



A review of Polynesian *Carposina* Herrich-Schäffer (Lepidoptera: Carposinidae), with descriptions of four new species

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Hawaiian *Carposina* represent over 17% of the known world fauna of Carposinidae. In contrast, only two species are known for all of French Polynesia in the South Pacific. Here we describe four new species: two from the Hawaiian Islands, *Carposina urbanae* sp. nov. and *C. gagneorum* sp. nov., and two from the Society Islands, *C. longignathosa* sp. nov. and *C. brevinotata* sp. nov. We further recognize another new Hawaiian species too worn to describe. Additionally, we present the first phylogeny for Polynesian *Carposina*, including 19 taxa, using one mitochondrial and two nuclear gene regions. The Hawaiian *Carposina* sampled thus far form a monophyletic clade. Lastly, we provide a framework to better understand the diversification and phylogeography of this group, and provide a summary of currently known host plant associations. Diversification appears to have resulted from interplay between host switching and geographic isolation across the Hawaiian Archipelago.

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INTRODUCTION

The family Carposinidae (Lepidoptera: Copromorpha) is known from only approximately 283 named species in 19 genera (Heppner, 2008; van Nieukerken *et al.*, 2011). Although worldwide in distribution, diversity is scant in most ecoregions, with the highest concentration in the Austral–Asian region, including 44 species in New Guinea (Diakonoff, 1989; Heppner, 2008). In the Hawaiian Islands, at least 39 named and ten unnamed species are known in the genus *Carposina* Herrich-Schäffer (c. 17% of the known Carposinidae world fauna), with many more species likely to be discovered (Meyrick, 1883, 1913, 1922; Walsingham, 1907;

Zimmerman, 1978). In sharp contrast, French Polynesia is depauperate of Carposinidae. Of the prominent archipelagos in the remote South Pacific (the Society, Marquesas, Tuamotu and Austral Islands), only two *Carposina* species are known from Rapa in the Austral Islands (Clarke, 1971).

Biological diversity is highly attenuated in the remote Pacific basin (Miller, 1996; Gillespie & Roderick, 2002). In the Hawaiian Archipelago, approximately 50% of insect orders and 15% of insect families successfully colonized the island chain (Howarth, 1990). But for those 254–400 lineages fortunate enough to become established (Zimmerman, 1948; Howarth, 1990), many have exhibited spectacular radiations, including nearly 1000 species of Drosophilidae (Diptera) (O’Grady *et al.*, 2011), 350+ species of *Hyposmocoma* Butler (Lepidoptera: Cosmopterigidae) (Haines, Schmitz & Rubinoff, 2014),

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80 species of *Nesosydne* Kirkaldy (Hemiptera: Delphacidae) (Goodman, Welter & Roderick, 2012) and 62+ species of *Nesophrosyne* Kirkaldy (Hemiptera: Cicadellidae) (Bennett & O'Grady, 2013). Moreover, diversification in each of these examples has coincided with novel host associations across disparate plant families.

For endemic Hawaiian phytophagous insects, host 'jumps' (in contrast to host shifts) do not represent a reciprocal diversification process or co-evolutionary 'arms race' whereby cladogenesis in herbivores roughly mirrors that of their host plants (e.g. Ehrlich & Raven, 1964; Pellmyr, 2003; Kawakita & Kato, 2009). Rather, host jumps span widely divergent plant families that came together secondarily in the Hawaiian Archipelago. For Hawaiian *Carposina*, host associations include nine plant families not recorded for *Carposina* elsewhere, with host associations yet unknown for many Hawaiian species (Swezey, 1954; Zimmerman, 1978; Robinson *et al.*, 2010). It is likely that the interplay between host jumps and infrequent inter-island dispersal plays a key role in the hyper-diversification of *Carposina* and other Hawaiian herbivorous insects.

Carposina of the Pacific Islands in general, and Hawaii in particular, is a group in need of revision and further study (Zimmerman, 1978). Although field and laboratory investigations are ongoing, there is urgent need to document the diversity and phylogeography of island species for which habitat degradation and possible extinction of host plants are occurring at an alarming rate (Medeiros *et al.*, 2013). Here we describe four new species of Hawaiian and Society Islands *Carposina* and propose a preliminary phylogeny for a subset of species based on one mitochondrial and two nuclear gene regions. We also review the distributions and host plant associations for Hawaiian *Carposina*, where known, and discuss a framework for understanding the phylogeography of the group.

METHODS

PHYLOGENETIC METHODS

We obtained adult *Carposina* by light trapping in the field, or from museum loan (Supporting Information Table S1). We extracted DNA from the legs of specimens up to 8 years old using the standard protocol described in Qiagen's DNeasy kits. We carried out PCRs to amplify segments of the protein-coding genes COI (mtDNA), wingless (nDNA) and EF1a (nDNA) (see Supporting Information Table S2 for PCR protocols), although in some cases we were not able to amplify each gene region for each specimen (Table S1). We purified PCR products using ExoSAP-IT (Affymetrix) and sequenced DNA on an Applied Biosystems 3730 DNA analyser. We obtained sequences for four outgroup species from GenBank (Table S1).

We visualized and edited sequences using 4Peaks (Griekspoor & Groothuis, 2006), assembling a final concatenated dataset with MEGA 6.06 (Tamura *et al.*, 2013). Our final dataset included 34 taxa and 2257 characters (including 853 bp COI, 968 bp EF1a and 436 bp wingless). We ran an initial maximum-likelihood analysis with 100 bootstrap pseudoreplicates using the RAxML Blackbox web service (Stamatakis, Hoover & Rougemont, 2008), while partitioning the dataset by gene region and codon position.

