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Angiostrongylus cantonensis in Hawai‘i: updated records and distributions of gastropod hosts on Maui, Moloka‘i and Lāna‘i[1]

Lauren A. Nguyen, Jaynee R. Kim, Kelli L. DeLeon
Hawaii Biological Survey, Bishop Museum, 1525 Bernice Street, Honolulu, Hawai‘i 96817-2704, USA; emails: lnguyen1@upenn.edu, jaynee@hawaii.edu, kelli.deleon@bishopmuseum.org

Tricia C. Goulding
Smithsonian Institution, National Museum of Natural History, P.O. Box 37012, MRC 163, Washington, D.C. 20013, USA; email: tc.goulding@gmail.com

Christopher S. Hobbs
Hawaii Biological Survey, Bishop Museum, 1525 Bernice Street, Honolulu, Hawai‘i 96817-2704, USA; email: cshobbs13@gmail.com

Hanna A. Kulvicki
University of Vienna, Center for Molecular Biology, Dr. Bohr-Gasse 9, 1030 Vienna, Austria; email: hanna@element-q.com

Norine W. Yeung, Kenneth A. Hayes
Hawaii Biological Survey, Bishop Museum, 1525 Bernice Street, Honolulu, Hawai‘i 96817-2704, USA; emails: norine@bishopmuseum.org, kenneth.hayes@bishopmuseum.org

Abstract. The Hawaiian Islands are a hotspot for the emerging infectious disease known as neuroangiostrongyliasis, caused by the parasitic nematode *Angiostrongylus cantonensis*. Gastropods are intermediate hosts of this parasite, and this study provides an update to the distributions of non-native gastropod hosts of *A. cantonensis* on the islands of Moloka‘i, Lāna‘i and Maui. Of the 29 gastropod species screened, *A. cantonensis* was detected in six (*Lissachatina fulica*, *Paropeas achatinaceum*, *Parmarion martensi*, *Cornu aspersum*, *Ambigolimax* cf. *nyctelius*, *Laevicaulis alte*), with *L. fulica* and *P. achatinaceum* as new host records for Moloka‘i and Lāna‘i, respectively. *Ambigolimax* cf. *nyctelius* is tentatively a newly recorded host of *A. cantonensis*. These updated data on the distributions of hosts for *A. cantonensis* contribute to the development of monitoring and effective management actions aimed at reducing the spread of the disease.

Keywords: Parasite, invasive species, snail, nematode, Pacific Islands, rat lungworm

INTRODUCTION

Angiostrongylus cantonensis, commonly referred to as the rat lungworm (RLW), is a parasitic nematode that causes the disease, eosinophilic meningitis in several groups of vertebrates, including humans (Diaz 2010). Rats are the definitive hosts and snails are intermediate hosts for the parasite, and both hosts are necessary in the life cycle of the parasite. Humans, other mammals, and birds are accidental hosts and can become ill after ingestion of the third-stage larvae, which are primarily found in the intermediate hosts.

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Infection typically occurs from accidental or intentional consumption of raw or undercooked gastropods (Cowie 2013, Kim *et al.* 2014), or less commonly from paratenic hosts such as fish, frogs, and crustaceans, carrying third-stage larvae (Qvarnstrom *et al.* 2007). Originally described from East Asia, *A. cantonensis* probably spread to the Hawaiian Islands via hosts that were unintentionally transported on ships (Kliks & Palumbo 1992) and was first documented in the Hawaiian Islands in 1961 (Horio & Alicata 1961) based on observations in 1960 reported by Ash (1962). Subsequently, the parasite has been reported from all six major Hawaiian Islands (Kim *et al.* 2014). Between 2007 and 2017, 82 cases of RLW were reported (Johnston *et al.* 2019) and an additional 17 more cases from 2017 to 2020 (Hawai‘i DOH).

