequence alignment (MUSCLE) and phylogenetic tree construction using the Neighbor-Joining (NJ) statistical method and the Kimura 2-parameter (K2P) model, with bootstrap values of 1000 replicates, were performed using MEGA version 11 software [26].

3. Results

BLAST comparison against GenBank, performed on the sequences reported by the BIOTECOOP laboratory, returned sequence identity values ranging from 94.38% to 100%, with query coverage between 80% and 100% and an E-value of 0.0 for most samples (full per-strain coverage, identity, and E-values are provided in Table S2). Five strains—T-3, T-11, T-14, T-15, and T-16—returned equal BLAST identity scores for both *T. harzianum* and *T. lentiforme*, indicating taxonomic ambiguity within the *Harzianum* species complex

that a single top-BLAST-hit comparison could not resolve. To resolve this ambiguity and refine identification beyond pairwise BLAST comparison, we constructed Neighbor-Joining phylogenetic trees using an expanded panel of GenBank reference sequences for each clade (see Sections 3.1 and 3.2). This phylogenetic placement resolved all five ambiguous strains within the *T. lentiforme* clade with strong bootstrap support, and additionally revealed that two strains initially matched to common species by BLAST top-hit in fact grouped with closely related sister species: strain T6-SR-B grouped with *T. azevedoi* rather than *T. harzianum*, and strain T12-R-A grouped with *T. parareesei* rather than *T. reesei*. The species names used throughout the Results and in Table S2 reflect this phylogenetically resolved identification; the taxonomic ambiguity encountered at the BLAST stage is discussed further as a limitation in the Study Limitations Section.

3.1. *Trichoderma* Isolates from Cacao

Two groups of *Trichoderma* isolates were found in cacao cultivation across different districts of the San Martín region (Figure 2). Group differentiation was based on a qualitative visual assessment of colony growth extension on PDA medium at the standard 5-day incubation endpoint, together with pigmentation, rather than on a quantitative growth-rate measurement; this criterion is acknowledged as a methodological limitation in the Study Limitations Section. The first group included strains T2-SR-A (*T. reesei*, Chazuta), T5-T-B (*T. longibrachiatum*, Sauce), T7-R-C (*T. spirale*, Chazuta), T10-SR-A (*T. longibrachiatum*, Cacatachi), and T15-R-C (*T. lentiforme*, San Martín), which showed visibly less colony extension on culture medium and produced yellow colonies. The second group showed similar colony types with visibly greater colony extension and produced green colonies; it comprised T1-R-A (*T. koningiopsis*, Chazuta), T3-R-B (*T. lentiforme*, Sauce), T6-SR-B (*T. azevedoi*, Sauce), T8-R-C (*T. harzianum*, Chazuta), T11-SR-A (*T. lentiforme*, Cacatachi), T14-H-B (*T. lentiforme*, Chazuta), and T16-H-C (*T. lentiforme*, San Martín). The full strain-to-species correspondence, including BLAST identity values and individual GenBank accession numbers, is provided in Table S2.

