New Records of *Neobenedenia girellae* (Hargis, 1955) (Monogenea: Capsalidae) in Marine Ornamental Fish Imported to Yucatan, Mexico

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**ABSTRACT:** We detected *Neobenedenia girellae* infections in 40 species belonging to 12 families of imported marine ornamental fish from a public aquarium in the Mexican state of Yucatan. A total of 348 fish specimens were examined for monogeneans in January 2018 to December 2020. Monogeneans were corroborated morphologically and molecularly with a partial sequence of 28S (region D1–D3) ribosomal DNA and analyzed in a molecular phylogenetic context in combination with other *N. girellae* sequences available in GenBank. The phylogenetic tree revealed that the specimen found consistently belonged to the *N. girellae* clade. High infection parameters were detected of *N. girellae* in most hosts. This identification is relevant to aquarists and aquaculturists in the Gulf of Mexico because *N. girellae* is considered highly pathogenic in confined fish. This work demonstrates that the importation of ornamental fish coupled with deficient sanitary measures (lack of quarantine areas in distribution centers) contributes to spread of parasites and their establishment within Mexico.

**KEY WORDS:** *Neobenedenia girellae*, monogenean, Capsalidae, marine ornamental fish.

Capsalid monogeneans comprise approximately 9 subfamilies, 57 genera, and more than 300 species, infecting predominantly the skin and gills of marine fish (Whittington, 2004; Ogawa et al., 1995; Gibson et al., 2010). Some genera (e.g., **Benedenia** Diesing, 1858, **Capsala** Bosc, 1811; **Entobdella** Blainville in Lamarck, 1818; **Neobenedenia** Yamaguti 1963) are considered highly pathogenic and responsible for severe outbreaks of epizootics, causing severe economic losses in farmed and aquarium fishes (Gaida and Frost, 1991; Whittington 2004). Shinn et al. (2015) reported economic losses from *Neobenedenia melleni* (MacCallum, 1927) Yamaguti, 1963, infections in cobia in Taiwan, with mortality of 40% and a loss of US$1.80 million. In Japan and Australia, *Benedenia seriolae* (Yamaguti, 1934) Meserve, 1938, affected Japanese amberjack (*Seriola quinquerradiata* Temminck and Schlegel, 1845) and greater amberjack (*Seriola dumerili* (Risso, 1810)), with losses of up to US$214 and 0.53 million, respectively. In particular, *Neobenedenia* has been reported to infect a large number of fishes, including more than 100 fish species representing about 30 families (Whittington and Horton, 1996; Whittington, 2004; Whittington and Chong, 2007; Magalhães-Cardoso et al., 2019; Sepúlveda and González, 2019). Outbreaks of *Neobenedenia* spp. have been widely detected in commercial species from Asia, (*Epinephelus coioides* (Hamilton, 1822), *Lutjanus argentimaculatus* (Forsskål, 1775), *Lutjanus johnii* (Bloch, 1792), *Pinjalo pinjalo* (Bleeker, 1850), *S. dumerili*, *S. quinquerradiata*, *Seriola rivoliana* Valenciennes in Cuvier and Valenciennes, 1833) (Seng, 1997; Hirayama et al., 2009; Ohno et al., 2009; Hirazaga, Ishizuka, et al., 2016, Hirazaga, Tsubone, et al., 2016; Sicuro and Luzzana, 2016); the northwestern Pacific (*Paralichthys olivaceus* (Temminck and Schlegel, 1846) and *Verasper variegatus* (Temminck and Schlegel, 1846)) (Seng, 1997; Hutson, 2007); Indonesia, Australia (*Barramundi Lates calcarifer* (Bloch, 1790)) (Seng, 1997), and Mexico (*Seriola lalandii* Valenciennes in Cuvier and Valenciennes, 1833) (Avilés-Quevedo and Castelló-Orvay, 2004), as well as