Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

FIRST REPORT OF LEAF SPOT OF *CHLOROPHYTUM COMOSUM* CAUSED BY *THIELAVIA TERRESTRIS* FROM PAKISTAN

[a]Khadija Ashraf, [a]Maryam Nawaz, [a]Nousheen Yousaf, [b]Najam-ul-Sehar Afshan
[a] *Department of Botany, Government College University Lahore, Katchery Road, 54000, Lahore, Pakistan.*
[b] *Institute of Botany, University of the Punjab, Lahore, Quaid-e-Azam Campus, Pakistan.*

ARTICLE INFO

Article history
Received: 23th September, 2022
Revised: 17th October, 2022
Accepted: 24th October, 2022

Keywords
Leaf spot
Necrosis
Taxonomy

ABSTRACT

In efforts to record pathogenic fungal diversity on ornamental plants of Pakistan, infected leaves of *Chlorophytum comosum* plants were collected from commercial nursery in Lahore, Pakistan. Investigation of leafspot symptoms led to identification of an ascomycete fungus, *Thielavia terrestris.* Analysis for fungal identification involved morphological, microscopic and molecular methods. ITS-nrDNA sequence data were used to construct molecular phylogenetic tree of *Thielavia* with allied species. The fungus was confirmed as *T. terrestris*. This is the first report of *T. terrestris* causing disease in *C. comosum* and it is also a new record for Pakistan.

Corresponding Author: Nousheen Yousaf
Email: dr.nousheenyousaf@gcu.edu.pk
© 2022 EScience Press. All rights reserved.

INTRODUCTION

Chlorophytum comosum (Thunb.) Jacques, is an evergreen perennial herb belonging to family Liliaceae. Due to its spider-like appearance, *C. comosum* is frequently referred to as spider plant, common spider plant, or spider ivy. Due to the beauty of its leaf, this native South African plant is grown both indoors and outdoors all over the world. *C. comosum* can fall several feet when used as a hanging plant, however it only reaches a height of around 60 cm. The long, thin leaves are around 6-25 millimetres broad and 20-45 cm long (McCune and Hardin, 1994). This plant has the unusual capacity to thrive in a broad range of light and temperature situations, and it does so especially successfully in areas with more daylight. Due to its hardiness, this plant species is simple to cultivate as a house plant and because of its sensitivity to fluoride in tap water, it frequently develops "burnt tips." The most common variations of this taxon are variegated (Howell et al., 1958). It is renowned for its high biomass output, easy cultivation, fierce competitiveness, high pollutants stress tolerance and elimination of gaseous waste (Ammonia, carbon dioxide, benzene, cigarette smoke, formaldehyde, nitrogen dioxide, ozone and toluene) (Liu, 2011).

Previously reported fungal pathogens on *C. comosum* include *Aecidium hartwegiae* from South Africa; *Alternaria alternata* from India; *Cercospora* sp., *Colletotrichum* sp., *Fusarium* sp. *Phyllosticta* sp., *Pythium splendens* and *Rhizoctonia solani* from Florida; *Mycoleptodiscus indicus* and *Pucciniopsis guaranicola* from Cuba; *Sclerotium rolfsii* from Argentina (Gutiérrez and Cúndom, 2006; Tou et al., 2019). The main objective of the sudy was to identify the fungal pathogen casuing leaf spot symptoms on ornamenal plants.

MATERIALS AND METHODS

Sampling sites and Morphological characterization

In December 2018, infected leaves of *C. comosum* plants were collected from commercial nursery in Lahore,

Pakistan. Approximately 20% to 55% of plants showed symptoms of leaf necrosis at the tips. For the isolation of related fungal pathogen, about 5 mm long infected leaves were surface sterilized with 2% sodium hypochlorite, washed with distilled water (sterile), dried in air and placed on Potato Dextrose Agar (PDA) in Petri plates at 27°C. The inoculated plates were left for 4-5 days. After obtaining fungal cultures, morphology of the colony was observed.

Slides were prepared using 5% KOH as mounting medium for microscopic examination of fungal taxon. Trypan blue was used as a staining agent for hyaline structures. Prepared slides were observed under different magnifications of Labomed compound microscope. Light micrographs were captured using camera. Dimensions of microscopic characters were calculated using ocular micrometer and drawings were made using camera Lucida. Detailed descriptions were prepared by combining characters of colony morphology and microscopic characters.

