

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 lungworm¹

ROBERT H. COWIE²

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: mcmedeir@hawaii.edu

CARL C. CHRISTENSEN³

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

1. Contribution No. 2019-005 to the Hawaii Biological Survey and No. 10722 of the University of Hawai‘i School of Ocean and Earth Science and Technology.

2. Research Associate, Hawaii Biological Survey, Bishop Museum, 1525 Bernice Street, Honolulu, Hawai‘i, 96827-2704, USA.

3. Research Affiliate, Pacific Biosciences Research Center, University of Hawai‘i, 3050 Maile Way, Gilmore 408, Honolulu, Hawai‘i 96822

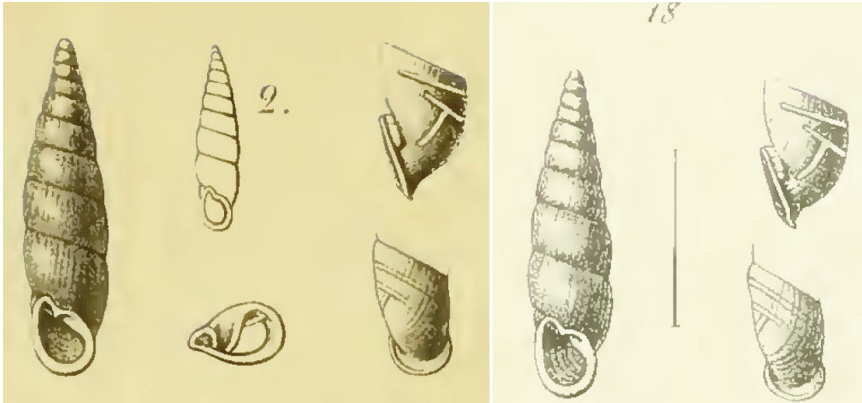


Fig. 1. Illustrations of *Clausilia tau* Boettger, 1877. **Left**, original illustration from Boettger (1878: pl. III, fig. 2). **Right**, illustration from Kobelt (1879: pl. VIII, fig. 18).

2013) and is widespread in the Hawaiian Islands (Kim *et al.* 2019). At two sites (Waiāhole 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 Waiāhole 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 Waiāhole 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 Phaesusinae (e.g., Ohtsuki & Takahashi 1982; Kato *et al.* 1989;



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.

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 (“*Speziell 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. 4–5. *Tauphaedusa tau* (Boettger, 1877). **4**, specimen collected in Mānoa Valley, O‘ahu, in 1962. One of four specimens in USNM 652985. Shell height 12.8 mm; **5**, specimens from O‘ahu, in the laboratory with offspring. The adults are preserved in BPBM Malacology 284894.

been represented by three lots cataloged as being from the United States. Lots 67597 and 67598 were identified as *Laminifera pauli* (Mabille, 1865) (now *Neniatlanta pauli*; see Shileyko 2000; Gargominy & Ripken 2011) with the locality “Bayonne, New Jersey”. *Neniatlanta 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-Drozd *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 μ l of buffer per 1 mg of tissue and 150 μ g 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 μ l qPCR reaction consisted of 5 μ l of Taqman Fast Advanced master mix (2X), 3.5 μ l of nuclease-free water, 0.5 μ l of the Taqman assay mix (20X), and 1.0 μ l 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 florescent 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); Wai‘āhole 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. -157.840157, 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

- Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. & Sayers, E.W.** 2017. GenBank. *Nucleic Acids Research* **45**: D37–D42.
- Boettger, O.** 1877. *Clausilienstudien*. Th. Fischer, Cassel. 122 pp., 4 pls.
- Boettger, O.** 1878. Neue recente Clausilien. I. *Jahrbücher der Deutschen Malakozoologischen Gesellschaft* **5**: 33–61, pls. II–IV.
- Boettger, O.** 1883. Zwei neue ostasiatische Clausilien. I. *Jahrbücher der Deutschen Malakozoologischen Gesellschaft* **10**: 270–272, pl. 8, figs. 8, 9.
- Budha, P.B., Naggs, F. & Backeljau, T.** 2015. Annotated checklist of the terrestrial gastropods of Nepal. *ZooKeys* **492**: 1–48.
- Chang, K.M.** 1982. New subspecies *Phaedusa (Euphaedusa) sheridani shihi* from Taiwan (Pulmonata: Clausiliidae). *Bulletin of Malacology R.O.C.* **9**: 15–21.
- Chen, H.-T.** 1935. Un nouveau nematode pulmonaire, *Pulmonema cantonensis*, n. g. n. sp. des rats de Canton. *Annales de Parasitologie Humaine et Comparée* **13**: 312–317.
- Cowie, R.H.** 1997. Catalog and bibliography of the nonindigenous nonmarine snails and slugs of the Hawaiian Islands. *Bishop Museum Occasional Papers* **50**: 1–66.
- Cowie, R.H.** 2013. Pathways for transmission of angiostrongyliasis and the risk of disease associated with them. *Hawai'i Journal of Medicine and Public Health* **72(6) Supplement 2**: 70–74.
- Cowie, R.H., Hayes, K.A., Tran, C.T. & Meyer, W.M., III.** 2008. The horticultural industry as a vector of alien snails and slugs: widespread invasions in Hawaii. *International Journal of Pest Management* **54**: 267–276.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R.** 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 291–299.
- Gargominy, O. & Ripken, T.E.J.** 2011. Une collection de référence pour la malacofaune terrestre de France. *MalaCo* (Hors Série) **1**: 1–108.
- Hamada, T. & Minato, H.** 1987. Historical and philological studies on “Shishangluosi”. *Venus* **44**: 275–281.
- Hayes, K.A., Tran, C.T. & Cowie, R.H.** 2007. New records of alien Mollusca in the Hawaiian Islands: nonmarine snails and slugs (Gastropoda) associated with the horticultural trade. *Bishop Museum Occasional Papers* **96**: 54–63.
- Hayes, K.A., Yeung, N.W., Kim, J.R. & Cowie, R.H.** 2012. New records of alien Gastropoda in the Hawaiian Islands: 1996–2010. *Bishop Museum Occasional Papers* **112**: 21–28.
- Kaneda, M. & Kitagawa, K.** 1990. Laboratory rearing method and biology of *Platydemus manokwari* de Beauchamp (Tricladida: Terricola: Rynchodemidae). *Applied Entomology and Zoology* **25**: 524–528.

- Kato, M., Matsumoto, M. & Kato, T.** 1989. Terrestrial malacofauna of Shizuoka Prefecture in Japan: biogeography and guild structure. *Contributions from the Biological Laboratory, Kyoto University* **27**: 171–215.
- Kawase, M., Nishio, K., Matsubara-Suzuki, M., Ichihara, T., Moriyama, A. & Kumazawa, Y.** 2018. Intraspecific variations of mitochondrial DNA sequences in *Stereophaedusa japonica* from central and western Japan and phylogenetic position of newly discovered specimens from Nagoya, central Japan. *Bulletin of Nagoya Biodiversity Center* **5**: 11–22.
- Kerney, M.P., Cameron, R.A.D. & Riley, G.** 1979. *A field guide to the land snails of Britain and north-west Europe*. Collins, London. 288 pp., 24 pls.
- Kim, J.R., Hayes, K.A., Yeung, N.W. & Cowie, R.H.** 2014. Diverse gastropod hosts of *Angiostrongylus cantonensis*, the rat lungworm, globally and with a focus on the Hawaiian Islands. *PLoS ONE* **9**(5): e94969.
- Kim, J.R., Wong, T.W., Curry, P.A., Yeung, N.W., Hayes, K.A. & Cowie, R.H.** 2019. Modelling the distribution in Hawaii of *Angiostrongylus cantonensis* (rat lungworm) in its gastropod hosts. *Parasitology* **146**: 42–49.
- Kobelt, W.** 1879. *Fauna molluscorum extramarinorum Japoniae. Nach den von Professor Rein gemachten Sammlungen*. C. Winter, Frankfurt am Main. 171 pp., 23 pls.
- Loosjes, F.E.** 1950. Some new gastropods of the family Clausiliidae from the Philippine Islands and Siam. *Proceedings of the United States National Museum* **100**: 539–545.
- Mabille, J.** 1865. Études sur la faune malacologique de Saint-Jean-de-Luz, de Dinan et de quelques autres points du littoral océanien de la France. *Journal de Conchyliologie* **13**: 248–265, pl. 14.
- Mitra, S.C., Dey, A. & Ramakrishna.** 2005. *Pictorial handbook—Indian land snails (selected species)*. Zoological Survey of India, Kolkata. iv + 344 pp.
- Möllendorff, O.F. von.** 1883. Materialien zur Fauna von China. *Jahrbücher der Deutschen Malakozoologischen Gesellschaft* **10**: 228–269, pl. 8, figs. 1–7.
- Möllendorff, O.F. von.** 1887. Die Landschnecken von Korea. *Jahrbücher der Deutschen Malakozoologischen Gesellschaft* **14**: 9–22, pl. 2, figs. 1–4.
- Motochin, R., Wang, M. & Ueshima, R.** 2017. Molecular phylogeny, frequent parallel evolution and new system of Japanese clausiliid land snails (Gastropoda: Stylomatophora). *Zoological Journal of the Linnean Society* **181**: 795–845.
- Nordsieck, H.** 2003. Systematic and nomenclatural notes on Phaedusinae with the description of new taxa. *Archiv für Molluskenkunde* **132**: 121–141.
- Nordsieck, H.** 2007. *Worldwide door snails (Clausiliidae), recent and fossil*. ConchBooks, Hackenheim. 214 pp., 20 pls.
- Ohtsuki, M. & Takahashi, S.** 1982. Studies on hermaphroditism of the ovoviviparous pulmonate, *Euphaedusa tau* (Böttger) — I. Shell whorls as a growth parameter. *Venus* **41**: 141–145.
- Pfeiffer, L.** 1846. *Symbolae ad historium heliceorum*. Sectio Tertia. Th. Fischer, Cassel. iv + 100 pp.
- Pfleger, V. & Chatfield, J.** 1988. *A guide to the snails of Britain and Europe*. Hamlyn, London. 216 pp.
- Pilsbry, H.A.** 1902. Additions to the Japanese land snail fauna—no. VII. *Proceedings of the Academy of Natural Sciences of Philadelphia* **54**: 517–533.

