

Research Paper

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Distribution of the newly invasive New Guinea flatworm *Platydemus manokwari* (Platyhelminthes: Geoplanidae) in Thailand and its potential role as a paratenic host carrying *Angiostrongylus malaysiensis* larvae

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Abstract

Invasive species constitute one of the most serious threats to biodiversity and ecosystems, and they potentially cause economic problems and impact human health. The globally invasive New Guinea flatworm, *Platydemus manokwari* (Platyhelminthes: Geoplanidae), has been identified as a threat to terrestrial biodiversity, particularly soil-dwelling native species (e.g. molluscs, annelids and other land planarians), and is listed among 100 of the world's worst invasive alien species. We report here, for the first time, *P. manokwari* occurrences in many locations throughout Thailand, using voluntary digital public participation from the social network portals associated with the Thailand Biodiversity Conservation Group and collections of living flatworm specimens. Mitochondrial cytochrome *c* oxidase subunit I (COI) sequences confirmed that all collected flatworms were *P. manokwari* and placed them in the “world haplotype” clade alongside other previously reported specimens from France, Florida (USA), Puerto Rico, Singapore, French Polynesia, New Caledonia, and the Solomon Islands. In addition, infective stage larvae (L3) of the nematode *Angiostrongylus malaysiensis* were found in the flatworm specimens, with a 12.4% infection rate (15/121 specimens examined). *Platydemus manokwari* occurrence in Thailand and its capacity to carry L3 of *Angiostrongylus* should be of concern to biodiversity conservation and human health practitioners, because this invasive flatworm species may be involved in the life cycle of angiostrongylid worms in Thailand.

Introduction

Globalization is bringing social, economic, cultural and technological benefits to human beings, but also facilitates biological invasions of alien species worldwide (Reaser *et al.*, 2007). Exotic, or alien, species can produce a variety of outcomes in the new environments in which they are introduced. Invasive species are species that have spread outside their natural distribution and threaten biodiversity (Convention on Biological Diversity, 2018). The lack of natural predators in new environments is recognized as a key factor that promotes the success of invasive species (Fridley and Sax, 2014). In addition to harming new environments, invasive organisms may potentially cause economic problems and impact public health (Beck *et al.*, 2008; Lowry *et al.*, 2013; Marbuah *et al.*, 2014). Biological invasion may cause two main effects on the epidemiology of parasites and infectious diseases: (1) spill-over of co-introduced parasites, and/or (2) spill-back of native parasites that infect introduced species (Dunn *et al.*, 2012; Morand, 2015).

New Guinea flatworms, *Platydemus manokwari* De Beauchamp 1963 (Platyhelminthes: Geoplanidae), are a known predator of land snails, slugs and other soil invertebrates (Kaneda *et al.*, 1990). They were successfully introduced into several places for use as biological control against the giant African snail, *Achatina fulica*, and have spread across many regions worldwide (Sugiura, 2009; Justine *et al.*, 2015). Two haplotypes based on the mitochondrial cytochrome *c* oxidase subunit I gene (COI) have been identified: the Australian haplotype, which is found in Australia and the Solomon Islands (the two countries located closest to Western New Guinea, the island of origin of *P. manokwari*), and the world haplotype, which is found in New Caledonia, French Polynesia, the Solomon Islands, Singapore, Florida, Puerto Rico and France (Justine *et al.*, 2015). The latter haplotype has been widely dispersed throughout the

world, and was probably transported by humans. This flatworm species is one of several examples of harmful invasive species that cause biodiversity reduction and extinction of native snail species on many islands in the tropical Pacific Ocean (Ohbayashi *et al.*, 2007; Sugiura and Yamaura, 2009). Consequently, the International Union for Conservation of Nature added *P. manokwari* to the list of 100 of the world's worst invasive alien species (Lowe *et al.*, 2000).

In addition to its impact on ecosystems, *P. manokwari* has been also recognized as a potential carrier of zoonotic parasites. This land planarian species potentially acts as paratenic (transport) host that harbours infective stage larvae (L3) of the rat lungworm, *Angiostrongylus cantonensis* (Asato *et al.*, 2004), which is the causative agent of angiostrongyliasis in the Pacific Islands and several Asian countries (Alicata and Jindrak, 1970; Graeff-Teixeira *et al.*, 2009; Eamsobhana, 2013). More than 2,800 registered cases of angiostrongyliasis have been documented worldwide, and many cases have certainly gone unreported or unrecognized (Wang *et al.*, 2008). Thailand is a hotspot of this disease, with over 1,300 cases recorded during 2000–2009 (Eamsobhana, 2013). Patients become infected by the nematode as a result of ingesting L3 larvae, by consuming raw molluscs or crustaceans in traditional food, or fresh vegetables, fruits or water contaminated with infected snails and slugs. Because humans are not a definitive host for this parasite, ingested larvae are unable to develop into adults, as happens in rats. The larvae usually migrate to extremities of the nervous system (e.g. the brain or eyes) and then die off. The main cause of illness is the effect of inflammatory granulomatous reaction around the dead parasites in the brain, which manifests as eosinophilic meningoencephalitis (Alicata and Jindrak, 1970; Wang *et al.*, 2008). Additionally, worms moving around or migrating to the brain could result in physical neurological damage (Wang *et al.*, 2008).

