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A TAXONOMIC UPDATE OF TAKAHASHI'S HISTORIC COLLECTION OF MEALYBUGS (HEMIPTERA: PSEUDOCOCCIDAE) FROM MALAYSIA AND SINGAPORE

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ABSTRACT

Dr. Ryoichi Takahashi collected mealybugs (Hemiptera: Coccomorpha: Pseudococcidae) from Peninsular Malaysia and Singapore in 1943–1944, and mounted them in 39 permanent slide mounts using Canada balsam. The slides are deposited in the Department of Agriculture, Kuala Lumpur, Malaysia. The objective of this research was to update the identification of Takahashi's specimens using recent taxonomic keys. Some preparations on cavity slides were re-mounted to facilitate

identification. The re-mounted specimens, and those on the original slides, were re-examined and 12 species were identified. The Takahashi samples of *Pseudococcus comstocki* (Kuwana) and *Rastrococcus mangiferae* (Green) are the earliest records of these species from Peninsular Malaysia.

Key words: Peninsular Malaysia, *Pseudococcus comstocki*, *Rastrococcus mangiferae*, earliest record, slide re-mounting.

ABSTRAK

Tiga puluh sembilan slaid tetap spesies Koya (Hemiptera: Coccomorpha: Pseudococcidae) dari Semenanjung Malaysia dan Singapura telah dikumpul sekitar tahun 1943-1944 serta disediakan oleh Dr. Ryoichi Takahashi dengan menggunakan larutan Canada Balsam. Slaid-slaid tersebut disimpan di Jabatan Pertanian Kuala Lumpur, Malaysia. Objektif kajian ini antaranya untuk mengemaskini pengecaman specimen oleh Takahashi dengan menggunakan kekunci taksonomi yang terkini. Sesetengah slaid asal telah dilekap balik untuk tujuan pengecaman. Sejumlah 12 spesies dari slaid asal dan yang dilekap semula telah dikenalpasti dan dicam semula. *Pseudococcus comstocki* (Kuwana) dan *Rastrococcus mangiferae* (Green) dari koleksi Takashashi telah dikenalpasti sebagai rekod awal dari Semenanjung Malaysia.

Kata kunci: Semenanjung Malaysia, *Pseudococcus comstocki*, *Rastrococcus mangiferae*, rekod terawal, slaid lekap semula.

INTRODUCTION

There is a collection of 39 permanent slides of mealybugs in the Plant Biosecurity Division, Department of Agriculture Malaysia (DoA), Jalan Gallagher 50480, Kuala Lumpur, Malaysia. The

specimens were collected and mounted by Dr Ryoichi Takahashi (1898–1963), a well-known insect researcher who studied Thysanoptera and Hemiptera (including mealybugs) from Japan, Taiwan and other Asian countries. Ito and Sorin (1963) listed Takahashi's publications and discussed the range of his research.

The Takahashi collection of mealybugs deposited at DoA were collected from the Malay Peninsula (now known as Peninsular Malaysia and Singapore) in 1943-1944 and all of the data labels are written in Takahashi's handwriting. The slides provide evidence of the presence of particular mealybug species in Peninsular Malaysia and Singapore at that time, but were not included in the list of economically important pests for West Malaysia compiled by Yunus and Ho (1980).

These 39 slides are part of a huge quantity of material collected by Takahashi. An additional 872 mealybug slides mounted and identified by Takahashi are deposited in the collection at TARI (Taiwan Agriculture Research Institute), including some designated by Takahashi as type specimens (Chen *et al*., 2012). More Takahashi slides are deposited at The University of Hokkaido, Japan (Williams 2004). According to Williams (In press), 14 Takahashi mealybug type specimens and some other slides of Malaysian mealybugs were transferred in 1955 from the Selangor Museum, Malaysia to The Natural History Museum, London, U.K. The Selangor Museum was established on 1889 but the building was destroyed in 1945 during the World War II (Department of Museums Malaysia 2017).

The objective of the present work was to update the identifications of Takahashi's mealybug specimens at DoA Malaysia using recent taxonomic publications, and to find early records of mealybug species from Malaysia and Singapore. The

slides consist of specimens mounted with or without staining (Fig. 1 and Fig. 2). Some specimens were mounted in cavity

slides, which caused difficulties for determination because the specimens were tilted, not flat; it was therefore necessery to re-mount them so that they could be re-identified.