We used BEAST 2.2.1 (Bouckaert *et al.*, 2014) to conduct our final phylogenetic analysis. First, we selected the best model of molecular evolution for each of our three codon positions for each of the three gene regions (a total of nine partitions), using PartitionFinder v. 1.1.1 (Lanfear *et al.*, 2012) and Akaike's information criterion (AIC). Models for each codon position for COI, EF1a and wingless, respectively, were GTR + I + G, GTR + I + G and GTR + I + G, GTR + G, GTR + I and GTR + G, and SYM + I + G, SYM + I + G and GTR + G. We then set up our BEAST analysis using the BEAUti interface, running four chains of 80 000 000 generations and sampling every 10 000 generations. Settings were default values except for the following: Site model menu – we used the models of evolution previously determined by PartitionFinder for each of our nine partitions; Clock model menu – Relaxed clock log normal (estimated rate); Priors menu – tree prior: Yule speciation. After completing our runs, we discarded the first 25% of the samples in each chain as 'burn-in', after evaluating the effective sample size and posterior convergence using Tracer 1.6 (<http://beast.bio.ed.ac.uk/Tracer>). We viewed the final tree estimate with FigTree 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Given that our sampling of *Carposina* to date is incomplete and that the phylogeny of the group is therefore not resolved, we did not feel confident in setting calibration points for a BEAST analysis based on assumptions about when taxa split from each other, as is commonly done with island radiations (e.g. Bess, Catanach & Johnson, 2014). However, as we were able to amplify COI for most specimens in the analysis (29 of 34 specimens have COI data, although this does not include either of the two new species from the Society Islands), we chose to estimate dates of divergence for the Hawaiian *Carposina* using commonly accepted rates of mutation in this gene region. As dates of divergence for Lepidoptera COI range from 2.3% pairwise divergence per million years (Brower, 1994) to 8.8% per million years (Haines *et al.*, 2014), using this method as the only way of calibrating a BEAST analysis is very uncertain. Nonetheless, we used both rates ('slow' and 'fast' in two separate BEAST analyses for COI only; Fig. 1) to obtain a rough estimate of the minimum age of the Hawaiian *Carposina*. Other than specifying the mutation rate and using only the three codon

