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From the dark side of paradise: a new natural replication of cave planthopper evolution from Hawaiian lava tubes (Hemiptera: Fulgoromorpha: Cixiidae)

Hannelore Hoch[1,*], Megan L. Porter[2], Christy M. Slay[3], Michael E. Slay[4], Mireille Steck[2], Rebecca A. Chong[2]

[1]Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Humboldt-Universität zu Berlin, Invalidenstrasse 43, 10115 Berlin, Germany
[2]School of Life Sciences, University of Hawaiʻi at Mānoa, Honolulu, HI 96822, USA
[3]The Sustainability Consortium, Julie Ann Wrigley Global Futures Laboratory, Arizona State University, Tempe, AZ 85281, USA
[4]Arkansas Field Office, The Nature Conservancy, Little Rock, AR 72201, USA

[*]Corresponding author. Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Humboldt-Universität zu Berlin, Invalidenstrasse 43, 10115 Berlin, Germany. E-mail: hhoch186@gmail.com

ABSTRACT

The Hawaiian Islands are known to harbour a rich and diverse fauna of troglobionts (obligate subterranean species). To date, 74 obligate cavernicolous arthropod species have been documented from across the main Hawaiian islands, the majority of which were from Hawaiʻi Island, and mostly from lava tubes of Kilauea volcano, the youngest volcano on the island. A recent bioinventory of the Kipuka Kanohina lava tube system on the south-western side of Mauna Loa volcano revealed the existence of previously unknown cave-adapted species. Among them is the first cave-adapted species of the planthopper genus *Iolania*, *Iolania frankanstonei* Hoch & Porter sp. nov. Morphological and molecular data suggest that the species is closely related to the epigean (i.e. surface-dwelling) species *Iolania perkinsi*, which occurs in surface environments on Hawaiʻi Island. Thus, parapatric speciation is assumed, further corroborating the assumption that adaptive shifts are the major evolutionary patterns underlying the evolution of troglobionts on young oceanic islands.

Keywords: adaptive shift; caves; parapatric speciation; root feeders; troglobionts; troglomorphy

INTRODUCTION

Geologically young, oceanic island archipelagos provide impressive examples of adaptive radiation in many groups of organisms and offer unique opportunities to study the evolutionary processes underlying rapid diversification of initial colonizers, driven by adaptation to novel ecological conditions and by geological dynamics. The Hawaiian Islands, with their known geological history, linear arrangement of islands, and great distance from mainland continents, provide an unparalleled opportunity to study how rapid evolution can result in high levels of species diversity and in ecological convergence across relatively young island habitats. The diverse insect fauna of Hawaiʻi, today comprising an estimated total of 10 000 species (Howarth and Mull 1992), can be traced back to only 350–400 original colonizing species (Howarth 1990). Among the more species-rich insect taxa across the Hawaiian Islands are the planthoppers (Hemiptera: Fulgoromorpha), namely the Delphacidae (with 143 endemic species; Asche 1997) and the Cixiidae (Zimmerman 1948), the latter including members of the genera *Oliarus* Stål, 1862 and *Iolania* Kirkaldy, 1902 (Fig. 1). Although *Oliarus* has undergone extensive adaptive radiation on all islands, with >60 endemic species, including at least seven cavernicolous species on Maui, Molokaʻi, and Hawaiʻi Island (Zimmerman 1948, Fennah 1973, Asche 1997, Hoch and Howarth 1999), to date only six surface-dwelling epigean species have been documented in *Iolania*, three of which are single-island endemics (Hoch 2006). These contrasting patterns of species diversity highlight how ecologically similar lineages can undergo different evolutionary trajectories. Thus, it remains unknown why *Iolania* comprises comparatively few, largely allopatrically distributed species with no apparent ecological specialization (Hoch 2006).

Low host plant specificity and high dispersal ability in *Iolania* species are suggested to have counteracted ecological specialization, and eventually, speciation (Hoch 2006). Specifically, Hoch (2006) argued that *Iolania* dispersal and speciation patterns

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appear closely linked to geological events, especially to the well-defined life cycle stages of volcanoes forming the Hawaiian Island chain (Moore and Clague 1992). *Iolania* speciation patterns proceed from older to younger islands by progressive inter-island dispersal (from Kaua'i to Maui Nui and O'ahu, respectively, and from O'ahu to Hawai'i Island), and divergence events leading to terminal taxa might be attributable to geological events at later stages of volcanic growth (characterized by erosion and landslides, and the subsidence stage). For example, the break-up of the Maui Nui island complex led to the divergence of *Iolania lanaiensis* Giffard, 1925 and *Iolania mauiensis* Giffard, 1925, and geological events (e.g. landslides and erosion) in the post-shield stage of the once large Wai'anae and Ko'olau volcanoes on O'ahu resulted in the speciation of *Iolania koolauensis* Giffard, 1925 and *Iolania oahuensis* Giffard, 1925 owing to reduction of suitable *Iolania* habitat (Hoch 2006). Remarkably, a previously unknown cavernicolous *Iolania* species, described below, has been recorded from recent biological studies in caves within the Kipuka Kanohina lava tube system on the Island of Hawai'i (Fig. 1, 2). This new discovery challenges the assumption that *Iolania* has diversified strictly by allopatric speciation and highlights that speciation can be driven by adaptation to novel ecological conditions.

MATERIALS AND METHODS

Collecting, preservation, and permanent storage

The specimens were collected by hand from lava tubes in the Kipuka Kanohina system, Hawai'i Island (Fig. 2), and transported to the laboratory for identification and preservation using 70% ethanol for specimens designated for morphological work or 95% ethanol for molecular work. Following dissection and examination, the abdomen and genitalia were transferred to polyethylene vials and individually associated with the specimen vial for permanent storage.

Morphological examination techniques and visualization

Measurements and examinations of external body features were made from the specimens in ethanol, without further manipulation. Measurements of body length refer to the distance between the anterior margin of the head and the tip of the anal segment in the male or the tip of the ovipositor in the female. Terminology of wing venation follows Bourgoin *et al.* (2015).

To prepare male genitalia for dissection, the genital capsule was removed from the specimen, macerated for 24 h in 10% KOH at room temperature, washed in water, and transferred to glycerin for storage or to glycerin jelly for drawings. Examinations and drawings were made using a Leitz stereomicroscope with a camera lucida attachment.

Photographs

Specimens were photographed live, *in situ* in lava tube root galleries and in the laboratory before sample processing. Images of habitus and head of *Iolania perkinsi* Kirkaldy, 1902 and *Iolania frankanstonei* (Fig. 3) were generated with a Leica Z16 microscope, with a Planapo 2.0 X/WD 39 mm objective and the stacking software Helicon Focus v.6.7.1, at the Centre

