*Zootaxa* 5116 (1): 107–122
https://www.mapress.com/zt/

**Article**

ISSN 1175-5326 (print edition)
**ZOOTAXA**
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5116.1.5
http://zoobank.org/urn:lsid:zoobank.org:pub:326AFAC4-6A8E-4F40-88F8-7D626210D810

# A new species of *Phymastichus* (Hymenoptera: Eulophidae: Tetrastichinae) parasitic on *Xyleborus* beetles (Coleoptera: Curculionidae: Scolytinae) in Hawai‘i, and aspects of its biology, life history, and behavior

DAVID N HONSBERGER[1,2] & MARK G WRIGHT[1,3]
[1]*Entomology Section, Department of Plant and Environmental Protection Sciences, College of Tropical Agriculture and Human Resources, University of Hawai'i at Mānoa, 3050 Maile Way, Honolulu, HI 96822, U.S.A.*
[2] *dnh8@hawaii.edu;* *https://orcid.org/0000-0002-3656-7258*
[3] *markwrig@hawaii.edu;* *https://orcid.org/0000-0002-1643-7560*

## Abstract

A third species in the genus *Phymastichus* LaSalle (Hymenoptera: Eulophidae), *Phymastichus holoholo* **sp. nov.**, is described from specimens endoparasitic on adult *Xyleborus* beetles in Hawai‘i. Plant and insect host records known for this species, and aspects of its searching, oviposition, and emergence behavior are discussed and illustrated with photographs and video. This new species, along with *Phymastichus xylebori* which is also present in Hawai‘i, has potential as a biological control agent against *Xyleborus* beetles where they are problematic. Of special interest is their potential as natural enemies of *X. ferrugineus*, *X. affinis*, and *X. perforans*, which have recently been implicated in possibly spreading Rapid ‘Ōhi‘a Death in Hawai‘i. Some aspects of their potential use to combat this disease, as well as some important but as of yet unanswered questions, are discussed. Two observed instances of parasitism by the newly described species of beetles in the *Euwallacea fornicatus* species complex, *Euwallacea fornicatus* (*sensu stricto*) and *Euwallacea perbrevis*, are also reported.

**Key words:** *Phymastichus holoholo*, Bark beetles, Rapid ‘Ōhi‘a Death, *Euwallacea fornicatus*

## Introduction

There are currently three known species in the genus *Phymastichus* LaSalle (Hymenoptera: Eulophidae: Tetrastichinae) including the one described here. *Phymastichus coffea* LaSalle, 1990 is an idiobiont endoparasitoid of the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) from Africa, and has been imported to much of the coffee growing world as a biocontrol agent with varying, but occasionally high levels of success (Vega *et al.* 2015). *Phymastichus xylebori* LaSalle, 1995 was described from specimens collected in Hawai‘i, South Carolina, and Costa Rica, the ones in Hawai‘i found emerging from *Xyleborus perforans* (Wollaston) in macadamia trees (*Macadamia integrifolia* Maiden & Betche) on Hawai‘i island (LaSalle 1995). The presently described species has so far been found in Hawai‘i on the islands of O‘ahu and Hawai‘i, and like *P. xylebori*, also uses *Xyleborus* beetles as hosts. All three species are thus parasitoids of scolytids. All three species also have the uncommon behavior among bark beetle parasitoids of parasitizing adult beetles. They are the only members of Tetrastichinae known to attack the adult stage of their hosts, a character which LaSalle (1995) proposed could potentially represent a synapomorphy that could be used as a diagnostic feature for this genus. All three also have the morphological character of a swollen parastigma of the forewing, a character found in only one other Tetrastichinae genus, *Paraspalangia* Girault, and lack a ventral plaque on the male scape (LaSalle 1995). Insect and plant hosts for this new species, and aspects of biology and behavior, will be further described in the biology section. All three have potential as biological control agents. Some of their hosts in their nonnative ranges are pests of agriculture or forest ecosystems, or vectors of plant disease.

*Accepted by N. Dale-Skey: 1 Feb. 2022; published: 17 Mar. 2022*

## Methods

### Molecular analysis

The two species of *Phymastichus* found to occur in Hawai‘i are morphologically similar, and share host range and habitat. To determine whether these species are indeed distinct, DNA was extracted from specimens of the presently described species emerged from octopus tree (*Schefflera actinophylla* (Endl.) Harms) in the Kahana Bay area of O‘ahu (21.5573 N, -157.8783 E) and specimens fitting the description of *P. xylebori* from Waimānalo, O‘ahu (21.3342 N, -157.7111 E), using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Inc., Valencia, Ca, USA). The CO1 gene was amplified using the primers of Folmer *et al.* (1994), and the D2 region of the 28S gene was amplified using the primers D2-3625 and D3-4283 in Gillespie *et al.* (2005). The resulting sequences for the newly described species are published on GenBank accession #MZ357963 and #OM327543–OM327547, and for *P. xylebori* on GenBank accession #MZ348365 and #OM327578.

### Species description

*Phymastichus* spp. were examined and photographed using a Leica MZ16 stereomicroscope. Specimens were also dissected, examined, and photographed using an Olympus CX31 compound microscope. Terminology relating to morphological characters follows Gibson *et al.* (1998).

### Biology

Methodology relating to biological and behavioral observations under field and laboratory conditions is described in detail in the respective sections.

## Results

### Molecular Analysis

CO1 sequences were obtained from five individuals of the presently described species and one individual of *P. xylebori*, and 28S sequences were obtained from one individual of each. Ends of the resulting sequences were clipped, and there was no variation detected within either of the proposed species. In CO1, out of 615 bp there were 53 substitutional differences between the species, giving a 91.4% similarity. For 28S, out of 536 bp there were 13 substitutional differences, giving a 97.3% similarity between the species. For both genes, and since the 28S gene is typically conserved, this amount of difference is consistent with the specimens belonging to two distinct species (Campbell *et al.* 1993).

## Key to known species of *Phymastichus* (females)

1 Syntergum without strong step-like carina. The cubital and subcubital setal lines of the forewing distinctly separate until approximately the apex of the trailing edge of the forewing (the longitudinal non-setose region at the very posterior of the forewing extends approximately the entire length of the trailing edge of the wing) (as in Figs 1d; 3a,d). Ovipositor not exposed. Coxae of all legs dark, of similar color to body . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . *P. coffea*

\- Syntergum with strong step-like carina, giving the apex of the gaster a crimped appearance dorsally (Figs 1a,b,e,f; 3 h). Non-setose region at the very posterior of the forewing between the cubital and subcubital setal lines extending between half the length of the wing to the full length of the trailing edge of the wing. Ovipositor exposed or not exposed in dry specimens. Procoxae of similar color to body, meso- and metacoxae distinctly lighter in color than body, metacoxae yellow to translucent white (Fig. 1a,e) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 2

2 Scale-like sculpture anterior to carina on the syntergum, and posterior to the carina, cuticle covered with a thick tuft of light-colored setae that extends to or past the end of the gaster (Fig. 1e,f). Pedicel yellow to brown, flagellum dark brown. Separation between the cubital and subcubital setal lines becomes indistinguishable at approximately half the length of the forewing, near the distal apex of the retinaculum (the longitudinal non-setose region at the very posterior of the forewing extends approximately half the length of the trailing edge of the wing) (Fig. 1g). Ovipositor usually exposed, and flattened laterally, blade-like (Fig. 1e,f) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . *P. xylebori* (Fig. 1e–g)

\- Cuticle smooth both anterior and posterior the carina on the syntergum, and with few setae medially (Figs 1a,b; 2a,b; 3h). Pedicel usually dark brown, similar in color to body and flagellum though can be lighter in some individuals. The cubital and subcubital setal lines of the forewing may become closer together at the distal apex of the retinaculum, but are distinctly separate until near the apex of the trailing edge of the forewing (the longitudinal non-setose region at the very posterior of the forewing extends approximately the entire length of the trailing edge of the wing) (Figs 1d; 3a,d). Ovipositor usually retracted in dry or alcohol preserved specimens, but if visible thin and needle-like (Fig. 1a–c; 2a) ....... *P. holoholo* **sp. nov.** (Figs 1a–d; 2–3)

## Description *of Phymastichus holoholo* sp. nov.

**Diagnosis:** Among described genera of Tetrastichinae, *Phymastichus holoholo* falls within the genus *Phymastichus* as redescribed by LaSalle (1995) given the characters of a swollen parastigma on the forewing; male scape lacking a sensory plaque; scutellum without submedian or sublateral sulci, and anterior pair of setae closer to anterior margin of scutellum than posterior setae (though only slightly in this species); mesoscutum without median sulcus.

Overall, females of this species can be distinguished within Tetrastichinae by the combination of the enlarged parastigma and hyaline forewing membrane (Figs 1d; 3a,d); a step-like carina on the syntergum, giving the apex of its gaster a crimped appearance dorsally, with sparse setae on either side of the carina and lacking a distinct tuft of setae posterior to the carina (Figs 1a,b; 2a,b; 3h); the subcubital and cubital setal lines of the forewing distinctly separated until near the apex of the trailing edge of the wing (Figs 1d; 3a,d); coloration and general habitus (Figs 1a; 2). They parasitize the adult stage of some *Xyleborus* beetles and potentially other Scolytinae as well.

**Differential diagnosis:** Among the other described species of *Phymastichus*, *P. holoholo* females can be distinguished from *P. coffea* by a strong step-like carina on the syntergum (Figs 1b; 3h) (this step-like carina absent in *P. coffea*); meso- and metacoxae lighter in color than the dark-brown to black body, metacoxae yellow to translucent white (Figs 1a; 2a) (all coxae of similar color to the dark-brown to black body in *P. coffea*).

