

A New Species of *Spelaeorchestia* (Crustacea: Amphipoda: Talitroidea) from Lava Tube Caves on the Island of Hawai i in the Hawaiian Archipelago

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Abstract. The Hawaiian islands are recognized as a hotspot of biodiversity, including both surface and subsurface habitats. Recent studies of Hawaiian lava tube fauna have continued to reveal new species. Here, a new species of cave dwelling talitroid amphipod in the genus *Spelaeorchestia* is described from lava tubes on the island of Hawai i. It is compared with the only other known cave amphipod from the Hawaiian archipelago and with a closely related cave talitroid from Japan in the genus *Minamitalitrus*.

INTRODUCTION

The superfamily Talitroidea is comprised of eight amphipod families (Myers & Lowry 2020) occupying habitats ranging from supralittoral/intertidal beaches, mangrove swamps, salt marshes, coastal meadows, freshwater riparian zones, forest leaf-litter and soils, and even moss-covered tree trunks (Lowry & Myers 2019). Obligate cave-dwelling ‘cave-hoppers’, however, are rare, with only four previously described species, each of which is in a separate genus: *Houlia bousfieldi* (Hou & Li 2003); *Minamitalitrus zoltani* White, Lowry & Morino 2013; *Palmorchestia hypogaea* Stock & Martin, 1988; and *Spelaeorchestia koloana* Bousfield & Howarth, 1976. With the exception of *H. bousfieldi*, for which precise collection locality and habitat information from within China is unknown (Hou & Li 2003), the remaining three species were all described from geograph-

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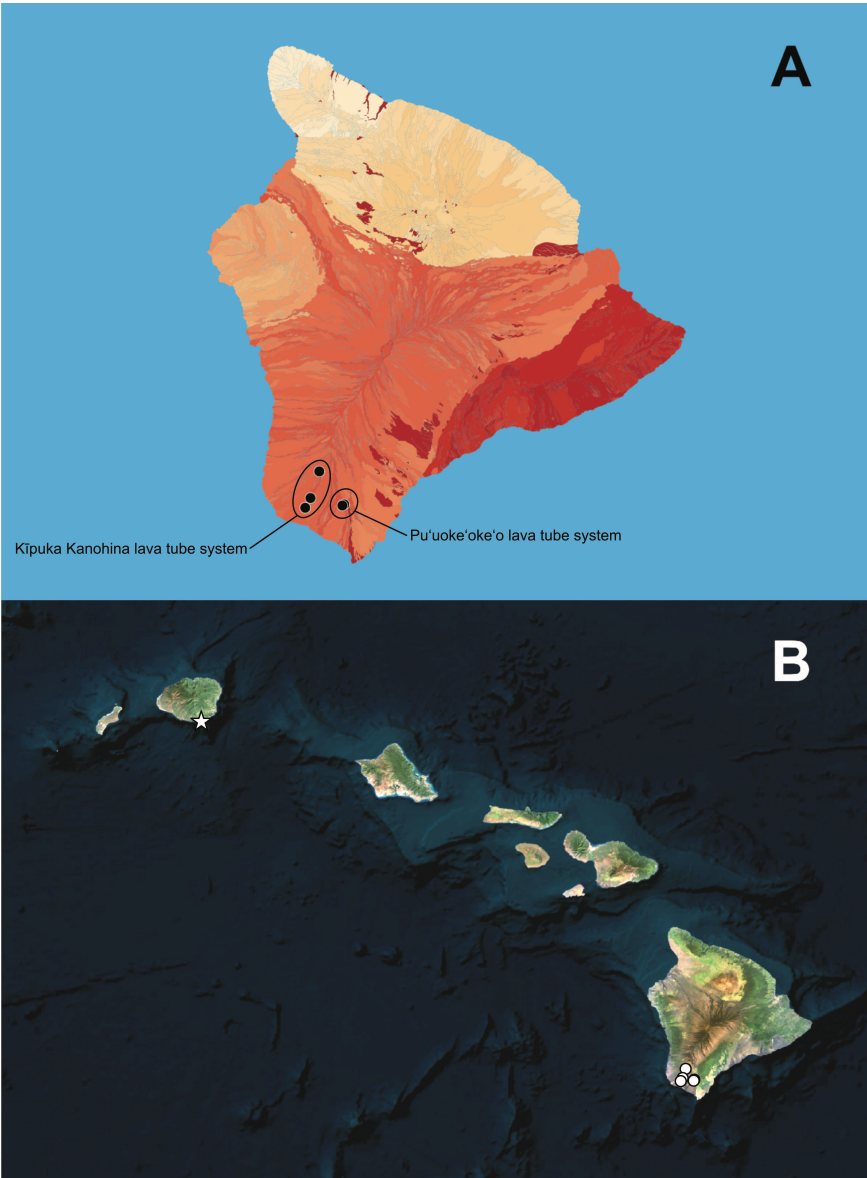