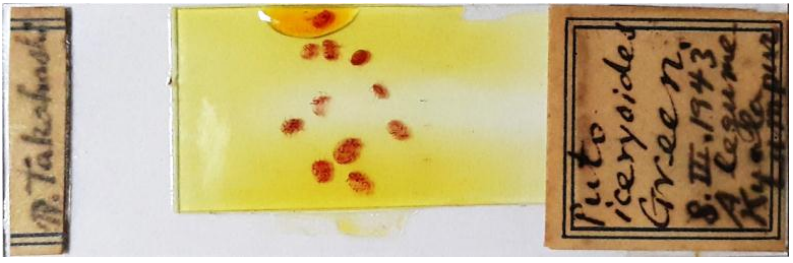

Figure 1. An original Takahashi slide containing 12 mealybug specimens that have been stained.

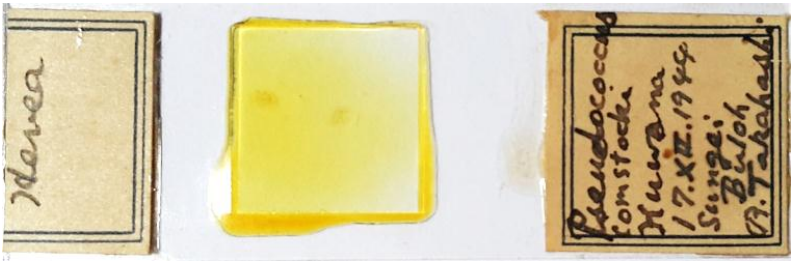

Figure 2. An original Takahashi slide containing two specimens that have not been stained.

MATERIALS AND METHODS

All 39 Takahashi slides were borrowed from DoA Malaysia and taken to the Plant Pest Diagnostic Center, California Department of Food and Agriculture, Sacramento, California, U.S.A. (CDFA) for study. After more than 70 years, the Canada balsam in most of the mounts had turned yellowish and the paper labels had become discoloured. In addition, evaporation of xylene from the mountant has left only a thin layer of Canada balsam under the coverslip, crushing the specimens so that the dorsal and ventral surfaces are closely adpressed. It is difficult to see on which surface particular structures are situated, making authoritative identification challenging. One sample was represented only by tilted and crumpled specimens in cavity slide mounts, making determination extremely difficult, so these slides werere-mounted and re-identified at CDFA. The rest of the slides were re-identified at the Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University, Bogor, Indonesia.

Removing specimens from old permanent slide mounts

Canada balsam is a mountant made from the resin of a tree, *Abies balsamea,* diluted with xylene (Brown, 1997). Takahashi mounted his specimens in Canada balsam and the first step in removing the specimens from each cavity slide mount was by soaking the slide in the appropriate solvent, xylene. Extreme care was necessary when handling specimens released from the old permanent mount, as they were far too fragile to attempt any dissection or re-shaping. The method used was as follows:

1. The slide was photographed in good light to preserve the data on the labels, in case it washed off during the process of re-mounting.

2. The edge of the cover slip was cleaned using a ball of tissue wet with 95% isopropanol, held in forceps, to remove any dirt or grease.

3. The slide was placed in water in a covered Petri dish and heated on a thermostatically controlled hotplate to 60^{o}C to dissolve the glue under the labels. In a short time, the labels floated off the slide. They were then retrieved, laid face-down on the lid of the dish to dry, and the slide was removed from the water and allowed to dry completely.

4. In a covered Petri dish, the slide was soaked in xylene in a fume hood for five days, to dissolve the Canada balsam and release the coverslip.

5. Once the coverslip floated off, soaking continued until the specimens floated free from the slide. Using a small spatula, specimens were transfered singly to xylene in a cavity block and covered. They were then ready to re-mount.

Re-mounting specimens in new permanent slide mounts

The specimens were re-mounted using a modified version of the slide preparation method given by Sirisena *et al*. (2013):

1. Using a small spatula, each specimen was tranferred from xylene to 100% alcohol in a covered cavity block and soaked for about 1 hour at room temperature, to remove the last traces of old Canada balsam.

