

# A Practical Method to Distinguish between *Neobenedenia girellae* and *Benedenia seriolae*

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**ABSTRACT**—A practical method to distinguish two pathogenic monogeneans of fish, *Neobenedenia girellae* and *Benedenia seriolae*, is described. *N. girellae* and *B. seriolae* were obtained from experimentally infected olive flounder *Paralichthys olivaceus* and yellowtail *Seriola quinqueradiata*, respectively, by freshwater bathing and fixed in 10% formalin. The two species were found to have a different shape at the anterior end in the midline of the body and are clearly distinguishable by this difference. Using this method, the ratio of these two parasite species was monitored in greater amberjack *Seriola dumerili* cultured in net cages at Amami-Oshima Island for one year. A seasonal shift of abundance between the two species was observed.

**Key words:** *Neobenedenia girellae*, *Benedenia seriolae*, morphological distinction, seasonal succession, *Seriola dumerili*, greater amberjack

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The skin-parasitic capsalid monogeneans *Neobenedenia girellae* (possibly synonymous to *Neobenedenia melleni*; see Whittington and Horton, 1996) and *Benedenia seriolae* cause problems on cultured marine fishes in Japan. The former parasitizes various fishes and shows low host specificity (Ogawa *et al.*, 1995), and the latter demonstrates high host specificity for members of the genus *Seriola* (Kearn *et al.*, 1992; Ogawa and Yokoyama, 1998). The greater amberjack *Seriola dumerili*, an important aquaculture species in Japan, is very susceptible to both of them. Currently, bath treatments with either freshwater or a hydrogen peroxide solution and oral administration of praziquantel are used to control these problematic monogeneans (Ogawa, 2004). Although *N. girellae* and *B. seriolae* are assumed to have different susceptibilities to these therapies, the two monogeneans are actually equated with each other in aquaculture sites.

In this study, we aimed to develop a practical method for distinguishing these two parasite species. Because freshwater bathing is frequently used for the control of them and is the easiest way to collect the skin-

parasitic monogeneans, flukes which were detached from hosts by freshwater bathing were used. In addition, the ratio of these two parasites on greater amberjack cultured on Amami-Oshima Island was monitored using this new method.

## Materials and Methods

### *Distinction between N. girellae and B. seriolae*

*N. girellae* specimens were obtained from olive flounder *Paralichthys olivaceus* which had been placed in a 150 L tank for 2–3 wk, where the parasite was experimentally propagated using greater amberjack as the host. Natural seawater was recirculated with a bio-filter at 21.8–22.2°C in the tank. Infected flounder (n = 2, total length = 142 and 167 mm) were bathed in freshwater, and detached *N. girellae* were collected and fixed in 10% formalin.

*B. seriolae* specimens were obtained from yellowtail *Seriola quinqueradiata* experimentally infected at 23.7–24.8°C at the Goto Station, National Center for Stock Enhancement, Fisheries Research Agency in July 2003. Yellowtail (n = 30, standard length = 64–159 mm) were exposed to *B. seriolae* oncomiracidia (within 30 min after hatching, average 161 larvae/fish) in still

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seawater in a 500 L tank for 3 h. Subsequently, flow-through seawater was supplied into the tank, and then 5 fish each were sampled and freshwater-bathed at appropriate intervals (3 h, 12 h, 24 h, 3 day, 1 wk and 2 wk after exposure) for the collection of *B. seriola*. The collected flukes were fixed in 10% formalin.

More than 30 specimens each of these two flukes were mounted on slides with a small amount of water and coverslips, and examined by light microscopy. Measurements (total body length including haptor (TL), body length, maximum breadth, haptor length, haptor width, anterior hamulus length, anterior sucker length and anterior sucker width) were made of the 15 flukes of various sizes from each species (*N. girellae*: TL = 390–5510  $\mu\text{m}$ , *B. seriola*: TL = 297–5010  $\mu\text{m}$ ) using an eyepiece micrometer on a light and/or dissecting microscope. The marginal valve of the haptor was not included for any measurements because it was sometimes distorted and we thought it not practical to labor to prevent it from being bent.