Figure 1. (A, B) Known distribution of *Spelaeorchestia pahoehoe* **sp. nov.** on the island of Hawai i (black or white circles) and (B) area where *Spelaeorchestia koloana* occurs on the island of Kaua i (white star). Darker red units on the island of Hawai i, correspond to younger lava flows (0.0 to 0.4 ka), while the lightest units on the island correspond to the oldest deposits (up to 12.4 ka) (Trusdell *et al.*, 2006).

ically distant insular cave habitats: *M. zoltani* from limestone caves on an uplifted coralline atoll in the Philippine Sea (White *et al.* 2013); *P. hypogaea* from volcanic lava tubes on the Canary Islands (Stock & Martin, 1988) and *S. koloana* from volcanic lava tubes on the Hawaiian island of Kaua i (Bousfield & Howarth 1976).

The Hawaiian Islands contain extensive subterranean habitats consisting of fractures, fissures, and human-accessible lava tubes. These lava tubes and associated habitats harbor diverse subterranean arthropod communities, with the best known obligate cavernicolous fauna including plant hoppers of the genus *Oliarus* Stål, 1862 (Hoch & Howarth 1999), the federally endangered Kaua i cave wolf spider *Adelocosa anops* Gertsch, 1973 (pe e pe e maka ole spider) and the federally endangered Kaua i cave amphipod *Spelaeorchestia koloana* Bousfield & Howarth, 1976 (uku noho ana).

In 2015, bioinventory investigations were initiated in the K puka Kanohina lava tube system on the southern flank of Mauna Loa, Hawai i Island (Fig. 1). These studies have resulted in the discovery of many undescribed species of cave-adapted arthropods (Liebherr, 2021; Hoch, H. *et al.*, *in litt.*), including a new species of *Spelaeorchestia* described here (Fig. 2). This recent work has built upon the foundational studies of Howarth (1973) and colleagues, who described communities from lava tubes in K lauea and northeastern Mauana Loa flows. Based on this work, Stone & Howarth (2005) estimated that over 44 species of cave species occur on Hawai i island. The new amphipod species described here demonstrates the potential that exists for further significant species discoveries from Hawaiian lava tubes.

Spelaeorchestia is now known to include two Hawaiian species and the new species described here extends the documented range of the genus in Hawai i to a second island. The new species brings the world total of ‘cave-hopper’ talitrids to five species.

MATERIAL AND METHODS

Collecting and preservation. Talitroid specimens were collected by hand from lava tubes found in two flows on the southern flank of Mauna Loa volcano (Fig. 1) and transported to field stations for identification and preservation using 70–75% ethanol for specimens designated for morphological work and 95–100% ethanol for specimens used for molecular work. Specimen collections were made under official permits from the Hawaii State Department of Land and Natural Resources (numbers FHM16-405; I1005; I1063) and Hawaii Volcanoes National Park (HAVO-2019-SCI-0050), as well as permission from all individual private landowners.

Specimen dissection, visualization, and permanent storage. The amphipods were dissected, and body parts were mounted on microscope slides using glycerine. Specimens were examined under a Zeiss Optiphot interference contrast microscope with an attached drawing tube. Material is deposited in the Bernice P. Bishop Museum, Honolulu (BPBM).

Abbreviations used in figures: A1, 2 = antenna 1, 2; Ep 1–3 = epimera 1–3; M = male; F = female; G1, 2 = gnathopods 1, 2; L = left; Md = mandible; Mx1, 2 = maxilla 1, 2; Mxp = maxilliped; P4–7 = pereopods 4–7; Plp 1–3 = pleopods 1–3; U1–3 = uropods 1–3; T = telson.

Mitochondrial COI sequencing and phylogenetic analysis. In order to provide a genetic barcode for the newly described species, one specimen from each lava flow where *S. pahoehoe* was collected (designated as CKM_1747A from the type locality, and

KKC_1168) were DNA-extracted using a Qiagen DNEasy Blood and Tissue kit following manufacturer's protocols. Polymerase chain reactions were performed using NEB Quick-Load® Taq 2x 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 sec, 46 °C annealing for 10 sec, 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 products were submitted to the University of Hawai i Advanced Studies in Genomics, Proteomics and Bioinformatics facility for sequencing of both strands.

