

New record in the Hawaiian Islands of *Orasema minutissima* (Hymenoptera: Eucharitidae), an ant-parasitic wasp and a potential biocontrol agent against the Little Fire Ant, *Wasmannia auropunctata* (Hymenoptera: Formicidae)

JOHN M. HERATY^{1*}, D. VALLE ROGERS², M. TRACY JOHNSON³, WILLIAM D. PERREIRA⁴,
AUSTIN J. BAKER¹, ELLYN BITUME³, ELIZABETH MURRAY⁵ AND LAURA VARONE^{6,7}

* corresponding author john.heraty@ucr.edu

Abstract. *Orasema minutissima* Howard (Hymenoptera: Eucharitidae) is recorded from the Hawaiian Islands for the first time. It has been established on the island of Hawai‘i since at least 2019. The wasp is a parasitoid of the immature stages of *Pheidole* and *Wasmannia* (Formicidae: Myrmicinae), both of which are significant pests on several of the Hawaiian Islands. Already found in substantial numbers, the wasp is a potential biological control agent for *Wasmannia auropunctata*, the Little Fire Ant.

INTRODUCTION

The Little Fire Ant (LFA) or electric ant, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae) is an increasingly important exotic pest. This species occurs throughout the warmer regions of the New World, with a potential native range from northern Argentina to Mexico and most of the Caribbean (Wetterer and Porter, 2003). The ant has been introduced into many different countries, including Australia, the Galapagos Islands, central West Africa, Melanesia (New Caledonia, Solomon Islands, Fiji), Polynesia (Tahiti, Wallis, Futuna and Hawai‘i), the mainland USA (Florida), and Israel (Wetterer & Porter 2003; Vonshak *et al.* 2009; Foucaud *et al.* 2010; Tindo *et al.* 2012; Wetterer 2013; Bousseyroux *et al.* 2018). The LFA is ranked as one of the 100 top most invasive species, and in alien (invasive) habitats is regarded as a serious threat to both native and agricultural ecosystems (Wetterer & Porter 2003; Vonshak *et al.* 2006). It is generally a pest of humans in urban landscapes, although in Cameroon its artificial spread was promoted as a means of controlling cacao pests (Bruneau De Mire 1969). The LFA was first recorded on the islands of Hawai‘i and Kaua‘i in 1999 (Conant & Hirayama 2000). By 2015, it had further spread to the islands of Maui and O‘ahu (Vanderwoude *et al.* 2015) and Lāna‘i (<http://stoptheant.org/lfa-in-hawaii/>). Based on molecular evidence, the proposed origin of the populations in Hawai‘i is from Florida (Mikheyev & Mueller 2007; Foucaud *et al.* 2010). Another exotic ant species, *Pheidole megacephala* (Fabricius) was first reported in Hawai‘i as early as 1879 and has been documented to have impacts on both native species and crop losses (Smith 1879; Vanderwoude *et al.* 2015). No para-

¹ Department of Entomology, University of California, Riverside, CA 92521, USA

² Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720, USA

³ USDA Forest Service, Pacific Southwest Research Station, Institute of Pacific Islands Forestry, Hilo, HI 96720

⁴ P.O. Box 61547, Honolulu, HI 96839-1547, USA

⁵ Department of Entomology, Washington State University, Pullman, WA 99164, USA

⁶ Fundación para el Estudio de Especies Invasivas, Hurlingham, Buenos Aires, Argentina

⁷ Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina

sitoids or specific natural enemies of either of these genera have been documented previously from the Hawaiian Islands.

There are few parasitoids of ant brood, but Eucharitidae (Hymenoptera: Chalcidoidea) are known to specialize on their immature stages. Eggs of Eucharitidae are deposited on or into plant tissue, away from the ant nest, and the minute first-instar larvae (planidia) use a variety of behaviors to associate with foraging ants and get carried back to the ant brood (Clausen 1941; Baker *et al.* 2020). The subfamily Oraseminae are specialists on ants in the subfamily Myrmicinae, which include *Pheidole*, *Wasmannia* and *Solenopsis* (Heraty 1994a,b, 2000; Murray *et al.* 2013; Baker *et al.* 2020). Species of *Orasema* are distributed throughout the New World, although they are most common in tropical regions (Baker *et al.* 2020). *Orasema* deposit single eggs away from the host into punctures made by their expanded ovipositor into plant tissue, with the active planidia attaching to or being picked up by foraging ants directly, or utilizing an intermediate Hemipteran or Thysanopteran host that is collected by the ants and provided as a food source to the ant larva (Clausen 1941; Das 1963; Wilson & Cooley 1972; Beshear 1974; Heraty 1994b, 2000; Herreid & Heraty 2017; Baker *et al.* 2020). Development proceeds on the pupa of their myrmicine ant host and adults must emerge from the nest, likely protected by acquired semiochemicals (Vander Meer *et al.* 1989). Species attacking the fire ant genera *Wasmannia* and *Solenopsis* occur in four distinct species groups: the *xanthopus* species group, which are parasitoids of *Solenopsis* (Heraty *et al.* 1993); the *stramineipes* species group, which are mostly parasitoids of *Pheidole* and *Wasmannia* (Heraty 1994b, 2000; Burks *et al.* 2018); the *bakeri* species group, which are mostly parasitoids of *Pheidole* with the exception of one species in Mexico also attacking *Solenopsis*; and the *coloradensis* species group, which includes a species that is a parasitoid of both *Pheidole* and *Solenopsis* (Baker & Heraty 2020).