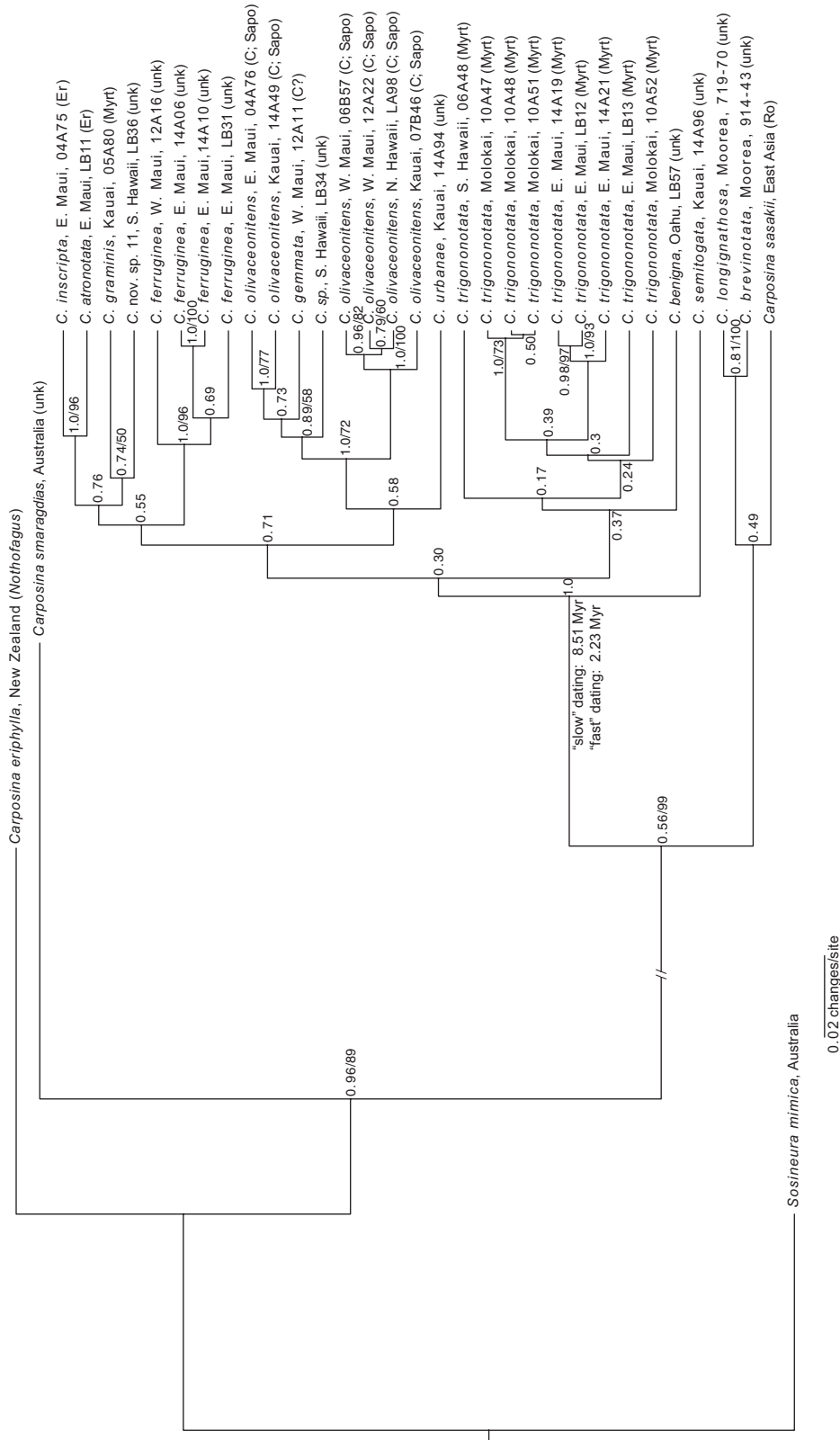


Figure 1. Phylogenetic tree using Bayesian inference for *Carposina* sampled in this study, based on two nuclear and one mitochondrial gene regions for almost every taxon (see Tables S1 and S2 for exceptions). Posterior probabilities are listed next to nodes; bootstrap support values are shown when > 50 and congruent with the Bayesian analysis. An estimate of time of colonization of Hawaii is given for both fast and slow estimates of COI evolution (see Methods). Tips are labelled with species, general localities, specimen codes and host-plant associations when known (C, Campanulaceae; Er, Ericaceae; Myrt, Myrtaceae; Ro, Rosaceae; Sapo, Sapotaceae; unk, unknown).

positions for COI, BEAST settings to date this node were identical to those used for the analysis of the concatenated dataset.

MORPHOLOGICAL METHODS

Genitalia dissections were carried out using the following protocol. We simmered abdomens in 10% KOH solution for 1 h, and transferred them to a 30% ethanol solution for 30 min. We removed the genitalia from the rest of the abdomen and stained them in a mixture of lactic acid and lignin pink for several minutes, followed by staining in cholorzol black for several seconds. We washed genitalia again in 30% ethanol before transferring to 100% ethanol for 30 min. Next, we transferred the genitalia to 100% isopropyl alcohol for 30 min, and Euparal Essence for several minutes before mounting on a slide using Euparal. Museum acronyms are as follows: BMNH: British Museum, Natural History, London, UK; BPBM: B.P. Bishop Museum, Honolulu, HI, USA; EMEC: Essig Museum of Entomology Collection, Berkeley, CA, USA; UHIM: University of Hawaii Insect Museum, Manoa, HI, USA.

RESULTS

The Hawaiian species we sampled form a monophyletic group [posterior probability (PP) = 1.0], as do the two Society Island species (Fig. 1). Two outgroup *Carposina* species from New Zealand and Australia appear distantly related to the rest of the *Carposina* represented in the tree. *Carposina sasakii* Matsumura, native to East Asia, is weakly grouped with the Society Islands species (PP = 0.49), and these three species together are tentatively grouped with the Hawaiian *Carposina* (PP = 0.56). The 'slow' method of dating the divergence of the Hawaiian clade resulted in an estimate of 8.51 Ma [95% highest posterior density (HPD) = 6.19–11.75 Ma] while the fast method of dating resulted in an estimate of 2.23 Ma (95% HPD = 1.59–2.88 Ma).

Two Hawaiian specimens (LB36 and LB34) appear as unique lineages in our molecular phylogeny (Fig. 1). LB36 is in a clade with *C. inscripta* (Walsingham), *C. atronotata* (Walsingham) and *C. graminis* (Walsingham). Although the genitalia and sequence data suggest this is a new species of Hawaiian *Carposina*, the wing patterns of the single specimen are difficult to discern because of rubbed scales (Fig. 2E), preventing adequate description, and so is noted as *C. new species 11*. Specimen LB34 is nested within a clade of *C. olivaceonitens* (Walsingham) (Fig. 1). Also nested within this clade is a specimen of *C. gemmata* (Walsingham). However, the poor physical condition of specimen LB34 prevents adequate morphological comparison with

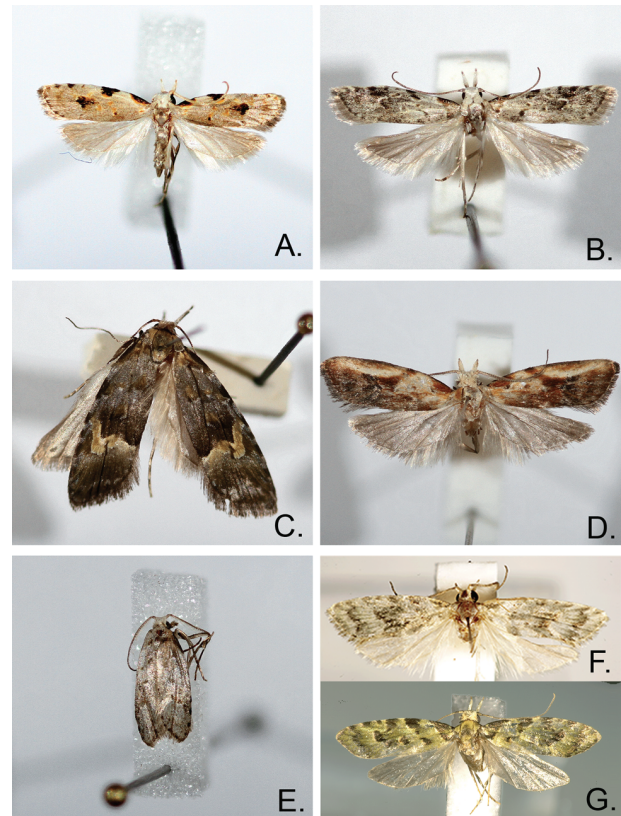


Figure 2. Adult *Carposina* (specimen codes in parentheses). A, *C. brevinotata* sp. nov. (PTO904-61). B, *C. longignathosa* sp. nov. (PTO918-80). C, *C. gagneorum* sp. nov. (LB60). D, *C. urbanae* sp. nov. (14A94). E, *C. new species 11* (LB36). F, *C. olivaceonitens* with nearly white forewing ground colour (12A22). G, *C. olivaceonitens* with green forewing ground colour (LA98).

known species, and is noted as *Carposina* sp. in the phylogeny (Fig. 1).

