Phytotaxa 725 (1): 071–081
https://www.mapress.com/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.725.1.6

First report of *Neopestalotiopsis clavispora* and *N. protearum* causing leaf spot in *Annona muricata* (soursop)

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Abstract

Fungal pathogens are increasingly recognized as major threats to global agriculture, with emerging species posing new challenges to crop health and productivity. This study reports, for the first time in Peru and in *Annona muricata* (soursop), the identification of *Neopestalotiopsis clavispora* and *N. protearum* as causal agents of leaf spot and fruit rot. Pathogenicity assays confirmed that both species infect foliar and fruit tissues in both commercially important cultivars ("common" and "Giant Criolla"), demonstrating their disease-causing potential under controlled conditions. Morphological characterization and multi-locus phylogenetic analyses (ITS, β-tubulin, and TEF1-α) confirmed the identity and taxonomic placement of both species within the *Neopestalotiopsis* clade. The findings represent a significant expansion of the known host range and geographic distribution of these fungi, which are already documented on a wide range of tropical and subtropical crops. Given their broad host plasticity and ecological adaptability, *Neopestalotiopsis* spp. may pose an increasing risk to diverse agricultural systems, especially under shifting environmental conditions. This study highlights the importance of developing species-specific diagnostic tools, implementing proactive surveillance, evaluating host resistance, and advancing research into epidemiology and control strategies to mitigate the risk of future outbreaks.

Key words: Annonaceae, plant pathogen, Pestalotioid fungi

Introduction

Soursop (*Annona muricata* L.) is a tropical fruit prized for its flavor, nutritional value, and industrial potential. It belongs to the Annonaceae family and originates in tropical regions of the Americas. Besides its geographic origin, this fruit species is currently cultivated in different parts of the world, such as Latin America, Africa, and Asia (Ramos *et al.* 2001). In Peru, soursop cultivation has gained importance in recent years. According to data from the Ministry of Agriculture, in 2022, more than 11,899 tons were produced across nearly 1249 hectares, with Junin and Ucayali being the main producing regions (MINAGRI 2022). Thanks to its versatility, this fruit is not only marketed in its natural form but also as frozen pulp, juice, nectar, or flour. The main export destinations include countries such as the United States, Chile, Uruguay, and several European countries, where demand is primarily concentrated in processed products (SIICEX 2024). Despite its growing commercial value, soursop cultivation faces various limitations, including phytosanitary problems. In districts such as Pichanaqui and San Martín de Pangoa, located in the central jungle of the Junín region of Peru, climatic conditions, especially high humidity and warm temperatures, create an environment conducive to the development of fungal diseases. Anthracnose, attributed to species of the genus *Colletotrichum*, is one of the most common and has already been widely documented in other producing countries such as Mexico and Brazil (Beltrán *et al.* 2023, Costa *et al.* 2019). However, other lesser-known pathogens that have not yet been thoroughly studied could be involved. One of these potential agents belongs to the group of fungi previously classified under the genus *Pestalotiopsis* (Jeewon *et al.* 2002, 2003, 2004) which in recent years was reorganized into three new

Accepted by Ruvishika Jayawardena: 30 Sept. 2025; published: 21 Oct. 2025

genera: *Neopestalotiopsis*, *Pseudopestalotiopsis*, and *Pestalotiopsis sensu stricto*. This new classification is based on phylogenetic studies using multiple genetic regions (internal transcribed spacer, β-tubulin 2 and translation elongation factor 1 α), in addition to morphological characteristics observed in conidia (Maharachchikumbura *et al.* 2014, Senanayake *et al.* 2020, Chaiwan *et al.* 2022). Among these newly described genera, *Neopestalotiopsis* has emerged as a significant pathogen affecting various crops worldwide. It is associated with a spectrum of diseases, ranging from leaf spots to more serious diseases like dieback and fruit rot (Jayawardena *et al.* 2015, Obregón *et al.* 2018, Gualberto *et al.* 2021). *Neopestalotiopsis clavispora* was first described through molecular analysis (Maharachchikumbura *et al.* 2014), and several investigations have shown that it can infect a wide range of hosts in different regions of the world. For example, in southeastern Bahia (Brazil), it has been associated with the appearance of leaf spots in *Macadamia integrifolia* Maiden & Betche (Santos *et al.* 2019), while in China it has been identified as the causal agent of a new foliar disease in *Musa acuminata* Colla (Qi *et al.* 2022). Similarly, in Malaysia, its presence was reported for the first time in *Cocos nucifera* L. seedlings, also linked to leaf lesions (Chong *et al.* 2023). A more recent relevant case was its detection in apple trees in China, found as the pathogen responsible for an emerging foliar disease, which was provisionally named "*Neopestalotiopsis* leaf spot of apple" (Shi *et al.* 2024). On the other hand, other species of the same genus, such as *Neopestalotiopsis protearum*, have also been identified as important phytopathogens on different continents. This species was originally reported in Zimbabwe, in affected leaves of *Leucospermum cuneiforme* (Burm. f.) Rourke cv. 'Sunbird' (Crous *et al.* 2011). In China, *N. protearum* was found in diseased tissues of species of the Orchidaceae family, collected in Guangxi Province, where its description was supported by both morphological characteristics and multilocus phylogenetic analysis (Ran *et al.* 2017). In Brazil, it was identified as part of a fungal complex associated with cladode brown spot in *Nopalea cochenillifera* (L.) Mill. (Conforto *et al.* 2019), and in China it was documented as a cause of seed rot in *Camellia oleifera* C.Abel (Tang *et al.* 2021). Recently, Huanaluek *et al.* (2021) identified *Pestalotiopsis hydeana* as the causative agent of leaf spots and fruit rot in several hosts, including *Annona squamosa* L. This marks the first documented occurrence of a pestalotioid fungus infecting this host. To date, no studies worldwide have confirmed the presence of *Neopestalotiopsis* species in *Annona muricata* crops. Given this knowledge gap, our study seeks to characterize *Neopestalotiopsis* species associated with leaf spot symptoms in soursop plants from the Junín region in Peru. The results could offer critical insights for early disease detection, accurate pathogen identification, and evidence of fungal pathogen diversity within a single crop system.

