Philippine Journal of Science
152 (4): 1433-1443, August 2023
ISSN 0031 - 7683
Date Received: 06 Oct 2022

Distribution of Bamboo Witches' Broom Disease in Various Bamboo Species in the Philippines and Molecular Identification of '*Candidatus* Phytoplasma luffae'-related Strain 16SrVIII

Lolita M. Dolores[1]*, Joanne A. Langres[3], Cris Q. Cortaga[1], and Merdelyn T. Caasi-Lit[2]

[1]Plant Pathology Laboratory, and [2]Entomology Laboratory,
Institute of Plant Breeding, College of Agriculture and Food Science,
University of the Philippines Los Baños, College, Laguna, 4031 Philippines
[3]College of Agriculture and Agri-Industries, Caraga State University,
Ampayon, Butuan City, Agusan Del Norte, 8600 Philippines

Bamboo is used for making structures, furniture, handicrafts, and ropes, as well as a source of food in the Philippines. One of the emerging diseases of bamboo in the country is the bamboo witches' broom (BWB), which has been occasionally noted in three genera of bamboo – including *Dendrocalamus*, *Gigantochloa*, and *Schizostachyum* from various provinces in the Philippines (Ilocos Norte, Laguna, Batangas, Quezon, Agusan del Sur, Bukidnon, and South Cotabato) since the 1990s. However, studies and information about BWB in the country remain lacking and largely unexplored. In this study, we report a similar disease affecting *Dendrocalamus* and *Gigantochloa* bamboo species from Bohol and Davao and – for the first time – in *Dendrocalamus merrillianus* ("bayog") and *Bambusa spinosa* ("kawayan-tinik") from Isabela and Nueva Vizcaya. As a result of the surveys conducted from 1999–2019, the disease is now identified in six species across four genera of bamboo – namely, *Gigantochloa* spp. (*G. levis* and *G. atter*), *Dendrocalamus* spp. (*D. asper* and *D. merrillianus*), *Schizostachyum lumampao*, and *Bambusa spinosa* recorded in 11 provinces in the Philippines. The BWB symptoms include clustering of leaves forming a rosette-like structure, leaf proliferation, excessive limb formation from a single node, and shortening of internodes. Nested PCR using the universal primers P1/P7 and R16MF2/R1 targeting the phytoplasma 16S ribosomal RNA gene revealed positive amplification in five symptomatic BWB samples from Isabela, Philippines. Subsequent sequencing (~ 1.3kbp) and phylogenetic analysis using the representative BWB isolates from Isabela revealed > 98.65% genetic similarity and clustering to *Candidatus* Phytoplasma luffae, which belongs to the 16SrVIII group (Loofah Witches' Broom Group). This paper determined the distribution of BWB in different species of bamboo in the Philippines, as well as the association of '*Ca*. Phytoplasma luffae'-related strain (16SrVIII) to BWB.

Keywords: bamboo, phytoplasma, witches' broom disease, 16S ribosomal RNA

*Corresponding author: lpmdolores@gmail.com

INTRODUCTION

Bamboo, comprised of at least 70 known species, is an economically and nutritionally important plant in the Philippines. It is mainly used for making handicrafts, furniture, and ropes; and for constructing buildings and other structures. Some edible bamboo can also be found in different provinces in the Philippines (Caasi-Lit *et al.* 2018). The Philippine bamboo industry ranked as the 6th largest exporter worldwide. Among the emerging diseases of bamboo that have been observed occasionally in the Philippines is the bamboo witches' broom (BWB). The disease is commonly reported in the genus *Dendrocalamus* and *Gigantochloa* plus few in *Schizostachyum* and *Phyllostachys* (Mohanan 1994; Dransfield and Widjaja 1995; Sinohin 1995; Caasi-Lit *et al.* 1999; Lit *et al.* 1999; Jung *et al.* 2006; Yadav *et al.* 2015). The infected plant exhibits symptoms of excessive branch proliferation at the nodal parts, smaller leaves, broom-like appearance, increased number of leaves than the normal, rosette-like structure, lighter infected leaves, shoot proliferation, and leaf malformation (Caasi-Lit *et al.* 1999; Jung *et al.* 2006; Yadav *et al.* 2015).

The disease is believed to be caused by *Candidatus* Phytoplasma (Mohanan 1994; Caasi-Lit *et al.* 1999), which is a phloem-inhabiting bacterium that lacks a cell wall. It is formerly called a mycoplasma-like organism due to its morphological similarity with mollicutes, which are common pathogens in animals (Dickinson *et al.* 2013). The unculturable nature of phytoplasmas makes it a challenging pathogen to study. Among the vital molecular techniques commonly used for the sensitive detection and identification of phytoplasmas are the polymerase chain reaction (PCR), sequencing of the highly conserved 16S ribosomal RNA (16S rRNA) gene, and restriction fragment length polymorphism (RFLP) analysis (Dickinson *et al.* 2013; Lee *et al.* 2010). In South Korea, the BWB-infected *Phyllostachys nigra* Munro var. *henonis* bamboo was associated with *Candidatus* Phytoplasma asteris 16SrI identified through sequence analysis of the 16S rRNA and intergenic spacer region between the 16S and 23S rRNA genes (Jung *et al.* 2006). On the other hand, *Candidatus* Phytoplasma aurantifolia 16SrII (peanut witches' broom group) was detected in *Dendrocalamus strictus* bamboo in India (Yadav *et al.* 2015).

Although the BWB disease was already reported in the Philippines since the 1990s, thorough studies and information about BWB and the affected bamboo species remain lacking and largely unexplored in the country. Thus, this study was conducted to determine the extent of BWB distribution among bamboo species in the Philippines, as well as to characterize the BWB symptoms and its causal phytoplasma. Phytoplasma was detected through two rounds of PCR (*via* nested PCR) for increased sensitivity and specificity, thus avoiding false positive/negative. The results of this study can aid in formulating effective disease management strategies to address this bamboo disease.