As of 2019, the total number of known snail hosts for *A. cantonensis* in Hawai‘i was 22 (Kim *et al.* 2014, Yeung *et al.* 2018, Cowie *et al.* 2019). The development of effective control measures for the parasite and thus reduction of incidences of disease must be predicated on an accurate understanding of the distributions of its hosts, particularly those that carry the infective third stage larvae (Kim *et al*. 2014). As such, continued monitoring and updated survey data for the gastropod hosts of *A. cantonensis* are imperative (Diaz 2010). An increase in the number of reported cases on Maui (Cowie *et al*. 2018) prompted a need for additional surveys on Maui Nui (i.e. Maui, Lāna‘i and Moloka‘i; excluding Kaho‘olawe), and herein we report the results from these surveys and updated information on the distribution of infected RLW intermediate hosts on Maui Nui.

METHODS

Specimen Collection

Between November 2018 and June 2019, 23 sites were surveyed for non-native gastropods; ten on Moloka‘i, seven on Lāna‘i and six on Maui. Sites targeted for surveys were all < 700 m in elevation, since range modeling indicates most snails infected with RLW are restricted to low-mid elevations (Kim *et al.* 2018). Survey protocols followed Cowie *et al.* (2008) for agricultural and horticultural facilities while those conducted outside these facilities (e.g. forest reserves) followed Durkan *et al.* (2013). All non-native snails at each site were collected, euthanized with boiling water (Fukuda *et al.* 2008), fixed and preserved in 95% ethanol and vouchered in the Bishop Museum Malacology collection (BPBM 287093–287105, 287107–287122, 287124, 287126–287130, 287139 –287149, 287158–287162, 287164–287172, 287174, 287176, 287179, 287180, 287182, 287183, 287186–287188, 287190, 287193–287200, 287202–287208, 287210, 287211, 287214, 287216–287218, 287222, 287342–287345, 287348, 287350–287358, 287363– 287377, 288380, 288381). An additional 15 specimens collected during seven previous surveys by Yeung *et al.* (2018) were also tested (BPBM 284181, 284186, 284189, 284195, 284196, 284213, 284214, 284232, 284237, 286897), bringing the total number of sites analyzed to 30.

DNA Extraction and Rat Lungworm Screening

Total genomic gDNA was extracted from up to eight specimens per species from each site, and these samples were screened for RLW using PCR of the ITS1 locus following Kim *et al.* (2014). All samples that tested positive, and a random sample of 20% of all negatives, were re-tested to verify results. Tissue and gDNA are vouchered in the cryorepository of the Pacific Center for Molecular Biodiversity at the Bishop Museum (PCMB50988, PCMB50989, PCMB53807–PCMB53810, PCMB53812, PCMB53816, PCMB56544–PCMB56933).

