Parasitology International 63 (2014) 473–478

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Insemination of the monogenean *Neobenedenia girellae* (Capsalidae, Benedeniinae)

K. Ogawa [a,*], S. Shirakashi [b], H. Ishitani [b]

[a] *Meguro Parasitological Museum, 4-1-1, Shimomeguro, Meguro-ku, Tokyo 153-0064, Japan*
[b] *Fisheries Laboratory, Kinki University, Wakayama 649-2211, Japan*

ARTICLE INFO

Article history:
Received 25 July 2013
Received in revised form 15 October 2013
Accepted 16 October 2013
Available online 24 October 2013

Keywords:
Neobenedenia girellae
Insemination
Spermatophore
Monogenea

ABSTRACT

In vitro spermatophore formation and insemination of *Neobenedenia girellae* (Monogenea: Capsalidae, Benedeniinae) were recorded on video and described for the first time. Upon contact of two individuals, the anterior adhesive discs of the donor firmly attached to the dorsal tegument of the recipient and the donor's fore body strongly contracted such that the genital pore region protruded and the penis was pushed anteriorly to protrude through the genital pore. It is hypothesised that the donor penis mechanically damaged the tegument of the recipient. The sperm and spermatophore matrix were released together through the penis, which was placed under the left anterior attachment disc immediately behind the adhesive pad. The spermatophore matrix containing the spermatozoa became solid and attached to the dorsal surface of recipient's body. When observed under scanning electron microscopy, the spermatophores were irregularly shaped, with a diameter of 52–83 μm. Under light microscopy they consisted of a proximal eosinophilic matrix portion and a distal thin-walled portion containing spermatozoa. Both parts were enclosed with a thin outer casing. Insemination occurred during and after spermatophore formation. Three types of insemination were recorded, unilateral and mutual insemination and self-insemination. The presence of self-insemination indicates that even a single *N. girellae* on a cultured fish may cause a significant parasite infection in the entire aquaculture system.

1. Introduction

There are several types of reproduction among benedeniine monogeneans (Capsalidae). Kearn & Whittington [1] described mutual cross-insemination with intromission, attachment of spermatophores to other individuals and self-insemination. Of the 14 genera in the subfamily Benedeniinae [2], *Neobenedenia* is unique in that they do not have a vagina [3]. Reproductive behaviour may be different from other benedeniine monogeneans because of the lack of a vagina, but no information regarding its reproduction is available except for a single case of *in vitro* observation of possible self-insemination in *Neobenedenia melleni* [4].

There have been discussions regarding the taxonomy of several *Neobenedenia* species [4,5]. In Japan, *Neobenedenia* collected from cultured marine fish has been identified as *N. girellae* according to Ogawa et al. [5], whereas this species was synonymised with *N. melleni* by Whittington & Horton [4]. More recently, it was suggested that *N. melleni*, which is known to infect more than 100 marine fish [4], comprises a species complex. This is based on molecular analysis of the large subunit ribosomal DNA [6]. Currently, it is not possible to identify *Neobenedenia* at the species level based solely on morphological characteristics. To avoid further confusion, here, we retain the scientific name *N. girellae,* instead of '*Neobenedenia* sp.' because this study deals solely with the reproductive biology of monogeneans.

When we observed live *Neobenedenia* specimens in a Petri dish with seawater under a stereomicroscope, we observed several specimens attached to other specimens for some time and leaving a spermatophore on the dorsal body surface of the latter. The objective of this study is to describe reproductive behaviour of *N. girellae* for the first time.

2. Materials and methods

Parasites were carefully removed with a scalpel from greater amberjack *Seriola dumerili* cultured at the Fisheries Laboratory, Kinki University, Shirahama, Wakayama Prefecture, Japan. A group of 10 to 20 specimens were transferred to Petri dishes containing clean sand-filtered seawater. They were allowed to move freely in the dish at room temperature and their behaviour was monitored under a stereomicroscope equipped with a digital camera. The insemination behaviour was video recorded and later shown on a computer screen to trace the position of the parasite onto paper. In addition, still pictures were recorded to determine the number of inseminated parasites and to record the location of spermatophores on the recipient body.

Within 1 h after insemination, the parasites were fixed for histology or scanning electron microscopy (SEM). The parasites were fixed in 10%

* Corresponding author.

1383-5769/$ – see front matter
http://dx.doi.org/10.1016/j.parint.2013.10.009

buffered formalin for histology. Briefly, the parasites were dehydrated, embedded in paraffin, cut into serial sections 4 μm in thickness, and stained with hematoxylin and eosin (H&E). For SEM, the parasites were fixed in 1.5% glutaraldehyde and dehydrated in an ethanol series.

3. Results

3.1. Observation of insemination

A total of 13 cases of insemination were recorded on video, in which an entire event was recorded on seven occasions, from the attachment of a donor to a recipient until the separation of the two after the donor planted a spermatophore on the recipient. In nine cases, the fore body of the donor was placed on the recipient (Fig. 1a). In another case in which the recipient was placed on the donor, the left lateral side of the donor's body was partially turned up and its fore body twisted ventrally to attach to the recipient from the dorsal side (Fig. 1b). In this case, the anterior attachment discs of the donor pinched the tegument of the recipient from both sides. Another type of insemination, mutual insemination, was also observed in which two individuals exchanged spermatophores (Fig. 1c). In this case, one worm attached to the other as in Fig. 1a, whereas the other worm maintained a posture similar to the worm in Fig. 1b but without pinching the recipient's tegument. Moreover, there was a case of self-insemination (Fig. 1d) in which the body proper was bent inward towards the middle and the anterior portion twisted and bent inwards again to hold the lateral part of its own body from the dorsal side.

