Unwanted ‘hitchhikers’ of ornamental snails: a case report of digeneans transported via the international pet trade

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Abstract
In the European Union, aquarium pets are organisms intended for closed places (e.g., pet shops, garden ponds, home aquariums). Until April 2021, regulations did not require veterinary inspection of these animals within the EU, although there is a potential risk of such organisms being released into the environment along with their symbiotes or parasites. Currently, a “disease-free” declaration is required, but no aquarium snail pathogen that needs attention in international trade has been included in the list of potential hazards. Here, we intended to check whether molluscs from the pet trade could be a source of parasites. We answered this question by using Anentome helena, a popular commodity in the ornamental pet industry, as a research model. Snail specimens were randomly collected from aquarium pet stocks imported from Bangkok (Thailand) to Warsaw (Poland) in March 2020. In total, three specimens were subjected to histological examination and 27 specimens were autopsied. Histological analysis revealed that one snail was infected with rediae (and the cercariae inside them). Our study is the first to show the presence of digenean larvae in A. helena originating from the ornamental pet industry. The spread of such “hitchhikers” in non-native areas will likely be associated with threats to environmental and public health. Therefore, it is necessary to constantly draw public attention to the possible consequences of releasing ornamental pets into the non-native environment.

Keywords: Aquarium trade, Anentome helena, non-native species, parasites, rediae

Introduction
Ornamental pets are potential vectors for numerous “hitchhikers”, often pathogen and parasite causing diseases, which can be overlooked and therefore spread worldwide via the pet trade (Tamukai et al. 2014; Patoka et al. 2016a; Maciaszek et al. 2020). In the European Union (EU), ornamental aquatic animals are intended for closed ornamental facilities (pet shops, garden centres, garden ponds, commercial aquaria or wholesalers keeping ornamental aquatic animals). At least until April 2021, they had not required veterinary inspection within the EU, as they had no direct contact with natural waters, and they did not pose significant risks to aquaculture, human health, or wild stocks. The inspectors were not competent to monitor the origin or quantity of the pets, which were often collected from local aquarium hobbyists or were bred in the stores (Commission Regulation (EU) 2010; Commission Implementing Regulation (EU) 2020). Unfortunately, this led to the uncontrolled spread of aquarium pets (including ornamental snails) within the trade as well as a frivolous attitude and their release into the environment (Domagała et al. 2004; Biondo 2017).
According to the legal regulations provided by the Commission Implementing Regulation (EU) (2020), aquatic ornamental pets in the EU are treated like other aquatic animals that are not dedicated to human consumption. Before the importation, declarations are issued for invertebrates that they are free from diseases mentioned in the animal health certificate model “aqua-entry-estab/release/other” (Model animal health certificate for the entry into the Union of aquatic animals intended for certain aquaculture establishments, for release into the wild or for other purposes, excluding human consumption). Despite the update of the regulations, still none of the freshwater mollusc diseases or pathogens is listed there. Therefore, there is a risk of various pathogens and parasites in molluscs obtained from the pet trade. The release of these host organisms into non-native environments is a potential threat not only to natural ecosystems but also to human health (Federspiel et al. 2020; Modrý et al. 2021; Rollins et al. 2021).

Snail-borne diseases may be dangerous helminthiasis due to their significant impact on human and animal health (Lu et al. 2018). Molluscs act a crucial role in the life cycles of digenean trematodes (Faltýnková et al. 2008). The most attractive ornamental pets most often originate from tropical countries, including areas endemic to numerous trematodes of medical and veterinary importance (Inobaya et al. 2014; Toledo & Esteban 2016; Pratumchart et al. 2019; Nguyen et al. 2021).

