

# Report on a New Invasive Species, *Pseudococcus dendrobiorum* Williams (Hemiptera: Pseudococcidae), in Taiwan

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## Abstract

Chen, S. P., Y. C. Chiu, C. C. Chen, and J. Y. Wong. 2015. Report on a new invasive species, *Pseudococcus dendrobiorum* Williams (Hemiptera: Pseudococcidae), in Taiwan. J. Taiwan Agric. Res. 64(1):1–9.

A new invasive dendrobium mealybug, *Pseudococcus dendrobiorum* Williams (Hemiptera: Pseudococcidae), through imported orchids (*Phalaenopsis* spp.) from Vietnam was confirmed by Taiwan Agricultural Research Institute (TARI) in 2013. In this study, morphological characteristics and ribosomal DNA internal transcribed spacer 1 (rDNA-ITS1) sequence were used for the species identification and confirmation of the introduction source. A standard process for elimination and subsequent detection survey of this pest to prevent its spreading was executed by the Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ) based on the Plant Protection Act. No individual of this pest was found again during the later surveys. The slide-mounted voucher specimens were deposited in the Insect and Mite Museum, Division of Applied Zoology, TARI.

**Key words:** *Pseudococcus dendrobiorum*, *Pseudococcus longispinus*, Mealybugs, Invasive pest, Pest identification.

## INTRODUCTION

Mealybugs (Hemiptera: Pseudococcidae) are important pests of a wide variety of agricultural and ornamental plants (Williams & Granara de Willink 1992; Ben-Dov 1994). They damage plants by sucking sap from the vascular tissues, and cause leaf yellowing and plant stunting and wilting. Furthermore, excreted honeydews can cause black sooty mold and impair plant quality (Williams 2004; Chen *et al.* 2011a). Mealybug species are currently found on orchids (Orchidaceae) worldwide, including *Dysmicoccus orchidum* Williams,

*Planococcus dendrobii* Ezzat & McConnel, *Planococcus philippinensis* Ezzat & McConnell, *Pseudococcus cryptus* Hempel, *Pseudococcus dendrobiorum* Williams, *Pseudococcus longispinus* (Targioni Tozzetti), and *Pseudococcus microcirculus* McKenzie (Ben-Dov & Miller 2014). Among these species, *Ps. longispinus* and *Ps. cryptus* are also distributed in Taiwan (Ben-Dov & Miller 2014). *Ps. longispinus* is a challenging pest causing serious damage to the aerial parts of orchids (Hsieh *et al.* 2011). *Ps. cryptus* is found on the aerial parts of *Ficus elastic* Roxb. and *Ficus microcarpa* L. (Tasi 2011), but never on orchids here. On the other

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Received: August 4, 2014; Accepted: October 24, 2014.

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hand, *Ps. dendrobiorum* is listed among the quarantine pests in many countries, but neither reported nor listed the same in Taiwan.

*Pseudococcus* spp. are small and similar in morphology, and distinguished only by their adult female morphology due to the variable patterns and inconspicuous characteristics of egg, nymphs and male adult (Williams 2004). Quick and correct identification of an invasive pest is important to initiate effective control measures. Molecular identification under these circumstances is handy as it is not constrained by life stage, polymorphism, and gender (Williams *et al.* 1990; Loxdale & Lushai 1998). Molecular data are very useful for improving our understanding of phylogenetic relationships within taxonomic groups, and help discover and confirm new taxa or new invasive species (Correa *et al.* 2011; Abd-Rabou *et al.* 2012; Correa *et al.* 2012; Mani *et al.* 2013).

Orchids (Orchidaceae: *Phalaenopsis* spp.) are economically important flower crops in Taiwan. Certain invasive mealybug species may cause significant economic impacts on this crop. A suspected invasive mealybug species was reported in April 2013, from an orchid nursery located in the Changhua County with the cultivated orchids imported from Vietnam. The purpose of this study was to identify the species and confirm the introduction source of this pest with morphological characteristics and the ribosomal DNA internal transcribed spacer 1 sequences (rDNA-ITS1). As the invasive status was confirmed, the Taiwan BAPHIQ, based on the Plant Protect Act, would trigger a standard process to eliminate and then regularly monitor this pest to protect local orchid cultivation environment.