Sequences from *Spelaeorchestia pahoehoe* (GenBank accession numbers OR195527-OR195528) were assembled using Geneious (v10.1.3) de-novo assembly software and were aligned using the MAFFT algorithm (v7.4.50; Katoh & Standley 2013) to published sequences from a representative set of amphipod species from within the Talitridae and Brevitalitridae. A maximum likelihood phylogeny was generated from the nucleotide alignment using the IQ-tree web server (Nguyen *et al.* 2015; Trifinopoulos *et al.* 2016) with default search parameters, allowing the software to determine the best-fit model of evolution with rate heterogeneity, and estimating branch support using both ultrafast bootstrap and SH-aLRT analyses (Guindon *et al.* 2010), each from 1,000 replicates.

SYSTEMATICS

Suborder Senticaudata Lowry & Myers, 2013

Infraorder Talitrida Serejo, 2004

Parvorder Talitridira Lowry & Myers, 2013

Superfamily Talitroidea Rafinesque, 1815

Family Brevitalitridae Myers & Lowry, 2020

Spelaeorchestia Bousfield & Howarth, 1976

Spelaeorchestia Bousfield & Howarth, 1976: 143.

Type species. *Spelaeorchestia koloana* Bousfield & Howarth, 1976, by original designation.

Included species. *S. koloana* Bousfield & Howarth, 1976 and *S. pahoehoe* **sp. nov.**

Diagnostic description. Head lacking eyes. *Antenna 1* short, reaching less than halfway along article 4 of antenna 2. *Antenna 2* about one third body length; *Mandible* left lacinia mobilis 2-3 cuspidate; maxilliped palp article 2 with or without distomedial lobe; palp with distinct, button-shaped article 4. *Gnathopod 1* palmate setae absent on merus, carpus and propodus *Gnathopod 2* mitten-like, merus with postero-distal lobe bearing palmate setae; carpus posterior margin with large patch of palmate setae; propodus with large posterodistal lobe bearing palmate setae. *Pereopods 3–7* cuspidactylate. *Pereopod 4* dactylus similar to that of pereopod 3. *Pleopods* biramous, uniramous or without rami. *Uropod 1* exopod without marginal setae. *Uropod 3* uniramus. *Telson* each lobe with single long marginal setae and a few terminal setae.



Figure 2. *Spelaeorchestia pahoehoe* **sp. nov.**, K ̄puka Kanohina lava tube system, CKM entrance, Hawai i A. Female, Holotype, (161G); B. Live animal of unknown sex; image by M.E. Slay.

Remarks. According to White *et al.* (2013), *Spelaeorchestia* is similar to *Minamitalitrus*, with the principal difference between these two genera being that the pleopods remain biramous in *Minamitalitrus* but are uniramous, vestigial or absent in *Spelaeorchestia*. This distinction was true at the time when only *S. koloana* was known. *Spelaeorchestia pahoehoe* **sp. nov.**, described here, has biramous pleopods and so this distinction no longer applies. However, *Minamitalitrus* also differs from *Spelaeorchestia* in its very short antenna 1 and 2 and in the weak development of palmate setae on the merus and carpus

