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Foreign Exploration for Natural Enemies of *Bemisia tabaci* from Southeast Asia

J. C. Legaspi[1], B. C. Legaspi, Jr.[2], R. I. Carruthers[3], J. Goolsby[4], W. A. Jones[2], A. A. Kirk[5], C. Moomaw, T. J. Poprawski[2], R. A. Ruiz[4], N. S. Talekar[6] and D. Vacek[4]

[1]Texas Agricultural Experiment Station, The Texas A&M University System, 2415 East Highway 83, Weslaco, TX 78596
[2]USDA-ARS Beneficial Insects Research Unit, 2413 East Highway 83, Weslaco, TX 78596
[3]National Program Staff, USDA-ARS-NPS, BARC-WEST, Beltsville, MD 20705
[4]Mission Biological Control Center, USDA-APHIS-PPQ, Mission, TX 78572
[5]USDA-ARS European Biological Control Laboratory, Montpellier, France
[6]Asian Vegetable Research and Development Center, PO Box 42, Shanhua, Tainan, Taiwan 74199

ABSTRACT

There is speculation that the sweetpotato whitefly *Bemisia tabaci* (Gennadius) (biotype 'B') (= B. *argentifolii*) may have originated in the orient because of the diversity of endemic *Bemisia* species, and effective natural enemies are likely to be found in the region. Parasitoids native to southeast Asia also may be adapted to intended areas of introduction in south Texas, because of climatic similarities. Accordingly, four separate explorations for natural enemies of *B. tabaci* were conducted in the Philippines, Taiwan, Malaysia, Thailand, and Indonesia over a period from November, 1993 to October, 1995. The collections resulted in the importation into the United States of 22 geographic strains of the aphelinid genera *Encarsia* spp. and *Eretmocerus* spp., 2 coccinellids, 1 unidentified syrphid, and several isolates of 3 species of entomopathogenic hyphomycetous fungi. One strain of *Eretmocerus* from Taiwan may represent an undescribed species; another strain of *Eretmocerus* from Thailand currently is being described as a new species. No natural enemy cultures were obtained from Malaysia. The aphelinids collected were classified according to DNA banding patterns using RAPD-PCR techniques. The classification revealed an interesting diversity of patterns in both genera from Taiwan. We discuss some possible implications of the DNA patterns, as well as efforts to evaluate the parasitoids as biological control agents of the sweetpotato whitefly in south Texas and other parts of the US.

RESUMEN

Se especula que la mosca blanca del camote *Bemisia tabaci* (Gennadius)(Biotipo 'B') (= B. *argentifolii*) pudo haberse originado en el oriente debido a la diversidad de especies de *Bemisia* endémicas, y que sus enemigos naturales efectivos se encuentren probablemente en esta región. Los parasitoides nativos del sureste de Asia también podrían adaptarse a áreas determinadas en el sur de Texas debido a las similitudes climáticas. De acuerdo a esto, se condujeron cuatro exploraciones separadas para buscar enemigos naturales de *B. tabaci* en Filipinas, Taiwán, Malasia, Tailandia e Indonesia por un período comprendido entre noviembre de 1993 a octubre de 1995. La colección resultó en la importación a los Estados Unidos de 22 variantes geográficas de los afelínidos *Encarsia* spp. y *Eretmocerus* spp., 2 coccinélidos, un sírfido no identificado y 3 especies de hongos entomopatógenos. Una de las variantes de *Eretmocerus* proveniente de Taiwán podría representar una especie no descrita; otra variante de *Eretmocerus* proveniente de Tailandia está actualmente siendo descrita como una especie nueva. Ningún cultivo de enemigos naturales se obtuvo de Malasia. Los afelínidos colectados se clasificaron de acuerdo a los patrones de bandeo del DNA usando técnicas de RAPD-PCR. La clasificación reveló una diversidad interesante de patrones en ambos géneros en Taiwán. Se discuten algunas posibles implicaciones de los patrones del DNA, también como los esfuerzos para evaluar a los parasitoides como agentes de control biológico de la mosca blanca del camote en el sur de Texas y otras partes de los Estados Unidos.