Material and methods

Field Sampling and Fungal Isolation

Leaf sample collection took place from February to July 2024 in *Annona muricata* orchards of the "Giant Criolla" variety located in the Pichanaqui district, Chanchamayo province (10°56'11"S, 74°53'02"W), and on farms cultivating the "common" variety in San Martín de Pangoa district, Satipo province (11°25'49"S, 74°28'32"W), both situated in the central jungle region of Junín, Peru. Leaves showing symptoms of irregular spots on the edges, characterized by gray and dark brown discoloration, were collected and transported to the laboratory. The plant material was cut into small pieces (5 mm) from both healthy and necrotic areas, and the surface sterilized by soaking in 70% ethanol (v/v) for one minute, followed by 2% sodium hypochlorite (v/v) for one minute. The samples were then rinsed three times with sterilized distilled water and air-dried on sterile absorbent paper inside a laminar flow hood. The cut leaf fragments were incubated on Potato dextrose agar (PDA) at 25 °C for 48 to 72 hours. Pure cultures were obtained from representative colonies (white to pale yellow, with aerial mycelium and lobulated edges), which were subsequently used for morphological and molecular characterization, as well as for pathogenicity testing. Symptomatic herbarium specimens were deposited in the Fungi Revolution Fungarium (Lima, Peru); while living cultures were preserved at the Laboratory of Biotechnology and Molecular Diversity (BIODIM), Universidad Nacional Agraria de la Selva, Tingo María, Peru.

Fungal and colony morphology

Morphological and cultural aspects of the colonies were examined by growing each isolate on PDA medium for 7 days. At this point, the following morphological characteristics of the conidia were recorded: length (µm), width (µm), septation, presence or absence of a basal appendage, and the number and length of apical appendages (µm). These

observations were made using a compound microscope (Leica DM 500). Slides were prepared with lactophenol. Additionally, the pigment of the median cells was noted and classified as "concolor" if the median cells exhibited uniform pigmentation, or "versicolor" if the median cells showed uneven pigmentation.

Pathogenicity test

In this study, pathogenicity tests were conducted on healthy leaves of approximately four-month-old *A. muricata* seedlings of the "Giant Criolla" and "common" varieties. The inoculation method involved placing mycelial plugs directly onto the leaves. The fungal isolates SMPP1F3 and PICHP3R2 were first subcultured on PDA medium and incubated in darkness at 25 °C for 7 days. For each seedling, three healthy leaves were selected and surface-sterilized using 70% ethanol. Inoculations were performed using two approaches: one involving superficial wounding and another without causing injury. In the wounded treatment, sterile needles were used to gently pierce the leaf surface before applying the mycelial plugs. For the control group, PDA plugs without fungal growth but of the same size were used. To maintain humidity and provide appropriate conditions for infection, sterile distilled water-moistened cotton was placed on top of the mycelial plugs over the inoculated leaves. Each fungal isolate was tested on three leaves, with three replicates per treatment. The inoculated samples were then kept under ambient greenhouse conditions. After 72 hours, the mycelial plugs were removed, and the development of symptoms was carefully monitored and documented. Consistent with leaf inoculation protocols, healthy detached fruits were inoculated with mycelial plugs under aseptic conditions and incubated in a humidity chamber (25±1 °C, >90% relative humidity, 12-h photoperiod) for 72 hours to evaluate pathogenicity. Symptomatic tissues were then used for fungal re-isolation, and the resulting cultures were compared morphologically with the original isolates to fulfill Koch's postulates.

