

Research Article

The origins of *Melanooides tuberculata* (Müller, 1774) in New Zealand's aquarium trade and non-indigenous population

Ian C. Duggan^{1,*} and Matthew A. Knox²

¹*Te Aka Mātuatua – School of Science, Environmental Research Institute, The University of Waikato, Hamilton, New Zealand*

²*School of Veterinary Science, Massey University, Private Bag 11 222 Palmerston North, 4442, New Zealand*

*Corresponding author

E-mail: ian.duggan@waikato.ac.nz

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Abstract

Melanooides tuberculata (Müller, 1774) is a widely distributed aquatic gastropod, primarily found in tropical regions, which has also invaded geothermally heated waters in temperate regions. The spread of *M. tuberculata* globally has primarily been in association with the aquarium trade and aquatic plants. In New Zealand, *M. tuberculata* is known from both the aquarium trade and from one non-indigenous population in a geothermal stream. To date, the origins of *M. tuberculata* in the aquarium trade in New Zealand—or elsewhere—has not been systematically examined genetically, and it has not been confirmed whether New Zealand's non-indigenous population is genetically similar to, or originated from, individuals sold in aquarium stores. We examined the origins of New Zealand *M. tuberculata*, in the aquarium trade, and in the established non-indigenous population using COI marker sequences. Representatives of *M. tuberculata* in New Zealand have not originated from a single source; two distinct genetic clades were identified for individuals, with the origins of each inferred to be from Asia and Africa. The non-indigenous population and individuals sourced from one private aquarium owner were found to originate in Asia, and are genetically similar to those collected from Singapore, one of the most significant countries globally for the culturing and distribution of aquarium species. The remaining collections, from a display aquarium and three online suppliers in various New Zealand locations, were most similar genetically to populations native to Lake Malawi and Tanganyika, and non-indigenous populations from Australia. Overall, as *M. tuberculata* is an obligate parthenogen, each independently introduced clone may be considered a distinct non-indigenous taxon. We recommend further systematic genetic analyses of individuals in the aquarium trade elsewhere, to assess more fully the diversity and origins of *Melanooides* being transported in the aquarium trade.

Key words: Thiaridae, parthenogenesis, exotic species, tropical aquaria, biosecurity

Introduction

The gastropod *Melanooides tuberculata* (Müller, 1774) (Gastropoda: Prosobranchia: Thiaridae) is a widely distributed snail in the freshwaters of tropical regions (Facon et al. 2003; Van Bocxlaer et al. 2015). Originally described from India, its native range has been difficult to determine (Facon et al. 2003). Early accounts of its distribution included tropical areas of Africa to south-east Asia (Pilsbry and Bequaert 1927). From there,

M. tuberculata has invaded widely, including other tropical areas in Central and South America, and in geothermal waters in temperate regions (Duggan 2002; Facon et al. 2003; Van Bocxlaer et al. 2015; Coelho et al. 2018). Recently invaded areas include North America, since the 1950s (Dundee 1974), and a geothermally heated stream in temperate Serbia in 2011 (Milenkovic and Gligorijevic 2012).

Introduction and establishment of *M. tuberculata* has primarily been in association with trade in aquatic plants, particularly through the aquarium trade (Madsen and Fradsen 1989; Pointier and Delay 1995). *Melanoides tuberculata* has been observed in aquarium stores in many countries, including Canada (Rixon et al. 2005), Brazil (Assis et al. 2014), Singapore (Ng et al. 2016) and Israel (Yanai et al. 2017). Further, Duggan (2010) found *M. tuberculata* to occur in around 30% of 55 aquaria sampled in New Zealand homes; in these homes, *M. tuberculata* was only found in freshwater aquaria where aquatic plants had been introduced. In recent years, *M. tuberculata* individuals have been sold via online auction websites (e.g., eBay; Preston et al. 2021), and are commonly advertised as being beneficial as scavengers, eating left-over food, plant debris, and as food for snail eating fishes (e.g., Harrison and Duggan 2019). Nevertheless, in most aquaria, *M. tuberculata* are unintentional inhabitants rather than intentional introductions.

