First Report of African Fig Fly, *Zaprionus indianus* Gupta (Diptera: Drosophilidae), on the Island of Maui, Hawaii, USA, in 2017 and Potential Impacts to the Hawaiian Entomofauna

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Abstract. This report confirms the first reported observation of *Zaprionus indianus* Gupta (Diptera: Drosophilidae), commonly known as African fig fly, on Maui (new island record). Adult specimens were collected in October and November 2017 while surveying for populations of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). Specimens were retrieved from four localities in Haiku and Kula among traps positioned at fruiting height in six host plant environments (orange, lemon, starfruit, banana, strawberry, and cherimoya). Historically, the earliest records of *Z. indianus* in the state were recorded on Oahu in 2013 (new state record, new island record), on Kauai in 2015 (new island record), and on the Big Island (Hawaii) in 2017 (new island record). Including this report, there are currently at least 33 introduced Drosophilidae species established in the state of Hawaii. Furthermore, it is the second member belonging to genus *Zaprionus* that has been identified on the Hawaiian Islands. Specimens were not only retrieved from farms and subdivisions but also within mountain ranges and state forest reserves, suggesting that further research is needed to evaluate potential impacts to endemic entomofauna.

Key words: Invasive drosophilids, *Zaprionus indianus,* African fig fly, new island record, Maui

The African fig fly, *Zaprionus indianus* Gupta (Diptera: Drosophilidae), is a red-brown vinegar fly with distinctive secondary coloring that is native to the Afrotropics (Gupta 1970). As illustrated in Figure 1, members of genus *Zaprionus* have longitudinal white stripes on the dorsal regions of the head and thorax (Yassin and David 2010). Genus *Zaprionus* contains two subgenera, *Anaprionus* and *Zaprionus*, which can be differentiated through examination of external morphology. While both groups have longitudinal white stripes, species belonging to subgenus *Anaprionus* have an odd number of white stripes whereas members of subgenus *Zaprionus*, including *Z. indianus*, have an even number of white stripes (van der Linde 2010). Furthermore, the black and white stripes on *Z. indianus* are of equal size with the stripe width maintained the full length of the head to the thorax (van der Linde 2010). The *vittiger* species group, of which *Z. indianus* is a member, is characterized by a row of composite spines fused with long bristles at the base of the forefemur (Yassin and David 2010). Once considered cryptic species,

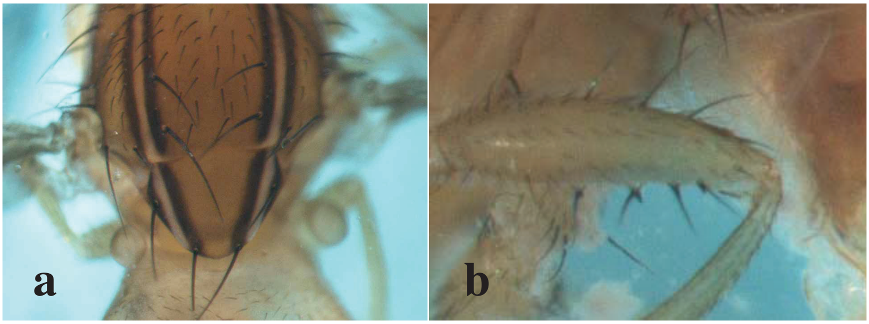

Figure 1. Thorax (a) and front tibia (b) of *Zaprionus indianus* collected on Maui. The lack of a white spot on the scutellum, and the presence of tibial spines, separates this species from *Zaprionus ghesquierei*.

members of the *indianus* species complex, including *Z. africanus*, *Z. gabonicus*, and *Z. indianus,* can be reliably distinguished by spermatheca shape with *Z. africanus* possessing a narrower spermatheca and an apically serrated aedeagal flap, *Z. gabonicus* with a basally smooth aedeagal flap and *Z. indianus* with a basally serrated aedeagal flap (Yassin and David 2010). Though morphologically similar, species belonging to the *indianus* species complex display a variety of ecological behaviors resulting in differences in pest status and invasive potential.