*Phymastichus holoholo* females can be distinguished from *P. xylebori* by the smooth cuticle anterior and posterior to the carina on the syntergum, and few setae medially posterior to the carina (Figs 1a,b; 2a,b; 3h) (scale like sculpture anterior to the carina on the syntergum, and posterior to the carina, a dense tuft of light colored setae extending past the end of the gaster in *P. xylebori* (Fig. 1e,f)); ovipositor thin and needle-like, usually retracted in dry or alcohol preserved specimens and not visible (Figs 1a–c; 2a) (ovipositor distinctly laterally flattened in *P. xylebori*, and usually visible in dry or alcohol preserved specimens (Fig. 1e,f)). Both sexes of *P. holoholo* can be distinguished from *P. xylebori* by the trailing edge of the forewing, where the cubital and subcubital setal lines of the forewing remain distinctly separate and with no setae between them until approximately the apex of the trailing edge of the wing (Figs 1d; 3a,d) (the cubital and subcubital lines become difficult to distinguish at approximately half the length of the forewing, near the distal apex of the retinaculum in *P. xylebori* (Fig. 1g)).

***Female*** (Figs 1a–d; 2a–c; 3a–c,g,h):

**Length**: 1.2–1.5 mm (Holotype 1.3 mm). Size in general correlates with the size of the beetle host. The length range recorded here is for wasps emerging from the beetles *Xyleborus ferrugineus* (Fabricius), *Xyleborus affinis* Eichhoff, and *Xyleborus perforans* (Wollaston), the currently confirmed hosts. If this wasp uses hosts other than these beetles, its size is expected to vary according to the size of the beetle, and may fall outside this range.

**Head**: Cuticle dark brown and shiny, with lightly reticulate sculpture visible under high magnification. Toruli located just above level of lower edge of eyes, scape reaching approximately to the level of the anterior ocellus. Scrobal depression shallow, its lateral edges smoothly rounded. Scrobal depression an inverted heart shape, widest at the toruli and tapering to a point below the anterior ocellus, a small triangular shaped interscrobal crest protruding between the toruli and reaching to approximately the level of the top of the toruli. The floor of the scrobal depression evenly rises to a shallow medial elevation that runs from the apex of the depression to the interscrobal crest. Frontofacial sutures consisting of two thin white lines, beginning at the outer margin of the two posterior ocelli and converging at the apex of the scrobal depression. Note that in air dried specimens much of the face from above the clypeus to the ocelli, including the entire scrobal area, is almost always collapsed and many of these characters are obscured. Antennae with scape, pedicel, a single anellus, 3 funicular segments, and a 3-segmented clava. Pedicel and flagellomeres apical of the anelli dark brown, similar in color to body, the scape lighter in color. The anellus typically appears more yellow than the rest of the antennae, and the pedicel can also be lighter in color than the flagellum in some individuals. Flagellum setose, and with sensilla including four apically converging terminal sensillar projections on the last segment of the clava that can be seen under high magnification. Setae on the pedicel and flagellum angled about 30–45 degrees from the apical direction of the antenna, and approximately 1/2 to 2/3 as long as the funicular segments are wide. Scape also setose, though the setae are shorter and thinner than on the pedicel and flagellum, and with a flattened area ventrally to receive the rest of the antenna when folded. Funicular segments longer than wide. Ratio of the length of the antennal segments, not including the anellus, approximately

Scape : Pedicel : F1 : F2 : F3 : F4 : F5 : F6 = 2.2 : 1.2 : 1.0 : 0.9 : 1.0 : 0.8 : 0.7 : 0.5. Eyes red to black with sparse, very small, short setae. Ocelli positioned in the form of an obtuse triangle, posterior ocelli closer to compound eyes than to each other, cuticle of the ocellar triangle raised relative to the rest of the vertex. Setae short on vertex, longer and of similar density on the face, shorter and of higher density on occiput. Margins of clypeus not delimited by a suture, but its upper corners with an anterior tentorial pit on each side. Malar sulcus complete. Mandibles tridentate and symmetrical, the ventral tooth pointed but not sharply, the middle tooth broadly rounded, the dorsal tooth differentiated from the overall curvature of the inner edge of the mandible by a small notch.

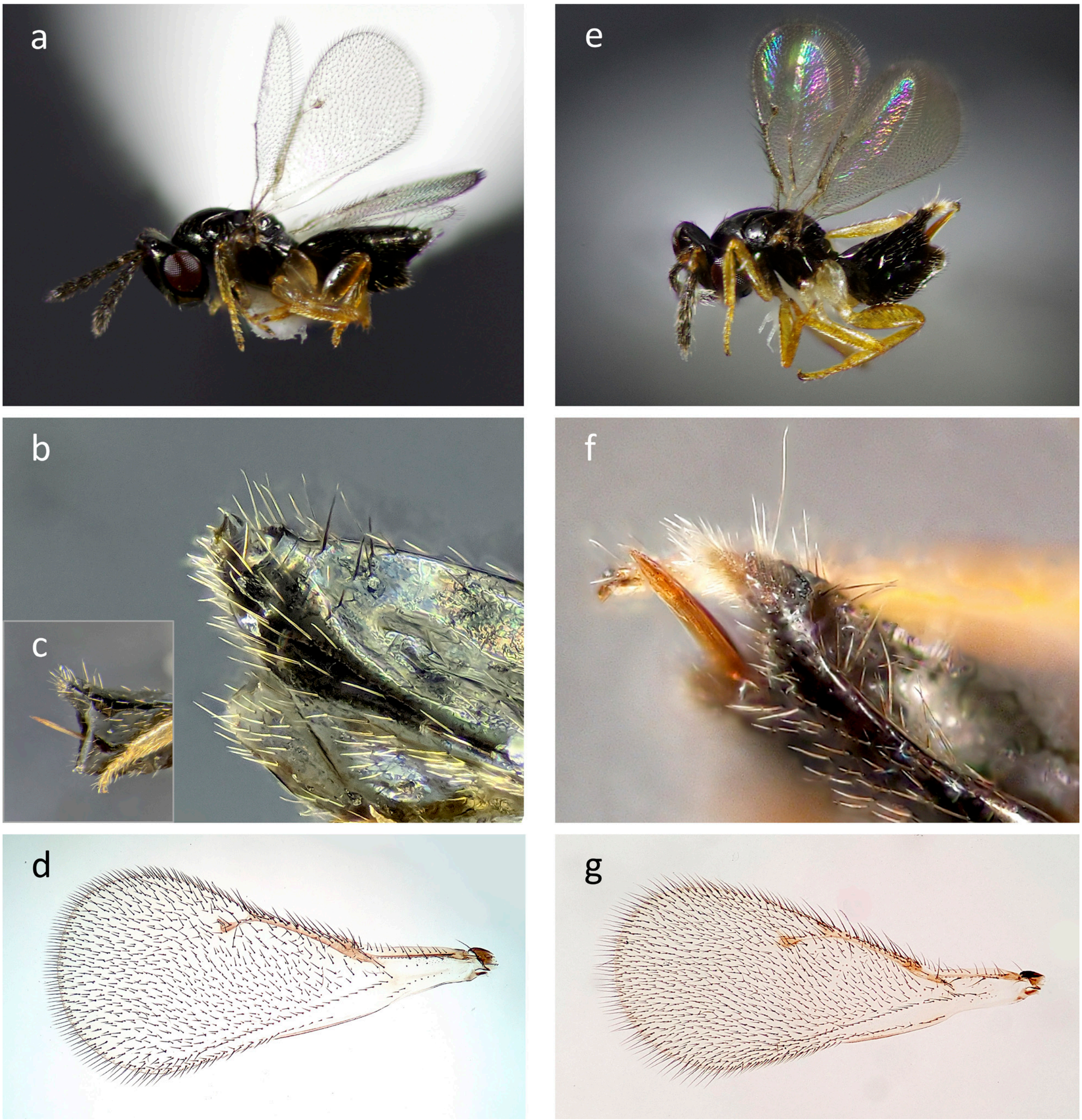

**FIGURE 1.** Comparison of *Phymastichus holoholo* **sp. nov.** (left column) and *Phymastichus xylebori* (right column). **a:** *Phymastichus holoholo* **sp. nov.** ♀ (ex *Xyleborus affinis* in *Macadamia integrifolia* trunk, Kea‘au, Hawai‘i island); **b:** *Phymastichus holoholo* **sp. nov.** ♀ (ex *Schefflera actinophylla* branch, Kahana Bay, O‘ahu), apex of metasoma showing the crimped syntergum with sparse setae; **c:** *Phymastichus holoholo* **sp. nov.** ♀ (same data as **(b)**), a specimen with ovipositor exposed and visible; **d:** *Phymastichus holoholo* **sp. nov.** ♀ (same data as **(b)**), forewing; **e:** a specimen fitting the description of *Phymastichus xylebori*, ♀, (ex *Macadamia integrifolia* trunk, ‘Āhualoa, Hawai‘i island); **f:** *Phymastichus xylebori* ♀ (ex *Macadamia integrifolia* trunk, Waimānalo, O‘ahu), apex of metasoma showing the crimped syntergum with a tuft of dense setae posteriorly and blade-like ovipositor; **g:** *Phymastichus xylebori* ♀ (same data as **(f)**), forewing.