Molecular characterization and phylogenetic analysis

Genomic DNA was extracted from fresh cultures by modified CTAB protocol. ITS-nrDNA region was amplified through PCR using fungal specific ITS1F and universal ITS4 primer pairs (Gardes and Bruns, 1993). Agarose gel electrophoresis was used to validate the PCR results. Afterwards, sequencing and purification of DNA products was done by Macrogen Inc. (South Korea). For construction of phylogenetic analysis, obtained forward and reverse DNA sequences were edited manually, and consensus sequence was created using BioEdit software. MUSCLE alignment software was used for Multiple alignment (Edgar, 2004). Using MEGA 7 software, phylogenetic tree was established with the Maximum Likelihood Algorithm (Kumar et al., 2016). A bootstrap consensus tree was inferred from 1000 replicates, and corresponding bootstrap values >50 % were cited in the tree. Generated ITS-nrDNA sequences of *Thielavia terrestris* was submitted to GenBank.

RESULTS

Taxonomy

***Thielavia terrestris* (Apinis) Malloch and Cain**

Colonies on PDA were green, white to grey at margins, globose, margins entire, surface raised, floccose, powdery, with white to grey aerial mycelium; reverse light yellow. **Cleistothecium** dark brown, spherical, thick-walled, surface rough, contains numerous asci and ascospores, 140-175 µm; **Appendages** dark brown, septate, unbranched, ornamented, ends tapering, intermingled, 8.5-125 × 1.9-4.2 µm; **Ascospores** brown, sub-globose, flat, limoniform; broader at middle, narrower towards tips, thick-walled, 8-11 × 5-8 µm. **Hyphae** thick-walled, light brown to hyaline, aseptate, encrusted, with tapering ends (Figure 1, 2 and 3).

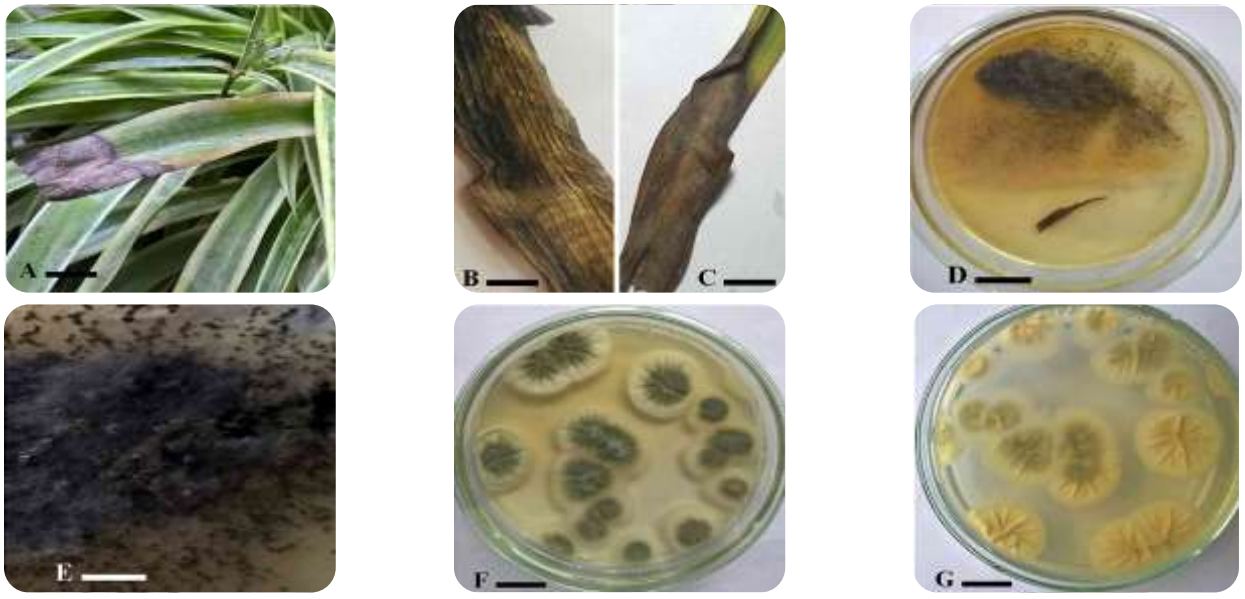

Figure 1: A-G: ***Thielavia terrestris*** **(A-C)** Infected leaves of *Chlorophytum comosum* **(D-F)** Pure culture **(E)** Spores appearing hyphal network on colony **(G)** Reverse of colony. Scale bars for A= 3cm, B-C= 2.7cm, D= 1.5cm, E= 1.3cm, F-G, 1.2cm.