As a polyphagous drosophilid, *Z. indianus* uses a wide range of host plants for opportunistic feeding and breeding (Lavagnino et al. 2008). This results in damage to agricultural crops as well as making eradication of the drosophilid challenging once established. Even though *Z. indianus* is typically regarded as a secondary pest, it has demonstrated the potential to cause direct injury to select cultivars of both fig (Matavelli et al. 2015) and strawberry (Bernardi et al. 2017) fruits. Oviposition and subsequent larval feeding on agricultural crops can contribute to decreased yields and rejected product. On intact ripe strawberry fruit, adult females can oviposit on the fruit surface where larvae emerge, penetrate the epidermis, and consume pulp and yeast vital for development (Bernardi et al. 2017). Unlike *Drosophila simulans* Sturtevant and other drosophilids that oviposit on decaying figs, adult *Z. indianus* females can oviposit on ripening figs, increasing economic damage incurred by farmers (Matavelli et al. 2015). As a secondary pest, adult *Z. indianus* females can oviposit into fruits that have mechanical injury from other insects, including oviposition injury by *Drosophila suzukii* (Matsumura) (Bernardi et al. 2017) making the presence of both drosophilids particularly concerning to farmers of any soft-skinned fruit.

Aided by international trade and commerce, *Z. indianus* has been introduced to a wide variety of localities outside of its native range including North and South America, Europe, and Asia (Westphal et al. 2008, Hulme 2009). Invasive range expansion for *Z. indianus* has been reported on the Asian continent in India (Gupta 1970, Fartyal et al. 2014), Saudi Arabia (Amoudi et al. 1991), Egypt (Yassin and Abou-Youssef 2004), Iraq (Al T'Oma et al. 2010), and Jordan (Al-Jboory and Katbeh-Bader 2012). In Europe, specimens have been reported in France (Kremmer, et al. 2017) and Madeira archipelago (Rego et al. 2017). In South America, *Z. indianus* has invaded Brazil (Vilela 1999), Uruguay (Goñi et al. 2001), and Argentina (Soto et al. 2006). In Central America, *Z. indianus* was confirmed in Panama (van der Linde et al. 2006). In North America, reports of *Z. indianus* were published in Mexico (Lasa and Tadeo 2015), Canada (Renkema et al. 2013), and in the United States including Florida (van der Linde et al. 2006), Virginia (Pfeiffer et al. 2012), Michigan (Van Timmeren and Isaacs 2014), and Pennsylvania (Joshi et al. 2014). We report here an additional range expansion to the island of Maui, Hawaii, USA in 2017, discovered during a survey to identify whether populations of the invasive drosophilid *D. suzukii* were present within several localities and host plants.

Materials and Methods

Survey localities and host plants. Four locations on Maui were selected for the qualitative survey: a private estate in Haiku, HI (20.899859°N, –156.283533°W, elevation 302 m), Kula Agricultural Park (20.797167°N, –156.368971°W, 341 m), Kula Country Farms (20.747138°N, –156.337140°W, 889 m), and University of Hawaii at Manoa Kula Research Station (20.757534°N; –156.319755°W, 980 m). Seven host plants were selected to survey including fruiting trees (orange, lemon, starfruit, banana, cherimoya) and fruits and vegetables (strawberry, pumpkin). Orange, lemon, and starfruit were surveyed at the Haiku estate. Bananas were surveyed at Kula Agricultural Park. Strawberries, pumpkin, and lemon were surveyed at Kula Country Farms. Cherimoya was surveyed at Kula Research Station.