The sweetpotato whitefly (SPWF), *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) is an extremely destructive pest of field, ornamental, and greenhouse crops throughout the world because of a combination of its wide geographical range (Cock, 1986; 1993), high fecundity, short life-cycle (Baumgartner and Yano, 1990), broad host range (Cock, 1986; Byrne et al., 1990), ability to serve as a vector for plant viruses (Brown et al., 1992), production of exudate which promotes growth of sooty mold and resurgence and resistance to conventional insecticides (Dittrich et al., 1990). Taxonomic uncertainty regarding *B. tabaci* and the recently described *B. argentifolii* Bellows and Perring (Bellows et al., 1994) makes it difficult to assess the global distribution of the whitefly as well as the relevance and accuracy of the literature purported to be on *B. tabaci* (see Brown et al., 1995). As a result, the geographical origin of *B. argentifolii* (= *B. tabaci* biotype "B") has been a matter of debate among taxonomists. Lopez-Avila (1986) (citing Mound 1965a,b; 1983) suggests that *B. tabaci* originated in the orient because of the presence of related indigenous species, and that human action resulted

Table 1. Insect natural enemies collected in Southeast Asia. Collections are tabulated by species identification, assigned identification number, DNA banding pattern, collection site, names of collector(s), date of collection, and original host plant

Species	ID	DNA	Collection site	Collectors[1]	Date	Host plant
En. transvena [2]	M94014	EN-11	Benguet, Philippines	L, C, P	3-94	white potato
En. transvena	M95073		Los Baños, Philippines	L	6-95	cassava
Syrphidae	M94015		Benguet, Philippines	L, C, P	3-94	*Alcea rosea*
En. transvena	M94016	EN-11	Shanhua, Taiwan	L, C, P	3-94	tomato
En. transvena	M94017	EN-3	Shanhua, Taiwan	L, C, P	3-94	poinsettia
En. transvena	M94019	EN-4	Shanhua, Taiwan	L, C, P	3-94	soybean
Eretmocerus sp. nov. (?)	M93055	ERET-3	Tainan, Taiwan	M	12-93	poinsettia
Eretmocerus sp.	M93058	ERET-1	Tainan, Taiwan	M	12-93	tomato
Eretmocerus sp.	M94020	ERET-3	Shanhua, Taiwan	L, C, P	3-94	soybean, tomato
Eretmocerus sp.	M95097	ERET-3, 11	Tainan, Taiwan	J, T	10-95	tomato
Er. nr. *furuhashii*	M95098	ERET-11	Tainan, Taiwan	J, T	10-95	tomato
Eretmocerus sp.	M94022		Tainan, Taiwan	L, C, P	3-94	soybean
Cheilomenes sexmaculata[3]	M94018		Shanhua, Taiwan	L, C, P	3-94	eggplant
Illeis koebelei[3]	M94021		Shanhua, Taiwan	L, C, P	3-94	soybean
En. transvena	M95049		Hua Hin, Thailand	L, C	5-95	lantana
En. transvena	M95051		Hua Hin, Thailand	L, C	5-95	unknown
En. transvena	M95053		Hua Hin, Thailand	L, C	5-95	cassava
En. transvena	M95055		Hua Hin, Thailand	L, C	5-95	unknown
En. transvena	M95059		Krabi, Thailand	L, C	5-95	cassava
Eretmocerus sp. nov.	M95054	ERET-3	Him Mui, Thailand	L, C	5-95	unknown
Encarsia sp.[4]	M95074		Medan, Indonesia	L, C, K	5-95	jasmine
En. nr. *strenua*	M95061		Cirebon, Indonesia	L, C, K	5-95	pumpkin
En. nr. *strenua*	M95063		Magelang, Indonesia	L, C, K	5-95	pumpkin
Encarsia sp.	M95070		Medan, Indonesia	L, C, K	5-95	jasmine
Encarsia sp.	M95075		Magelang, Indonesia	L, C, K	5-95	pumpkin

[1]Collectors' identifications: L = Jesusa C. Legaspi; C = Raymond I. Carruthers; P = Tadeusz J. Poprawski; M = Charles Moomaw; K = Alan A. Kirk; J = Walker A. Jones; T = Narayan S. Talekar

[2] Collected from host insect *Trialeurodes* (Homoptera: Aleyrodidae) spp.

