

## Research Article

# First report of the non-native freshwater nereidid polychaete *Namalycastis hawaiiensis* (Johnson, 1903) from a private goldfish aquarium in eastern Japan

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## Abstract

The aquarium trade poses a great risk of aquatic invasion worldwide, but little attention has been paid to unintentionally introduced species. The present study reports the occurrence of the freshwater polychaete *Namalycastis hawaiiensis* for the first time from a private home aquarium in Japan, which was a considerable distance away from the native distribution of the species (tropical/subtropical regions). We concluded that the species was unintentionally introduced via the aquarium trade. As the specimens were collected from an aquarium of the common goldfish, which is one of the most popular ornamental fish in the world, it is possible that a cryptic invasion has already expanded worldwide.

**Key words:** home aquarium, aquarium trade, cryptic invasion, Nereididae, Namanereidinae, aquatic conservation

## Introduction

The aquarium trade has a long history of transport and introduction of many aquatic species into non-native regions worldwide (Duggan 2010). Some attention has been paid to the role of the aquarium trade in transporting and introducing intentionally carried species. However, unintentionally carried species, such as small invertebrates from the water column of aquaria, in the substrate, and associated with aquatic plants, usually go unnoticed, although a number of such taxa have been noted in private home aquaria (Duggan 2010; Kajihara et al. 2016; Patoka et al. 2016a, b). Despite biological invasions being increasingly recognized as a primary threat to global biodiversity and ecosystems (Bax et al. 2001; Padilla and Williams 2004; Ricciardi 2015), the risk of invasion by non-native invertebrates in aquaria has rarely been analyzed.

The subfamily Namanereidinae (Annelida: Nereididae), which consists of three genera, *Lycastoides*, *Namanereis*, and *Namalycastis*, is one of the most successful polychaete groups to colonize brackish and freshwater environments worldwide (Glasby and Timm 2008; Alves and Santos 2016). Namanereid polychaetes have been recorded from the upper reaches of streams and rivers, subterranean caves, and even in arboreal waters, despite such habitats being uncommon for polychaetes, of which the vast majority being marine or estuarine species (Glasby 1999; Glasby et al. 2014). Some species of Namanereidinae are listed as non-indigenous in some regions; however, the status of most of these species is cryptogenic or questionable (Carlton and Eldredge 2009; Çınar 2013).

Some unfamiliar polychaete worms were detected during a regular cleaning of a home aquarium for goldfish in July 2016. The worms were an apparently unintentional component of the aquarium; subsequent



**Figure 1.** Map of the specimen collection site (private home aquarium tank) in the present study (white circle), and known geographical distribution sites of *Namalycastis hawaiiensis* (black squares) based on Glasby (1999).

morphological inspection identified all specimens as the namanereid polychaete *Namalycastis hawaiiensis* (Johnson, 1903) following the key of Glasby (1999). This species is widely distributed in the tropical Indo-Pacific, ranging latitudinally from Kikai Island, Ryukyu Islands to Milne Bay, Papua New Guinea and longitudinally from the Hawaiian Islands to Sumatra (Glasby et al. 2003; Sato and Sakaguchi 2016; Figure 1). The species has been found in several freshwaters and estuaries in southern Japan (Sato and Sakaguchi 2016; Sato 2017). The present study is the first report of the occurrence of *N. hawaiiensis* from a private home aquarium in Japan. To facilitate future identification and detection, we provide partial sequences of the cytochrome *c* oxidase subunit I (COI), 16S ribosomal RNA (16S), 18S ribosomal RNA (18S), and 28S ribosomal RNA (28S) genes, as well as a brief morphological description of the specimens.