Trap assembly. The trapping device used in this experiment was a novel variation of the traditional deli cup design where a Mason jar, serving as a removable trap base, houses the drowning solution while a Solo cup permits entry and passive diffusion of the attractant within the headspace of the trap. Each trap was composed of a 236 ml Mason jar and a 473 ml red Solo cup and assembled with epoxy, sandpaper, an unfolded paperclip, and a soldering tool (Cold-Heat®). The first 2.5 cm of the Solo cup interior was scoured with sandpaper, then coated with a thin layer of epoxy. The silver band from the Mason jar lid was then firmly pressed down into the cup interior. The Solo cup was allowed to cure for 24 hours. An unfolded paperclip was pressed against the ceramic tip of the soldering tool to conduct heat. Then, the ceramic tip was used to pierce the solo cup in eight places (two on each side) to create 5 mm diameter entry holes. Nylon rope was strung through the top two holes of the Solo cup to enable trap installation (Figure 2).

Attractants. Attractants were selected based on drosophilids innate attraction to fermentation volatiles (Stensmyr et al. 2003, Stökyl et al. 2010, Faucher et al. 2013) and historical success in field capture using vinegars and wine as olfactory baits (Landoldt et al. 2012, Cha et al. 2015). The five attractants used in this survey were: apple cider vinegar (ACV, supplied by Marukan Vinegar Co. Ltd.), brown rice vinegar (BRV, supplied by Marukan Vinegar Co. Ltd.), red wine (RW, Oak Leaf Vineyards, Merlot), ACV+RW, and BRV+RW. The latter two attractant blends were prepared at a 60:40% concentration of vinegar to wine. Acetic acid (AcOH) concentrations were quantified by Dr. Naoki Akasaka with high-performance liquid chromatography (HPLC, Organic Acid Analysis System Prominence, Shimadzu) as previously described in Akasaka et al. (2017). The AcOH concentrations of undiluted BRV and ACV were 4.23% and 4.37% (% wt/vol), respectively. Once diluted with red wine, the AcOH concentrations of 60% BRV and 60% ACV were 2.54% and 2.62% (% wt/vol), respectively. Attractants were aliquoted (40 ml per trap) into Mason

Figure 2. Trap installation: example of trap positioning on a lemon tree

jars, a drop of unscented dish soap was added to each jar to break the surface tension, and the lids and bands were affixed for transport.

Trap installation and retrieval. Five traps were used (one of each attractant type) for each host plant at each location for a total of 40 traps. Once trap locations were delineated, the traps were installed at fruiting height, which varied by crop type. For fruiting trees (orange, lemon, banana, cherimoya, and starfruit), traps were hung at a ca. 1 m height. For fruit and vegetable crops (strawberry and pumpkin), traps were tied to a gardening stake that was secured in the ground at fruit height. After nylon rope was used to hang the trap to fruiting height, a Mason jar containing attractant solution was screwed on to the bottom of the Solo cup. On October 23rd 2017, 15 traps were deployed on orange, lemon, and starfruit trees at the Haiku estate. On 31 Oct 2017, 5 traps were deployed at the cherimoya grove at Kula Research Station. On 1 Nov 2017, 5 traps were deployed on the bananas at Kula Agricultural Park. On 2 Nov 2017, 15 traps were deployed on the strawberry, pumpkin, and lemon at Kula Country Farms. After one week, traps were removed and the specimens collected. Specimens were strained by pouring the attractant solution over a fine mesh filter (1 mm) and were stored in 70% isopropyl alcohol for three days prior to examination.

Identification and voucher specimens. Though the original objective of the survey was to identify whether *D. suzukii* populations were present (Yes/No) in the localities and host-plants surveyed, this objective was expanded to include *Z. indianus* after the first specimen was recognized. Specimens were identified as *D. suzukii*, *Z. indianus*, or non-target. Once *D. suzukii* and *Z. indianus* specimens were identified for a particular attractant type and host plant combination, no further quantification was performed. Identification as *Z. indianus* was confirmed through examination of external morphology by BW, using standard keys (van der Linde 2010, Yassin and David 2010). Voucher specimens were submitted to DP and LL, for imaging, identification, and collection submission. Taxonomic confirmation was provided by LL and AY. Specimens were deposited at the Systemic Entomology Lab, USDA ARS (Beltsville, MD), the Department of Entomology at Virginia Tech, the Muséum d'Histoire Naturelle de Paris, the Hawaii Department of Agriculture, the Bishop Museum, and the Insect Museum at the University of Hawaii at Manoa.