**Mesosoma:** Cuticle, with the exception of the legs (see below) and tegula which are typically translucent yellow, dark brown to black and shiny with light reticulate sculpture visible under high magnification. Pronotum with a transverse row of posterior pointing setae near the border with the mesoscutum, except near the midline which is free of setae, the first one or two setae lateral of the midline longer than the others. Additional setae often present anterior to this row. Mesoscutum lacking a medial sulcus on cuticle surface, and with three adnotaular setae, the anterior one approximately even with most anterior reach of the axillae, the two posterior setae near the scutellum, close together, and longer than the anterior seta. Mesoscutum mesal of notauli otherwise free of setae. Mesoscutal lateral lobe with setae pointing in various directions, of similar density to those on the posterior region of the pronotum. Scutellum with two pairs of setae near the lateral margins of the disk, one pair slightly anterior of the middle and the other pair near the posterior margin, a pit also present between the anterior and posterior setae. Lateral to the scutellar disk, the scutellum transitions smoothly to the impressed axillula where the cuticle is slightly wrinkled but lacks the set of distinct carinae present in *P. xylebori*. Propodeum strongly indented posteriorly to receive the small petiole, its margin also slightly concave where it comes up against the dorsellum. Median propodeal carina present but wide and somewhat inconspicuous. Plicae absent, callus with setae posterior and lateral to the spiracle, including on the convex region where the inclination of the sclerite becomes almost vertical.

**Legs:** Yellow to translucent yellow below coxae, though in some individuals the femora also have a black tint. Procoxae dark brown to black, similar in color to the body. Meso- and metacoxae translucent yellow, often lighter in color than the rest of the leg. Mildly curved tibial spurs on all legs. Tarsi and tibiae setose, femora with some setae but largely bare. Pro- and mesofemora of similar shape and thickness, metafemur substantially thicker. First through third tarsal segments subequal in length in all legs, fourth not including the pretarsus slightly longer. Tarsal claws brown in color, more robust in the mid and hind legs than in the foreleg.

**Wings:** Forewing: Parastigma slightly to distinctly swollen, as is characteristic for this genus. Cubital and subcubital setal lines join near the apex of the trailing edge of the wing, though the non-setose region between them becomes narrower distal to the retinaculum. Postmarginal vein protrudes only slightly if at all from the junction of the marginal and stigmal veins, and is much shorter than the stigmal vein. Marginal and submarginal veins subequal in length. Submarginal vein usually with three strong dorsal setae, and other smaller setae on the membrane anterior to the vein. Marginal vein with many strong setae. Veins somewhat uniformly yellow-brown in color, except for the stigmal vein which is transparent basal of the stigma. Stigma yellow-brown, and uncus present with four sensilla. Membrane subhyaline, apical of the small speculum and anterior to the cubital setal line covered approximately evenly over its surface with short setae. These setae are uniformly produced on the dorsal surface of the wing. On the ventral side, in the apical region of the wing they are similar in length to those on the dorsal side, but become strongly reduced in the medial and basal regions, especially closer to the trailing edge.

Hindwing: Membrane hyaline, the density and size of setae on the dorsal surface similar to that of the forewing; setae on the ventral surface reduced to dark spots. Trailing edge with long marginal setae consistently about 2/5 the maximum width of the hindwing. Veins yellow-brown in color. Three hamuli spaced closely together extend forward from the venation near its apex.

**Gaster:** Cuticle shiny dark brown to black, with somewhat reticulate sculpture visible under high magnification. Each gastral segment except the last with a transverse line of setae, setae longer and more upright on posterior segments. Gaster with hypopygium often distinct in dried specimens, extending near to the end of the gaster, cavernous posteriorly. The hypopygium is, however, not clearly distinguished in some individuals, and the shape of the gaster as a whole can vary. Relative to the rest of the gaster, setae increase in density on the hypopygium around the margins of the cavity and on the ventral part of the gaster posterior to the hypopygium. Syntergum with distinct step-like carina, similar to that of *P. xylebori*, giving the very end of the gaster a crimped appearance. Cerci located directly posterior to the carina. Anterior to the carina, the cuticle is smooth, without scale-like sculpture as in *P. xylebori*, and medially free of setae. Posterior to the carina, the cuticle is also smooth and shiny, the same color as the rest of the gaster and with at most a few short setae medially, free of long setae other than those emerging from the cerci. Ovipositor almost always retracted and only rarely projecting in dry or alcohol preserved specimens, but if visible, straight and needle-like, not especially flattened laterally in cross section as it is in *P. xylebori*.

***Male*** (Figs 2d–f; 3d–f)**:** Similar in overall shape and coloration to female, but with the following differences:

**Size:** 0.7–0.8 mm long (Allotype 0.75 mm). Typically much smaller than the females, and always smaller than a female emerging from the same host beetle.

**Head** as in female except: Antennae substantially thinner than in the female and with one more flagellar segment, consisting of scape, pedicel, a single anellus, and 7 flagellomeres, the last 3 of which form a clava. Ratio of the length of the antennal segments, not including the anellus, is approximately Scape : Pedicel : F1 : F2 : F3 : F4 : F5 : F6 : F7 = 2.9 : 1.8 : 1.0 : 1.0 : 1.1 : 1.3 : 1.2 : 1.1 : 0.8. Antennae setose and with sensilla, setae longer than the width of the funicular segments. Last antennal segment ends in a single terminal sensillum. As in the two other known species of *Phymastichus*, and unlike all other known members of Tetrastichinae, males lack a ventral sensory plaque on the scape.

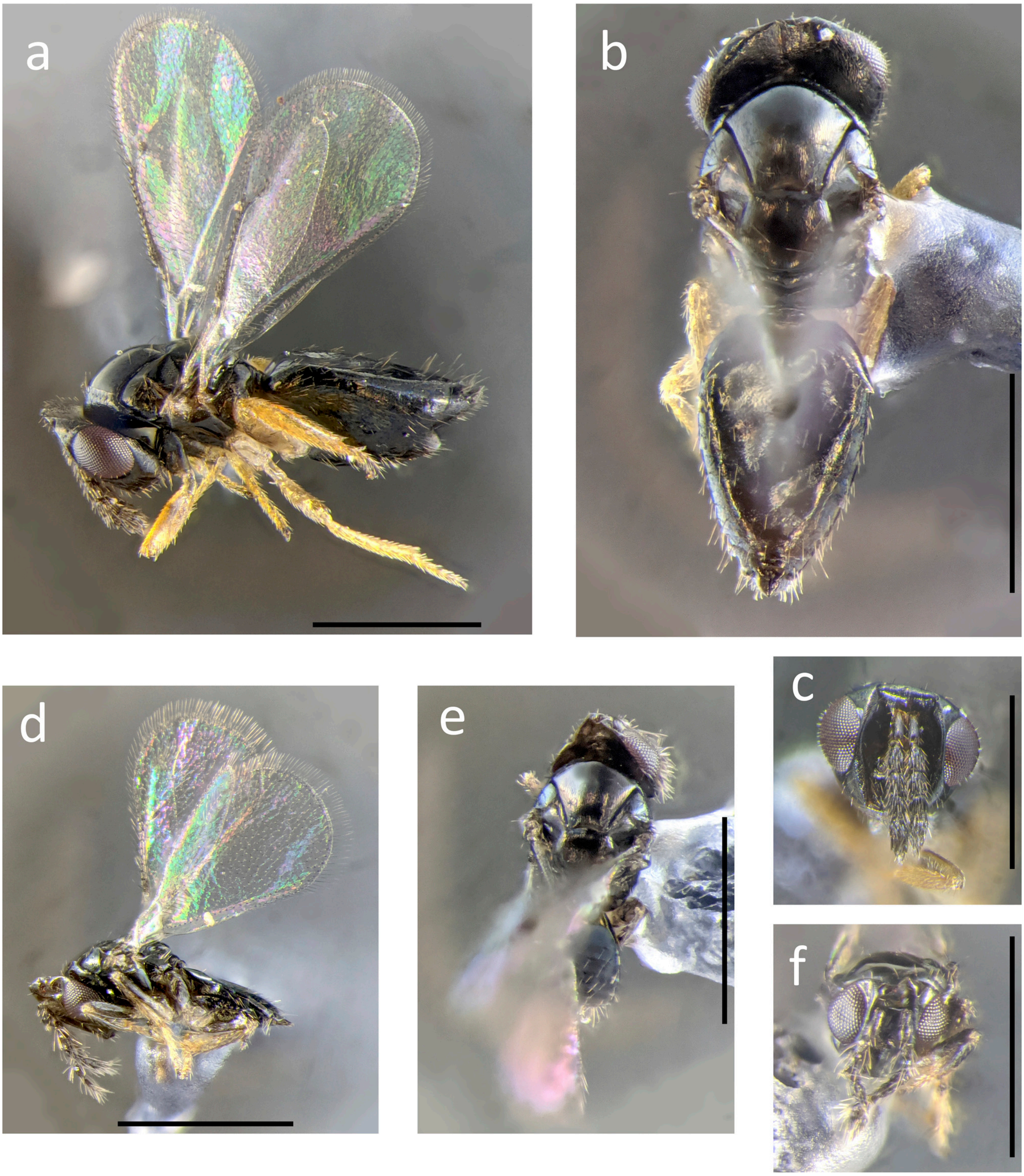

**FIGURE 2.** *Phymastichus holoholo* **sp. nov.** adults, holotype (♀) and allotype (♂). **a:** holotype ♀, side view; **b:** holotype ♀, dorsal view; **c:** holotype ♀, head, note that the scrobal area is somewhat collapsed due to drying of specimen; **d:** allotype ♂, side view; **e:** allotype ♂, dorsal view; **f:** allotype ♂, head. Scale bars: 500µm