MATERIALS AND METHODS

Survey and Collection of Infected Samples

The bamboo plants showing typical symptoms of BWB disease were surveyed, characterized, and collected from different provinces in the Philippines. Specifically, BWB was surveyed in Isabela, Nueva Vizcaya, Bohol, and Davao (in this study) and in Ilocos Norte, Laguna, Batangas, Quezon, Agusan del Sur, Bukidnon, and South Cotabato [initially reported by Caasi-Lit *et al.* (1999)]. Bamboo species were identified based on their characteristics (Caasi-Lit *et al.* 2010, 2018; Pelser *et al.* 2020). To detect and identify the causal phytoplasma strain, leaf samples from five BWB-infected bamboo plants from Brgy. Pintor, Gamu, Isabela, Philippines were collected and brought to the Plant Pathology Laboratory, Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños for molecular analysis.

DNA Extraction

Leaves (with prominent midrib and petiole) from symptomatic, BWB-infected samples were randomly obtained and cut into small pieces. About 200 mg was used for DNA extraction through a modified CTAB extraction protocol (Doyle and Doyle 1987). The cut sample pieces were manually ground in CTAB extraction buffer (0.1M Tris-HCl pH 8.0: 0.02M EDTA: 1.4M NaCl: 2% CTAB: distilled water) using a sterile mortar and pestle. The ground samples were transferred to sterile 1.5-mL microcentrifuge tubes, incubated at 65 °C for 10 min, and then centrifuged at 13,000 rpm for 10 min. About 700 µL of supernatant was transferred to a new 1.5-mL microcentrifuge tube, added with an equal volume of chloroform:isoamyl alcohol (24:1), mixed, and centrifuged at 13,000 rpm for 10 min. This step was repeated twice. Afterward, the aqueous layer containing the DNA was transferred to a new 1.5 mL microcentrifuge tube, wherein 5 M NaCl (0.5 vol) and ice-cold isopropanol (1 vol) were added. The tube was incubated overnight in the freezer at –20 °C and centrifuged at 13,000 rpm for 10 min. The supernatant was carefully discarded, retaining only the DNA pellet at the bottom of the tube. The pellet was washed three times using 500 mL of ice-cold 70% ethyl alcohol, air-dried, and the DNA was resuspended using 100 µL diethyl pyrocarbonate (DEPC) water. Before PCR amplification, the concentration and purity ratios

(A260/280) of the extracted DNA from symptomatic samples were determined using Epoch™ Microplate Spectrophotometer run with Gen5™ Microplate Reader and Imager Software. Meanwhile, the quality of the DNA was checked *via* gel electrophoresis (Lin 2012) using solidified 1.5% agarose gel viewed under a gel documentation system (Axygen Gel Documentation system).

Nested-PCR Amplification

The 50 ng/µL good quality DNA was used as a template for the nested PCR amplification using a set of universal primers P1/P7 (Deng and Hiruki 1991; Schneider *et al.* 1995; Smart *et al.* 1996) in the first round of PCR, followed by a second round of PCR using R16MF2/R1 primer pairs (Gundersen and Lee 1996) to amplify the ~ 1.8 and 1.3kbp fragments of the phytoplasma 16S rRNA gene, respectively (Table 1). PCR was carried out in a 15 µL reaction volume containing 2 µL DNA template, 1x PCR Buffer, 2 mM $MgCl_2$, 0.2 mM (first PCR)/ 0.4 mM (second PCR) deoxyribonucleotide triphosphate (dNTPs), 0.4 µM of each forward and reverse primer, 1 U Taq DNA Polymerase (Invitrogen), and 9.35 µL (first PCR) or 9.05 µl (second PCR) DEPC water. PCR amplification was performed using G-Storm (model GS04822) thermal cycler with the following settings: initial denaturation at 94 °C for 2 min; followed by 45 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 2 min, extension at 72 °C for 1 min; and then a final extension at 72 °C for 7 min. The PCR products were resolved using 1.5% agarose gel subjected to a gel electrophoresis system at 230V for 26 min with 0.5X TBE as a running buffer and then viewed under the gel documentation system. During PCR analysis, the DNA from phytoplasma-infected loofah plants (exhibiting loofah witches' broom) and DNA from a healthy bamboo plant served as the positive and negative controls, respectively. PCR reaction with DEPC water as a template (instead of DNA) was also included as a negative control, as well as to check for PCR contamination.

Sequencing and Molecular Analysis

The target band produced from nested PCR (~ 1,300 bp), which corresponds to the 16S rRNA gene, was carefully excised from the gel and subsequently purified using PureLink™ Quick Gel Extraction Kit (Invitrogen). The purified amplicons from gel excision were sent for DNA sequencing (1st Base, Malaysia) with additional pre-treatment (clean-up) services. The BioEdit Sequence Alignment Editor version 7.3.6 (Hall 1999) was used to analyze the forward and reverse sequences. The sequences were subjected to nucleotide BLAST (BLASTn) (Altschul *et al*. 1990) and the percent genetic similarity was recorded. To construct the phylogenetic tree, the phytoplasma 16S rRNA sequences generated in this study and the reference sequences from NCBI (other phytoplasma strains) (Table 3) were aligned using Clustal W (Higgins *et al*. 1994) installed in MEGA (version 7.0.26) (Kumar *et al.* 2016). Based on the alignment, the best-fit model was identified and applied for the construction of the phylogenetic tree. The strength of the tree was evaluated by 1,000 bootstrap replications.

RESULTS

Symptoms of Bamboo Witches' Broom (BWB) disease

The infected bamboo plants in the Philippines exhibit moderate to severe symptoms of witches' brooming, with infected branches showing leaf proliferation and clustering of leaves, sometimes appearing so heavy on the shoot part) – as shown in the bamboo species *Dendrocalamus merrillianus* observed in Gamu, Isabela in 2019 (Figure 1a). The formation of small clustered leaves between the shortened internodes is also evident, which sometimes form a rosette-like structure (Figure 1b). There is also an excessive limb formation on the infected node resulting in the clustering of the leaves (Figure 1c–d).