Orasema minutissima is a common and abundant parasitoid of *W. auropunctata* in both the Caribbean and mainland South America (Heraty 1994a,b; Burks *et al.* 2018). It belongs to the *Orasema stramineipes* species group, which was recently revised (Burks *et al.* 2018). The species has three distinct size morphs: the smallest morph attacks the brood of *Wasmannia*, while the two larger morphs likely attack different castes of species of *Pheidole* (Heraty 1994a; Burks *et al.* 2018). *Orasema minutissima* is extremely common on most of the islands in the Caribbean, but notably has not been found in Florida and it is less common on mainland Central America and northern South America (cf. map in fig. 3 of Burks *et al.* 2018). The species is heavily female biased. Of the three different size morphs, Burks *et al.* (2018) examined a total of 1,032 females and 19 males (small morph), 97 females and three males (medium morph), and only one female of the large morph. Given that these are from museum collections and represent a variety of collection methods, this would suggest that the bias is real, and that the species may be largely parthenogenetic. The medium and large-sized morphs identified as *O. minutissima* are known only from islands ranging between Hispaniola and Trinidad. Importantly, the small and larger morphs with geographic samples from Colombia (Gorgona Island), Cuba, Dominica, St. Lucia, Tortola and Trinidad all share an identical haplotype for the 28S-D2, COI gene, which is distinct from all other species of *Orasema* sampled (Burks *et al.* 2018). Specimens have been collected in nests of both *Wasmannia* (Gahan 1940; Heraty 1994a) and *Pheidole* (Burks *et al.* 2018) confirming the host associations. Thus, we have a single widespread and common species that attacks two different genera of Myrmicinae.

From field studies in Dominica in 2009 and 2010, we know that the small form prefers to oviposit into the leaf surface of broadleaf ferns, and the larger morphs prefer short emergent broad-leaf dicot plants. In contrast to other species of *Orasema*, all morphs of *O. minutissima* are readily sampled in yellow pan traps (YPTs), which make them easier to survey. The potential of this parasitoid for biological control of the LFA has been discussed (Heraty, 1994a), but never attempted. A new establishment on Hawai'i and not on other islands with *Wasmannia* may offer an opportunity to monitor their impact on ant populations. Furthermore, it will be interesting to observe if they attack *P. megacephala*, which has been recorded as a host of *Ivieosema fraudulenta* (Reichensperger) (Oraseminae) from Ethiopia (Reichensperger 1913), but never by a New World species of *Orasema*.

MATERIALS AND METHODS

Sampling. The first observation of *O. minutissima* was made by DVR in Hilo using Yellow Pan Traps (YPT) that consisted of a yellow plastic bowl [i.e., 13.5 oz (0.4 liter), 7" × 1 3/4" (17.5 cm × 4.5 cm)], with water and a few drops of non-scented liquid soap, that was placed on the ground. Similar YPT traps were used by MTJ and EB at Hilo and the Keauohana Forest Reserve, two sites known to be infested by *Wasmannia*. WDP had been monitoring several sites in Hawai'i (Fig. 2), Maui, and O'ahu as part of a separate insect survey project. On Maui and O'ahu, *Wasmannia* was not present at either site being sampled, but *Pheidole* were present. His YPTs consisted of yellow 8 ounce (0.24 liter) plastic "shave ice" flower cups, readily available at local restaurant supply stores and online, filled with water and about 5 ml of yellow colored dish soap; traps were placed in the field for two weeks and the specimens air-dried. Bowl size (larger or smaller) is not likely to affect monitoring for presence or absence of *O. minutissima*. WDP also sampled with Yellow Sticky Board Traps (YSBT) consisting of 10" × 12" (25.40 cm × 30.48 cm) cards from HTG Supply (htgsupply.com; Callery, PA) cut into 10" × 3" (25.4 cm × 7.5 cm) strips and hung from branches approximately 3–4 ft (0.91–1.22 m) above ground at each field location (Fig. 2). After two weeks, the YSBTs were replaced with new traps and specimens stuck to the exposed traps were removed using Aliphatic Naphtha (Crown Brand V.M.&P. NAPHTHA®), soaked in same solutions for about one hour until all adhesive materials were dissolved, and then air-dried. Specimens of *Orasema* were either air-dried or chemically dried from ethanol using HMDS (Heraty & Hawks 1998) and then point-mounted and deposited in either the Entomology Research Museum, University of California, Riverside, CA (UCRC_ENT) or the Bernice P. Bishop Museum, Honolulu, HI (BPBM) (Appendix). Individuals were assigned a unique barcode and databased in the Heraty lab FileMaker Pro database.