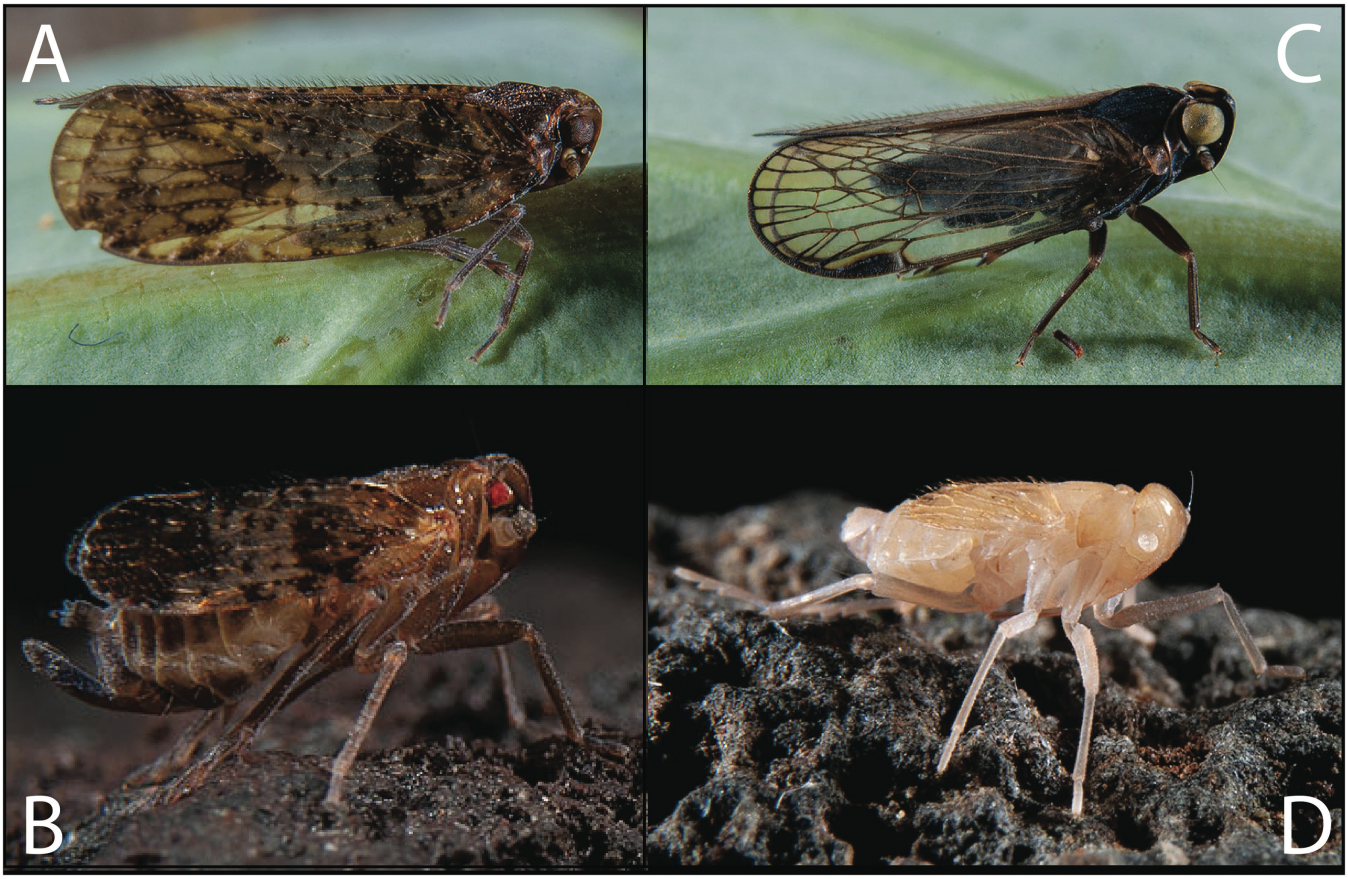

Figure 1. Habitus of closely related epigean and cavernicolous planthopper species. A, *Iolania perkinsi* (epigean). B, ***Iolania frankanstonei*** (cavernicolous). C, *Oliarus* cf. *filicicola* (epigean). D, *Oliarus polyphemus* (cavernicolous). Images of live specimens by M.E. Slay.

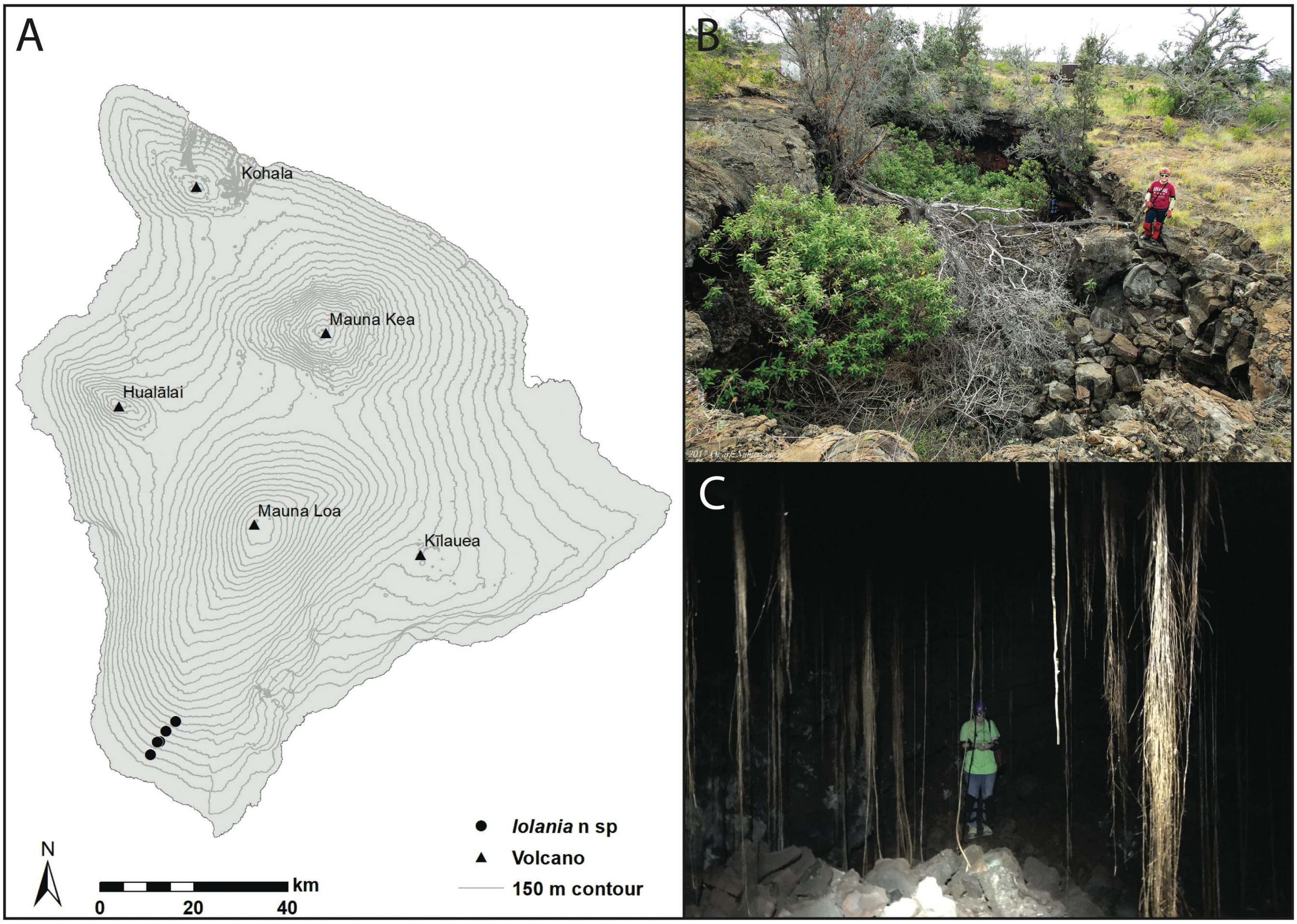

Figure 2. A, map of Hawai'i Island illustrating the peaks (black triangles) of each of the two active (Mauna Loa and Kīlauea) and three dormant (Mauna Kea, Hualālai, and Kohala) volcanoes, in addition to the general locations of the entrances (black circles) sampled within the Kipuka Kanohina cave system, to illustrate the distribution and elevational range of ***Iolania frankanstonei***. B, C, images depicting the typical landscape and vegetation of surface (B) and subterranean (C) habitats. Photographs by M.E. Slay (B) and R.A. Chong (C).

for Integrative Biodiversity Discovery (CIBD), Museum für Naturkunde, Berlin, Germany.