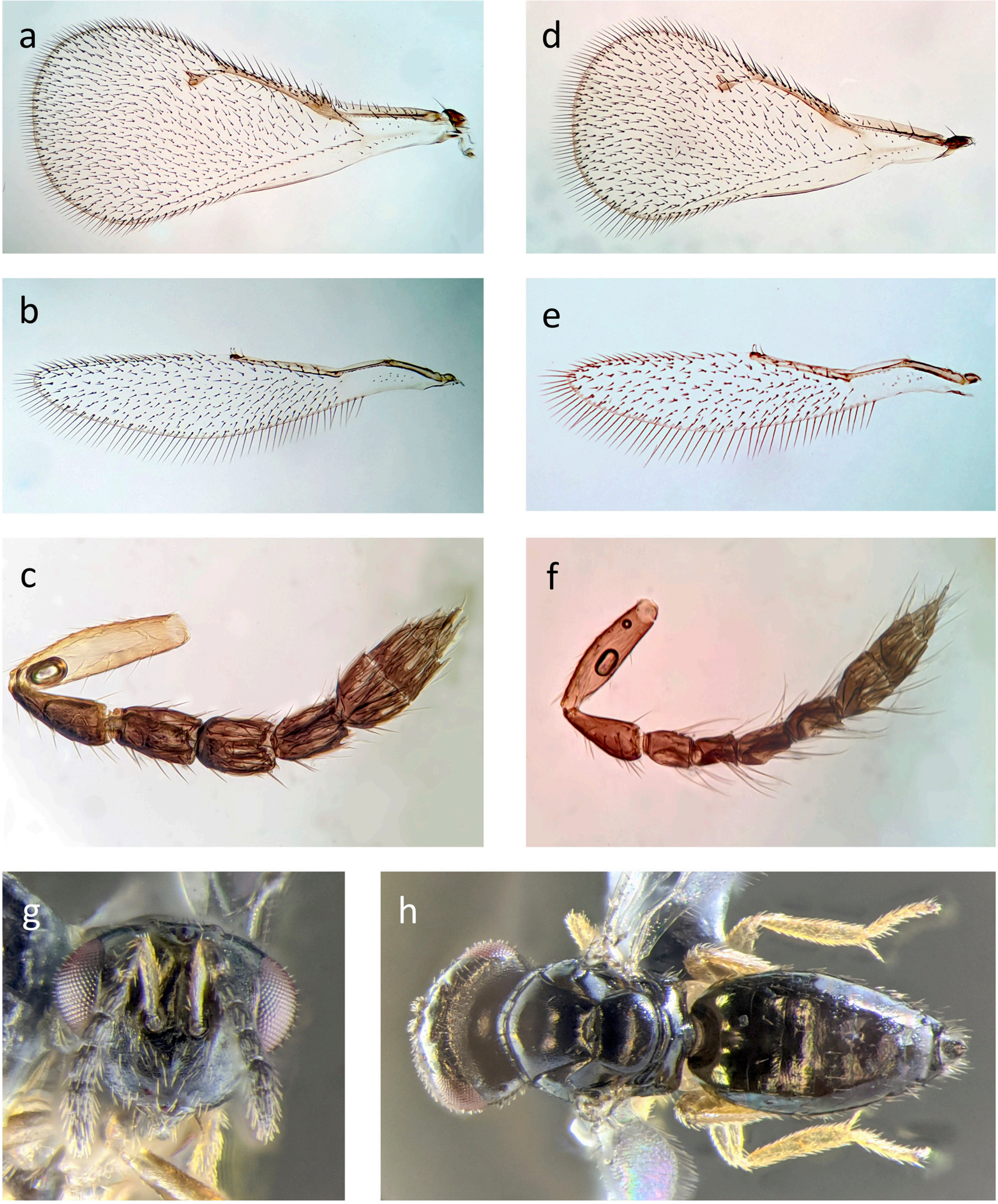

**FIGURE 3.** *Phymastichus holoholo* **sp. nov. a:** forewing ♀; **b:** hindwing ♀; **c:** antenna ♀; **d:** forewing ♂; **e:** hindwing ♂; **f:** antenna ♂; **g:** head (uncollapsed) ♀; **h**: dorsal view ♀.

**Mesosoma** as in female except: **Legs** with all coxae dark brown at least basally and medially, of similar color to the body, often lighter apically; femora and tarsi also typically somewhat darker than in the female, though this varies among individuals. **Wings** similar in overall structure to female except forewing with parastigma somewhat swollen but variable and typically not as swollen as in the female; submarginal vein often with only two long setae in addition to shorter setae.

**Gaster:** Overall somewhat oval in shape in dorsal view; setae in a similar pattern though overall less setose than in females; last tergite free of setae except for those emerging from the cerci.

## Repositories

UHIM—University of Hawai‘i Insect Museum, Honolulu, Hawai‘i, USA
BPBM—Bernice Pauahi Bishop Museum, Honolulu, Hawai‘i, USA
NHMUK—The Natural History Museum, London, UK

**Holotype:** O‘ahu, Kahana Bay (21.5573 N, -157.8783 E, 15 m), 22.vii.2020, ♀, ex *Xyleborus* beetles from *Schefflera actinophylla* (deposited in UHIM).

**Allotype:** O‘ahu, Kahana Bay (21.5573 N, -157.8783 E, 15 m), 22.vii.2020, ♂, ex *Xyleborus* beetles from *Schefflera actinophylla* (UHIM).

**Paratypes:** O‘ahu, Kahana Bay (21.5573 N, -157.8783 E, 15 m); 10.ix.2020; 11 ♀, 2 ♂; ex *Xyleborus* beetles from *Schefflera actinophylla*; (6 ♀, 2 ♂ UHIM; 5 ♀ BPBM) • O‘ahu, Kahana Bay (21.5573 N, -157.8783 E, 15 m); 22.vii.2020; 18 ♀, 5 ♂; ex *Xyleborus* beetles from *Schefflera actinophylla*; (8 ♀, 1 ♂ UHIM; 5 ♀, 2 ♂ BPBM; 5 ♀, 2 ♂ NHMUK) • O‘ahu, Kahana Bay (21.5604 N, -157.8765 E, 15 m); 31.i.2020; 3 ♀, 2 ♂; ex *Xyleborus ferrugineus* from *Mangifera indica*; (UHIM) • Hawai‘i island, ‘Āhualoa; 30.x.2019; 1 ♀, 1 ♂; ex *Xyleborus perforans* from *Macadamia integrifolia*; (UHIM) • Hawai‘i island, Kea‘au; 1.xi.2019; 4 ♀, 4 ♂; ex *Xyleborus ferrugineus* from *Macadamia integrifolia*; (2 ♀, 2 ♂ UHIM; 1 ♀, 1 ♂ BPBM; 1 ♀, 1 ♂ NHMUK) • 2 ♀, 1 ♂; same data as previous except ex *Xyleborus affinis* from *Macadamia integrifolia*; (BPBM) • 1 ♀, 1 ♂; same data as previous except ex *Xyleborus perforans* from *Macadamia integrifolia*; (NHMUK) • O‘ahu, Mānoa valley (21.3293 N, -157.7972 E); 15.ix.2020; 3 ♀, 4 ♂; ex *Xyleborus affinis* from *Cecropia obtusifolia*; (1 ♀, 1 ♂ UHIM; 1 ♀, 2 ♂ BPBM; 1 ♀, 1 ♂ NHMUK).

**Etymology.** This wasp strolls along the surface of wood to search for its hosts. The species name is Hawaiian, meaning “to go out for a walk or to see the sights” and is to be treated as an indeclinable noun in apposition.

## Biology

### Known host range

*Phymastichus holoholo* **sp. nov.** has been reared from *Xyleborus ferrugineus*, *X. perforans*, and *X. affinis* adult beetles in octopus tree (*Schefflera actinophylla*) in the Kahana Bay area of O‘ahu; the same three beetle species in macadamia (*Macadamia integrifolia*) in the areas of ‘Āhualoa, Kapa‘au, and Kea‘au on the island of Hawai‘i; from *X. ferrugineus* in mango (*Mangifera indica* L.) branches in the Kahana Bay area of O‘ahu; and from *X. affinis* in trumpet tree (*Cecropia obtusifolia* Bertol.) branches near the upper reaches of Mānoa valley on O‘ahu.

Emergence holes on the elytral declivity of the beetle, typical for this genus as observed in LaSalle (1995), Espinoza *et al.* (2009), and the present study, were found on two Hawaiian endemic *Xyleborus lanaiensis* Perkins beetles extracted from a fallen ‘āla‘a (*Planchonella sandwicensis* (A. Gray) Pierre) tree in the Wai‘anae mountains of O‘ahu (Fig. 4i). We expect these beetles were parasitized by *P. xylebori* or *P. holoholo*, or perhaps another unknown *Phymastichus* species, but cannot know with certainty whether one or both of these species include any members of the Hawaiian endemic radiation of *Xyleborus* beetles in their host range. Hundreds of *X. lanaiensis* individuals were collected from the same tree and no emergence of parasitoids was observed, suggesting the percent parasitism of these beetles at this location in this tree was low. Another similar emergence hole was also found on an *X. ferrugineus* beetle in *Acacia koa* A.Gray on Wa‘ahila Ridge on O‘ahu, though again we do not know by which species it was parasitized.

We also observed two species of Scolytinae that this wasp may use under some circumstances but does not seem to prefer, at least in the environments we have observed it so far. An emergence hole typical of *Phymastichus* was found on a single *Xylosandrus crassiusculus* Motschulsky beetle inhabiting the octopus tree branches used in the calculation of percent parasitism (described later) (Fig. 4j). Since all wasps emerging from beetles within this wood were *P. holoholo* we assume the beetle was parasitized by this species,. Though *X. crassiusculus* is a somewhat common inhabitant of the octopus tree wood in that area, and we have extracted a good number of individuals from those trees during this study (in excess of 100), this is the only individual we have seen that was clearly parasitized.