[3] Coccinellidae

[4] Collected from unknown species of whitefly host

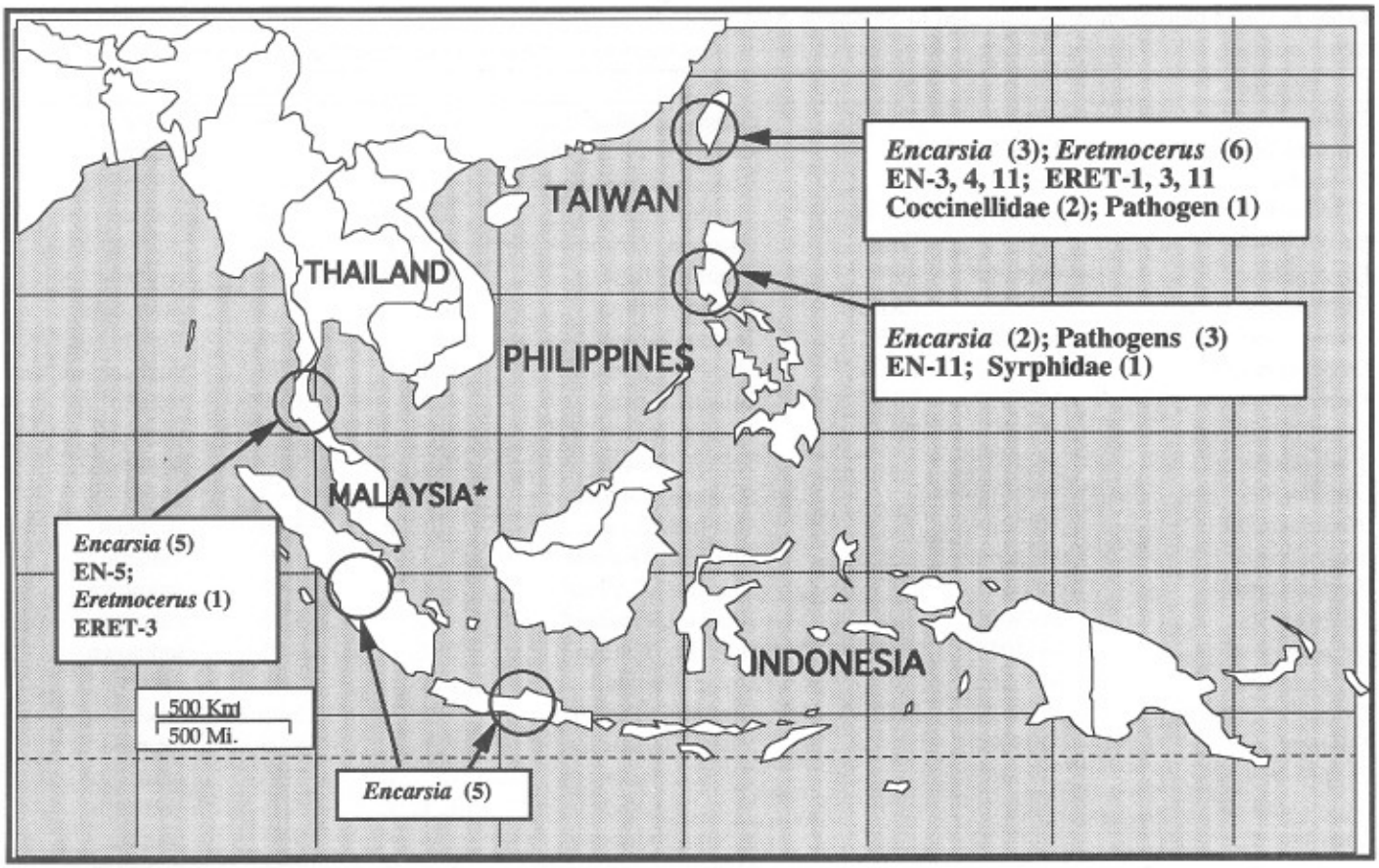
in subsequent dispersal to India, America, and Africa. Greathead and Bennett (1981) (using the data of Mound and Halsey, 1978) found greatest diversity of *Bemisia* species, excluding *tabaci*, in the Palearctic and adjoining oriental regions. They argue that these regions constitute the center of diversity for the genus *Bemisia* and areas where effective natural enemies are likely to be found. Furthermore, parasitoids native to southeast Asia may be adapted to intended areas of introduction in south Texas, because of climatic similarities. Accordingly, four separate explorations for natural enemies of *B. tabaci* were conducted in the Philippines, Taiwan, Malaysia, Thailand, and Indonesia over a period from November, 1993 to October, 1995. We cite the natural enemies imported into the US against the whitefly which resulted from these explorations.

