

RESEARCH ARTICLE

Ooencyrtus pitosina (Hymenoptera: Encyrtidae)—A natural enemy of Samoan swallowtail butterfly *Papilio godeffroyi* (Lepidoptera: Papilionidae)

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Abstract

A new species of encyrtid wasp, *Ooencyrtus pitosina* Polaszek, Noyes & Fusu **sp. n.**, (Hymenoptera: Encyrtidae: Encyrtinae) is described as a gregarious parasitoid in the eggs of the endemic Samoan swallowtail butterfly *Papilio godeffroyi* (Lepidoptera: Papilionidae) in the Samoan archipelago. It is described here because it is an important natural enemy of this butterfly, and to facilitate identification for future work with this parasitoid and its host.

Introduction

The Samoan swallowtail butterfly (*Papilio godeffroyi* Semper 1866), is one of seven endemic butterfly species in the Samoan Archipelago [1, 2] and is classified as “Endangered” due to declining range, habitat, and population [3]. The butterfly, which inhabits relatively undisturbed rainforest, oviposits on *Micromelum minutum* (family Rutaceae), a small tree indigenous to the Samoan Archipelago, and distributed widely in the Indo-Pacific [4]. Nearly 140 years ago, Mathew [5] observed that the eggs of the Samoan swallowtail were attacked by an unknown parasitoid wasp. He also noted heavy parasitism of the eggs of the Fijian swallowtail butterfly, *Papilio schmeltzi* (Herrich-Schäffer 1869), which today is classified as “Near Threatened” [6]. Of the Fijian swallowtail, he said (page 358), “These ova were terribly subject to the attacks of a minute hymenopterous parasite. Only about one in a dozen produced a larva, the remainder giving birth to three or four ichneumons, so small that they were barely visible to the naked eye.” Of the Samoan swallowtail he wrote (page 362), “They were also terribly attacked by the same kind of parasite.” Mathew did not report on the number of eggs he attempted to rear for either butterfly species, but his observations clearly indicated high rates of attack, possibly by the same or similar parasitoid wasp species.

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Competing interests: The authors have declared that no competing interests exist.

As part of a life history investigation of *P. godeffroyi*, conducted on Tutuila Island, American Samoa from November 2013 to June 2014 [7], we frequently observed an undescribed species of wasp, *Ooencyrtus* (Hymenoptera: Encyrtidae), emerging from field collected eggs [8]. Up to five wasps were observed emerging from a single butterfly egg. We collected *M. minutum* leaflets bearing eggs of *P. godeffroyi*, and transported them to the laboratory at American Samoa Community College (ASCC) for rearing. In the laboratory, excised leaf pieces bearing intact eggs were placed in 1-cm diameter, 3.5 cm-tall glass shell vials with plastic caps containing 0.5 cm-diameter holes covered with 100 mesh stainless wire cloth. The eggs were maintained in an incubation chamber at 27°C with a photoperiod of 14 h light and 10 h dark until larvae or parasitoids emerged. Parasitoids were preserved in molecular grade absolute ethanol for molecular analysis.

Our observations, together with Mathew's [5] historical account, have motivated this taxonomic determination to facilitate identification in the future and to provide the formal nomenclature critical for additional work with this parasitoid and its hosts. Although we are not aware of any wasp specimens that Mathew may have collected from Fijian or Samoan swallowtail eggs, we expect that the new species we describe here may be the same, at least in the case of the Samoan swallowtail. We encourage others to identify parasitoids associated with the Fijian and other Pacific swallowtails to determine whether they are the same species as the one we describe here, which would indicate its distribution beyond the Samoan Archipelago.

Materials and methods

Specimen depositories: Abbreviations

AICF: Lucian Fusu collection, Al. I. Cuza University.

BPBM: Bernice P. Bishop Museum, Honolulu, Hawai'i, USA.

NHMUK: Natural History Museum, London, UK.

USNM: United States national Museum, Washington D.C., USA.

Morphological study

Morphological terminology and the format for the species description follow Noyes [9].

Abbreviations are as follows: AOD = largest diameter of anterior ocellus; AOL = minimum distance between posterior ocellus and anterior ocellus; EL = eye length; EW = eye width; FV = minimum width of frontovertex; FVL = length of frontovertex from occipital margin to top of antennal scrobes as seen in dorsal view; FVS = width of frontovertex a little above top of scrobes at a point where eye margin changes from being virtually straight to distinctly curved; FWL = fore wing length; FWW = fore wing width; GL = gonostylus length; HW = head width measured in facial view; HWL = hind wing length; HWW = hind wing width; MS = malar space (minimum distance between eye and mouth margin); MT = mid tibia length; OCL = minimum distance between posterior ocellus and occipital margin; OL = ovipositor length; OOL = minimum distance between eye margin and adjacent posterior ocellus; POD = largest diameter of posterior ocellus; POL = minimum distance between posterior ocelli; SL = scape length; SW = scape width.