Though *Euwallacea fornicatus* (*sensu lato*) beetles are also common inhabitants of the octopus tree wood from which we found this species emerging, we have not observed any individuals with a parasitoid emergence hole.

*Phymastichus holoholo* individuals have also been observed to actively walk over *E. fornicatus* (*sensu lato*) beetles naturally infesting octopus tree wood without showing any sign of interest, while showing substantial interest in and parasitism of *Xyleborus* beetles in the same stage of infestation. Despite this, two instances of parasitism have been observed in dissected *E. fornicatus* (*sensu lato*) individuals: one parasitoid larva, confirmed by molecular analysis to be *P. holoholo*, and one seemingly developed adult that eclosed but had not yet emerged from the beetle, were found inside beetles extracted from naturally infested octopus tree wood and identified by DNA sequences as *E. perbrevis* (Schedl) and *E. fornicatus* (Eichhoff), respectively (using the primers of Folmer *et al.* (1994); the CO1 gene matched with specimens on GenBank referenced in Smith *et al.* (2019)). Thus at least some *P. holoholo* individuals may under some circumstances take marginal interest in members of this species complex and are able to complete their development inside them, though such instances seem to be rare.

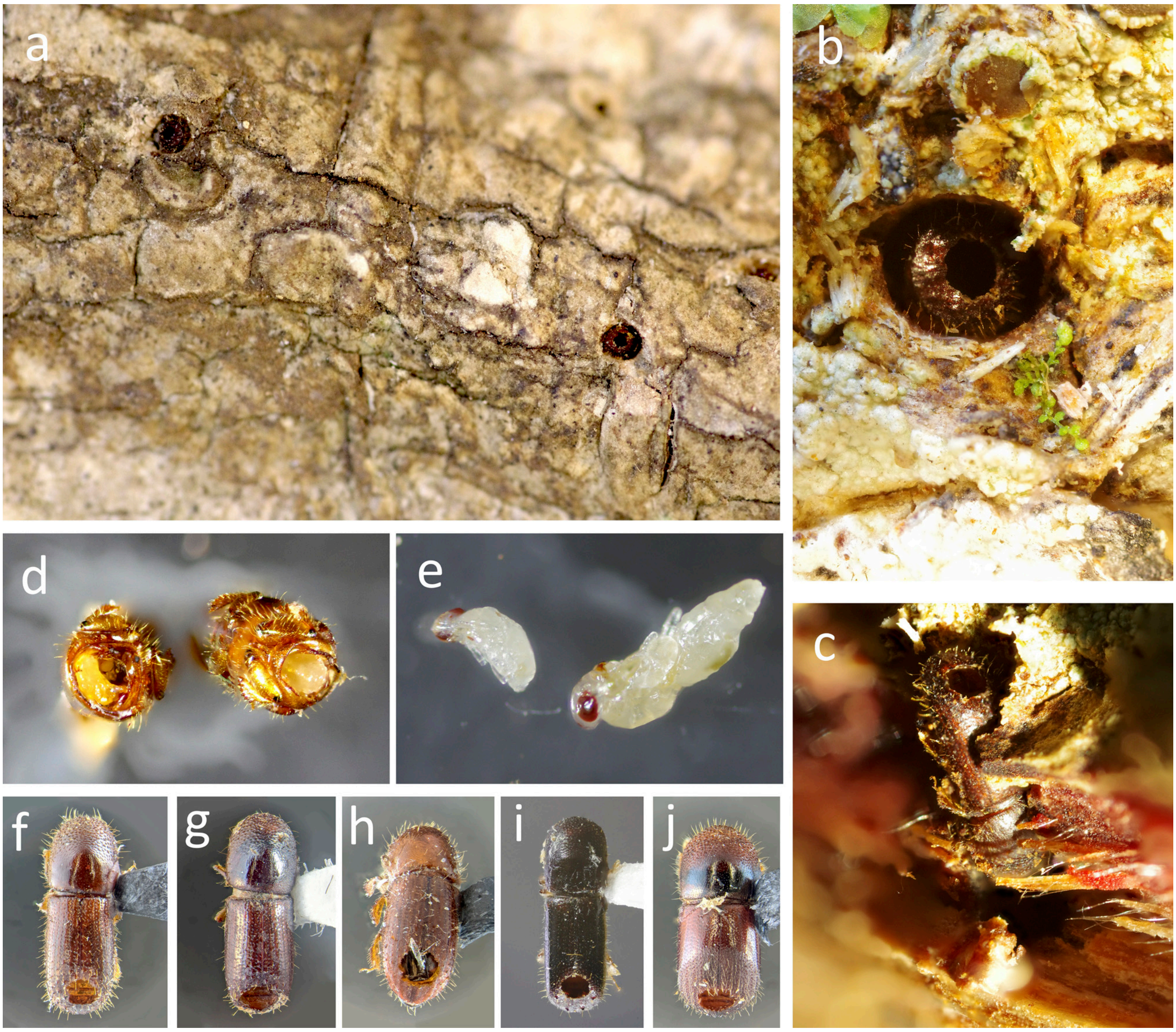

**FIGURE 4.** Parasitism of bark beetles by *Phymastichus*. **a:** Two *Xyleborus ferrugineus* beetles in octopus tree from Kahana Bay, Oʻahu with typical *Phymastichus* emergence holes. **b:** *Xyleborus ferrugineus* beetle in *Acacia koa* from Waʻahila ridge on Oʻahu with a (presumably) *Phymastichus* emergence hole. **c:** The same beetle as in (**b**) partially extracted from wood to show its position; **d:** *X. ferrugineus* from octopus tree near Kahana Bay, Oʻahu, broken open between the pro- and mesothorax to show *Phymastichus* pupae developing inside. On the left is the anterior part of the beetle with the male *P. holoholo* pupa positioned transversely inside. On the right is the rest of the beetle, the female pupa positioned with its head towards the back of the beetle; **e:** The pupae in (**d**) removed from the beetle, female on the right, male on the left; **f,g:** *Xyleborus ferrugineus* from octopus tree near Kahana Bay, Oʻahu with *P. holoholo* emergence holes; **h:** *Xyleborus affinis* from octopus tree near Kahana Bay, Oʻahu with a partially emerged *Phymastichus holoholo* adult female still inside. **i:** Hawaiian endemic *Xyleborus lanaiensis* from ʻālaʻa (*Planchonella sandwicensis*) with a (presumably) *Phymastichus* emergence hole; **j:** *Xylosandrus crassiusculus* from octopus tree near Kahana Bay, Oʻahu with a *Phymastichus holoholo* emergence hole.

We also report a species of Scolytinae that we infer is likely not used as a host by this species. The coffee berry borer, *Hypothenemus hampei* (Ferrari), a very significant pest of coffee in almost all major growing areas outside its native range, also seems typically to be rejected as a host by both *P. holoholo* and *P. xylebori*. *Hypothenemus hampei* individuals (n = 24) were glued into holes both artificially drilled and naturally bored by *X. affinis* beetles in octopus tree wood, and these logs were placed in an area of Kahana Bay on Oʻahu where substantial parasitism of other bark beetles by *P. holoholo* was consistently observed. Upon subsequent rearing and dissection, none were found to have been parasitized. And though *P. holoholo* is known through other observations to readily attack its preferred hosts *X. ferrugineus* and *X. affinis* when they are placed together in small containers, laboratory experiments that exposed *H. hampei* adult females to *P. holoholo* and *P. xylebori* females in a Petri dish, following the methods of Yousuf *et al*. (2021) for host specificity testing of *P. coffea*, did not result in any successful parasitism of *H. hampei* (n = 10 for *P. holoholo*, n = 2 for *P. xylebori*). In another laboratory test, 95 *P. holoholo* individuals were exposed to *H. hampei*, some drilling into coffee berries and others loose on the bottom of the test container. One *P. holoholo* individual was observed attempting to parasitize an *H. hampei* adult, though no progeny emerged from the beetle. *Hypothenemus hampei* is substantially smaller than the *Xyleborus* beetles which are confirmed hosts of *P. holoholo* and *P. xylebori*, and the wasp observed attempting parasitization was an atypically small female. Besides this one instance, no interest in *H. hampei* was observed.

The insects and plants recorded here as hosts of *P. holoholo* are not predicted to be a comprehensive list. More exploration will almost certainly bring records of additional plant species on which this species attacks *Xyleborus* beetles, and possibly of additional hosts. We do not know the native range of this wasp, though two of its hosts in Hawaiʻi, *X. ferrugineus* and *X. affinis*, are thought to be neotropical in origin, while the native range of *X. perforans* is not as clear (Wood 2007).

### Lifespan

Of 52 *P. holoholo* females and males placed in a cubic plexiglass box with sides approximately 30 cm in length and provided with honey as an energy source, 96% were dead between 48 and 72 hours after emergence, and 100% were dead between 72 and 96 hours after emergence. Their lifetime thus seems to be about 2 or 3 days under these conditions. A similar lifespan has been reported for adult *P. coffea* (Espinoza *et al*. 2009).