## Material and methods

Specimens of *N. hawaiiensis* were collected from the bottom gravel of a private home aquarium owned by one of the authors (YU) in Narashino City, Chiba Prefecture, Japan, which is approximately 1300 km northeast from the previous northernmost record of this species (Kikai Island, the Ryukyu Islands; Figure 1). Two, fifteen, and three specimens were collected on 28 July, 25 August, and 12 October 2016, respectively. On August 25, polychaetes were searched for and collected throughout half of the aquarium; the remainder of the aquarium was not searched, so as to reduce the probability of extirpating the population from the aquarium.

The aquarium was set up in July 2014 and had been maintained for two years before *N. hawaiiensis* was found. It consisted of a glass tank 60 cm wide, 36 cm high, and 30 cm deep, a cycle filtration system, bottom sediment, and purified tap water (Figure 2). The bottom sediment consisted of purchased fine river gravel and a small amount of detritus. The common goldfish, *Carassius auratus* (Linnaeus, 1758) and a waterweed, *Ceratophyllum demersum* Linnaeus were the only biota intentionally kept in the aquarium (Figure 2). The common goldfish were purchased from an aquarium store in Tsuchiura City, Ibaraki Prefecture, Japan, where located approximately 48 km north from the home aquarium, in July and August 2014 and in July and September 2015. The waterweed was purchased from the same aquarium store, approximately every two months from August 2014 to January 2016. Dried feed and frozen bloodworm (chironomid larvae) were provided as goldfish food. The temperature of the aquarium water was measured continuously using a digital water temperature meter (Multi Thermometer; Japan Pet Design, Tokyo, Japan) and was maintained at approximately 28°C with a thermostatically controlled heater. The salinity was 0.7–1.2 PSU when it was measured with an electrical conductivity meter (CM-31P; DKK-TOA, Tokyo, Japan) at the same time as the specimens were collected. Diameters (mean ± SD) of major and minor axes of 50 particles sampled randomly from the bottom gravel were  $6.0 \pm 1.3$  mm (range 3.6–9.5 mm) and  $4.3 \pm 0.8$  mm (2.7–5.9 mm), respectively.

The morphological characteristics of the polychaete individuals were observed under a stereomicroscope (SZX16; Olympus, Tokyo, Japan) and an optical

**Figure 2.** Photograph of the private home aquarium (60 cm W × 36 cm H × 30 D cm) for goldfish keeping, where the *Namalycastis hawaiiensis* specimens were found and collected. Common goldfish, *Carassius auratus*, and waterweed, *Ceratophyllum demersum*, were the intentionally introduced members of the aquarium. Photo by HA.



microscope (DM4000 B; Leica Microsystems, Tokyo, Japan), both for live (anesthetized in 7% magnesium chloride solution) and fixed (in absolute ethanol or 10% neutral formalin and stored in 70% ethanol) specimens. Light micrographs were taken using a digital camera (α6000; Sony, Tokyo, Japan) attached to the microscope using a c-mount camera adapter (SA20; Wraymer, Osaka, Japan). Voucher specimens were deposited in the National Museum of Nature and Science, Tsukuba (NSMT), Japan, under the museum registration numbers NSMT-Pol 113208–113218.

Molecular genetic analyses for the nuclear 18S, 28S, and mitochondrial 16S and COI genes were performed on five *N. hawaiiensis* specimens collected on July 28 and October 12, 2016. Genomic DNA was extracted from live or absolute ethanol-preserved tissues by grinding in 50 µl of 10% Chelex®100 (Bio-Rad Laboratories Inc., Richmond, CA, USA), followed by heating at 95 °C for 20 min (Richlen and Barber 2005). Polymerase chain reaction (PCR) amplification and sequencing of nuclear 18S, 28S, and mitochondrial 16S rRNA genes were performed following the methods described in Sato-Okoshi and Abe (2012, 2013), Teramoto et al. (2013), and Sato-Okoshi et al. (2016). Partial sequences of the COI gene were amplified using the LCOmod/HCOmod (Kano 2008) or LCO1490 (Folmer et al. 1994) / HCOmod primer sets. The PCR cycling conditions for the COI gene were 94 °C for 120 s, followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 40 s at 45 or 48 °C, and extension for 300 s at 72 °C. The partial sequences of each gene were compared

with those of *Namalycastis abiuma* (Grube, 1872), *N. elobeyensis* Glasby, 1999, *N. glasbyi* Fernando and Rajasekaran, 2007, *N. indica* (Southern, 1921), *N. jaya* Magesh, Kvist, and Glasby, 2012, and unidentified *Namalycastis* spp., which were available in the GenBank database. The gene sequence data obtained in the present study has been deposited in the GenBank with accession numbers LC213726–213729.