in several ornamental marine fish from Brazil (*Chaetodon semilarvatus* Cuvier in Cuvier and Valenciennes, 1831, *Pomacanthus asfur* (Forsskål, 1775), *Pomacanthus maculosus* (Forsskål, 1775), *Pygopliches diacanthus* (Boddart, 1772)) (Magalhães-Cardoso et al., 2019); Mexico (*Canthigaster bennetti* (Bleeker, 1854); Australasia (*Nemateleotris decora* Randall and Allen, 1973, *Neocirrhites armatus* Castelnau, 1873, *Pseudechilinus hexataenia* (Bleeker, 1857), *Pseudochromis fridmani* Klausewitz, 1968); Africa (*Gnathanodon speciosus* (Forsskål, 1775)) (Brazenor, Bertozzi, et al., 2018). Specifically, *Neobenedenia girellae* (Hargis, 1955)
Yamaguti, 1963, is a lethal and persistent species, with a wide geographic distribution and a high infective capacity in several hosts (Hiramaya et al., 2009; Brazenor, Bertozzi, et al., 2018, Brazenor, Saunders, et al., 2018; Tedesco et al., 2021). It has been recorded in approximately 30 host species, including several tropical reef fish families Carangidae, Cirrhitidae, Coryphaenidae, Labridae, Latidae, Microdesmiidae, Pleuronectidae, Pseudochromidae, Rachycentridae, Serranidae (Brazenor, Bertozzi, et al., 2018, Brazenor, Saunders, et al., 2018), among others. Because of its short life cycle and low host specificity, it can become highly prevalent and cause large numbers of mortalities of aquarium fish (Nam et al., 2020). The elevated abundance of *N. girellae* causes considerable irritation to fish (e.g., weakness, anorexia, dyspnea, mild hemorrhages on the skin and eyes, mucous hypersecretion, blindness, and death from secondary infections [mainly bacterial infections] if not treated) (Hirazaga, Ishizuka, et al., 2016, Hirazaga, Tsubone, et al., 2016). This parasite was described in *Girella nigricans* (Ayres, 1860) for the first time in California, U.S.A. (Hargis, 1995). Subsequently, it was described in several localities in Mexico (among other regions of the world) (Bravo-Hollis and Deloya, 1973; Moser and Haldorson, 1982; Gaida and Frost, 1991; Ogawa et al., 2006), including in *Mycteroperca pardalis* (Streets, 1877) and *Scarus perrico* Jordan and Gilbert, 1882, in La Paz, Baja California, Mexico, and Nayarit, Mexico, respectively (Bravo-Hollis, 1958). The export and import of fish for aquaculture is likely one of the most important sources of its dispersion (Salgado and Rubio, 2014). However, the role of the ornamental fish trade in the translocation and establishment of wild and farmed fish has received limited attention in Mexico. The present research thus focused on the detection and identification of *N. girellae* in traded marine ornamental fish into the state of Yucatan, Mexico.

**MATERIALS AND METHODS**

The marine ornamental fish examined in this study were donated from a commercial aquarium in Merida, Yucatan, Mexico, between January 2018 and December 2020. A total sample of 348 ornamental fish were collected (Table 1). Most of the fish were originally captured from the natural environment of the Indo-Pacific region, although the exact capture locations were not available to the importer. Upon arrival in Mexico, the imported fish is inspected by the Agricultural Health Inspection Office (OISA), which issues a health certificate of fish. Subsequently, the ornamental fish are distributed to several regions of Mexico (specifically, until their point of sale, e.g., Merida, Yucatan) or transferred to the market Morelos in Mexico City, Mexico, which represents one of the main commercialization and distribution centers for ornamental fish from Mexico.