In October 2017, *P. manokwari* was reported for the first time in Thailand. The flatworms were first seen preying on a giant African snail in a house garden in Lam Luk Ka District, Pathum Thani Province, and were first officially reported in Thailand in a press release of the Thailand Biodiversity Conservation Group (Panitvong, 2017). Subsequently, the presence of New Guinea flatworms became a hot topic in newspapers, television programmes and online media. The presence of this invasive flatworm species together with the news broadcast by local mass media caused severe societal concern; people started to worry about effect of the alien flatworm species on public health, particularly because it is a potential cause of eosinophilic meningoencephalitis.

The invasion status and public health impact of New Guinea flatworms in Thailand are still not known, and further investigation is required. Accordingly, the objectives of this study were to (1) confirm New Guinea flatworm identification based on genetic information; (2) assess the current distribution and invasion status of the New Guinea flatworm in Thailand; and (3) investigate whether the invasive New Guinea flatworm is able to harbour *Angiostrongylus* nematodes and plays a role in angiostrongyliasis epidemiology.

Materials and methods

Platydemus manokwari occurrence records in Thailand

Information regarding *P. manokwari* occurrence in Thailand was extracted from the nationwide social network portals associated

with the Thailand Biodiversity Conservation Group (i.e. the Siamensis.org Facebook page and New Guinea Flatworm Line ID @sde5284v). *Platydemus manokwari* observations across Thailand were reported by members of the two social network groups. From October 2017 onwards, citizens were asked to submit photographic or video clip evidence and information on the location (province or district) where they encountered the alien flatworm species. *Platydemus manokwari* was identified morphologically from the submitted evidence by the social network group administrators and flatworm taxonomists to verify the records from non-taxonomist citizens. Only the records that clearly showed morphological characteristics of *P. manokwari* (see Justine *et al.*, 2014) were included in the database (supplementary table S1). The submitted images or video clips that were not of sufficient quality for accurate identification were excluded from further analysis.

To illustrate potential invasion of *P. manokwari* across the country, a flatworm distribution map was generated using the *maptools* package (Bivand and Lewin-Koh, 2017) implemented in R v3.3.0 (R Core Team, 2016).

Platydemus manokwari specimen collection

To obtain fresh flatworm samples, our field research team first contacted citizens who had reported *P. manokwari* occurrence on the social network portals. Then, an appointment was made by the field research team to visit where the samples were collected, or arrangement was made for those citizens to send the flatworm specimens by post. In the latter case, the specimen providers were instructed to prepare live flatworms in a plastic container with the lid closed tightly, and include cotton wool/tissue paper soaked in water to maintain a high moisture level during transportation (see the laboratory rearing method described in Kaneda *et al.*, 1990). GPS coordinates were recorded for each sampling location where *P. manokwari* was obtained.

Investigation of angiostrongylid larvae in *Platydemus manokwari*

Parasitic infection was investigated from *P. manokwari* tissue using the artificial tissue digestion technique (pepsin digestion) and microscopic examination. Whole flatworm bodies were digested individually in 35 ml of 1% pepsin-HCl solution for 1 h at 37°C. *Angiostrongylus* larvae (L3) were counted and isolated by classic sedimentation technique, as follows. The samples were washed in 0.85% normal saline solution to remove and stop pepsin activity, and then let stand for 20 minutes to allow sedimentation of L3 larvae. For each sample, supernatant was discarded, to retain only sediment for subsequent microscopic inspection. The prevalence, mean abundance and range of angiostrongylid larvae infection in *P. manokwari* were estimated using Quantitative Parasitology v3.0 (Rozsa *et al.*, 2000).

Molecular procedures

Genetic materials of *P. manokwari* were obtained from living samples from each location. They were extracted individually using a DNeasy Blood & Tissue kit (QIAGEN, Hilden, Germany). A small piece of each flatworm was cut (*c.* 0.5 cm³) and crushed with pellet pestles (polypropylene stick) in a 1.5 ml microcentrifuge tube that contained ATL buffer and proteinase

K solution. All of the subsequent steps followed the manufacturer's protocol.