DNA extraction, PCR and amplicon sequencing

Genomic DNA was isolated following a modified CTAB method originally described by Doyle and Doyle (1990), utilizing a 2.8% CTAB buffer and incorporating minor protocol adjustments to optimize yield and purity. Amplification of target gene regions was performed via PCR using specific primer sets: ITS1/ITS4 for the internal transcribed spacer (White *et al.*1990), Bt2a/Bt2b for the β-tubulin 2 gene (O'Donnell & Cigelnik 1997), and EF1-728F/EF-2R for the partial translation elongation factor 1 α (Carbone & Kohn 1999, O'Donnell *et al.* 1998). PCR conditions for each marker followed established protocols: ITS amplification parameters were adapted from Fuentes-Aragón *et al.* (2018), while the thermal cycling conditions for tub2 and TEF1-α followed Gualberto *et al.* (2021). Specifically, the thermocycling profile for the ITS region included an initial denaturation at 94 °C for 5 minutes, followed by 30 cycles of 45 seconds at 94 °C, 60 seconds at 55 °C, and 60 seconds at 72 °C, with a final extension step of 7 minutes at 72 °C. For the tub2 gene, the protocol began with 2 minutes at 96 °C, followed by 30 cycles comprising 30 seconds at 94 °C, 30 seconds at 56 °C, and 30 seconds at 72 °C, ending with a final extension of 7 minutes at 72 °C. The TEF1-α reaction started with 3 minutes at 94 °C, followed by 40 cycles of 60 seconds at 94 °C, 30 seconds at 54 °C, and 30 seconds at 72 °C, concluding with a 5-minute extension at 72 °C. All amplified products were subsequently submitted for purification and sequencing through the services of Macrogen Inc. (Chile).

Phylogenetic tree construction

Raw DNA sequence data were manually curated using *Geneious* software version 11.1.5 (Kearse *et al.* 2012). Sequence identities were initially assessed through BLAST searches (Altschul *et al.* 1990). To conduct phylogenetic comparisons, reference sequences corresponding to the ITS, tub2, and TEF1-α nucleotide sequences for multiple *Neopestalotiopsis* species were retrieved from GenBank (NCBI 1988), based on datasets cited in previous studies (Maharachchikumbura *et al.* 2012, 2014; Jayawardena *et al.* 2015). The species *Pestalotiopsis colombiensis* was designated as the outgroup for the analysis. Accession numbers and taxonomic details for all included sequences are presented in Table 1. Alignments were carried out using the MAFFT algorithm (Katoh *et al.* 2002) applying the 200PAM/k=2 scoring matrix, with a gap opening penalty of 1.53 and an offset value of 0.123. Terminal regions of the alignments were trimmed to remove poorly aligned ends. The three individual alignments were then concatenated in the following order: ITS, tub2, TEF1-α. Phylogenetic inference was conducted using three complementary approaches: Maximum Likelihood (ML), Maximum Parsimony (MP), and Bayesian Inference (BI). All analyses were performed on the concatenated dataset of the three gene regions. ML trees were inferred using the RAxML algorithm (Stamatakis 2014) with the GTR+GAMMA substitution model, employing 1,000 bootstrap replicates and initiating the search from a randomized

tree topology. For the MP analysis, alignments were converted to MEGA format and carried out using the MEGA X software suite (Kumar *et al.* 2018). Prior to BI, the best-fit nucleotide substitution models for each gene partition were determined using JModelTest v2.1.10 (Darriba *et al.* 2012). This model selection process considered 11 substitution schemes, including estimations of base frequencies (+F), rate heterogeneity (+I and +G), and likelihood optimization via SPR-based tree searches. Bayesian inference was implemented using MrBayes (Huelsenbeck & Ronquist 2001), executed as a plugin within Geneious software. The analysis employed the GTR+GAMMA model, with four heated chains (temperature = 0.15) run over 1,500,000 generations. Trees were sampled every 1,000 generations, and the first 150,000 generations were discarded as burn-in. The final Bayesian tree was generated using the combined alignment across ITS, TUB, and TEF1-α regions, with appropriate partitioning defined within the NEXUS file.