The imported fish were transported in isolated plastic bags with artificial aeration. Once at their point of sale (i.e., the aquarium in Merida), the dead or dying fish were separated and kept in coolers, posteriorly donated, and transported to the Aquatic Pathology Laboratory at CINVESTAV-IPN Unidad Mérida for parasitological examination. Once at the laboratory, fish were measured to obtain total length, standard length, and total weight. The surface of the skin and eyes, gills, scales from the lateral line, and fins were examined under a stereomicroscope (Stemi 305, Carl Zeiss) for ectoparasites. Whenever parasites were found, they were counted, preliminarily identified to the genus level, and fixed depending on the taxonomic group (Whittington, 2004). Capsalid monogeneans were isolated, counted in situ, cleaned with physiological saline, and preserved in 4% formalin or 96% alcohol labeled vials for subsequent morphological or molecular studies, respectively (Brazenor, Bertozzi, et al., 2018, Brazenor, Saunders, et al., 2018). Monogeneans were removed with fine paintbrushes, stained with ammonium picrate, and identified to the species level according to suitable literature (e.g., Whittington and Kearn, 1993; Hargis, 1995; Ogawa et al., 2006). Infection parameters such as prevalence, mean abundance, and mean intensity were those proposed by Bush et al. (1997). Standard measurements were made with an Olympus BX50 compound microscope (Olympus, Tokyo, Japan) and ImageJ software (Wayne Rasband Scientific Software, Kensington, Maryland, U.S.A.). Drawings were prepared by Adobe Illustrator software (Adobe Inc., San Jose, California, U.S.A.). A full-body view of *N. girellae*, as well as a ventral view of the accessory sclerite, anterior hamulus, posterior hamuli, and marginal hooks, were illustrated (Figs. 1, 2). The following features were measured for morphological and morphometric description: body, length and width; pair of anterior attachment organs, length by width; haptor, length; anterior hamuli, length, posterior hamuli, length, accessory sclerites, length; pair of testes, length by width; ovary, length by width; egg, length by width (Whittington and Kearn, 1993; Whittington, 2004) (Table 2). All measurements are...
Table 1. Host species diversity and Neobenedenia girellae parasite numbers per infected host, imported to Yucatan, Mexico.