We recognize three new species of *Carposina* based on unique male genitalia, wing patterns and sequence data, including two species from the Society Islands and one from the Hawaiian Islands (Fig. 1). We also recognize a second Hawaiian species based on morphology, not represented in the molecular phylogeny.

TAXONOMY

FAMILY CARPOSINIDAE

GENUS *CARPOSINA* HERRICH-SCHÄFFER, 1853

Carposina are typical of the Carposinidae with up-turned or porrect labial palpi, often longer in the female, absence of chaemata and patches of raised scales on the dorsal surface of the forewing. In *Carposina*, male

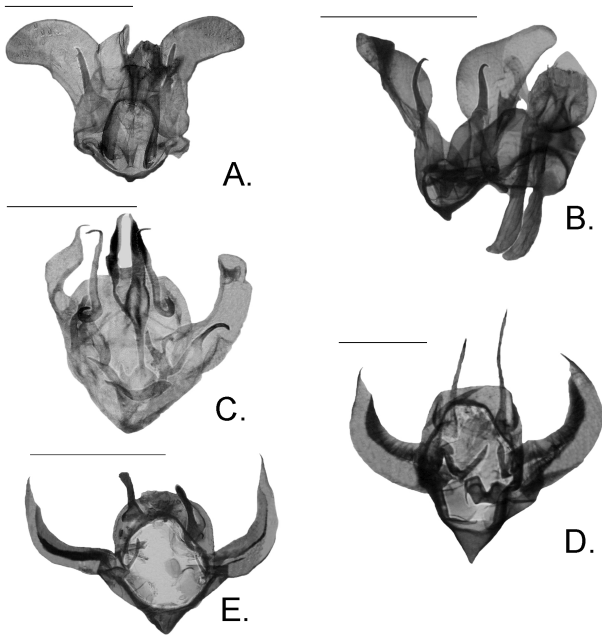


Figure 3. Genitalia of *Carposina*, males (specimen codes in parentheses). Scale bars indicate 0.5 mm. A, *C. brevinotata* sp. nov. (PTO914-43). B, *C. longignathosa* sp. nov. (PTO719-70). C, *C. gagneorum* sp. nov. (LB01; aedeagus *in situ*). D, *C. urbanae* sp. nov. (14A94). E, *C. new species 11* (LB36).

genitalia have uncus greatly reduced or absent, absence of socii and well-developed gnathos arms.

***CARPOSINA BREVINOTATA* sp. nov.**, MEDEIROS & OBOYSKI (FIGS 2A, 3A, 4A)

Holotype: French Polynesia: Society Islands: Moorea: Mt Mouaputa, 800 m, S17.52654 W149.80339. 15.ix.2009. ♂. PT Oboyski. PTO-904.61. Essig Museum of Entomology, Berkeley (EMEC).

Paratypes: French Polynesia: Society Islands: Tahiti: Mt Marau, 1189 m, S17.60822 W149.5511. 26.iv.2010. 1♂. PT Oboyski. Moorea: Mt Mouaputa, 800 m, S17.5265 W149.8034. 15.ix.2009. 3♂. PT Oboyski. Mt Rotui, ridge trail, 822 m, S17.50740 W149.84012. 5.ix.2008. PT Oboyski. Mt Tohiea ~summit, 1190 m, S17.55076 W149.82277. 24.ix.2009. 5♂, 1♀ (slide PTO-914.43♂). PT Oboyski, A Yang. Mt Tohiea ~summit, 1120 m, S17.55191 W149.82112. 23.ix.2009. 2♂, 1♀. PT Oboyski. Mt Tohiea trail, 940 m, S17.55337 W149.81860. 26.ix.2009. 1♂, 1♀ (slide PTO-918.65♀). PT Oboyski. BMNH; BPBM; EMEC; UHIM.

Localities of additional material examined (not part of the type series): French Polynesia: Society Islands:

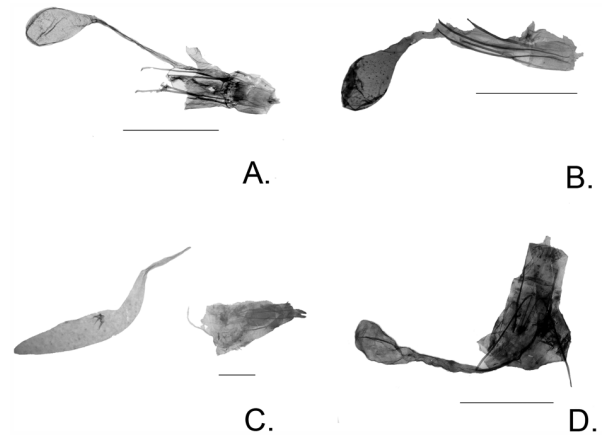


Figure 4. Genitalia of *Carposina*, females (specimen codes in parentheses). Scale bars indicate 1.0 mm. A, *C. brevinotata* sp. nov. (PTO918-65). B, *C. longignathosa* sp. nov. (PTO918-80). C, *C. gagneorum* sp. nov. (LB60; note that ductus bursae is broken). D, *C. urbanae* sp. nov. (LB61).

Huahine, Avea Baie ridge, 75m, UVL, 19.VII.2015, PT Oboyski. Moua Tapu, 400m, UVL, 21.VII.2015, PT Oboyski. EMEC.

Diagnosis: A distinctive wing pattern among the known French Polynesian *Carposina*, with dark black spots against a light brown background (Fig. 2A). The male genitalia are remarkably unornamented compared with other Polynesian *Carposina*, with the gnathos barely developed (Fig. 3A).