Results

Phylogenetic analysis

Phylogenetic analyses were performed using concatenated sequences of the ITS, tub2, and TEF1-α partial regions. The sequences obtained in this study were submitted to GenBank. All sequences from the two isolates showed 95–100% identity with previously published sequences available in the NCBI GenBank database. For the phylogenetic analysis, a tree was constructed using these two isolates along with 18 reference strains of *Neopestalotiopsis* spp. retrieved from GenBank (Table 1). *Pestalotiopsis colombiensis* was included as the outgroup. Tree reconstruction was conducted using three independent methods: Maximum Likelihood (ML), Maximum Parsimony (MP), and Bayesian Inference (BI), all of which yielded congruent topologies. The consensus tree (Figure 1) presents the ML topology, with nodal support values from all three methods (ML bootstrap ≥ 50%, MP bootstrap ≥ 50%, and BI posterior probabilities ≥ 0.50) indicated at branch nodes. Isolate SMPP1F3 clustered robustly within the *N. clavispora* clade, while isolate PICHP3R2 grouped unequivocally with *N. protearum* strains.

TABLE 1. Sequences used for phylogenetic analysis, along with their corresponding GenBank accession numbers, are listed. Sequences obtained in this study are indicated in bold. Reference sequence data were sourced from the following references: Maharachchikumbura *et al.* (2014), Jayawardena *et al.* (2016), Akinsanmi *et al.* (2017), Razaghi *et al.* (2024), and Zhang (2024, unpublished).

				Genbank accession numbers		
Species	**strain**	**Host**	**Country**	**ITS**	**tub2**	**TEF1-α**
Pestalotiopsis colombiensis	CBS 118553	*Eucalyptus eurograndis*	Colombia	KM199307	KM199421	KM199488
Neopestalotiopsis clavispora	**SMPP1F3**	***Annona muricata***	**Peru**	**PV549392**	**PV696244**	**PV696242**
Neopestalotiopsis protearum	**PICHP3R2**	***Annona muricata***	**Peru**	**PV549391**	**PV696243**	**PV696241**
Neopestalotiopsis iranensis	CBS 137768	*Fragaria x ananassa*	Iran	OR230041	OR381098	OR380984
Neopestalotiopsis surinamensis	CBS 450.74	Soil under *Elaeis guineensis*	Suriname	KM199351	KM199518	KM199465
Neopestalotiopsis mesopotamica	CBS 299.74	*Eucalyptus* sp.	Turkey	KM199361	KM199435	KM199541
Neopestalotiopsis vitis	JZB340023	*Vitis vinifera* cv.“kyoho”	China	KU140699	KU140690	KU140681
Neopestalotiopsis javaensis	CBS 257.31	*Cocos nucifera*	Indonesia: Java	KM199357	KM199543	KM199437
Neopestalotiopsis rosae	CBS 101057	*Rosa* sp.	New Zealand	KM199359	KM199523	KM199429
Neopestalotiopsis piceana	CBS 394.48	*Picea* sp.	UK	KM199368	KM199453	KM199527
Neopestalotiopsis eucalypticola	CBS 264.37	*Eucalyptus globulus*	-	KM199376	KM199431	KM199551
Neopestalotiopsis honoluluana	CBS 111535	*Telopea* sp.	USA: Hawaii	KM199363	KM199461	KM199546
Neopestalotiopsis saprophytica	CBS 115452	*Litsea rotundifolia*	Hong Kong	KM199345	KM199538	KM199433
Neopestalotiopsis cubana	CBS 600.96	Leaf litter	Cuba	KM199347	KM199521	KM199438
Neopestalotiopsis protearum	GUCC 23-0259	-	-	PQ129343.1	PQ148928.1	PQ140978.1
Neopestalotiopsis protearum	GUCC 23-0323	-	-	PQ129357.1	PQ148942.1	PQ140992.1

.....continued on the next page

TABLE 1. (Continued)

				Genbank accession numbers		
Species	**strain**	**Host**	**Country**	**ITS**	**tub2**	**TEF1-α**
Neopestalotiopsis macadamiae	BRIP 63737c	*Macadamia integrifolia*	Australia	KX186604.1	KX186654.1	KX186627.1
Neopestalotiopsis saprophytica	MFLUCC12-0282	*Magnolia* sp.	China	JX398982	JX399017	JX399048
Neopestalotiopsis foedans	CGMCC3.9123	Mangrove plant	China	JX398987	JX399022	JX399053
Neopestalotiopsis clavispora	MFLUCC12-0280	*Magnolia* sp.	China	JX398978	JX399013	JX399044
Neopestalotiopsis clavispora	MFLUCC12-0281	*Magnolia* sp.	China	JX398979	JX399014	JX399045

