Parasitology Research (2019) 118:3267–3277
https://doi.org/10.1007/s00436-019-06460-1

FISH PARASITOLOGY - ORIGINAL PAPER

Effects of temperature on the life cycle of *Neobenedenia* sp. (Monogenea: Capsalidae) from *Seriola rivoliana* (Almaco jack) in Bahía de La Paz, BCS Mexico

Isabel Valles-Vega[1] · Felipe Ascencio[1] · Teresa Sicard-González[1] · Carlos Angulo[1] · Emma J. Fajer-Avila[2] · Roxana Bertha Inohuye-Rivera[1] · Juan Carlos Pérez-Urbiola[1]

Received: 12 December 2018 / Accepted: 11 September 2019 / Published online: 31 October 2019

Abstract

Seriola rivoliana cultivated in Mexico are infected by *Neobenedenia* sp. (Monogenea: Capsalidae), resulting in dermal ulceration and subsequent bacterial invasion that can cause fish death. This study assesses the effects of temperature over hatching success, oncomiracidia longevity, and infection success. The experimental design consisted of culturing the parasite at temperatures ranging between 16 and 32 °C. The oncomiracidia infection success, time to sexual maturity, and size at sexual maturity of *Neobenedenia* sp. were examined only at three temperatures (20 °C, 24 °C, and 30 °C). Experiments were conducted under controlled conditions in the laboratory. The oncomiracidia development was found to be faster at warmer temperatures (4–5 days between 24 and 30 °C) than in colder treatments (7–11 days between 18 and 20 °C). Hatching success and oncomiracidia longevity were higher at 24 °C and 26 °C. At 20 °C, 24 °C, and 30 °C, infection success was greater than 90%. Additionally, the laid eggs were observed at 9, 12, and 15 days at 30 °C, 24 °C, and 30 °C, respectively. The results of this study will allow for improving the temporal schedule of applications of treatments against *Neobenedenia* sp. by the function of temperatures. In conclusion, it is recommended to treat fish more frequently if the temperature in cultures is higher than 24 °C, because *Neobenedenia* sp. development is faster. As an alternative, the fish could be moved to deeper and cooler waters.

Keywords Capsalidae · *Neobenedenia* · Early development · Sexual maturity · Water temperature · *Seriola rivoliana*

Introduction

Monogeneans Capsalidae are marine parasites (Ogawa 1996; Whittington et al. 2001; Tubbs et al. 2005). Within these species, the genus *Neobenedenia* has been recognized as an ectoparasite of farmed fish around the world (Ogawa et al. 1995; Hirazawa et al. 2010; Trasviña-Moreno et al. 2017). Along the Pacific and Atlantic coast of Mexico, several fish species have been recorded to be infected by *Neobenedenia* species, (Rubio-Godoy et al. 2011; Brazenor et al. 2018), including *S. rivoliana* (Valenciennes, 1833) which is a recently exploited species of Mexican aquaculture farms (Avilés-Quevedo and Castello-Orvay 2004).

Infection of *Seriola* spp. by capsalids like *N. girellae* (Hargis 1955) can cause hemorrhage, inflammation, and mucus hyperproduction (Paperna 1991). Heavily infected fish may also stop feeding and in some cases, the fish may rub against the sea-cage nets resulting in dermal ulceration and subsequent bacterial invasion (Leong and Colorni 2002). Hence, *Neobenedenia* spp. can cause serious health problems in farmed fish.

Neobenedenia spp., as well as other monogenean, have a direct life cycle that does not require an intermediate host in order to complete their biological cycle (Rohde 1993). They are able to reproduce rapidly, and produce eggs that often become entangled in the sea-cage netting, leading, in many cases, to re-infection of the farmed fish (Ernst et al. 2002).

Section Editor: Guillermo Salgado-Maldonado

✉ Juan Carlos Pérez-Urbiola
jperez@cibnor.mx

[1] Centro de Investigaciones Biológicas del Noroeste (CIBNOR) S.C., Km. 1, Carretera a San Juan de La Costa "El Comitán", 23097 La Paz, Baja California Sur, Mexico

[2] Centro de Investigación en Alimentación y Desarrollo, A. C., Unidad Mazatlán en Acuicultura y Manejo Ambiental, Av. Sábalo Cerritos s/n, C.P., 82010 Mazatlán, Sinaloa, Mexico

It is well-known that eggs are more resistant than other developmental stages to anti-helmintic agents such as praziquantel or other cleaning treatments including baths of freshwater, hydrogen peroxide, and formalin (Thoney and Hargis 1991; Morales-Serna et al. 2018). Due to this high resistance of eggs, fish are at risk of being re-infected and it is difficult to control species of *Neobenedenia* in aquaculture systems (Diggles et al. 1993; Yoshinaga et al. 2000).

Neobenedenia spp. infections commonly occur with the onset of summer when higher temperatures reduce the time of hatching and age of maturity (Bondad-Reantaso et al. 1995; Kinami et al. 2005; Hirazawa et al. 2010). In this regard, Brazenor and Hutson (2015) have shown that hatching time for *Neobenedenia* sp. from *Lates calcarifer* varied between 6–8 days at 22 °C and 4–6 days at 24 °C and 30 °C. In addition, *N. girellae* eggs hatched in a temperature range between 18 and 30 °C, but did not hatch at 15 °C (Bondad-Reantaso et al. 1995). Thus, temperature has an effect on the development of the early stages of *N. girellae* (based on Brazenor et al. 2018) collected in Japan and Australia.

N. girellae can infect a wide variety of hosts (i.e., *Lates calcarifer* (Bloch, 1790), *Paralichthys olivaceus* (Temminck & Schlegel, 1846), *S. lalandi* (Valenciennes, 1833), *S. quinqueradiata* (Temminck & Schlegel, 184), *Rachycentron canadum* (Linnaeus, 1766), and other species), within tropical regions of the Pacific and Atlantic Oceans (Brazenor et al. 2018). This is unusual because ~80% of all monogeneans infect a single species and they are usually distributed in one ocean basin (Rohde 1979; Byrnes and Rohde 1992; Whittington 1998).

A recent study based on genetic analysis showed that species other than *N. girellae* have infected cultured *S. lalandi* and wild fish off the Northern coast of Chile (Sepúlveda and González 2019). This allows for the possibility that *Neobenedenia* form a complex of cryptic species that may adapt to their local conditions (Whittington 2004). This is relevant because a misunderstanding of the true delineation of parasitic species may affect diagnostic, control, and eradication programs. Thus, an accurate knowledge of the life cycle of *Neobenedenia* sp. and the influence of temperature will help to design specific temporal schedules of treatments by the function of temperature to better combat this parasite (Tubbs et al. 2005; Nadler and Perez-Ponce De León 2011).

The carangid *Seriola rivoliana* (Almaco jack) is currently being cultured in Bahía de La Paz, BCS, Mexico, where the surface water temperature varies during the annual cycle from 20.5 °C in the winter to 31 °C in the summer (Obeso-Nieblas et al. 2008; Guevara-Guillén et al. 2015). However, the influence of water temperature on oncomiracidia development, hatching success, oncomiracidia longevity, and infection success and time to sexual maturity of *Neobenedenia* sp. from *S. rivoliana* has not been previously investigated. This information is useful for developing strategies to better control *Neobenedenia* sp. in aquaculture systems. Hence, the present study describes temperature effects on the life cycle of *Neobenedenia* sp. from *S. rivoliana*.