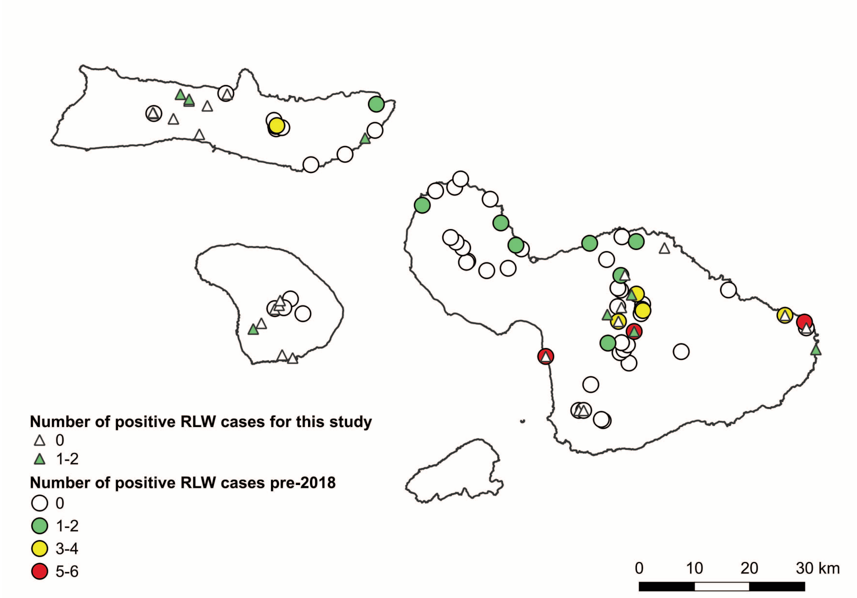

Figure 1. Map of sites surveyed on Maui Nui for non-native snail hosts of rat lungworm (RLW). Circles are historical data from Kim *et al.* (2014) and Yeung *et al.* (2018), and triangles are data from the present study. Map created in QGIS v. 3.10.

RESULTS

Surveys of 30 sites across the three islands (Fig. 1) yielded 979 non-native snails representing 29 species, of which 398 specimens were screened (Table 1). One of the 30 sites had previously been surveyed in October 2004 and was revisited in November 2018. Eight of the 30 sites contained snails that tested positive for RLW: three on Moloka‘i, one on Lāna‘i and four on Maui (Fig. 1). Twelve individuals representing six species (*Lissachatina fulica*, *Paropeas achatinaceum*, *Parmarion martensi*, *Cornu aspersum*, *Ambigolimax* cf. *nyctelius*, *Laevicaulis alte*) tested positive for *A. cantonensis*, and all six species except *A.* cf. *nyctelius* were previously reported as hosts of the nematode (Kim *et al.* 2014, Yeung *et al.* 2018). However, *L. fulica* and *P. achatinaceum,* as RLW intermediate hosts, are new island records for Moloka‘i and Lāna‘i, respectively, and *A.* cf. *nyctelius* is a potentially newly recorded host of *A. cantonensis* and new state record for the occurrence of this species, pending confirmation of the identification. Accounts of the six species that tested positive are provided below.

Achatinidae

Lissachatina fulica (Bowdich, 1822) **New Island Record for Gastropod Host on Moloka‘i**

Lissachatina fulica was first recorded in Hawai‘i in 1936 and is present across the main Hawaiian Islands (Cowie 1998, Gerlach *et al.* 2021). Kim *et al.* (2014) reported 11% of

Table 1. List of all species that were tested for *Angiostrongylus cantonensis* in this study. Total number of individuals collected for each species with number of sites in parentheses are given, as well as total number of individuals screened, with the number of individuals that tested positive for *A. cantonensis* in parentheses. Catalog numbers for each lot (BPBM) are provided next to the number of specimens collected, screened, and individuals that tested positive for *A. cantonensis* (collected/screened/postive).