MATERIALS AND METHODS

Collections site and dates. Four trips were made to southeast Asian countries, over a period from November 1993 - October 1995: 1) November - December 1993: Philippines and Taiwan; 2) March 1994: Philippines and Taiwan; 3) May - June 1995: Philippines, Malaysia, Thailand and Indonesia; 4) September - October 1995: Taiwan.

In the first trip to Taiwan and the Philippines, collections were made from 4 Taiwanese provinces: Tao-Yuan (north; ~24° 50'N, 121° 30' E), Tai-Chung (central; ~24° 10' N, 120° 50' E), Tainan (south; ~22° 50' N, 120° 30' E) and Ping-Tung (south; ~22° 45' N, 120° 35' E). Host plants were predominantly greenhouse poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) and field eggplant (*Solanum melongena* L.), although some collections were also made from field tomato (*Lycopersicon esculentum* Miller) and soybean (*Glycine max* (L.) Merr.), as well as greenhouse soybean. In the Philippines, collections were taken from the provinces of Cavite (~14°28' N, 120° 50' E) and Quezon (~14° 25' N, 121° 30' E), situated in the southern parts of island of Luzon.

In the second series of collections from the Philippines parasites of *B. tabaci* were collected from cassava (*Manihot esculenta* Crantz) in the towns of Puypoy, Bay and Los Baños in the province of Laguna (~14° 10' N, 121° 15' E). Collections were also made in the provinces of Pangasinan (~16°N, 120° 25' E), Tarlac (~15° 40' N, 120° 30' E) and Benguet (~15° 30' N, 120° 45' E). The climate in Pangasinan and Tarlac is usually hot and dry in March (mean temperature ≈27° C). Benguet is often cooler and more humid (mean tem-



* Collections made, but no natural enemies were obtained

Fig. 1. Map of natural enemies collected in Southeast Asia. Where appropriate, DNA banding pattern is indicated.

perature ≈21° C) during the day because of its high elevation (~1800 m.).

In the third trip, a single collection was made from the Philippines (Los Baños, Laguna). A single collection was also made from Kuala Lumpur, Malaysia (~30° N, 102° E) on cassava. Thirteen collections were made in Indonesia (~7° S, 107° E), mostly in Cirebon, Magelang, central and western Java, and northern Sumatra. Whitefly parasites were collected on pumpkin (*Cucurbita pepo* L.), cassava, cucumber (*Cucumis sativus* L.), and jasmine (*Jasminum* sp. L).

The fourth trip consisted of more collections from tomato host plants taken in Tainan, Taiwan.

Collection protocol. Collections for parasites were made following the methods described by Rose and DeBach (1990). Immature whiteflies, especially parasitized individuals, were excised from leaves in groups of ≈5-10 whiteflies. The leaf cuttings were then placed into 0.25 dram glass vials. A thin streak of honey was applied to the inside of each vial using a camel hair brush. The vials were sealed with cotton and placed into a plastic container containing individual cells, similar to those used to hold bullet cartridges. Leaves with very high whitefly densities were placed in paper or plastic containers and separated using thin tissue. The samples were sorted by location, host plant and genera. Predators were kept individually in vials with leaves infested with whiteflies. Collections for entomopathogens were made only in the Philippines and Taiwan. To collect entomophathogens, host cadavers were transferred individually using sterile techniques onto Sabouraud dextrose agar, supplemented with 1% yeast extract (SDAY) and 0.1% of the antibiotic, gentiacin.

All samples collected were placed in homemade humidity controlled packages together with detailed collection notes and then transported to the Beneficial Insects Research Unit, USDA-ARS Subtropical Agricultural Research Center, Weslaco, Texas or APHIS-MBCC.

Insect identifications. Voucher specimens of the parasites collected were sent to M. Rose (currently at Montana State Univ.), G. Zolnerowich, and J. Woolley (Texas A&M, College Station, TX) and to M. Shauff (USDA Systematics Lab.) for identification. A coccinellid was sent to N. van den Berg (USDA Systematics Lab.) for identification. Samples of the whiteflies collected were sent to J. Brown (U. Arizona) and R. Gill (Univ. California) for genetic, enzymatic, and morphological identification and comparison to populations found in the US.