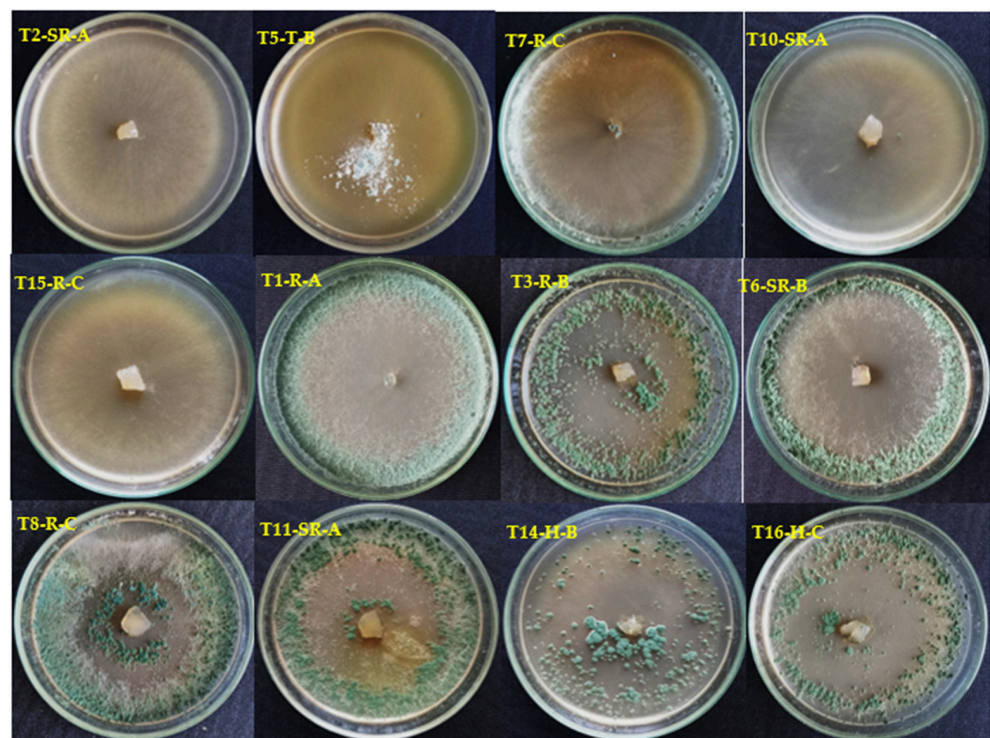


Figure 2. *Trichoderma* species isolated from cacao (*Theobroma cacao*) rhizospheric soil and plant tissue in the San Martín region, Peru. Colony morphology on PDA medium at 25 °C after 5 days of incubation.

Through analysis of *tef1* gene nucleotide sequences from the 12 strains studied for cacao cultivation, seven putative *Trichoderma* species were identified: *T. koningiopsis*, *T. reesei*, *T. lentiforme*, *T. longibrachiatum*, *T. harzianum*, *T. spirale*, and *T. azevedoi*. Most species obtained sequence identity values between 94.38% and 100% compared to sequences deposited in GenBank (NCBI), UNITE, and BOLD SYSTEMS databases (Figure 3). The topology in Figure 3 places our isolates within seven well-supported clades (bootstrap values 76–100), each anchored by reference sequences of a single named species. Strain T6-SR-B clusters with high support (bootstrap 98) alongside four *T. azevedoi* reference sequences rather than within the neighboring *T. harzianum* clade, despite an initial BLAST top-hit match to *T. harzianum*. The five strains with BLAST-level ambiguity (T-3, T-11, T-14, T-15, T-16) all fall within the *T. lentiforme* clade (bootstrap 93–99), distinct from the sister *T. harzianum*/*T. azevedoi* clade—the phylogenetic placement that resolves the identification discussed in Section 3 above. The *T. koningiopsis*, *T. reesei*/*T. longibrachiatum*, and *T. spirale* clades each form separate, well-supported branches with no overlap with the Harzianum-complex clades.

