A Survey of Fruit Flies (Diptera: Tephritidae: Dacinae) and their Opiine Parasitoids (Hymenoptera: Braconidae) in Palau

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Abstract. Surveys for fruit flies and their parasitoids, conducted by male lure trapping and host fruit sampling in 2001, 2013, and 2014, demonstrate that the agricultural pests *Bactrocera dorsalis*, *B. frauenfeldi*, and *B. umbrosa* and non-economic *B. calophylli* (Diptera: Tephritidae: Dacinae) are present and widespread in Palau. The *COI* gene haplotype networks and aedeagus measurements of *B. dorsalis*, detected in Palau in 1996, suggest that it is most likely of Philippine origin. *Bactrocera occipitalis*, previously reported from Palau, was not collected during these surveys, and is probably absent. *Fopius arisanus* (Hymenoptera: Braconidae: Opiinae) was reared from fruit containing parasitized larval fruit flies. Parasitism was low (3.4-11.7%), compared to Hawaii or French Polynesia, where *F. arisanus* has lowered populations of *B. dorsalis*.

Fruit production in the Republic of Palau is affected by native and invasive pest fruit flies (Diptera: Tephritidae: Dacinae). This Pacific Island nation comprises 343 islands (488 km^2), located 900 km east of the Philippines. The largest island is 396 km^2 Babeldaob, but most of the population is concentrated on Koror Island. Hardy and Adachi (1956) listed the species of *Bactrocera* on Palau as *B. frauenfeldi* (Schiner), a generalist pest of fruit, *B. umbrosa* (Fabricius), a pest of breadfruit and jackfruit, and *B. calophylli* (Perkins & May), a non-economic species breeding on Indian laurel (*Calophyllum inophyllum* L.). Limited trapping and host fruit surveying in 1988-90 confirmed the presence of the first two species (Allwood et al. 1999).

The presence of a suspected new immigrant pest species, causing significant damage on starfruit (*Averrhoa carambola* L.), was confirmed in September 1996, and the fly was identified as *Bactrocera dorsalis* (Hendel) by R.A.I. Drew (Leblanc 1997). In response, methyl eugenol-baited detection traps were deployed in 1999–2000, and *B. dorsalis* was found to be widespread over all the main islands (Kayangel, Babeldaob, Koror, the Rock Islands, Peleliu, and Angaur). In technical feasibility and socioeconomic study reports (Allwood et al. 1999, McGregor 2000), it was concluded that *B. dorsalis* and *B. umbrosa* could be eradicated by using the male annihilation technique (MAT) and protein bait spraying at a cost of USD 1.2 million, but the planned eradication program did not occur. Based on the examination of specimens obtained from infested fruits, Drew determined in early 2001 that the invasive species in Palau were actually *B. philippinensis* Drew & Hancock and *B. occipitalis* (Bezzi), both members of the *B. dorsalis* species complex, native to and common in the Philippines (SPC 2001). A decade later, Drew re-examined specimens bred from fruit samples in 2001 and declared that *B. philippinensis*, which was to become a synonym of *B. papayae* (Drew and Romig 2013), was the only *B. dorsalis* complex species present (Leblanc et al. 2012). More recently, *B. papayae* was found to be conspecific with and declared junior synonym of *B. dorsalis* (Schutze et al. 2015).

In a preliminary host fruit survey carried out in 2001, fruit flies and parasitoids tentatively identified as *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae: Opiinae), were recovered from samples of starfruit, common guava (*Psidium guajava* L.) and several species of *Syzygium* apples. *Fopius arisanus* is native to South and East Asia and was introduced to Hawaii, in 1948, to control recently introduced *B. dorsalis*, resulting in a dramatic drop in fly populations (Vargas et al. 2012a). From Hawaii, *F. arisanus* was subsequently introduced to several other Pacific Island nations, Australia, Central America and Africa (Vargas et al. 2012a), though no record exists of its introduction to Palau. More recently, it was introduced and became established in French Polynesia, resulting in a drastic reduction of *B. dorsalis* populations, after a failed *B. dorsalis* eradication attempt by using MAT (Vargas et al. 2012b, Leblanc et al. 2013b).