			Moloka'i			**Lāna'i**			**Maui**		
Family	**Genus**	**Species**	*n* = collected (*n* = sites)	BPBM	*n* = screened (*n* = positive)	*n* = collected (*n* = sites)	BPBM	*n* = screened (*n* = positive)	*n* = collected (*n* = sites)	BPBM	*n* = screened (*n* = positive)
Achatinidae	*Allopeas*	*clavulinum*	23 (3)	287103 (5/4/0) 287120 (6/3/0) 287139 (12/4/0)	11 (0)	11 (3)	287165 (6/3/0) 387270 (2/1/0) 287203 (3/3/0)	7 (0)	8 (1)	287376 (8/6/0)	6 (0)
Achatinidae	*Lissachatina*	*fulica*	69 (6)	287093 (19/5/0) 287100 (7/5/1) 287118 (27/5/0) 287126 (12/5/0) 287130 (2/2/2) 287140 (2/2/0)	24 (3)	48 (4)	287158 (4/4/0) 287174 (6/5/0) 287186 (2/2/0) 287199 (36/5/0)	16 (0)	4 (1)	287342 (4/4/1)	4 (1)
Achatinidae	*Opeas*	*hannense*	1 (1)	288381 (1/1/0)	1 (0)	6 (4)	287171 (1/1/0) 287193 (2/1/0) 287204 (1/1/0) 287211 (2/2/0)	5 (0)	3 (2)	287358 (2/1/0) 287372 (1/1/0)	2 (0)
Achatinidae	*Paropeas*	*achatinaceum*	170 (7)	287095 (10/5/0) 287116 (69/5/0) 287128 (80/5/0) 287142 (1/1/0) 287146 (1/1/0) 288380 (1/1/0) 287096 (1/1/0) 287109 (7/5/0)	20 (0)	32 (5)	287166 (13/5/0) 287172 (5/4/1) 287179 (3/3/0) 287194 (4/3/0) 287205 (7/5/0)	20 (1)	17 (5)	284189 (2/2/0) 287353 (3/2/0) 287363 (8/8/0) 287371 (1/1/0) 287377 (3/3/0)	16 (0)
Achatinidae	*Subulina*	*octona*	13 (4)	287108 (8/2/0) 287121 (4/4/0) 287147 (1/1/0)	11 (0)	1 (1)	287180 (1/1/0)	1 (0)	5 (2)	284232 (1/1/0) 287364 (4/3/0)	4 (0)
Agriolimacidae	*Deroceras*	*laeve*	13 (2)	287113 (10/5/0) 287144 (3/3/0)	8 (0)	1 (1)	287159 (1/1/0)	1 (0)	24 (3)	287343 (9/5/0) 287350 (1/1/0) 287354 (14/5/0)	11 (0)
Agriolimacidae	*Deroceras*	*reticulatum*	0 (0)		0 (0)	0 (0)		0 (0)	7 (1)	284195 (7/1/0)	1 (0)
Ampullariidae	*Pomacea*	*canaliculata*	0 (0)		0 (0)	11 (1)	287216 (11/2/0)	2 (0)	0 (0)		0 (0)
Ariophantidae	*Parmarion*	*martensi*	0 (0)		0 (0)	0 (0)		0 (0)	13 (1)	287366 (13/5/2)	5 (2)
Assimineidae	*Cyclotropis*	sp.	15 (1)	287114 (15/5/0)	5 (0)	45 (4)	287160 (2/1/0) 287168 (1/1/0) 287187 (21/5/0) 287200 (21/5/0)	12 (0)	1 (1)	287367 (1/1/0)	1 (0)
Bradybaenidae	*Bradybaena*	*similaris*	53 (8)	287094 (16/5/0) 287101 (7/3/0) 287107 (17/5/0) 287115 (1/1/0) 287119 (2/2/0) 287127 (2/2/0)	24 (0)	60 (3)	287161 (25/5/0) 287169 (3/3/0) 287188 (32/5/0)	13 (0)	34 (4)	284181 (13/3/0) 287344 (14/7/0) 287351 (2/2/0) 287355 (5/5/0)	17 (0)

Table 1. List of all species that were tested for *Angiostrongylus cantonensis* in this study (continued)...