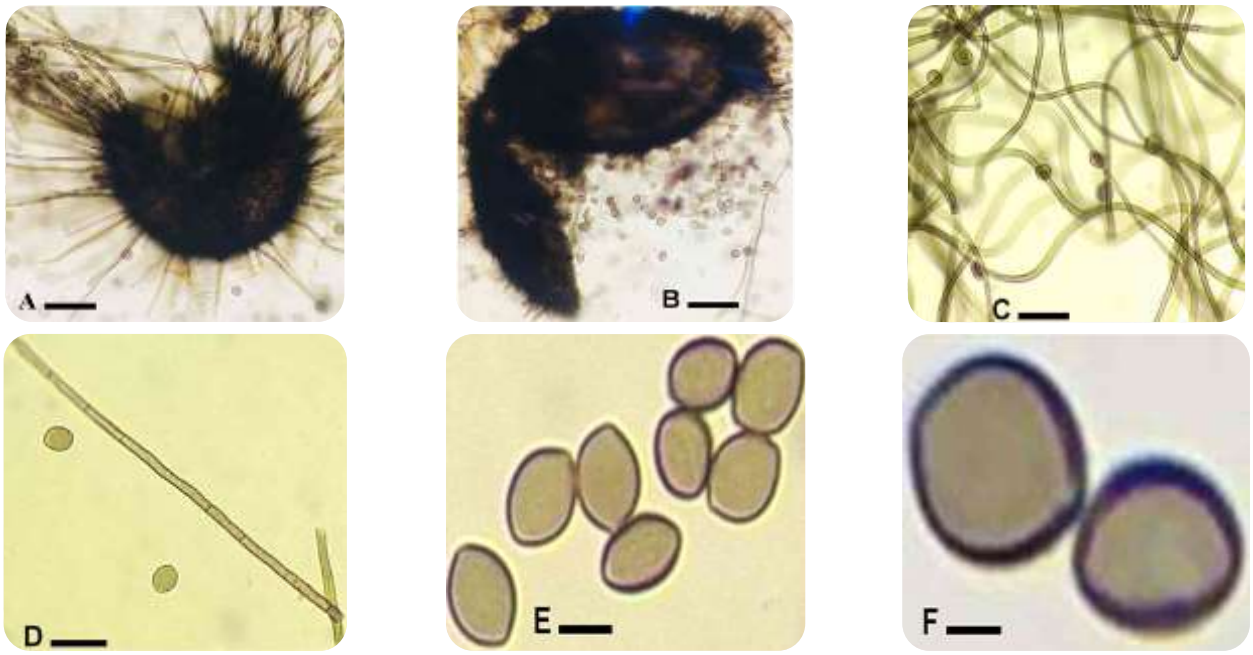

Figure 2: A-F: Light micrographs of microscopic features of *Thielavia terrestris* (A and B) Mature and ruptured cleistothecium **(C)** Aseptate unbranched hyphae **(D)** Septate, unbranched appendages of cleistothecium **(E and F)** Thick-walled ascospores. Scale bars for A= 37µm, B= 29µm, C= 7µm, D= 6µm, E= 7µm, F= 3µm.