Description (N = 17) (Fig. 2A): Wing expanse 11–15 mm. Head light cream colour. Haustellum unscaled. Labial palpus longer than width of eye in male, nearly 2× width of eye in female, dark brown near base of second segment, transitioning to orange then cream colour or light brown by apex of third segment. Antennae of male with long, fine cilia underneath. Thorax, tegula and abdomen cream colour. Foreleg dark brown. Midleg brown with tufts of lighter scales near joints, spurs present. Hindleg entirely very light brown, spurs present. Forewing ground colour very light brown; dark brown subbasal and antemedial spots present along costal margin; these medial spots associated with clusters of raised scales; several smaller orange, brown and dark brown spots present near cell and along terminal margin; fringe minimal. Hindwing and fringe uniformly light pale brown.

Male genitalia (Fig. 3A): Valvae large, broad, rounded at apex. Uncus nearly absent. Annular lobes projecting sharply caudal, straight, nearly length of valva.

Saccus broadly U-shaped with small central lobe. Aedeagus long, slender, widened distally, cornuti present just below apex.

Female genitalia (Fig. 4A): Papillae anales short. Apophyses thin and straight; posterior apophyses relatively long, similar in length to anterior apophyses, both approximately length of ductus bursae. Corpus bursae oval, about 0.5× length of apophysis; signum absent.

Distribution: This species has been collected in areas of native vegetation from 75 m to 1190 m on the islands of Tahiti, Moorea and Huahine, Society Islands, French Polynesia. Sympatric with *C. longignathosa* sp. nov.

Remarks: The male genitalia of this species are relatively unornamented compared with other Polynesian *Carposina*. Larval biology and host plant unknown.

Etymology: Forewings of this species have small dark spots on a pale background.

CARPOSINA LONGIGNATHOSA sp. nov., MEDEIROS & OBOYSKI (FIGS 2B, 3B, 4B)

Holotype: French Polynesia: Society Islands: Moorea: Mt Rotui, ridge trail, 822 m, S17.50740 W149.84012. 5.ix.2008. ♂. PT Oboyski. PTO-719.35. EMEC.

Paratypes: French Polynesia: Society Islands: Moorea: Mt Rotui, ~ 800 m. 19.x.2002. 2♂, 1♀. PT Oboyski. Mt Rotui, ridge trail, 822 m, S17.50740 W149.84012. 5.ix.2008. 7♂ (slides PTO-719.70♂ & PTO-719.71♂). PT Oboyski. Mt Tohiea, trail, 840 m, S17.55352 W149.81747. 26.ix.2009. 1♂. PT Oboyski. Mt Tohiea, trail, 940 m, S17.55337 W149.81680. 2♀ (slide PTO-918.80). PT Oboyski. BMNH; BPBM; EMEC; UHIM.

Localities of additional material examined (not part of the type series): French Polynesia: Society Islands: Raiatea: Temehani Rahi, 465 m, UVL, 7.vii.2015, PT Oboyski. Temehani Rahi, 740 m, UVL, 6.vii.2015, PT Oboyski. Tahiti: Matofefe captage, 550 m, UVL, 27.vi.2015, PT Oboyski. Matofefe ridge, 650 m, UVL, 26.vi.2015, PT Oboyski. Mt Aorai, 1425 m, UVL, 22.VI.2015, PT Oboyski. Temaruata ridge, 805 m, UVL, 20.vi.2015, PT Oboyski. Temaruata ridge, 1130 m, UVL, 19.vi.2015, PT Oboyski. BMNH; BPBM; EMEC; UHIM.

Diagnosis: Although this species has a wing pattern similar to that of *C. apousia* Clarke, from Rapa, the male genitalia of *C. longignathosa* are distinctive: the gnathos arms are very long, noticeably longer than that of any other known *Carposina* (Fig. 3B).

Description (N = 15) (Fig. 2B): Wing expanse 11–14 mm. Head light cream colour. Haustellum unscaled. Labial palpus longer than width of eye in male, nearly 2× width of eye in female, dark brown near base of second segment, transitioning to cream colour back to brown by apex of third segment. Antennae of male with long, fine cilia underneath. Thorax, tegula and abdomen light brown to brown. Foreleg nearly black. Midleg dark brown with tufts of lighter scales near joints, spurs present. Hindleg entirely very light brown, spurs present. Forewing ground colour very light brown; orange to dark brown to black basal band, and somewhat broken ante- and postmedial bands present; these ante- and postmedial bands associated with clusters of raised scales; several brown spots present along costal and terminal margins; fringe minimal. Hindwing and fringe uniformly light pale brown.

Male genitalia (Fig. 3B): Valvae large, broad, tapering to rounded apex. Uncus apparently absent. Process of sacculus long, projecting sharply caudal. Saccus broadly U-shaped with small central lobe. Gnathos arms thick, broad, projecting cephalad. Aedeagus long, slender, widened distally, cornuti present just below apex.

Female genitalia (Fig. 4B): Papillae anales short. Apophyses thin and straight; posterior apophyses relatively long, approximately 1.5× length of anterior apophyses, the posterior apophyses approximately length of ductus bursae. Corpus bursae oval, about 0.5× length of ductus bursae; signum absent.

Distribution: This species has been collected on Tahiti (550–1425 m), Moorea (800–940 m), and Raiatea (465–740 m), Society Islands, French Polynesia. Sympatric with *C. brevinotata* sp. nov.

Remarks: The genitalia of this species are similar to those of Zimmerman's 'new species 5' (Zimmerman, 1978, p. 832), probably the result of convergence and not close phylogenetic relationship. While the wing pattern and female genitalia are reminiscent of *C. apousia* Clarke, from Rapa (no male was included in Clarke's description), this species has a significantly longer wing expanse. Larval biology and host plant unknown.