Melanoides tuberculata is polymorphic, with a number of shell morphs described in the literature. Despite distinct morphologies being heritable over generations in laboratory settings (Pointier et al. 1992), different morphs are commonly not monophyletic (Facon et al. 2003; Sorensen 2005). Further, *M. tuberculata* is not a genetically homogeneous taxon, but contains divergent clades (Facon et al. 2003; Van Bocxlaer et al. 2015). As such, morphological characteristics are poor determinants for differentiating *Melanoides* taxa and their origins. Although many authors have assigned morphs to non-indigenous populations, variation in shell morphology—including great plasticity in non-native populations—can sometimes make it difficult to determine invaders from native populations where invasions have occurred (Van Bocxlaer et al. 2015). Facon et al. (2003) determined that genetic clades of *M. tuberculata* appear to originate from different parts of the world; for example, distinct clades were identified representing populations originating from Africa and the Middle East, the Pacific, and south-eastern Asia. Nevertheless, they also found “invasive clones” to be distributed throughout much of the phylogenetic tree, indicating that non-native populations do not originate from a single region. Similarly, Van Bocxlaer et al. (2015) identified two major clades among the *Melanoides* genus; the first contained *M. polymorpha*, endemic to Lakes Malawi and Mweru, Asian *Tarebia granifera* (Lamarck, 1822), *M. tuberculata* from Asia, and *M. tuberculata* invaders from Asia into Africa and America; the second major clade contained *Melanoides* taxa endemic to the Congo Basin

(e.g., *M. cf. liebrechtsi* (Dautzenberg, 1901)), African *M. tuberculata*, subclades of Asian and Polynesian *M. tuberculata*, and individuals in Lake Tanganyika that had invaded from Asia. Overall, the phylogenetic analyses of Van Bocxlaer et al. (2015) further confirmed that the genus *Melanoides* is paraphyletic, while *M. tuberculata* is polyphyletic.

In New Zealand, *M. tuberculata* is known from the aquarium trade (Duggan 2010; Harrison and Duggan 2019) and from one non-indigenous population surviving in a geothermally heated stream (Duggan 2002; Duggan et al. 2007). Nevertheless, the origins of *M. tuberculata* in the aquarium trade in New Zealand—or elsewhere, in any systematic manner—have not been examined genetically, and it has not been confirmed whether the non-indigenous population at Golden Springs is genetically similar to, or originated from, individuals sold in aquarium stores. Here, we examine the origins of *M. tuberculata* in New Zealand, in the aquarium trade and in the established non-indigenous population in a geothermal stream.

Materials and methods

Collection

We collected *M. tuberculata* from three commercial internet suppliers selling via New Zealand auction website TradeMe (two in Auckland, on 1 May 2016 and 29 May 2016, and one in Christchurch on 22 April 2016). Further collections were made from a display aquarium at The University of Waikato on 2 May 2016 and a private home aquarium in Hamilton on 12 May 2016. Finally, individuals were collected from the one known established population of the species at Golden Springs on 11 February 2016.

DNA analysis

Tissue from specimens was dissected and 2 mm³ used for molecular analyses. DNA was extracted and amplified using the REDExtract-N-Amp™ Tissue PCR Kit following manufacturer's instructions. COI sequences were amplified using 0.2 μM of the primers CrustDF1: GGTCWACAAAYCATAAAGAYATTGG and CrustDR1: TAAACYTCAGGRTGACCRAARAAYCA (Steinke et al. 2016). PCR consisted of 94 °C for 2 min followed by 5 cycles of 94 °C for 40 s, 45 °C for 40 s and 72 °C for 1 min, then 35 cycles of 94 °C for 40 s, 51 °C for 40 s and 72 °C for 1 min and a final step of 72 °C for 5 min. PCR product was cleaned using 0.1 μL EXO I enzyme (10 U/μL), 0.2 μL shrimp alkaline phosphate (1 U/μL) and 2.7 μL sterile H₂O incubated at 37 °C for 30 min and 80 °C for 15 min. Samples were sequenced in both directions using ABI 3130xl automated DNA sequencers. All sequences and supporting information have been deposited in BOLD (project NZAQT) and GenBank® (accession numbers OM616955–OM616988).

COI sequences were supplemented with previously published material available on GenBank (n = 161 sequences 2 February 2022) using the search

