

## First Confirmed Record of Leaf Mining in the Fruitworm Moths (Carposinidae): A New Species Feeding on an Endemic Hawaiian *Clermontia* (Campanulaceae)

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**Abstract.** We discovered an unknown insect mining the leaves of *Clermontia fauriei* (Campanulaceae) in the Alakai Swamp, Kauai. Although the leaf mines superficially resembled those of the Hawaiian endemic genus *Philodoria* (Gracillariidae), or possibly *Euperissus* (Cosmopterigidae), rearing revealed an undescribed species of *Carposina* (Carposinidae). We describe it here as *Carposina hahaiella* **sp. n.**, and include detailed information on the morphology, leaf mines, cytochrome c oxidase I sequences, and a parasitoid of the new species. *Carposina hahaiella* represents the first confirmed record of leaf mining in the fruitworm moth family, adding to a remarkable variety of larval habits in Carposinidae.

**Key words:** taxonomy, plant-insect, Kauai, diversification, endangered

### Introduction

*“I regret most deeply that I have not been given the opportunity to study this genus with the care which it deserves.”*

—Elwood C. Zimmerman on *Carposina*, 1978.

The family Carposinidae, commonly known as fruitworm moths, comprises nearly 300 described species found in all major biogeographical regions, but the family is most diverse in the Indo-Pacific region (van Nieukerken et al. 2011, Dugdale et al. 1999, Zimmerman 1978). The 41 described and 11 undescribed Hawaiian species are all assigned to the genus *Carposina*, and most are endemic to one or a few islands (Medeiros et al. 2016, Zimmerman 1978). The group is especially remarkable for its diversity of larval habits; many feed in berries or fruits as their com-

mon name implies, but some larvae feed on terminal buds, some are stem borers, others are gall formers, and still others feed externally on foliage. Larval hosts include at least 15 different plant families in Hawaii alone (Zimmerman 1978).

Whether or not leaf mining, where larva feed internally in a leaf, occurs in Carposinidae has been a point of contention. There are two historical records of leafmining carposinids, both from Hawaii. One was misidentified as *Heterocrossa* (= *Carposina*) *crinifera* (Walsingham) by Swezey (1913) and subsequently lost (Zimmerman 1978). The larvae were reported to mine the leaves of *Cyanea humboldtiana* (Gaudich.) Lammers, Givnish & Sytsma (Campanulaceae) on Mt. Olympus, Oahu. Swezey (1954) stated that his original identification was “based on an error in determination.” Zimmerman (1978) referred to this leafmining species as “*Carposina* new species 3.” A

second undescribed species was reared from leaf mines on *Clermontia kakeana* and *C. arborescens* from Haelaau, Maui (Swezey 1954). We were unable to locate these specimens which Zimmerman (1978) referred to as “*Carposina* new species 6.” Several papers (e.g., Meyrick 1922, Davis 1969, Connor and Taverner 1997, Ponomarenko 1999) suggest that Carposinidae are known to be leaf miners, presumably referring to Swezey (1913) or Swezey (1954). However, these two records are unverified and incomplete, even though it has been repeated multiple times in the literature. Despite the close proximity of Mt. Olympus to the University of Hawaii at Manoa campus, the leaf miner reported by Swezey (1913) has never again been seen, possibly a victim of the severe impacts of invasive species on Hawaii’s native insect fauna (Medeiros et al. 2017). The same may be true for the Maui species. We here describe a new species of *Carposina* from the Alakai Swamp on Kauai that was incidentally discovered and represents the first confirmed record of the leaf mining habit in Carposinidae.

### Material and methods

**Sampling.** We collected *Clermontia fauriei* leaves with mines during fieldwork along the Alakai Swamp Trail, Kauai, in July 2020. We placed tenanted leaf mines in a 32-oz rearing container and monitored them daily. Larvae that exited the mine were reluctant to pupate, often wandering for days. Some were moved to a smaller container with several potential pupation substrates, including tissue, moss, and soil. Three larvae were preserved in 95% ethanol in a  $-20^{\circ}$  freezer; emerged adults were pinned and spread. Vacated leaf mines were pressed and dried. We used an Epson V600 Photo flatbed scanner to create transmitted light scans of the dried leaf mines. All voucher material is deposited in the University of Hawaii Insect Museum (UHIM).