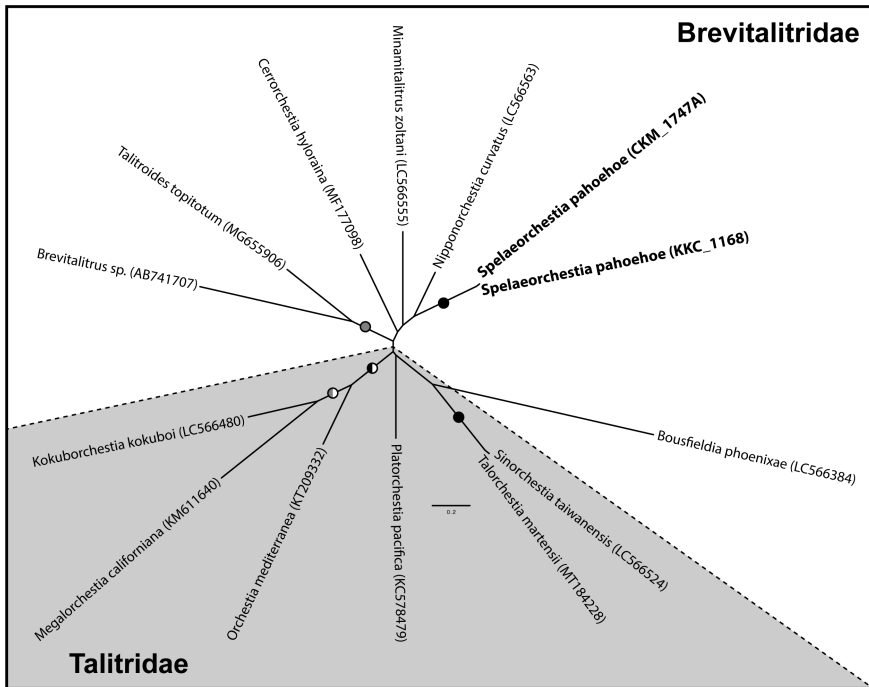


Figure 3. Unrooted maximum likelihood phylogeny of cytochrome oxidase I sequences from representative Talitridae and Brevitalitridae amphipod species to provide context for newly generated *Spelaeorchestia pahoehoe* **sp. nov.** sequences (in bold). GenBank accession numbers are provided in parentheses for each species. Branch support values are shown on corresponding branches as circles, with the left half of the circle indicating support from bootstrap analyses, and the right half of the circle representing support from SH-aLRT branch tests. The color of each half of the circle indicates the level of support, with black indicating support values $\geq 90\%$, grey values from 80.0–89.9%, and white circles values from 70.0–79.9%; support values less than 70% in one or both metrics not shown.

of gnathopod 2. There is also a significant geographic distance between the occurrence of *Spelaeorchestia* in Hawai i and that of *Minamitalitrus* in Japan. Given the recovered COI phylogenetic relationships (Fig. 3), these taxa are still considered to be separate genera.

***Spelaeorchestia pahoehoe* Myers & Lowry, sp. nov.**

(Figs. 2, 4–6)

Type Material. Holotype female (BPBM 2008035027) and 4 female paratypes from HAWAIIAN ISLANDS: **Hawai i I:** K puka Kanoehina lava tube system, CKM entrance (HI 00161G), 1,096 m a.s.l., 24 Nov 2016, M.E. Slay, C.A.M. Slay, M.L. Porter coll.

Other material. There are 31 individuals from 13 sites collected for study including juveniles and eggs documenting reproduction in *S. pahoehoe*: HAWAIIAN ISLANDS: **Hawai i I:** Pu uoke oke o lava tube system: 2 individuals, HHB entrance (HI 00030), 613 m a.s.l., 2 Dec 2015, M.E. Slay, C.A.M. Slay, M.L. Porter coll.; 1 individual, ECC entrance (HI 00035), 613 m a.s.l., 2 Dec 2015,

M.E. Slay, C.A.M. Slay, M.L. Porter coll. K ʔuka Kanohina lava tube system: 1 individual, SRF entrance (HI 00075), 1,355 m a.s.l., 21 Nov 2016, M.E. Slay, C.A.M. Slay, M.L. Porter coll.; 7 individuals, CKM entrance (HI 00162), 1,096 m a.s.l., 24 Nov 2016, M.E. Slay, C.A.M. Slay, M.L. Porter coll.; 4 individuals, ELI entrance (HI 00296), 288 m a.s.l., 25 Nov 2017, M.E. Slay, S. Engel, M.L. Porter coll.; 2 individuals, HLC entrance (HI 00330), 539 m a.s.l., 25 Mar 2018, M.E. Slay, A.S. Engel, S. Engel, V. Hackell, T. Gracanin coll.; 3 individuals, ELI entrance (HI 00398), 288 m a.s.l., 27 Mar 2018, A.S. Engel, S. Engel coll.; 2 individuals, HLC entrance (HI 00523), 539 m a.s.l., 19 Nov 2018, R.A. Chong, A.S. Engel, M.L. Porter coll.; 2 individuals (1 adult, 1 juvenile), CWD entrance (HI 00655), 318 m a.s.l., 20 Nov 2018, C.A.M. Slay, A.G. Hudson, V. Hackell, M.L. Porter coll.; 1 individual, CWD entrance (HI 00659), 318 m a.s.l., 20 Nov 2018, C.A.M. Slay, A.G. Hudson, V. Hackell, M.L. Porter coll.; 2 individuals, CWD entrance (HI 00660), 660 m a.s.l., 20 Nov 2018, C.A.M. Slay, A.G. Hudson, V. Hackell, M.L. Porter coll.; 2 individuals, BGV entrance (HI 00847), 556 m a.s.l., 24 Nov 2018, M.L. Porter, A.S. Engel, A.G. Hudson, V. Hackell coll.; 2 individuals (one female with embryos), BGV entrance (HI 00855), 556 m a.s.l., 24 Nov 2018, M.L. Porter, A.S. Engel, A.G. Hudson, V. Hackell coll. (all BPBM).