## Results

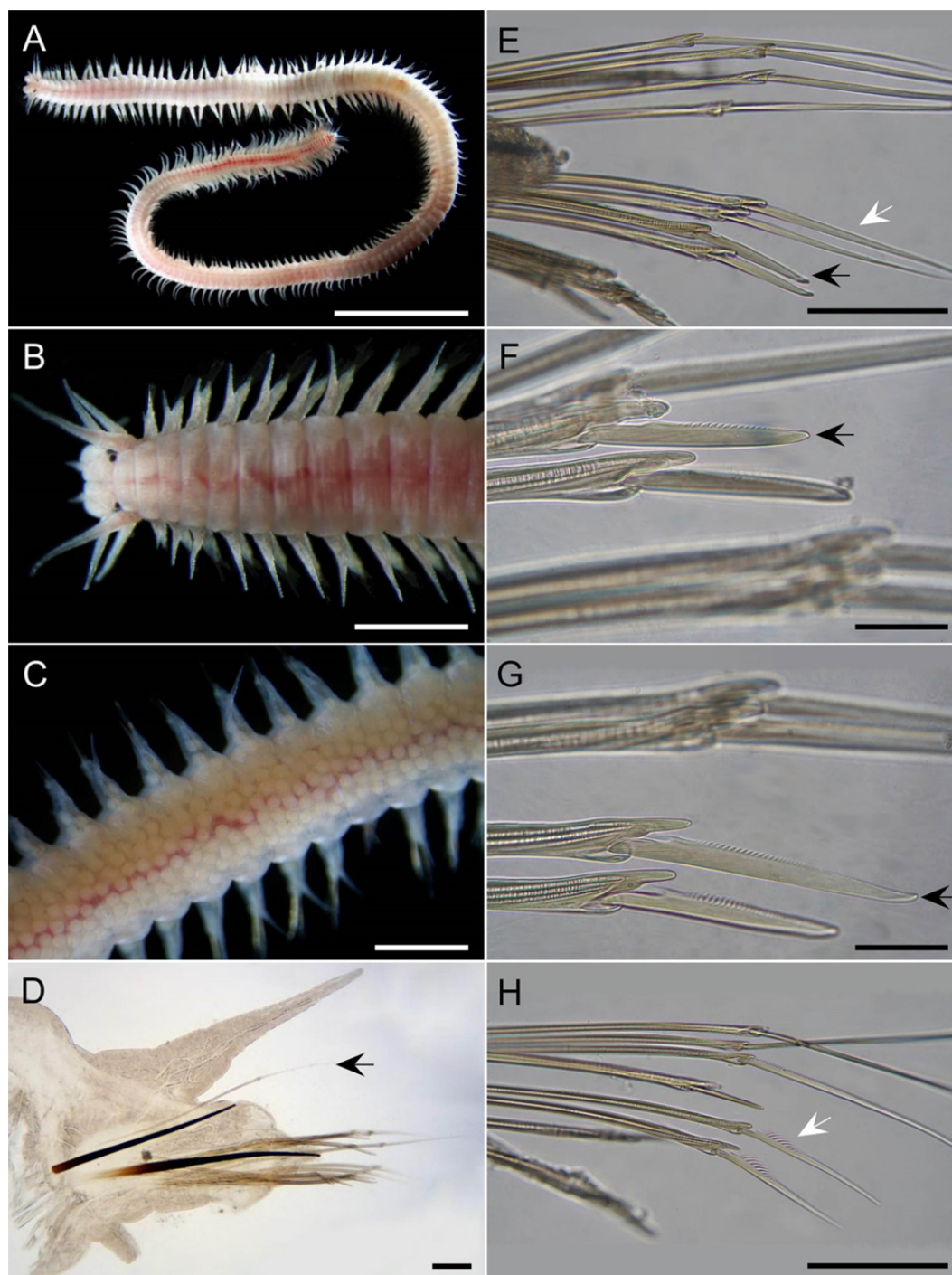
### *Species identification*

Family Nereididae Blainville, 1818  
Subfamily Namanereidinae Hartman, 1959  
Genus *Namalycastis* Hartman, 1959  
*Namalycastis hawaiiensis* (Johnson, 1903)  
(Figure 3)

For synonymy see Glasby (1999)

**Material examined.** Private home aquarium, Narashino, Chiba, Japan, Jul. 28, 2016, NSMT-Pol 113208–113209, two inds, collector Y. Ueno; Aug. 25, 2016, NSMT-Pol 113210–113215, 11 inds, collector Y. Ueno, M. Tanaka, and H. Abe; Oct. 12, 2016, NSMT-Pol 113216–113218, three inds, collector Y. Ueno.

**Brief description.** Specimens 31.7–69.4 mm total body length, 159–187 chaetigers (only complete specimens, n = 13), 0.8–1.38 mm wide at chaetiger 10 excluding parapodia (all specimens). Body uniform in width anteriorly, tapering gradually posteriorly. Color uniformly pale pink in living specimens (Figure 3A), yellowish white in preserved ones.



**Figure 3.