**Dissection, DNA extraction, and sequencing.** We used a non-destructive DNA extraction method on one larva and both emerged adults, to maximize the retention of morphological characters. The ethanol-preserved larva was prepared for lysis by using a sharpened #2 pin to make small perforations near the head and segment A10, and a cut along middle segments. We removed the abdomens of the adults by gently tilting them upwards. We then used a Qiagen DNeasy blood and tissue spin-column kit (Qiagen, Inc., Valencia, CA) to extract DNA following manufacturer’s protocols. The abdomens and larval pelt were removed from the lysis buffer after incubation using a minutien and mounted on a microscopic glass slide, using standard procedures for small Lepidoptera (Robinson 1976). Phenosafranin-O was used to stain the male genitalia and pelt, and Eosin Y and chlorazol-black to stain the female genitalia before slide-mounting in euparal. Photographs of the slides were taken using a Zeiss Discovery v8 microscope mounted with a Sony alpha a6300 camera. Morphological terminology for adult genitalia follows Klots (1956) except for “ostium,” which we use instead of “ostium bursae.”

To establish a genetic reference we amplified a section of the cytochrome c oxidase I gene with forward primer LCO 5’ GCTCAACAAATCATAAAGATATTGG 3’ and reverse primer Pat2 5’ TC-CATTACATATAATCTGCCATATTAG 3’ (Simon 1994). We used Q5 hot-start high fidelity 2x DNA polymerase (New England BioLabs, Ipswich, MA) with the manufacturer’s recommended PCR program and reagent mix. PCR products were visualized on a 1% agarose gel, followed by purification with QIAquick spin columns (Qiagen, Inc., Valencia, CA) according to the manufacturer’s protocol. For each sample, sense and anti-sense strands of the PCR products were sequenced via the Sanger method

in two parts, outsourced to Eurofins (KY, USA). The first part (COI-5P) was sequenced with LCO and HCO 5' TA-AACTTCAGGGTGACCAAAAAATCA 3' (Simon 1994). The second part (COI-3P) was sequenced with Jerry 5' CAACATT-TATTTTGATTTTTTGG 3' (Simon 1994) combined with Pat2. Sample data and COI sequences are available on the Barcoding of Life Datasystems (BOLD; Dataset DOI <https://dx.doi.org/10.5883/DS-CAROHA>) and in NCBI GenBank under accessions MW698684–6.

To better understand the identity and potential novelty of the species we had reared, we aligned the COI-3P section of our specimens to published sequences of Hawaiian *Carposina* from Medeiros et al. (2016), creating a 724 base pair alignment. We used IQ-Tree v2.0.3 (Minh et al. 2020) to infer a maximum likelihood tree, with model selection through the integrated ModelFinder, which used the Bayesian information criterion to determine TPM2+F+I+G4 as the best fit. We performed 50 independent runs to find the optimal tree and support values for the branches were estimated with 5,000 ultra-fast bootstrap replicates. While this approach is not appropriate for establishing phylogenetic relationships among species (Rubinoff and Holland 2005), in combination with the morphology and ecology it can provide insight into species identity (e.g., Rubinoff and Powell 2004).

## Results

Larvae typically exited the mine to pupate and wandered in the rearing containers. Although most larvae dug into the provided soil, and disregarded the offered paper tissue, moss, and detritus, we did not find any pupae after several weeks, so we assume the larvae died. We suspect they may have sought specific environmental conditions that we did not provide in the lab for pupation. Of the approximately 15