DNA barcoding and phylogenetic analysis

Nymphs collected in close proximity to adults from four distinct lava tube sections (designated as CKM, FFL, HLC, and OHS) within the Kipuka Kanohina system underwent DNA extraction using a Qiagen DNEasy Blood and Tissue kit following the manufacturer's protocols. Fragments of mitochondrial cytochrome *c* oxidase I (*COI*) were amplified by PCRs performed using NEB Quick-Load® Taq 2× master mix (M0271L), in 20 µL reactions using 0.4 mM Folmer primers (LCO1490, 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198, 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) and 1–12 ng DNA (Folmer *et al.* 1994). A thermocycling protocol with a 2 min initial denaturation at 94°C was followed by 50 cycles of 94°C denaturation for 20 s, 46°C annealing for 10 s, and 65°C elongation for 1 min, and completed with a 7 min final extension at 65°C. Sequences were cleaned using five units of exonuclease I and 0.5 units of shrimp alkaline phosphatase (ExoSAP) at 37°C for 30 min, followed by inactivation at 80°C for 15 min. Cleaned PCR products were submitted to the University of Hawai'i Advanced Studies in Genomics, Proteomics, and Bioinformatics facility for sequencing of both strands.

Sequences from cave-adapated species (GenBank accession numbers: *Oliarus polyphemus* Fennah, 1973 MZ048272-75; *Iolania frankanstonei* MZ048276-81) and two endemic surface species (accession numbers: *Iolania perkinsi* MZ048282; *Oliarus filicicola* Kirkaldy, 1909 MZ048271) were assembled using Geneious (v.10.1.3) *de novo* assembly software and aligned using the MAFFT algorithm (v.7.4.50; Katoh and Standley 2013) to published sequences from closely related members of Cixiidae, in addition to a representative hemipteran species from Aphrophoridae [*Philaenus spumarius* (Linnaeus, 1758) NC_005944] and Cercopidae [*Callitettix versicolor* (Fabricius, 1794) NC_020031] for context. A sequence from the aphid species *Acyrthosiphon pisum* Harris, 1776 was used as an outgroup. A maximum likelihood phylogeny was made using RAxML (v.8.2.12; Stamatakis 2014) generating 1000 bootstrap replicates to estimate branch support values.

Depository

All specimens, including locality information, are deposited in the Howarth Hawaiian Cave Arthropod Research Collection of

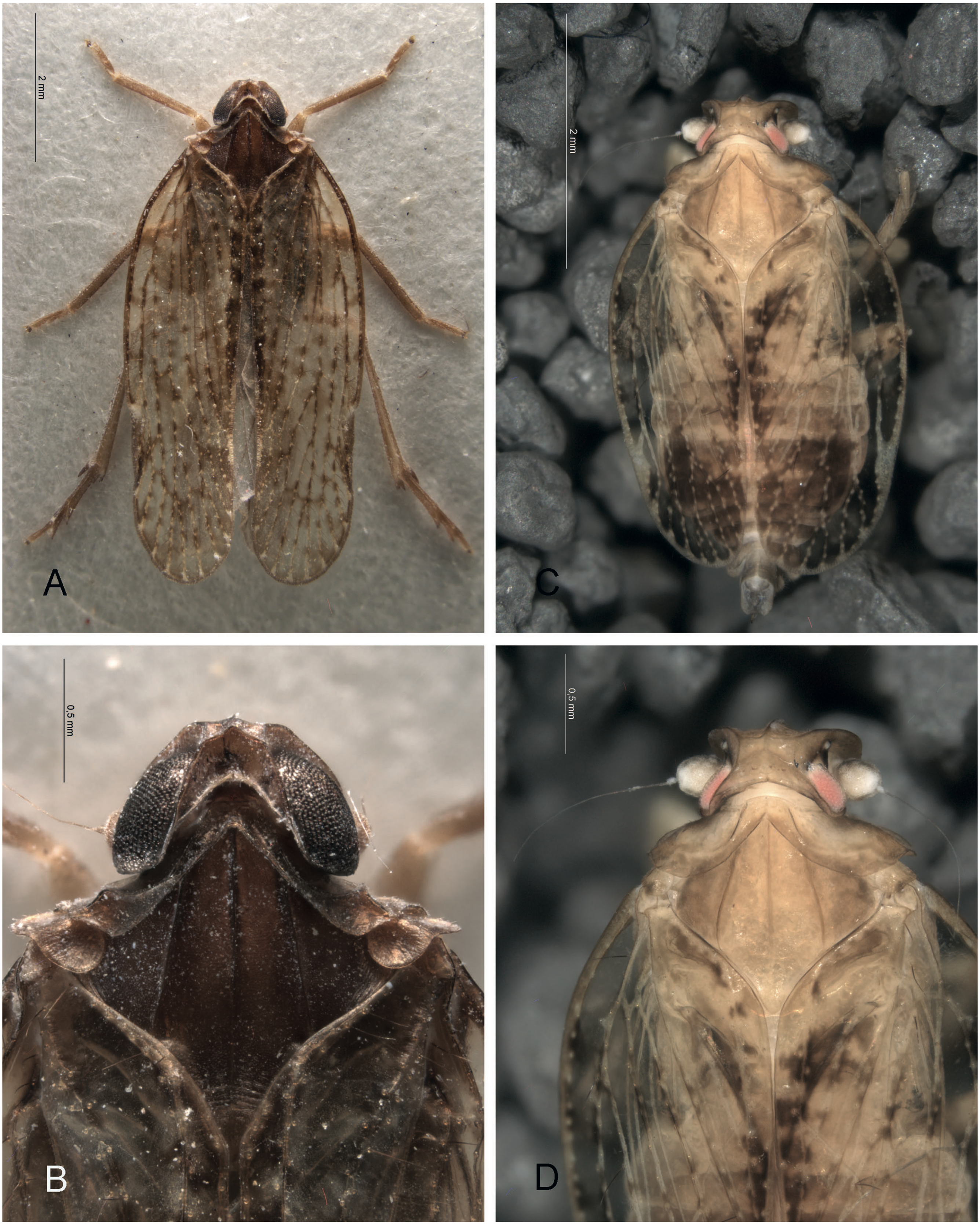

Figure 3. Images of epigean (*Iolania perkinsi*) and cavernicolous (***Iolania frankanstonei***) *Iolania* species, illustrating external morphological features. A, B, *Iolania perkinsi* Kirkaldy habitus (A) and head and thorax (B), dorsal aspect. C, D, *Iolania frankanstonei* habitus (C) and head and thorax (D), dorsal aspect. Specimen from OHS entrance (images by B. Schurian).

the Bernice Pauahi Bishop Museum (BPBM), Honolulu, HI, USA. Additional information on accessing collection localities is maintained by the Hawaiʻi Speleoglocial Society and the Cave Conservancy of Hawaiʻi.

RESULTS

TAXONOMY

Cixiidae Spinola, 1839

Cixiini Muir, 1923

Iolania Kirkaldy 1902: 118

Type species: Iolania perkinsi Kirkaldy 1902: 119.

Genus diagnosis

Hoch (2006) provided a detailed diagnosis of the genus *Iolania*, which then contained exclusively epigean species. The cavernicolous species *I. frankanstonei* from Hawaii Island (described below) features characters pertaining to its external morphology that have evolved in the course of adaptation by the species to its subterranean habitat (troglomorphies). These include the reduction of compound eyes, tegmina and hindwings, and bodily pigmentation. As has been shown in other cavernicolous planthopper taxa (Hoch and Howarth 1989), these reductions of single traits can influence the configuration of other traits, such as head width, sculpturing (e.g. carination), and general body shape. It therefore seems appropriate to provide a separate diagnosis for cavernicolous *Iolania* species.

All species of the genus *Iolania* are easily distinguishable from the other Hawaiian cixiid genus, *Oliarus*, by their tricarinate pronotum (vs. pentecarinate in *Oliarus*) and from all other known Cixiini by the morphological configuration of the male copulatory organ, the aedeagus (shaft bilaterally symmetrical, smooth; flagellum rigid, short, not movable against shaft; phallotreme near base of flagellum, exposed dorsally).