## MATERIALS AND METHODS

### Morphological study

The new invasive mealybugs were collected by visual inspection of infested orchid plants (*Phalaenopsis* spp.), and placed in

plastic net bags (30 cm × 45 cm), then labelled with collection data (localities, dates, and collectors). In the laboratory, morphological characteristics of the collected mealybugs were observed under a dissecting stereo microscope (Leica Z16 Apo Stereomicroscope, Switzerland) with digital charge-coupled device (Leica DFC 420 Digital Microscope Cameras, Switzerland), and compared with the *Ps. dendrobiorum* specimens collected from Vietnam. Slide-mounted female and immature specimens were made with Canada balsam using the methods described in Chen *et al.* (2011b). The adult female specimens were examined using an optical microscope (Leitz DMRB, Leica, Milton Keynes, U.K.) and digital images of morphological characteristics were taken using Leitz DMRB light microscope with Automatic Piezo stepping motor (Leica Piezo Z-drive, Switzerland) and Leica DFC 420 digital microscope camera (Leica, Switzerland). The morphological terms were based on the descriptions of Williams (2004) and Williams & Granara de Willink (1992). The slide-mounted specimens were deposited as vouchers in the Insect and Mite Museum, Division of Applied Zoology, TARI.

### Molecular study

**DNA extraction, polymerase chain reaction (PCR), and sequencing:** Sixteen specimens of the new invasive mealybug, 10 specimens of *Ps. dendrobiorum* from Vietnam, 18 specimens of *Ps. longispinus*, and 2 specimens of *Dysmicoccus brevipes* (served as an outgroup) were sampled. Live specimens were collected and stored in 95% ethanol. All specimens were identified to species based on morphological characteristics and the pertinent information listed in Table 1.

Genomic DNA was obtained from the individual whole specimen using the Blood and Tissue Genomic DNA Miniprep System Kit (Viogene Inc., Taiwan) following the extraction protocol established by the manufacturer. Precipitated DNA was resuspended in 100 µL

**Table 1.** Specimen names, collection data, lengths of ribosomal DNA internal transcribed spacer 1 (rDNA-ITS1), and GenBank accession numbers of the mealybugs studied.

Specimen	Collection locality	Collection date	Host plant	Stage (No. of specimens)	Length of rDNA-ITS1 (bp)	GenBank accession numbers <sup>z</sup> rDNA-ITS1 variants (No. of specimens)
New invasive mealybug	Changhua, Taiwan	04/08/2013	<i>Phalaenopsis</i> sp.	Adults ♀ (8)	639	KJ767503 (6) KJ767504 (2)
		04/25/2013	<i>Phalaenopsis</i> sp.	Adults ♀ (4)	639	KJ767505 (3) KJ767506 (1)
				2 <sup>nd</sup> instar (4)	639	KJ767505 (2) KJ767507 (1) KJ767508 (1)
Pseudococcus dendrobiorum	Lamdong, Vietnam	05/01/2013	<i>Phalaenopsis</i> sp.	Adults ♀ (2)	639	KJ767505 (1) KJ767509 (1)
				2 <sup>nd</sup> instar (4)	639	KJ767510 (1) KJ767511 (1) KJ767512 (1) KJ767513 (1)
				1 <sup>st</sup> instar (4)	639	KJ767505 (3) KJ767514 (1)
<i>Pseudococcus longispinus</i>	Taichung, Taiwan	03/16/2010	<i>Phalaenopsis</i> sp.	Adults ♀ (2)	590	KJ767500 (2)
		04/13/2010	<i>Phalaenopsis</i> sp.	Adults ♀ (2)	590	KJ767500 (2)
		04/29/2010	<i>Asplenium nidus</i>	Adults ♀ (2)	590	KJ767501 (2)
	Nantou, Taiwan	12/24/2010	<i>Rohdea japonica</i>	Adults ♀ (2)	592	KJ767502 (2)
	Yunlin, Taiwan	10/07/2011	<i>Anthurium scherzerianum</i>	Adults ♀ (2)	590	KJ767500 (2)
		04/26/2012	<i>Phalaenopsis</i> sp.	Adults ♀ (3)	590	KJ767500 (3)
	Taipei, Taiwan	05/25/2012	<i>Asplenium nidus</i>	Adults ♀ (2)	592	KJ767502 (2)
	Yunlin, Taiwan	05/07/2013	<i>Anthurium scherzerianum</i>	Adults ♀ (3)	590	KJ767500 (3)
Outgroup						
<i>Dysmicoccus brevipes</i>	Tainan, Taiwan	04/22/2007	<i>Musa sapientum</i>	Adults ♀ (2)	628	EU307273 (2)