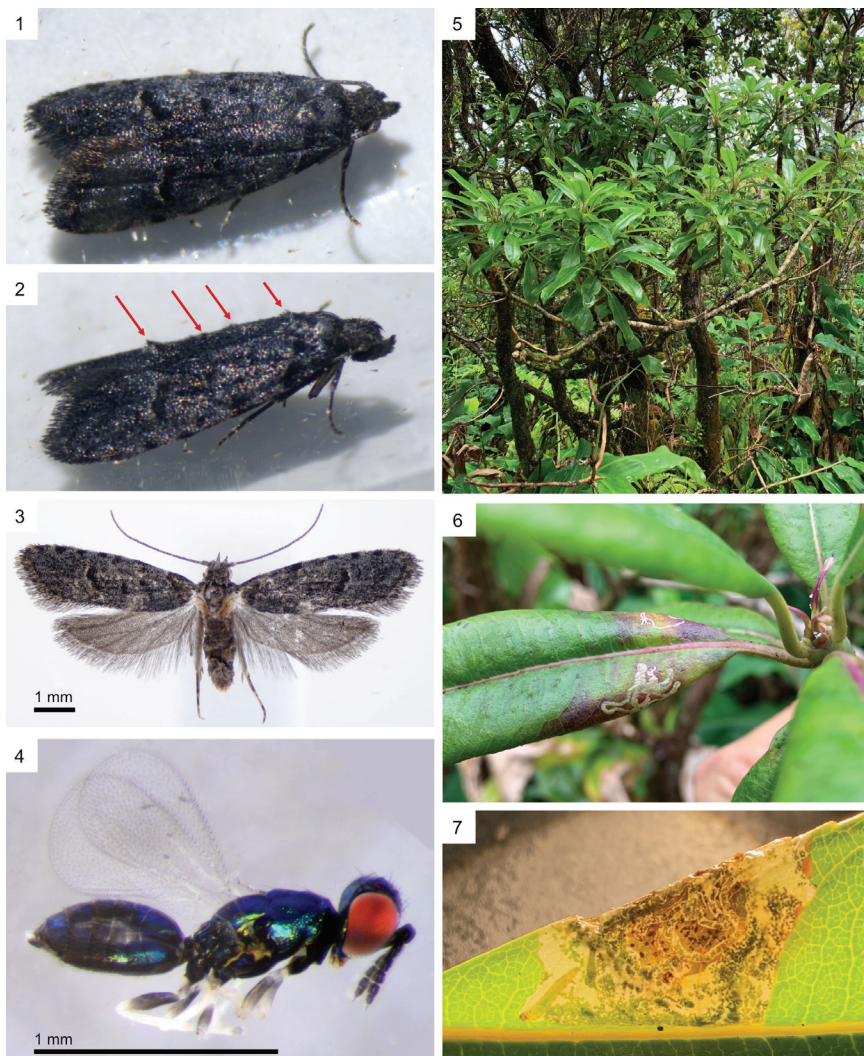
larvae we collected, only two produced adult moths.

### *Carposina hahaiella* sp. nov.

**Holotype.** Figs. 1–3. Female. Pinned, labelled: “United States: Hawaii: Kauai: Alaka'i x Pihea trail. N 22.1494 E –159.6153. 21.vii.2020. Leaf mines on *Clermontia fauriei*. e.l. 14.viii.2020. Leg. D., R. Rubinoff and C. Doorenweerd. DNA sample UHIM.DNA00096, genitalia slide KAA #0390.” Deposited in the University of Hawaii Insect Museum (UHIM).

**Paratype.** One female, pinned, same data as holotype, except em: 13.viii.2020. DNA sample UHIM.DNA00022. Deposited in UHIM.

**Differential diagnosis.** *Carposina hahaiella* is among the smallest described species of *Carposina* in Hawaii. Only *C. pusilla* (Walsingham), described from Oahu, and *C. pygmaeella* (Walsingham), described from Hawaii island, have a shorter forewing length. Both of these species are known only from males but can be distinguished from *C. hahaiella* externally by the following: the forewing of *C. pusilla* is yellowish white and the forewing of *C. pygmaeella* is dark brown, whereas the forewing of *C. hahaiella* is chalky gray-blue. The small size of *C. hahaiella* (female FWL 4.1–4.3 mm) and its chalky gray-blue forewing ground color separate it from all other known *Carposina* in Hawaii. *Carposina hahaiella* may be superficially confused with *C. gemmata* (Walsingham) based on the wing patterning, color, and iridescence, but the latter has a horizontally oriented medial spot, which is vertically oriented in *C. hahaiella*, and the former is significantly larger with a wingspan of 22 mm. The leaf-mining life history on *Clermontia* also separates *C. hahaiella* from all other described *Carposina* in Hawaii with known larval habits. Like most, but not all, Hawaiian *Carposina*, *C. hahai-*