Diagnosis of epigean species of Iolania *(see also Hoch 2006: 303–304)*

Total length (tip of head to distal margin of tegmina): males, 4.8–6.3 mm; females, 6.2–7.1 mm. Moderately large cixiids of robust appearance (Fig. 3A, B); tegmina ~2.6× longer than maximally wide, more or less shallowly tectiform, surpassing tip of abdomen with approximately one-third of total length. Costal veins of tegmina nearly parallel in dorsal aspect. Tegmina and hindwings translucent to hyaline. Tegmina with colour pattern variable within populations, ranging from hyaline with little recognizable patterning to those with distinct dark brown spots or stripes, especially at the tegmen base and in the distal third. Granules on veins usually brownish. Body coloration inconspicuous; intraspecific variation ranging from yellowish brown to darker brown. Coloration of body and tegmina usually slightly darker in females. Vertex short, with lateral margins diverging slightly posteriorly. Hind tibiae laterally with three minute spines.

Diagnosis of cavernicolous species of Iolania *(Figs 3C, D, 4, 5), as represented by I. frankanstonei*

Total length: males, 3.5–4.5 mm; females, 4.4–4.6 mm. Tegmina 2× longer than maximally wide, shallowly tectiform, not surpassing tip of anal segment. Costal veins of tegmina in dorsal aspect convex, together creating an oval outline (Fig. 4A). Vertex with lateral margins strongly diverging posteriorly and with posterior margin shallowly concave; compound eyes small, pronotum in dorsal aspect medially narrow and laterally expanded. Compound eyes with light red pigmentation, coloration of body yellowish to light brown, tegmina with darker spots and stripes, variable among specimens studied. Hind tibiae laterally with three minute spines.

Key to *Iolania* species (modified from Hoch 2006)

(1) Tegmina longer than twice their maximal width, well surpassing tip of abdomen; compound eyes large and darkly pigmented; vertex with lateral margins slightly diverging posteriorly and with posterior margin deeply concave; pronotum in dorsal aspect narrow throughout; body size of males 4.8–6.3 mm, females 6.2–7.1 mm 2

\- Tegmina twice as long as maximally wide, not surpassing tip of anal segment; compound eyes small and reddish; vertex with lateral margins strongly diverging posteriorly and with posterior margin shallowly concave; pronotum in dorsal aspect medially narrow and laterally expanded; body size of males 3.5–4.5 mm, females 4.4–4.6 mm .... ***Iolania frankanstonei* sp. nov.**

(2) Male genitalia with aedeagus symmetrical; anal segment without spinose processes 3

\- Aedeagus with asymmetrical paired spines; anal segment caudally bent ventrad, with two distinct ventrolateral spinose processes .... *Iolania kraussohana* Hoch, 2006

(3) Shaft of aedeagus in lateral aspect curved dorsad; flagellum without sculpturing; gonostyli not ventrally ridged 4

\- Shaft of aedeagus straight; flagellum sculptured with numerous distinct tuberculous protuberances; gonostyli ventrally ridged 5

(4) Paired spines of aedeagus foliately dilated .... *Iolania mauiensis*

\- Paired spines of aedeagus not dilated, arm-like, curved dorsad at midlength .... *Iolanaia lanaiensis*

(5) Gonostyli with caudal margin triangular; tips of aedeagal spines directed straight basad or mediobasad 6

\- Gonostyli with caudal margin rounded; tips of aedeagal spines directed dorsad .... *Iolania perkinsi*

(6) Male anal segment distally with produced lateroventral corners; shaft of aedeagus in lateral aspect with ventral margin distinctly narrowing in distal half; tips of aedeagal spines curved mediad .... *Iolania oahuensis*

\- Male anal segment in caudal aspect ventrally rounded; shaft of aedeagus in lateral aspect not narrowing towards apex; tips of aedeagal spines directed straight basad .... *Iolania koolauensis*

Iolania frankanstonei Hoch & Porter sp. nov.
(Figs 1B, 3C D, 4–5)

Description

Slightly troglomorphic species with compound eyes present, but small; tegmina covering most of the abdomen, barely attaining but not surpassing tip of abdomen; hind wings well developed but slightly reduced in length in comparison to epigean *Iolania* species; coloration generally lighter than in epigean species, with darker portions (see '*Coloration*' below).

Body length: Male, 3.5–4.5 mm (*N* = 6); female, 4.4–4.6 mm (*N* = 2).

Coloration: Body and legs yellowish. Compound eyes reddish; antennae yellowish-white. Head and mesothorax laterally sordid light brown. Tergites and sternites of abdominal segments slightly darker brown. Tegmina translucent, light yellowish, with irregularly defined brownish portions distally of nodal line and faint brown transverse stripes across postcostal cell; brownish markings also accompanying median branch of Y-vein (= Pcu) and common stem of Y-vein (= Pcu + A1), in dorsal aspect creating a median 'V' across the tegmina. Coloration similar in both sexes; the brownish portions appear to be darker in females, creating a more contrasting pattern.

Head: Vertex short, ~3.6× wider posteriorly than medially long, broadly triangular, concave; posterior margin shallowly incised medially, lateral margins ridged; transverse carina medially forming an obtuse angle. Areolets (or 'fossette' *sensu* Giffard 1925) indistinctly divided medially by a short obtuse carina. Frons separated from anterior margins of areolets by an obtuse transverse carina, and with a median longitudinal carina that is distinctly ridged and running from apical transverse carina to frontoclypeal suture. Frons widest at base of antennae, 0.8× shorter medially than maximally wide. Lateral margins of frons in ventral portion foliately ridged. Postclypeus almost smooth, with a very faint obtuse median carina in upper portion; anteclypeus with a median faint carina; post- and anteclypeus