<sup>z</sup> DNA sequences from different specimens with the identical nucleotides arrangement were incorporated into the same accession numbers.

of double-distilled (dd) H<sub>2</sub>O, and then used to amplify the partial ribosomal RNA gene sequences with PCR.

Each PCR was carried out in a final volume of 50 µL with 25 µL of PCR Master Mix 2× (including 0.05 units µL<sup>-1</sup> *Taq* DNA Polymerase in reaction buffer, 4 mM MgCl<sub>2</sub>, 0.4 mM dNTP) (MBI Fermentas Inc., EU), 5.0 µL of each 1.5 µM of primer, and 5 µL of extracted genomic DNA solution. PCR conditions were as follows: an initial denaturation step

of 95°C (7 min), then 40 cycles of denaturation at 95°C (1 min), annealing at 56°C (1 min), and extension at 72°C (2 min), followed by a final extension step at 72°C (7 min). The primers used in this study were Amur18S-3 (5'-CGTTGAT-TACGTCCCTGCCCTTTG-3') and ITS6 (5'-GAGCCGAGTGATCCACCGCT-3') (Chiu *et al.* 2001). The PCR products were run on 1.2% agarose gels in 1× TAE buffer to ensure correct amplification.

Amplified products were sent to the Mission Biotech Ltd. (Taipei, Taiwan) for purification and sequencing. Sequencing reactions were conducted using a 96-well Gel/PCR Clean Up Kit (Geneaid Biotech Ltd., Taiwan) on an ABI 3730 DNA analyzer. The PCR products were sequenced in both directions and subsequently edited with Vector NTI Advance software v10.3 (Life Technologies Inc., USA). Sequence similarity analyses were conducted using the on-line programs (SeqWeb v3.1.2, <http://bioinfo.nhri.org.tw:8003/mgr.shtml>). Sequences were sent to the GenBank, and the accession numbers were given in Table 1.

**Phylogenetic analyses:** In order to determine the relationship between the 2 *Pseudococcus* taxa and the invasive mealybug, variants were used to infer the phylogenies with the neighbor-joining (NJ) method. The Phylip Version 3.695 (Felsenstein 2013) and the Kimura two-parameter model (Kimura 1980) were used to perform the NJ analyses. *D. brevipes* was used as an outgroup, and the bootstrapping values (Felsenstein 1985) determined with 1,000 repetitions were used to evaluate the clade support.

## RESULTS AND DISCUSSION

A new invasive mealybug pest *Ps. dendrobium* Williams, which caused damages to the roots and aerial parts of orchids in Changhua County, was confirmed through a case study of TARI agricultural pest diagnostic service in April 2013 (Fig. 1). All specimens examined for morphology were as follows: Taiwan (Changhua County), 7♀, 08-IV-2013, TARI ex *Phalaenopsis* sp.; Taiwan (Changhua County), 7♀, 25-IV-2013, TARI ex *Phalaenopsis* sp.; Vietnam, 5♀, 01-V-2013, TARI ex *Phalaenopsis* sp.

Adults and immatures of this pest are grayish-pink in color, thin filaments around the body, caudal pair shorter than one half of the body length (Fig. 2). This pest does not possess long wax filaments on the tip of the abdo-



Fig. 1. *Phalaenopsis* orchids with infestation of the new invasive pest *Pseudococcus dendrobium* on roots.