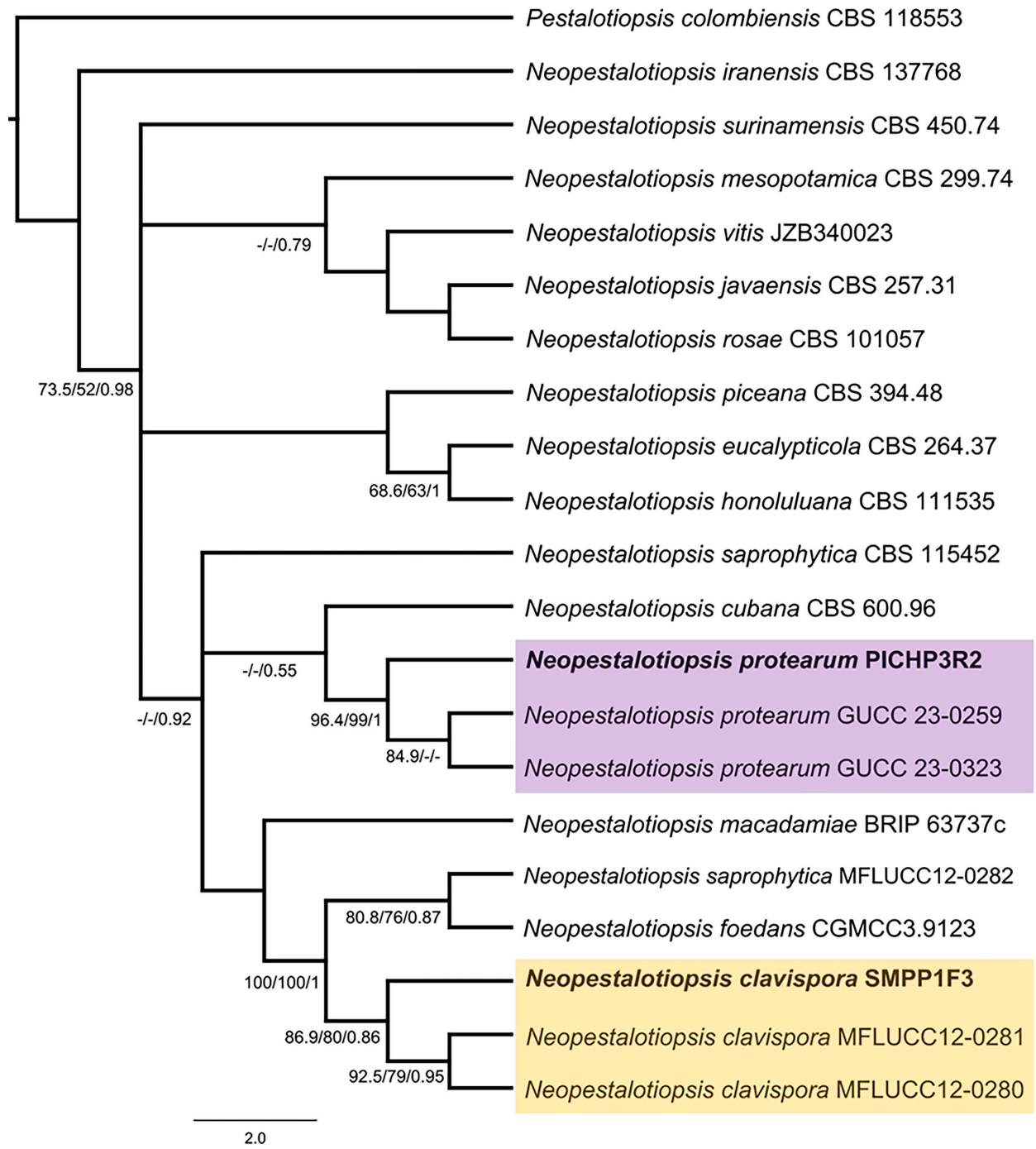

FIGURE 1. Phylogenetic tree showing the relationships within *Neopestalotiopsis*, based on a concatenated dataset of ITS, tub2, and TEF1-α partial gene sequences. The two *Neopestalotiopsis* species under study are shown in bold and highlighted with purple (*N. protearum*) and orange (*N. clavispora*). Topology was inferred using maximum likelihood (ML) analysis, and support values (>50%) from maximum likelihood (ML), maximum parsimony (MP) and Bayesian Inference (BI) are shown at each node (in the order: ML/MP/BI).

Pathogenicity

Typical necrotic symptoms were observed on soursop leaves 3–4 days after inoculation with *N. clavispora* (isolate SMPP1F3) and *N. protearum* (isolate PICHP3R2). These symptoms closely resembled those found under natural field conditions (Figure 2A, F) and were consistently reproduced in all inoculated tissues. In the "common" variety, *N. clavispora* produced characteristic lesions (Figure 3A), while *N. protearum* caused similar necrotic spots (Figure 4A); in the "Giant Criolla" variety, both pathogens elicited comparable necrosis (Figures 3B and 4B). Pathogenicity was confirmed through successful re-isolation of the fungi, thus fulfilling Koch's postulates. Fruit inoculations further supported their pathogenic role, as *N. clavispora* induced necrosis in both "common" (Figure 3C, D) and "Giant Criolla" (Figure 3E, F) varieties, and *N. protearum* produced analogous lesions in fruits of both varieties (Figure 4C–F).