Etymology: Males of this species have an unusually long pair of gnathos.

CARPOSINA GAGNEORUM sp. nov., MEDEIROS & OBOYSKI (FIGS 2C, 3C, 4C)

Holotype: United States: Hawaiian Islands: Molokai: Kamakou Preserve, Pepeopae Trail. 19.v.2004. ♂ (slide LB01♂). D Rubinoff *et al.* UHIM.

Paratype: United States: Hawaiian Islands: Maui: Haleakala National Park, bog E of Kipahulu Val., 1859 m. 22–25.vi.1981. 1♀ 9 (slide LB60♀). WC Gagné. BPBM.

Diagnosis: No other Hawaiian *Carposina* has a similar wing pattern: a dark brown medial band, abutted by a very light brown band just distal to it (Fig. 2C).

Description (N = 2) (Fig. 2C): Wing expanse 17–29 mm. Head grey-brown. Haustellum unscaled. Labial palpus approximately width of eye in male, nearly 3× width of eye in female, second segment brown, third segment dark brown. Antennae of male with long, fine cilia underneath. Thorax and tegula grey-brown to olive (abdomens of both specimens cleared and mounted on slides). Foreleg black. Midleg dark brown, spurs present. Hindleg brown, spurs present. Forewing ground colour grey-brown; a curving very dark brown medial band present, abutted by a very light brown band just distal to it, these bands consisting largely of raised scales; additional clusters of raised scales scattered proximally to the medial bands, these clusters more numerous and pronounced in the female specimen; fringe brown. Hindwing and fringe uniformly light pale brown.

Male genitalia (Fig. 3C): Valvae long, narrow, tapering to an acute hooked apex. Uncus prominent, flanked by short dense setae. Arms of gnathos projecting upward sharply, tipped with outwardly projecting setae. Annular lobes short, straight, approximately 0.5× length of valva. Saccus V-shaped. Process of sacculus broad, curving outward, apex two-pronged. Aedeagus (*in situ*, Fig. 3C) long, broad, widened distally, cornuti present along entire posterior section.

Female genitalia (Fig. 3C): Papillae anales short. Apophyses thin and straight; posterior apophyses relatively long, 2× total length of anterior apophyses. Ductus bursae long, almost 2× length of posterior apophyses. Corpus bursae long, nearly length of ductus bursae; two large V-shaped signa present.

Distribution: This species has been collected on the islands of Molokai and Maui, Hawaiian Islands.

Remarks: The male genitalia of this species is similar to that of *C. graminicolor* (Walsingham) and *C. crinifera* (Walsingham), also from the Hawaiian islands, but the wing pattern is extremely divergent, unlike any other Hawaiian *Carposina*. This species has not been collected since 2004, and attempts to amplify DNA were unsuccessful. New material is needed to place it within the phylogeny of *Carposina*. Larval biology and host plant unknown.

Etymology: *Carposina gagneorum* is named in honor of Betsy Gagné, and her late husband Wayne Gagné. Wayne collected the first specimen of this species, and was greatly admired and respected for his work in Hawaiian entomology and conservation. Likewise, Betsy, through her work at the State of Hawaii Division of Forestry and Wildlife for many years, is an ally to the Hawaiian insect fauna, and a dear friend of MJM and PTO.

***CARPOSINA URBANAE* sp. nov., MEDEIROS & OBOYSKI (FIGS 2D, 3D, 4D)**

Holotype: United States: Hawaiian Islands: Kauai: Kokee St Park, Pihea Tr, 22.149, -159625. 21.vii.2014. ♂ (slide 14A94♂). MJ Medeiros & AX Nguyen. BPBM.

Paratypes: United States: Hawaiian Islands: Kauai: Na Pali – Kona FR, Alakai Swamp at Junction of Pihea Trail. 18.v.2005 & 23.vii.2006. 1♂, 1♀ (slide LB61♀). D Rubinoff. UHIM.

Diagnosis: *Carposina urbanae* has a unique wing pattern in comparison with all other Hawaiian *Carposina*: an orange-brown medial band running from the costa to middle of wing, diagonally toward the tornus (Fig. 2D).

Description (N = 3) (Fig. 2D): Wing expanse 17–20 mm. Head light brown. Haustellum unscaled. Labial palpus longer nearly 2× width of eye in male, over 2× width of eye in female, dark brown near base of second segment, transitioning to lighter brown by apex of third segment. Antennae of male with long, fine cilia underneath. Thorax, tegula and abdomen light brown. Foreleg and midleg very dark brown, with rings of lighter scales near joints of tarsi; spurs present in midleg. Hindleg similar but somewhat lighter in colour, spurs present. Forewing ground colour very light brown; orange-brown medial band running from costa to middle of wing, diagonally toward tornus, with several clusters of raised scales; posterior half of wing darker in colour than anterior half; orange-brown basal spot present; orange and black spots present along costal margin; fringe grey. Hindwing and fringe uniformly light brown.

Male genitalia (Fig. 3D): Valvae long, somewhat broad, tapering to an acute apex. Uncus nearly absent. Annular lobes projecting caudal, straight, less than 0.5× length of valva. Arms of gnathos long, projecting sharply upward, topped with short setae. Process of sacculus broad, tipped with two short lobes. Saccus V-shaped. Aedeagus long, slender, widened distally, cornuti present at apex.

Female genitalia (Fig. 4D): Papillae anales short. Apophyses thin and straight; posterior apophyses

relatively long, approximately 1.5× length of anterior apophyses. Ductus bursae long, approximately 1.5× length of posterior apophyses. Corpus bursae oval, short, about 0.5× length of anterior apophyses; signum absent.

Distribution: This species has been collected only from near the summit of the island of Kauai, Hawaiian Islands.