** *Namalycastis hawaiiensis* (Johnson, 1903) obtained from the private home aquarium tank shown in Figure 2. A: Dorsal view of the entire body of a living specimen. B: Anterior, dorsal view of living specimen (NSMT-Pol 113213). C: Dorsal view of the middle part of the body, mature individual with numerous, spherical oocytes in each chaetiger (NSMT-Pol 113214). D: Parapodium from 10th chaetiger with notopodial supra-acicular sesquigomph spiniger (arrowed) (NSMT-Pol 113214). E: Supra- and sub-neuroacicular fascicles of parapodium in 10th chaetiger, with sub-neuroacicular spinigers (white arrowed) and falcigers (black arrowed) with finely serrated blades (NSMT-Pol 113214). F: Supra-neuroacicular falcigers in 10th chaetiger (arrowed) with finely serrated blades (NSMT-Pol 113215). G: Sub-neuroacicular falcigers in 10th chaetiger (arrowed) with finely serrated blades (NSMT-Pol 113215). H: Supra- and sub-neuroacicular fascicles of parapodium in chaetiger 120, with sub-neuroacicular spinigers with coarsely serrated blades (white arrowed) (NSMT-Pol 113215). Scale bars: A = 5 mm, B, C = 1 mm, D, E, H = 100  $\mu$ m, F, G, = 25  $\mu$ m. Photomicrographs by HA and MT.