The partial mitochondrial COI sequence was selected as a gene target for polymerase chain reaction (PCR) with the primers COI-ASmit1 and COI-ASmit2 (Littlewood *et al.*, 1997; Justine *et al.*, 2014). PCR amplification was carried out in 20 µl reactions that contained 1 µl of DNA template, 2× KAPA Taq ReadyMix (KAPA Biosystem, Cape Town, South Africa), 3 mM MgCl₂, and 0.2 µM of each primer. PCR was run as follows: initial denaturation at 95°C for 5 minutes; 40 cycles of 95°C for 30 s, 48°C for 40 s and 72°C for 50 s; and finishing with final extension at 72°C for 7 minutes. PCR products were visualized in 1.5% agarose gel electrophoresis at 150 V for 30 minutes.

To confirm the nematode parasite species, L3 angiostrongylid larvae from flatworm hosts were arbitrarily selected for molecular identification using the mitochondrial COI marker. The larvae were pooled (five individuals) in absolute ethanol. Under stereomicroscope, larvae were submerged in 10 µl nuclease-free water (Life Technologies, Carlsbad, California, USA), and cut into small pieces using the tip of a 21G needle. Subsequently, genomic DNA was prepared using the HotSHOT DNA extraction method following the protocol described by Montero-Pau *et al.* (2008).

To confirm genetic information of the isolated larvae, PCR amplification of angiostrongylid mitochondrial COI DNA was performed in a 50 µl reaction mixture that included c. 1 ng of genomic DNA template of the L3 larvae, 5 units/µl of TaKaRa Ex Taq (Clontech, Palo Alto, California, USA), 1× Ex Taq Buffer, 2 mM MgCl₂, 0.2 mM dNTPs mixture, and 1 µM of each primer: LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HC02198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.*, 1994). PCR was run as follows: initial denaturation at 94°C for 1 minute; 5 cycles of 94°C for 1 minute, 45°C for 90 s, 72°C for 90 s; 35 cycles of 94°C for 1 minute, 50°C for 90 s, and 72°C for 1 minute; and a final extension at 72°C for 5 minutes. The PCR products were visualized by 1.5% agarose gel electrophoresis at 150 V for 30 minutes.

PCR products of *P. manokwari* and angiostrongylid larvae were cleaned and subjected to DNA sequencing on an ABI 3730xl DNA Analyzer (Bioneer, Daejeon, South Korea). Sequencing data were edited using Bioedit v7.1 (Hall, 2011) and compared using the sequence database on Nucleotide Basic Local Alignment Search Tool with BLASTn online platform (<https://blast.ncbi.nlm.nih.gov>). DNA sequences were aligned with CLUSTALX v2.0 (Larkin *et al.*, 2007). Maximum likelihood phylogenetic trees were then constructed based on the Hasegawa–Kishino–Yano substitution model (for *P. manokwari*) and Tamura–Nei substitution model (for angiostrongylid larvae) with 1000 pseudo-replications in MEGA v7.0 (Kumar *et al.*, 2016).

Results

Platydemus manokwari distribution records in Thailand

The number of *P. manokwari* observations was reported with photographic evidence taken by volunteer members of the online social networks: the Siamensis Facebook group and New Guinea Flatworm Line ID @sde5284v, throughout Thailand (fig. 1). In total, there were 66 verified cases in 40 districts of 31 provinces (Bangkok, Buriram, Chaiyaphumi, Chanthaburi, Chaing Mai, Chonburi, Chumphon, Kamphaeng Phet, Kanchanaburi, Khon Kaen, Lampang, Loei, Nakhon Ratchasima, Nakhon Si

Thammarat, Narathiwat, Nonthaburi, Pathum Thani, Phetchaburi, Phitsanulok, Phuket, Prachin Buri, Rayong, Samut Prakarn, Samut Sakorn, Sara Buri, Songkhla, Surat Thani, Trat, Ubon Ratchathani, Udon Thani and Yala) (supplementary table S1). This flatworm was frequently reported in several districts in Bangkok and its neighbouring provinces, and provinces on the east coast (i.e. Chonburi, Rayong, Chanthaburi and Trat). In addition to the mainland, this flatworm was also reported on islands (i.e. Muang and Kathu Districts, Phuket Province and Koh Kut District, Trat Province). The oldest evidence of *P. manokwari* occurrence in Thailand was a photograph of the flatworm taken at Kasetsart University, Chatuchak District, Bangkok on 7 January 2010 (credit: Mr Chayajit Deekrachang) (fig. 2).