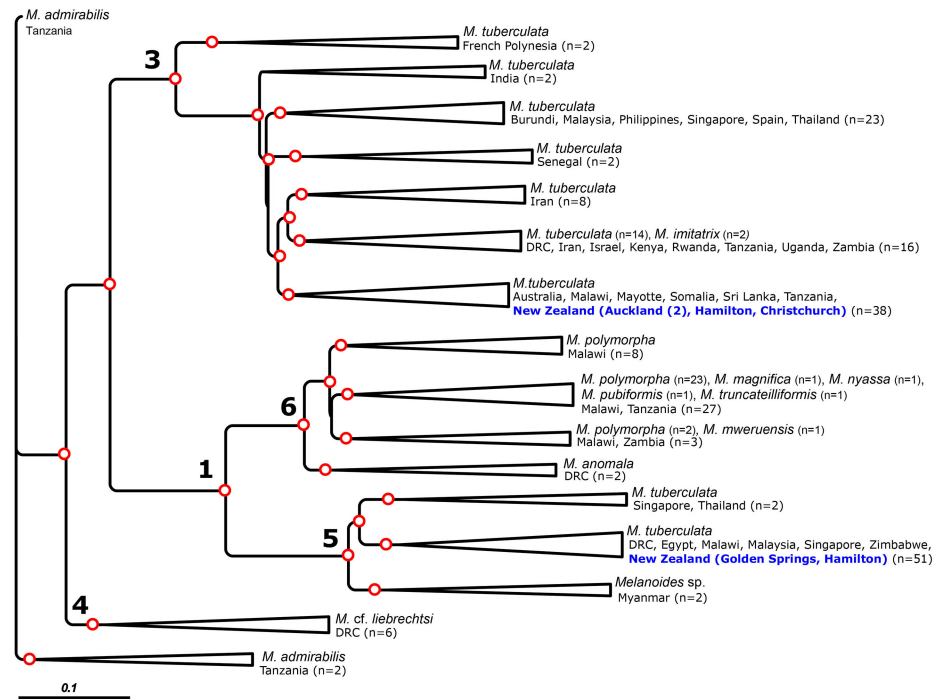


Figure 1. *Melanoides* phylogenetic tree, based on the COI gene. Sequences from this study were aligned with others from NCBI. Branch support > 90% is indicated by red circles at nodes. Collapsed nodes are indicated with triangles. Clade numbers correspond to those identified by Van Bocxlaer et al. (2015). Scale bar corresponds to 0.1 substitutions per site.

term “(Melanoides[Organism]) AND (COI[All Fields] OR cytochrome c oxidase OR cytochrome oxidase)”. DNA sequences were edited and aligned in Geneious R10 (Kearse et al. 2012) and trimmed to include only regions with > 50% coverage, resulting in a 643 bp nucleotide alignment. A maximum likelihood phylogenetic tree was built using PHYML (Guindon et al. 2010) with parameters determined using Smart Model Selection (Lefort et al. 2017).

In our analyses, we rely heavily on the analysis and interpretation of clades given by Van Bocxlaer et al. (2015), the most recent examination of *Melanoides tuberculata* phylogeny. The average evolutionary divergence over sequence pairs within and between (sub)clades 3–6 was estimated in MEGA (Tamura et al. 2004, 2021). We also estimate divergence over sequence pairs within the two subclades containing sequences from our study.

Results

We obtained 5 sequences each from two suppliers in Auckland, from a Christchurch supplier, the display aquarium at The University of Waikato and a private home aquarium in Hamilton, and 9 from the established population of the species at Golden Springs, resulting in 34 sequences.

Our tree, based on our New Zealand COI nucleotide sequences as well as representatives from GenBank (n = 161; Figure 1), exhibited a similar topology to that of Van Bocxlaer et al. (2015), who used concatenated COI + 16S. As with their study, two distinct clades were recognised, with both clades having occurrences of Asian and African representatives. However,

M. liebrechtsi, in clade 2 in Van Bocxlaer et al. (2015), form an outgroup in our analysis. Nevertheless, the subclades distinguished are all similar, providing confidence that our results can be discussed in the context of the interpretation of Van Bocxlaer et al. (2015). We have labelled our tree with the same clade numbers as Van Bocxlaer et al. (2015). New Zealand specimens were distributed within both of these two major clades.

Clade 1 of Van Bocxlaer et al. (2015) contained *Melanooides polymorpha*, endemic to Lakes Malawi and Mweru (sub-clade 6), *M. tuberculata* from Asia, and *M. tuberculata* invaders from Asia (e.g., Singapore and Malaysia) into Africa and America (sub-clade 5), which was reflected in our analysis. A number of our New Zealand sequences fall into the latter sub-clade (Clade 5), including the established non-indigenous population in Golden Springs, and from the private aquaria at a residence in Hamilton, indicating that these populations are derived from populations originating in Asia.

Clade 2 of Van Bocxlaer et al. (2015), divided into sub-clade 3 (containing *Melanooides* taxa endemic to the Congo Basin; e.g., *M. cf. liebrechtsi*) and 4 (African *M. tuberculata*, subclades of Asian and Polynesian *M. tuberculata*, and individuals from Lake Tanganyika that had invaded from Asia), were distinctly split in our analysis. The remaining four New Zealand bought and display aquarium collected specimens clustered within clade 3, and were most closely associated with populations from Lake Malawi and Tanganyika, and non-indigenous populations from northern Australia. However, the New Zealand individuals were not closely related to those from Lake Tanganyika that have invaded Asia or any other non-indigenous sequences.