#### Seasonal change of the two parasites

We identified monogeneans collected from greater amberjack using the method described here. The flukes were collected from the two groups of greater amberjack in a fish farm at Amami-Oshima Island, where both parasites were naturally present, from October 2003 (mean fork length = 358 mm) to August 2004 (mean fork length = 573 mm). These fish groups were introduced from China in March and April 2003 and reared in different net cages suspended in the sea. The fish were bathed in freshwater *ad libitum* (1–3 times a month) and the bathwater from 4–5 fish was filtered with 0.5 mm nylon mesh to obtain the parasites. Trapped matter was fixed in 10% formalin, transported to the University of Tokyo and observed under a dissecting microscope. The flukes were sorted out from the trapped debris and identified as *N. girellae* or *B. seriola* using the method developed in the present study. As the experimental two fish groups were shipped to markets in August 2004, additional samples were taken on

17 September 2004 from other two fish groups (mean fork length = 586 mm) of the same age (2 fish each). 50 specimens of each identified parasite species (*N. girellae*: TL = 1010–6950  $\mu\text{m}$ , *B. seriola*: TL = 873–6520  $\mu\text{m}$ ) from Amami-Oshima Island were examined again under a light microscope, and the body parts were measured as described above to confirm the suitability of the distinction method used.

## Results

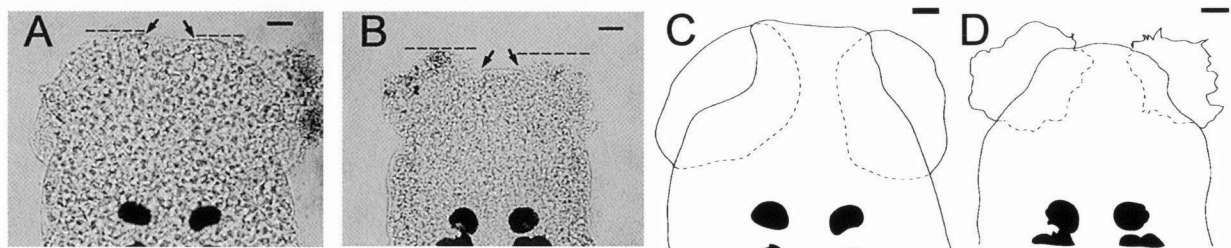
#### Distinction between *N. girellae* and *B. seriola*

A clear morphological difference was found between the two parasite species. We observed a difference in the shape at the anterior end in the midline of the body (the structure between two anterior suckers). In *N. girellae*, the anterior structure is slightly concave, or flat, starting with the horizontal top of their anterior suckers, which had been described as 'hooded' (body margin extending to the anterior edge of anterior suckers) by Whittington and Horton (1996). On the contrary, in *B. seriola*, the corresponding structure is convex, starting with the lower portion of their anterior suckers. This difference was apparent even amongst the smallest samples we examined (Fig. 1), and it is proposed as the characteristic for the practical distinction method of the two flukes.

Among the measurements or measurement indices, the ratio of anterior sucker length (AS) to TL showed the clearest difference between the two species of various sizes (data not shown). However, the smallest sample of *B. seriola* (TL = 297  $\mu\text{m}$ ) could not be distinguished by this index or other measurements, and it seemed that no measurements were useful for the consistent distinction of the two flukes.

#### Seasonal change of the two parasites

The abundances and sizes of *N. girellae* and *B. seriola* which parasitized greater amberjack cultured in net cages at Amami-Oshima Island varied widely throughout the year, and no seasonal trends were