Sequencing. Specimens from 95% ethanol were extracted using DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) with 1 µL RNase A added after incubation. PCR products were purified with DNA Clean & Concentrator-5 kits (Zymo Research, Irvine, CA, USA). PCR product concentrations were determined using Nanodrop 2000c (Thermo Scientific, Waltham, MA, USA). Two regions of ribosomal DNA were PCR amplified individually and Sanger-sequenced with the following primer sequences: 28S D2 rDNA (D2-F: CGGGTTGCTTGAGAGTGCAGC; D2-Ra: CTCCTTGGTCCGTGTTTC) and ITS2 (ITS2-F: TGTGAAGTGCAGGACACATG; ITS2-R2: TCTCGCCTGCTCTGAGGT). The following thermocycler protocol was used: initial denaturization: 94 °C 3 min., 34 cycles

Table 1. *Oreasema minutissima* specimens sequenced.

specimen id	28S-D2	ITS2	Country	size class	D number
235958	KY349472*	MW575764	Brit. Virgin Islands	small	0424
235964	KY349465*	MW575761	St. Lucia	small	0437
235966	KY349458*	MW575757	Dominica	medium	2766
235967	—	identical	Dominica	medium	2765
235968	KY349457*	MW575758	Dominica	medium	2764
235969	KY349463*	identical	Dominica	small	2763
235970	KY349462*	MW575760	Dominica	small	2762
271392	KY349464*	MW575762	Dominica	small	2830
271393	KY349459*	identical	Dominica	medium	2831
271395	KY349456*	MW575756	Dominica	large	2833
412118	KY349466*	identical	Trinidad	small	3800
412119	KY349467*	MW575763	Trinidad	small	3801
412127	KY349469*	identical	Trinidad	small	3809
412131	KY349471*	identical	Trinidad	small	3814
412132	KY349470*	—	Trinidad	small	3810
447074	KY349473*	—	Colombia	small	4207
447075	KY349455*	MW575755	Colombia	small	4208
456205	identical	identical	Puerto Rico	small	2808
468543	MW357878	MW357873	Hawai‘i	small	6943
468544	MW357879	identical	Hawai‘i	small	6944

28S-D2 GenBank accession numbers marked by an asterisk were used in Burks *et al.* (2018); ITS2 accessions are all new. Specimen ID numbers are associated with a prefix UCRC_ENT00 and deposited in UCRC, with full collection data presented in Appendix 1. D numbers are the DNA voucher codes. Identical refers to sequences that were captured but not deposited in GenBank.

(94 °C 1 min.; 55 °C 1 min., 72 °C 1 min.), final extension: 75 °C 7 min. PCR samples were sent to Retrogen Inc (San Diego, CA, USA) for Sanger sequencing on an Applied Biosystems 3730xl DNA Analyzer. Deposition of molecular vouchers is indicated in the Material Examined sections. GenBank accession numbers are listed in Table 1.

Measurements. To investigate the different size classes within *O. minutissima* and infer the ant host, measurements were taken for 31 specimens of the total body length (anterior margin of head to apex of gaster) and fore wing length (apex of humeral plate to most distal margin of wing membrane). For specimens from Hawai‘i, all of the small specimens were measured from material that was dried using the HMDS method; the two larger specimens were air-dried but this appeared to have minimal impact on body length. Identification numbers for specimens measured are UCRC_ENT00422315–16, 422320, 468555–67, 468588 (see Appendix for details). Measurements of the Neotropical material included specimens of three size classes (small, medium, large) from across the range of *O. minutissima*, including the British Virgin Islands, Colombia (Gorgona Island), Dominica, Puerto Rico, and Trinidad (Appendix); all of these specimens were dried with HMDS. For the Neotropical specimens, we chose only material that had been sequenced for either 28S-D2 or ITS2 (all with identical sequences) to guarantee that they were all forms with the identical haplotype of *O. minutissima*.

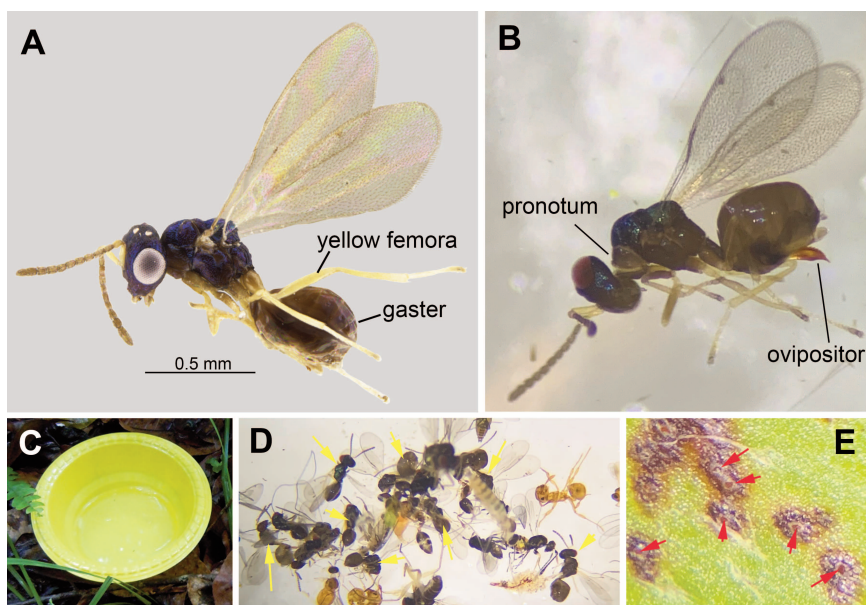


Fig. 1. *Orasema minutissima*. **A**, habitus of dry-mounted specimen. **B**, habitus of ethanol-preserved specimen. **C**, yellow pan trap. **D**, portion of YPT sample with numerous *O. minutissima* females (yellow arrows). **E**, egg punctures (red arrows) in surface of swordfern leaf.