Distribution of Bamboo Species Infected with BWB

The occurrence and symptoms of BWB have been observed in the 1990s in the provinces of Ilocos Norte, Laguna, Batangas, Quezon, Agusan del Sur, Bukidnon, and South Cotabato on four bamboo species – namely, *Gigantochloa levis* (Blanco) Merrill, *Gigantochloa atter* (Hassk.) Kurz, *Dendrocalamus asper* (Schult. & Schult. F.) Backer, and *Schizostachyum lumampao* (Blanco)

Table 1. Primers used for the amplification of 16S rRNA gene of *Ca.* Phytoplasma.

Primers	Primer sequences (5' → 3')	Expected size (kbp)	References
P1	AAGAGTTTGATCCTGGCTGAGGATT	~ 1.8	Deng and Hiruki (1991); Schneider *et al.* (1995); Smart *et al.* (1996)
P7	CGTCCTTCATCGGCTCTT		
R16MF2	CATGCAAGTCGAACGCA	~ 1.3	Gundersen and Lee (1996)
R16R1	CTTAACCCCAATCATCGAC		

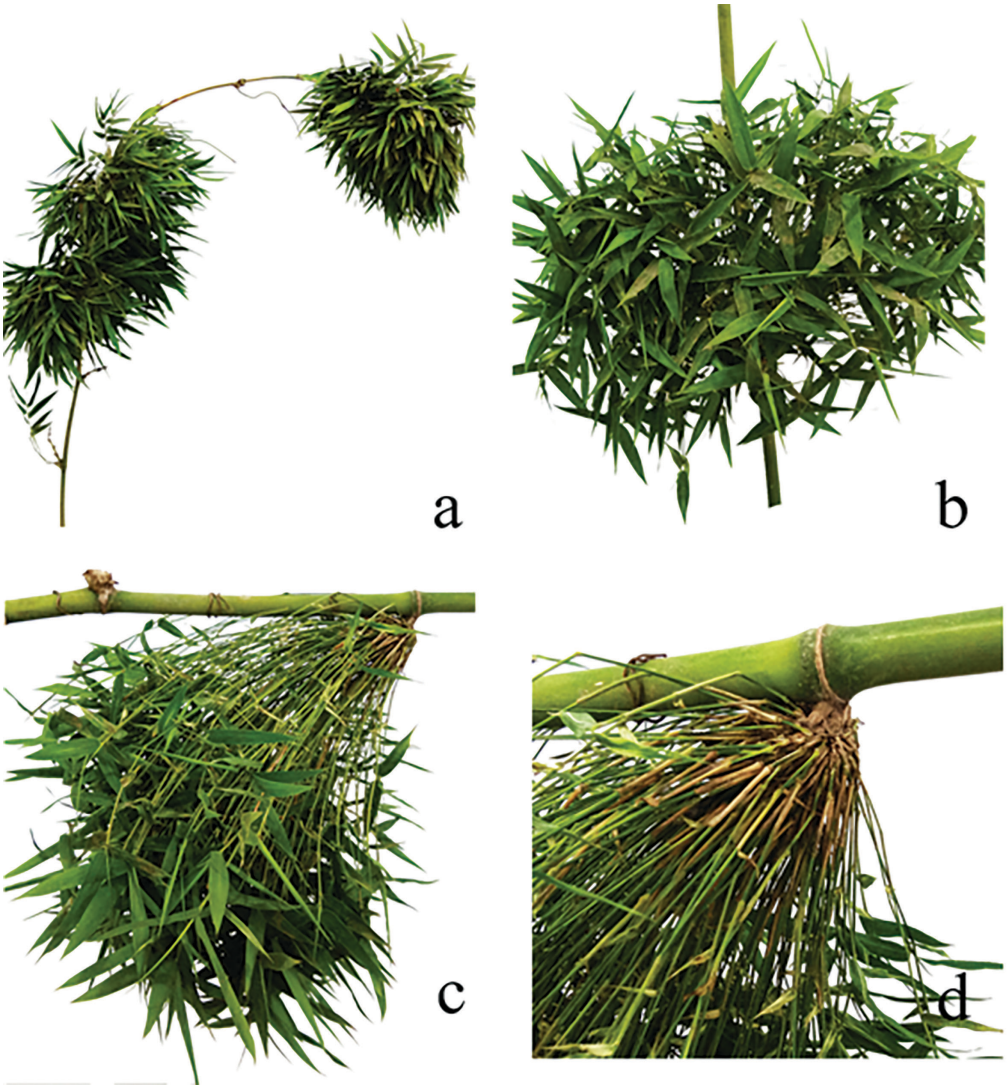

Figure 1. Symptoms of witches' broom in bamboo (*Dendrocalamus merrillianus*): [a] shoot and shortened internodes with excessive clustered small leaves, [b] rosette-like structure, [c] leaf proliferation from a single node, and [d] excessive limb formation from a node.

Merr (Figure 2; Table 2). The BWB is most prevalent in the provinces of Laguna, Quezon, and South Cotabato (Figure 2; Table 2). In Loboc and Sikatuna towns in Bohol province, the disease was recorded in 2018 infecting *G. levis* and *G. atter,* respectively (Figures 3a and b, respectively; Table 2). The following year, the disease was observed in *Dendrocalamus merrillianus* (Elmer) Rojo & Roxas (locally known as "bayog") (Figure 3c; Table 2) in Gamu, Isabela and *Bambusa spinosa* J.A. and J.H. Schultes (locally known as "kawayan-tinik") (Figure 3d; Table 2) in Roxas, Isabela and Bagabag, Nueva Vizcaya. The disease was also observed on an unidentified bamboo in Bay, Laguna (Figure 3e) and in *D. asper* (Figure 3f) in Davao City.