Material and methods

Initial experimental settings

Adults of capsalids were collected from Almaco jack (*S. rivoliana*) juvenile fish maintained in ponds at the Northwest Biological Research Centre (CIBNOR). The fish were anesthetized with a solution of eugenol (3 ml × 100 l^{-1}) as recommended by Boijink et al. (2016). Living parasites were carefully removed from the fish skin and some selected specimens were fixed with formaldehyde and changed to 70% ethanol and stained with Gomori's trichrome, cleared with salicylate and mounted in permanent preparations with synthetic resins, for identification purposes. Adults of capsalids were identified as *Neobenedenia* sp. based on the description of Whittington and Horton (1996) and some of them were deposited in the Parasitological Collection of the Natural History Museum at Universidad Autónoma de Baja California Sur (CPMHN-UABCS) with the accession number 725 (Fig. 1).

To collect enough parasites for the experiments, we first transferred 35 adults of *Neobenedenia* sp., from the skin of Almaco jack to a six-well flat-bottom culture vessel where

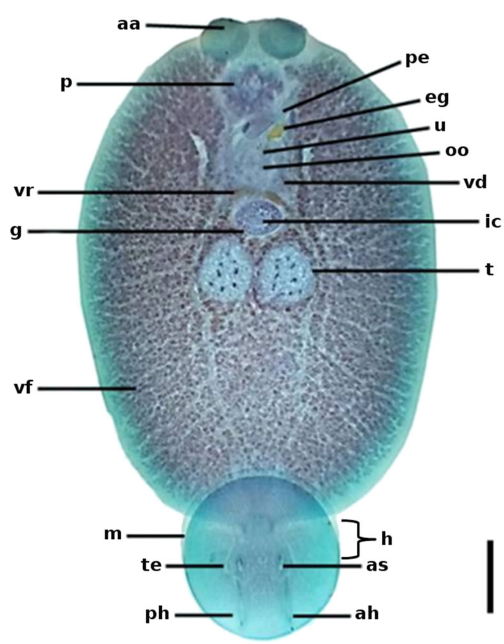

Fig. 1 Ventral view of an adult *Neobenedenia* sp., stained with Gomori's trichrome. aa, anterior attachment organ; ah, anterior hamulus; as, accessory sclerite; e, egg; g, germarium; h, haptor; m, marginal valve; ic, internal fertilization chamber; oo, ootype; p, pharynx; pe, penis; ph, posterior hamulus; t, testis; te, tendon; u, uterus; vd, vas deferens; vf, vitelline follicle; vr, vitelline reservoir. Scale bars represent 200 μm

they deposited eggs. In the culture vessels, the eggs developed into oncomiracidia larvae and, then they were used for infecting Almaco jack juveniles (average weight 150 ± 50 g) in the laboratory. The infections were carried out in two tanks of 600 l. In each tank, three fish were placed and conditions were kept constant (salinity 36 ± 1 ppt, dissolved oxygen 5.5 ± 0.5 mg O_2/l, temperature 25 ± 1 °C, and natural photoperiod). Fish were fed twice a day with a commercial diet (EWOS® Canada Ltd., Vancouver, British Columbia, Canada) and at daily feeding rate of 3% of the body weight.

All experiments were made in a water bath system and thermal control was by the thermic simulator called SITMA (from the Spanish "Marine Thermic Simulator"), which was developed at CIBNOR (Sicard-Gonzalez 2006). SITMA allows for the control of the temperature in each independent tank with an error margin of ± 0.02 °C. Also, the temperatures were recorded with Hobo sensors as an internal control (Onset Computer Corporation, Bourne, MA, USA) and the photoperiod was programed at 12:12 (light/dark) with a timer placed on a light switch.

Egg collection

Six infected fish from the laboratory were selected and moved to a clean tank with 600 l of seawater and they were kept without water flow for 3 h at 25 °C. The oxygen concentration was kept above 5 mg O_2/l, salinity at 36 ppt, and a natural photoperiod. *Neobenedenia* sp. eggs were collected on nylon threads tied to the air pipe. The nylon threads were cut into 1 cm segments under a stereomicroscope and transferred to test tubes with 6 ml of sterile seawater at 35 ppt. These nylon segments were used for three experiments (i) to follow the oncomiracidia development at 24 °C, (ii) to determine the temperature effects in hatching success and time and (iii) in the oncomiracidia longevity assessment.

Oncomiracidia development

In order to follow the oncomiracidia development, six test tubes with nylon threads with 30 eggs were incubated at 24 °C. The eggs were observed every 24 h until we recorded the first hatching event and the time used to describe the development, which was expressed as hours post-deposition (hpd) or days post-deposition (dpd). Morphological changes were documented by photographs taken from microscopic observations and analyzed with Image Pro Premier software 9.2 (Media Cybernetics, Rockville, MD).

Embryo development was classified according to the three development stages. Stage 1 or the embryo was assigned to eggs having dark brown color with evidence of initial cellular division; stage 2 encompassed eggs with oncomiracidia presenting haptor sclerites and visible eyespots; and stage 3 was determined when the eggs appeared empty and were characterized by a clear color and an open operculum (see Fig. 2).

Hatching success and times

Hatching success was evaluated in another set of tubes with the nylon threads with 35 eggs, which were incubated at 16 °C, 18 °C, 20 °C, 22 °C, 24 °C, 26 °C, 28 °C, 30 °C, and 32 °C (three replicates per temperature) in SITMA. Tubes were monitored every 24 h under a stereomicroscope with both incident and transmitted light and a third of the water was replaced daily.

When a stage 3 egg was detected, we looked for the oncomiracidium and it was removed from the sample with a pipette to avoid over count of larvae. In this step, the time of hatching was recorded and the monitoring stopped after 48 h if no newly hatched oncomiracidia were detected and also if the remaining eggs did not show evidence of development (cellular division, eye spots). Hatching success was estimated following Brazenor and Hutson (2015), as the ratio of oncomiracidia over the total number of eggs at the beginning of the experiment (35 eggs).

Oncomiracidia longevity

This experiment was done incubating test tubes in SITMA at temperatures ranging from 18 to 30 °C with 2 °C increments. In each temperature, 100 eggs were used.

Ten oncomiracidia showing vigorous swimming behavior were selected just after hatching. They were gently aspirated with a glass pipette and slowly ejected into tubes containing 6 ml of sterile seawater at 35 ppt. Three replicates were made for each temperature that were monitored every 2 h to evaluate survival. "Time 0" was artificially established as the moment when the oncomiracidia larvae were deposited in test tubes. Oncomiracidia were considered dead when they did not show signs of motion. Oncomiracidia longevity was considered as the elapsed time from time 0 until the time of death.