RESULTS AND DISCUSSION

Distribution in Hawai‘i

To date, *O. minutissima* has only been collected in the vicinity of Hilo on the island of Hawai‘i at or near sites 5, 6, 8, 10 and 13 despite continuous trapping at the other sites (Fig. 2, Appendix). *Orasema minutissima* (Howard) was first discovered on the island of Hawai‘i near Hilo in October 2019 (Fig. 1) using yellow pan traps (YPTs, Fig. 1C). Subsequent sampling in September 2020 at two sites on Hawai‘i Island by MTJ found *O. minutissima* in at least half of 24 YPTs at one site and in most of 10 traps at another, with as many as 10 wasps collected in an individual trap, suggesting that the species is well established (Fig. 1D). Additional collections made during a survey initiated across Hawai‘i in April 2019 using yellow sticky board traps (YSBT) and a different type of YPT recovered *O. minutissima* at 5 out of 17 sites where *Wasmannia* was also present (Fig. 2). Of the sites where no *Orasema* were collected, only site 7 (Hilo) and 17 (Whittington Beach Park) are low enough in elevation to also have *Wasmannia*; the other sites with no *Orasema* are likely too high (>1800 m) to have *Wasmannia*. From this limited sampling, it is unclear if the *Orasema* is distributed only in eastern Hawai‘i, or if it may be more widespread across the island. Mostly females have been recovered, but one male was sampled on a YSBT at site 8. Sampling using both YPTs and YSBTs failed to recover these wasps from either O‘ahu or Maui, suggesting that it is established only on the island of Hawai‘i, although none of the sites sampled had *Wasmannia* present. This is the first

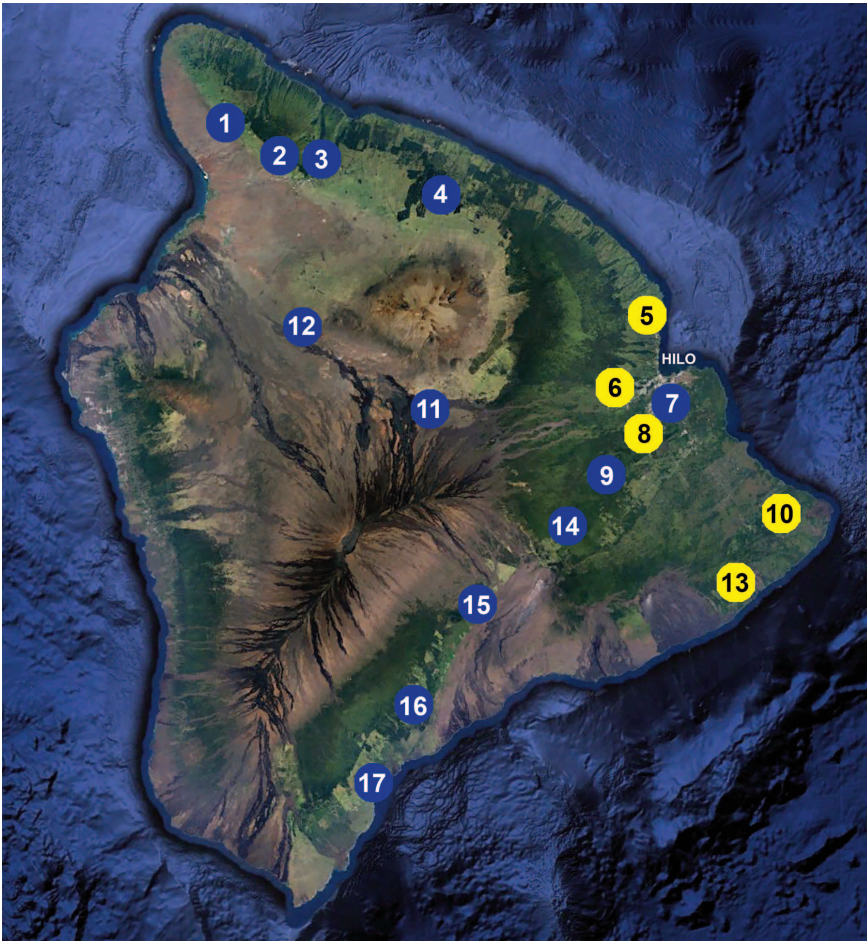


Fig. 2. Yellow Sticky Board sampling sites on Hawai‘i Island: (1) Koai‘a Tree Sanctuary; (2) Waimea Town; (3) UH Mealani Research Station; (4) Kalōpā State Recreation Area; (5) Kawainui Stream; (6) Kaumana; (7) Hilo Town; (8) Pana‘ewa Zoo; (9) Kurtistown; (10) Pahoa District Park; (11) Pu‘u Huluhulu; (12) Route 200 Saddle Road; (13) Kaimu-Chain of Craters Road; (14) Wright Road, Volcano Village; (15) Hawai‘i Belt Road; (16) Pahala and (17) Whittington Beach Park. *Orasema minutissima* were sampled at sites 5, 6, and 10 in 2019; at site 8 in 2019-2021; and at site 13 in 2020-2021 (yellow circles). Satellite image credited to Google Earth.

record of a eucharitid wasp in the Hawaiian Islands, and its accidental introduction has the potential to offer some form of biological control over the invasive LFA.