Card-mounted specimens were observed with a Leitz binocular microscope at magnifications ranging from 20x to 80x. Slide-mounted specimens were observed with a Leitz Dialux 20 microscope at magnifications ranging from 40 to 400x.

Images were generated as follows: Figs 1 and 2 Canon DSLR with 10x Mitutoyo objective, processed with Helicon Focus© stacking software with final editing in Adobe Photoshop© CC. Figs 3–8 were obtained using a Jenoptik Progres Gryphax NAOS digital camera attached to a Dialux 20 compound microscope using bright-field illumination. From 10 to 80 images, at



Fig 1. *Ooencyrtus pitosina* holotype female dorsal and lateral habitus.

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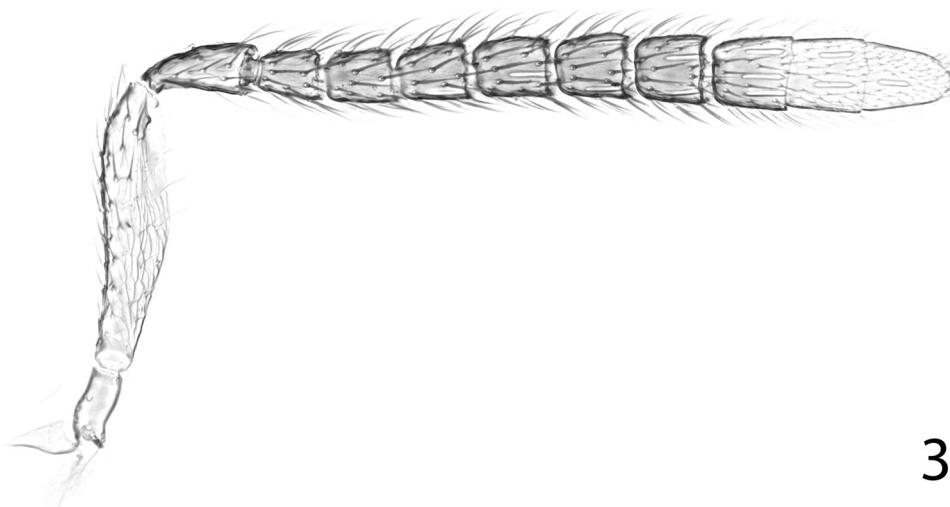
Fig 2. *Ooencyrtus pitosina* holotype female head.

<https://doi.org/10.1371/journal.pone.0288306.g002>

different focal depths, were taken of each body part to be illustrated. Each series of images was stacked and merged into a single in-focus image using Helicon Focus ©. Where possible, extraneous micro artifacts (dust, etc.) were removed physically before imaging or the images were digitally cleaned and enhanced using PhotoShop©.

DNA sequencing

Genomic DNA extraction was undertaken from 10 specimens using the protocol in Polaszek *et al.* [10] and Cruaud *et al.* [11], which leaves the sclerotized parts of the specimen intact.



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Fig 3. *Ooencyrtus pitosina* paratype female, antenna.

<https://doi.org/10.1371/journal.pone.0288306.g003>

Specimens were then critical point dried and card-mounted, with selected individuals then dissected and mounted in Canada balsam on microscope slides.

As the Folmer primer pair LCO1490/ HCO2198 [12] does not perform well in many chalcid wasp taxa [13–15], especially in those with suboptimal DNA extracts [16], to get the full-length Cytochrome c Oxidase I (COI) barcode region, the internal primers MChaR1 and MChaF1 [16] were used in combination with the Folmer primers. The 28S D2 fragment was amplified with the primers D23F [17] and 28Sb [18, 19]. The PCR reactions and sequencing were carried out as described in [16]. Forward and reverse sequences were assembled and corrected using Pregap4 and Gap4 software in the Staden package [20]. The p-distance between sequences was computed in MEGA 11 [21]. In our BLAST searches we favoured identity over max score, the last one being influenced by query cover. The sequences have been deposited in GenBank under accession numbers OQ648163–OQ648167 (COI) and OQ650244–OQ650251 (28S), respectively.



4

Fig 4. *Ooencyrtus pitosina* paratype male, antenna.