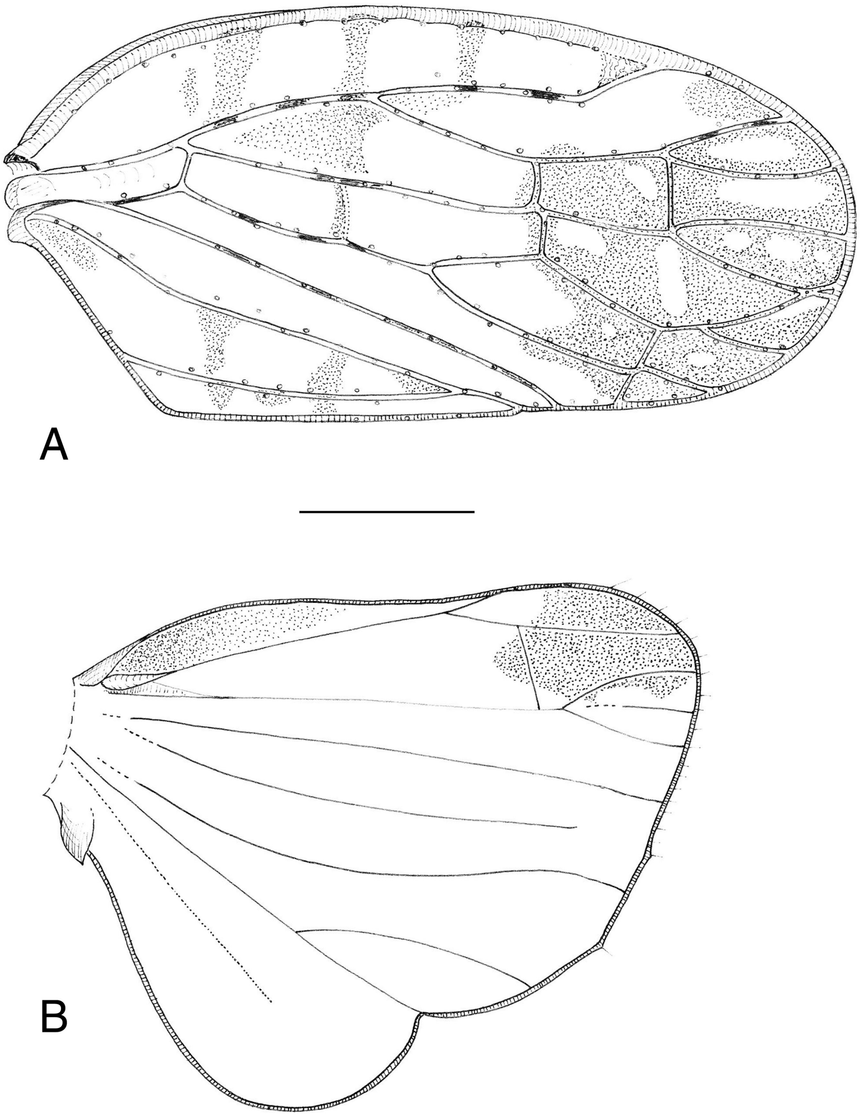

Figure 4. ***Iolania frankanstonei***, male, wing morphology. A, right tegmen. B, right hind wing. Scale bar: 0.5 mm. Male from CKM entrance.

together medially ~1.6× longer than frons. Frontoclypeal suture highly vaulted. Compound eyes small; lateral ocelli present, vestigial; median frontal ocellus absent. First antennal joint (scape) short, ring-like; second antennal joint (pedicel) cylindrical, slightly longer than wide, with sensory plaque organs distinctly recognizable. Rostrum slightly surpassing posterior margin of hind coxae.

Thorax: Pronotum short, ~15× wider than medially long, and 1.6× wider than maximum width of head including eyes; posteriorly deeply incised, with obtuse median and two sharply ridged lateral carinae. Mesonotum tricarinate; carinae distinctly ridged; lateral carinae slightly diverging posteriorly. Mesonotum medially slightly longer than maximally wide, with lateral portions oblique. Tegulae small, without carina. Tegmina short, ~2× longer than maximal width, not surpassing tip of abdomen; in males with contracted abdomen attaining cephal margin of genital segment; in males with expanded abdomen attaining cephal margin of abdominal tergite VII; in females not surpassing tip of ovipositor; distal margin rounded, pterostigma indistinct, veins densely covered with bases of setae. Venation with proximal branching pattern as in epigean *Iolania* species, but postcostal cell relatively wider; venation distally of nodal line varying among individuals, with variation concerning the branching pattern of posterior radius and media and that of anterior cubitus (Fig. 4). Claval vein (CuP) and Y-vein (Pcu, A1 and Pcu + A1) as in epigean *Iolania* species. Hind wings well developed (Fig. 4); in comparison to epigean *Iolania* species, relatively shorter. Legs moderately long; metatibiae laterally with three minute spines, and five to seven strong apical spines, bilaterally and individually variable (5/6; 5/7; 6/6). Metabasitarsus elongate, approximately as long as second and third tarsomere together, with five apical spines. Second tarsomere with four or five apical spines. Pretarsal claws small, arolia pad-like.

Male genitalia: Genital segment in caudal aspect slightly higher than wide, laterodorsal margin rounded, medioventral process triangular. Anal segment distally of anal style on both sides broadly rounded, ventrocaudal margin medially straight; anal segment in dorsal aspect with lateral margins more or less parallel, in lateral aspect straight, distally of anal style not bent ventrally. Gonostyli proximally slender, ventrally ridged, ridge finely striate, distal portion slightly dilated, slender, produced dorsally, caudal margin rounded, dorsal margin slightly concave. Aedeagus bilaterally symmetrical; shaft with enhanced sclerotization in basal half, in lateral aspect distinctly narrowing in apical third (Fig. 5G, arrow); shaft ventrally in basal portion finely rugose; flagellum short, with surface distinctly sculptured, dorsal surface medially concave, with conspicuous groove distally of phallotreme; with two movable spinose processes near apex, processes arm-like, proximally taeniform, distally terete and tapering in an acute tip, in repose directed mediobasally, not attaining basal (proximal) third of shaft. Gonoduct with conspicuous protuberance at apex of shaft.

Female genitalia: External structures of female genitalia as in epigean *Iolania* species: ovipositor ensiform, caudally slightly surpassing anal segment. Tergite IX caudally truncate, very slightly concave, with wax-secreting area inconspicuously delimited. Anal segment slender, ventral margin caudally slightly produced.

Molecular identification

Mitochondrial *COI* sequences of *I. frankanstonei* were deposited in GenBank under accession numbers MZ048276–MZ048281.

Species diagnosis

Iolania frankanstonei differs from all epigean *Iolania* species in external characters related to cave adaptation (reduction of compound eyes, configuration of head carination and proportions, length and venation of tegmina and wings, pigmentation pattern; see above). Concerning the male genital structures, *I. frankanstonei* is most similar to *I. perkinsi*, but differs in the shape of the anal segment (distal portion straight vs. bent ventrally in *I. perkinsi*) and of the aedeagus (shaft with enhanced sclerotization in basal half vs. in basal third in *I. perkinsi*; enhanced sclerotized part ventrally rugose vs. smooth in *I. perkinsi*; shaft in lateral aspect distinctly narrowing in apical third vs. straight in *I. perkinsi*; arm-like paired processes at transition of shaft and flagellum taeniform throughout vs. distally terete in *I. perkinsi*, and their tips in repose directed straight basomedially vs. pointed dorsally in *I. perkinsi*; dorsal surface of flagellum with phallotreme wide but without longitudinal conspicuous groove distal of it as in *I. perkinsi*).

Remarks

The description of *I. frankanstonei* is based on comparatively few specimens (six males and two females). The range of variation within and between populations from different passages of the Kipuka Kanohina lava tube system can therefore not be assessed fully. Variation exists, however slight, in the size of the compound eyes, relative length and venation of the tegmen, and male genital characters (e.g. the shape of gonostyli) within and among specimens from different populations; therefore, it cannot be excluded that the specimens here, hypothesized to present a single species, might belong to separate reproductive units. We therefore decided to assign the holotype and paratypes from a single population and chose the original collection location, the CKM entrance, as the type locality, because it was the first location in the Kipuka Kanohina system where specimens were collected.

Material examined

Holotype: Male, Hawai'i/USA: Hawai'i Island, Kipuka Kanohina lava tube system, CKM entrance (HI 00153), 1096 m a.s.l., 24 November 2016, M.E. Slay, C.A.M. Slay, and M.L. Porter coll., BPBM (type number: *BPBMENT 2008035007*).

Paratypes: Two males, same data as holotype, BPBM.

Additional material: One male, two females, Hawai'i/USA: Hawai'i Island, Kipuka Kanohina lava tube system, OHS entrance (HI 00809), 730 m a.s.l., 24 November 2018, M.E. Slay, C.A.M. Slay, A. Katz, J. Gunter, K. Yelverton, and P. Bosted coll., BPBM.

One male, Hawai'i/USA: Hawai'i Island, Kipuka Kanohina lava tube system, HLC entrance (HI 00323), 539 m a.s.l., 25 March 2018, M.E. Slay, A.S. Engel, S. Engel, V. Hackell, and T. Gracanin coll., BPBM.