Sequencing

Two gene regions, 28S-D2 and ITS2, were sequenced for two of the specimens from Hilo following the protocols and primers outlined in Baker *et al.* (2020) and Burks *et al.*

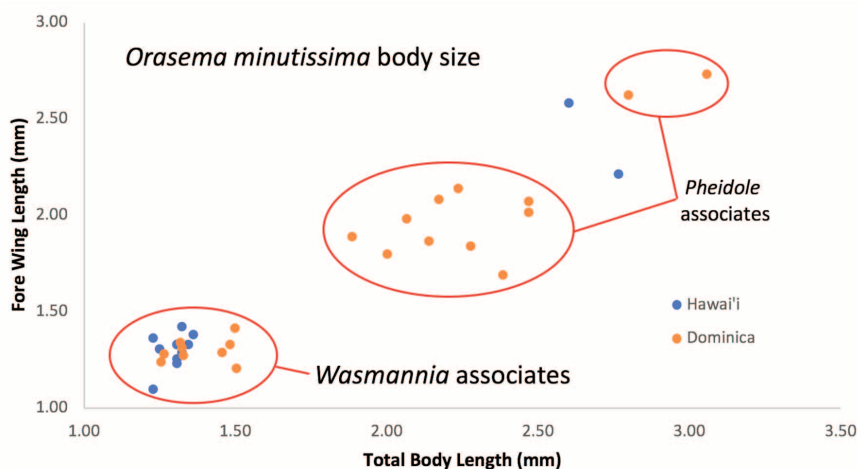


Fig. 3. Size classes of *Orasema minutissima* females. Neotropical refers to samples from various islands in the Caribbean and in the Pacific Ocean from Gorgona Island off the coast of Colombia (see Appendix 1). All Neotropical specimens measured were sequenced for 28S-D2, COI and ITS2, and have an identical haplotype (Burks *et al.* 2018 and this study).

(2018). The second ITS2 read for D6944 was not of high enough quality to deposit on GenBank but was identical for the sequence obtained. The 28S-D2 sequences were identical to other specimens of *O. minutissima* that have been sequenced from the Caribbean and Colombia (Gorgona Island) (Burks *et al.* 2018), and for the ITS2 obtained in this study (Table 1).

Origin of *Orasema minutissima*

There have been no purposeful introductions of this wasp to Hawai'i. It is most likely that they were imported along with a new accidental importation of *Wasmannia*. Given that *O. minutissima* is not known to occur in Florida, it is not likely to have come with the initial population from Florida, which was the place of origin proposed by Mikheyev & Mueller (2007) and Foucaud *et al.* (2010). Thus, a separate introduction of *Wasmannia* from one of the localities where it is currently distributed, along with its parasitoid, is inferred. However, given that the current gene regions of the wasp have identical haplotypes across the range of the species, no further inference of origin can be made at this time.

Measurements and size classes

A total of 13 specimens were measured from Hawai'i and another 18 from various Neotropical islands (Fig. 3). All but two of the specimens from Hawai'i fell into the small size morph class, which is associated with parasitism of *Wasmannia* (Burks *et al.* 2018). The two larger sized specimens appear to be intermediate in size to the medium and large morphs from the Neotropical material (Fig. 3). The number of measurements for the Neotropical specimens is meager, but we felt it important to only measure sequenced specimens, as closely related species in the complex can be difficult to separate based on morphology alone (Burks *et al.* 2018). Larger samples from the Caribbean support the

separation of size classes, but the large and medium classes are rarely sampled. Pupae of the small size class have been found only with brood of *Wasmannia* in the Caribbean. A single pupa of a medium-size morph was recovered from a nest of *Pheidole* in Dominica, and in an area where both the medium and large morph specimens were collected. We assume that the medium and large size classes are associated with different castes of *Pheidole*. On Hawai'i, we assume that the larger size class also indicates an association with *Pheidole*; however, it is also possible that they are developing on the queen brood of *Wasmannia*.

Host plants

In Jamaica, *Orasema minutissima* have been collected from *Chamissoa altissima* (Jacq.) Kunth (Amaranthaceae), *Gynerium sagittatum* (Aubl.) Beauv. (Poaceae) and *Zapoteca ?formosa* (Kunth) H.M. Herm. (Fabaceae), although no oviposition was observed (Heraty 1994a; Burks *et al.* 2018). In Dominica, the small morph has been observed to oviposit on ferns (*Cyathea tenera* (J.E. Smith) Moore, Cyathaceae; *Nephrolepis biserrata* Schott, Polypodiaceae; *Thelypteris opposita* (Vahl) Ching, Thelypteridaceae), whereas the larger size morphs preferentially oviposit on a variety of short (6–12" high) broad-leaf plants (*Coccoloba uvifera* L., Polygonaceae; *Simarouba amara* Aublet, Simaroubaceae; miscellaneous Fabaceae and Rubiaceae). Single eggs are deposited into excavations made into the leaf surface by the enlarged ovipositor (Fig. 1B), with the punctures usually surrounded by a brown scarring of the leaf tissue. In east Hawai'i, egg punctures, eggs and planidia have been found on alien swordfern (*Nephrolepis brownii* (Desv.) Hovenkamp & Miyam., Polypodiaceae) (Fig. 1E), a ground fern with large-lobed fronds (*Microsorium grossum* (Langsdorff & Fischer) S.B. Andrews, Polypodiaceae), and strawberry guava (*Psidium cattleianum* Sabine, Myrtaceae) at the Keauohana Forest Reserve site. While the oviposition scars may cause some cosmetic damage when numbers are high, the leaves usually recover as they mature.