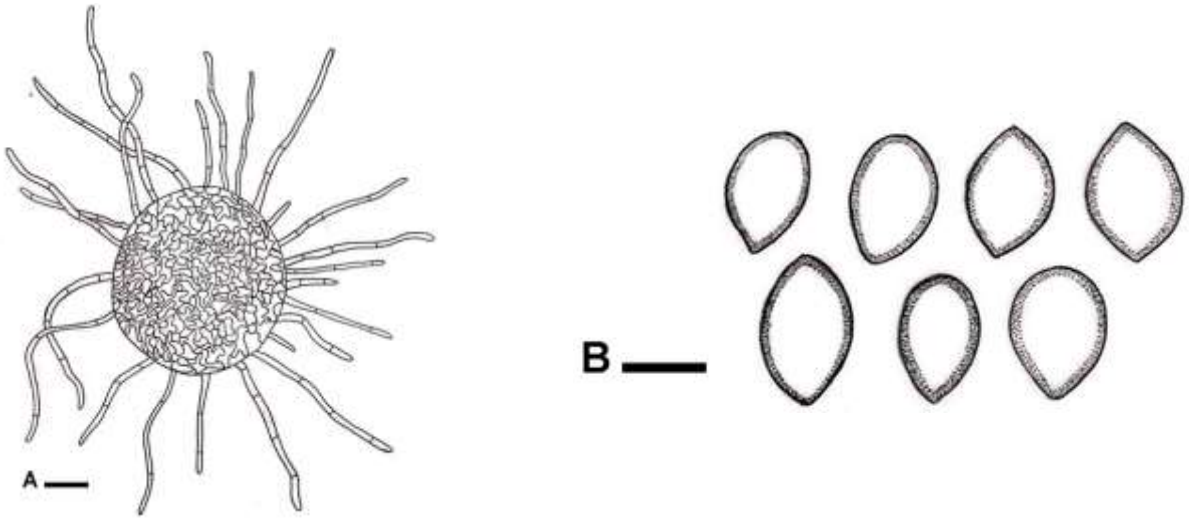

Figure 3: A and B: Illustrations of *Thielavia terrestris* (A) Cleistothecium **(B)** Ascospores. Scale bars for A= 13.8µm, B= 5µm.

Material examined

Pakistan: Punjab, district Lahore, isolated from leaf of *C. comosum,* collected from Firdous Park, Lahore, 18 December 2018; Collector (K. Ashraf); Sample voucher code (PK14).

Pathogenicity Test

To demonstrate pathogenicity, 10 seedlings of healthy *S. saligna* plants were sprayed with 50 µl of the fungal conidial suspension (10^7 conidia/ml, 10 ml/plant). Sterile distilled water was used to treat five control plants. All the plants were kept at a temperature of 15 ± 5°C and 90% humidity. After 4 weeks of inoculation, distinctive leaf spots appeared on the leaves. From all the inoculated plants, *T. terrestris* was consistently reisolated. On control

plants, there were no symptoms seen.

Molecular Phylogenetic Analyses

The pathogen was identified as *Thielavia terrestris* Apinis, based on morphological and molecular phylogenetic characteristics. The Internal Transcribed Spacer (ITS1-5.8S-ITS2) region of nrDNA was amplified using ITS1F/ITS4 primer pair (White et al., 1990). The obtained sequence (MN879311) had 99% similarity with *T. terrestris* (MH305269). The taxon isolated in this study clustered with six other published sequences of *T. terrestris* by forming a separate clade from other sequences of Sordariales, thereby confirming its identity. Tree topology is in consistent with the published phylogram of Thanh et al. (2019) (Figure 4).