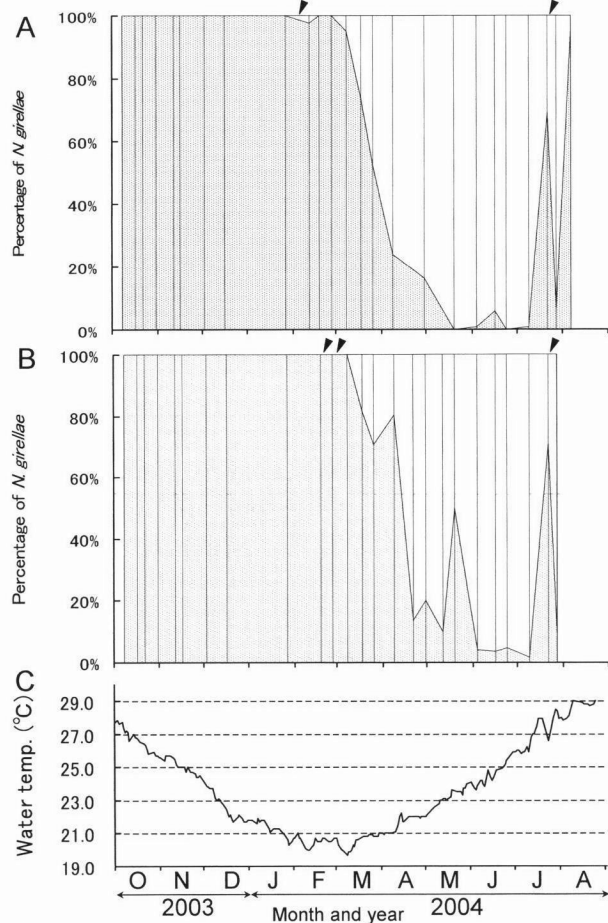
**Fig. 1.** Anterior portion of the smallest *Neobenedenia girellae* and *Benedenia seriola* examined (dorsal view). Bars = 10  $\mu\text{m}$ . **A**, photo of *N. girellae* (total body length including haptor: TL = 390  $\mu\text{m}$ ); **B**, photo of *B. seriola* (TL = 297  $\mu\text{m}$ ). In **A** and **B**, broken lines indicate horizontal ends of anterior suckers and arrows the starting point of the anterior end between two anterior suckers; **C**, drawing of the photo **A**; **D**, drawing of the photo **B**. The rough outline of the anterior suckers in **D** is an artifact due to the freshwater bathing procedure.

found. This is probably because the frequency of bath treatments for the skin flukes also varied according to water temperature. However, using the morphological distinction method, clear seasonal changes were observed in the abundance ratios of the two flukes. Almost all flukes observed from October 2003 to February 2004 were *N. girellae*. Subsequently the percentage of *B. seriolae* increased and reached above 90% in June 2004 (Fig. 2). In July 2004, the ratios of *N. girellae* began to increase again. In the additional two samples taken on 17 September 2004, all but one of flukes were *N. girellae*; one group was composed of 184 *N. girellae* and no *B. seriolae*, and the other 101 *N. girellae* and one *B. seriolae*.

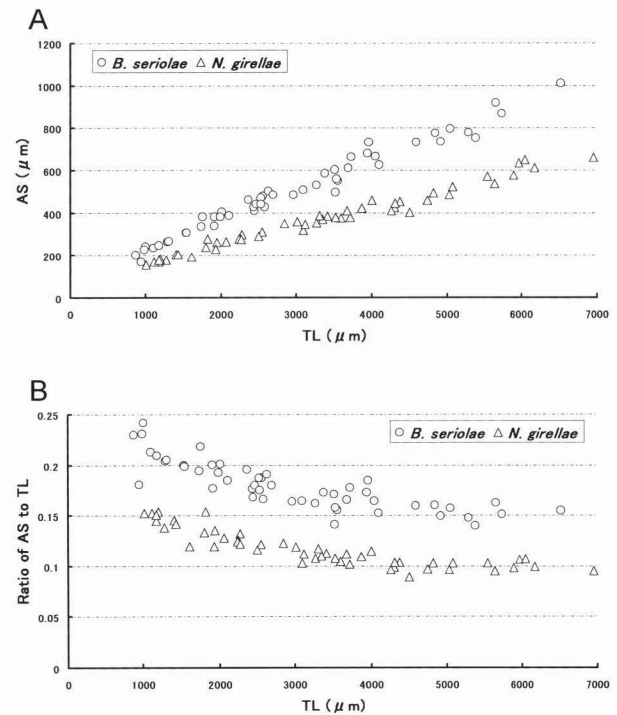
50 specimens each of *N. girellae* and *B. seriolae*, which were obtained from the fish farm at Amami-

Oshima Island and identified by the present method, were measured by microscopy. The ratios of AS to TL in these 100 flukes showed a clear difference between *N. girellae* and *B. seriolae* identified by the morphological feature of the anterior end (Fig. 3), except for one sample (*B. seriolae*: TL = 945  $\mu\text{m}$ ).