Platydemus manokwari collection

A total of 121 living *P. manokwari* samples were collected from six localities in Thailand, including Bangkok (Saimai District, latitude: 13.901853 and longitude: 100.681765), Khon Kaen (Kranuan District, latitude: 16.707427 and longitude: 103.079975), Narathiwat (Cho-airong District, latitude: 6.2422833 and longitude: 101.426388), Nontaburi (Bang Bua Thong District, latitude: 13.899101 and longitude: 100.428332), Pathum Thani (Lam Luk Ka District, latitude: 13.949563 and longitude: 100.738588) and Samut Sakorn (Ban Phaeo District, latitude: 13.591572 and longitude: 100.111636) (table 1).

The flatworms were uniform in external appearance, long and flattened, and dark brown or black in body colour, with a light brown line running dorsally from the anterior tip to posterior end. The ventral part was a pale cream, grey or light brown, with a white cylindrical pharynx located at the middle part of the body. The collected flatworms differed in size, and ranged from 12–98 mm long and 1–8.5 mm wide. All flatworms were collected alive and survived until arrival at the laboratory for further investigation.

Genetic confirmation of Platydemus manokwari in Thailand

COI sequences (424 bp) of the flatworms from five localities were analysed, except the specimens from Bangkok, as there were only two individuals and those were used in the experiment for angiostrongylid larva investigation. The COI sequences from all analysed specimens were 100% identical (E-value = 0) to *P. manokwari* sequences in the NCBI database (e.g. accession nos KR349595.1, KF887958.1 and KR349610.1). Phylogenetic relationships showed that the five representative Thai *P. manokwari* samples grouped in the world haplotype clade alongside other *P. manokwari* sequences from France (KF887958.1), Florida (KR349598.1), Puerto Rico (KR349610.1), Singapore (KR349579.1), French Polynesia (KR349595.1), New Caledonia (KR349596.1) and the Solomon Islands (KR349608.1) (fig. 3).

Evidence of Angiostrongylus larvae in Platydemus manokwari

In total, 317 L3 larvae of *Angiostrongylus* nematodes were recovered from the New Guinea flatworms. All of the larvae microscopically exhibited the key characteristics of *Angiostrongylus* larvae: a pair of chitinous rods at the cephalic part, and caudal constriction (tail-pointed tip) (fig. 4). The larvae were 468–489 mm long and 25.9–32.6 mm wide (n = 10), and resembled the L3 stage of *Angiostrongylus* spp. (Alicata and Jindrak, 1970). The overall rate of infection was 12.4% (15/121 flatworms). Up to 81 larvae