Detection and Molecular Identification of Causal Phytoplasma Strain

Through nested PCR, the expected phytoplasma 16S rRNA gene was amplified from all of the five symptomatic *D. merrillianus* plants sampled from Gamu, Isabela, thus confirming the presence of phytoplasma. Aside from this, positive PCR amplification was also observed in the positive control (a phytoplasma-infected loofah), whereas no PCR amplification was observed in the negative controls. Subsequently, from the positive samples, two representative BWB isolates were sequenced to identify the causal phytoplasma strain. The BLASTn analysis of the 16S rRNA sequence from two Philippine BWB isolates – the pbwb1a (GenBank Accession MN332189) and pbwb2a (GenBank Accession MN332190) – showed

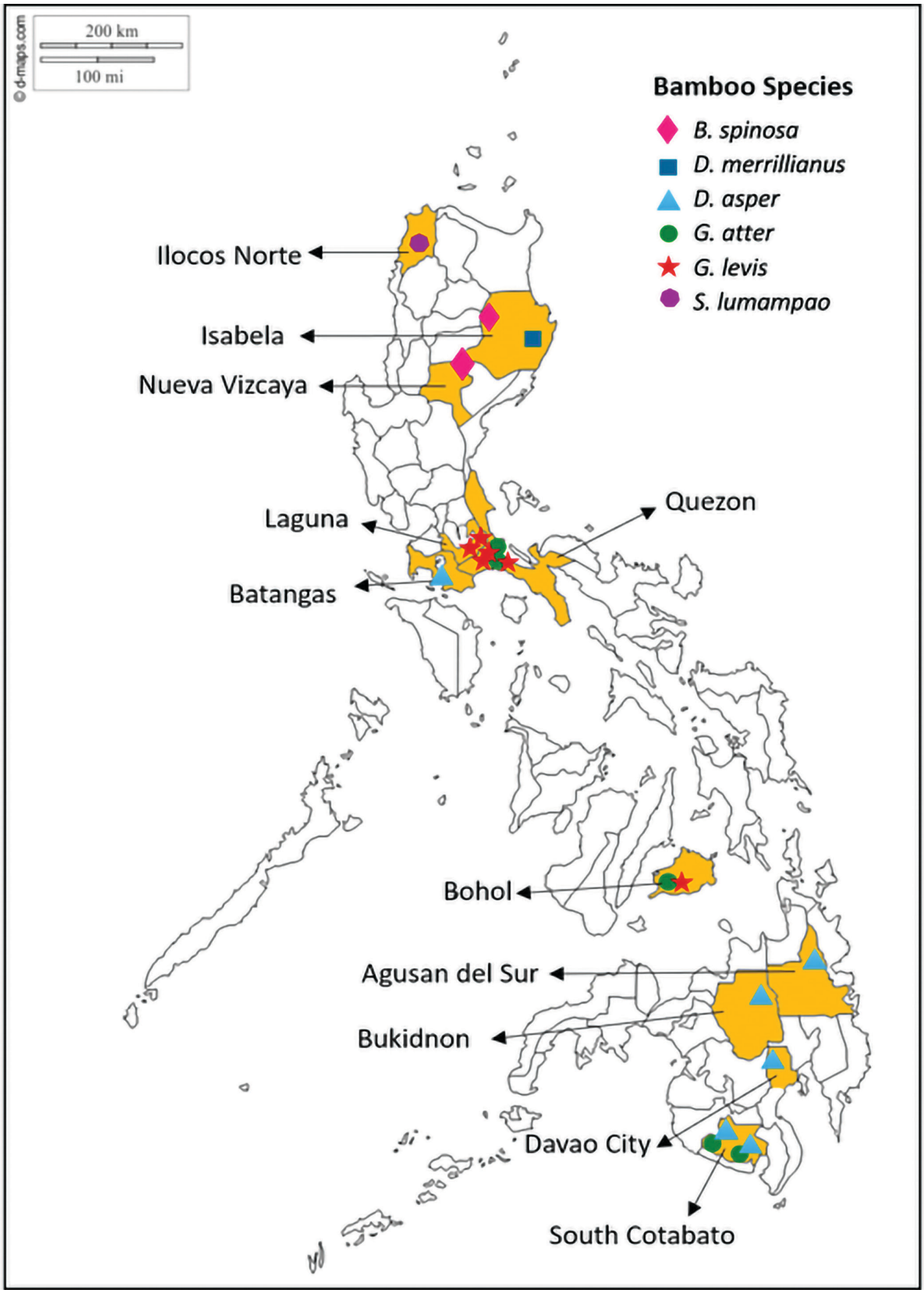

Figure 2. Distribution of bamboo species infected with witches' broom disease in the Philippines.

Table 2. Field surveys of bamboo species infected with bamboo witches' broom disease in the Philippines.

Bamboo species	Common/ local name	Location	Year observed	Reference
Gigantochloa levis (Blanco) Merrill	"Bolo"	Cavinti, Laguna	1995 1999	Sinohin (1995) Caasi-Lit *et al.* (1999)
		Luisiana, Laguna	1995 1999	Sinohin (1995) Caasi-Lit *et al.* (1999)
		Pagbilao, Quezon	1995 1999	Sinohin (1995) Caasi-Lit *et al.* (1999)
		Tayabas, Quezon	1999	Caasi-Lit *et al.* (1999)
		Lucban, Quezon	1999	Caasi-Lit *et al.* (1999)
		Loboc, Bohol	2018	This paper
Gigantochloa atter (Hassk.) Kurz	"Kayali"	Tayabas, Quezon	1999	Caasi-Lit *et al.* (1999)
		Lucban, Quezon	1999	Caasi-Lit *et al.* (1999)
		T'Boli, South Cotabato	1999	Caasi-Lit *et al.* (1999)
		Lake Sebu, South Cotabato	1999	Caasi-Lit *et al.* (1999)
		Sikatuna, Bohol	2018	This paper
Dendrocalamus asper (Schult. & Schult. F.) Backer	Giant bamboo	Cuenca, Batangas	1999	Caasi-Lit *et al.* (1999)
		Malaybalay, Bukidnon	1999	Caasi-Lit *et al.* (1999)
		T'Boli, South Cotabato	1999	Caasi-Lit *et al.* (1999)
		Agusan del Sur	1999	Lit *et al.* (1999)
		Lake Sebu, South Cotabato	1999	Lit *et al.* (1999)
		Marilog, Davao City	2019	This paper
Schizostachyum lumampao (Blanco) Merr	"Buho"	Ilocos Norte	1999	Caasi-Lit *et al.* (1999)
Dendrocalamus merrillianus (Elmer) Elmer	"Bayog"	Gamu, Isabela	2019	This paper
Bambusa spinosa Roxb.	"Kawayan-tinik"	Roxas, Isabela	2019	This paper
		Bagabag, Nueva Vizcaya	2019	This paper

98.98 and 99.85% genetic similarity, respectively, to the *Ca.* Phytoplasma luffae 16SrVIII reference isolate from Taiwan (GenBank Accession AF248956) (Table 3). On the other hand, the other phytoplasma groups had 89.45–95.93% homology to the Philippine BWB isolates. The phylogenetic tree constructed *via* the maximum likelihood method (tested with 1,000 bootstrapping iterations) resulted in the clustering of the Philippine BWB isolates to the *Ca.* Phytoplasma luffae 16SrVIII clade, whereas all other reference sequences formed separate clades (Figure 4).