2. The specimen was transferred into 95% isopropanol in a covered cavity block for one hour, to begin re-hydration.

3. The specimen was transferred to acidified 80% alcohol in a covered cavity block for about one hour, to further hydrate and to acidify the cuticle.

4. The specimen was transferred to stain (acid Fuchsin mixed with Essig's Aphid Fluid) in a covered cavity block, and heated at about 35º C for 2–12 hours.

5. The specimen was transferred from stain to acidified 80% alcohol in a cavity block briefly, to quickly wash off surplus stain, and then immediately transferred into 95% isopropanol in a covered cavity block for 20 minutes to fix the stain. When staining mealybugs, the darkest stain should be in heavily sclerotized structures like the legs and antennae; the membranous cuticle should be light pink.

6. The specimen was transferred from 95% isopropanol into clove oil in a covered cavity block for 20–30 minutes to clear the cuticle. The time spent in clove oil was limited because if left longer, the cuticle began to stiffen and become even more fragile.

7. A slide was labeled with a brief identifier code using a fine felt-tip pen, and a drop of clove oil was placed at its centre.

8. The specimen was transferred from the cavity block into the clove oil drop on the slide, and positioned for mounting. Surplus clove oil was then removed by capillarity, using a folded tissue.

9. A very small drop of Canada balsam was placed on the specimen and quickly spread with a mounted needle until it formed a very shallow pool with a flat surface around the specimen.The slide was left on a warm horizontal surface for 20 minutes to dry, so that the balsam glued the specimen in position.

10. A clean coverslip was positioned nearby, ready for use. A large drop of Canada balsam was placed on the specimen and quickly spread into a pool around it.The coverslip was

positioned so that the specimen was in the centre and then slowly lowered on to the Canada balsam.

11. The slide was placed on a horizontal shelf in an oven at 40ºC for three months to dry.

12. Once dry, the slide was ready for labels to be attached using non-water-soluble glue. If only one slide had been produced, provided they were still legible the original labels were attached to the new mount. However, if additional slides had been made, then the original labels were attached to the best mount and the other slides were labeled using replica labels printed from the photograph of the original slide.

13. The re-mounted specimens were identified using the keys in Cox (1989), Williams (1989; 2004) and Williams and Watson (1988).

RESULTS AND DISCUSSION

Of the 39 slides examined, 16 were listed in Takahashi's (1951) work on mealybugs from the Malay Peninsula. Five of the slides, containing only immature stages, males or adult females in poor condition, could only be identified to genus level. From the remaining 34 slides, 12 mealybug species were identified and are discussed below. All the samples were collected by R. Takahashi.

Dysmicoccus brevipes (Cockerell)

Original label information: *Pseudococcus brevipes* (1 slide): MALAYSIA, Ulu Gombak, on pineapple, 2.xii.1944.

Pseudococcus brevipes (Cockerell) was placed in a new nomenclatural combination, *Dysmicoccus brevipes* (Cockerell), by Ferris (1950). This specimen was listed in Takahashi (1951). The earliest record of the presence of *D. brevipes* from Malaysia is a plant quarantine interception made in 1917 at U.S.A., Washington DC, on *Ananas* sp. (Williams, 2004).

Dysmicoccus debregeasiae (Green)

Original label information: *Pseudococcus comstocki* Kuwana (1 slide): MALAYSIA, Kuala Lumpur, on unknown host, 9.ii.1943.

The slide contained two specimens; on preliminary observation, both lacked the oral rim tubular ducts characteristic of the genus *Pseudococcus*. Re-mounting and re-examination resulted in a new identification as *D. debregeasiae*. Figure 3 shows the re-mounted slide (Figure 3A) and key taxonomic characters of *D. debregeasiae*. The specimen has thoracic cerarii each containing two enlarged conical setae (Figure 3B); oral collar tubular ducts on the venter of the thoracic margin (Figure 3C); anal lobe cerarii each containing two conical setae together with auxiliary setae (Figure 3D); and a circulus notched on each side and divided by an intersegmental line (Figure 3E). The earliest Malaysian record of this species was by R.H. Le Pelley, from Malaysia, Serdang, on *Manilkara zapota*, 13.vi.1937 (Williams, 2004).