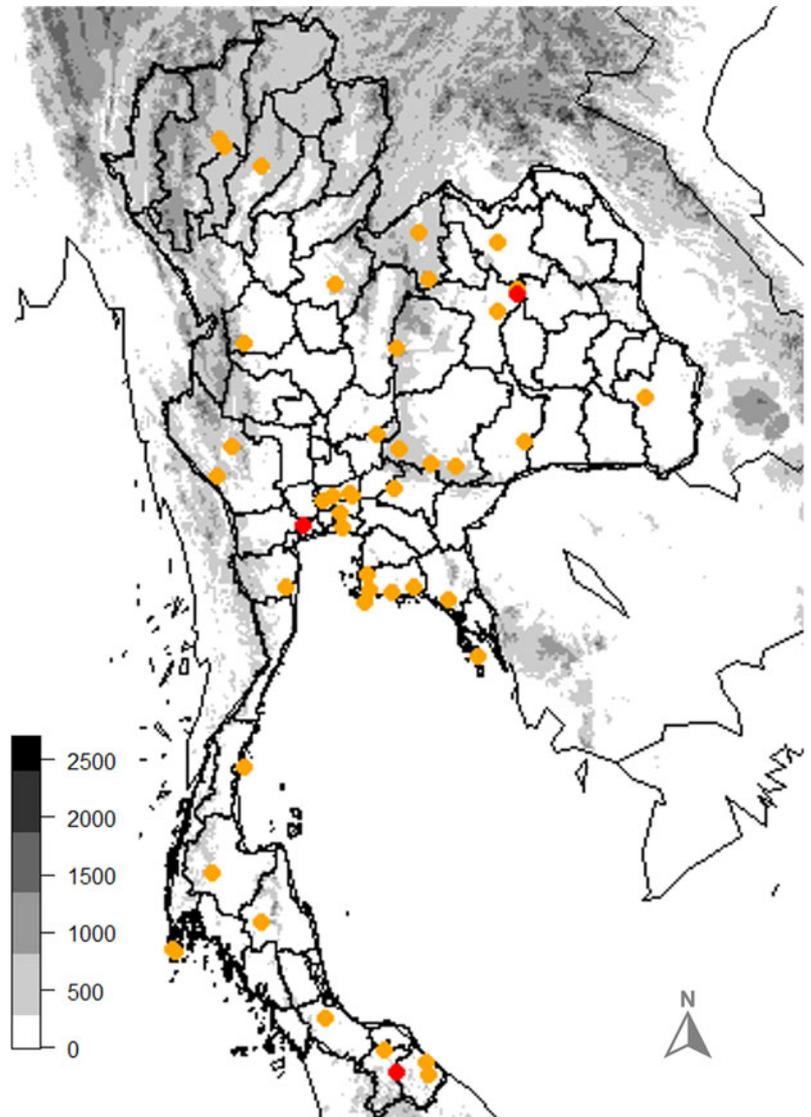


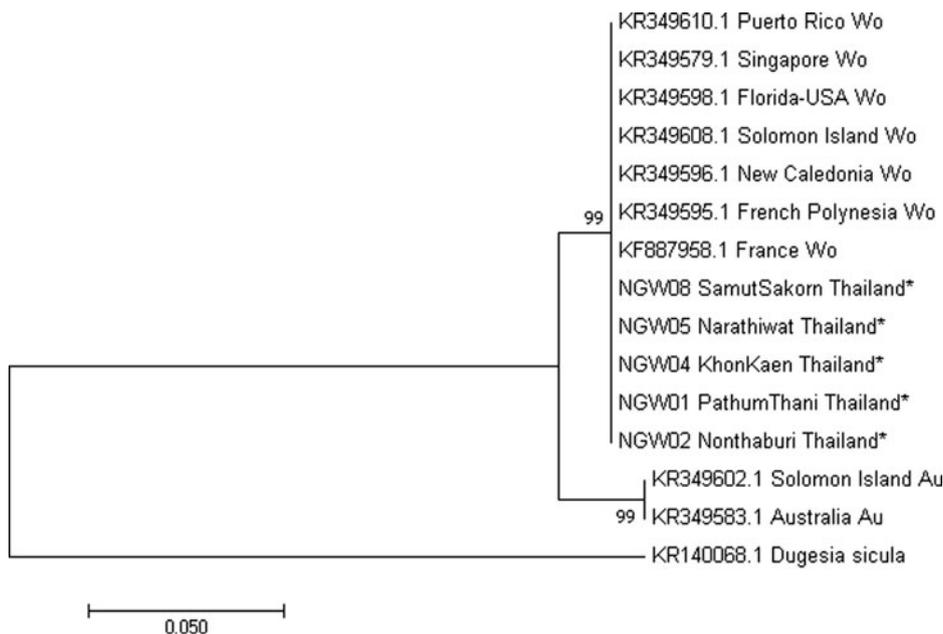
Fig. 1. Distribution of the New Guinea flatworm (*Platydemus manokwari*) in Thailand. Mapping data are based on photographic evidence of *P. manokwari* reported by public volunteers on the online social network portals the Siamensis.org Facebook page and New Guinea Flatworm Line ID@sde5284v (orange dots); red dots indicate L3 *Angiostrongylus*-positive *P. manokwari* specimens in the present study. The grey scale bar represents the altitudinal gradient.



Fig. 2. The oldest photographic evidence of *Platydemus manokwari* occurrence in Thailand (credit: Chayajit Deekrachang, 2010). This New Guinea flatworm specimen was found in a small garden around a monument at Kasetsart University, Bangkok.

Table 1. Angiostrongylid (L3) infection rate, mean abundance, and range in *Platydemus manokwari* from various locations in Thailand.

Location (Province)	No. of flatworms collected	No. of angiostrongylid-infected flatworms	Rate of infection, % (95% CI)	Mean abundance	Range of infection
Bangkok	2	–	–	–	–
Khon Kaen	12	1	8.3 (0.43–37.0)	5.5	66
Narathiwat	30	11	36.7 (21.3–55.1)	4.13	1–81
Nonthaburi	6	–	–	–	–
Pathum Thani	56	–	–	–	–
Samut Sakorn	15	3	20 (5.7–46.6)	8.47	17–75
Total	121	15	12.4 (7.3–19.7)	2.62	1–81

**Fig. 3.** Phylogenetic analysis based on mitochondrial COI sequences of *Platydemus manokwari* collected from Thailand compared with the world (Wo) and Australian (Au) haplotypes from previous studies (Justine *et al.*, 2014, 2015). A sequence of an aquatic planarian, *Dugesia sicula*, was used as the outgroup. The phylogenetic tree was constructed using the maximum likelihood method based on the Hasegawa–Kishino–Yano model. The tree is drawn to scale, with branch lengths indicating the number of substitutions per site.