<https://doi.org/10.1371/journal.pone.0288306.g004>

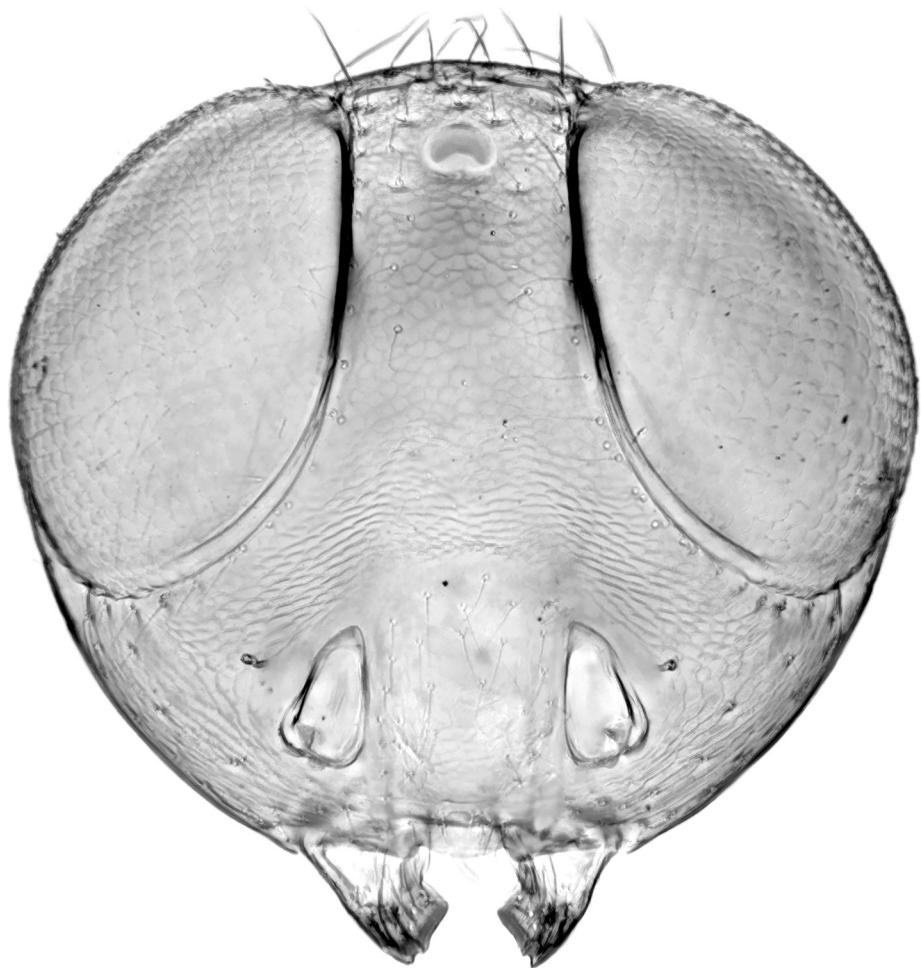


Fig 5. *Ooencyrtus pitosina* paratype female head.

<https://doi.org/10.1371/journal.pone.0288306.g005>

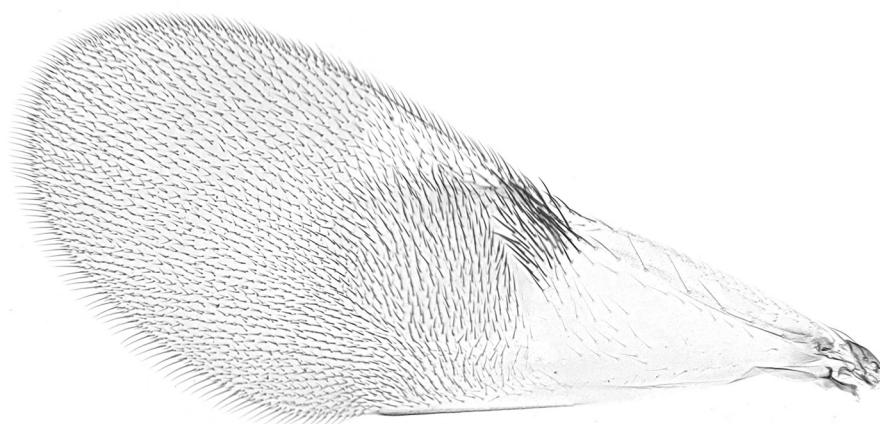


Fig 6. *Ooencyrtus pitosina* paratype female, dorsal mesosoma.