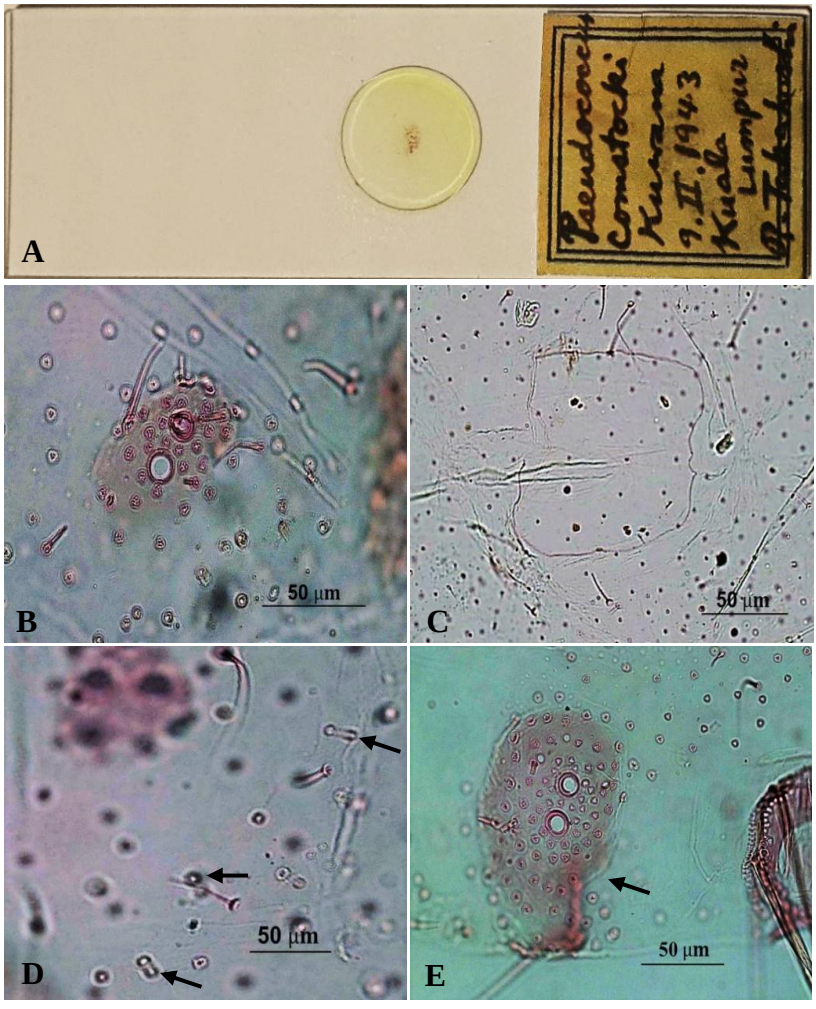

Figure 3. *Dysmicoccus debregeasiae*: (A) a specimen originally identified by Takahashi as *Pseudococcus comstocki*, re-mounted and re-identified as *D. debregeasiae*; (B) abdominal cerarius with two conical setae, one of which has fallen off; (C) circulus; (D) oral collar tubular ducts on venter (arrows); and (E) an anal lobe cerarius (arrow) whose conical setae have fallen off.

Dysmicoccus lepelleyi (Betrem)

Original label information: *Pseudococcus hispidus* Morrison (1 slide): MALAYSIA, Ulu Gombak,on pineapple,10.vii.1944.

Pseudococcus hispidus is a junior synonym of *Exallomochlus hispidus* (Morrison). However, the specimen has dorsal oral collar tubular ducts, each about twice the diameter of a trilocular pore, present singly on most segments around the margin, and was re-identified as *D. lepelleyi*. Figure 4A shows the Takahashi slide which was re-identified as *D. lepelleyi*; Figure 4B shows an oral collar tubular duct between two posterior abdominal cerarii. According to Williams (2004), the earliest record of *D. lepelleyi* from Malaysia was collected at Georgetown, on *Annona muricata*, 19.vi.1937, by R.H. Le Pelley.