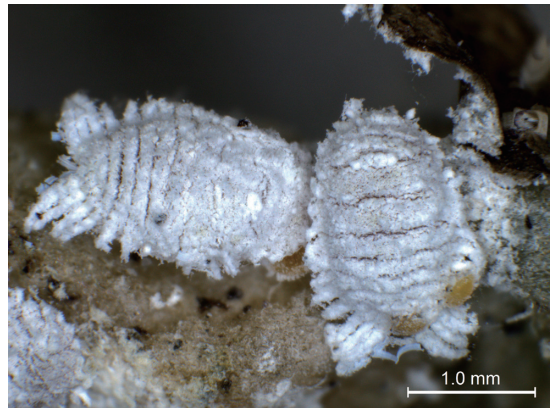


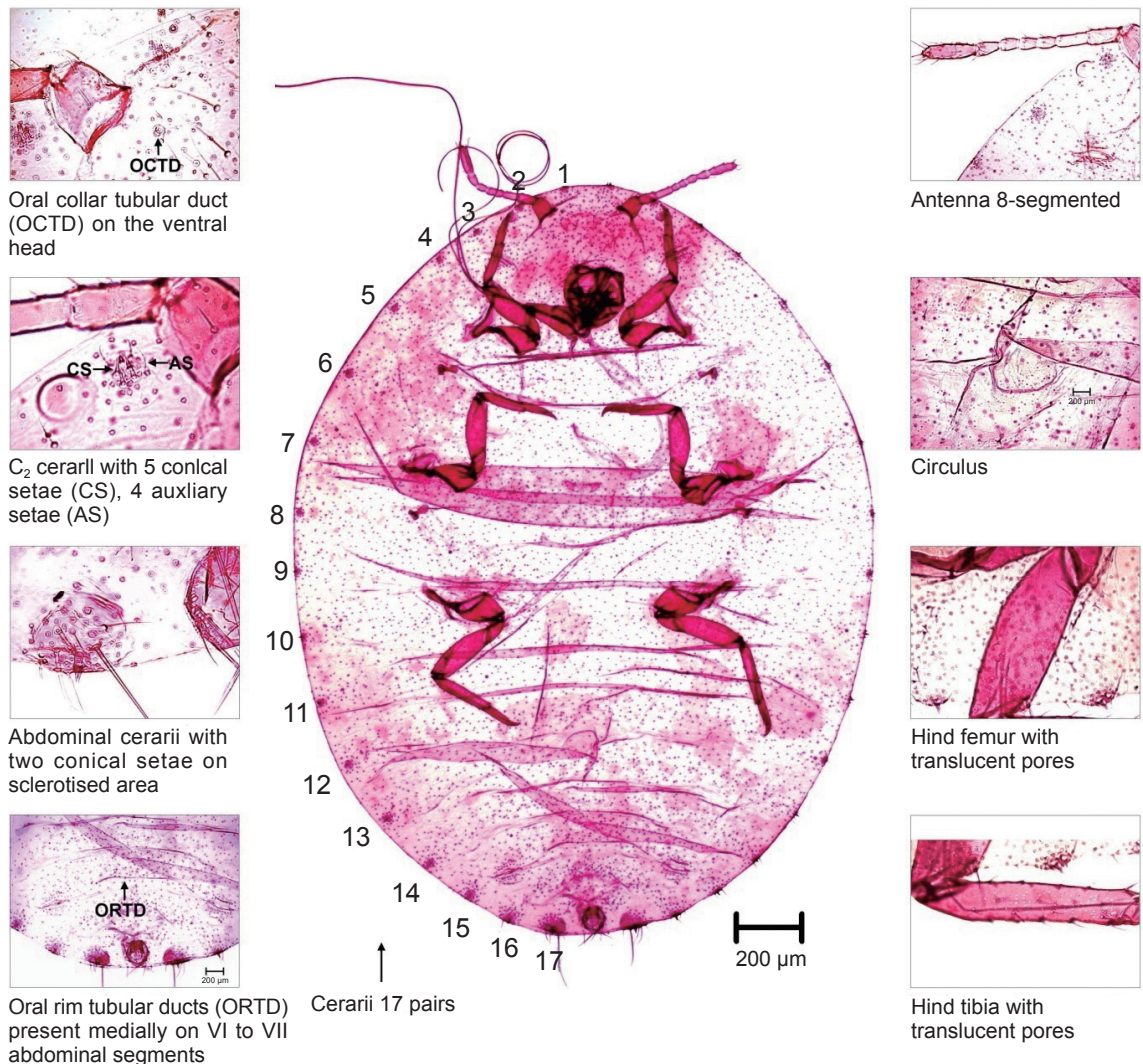
Fig. 2. Adult female of the new invasive pest *Pseudococcus dendrobium* Williams.