The objectives of this study were to: (1) Determine the origin of the *B. dorsalis* invasion in Palau; (2) Determine whether *B. occipitalis* is present in Palau; (3) Gather preliminary data on the comparative densities of the pest species across Babeldaob and Koror islands; (4) Establish baseline data on fruit fly infestation and parasitism rates on the most common fruit fly host fruits in Palau; and (5) Confirm the presence of *F. arisanus* in Palau. To achieve those objectives adult and larval survey was conducted.

Materials and Methods

Traps baited with male lures were established in December 2013 in residential and village areas on Koror and Babeldaob Islands as long-term trapping stations to monitor populations and detect invasions of new species. Thirty-five sites included methyl eugenol-baited traps; cue-lure-baited traps were maintained at a subset of 10 of these sites. Multi-Lure traps (Better World Manufacturing, Fresno, CA) were used, with a plug containing 2 g of male lure (Scentry Biologicals, Billings, MT) inserted in the receptacle built in the trap roof, and a 25 x 90-mm strip containing 10% dichlorvos (Vaportape, Hercon Environmentals, Emingsville, PA) was placed inside the trap to kill flies. Trapping data presented in this paper are based on the first sample collection, three days following the establishment of the trapping sites.

A one-week snap-shot survey with temporary traps was also carried out in December 2014, on Koror and Babeldaob, to collect and preserve flies for DNA sequencing, in order to determine the probable origin of *B. dorsalis* and investigate whether *B. occipitalis* is absent from Palau. Pairs of temporary traps, baited with each of the two male lures, were maintained for 3–5 days at ten trapping sites. Traps were made of 5-oz urine sample cups, with the male lure plug and a 10 x 10-mm piece of dichlorvos strip attached to the trap's inner ceiling through a hook made of tie wire (Fig. 1). A solution of 25% propylene glycol (Better World

Figure 1. Male lure trap crafted from urine sample cup used in the 2014 survey.

Manufacturing, Fresno, CA) was used to preserve captured flies, which were transferred to 95% ethanol and kept in a freezer for DNA extraction. Number of trapped flies was adjusted to flies per trap per day, and results from the 2013 and 2014 trapping are presented on the same map for both *B. dorsalis* and *B. frauenfeldi*.

Samples of ripe fruits of potential host plants were collected in May 2001 (59 sites on Koror, Babeldaob, Peleliu, and the Rock Islands, yielding 135 samples and 4787 fruits, weighing 133 kg) and December 2014 (17 sites on Koror and Babeldaob, yielding 42 samples and 398 fruits, weighing 15 kg), covering 50 species in 33 families. Samples were incubated in plastic containers, covered with fine gauze fabric for ventilation, and lined with vermiculite at the bottom to allow larvae emerging from fruits to pupate. Fruits were either placed on petri dishes over the sawdust, or fruits likely to break down and release juice were placed on gauze fabric resting on chicken wire mesh over a plastic container, placed on the sawdust lining, to collect juice and prevent wetting the vermiculite. After one and two weeks of incubation, the vermiculite was sifted to collect pupae, and these were counted and placed in fresh vermiculite in emergence containers. Emerged flies and parasitoids were maintained alive for five days, fed with sugar and water, to ensure that adult colour patterns developed fully to allow identification, before being killed by freezing and preserved in 95% ethanol.

All insects collected in traps and emerged from fruit samples during the 2014 survey were individually examined under a dissecting microscope, to identify them to species level and select typical and unusual specimens for DNA sequencing.

To confirm species identity of flies and determine the probable origin of the *B. dorsalis* invasion, DNA was extracted from 49 specimens of *B. dorsalis* from three populations (23 from Palau, 7 from the Philippines and 19 from Taiwan), and a 780 bp section of the mitochondrial *COI* gene was sequenced, following the procedure described by San Jose *et al.* (2013). We selected typical specimens, but also variants with more extensive dark markings on their abdomens, to compare their *COI* sequences with those from *B. dorsalis* collected in the Philippines (Luzon: Batangas) and Taiwan (12 different localities). The *COI* dataset was used to reconstruct a haplotype network using statistical parsimony (Templeton et al. 1992), as implemented in TCS version 1.21 (Clement et al. 2000), in order to visualize genetic variation. To further confirm the most likely origin of *B. dorsalis*, male aedeagi were dissected, cleared in a 25% potassium hydroxide overnight, mounted on a slide with a 1-mm grid (Fig. 3a) and measured, for 20 specimens collected in methyl eugenol traps at each of three populations (Palau: Koror Island; Philippines: Cebu Island, Moalboal; Taiwan: Lanyu Island). Aedeagus length has been documented to vary geographically and was used to help distinguish species now synonymous with *B. dorsalis* (Iwahashi 2000, Drew et al. 2008, Krosch et al. 2013).