were found in an infected flatworm (total mean abundance 2.62). The infected *P. manokwari* were from Narathiwat, Samut Sakorn and Khon Kaen Provinces, with infection rates of 36.7%, 20% and 8.3%, respectively (see table 1 and fig. 1). The COI sequence (579 bp) of pooled *Angiostrongylus* larvae was translated based on translation table 5 (the invertebrate mitochondrial DNA code) in MEGA v7.0 (Kumar *et al.*, 2016), and there were no premature stop codons, which ensured that the sequence was not a pseudogene. In addition, we compared the sequence to COI sequences from the complete mitochondrial genome samples in GenBank (i.e. NC_013065, *A. cantonensis*; NC_030332, *A. malaysiensis*). The maximum likelihood phylogenetic tree showed that the L3 larvae of *Angiostrongylus* nematodes grouped with the AM6 isolate of *A. malaysiensis* (accession no. KT947979) (fig. 5).

Discussion

The invasive New Guinea flatworm is reported here for the first time in Thailand. Specimens collected from several places were confirmed to be *P. manokwari* by mitochondrial COI sequence.

This species occurs widely throughout the country. There was also evidence of this flatworm harbouring infective-stage larvae of *Angiostrongylus* nematodes. Here, distribution of this flatworm species and its potential impacts on the environment and public health are discussed.

This is also the first report of *P. manokwari* in mainland South-east Asia, although it was previously found in two island countries of this region: the Philippines and Singapore (Justine *et al.*, 2014, 2015). It appears that *P. manokwari* is already distributed throughout Thailand, and was found on both the mainland and islands (i.e. Koh Kut District, Trat Province; Koh Samui District, Surat Thani Province; and Phuket Province) (fig. 1). The oldest photographic evidence dates back to 2010 (fig. 2), which indicates that *P. manokwari* was introduced to Thailand at least 8 years ago, but was not publicly reported until October 2017 (Panitvong, 2017). There is some speculation regarding the possible ways that this flatworm was introduced into Thailand. International commercial trade appears to be the main route that has facilitated invasion of this flatworm (the worm itself, or its fragment or cocoon) across several countries and continents. Materials, particularly pottery and commercially



Fig. 4. Microscopic illustration of an infective stage larva (L3) of *Angiostrongylus* sp. isolated from *P. manokwari* after a tissue digestion experiment. The whole body (a), and cephalic (b) and caudal ends (c) of the larva are presented with key morphological characteristics (CR = chitinous rods, TPT = tail-pointed tip) of angiostrongylid larvae.

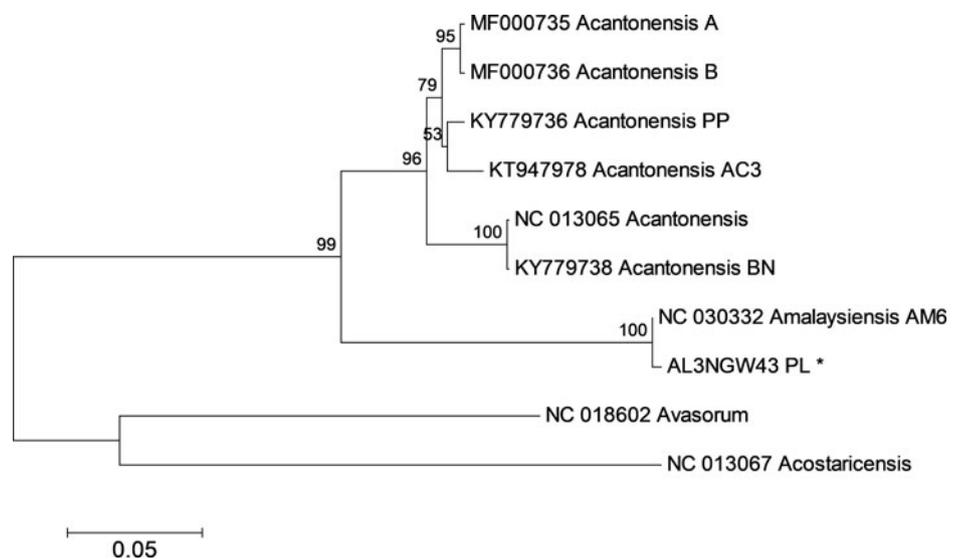


Fig. 5. Phylogenetic analysis of mitochondrial COI sequences of pooled L3 *Angiostrongylus* isolated from *P. manokwari* in Samut Sakorn Province, Thailand (AL3NGW43-PL). Molecular analysis confirmed larval identification as *A. malaysiensis*. The phylogenetic tree was constructed using the maximum likelihood method based on the Tamura–Nei model. The tree is drawn to scale, with branch lengths indicating the number of substitutions per site.

imported plants (either the soil or the plants themselves), could be contaminated (Sugiura *et al.*, 2006; Sugiura and Yamaura, 2009; Justine *et al.*, 2014). Subsequent within-country movement of contaminated plants and other types of materials associated

with soil can easily transport the flatworm to new places (Sugiura, 2009).