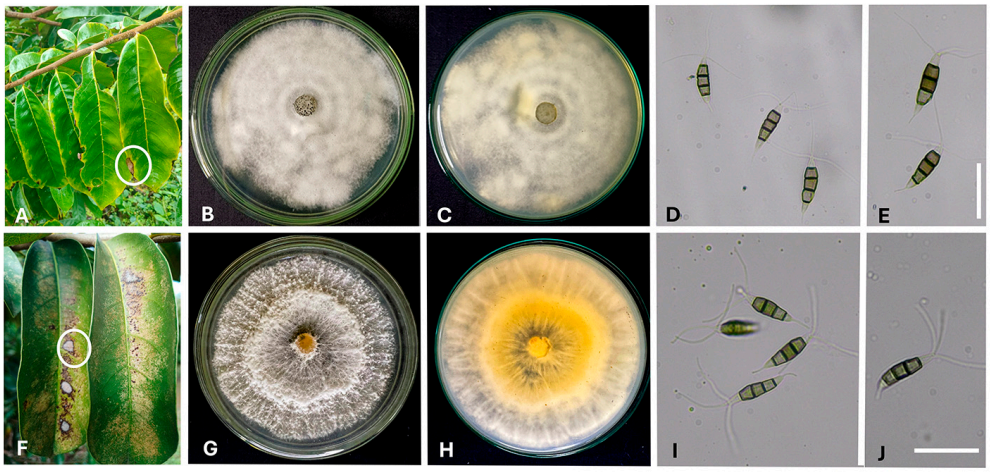

FIGURE 2. *Neopestalotiopsis clavispora* (A–E); (A) Field symptoms of leaf spot on soursop leaves; (B) Colony aspect on PDA medium (top view); (C) Colony aspect (bottom view); (D, E) Conidia. *Neopestalotiopsis protearum* (F–J); (F) Field symptoms of leaf spot on soursop leaves; (G) Colony aspect on PDA medium (top view); (H) Colony aspect (bottom view); (I, J) Conidia. Scale bars: D, E, I, J = 20 μm.

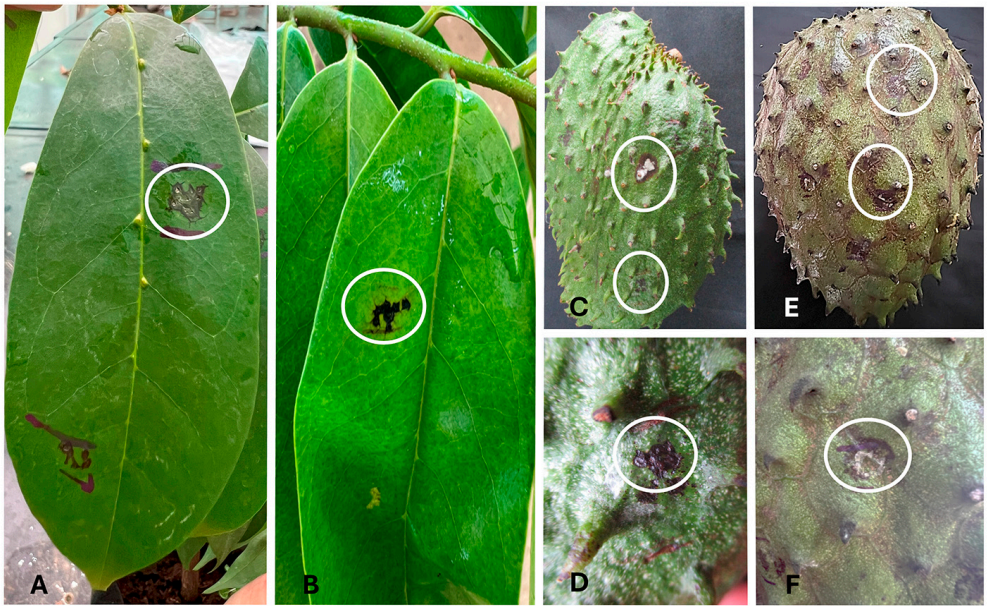

FIGURE 3. Pathogenicity assay of *Neopestalotiopsis clavispora* in soursop (*Annona muricata* L.). Symptoms on seedling leaves of the "common" (A) and "Giant Criolla" (B) varieties post-inoculation. Necrotic lesions on wounded detached fruits at 72 hours post-inoculation with mycelial plugs from "common" variety (C, D) and "Giant Criolla" variety (E, F).

