Mating in the Capsalid Monogenean *Benedenia seriolae*, a Skin Parasite of the Yellowtail, *Seriola quinqueradiata*, in Japan

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With Text-figures 1–2

Abstract Mating is described for the first time in a member of the genus *Benedenia*, namely in *Benedenia seriolae* from the skin of the yellowtail *Seriola quinqueradiata*, and is compared with mating in the related monogenean, *Entobdella soleae*. In adult specimens of *B. seriolae* separated from the host and attached to glass, mating is brief, the penis of one hermaphrodite individual being inserted into the relatively spacious vestibule of the vagina of the co-copulant and *vice versa*. There is no spermatophore similar to that attached externally during mating in *E. soleae*. After separation, the same two individuals of *B. seriolae* can sometimes be induced to mate for a second time within a few minutes, by pushing the parasites together. The reproductive system of the parasite is re-described.

Introduction

Monogeneans of the genus *Benedenia* and their close relatives *Entobdella* spp. are skin parasites of marine fishes, the former generally parasitizing round-bodied teleosts and the latter, teleost and elasmobranch flatfishes. Mating in *Entobdella soleae* was observed by Kearn (1970) and was found to involve the mutual exchange of spermatophores, but mating in *Benedenia* has not been described. The opportunity arose to study interactions between living specimens of *Benedenia seriolae* (Yamaguti, 1934) Price, 1939 during a study visit to the Seto Marine Biological Laboratory of Kyoto University at Shirahama, Wakayama Prefecture, Japan in 1990. Although the general arrangement of the reproductive organs and ducts of *B. seriolae* was illustrated by Yamaguti (1934) and later by Hoshina (1968), an examination of living parasites revealed that these authors had overlooked many details or had described other features inadequately. Consequently the reproductive system of the parasite was re-examined. Throughout the study, comparison was made with the mating behaviour and reproductive system of *E. soleae*.

Materials and Methods

Living specimens of *Benedenia seriolae* were removed with a scalpel blade from the skin of cultured yellowtail (Japanese name: hamachi or buri), *Seriola quinqueradiata*, at fish farms in Shirahama belonging to the Inari Fish Culture Company and the Katada Fishermen's Association. The parasites were transferred to a small Petri dish containing filtered sea water at 20°C. Undamaged parasites attached immediately to the glass surface and were transported to the Seto Laboratory in an insulated container. Observations on behaviour were made in a constant temperature room at about 20°C.

Locomotion took place frequently in the containers but rarely resulted in contact between two individuals, but it was found that the haptor, attached by suction to the glass, would slide readily across the glass surface when gently pushed with a brush. This technique was used to move parasites close to each other to provide an opportunity for mating.

Observations on the reproductive system of *B. seriolae* were made using phase contrast or interference contrast microscopy on living parasites or on flattened individuals preserved in 10% formaldehyde and dehydrated, cleared and mounted unstained.

Results

(1) The reproductive system (Text-Fig. 1)

Each testis is perforated by 10–15 vertical columnar structures (muscles?). The vasa efferentia are narrow ducts that soon unite. The region of the vas deferens between the testes and the germarium expands abruptly to produce a conspicuous seminal vesicle, not clearly described by either Yamaguti (1934) or by Hoshina (1968). The vas deferens leaves the seminal vesicle distally, travels around the left side of the germarium and coils extensively before penetrating the lateral wall of the penis sac about half way along its length. Within the penis, the vas deferens follows a longitudinal path towards the distal tip of the organ. Proximally, the penis sac accommodates a conspicuous accessory gland reservoir. The contents of this reservoir are coarsely granular, the granules being spherical or ovoid and ranging in size from 4 to 8 μm. The source of these granules outside the penis sac was not found in the living animal, but, in formaldehyde-preserved, unstained relatively small adults viewed with phase contrast illumination, ducts were seen entering the penis sac from two directions. What appears to be a bundle of ducts from the right side of the body penetrates the proximal end of the penis sac, while a similar duct bundle from the left side of the body enters the penis sac close to the point of penetration of the vas deferens. These ducts contain a finely granular secretion which presumably condenses to form coarser material on entering the reservoir.

The accessory gland reservoir inside the penis sac gives rise to a duct which runs along the penis roughly parallel with the vas deferens. It seems most likely that these two ducts join at the extreme distal tip of the penis and share a common aperture, but they may have separate openings lying close together. The uterus and the penis share a common genital opening close to the left lateral margin of the parasite on the ventral surface. During the contractions involved in egg-laying, the penis is often partly protruded through the common genital opening.