Type locality. The type locality is an upper elevation section of the K ʔuka Kanohina lava tube system, although it is not directly connected by humanly-accessible passages to any other caves in the same flow. The lava tube has just over 2.9 km of passage with multiple entrances. There are numerous, dense patches of roots throughout the extent of the lava tube that serve as habitat for cave-adapted species; *Spelaeorchestia pahoehoe* **sp. nov.** were often found in wet but bare rock in the vicinity of roots.

Etymology. A p ʔoehoe is a smooth, unbroken type of lava in ‘Ōlelo Hawai i

Description. (based on female 9.7 mm)

Head. Lacking eyes. *Antenna 1* short, reaching less than halfway along article 4 of antenna 2; peduncular articles 2 and 3 subequal in length; flagellum a little longer than combined length of articles 2 and 3 with 4 articles. *Antenna 2* about one third body length; peduncular article 5 a little longer than article 4; article 3 about one third length of article 5; flagellum longer than peduncle with 13 articles, the terminal article with apical cluster of imbricated setae. *Mandible* left lacinia mobilis 3-cuspidate; maxilla 1 inner plate slender with 3 terminal pectinate setae; maxilla 2 without oblique setal row; maxilliped palp article 2 with small distal lobe; palp with distinct, button-shaped article 4.

Pereon. *Gnathopod 1* palmate setae absent on merus, carpus and propodus; basis slender, uniform width, about 3 times as long as broad; carpus wedge shaped, about 4x as long as broad; propodus subrectangular half the length of the carpus; palm substraight; dactylus short, fitting palm, posterodistal margin with strong spines. *Gnathopod 2* larger than gnathopod 1, merus with postero-distal lobe bearing palmate setae; carpus and propodus subequal length; carpus posterior margin with large patch of palmate setae; propodus with large posterodistal lobe bearing palmate setae. *Pereopods 3–7* cuspidactylate. *Pereopod 4* dactylus similar to that of pereopod 3. *Pereopods 5–7* becoming progressively longer; *Pereopod 7* basis subovoid with distinct posterodistal flange.

Pleon. *Epimera 1–3* posterodistally acute. *Pleopods* biramous, peduncles each with 2 retinaculæ (coupling hooks), each ramus consisting of single article. *Pleopod 1* rami subequal in length; *Pleopods 2, 3*, with inner ramus the longer. *Uropod 1* peduncle a little longer than rami, with distal robust seta; endopod a little longer than exopod, exopod without marginal setae. *Uropod 2* rami longer than peduncle. Both rami with marginal