Biological control potential

Heraty (1994a) proposed that *O. minutissima* was one of the few species that may have an impact as a biological control agent against *Wasmannia*. When present, especially in moist island habitats in the Caribbean, *O. minutissima* can be extremely common. However, their impact on the ant population numbers has not yet been assessed within an experimental framework. Their purposeful importation as a biological control agent to other islands, especially to islands in the Pacific, has not been implemented. The primary issues for treating this as a coordinated biological control effort lie with the difficulty of transport and a need for quarantining with the capability for continuous rearing to test for negative impacts against native species. With regard to the Hawaiian introduction, there are no native myrmicine ants on any of the Hawaiian Islands, and in any case Oрасемінае are specific only to that ant subfamily (Murray *et al.* 2013; Baker *et al.* 2020). The impacts of cosmetic damage to native vegetation will likely be minimal, but this should be assessed in the future. It will be necessary to sample for presence of the wasp on island locations that have *Wasmannia* present to assess the presence and spread of *Oрасема*. However, *O. minutissima* has been discovered at a time where the spread of the parasitoid and its impact on *Wasmannia*, and potentially *Pheidole*, can be monitored to see if they have any effect on lowering population densities.

ACKNOWLEDGMENTS

We thank Chrysalyne Dominguez and Luke Kresslein for comments on an earlier draft of this paper. This research has been supported in part by USDA National Institute of Food and Agriculture Hatch project 1015803 to JMH. Funding of yellow sticky card sampling was generously provided to WDP by The Atherton Family Foundation and The Dana Anne Yee Foundation. We are grateful to Clinton Yamada and Robert Lee, State of Hawai'i Department of Transportation, Highways Division and the County of Hawai'i, Department of Parks and Recreation for allowing us to collect on their lands.

REFERENCES

- Baker, A., Heraty, J.M., Mottern, J., Zhang, J., Hines, H.M., Lemmon, A.R. & Lemmon, E.M.** 2020. Inverse dispersal patterns in a group of ant parasitoids (Hymenoptera: Eucharitidae: Oraseminae) and their ant hosts. *Systematic Entomology* **45**: 1–19.
<https://doi.org/10.1111/syen.12371>
- Baker, A.J. & Heraty, J.M.** 2020. The New World ant parasitoid genus *Orasema* (Hymenoptera: Eucharitidae). *Zootaxa* **4888**: 1–84.
<https://doi.org/10.11646/zootaxa.4888.1.1>
- Beshear, R.J.** 1974. A chalcidoid planidium on thrips larvae in Georgia. *Journal of the Georgia Entomological Society* **9**: 265–266.
- Bousseyroux, A., Blanvillain, C., Darius, T., Vanderwoude, C. & Beune, D.** 2018. Ecological impacts of the little fire ant (*Wasmannia auropunctata*) in Tahiti. *Pacific Conservation Biology* **25**(3): 299–307.
<https://doi.org/10.1071/PC18035>
- Bruneau de Mire, P.** 1969. Une fourmi utilisée au Cameroun dans la lutte contre des mirides du cacaoyer *Wasmannia auropunctata* Roger. *Café,-Cacao-The* **13**: 209–212.
- Burks, R.A., Heraty, J.M., Dominguez, C. & Mottern, J.L.** 2018. Complex diversity in a mainly tropical group of ant parasitoids: Revision of the *Orasema stramineipes* species group (Hymenoptera: Chalcidoidea: Eucharitidae). *Zootaxa* **4401**: 1–107.
<https://doi.org/10.11646/zootaxa.4401.1.1>
- Clausen, C.P.** 1941. The habits of the Eucharitidae. *Psyche* **48**: 57–69.
<https://doi.org/10.1155/1941/21539>
- Conant, P. & Hirayama, C.** 2000. *Wasmannia auropunctata* (Hymenoptera: Formicidae): established on the Island of Hawai'i. *Bishop Museum Occasional Papers* **64**: 21–22.
- Das, G.M.** 1963. Preliminary studies on the biology of *Orasema assectator* Kerrich (Hymenoptera: Eucharitidae) parasitic on *Pheidole* and causing damage to leaves of tea in Assam. *Bulletin of Entomological Research* **54**: 393–398, pl.x.
<https://doi.org/10.1017/S0007485300048884>
- Foucaud, J., Orivel, J., Loiseu, A., Delabie, J.H.C., Jourdan, H., Konghouleux, D., Vonshak, M., Tindo, M., Mercier, J.-L., Fresneau, D., Mikissa, J.-B., McGlynn, T., Mikheyev, A. S., Oettler, J. & Estoup, A.** 2010. Worldwide invasion by the little fire ant: routes of introduction and eco-evolutionary pathways. *Evolutionary Applications* **10**: 363–374.
<https://doi.org/10.1111/j.1752-4571.2010.00119.x>