Infection success, time, and size to sexual maturity

Initially, 1000 eggs of *Neobenedenia* sp. were incubated at 20 °C, 24 °C, and 30 °C in tanks of the SITMA system. For each temperature, 300 vigorous swimming oncomiracidia were aspirated with a glass pipette and transferred to a 100-ml Erlenmeyer flask containing 60 ml of sterile seawater at 35 ppt and protected from light. These larvae were added to tanks with 21 uninfected juveniles of *S. rivoliana* (Almaco jack) with an average weight of 26.5 ± 5.5 g. During this step, the water re-circulating system was turned off for 30 min following Hirazawa et al. (2010). After the 30 min, the re-circulation system was activated, oxygen concentration was conserved above 5 mg O_2/l, salinity was preserved at 35 ±

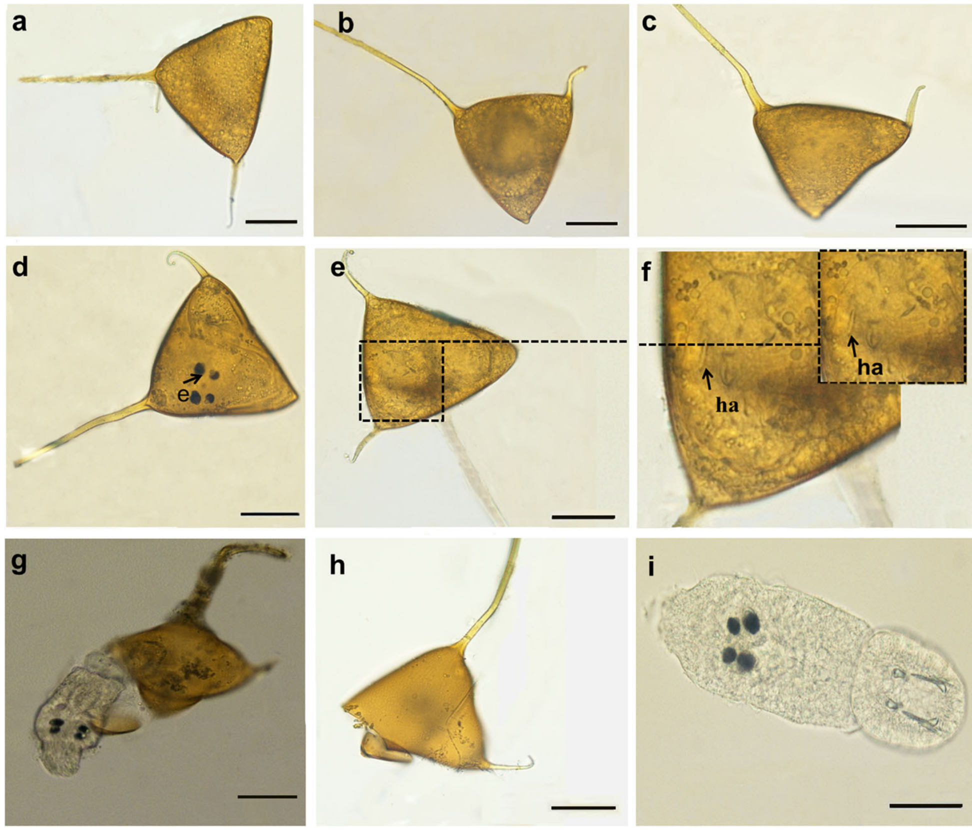

Fig. 2 Oncomiracidial development of *Neobenedenia* sp. Egg development was scored as stage 1: **a** Recently deposited eggs. **b** Eggs after 24 h. **c** Whole embryos; stage 2: **d** Defined eyespots. **e** Images of eggs after 96 h. **f** Details of primordia of hamuli; and stage 3: **g** Oncomiracidia recently emerged. **h** Eggs hatched. **i** Oncomiracidia. Abbreviations: e, eyespot; ha, hamulus. Scale bars represent 50 μm

1 ppt, and the water temperature was adjusted by SITMA at 20 °C, 24 °C, and 30 °C. Also, the photoperiod was programed at 12:12 light/dark cycle and three replicates were made for each temperature. The fish were fed once a day based on 2% of body weight with a commercial diet (EWOS®).

A single multifilament nylon thread was placed into each tank and inspected daily under a stereomicroscope to look for eggs: an indication of the presence of sexually mature parasites. Three infected fish with sexually mature parasites were sampled from each temperature and anesthetized with a solution of eugenol (0.5 ml l^{-1}) and bathed in freshwater for 5 min. The epithelial surface was gently rubbed in order to dislodge attached parasites. Infection success was determined following Brazenor and Hutson (2015). It was the number of parasites collected from all fish in a given temperature divided by the number of oncomiracidia introduced to the tank (300 oncomiracidia). In addition, mean infection intensity was obtained for each temperature (Bush et al. 1997).

Neobenedenia sp. were fixed in 10% formaldehyde and changed to 70% ethanol and then stained with Gomori's trichrome, cleared with salicylate, and mounted in permanent preparations with synthetic resin. Parasites were measured on the day that sexual maturity was observed. Total length, total width, anterior hamuli, and accessory sclerites were measured using a micrometer because they are taxonomically relevant characters for *N. girellae* (see Hargis 1955; Whittington 1996). Measurements are given as mean (minimum–maximum range) in millimeters.

Molecular identification

Due to the lack of morphologically distinguishable characters between species, some adults were genetically identified. The DNA extraction was based on the protocol of Ausubel et al. (2002). PCR amplifications of partial sequence reactions were

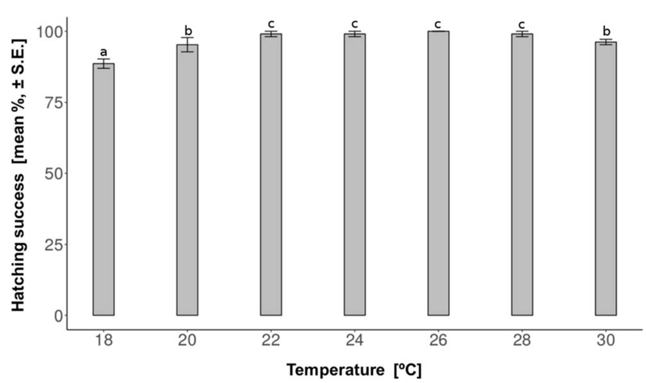

Fig. 3 *Neobenedenia* sp. hatching success in temperature treatment (16 °C and 32 °C not shown; no hatching observed). a, b, and c differences between pairs of means determined using Fisher's exact test, $p < 0.05$

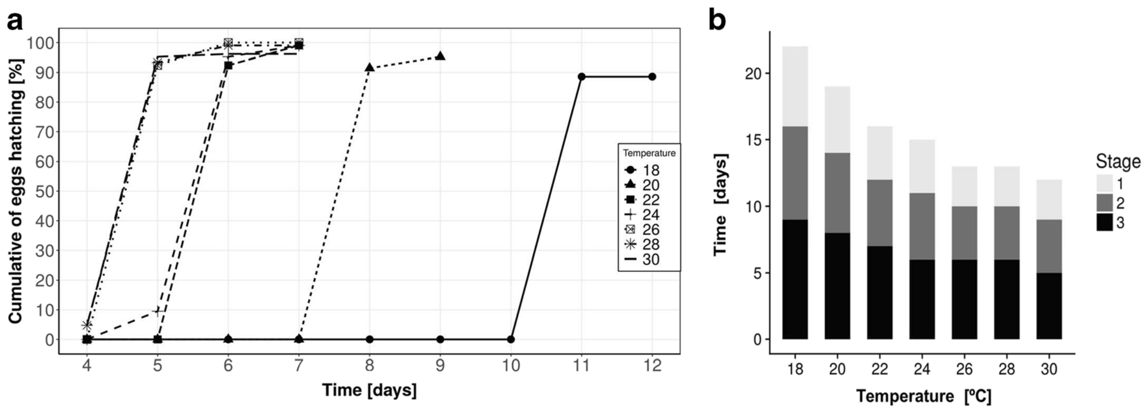

Fig. 4 **a** Cumulative proportion of hatched *Neobenedenia* sp. at different temperatures. **b** Egg development for *Neobenedenia* sp. at different temperatures: 1, number of days before 60% of the developing eggs hatched; 2, number of days before 60% of the developing eggs with eyespots present; 3, eggs clear with opened operculum

carried out in 25 μl using primers forward C1 (5′-ACCC GCTGAATTTAAGCAT-3′) and reverse EC-D2 (5′-CCTT GGTCCGTGTTTCAAGACGGG-3′) for 28S rDNA and forward primer M1676 (5′-TGAGTTATTATTGATGTAGAGG-3′) and reverse M1677 (5′-AAAATATCAKTCAGGCTTWA-3′) for cytochrome b (Cytb), as described by Brazenor et al. (2018).