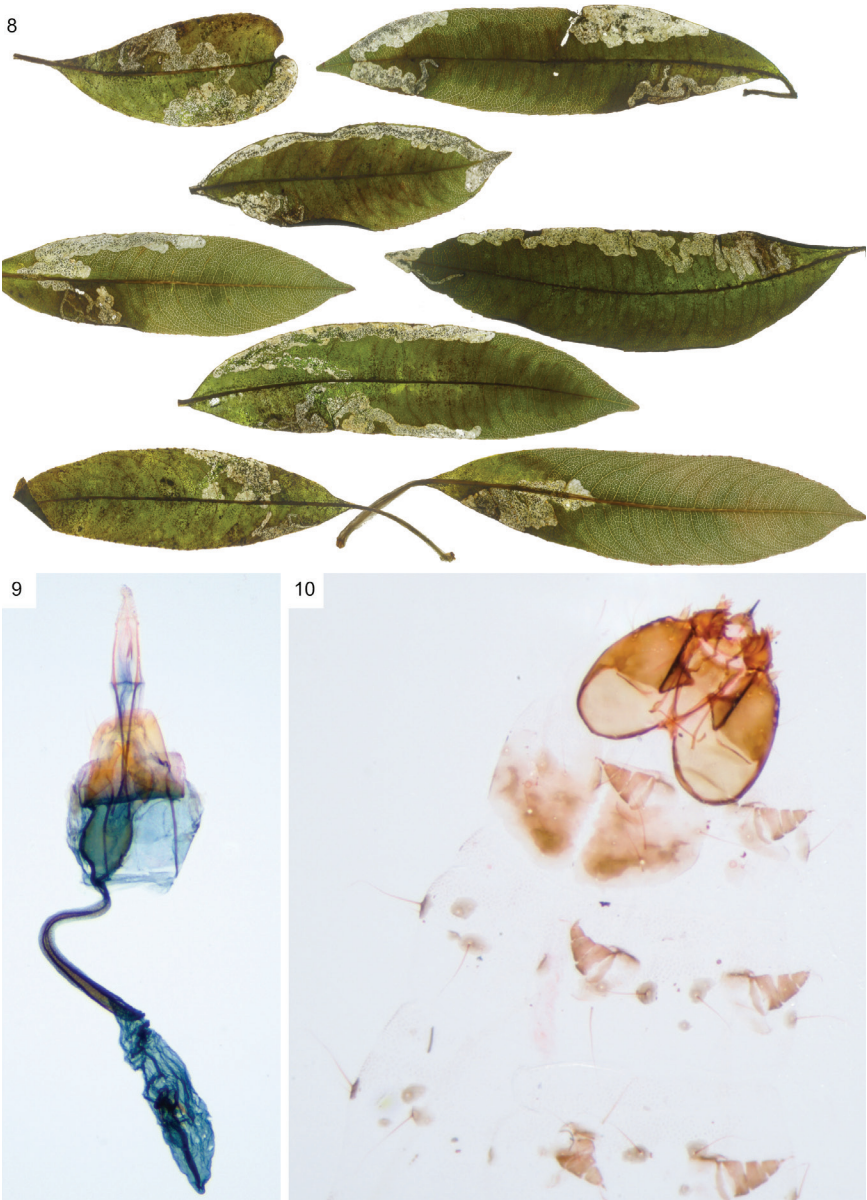
**Figures 1–7.** 1. Dorsal view of holotype adult of *Carposina hahaiella* in resting position. 2. Angled view of holotype in resting position; red arrows indicate patches of black raised scales. 3. Pinned holotype. 4. *Pediobius* sp. parasitoid wasp (UHIM.DNA00075); identification by David Honsberger. 5. Host plant in its native habitat, note invasive kahili ginger plants dominating the undergrowth. 6. Leaf mines on *Clermontia* in the field. 7. Transmitted light view of live larva feeding in leaf mine.

*ella* possesses a pair of deeply furcate two-pronged signa in the corpus bursae (signa are absent in *C. gemmata*). Except for their comparatively smaller size, little else separates the female genitalia of *C.*

*hahaiella* from other species of Hawaiian *Carposina*.

**Molecular diagnostics.** Fig. 11. The COI (3-P fragment) sequences are most similar to *Carposina olivaceonitens*





**Figures 8–10.** 8. Composition of transmitted-light scans of dried leaf mines of *Carpocina hahaiella* on *Clermontia fauriei*. Each leaf has one to three mines, some with a dead larva inside. 9. Holotype female genitalia, specimen UHIM.DNA00096, genitalia slide KAA #0390. 10. Slide mounted larva after DNA-extraction, UHIM.DNA00072, dorsal view of section with head capsule and prolegs.