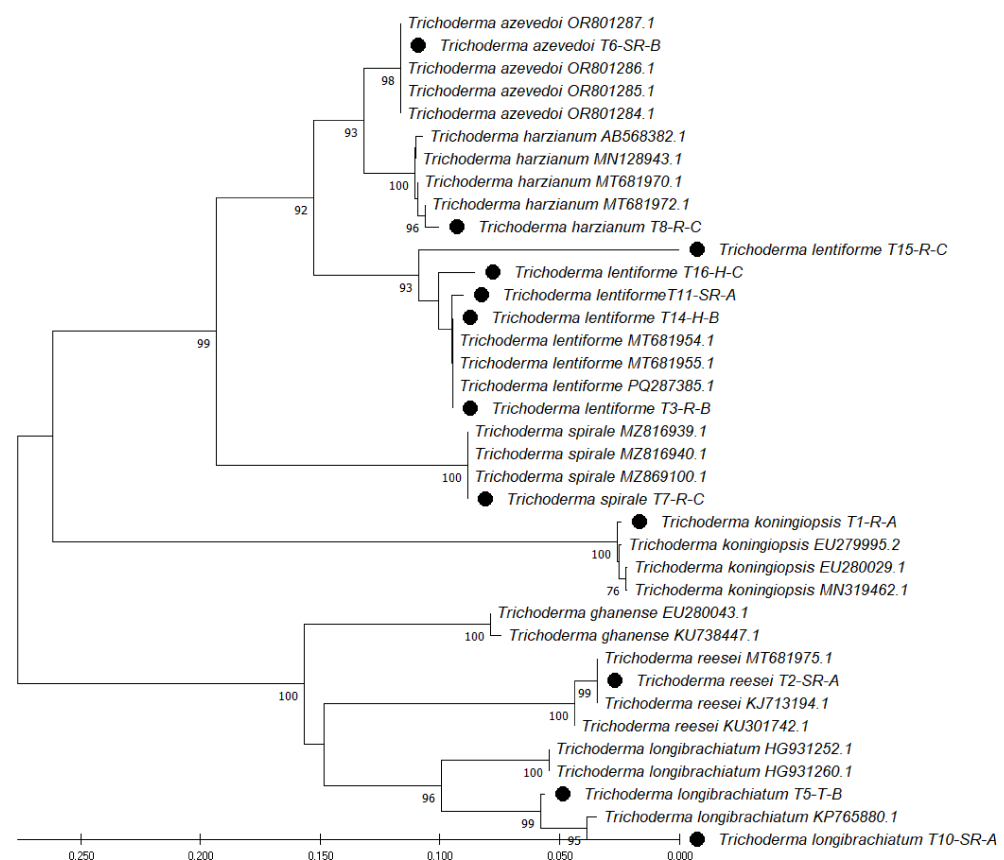


Figure 3. Phylogenetic tree of identified *Trichoderma* isolates based on the *tef1* sequence dataset in cacao cultivation. Numbers below the branches indicate bootstrap values. (●) indicates strains isolated in this study, in contrast to reference sequences retrieved from GenBank.

3.2. *Trichoderma* Isolates from Orange

Two groups of *Trichoderma* isolates were found in orange cultivation (Figures 4 and 5), differentiated using the same qualitative colony-extension and pigmentation criteria described in Section 3.1. The first group consisted of strain T13-R-B (*T. longibrachiatum*, Cacatachi), which showed visibly less colony extension and produced light-yellow colonies. The second group showed visibly greater colony extension and produced green colonies; it comprised T9-NSR (*T. erinaceum*, Cabo Alberto Leveau), T12-R-A (*T. parareesei*, Cacatachi), T17-SR-A (*T. virens*, Cabo Alberto Leveau), T18-NR (*T. erinaceum*, Cabo Alberto Leveau), and T19-NSR (*T. erinaceum*,

Chazuta). The full strain-to-species correspondence, including BLAST identity values and individual GenBank accession numbers, is provided in Table S2.

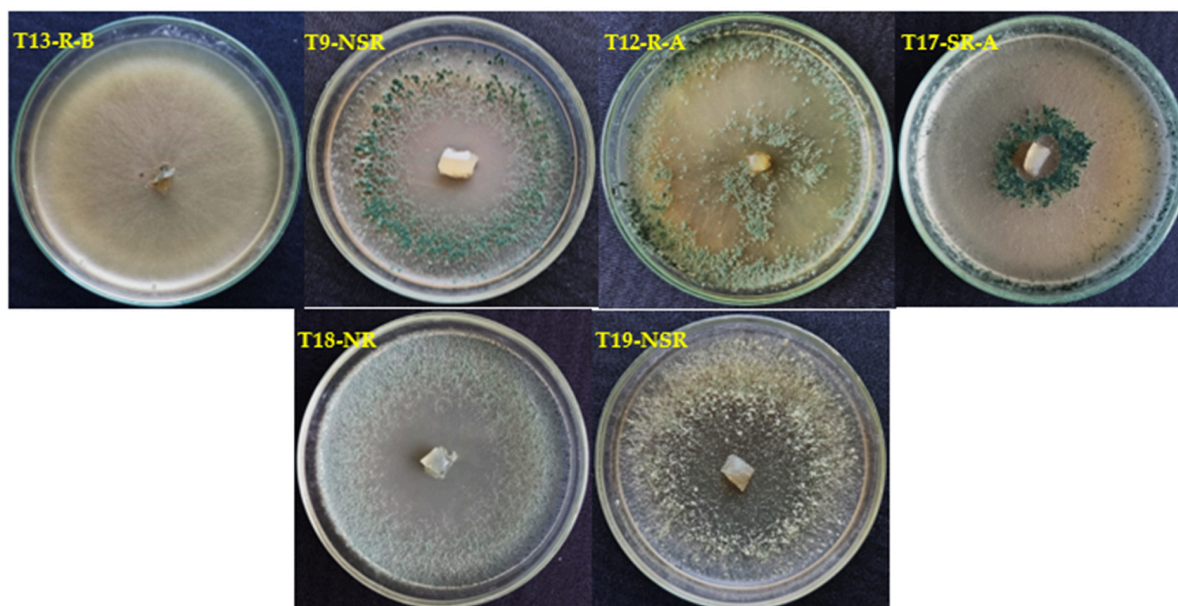


Figure 4. *Trichoderma* species isolated from orange (*Citrus sinensis*) rhizospheric soil in the San Martín region, Peru. Colony morphology on PDA medium at 25 °C after 5 days of incubation.