Results

Adult *Z. indianus* specimens were captured within traps installed in six of the seven host plant fruits surveyed (banana, cherimoya, lemon, orange, starfruit, and strawberry) and at all four localities surveyed. Survey results are presented (Table 1) simply to show that *Z. indianus* was attracted to the various baits and in different fruit systems, each with a different olfactory environment. At the private estate in Haiku, all three host plants surveyed including lemon, orange, and starfruit contained both *Z. indianus* and *D. suzukii* specimens. At Kula Agriculture Park, only *Z. indianus* specimens were retrieved from the traps placed in the banana grove. At Kula Country Farms, *D. suzukii* specimens were retrieved from all three host plants surveyed, while *Z. indianus* specimens were retrieved from the lemon and strawberry, but not the pumpkin traps. At Kula Research Station, the traps positioned in the Cherimoya grove contained both *D. suzukii* and *Z. indianus*.

Five attractants were used (ACV, BRV, RW, ACV+RW, BRV+RW) in the field capture of two exotic drosophilids (*Z. indianus*, *D. suzukii*) with results that varied by host plant and locality. At the private estate in Haiku, every attractant examined resulted in captures of *Z. indianus* and *D. suzukii*. At Kula Agricultural Park, every attractant was effective in capturing *Z. indianus* adults, but no *D. suzukii* specimens were retrieved. At Kula Country Farms, both *Z. indianus* and *D. suzukii* specimens were retrieved from all attractant types used in the lemon grove. In the strawberry fields, no target drosophilids were captured when RW was used as an attractant. However, BRV and ACV, alone and blended with RW, were effective in the field capture of both target drosophilids. For the pumpkin traps, *D. suzukii* specimens were captured using ACV+RW or BRV+RW attractants. No *Z. indianus* specimens were retrieved, regardless of attractant used. At Kula Research Station, every attractant used was effective in capturing *D. suzukii*, whereas *Z. indianus* specimens were retrieved only in RW, ACV+RW, and BRV+RW baited traps. It is noteworthy that BRV and BRV+RW were effective at field captures of both target drosophilids, as previous research has established BRV as an effective attractant to *D. suzukii* in laboratory trapping experiments (Akasaka et al. 2017). The attractants used in this survey and the resulting captures were reported simply to provide a detailed record of the circumstances from which target specimens were attracted. Whether an attractant resulted in captures in this survey, does not provide evidence that these results would be applicable to other surveys or experiments. Further research would be needed to discern any trends comparing attractant efficacy with confidence, as the lack of replicates and quantification limit the extrapolation of trends outside of this survey.

Discussion

This report established a new island record for *Z. indianus* on Maui, compiled collection data throughout the state (Table 2), and provided an update for introduced Drosophilidae established in Hawaii. Historically, the earliest *Z. indianus* specimens in the state were collected on Oahu in 2013 (new state record, new island

Table 1. Qualitative reporting of *Drosophila suzukii* (SWD) and *Zaprionus indianus* (AFF) and by attractant, survey locality, and host-plant. Attractant Abbreviations: ACV (Apple cider vinegar), BRV (Brown rice vinegar), RW (Red wine). Minus sign denotes zero captures; Plus sign denotes one or more captures.