Remarks: The male genitalia of this species is similar to that of *C. ferruginea* (Walsingham) and Zimmerman's 'new species 2' (Zimmerman, 1978, p. 830), also from the Hawaiian Islands, but the wing pattern is extremely divergent, unlike any other Hawaiian *Carposina*. Larval biology and host plant unknown.

Etymology: *Carposina urbanae* is named in honour of the Urban School of San Francisco, where MJM has been a science teacher for 5 years, and many of his Urban Advanced Studies Genetics students performed PCRs used in this study. Urban has supported MJM's research programme in multiple ways. Lastly, Urban has shown a commitment to entomology, with the formation of a new class, Entomology: Bugs & Biodiversity, which provides an education in general entomology to high school students.

CARPOSINA NEW SPECIES 11 (FIGS 2E, 3E)

Material examined: United States: Hawaiian Islands: Hawaii: Hawaii Volcanoes National Park, Desolation Trail, 929 m, N19.36880 W155.3674. ♂ (slide LB36♂). D Rubinoff & A Kawahara. UHIM.

Remarks: This specimen has unique genitalia and a wing pattern unlike the other species near it in Figure 1, but the specimen is somewhat rubbed. Without additional material, we do not feel a full description is warranted at this time. The designation as new species 11 follows the sequence initiated by Zimmerman (1978).

DISCUSSION

Two new species, *C. longignathosa* sp. nov. and *C. brevinotata* sp. nov., from the Society Islands, French Polynesia, appear more closely related to the Asian *C. sasakii* Matsumura than the Hawaiian species (Fig. 1). Clarke (1971) named *C. paracrinifera*, a species from Rapa, for its superficial similarity to *C. crinifera* (Walsingham) from Hawaii. However, given the genetic distance and weak node support between the North and South Pacific species in this study, it is likely that Hawaiian *Carposina* derived from a northern temperate ancestor (Zimmerman, 1978), while the French Polynesia species appear to represent an independent incursion of the genus into the Pacific from the Austral-

Asian region. However, greater outgroup sampling is necessary to test this hypothesis. A similar pattern of multiple colonizations in the Pacific was also found in *Tetragnatha* spiders (Gillespie, 2002), *Misumenops* spiders (Garb, 2006), *Ptycta* bark lice (Bess *et al.*, 2014) and *Cydia* moths (Oboyski, 2011). Unfortunately, there are very few well-resolved phylogenies for Polynesian arthropods that broadly sample Pacific Islands as well as potential mainland source populations to fully evaluate how widespread this pattern is.

Three new species, *Carposina urbanae* sp. nov., *C. gagneorum* sp. nov. and *C. new species 11*, appear nested well within the Hawaiian clade, indicating they are part of the Hawaiian radiation and not recent immigrants. *Carposina urbanae* sp. nov. (host unknown) from Kauai appears weakly connected to an *olivaceonitens* clade that is distributed across the high islands feeding on *Clermontia* (Campanulaceae) and *Pouteria* (Sapotaceae). Although the *olivaceonitens* clade is well supported, the tentative placement of this species is likely to change with further sampling. *Carposina gagneorum* sp. nov. is known from only single male and single female specimens, with no sequence data. However, the wing pattern is so unique for Hawaiian *Carposina* (Fig. 2C) that we are confident they represent a single species separate from *C. crinifera* and *C. graminicolor* with which it shares similar male genital morphology. *C. new species 11* (host unknown) from Hawaii is in a moderately supported clade with *C. graminis* from Kauai which feeds on *Metrosideros* (Myrtaceae). The genetic and geographical distance between these specimens suggest other lineages within this clade exist on the intervening islands.

The distribution and host-plant associations for Hawaiian *Carposina* are confusing at best (Table 1). Species descriptions (Meyrick, 1883, 1913; Walsingham, 1907) were based on short series (in some cases single specimens) of adult moths, largely collected by R. C. L. Perkins during the Fauna Hawaiiensis project (Perkins, 1913). Confusion was further compounded by the high degree of wing pattern polymorphisms in several Hawaiian microlepidoptera groups. And although male genital characters are particularly useful for *Carposina*, their widespread use in Lepidoptera taxonomy began after Walsingham and Meyrick's work on Pacific Islands taxa. Larval host-plant records for several species were subsequently gained through extensive rearing efforts by O. H. Swezey (summarized in Swezey, 1954). However, Zimmerman (1978) questioned many of Swezey's identifications and recognized *Carposina* new species 1 to 10 to account for discordant host and island records (Table 1). In particular, Zimmerman (1978) questioned records for *C. olivaceonitens*, which included plants in the distantly related families Campanulaceae and Sapotaceae. Our phylogeny shows two well-supported clades of *C. olivaceonitens* that could