### An approximation of the fecundity of *P. holoholo*

Mated adult *P. holoholo* females without a chance to have oviposited in any host were dissected and the number of eggs within their gasters were counted. Given their short lifespan, these wasps presumably would not be able to produce new eggs during their lifetimes and are likely proovigenic, so that the number of eggs counted likely represents an accurate estimate of their maximum fecundity. Ten individuals were dissected yielding a mean of 33.5 (SD = 7.0) eggs per individual. Two eggs are typically laid per host beetle (see the section: *Development, emergence, and mating*), which implies the mean maximum number of beetles a single female could parasitize is 16.75 (SD = 3.5).

## Behavior

### Searching and oviposition

Parasitism of *Xyleborus* beetles by *P. holoholo* was observed under field conditions periodically over approximately a one year period. Octopus tree wood bolts approximately 60 cm long and 5 to 8 cm in diameter were cut from living trees and suspended off conspecifics in an octopus tree forest in Kahana Bay on Oʻahu; after approximately 6 weeks they had become infested by a variety of wood associated insects including bark beetles. *Phymastichus holoholo* wasps were regularly observed searching and parasitizing *Xyleborus* beetles on these branches. Their behavior in this environment seems to follow this general pattern:

*Phymastichus holoholo* females fly near the surface of wood containing potential hosts, generally within about 20 cm in an undulating flight pattern, as if scanning the wood to determine its quality. They may then land on the surface of the wood, not necessarily in the direct vicinity of a potential host, and commence searching by walking over the wood surface. This stage of searching seems to be largely visually and tactically mediated, as opposed to using long range olfactory cues, based on the following observations: the wasps walk in a generally linear pattern with few abrupt changes in direction except when taking interest in a nearby feature on the surface of the wood or reaching an obstacle. While they often move in the direction of bark beetle holes that appear to be visible from their position (either the hole itself or a beetle frass pile coming out of it), they also seem not to be aware of ostensibly healthy host tunnels that are spatially close but separated from them by a visual barrier such as moss or a bump on the bark. They sometimes change direction to move towards visible protrusions in the wood, even when there is no beetle hole present. While these wasps do not seem to be guided to attractive beetle holes by long range olfactory cues when walking on the wood, these observations do not preclude the use of short range olfactory cues of a centimeter or so in host finding. More than fifty *P. holoholo* were observed searching on the wood surface. An example of this searching behavior is shown in Supplementary Video 1.

Upon discovery of a bark beetle hole, *P. holoholo* females were observed to either 1) take interest in it, visible to the human observer through slowing or arrestation of walking motion and antennation of the beetle itself or the entrance to the hole, or 2) show a lack of ostensible reaction by walking over, around, or past the hole without a clear change in pace. *Xyleborus* beetles are often observed to sit with the back of their elytra flush with the surface of the wood, presumably to protect their tunnels. A *P. holoholo* wasp that takes interest in a beetle in such a position will briefly antennate its elytra, and if it finds it acceptable quickly move onto the back of the beetle, raise its wings, insert the ovipositor into the declivity of the beetle between the elytra, and commence oviposition. The position it holds its wings during oviposition is similar to what has been recorded for *P. coffea* (Espinoza *et al*. 2009). *P. holoholo* has also been observed to enter a beetle tunnel to which it has taken interest if the beetle is not near the surface of the wood. It does this by backing down into the tunnel, digging its way through any frass or dust with its legs as it does so. This behavior of entering the host plant to find the beetle is in contrast with what is known for *P. coffea*, which is thought to only attack beetles exposed on the outside of the plant material while boring into coffee fruits (Jaramillo *et al*. 2005).

Once oviposition commences, the beetle becomes agitated. It rotates itself in its tunnel and moves in and out, as if trying to shake off the ovipositing wasp. If the beetle was inside the tunnel when the wasp found it, the two are often observed coming back to the surface of the wood during this process as the beetle seems to attempt to clear the wasp out of the hole. This agitation does not often seem to much improve the beetle's chances, as the wasp remains safely perched on the back end of the beetle, the beetle unable to abrade it against anything. In this effort, one beetle was observed falling onto the ground with the wasp attached to it, but in all other observations the beetle remained in its tunnel. This movement has also been observed to attract the attention of spiders and ants in the vicinity, though whether they might disturb the wasps is unknown.

Once oviposition has presumably concluded, the wasp removes its ovipositor and walks off the beetle. Searching by walking over the surface of the wood then often resumes, or the wasp flies off. Oviposition seems to last typically 2 or 3 minutes, though longer times up to 20 minutes have been observed. Oviposition times less than 30 seconds have also been observed, though it is unclear if these were successful ovipositions or aborted attempts. Full oviposition events were observed and described in detail 10 times, and numerous additional such interactions were also seen to occur. We know that *P. holoholo* will somewhat readily parasitize its hosts outside of wood tunnels in laboratory containers, but oviposition attempts on free-living beetles have not been observed in nature. See Supplementary Videos 2 and 3 for host acceptance and oviposition events. Male *P. holoholo* have not been observed on these bolts of wood.

Individual wasp females encountering each other on the surface of the wood seemed to show little interest in each other. *Phymastichus holoholo* individuals were also observed to come across other actively ovipositing *P. holoholo* while searching on wood. In most observed instances, they would briefly antennate the area and then move on to continue searching apparently without affecting the other wasp's behavior, though on a few occasions the ovipositing wasp appeared disrupted and flew away.

*Phymastichus holoholo* seems potentially to be able to distinguish parasitized from unparasitized beetles. We have not confirmed this, but *P. holoholo* has been observed to walk directly over apparently alive and healthy host beetles without showing any sign of interest, and then go on to parasitize others of the same species. Ability to recognize hosts previously oviposited in by a conspecific and a preference for unparasitized hosts has also been observed for *P. coffea* (Castillo *et al.* 2004). Additionally, some frass piles from active beetle tunnels arrested the wasps while others were largely ignored, suggesting that *P. holoholo* might be able to distinguish host species using cues from the fresh frass around their tunnels.

### Development, emergence, and mating

After hatching from their eggs, larval *P. holoholo* cleanly consume the insides of their host. Pupation was observed to typically occur with a larger female occupying the posterior of the beetle, its head facing the posterior end and its body extending to approximately the junction between the pro- and mesothorax, and a smaller male curled up facing transversely inside the prothorax (Fig. 4 d,e). Similar location of pupae inside their host has been observed for *P. coffea* (Espinoza *et al.* 2009).

Emergence behavior was observed by collecting paralyzed (and thus potentially parasitized) *X. ferrugineus* and *X. affinis* beetles from octopus tree branches, gluing them close together in a Petri dish, and placing a video camera above to record emergence events.

Like *P. coffea* and *P. xylebori*, *P. holoholo* emerges from its host beetle by chewing a round exit hole in the middle of the elytral declivity (Fig. 4 a,f,g,h). This process was observed to take between 61 and 256 minutes with a mean of 140 minutes (n = 7). After chewing a hole just large enough to emerge through, the female crawls out of the beetle and remains close to it. There is typically one male and one female wasp in each host, and the female emerges first, though sometimes only a single female would emerge. Out of 15 emergence events observed, 11 had one male and one female, and 4 had only a single female. A single male was never observed emerging without a female. Some time later, between 17 and 990 seconds with a mean of 330 seconds (n = 4), the smaller male crawled out of the hole and walked away from its host and the previously emerged female, as if exploring the area. This behavior is presumably to find a mate that is not its sister. In our setup, however, the only option was its sister, and after 8 to 246 seconds with a mean of 70 seconds (n = 4), the male returned to its emergence location, where the sister was still waiting. The pair did a courtship routine followed by mating. After this, both the male and female flew off. If no male emerged, the female was observed to wait 36 to 45 minutes with a mean of 41 minutes (n = 3) next to its emergence site before flying off. This mating behavior contrasts with that of *P. coffea*, which is thought to have sibling mating inside its host prior to emergence (Espinoza *et al.* 2009). It is also not common among parasitoids that the female waits for the male to emerge for mating, but maybe understandable for this species given its short lifespan and the consequent necessity to mate quickly to oviposit in a new host. See Supplementary Video 4 for an example of emergence and mating.

### Percent parasitism in octopus tree wood in Kahana Bay, O‘ahu

Six octopus tree wood bolts 5 to 8 cm in diameter and 60 cm long were cut fresh and suspended from a metal hanger approximately 2 m off the ground in an octopus tree forest in Kahana Bay, O‘ahu (21.5573 N, -157.8783 E, 15 m). After 6 weeks, on 22 July 2020, the wood was taken down and dissected completely. Every beetle tunnel was followed to its end, first by peeling the bark and thoroughly searching it, and then by splitting the xylem apart with a hammer, chisels, and pliers. All *Xyleborus* adult beetles were removed and placed in screened boxes for parasitoid emergence. After 5 weeks, beetles with wasp emergence holes were counted and emerged wasps were collected. Emergence conditions were likely less than ideal, so all remaining beetles were dissected to find *Phymastichus* immatures or adults inside that had not fully developed or emerged. All *Phymastichus* emerging from these bolts were *P. holoholo*, so immatures inside the beetles were assumed to also be *P. holoholo*.

From past observations cutting and suspending similar octopus tree logs from trees in the same area, bark beetles typically begin to enter the wood approximately 4 weeks after it is cut: a second generation of adults could thus not have been produced in the 6 weeks the wood was exposed to the environment before it was dissected. The calculated percent parasitism of beetles collected from the wood can thus be considered an accurate measure of the percent of beetles colonizing the wood that were parasitized by *P. holoholo*.

In total, 53 of 86 (62%) *X. ferrugineus* adult beetles collected from the wood were parasitized. 124 of 152 (82%) *X. affinis* adult beetles were parasitized. A single *X. perforans* beetle was present and it was parasitized. Thus the total percent parasitism for all *Xyleborus* beetles was 74%.