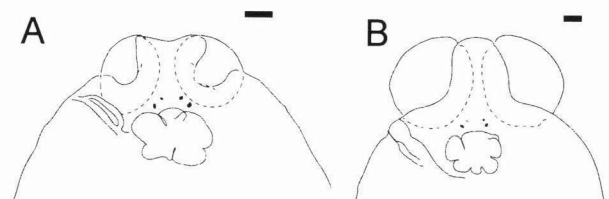
As an example to show the difference between the two monogeneans of a relatively bigger size, the anterior end of an individual worm of each species with a similar TL (*N. girellae*: TL = 3110  $\mu\text{m}$ , *B. seriolae*: TL = 3190  $\mu\text{m}$ ) was drawn in Fig. 4.



**Fig. 2.** Seasonal succession of *Neobenedenia girellae* and *Benedenia seriolae* in greater amberjack cultured at Amami-Oshima Island. **A** and **B**, seasonal changes in the percentage of *N. girellae* in all specimens obtained from different two fish groups. Sample points which had comprised <10 flukes were excluded from analysis. Vertical lines indicate the days of sampling and arrowheads the days of cage changing; **C**, Sea-water temperature of 5 m below sea level at culture site at Amami-Oshima Island.



**Fig. 3.** Relationship between anterior sucker length (AS) and total body length including haptor (TL) in *Neobenedenia girellae* and *Benedenia seriolae* from Amami-Oshima Island, which were identified using the newly developed method. **A**, AS against TL in each species; **B**, ratio of AS to TL in each species and their relations to TL.



**Fig. 4.** Anterior portion of *Neobenedenia girellae* and *Benedenia seriolae* specimens from Amami-Oshima Island (dorsal view). Bars = 20  $\mu\text{m}$ . **A**, drawing of *N. girellae* (total body length including haptor: TL = 3110  $\mu\text{m}$ ); **B**, drawing of *B. seriolae* (TL = 3190  $\mu\text{m}$ ).

## Discussion

In the present study, a practical method to distinguish between *N. girellae* and *B. seriolae* was developed. The difference in the anterior shape on the midline could be observed even between the smallest specimens of experimentally propagated *N. girellae* (TL = 390  $\mu$ m) and *B. seriolae* (TL = 297  $\mu$ m) examined. In addition, Amami-Oshima samples, which were examined and identified by this method (50 flukes each), showed a clear difference in the AS-TL ratio (see Fig. 3). This ascertained the validity of the developed method to distinguish of these two parasite species in aquaculture sites.

Previously described morphological characteristics for the distinction of these two flukes by Yamaguti (1934, 1963), Hargis (1955), Kearns *et al.* (1992) and Ogawa *et al.* (1995) (shape and/or length of hamulus, shape of body and haptor, presence or absence of vaginal opening, and so on) can not be practically applied for the distinction of these parasites materials in the field. Generally, the way to distinguish these flukes on the same fish is to make flattened preparations to observe the above structures. However, this requires live flukes and the process is time-consuming and cumbersome. Moreover, these features are sometimes unapparent in the freshwater bathed or immature flukes.

Using the proposed method, these two parasite species on greater amberjack cultured at Amami-Oshima Island were monitored for almost one year. Although the smaller flukes which had passed through the 0.5 mm mesh were not collected, clear seasonal changes were observed in the ratio of the two flukes. Kasahara (1967) reported the high-temperature limit for *B. seriolae* infection as 29°C. The disappearance of *B. seriolae* in August and September may be due to high water temperature. However, considering that *B. seriolae* did not reappear until early spring, temperature was not the only factor for the fluctuation of the two flukes. Further study is needed to clarify the causes of this variation.

It seems likely that *N. girellae* and *B. seriolae* have different susceptibilities against control treatments such as bathing with either freshwater or hydrogen peroxide solution and orally administered medicines. The manufacturer's instructions for the hydrogen peroxide drug (Marine Sour SP-30, Katayama Chemical Inc., Osaka, Japan) recommend different bathing periods for the treatment of *B. seriolae* in Perciformes (e.g. *Seriola* species) and *N. girellae* in Tetraodontiformes (e.g. tiger

puffer *Takifugu rubripes*). However, these two flukes are currently equated with each other on farming sites. Therefore, on-site distinction of the two parasites is needed to take proper countermeasures which will lead to increased efficacy of treatment, reduce excess cost, be less damaging for fish, and be better for the environment. As the proposed distinction is convenient, it can also be applied to further studies on the skin parasites as well as the practical usage.

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