| Family              | Host                          | Infected host | Total host | Total parasites | Prevalence (%) | Mean abundance (±SD) | Mean intensity (±SD) | Original distribution       |
|---------------------|-------------------------------|---------------|------------|----------------|-----------------|----------------------|------------------------|----------------------------|
| Acanthuridae        | Acanthurus japonicus          | 3             | 5          | 108            | 60              | 21.6 ± 17.38        | 36 ± 28.39             | Indo–West Pacific         |
|                     | Acanthurus pyroferus          | 3             | 7          | 42             | 42              | 0.42 ± 4.21         | 1 ± 5.89               | Indo–Pacific             |
|                     | Acanthurus triostegus         | 1             | 2          | 43             | 50              | 21.5 ± 17.28        | 43 ± 35.95             | Indo–Pacific             |
|                     | Acanthurus lineatus           | 2             | 15         | 12             | 13              | 0.8 ± 3.41          | 6 ± 0.33               | Indo–Pacific             |
| Paracanthurus hepatus| 2                             | 5             | 1          | 40             | 40              | 0.2 ± 4.01          | 0.5 ± 5.67             | Indo–Pacific             |
| Zebrasoma flavescens | 5                             | 7             | 14         | 71             | 71              | 2 ± 2.21            | 3 ± 3.09               | Northwest and central Pacific Ocean |
|                     | Zebrasoma velifer             | 3             | 14         | 35             | 22              | 3 ± 2.11            | 12 ± 5.70              | Eastern Indian Ocean to the Pacific Ocean |
| Apogonidae          | Pterapogon kauderni           | 4             | 5          | 2              | 80              | 0.4 ± 3.81          | 0.5 ± 1.25             | Western Pacific          |
| Balistidae          | Ballesta niger                | 3             | 5          | 1              | 60              | 0.2 ± 0.41          | 0.33 ± 0.27            | Indo–Pacific             |
|                     | Rhinecanthus aculeatus        | 10            | 17         | 28             | 59              | 3 ± 1.21            | 3 ± 0.78               | Indo–West Pacific        |
|                     | Rhinecanthus verrucosus       | 9             | 23         | 18             | 39              | 0.78 ± 3.43         | 2 ± 1.82               | Indo–West Pacific        |
| Bleniidae           | Ecsenias bicolor              | 3             | 12         | 6              | 8               | 0.5 ± 3.71          | 6 ± 0.33               | Indo–Pacific             |
|                     | Salarias ramosus              | 1             | 3          | 12             | 33              | 4 ± 6.78            | 12 ± 5.61              | Western central Pacific  |
| Callymonidae        | Synchronus splendidus         | 3             | 12         | 16             | 25              | 1.33 ± 2.88         | 5.33 ± 7.89            | Western Pacific          |
| Chaetodontidae      | Chelmon rostratus             | 7             | 7          | 13             | 100             | 2.2 ± 2.01          | 2 ± 1.82               | Western Pacific          |
|                     | Forcipiger longirostris       | 10            | 11         | 13             | 90              | 1.18 ± 3.03         | 1.3 ± 2.73             | Indo-Pacific             |
|                     | Heniochus acuminatus         | 4             | 6          | 28             | 67              | 5 ± 0.78            | 7 ± 3.07               | Indo-Pacific             |
| Grammatidae         | Gramma loreto                 | 5             | 6          | 12             | 83              | 2 ± 2.21            | 2.4 ± 1.86             | Western central Atlantic |
| Labridae            | Centrolabrus exoletus         | 1             | 3          | 9              | 33              | 3 ± 1.23            | 9 ± 11.23              | Eastern Atlantic         |
|                     | Halichoeres chrysus           | 2             | 4          | 17             | 50              | 4.25 ± 7.90         | 8.5 ± 7.34             | Eastern Indian Ocean     |
| Microdesmidae       | Nemateleotris magnifica       | 3             | 6          | 1              | 17              | 0.16 ± 4.05         | 0.33 ± 3.68             | Indian Ocean             |
| Pomacanthidae       | Pomacanthus imperator         | 3             | 6          | 2              | 50              | 0.33 ± 3.88         | 0.66 ± 5.59             | Indo-Pacific             |
|                     | Pomacanthus paru              | 4             | 5          | 7              | 80              | 1.4 ± 2.81          | 2 ± 4.12               | Western and eastern Atlantic |
| Pomacentridae       | Pomacentrus zonipectus        | 1             | 2          | 32             | 50              | 16 ± 11.78          | 32 ± 25.56             | Eastern Pacific          |
|                     | Abudefduf vaigiensis          | 14            | 18         | 25             | 78              | 1.3 ± 2.91          | 2 ± 3.16               | Indo-Pacific             |
|                     | Ampiprion ocellaris           | 8             | 9          | 16             | 89              | 1.77 ± 2.44         | 2 ± 2.39               | Indo–West Pacific        |
|                     | Ampiprion perula              | 22            | 23         | 21             | 96              | 1 ± 3.21            | 1 ± 3.39               | Western Pacific          |
|                     | Ampiprion polymusus           | 2             | 5          | 13             | 40              | 3 ± 1.21            | 7 ± 2.12               | Western Pacific          |
|                     | Chromis xanthurra            | 11            | 15         | 2              | 73              | 0.13 ± 2.05         | 0.18 ± 4.38             | Pacific Ocean            |
|                     | Chrysiptera parasema          | 8             | 15         | 21             | 53              | 1.4 ± 2.81          | 3 ± 1.95               | Western Pacific          |
|                     | Dacillus trimaculatus         | 4             | 4          | 8              | 100             | 2 ± 2.21            | 2 ± 2.68               | Indo-Pacific             |
| Pseudochromidae     | Plectichromis paccagnellae     | 9             | 15         | 23             | 50              | 1.5 ± 2.71          | 3 ± 4.55               | Western Pacific          |

given (mm) with the range followed by the mean in parentheses (Table 2).

