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First report of *Bradysia difformis* (Diptera: Sciaridae) Damage to *Phalaenopsis* orchid in China

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ABSTRACT

Phalaenopsis orchid is among the most valuable ornamental flowering plants in the world. Since visible damage substantially decreases its amenity, limited damage is allowed in its production. An unknown insect species (Diptera: Sciaridae) was found to cause serious damage to the seedling of the *Phalaenopsis* orchid in greenhouses in Guangdong, China. The insect occurred in high populations in almost all greenhouses that grow *Phalaenopsis* orchid and the number of sciarid adults trapped on a yellow sticky card could reach as many as 303 in 24 h. An effective management strategy on any pest requires an accurate identification. Therefore, it is urgent to identify this pest correctly to mitigate its damage to the industry. Damage to *Phalaenopsis* orchid and morphological characteristics of the pest was described in this study. Molecular analyses based on the 488-bp portion of the mitochondrial DNA from the cytochrome oxidase I(mt *COI*) region were conducted to supplement morphological characteristics in identifying this pest. Both morphological characteristics and phylogenetic tree constructed with mt *COI* genes identified this sciarid as *Bradysia difformis* Frey, 1948 (= *Bradysia paupera* Tuomikoski, 1960) (Diptera: Sciaridae). A literature search indicated that *B. difformis* has been a common pest of greenhouse and forestry nurseries in Europe and South Africa. Our study is the first record of *B. difformis* damaging *Phalaenopsis* orchid in China.

Introduction

The *Phalaenopsis* orchid is among the most valuable flowering plants that are commercially produced throughout the world with the highest degree of industrialization. It is a perennial monopodial monocotyledon, and its flowers mimic the shapes of butterflies in appearance. It has a long blooming period with diverse flower colors. The commercial cultivation of *Phalaenopsis* is an important part of the Chinese floriculture industry. Insect pests *Saissetia coffeae* (Walker) (Hemieptera: Coccidae), *Parlatoria proteus* (Curtis) (Hemiptera: Diaspididae), and *Pseudococcus longispinus* (Targ.) (Hemiptera: Pseudococcidae) and mite pests *Tenuipalpus pacificus* Baker (Trombidiformes: Tenuipalpidae) and *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) occur and can attack *Phalaenopsis* orchids throughout the year (Yang, 1997; Rampal et al., 2013). The authors have surveyed pests of *Phalaenopsis* orchid in Guangdong province, China, since 2009 and found that a sciarid (Diptera: Sciaridae) was damaging seedlings of *Phalaenopsis* orchid and the damage was observed in nearly all *Phalaenopsis* orchids greenhouses.

Sciarids are mostly small, dark-colored gnats with a slender body, long legs and unmarked wings with characteristic venations (Menzel et al., 2006). The larvae feed on organic matter, fungi, algae, and damage roots of cuttings or small plants by tunneling through stems. Sciarid commonly infests mushroom, glasshouse ornamental, and forestry nurseries (Gouge and Hague, 1995; White et al., 2000; Shen et al., 2009; Hurley et al., 2010). Sciarid damage to orchids has not been reported in China. The objectives of the present study were (1) to identify the unknown sciarid species attacking the seedlings of *Phalaenopsis* orchid by combining traditional morphological characteristics and phylogenetic analyses and (2) to obtain biological data on this pest for development of effective control strategies in *Phalaenopsis* greenhouse.

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Materials and methods

Insect sampling and rearing

Adult insects were collected from *Phalaenopsis* orchid greenhouses in Shantou and Guangzhou, Guangdong province, using common suction traps (Yulin Edu. Project Co., LTD., Henan, China) and brought to laboratory for rearing. The insects were kept in cages covered with 60- mesh screen under 27 ± 1 °C and 65 ± 5% relative humidity. Wet ground dregs of soybean (*Glycine max*) were used as a substrate. The mycelia that subsequently grew on the substrate provided ideal sites for oviposition. The larvae feed on the wet ground dregs of soybean.

Morphological identification

The morphology of adults, eggs, larvae, and pupae was examined under a Leica S4E stereo zoom microscope (Leica Microsystems Inc., Buffalo Grove, IL, USA). Adult specimens were glass slide-mounted following the method of Zhang et al. (2010) before observation and measurements under a Nikon microscope (Eclipse 80I, Nikon Instruments Inc., Melville, NY, USA). Identification terminology followed Hippa and Vilkamaa (2007) and Zhang et al. (2010).

Mt COI gene sequence-based phylogenetic tree analysis

For each sciarid adult, 50 μL of total DNA was extracted using a UNIQ-10 Animal Genomic DNA extraction kit (Sangon Biotech, Shanghai, China). A portion of the mitochondrial cytochrome *c* oxidase I (mt *COI*) gene was amplified by PCR using the forward primer 5′-GGAGCTCCTGACATAGCATTCCC-3′ and reverse primer 5′-CCCGGT AAAATTAAAATATAAACTTC-3′ (Simon et al., 1994). PCRs were conducted in a 25 μL reaction mixture containing 2.5 μL 10 × PCR buffer (Mg^{+}), 2 μL dNTP (2.5 mmol/L), 1 μL forward and 1 μL reverse primers (10 mmol/L), 1 μL DNA template, and 0.5 μL Taq polymerase (5 U/μL) (Takara, Kyoto, Japan). The PCR program included in order an initial denaturation step of 3 min at 94 °C, 35 cycles of 94 °C for 30 s, annealing at 55 °C for 30 s and 72 °C for 30 s, and a final extension at 72 °C for 10 min. PCR products were checked by electrophoresis on a 1.5%W/V agarose gel in TAE buffer (40 mmol/L Tris-acetate, 2 mmol/L Na_2 EDTA·$2H_2O$), and the resulting bands were visualized by ethidium bromide staining (Wang et al., 2014). The target bands were purified from the gel and cloned into a pGEM-T easy vector (Invitrogen, Guangzhou, China). Recombinant plasmids were sequenced with primers for both strands (Invitrogen, Guangzhou, China).