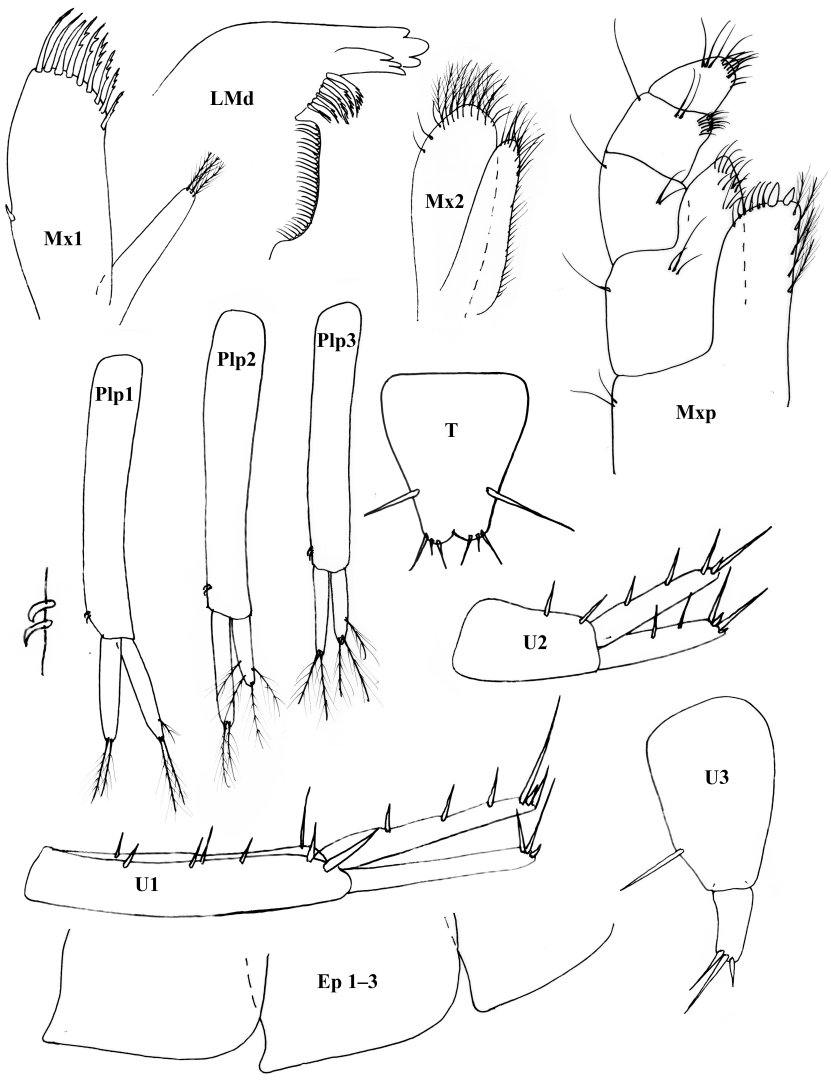


Figure 4. *Spelaeorchestia pahoehoe* sp. nov., K ̄puka Kanohina lava tube system, BGV entrance, Hawai i. Female paratype, (855C), mouthparts, pleopods, uropods, telson, and epimera.

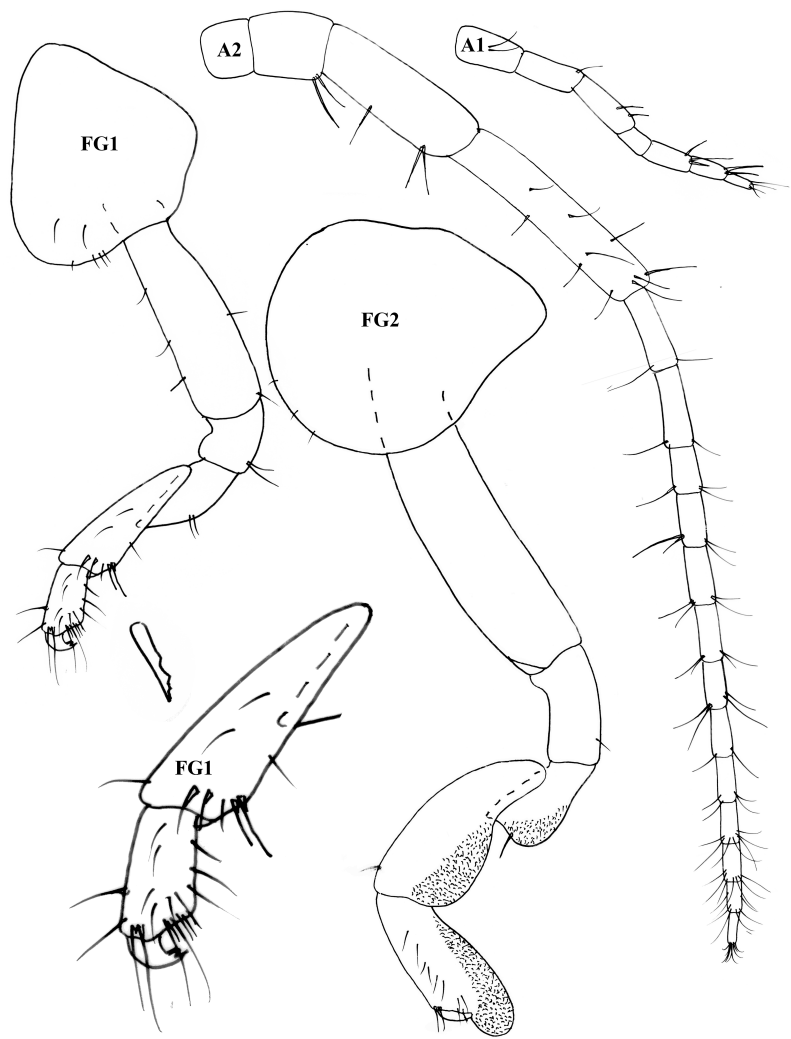


Figure 5. *Spelaeorchestia pahoehoe* sp. nov., K ̄uka Kanohina lava tube system, CKM entrance, Hawai i. Female paratype, antennae and gnathopods.