In this report, we focused on *Aentome helena* (von dem Busch in Philippi, 1847) (Gastropoda: Nassariidae), known as the assassin snail, killer snail, or bumblebee snail (Stegeman et al. 2020), which commonly occurs in the lower reaches of coastal rivers, lakes, and ponds throughout Southeast Asia (CABI 2021). Currently, the snail is a popular commodity in the ornamental pet industry (Yanai et al. 2017; Hossain & Mohsin 2020), especially due to its curious looking shell – fusiform, prominently ribbed and spirally banded (Strong et al. 2017). The interest in this mollusc is intense among aquarium enthusiasts because of its voracious appetite for other snails of different species, including *Helisoma* sp., *Melanoides* sp., *Physa* sp., *Planorbella* sp., which easily breed and often become major pests in aquaria (Bogan & Hanneman 2013). However, it should be emphasized that *A. helena* is a non-selective predator and scavenger that preys on both molluscs and fish eggs as well as on crustaceans (Bogan & Hanneman 2013). Therefore, there is growing concern about the potential threat to native aquatic animal populations if *A. helena* is introduced into the natural environment (Bogan & Hanneman 2013; Ng et al. 2016a), especially that there are frequent reports of the illegal releases of aquatic pets into natural waters, where they can become invasive (Chucholl 2013; Ng et al. 2016b; Patoka et al. 2018; Banha et al. 2019). Moreover, it has not been controlled so far whether this very popular snail among aquarium enthusiasts can be a source of non-native parasites for Europe. Here, using *A. helena* as a research model, we report that molluscs obtained from a shipment of aquarium fauna imported from Southeast Asia to Europe could be the source of trematodes.

**Material and methods**

A total of 30 adults of *A. helena* were randomly collected from aquarium pet stocks imported from Bangkok (Thailand) to Warsaw (Poland) in March 2020. In the laboratory, snails were immediately placed individually in transparent glass beakers with a small amount (approx. 50 ml) of dechlorinated tap water and exposed to artificial light (desk lamp) for 24 h to stimulate the release of cercariae (Blankespoor & Reimink 1991). Then, the water was checked for cercariae presence with a stereoscopic microscope (Science ETD-101, Bresser, Rhede, Germany). Next, we looked for the presence of trematodes in the snail tissues.

The whole soft body of three randomly selected snails was fixed in Bouin’s solution for histological examination. Following the fixation, the samples were dehydrated in a graded ethanol series (Chempur, Pieacky Slaskie, Poland), cleared with xylene (POCH, Gliwice, Poland), and embedded in paraffin wax (Chempur). Serial cross-sections (4–6 μm thick) were cut with a rotary microtome (Hyrax M55, Zeiss, Oberkochen, Germany). Histological slides were deparaffinized, rehydrated, and stained with Ehrlich hematoxylin (Carl Roth, Karlsruhe, Germany) for five minutes and with a 1% ethanol solution of eosin Y (Analab, Warszawa, Poland) for five minutes. Then, slides were dehydrated in 96% ethanol and twice in isopropyl alcohol (Leica, Wetzlar, Germany), cleared in Clearene (Leica), and embedded in CV Ultra (Leica). Snail tissues on the glass slides were analysed under the light microscope (Eclipse 80i, Nicon, Tokyo, Japan) in a bright field using 4, 10, 20, 100× objective magnification and photographed using a digital camera (Axio Cam MRC5, Zeiss) and image acquisition software (ZEN, Zeiss).

The remaining 27 soft bodies of snails were cut up into smaller sections, which were gently crushed between a glass slide and a coverslip (Reddy et al. 2004). To search for sporocysts, rediae, cercariae, and metacercariae of digenean trematodes, freshly
squeezed tissues were examined under a light microscope (Primostar, Zeiss) at various objective magnifications (5, 10, 40, and 100×).

Results

The non-invasive diagnostic method used in live snails showed no digenean cercariae in the water. In histological assessment, one of the three examined snails showed the prepatent infection of digenean larvae. More precisely, numerous rediae and cercariae inside them were observed in the hepatopancreas of the infected individual of Anentome helena (Figure 1). Autopsy of the remaining 27 specimens revealed no developmental stages of digenean trematodes. Our research showed that digenean infection was recorded in 3.33% of all snails investigated.