The average number of base substitutions per site between clades 3–6 (see Figure 1) ranged from 0.08–0.10, reflecting the relatively high levels of COI nucleotide sequence diversity within *M. tuberculata*. Within these clades, the average number of base substitutions per site was lower, 0.021, 0.014, 0.021 and 0.007, for clades 3–6 respectively. The two subclades containing sequences from New Zealand (see Figure 1) were analysed and here the diversity was very low, with 0.0041 and 0.0027 base substitutions per site for the subclades containing New Zealand sequences within clades 3 and 5, respectively, typically corresponding to 0–4 nucleotide differences between sequences (SI). Translating nucleotide sequences to amino acid revealed a high rate of synonymous substitutions, common among mollusc species complexes elsewhere (Wilding et al. 2000; Reunov et al. 2021). For instance, the two groups of New Zealand *M. tuberculata* have distinct (86%) nucleotide similarity, but almost identical amino acid sequences (> 99%).

Individuals among populations sampled showed a great variety in size, shape and shell wear, within and between individuals from identified clades (Figure 2). Overall, our results indicate that the New Zealand aquarium trade carries *M. tuberculata* originating from both Asia and Africa, while those of the non-indigenous population at Golden Springs are derived from Asia; this is seemingly the less common genotype in the New Zealand aquarium trade.

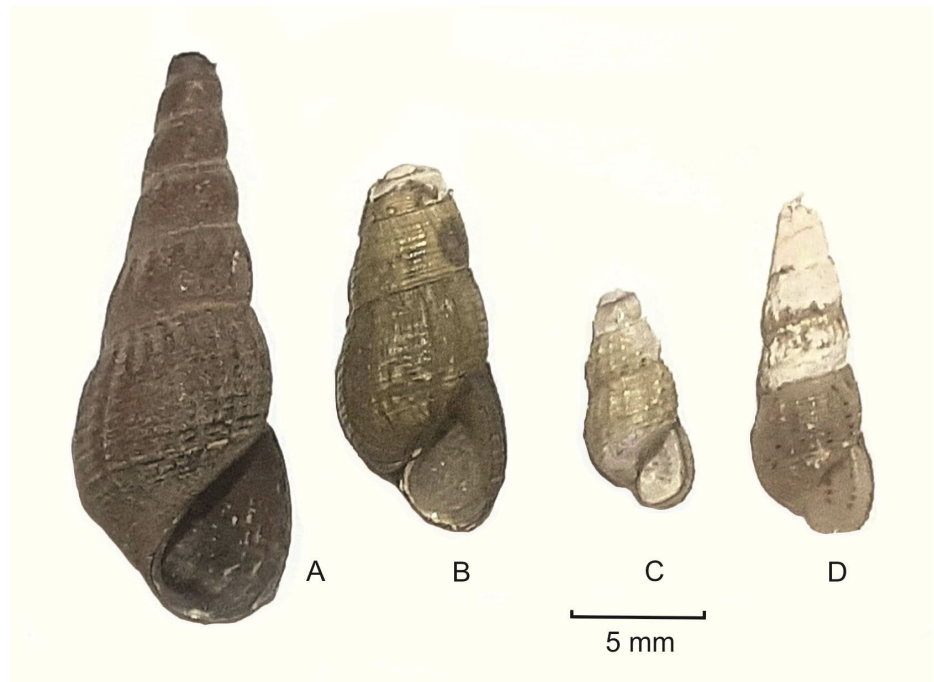


Figure 2. *Melanoides tuberculata* individuals from: A) a non-indigenous population at Golden Springs, B) an aquarium owner, Hamilton, C) a display aquarium, The University of Waikato, and D) an online supplier, Christchurch. A and B group in subclade 5, and C and D in subclade 3, of the Van Bocxlaer et al. (2015) analysis. Photographs by Ian Duggan.

Discussion

Representatives of *M. tuberculata* in New Zealand, and from the aquarium trade in general, do not originate from a single source. Overall, New Zealand individuals collected fitted into two distinct groups, representing both of the major clades of *Melanoides* identified by Facon et al. (2003) and Van Bocxlaer et al. (2015). Using the interpretation of Van Bocxlaer et al. (2015), New Zealand individuals are considered to have originated from both Asia and Africa, with the non-indigenous population at Golden Springs originating from Asia. As *M. tuberculata* is an obligate parthenogen, each independently introduced clone may be considered as a distinct non-indigenous taxon (*sensu* Facon et al. 2003).