Attractant:	**ACV**		**BRV**		**RW**		**ACV+RW**		**BRV+RW**	
Species:	**SWD**	**AFF**	**SWD**	**AFF**	**SWD**	**AFF**	**SWD**	**AFF**	**SWD**	**AFF**
Private Haiku Estate										
Lemon	+	+	+	+	+	+	+	+	+	+
Starfruit	+	+	+	+	+	+	+	+	+	+
Orange	+	+	+	+	+	+	+	+	+	+
Kula Agricultural Park										
Banana	–	+	–	+	–	+	–	+	–	+
Kula Research Station										
Cherimoya	+	–	+	–	+	+	+	+	+	+
Kula Country Farms										
Lemon	+	+	+	+	+	+	+	+	+	+
Strawberry	+	+	+	+	–	–	+	+	+	+
Pumpkin	-	–	–	–	–	–	+	–	+	–

Table 2. *Zaprionus indianus* records in the State of Hawaii. N.S. = new state record; N.I. = new island record. Collection abbreviations: HDOA (Hawaii Department of Agriculture), USDA-SEL (United States Department of Agriculture – Systemic Entomology Lab), BM (Bishop Museum), VT-ENT (Virginia Tech – Entomology Department), MNH-P (Muséum d'Histoire Naturelle de Paris), IM-UH (Insect Museum – University of Hawaii). [1](Evenhuis et al., 2017). [2](K. Magnacca, personal communication, 2018).

Year	Record	Locality	GPS N	GPS W	Elev. (m)	Habitat	Host plant environment	Collector	Collection
Oahu									
2013	N.S., N.I.	Pahole Gulch				Gulch	Unknown*	K. Magnacca	Personal[1]
2013		Central Kaluaa Gulch				Gulch	Unknown*	K. Magnacca	Personal
2013		Peahinaia Trail			610	Mountains	Unknown*	K. Magnacca	Personal
2014		Kaneohe				Residence	Jaboticaba	B. Azama	HDOA, USDA-SEL
2016		Lualualei	21.42582	–158.10302	1342	Mountains		C. Imada	BM
2017		Lualualei	21.42582	–158.10284	1356	Mountains		N. Evenhuis et. al.[2]	BM
2017		Lualualei	21.42475	–158.10360	1383	Mountains		N. Evenhuis et. al.	BM
2017		Lualualei	21.42457	–158.10357	1397	Mountains		N. Evenhuis et. al.	BM
2017		Lualualei	21.42480	–158.10370	1387	Mountains		N. Evenhuis et. al.	BM
Kauai									
2015	N.I.	Lihue				Residence	Litchi	L. Ishii	HDOA
2018		Kokee State Park	22.09820	–159.69015	905.3	Forest reserve		K. Adachi; Ruabora, A.	HDOA
Hawaii									
2017	N.I.	Kainaliu				Farm	Surinam cherry	R. Curtiss et. al.	HDOA
Maui									
2017	N.I.	Haiku	20.89986	–156.28353	302	Residence	Orange, lemon, starfruit	B. Willbrand et. al.	VT-ENT, USDA-SEL, BM
2017		Kula	20.79717	–156.33690	341	Farm	Banana	B. Willbrand et. al.	MNH-P
2017		Kula	20.74714	–156.33714	889	Farm	Strawberry, lemon	B. Willbrand et. al.	HDOA
2017		Kula	20.75753	–156.33714	980	Research station	Cherimoya	B. Willbrand et. al.	IM-UH

* captured with banana bait

record), on Kauai in 2015 (new island record), and on the Big Island in 2017 (new island record). Adding to the detailed list of introduced Drosophilidae compiled by Leblanc et al. (2009), there can now be considered at least 33 introduced Drosophilidae species established in the state of Hawaii.