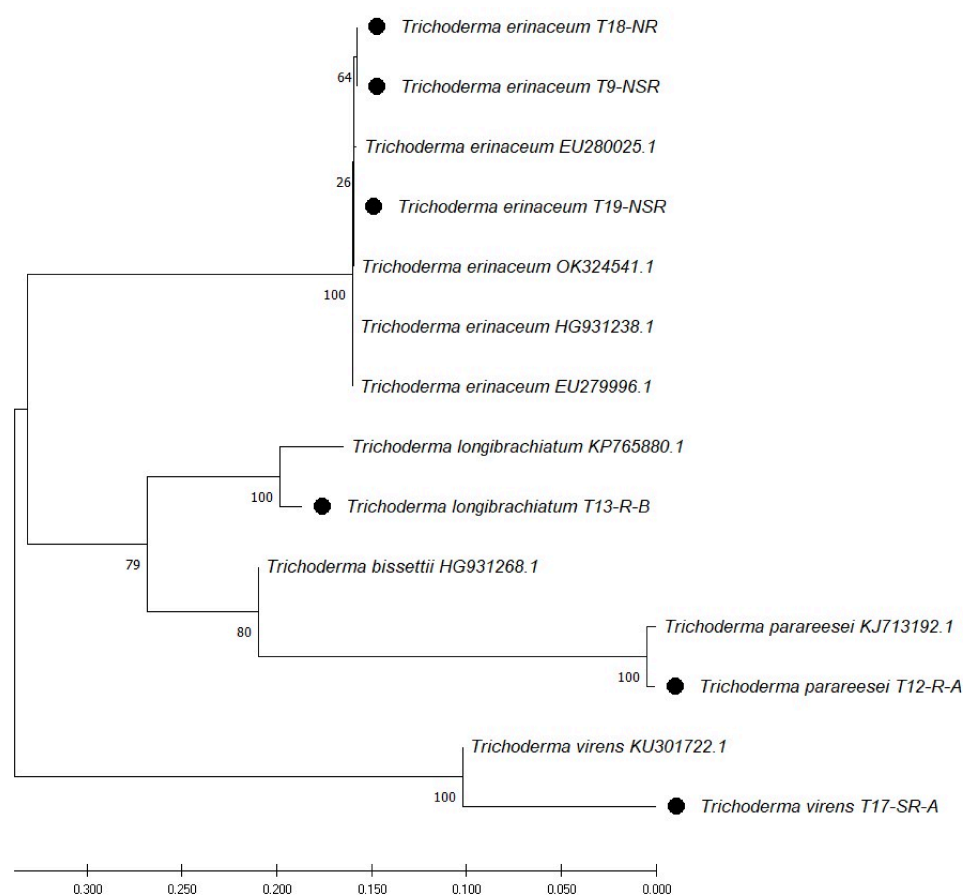


Figure 5. Phylogenetic tree of identified *Trichoderma* isolates based on the *tef1* sequence dataset in orange cultivation. Numbers below the branches indicate bootstrap values. (•) indicates strains isolated in this study, in contrast to reference sequences retrieved from GenBank.

Through analysis of *tef1* gene nucleotide sequences from the six strains studied for orange cultivation, four putative *Trichoderma* species were identified: *T. erinaceum*, *T. parareesei*, *T. longibrachiatum*, and *T. virens*. Most species obtained sequence identity values between 94.38% and 100% compared to sequences deposited in the GenBank (NCBI), UNITE, and BOLD SYSTEMS databases, consistent with the identity range observed for the cacao-associated isolates (Section 3.1). As in Figure 3, the topology in Figure 5 places each strain within a well-supported clade (bootstrap 64–100) anchored by named reference sequences. Notably, strain T12-R-A, which BLAST matched to *T. reesei* (99.11% identity), clusters instead with *T. parareesei* reference sequences (bootstrap 100)—a sister species within the same species complex that BLAST top-hit comparison alone did not distinguish, again illustrating the added resolving power of phylogenetic placement over single-marker BLAST identification (Study Limitations Section).