Discussion

The reports on the presence of digenean larvae in Southeast Asia snails, including Anentome helena are scarce and insufficient (Krailas et al. 2012; Anucherngchai et al. 2016; Haruay & Piratae 2019; Dunghungzin & Chontananarth 2020, 2021; Wiroonpan et al. 2020; Yooyen 2020). Among the cases recorded in the native area, A. helena acts the role of the first and second intermediate host for digenean trematodes (Chantima et al. 2013, 2018; Chomchoei et al. 2018; Haruay & Piratae 2019; Buuttonchoo et al. 2020). However, only single reports indicate that redia-born digenean species use A. helena as the first intermediate host (Wiroonpan et al. 2020). Here, we are not able to carry out species identification for the detected Digenea, we can only claim that it belongs to the order Echinostomida, due to the presence of rediae. Nevertheless, our research proves the possibility that imported ornamental pet snails, such as A. helena, may be a source of digenean trematodes. There is some risk to the health of the aquarium enthusiasts or the animals co-inhabiting aquariums with the introduced infected snail. Additionally, the irresponsible release of infected ornamental pets into the non-native environment may have far-reaching consequences. The basis for the pessimistic scenario is primarily the adaptability of aquatic snails introduced to non-native areas. Admittedly, A. helena is

Figure 1. Digenean larvae in the hepatopancreas of Anentome helena. A. Redia. B. Redia filled with cercariae and germ balls. C. Cercaria in a body of redia. D. Free cercaria between hepatopancreatic acini. Abbreviations: bw, body wall of redia; c, cercaria; ev, excretory vesicle; gb, germ balls; hp, hepatopancreas; in, intestine; os, oral sucker; ph, pharynx; tc, tail of cercaria; vs, ventral sucker.
a thermophilic species with an optimal temperature range of 22 to 28°C (CABI 2021), and at higher latitudes it can survive in heat islands such as greenhouses and botanical garden ponds. From these anthropogenic habitats, alien snail species began the expansion to wild areas (Alexandrowicz 1993; Strzelec et al. 2006). Also, thermally polluted water bodies connected to lakes or rivers with a natural thermal regime promote the spread of non-native species (Piechocki et al. 2003; Domagala et al. 2004, 2007; Labecka et al. 2005; Maciaszek et al. 2020, 2021b). Furthermore, the global warming scenario should also be considered (Rahel & Olden 2008; Fenoglio et al. 2010).

The pet trade is a key driver of non-native aquatic species introductions (Patoka et al. 2020). There are numerous reports of the presence of ornamental aquatic animals in natural environments outside their native range (Weiperth et al. 2019; Mabrouki et al. 2020; Tarkan et al. 2021; Maciaszek et al. 2021a), most likely due to an intentionally release and/or as an escape from captivity (Hulme et al. 2008). Additionally, numerous non-native species, including molluscs, are not deliberately transported but arrive as commodity contamination (Patoka et al. 2016b, 2017; Patoka & Patoková 2021). It should also be emphasized that illegal or unsustainable wildlife trade is developing at a global level (Cardoso et al. 2021). Many of the most dangerous invaders come from the pet trade, and their invasive status is due to the disease they spread (Hatcher et al. 2012). New reports of pathogens or parasites in imported ornamental animals are constantly emerging, including those of veterinary and medical importance (Mehrdana et al. 2014; Haenen et al. 2020; Pace et al. 2020). In Europe, reports on “hitchhikers” from the aquatic pet trade have focused mainly on their presence in fish (Mehrdana et al. 2014; Śmiga et al. 2016; Haenen et al. 2020; Pace et al. 2020) and decapod crustaceans (Mrugala et al. 2015; Patoka et al. 2016a; Maciaszek et al. 2020, 2021b; Lożek et al. 2021). The epidemiological threat of introducing an ornamental mollusc into a non-native environment is likely to be downplayed because the main “hitchhikers” are likely to be digeneans and most often require the presence of other non-native species to complete their complex life cycle and become established. Nevertheless, Mehrdana et al. (2014) emphasize that the possibility of the establishment of digeneans transported by ornamental pets to Europe should be considered a real risk. These scientists base their opinion on the detection of metacercariae of Centrocestus sp. in ornamental fish while emphasizing that the first intermediate host of this zoonotic trematode is M. tuberculata, a snail species of great interest among aquarium enthusiasts, simultaneously, its presence in European water bodies has been recorded.

Our report highlights that greater caution is needed in the procedure of international trade in ornamental animals, including aquarium molluscs. The possible spread of “hitchhikers” to non-native areas may have a cascading effect on the environment and be of public health importance. It is important to constantly draw public attention to the consequences of releasing aquarium animals into the non-native environment.

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