DNA amplification, sequencing, and phylogenetic analyses

Genomic DNA was extracted from each specimen of Neobenedenia with a DNeasy TM Blood & Tissue Kit (Qiagen, Hilden, Germany) following the standard manufacturer’s protocol. Specimens of different host species were chosen for extraction. Given that the 28S ribosomal gene has been used in other studies to identify species of Neobenedenia (Brazenor, Bertozzi, et al., 2018, Brazenor, Saunders, et al., 2018), we also amplified the D1, D2, and D3 regions of this gene. The amplification was carried out with the primers 391 (Nadler and Hudspeth, 1998) and 536 (Garcia-Varela and Nadler, 2005), and the conditions of the polymerase chain reaction amplification were: 94°C
Table 2. Comparative measures for *Neobenedenia girellae* found infecting imported fish

| Measure (mm)       | Acanthurus japonicus, n = 2 | Acanthurus lineatus, n = 1 | Ballesta niger , n = 2 | Rhinecanthus verrucosus, n = 4 | Forcipiger longirostris, n = 5 | Centropyge multispinis, n = 5 | Chrysiptera parasema, n = 5 | Centropyge loricula, n = 2 |
|--------------------|------------------------------|----------------------------|------------------------|---------------------------------|-------------------------------|-----------------------------|-----------------------------|----------------------------|
| Maximum width      | 3.01–3.04                      | 3.04                       | 3.04                    | 3.21                            | 3.80                          | 3.65                        | 3.75                        | 3.65                       |
| Anterior attachment organs | 0.44                          | 0.46                       | 0.45                    | 0.45                            | 0.47                          | 0.44                        | 0.46                        | 0.45                       |
| Marginal hooks     | 0.8                            | 0.9                        | 0.9                     | 0.9                             | 0.9                           | 0.9                         | 0.9                         | 0.9                        |
| Number of marginal hooks | 7                             | 7                          | 7                       | 7                               | 7                             | 7                           | 7                           | 7                          |
| Pharyngeal length  | 0.19–0.20                      | 0.20                       | 0.20                    | 0.20                            | 0.20                          | 0.20                        | 0.20                        | 0.20                       |
| Eggs               | 0.10–0.25                      | 0.14                       | 0.15                    | 0.20                            | 0.18                          | 0.33                        | 0.32                        | 0.32                       |
| Outer length       | 0.26–0.27                      | 0.26                       | 0.26                    | 0.26                            | 0.26                          | 0.25                        | 0.25                        | 0.25                       |

**Notes:**
- n = number of specimens.
- Measurements are given in millimeters (mm).
Figures 1, 2. Neobenedenia girellae (Hargis, 1955) Yamaguti, 1963, from the external surfaces from marine ornamental fish. 1. Full body. 2. Ventral view, (a) accessory sclerite; (b) anterior hamulus; (c) posterior hamuli; (d) marginal hooks.

for 5 min, 35 cycles at 94°C for 1 min, 50°C for 1 min, 72°C for 1 min, and a postamplification extension at 72°C for 10 min. For sequencing, the 2 amplification primers plus 503 (Stock et al., 2001) and 504 (García-Varela and Nadler, 2005) were used. Sequencing was carried out in GENEWIZ (South Plainfield, New Jersey, U.S.A.). The sequences obtained from each primer were read, edited, and assembled into a consensus sequence for each extracted specimen by Geneious Pro 4.8.4® (Biomatters Ltd.). The new sequences were submitted to GenBank for publication and public access. For phylogenetic analyses, the new sequences were aligned with other 28S sequences from Neobenedenia available in GenBank. The alignment was performed by ClustalW (Thompson et al., 1994), implemented in “SLOW/ACCURATE” and “CLUSTALW (for DNA)” (Kyoto University Bioinformatics Center, 2019). The nucleotide evolution model was estimated in jModelTest v.2 (Darriba et al., 2012). A maximum likelihood (ML) analysis was performed to obtain the phylogenetic tree with RAxML v.7.0.4 (Stamatakis, 2006), and 1,000 bootstrap repetitions (bt) were implemented. The ML tree was visualized in FigTree v.1.4.3. (Rambaut, 2000). The genetic distances of the 28S gene were calculated with uncorrected P-value (p-distances) in MEGA v.6.0 (Tamura et al., 2013).