DISCUSSION

The BWB disease has been observed in various areas in the Philippines. The BWB found in Bohol, Isabela, Nueva Vizcaya, Laguna, and Davao reported in this study, and the BWB-infected bamboo initially reported (Caasi-Lit *et al.* 1999; Table 2) exhibited similar disease symptoms to the witches' broom-infected bamboo plants described in India (Yadav *et al.* 2015) and South Korea (Jung *et al.* 2006). The symptoms and occurrence of BWB have been previously observed in three genera of bamboo (*i.e. Dendrocalamus*, *Gigantochloa*, and *Schizostachyum*) found in various provinces in the Philippines since the 1990s (Table 2). However, no BWB-infected *Bambusa* spp. was reported until this study. Recently, species of *Dendrocalamus merrillianus* ("bayog") from Isabela and *Bambusa spinosa* ("kawayan-tinik") from Isabela and Nueva Vizcaya were observed to display the typical witches' broom disease symptoms for the first time (Figure 3; Table 2). Thus, the BWB is now distributed in *Gigantochloa* spp. (*G. levis* and *G. atter*), *Dendrocalamus* spp. (*D. asper* and *D. merrillianus*), *Schizostachyum lumampao*, and *Bambusa spinosa* bringing to a total of

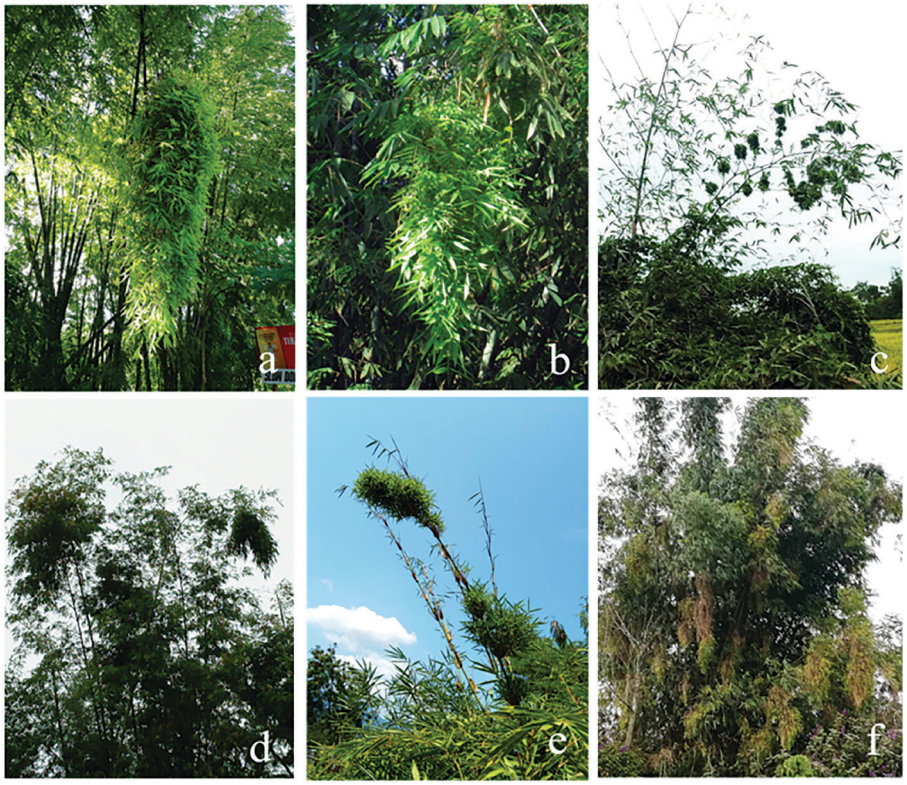

Figure 3. Bamboo species infected with BWB: [a] *Gigantochloa levis* in Loboc, Bohol; [b] *Gigantochloa atter* in Sikatuna, Bohol; [c] *Dendrocalamus merrillianus* in Gamu, Isabela; [d] *Bambusa spinosa* in Roxas, Isabela; [e] unidentified bamboo species in Bay, Laguna; and [f] *Dendrocalamus asper* in Marilog, Davao City.

Table 3. The percent similarity of the 16S rRNA sequences (~ 1.3kbp) of the detected BWB isolates analyzed through BLASTn.

Taxon	Strain	Host	Country	Query cover	E-value	Similarity to MN332189 (pbwb1)	Similarity to MN332190 (pbwb2a)	Accession
Ca. Phytoplasma 16SrI	–	Henon bamboo	South Korea	100%	0.0	89.63%	90.39%	AB242433
Ca. Phytoplasma 16SrI	BWB-TA	Bamboo	China	94%	0.0	89.84%	90.61%	FJ853161
Ca. Phytoplasma 16SrI	Shaanxi	*Sasa fortunei*	China	100%	0.0	90.02%	90.73%	FJ501958
Ca. Phytoplasma 16SrI	WBW-RZ	Bamboo	China	99%	0.0	90.44%	91.16%	FJ853158
Ca. Phytoplasma 16SrII	BB10	*Dendrocalamus strictus*	India	95%	0.0	89.53%	90.40%	LN811707
Ca. Phytoplasma 16SrII	BB15	*Dendrocalamus strictus*	India	95%	0.0	89.45%	90.32%	LN811708
Ca. Phytoplasma 16SrII	Ry2	Cassava	Thailand	99%	0.0	90.74%	91.52%	LN897457
Ca. Phytoplasma 16SrII	–	*Catharanthus roseus*	–	99%	0.0	89.77%	90.79%	L33765
Ca. Phytoplasma fraxini 16SrVII-B	CfS-Br10	*Brassica oleracea* var. botrytis	Brazil	100%	0.0	94.66%	95.41%	KR270802
Ca. Phytoplasma pruni 16SrIII	–	–	–	100%	0.0	92.47%	93.20%	L04682

Table 3 Cont.