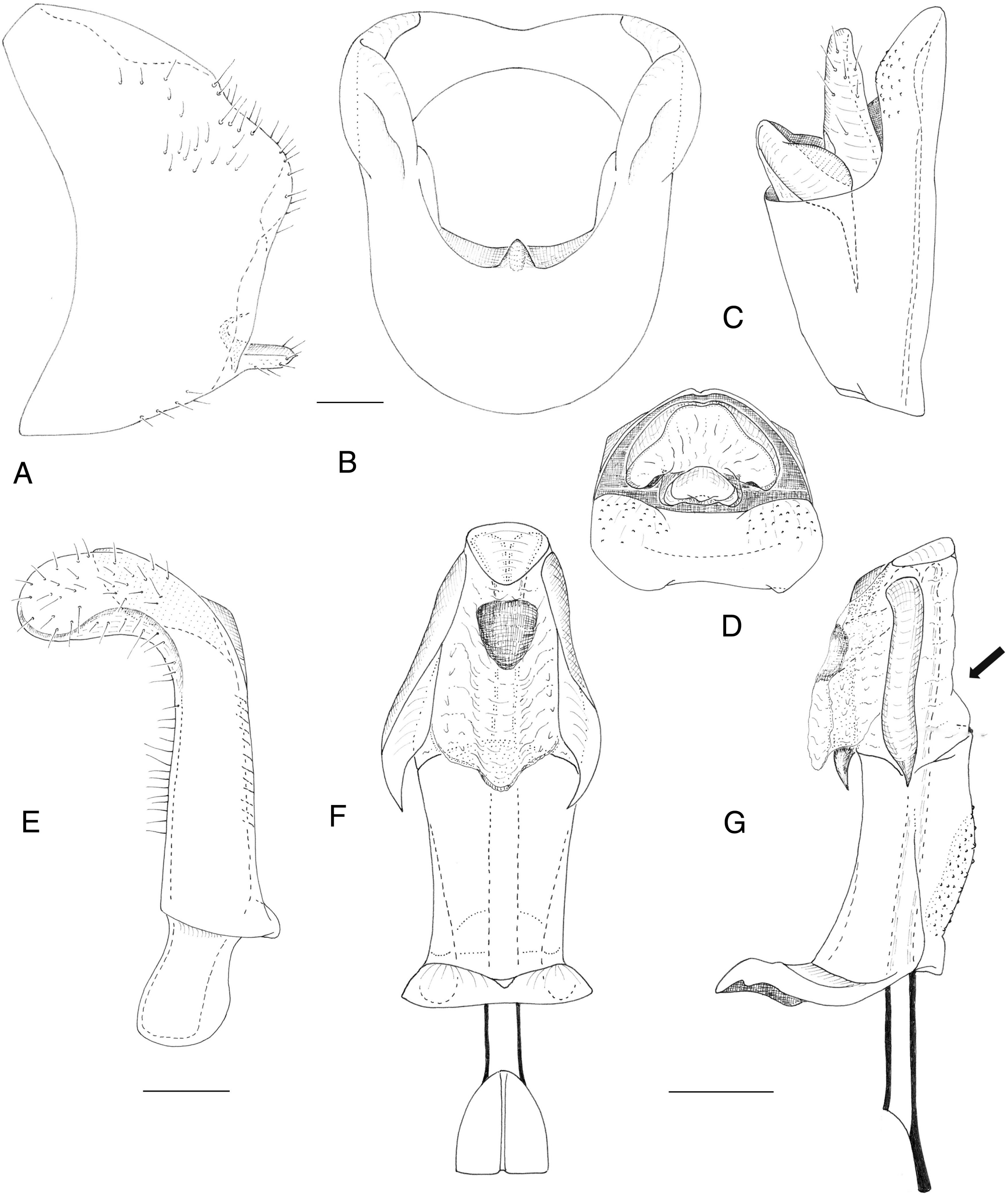

Figure 5. ***Iolania frankanstonei***, male genitalia. A, genital segment, left lateral aspect. B, same, caudal aspect. C, anal segment, right lateral aspect. D, same, caudal aspect. E, left gonostyle, maximal aspect. F, aedeagus, dorsal aspect. G, same, left lateral aspect. Scale bars: 0.1 mm. Male from CKM entrance, same as in Figure 4.

One male, Hawai'i/USA: Hawai'i Island, Kipuka Kanohina lava tube system, HLC entrance (HI 00524), 539 m a.s.l., 19 November 2018, R.A. Chong, A.S. Engel, and M.L. Porter coll., BPBM.

Cixiid nymphs belonging to *Iolania* were also collected from cave sections within the Kipuka Kanohina lava tube system in close proximity to adult specimens used for this description. However, nymphs of epigean *Iolania* are 'hitherto unknown – they most likely live (like the nymphs of all cixiids) close to or inside the soil, and are not documented in collections' (Hoch 2006: 317). Based on morphology alone, we therefore cannot assign the nymphs with certainty to either *I. perkinsi* or *I. frankanstonei*, hence we have opted to refrain from accessing these specimens here.

Etymology

The species epithet is chosen to honour the friendship and collaboration between the pioneers of Hawaiian cave biology, the late Fred D. Stone (1938–2018) and Frank G. Howarth, Bishop Museum. Over the course of nearly six decades, they explored hundreds of caves, discovered dozens of new species, and inspired and supported countless people (students in addition to novice and senior scientists). Known to fellow cavers as 'Frank 'n Stone', they are held in high esteem as reliable buddies in the field and valued discussion partners. Their scientific expertise, combined with personal integrity and their notorious sense of (occasionally dark) humour, renders them unforgettable to anyone who has had the good fortune to meet and spend time underground with them.

Distribution

Specimens of *I. frankanstonei* have been documented from multiple lava tubes across a range of elevations in the Kipuka Kanohina Cave System (Bosted 2017), on the south-western side of Mauna Loa volcano in 750- to 1500-year-old lava flows. It is a highly braided and mazy system, with multiple levels and interconnected passages, located in the desert of Ka'u near the community of Ocean View, ~72 km south of Kailua-Kona and ~48 km north of South Point (the southernmost point in the USA). So far, >29 km of continuous cave passages have been explored within the system, with >42 km in the entire system. It has a vertical extent of >1300 m, with passages at almost sea level up to >1400 m a.s.l. The cave system is currently listed as the third longest lava tube in the world, one of only three to surpass 26 km of uninterrupted passage in length. Despite the significant amount of subterranean habitat found within the Kipuka Kanohina system, *I. frankanstonei* has been collected from only four cave sections (of >30 surveyed) with an elevational range from 539 to 1096 m, suggesting this species might be restricted to the higher-elevation passages.

Ecology and behaviour

There is little documented information on the ecology and behaviour of *I. frankanstonei*. Adults and nymphs have been observed on and around roots and on cave walls near roots anywhere from twilight into the deep cave zone of lava tubes (for lava tube zonation, see Howarth 1983), although anecdotally *I. frankanstonei* appears to be more abundant close to entrance and twilight zones. In two collection locations, the roots have been confirmed to be from the native tree *Metrosideros polymorpha* Gaudich. using barcoding techniques (unpublished data B. Chong and M. Porter). Adults have not been observed flying, although the morphology of the hind wings suggests that the species might be capable of some flight, although perhaps not sustained. In all locations sampled, *I. frankanstonei* individuals have been collected from dense root mats and have been observed to co-occur with *Oliarus polyphemus* Fennah, 1973 s.l.

Mitochondrial COI phylogeny

Phylogenetic relationships inferred from *COI* sequence data support monophyly of *I. frankanstonei* and strongly support the sister relationship between *I. frankanstonei* and *I. perkinsi*, which together form a distinctly different clade from the Hawaiian *Oliarus* samples (Fig. 6; Chong *et al.* 2022).