Table 1. Host-island matrix of *Carposina* species in Hawaii

	Kauai	Oahu	Molokai	Lanai	Maui	Hawaii	Total
Host unknown	<i>cervinella corticella</i> <i>distincta divaricata</i> <i>piperatella</i> <i>plumbeonitida</i> <i>semitogata tincta?</i> <i>urbane</i>	<i>benigna crinifera? glauca</i> <i>herbarum? lacerata</i> <i>nigromaculata pusilla</i> <i>saurates</i>	<i>bicincta crinifera</i> <i>ferruginea punctulata</i> <i>gagneorum</i>	<i>herbarum irrorata</i> <i>subolivacea tincta?</i>	<i>mauii togata</i> <i>gagneorum</i>	<i>achroana herbarum?</i> <i>dispar latifasciata</i> <i>plumbeonitida?</i> <i>pygmaeella tincta</i> new sp. 11	31
Campanulaceae	<i>olivaceonitens</i>	<i>gemmata? olivaceonitens?</i> new sp. 3	new sp. 9		new sp. 6 <i>olivaceonitens?</i>	<i>gemmata olivaceonitens?</i>	4
<i>Clermontia</i>		new sp. 5					1
<i>Cyanea</i>		<i>gracillima</i> new sp. 1	<i>gracillima</i>		<i>gracillima atronotata</i>	<i>gracillima inscripta</i>	1
Elaeocarpaceae		<i>viridis?</i>					3
<i>Elaeocarpus</i>		<i>subumbrata</i>					1
Ericaceae		new sp. 2					1
<i>Leptecophylla</i>		<i>nigronotata?</i>					1
<i>Vaccinium</i>		new sp. 4 new sp. 5	<i>trigononotata</i>				3
Gesneriaceae		<i>graminis</i>					1
<i>Cyrtandra</i>		<i>graminicolor</i>					1
Goodeniaceae		new sp. 10					1
<i>Scaevola</i>							1
Hydrangeaceae							1
<i>Broussaisia</i>							1
Myrsinaceae							1
<i>Myrsine</i>							3
Myrtaceae							2
<i>Metrosideros</i>							1
<i>Syzygium</i>							1
Oleaceae							1
<i>Nestegis</i>							1
Pentaceae							1
<i>Plectronia</i>							1
Rhamnaceae							1
<i>Alphitonia</i>							1
Rubiaceae							1
<i>Bobea</i>							1
<i>Kadua</i>							2
<i>Canthium</i>							1
Sapotaceae							1
<i>Pouteria</i>							1
Total	16	22	8	4	8	16	1

? indicates host or island records from O. H. Swezey (1954) needing confirmation, according to Zimmerman (1978). All species described in the genus *Heterocrassa* by Walsingham, 1907, except for *C. achroana* Meyrick, 1883; *C. glauca*, *C. lacerata*, *C. saurates* and *C. benigna* Meyrick, 1913; and *C. gagneorum* sp. nov. and *C. urbanae* sp. nov. Medeiros & Obayski. New species 1–10 are those noted by Zimmerman (1978). New species 11 (this paper).

represent cryptic species, or host races in the process of diverging. Moreover, polymorphism in this clade (compare Fig. 2F and G) makes species assignment difficult based on superficial morphology. This uncertainty can only be resolved by comparing the morphology and molecules of specimens reared from each host across the archipelago.

Presently, no new host associations are proposed, but some island records are confirmed or noted as new (Table 1). *Carposina atronotata* is reported from Maui; *C. ferruginea* (Walsingham), known only from Molokai, is reported from Maui; *C. gemmata*, known from Hawaii (and possibly Oahu), is reported from Kauai; and *C. olivaceonitens*, that Zimmerman (1978) restricted to Kauai, is confirmed on Maui and Hawaii.

Our analyses support the monophyly of Hawaiian *Carposina* (Fig. 1). Using typical and accelerated mutation rates for Lepidoptera (Brower, 1994; Haines *et al.*, 2014), our results predict a period of 2.23–8.51 Myr (95% HPD 1.59–11.75 Myr) since the arrival of *Carposina* in Hawaii. The current high islands were formed 0.5 Mya (Hawaii) to 5 Mya (Kauai) (Carson & Clague, 1995; Price & Clague, 2002), which places *Carposina* colonization sometime during the formation of Nihoa, Niihau, Kauai, Oahu or the Maui Nui complex. However, these preliminary findings are likely to change with further taxon sampling, additional molecular data and more refined estimates of mutation rates.

Although the basal species in our limited sampling of the Hawaiian clade, *C. semitogata*, was collected from Kauai, the overall topology does not lend obvious support to a progression rule pattern of diversification (Funk & Wagner, 1995). Instead, subclades appear to include representatives feeding on the same host on both old and young islands. Similar patterns of diversification were shown for Hawaiian *Cydia* Hübner (Lepidoptera: Tortricidae), whereby jumps to new host genera in disparate subfamilies of Fabaceae were accompanied by filling those host niches across the archipelago (Oboyski, 2011), and for *Nesophrosyne* Kirkaldy (Hemiptera: Cicadellidae) with host jumps between plant families (Bennett & O'Grady, 2012, 2013). In this scenario, some species are likely to become paraphyletic as a result of differential dispersal between land masses – more isolated populations will develop evolutionary trajectories independent of their containing clade. This appears to be the case for *C. olivaceonitens* in the current study, which is rendered paraphyletic by *C. gemmata* and LB34 (a damaged specimen that we currently are not able to identify with certainty) (Fig. 1), both of which have distinctly different genital morphology from *C. olivaceonitens*.

The Hawaiian *Carposina* clade is separated from outgroup taxa by a relatively long branch, while several interior branches have only modest support (Fig. 1). Several factors may contribute to this, including limited

outgroup sampling, limited ingroup sampling, choice of genetic markers, a long period of isolation for the Hawaiian clade, extinction and/or accelerated evolutionary rates. As a result, long branches make Hawaiian *Carposina* difficult to place in the world fauna. Although extinction is difficult to account for in phylogenetic reconstruction (e.g. Morlon, Parsons & Plotkin, 2011), these other factors can be addressed directly with continued investigation.

Carposina present an opportunity to test competing hypotheses about Hawaiian phylogeography and phylogeology. While several species are known for each island, host associations remain obscured for most (Table 1). Moreover, host/habitat loss, extinctions, climate change, and competition and predation from alien species are likely to hinder collection of essential ecological and evolutionary data (Medeiros *et al.*, 2013). Therefore, identifying larval hosts, particularly from critically endangered habitats, and constructing a well-resolved phylogeny for the entire clade is the highest priority for this group.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Specimens included in the phylogenetic analysis.

Table S2. Primers used in this study.