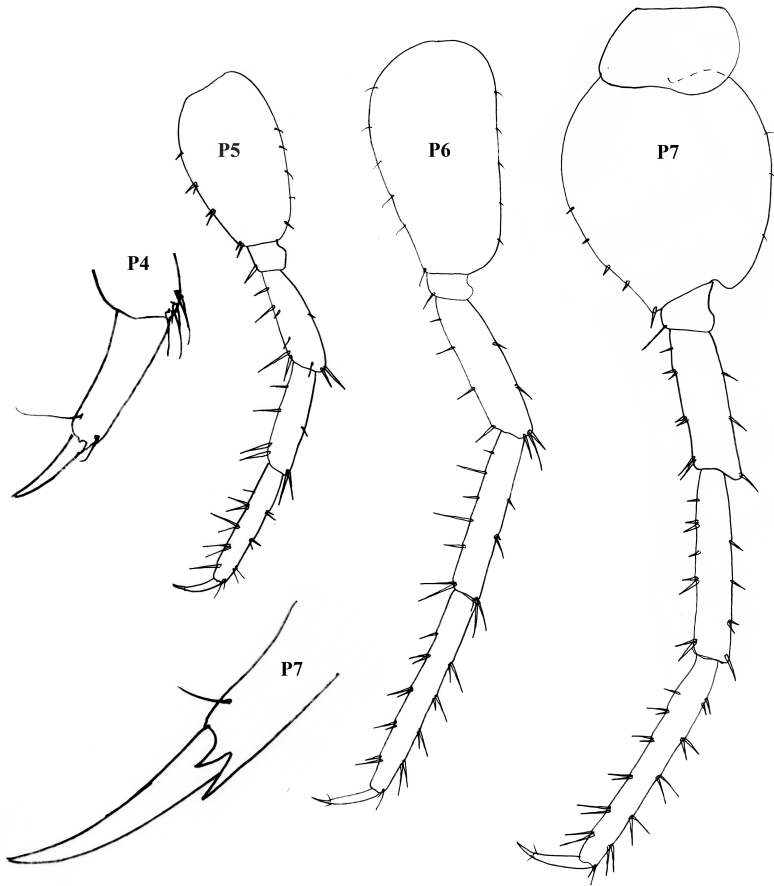


Figure 6. *Spelaeorchestia pahoehoe* **sp. nov.**, K ʻŀuka Kanohina lava tube system, CKM entrance, Hawai i. Female paratype, pereopods 4–7.

robust setae. *Uropod 3* uniramus, peduncle much longer than ramus, inner margin with long robust seta, ramus without marginal setae and with 3 terminal setae. *Telson* each lobe with single long marginal setae and 3 terminal setae.

Male. No significant sexual dimorphism.

Remarks. *Spelaeorchestia pahoehoe* **sp. nov.** is morphologically very similar to *S. koloana* Bousfield & Howarth. The primary difference is in the pleopods that are fully biramous in *S. pahoehoe* but variously reduced in *S. koloana*. In other respects, the difference is mainly in the relative proportions of podomeres. The carpus of gnathopod 1 is more slender in *S. pahoehoe* than in *S. koloana* and gnathopod 2 is less elongate in *S. pahoehoe* than in *S. koloana*.

Myers & Lowry (2020) tentatively placed *Spelaeorchestia* in the Brevitalitridae stating that the relationship remained uncertain; this tentative placement is now supported by our molecular results (Fig. 3). Takahashi *et al.* (2021), on the basis of a molecular study, placed *Minamitalitrus zoltani* (White, Lowry & Morino, 2013) as a sister taxon to species of the forest litter talitrid genus *Nipponorchestia* Morino & Miyamoto, 2015 (Talitridae). This relationship is also supported in the present molecular results (Fig. 3). The molecular results cluster *Minamitalitrus* and *Nipponorchestia* within the family Brevitalitridae although they have previously been considered to be members of the Talitridae (Myers & Lowry 2020). The allocation of *Nipponorchestia* to the family Talitridae has rested on the presence in that taxon of a distomedial lobe on article 2 of the maxilliped palp. In other character states such as the setation of the telson and form of the uropods, *Nipponorchestia* aligns well with members of the Brevitalitridae. The distomedial lobe on the maxilliped palp is likely to be a homoplasy. According to Morino & Miyamoto (2015), *Nipponorchestia* is allied to *Bousfieldia* Chou & Lee, 1996, which is a member of the family Brevitalitridae (Fig. 3). Additionally, the morphological cladistic analysis of Myers & Lowry (2020) supports the close relationship of *Minamitalitrus* to both *Nipponorchestia* and *Spelaeorchestia*. We therefore transfer both *Minamitalitrus* and *Nipponorchestia* from the family Talitridae to the family Brevitalitridae.

Distribution of *Spelaeorchestia* in Hawai i. *Spelaeorchestia pahoehoe* **sp. nov.** are found on the southern slopes of Mauna Loa volcano in lava tubes formed in at least two different flows, K ʻŷuka Kanohina and Pu u oke oke o (Fig. 1A), ranging in age from 750 to 1800 yr (Sherrod *et al.* 2021). This expands the distribution of the genus to a second Hawaiian island, with the first described species, *Spelaeorchestia koloana* **sp. nov.**, distributed across five populations from a small geographic region on southern Kauaʻi when it was federally listed as endangered (U.S. Fish and Wildlife Service, 2000).