RAPD-PCR DNA analysis. The parasites quarantined at MBCC were assayed for their randomly amplified polymorphic DNA (RAPD) patterns using the techniques described in Black et al. (1992) to assist in taxonomic determinations. Individual insects are assigned to DNA pattern types (Vacek et al., 1996, 1997). The DNA of individual insects was amplified in a GeneAmp™ PCR System 9600 thermal cycler with

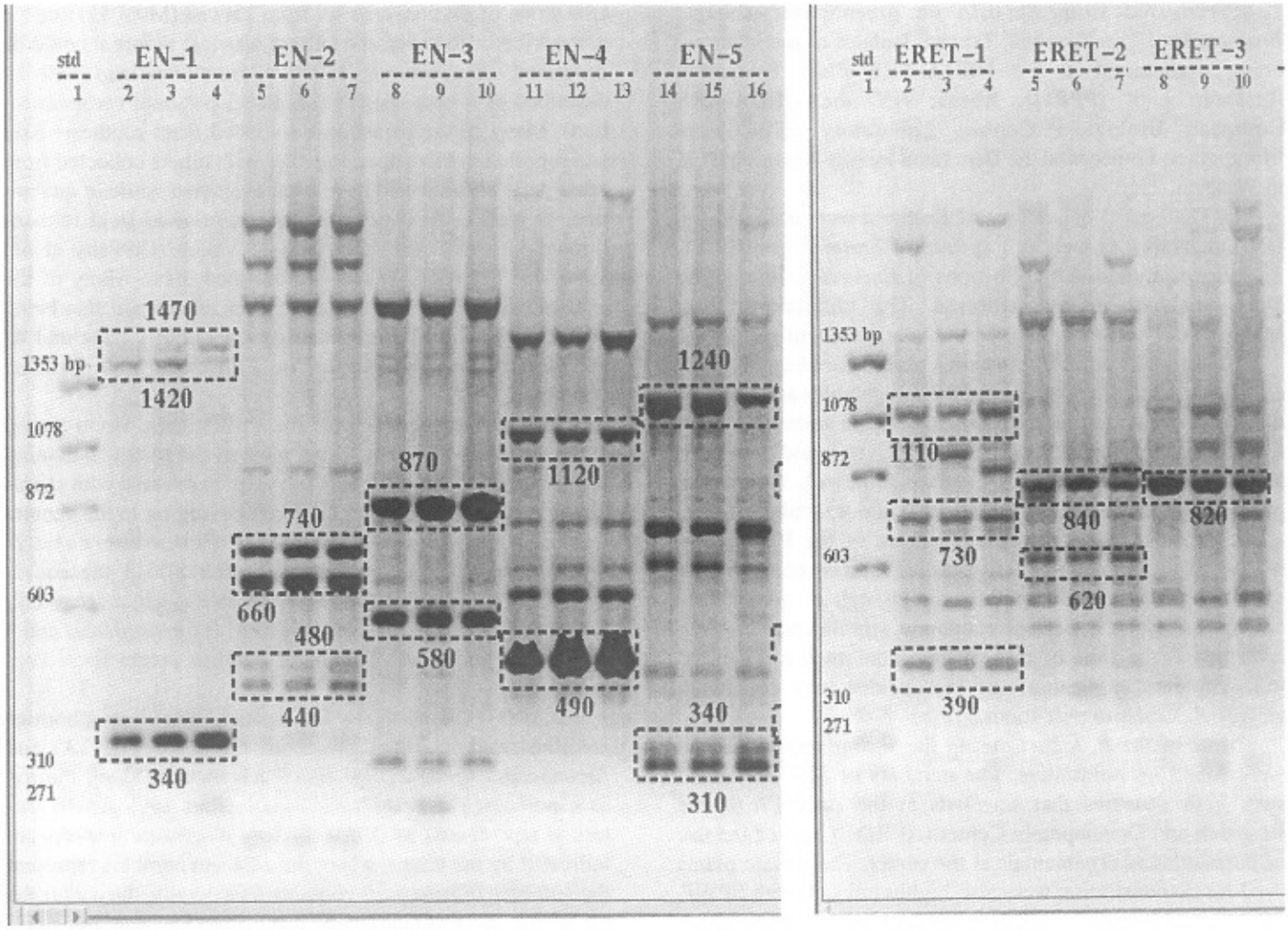


Fig. 2. Genetic fingerprints for selected *Encarsia* and *Eretmocerus* patterns. Each genetic pattern is represented by 3 insects, numbered across the top. Key diagnostic markers are indicated by the boxes, where the adjacent numbers represent the numbers of base pairs contained. The patterns EN-1 (India), EN-2 (Africa, Egypt, Greece, Thailand, USA), and ERET-2 (India) were obtained from studies independent to those described in this paper. The banding patterns for EN-11 showed key markers for 1450, 505, and 350 base pairs (figure not shown); and the pattern for ERET-11 showed key markers at 1250, 1110, 870, and 800 base pairs (figure not shown) (see text for details).