(Walsingham) samples from Maui, at 6.1% pairwise distance. However, *C. olivaceonitens* is polyphyletic in the phylogenetic tree in Medeiros et al. (2016). The sequence falls within a cluster of Campanulaceae feeders (Fig. 11).

**Description of adult. Male.** Unknown. **Female** ( $n=2$ ). **Head.** Frons, vertex clothed in gray-blue, smoothly appressed scales; vertex partially to completely obscured by large, concolorous supraocular scale tufts; ocellus, chaetosemata not observed; proboscis well-developed, naked; labial palpus approximately 1.5 $\times$  width of compound eye, lateral surface with dark gray to gray-blue scales, second segment apically expanded, apex of third segment with white scales. Antenna approximately 0.7 $\times$  length of forewing, filiform, dark gray; scape concolorous or slightly paler. **Thorax.** Scales on pronotum dark gray to black, gray-blue on posterior third; tegula similarly colored. Legs dark gray to black; spurs and joints of tarsomeres pale. Dorsal surface of forewing (FWL: 4.1–4.3 mm) with ground color chalky gray-blue with scattered black scaling; patches of uniformly black scales present at base, as a raised patch at end of discal cell (flattened in pinned specimens), and at regular intervals along distal half of costa; fringe dark gray. Ventral surface of forewing uniformly gray. Dorsal surface of hindwing gray, fringe gray; frenulum with two bristles. Ventral surface of hindwing gray. Venation similar to species figured in Zimmerman (1978) except for a more strongly developed M-vein in the discal cell of the forewing. **Abdomen** ( $n=1$ ). Vestiture of abdomen entirely silvery gray. Genitalia (Fig. 9) with ovipositor telescopic; papillae anales slender, short, ventral surface slightly roughened, covered in short- to moderate-length setae; posterior apophyses slender, long, approximately 2 $\times$  length of sternum VII; anterior apophyses approximately length of sternum VII; sclerotized por-

tion of sternite VIII incomplete medially, instead with a pair of posteriorly directed arm-like lobes which approach near each other distally but are not joined or fused; ostium broad, globose; ductus bursae with cestum-like structure present throughout entire length; wall of ductus bursae finely scobinate, appearing minutely reticulate; corpus bursae membranous with a pair of deeply furcate, two-pronged signa.

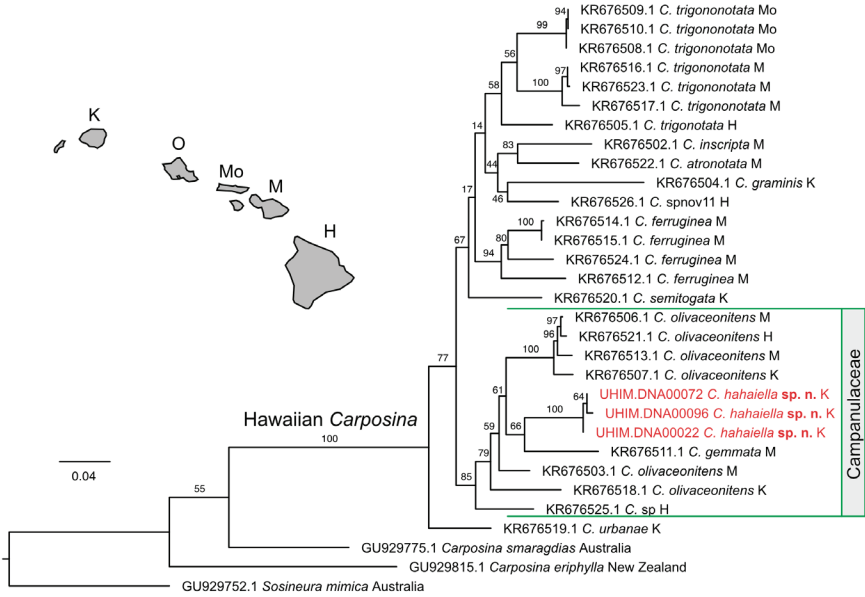
**Larva.** Figs. 7, 11. Body pale green, head capsule light brown. Chaetotaxy closely matching the family diagnosis (Swatschek 1958): prothoracic shield with the L-group bisetose (as opposed to trisetose in Tortricidae); abdominal segments with SV-group quadrisetose (as opposed to unisetose or bisetose in Pyraloidea); A8 with SD1 anterodorsal from the spiracle; and A9 with D1 closer to D2 than SD1; A9 without L2, L3. Prolegs sclerotized.