The cycling conditions change depending on the genetic marker. For amplification of the 28S rDNA, an initial denaturation step of 95 °C (2 min) was used, followed by 35 cycles of 95 °C (1 min), 55 °C (40 s), and 72 °C (1.2 min), followed by a final extension at 72 °C (10 min). Cycling conditions for Cytb were an initial denaturation step of 95 °C (5 min), followed by 5 initial cycles of 95 °C (30 s), 50 °C (30 s), and 72 °C (30 s), with an addition of 30 cycles of 95 °C (30 s), 55 °C (1 min), and 72 °C (1 min), followed by a final extension phase at 72 °C (10 min). PCR products were run through electrophoresis gels of agarose (SYNERGEL™) prepared at 1% and visualized with Uview 6× loading dye and a transilluminator (Chemi Doc TM MP Imaging System BIORAD). PCR products were purified and sequenced by GENEWIZ (USA).

All *Neobenedenia* 28S rDNA and Cytb sequences from the GenBank were downloaded and aligned with Clustal X (version 2.0). Trees were constructed using neighbor-joining (NJ) based on P-distance values, using MEGA (version 5.05). The reliability of the measurements of stability of the branches was evaluated using non-parametric bootstrap (Felsenstein 1985) for the NJ tree, with 1000 replicates.

Statistical analyses

Fisher's exact test for 2 × 2 contingency tables was used to evaluate statistical differences in the proportion of hatched eggs as a function of the different temperatures. This test was chosen because data were not normally distributed, and they were not homoscedastic. The relationship between hatching time and temperature was determined through a Spearman correlation with a confidence level of 95%.

Finally, Kruskal-Wallis test was used to determine if there was a statistical difference in oncomiracidia longevity and size at sexual maturity between temperatures. Posteriori comparisons of group means were performed using Dunn's test.

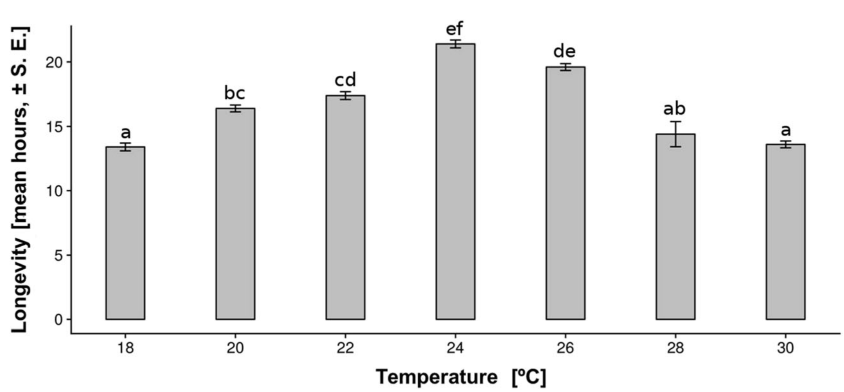

Fig. 5 *Neobenedenia* sp. oncomiracidia longevity (in hours) in temperature treatment a, ab, bc, cd, de, and ef differences between pairs of means determined using Kruskal-Wallis and post hoc Dunn's test, $p < 0.05$

Table 1 Life cycle parameters of *Neobenedenia* sp. infecting *Seriola rivoliana* in three different temperatures

Temperature (°C)	F/LH (days)	OL (hours)	SM (days)	LC (days)	Infection success (%)	Mean intensity
20	8–9	16	15	23	100	11
24	6–7	22	12	18	97	8
30	4–5	14	9	13	94	7

F/LH time to first and last hatch; *OL* oncomiracidia longevity; *SM* minimum time to sexual maturity; *LC* minimum time to completion of life cycle (LC)

Significance was accepted when $p < 0.05$. All the statistical tests were carried out in R version 3.0.2 (R Core Team 2016).

Results

Oncomiracidia development

The description of oncomiracidia development of *Neobenedenia* sp. was based on observations at 24 °C. The same stages were observed at other temperatures (18 to 30 °C), but the elapsed time between stages changed (Fig. 2).

Eggs presented three polar filaments: two short with hooks and another long and gradually thicker. At the beginning of the study (time 0), the eggs were completely filled with vitelline material and the embryo could not be distinguished (Fig. 2a). After 24 to 48 hpd (1 to 2 dpd), most vitelline material progressively migrated to the egg poles and the embryo became visible in the middle of the egg (Fig. 2b, c).

The vitelline material decreased during 72 to 96 hpd (3 to 4 dpd) when the oncomiracidia was distinguishable within the egg. During this stage, the eyespots became visible as a small and scattered accumulation of pigments that progressively were more defined (Fig. 2d). The sclerites of the haptor developed until becoming well defined and visible (Fig. 2e, f).