- Gahan, A.B.** 1940. A contribution to the knowledge of the Eucharidae (Hymenoptera: Chalcidoidea). *Proceedings of the United States National Museum* **88**: 425–458.
<https://doi.org/10.5479/si.00963801.88-3086.425>.
- Heraty, J.M.** 1994a. Biology and importance of two eucharitid parasites of *Wasmannia* and *Solenopsis*, pp. 104–120. In: Williams, D.F. (ed.) *Exotic Ants: Biology, Impact and Control of Introduced Species*. Westview Press, Boulder, Colorado, 332 pp.
- Heraty, J.M.** 1994b. Classification and evolution of the Oraseminae in the Old World, with revisions of two closely related genera of Eucharitinae (Hymenoptera: Eucharitidae). *Life Sciences Contributions, Royal Ontario Museum* **157**: 1–174.
- Heraty, J.M.** 2000. Phylogenetic relationships of Oraseminae (Hymenoptera: Eucharitidae). *Annals of the Entomological Society of America* **93**: 374–390.
[https://doi.org/10.1603/0013-8746\(2000\)093\[0374:PROOHE\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2000)093[0374:PROOHE]2.0.CO;2).
- Heraty, J.M. & Hawks, D.** 1998. Hexamethyldisilazane: A chemical alternative for drying insects. *Entomological News* **109**: 369–374.
<https://doi.org/10.5962/BHL.PART.13421>
- Heraty, J.M., Wojcik, D.P. & Jouvenaz, D.P.** 1993. Species of *Orasema* parasitic on the *Solenopsis saevissima* complex in South America (Hymenoptera: Eucharitidae, Formicidae). *Journal of Hymenoptera Research* **2**: 169–182.
- Herreid, J.S. & Heraty, J.M.** 2017. Hitchhikers at the dinner table: a revisionary study of a group of ant parasitoids (Hymenoptera: Eucharitidae) specializing in the use of extrafloral nectaries for host access. *Systematic Entomology* **42**: 204–229.
<https://doi.org/10.1111/syen.12206>
- Mikheyev, A.S. & Mueller, U.G.** 2007. Genetic relationships between native and introduced populations of the little fire ant *Wasmannia auropunctata*. *Diversity and Distributions* **13**: 573–579.
<https://doi.org/10.1111/j.1472-4642.2007.00370.x>
- Murray, E., Carmichael, A.E. & Heraty, J.M.** 2013. Ancient host shifts followed by host conservatism in a group of ant parasitoids. *Proceedings of the Royal Society, B* **280**: 20130495.
<https://doi.org/10.1098/rspb.2013.0495>
- Reichensperger, A.** 1913. Zur Kenntnis von Myrmecophilen aus Abessinien. I. *Zoologische Jahrbücher* **35**: 185–218.
- Smith, F.** 1879. Descriptions of new species of aculeate Hymenoptera collected by the Rev Thos. Blackburn in the Sandwich Islands. *Journal of the Linnean Society of London* **4**: 674–685.
<https://doi.org/10.1111/j.1096-3642.1879.tb02459.x>
- Tindo, M., Mhenoun Masse, P.S., Kenne, M., Mony, R., Orivel, J., Doumstop Fotio, A., Fotoso Kuaté, A., Djiéto-Lordon, C., Fomena, A., Estoup, A., Dejean, A. & Foucaud, J.** 2012. Current distribution and population dynamics of the little fire ant supercolony in Cameroon. *Insectes Sociaux* **59**: 175–182.
<https://doi.org/10.1007/s00040-011-0202-x>
- Vander Meer, R.K., Jouvenaz, D.P. & Wojcik, D.P.** 1989. Chemical mimicry in a parasitoid (Hymenoptera Eucharitidae) of fire ants (Hymenoptera: Formicidae). *Journal of Chemical Ecology* **15**: 2247–2261.
<https://doi.org/10.1007/BF01014113>.

- Vanderwoude, C., Montgomery, M., Forester, H., Hensley, E. & Adachi, M.K.** 2015. The history of Little Fire Ant *Wasmannia auropunctata* Roger in the Hawaiian Islands: spread, control, and local eradication. *Proceedings of the Hawaiian Entomological Society* **48**: 39–50.
- Vonshak, M., Dayan, T. & Hefetz, A.** 2006. The little fire ant (*Wasmannia auropunctata*) in Israel. Available at: http://www.tau.ac.il/lifesci/zoology/members/dayan_files/articles/merav_ziv_2006.pdf. (last accessed 02/26/2021).
- Vonshak, M., Dayan, T., Ionescu-Hirsh, A., Friedberg, A. & Hefetz, A.** 2009. The little fire ant *Wasmannia auropunctata*: a new invasive species in the Middle East and its impact on the local arthropod fauna. *Biological Invasions* **12**: 1825–1837. <https://doi.org/10.1007/s10530-009-9593-2>.
- Wetterer, J.K.** (2013) Worldwide spread of the little fire ant, *Wasmannia auropunctata* (Hymenoptera: Formicidae). *Terrestrial Arthropod Reviews* **6**: 173–184. <https://doi.org/10.1163/18749836-06001068>
- Wetterer, J.K. & Porter, S.D.** 2003. The Little Fire Ant, *Wasmannia auropunctata*: distribution, impact and control. *Sociobiology* **41**: 1–41.
- Wilson, T.H. & Cooley, T.A.** 1972. A chalcidoid planidium and an entomophilic nematode associated with the western flower thrips. *Annals of the Entomological Society of America* **65**: 414–418. <https://doi.org/10.1093/aesa/65.2.414>

Appendix. Material examined for this study.