4. Discussion

Trichoderma endophytic fungi represent an alternative for organic production of cacao [20] and orange, as they contribute to crop sustainability and adaptation by conserving fungal biodiversity. The present findings extend this line of evidence to the San Martín region, adding a locality-level baseline that was previously absent for both crops, and pointing to biofertilization and biocontrol applications relevant to disease management in local production systems [18].

Three of the cacao-associated isolates in this study (T3-R-B, T11-SR-A, T14-H-B, T15-R-C, T16-H-C, Sauce, Chazuta, Cacatachi, and San Martín districts) grouped within the *T. lentiforme* clade following phylogenetic resolution of an initial BLAST-level ambiguity with *T. harzianum* (Section 3). A sixth strain, T6-SR-B, grouped instead with the closely related *T. azevedoi*, despite an initial BLAST top-hit to *T. harzianum*. This is consistent with reports that the Harzianum clade is morphologically heterogeneous and phylogenetically complex, encompassing several closely related species that are difficult to resolve with single-locus markers or pairwise BLAST comparison alone [19,27]. The recurrence of this difficulty across six of our strains (Section 3) mirrors similar challenges reported for Harzianum-clade isolates from other geographic origins and hosts, which combine ecological and economic importance with relevant prospects for biological control use [27–29]—underscoring, as discussed in the Study Limitations Section, that confirming these identifications with additional markers remains a priority for strains of applied interest.

The presence of *T. reesei* in cacao (Chazuta district) and of its sister species *T. parareesei* in orange (Cacatachi district, strain T12-R-A) in this study is consistent with the recognized ecological niche of the Reesei/Parareesei species complex: decaying plant matter in tropical rainforest environments [30]. As a member of the Longibrachiatum clade—a group of roughly 26 species [31] that also includes *T. longibrachiatum*, recovered here from both crops—most previously reported isolates of this complex likewise originate from tropical or subtropical regions such as the Solomon Islands, French Guiana, Brazil, or Peru [31]. That BLAST top-hit comparison alone matched T12-R-A to *T. reesei* while phylogenetic placement resolved it as *T. parareesei* illustrates how closely related these two species are at the *tef1* locus—*T. reesei* remains among the best-studied species in the genus, largely owing to its wide application in industrial biotechnology [32,33], and the recovery of its sister species from a Peruvian citrus agroecosystem adds a further locality record for the complex.

T. koningiopsis and *T. spirale*, both recovered from cacao in the Chazuta district in this study, have previously been described as endophytes of other tropical woody plants, including cacao and rubber [34]. *T. koningiopsis* is a cosmopolitan species with documented plant-protection activity in cacao and other biocontrol applications, and has also been reported as an endophyte of *Coffea canephora* in Cameroon [28], while *T. spirale* is most

frequently isolated from tropical environments generally [35,36]—a pattern the San Martín cacao samples reproduce at the local scale.

T. erinaceum, recovered here from orange rhizosphere in the Cabo Alberto Leveau and Chazuta districts, has previously been isolated mainly from forest rhizosphere [21] and maize rhizosphere [37]; its detection in a citrus agroecosystem in this study broadens the set of crop systems in which the species has been documented. Across all species reported here, correct isolation and use of monoconidial cultures is essential for reliable molecular identification, since DNA extraction and PCR amplification from mixed cultures can amplify multiple alleles of a gene, producing unreliable sequences and questionable species assignments [38]—a consideration directly relevant to the limitations discussed below.

Study Limitations

This study presents methodological limitations that must be acknowledged. Molecular identification relied on a single locus, the *tef1* gene marker, because this was the marker offered by the contracted molecular biology laboratory (BIOTECOOP) at the time of processing; multilocus identification combining ITS, *tef1*, and *rpb2* in conjunction with specialized databases such as trichoderma.info, maintained by the International Subcommittee on *Trichoderma* Taxonomy [39], was not available within the scope of the original laboratory service and remains a priority for future confirmatory work. Phylogenetic placement using an expanded GenBank reference panel (Figures 3 and 5) partially mitigated this single-marker limitation by resolving the BLAST-level *T. harzianum*/*T. lentiforme* ambiguity for six strains and by distinguishing *T. reesei* from its sister species *T. parareesei* for strain T12-R-A (Section 3); however, phylogenetic placement from a single locus does not substitute for independent confirmation with additional markers, and the species identifications reported here should still be considered tentative.