COI sequencing. The interpretation of phylogenetic relationships from CO1 barcoding is not straightforward. Since mitochondrial genes are inherited entirely from the female line, it has been assumed that phylogenetic relationships will not have been obscured by the recombination that occurs in nuclear genes. However, the degree of homoplasy that may occur in genes has not been established and Rodriguez-Pena *et al.* (2020) have demonstrated a high incidence of mitochondrial gene heteroplasmy in a natural population of spider crabs. In the present analysis the close relationship between *Spelaeorchestia*, *Minamitalitrus* and *Nipponorchestia* revealed by morphological cladistic analysis (Myers & Lowry 2020) is supported by the CO1 sequencing (Fig. 3).

Evolution of *Spelaeorchestia* in Hawai i. The two species of *Spelaeorchestia* on the Hawaiian islands are divergent in unexpected ways. Biramous pleopods with long peduncles, each ramus with multiple articles is the plesiomorphic state in the Amphipoda. Reduction in pleopod size, reduction in article number on rami or elimination of one or more rami is therefore the apomorphic condition. In Hawai i, *Spelaeorchestia koloana* from Kaua i has pleopods with rudimentary rami and is therefore a more derived lineage than *S. pahoehoe* **sp. nov.** from Hawai i, which has pleopods that are biramous. This would appear to be counter to the generally recognised progression rule, that would predict colonisation from the older Kaua i (4.7 my), to the younger Hawai i (0.5 my) (Clague 1996). However, when the islands of Hawai i were first colonised, the ancestral *Spelaeorchestia* may have possessed biramous pleopods. During the following period of isolation between the populations on Kaua i and Hawai i, the two populations would then have diverged, with *S. koloana* undergoing pleopod reduction, while *S. pahoehoe* experienced stabilising selection with little pleopod modification from the ancestral form.

With this description, there are two species of *Spelaeorchestia* that are now known to occur on two islands that are nearly 500 km apart in the Hawaiian archipelago. While there are several possible hypotheses that may explain this distribution, the detailed morphological descriptions of these two species does not alone provide sufficient information to be able to formulate the evolutionary route leading to the distribution of the observed species. The addition of more in-depth molecular data, including comparative sequence data from *Spelaeorchestia koloana* and other Hawaiian talitrids, will be required before further speculation can be made on the most likely scenarios for the occurrence of cave-inhabiting *Spelaeorchestia* on two widely separated islands within the Hawaiian archipelago.

Biogeography of *Spelaeorchestia*. The closest known sister taxon to the two Hawaiian *Spelaeorchestia* species lives in caves on the Daito Islands of Japan in the Northwestern Pacific Ocean. Both morphological and molecular studies have suggested a close phylogenetic relationship between the Japanese forest inhabiting *Nipponorchestia* and the cavernicolous *Minamitalitrus* from Japan and *Spelaeorchestia* from Hawai i. A surface living ancestor of *Nipponorchestia* may have colonized the Hawaiian islands and entered the hypogeum giving rise to *Spelaeorchestia*. While no explanation has yet been hypothesised to explain the existence of similar taxa between Japan and Hawai i, a relationship between these two regions has been reported previously. Myers (1991) demonstrated that two species of endemic Hawaiian marine amphipods had their closest sister taxon in Japan. Similarly, a molecular phylogeny and biogeography of Hawaiian endemic land snails of the family Succineidae (Rundell *et al.* 2004), has suggested that these endemic land snails may have had a Japanese, or at least Asian origin, while *Nesoprosope*, a Hawaiian endemic subgenus of *Hylaeus* bees, otherwise known primarily from Japan, is also known from China and one species extends into Europe (Cowie & Holland 2008).

Conservation Implications. The island of Hawai i as the youngest island in the chain has the highest concentration of lava tubes, and correspondingly the largest recorded number of cavernicolous species (Stone & Howarth 2005). Given that the only other species of *Spelaeorchestia* in Hawai i is listed as federally endangered due to limited range and significant threats from development (U.S. Fish and Wildlife Service 2000), the discovery of a second species on the island of Hawai i has significant conservation implications for this genus. At a minimum, further inventories of *S. pahoehoe* are needed to delineate fully its distribution and general abundance across the island of Hawai i. The Kūpuka Kanohina system in which *S. pahoehoe* is found has faced increased threats from residential development in the form of clearing of native forest on the surface, replacement of native vegetation by non-native invasive species, as well as increased impacts from pollution in the form of pesticide use and potential septic system leaks. Because of the significant impacts and recent potential loss of habitat experienced by *S. koloana* from development (Gutierrez 2022), a full conservation assessment of *S. pahoehoe* is also warranted to determine the habitat stability and potential threats it also may face across its distribution.

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