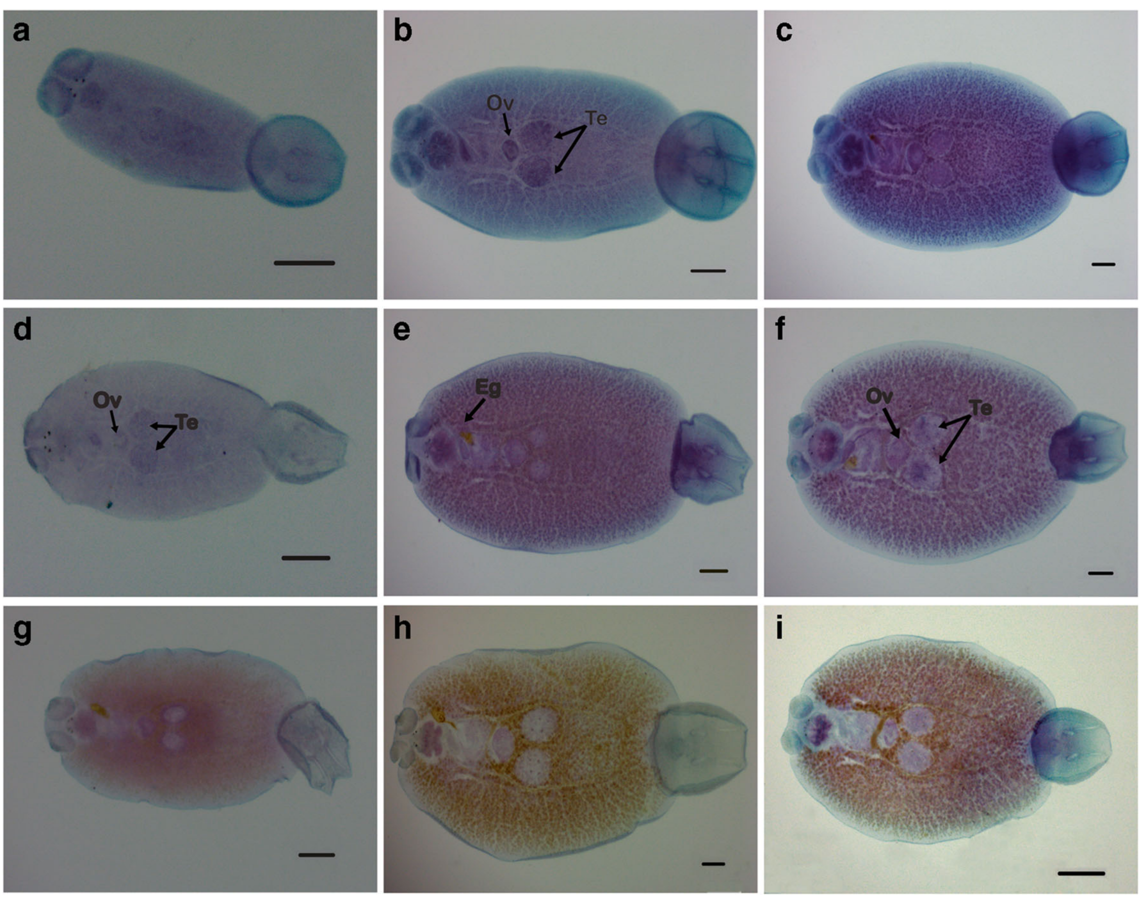

Fig. 6 Growth of *Neobenedenia* sp. from *S. rivoliana* (Almaco jack) at 20 °C, 24 °C, and 30 °C. **a–c** Parasites at 8 days while water temperature at **a** 20 °C, **b** 24 °C, and **c** 30 °C. **d–f** Parasites at 12 days at **d** 20 °C, **e** 24 °C, and **f** 30 °C. **g–i** Parasites at 15 days at **g** 20 °C, **h** 24 °C, and **i** 30 °C. Abbreviations: Te, testis; Ov, ovary; Eg, egg. Scale bar is 200 µm for **a** to **h** and 500 µm for **i**

The oncomiracidia began to rotate inside the eggs and hatched after 5 dpd through the opercular opening (Fig. 2g), doing a series of body contractions and using their cilia to propel themselves (Fig. 2i).

Hatching success and times

Hatching success was greater than 80% in the temperature range from 18 to 30 °C. At 18 °C, oncomiracidia had the lowest hatching rate (85%) that was statistically different from the other treatments (Fig. 3). In addition, at 20 °C and 30 °C, hatching success was also significantly different, and at temperatures from 22 to 28 °C, the hatching rate at > 99% being statistically homogeneous (Fig. 3). At 16 °C and 32 °C, hatching did not occur.

The Spearman correlation between temperature and hatching time was negative indicating that at higher temperature, the hatching times were shorter ($\rho = -0.90$, $p < 0.05$, $n = 35$). This result can be seen in Fig. 4a. At temperatures of 24 to 30 °C, the hatching time was 4–5 days. By contrast, at temperatures < 22 °C, hatching time required more time (> 7 days at 22 °C). This relationship was also observed in the other developmental stages of *Neobenedenia* sp. (Fig. 4b) and hence, the development is faster as higher temperatures.

Oncomiracidia longevity

Oncomiracidia survived longer at 24 °C and 26 °C (22 and 18 h; see Fig. 5). Oncomiracidia longevity was significantly reduced at 18 °C, 28 °C, and 30 °C (14 h). These differences of the longevity between temperature treatments were significant as indicated by the Kruskal-Wallis test (kw = 61.94; df = 6; $p = < 0.05$).

Infection success, time, and size to sexual maturity

At 20 °C, 24 °C, and 30 °C, infection success was > 90% and an infection intensity greater than 7 parasites per fish (Table 1), highlighting the effects of temperature in adults. Additionally, the laid eggs were observed at 9 days at 30 °C but they were observed until days 12 and 15 at 24 °C and 20 °C (Fig. 6). It was noticeable that at 24 °C and 20 °C, the deposited eggs took more time even so testis and ovaries were formed and observable at the ninth day of the experiment (Fig. 6).

The statistical comparison of the length at sexual maturity resulted in significant differences (kw 20.75, df = 2, $p = < 0.05$), with larger parasites at 30 °C than at 24 °C and 20 °C (Fig. 7a). The measurements of other body structures showed the same trend increasing at greater temperatures (Fig. 7b). The statistical differences were for the total width (kw 33.24, df = 2, $p = < 0.05$) and the length of the anterior hamulus (kw 23.23, df = 2, $p = < 0.05$) and accessory sclerite (kw 15.22, df = 2, $p = < 0.05$).

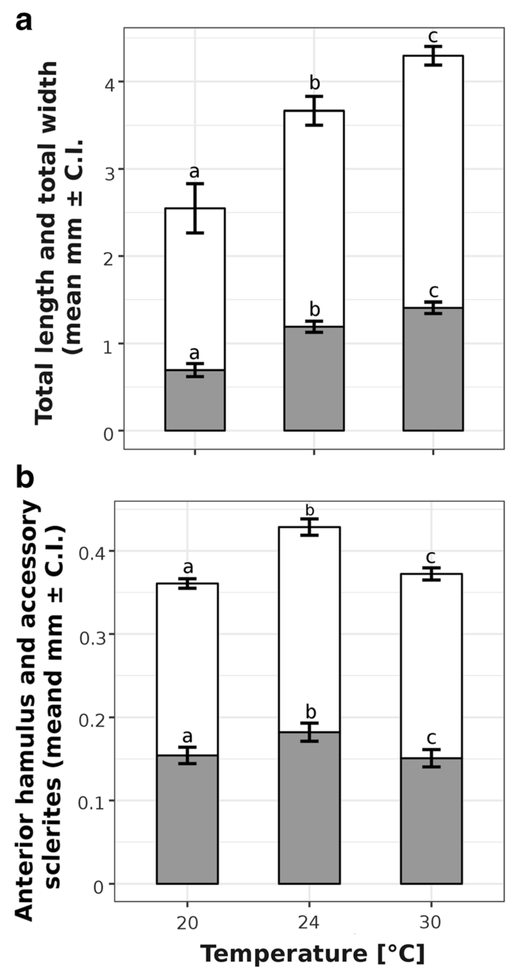

Fig. 7 **a** *Neobenedenia* sp. total length (total column height) and total width (dark gray column) and **b** anterior hamulus (total column height) and accessory sclerite length (dark gray column) of day to sexual maturity with respect to temperature. a, b, and c differences between pairs of means determined using Kruskal-Wallis and post hoc Dunn's test, $p < 0.05$

Phylogenetic analyses

The genetic analyses of the 28S rDNA grouped our specimens with sequences belonging to a single clade within *Neobenedenia girellae*. The clade had a bootstrap support of 89% (Fig. 8). This clade included *Neobenedenia* obtained from different hosts and localities. Nonetheless, the topology of the Cytb neighbor-joining tree was different. In this tree, the sequences obtained in our study belong to a clade distinguishable from *N. girellae*. This new clade had a bootstrap value of 34% (Brazenor et al. 2018; see Fig. 9).