The DNA was extracted also from parasitoids bred from fruit samples in Palau following the method described in San Jose et al. (2013). We used the primers LCO-1490 and HCO-2198 from Folmer et al. (1994) to PCR and sequence the barcode region (651 bp) of the *COI* gene. The sequences were compared to those from specimens of *Fopius arisanus*, *Diachasmimorpha longicaudata* (Ashmead) and *Psyttalia fletcheri* (Silvestri) (Hymenoptera: Braconidae: Opiinae), reared on *Bactrocera* fruit flies in laboratory colonies maintained in Hawaii. We also obtained a sequence of *F. arisanus* from Australia from Genbank (KC857548). Maximum Likelihood analysis was implemented using the RaxML web-server (Stakamakis 2008) and visualized using FigTree v1.4.0 (Rambaut 2012).

All specimens are deposited as vouchers in the University of Hawaii Insect Museum and all genetic sequences are available on GenBank (See Appendix for details).

Results and Discussion

Results from the 2013 and 2014 snapshot trapping were consistent, and similar to trapping data from 1999–2000 (F. Sengebau, unpublished). *Bactrocera dorsalis* was abundant on Koror, where large concentrations of host fruit trees are found in backyards, in addition to littoral host trees, such as tropical almond (*Terminalia catappa* L.) and *C. inophyllum* (Fig. 2a). It was much less common on Babeldaob, likely because the density of settlements, and their associated backyard host fruit trees, is very low, and agriculture is mostly limited to small family plots. *Bactrocera frauenfeldi*, on the other hand, was less common and more uniformly distributed over the territory (Fig. 2b). *Bactrocera umbrosa*, a breadfruit specialist, was collected in small numbers (< 50 in 2013 and 21 specimens in 2014) in localized areas, consistent with the observed scarcity of breadfruit trees on Koror and Babeldaob.

The known distribution in Palau of the four fruit fly species, based on trapping data, emergence from fruit surveys, literature records and label data from museum specimens, is summarized in Table 1.

Fruit flies were recovered from 17 species of hosts (Leblanc et al. 2012, 2013a). Among the hosts sampled in largest numbers (Table 2), *B. dorsalis* was mostly bred from starfruit, kalamansi (*Citrus x microcarpa* (Bunge) Wijnands), and common guava (*Psidium guajava* L.), whereas *B. frauenfeldi* was bred mostly from guava, *Syzygium* apples, and tropical almond. Among the other hosts, of which fewer samples were collected but from which flies were recovered, *B. dorsalis* emerged from samples of Indian laurel, sour orange (*Citrus aurantium* L.), tangerine (*C. reticulata* Blanco), ripe banana (*Musa* sp.), giant granadilla (*Passiflora quadrangularis* L.), and jujube (*Ziziphus jujuba* Mill.). *Bactrocera frauenfeldi* was also reared from Tahitian chestnut (*Inocarpus fagifer* (Parkinson) Fosberg), *Ochrosia oppositifolia* (Lam.) K.Schum., and jujube. Additionally, two specimens of *B. calophylli* emerged from ripe Indian laurel; the first record of this species, not attracted to male lures, in Palau since 1954.

All the *B. dorsalis*-like specimens collected by trapping (3,884 specimens) and bred from fruit samples (464 specimens) in December 2014 were consistent with *B. dorsalis* in appearance. None even