Interestingly, *P. manokwari* occurrences tended to be well aggregated in/around the capital city, Bangkok, and nearby

provinces, and some other industrialized cities and ports on the east coast (i.e. Chonburi and Rayong) (fig. 1). These cities could be hypothesized to be the first portals of *P. manokwari* introduction via seaports or international airports. Alternatively, this could be interpreted as possible bias from using a citizen science approach. The majority of the records may come from highly developed areas where people have easy access to technology (e.g. they more commonly use smart phones or the internet) compared with rural areas.

In addition, local observations revealed that *P. manokwari* occurrences seemed to be limited to human-made habitats (e.g. orchards, military camps, private home gardens, kitchen walls and bathrooms, including toilets). There is still no information about occurrence of this flatworm in rural agriculture lands, woodlands, community forests, national parks and other protected areas. Elucidating the occurrence of these species in more rural areas could help reveal if this species was accidentally imported into the country by humans. However, this invasive flatworm species may not be able to survive in these environments for unknown reasons, such as unsuitable environmental conditions (biotic and abiotic resistance), propagule pressure, unknown native resident predation, genetic constraint, or lack of mutualism (Blackburn and Duncan, 2001; Keane and Crawley, 2002; Zenni and Nuñez, 2013). Further investigation is required to more thoroughly understand *P. manokwari* invasion and associated consequences.

Mitochondrial COI sequences of the flatworm specimens confirmed *P. manokwari* taxonomic identification. The five representative *P. manokwari* in Thailand all grouped in the world haplotype, with low genetic variation. This finding could result from limitations of using a partial single gene marker (424 bp of COI in this case) that may be too low in resolution to draw conclusions regarding the population structure of this species, and/or this species is currently in the early stage of invasion. This flatworm may not be able to establish populations easily, particularly in the tropical mainland, where high biodiversity or environmental heterogeneity potentially act as ecological buffers that determine response to external pressures (Melbourne *et al.*, 2007; Freestone *et al.*, 2013). Further fine-scale investigation of population genetics (e.g. DNA microsatellite markers) and ecological parameters in these areas would help elucidate the population structure of the invasive New Guinea flatworm in Thailand.

Among the *P. manokwari* specimens collected in Thailand, infective stage larvae (L3) of *Angiostrongylus* had a total infection rate of 12.4%. The infection rate in Thailand was quite high, but slightly lower than those in other areas, such as *A. cantonensis* larvae in *P. manokwari* (14.1%) from Okinawa, Japan (Asato *et al.*, 2004). In Hawaii, Qvarnstrom *et al.* (2013) reported very high prevalence of *A. cantonensis* infection (67%, 8/12 specimens examined) in land planarian species (some of which were *P. manokwari*). Epidemiological research on angiostrongyliasis in its endemic areas, including Thailand, has largely focused on the risk of infection from ingesting raw or undercooked intermediate host snails (i.e. *Pila* spp., *Pomacea canaliculata*, *Cryptozonia siamensis*, and *Achatina fulica*) (Pipitgool *et al.*, 1997; Tesana *et al.*, 2009; Vitta *et al.*, 2011, 2016). However, information regarding the potential risk of terrestrial flatworms, such as *P. manokwari* as a paratenic host (carrier) of the parasite, is limited, particularly in Thailand. Transmission of L3 *Angiostrongylus* to humans includes both eating intermediate or paratenic hosts, and ingesting vegetables or water contaminated with infected slugs or snails (Heyneman and Lim 1967; Alicata and Jindrak

1970; Ash, 1976; Alto, 2001; Cowie, 2013). Shedding of L3 *Angiostrongylus* in the mucus of intermediate/paratenic hosts (e.g. snails, slugs and land planarians) potentially results in parasite contamination of water and food, particularly in leafy salad, vegetable juice, and fruit. Damaged or decayed intermediate hosts could also release numerous L3 *Angiostrongylus* that can survive up to 72 hours in a moist environment (Cheng and Alicata, 1964; Cowie, 2013) and subsequently contaminate water and vegetables. Qvarnstrom *et al.* (2013) also speculated that the flatworms could hide in and contaminate fresh vegetables with the parasitic nematode through their secretions and/or when they are chopped during food preparation. Moreover, with their exceptional capacity to survive (e.g. their regeneration ability) and expand their populations quickly and globally as an invasive species, *P. manokwari* could potentially act as an important reservoir that harbours and spreads *Angiostrongylus* to new places via rat infection. Based on previous literature and the results of our study, we emphasize the important role of *P. manokwari* in angiostrongyliasis epidemiology in Thailand and nearby countries.