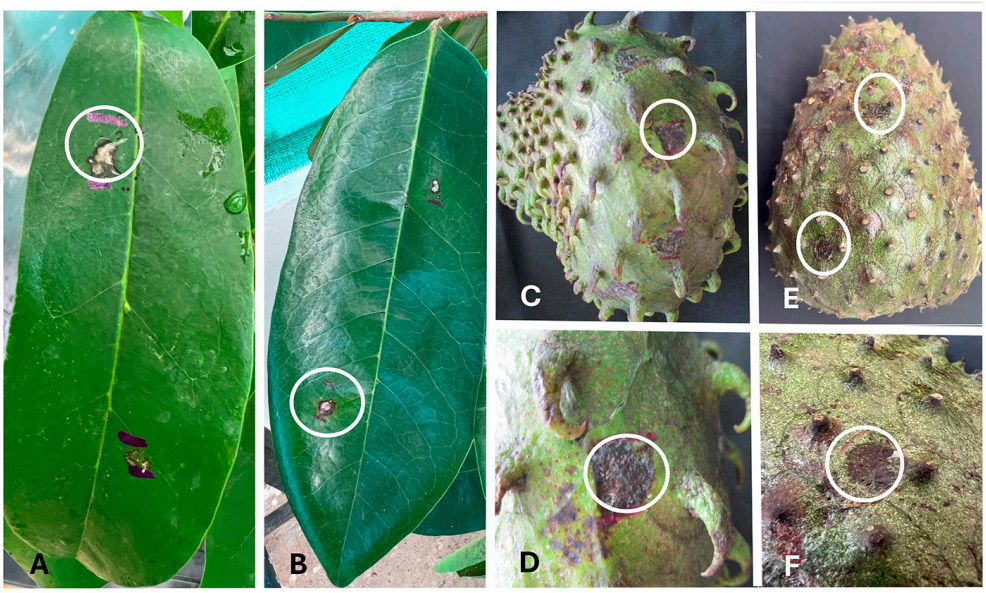

FIGURE 4. Pathogenicity evaluation of *Neopestalotiopsis protearum* in soursop (*Annona muricata* L.). Symptoms on seedling leaves of the "common" (A) and "Giant Criolla" (B) varieties after inoculation. Necrotic lesions on wounded detached fruits at 72 hours post-inoculation with mycelial plugs from the "common" variety (C, D) and "Giant Criolla" variety (E, F).

Taxonomy

Neopestalotiopsis clavispora (G.F. Atk.) Maharachch., K.D. Hyde & Crous, in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, Stud. Mycol. 79: 138 (2014).

Culture characteristics: Circular colony with irregular wavy margins; cottony, white aerial mycelium with pale yellow underside; 80 mm diameter after 7 days at 25 °C.

Conidia straight, fusoid, to slightly curved, 4-septate, 21–28 × 5–8 µm (n = 30); basal cells broad and conical with a truncated base, hyaline and thin-walled, 3–5 µm long; Three medium-sized cells, 14–18 µm, rough wall, versicolor, septa darker than the rest of the cells, second cell from the base light brown, 4–6 µm long; third cell brown, 4–6 µm long; fourth cell brown, 4–5 µm; apical cell 3.5–5 µm long, hyaline, conical, with 2–3 tubular apical appendages arising from the apical tip, unbranched, filiform, flexuous, 15–25 µm long; basal appendage present, tubular, unbranched, centric, 4–7 µm long.

Material examined: Peru, Junin State, Satipo province, San Martin de Pangoa district, on leaves of soursop (*Annona muricata* L.), February 17, 2024, G.C.C.Yabar, FRF-2025-000001 Fungi Revolution Fungarium, ex-type living culture (BIODIM 2024)

Neopestalotiopsis protearum (Crous & L. Swart) Maharachch., K.D. Hyde & Crous, Studies in Mycology 79: 147 (2014)

≡ *Pestalotiopsis protearum* Crous & L. Swart, Persoonia 27: 34 (2011)

Culture characteristics: Colony on PDA at 25 °C, circular with wavy margins; with woolly, white aerial mycelium, the reverse of the plate honey-yellow; 76 mm in diameter after 7 days at 25 °C. With conidiomata developing over the entire surface of the colony, producing masses of moist, black spores.