			Moloka‘i			**Lānaʻi**			**Maui**		
Family	**Genus**	**Species**	*n* = collected (*n* = sites)	BPBM	*n* = screened (*n* = positive)	*n* = collected (*n* = sites)	BPBM	*n* = screened (*n* = positive)	*n* = collected (*n* = sites)	BPBM	*n* = screened (*n* = positive)
Chronidae	*Kaliella*	*doliolum*	2 (1)	287141 (1/1/0) 287145 (7/5/0) 287102 (2/2/0)	2 (0)	8 (1)	287162 (8/5/0)	5 (0)	0 (0)		0 (0)
Euconulidae	*Kororia*	cf. *palaensis*	0 (0)		0 (0)	0 (0)		0 (0)	14 (2)	284213 (6/2/0) 287369 (8/5/0)	7 (0)
Gastrodontidae	*Zonitoides*	*arboreus*	5 (3)	287099 (2/2/0) 287124 (1/1/0) 287149 (2/2/0)	5 (0)	2 (1)	287198 (2/2/0)	2 (0)	0 (0)		0 (0)
Helicarionidae	*Ovachlamys*	*fulgens*	0 (0)		0 (0)	0 (0)		0 (0)	11 (2)	287368 (1/1/0) 287374 (10/5/0)	6 (0)
Helicidae	*Cornu*	*aspersum*	0 (0)		0 (0)	0 (0)		0 (0)	15 (5)	284196 (4/1/1) 284237 (5/1/0) 287345 (2/2/0) 287352 (3/3/0) 287356 (1/1/0)	8 (1)
Limacidae	*Ambigolimax*	cf. *nyctelius*	0 (0)		0 (0)	0 (0)		0 (0)	3 (1)	287357 (3/3/1)	3 (1)
Philomycidae	*Meghimatium*	*bilineatum*	0 (0)		0 (0)	0 (0)		0 (0)	5 (1)	287375 (5/5/0)	5 (0)
Philomycidae	*Pallifera*	sp.	0 (0)		0 (0)	1 (1)	287190 (1/1/0)	1 (0)	12 (3)	286897 (6/1/0) 284214 (4/1/0) 287370 (2/2/0)	4 (0)
Physidae		sp.	0 (0)		0 (0)	9 (1)	287202 (9/5/0)	5 (0)	0 (0)		0 (0)
Planorbidae	*Planorbella*	*trivolvis*	0 (0)		0 (0)	5 (1)	287217 (5/4/0)	4 (0)	0 (0)		0 (0)
Pristilomatidae	*Hawaiia*	*minuscula*	7 (3)	287098 (4/3/0) 287112 (2/1/0) 287129 (1/1/0)	5 (0)	3 (3)	287183 (1/1/0) 287197 (1/1/0) 287214 (1/1/0)	3 (0)	0 (0)		0 (0)
Sagdidae	*Lacteoluna*	*selenina*	0 (0)		0 (0)	2 (1)	287176 (2/2/0)	2 (0)	0 (0)		0 (0)
Spiraxidae	*Euglandina*	*rosea*	0 (0)		0 (0)	31 (1)	287210 (14/3/0) 287222 (17/5/0)	8 (0)	0 (0)		0 (0)
Succineidae	*Succinea*	*unicolor*	7 (2)	287110 (1/1/0) 287117 (6/1/0)	2 (0)	18 (2)	287195 (17/5/0) 287206 (1/1/0)	6 (0)	0 (0)		0 (0)
Thiaridae	*Melanoides*	*tuberculata*	0 (0)		0 (0)	24 (2)	287207 (11/5/0) 287218 (13/5/0)	10 (0)	0 (0)		0 (0)
Veronicellidae	*Laevicaulis*	*alte*	11 (3)	287097 (7/5/2) 287104 (3/3/1) 287111 (1/1/0)	9 (3)	0 (0)		0 (0)	9 (1)	287348 (9/5/0)	5 (0)
Veronicellidae	*Veronicella*	*cubensis*	13 (4)	287105 (3/3/0) 287122 (1/1/0) 287143 (3/3/0) 287148 (6/5/0)	12 (0)	22 (4)	287167 (5/5/0) 287182 (4/4/0) 287196 (11/5/0) 287208 (2/2/0)	16 (0)	19 (3)	284186 (5/3/0) 287365 (2/2/0) 287373 (12/5/0)	10 (0)
Vertiginidae	*Gastrocopta*	*servilis*	0 (0)		0 (0)	33 (1)	287164 (33/5/0)	5 (0)	0 (0)		0 (0)
Total			402 (10)		139 (6)	373 (7)		144 (1)	204 (13)		115 (5)

individuals (2 individuals from Kaua‘i, 3 from O‘ahu and 2 from Maui) tested positive for RLW across the six major Hawaiian Islands. Similar levels of infection, 10% (1 of 10), were reported for *L. fulica* on Maui (Yeung *et al.* 2018). In this study 121 *L. fulica* were collected from six sites on Moloka‘i (BPBM 287093, 287100, 287118, 287126, 287130, 287140), four sites on Lāna‘i (BPBM 287158, 287174, 287186, 287199) and one site on Maui (BPBM 287342). Of these, 44 *L. fulica* were screened for RLW, and 4 (9%; BPBM 287100, 287130, 287342) tested positive. The prevalence of infection differed among islands (Table 1): 13% on Moloka‘i (3 of 24), 0% on Lāna‘i (0 of 16), and 25% on Maui (1 of 4).