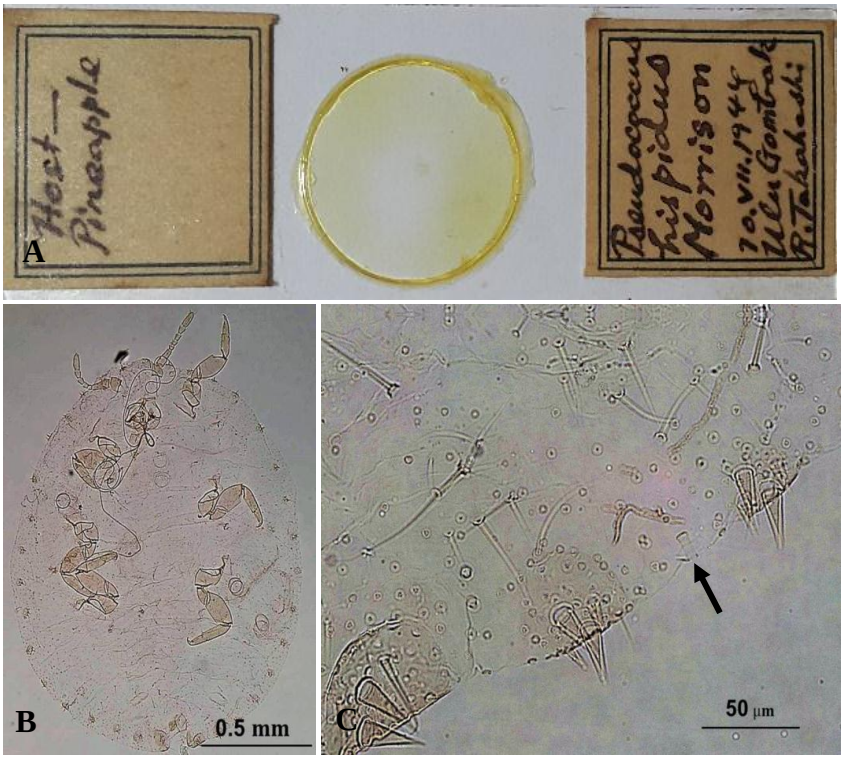

Figure 4. *Dysmicoccus lepelleyi*: (A) a specimen originally identified by Takahashi as *Pseudococcus hispidus*, now re-identified as *D. lepelleyi*; (B) one adult female specimen; and (C) an oral collar tubular duct (arrow) situated between two abdominal cerarii.

Ferrisia virgata (Cockerell)

Original label information: *Ferrisiana virgata* Ckll. (1 slide): MALAYSIA, Selangor, Serdang, on unknown host, 6.vi.1944.

Ferrisiana virgata is now known as *Ferrisia virgata* (Cockerell). The earliest record of *F. virgata* from Malaysia was collected at Kuala Lumpur on *Acacia farnesiana*, 1924 (Williams, 2014). García Morales *et al.* (2016) lists the great diversity of host-plants of this highly polyphagous mealybug.

Nipaecoccus viridis (Newstead)

Original label information: *Pseudococcus filamentosus* Ckll. (1 slide): MALAYSIA, Sungai Buloh, on *Artocarpus*, 23.vi.1944.

Pseudococcus filamentosus is now known as *Nipaecoccus filamentosus*. However, the specimens were re-identified as *N. viridis* because they have distinctly lanceolate dorsal setae, similar to the cerarian setae but shorter and of many different sizes, as described in Williams (2004). The specimens on Takahashi's slide are purple. This may be the original cuticle color. Figure 5A shows the original Takahashi slide and Figure 5B shows two different sizes of dorsal lanceolate setae next to an abdominal cerarius, a key diagnostic character. Each abdominal cerarius consists of two lanceolate setae situated on a membranous area. Another diagnostic character is that the circulus is not divided by an intersegmental line (Figure 5C). The specimen data was mentioned in Takahashi (1951). Williams (2004) included Malaysia and Singapore in the geograpical distribution of this species.