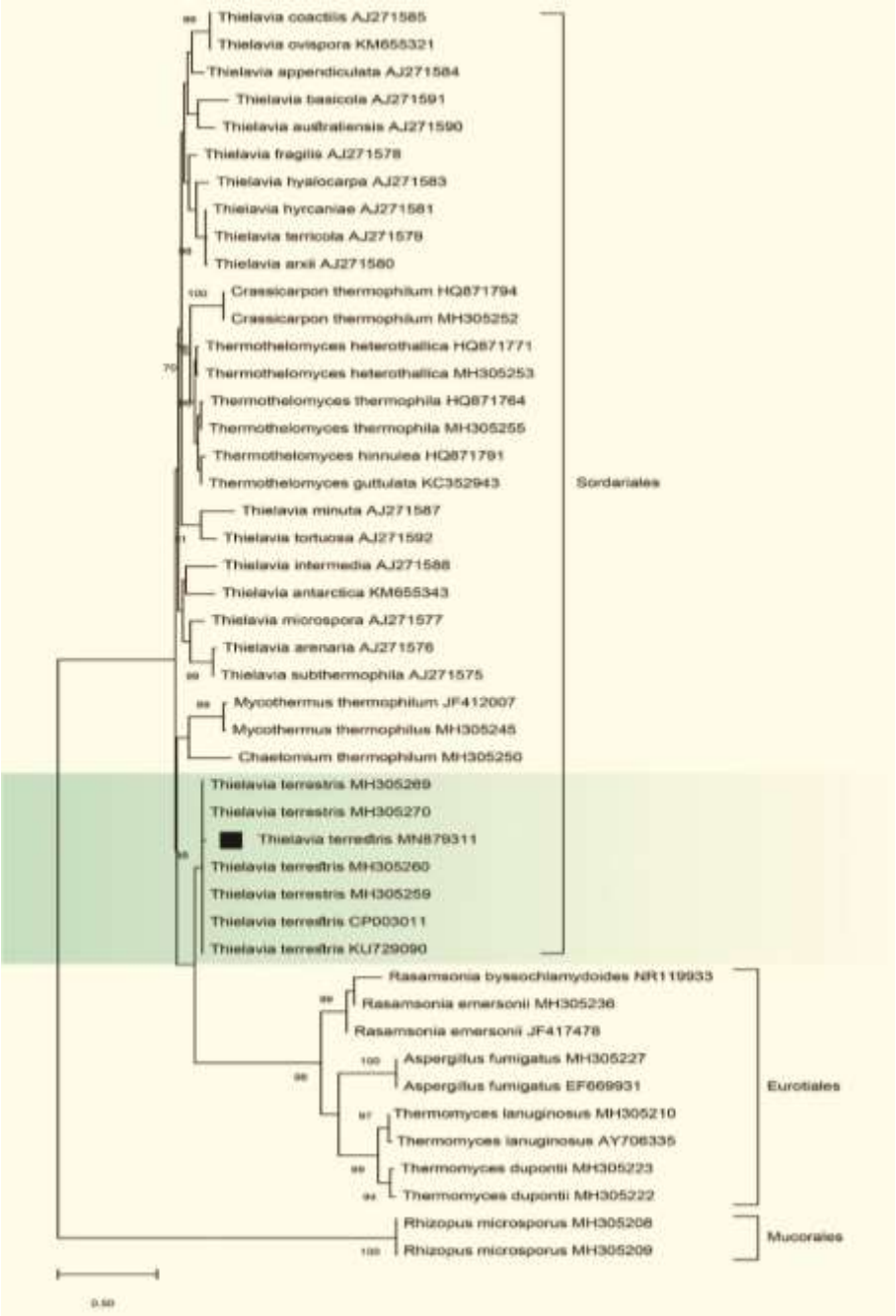

Figure 4: Molecular Phylogenetic analysis and ITS sequences of *Thielavia terrestris* by Maximum Likelihood method. Our sequence is indicated with bullet.

DISCUSSION

Theilavia, a genus of family chaetomiaceae comprised of about 31 species world-wide (Kirk et al., 2008). It is a pathogenic fungus and can cause disease in plants and humans. Morphologically, this genus can be identified by the presence of closed ascocarp i.e. cleistothecium, and dark brown, smooth ascospores. From Pakistan, about 8 species of this genus have been reported up till now (Ahmad et al., 1997). During the present study, *T. terrestris* was isolated from the leaves of *C. comosum* collected from Firdous Park, Lahore. It was characterized by black cleistothecium with unbranched, septate appendages and smooth, thick walled, brown ascospores. The pure culture of fungus on PDA appeared olive green. The fungus was identified by using a combination of morpho-anatomical and molecular characters.

C. cosmosum Jacques is a species of perennial flowering plants and was found as an ornamental plant as well. Different fungal pathogens and diseases have been reported from all over the world specifically on this plant i.e. *Aecidium hartwegiae* from South Africa (Crous et al., 2000; Doidge, 1950; Gorter, 1981), *Alternaria alternata* from India (Muthukumar and Venkatesh, 2013), *Alternaria* sp., causing leaf spot from Florida (Alam et al., 2017; Alfieri et al., 1984), *Athelia rolfsii* from Sri Lanka (Adikaram and Yakandawala, 2020), *Cercospora* sp. and *Colletotrichum* sp. causing leaf spot from Florida (Alfieri et al., 1984), *Fusarium* sp. causing leaf necrosis in Florida (Alfieri et al., 1984), *Mycoleptodiscus indicus* from Cuba (Urtiaga, 1986), *Phyllosticta* sp. causing leaf spot from Florida (Alfieri et al., 1984) and Japan (Motohashi et al., 2009), *Pucciniopsis guaranicola* from Cuba (Urtiaga, 1986), *Pythium splendens* causing root rot from Florida (Alfieri et al., 1984), *Rhizoctonia solani* causing root rot from Florida (Alfieri et al., 1984) and *Sclerotium rolfsii* causing Southern blight from Argentina (Gutiérrez and Cúndom, 2006). In the present study, *Thielavia terrestris* was reported as a new pathogen of *Chlorophytum comosum* from Pakistan. There are no previous records of any fungi reported on *C. comosum* from Pakistan.