Discussion

Taxonomical identification of the parasite is key to describing its life cycle. To achieve this in our work, we first identified morphological characteristics that lead to the genus *Neobenedenia* sp. This genus includes *N. melleni* and

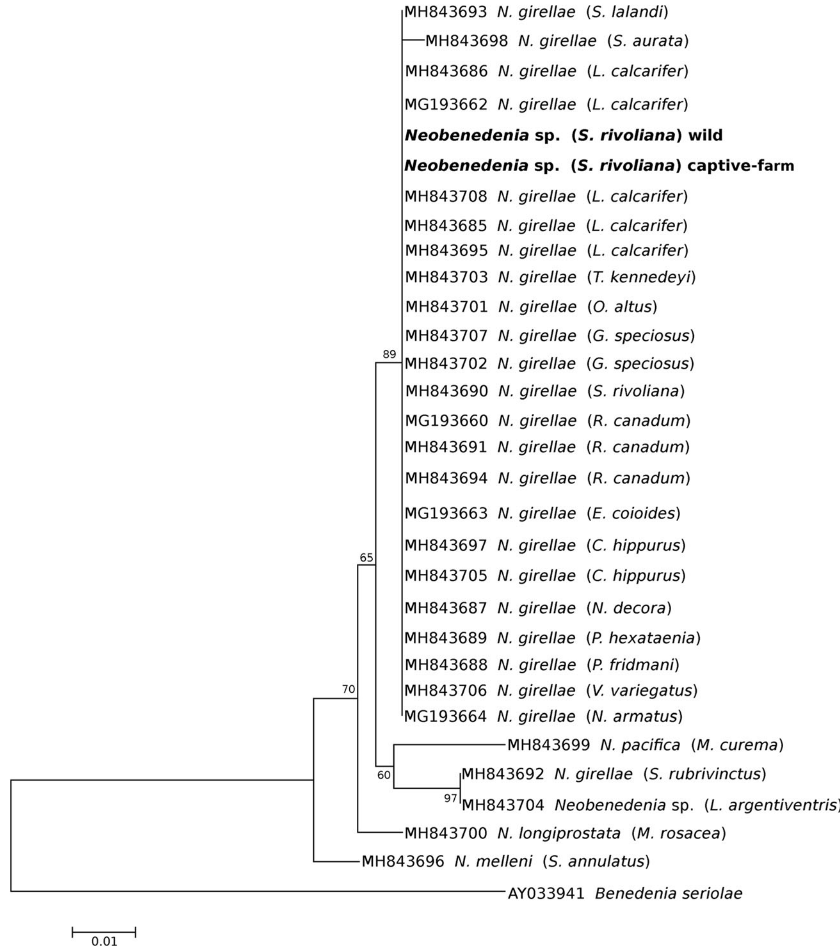

Fig. 8 Neighbor-joining reconstruction between partial 28S rDNA sequences of *Neobenedenia* sp. obtained in this study (in bold, also showing the host) and sequences of *Neobenedenia* species collected from fishes obtained from the NCBI. The numbers on the tree branches represent the percentage of bootstrap resampling (with 1000 replicates). *Benedenia seriolae* was used as an outgroup. GenBank accession numbers are in front of species names

N. girellae, two species considered as synonymous or cryptic species because they are genetically distinct but morphologically equal (Whittington 2004; Brazenor et al. 2018).

Brazenor et al. (2017) suggested that the cytochrome b is an adequate molecular marker to determine intraspecific differences in *Neobenedenia* spp. Considering this, our results indicated that the monogeneans collected from Almaco jack in Bahía de La Paz, Mexico, belong to a different population within *N. girellae*. However, we recommend reviewing the taxonomy of this genus using a multilocus approach as in Brazenor et al. (2018). Because, it had been indicated that other species of *Neobenedenia* other than *N. girellae* can infect farmed fish (Sepúlveda and González 2019). In this work, we considered that our parasite is *Neobenedenia* sp. infecting to *S. rivoliana*.

This parasite is affected by temperature throughout its life cycle and we started with description of the early stages development at 24 °C. This development followed the common pattern of monogeneans like *N. girellae*, *Benedenia seriolae*, and *Zeuxapta seriolae* (Bondad-Reantaso et al. 1995; Tubbs et al. 2005; Brazenor and Hutson 2015). The first visible structure was the primordia hook, followed by the eyespots, cilia, and opercula of the egg. In other temperatures, the early stages development also followed this pattern and no deformation was observed at the temperature extremes tested in this study.

Hatching time took 4 days at 30 °C and 11 days at 18 °C, indicating a temperature dependence, which has been reported for other species like *B. seriolae* and *Neobenedenia* sp. infecting *Lates calcarifer*. Some authors have attributed it to changes in the metabolic rate that increase at higher temperatures (Roubal and Diggles 1993; Bondad-Reantaso et al. 1995; Hirazawa et al. 2010). In addition to this temperature dependence, we noted a complete inhibition of the hatching process of *Neobenedenia* sp. eggs at temperatures at 16 °C and 32 °C that had been reported for *Neobenedenia girellae* infecting *Paralichthys olivaceus* which do not hatch at 15 °C and other species like *Neoheterobothrium hirame* had reduced hatching success at the higher temperature (30 °C) (Bondad-Reantaso et al. 1995; Yoshinaga et al. 2000).

The mean percentage of hatched eggs in this study (> 85%; Fig. 3) was higher than the maximum value (80%) reported for other capsalids (Bondad-Reantaso et al. 1995; Tubbs et al. 2005; Brazenor and Hutson 2015). This can be attributed to the eggs collection method. In our case, we collected the eggs on nylon threads placed within the tanks containing the