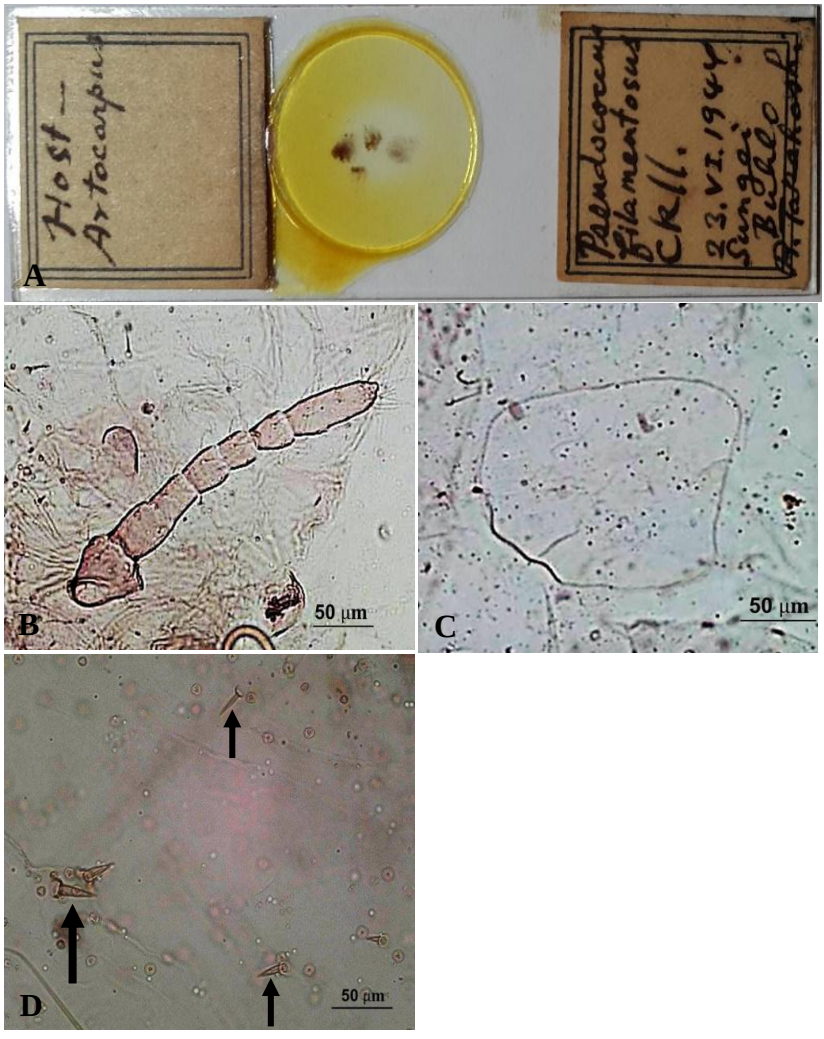

Figure 5. *Nipaecoccus viridis*: (A) specimens originally identified by Takahashi as *Pseudococcus filamentosus*, now re-identified as *N.viridis*; (B) 7-segmented antenna; (C) circulus not divided by intersegmental line; and (D) lanceolate dorsal setae (thin arrows) near to a cerarius containing two lanceolate setae (thick arrow).

Planococcus lilacinus (Cockerell)

Original label information: *Pseudococcus tayabanus* Cockerell (1 slide): Malaysia, Kuala Lumpur, on *Ficus*, 12.iii.1943.

Pseudococcus tayabanus Cockerell was placed by Morrison (1920) as a junior synonym of *Pl. lilacinus* (Cockerell). The sample data on this single slide was listed byTakahashi (1951). Both Cox (1989) and Williams (2004) listed material of *Pl. lilacinus* collected from Malaysia but, as neither publication provided dates of collection, it is not possible to tell whether the Takahashi sample is the earliest known record.

Planococcus minor (Maskell)

Original label information: *Pseudococcus citri* Risso (4 slides): MALAYSIA, Kuala Lumpur**,** on unknown host, 11.iii.1943; on a plant of Lauraceae, 24.xii.1944; Sungai Buloh, on unknown host, 10.iv.1943; Fraser's Hills**,** on *Artemisia*, 6.vi.1943.