Ca. Phytoplasma palmae 16SrIV	TPD	Canary Island Date Palms	Texas	98%	0.0	93.76%	94.51%	AF434989
Ca. Phytoplasma ulmi 16SrV	EY1	–	–	100%	0.0	94.57%	95.34%	AY197655
Ca. Phytoplasma trifolii 16SrVI	CP	*Trifolium hybridum*	Canada	100%	0.0	95.18%	95.93%	AY390261
***Ca.* Phytoplasma luffae 16SrVIII**	**LfWB**	**Loofah**	**Taiwan**	**100%**	**0.0**	**98.98%**	**99.85%**	**AF248956**
Ca. Phytoplasma phoenicium 16SrIX	A4	Almond trees	Lebanon	100%	0.0	92.54%	93.27%	AF515636
Ca. Phytoplasma 16SrX-B	LNp	Plum cv. Orzark Premier	Italy	99%	0.0	90.94%	91.72%	JQ868450
Ca. Phytoplasma oryzae 16SrXI	Minab4	*Cyperus* sp.	Iran	99%	0.0	93.54%	94.28%	MF136620

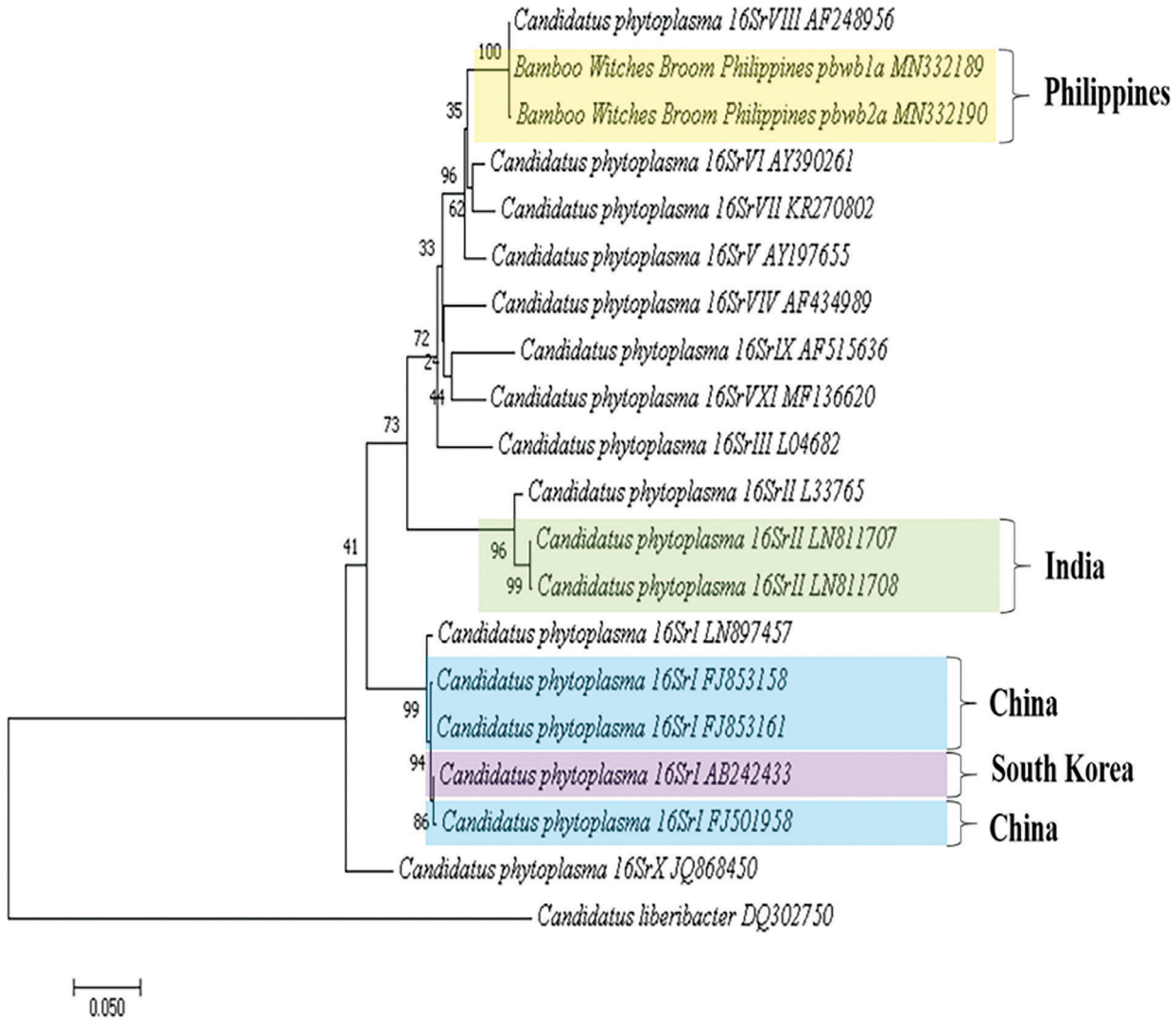

Figure 4. Maximum likelihood phylogenetic tree constructed based on the lowest Bayesian Information Criterion (BIC) score generated from the 16S rRNA sequences of *Ca.* Phytoplasma. Gaps were treated as missing data with 1,000 bootstrap replications using the Tamura 3-parameter in MEGA 7 (Kumar *et al.* 2016). *Ca*. Liberibacter (DQ302750) served as the outgroup taxa.

six species across four genera of bamboo recorded in 11 provinces in the Philippines (Figure 2; Table 2). Although the disease was already observed before in *G. atter* and *G. levis* bamboos, this is the first time that BWB was recorded in these bamboo species in Bohol. Likewise, the infected *D. asper* in Davao City is the first BWB record in the Davao region.