Paropeas achatinaceum (Pfeiffer, 1846) **New Island Record for Gastropod Host on Lāna‘i**

Paropeas achatinaceum was first recorded in Hawai‘i in 1904 (Cowie 1997) and is now found throughout the main Hawaiian Islands (Hayes *et al*. 2007). Kim *et al*. (2014) reported 4% of individuals (2 from Kaua‘i and 1 from Hawai‘i) tested positive for RLW across the six major Hawaiian Islands. None of the specimens that were screened by Yeung *et al*. (2018) tested positive. In the current study, 219 *P. achatinaceum* were collected from seven sites on Moloka‘i (BPBM 287095, 287108, 287116, 287128, 287142, 287146, 288380) and five sites each on Lāna‘i (BPBM 287166, 287172, 287179, 287194, 287205) and Maui (BPBM 284189, 287353, 287363, 287371, 287377). Of these, 56 individuals were screened for RLW with only 1 of 20 (5%; BPBM 287172) from Lāna‘i testing positive and 0% from Maui (0 of 16) or Moloka‘i (0 of 20).

Ariophantidae

Parmarion martensi (Simroth, 1893)

Thirteen *P. martensi* were collected from one site on Maui (BPBM 287366). This species was first recorded on O‘ahu in 1996 and has only been observed on the islands of Hawai‘i, O‘ahu, and Maui (Cowie 1998, Cowie *et al.* 2008, 2018). Kim *et al.* (2014) reported that 68% of individuals from O‘ahu and Hawai‘i (2 from O‘ahu and 11 from Hawai‘i) tested positive for *A. cantonensis,* and Yeung *et al.* (2018) reported that 31% of individuals (5 of 16) on Maui tested positive for the parasite. In this study, 40% (2 of 5) tested positive (BPBM 287366).

Helicidae

Cornu aspersum (Müller, 1774)

Cornu aspersum was first recorded in Hawai‘i in 1952 (Cowie 1997) and is now known to be established only on Maui and Hawai‘i (Yeung *et al.* 2018). None of the specimens that were screened by Kim *et al.* (2014) tested positive, while Yeung et al. (2018) reported 20% of individuals (2 of 10) as positive for the parasite. For this study, a total of 15 specimens were collected from five sites on Maui (BPBM 284196, 284237, 287345, 287352, 287356), of which 13% (1 of 8) tested positive (BPBM 284196).

Limacidae

Ambigolimax cf. ***nyctelius*** (Bourguignat, 1861) **Possible New Record of Gastropod Host**

The specimens screened for this study were tentatively identified as *Ambigolimax* cf. *nyctelius*, and once confirmed this will make it a new host of rat lungworm and constitute a new state record for this non-native snail. In this study, three specimens from one site on Maui were collected, and one tested positive (33%; BPBM 287357).

Veronicellidae

Laevicaulis alte (Férussac, 1822)

Laevicaulis alte was first recorded in the Hawaiian Islands in 1900 (Cowie 1998) but can now be found on six of the largest main Hawaiian Islands, excluding Niʻihau or Kahoʻolawe (Hayes *et al.* 2007, Cowie *et al.* 2008). Kim *et al.* (2014) reported that 30% of individuals (1 from Kauaʻi, 4 from Oʻahu, 1 from Molokaʻi, 3 from Maui and 4 from Hawaiʻi) tested positive across the six major Hawaiian Islands and Yeung *et al.* (2018) reported that only 22% of individuals (2 of 9) on Maui tested positive for *A. cantonensis*. In the current study, 21% (3 of 14) of individuals screened tested positive for *A. cantonensis* (BPBM 287097, 287104) out of 20 total individuals collected (BPBM 287097, 287104, 287111, 287348). The percentage of individuals found to be infected with *A. cantonensis* varies by island: all 3 individuals that tested positive were from Molokaʻi with 33% of individuals (3 of 9) testing positive, and none of the specimens (0 of 5) collected from Maui were found to carry *A. cantonensis*. Although *Laevicaulis alte* is established on Lānaʻi, no individuals were found there during the recent surveys.