RESULTS

A total of 348 fish specimens of 40 species representing 12 families were examined for monogeneans (Table 1). A total of 213 fish were infected, and 803 N. girellae were collected, infecting the skin, the surface of the eyes, the gills, or a combination of samples from host species of all the families mentioned (Table 1). Macroscopic external lesions were observed in parasitized fish epidermal damage associated with the site of haptor attachment, mild hemorrhages on the skin and eyes, exophthalmia, presence of dyspnea, and anorexia. All monogeneans were identified with morphological characteristics of the genus Neobenedenia described by Whittington and Kearn (1993) and Whittington (2004) with a morphologically flattened, leaflike body shape, absence of haptoral septa, and a vagina, although having accessory sclerites, haptoral hamuli, paired anterior circular discs, and 2 juxtaposed testes, which is a unique combination in species of this genus (Table 2). Monogeneans were collected from species of all the families, therefore representing new host and geographical records. Prevalence ranged from 8 to 100% in the hosts, with mean abundance and intensity ranging from 0.13 ± 2.05 to 32 ± 27.78 and 32 ± 27.78 to 48 ± 42.85, respectively (Table 1). The morphological measurements of the host species are presented in Table 2.

Phylogenetic analyses

Only 6 new sequences were successfully obtained from all the specimens collected from 6 different host species, with a length of 1,123 to 1,203 base pairs (bp). The length of the final alignment was 1,249 bp. The estimated substitution model was GTR + GAMMA and the nucleotide frequencies were 0.259 (A), 0.178 (C), and 0.300 (T). The ML value was –s3209.140011. The phylogenetic tree showed a major clade with a high support value (bt = 86), where N. girellae was
grouped with 2 other species of the genus, which were only recognized as Neobenedenia sp. (Fig. 3). In particular, within the clade of *N. girellae* (bt = 73), most of the specimens identified for this species were grouped, including the specimens of our study. The exception was the specimen MH843692, which despite being named *N. girellae* was grouped in a different independent clade of the tree. The genetic distance was null among the specimens collected in this study. The intraspecific genetic distance of the clade *N. girellae* ranged from 0 to 0.9%. The distance between the specimens of the clade *N. girellae* and the specimen that was grouped in a different independent clade ranged from 6.8 to 7.2%. Finally, the genetic distance between *N. girellae* and the other species of the genus represented in our phylogenetic analysis ranged from 1.1 to 11.2%.

**DISCUSSION**

Presented here are the first confirmed molecular and morphological data of *N. girellae* in Yucatan, Mexico. This monogenean represents both new host and new geographical records and shows the wide range of aquarium fish that this parasite can infect (see Table 1). We consider our findings relevant for aquaculturists and pet shop owners in the Gulf of Mexico because *N. girellae* is an emerging parasitic infection and a potential threat to the trade of ornamental fish. Although this monogenean species is well established in Mexico (Bravo-Hollis and Deloya, 1973), our findings indicate that constant reintroductions of the parasite occur in different regions, possibly following market routes.

We suggest that the parasite has at least 2 possible origins, although neither is conclusive: the movement of imported infected fish and the possible acquisition of infections within reservoir centers (e.g., Morelos markets), where the fish are kept in confinement without adequate sanitary measures before being distributed to various regions of Mexico. The importation of ornamental fish is one contribution to the introduction of parasites and their dispersal and establishment within Mexico.