Pycnidial conidiomata are globose, solitary, and black. Conidia ellipsoidal, straight to slightly curved, 4-septate, 20–25 × 6–8 µm (n = 30), basal cell obconic with a truncated base, hyaline, slender 4–5 µm long, 2nd cell from base subcylindrical, verruculose, 4–5 µm, 3rd and 4th cells doliform to subcylindrical, dark brown, verruculose, 4–5 and 5–7 µm long, respectively, 3-celled medium 14–17 µm, apical cell hyaline, obconic to cylindrical, 4–5 µm, with 1 to 3(4) tubular apical appendage, arising from apical ridge, 15–32 µm, basal appendage occasionally absent, filiform, flexuous, thin, hyaline, 3–5 µm.

Material examined: Peru, Junin State, Chanchamayo Province, Pichanaqui District, on leaves of soursop (*Annona muricata* L.), July 22, 2024, G.C.C.Yabar, FRF-2025-000002, Fungi Revolution Fungarium, ex-type living culture (BIODIM, 2024)

Discussion

Fungal diseases have become increasingly prevalent in forest and agricultural systems worldwide, with emerging pathogens posing new challenges to crop protection (Fisher *et al.* 2012, Liang *et al.* 2021, Gu *et al.* 2018, Gomdola *et al.* 2022). Among these, *Neopestalotiopsis* species have gained attention for their capacity to induce a broad spectrum of symptoms across multiple plant tissues, including characteristic leaf spots, fruit rot, and dieback symptoms (Jayawardena *et al.* 2016, Gualberto *et al.* 2021). To our knowledge, this is the first report identifying *N. clavispora* and *N. protearum* as causative agents of leaf spot and fruit rot in *Annona muricata* (soursop), and this represents a significant expansion of their known host range and geographical distribution. Pathogenicity assays confirmed that both fungal species can infect both leaf and fruit tissues, indicating their potential to cause disease under natural conditions. These experiments showed consistent pathogenic behavior in both commercially important soursop cultivars ("common" and "Giant Criolla"), with successful tissue colonization observed under controlled experimental settings. The successful isolation and characterization of these pathogens through combined morphological and molecular approaches (ITS, β-tubulin, and TEF1-α sequencing) provides reliable diagnostic markers for future surveillance efforts. Moreover, the observed conidial morphology and pigmentation patterns align precisely with previous taxonomic descriptions (Maharachchikumbura *et al.* 2012), while phylogenetic analysis confirms their placement within the *Neopestalotiopsis* clade. A related species, *Pestalotiopsis hydeana*, was recently reported to infect *Annona squamosa* (Huanaluek *et al.* 2021), further featuring the capacity of pestalotioid fungi to infect diverse hosts within the Annonaceae family. While current field observations indicate relatively limited disease impact in Peruvian orchards, the experimental evidence of pathogenic capability raises important concerns. These organisms are potentially to emerge as important pathogens when environmental conditions become more favorable or agricultural practices undergo modification (Yang *et al.* 2024). The most concerning ecological aspect is their proven ability to jump to additional host plants under suitable environmental conditions (Jayawardena *et al.* 2020). *Neopestalotiopsis* exhibit notable ecological adaptability and broad host plasticity. *Neopestalotiopsis clavispora* has been documented in a wide range of crops including macadamia, banana, coconut, and apple (Santos *et al.* 2019, Qi *et al.* 2022, Chong *et al.* 2023, Shi *et al.* 2024). Similarly, *N. protearum* has been isolated from diverse ornamental and agricultural hosts such as proteas, orchids, prickly pear, and camellia (Crous *et al.* 2011, Ran *et al.* 2017, Conforto *et al.* 2019, Tang *et al.* 2021). These findings underscore the growing importance of these fungi in tropical and subtropical cropping systems and highlight their potential to affect a wide variety of economically significant plant species. The possibility of pathogen spillover between adjacent agricultural systems also suggests a risk of infection to non-host species. The findings emphasize the urgent need for the development of species-specific diagnostic protocols, enhanced surveillance for emerging pathogens, systematic evaluation of cultivar resistance under field conditions, and investigation of potential yield impacts. Future research directions should prioritize comprehensive assessments of these pathogens' epidemiological characteristics, particularly their environmental optimal conditions, dispersal mechanisms and control methods, to inform effective management strategies before potential disease escalation occurs.

Acknowledgements

We would like to express our gratitude to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior) for providing financial support for this research. We also thank BIODIM (Biotechnology and Molecular Diversity Laboratory) for providing the infrastructure for molecular and phylogenetic analyses. We also thank the UNAS (Universidad Nacional Agraria de la Selva) for funding the sequencing. We also thank Engineer Ronald Cesar Ingaruca Lopez, head of the avocado and soursop project of the Junin state government, for helping us to collect samples in the experimental fields of said project. We gratefully acknowledge the Fungi Revolution Fungarium for the acceptance and curation of the herbarium specimens. Finally, we extend our sincere thanks to José Luiz Bezerra for his excellent review of this manuscript.

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