DISCUSSION

The Hawaiian Islands, with their warm, tropical climate, are vulnerable to outbreaks of emerging infectious diseases like eosinophilic meningitis (Diaz 2010, Horio & Alicata 1961). As large numbers of gastropod species continue to be inadvertently transported around the world, it is expected that *A. cantonensis* will continue to spread to new places along with its intermediate hosts (Lafferty 2009, Iwanowicz *et al*. 2015). Snails at three new sites on Molokaʻi, one on Lānaʻi and three on Maui tested positive for *A. cantonensis*, and while infected *Lissachatina fulica* and *Paropeas achatinaceum* have previously been recorded in the Hawaiian Islands, this is the first record for Molokaʻi and Lānaʻi, respectively. Yeung *et al.* (2018) first recorded *Cornu aspersum* as a host, with two specimens from Maui testing positive for the parasite. In this study, an additional *C. aspersum* specimen from the same site tested positive. There were no instances of snails testing positive from sites on Maui where no snails had tested positive before this study. However, surveys of new sites revealed additional rat lungworm hosts. The detection of the spread geographically and to new hosts was possible only through ongoing and expanded surveys and testing, highlighting the critical need for continued monitoring across the islands. Such data are necessary for accurate estimates of the distribution of this parasite and for understanding its potential impacts.

Surprisingly, although there are several snail species on Lānaʻi that are known to carry the parasite on other islands, our current surveys and testing detected *Angiostrongylus cantonensis* in only one of the snails from Lānaʻi. Although we have no well-supported explanation for its low prevalence there, it may be because Lānaʻi is in the rain shadow of Maui, and receives less rainfall than other islands (Leopold 1948), and may be less suitable for *A. cantonensis*, as the parasite appears to proliferate in wetter, warmer conditions (Kim *et al.* 2018). Environments with higher precipitation facilitate the transfer of the nematode parasite to its hosts better because the wetter conditions support parasite larvae persistence in rat feces, possibly protecting them from desiccation (Kim *et al.* 2018, Hollingsworth *et al.* 2007). Alternatively, our limited sampling may affect our ability to detect the parasite at low thresholds, and additional testing is needed to evaluate the presence/absence and distribution of *A. cantonensis* hosts on Lāna‘i. In this study, *Ambigolimax* cf. *nyctelius* is tentatively identified as a new gastropod host of *A. cantonensis* pending confirmation of the identification, increasing the total in the state to 23 known species of snails that serve as intermediate hosts for the parasite. These results should be supported with additional testing to ensure an up-to-date record of gastropod vectors, as it is impossible to determine whether the lack of new species is due to limited spread of the parasite across species or limited testing capability.

As the number of sites containing hosts infected with *A. cantonensis* increases, the probability of eosinophilic meningitis outbreaks also increases. Furthermore, rising global temperatures will increase the availability of suitable habitat, expanding the range in which intermediate hosts and the parasite can thrive (Kim *et al.* 2018). In the Hawaiian Islands, and globally, it is essential to build on our knowledge of non-native molluscs and their distribution to inform proper management of these species, as this information is vital to maintaining public health and functioning ecosystems. Many of the sites containing snails infected with *A. cantonensis* include farms, nurseries, and places with high human traffic, and if food products from these facilities are not sanitized appropriately, accidental hosts (e.g. humans) risk ingesting carriers of *A. cantonensis* and contracting the rat lungworm disease (Qvarnstrom *et al.* 2007).

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