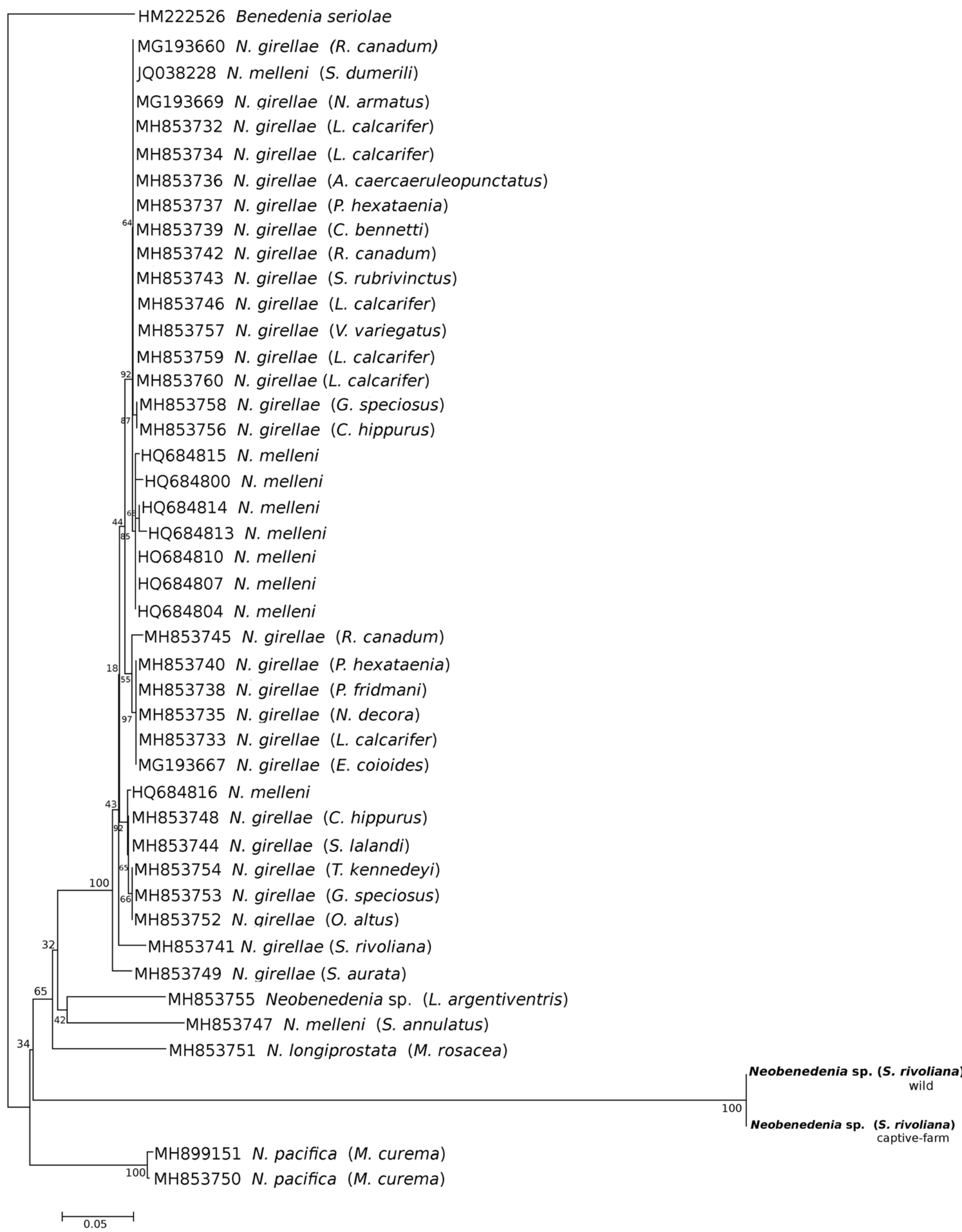

Fig. 9 Neighbor-joining reconstruction between partial *cytochrome b* sequences of *Neobenedenia* sp. collected from Almaco jack (in bold, indicating the host) used for this study and sequences of *Neobenedenia* species of fishes from the NCBI database. The numbers on the tree branches represent the percentage of bootstrap resampling (with 1000 replicates). *Benedenia seriolae* was used as an outgroup. The GenBank accession numbers are in front of species names

infected fish. Thus, the eggs were deposited by adults attached to their host, causing a minimal disturbance. In other studies, the adults were removed from the host and the eggs' deposition occurs in vitro. According to Ernst and Whittington (1996), this procedure can harm the adults yielding lower recovery rates of larvae.

The maximum longevity of the oncomiracidia larvae was 22 h at 24 °C and 26 °C (Fig. 5). Below (18 to 22 °C) and above (28 to 30 °C), longevity was reduced indicating that there is an optimal thermal range favoring larval longevity (Fig. 5). This result, with the highest longevity at 22 to 26 °C, differed from those reported for *Neobenedenia girellae* in Australian waters. Brazenor and Hutson (2015) reported a maximum longevity of 37 h at 22 °C and 35 ppt, which decreased at higher temperatures. This difference can arise from the adaption of populations to local conditions.

Temperature also affected the time of sexual maturity, taking longer at 20 °C relative to 24 °C and 30 °C (Fig. 6; Table 1). This trend is similar to those reported for *N. girellae* infecting *S. dumerili* where time to sexual maturity was observed at 6 days at 30 °C, 8 days at 25 °C, and 13 days at 20 °C (Hirazawa et al. 2010). Another relevant result was that 20 °C lead to smaller sexually mature organisms in comparisons to those at 24 °C and 30 °C (Fig. 7). Thus, an increase in temperature will reduce the time to sexual maturity and will increase their size.

Neobenedenia sp. hatched in a temperature range that overlapped the maximum survival of *S. rivoliana* (Fig. 10) and

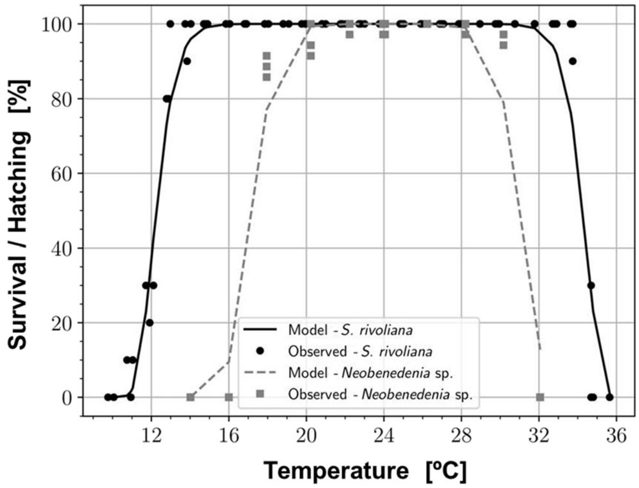

Fig. 10 Thermal tolerance of juveniles of *S. rivoliana* and cumulative proportion of hatched *Neobenedenia* sp. based on the modified Gauss model

within the seasonal variability in Bahía de La Paz, BCS, Mexico. Hence, infections cannot be avoided. However, we suggest making treatments more frequently in summer than in winter due to the acceleration in all the stages of the life cycle. Another option is to move the fish to deeper and cooler waters after a therapeutic treatment.

Acknowledgments The authors are grateful to Rosa Isela Vázquez Sánchez and Gilberto González Soriano for laboratory assistance. Delia Rojas Posadas for help with the genetic identification of the parasite and Amaru Márquez Artavia for statistical advice. Kampachi Farm for providing juvenile fish.

Funding information This work has been funded by the CIBNOR (PAC to F.A and J.C.P.U.). I.V.V. is a recipient of doctoral fellowship from the Consejo Nacional de Ciencia y Tecnología (CONACYT).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Ausubel FM, Brent R, Kingston RE, Moore DD et al (2002) Short protocols in molecular biology: a compendium of methods from current protocols in molecular biology. John Wiley & Sons, New York, NY

Avilés-Quevedo A, Castelló-Orvay F (2004) Manual para el cultivo de Seriola lalandi (Pisces: Carangidae) en Baja California Sur, México. Instituto Nacional de la Pesca. ISBN: 968-800-580-0

Boijink CL, Maciel PO, Tavares-Dias M, Iwashita MKP, Morais MS, Hide DMV, Souza NC, Couto MVS, Meneses JO, Cunha FS, Fujimoto RY (2016) Anesthesia by sprinkling method in the gills of tambaqui Colossoma macropomum does not influence intensity and morphology of monogeneans. Braz J Biol 77:367–371

Bondad-Reantaso M, Ogawa K, Fukudome M, Wakabayashi H (1995) Reproduction and growth of Neobenedenia girellae (Monogenea: Capsalidae), a skin parasite of cultured marine fishes of Japan. Fish Pathol 30(3):227–231

Brazenor AK, Hutson SK (2015) Effects of temperature and salinity on the life cycle of Neobenedenia sp. (Monogenea: Capsalidae) infecting farmed barramundi (Lates calcarifer). Parasitol Res 114:1875–1886. https://doi.org/10.1007/s00436-015-4375-5

Brazenor AK, Saunders RJ, Miller TL, Hutson KS (2017) Morphological variation in the cosmopolitan fish parasite Neobenedenia girellae (Capsalidae: Monogenea). Int J Parasitol 48:125–134. https://doi.org/10.1016/j.ijpara.2017.07.0090020-7519