Morphological characterization was intentionally limited to macroscopic colony traits—colony extension, pigmentation, and margin, documented in Figures 2 and 4—because this study prioritized molecular identification over detailed morphological characterization as its primary diagnostic approach. Consequently, key morphological traits used in classical *Trichoderma* taxonomy, such as conidial and phialide morphology, and quantitative growth-rate measurements, were not analyzed. We explicitly acknowledge this as a limitation of the morphological documentation and recommend that future surveys pair molecular identification with conidial and phialide photomicrography and replicated quantitative growth-rate assays (e.g., colony diameter measured over multiple time points) from the outset, rather than relying on qualitative visual assessment as was done here for the growth-rate groupings in Sections 3.1 and 3.2.

Additional laboratory-reporting limitations should also be noted. DNA quantity and quality following extraction were not measured (e.g., by spectrophotometry or fluorometry) as part of the contracted laboratory service, so this could not be verified independently of successful amplification and sequencing. Likewise, the amplification product length and the PCR thermal cycling profile (denaturation, annealing, and extension temperatures and durations) were not included in the laboratory report provided to the authors and could not be reproduced here; this information has been requested from BIOTECOOP for a future version of this manuscript, and is noted here as a reporting limitation rather than a change to the methodology actually used.

Additionally, the cultures used were not monoconidial, which may have affected sequence quality and species identification reliability, consistent with the general risk of mixed-allele amplification discussed above [38]. The classification of isolates into faster- and slower-growing groups (Sections 3.1 and 3.2) was likewise based on qualitative visual assessment at a single incubation endpoint rather than on replicated quantitative growth-

rate measurements, and should be interpreted as a descriptive, not a statistically validated, distinction. Therefore, the species identifications reported here should be considered tentative and require confirmation through polyphasic approaches. Future studies should employ monoconidial cultures, multilocus sequence analyses, quantitative growth-rate assays, conidial micromorphology, and deposit sequences in public databases to allow independent verification.

5. Conclusions

This study provides the first documented report of *Trichoderma* diversity associated with cacao and orange crops in the San Martín region of the Peruvian Amazon. Seven putative species of the genus *Trichoderma* (*T. koningiopsis*, *T. reesei*, *T. lentiforme*, *T. longibrachiatum*, *T. harzianum*, *T. spirale*, and *T. azevedoi*) were identified from cacao trees, while four putative species (*T. erinaceum*, *T. parareesei*, *T. longibrachiatum*, and *T. virens*) were identified in rhizospheric soil of orange trees. These species show potential for disease management and pathogen bioprospection in cacao and orange crops. The genus *Trichoderma* exhibits species with broad ecological plasticity, adapting to different districts and localities across an altitudinal range from 195 to 785 m a.s.l. Despite the acknowledged methodological limitations—single-marker molecular identification, morphological characterization limited to macroscopic traits, non-monoconidial cultures, and qualitative growth assessment—this work establishes a baseline for future, more rigorous taxonomic studies of native *Trichoderma* diversity in the Peruvian Amazon.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/d18090559/s1>, Table S1: Geographical location and environmental conditions of sampling sites; Table S2: Correspondence between strain code, putative species identification, and individual GenBank accession number for the 18 sequenced strains.

Author Contributions: Conceptualization, R.P.-G. and G.V.-T.; methodology, R.P.-G. and J.C.-T.; validation, R.P.-G., G.V.-T. and J.C.-T.; formal analysis, R.P.-G. and G.V.-T.; investigation, R.P.-G. and J.C.-T.; resources, G.V.-T. and J.C.-T.; data curation, R.P.-G.; writing—original draft preparation, R.P.-G. and G.V.-T.; writing—review and editing, R.P.-G., G.V.-T. and J.C.-T.; visualization, R.P.-G.; supervision, G.V.-T.; project administration, R.P.-G. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable. This study involved the collection of rhizospheric soil samples and plant material from agricultural fields, and did not involve human participants or animal experimentation.

Informed Consent Statement: Not applicable.

Data Availability Statement: The *tef1* gene sequences generated during this study are deposited in GenBank (NCBI) under accession numbers PZ233892–PZ233909. The sequences will be publicly available upon article publication. All other data generated or analyzed during this study are included in this article and its Supplementary Materials.

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All AI-assisted text was reviewed and verified by the corresponding author, who takes full responsibility for the accuracy and integrity of the final manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

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