## Discussion

*Phymastichus holoholo*, and potentially also *P. xylebori*, may have potential to control populations of the species they parasitize. A percent parasitism in octopus tree branches for *P. holoholo* was calculated to be 62% on *X. ferrugineus* adults and 82% on *X. affinis* adults. Though this was only formally calculated one time, observations have often shown large numbers of wasps emerging from octopus tree wood and large numbers of dead beetles in the wood with the characteristic exit hole, suggesting that the proportion parasitized is often high.

Some of the *Xyleborus* beetles utilized as hosts by *P. holoholo* and *P. xylebori* may contribute to the spread of Rapid ‘Ōhi‘a Death, a very serious disease killing Hawaiian endemic ‘ōhi‘a lehua (*Metrosideros polymorpha* Gaudich.) trees on Hawai‘i Island, and recently found also on Kaua‘i, Maui, and O‘ahu (Keith *et al*. 2015). Roy *et al.* (2018) and Roy *et al.* (2020) found *Xyleborus ferrugineus, X. affinis*, *X. perforans, X. simillimus* Perkins*,* and *Xyleborinus saxesenii* (Ratzeburg) to inhabit ‘ōhi‘a trees and to excavate frass containing viable fungal spores, and though the mechanisms of spread of this disease in forests are not yet fully determined, bark beetles and their frass are suggested as a likely important factor. *Phymastichus holoholo* uses at least three of these *Xyleborus* beetles that infest ‘ōhi‘a lehua as hosts, and along with *P. xylebori* may have the potential to be used as a biological control agent for augmentative release in forests where this disease is spreading or may spread in the future. To explore the potential for this idea, we need to address some as of yet unanswered questions and possible concerns.

1. We do not know yet if these *Phymastichus* species will seek and attack their hosts in ‘ōhi‘a trees.
2. We do not yet know the full host range of *P. holoholo* and *P. xylebori*. There are 21 species of endemic *Xyleborus* beetles described from Hawai‘i (Nishida 2002), and it is important to note the discovery of two native *Xyleborus lanaiensis* individuals with the characteristic exit hole typical of *Phymastichus* in an ‘āla‘a (*Planchonella sandwicensis*) tree on O‘ahu. Since we do not know of any other species that would produce such an exit hole, it seems reasonable to suggest that it was parasitized by one of these *Phymastichus* species. We do not, however, know the difference in host range between *P. holoholo* and *P. xylebori* at this time, and thus we do not know if neither, only one, or both of these species parasitizes native Hawaiian species. The importance of slowing or stopping this disease for the health of Hawaiian forests, especially given that these wasps already occur in Hawai‘i, may somewhat mediate this concern.
3. Even if it is true that these beetles are an important component in the spread of this disease through the excavation of spores into the environment in their frass, and greater populations of beetles in infected ‘ōhi‘a trees would presumably result in more propagules being excavated, it is not immediately clear that mortality induced by a biological control agent will reduce the total amount of frass produced or the prevalence of the disease. Bark beetles are not currently known as direct vectors of Rapid ‘Ōhi‘a Death, but such considerations are better understood for vectors as opposed to indirect spreaders, and some of the dynamics may be related. Potential factors include the density-dependence of a vector on disease transmission (Okamoto and Amarasekare 2012); the relationship between the amount of time a vector actively inhabits the plant material and its ability to transmit the disease (Perring *et al*. 1999); and the ability of vectors to fly into an area of concern from outside of a treated region (Peters 1987; Sharaf and Allawi 1980; Bacon *et al*. 1976). For examples of reduction of vector populations resulting in reduction of disease, see a discussion of the substantial reduction in huanglongbing disease in orange trees on Reunion Island after introduction of a parasitoid that attacks the psyllids that vector the disease (Aubert *et al*. 1996), the substantial decrease in pine wilt disease in China through biological control of the cerambycid *Monochamus alternatus* Hope that vectors the disease (Yang *et al.* 2014), and a discussion of intensive programs of dead wood removal and debarking which reduced the prevalence of Dutch elm disease by decreasing populations of its bark beetle vectors (Scheffer *et al*. 2008).

Though these and likely other considerations are unanswered at this time, *P. holoholo* and *P. xylebori* may be worth exploring as an option to reduce the transmission of Rapid ‘Ōhi‘a Death, especially as of this writing, when there are few other known options and forests that are environmentally and culturally very important are rapidly being altered through sudden tree deaths.

Though we have not observed any adults with an exit hole, we have observed two instances of parasitism of beetles in the *Euwallacea fornicatus* species complex by *P. holoholo*, albeit neither with successful emergence of the parasitoids from the host. One *P. holoholo* adult that eclosed but did not emerge from the beetle was found inside a dead *E. fornicatus* (*sensu stricto*) individual, and one larva was dissected out of an *E. perbrevis* individual. Both beetles were inhabitants of naturally infested octopus tree branches. Other observations of *P. holoholo* on octopus tree branches inhabited by these beetles suggest that this wasp does not seem to be especially attracted to *E. fornicatus* (*sensu lato*), at least in that specific environment. There may be, however, marginal interest, and it is of note that a fully eclosed and healthy looking adult female was found inside a beetle, which implies that *P. holoholo* is physiologically able to fully develop inside at least *E. fornicatus* (*sensu stricto*). Similar fully eclosed and healthy looking *P. holoholo* adults that did not emerge from their host were observed occasionally during the dissection of *Xyleborus* beetles that *P. holoholo* less equivocally uses as a host, and thus the discovery of an adult wasp inside an *E. fornicatus* (*sensu stricto*) that did not chew an exit hole in the beetle does not imply its inability to do so. It is possible that these instances of parasitism were the result of mistaken identity by the wasps, with *Xyleborus* beetles also present in the wood and the resultant possibility of frass or other substances containing host cues from one of its preferred hosts falling onto a non-host beetle.

*Euwallacea fornicatus* (*sensu lato*) has recently been found to actually be a complex of somewhat cryptic species, some of which can in some places and situations be very serious pests of agriculture and forests (Stouthamer *et al.* 2017; Boland 2016; García-Avila *et al.* 2016; Eskalen *et al.* 2013; Eskalen *et al.* 2012; Mendel *et al.* 2012). Though the observations reported here are not especially promising in terms of biological control potential of *P. holoholo* on these beetles, they represent one of the few confirmed instances of parasitism of any member of the *E. fornicatus* species complex; the other known parasitoids were discovered during exploration in Taiwan by Husein (2019) and Husein *et al*. (2018). Further studies could explore the attack rate on other plant hosts or in other environments, and if indeed this wasp does have marginal interest, it could potentially be conditioned or bred to encourage its interest in these species.

*Phymastichus holoholo* has been observed to parasitize *X. ferrugineus* and *X. affinis* beetles in small containers in a laboratory environment, and to do so especially readily when the beetles are sprinkled with their frass and given a rough substrate on which to walk and to right themselves if they flip over, presenting their elytra for parasitism. Observations that show a lack of aggressive behavior that would obstruct oviposition of conspecifics, combined with observations that this wasp may avoid superparasitism, suggest that there is little active competition between these wasps and their behavior may be little affected by their density in a potential rearing environment. Though mass rearing of this species has not yet been attempted, it seems as though such efforts could potentially be successful.

## Acknowledgements

We thank Maya Honsberger for her observations, insights, and support. Dr. Mohsen Ramadan was also an invaluable assistance, as he among many other things first noticed the species described here was distinct from other known species and undescribed. We thank both Dr. Conrad Gillett and Jared Bernard for their advice and beetle identification, Dr. Karl Magnacca for first finding a native Hawaiian bark beetle with an exit hole and for drawing our attention to logs containing native Hawaiian beetles, Michelle Au and Scott Nikaido for their help with the genetic analysis, and our other coworkers including Dr. Abdullah Ali, Ali Miarkiani, and Robert Sakuda for their assistance. We are also very thankful to Natalie Dale-Skey, Dr. Roger Burks, Dr. JB Friday, and an anonymous reviewer whose comments and expertise much improved the manuscript. We are very grateful to Hawai'i Department of Agriculture, USDA-APHIS, USDA-ARS, and NIFA Hatch project HAW09041-H, administered by CTAHR, for their funding.

## References

Aubert, B., Grisoni, M., Villemin, M. & Rossolin, G. (1996) A case study of huanglongbing (greening) control in Reunion. *International Organization of Citrus Virologists Conference Proceedings (1957–2010)*, 13 (13), 276–278.
https://doi.org/10.5070/C58024T7FJ

Bacon, O.G., Burton, V.E., McLean, D.L., James, R.H., Riley, W.D., Baghott, K.G. & Kinsey, M.G. (1976) Control of the green peach aphid and its effect on the incidence of potato leaf roll virus. *Journal of Economic Entomology*, 69 (3), 410–414.
https://doi.org/10.1093/jee/69.3.410

Boland, J.M. (2016) The impact of an invasive ambrosia beetle on the riparian habitats of the Tijuana River Valley, California. *PeerJ*, 4, e2141.
https://doi.org/10.7717/peerj.2141

Campbell, B.C., Steffen-Campbell, J.D. & Werren, J.H. (1993) Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. *Insect Molecular Biology*, 2 (4), 225–237.

https://doi.org/10.1111/j.1365-2583.1994.tb00142.x

Castillo, A., Infante, F., Vera-Graziano, J. & Trujillo, J. (2004) Host-discrimination by *Phymastichus coffea*, a parasitoid of the coffee berry borer. *BioControl*, 49 (6), 655–663.
https://doi.org/10.1007/s10526-004-5277-0

Eskalen, A., Gonzalez, A., Wang, D.H., Twizeyimana, M., Mayorquin, J.S. & Lynch, S.C. (2012) First report of a *Fusarium* sp. and its vector tea shot hole borer (*Euwallacea fornicatus*) causing Fusarium dieback on avocado in California. *Plant Disease*, 96 (7), 1070.
https://doi.org/10.1094/PDIS-03-12-0276-PDN

Eskalen, A., Stouthamer, R., Lynch, S.C., Rugman-Jones, P.F., Twizeyimana, M., Gonzalez, A. & Thibault, T. (2013) Host range of Fusarium dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in Southern California. *Plant Disease*, 97 (7), 938–951.
https://doi.org/10.1094/PDIS-11-12-1026-RE

Espinoza, J.C., Infante, F., Castillo, A., Pérez, J., Nieto, G., Pinson, E.P. & Vega, F.E. (2009) The biology of *Phymastichus coffea* LaSalle (Hymenoptera: Eulophidae) under field conditions. *Biological Control*, 49, 227–233.
https://doi.org/10.1016/j.biocontrol.2009.01.021

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit 1 from diverse metazoan invertebrates. *Molecular marine biology and biotechnology*, 3 (5), 294–299.