Brazenor AK, Bertozzi T, Miller TL, Whittington ID, Hutson KS (2018) DNA profiling reveals Neobenedenia girellae as the primary parasitic monogenean in global fisheries and aquaculture. Mol Phylogenet Evol 129:130–137. https://doi.org/10.1016/j.ympev.2018.05.012

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83(4):575–583

Byrnes T, Rohde K (1992) Geographical distribution and host specificity of ectoparasites of Australian bream, Acanthopagrus spp. (Sparidae). Folia Parasitol 39:249–264

Diggles BK, Roubal FR, Lester RJG (1993) The influence of formalin, benzocaine and hyposalinity on the fecundity and viability of Polylabroides multispinosus (Monogenea: Microtylidae) parasitic on the gills of Acanthopagrus australis (Pisces: Sparidae). Int J Parasitol 23(7):877–884

Ernst I, Whittington ID (1996) Hatching rhythms in the capsalid monogeneans Benedenia lutjani from the skin and B. rohdei from the gills of Lutjanus carponotatus at Heron Island, Queensland, Australia. Int J Parasitol 26(11):1191–1204

Ernst I, Whittington ID, Corneillie S, Talbot C (2002) Monogenean parasites in sea-cage aquaculture. Austasia Aquacult 16:46–48

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Guevara-Guillén C, Shirasago-Germán B, Pérez-Lezama EL (2015) The influence of large-scale phenomena on La Paz Bay hydrographic variability. Open J Mar Sci 5:146–157. https://doi.org/10.4236/ojms.2015.51012

Hargis WJ (1955) A new species of Benedenia (Trematoda: Monogenea) from Girellani gricans, the opaleye. J Parasitol 41:48–50

Hirazawa N, Takano R, Hagiwara H, Noguchi M, Narita M (2010) The influence of different water temperatures on Neobenedenia girellae (Monogenea) infection, parasite growth, egg production and emerging second generation on amberjack Seriola dumerili (Carangidae) and the histopathological effect of this parasite on fish skin. Aquaculture 299:2–7

Kinami R, Miyamoto J, Yoshinaga T, Ogawa K, Nagakura Y (2005) A practical method to distinct between Neobenedenia girellae and Benedenia seriolae. Fish Pathol 40(2):63–66

Leong TS, Colorni A (2002) Infection diseases of warmwater fish in marine and brackish waters. In: Woo PTK, Bruno DW, Lim LHS (eds) Diseases and disorders of finfish in cage culture. CABI Publishing, London, pp 193–230

Morales-Serna FN, Chapa-López M, Martínez-Brown JM, Ibarra-Castro L, Medina-Guerrero RM, Fajer-Ávila EJ (2018) Efficacy of praziquantel and a combination anthelmintic (Adecto®) in bath treatments against Tagia ecuadori and Neobenedenia melleni (Monogenea), parasites of bullseye puffer fish. Aquaculture 492:361–368

Nadler S, Perez-Ponce De León G (2011) Integrating molecular and morphological approaches for characterizing parasite cryptic species: implications for parasitology. Parasitology 138:1688–1709

Obeso-Nieblas M, Shirasago B, Gaviño-Rodríguez J, Pérez-Lezama E, Obeso-Huerta H, Jiménez-Illescas Á (2008) Variabilidad hidrográfica en Bahía de La Paz, Golfo de California, México (1995-2005). Rev Biol Mar Oceanogr 43:559–567

Ogawa K (1996) Marine parasitology with special reference to Japanese fisheries and mariculture. Vet Parasitol 64:95–105

Ogawa K, Bondad-Reantaso MG, Fukudome M, Wakabayashi H (1995) Neobenedenia girellae (Hargis, 1955) Yamaguti, 1963 (Monogenea: Capsalidae) from cultured marine fishes of Japan. J Parasitol 81(2): 223–227

Paperna I (1991) Diseases caused by parasites in the aquaculture of warm water fish. Annu Rev Fish Dis 1:155–194

R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL https://www.R-project.org/

Rohde K (1979) A critical evaluation of intrinsic and extrinsic factors responsible for niche restriction in parasites. Am Nat 114:648–671

Rohde K (1993) Ecology of marine parasites: an introduction to marine parasitology, 2nd edn. Cab International, Wallingford, UK

Roubal FR, Diggles BK (1993) The rate of development of Polylabroides multispinosus (Monogenea: Microcotylidae) parasitic on the gills of Acanthopagrus australis (Pisces: Sparidae). Int J Parasitol 23(7): 871–875

Rubio-Godoy M, Montiel-Leyva A, Martínez-Hernández JA (2011) Comparative susceptibility of two different genetic types of tilapia to Neobenedenia sp. (Monogenea). Dis Aquat Org 93:171):171–171):177

Sepúlveda FA, González MT (2019) DNA barcoding evidence for the first recorded transmission of Neobenedenia sp. from wild fish species to Seriola lalandi cultured in an open recirculating system on the Coast of Northern Chile. Aquaculture 501:239–246

Sicard-Gonzalez MT (2006) Efecto de la oscilación Térmica en la Fisiología de la almeja mano de león (*Nodipecten subnodosus* Sowerby, 1835). Dissertation, Universidad Autónoma de Nuevo León, México

Thoney DA, Hargis WJ (1991) Monogenea (Platyhelminthes) as hazards for fish in confinement. Annu Rev Fish Dis 1:133–153

Trasviña-Moreno AG, Ascencio F, Angulo C, Hutson KS, Avilés-Quevedo A, Inohuye-Rivera RB, Pérez-Urbiola JC (2017) Plant extracts as a natural treatment against the fish ectoparasite Neobenedenia sp. (Monogenea: Capsalidae). J Helminthol 93:57–65. https://doi.org/10.1017/S0022149X17001122

Tubbs LA, Poortenaar CW, Sewell MA, Diggles BK (2005) Effects of temperature on fecundity in vitro, egg hatching and reproductive development of Benedenia seriolae and Zeuxapta seriolae (Monogenea) parasitic on yellowtail kingfish Seriola lalandi. Int J Parasitol 35:315–327

Whittington ID (1996) Benedeniine capsalid monogeneans from Australian fishes: pathogenic species, site-specificity and camouflage. J Helminthol 70:177–184

Whittington ID (1998) Diversity "down under": monogeneans in the Antipodes (Australia) with a prediction of monogenean biodiversity worldwide. Int J Parasitol 28:1481–1493

Whittington ID (2004) The Capsalidae (Monogenea: Monopisthocotylea): a review of diversity, classification and phylogeny with a note about species complexes. Folia Parasitol 51:109–122

Whittington ID, Horton MA (1996) A revision of Neobenedenia Yamaguti, 1963 (Monogenea: Capsalidae) including a redescription of N. melleni (MacCallum, 1927) Yamaguti, 1963. J Nat Hist 30: 1113–1156

Whittington ID, Corneillie S, Talbot C, Morgan JAT, Adlard RD (2001) Infections of Seriola quinqueradiata Temminck and Schlegel and S. dumerili (Risso) in Japan by Benedenia seriolae (Monogenea) confirmed by morphology and 28S ribosomal DNA analysis. J Fish Dis 24(7):421–425

Yoshinaga T, Segawa I, Kamaishi T, Sorimachi M (2000) Effects of temperature, salinity and chlorine treatment on egg hatching of the monogenean Neoheterobothrium hirame infecting Japanese flounder. Fish Pathol 35(2):85–88