This is the second member of genus *Zaprionus* that has been identified on the Hawaiian Islands. In 2005, *Z. ghesquierei* Collart was identified on the island of Hawaii and a year later specimens were identified throughout Kula, Maui (Leblanc et al. 2009). *Zaprionus ghesquierei* can be easily distinguished from *Z. indianus*, as specimens have a white spot on the tip of the scutellum of *Z. ghesquierei* (lacking in *Z. indianus* (Figure 1a) and the presence of spurs on the foretibiae of *Z. indianus* (Figure 1b) (Yassin and David 2010). Following this report of *Z. indianus*, earlier *Zaprionus* flies captured in Kula in 2006 were re-examined by L.L., and their identity as *Z. ghesquierei* was re-confirmed. Furthermore, four *Z. ghesquierei* vouchers at the University of Hawaii Insect Museum were re-identified by Camiel Doorenweerd to confirm the identification. No *Z. ghesquierei* specimens were identified in this survey.

The effectiveness of a given compound to elicit an attraction response is influenced by environmental cues (Faucher et al. 2013) and physiological adaptations (Matavelli et al. 2015, Nguyen et al. 2016). Thus, attractant efficacy is mediated not only by the species unique preferences, but also by environmental co-factors, such as olfactory and visual cues, that vary between host plant environments. In this survey, no *D. suzukii* specimens were retrieved from the banana grove at Kula Agricultural Park and no *Z. indianus* specimens were retrieved in the pumpkin field at Kula Country Farms. In both instances, it is possible that the attractants used were unsuitable for the given combination of target species and host plant environment. However, other factors such as climate, elevation, fruiting stage, and population dispersal may have contributed to the results. The absence of *Z. indianus* in the pumpkin fields of Kula Country Farms may be attributed to population dispersal to preferred hosts, such as the adjacent lemon grove. Even though *Z. indianus* specimens were retrieved from the majority of host plant environments surveyed (lemon, orange, starfruit, banana, cherimoya, strawberry), the presence of an insect is not sufficient evidence of economically significant damage. Further research is needed to determine economic thresholds by crop and to characterize ecological impacts to endemic species.

Unfortunately, the discovery of *Z. indianus* at four localities throughout Maui provokes more questions than answers—Is the range expansion limited to agricultural ecosystems, or are they also present in native forests? Throughout the state, specimens have not only been retrieved from farms and subdivisions but also within mountain ranges and state forest reserves, which suggests that the species is able to thrive in a wide variety of environments. Based on our results, it appears that further surveying on Maui is needed in regions producing soft-skinned fruit and berries, native forest reserves, and within diverse microclimates. Will the combined presence of *D. suzukii* and *Z. indianus* have deleterious impacts for Maui farmers? Considering that *Z. indianus* can cause direct injury to fig and strawberry (Matavelli et al. 2015, Bernardi et al. 2017), and that these crops are produced by Maui farmers, the pest potential of this introduced species should be examined and economic thresholds defined. Are there any adverse impacts to endemic drosophilids? *Zaprionus indianus* has led to declines of native drosophilids in other locations (Castro and Vilente 2001, Tidon et al. 2003, da Silva et al. 2005b, da Silva et al. 2005a) and the Hawaiian Islands are already struggling to cope with the appearance of many invasive species, including insects. On Hawaii and Maui, even though researchers found a strong association between exotic drosophilids with introduced host plant environments and endemic drosophilids with native plant species, invasive drosophilids were still encountered in native forests and endemic drosophilids in disturbed or nonnative environments (Leblanc et al. 2013).

This finding not only suggests ecological interactions between endemic and introduced species but also adaptations within a dynamic environment, both of which warrant characterization. Additional research is needed to quantify the economic and ecological significance of this finding and to examine whether eradication efforts are necessary and feasible. The first step would be to delineate the range of *Z. indianus* across Maui. Secondly, interspecies interactions need to be examined, not only between *Z. indianus* and other exotic drosophilids, but also between exotic and endemic drosophilids. Finally, economic thresholds for both species should be defined for use in survey protocols for integrated pest management programs. Nonetheless, this work has identified the first report of *Z. indianus* on Maui which requires further inquiries to identify the extent of the introduced species effect on the local ecology and agriculture economy.

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