New records of Clausiliidae: Tauphaedusa tau (Boettger, 1877) (Gastropoda: Heterobranchia) on O‘ahu, Hawaiian Islands, and the first global record of infection of a clausiliid land snail with Angiostrongylus cantonensis (Chen, 1935), the rat lungworm

ROBERT H. COWIE
Pacific Biosciences Research Center, University of Hawai‘i, 3050 Maile Way, Gilmore 408, Honolulu, Hawai‘i 96822, USA; email: cowie@hawaii.edu

RANDI L. ROLLINS
Pacific Biosciences Research Center, University of Hawai‘i, 3050 Maile Way, Gilmore 408, Honolulu, Hawai‘i 96822, USA
Department of Biology, University of Hawai‘i, 2538 McCarthy Mall, Edmondson 216, Honolulu, Hawai‘i 96822, USA; email: rrollins@hawaii.edu

MATTHEW C. I. MEDEIROS
Pacific Biosciences Research Center, University of Hawai‘i, 1993 East West Road, Honolulu, Hawai‘i 96822, USA; email: mmredeiro@hawaii.edu

CARL C. CHRISTENSEN
Hawaii Biological Survey, Bishop Museum, 1525 Bernice Street, Honolulu, Hawai‘i, 96827-2704, USA; email: carl@bishopmuseum.org

Clausiliidae
Tauphaedusa tau (Boettger, 1877) New state record, new US record
The only published record of clausiliids in the Hawaiian Islands (Cowie, 1997) is of two lots of unidentified specimens collected in 1965 at two localities on O‘ahu, one in Nu‘uanu Valley (including one live-collected specimen) and one in Makiki Valley (see material examined). This material was deposited in the Bishop Museum and has until now remained unidentified. Prior to these collections, unidentified clausiliids (shells only) were collected in Mānoa Valley, O‘ahu, in 1962 and deposited in the U.S. National Museum of Natural History (see material examined). The Mānoa specimens have not previously been reported in the literature, and no additional records of clausiliids in the Hawaiian Islands have been published until now. This is only the second clausiliid species to have been recorded anywhere within the United States and its territories, the other being Nenia tridens (Schweigger, 1820), which is native to Puerto Rico (van der Schalie 1948; Thompson 1998; Uit de Weerd & Gittenberger 2013).

In 2018, surveys were undertaken across O‘ahu as part of a project to screen snails of all species encountered for Angiostrongylus cantonensis (Chen, 1935), the rat lungworm. This nematode is the primary cause of eosinophilic meningitis in humans (Cowie
and is widespread in the Hawaiian Islands (Kim et al. 2019). At two sites (Wai‘āhole and Kalihi Valleys) specimens of an unidentified species of Clausiliidae were found and identified as follows based on DNA sequences and shell morphology.

A 439 bp portion of the mitochondrially encoded cytochrome c oxidase subunit I (MT-COI) of one of the two living specimens found in Wai‘āhole Valley (see material examined) was amplified and sequenced using the universal mtDNA primers of Folmer et al. (1994). A GenBank BLAST (Benson et al. 2017) search indicated that at 100% query cover the sequence was 99.51% identical to a section of a 697 bp sequence (LC335870) from Japan, identified as *Euphaedusa tau* (Boettger, 1877) by Kawase et al. (2018).

The shells of the specimens collected from both Wai‘āhole and Kalihi Valleys were compared with the original description of this species (Boettger 1877) and with illustrations of this and other clausiliids from eastern Asia in subsequent publications (Boettger 1878, 1883; Kobelt 1879; Möllendorff 1883, 1887; Pilsbry 1902). In particular, the overall shape and size of the shells and the appearance of the transverse shell ribbing and of the apertural lamellae viewed both from the aperture and as visible through the somewhat translucent abapertural part of the shell confirmed this identification (Figs. 1–3). In addition, a specimen collected in 2018 by J.R. Kim and others in Nu‘uanu Valley was identified as belonging to this species, as were the specimens collected in 1962 (Fig. 4) and 1965 (see material examined). All these collected specimens were confirmed as belonging to a single species based on the morphological features noted above. A single live specimen was also found by State of Hawai‘i Department of Land and Natural Resources personnel in 2018 in Makiki Valley; it was photographed but not preserved.