Hawaiian Material Examined (deposited in UCRC or BPBM, with UCRC_ENT00 code [abbreviated UC]; DNA number = Dxxxx): **USA: Hawaiian Islands: Hawai'i I:** Hilo, 112 m, 19°41'52"N 155°05'44"W, 14–17 Oct 2019, Valle Rogers, along boundary between lawn and forest, YPT, 0406.19 [6♀, BPBM: UC422320, UCRC: UC468543 (**D6943**), UC468544 (**D6943**), UC468555–57]. Hilo, 108 m, 19°41'56"N 155°05'45"W, 18 Sep 2020, M.T. Johnson, vegetation along outside fence, YPT [23♀, BPBM: UC422386–95, UCRC: UC499508–20]. Kalapana, Kaimu, Chain of Craters Road, mi 21, 18 m, 19°21'40"N 154°58'38"W, 28 Nov–12 Dec 2020, W.D. Perreira, YBST [17♀, UCRC: UC4995722–38]. Kaumana Dr., 434 m, 19°40'52"N 155°09'20"W, 28 May–8 Jun 2020, W.D. Perreira, YSBT [1♀, UCRC: UC468563 (large form)]. Kawainui Stream, 65m, 19°49'13"N 155°05'42"W, 26 Oct–9 Nov 2019, W.D. Perreira, YSBT [1♀, UCRC: UC468558]. Pahoa District Park, 200 m, 19°29'35"N 154°56'51"W, 29 Feb–14 Mar 2020, W.D. Perreira, YSBT [2♀, BPBM: UC422315–16 (16 = large form)]. Pahoa District Park, 200 m, 19°29'35"N 154°56'51"W, 8–22 Jun 2020, W.D. Perreira, YSBT [3♀, UCRC: UC468559–61]. Pana'ewa Zoo, 35 m, 19°19'16"N 155°04'16"W, 27 Dec–8 Jan 2020, W.D. Perreira, YSBT [40♀ 1♂, UCRC: UC499539–79]. Pana'ewa Zoo, 35 m, 19°19'16"N 155°04'16"W, 8–20 Jun 2020, W.D. Perreira, YSBT [1♀, UCRC: UC468562]. Hwy 130, Keauohana For. Res., 240 m, 19°24'51"N 154°57'08"W, 25 Sep 2020, M.T. Johnson, along forest edge, YPT [10♀, BPBM: UC422317–19, UCRC: UC499501–507].

Neotropical Material Examined (deposited in UCRC, with UCRC_ENT00 code [abbreviated UC], unless otherwise indicated; DNA number = Dxxxx followed by size class: s = small, m = medium, l = large; coordinates in italics estimated from google earth): **British Virgin Islands: Tortola:** Mt. Sage Nat. Pk., Sage Mt., 18°24'14"N 64°39'39"W, 10 Dec–8 Jan 1993, M.A. Ivie & T. Hughes, nr. toilets, flight intercept trap #4 [1♀, UC235958 (**D0424s**)]. **Colombia: Cauca:** PNN Gorgona, El Saman, 5m, 2°58'0"N 78°11'0"W, 6–22 Mar 2001, H. Torres, Malaise trap, M.1476 [1♀, UC447075 (**D4208s**)]. PNN Gorgona, El Saman, 5 m, 2°58'0"N 78°11'0"W, 7–25 May 2001, H. Duque, malaise trap, M.1844 [1♀,

UC447074 (**D4207s**)]. **Dominica**: Central Forest Reserve, 337 m, 15°26'29"N 61°19'40"W, 15 May 2009, J. Heraty, rainforest, sweep, H09-018 [1♀, UC235970 (**D2762s**)]. Northern Forest Reserve Sympa trail (upper site), 390 m, 15°31'55"N 61°21'35"W, 14 May 2010, J. Heraty, H10-016 [3♀, UC271392 (**D2830s**), UC271393 (**D2831m**), UC271395 (**D2833l**)]. Sulphur Springs, pools trail, 290 m, 15°14'21"N 61°20'50"W, 16 May 2009, J. Heraty, rainforest, sweep, H09-019 [2♀, UC235966 (**D2766m**), UC235967 (**D2765m**)]. Sulphur Springs, pools trail, 290 m, 15°14'21"N 61°20'50"W, 20 May 2009, J. Heraty, rainforest, sweep, H09-025 [2♀, UC235968 (**D2764m**), UC235969 (**D2763s**)]. **Puerto Rico: Maricao**: Maricao Forest, rd 120 km 17.9, 18°10'51"N 66°58'48"W, 22 Oct 2002, Gates, rd edge and shrubs, sweep [1♀, UC456205 (**D2808s**)]. **St. Lucia: W.I.**: Barre del Isle, 13°52'23"N 60°58'1"W, 2 Mar 2000, L. Masner, forest trail, ss [1♀, UC235964 (**D0437s**)]. **Trinidad**: Gasparillo, 10°18'57"N 61°25'7"W, 5–15 Nov 1987, R. Borneo, grass/forest edge, malaise trap, ROM 870031 [1♀, ROME: UC364799 (**D2810s**)]. Maracas Falls, 190 m, 10°43'28"N 61°24'32"W, 16 Jul 2013, Heraty, forest, swp, H13-044 [2♀, UC412118 (**D3800s**), UC412119 (**D3801s**)]. Maracas Falls, 190 m, 10°43'28"N 61°24'32"W, 23 Jul 2013, Heraty&Baker, forest, YPT/PB bait, H13-073 [3♀, UC412127 (**D3809s**), UC412131 (**D3814s**), UC412132 (**D3810s**)].