men, which is similar to the pineapple mealybug *D. brevipes* (Cockerell), but different from the longtailed mealybug *Ps. longispinus*. Other morphological characteristics include body of the female oval to elongate, antenna 8 segmented, oral collar tubular duct on the ventral head, legs well developed, translucent pores present on hind femur and hind tibia, cerarii 17 pairs,  $C_2$  cerarii with 5 conical setae, 4 auxiliary setae and a group of trilocular pores, anal lobe cerari with 2 conical setae, and many trilocular pores on sclerotised area, sparse oral rim tubular ducts present medially on VI to VII abdominal segments, and multilocular disc

pores present on V to VII abdominal segments (Fig. 3). These morphological characteristics of the adult female match the descriptions of *Ps. dendrobiorum* given by Williams (2004), but differ from all other *Pseudococcus* spp. recorded in Taiwan (Tsai 2011; Ben-Dov & Miller 2014) in possessing oral collar tubular duct on the ventral head.

While *Ps. longispinus* (already distributed in Taiwan) infested only the aerial parts of orchids, this study found that the new in-

vasive mealybug infested orchid roots and leaves. It has previously been recorded that Orchidaceae: *Coelogyne dayana*, *Dendrobium* sp., *Phalaenopsis amatilis*, *Raphioperdalum bellatulum*, *Spathoglottis* sp., *Spathoglottis plicata*, *Vanda teres*, in addition to Oleaceae: *Jasminum*; Pandanaceae: *Pandanus* sp., *Pandanus upoluensis*; and Passifloraceae: *Passiflora foetida*, and were among the host range of *Ps. dendrobiorum* (Ben-Dov & Miller 2014). The distributions of this mealybug species included



**Fig. 3.** Morphological characters used for identification of the new invasive pest *Pseudococcus dendrobiorum* Williams collected on orchids in Taiwan.

Australia (Northern Territory, Queensland), Guam, Hawaiian Islands (Hawaii, Oahu), India, Indonesia, Malaysia, Papua New Guinea, Philippines, Sri Lanka, South Korea, Thailand (Ben-Dov & Miller 2014), and Vietnam (newly recorded). These suggest that the suspected mealybug is not the local species of *Ps. longispinus*, but most likely is being introduced together with the imported orchids from Vietnam.

In the molecular study, ribosomal DNA gene sequences were successfully amplified from all the specimens of adults and nymphs. The length of the internal transcribed spacer 1 (ITS1) in both the invasive mealybug and the *Ps. dendrobiorum* from Vietnam was identical (639 aligned bp), while that in the *Ps. longispinus* was 590 (14 specimens) or 592 (4 specimens) aligned bp. With 15 variants identified, *Ps. dendrobiorum* (12 variants) was more variable than *Ps. longispinus* (3 variants) (Table 1). The ITS1 length was 628 bp for *D. brevipes*. This study showed that *Ps. dendrobiorum* could be distinguished from *Ps. longispinus* by the species-specific length of rDNA-ITS1.

Sequence similarity analysis of rDNA-ITS1 between inter- or intraspecies specimens indicated that the new invasive mealybug was highly similar to the *Ps. dendrobiorum* from Vietnam (similarity = 99.61%, parallel to the respective intraspecies similarities), but much less similar with the *Ps. longispinus* in Taiwan (similarity = 92.64%). The outgroup *D. brevipes* had the relatively lowest similarities with the samples of *Ps. dendrobiorum* (83.87%), the invasive mealybug (83.94%), and *Ps. lon-*