Boettger (1877) described this species from Japan in the genus *Clausilia* section *Phaedusa*, and within *Phaedusa* in the “Gruppe der shangaiensis Pfr. (*Euphaedusa*)”. This constituted the original establishment of the genus-group taxon *Euphaedusa*, which has been treated variously as a section of *Clausilia* (e.g., Pilsbry 1902), a subgenus of *Phaedusa* (e.g., Loosjes 1950; Zilch 1959; Chang 1982), or as a full genus. Boettger’s species has been placed consistently in *Euphaedusa*, generally treated as a full genus, within the subfamily Phaedusinae (e.g., Ohtsuki & Takahashi 1982; Kato et al. 1989;
Kaneda & Kitagawa 1990; Schileyko 2000; Ueshima et al. 2014; Waki 2017). However, Nordsieck (2003) introduced Tauphaedusa as a new subgenus of Euphaedusa, with Clausilia tau Boettger, 1877 as type species, an arrangement followed by Nordsieck (2007) and Uit de Weerd & Gittenberger (2013: Supplementary data on-line). Tauphaedusa was raised to a full genus by Motochin et al. (2017), whose classification was followed by Sulikowska-Drozd et al. (2018) and is also followed here.

Möllendorff (1883) listed a number of varieties of Clausilia tau from central and southern China but considered them to exhibit only minor differences in various shell morphology features that were gradual in nature, presumably grading into each other, which was why he treated them as varieties and not separate species (“Speiell bei unsern Clausilienformen sind die Unterschiede durchgängig so geringfügiger und gradueller Natur, dass es völlig hinreichend ist, die verschiedenen Lokalformen als Varietäten zu scheiden”). The species was subsequently recorded from south-west Korea by Möllendorff (1887), who considered this form to be sufficiently different from Japanese and Chinese specimens to be a distinct, but un-named variety. However, the species has been recorded most extensively in Japan (e.g. Pilsbry 1902), where it is widespread and even used in folk medicine (Hamada & Minato 1987).

The greatest diversity of clausiliids is centered in three regions, western Eurasia, eastern Asia, and the Neotropics (including the Caribbean islands of Puerto Rico and Hispaniola), with a notable absence in North America (Kerney et al. 1979; Pfleger & Chatfield 1988; Thompson 1998; Uit de Weerd & Gittenberger 2013). Supposed North American records for clausiliids in the U.S. National Museum of Natural History have

**Figs. 2–3.** Tauphaedusa tau (Boettger, 1877) collected in Kalihi Valley, Oahu, in 2018. BPBM 284894. Shell height: 12.5 mm. 2, shell in apertural and right lateral view; 3, Abapertural close-up of the specimen in Fig. 2 with the internal lamellae visible through the shell (arrowed) and corresponding to the illustrations in the original description.
been represented by three lots cataloged as being from the United States. Lots 67597 and 67598 were identified as *Laminifera pauli* (Mabille, 1865) (now *Nenia pauli*; see Shileyko 2000; Gargominy & Ripken 2011) with the locality “Bayonne, New Jersey”. *Nenia pauli* is native to the western Pyrenees south of Bayonne, France, and adjacent areas of Spain (Mabille 1865; Kerney *et al.* 1979; Shileyko 2000; Gargominy & Ripken 2011); the locality in the USNM records has been corrected to “Bayonne, France”. Lot 31384 was identified as *Hemiphaedusa cylindrica* (Pfeiffer, 1846) (now *Cylindrophaedusa cylindrica*; Shileyko 2000; Budha *et al.* 2015) from “Western Hills Indiana”. However, *C. cylindrica* is native to northern India and Nepal (Pfeiffer 1846; Mitra *et al.* 2005; Budha *et al.* 2015), “Indiana” clearly being an error for “India”. Nonetheless, there is nothing in the USNM records to support this inference and the record has been retained in the USNM as “Indiana”, which we consider erroneous. Accordingly, we conclude that the present records, including those reported by Cowie (1997), which are referred to *Tauphaedusa tau* herein, along with *Nenia tridens* in Puerto Rico, as noted above, are the only valid records of Clausiliidae in the United States and its territories.

The circumstances of the introduction of *Tauphaedusa tau* to the Hawaiian Islands are not known. Numerous non-native species have been introduced to the Islands accidentally via the horticultural trade but this species has not been found in surveys of horticultural nurseries (Hayes *et al.* 2007, 2012; Cowie *et al.* 2008; Yeung *et al.* 2019). As these records from O‘ahu appear to be the first records of *T. tau* outside what is presumed to be its native range in Japan, China and Korea (Pilsbry 1908), it is plausible that it may have been brought in by the Asian community in Hawai‘i, either inadvertently or perhaps because of its use as a medicine in Japan (Hamada & Minato 1987), but this remains unknown. As the species has now been found in multiple locations on O‘ahu, spanning
approximately 20 km of the Ko‘olau range, and over a period in excess of 50 years, it is here regarded as established in the Hawaiian Islands.