Epidermal pigment absent. Prostomium anteriorly shallowly cleft, with two pairs of black eyes arranged obliquely (Figure 3B). Paired antennae short, subconical, extending to tip of palpophore. Four pairs of tentacular cirri with tentaculophores, unequal in length; postero-dorsal ones longest (Figure 3B), extending posteriorly to chaetigers 4–5. Paired blown jaws with single terminal tooth, and seven subterminal teeth ( $n = 2$ ). Dorsal cirri with cirrophore, increasing in length posteriorly (Figure 3A). Notopodial lobes absent. Usually one (rarely two) notopodial supra-acicular sesquigomph spiniger present (Figure 3D). Neuropodia with single acicular ligule. Neurochaetal arrangement type A (Glasby 1999): sesquigomph spinigers, heterogomph falcigers in neuropodial supra-acicular fascicles; heterogomph spinigers, heterogomph falcigers in sub-acicular fascicles. Neuropodial supra-acicular sesquigomph spinigers numbered less than 5. Supra-neuroacicular falcigers in chaetiger 10 with blades 4.6–5.5 times longer than width of shaft head ( $n = 2$ ), finely serrated, 13 teeth (Figure 3F). Sub-neuroacicular spinigers in anterior chaetigers with finely serrated blades (Figure 3E), posteriorly blades having coarse serrations proximally (Figure 3H). Sub-neuroacicular falcigers in chaetiger 10 dorsally with blades 6.9–7.9 times longer than width of shaft head ( $n = 2$ ), finely serrated, more than 35 but less than 70 teeth (Figure 3G); in middle to posterior chaetigers blades increasingly coarsely serrated proximally. Neuropodial sub-acicular fascicles with many falcigers more than spinigers in number. Pygidium button-shaped, multi-incised, with paired conical anal cirri. One mature female specimen (NSMT-Pol 113214) with numerous, spherical oocytes (ca. 140–160  $\mu\text{m}$ ) throughout body (Figure 3C).

**Remarks.** The individuals examined were consistent with the neotype description of *N. hawaiiensis* (Glasby 1999). However, one morphological characteristic of our material is worth noting: Glasby (1999) described that notochaetae of this species are usually absent, while all our specimens had notochaetae on most chaetigers as far back as the middle of the body. Horst (1909) reported that one of his specimens (as *Lycastis hawaiiensis*) from a freshwater pond at the Botanical Garden in Buitenzorg (= Bogor), Indonesia, had a weakly developed notochaeta in the 41st parapodium. Furthermore, Augener (1933) also reported the presence of notochaetae from anterior to the 57th–59th parapodia of his specimen (as *Lycastis ranauensis*[sic]) collected from the same location. Thus, their materials, as well as those of the present study, agreed well with Feuerborn's (1931a, b) original description of *Lycastis ranauensis*, which is

now regarded as a junior synonym of *N. hawaiiensis* (Glasby 1999), particularly in terms of the presence of notochaetae. We could not evaluate at this time whether this morphological variation within the species represents an ontogenic, interpopulational variation, or is actually a different species. However, the possibility of cryptic speciation in *N. hawaiiensis* has been pointed out by Sato (2017); if this is true, *N. hawaiiensis* may include several cryptic species, indicating that *L. ranauensis* is valid. Future taxonomic studies are required to solve this problem.

### Molecular analyses

The nuclear 18S (1781 bp), 28S (816 bp), and mitochondrial 16S (521 bp) and COI (661 bp) gene sequences were successfully obtained. No variation was detected in any of the four gene sequences from the five *N. hawaiiensis* specimens analyzed in the present study. The sequences were distinct from those of *N. abiuma*, *N. elobeyensis*, *N. glasbyi*, *N. indica*, *N. jaya*, and unidentified *Namalycastis* spp., which are available in the GenBank database. BLAST searches (Altschul et al. 1990) of the partial 18S (LC213729), 16S (LC213728), and COI (LC213726) sequences from our specimens at the NCBI website (<http://blast.ncbi.nlm.nih.gov>) yielded the highest maximum scores with *N. abiuma* group sp. (HQ157237; 97% query cover and 97% identity) in 18S, *N. abiuma* (KU351089; 100% query cover and 83% identity) in 16S, and *N. glasbyi* (JX843802; 100% query cover and 82% identity) in COI; there is no 28S sequence data for *Namalycastis* in the GenBank database, and the 28S partial sequence (LC213727) hit the phyllodocid polychaete *Notophyllum foliosum* (AY996100; 70% query cover and 85% identity).