Re-identification of these specimens using Cox's (1989) discriminant function scoring system identified these samples as *Pl. minor*; all the specimens scored under 35. Takahashi's collection data indicates that *Pl. minor* was already present in Malaysia in 1943. Two of the above samples, collected on Lauraceae and *Artemisia*, were mentioned in Takahashi (1951). Figure 6 shows one of the original slides of *Pl. minor* in the Takahashi collection. Both Cox (1989) and Williams (2004) listed material of *Pl. minor* collected from Malaysia but, as neither publication provided dates of collection, it is not possible to tell whether the Takahashi samples are the earliest known records.

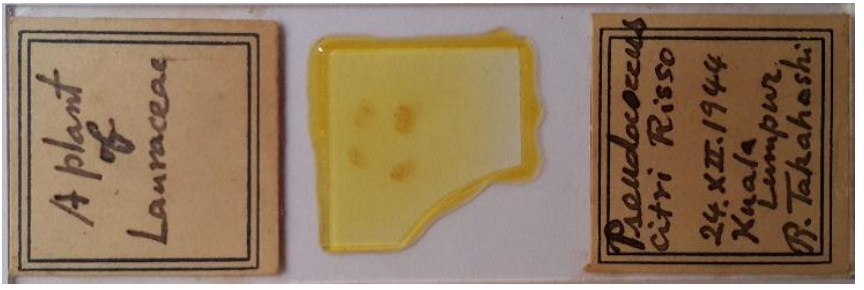

Figure 6. Specimens originally identified by Takahashi as *Pseudococcus citri*, now re-identified as *Planococcus minor*.

Out of 79 slides labeled as *Ps. citri* (Risso) collected by Takahashi from Micronesia, Palau, Taiwan and U.S.A., Chen *et al*. (2012) recognized 7 as containing *Pl. minor*.

On re-examination, one additional Takahashi slide is also considered to be *Planococcus minor*. Original label information: *Pseudococcus* (1 slide): MALAYSIA, Selangor, Puchong, on unknown host, 24.vii.1944. The original slide is shown in Figure 7.

Figure 7. Specimens originally identified by Takahashi as *Pseudococcus* sp., now re-identified as *Planococcus minor*.

Planococcus sp.

Original label information: *Pseudococcus* (1 slide): MALAYSIA, Kuala Lumpur, on Clerodendrum, 18.iv.1944.

The slide contains four specimens in insufficiently good condition for identification; they were not re-mounted to avoid further damage. The specimens could be identified to genus level only, as *Planococcus*, because they have distinct anal lobe bars and bar setae, and cerarii numbering 18 pairs bearing conical setae.

Pseudococcus comstocki (Kuwana)

Original label information: *Pseudococcus comstocki* Kuwana. (7 slides): MALAYSIA, Kuala Lumpur, on Palm, 12.ii.1944; on unknown host, 8.x.1943; on unknown host, 1.xii.1943; Ulu Gombak, on Coconut, 10.vii.1944; Sungai Buloh, 17.xii.1944 on *Hevea*; Selangor, on unknown host, 2.v.1943; Selangor, Morib, on mangrove, 30.i.1944.

The three slides collected on palm, coconut and *Hevea* respectively are important, since Williams (2004) reported no specimens had been available to him to confirm Takahashi's (1951) records of this species on coconut, palm and *Hevea*. Our discovery of Takahashi's original slides of *Ps. comstocki* supports his record of this species from Peninsular Malaysia (the earliest) and provides evidence of the presence of this species in southern Asia since 1943. Moreover, Williams (2004) recorded that this species was intercepted from Malaysia at Los Angeles, U.S.A. on fruit of *Nephelium lappaceum* (Sapindaceae) on 20.iv.1981.

Rastrococcus iceryoides (Green)

Original label information: *Puto iceryoides* Green (7 slides): MALAYSIA, Kuala Lumpur, on legume, 8.iii.1943; on Durian, 16.vii.1943; on unknown host, 16.vii.1943; on *Ficus*, 18.vii.1943; on *Gardenia*, no date of collection; Sungai Buloh, on legume, 10.iv.1943; Kuala Lumpur SINGAPORE, on *Ficus*, 2.iii.1944.

Takahashi collected this species from Malaysia in 1943 and from Singapore in 1944. The earliest record of *R. iceyioides* in Malaysia was recorded by Williams (2004) as 1924 at Malaysia, Perak, Ipoh. Takahashi slide mounts of additional samples of this species from India, Malaysia and Singapore are deposited at TARI in Taiwan (Chen *et al.*, 2012).