AmpliTaq® DNA polymerase. Insect DNA preparation, specific DNA primers, PCR reaction mixtures, thermal cycler program, and electrophoresis followed those of Black et al. (1992), who identified discrete genetic markers in aphids and 2 micro-hymenopterans. The following primers were synthesized by Operon Technologies Inc. for use in this study: C04: 5'-CCGCATCTAC-3', C01: 5'-TTCGAGCCAG-3', BAM: 5'-ATGGATCCGC-3', and ECO: 5'-ATGAATTCGC-3'. The primers bind randomly to sections of insect DNA which are amplified and separated by electrophoresis according to numbers of base pairs amplified. In conjunction with classical taxonomic methods, these genetic "fingerprints" are used as key diagnostic tools for identification and classification of insects.

RESULTS AND DISCUSSION

Collection results. The collections from southeast Asia resulted in 22 geographic strains of parasites belonging to the genera *Encarsia* or *Eretmocerus*, 2 coccinellids, 1 unidentified syrphid and 3 entomopathogenic fungi (Table 1 and Fig. 1). In Table 1, collections are tabulated by species identification, identification number, DNA banding pattern, collection site, names of collector(s), date of collection, and original host plant. Taiwan yielded 3 collections of *Encarsia*, representing 3 DNA banding patterns (EN-3, 4, 11), 6 collections of *Eretmocerus*, representing 3 banding patterns (ERET-1, 3, 11), and 2 predatory coccinellids, *Cheilomenes sexmaculata* (F.) and *Illeis koebelei* Timberlake obtained from eggplant and soybean, respectively. In the Philippines, 2 collections of *Encarsia* were obtained, 1 possessed EN-11 pattern and no DNA analysis was performed on the other. In addition, an unidentified syrphid was collected, as well as the entomopathogenic fungi *Paecilomyces fumosoroseus* (Wize) Brown & Smith, *Fusarium coccophilum* (Desmazieres) Wollenweber and Reinking, and *Beauveria bassiana* (Balsamo) Vuillemin (Fig. 1). An isolate of *Paecilomyces* was

also recovered from *Bemisia* on greenhouse cabbage, *Brassica* sp. L., in Shanhua, Taiwan. Isolates of the latter are being maintained at the USDA-ARS Plant Protection Research Unit (PPRU), Ithaca, NY, the USDA-ARS European Biological Control Laboratory (EBCL) in Montpellier, France, and the Beneficial Insects Research Unit, in Weslaco, TX.

In Thailand, 5 collections of *Encarsia* were obtained (no DNA analyses), as well as 1 species of *Eretmocerus* (ÉRET-3). Indonesia yielded 5 collections of *Encarsia*, although no DNA analyses were performed. The collections from Malaysia did not result in any insect natural enemies.

Sweetpotato whitefly was not a pest in Taiwan at the time of the first visit, except in greenhouse vegetables and poinsettia. The dominant whitefly problem was the spiraling whitefly, *Aleurodicus dispersus* Russell, which is found on a variety of hosts including guava (*Psidium guajava* L.), plumaria, poinsettia, papaya (*Carica papaya* L.) and a number of vegetable crops. A similar situation existed in the Philippines, where the sweetpotato whitefly was less of a problem, but the spiraling whitefly was ubiquitous. Although *B. tabaci* does not appear to be a pest of economic significance in these countries at the time of this writing, continued routine and indiscriminate applications of insecticides may eventually elevate *B. tabaci* to pest status.