- Pilsbry, H.A.** 1908. A comparison of the land-snail fauna of Korea with the faunas of Japan and China. *Proceedings of the Academy of Natural Sciences of Philadelphia* **60**: 452–455.
- Qvarnstrom, Y. , da Silva, A.C.A., Teem, J.L., Hollingsworth, R., Bishop, H., Graeff-Teixeira, C. & da Silva, A.J.** 2010. Improved molecular detection of *Angiostrongylus cantonensis* in mollusks and other environmental samples with a species-specific internal transcribed spacer 1-based TaqMan assay. *Applied and Environmental Microbiology* **76**: 5287–5289.
- Schileyko, A.A.** 2000. Treatise on Recent terrestrial pulmonate mollusks. Part 5. Clausiliidae. *Ruthenica Supplement* **2**: 565–729.
- Schweigger, A.F.** 1820. *Handbuch der Naturgeschichte der skelettlosen ungegliederten Thiere*. Verlag der Dyk'schen Buchhandlung, Leipzig. xvi + 776 pp.
- Sulikowska-Drozd, A., Hirano, T., Wu, S.-P. & Páll-Gergely, B.** 2018. High fecundity, rapid development and selfing ability in three species of viviparous land snails Phaedusinae (Gastropoda: Stylommatophora: Clausiliidae) from East Asia. *Zoological Studies* **5**: 38. doi:10.6620/ZS.2018.57-38
- Thompson, F.G.** 1998. Clausiliid land snails from Hispaniola and their relationships to other New World genera (Gastropoda: Pulmonata: Clausiliidae). *Archiv für Molluskenkunde* **127**: 33–41.
- Ueshima, R., Motochin, R., Hasegawa, K. & Saito, H.** 2014. Land Snails of the Imperial Palace, Tokyo. *Memoirs of the National Museum of Nature and Science* **50**: 537–540.
- van der Schalie, H.** 1948. The land and fresh-water mollusks of Puerto Rico. *University of Michigan Museum of Zoology Miscellaneous Publications* **70**: 1–134.
- Waki, T.** 2017. Diversity of terrestrial mollusks and their helminths in artificial environments in Yoyogi Park, Tokyo, Japan. *Journal of Asia-Pacific Biodiversity* **10**: 254–256.
- Yeung, N.W., Meyer, W.M., III, Hayes, K.A., Kim, J.R., Skelton, T.J. & Cowie, R.H.** 2019. Non-native gastropods in high elevation horticultural facilities in Hawaii: a threat to native biodiversity. *Biological Invasions* **21**: 1557–1566.
- Zilch, A.** 1959. *Handbuch der Paläozoologie*. Band 6. Gastropoda. Teil 2: Euthyneura. Gebrüder Borntraeger, Berlin. xii + 835 pp.