<https://doi.org/10.1371/journal.pone.0288306.g006>

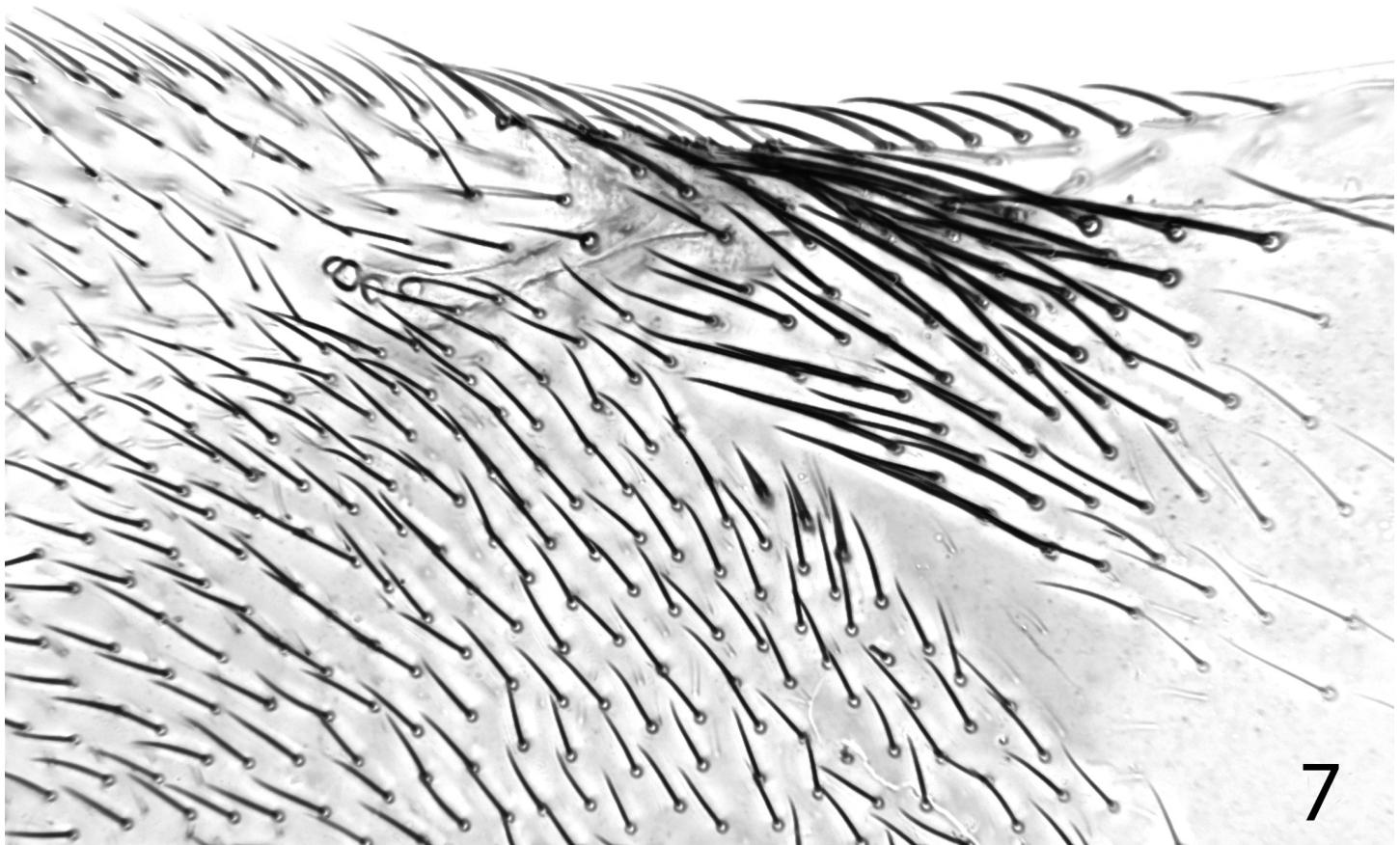


Fig 7. *Ooencyrtus pitosina* paratype female, fore wing.

<https://doi.org/10.1371/journal.pone.0288306.g007>

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under the Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>. The LSID for this publication is: urn:lsid:zoobank.org:pub:94EC4-C18-EEFD-49F6-B4CB-624574A5ABA. The LSID for this publication is: urn:lsid:zoobank.org:pub:94EC4-C18-EEFD-49F6-B4CB-624574A5ABA

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Results

Description

***Ooencyrtus* Ashmead.** *Ooencyrtus* Ashmead, 1900:381–382 [22]. Type species: *Encyrtus clisiocampae* Ashmead, by original designation.