The international trade of ornamental fishes has been identified as a threat to transboundary biosecurity, biodiversity, and future aquaculture development (Whittington and Chong, 2007; Salgado-Rubio, 2014; Mendoza et al., 2015). The presence of *N. girellae* has been reported in various ornamental marine species (e.g. *Chaetodon, N. decora, N. armatus, P. hexataenia, P. fridmani, P. asfur, P. maculosus, P. diacanthus, Trachinotus blochii* (Lacépède, 1801)) in Brazil, Australia, and Korea. However, few studies have reported infection parameters (Gaida and Frost, 1991; Ogawa et al., 1995; Ogawa et al., 2006; Hirayama et al., 2009; Hirazaga, Ishizuka, et al., 2016; Brazenor, Bertozzi, et al., 2018, Brazenor, Saunders, et al., 2018; Nam et al., 2020).

In this study, high prevalences were found in most hosts. Elevated infection rates are commonly observed in aquarium fish owing to high stocking density and sometimes inadequate water quality maintenance (Magalhães-Cardoso et al., 2019). On the other hand, the stress associated with the capture, handling, and transport of ornamental fish from their origin, coupled with deficient sanitary measures (lack of quarantine areas in distribution centers) and mishandling, facilitates these parasites’ dispersal with high infections parameters. During transport, the fish are handled in excess, being placed in overcrowded plastic bags with low oxygen levels and increasing amounts of excreted nitrogenous waste (ammonium). These deteriorating conditions pave the way for the establishment of this monogenean.

Putri et al. (2020) reported a prevalence of 60% of *N. girellae* in *Rachycentron canadum* (Linnaeus, 1766) from Indonesia, and Gaida and Frost (1991) reported a prevalence of 75% in *Medialuna californiensis* (Steindachner, 1876) from California. The life cycle of *N. girellae* is short, a smaller body size being needed to attain maturity. Bondad-Reantaso et al. (1995) identified the rapid development of *N. girellae* in Japanese flounder, reaching sexual maturity in 10–11 days at 25°C from oncomiracidia. Egg to maturation was 15–17 days. In the present study, this parasite was particularly abundant on the eyes, causing corneal opacity and skin irritation. *Neobenedenia girellae* harm fish by mechanical attachment of the haptor: Ogawa et al. (2006) found particular histological damage in the cornea of infected fish displaying hyperplasia of squamous epithelial cells and mucous cells. The *N. girellae* ectoparasite is well-adapted to tropical regions, so successful establishment in wild native fauna and cultured fish in Yucatan can be foreseen if they reach the open environments. Brazenor, Bertozzi, et al. (2018) and Brazenor, Saunders, et al. (2018) found that this parasite completed its life cycle almost twice as quickly in warm, high-saline conditions compared with cooler temperatures (i.e., oncomiracidia’s longevity is significantly lower in salinities below 22% compared with higher saline conditions (35–40%)). Moreover, at 20–25°C, the parasite attained sexual maturity and produced eggs more slowly than at 30°C. In this sense, the
Figure 3. Phylogenetic relationships of *Neobenedenia* species found in several host species resulting from the maximum likelihood analysis with the 28S rDNA gene. The newly generated sequences are highlighted in red. Numbers near the tree nodes represent the bootstrap support values, and the scale bar indicates the number of substitutions per site.

Yucatan marine environment provides suitable habitats for this parasite’s establishment and reproduction, given its high temperatures, high salinity, and multiple reef spots.

Unfortunately, in Mexico few regulations exist for the importation and introduction of ornamental species to the market, allowing practically any aquatic organism of this sort to be introduced with limited sanitary control (Contreras et al., 1998; Cedillo et al., 2001). A health certificate declaring that imported fish into Mexico are free from World Organisation for Animal Health (OIE) listed diseases is compulsory; otherwise, the entry of such goods is denied. Although *N. girellae* is a dangerous pathogen, it is not currently included on the list of diseases. Furthermore, even though health authorities conduct physical inspections at some border points, marine ornamentals, as a valuable commodity, must be transferred swiftly; therefore, fish carrying parasites or disease are practically unnoticed.

We consider it equally important that sanitary agents be trained to recognize significant pathogens besides the listed diseases that can be problematic for aquaculture and the aquarium industry. Containment measures such as quarantine may be worth reviewing in terms of their effectiveness in preventing parasite detection, with the aim of reducing the spread of disease.

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