DISCUSSION

Ecological classification

How should *I. frankanstonei* be classified ecologically? Traditionally, cave-dwelling organisms were classified ecologically into three categories: as trogloxenes (accidentals) that only occasionally enter the caves, facultative cavernicoles (troglophiles) that spend part of their life cycle in caves, or obligate cavernicoles (troglobionts) that are restricted to caves (Racovitza 1907). In order to achieve a more detailed classification of cavernicoles, Sket (2008) and, more recently, Howarth and Moldovan (2018a, b) developed a catalogue of characteristics pertaining to morphology, behaviour, and ecology. *Iolania frankanstonei* displays several troglomorphic characters, such as the reduction of compound eyes, light pigmentation of body and tegmina, and reduction of tegmina and wings. Its degree of troglomorphy is intermediate between epigean representatives of Cixiidae, which have large compound eyes, well-developed functional wings, and display dark bodily pigmentation, and highly troglomorphic (i.e. white, eyeless, and flightless) species. Although very little is known about the behaviour and ecology of *I. frankanstonei*, the phenotypical configuration of eyes and wings suggests that it is probably restricted to a subterranean environment and is likely to complete its life cycle underground. We therefore regard it preliminarily as an obligate cavernicole, or troglobiont.

Epigean relatives

Evidence from morphology (special configuration of male genitalia) and molecular data (*COI* sequence information) indicates a close phylogenetic relationship between the cave-dwelling *I. frankanstonei* and the epigean *I. perkinsi* (Fig. 6). The two species occur sympatrically on the same island, and their distributional ranges might even overlap, in part. *Iolania perkinsi* is widely distributed on Hawai'i Island, and adults have been collected from rainforest habitats, such as *M. polymorpha/Cibotium* fern forests (Hoch 2006). Although *I. perkinsi* specimens have not (yet) been documented from the surface vegetation above the Kipuka Kanohina system, it is likely that they occur in close vicinity to the caves, because the upper part of the elevational range is mostly *Metrosideros* forest.

The discovery of a cavernicolous *Iolania* species obviously challenges the assumption that *Iolania* has diversified by allopatric speciation (Hoch 2006), a hypothesis that had been

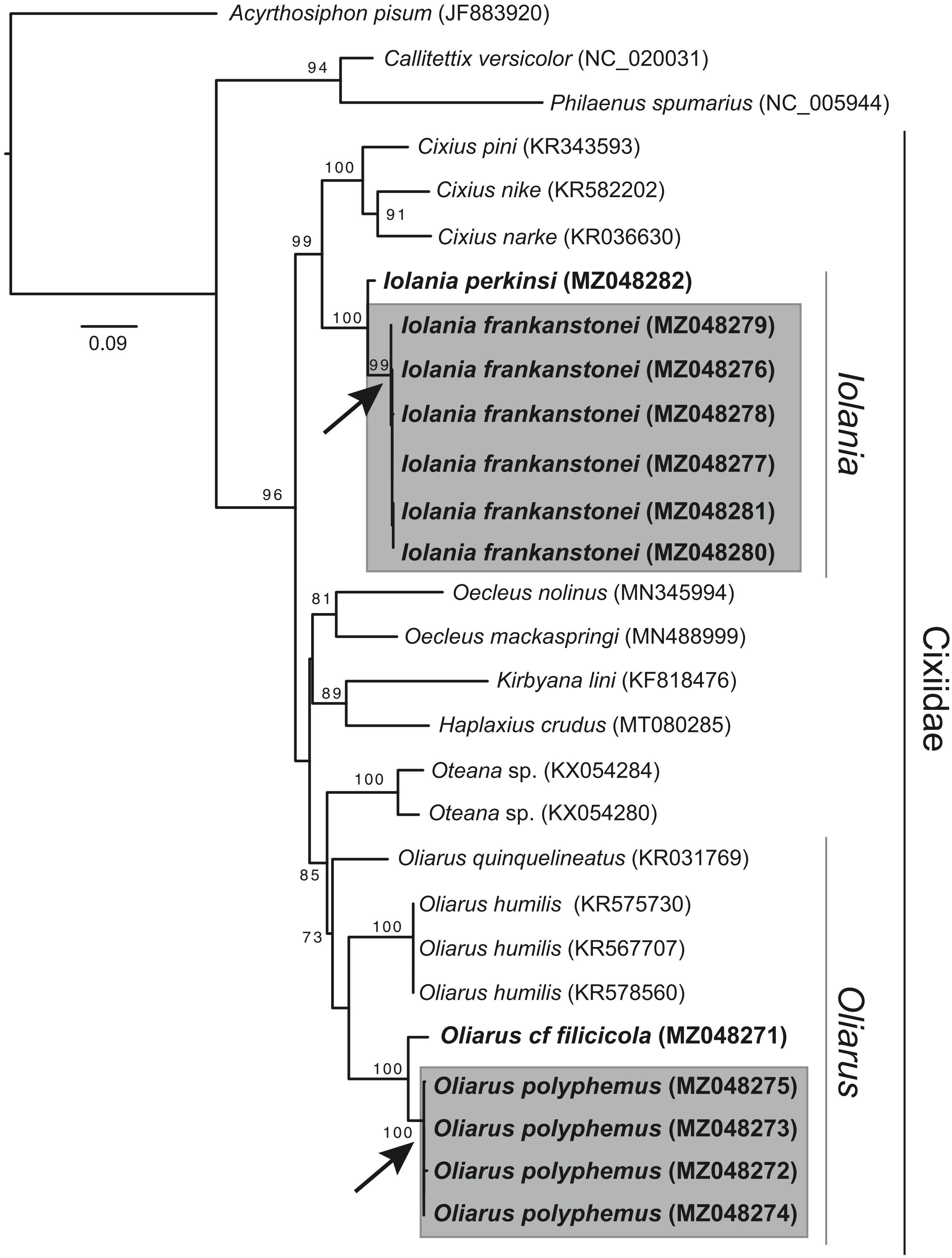

Figure 6. Maximum likelihood phylogeny of representative cixiid species of the two separate lineages that have colonized Hawaii: *Iolania* and *Oliarus*, Aphrophoridae (*Philaenus spumarius*), and Cercopidae (*Callitettix versicolor*), and the aphid *Acyrthosiphon pisum* used as an outgroup. Bootstrap values from 1000 replicates that were >70% are included to indicate branch support. GenBank accession numbers for all sequences used are provided in parantheses after species names. New sequences generated for this study are in bold, and troglobiont species are indicated by grey boxes. Arrows indicate separate cave colonization events in both cixiid lineages on Hawaiʻi Island.

derived from distribution patterns of epigean *Iolania* species. If *I. perkinsi* and *I. frankanstonei* were indeed sibling species, Howarth's (1987) model of parapatric speciation to explain the evolution of terrestrial troglobites on young oceanic islands would be further corroborated. Also, the degree of troglomorphy observed in *I. frankanstonei* might be indicative of the species representing the early stage of an adaptive shift from epigean to subterranean habitat (see below).

Evolutionary implications

According to the adaptive shift model as suggested by Howarth (1982, 1983), populations of epigean species invade subterranean habitats to exploit novel resources, and the drastic change in habitat is the driving force for genetic divergence and speciation (Howarth *et al.* 2019). Divergence by adaptive shifts is usually envisioned as sympatric or parapatric; that is, the diverging populations remain in contact along a narrow boundary during the split. It now appears that many cave-adapted animals, including the new species, *I. frankanstonei*, described here, have evolved by the process of adaptive shifts from representatives of the local surface fauna (Howarth *et al.* 2019).

We hypothesize that founder events play a major role in initiating an adaptive shift along a steep ecological gradient, such as from surface to cave environments, by increasing generation of novel genetic variants in bottlenecked populations, potentially increasing the production of adaptive phenotypes. The 'founder-flush' concept (Carson 1968, Carson Templeton 1984) proposes that a founder population will expand rapidly to occupy a new habitat (the 'founder flush') after the initial founder event and associated reduction in effective population size. During this phase, a strong increase in genotypic and phenotypic variation occurs (Carson and Wisotzky 1989) owing to the relaxed selection resulting from reduced competition for resources, and in particular, the relaxed sexual selection on both sexes resulting from low availability of potential mating partners (Kaneshiro 1989). In the 'founder-flush' model, co-adapted gene complexes can be broken up in the 'flush' phase, leading to new genetic combinations, some of which might yield enhanced phenotypic adaptation to the new environment.