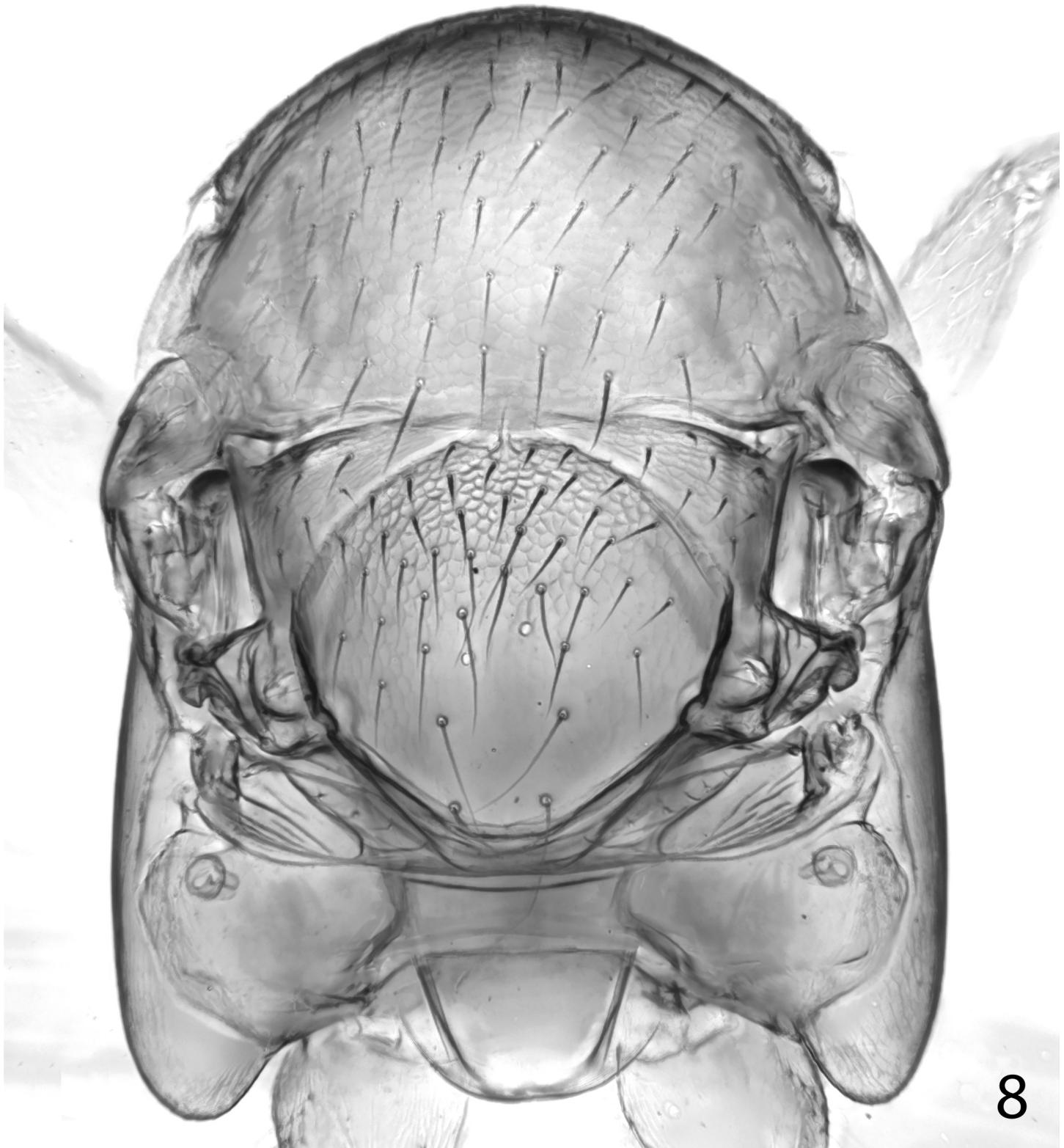


Fig 8. *Ooencyrtus pitosina* paratype female, detail of fore wing.

<https://doi.org/10.1371/journal.pone.0288306.g008>

For a complete synonymy list see [23]

Diagnosis. Female (Fig 1). Length about 0.5–2.1mm. Moderately robust, rarely elongate; head and thorax usually dark brown with a metallic sheen, rarely yellow or orange; gaster varying from yellow or orange to dark brown with a metallic sheen; fore wing hyaline, rarely conspicuously infuscate; legs completely orange to brown.

Head (Figs 2, 5) with frontovertex varying from less than one-fifth to about two-fifths head width; occipital margin usually rounded; eye often overreaching occipital margin; antennal torulus separated from mouth margin by about its own length, dorsal margin usually well below lower eye margin; antenna (Fig 3) with funicle 6-segmented; clava 3-segmented, rarely solid or 2-segmented, apex usually rounded; mandible variable, usually with one or two teeth and a truncation, sometimes with 3 acute teeth, rarely with only 2 teeth; palp formula 4–3.

Mesoscutum (Fig 6) with notaular lines absent; axillae narrowly meeting, clearly overlapped medially by posterior margin of mesoscutum; mesopleuron enlarged so that posteriorly it touches base of gaster, in lateral view clearly separating metapleuron and propodeum from hind coxa; fore wing usually fully developed; marginal vein usually more or less punctiform very rarely as much as 4X as long as broad; postmarginal vein mostly very short, sometimes absent, rarely as long as stigmal vein; propodeum with not more than 4 or 5 setae outside spiracle.