Since the early report of BWB in the country, identification of the causal phytoplasma strain has become a great challenge due to its unculturable nature. The utilization of molecular techniques such as PCR, DNA sequencing, and RFLP has been recently sought for phytoplasma identification (Dickinson *et al.* 2013; Lee *et al.* 2010; IPPC 2016; Davis *et al.* 2017). The PCR detection using universal primers (*e.g.* P1/P7 and R16MF2/R1) designed to amplify the 16S rRNA gene of phytoplasma (Duduk *et al.* 2013) enabled successful amplification of the target gene region of the causal BWB phytoplasma strain. In phytoplasma, a genetic similarity of at least 98.65% using the 16S rRNA sequence may implicate conspecificity, according to the species demarcation criteria by Bertaccini *et al.* (2022). In this study, the BLASTn results of the 16S rRNA sequences of the representative Philippine BWB isolates (from infected *B. merrilliana* samples collected from Isabela province) showed a greater genetic similarity value of more than 98.65% to the *Ca.* Phytoplasma luffae 16SrVIII (loofah witches' broom group) reference isolate, as supported also by the successful clustering in the 16SrVIII clade in the phylogram (Table 3; Figure 4). No direct relatedness to the Philippine BWB isolates was observed in other phytoplasma reference sequences. In addition, the same phytoplasma strain was recently detected in bamboo samples infected with BWB from the Philippine province of Laguna (Sta. Cruz *et al.* 2021), thus confirming the presence of this phytoplasma strain in BWB-infected samples. Aside from bamboo, this phytoplasma strain was also reported to cause witches' broom disease in vegetables (*e.g.* loofah, bitter gourd, cucumber) (Sta. Cruz *et al.* 2021; Borines *et al.* 2020) and cassava (Dolores *et al.* 2023) in the Philippines. *Ca.* Phytoplasma luffae, a strain belonging to the 16SrVIII group, was first identified in loofah plants showing witches' broom disease in Taiwan (Yang *et al.* 1974; Chung *et al.* 1975; Davis *et al.* 2017).

Previous BWB studies from other countries reported different phytoplasma strains such as in Korea (Jung *et al.* 2006) and China (Zhang *et al.* 2009), which reported similarities to the *Ca.* Phytoplasma asteris (16SrI group), whereas *Ca.* Phytoplasma aurantifolia (16SrII or peanut witches' broom group) was reported in India (Yadav *et al.* 2015) (Figure 4). Recently, Ravi *et al.* (2022) reported wide genetic diversity of phytoplasma, *i.e. Ca.* Phytoplasma australasia related strains (16SrII-C or 16SrII-D group), *Ca*. Phytoplasma asteris (16SrI-B group), and *Ca. Phytoplasma cynodontis* (16SrXIV-A group) associated with witches' broom disease of different bamboo species in different states of India.

The '*Ca.* Phytoplasma luffae'-related strain (16SrVIII) detected in *D. merrillianus* can be used as a reference to determine the relationship between phytoplasma species infecting bamboo in the Philippines and other countries. In the case of *D. merrillianus*, which has a somewhat complicated taxonomic classification (Caasi-Lit *et al.* 2018), it would also be interesting to correlate its susceptibility to phytoplasma for taxonomic identification.

CONCLUSION

The surveys conducted from 1999–2019 showed that BWB symptoms were observed in six bamboo species in 11 provinces in the Philippines, with the first country record of BWB in two bamboo species (*Dendrocalamus merrillianus* and *Bambusa spinosa*) reported in this paper. The results obtained also confirmed the identity of '*Ca*. Phytoplasma luffae'-related strain (16SrVIII) (loofah witches' broom group) as the phytoplasma strain associated with BWB in the Philippines, which is a different strain from other countries. While the economic impact of BWB in the Philippine bamboo industry is still unknown, its presence poses a risk in increasing the potential reservoirs of witches' broom disease in the Philippines, which can affect some of the major crops. Future studies related to vectors, host range, and biological transmission of phytoplasma to other bamboo species are deemed relevant to unravel the epidemiology and impact of BWB disease in the country. Overall, this paper demonstrated the association of '*Ca.* Phytoplasma luffae' -related strain (16SrVIII) with BWB and determined the distribution of BWB in different species of bamboo in the Philippines.

ACKNOWLEDGMENTS

The authors thank Yron M. Retuta, Bernard A. Panabang, Ruby Ana P. Laude, Joseph Aeron P. Pia, and Reyneluz P. Lubigan for the technical assistance; and the Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños and the Department of Agriculture Biotechnology Program Office for the continued support.

STATEMENT ON CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

ALTSCHUL SF, GISH W, MILLER W, MYERS EW, LIPMAN DJ. 1990. Basic local alignment search tool. J Mol Biol 215: 403–410.

BERTACCINI A, AROCHA-ROSETE Y, CONTALDO N, DUDUK B, FIORE N, MONTANO HG, ZAMORANO A. 2022. Revision of the '*Candidatus* Phytoplasma' species description guidelines. Int J Syst Evol Microbiol 72(4).

BORINES LM, NUÑEZ JAC, DUERO ND, SAGARINO-BORINES R, GERONA RG. 2020. Detection and molecular characterization of phytoplasma affecting vegetables in Eastern Visayas, Philippines. Ann Trop Res 42(2): 1–20.

CAASI-LIT M, LIT IL JR., SINOHIN VO. 1999. Is witch's broom sweeping Philippine Bamboos? Canopy International. 25(1): 4–5.

CAASI-LIT MT, MABESA LB, CANDELARIA RA. 2010. Bamboo shoot resources in the Philippines: I. Edible shoots and the status of local bamboo shoot industry. Philippine Journal of Crop Science 35(2): 54–68.

CAASI-LIT MT, MATEO JM, VILLAREAL RL. 2018. Promoting endemic *Bambusa* spp. (*B. merrilliana* and *B. philippinensis*) as sources of edible bamboo shoots in the Philippines. Proceedings of the 11th World Bamboo Congress (WBC), Mexico. 16p.

CHUNG SJ, HUANG PY, YANG SJ, CHOU TG. 1975. Studies on loofah (*Luffa cylindrica* Roem.) witches' broom in Taiwan. II. Electron microscopic observation of the diseased tissue. Plant Prot Bull 17: 329–332.