Iolania frankanstonei clearly represents a cave colonization event separate from those within the other native cixiid lineage, *Oliarus*. Comparison of the two taxa might yield insights regarding the factors that determined the evolution of cave planthoppers in Hawaiʻi. In *Oliarus*, hitherto seven independent cave colonization events have been documented: one on Molokaʻi, three on Maui, and at least three on Hawaiʻi Island (Hoch and Howarth 1999). The cave-dwelling *Oliarus* species show varying degrees of troglomorphy, ranging from taxa with moderately reduced compound eyes and wings to completely eyeless and unpigmented species with vestigial wings, such as *Oliarus priola* Fennah, 1973 and *Oliarus waikau* Hoch & Howarth, 1999 from Maui and *Oliarus polyphemus* on Hawaiʻi Island (Fig. 1D). Hoch and Howarth (1999) found that the degree of troglomorphy was correlated better with the physical parameters of the caves than (as appears logical to assume) geological or evolutionary time. Interestingly, all three highly troglomorphic *Oliarus* species occur in the deep cave zone of geologically young lava tubes, feeding on roots of *M. polymorpha*, a pioneering native tree that on young lava flows produces large root masses that penetrate the rock. In contrast, the least cave-adapted *Oliarus* species live in old, eroded lava tubes in the lowlands, where they feed on roots of lowland drought-adapted shrubs that do not produce significant amounts of roots. Hoch and Howarth (1999) assumed that older, more eroded caves and crevices filled with soil, meaning that roots would be available for cave cixiids nearer to the surface. Consequently, selective pressure would be greater to retain functional (even if small) eyes to recognize hostile surface environments. Similar phenomena have been observed in cavernicolous cixiid species on the Canary Islands (Hoch and Asche 1993) and Australia (Hoch and Howarth 1989).

One species, however, did not fit this pattern: *Oliarus makaiki* Hoch & Howarth, 1999 from lava tubes on Hualālai, is intermediate in troglomorphy, but occurs in a humid cave at a higher elevation (~1300 m) within a lava flow estimated to be between 1500 and 3000 years old, which is situated in wet *Metrosideros* forest with roots of *Metrosideros* being the presumed host plant. Hoch and Howarth (1999) argued that *O. makaiki* might be a relatively recent invader and in the early stages of cave adaptation, further corroborated by the fact that the presumed epigean sister species of *O. makaiki*, *Oliarus koanoa* Kirkaldy, 1902, occurs parapatrically on the surface and is abundant on the surface around the cave entrance. Inside the cave, *O. makaiki* was found to co-occur with a species of the *O. polyphemus* complex (Hoch and Howarth 1999). The situation is similar in *I. frankanstonei*. The species occurs in the Kipuka Kanohina lava tube system, on the south-western side of Mauna Loa volcano, in 750- to 1500-year-old lava, and specimens have been found in passages leading off entrances along an elevational gradient, ranging from 539 to 1096 m a.s.l. The upper elevations of the Kipuka Kanohina system, including entrances CKM and OHS, are in wet *Metrosideros* forest.

In all locations where *I. frankanstonei* has been found, it occurs together with the highly troglomorphic *O. polyphemus*, generally in the same root chambers. In two of the four cave sections where *I. frankanstonei* has been found (Kipuka Kanohina entrances FFL and HLC), *O. polyphemus* was by far the more abundant of the two species. In Kipuka Kanohina entrance OHS, where there is very little deep cave habitat, *Iolania* adults were the only planthopper collected, with no observations of *O. polyphemus* noted. In Kipuka Kanohina entrance CKM, *Iolania* adults were more frequent in the twilight zone, with *O. polyphemus* individuals becoming more abundant in deeper cave zones. We therefore hypothesize that *I. frankanstonei* represents a recent invader to the cave environment and is in the early stages of an adaptive shift to a novel habitat. The fact that *I. frankanstonei* was most abundant only in the upper elevational ranges (CKM and OHS) suggests that passages off the lower elevation entrances (FFL and HLC) under drier surface habitats might represent marginal habitats for this species.

Conservation status

Iolania frankanstonei is limited to two main cave sections and two marginal cave locations within the larger Kipuka Kanohina system, which is mostly limited to a single lava flow. Based on the documented limited distribution, high endemicity, and specialized habitat of *I. frankanstonei*, in addition to the presumed small population size, we suggest preliminary conservation statuses for the species using International Union for Conservation of Nature (IUCN) and NatureServ ranks. According to the IUCN Red Data Book categories, *I. frankanstonei* must be regarded at least as Vulnerable, if not Endangered (IUCN 2022). Likewise, based on NatureServ ranks, *I. frankanstonei* would have a conservation status of Critically Imperiled/Imperiled at both the global (G1G2) and state (S1S2) scales.

Factors influencing our preliminary conservation status designations for this species include both surface and subterranean threats that impact its limited and highly specialized lava tube habitat (Stone and Howarth 2005). Alterations and removal of native vegetation on the surface can have large impacts on the availability of the roots in lava tubes, which serve as the food source for *I. frankanstonei*. In the Kipuka Kanohina system, the largest cause of surface alterations is development for housing, which often leads to both loss of native vegetation owing to land clearance and changes in water drainage from surface to subsurface environments. In some cases, clearance of land for development might lead to the entire removal of caves or alteration of entrance features that impact the microclimate of the subsuface habitat. Another impact of development, particularly in areas without access to city-provided utilities, is the risk of underground pollution from septic systems, cesspools, fertilizers, and pesticides. Increased human presence in a region is also associated with increased pressures from human-associated invasive species, such as the roof rat, *Rattus rattus* (Linneaus, 1758), which can degrade lava tube habitat quality and use cave-adapted species as a food source (Howarth and Stone 2020). With population growth and significantly increased demand for housing in this area over the last 5 years, all these threats have increased and might lead to significant declines in population sizes and suitable habitat for this species.

Perspectives

The discovery of an obligately cavernicolous species in the genus *Iolania* offers a unique opportunity to investigate the processes underlying the parallel evolution of cave adaptation in two separate lineages within the planthopper taxon Cixiidae, which co-occur on only one island in the world. Comparisons of the two systems might allow us to draw more general conclusions on which factors drove the adaptive shift to novel ecological conditions. The cavernicolous fauna of the lava tubes on Hawai‘i Island has become known only some 60 years ago and has since been studied by many researchers. The fact that a new species of *Iolania* has been found only recently might be indicative of the richness of species that await discovery. Other volcanic systems on Hawai‘i Island (Kīlauea, Hualālai, and Mauna Kea) and on the other islands of the archipelago where epigean *Iolania* species are present (Maui, Lāna‘i, Moloka‘i, O‘ahu, and Kaua‘i) might also hold cavernicolous *Iolania* species. Systematic bioinventories are mandatory to make full use of the research opportunities offered by the unique biota of Hawai‘i and to support conservation efforts for these species.

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AUTHOR CONTRIBUTIONS

The authors contributed equally to the manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

None declared.

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DATA AVAILABILITY

All specimens, including locality information, are deposited in the Howarth Hawaiian Cave Arthropod Research Collection of the Bernice Pauahi Bishop Museum (BPBM), Honolulu, HI, USA. Additional information on accessing collection localities is maintained by the Hawai‘i Speleoglocial Society and the Cave Conservancy of Hawai‘i. Mitochondrial *COI* sequences are deposited in GenBank under the following GenBank accession numbers: *Iolania frankanstonei* (MZ048276–MZ048281), *Iolania perkinsi* (MZ048282), *Oliarus polyphemus* (MZ048272–MZ048275), and *Oliarus filicicola* (MZ048271).

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