CONCLUSION

This research work provides the first report of leaf spot disease of *Chlorophytum comosum* caused by *Thielavia terrestris* in Pakistan. Single-spore culturing of the fungus resulted *T. terrestris*, based on morphological characterization. Phenotypic characters and molecular analysis confirmed the fungal identification. Phylogenetic tree was generated with ITS sequence data analysis by Maximum Likelihood demonstrates the accurate position of *T. terrestris*. The search of the literature revealed that *C. comosum* is the new host for the pathogen from Pakistan. Identification of the fungal pathogen on plant is the first step for disease removal. It can prove helpful for the introduction and selection of new and effective fungicides for the pathogen's removal from important commercial plants.

AUTHORS' CONTRIBUTION

KA designed the study and conducted field surveys for the collection of samples and performed morphological and molecular analysis. MN helped in research work and manuscript writeup. NY provided the lab materials for conducting the research analysis, guided and supervised all the lab work. KA and NY wrote and edited the manuscript. NSA co-supervised the molecular analysis and conducted different identification tests for this pathogen.

ACKNOWLEDGEMENT

Authors are thankful to Prof. Dr. Abdul Nasir Khalid (Institute of Botany, University of the Punjab, Lahore, Pakistan) for providing resources for molecular analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Adikaram, N., Yakandawala, D., 2020. A checklist of plant pathogenic fungi and Oomycota in Sri Lanka. Ceylon Journal of Science 49, 93-123.

Ahmad, S., Iqbal, S.H., Khalid, A.N., 1997. Fungi of Pakistan. Mycological Society of Pakistan, Department of Botany, University of the Punjab, Lahore, Pakistan.

Alam, M., Rehman, A., Gleason, M., Khan, A., Amin, M., Ali, S., Fiaz, M., Ahmed, R., 2017. First report of *Alternaria alternata* causing postharvest fruit rot of lychee in Pakistan. Plant Disease 101, 1041.

Alfieri, S.A.J., Langdon, K.R., Wehlburg, C., Kimbrough, J.W., 1984. Index of plant diseases in Florida. Florida Department of Agriculture and Consumer Services, Division of Plant Industry. Bulletin 11, 1-

389.

Crous, P.W., Phillips, A.J.L., Baxter, A.P., 2000. Phytopathogenic Fungi from South Africa. University of Stellenbosch, Department of Plant Pathology Press, p. 358.

Doidge, E.M., 1950. The South African fungi and lichens to the end of 1945. Bothalia 5, 1-1094.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32, 1792-1797.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology 2, 113-118.

Gorter, G.J.M.A., 1981. Index of plant pathogens, II, and the diseases they cause in wild growing plants in South Africa. Department of Agricultural Technical Services. Science Bulletin 398, 1-84.

Gutiérrez, S., Cúndom, M., 2006. First record of *Sclerotium rolfsii* on *Chlorophytum comosum* in Argentina. Australasian Plant Disease Notes 1, 11-12.

Howell, J.T., Raven, P.H., Rubtzoff, P., 1958. Flora of San Francisco. Wasmann Journal of Biology 16, 1-155.

Kirk, P.M., Cannon, P.M., Minter, D.W., Stalpers, J.A., 2008. Ainsworth & Bisby's Dictionary of the Fungi, 10th ed. CABI Europe, UK.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33, 1870-1874.

Liu, D., 2011. *Myceliopthora and Thielavia*. Molecular detection of human bacterial pathogens. CRC press, p. 445.

McCune, S., Hardin, D.W., 1994. *Chlorophytum comosum* (Thunb.) Jacques. PlantNet 2, 3-10.

Motohashi, K., Inaba, S., Anzai, K., Takamatsu, S., Nakashima, C., 2009. Phylogenetic analyses of Japanese species of *Phyllosticta sensu stricto*. Mycoscience 50, 291-302.

Muthukumar, A., Venkatesh, A., 2013. A new record of leaf blight of ribben plant caused by *Alternaria alternata* in India. Journal on New Biological Reports 2, 228-230.

Thanh, V., Thuy, N., Huong, H., Hien, D., Hang, D., Anh, D., Hüttner, S., Larsbrink, J., Olsson, L., 2019. Surveying of acid-tolerant thermophilic lignocellulolytic fungi in Vietnam reveals surprisingly high genetic diversity. Scientific Reports 9, 3674.

Tou, I., Azri, Y., Sadi, M., Lounici, H., Kebbouche-Gana, S., 2019. Chlorophytum microbial fuel cell characterization. International Journal of Green Energy 16, 947-959.

Urtiaga, R., 1986. Index of plant diseases in Venezuela and Cuba. Printed in Printed New Century. SRL, Barquisimeto, Venezuela, p. 202.

White, T.J., Bruns, T., Lee, S., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp. 315–332.