García-Avila, C.D.J., Trujillo-Arriaga, F.J., López-Buenfil, J.A., González-Gómez, R., Carrillo, D., Cruz, L.F., Ruiz-Galván, I., Salinas-Quezada, A. & Acevedo-Reyes, N. (2016) First report of *Euwallacea* nr. *fornicatus* (Coleoptera: Curculionidae) in Mexico. *Florida Entomologist*, 99 (3), 555–556.
https://doi.org/10.1653/024.099.0335

Gibson, G.A.P., Read, J.D. & Fairchild, R. (1998) Chalcid wasps (Chalcidoidea): illustrated glossary of positional and morphological terms. Available from: http://www.canacoll.org/Hym/Staff/Gibson/apss/chalglos.htm (accessed 30 June 2021)

Gillespie, J.J., Munro, J.B., Heraty, J.M., Yoder, M.J., Owen, A.K. & Carmichael, A.E. (2005) A secondary structural model of the 28S rRNA expansion segment D2 and D3 for chalcidoid wasps (Hymenoptera: Chalcidoidea). *Molecular Biology and Evolution*, 22 (7), 1593–1608.
https://doi.org/10.1093/molbev/msi152

Husein D. (2019) A “boring” problem that’s generating big interest in southern California. *Topics in Subtropics Newsletter*, 20, 2–7.

Husein, D., Stouthamer, R. & Rugman-Jones, P.F. (2018) Hidden treasures of Taiwan: promising natural enemies of the polyphagous shot hole borer. *102nd Annual Meeting of the Pacific Branch of the Entomological Society of America, Reno*, 10–13 June 2018. [conference presentation]

Jaramillo, J., Bustillo, A.E., Montoya, E.C. & Borgemeister, C. (2005) Biological control of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae) by *Phymastichus coffea* (Hymenoptera: Eulophidae) in Colombia. *Bulletin of Entomological Research*, 95, 467–472.
https://doi.org/10.1079/BER2005378

Keith, L.M., Hughes, R.F., Sugiyama, L.S., Heller, W.P., Bushe, B.C. & Friday, J.B. (2015) First report of *Ceratocystis* wilt on ‘ōhi‘a (*Metrosideros polymorpha*). *Plant Disease*, 99, 1276.
https://doi.org/10.1094/PDIS-12-14-1293-PDN

LaSalle, J. (1990) A new genus and species of Tetrastichinae (Hymenoptera, Eulophidae) parasitic on the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera, Scolytidae). *Bulletin of Entomological Research*, 80, 7–10.
https://doi.org/10.1017/S0007485300045843

LaSalle, J. (1995) A new species of *Phymastichus* (Hymenoptera: Eulophidae) parasitic on adult *Xyleborus perforans* (Coleoptera: Scolytidae) on Macadamia trees in Hawai‘i. *Proceedings of the Hawaiian Entomological Society*, 32, 95–101.

Mendel, Z., Protasov, A., Sharon, M., Zveibil, A., Ben Yehuda, S., O’Donnell, K., Rabaglia, R., Wysoki, M. & Freeman, S. (2012) An Asian ambrosia beetle *Euwallacea fornicatus* and its novel symbiotic fungus *Fusarium* sp. pose a serious threat to the Israeli avocado industry. *Phytoparasitica*, 40, 235–238.
https://doi.org/10.1007/s12600-012-0223-7

Nishida, G.M. (2002) Hawaiian terrestrial arthropod checklist, fourth edition. *Bishop Museum Technical Report*, 22, 1–313.

Okamoto, K.W. & Amarasekare, P. (2012) The biological control of disease vectors. *Journal of Theoretical Biology*, 309, 47–57.
https://doi.org/10.1016/j.jtbi.2012.05.020

Perring, T.M., Gruenhagen, N.M. & Farrar, C.A. (1999) Management of plant viral diseases through chemical control of insect vectors. *Annual Review of Entomology*, 44, 457–481.
https://doi.org/10.1146/annurev.ento.44.1.457

Peters, D. (1987) Control of virus spread. *In*: de Bokx, J.A. & van der Want, J.P.H. (Eds.), *Viruses of potatoes and seed-potato production. 2nd Edition*. Wageningen, Pudoc, pp. 171–174

Roy, K., Ewing, C.P., Hughes, M.A., Keith, L. & Bennett, G.M. (2018) Presence and viability of *Ceratocystis lukuohia* in ambrosia beetle frass from Rapid ‘Ōhi‘a Death-affected *Metrosideros polymorpha* trees on Hawai‘i Island. *Forest Pathology*, 49, 1.
https://doi.org/10.1111/efp.12476

Roy, K., Jaenecke, K.A. & Peck, R.W. (2020) Ambrosia Beetle (Coleoptera: Curculionidae) Communities and frass production in ʻōhiʻa (Myrtales: Myrtaceae) infected with *Ceratocystis* (Microascales: Ceratocystidaceae) fungi responsible for Rapid ʻŌhiʻa Death. *Environmental Entomology*, 49 (6), 1345–1354.
https://doi.org/10.1093/ee/nvaa108

Scheffer, R.J, Voeten, J.G.W.F. & Guries, R.P. (2008) Biological control of Dutch elm disease. *Plant Disease*, 92 (2), 192–200.
https://doi.org/10.1094/PDIS-92-2-0192

Sharaf, N.S. & Allawi, T.F. (1980) Studies on whiteflies on tomato in the Jordan Valley III. Laboratory and field experiments on control of whitefly (*Bemisia tabaci* Genn., Homoptera: Aleyrodidae) populations with organophosphorus insecticides and the incidence of the tomato yellow leaf curl virus. *Journal of Plant Diseases and Protection*, 87 (3), 176–184.

Smith, S.M., Gomez, D.F., Beaver, R.A., Hulcr, J. & Cognato, A.I. (2019) Reassessment of the species in the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) complex after the rediscovery of the “lost” type specimen. *Insects*, 10 (9), 261.
https://doi.org/10.3390/insects10090261

Stouthamer, R., Rugman-Jones, P., Thu, P.Q., Eskalen, A., Thinbault, T., Hulcr, J., Wang, L.J., Jordal, B.H., Chen, C.Y., Cooperband, M., Lin, C.S., Kamata, N., Lu, S.S., Masuya, H., Mendel, Z., Rabaglia, R., Sanguansub, S., Shih, H.H., Sittichaya, W. & Zong, S.X. (2017) Tracing the origin of a cryptic invader: phylogeography of the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) species complex. *Agricultural and Forest Entomology*, 19, 366–375.
https://doi.org/10.1111/afe.12215

Vega, F.E., Infante, F. & Johnson, A.J. (2015) The genus *Hypothenemus*, with emphasis on *H. hampei*, the coffee berry borer. *In*: Vega, F.E., Hofstetter, R.W. (Eds.), *Bark beetles: biology and ecology of native and invasive species*. 1st ed. London: Academic Press, pp. 427–494.
https://doi.org/10.1016/B978-0-12-417156-5.00011-3

Wood, S.L. (2007) *Bark and ambrosia beetles of South America (Coleoptera, Scolytidae)*. Monte L Bean Life Science Museum, Brigham Young University, Provo, Utah, 900 pp.

Yang, ZQ., Wang, XY., & Zhang, YN. (2014) Recent advances in biological control of important native and invasive forest pests in China. *Biological Control*, 68, 117–128.
https://doi.org/10.1016/j.biocontrol.2013.06.010

Yousuf, F., Follett, P.A., Gillett, C.P.D.T., Honsberger, D.N., Chamorro, L., Johnson, M.T., Giraldo Jaramillo, M., Benavides Machado, P. & Wright, M.G. (2021) Limited host range in the idiobiont parasitoid *Phymastichus coffea*, a prospective biological control agent of the coffee pest *Hypothenemus hampei* in Hawaii. *Journal of Pest Science*.
https://doi.org/10.1007/s10340-021-01353-8

## SUPPLEMENTARY MATERIAL

**Video 1:** Searching behavior of *Phymastichus holoholo* **sp. nov.**
https://vimeo.com/599806458

**Video 2:** Host acceptance and oviposition behavior of *Phymastichus holoholo* **sp. nov.**
https://vimeo.com/599818826

**Video 3:** Host acceptance and oviposition behavior of *Phymastichus holoholo* **sp. nov.**
https://vimeo.com/599823504

**Video 4:** Emergence and mating behavior of *Phymastichus holoholo* **sp. nov.**
https://vimeo.com/599835585