**Host plant.** Figs. 6, 7. *Clermontia fauriei* H. Lev. (Campanulaceae). Hawaiian name: Hāhā ‘aiakamanu.

**Leaf mine.** Figs. 6, 7, 9. The larva creates an irregularly shaped mine that crosses itself in early stages and often has tight coils with merging margin corridors, the final section is mostly linear. Dried leaves stored in UHIM. We found one to three larvae per leaf, the larva appears to enter the leaf from the underside, with some tearing in the lower epidermis adjacent to the point of oviposition. Frass pellets are irregularly deposited in the mine. The leaf mines resemble those created by the endemic moth genus *Philodoria* (Gracilariidae), but there are no species of that genus known to feed on Campanulaceae (Johns et al. 2018).

**Parasitoid.** Fig. 4. We reared a single parasitoid wasp, tentatively identified as *Pediobius* sp. (Eulophidae). The specimen is preserved in 95% ethanol, UHIM. DNA00075.

**Etymology.** The species epithet is a noun in apposition, composed of the



**Figure 11.** Maximum likelihood tree inference of 724 base pair alignment of COI-3P sequences. KR and GU sample codes are NCBI GenBank accessions, UHIM codes refer to samples added here. K = Kauai, O = Oahu, Mo = Molokai, M = Maui, H = Hawaii.

Hawaiian name for the Kauai species of *Clermontia*, hāhā, and the suffix ‘-ella’ to denote its relatively small size and as a reference to the common use of this suffix in leaf mining moth families such as Gracillariidae and Nepticulidae.

## Discussion

**Confirmed leaf mining in *Carposinidae*.** Based on the distinct ecology, morphology, and COI sequence data, we determined that this represents a new species of carposinid in the genus *Carposina*. The leaf mine record of *Carposina* “new species 3” (see Zimmerman 1978) reared by Swezey (but the specimens were lost and never described) was described to follow the midrib of the leaf outward with lateral tunnels on both sides, on *Cyanea humboldtiana* plants on Oahu. Based on the description of the mine, the different host and different island of collection, it is

unlikely that this record refers to *Carposina hahaiella*. Two other species of *Carposina* have been reared from *Clermontia*: *Carposina olivaceonitens* (Walsingham) from fruits and flower buds of *Clermontia kakeana* Meyen on O’ahu and *Carposina gemmata* (Walsingham) from *Clermontia* fruits on Oahu (Swezey 1919). The type localities of *C. olivaceonitens* and *C. gemmata* are Kauai and Hawaii island, respectively; we find Swezey’s determinations to be questionable, as did Zimmerman (1978). *Carposina olivaceonitens* also appears paraphyletic based on the limited genetic data that is available (Medeiros et al. 2016); *Carposina* clearly is in dire need of a modern systematic revision. The COI gene-tree further suggests that the Campanulaceae-feeding *Carposina* may form a monophyletic group, having resulted from a single shift to this family with subsequent dispersal to different

islands, use of different plant parts, and speciation. A potential synapomorphic character for this group is the presence of metallic/iridescent scales (Medeiros, pers. comm.). Such hypotheses, as well as those on biogeographic dispersal and speciation, remain to be tested with additional sampling and robust species trees from nuclear genomic approaches.

**Conservation status.** The new species is known only from its type locality, and it is likely that it is endangered. The host plant, like most native forest plants, is threatened in particular by feral pigs and invasive kahili ginger (*Hedychium gardnerianum* Sheppard ex Ker Gawl.), the latter of which is taking over the understory of Hawaiian rainforests, eliminating a diverse native fauna (Allison and Vitousek 2004). Fencing and active removal of ginger in the Alakai Swamp would be feasible and important steps for the conservation of *Clermontia* and other native Hawaiian plants there as well as the insects that rely on them.

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