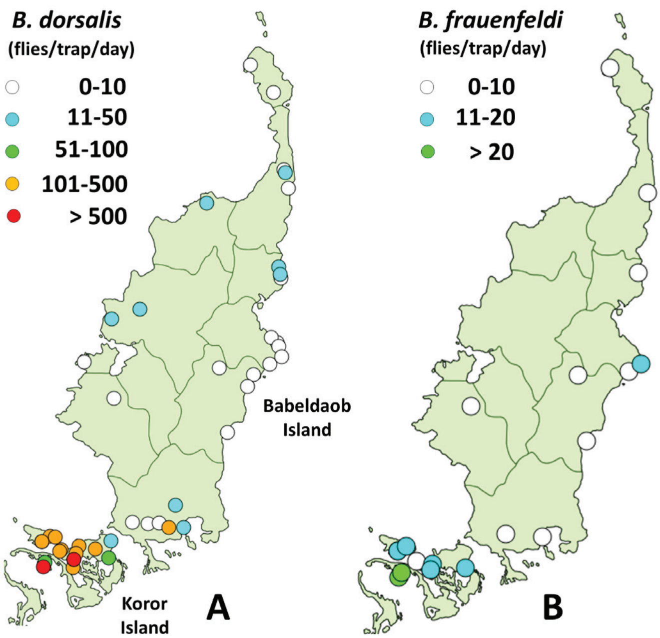

Figure 2a–b. Number of fruit flies collected during three days of male lure trapping in December 2013 and December 2014 on Koror and Babeldaob Islands, Palau. MultiLure traps were used in 2013 and traps crafted from urine sample cups (Fig. 1) were used in 2014.

Table 1. Species of fruit flies present in Palau and their confirmed distribution records.

Species:	*B. dorsalis*	*B. frauenfeldi*	*B. umbrosa*	*B. calophylli*
Lure:	Methyl eugenol	Cue-lure	Methyl eugenol	No lure
Hosts[1]:	40 spp. fruits	73 spp. fruits	Breadfruit, jackfruit	*Calophyllum inophyllum*
Location				
Kayangel	Present	Present	Present	Not confirmed
Babeldaob	Present	Present	Present	Present[2]
Koror	Present	Present	Present	Not confirmed
Rock Islands	Present	Probably[3]	Probably[3]	Not confirmed
Peleliu	Present	Present	Present	Not confirmed
Angaur	Present	Probably[3]	Probably[3]	1954 collection record[4]
Southwest Islands	?	Pulo Anna[4]	?	?

[1]Number of hosts recorded in the Pacific Islands region, as published in Leblanc et al. (2013a)
[2]Presence confirmed by host fruit survey in 2014
[3]Probably present: no official record of presence, but widespread species
[4]Probably widespread; record from Hardy and Adachi (1956)

Table 2. Emergence of fruit flies (*Bactrocera dorsalis* and *B. frauenfeldi*) and parasitoids (*Fopius arisanus*) from field-collected samples of ripe host fruits in May 2001 and December 2014.

Host	Survey year	No. samples	No. fruits	Weight (kg)	*B. dorsalis* per kg	*B. frauenfeldi* per kg	*F. arisanus* per kg	Parasitism %
Averrhoa carambola	2001	4	108	4.0	141.8	5.2	7.5	4.8
	2014	6	67	2.7	127.1	0	4.4	3.4
Carica papaya	2001	12	25	9.6	0.4	0	0	0
	2014	2	5	1.1	0	0	0	0
Citrus microcarpa	2001	7	237	5.0	6.7	3.0	0	0
	2014	1	34	0.7	10.4	0	0	0
Psidium guajava	2001	3	33	4.5	27.2	134.1	9.7	5.7
	2014	4	16	1.8	7.8	0	0.6	6.7
Syzygium aqueum	2001	4	126	1.1	0	220.7	29.1	11.7
	2014	2	40	0.4	0	0	0	0
Syzygium malaccense	2001	11	279	10.6	0	100.7	0	0
	2014	None	-	-	-	-	-	-
Syzygium samarangense	2001	3	118	5.0	0	206.0	0	0
	2014	3	54	2.2	6.9	7.8	0	0
Terminalia catappa	2001	8	107	3.1	0	448.7	0	0
	2014	4	29	1.0	6	6	0	0