DAVIS RE, ZHAO Y, WEI W, DALLY EL, LEE IM. 2017. '*Candidatus* Phytoplasma luffae', a novel taxon associated with witches' broom disease of loofah, *Luffa aegyptica* Mill. Int J Syst Evol Microbiol 67(8): 3127–3133.

DRANSFIELD S, WIDJAJA EA. 1995. Plant Resources of Southeast Asia, No. 7 Bamboos. Backhuys Publishers, Leiden. 189p.

DENG S, HIRUKI C. 1991. Genetic relatedness between two nonculturable mycoplasmalike organisms revealed by nucleic acid hybridization and polymerase chain reaction. Phytopathology 81: 1475–1479.

DICKINSON M, TUFFEN M, HODGETTS J. 2013. The Phytoplasma: an Introduction. In: Phytoplasma: Methods and Protocols. Dickinson M and Hodgetts J eds. Methods in Molecular Biology, Vol. 938. Springer Science + Business Media, LLC. p. 1–14. DOI: 10.1007/978-1-62703-089-2_1

DOLORES LM, LANGRES JA, PINILI MS, CAASI-LIT MT, CORTAGA CQ, RETUTA YM, DELA CUEVA FM. 2023. Incidence, distribution, and genetic diversity of '*Candidatus* Phytoplasma luffae'-related strain (16SrVIII) associated with the cassava witches' broom (CWB) disease in the Philippines. Crop Prot https://doi.org/10.1016/j.cropro.2023.106244

DOYLE JJ, DOYLE JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

DUDUK B, PALTRINIERI S, LEE IM, BERTACINNI A. 2013. Nested PCR and RFLP Analysis Based on the 16S rRNA gene. In: Phytoplasma: Methods and Protocols. Dickinson M, Hodgetts J eds. Methods in Molecular Biology, Vol. 938. Springer Science + Business Media, LLC. p. 159–172. DOI: 10.1007/978-1-62703-089-2_1

GUNDERSEN DE, LEE IM. 1996. Ultrasensitive detection of Phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathol Mediterr 35: 114–151.

HALL TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95–98.

HIGGINS D, THOMPSON J, GIBSON J, THOMPSON JD, HIGGINS DG, GIBSON TG. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequences alignment through sequence weighting positioning-specific gap penalties and weight matrix choice. Nuclei Acid Res 22: 4673–4680.

[IPPC] International Plant Protection Convention. 2016. DP 12: Phytoplasmas. In: Diagnostic protocols for regulated pests. International Standards for Phytosanitary Measures 27 (ISPM 27), Annex 12.

JUNG HY, CHANG MU, LEE JT, NAMBA S. 2006. Detection of ''*Candidatus* Phytoplasma asteris'' associated with henon bamboo witches' broom in Korea. J Gen Plant Pathol 72: 261–263.

KUMAR S, STECHER G, TAMURA K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870–1874.

LEE IM, ZHAO Y, DAVIS RE. 2010. Prospects of Multiple Gene-based Systems for Differentiation and Classification of Phytoplasmas. In: Phytoplasmas: Genomes, Plant Hosts, and Vectors. Weintraub PG, Jones P eds. CAB International. p. 51–63.

LIN P. 2012. Genomic DNA QC using standard gel electrophoresis (for collaborators). Doe Joint Genome Institute. United States. 11p.

LIT IL JR., CAASI-LIT M, CAPRICHO MAA. 1999. A survey of insects found on witch's broom-diseased bamboos in the Philippines. Philipp Entomol 13: 173–175.

MOHANAN C. 1994. Little leaf disease of bamboo in Kerala, India. Bamboo Information Centre India Bulletin 4: 30–37.

PELSER PB, BARCELONA JF, NICKRENT JL. 2020. "Poaceae". Co's Digital Flora of the Philippines. Retrieved on 29 Mar 2023 from https://www.philippine-plants.org/Families/Poaceae.html

RAVI M, RAO GP, MESHRAM NM, SUNDARARAJ R. 2022. Genetic diversity of phytoplasmas associated with several bamboo species in India. For Pathol 52. e12741.

SCHNEIDER B, SEEMULLER E, SMART C, KIRKPATRICK C. 1995. Phylogenetic classification of plant pathogenic mycoplasmalike organisms or phytoplasmas. In: Molecular and Diagnostic Procedures in Mycoplasmology. Razin R, Tully JG eds. San Diego, California: Academic Press. p. 369–380.

SINOHIN VO. 1995. Unidentified disease of Bamboo. Forest Pest Watch 1(2): 5.

SMART CD, SCHNEIDER B, BLOMQUIST CL, GUERRA LJ, HARRISON NA, AHRENS U, ..., KIRKPATRICK BC. 1996. Phytoplasma-specific PCR primers based on sequences of the 16S-23S rRNA spacer region. Appl Environ Microbiol 62(8): 2988–2993.

STA. CRUZ FC, HABILI N, WU Q, SAGARINO RB, RANDLES JW. 2021. Detection of '*Candidatus* Phytoplasma luffae' in Sponge Gourd, Bitter Gourd, and Bamboo from Laguna in Luzon, Philippines. Philipp Agric Sci 104(2): 181–185.

YADAV A, THORAT V, SHOUCHE Y. 2015. *Candidatus* Phytoplasma aurantifolia (16SrII Group) associated with witches' broom disease of bamboo (*Dendrocalamus strictus*) in India. Plant Dis. https://doi.org/10.1094/PDIS-05-15-0534-PDN

YANG SJ, CHOU TG, CHANG SJ, HUANG PY. 1974. Studies on loofah (*Luffa cylindrica* Roem.) witches' broom in Taiwan. I. Occurrence, symptoms, and transmission test. Plant Prot Bull 16: 162–166.

ZHANG CP, MIN H, ZHENG X, YU XQ, ZHANG H, WU, YF. 2009. Detection and molecular characterization of a '*Candidatus* Phytoplasma asteris'-related strain infecting *Sasa fortunei* in China. Plant Dis 93: 55.