Rastrococcus mangiferae (Green)

Original label information: *Puto mangiferae* Green (1 slide): MALAYSIA, Kuala Lumpur, on unknown host, 19.ix.1943. This specimen was mentioned in Takahashi (1951). He remarked that this species had not been recorded previously from Malaya.

Puto mangiferae is now known as *Rastrococcus mangiferae.* The slide contains only one specimen, which can be identified based on the shape of the multilocular disc pores, which appear to be wagon-wheel shaped, and their distribution on the venter as far foward as the anterior part of the body. The specimen was listed in Takahashi (1951), and is the earliest record of the species from Malaysia. Williams (2004) recorded *R. mangiferae* from India and Sri Lanka, and Tang (1992) added China to its distribution. Chen *et al.* (2012) mentioned three slides of *R. mangiferae* collected by Takahashi from Taiwan.

Rastrococcus spinosus (Robinson)

Original label information: *Puto spinosus* Robinson (5 slides): MALAYSIA, Kuala Lumpur, on Mango, 1.i.1943; on unknown host, 26.iii.1944; Kuala Selangor, on unknown host, 18.vii.1943. SINGAPORE, on *Artocarpus*, 2.iii.1944; on *Ficus*, 2.iii.1944.

The Takahashi specimens are not the earliest records from Malaysia or Singapore. In 1925, *R. spinosus* was found in Malaysia, Melaca on an unknown host (Williams, 2004). Takahashi's record expanded the known distribution of *R. spinosus* in Malaysia at that time. The two slides collected from Singapore were mentioned in Takahashi (1951).

Rastrococcus sp.

Original label information: *Phenacoccus* (3 slides): MALAYSIA, Kuala Lumpur, on *Dischidia*, vii.1943 (2 cavity slides); on *Dischidia*, 16.vii.1943 (1 slide).

At the time Takahashi was working, the genus *Rastrococcus* Ferris, 1954 had not been described and the species were assigned to *Phenacoccus.* The specimens in the two cavity slide mounts were remounted as 16 new slide mounts. This revealed that all the specimens were immature males, so identification was only possible to generic level.

Original label information: *Puto spinosus* Robinson (1 slide): MALAYSIA, Cameron Highlands, on unknown host, 6.x.1944. The slide contained immature *Rastrococcus* that could only be identified to genus level with confidence.

Original label information: *Puto* (Nympha) (1 slide): MALAYSIA, Malacca, on mangosteen, 25.iv.1943. It was only possible to identify this poor, immature material to genus level.

Saccharicoccus sacchari (Cockerell)

Original label information: *Trionymus sacchari* Ckll. (2 slides): MALAYSIA, Selangor, Gombak, on Sugar cane, 17.iv.1944; Sungai Tua, on Sugar cane, 11.vii.1944.

Trionymus sacchari (Cockerell) was placed in a new nomenclatural combination, *Saccharicoccus sacchari* (Cockerell), by Ferris (1950). These two slides were listed inTakahashi (1951). Williams (2004) noted the earliest collection of *S. sacchari* from Malaysia was in 1923, in Melaka.

CONCLUSION

Twelve species of mealybugs were re-identified in Takahashi's collection: *Dysmicoccus brevipes, D. debregeasiae, D. lepelleyi, Ferrisia virgata, Nipaecoccus viridis, Planococcus lilacinus, Pl. minor, Pseudococcus comstocki, Rastrococcus mangiferae, R. iceryoides, R. spinosus* and *Saccharicoccus sacchari.* Of the 39 original slides, 16 were listed in Takahashi (1951): *D. brevipes* (1), *D. lepelleyi* (1), *Ps. comstocki* (3), *Pl. lilacinus* (1), *Pl. minor* (2), *R. iceryoides* (3), *R. mangiferae* (1), *R. spinosus* (2) and *S. sacchari* (2). Six other Takahashi slides could only be identified to genus level. Takahashi's mealybug collection at DoA Malaysia contains the earliest known records from Malaysia for *Ps. comstocki* and *R. mangiferae.*

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