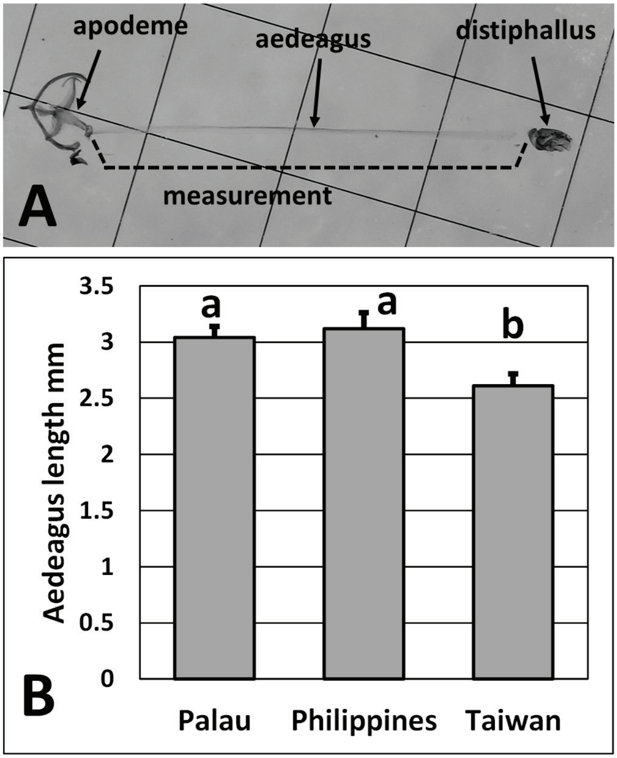

Figure 3a–b. Length of aedeagus (a) for populations of *Bactrocera dorsalis* from Palau, the Philippines and Taiwan (b). Bars labelled by the same letter on the graph are not significantly different at the $P < 0.05$ level; $F_{2,57} = 108.95$, $P < 0.001$; n = 20 measurements for each population.

remotely or superficially resembled *B. occipitalis*. These two species are easily discernible based on abdomen coloration and the extent of wing infuscations (Drew and Romig 2013), and are genetically distinct. Although only *B. dorsalis* was observed in samples, the possibility that *B. occipitalis*, if it ever occurred in Palau, may subsist as very small populations cannot be entirely ruled out.

Results from DNA sequencing and the haplotype network (Fig. 4) support the Philippines as the possible source of *B. dorsalis* in Palau. Palau shares haplotypes with the Philippines whereas samples from Taiwan, although very diverse genetically, cluster in a separate part of the network. A future study will include additional samples of *B. dorsalis* from numerous world localities, where *B. dorsalis* is native and invasive, and results will help pin point the sources of *B. dorsalis* invasions throughout the world.

Aedeagus measurements confirm the probable Philippine origin of the *B. dorsalis* populations in Palau. Aedeagi dissected from flies from these two countries were of equal length (3.04 mm for Palau and 3.12 mm for Philippines), and significantly longer than aedeagi from the Taiwan samples (2.61 mm) (Fig. 3b). They were also comparable to measurements published

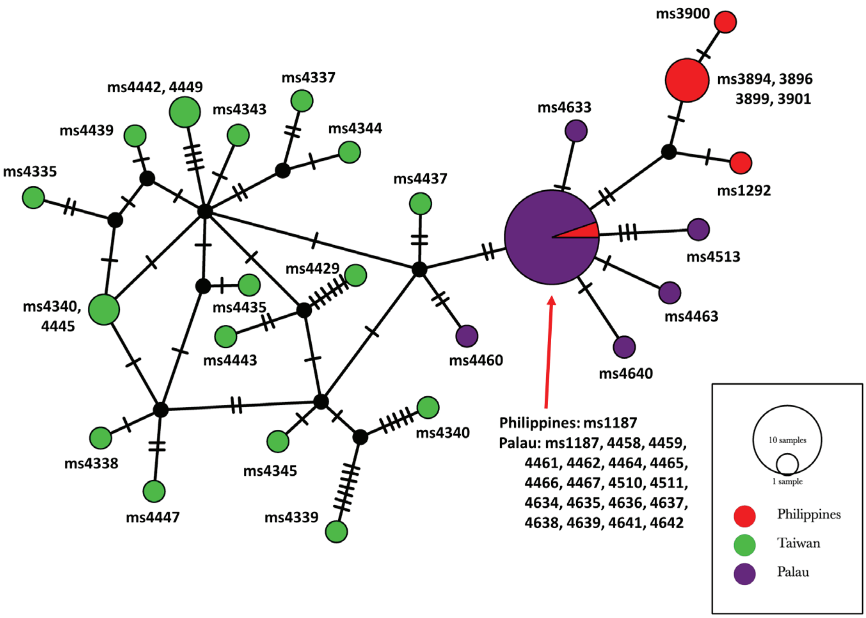

Figure 4. Statistical parsimony haplotype network based on *COI* (780 bp) data for 48 specimens of *B. dorsalis* collected in Palau, the Philippines and Taiwan. Circles represent the 25 unique haplotypes and their sizes are proportional to the number of specimens with those haplotypes. Black dots and bars represent missing intermediate haplotypes (mutational steps). Voucher numbers are indicated in each circle.

by Iwahashi (2000) (2.61 and 3.09 mm for Taiwan and Philippines populations, respectively).