Prior to their preservation, live-collected individuals were kept in Petri dishes in the laboratory for photography and unexpectedly produced large numbers of live-born offspring, ~2 mm in shell height at birth (Fig. 5), confirming the observations of Sulikowska-Drozdz et al. (2018) that Tauphaedusa tau is viviparous.

Six of the specimens from Kalihi Valley (see material examined) were screened for Angiostrongylus cantonensis. Tissue digestion was done in Longmire’s lysis buffer (0.1 M Tris, 0.1 M EDTA, 0.01 M NaCl, 0.5 % sodium dodecyl sulfate), 24 μl of buffer per 1 mg of tissue and 150 μg of proteinase K. DNA was extracted from the digested lysate with Qiagen Blood and Tissue Spin Column Kits following the manufacturer’s general protocol. The DNA was screened for A. cantonensis with a Taqman qPCR assay (ACANITS1, Life Technologies assay ID #AI39RIC) with oligonucleotides specific for the parasite’s internal transcribed spacer 1 (ITS1) gene (Qvarnstrom et al. 2010). Each 10 μl qPCR reaction consisted of 5 μl of Taqman Fast Advanced master mix (2X), 3.5 μl of nuclease-free water, 0.5 μl of the Taqman assay mix (20X), and 1.0 μl of template DNA. The qPCR run conditions consisted of a 2 min incubation at 50 °C, a 20 s denaturation period at 95 °C, and 40 cycles of 95 °C for 1 s and 60 °C for 20 s. A sample with an exponential amplification curve crossing a threshold of 0.2 fluorescent units was to be interpreted as positive for the presence of A. cantonensis. One of the six specimens was positive for the parasite. Based on the global compilation by Kim et al. (2014) of records of A. cantonensis in gastropods, it appears that this is the first record of infection, natural or experimental, in a clausiliid.

Material examined: O‘AHU: “W. side Manoa Valley, Oahu, Hawaiian Is.; rotten log, Round Top Forest Reserve, Round Top Drive” [USNM label], 7 Jun 1962, R. W. Husband (USNM 652985, 4 dry shells, Fig. 4); “Nuuanu Valley about 200 yards from highway on left side of stream in the dirt under dead leaves” [CCC field notes], 5 Sep 1965, William R. Hay & CCC (BPBM Malacology 207626, 1 collected alive, 2 dry shells); “Makiki Valley below Poloke Pl., c. 1200 ft. elevation” [CCC field notes], 23 Mar 1965, CCC (BPBM Malacology 252169, 4 dry shells); Nu‘uanu Valley, lat. 21.349233, long. -157.821900, 29 March 2018, J. R. Kim, T. C. Goulding, R. S. Kong (BPBM Malacology 284639, 1 dry shell); Waiāhole Valley, lat. 21.482094, long. -157.863509, 25 May 2018, RHC, RLR, MCIM (BPBM Malacology 284892; 2 collected alive, of which 1 sequenced; shells only preserved); Kalihi Valley, lat. 21.361389, long. -157.863509, 14 Aug 2018, RHC, RLR, MCIM (BPBM Malacology 284893; 4 dry shells); Kalihi Valley, lat. 21.361389, long. -157.840157, 5 Sep 2018, RHC, MCIM (BPBM Malacology 284894; 12 collected alive, of which 6 screened for A. cantonensis, 2 died, 4 with bodies preserved).

ACKNOWLEDGMENTS

We thank Norine Yeung for sharing information regarding the specimens found in Nu‘uanu and Makiki Valleys in 2018. She, Jaynee Kim and Regina Kawamoto helped in depositing the newly collected specimens in the Bishop Museum Malacology Collection. Nicole Yoneishi helped with screening for A. cantonensis. We thank Ellen Strong for providing photographs of the shells in USNM 652985 and for discussion of the records of USNM 67597, 67598 and 31384, and Bill Moser, Geoff Keel and Karen Reed for facilitating access to USNM 652985 at the Smithsonian Museum Support Center. Tricia Goulding took the photograph in Fig. 3. Fig. 4 was provided by the Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution. John
Slapcinsky and Kenneth Hayes commented on the manuscript and the former helped with obtaining literature. We also thank the landowners for facilitating access to the collection localities. Specimens collected by RHC, RLR, and MCIM were collected in conjunction with a State of Hawai‘i Department of Health (DoH) project to train DoH personnel about rat lungworm.

**LITERATURE CITED**