Some of our New Zealand sequences fall into subclade 5 of Van Bocxlaer et al. (2015); the established non-indigenous population at Golden Springs, and individuals from the private aquaria of an individual in Hamilton. Based on the interpretation of Van Bocxlaer et al. (2015), these individuals are derived from Asian populations. Included among specimens in this group are those collected from Singapore, one of the most significant countries globally for the culturing of aquarium species (Ng et al. 2016). Indeed, Ng et al. (2016) identified *M. tuberculata* from molluscs collected from pet stores and major ornamental exporters in this country. As such, it is unsurprising that some of the individuals in the New Zealand aquarium trade correspond with these. Individuals from the non-indigenous population at Golden Springs are genetically identical to those of the one aquarium

owner surveyed. Accordingly, this non-indigenous population likely originated from individuals from the aquarium trade. Supporting this hypothesis, Golden Springs was also found to sustain populations of the guppy (*Poecilia reticulata* Peters, 1859), a popular tropical aquarium fish (Duggan 2002). Further, individuals of *M. tuberculata* from Florida, USA, likely introduced from the aquarium trade, also fit within clade 5. Similarly, individuals in this clade include those that have invaded Lake Malawi and sites in the Democratic Republic of Congo, which are considered to have originated from Asia (Genner et al. 2004; Van Bocxlaer et al. 2015), strengthening the assertion that this invasion was derived from the aquarium trade.

Our other specimens, from three populations bought online and from a display aquarium, clustered within subclade 3 of Van Bocxlaer et al. (2015), and were most closely associated with populations native to Lake Malawi and Tanganyika, and populations non-indigenous to northern Australia. Nevertheless, the New Zealand individuals were not closely associated with individuals from Lake Tanganyika that have invaded Asia. That is, there is no clear link for these individuals to be importations from Asia by the aquarium trade. This suggests, with our current state of knowledge, that individuals within the New Zealand and Australian aquarium trade may have been uniquely imported from Africa. However, New Zealand and Africa do not possess strong trade routes. A more likely alternative is that with greater sequencing of *M. tuberculata* individuals from aquaria globally, we may elucidate their origins more fully. Interestingly, Australian populations of *M. tuberculata* were for many years assumed to be native; however, systematic sampling has revealed these are restricted there to human population centres (Glaubrecht 2000). Our analysis suggests that many individuals in the New Zealand aquarium trade, and those that have invaded Australia, have an identical origin, thus strengthening the aquarium trade being the origin of the Australian populations.

Our specimens showed great variation in size, shape and shell wear, within and between the identified clades. As such, there does not appear to be clear and consistent morphological traits shared by specimens within genotypes that allows them to be differentiated between morphs. While Facon et al. (2008) found different invading genotypes to have identical morphologies in Martinique, West Indies, this is not a universal truth. For example, Van Bocxlaer et al. (2015) found Asian invasive lineages to resemble each other in overall shell morphology, despite belonging to deeply divergent clades. As such, we do not recommend the use of morphological characteristics alone for differentiating *Melanoides* taxa and their origins.

Systematic sampling and genetic analyses of *Melanoides* in the aquarium trade has not previously been undertaken elsewhere. While we identified two major haplotypes in New Zealand's aquarium trade, it appears internationally that there are other haplotypes present in the trade. For

example, Facon et al. (2003) included one individual in their analysis of a specimen from an aquarium store in Douai, France, which matched haplotypes from populations in Indonesian, Caribbean and South American waters. As such, we recommend further genetic analyses of individuals in the aquarium trade elsewhere, to assess more fully the diversity of *Melanooides* being transported in the aquarium trade. One manner in which populations may differ among trades is that importation of macrophytes, seemingly the dominant introduction vector for *Melanooides*, has been limited into New Zealand since the 1990s, with extant macrophytes now domestically cultivated (Champion and Clayton 2001). As such, any new clones that have entered the trade will have had a low probability of being introduced into New Zealand. In European countries, in contrast, aquatic plants sold in the aquarium trade have continued to be imported from Singapore, Indonesia and Thailand (Brunel 2009) since this time (Champion and Clayton 2001).

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Authors' contribution

ICD was responsible for research conceptualization, sample design and methodology, sample collection, results interpretation and writing; MAK was responsible for genetics analyses, interpretation and writing.

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