Parasitoids were reared out of the fruit samples collected in 2001 and 2014 (Table 2). The tentative original *F. arisanus* determination was confirmed based on an exact match with sequences of the *COI* gene (barcode section) of the Palau specimens with those from specimens kept in laboratory colonies in Hawaii, based on Maximum Likelihood analysis (Fig. 5).

Historical records of *F. arisanus* introductions to Palau are lacking. Its presence was first reported by F. Sengebau, who bred the wasps from infested starfruit in August 2000. Scanty evidence suggests that *F. arisanus* may have been introduced in Palau and was breeding on *B. frauenfeldi* during the time the nation was part of the Trust Territory in the Pacific, long before the *B. dorsalis* invasion. Allwood et al. (2001) reported examining two specimens of *Opius oophilus* (= *F. arisanus*) in the Belau National Museum insect collection, dating from April 1969, with D.L. Moody as collector. *Bactrocera frauenfeldi* is a suitable host for *F. arisanus*, as demonstrated by its introduction and recovery from field-collected fruit on Pohnpei Island in 1997 (L. Leblanc, unpublished). Additionally, *B. frauenfeldi* was also demonstrated to be a suitable host in the laboratory in Pohnpei (L. Leblanc, unpublished).

Levels of parasitism by *F. arisanus* in Palau (3.4–11.7%) are markedly lower than in Hawaii or French Polynesia, where

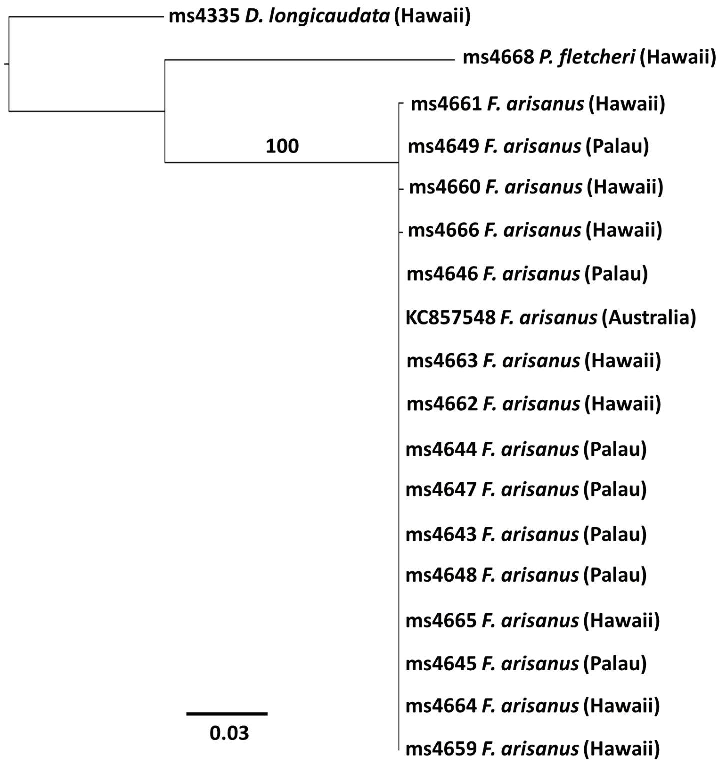

Figure 5. Phylogenetic relationships among fruit fly parasitoids (*Fopius arisanus*, *Diachasmimorpha longicaudata*, *Psyttalia fletcheri*) (Hymenoptera: Braconidae: Opiinae) based on the DNA barcode section of COI gene (651 bp).

its impact was spectacular (Vargas et al. 2012a,b, Leblanc et al. 2013b.). The introduction of the strain of this species kept in culture in Hawaii, which may be better adapted to *B. dorsalis*, should be considered and may help increase parasitism in Palau.

However effective parasitoids may be at reducing the overall population of *B. dorsalis* and number of larvae per kg fruit, reductions in the percentage of infested fruits are not nearly as substantial, though significant and also impressive. In French Polynesia, infestations on guava were decreased from 250 to < 25 larvae per kg, but percent of individual fruits infested was decreased from >80% to about 30% (Leblanc et al. 2013b). In order to achieve profitable production, with fruit infestation below 5%, application of area-wide suppression is required, in addition to biological control.