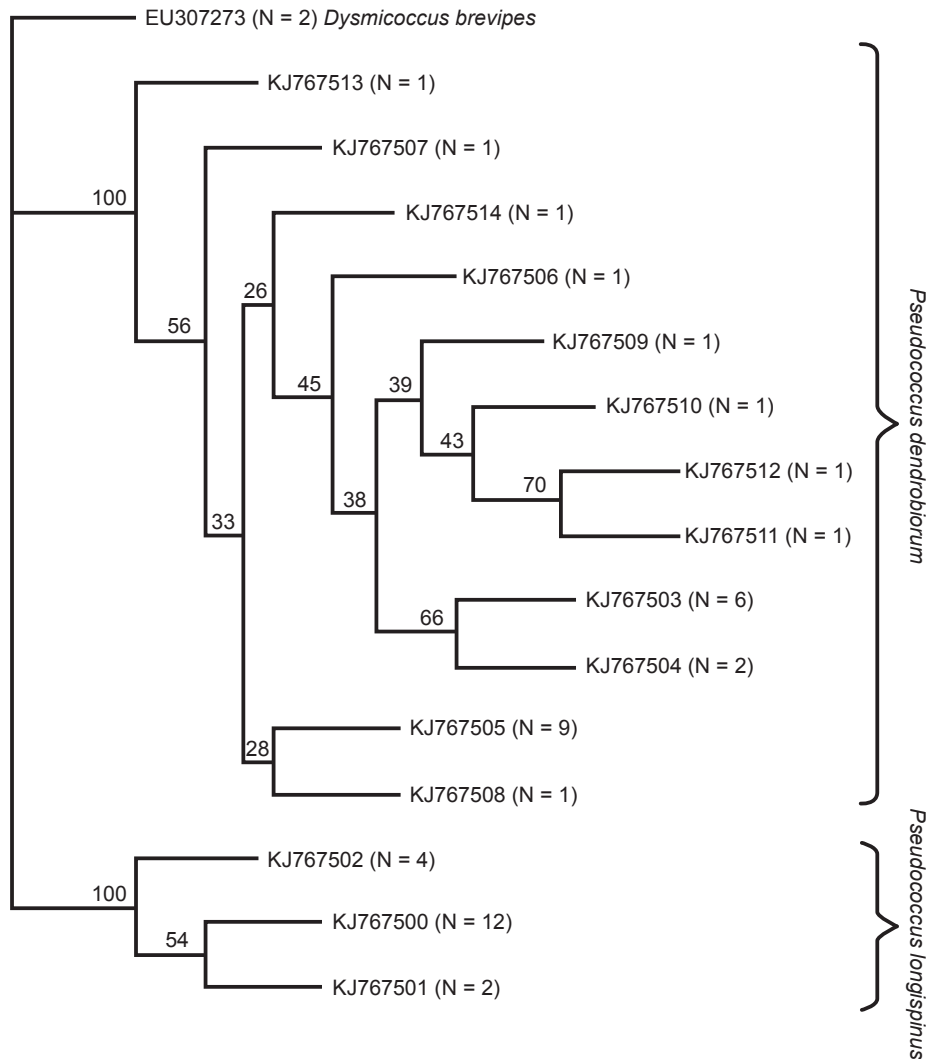
*gispinus* (84.00%) (Table 2). The phylogenetic tree (neighbor-joining tree) for gene sequences of the 3 mealybug species studied is shown in Fig. 4. All the *Ps. dendrobiorum* and *Ps. longispinus* sequences formed a single group, respectively. These phylogenetic results consistent with the morphological resolution suggest that the new mealybug pest and the *Ps. dendrobiorum* collected from Vietnam are the same species.

The frequency of mealybugs detected in the imported agricultural products through inspection programs conducted by Taiwan BAPHIQ is high (Chen *et al.* 2011b). In this study, the morphological characteristics and the rDNA-ITS1 sequences similarity analysis techniques were established to identify the species and confirm the introduction source of an invasive mealybug pest, which could cause damages to orchids. The combination of morphological and molecular characteristics has been broadly used as a robust technique for pest identification in recent years.