Gaster mostly about as long as thorax, occasionally longer than head and thorax combined; paratergites absent; hypopygium not reaching apex of gaster; ovipositor varying from hidden to strongly exserted; gonostylus freely articulated with second valvifer.

Male. Length about 0.6–1.7mm. Sexual dimorphism moderate to extreme, usually similar to female except for structure of antenna and genitalia; sometimes males with conspicuously deeper sculpture and brighter and more metallic than female, rarely thoracic structure very different from that of female; head with torulus separated from mouth margin by at least its own length, lower margin at least about level with lower eye margin; antenna (Fig 4) with all funicle segments longer than broad and clothed in long setae that are generally at least as long as diameter of segments; clava solid, rarely two-segmented; phallobase fairly slender, parameres short, about one-third to half-length of digitus, with a single apical seta; digitus about 2.5× as long as broad with one apical tooth; aedeagus slender, about half to three-quarters as long as mid tibia, with apex acute, rounded or truncate.

Distribution. Cosmopolitan.

Hosts. Most *Ooencyrtus* are primary parasitoids of the eggs of Hemiptera or Lepidoptera, but some species are known to attack lac insects (Hemiptera: Kerriidae), the prepupae of Lepidoptera, braconid (Hymenoptera) primary parasitoids of caterpillars, nymphal stages of Aphididae (Hemiptera), immature stages of Syrphidae (Diptera), pupae of Chloropidae (Diptera), immature stages of Dryinidae (Hymenoptera) attacking achenorrhynchous Hemiptera, or Coccinellidae (Coleoptera) feeding on aphids, see [23–27].

Identification. [28, 29] catalogues of Nearctic species; [23, 30, 31] Neotropical species; [27] Palaearctic species; [24] Afrotropical species; [25, 26] Indian species; [32] Indo-Pacific species.

***Ooencyrtus pitosina* Polaszek, Noyes & Fusu sp. n.** urn:lsid:zoobank.org:act:B11645AD-7830-4028-B938-539F2AE60812

Figs 1–8

Morphology. **Female** (holotype) length: 1.21mm, including ovipositor; 1.16mm, excluding ovipositor.

Head (Fig 2) pale orange with weak brassy reflections, occiput with a small brown mark above foramen; 8 brown setae along occipital margin, 1 behind each posterior ocellus; 4 or 5 translucent pale setae in ocellar area, 8 between anterior ocellus and top of scrobes and a line

along inner eye margin, about 25 on interantennal prominence and several short, inconspicuous setae on gena; antenna with radicle and scape very pale orange; pedicel mostly pale orange-brown, very pale orange ventrally; funicle dark brown; clava very pale yellow, basal segment proximally dark brown; thorax pale orange, neck of pronotum mixed brown; mesoscutum with a slight brassy sheen; tegula very pale yellow with weak coppery reflections; axilla slightly dusky with a slight brassy sheen; scutellum dusky, a little darker than axilla and medially weakly coppery, apical one-third or so with a slight, distinct metallic green and golden sheen; axillula and metanotum laterally dark brown, metanotum medially pale orange; prepectus pale orange; mesopleuron darker orange; prosternum pale orange; mesosternum orange; fore coxa very pale orange, fore leg otherwise yellow, extreme apex of tarsus brown; mid coxa pale orange, mid femur yellow, tibia white, apex and spur yellow, tarsus yellow, extreme apex brown; hind coxa pale orange, hind femur pale orange, a little dusky towards apex dorsally, tibia narrowly pale yellow at base, otherwise dark brown, tarsus yellow; fore wing hyaline with a circular, infuscate area below venation in middle one-third from marginal vein to posterior margin; submarginal vein white, dusky basally; marginal, postmarginal vein and stigmal veins pale orange; propodeum slightly dusky pale orange; about 10 translucent, silvery setae on side near spiracle; gaster with TI dorsally white, side pale orange; venter of gaster pale orange proximally; TII to syntergum of gaster dark brown; gaster with a slight brassy sheen with metallic green and coppery purple reflections, darker areas with a stronger sheen; visible part of gonostyly pale orange.

Head with occipital margin weakly rounded, almost sharp, eyes slightly overreaching occipital margin and clothed in conspicuous, dense, pale setae, each slightly longer than diameter of an eye facet; ocelli forming an angle of about 65°; frontovertex with very fine, polygonally reticulate sculpture of mesh size slightly less than diameter of eye facet; temple and gena with longitudinally elongate, polygonally reticulate sculpture; interantennal prominence with similar sculpture to frontovertex, but shallower; scrobes moderately deep, \cap -shaped, margins rounded, interantennal prominence dorsally rounded; antennal torulus separated from mouth margin by about its own length. Antenna with funiculars longer than wide except F6 about as long as wide. Relative measurements: HW 88.5, FV 20.5, FVL 51, POL 9.0, OOL 1.0, OCL 6.5, AOL 9, EL 54, EW 49, MS 33.5, SL 39, SW 10.