Acknowledgments

We are grateful to Harlan Derbai, J.B. Nagata, Pasqual Ongos, Hilda Etpison, Carter Ngiralbong and John K. Pedro, Quarantine Officers of the Palau Bureau of Agriculture, for their assistance in servicing traps and collecting and processing fruit samples. Michael McKenney (USDA-ARS, Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center, Hilo, Hawaii, USA) provided specimens of parasitoids from their laboratory colonies for genetic sequencing. Travel to Palau was sponsored by the International Atomic Energy Agency through technical cooperation expert missions in 2013 and 2014 under project PLW5001. The feasibility studies and training in 1999-2001 were funded by the FAO/AusAID/UNDP/SPC Project on Regional Management of Fruit Flies in the Pacific, and conducted by Chief Technical Advisor Allan Allwood. Additional support was provided by USDA-NIFA Hatch projects HAW00942-H and HAW00956-H, and USDA Farm Bill funding (project 3.0251), administered by the University of Hawaii College of Tropical Agriculture and Human Resources (CTAHR).

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Appendix
List and GenBank accession numbers for specimens of *Bactrocera dorsalis* and *Fopius arisanus* sequenced for this paper

Bactrocera dorsalis from Palau: Babeldoab, airport quarantine office: KT595028 (ms4461), KT595033 (ms4466), KT595042 (ms4640). Babeldoab, Melekeok State office: KT595029 (ms4462), KT595034 (ms4467), KT595043 (ms4641). Koror, Malakal Central Store: KT595037 (ms4635). Koror, Medalaii: KT595031 (ms4464), KT595038 (ms4636). Koror, Meyuns: KT595032 (ms4465), KT595036 (ms4634). Koror, Ngarkebsang: KT595035 (ms4633). Koror, Belau national Museum: KT595039 (ms4637). Koror, Palau Penthouse hotel: KT595025 (ms4458), KT595026 (ms4459), KT595027 (ms4460), KT595030 (ms4463), KT595040 (ms4638), KT595041 (ms4639), KT595044 (ms4642).

Bactrocera dorsalis from the Philippines: Luzon, Anilao Batangas: KT595047 (ms3894), KT595048 (ms3896), KT595049 (ms3899), KT595050 (ms3900), KT595051 (ms3901). Specimens from IAEA Lab colonies in Vienna: KT595045 (ms1187), KT595046 (ms1292).

Bactrocera dorsalis from Taiwan: Chayi Co.: KT595013 (ms4344), KT595014 (ms4345). Matsu Island group: KT595015 (ms4429). Nantou Co.: KT595008 (ms4337), KT595009 (ms4338), KT595010 (ms4339), KT595020 (ms4442). Pingtung Co.: KT595011 (ms4340). Sinchu Co.: KT595018 (ms4439). South Cross-Island Hwy: KT595023 (ms4445). Taipei: KT595007 (ms4335). Taitung Co.: KT595012 (ms4343), KT595024 (ms4449). Wulai Township: KT595017 (ms4437). Yangmingshan Nat. Pk.: KT595016 (ms4435). Yilan Co.: KT595019 (ms4440). Yushan N.P.: KT595021 (ms4443), KT595022 (ms4444).

Fopius arisanus from Palau: Koror, Meketii: KT595055 (ms4646). Koror, Meyuns: KT595053 (ms4644), KT595054 (ms4645). Koror, Ngarkebsang: KT595052 (ms4643). Koror, Ngerbeched: KT595056 (ms4647). Koror, Ngerchemai: KT595057 (ms4648), KT595058 (ms4649).

Fopius arisanus bred on *B. dorsalis* in Hawaii: KT595059 (ms4659), KT595060 (ms4660), KT595061 (ms4661), KT595062 (ms4662). *Fopius arisanus* bred on *Ceratitis capitata* in Hawaii: KT595063 (ms4663), KT595064 (ms4664), KT595065 (ms4665), KT595066 (ms4666).

Diachasmimorpha longicaudata bred on *B. dorsalis* in Hawaii: KT595067 (ms4667).

Psyttalia fletcheri bred on *B. cucurbitae* in Hawaii: KT595068 (ms4668).