### Discussion

The present study is the first report of the occurrence of *N. hawaiiensis* from a private home aquarium in eastern Japan, which is a considerable distance from the native distribution of the species (tropical/subtropical regions) (Figure 1). As our material was collected from the bottom gravel in an aquarium of the common goldfish, which is one of the most popular ornamental fish worldwide (Viji et al. 2011), it is possible that the silent invasion of this species via the freshwater aquarium trade has already expanded worldwide. The possibility of human-assisted translocation of populations of this species via the aquarium trade has been pointed out previously (Glasby et al. 2003, 2009), and the species has been intercepted as an aquarium contaminant in Australia

(Glasby and Dane 2008). Further, some information about the occurrence of unidentifiable nereidid polychaetes from freshwater aquaria in Japan has appeared on internet websites ([http://blog.goo.ne.jp/gekkabijin\\_sp/e/d4245edb878f9965d2eaa86a0965c05c](http://blog.goo.ne.jp/gekkabijin_sp/e/d4245edb878f9965d2eaa86a0965c05c); accessed on 21 December 2016).

*Namalycastis hawaiiensis* normally inhabits fresh or brackish waters in various coastal riparian areas, such as streams, swamps, and even plant container habitats called phytotelmata (Glasby 1999; Glasby et al. 2003). A report that this species was collected in phytotelmata about 1 km from shore (Glasby et al. 2003) suggests that it can maintain a population in a landlocked environment without the need for salt-water. Although the complete life history of this species is unknown, it has been reported that a southern Sumatran population of this species (as *Lycastis ranauensis*) is hermaphroditic, i.e., both eggs and immature sperm occur in the same individual (Feuerborn 1931b; Glasby et al. 2003). This biological feature may allow a population to survive and reproduce even in a freshwater home aquarium; Feuerborn (1931b) succeeded in transporting live worms kept in a wide bottle from southern Sumatra to Europe and was able to increase the number of them in an “Aquarien mit Leitungswasser” (= aquarium filled with tap water). In the present study, the eggs were 140–160 µm in diameter, which was comparable to mature oocytes of *N. hawaiiensis* (140–155 µm; Glasby 1999). However, the eggs were detected in the coelom, where no spermatozoa were found (Figure 3C). Sato (2017) reported that laboratory-reared worms collected from the Ryukyu Islands were likely dioecious, not hermaphroditic. In this regard, it is also an important issue to verify whether this species has a planktonic larval stage. If the worm has a planktonic larval stage, we cannot rule out the possibility of individuals being released to outdoor habitats via waste water during regular cleaning. Thus, further studies are needed to fully understand the reproductive features and larval development of the species.

The introduction pathway of the present material into the private home aquarium is unclear, but we infer that *Namalycastis* were unintentionally introduced into the aquarium via waterweed purchased at an aquarium shop in Tsuchiura City, Japan, because there were no other possible vectors for the introduction (Figure 2): substrate was completely desiccated before it was deposited on the bottom of the aquarium. The report that this species prefers plant-associated habitats (Glasby et al. 2003) supports the assumption of the introduction via waterweed. It is noteworthy that totally 20 individuals of *N. hawaiiensis* were found in a single small aquarium

in the present study. We briefly searched for *Namalycastis* in about half the area of the bottom gravel in the aquarium on 25 August 2016 and collected 15 individuals, so it is estimated that there should be approximately twice that number in the tank. Such a high number of individuals is unlikely to have been introduced to the aquarium at any one time, at least as adults. The fact that one individual had eggs in its coelom (Figure 3C) suggests that they are capable of maturing in the aquarium. However, it is unknown whether the worms reproduced successfully, or are derived from a single or a few individuals introduced to the aquarium. Another possibility is that many small juveniles were introduced via waterweeds or sediment at the same time. The gene sequence data of *N. hawaiiensis* provided in the present study is made available so as to potentially determine their source of origin and the route of introduction in the future. We hope that the present study stimulates future detection of this species from similar aquarium environments around the world, and that they are treated carefully as a non-indigenous species.

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