Most of the *B. tabaci* during the second trip to Taiwan were found on poinsettias. The numbers of SPWF attained such high densities that scientists at the Asian Vegetable Research and Development Center (AVRDC) banned the use of poinsettias as ornamentals at the center. The tomato plants used for demonstration were also highly infested with SPWF. Many of the commercial fields were sprayed with insecticides although some field crops such as soybean were found to have parasitized whiteflies. Most of the plant hosts with parasitized whiteflies came from plants in the greenhouses that were not sprayed with insecticides. According to researchers at Taiwan Agricultural Research Institute, *B. tabaci* attacks at least 16 known host plants in Taiwan: florist's chrysanthemum (*Chrysanthemum morifolium* Ramat.), poinsettia, sunflower *(Helianthus annuus* L.), gerbera (*Gerbera jamesonii*), sweet potato (*Ipomoea batatas* (L.) Lam.), Indian lettuce (*Lactuca indica* L.), *Leonurus sibiricus*, eggplant, *Ludwigia octovalvis*, white mulberry (*Morus alba* L.), guava, *Pueraria lobata* (Willd.) Ohwi (Kudzu), an herb (*Rhinacanthus nasutus*), Cuba jute (*Sida rhombifolia* L.), *Solanum capsicastrum*, and *Tithonia diversifolia*.

In the Philippines, parasite larvae were observed in *B. tabaci* collected in Quezon on eggplant, sweet potato and non-cultivated squash (*Cucurbita* spp.). Whitefly were collected in Cavite from sweet potato and cultivated squash, but no parasites were observed. Collecting was hampered because of excessive rainfall during typhoon season in the Philippines. The cassava plants are usually not treated with insecticides and were grown mainly for subsistence rather than commercial production. Commercial plots which were sprayed were not good collection sites. The collections yielded 2 cultures which may represent undescribed species of *Eretmocerus*. One strain of *Eretmocerus* sp. from Taiwan (M93055) may be undescribed. However, the culture was lost before it could be described. *Eretmocerus* M95054 from Thailand will be described as a new species (M. Rose, personal communication). Many of the parasitoids collected from southeast Asia and reported in this paper, together with others collected from other parts of the world have been evaluated while in quarantine, as well as in cage experiments, prior to field releases throughout the Lower Rio Grande Valley (Goolsby et al., 1996, 1997; J.A.G., W.A.J. unpublished data). Many of the exotics which have been permitted for release are also being evaluated by the Mission Biological Control Center and its cooperators in field cages in California and Arizona (Hoelmer, 1997).

Entomopathogens from the Philippines. Upon arrival at the BIRU in Weslaco, Texas, most of the plates containing the pathogen collections were found to be covered with saprophytic and/or phytopathogenic fungi belonging to the genera: *Cladosporium, Fusarium, Aspergillus, Penicillium, Fumago, Scorias* and *Capnodium*. However, after 3 to 5 subsequent transfers onto fresh SDAY, we were able to obtain pure cultures of 16 isolates of *B. bassiana*, 5 of *P. fumosoroseus* and 1 of *F. coccophilum*, all from adult *Bemisia* except for *F. coccophilum*.

RAPD-PCR analysis. Examples of the electrophoreses are illustrated in Fig. 2, for both *Encarsia* (Fig. 2A) and *Eretmocerus* species (Fig. 2B). Each number along the top axis indicates a separate insect sample; thus, each genetic pattern is represented by 3 insects. Key diagnostic markers are indicated by the boxes, where the adjacent numbers represent the numbers of base pairs contained. Generally, the darker the mark, the more reliable the fingerprint. The patterns EN-1 (India), EN-2 (Africa, Egypt, Greece, Thailand, USA), and ERET-2 (India) were obtained from studies independent to those described in this paper. The banding patterns for EN-11 showed key markers for 1450, 505, and 350 base pairs (figure not shown); and the pattern for ERET-11 showed key markers at 1250, 1110, 870, and 800 base pairs (figure not shown).

When the collections reported in this paper are pooled with those of other workers, RAPD-PCR genetic analyses revealed an interesting abundance of distinct DNA banding patterns in Taiwan. Only 1 pattern for *Encarsia* (EN-11) was found in the Philippines, whereas 4 each for *Encarsia* (EN-3, 4, 5, 11) and *Eretmocerus* (ERET-1, 3, 9, 11) were found in Taiwan. The *Encarsia* pattern found in the Philippines was found also only in Taiwan. *Encarsia* banding patterns EN-3 and EN-4 are currently unique to Taiwan. The *Eretmocerus* patterns ERET-9 and ERET-11 are currently unique to Taiwan, and ERET-3 has also been found in Thailand. In contrast, the pattern ERET-1 is widely distributed, having also been found in populations collected from: Egypt, India, Spain, Italy, and Israel. It is interesting to speculate whether the diversity of genetic patterns discovered in a given country or geographical area simply reflects greater collection efforts relative to other localities or suggests an underlying evolutionary diversification.

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