Thorax with polygonally reticulate sculpture on mesoscutum that is of slightly larger mesh than that on frontovertex and a little shallower; scutellum medially in basal half with very similar sculpture to frontovertex, laterally and posteriorly of slightly larger mesh and a little more shiny; mid tibial spur slightly shorter than basitarsus; fore wing venation and setation as in Figs 7 and 8; marginal vein about 2.5× as long as broad; postmarginal vein about 0.4× as long as stigmal vein. Relative measurements: FWL 204, FWW 78.5, HWL 135, HWW 33.

Gaster slightly shorter than thorax; hypopygium reaching about 0.4× along gaster; syntergum about 0.8× as long as mid tibia, apex rounded; ovipositor slightly exserted, the exserted part about 0.4× as long as mid tibial spur.

Paratype. Funicle with linear sensilla on F2–F6; mandible with one short, acute tooth and broad, very slightly convex truncation. Relative measurements: OV 111, GS 26.5 [MT 91.5].

Variation. The overall length of the female varies from 0.93 to 1.46mm; the pronotum immediately in front of mesothoracic spiracle may be marked dark brown, the posterior half of the scutellum may be orange-brown, the base of the hind tibia may be white and the head varies from 3.8 to 4.4× as wide as frontovertex.

Male (length 0.66–1.16mm): very similar to female but for slightly wider frontovertex, structure and colour of antenna and structure of genitalia. Head about 2.7–3.4× as wide as frontovertex; antenna with funicle dusky orange; funicle with all segments longer than broad; clava 2-segmented, completely white; phallobase with digitus about 3.6× as long as broad and

each with a single apical tooth, parameres developed, each with a basal and a subapical seta; aedeagus slender with apex strongly acute, Relative measurements: MT 61.5, AL 36.5.

Molecular analysis. We obtained five full length DNA barcodes (COI) and eight 28S sequences 858 bp in length (not all 10 extracted specimens had high quality DNA). The COI sequences revealed three haplotypes differing at two variable sites. The 28S sequences were all identical. The paucity of DNA sequences for *Ooencyrtus* species in GenBank or in Barcode of Life Data System (BOLD) have precluded the need for any phylogenetic analyses. A GenBank BLAST of our COI sequences resulted in *Ooencyrtus plautus* Huang & Noyes (assuming correct identification), from a recently published complete mitochondrial genome study [33] as the top hit. The following 10 similar sequences are as follows: two of *Ooencyrtus nezarae* from [34], five of *Metaphycus flavus*, and three of Encyrtidae sp. or Hymenoptera sp. The last three sequences (KJ208550.1, KY831595.1, KY845087.1) are derived from BOLD and based on their images can be identified as *Ooencyrtus* sp. and *Lamennaisia* sp. Even *O. plautus*, the most similar species present in GenBank, is 13.9–14.1% divergent from *Ooencyrtus pitosina*.

Using BLAST our 28S sequences were found to have a 90.4% identity with *Ooencyrtus* sp. 1 YZZ-2014 (accession KF850145.1), a sequence derived from a paratype of *O. protohermes* Zhang & Zhang [35]. The *p*-distance between this species and *O. pitosina* is 7.9%.

Hosts. A gregarious parasitoid of the eggs of *Papilio godeffroyi* on leaves of *Micromelum minutum*.

Distribution. American Samoa.

Material examined. Holotype ♀: AMERICAN SAMOA, Tutuila Island, Afono, ex *Papilio godeffroyi* egg on *Micromelum minutum* leaf, ASPG0824, Host coll 16.i.2014 (M. Schmaedick). Paratypes: AMERICAN SAMOA, 2♀, 2♂, Tutuila Island, Vatia, ex *Papilio godeffroyi* egg on *Micromelum minutum* leaf, Host coll. 9.i.2013 (M. Schmaedick); 12♀, 4♂, Tutuila Isl., Vatia, ex egg of *Papilio godeffroyi* on *Micromelum minutum*, coll. 21–22.i.2022 (N. Leifi); 4♀, 3♂, Tutuila Island, Masausi, ex *Papilio godeffroyi* egg on *Micromelum minutum* leaf, Host coll. 11.i.2013 (M. Schmaedick); 2♀, Tutuila Island, Aoa, ex *Papilio godeffroyi* egg on *Micromelum minutum* leaf, ASPG0081, Host coll. 24.vii.2013 (M. Schmaedick); 46♀, 26♂, Tutuila Isl., Afono, ex egg of *Papilio godeffroyi* on *Micromelum minutum*, Host coll. 19.xii.2013, 16.i.2014, 21.ii.2014, 13. vi.2014, 19.vi.2014 (M. Schmaedick); 47♀, 18♂, Tutuila Isl., Maloata, ex egg of *Papilio godeffroyi* on *Micromelum minutum*, Host coll. 24.xii.2013, 30.i.2014, 21.ii.2014, 7.iii.2014, 25. iv.2014, 23.v.2014 (M. Schmaedick); 7♀, 1♂, Tutuila Isl., Malaeimi, ex egg of *Papilio godeffroyi* on *Micromelum minutum*, Host coll. 18–20.i.2022 (N. Leifi). Holotype in NHMUK, paratypes in AICF, BPBM, NHMUK and USNM.