In histological sections, both the sperm reservoir and spermatophore matrix reservoir [as accessory gland reservoir [4,5] in the penis sac of the adult parasite is surrounded by well-developed muscle bundles (Fig. 2). When the muscle contracts, it is likely that the spermatozoa and spermatophore matrix are mixed and released together.

Throughout the insemination process, the anterior attachment discs of the donor firmly attach to the dorsal body surface of the recipient. Although it was not possible to observe the precise events of each insemination process in the video clips, it is assumed that the genital pore region of the donor protruded and was inserted under the left anterior attachment disc, maintaining the genital pore in a fixed position immediately behind the adhesive pad of the disc. The fore body strongly contracted and the penis sac was pushed anteriorly, such that the distal end of the penis reached the protruding terminal region (Fig. 3a). Then, both the sperm and spermatophore matrix were released from the penis (Fig. 3b–c). The spermatophore matrix containing the spermatozoa became solid and attached to the dorsal surface of recipient's body. Subsequently, the fore body was relaxed and the penis sac returned to the original position (Fig. 3d). After the two parasites separated, the spermatophores, which were shaped irregularly and cylindrical and consisted of a light-coloured proximal part and a larger yellow- to brown-coloured distal part in the video recordings, in no case disintegrated or detached from the recipient. After separation, an oblong mark was left in the donor's left adhesive pad, where the spermatophore was held (Fig. 3e). Both the donors and recipients remained stationary in the Petri dish during insemination.

The time required for the entire insemination process ranged from 36 s to 86 s (mean: 56.6 s). A total of 26 spermatophores attached to

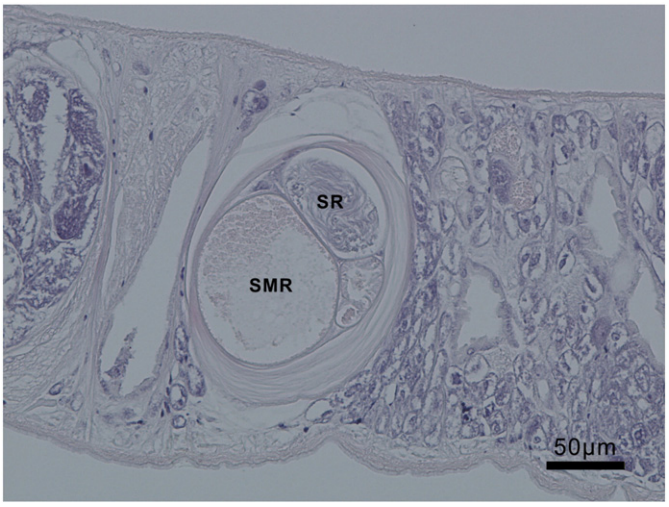

Fig. 2. A transverse section of the proximal portion of the penis sac. Well-developed muscle bundles encircle both the sperm reservoir (SR) and spermatophore matrix reservoir (SMR).

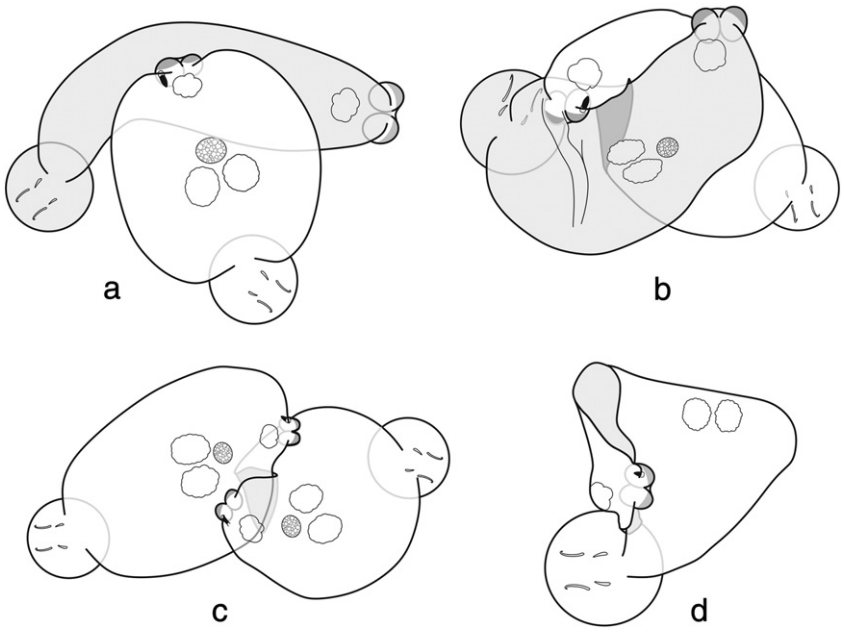

Fig. 1. Semi-diagrammatic drawings of insemination in *Neobenedenia girellae*. Donor parasites (in white) plant a spermatophore on the dorsal side of the recipients (in grey). a, b: unilateral insemination; c: mutual insemination in which parasites (both in white) are donors and recipients; d: self-insemination.