In our surveys, the damage of the invasive *Ps. dendrobiorum* to the orchids in Taiwan was limited to a single greenhouse in Changhua County. Although the economic impacts of this mealybug is difficult to estimate at this point as the information on its local ecology is lacking, *Ps. dendrobiorum* could be a challenging species and affect local export orchids (*Phalaenopsis* spp.) if they become established. In order to avoid proliferation of this invasive mealybug, Taiwan BAPHIQ has carried out a standard process in which the entire infested areas were sprayed with insecticide (44% Di-

**Table 2.** Mean percent similarities ( $\pm$  SD) among the new invasive mealybug, *Pseudococcus dendrobiorum* from Vietnam, *Pseudococcus longispinus* and *Dysmicoccus brevipes*, in the ITS1 sequence.

	New mealybug (Taiwan) (n = 16)	<i>Ps. dendrobiorum</i> (Vietnam) (n = 10)	<i>Ps. longispinus</i> (Taiwan) (n = 18)	<i>Dy. brevipes</i> (Taiwan) (n = 2)
New mealybug (Taiwan) (n = 16)	99.75 $\pm$ 0.18	99.61 $\pm$ 0.26	92.64 $\pm$ 0.19	83.94 $\pm$ 0.08
<i>Ps. dendrobiorum</i> (Vietnam) (n = 10)		99.53 $\pm$ 0.32	93.04 $\pm$ 0.67	83.87 $\pm$ 0.24
<i>Ps. longispinus</i> (Taiwan) (n = 18)			99.91 $\pm$ 0.10	84.00 $\pm$ 0.21
<i>Dy. brevipes</i> (Taiwan) (n = 2)				100.00 $\pm$ 0.00



**Fig. 4.** Neighbor joining (NJ) tree with bootstrap support (1,000 replicates) showing clustering of *Pseudococcus* species for rDNA-ITS1 sequences. All *Ps. dendrobiorum* and *Ps. longispinus* sequences formed a single group, respectively. Bootstrap values are shown above the branches. “N” in the parenthesis indicates number of specimens. *Dysmicoccus brevipes* (EU307273) were used as an outgroup.

methoate EC 1,000×), and the infested plants, media, and containers were burned and destroyed to eliminate this pest. Regular detection surveys were also made subsequently to ensure its eradication, and no individuals were found again thereafter (Wu *et al.* 2014).

Farmers alertness combined with the TARI agricultural pest diagnostic service has been one of the major sources for gathering

information about invasive pests in Taiwan, assisting official control programs to eliminate the pests before their establishment. In addition, this study provides basic information and digital archives of *Ps. dendrobiorum* for the TARI Digital Insects website (<http://DigiPest.tari.gov.tw/>), which is helpful to the taxonomic researchers, quarantine inspectors, and farmers in pest identification.

## ACKNOWLEDGEMENTS

This study was funded by Council of Agriculture, Executive Yuan, Taiwan ROC (grants 103AS-7.1.1-C1-C1(6) and 102AS-10.2.2-BQ-B6).

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# 台灣新入侵石斛粉介殼蟲 (半翅目：粉介殼蟲科) 初報

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## 摘要

陳淑佩、邱一中、陳健忠、翁振宇。2015。台灣新入侵石斛粉介殼蟲 (半翅目：粉介殼蟲科) 初報。台灣農業研究 64(1):1-9。

本研究於 2013 年以形態特徵及核醣體 RNA 基因之 ITS1 (rDNA-ITS1) 序列相似性分析的多重比對資料，鑑定出蝴蝶蘭的新入侵害蟲石斛粉介殼蟲 [*Pseudococcus dendrobiorum* Williams (半翅目：粉介殼蟲科)]，並確認其來源地為越南。本粉介殼蟲玻片標本存放於行政院農委會農業試驗所應用動物組昆蟲標本館，作為我國入侵害蟲之存證標本，其資料可作為日後監測此種害蟲的依據。根據植物保護法，行政院農業委員會動植物防疫檢疫局進行滅絕及監測此害蟲的工作，迄今未再發現此害蟲。

**關鍵詞：**石斛粉介殼蟲、長尾粉介殼蟲、粉介殼蟲、入侵害蟲、害蟲鑑定。

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投稿日期：2014 年 8 月 4 日；接受日期：2014 年 10 月 24 日。

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