Based on genetic information, our results showed that L3 *Angiostrongylus* recovered from *P. manokwari* in Thailand were *A. malaysiensis*. Although *A. cantonensis* is believed to be the main agent of angiostrongyliasis, which causes eosinophilic meningitis worldwide, the closely related *A. malaysiensis* may also be a human pathogen in endemic areas, including Thailand and other countries in South-east Asia (Prociv *et al.*, 2000; Rodpai *et al.*, 2016). *Angiostrongylus malaysiensis* can cause neurological disease in monkeys under experimental conditions (Cross, 1979). Reports from Malaysia, Indonesia and Thailand indicate that *A. malaysiensis* also potentially causes human disease (Carney and Stafford, 1979; Lim and Ramachandran, 1979; Rodpai *et al.*, 2016). However, *A. malaysiensis* has still not been isolated from humans (Prociv *et al.*, 2000).

Recent evidence revealed that *A. cantonensis* and *A. malaysiensis* widely co-occur throughout the countries of the Indochinese Peninsula (Rodpai *et al.*, 2016; Dusitsittipon *et al.*, 2017; Eamsobhana *et al.*, 2018). Apart from the two *Angiostrongylus* spp. mentioned above, there are also some other metastrongylid nematodes in wild murine rodents from this region (i.e. *A. siamensis*, *Thaistrongylus harinasutai* and *Malayometastrongylus diardinematus*) (Ohbayashi *et al.*, 1979; Gibbons and Krishnasamy 1986; Veciana *et al.*, 2014). L3 infective stage larvae of these metastrongylids ideally circulate in the environment and are picked up by their mollusc intermediate hosts, or even paratenic hosts like *P. manokwari*, to continue their life cycle. In angiostrongyliasis epidemiology, it is still largely unknown whether L3 of these metastrongylids, similar to the well-known agent *A. cantonensis*, can cause eosinophilic meningoencephalitis in humans; additional collaborative studies on disease surveillance in the environment and clinical studies are needed to elucidate this issue. Improvement of clinical (particularly molecular) diagnostic methods would also help improve our understanding of angiostrongyliasis epidemiology in South-east Asia.

In ecology, host–parasite relationships have been recognized as important interactions in ecosystem functioning, with various effects of individuals on populations and communities (Morand, 2015). There are two main epidemiological hypotheses regarding the role of invasive host species in parasite transmission: (1) parasite spill-over, in which invasive host species co-introduce novel parasites to local native hosts; and (2) parasite spill-back, in which a native parasite infects competent invasive

host species, which produces a wider host range that promotes parasite survival (Kelly *et al.*, 2009; Strauss *et al.*, 2012; Morand, 2015). In the present case of a new invasive species in Thailand, *P. manokwari*, the invasive flatworms appeared to be infected by *A. malaysiensis* as a result of their predation on native intermediate host molluscs. This finding indicates potential evidence of parasite spill-back. The invasive flatworms may act as a new host for *Angiostrongylus* spp., which could potentially amplify the transmission of the parasite in the invaded areas.

Globally, humans have entered into the “Information Age”, with increasing use of modern digital technologies for communication. This technological revolution has resulted in changes in communication methods in a wide range of societal disciplines, including ecological and environmental sciences (Arts *et al.*, 2015). For example, volunteers that participate in a biodiversity exploration campaign report their local information via digital devices, such as smart phones and related technology (a citizen science approach), and this is rapidly becoming an alternative method in ecological research and nature conservation (Dickinson *et al.*, 2010; Conrad and Hilchey, 2011). Gathering data in this manner would promptly and cost-effectively produce information on the distribution of the invasive flatworm species at the national level. However, the information obtained would require careful verification, particularly the identification of flatworm species by non-taxonomist volunteers, as some endemic organisms (e.g. land planarians: *Bipalium javanum*, *Diversibipalium haasei* and *Anisorhynchodemus* spp.; and slugs: *Pamaron* spp. and *Semperula* spp.) could easily be misidentified, and could be eliminated unintentionally.

In summary, the globally invasive flatworm *P. manokwari* was reported for the first time in Thailand. The flatworms were also found harbouring infective stage larvae of *Angiostrongylus malaysiensis*. These findings provide updated information on the role of this invasive species in the life cycle of a parasitic nematode. Continued monitoring of the distribution status of this invasive flatworm and additional research on its biology would help provide an enhanced understanding of infection by this nematode in its endemic areas and contribute to our understanding of the importance of invasive species in biodiversity conservation worldwide.

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.1017/S0022149X18000834>

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