Etymology. “pito” = “tip” and “sina” = “white” in Samoan, referring to the pale-tipped antenna. Noun in apposition.

Comments. *Ooencyrtus pitosina* can be distinguished from all known species of the genus by the unique combination of the generally orange colour of the body, mandible with a small tooth and broad truncation, infuscate fore wing and elongate marginal vein of the fore wing. In the key to Indo-Pacific species [32] it runs easily to *Ooencyrtus lucina* Huang & Noyes at couplet 52 which shares a similar general coloration. No almost completely orange species of *Ooencyrtus* are known in other parts of the world. The two species can be distinguished in both sexes by the relative length of the marginal vein which is about 2.5× as long as broad in *pitosina* and punctiform in *lucina*. In addition, females of *pitosina* have the frontovertex about one-quarter head width, F1 smaller than remaining funicle segments which are subequal in size, the hind tibia dark brown, fore wing conspicuously infuscate and ovipositor slightly exserted. In females of *lucina* the frontovertex is one-third head width, F1–F2 conspicuously smaller than remainder which are not subequal, the hind tibia is yellow, the fore wing is completely hyaline, and the ovipositor is not exserted. On the other hand, males of *pitosina*

have the head orange, clava completely white, frontovertex about one-third head width, fore wings weakly infuscate and hind tibia dark brown, whilst in males of *lucina* the head has a dull dark green and purple sheen, the basal half of the clava is brown, the frontovertex is about half head width and the fore wing is completely hyaline. In the key to Indian species [25] *pitosina* runs to couplet 62 and clearly differs from any of the species included after that point because none is orange, and all have hyaline wings. Other regional comprehensive keys to the species of *Ooencyrtus*—Costa Rica [23], southern Africa [24] and Palaearctic [27] do not include any species that are yellow or orange in colour, and very few species included in those treatments have a marginal vein that is more than 1.5× as long as broad. The only group of species currently placed in *Ooencyrtus* that have a completely orange (or yellow) head and thorax and long marginal vein are those species that are known to parasitize lac insects (Kerriidae). Two of these species also have the fore wing weakly partially infuscate, viz: *O. kerriae* Hayat and *O. paratachardinae* Hayat, Schroer & Pemberton. These species are superficially somewhat similar to *pitosina* but differ in the mandible having three uniform acute teeth.

Because of the unusually elongate marginal vein of the fore wing, we considered it worth comparing *Ooencyrtus pitosina* with species currently placed in similar or closely related genera of Encyrtidae. The most similar of these is *Hengata spinosa* Noyes & Hayat [36] described from Sulawesi (Indonesia) with an unknown biology. The species are superficially very similar in that they are both generally orange in colour and have similar antennae. However, *Ooencyrtus pitosina* differs from *Hengata* in lacking a median longitudinal ridge on the mesoscutum, having a mandible with one short tooth and a very broad truncation and the male scape lacking a spine on its ventral margin. In *Hengata* the mesoscutum has a median, longitudinal, posterior ridge, the mandible is tridentate with the upper tooth truncate and the male scape has an elongate, distinct spine on its ventral margin. Further to this in *Ooencyrtus pitosina* the clava is proximally brown and the fore wing strongly infuscate medially whereas in *Hengata spinosa* the clava is completely white and the fore wing hyaline.

Discussion

It appears that *O. pitosina* is not closely related to any species with sequences currently deposited in Genbank. Neither the unidentified species of *Ooencyrtus* nor *O. plautus* or *O. nezarae* (the closest matches based on COI) bear any resemblance to *O. pitosina*. The most similar species based on 28S, *O. protohermes*, has completely yellow legs, a yellow acropleuron and possibly a yellow prosternum [35] but otherwise they are dissimilar. The fact that we found three haplotypes out of five COI sequences indicates that *O. pitosina* might be a long-established species in American Samoa, though a recent human-mediated introduction cannot be excluded. Accidentally introduced Hymenoptera species frequently have reduced genetic diversity resulting from the small number of colonising individuals (usually only one haplotype is present in the recently colonised areas) [37–39].

Acknowledgments

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Author Contributions

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