A BLAST search was conducted in GenBank to determine the closest sequence match. Sequence data were analyzed using Seqman software (DNASTAR, Inc.). A phylogenetic tree was constructed by using the maximum parsimony with MEGA 5 software (Tamura et al., 2011). The inferred phylogeny was tested by a bootstrap analysis with 1000 replicates.

Biology of the sciarid

Different developmental stages (egg, larva, pupa and adult) and the biology of this pest were studied in laboratory of Plant Protection in Zhongkai University of Agriculture and Engineering located in Guangzhou, Guangdong province, China, under temperatures 27 ± 1 °C and 65 ± 5% relative humidity.

Yellow sticky cards (20 cm × 25 cm) (Pherobio Technology Co., LTD., Beijing, China) were placed among and just 1 cm above the seedlings of the *Phalaenopsis* orchid cultivar ("Mantianhong") in the greenhouse in Shantou, Guangdong province, on April 19, 2012. The density of yellow cards was approximately one card per 2 m^2 area. The number of sciarid adults trapped on each yellow sticky card was counted and recorded 24 h later. Damage to seedlings caused by the sciarid larvae was recorded and photos were taken by DMC–LX3GK Panasonic digital camera (Panasonic Corporation, Japan).

Results

Morphological identification

Adults of the collected sciarid were small, dark-colored flies. They had long and slender legs with a pair of clear and iridescent forewings. Antennae were 16-segmented.

Male: body length, 1.80–2.02 mm; wing length, 1.33 mm; width, 0.59 mm; antennae length, 1.06 mm; eye bridge, 2–3 facets wide; compound eyes with microtrichia; fourth flagellomere, 1.5 times as long as it is wide. Palpus three-segmented: basal segment club shaped with 4 bristles (1–2 obviously longer than the others) and with a deep sensory pit; second segment oval with 6 bristles; last segment with 7–10 bristles, 1.3–1.5 times as long as the second segment (Fig. 1A). M and Cu veins without macrotrichia. The stem of M is longer than the fork of M; the fork of M short, R1 short = 0.7R (Fig. 1C). Fore tibia with one spur; mid and hind tibiae with two yellowish, thin, and subequal spurs; and inner side of fore tibia with comb-like row of 6–7 strong bristles. A row of bristles on the back of hind tibia. Tarsal claws untoothed. Tergite IX trapezoid, slightly emarginated apically and edged with bristles. Gonostyle approximately 2.5 times as long as wide. Hypopygium compact and almost as high as wide. Tip of gonostyle with thicker and coarser bristles, apex with distinct raised, thin, hooked tooth, and 5–7 subequal curved spines directed ventromedially (Fig. 1B).

Female: body length, 1.90–2.30 mm; antennae length, 1.20 mm; fourth flagellomere, 1.6 times as long as it is wide; vaginal fork narrow with stubby handle. All other characteristics are the same as those in the male.

Based on the morphology, this insect was identified as *Bradysia difformis* Frey.

Molecular identification constructed with mt COI nucleotides

The 488-bp portion of the mt *COI* gene of a sciarid adult was amplified by PCR and sequenced, and the mt *COI* sequence was deposited in the GenBank database (Accession No. KF478669). The alignment of mt *COI* gene of *B. difformis* (FN868634), *B. difformis* K2 (DQ060446), *B. difformis* S2-6 (EU450782), *B. difformis* K7 (DQ060451), *B. difformis* S9 (JN378638), *B. difformis* M1 (DQ060460), *B. difformis* R1 (DQ060488), *Corynoptera saetistyla* 876 (JQ613847), *C. vagula* 959 (JQ613867), *Anopheles campestris* NSK01 (JQ003058), *A. rangeli* COL182-2 (JX205120), *A. rangeli* MG30-1 (HM022390), *A. benarrochi* B COL42-12 (JX205105), *Ctenosciara insolita* 803 (JQ613835), *Aleochara laticornis* (AJ293048), *Bradysia procera* 695 (JX418153), and *Sciara kitakamiensis* (HQ979114) was generated by using the maximum parsimony with MEGA 5 software (Tamura et al., 2011).

The phylogenetic analyses of this insect with other related species indicated that this sciarid had the highest similarity (100%) to *B. difformis* Frey, 1948 (= *B. paupera* Tuomikoski, 1960) sequences (Fig. 2).

Biology of the sciarid

Newly emerged *B. difformis* adults were soft and pale, turned to black after about 30 min. Adults mated within 24 h of emergence. Males flapped their wings and chased females with the posterior abdomen bending forward. After managing to approach female, male clamped female with his hypopygium and started to mate, with their bodies being in a line. The pair of female wings was always unfolded, whereas the wings of males were folded over its body during the mating. The male could be dragged by the female to run during copulation or they