The germarium contains a conspicuous central sac, not described by Yamaguti or by Hoshina. This sac contains what appear to be fully-developed oocytes (or zygotes?) together with spermatozoa and opens anteriorly into the oviduct. Vertical columnar structures similar to those in the testes (see above) were also observed in the germarium. The vagina runs obliquely from the vitelline reservoir to a relatively large aperture on the dorsal surface close to the left margin of the body and just posterior to the common genital opening. The wide vaginal aperture gives access to a roughly cone-shaped vestibule (Fig. 1, dv), which tapers proximally to a
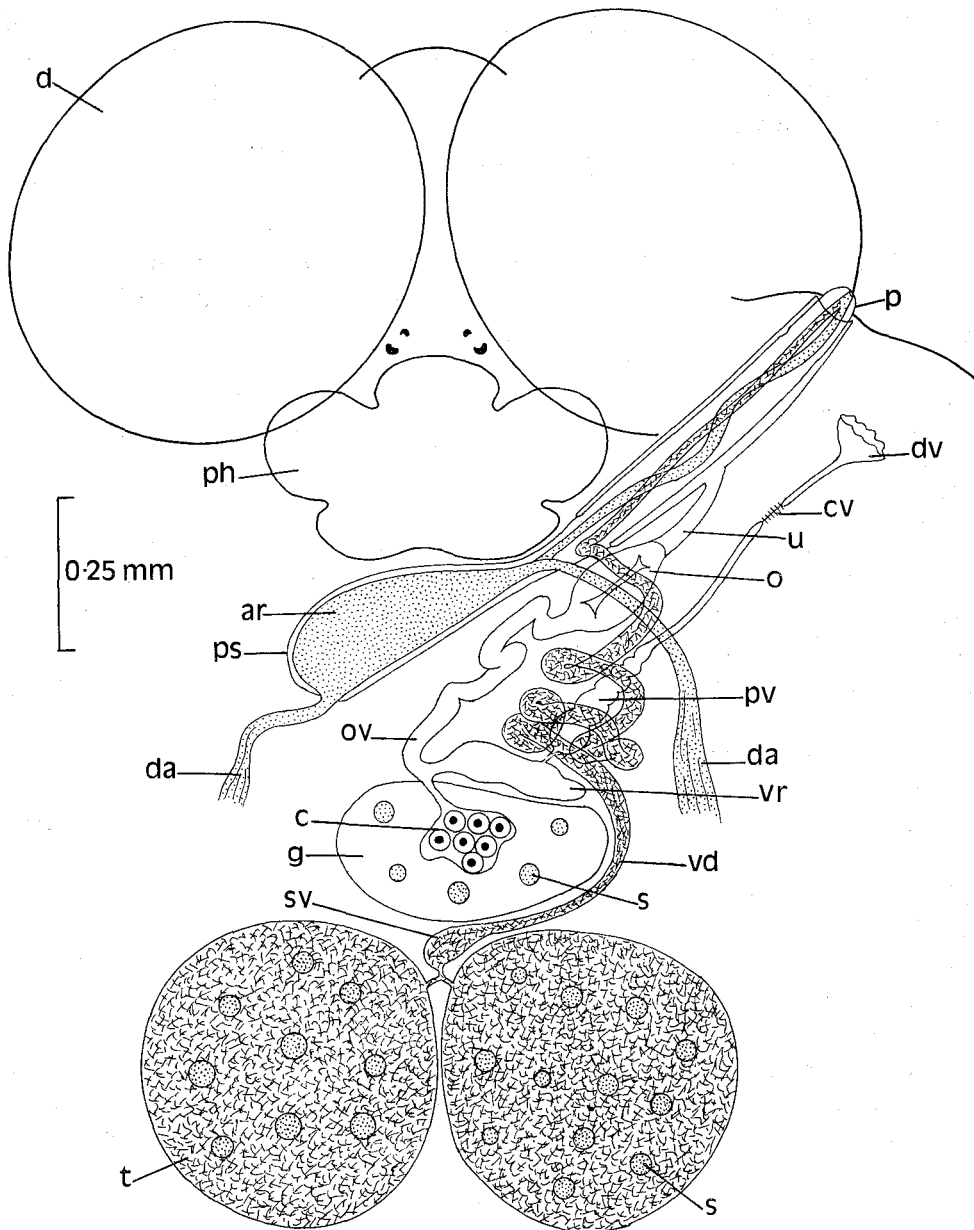
Fig. 1. Reproductive system of *Benedenia seriolae* in ventral view, with vitellarium omitted. ar, Accessory gland reservoir; c, central chamber of germarium; cv, constricted region of vagina; d, anterior attachment disc; da, ducts of accessory gland; dv, distal vestibule of vagina; g, germarium; o, ootype; ov, ovo-vitelline duct; p, penis; ph, pharynx; ps, penis sac; pv, proximal storage region of vagina; s, columnar structure (vertical muscle?); sv, seminal vesicle; t, testis; u, uterus; vd, vas deferens; vr, vitelline reservoir.

tube with a diameter of about 4–5 μm. This vestibule is separated from the proximal tubular region of the vagina (Fig. 1, pv) by a short straight duct with an extremely narrow lumen about 1–2 μm in diameter (Fig. 1, cv). There appear to be circular muscle fibres spaced at regular intervals along this constricted region of ducting and proximal to it the vaginal lumen again increases in size and continues for some distance as a more or less straight tube of fairly uniform diameter. This tube then increases progressively in size, develops folded saccular walls and contains material which is presumably spermatozoa. At the proximal end this saccular region communicates with the vitelline reservoir by another short length of extremely narrow ducting. There are no seminal receptacles, similar to those communicating with the ovo-vitelline duct of *E. soleae* (see Kearn, 1970).

(2) Mating

Parasites separated from the host spend much of their time attached by the haptor to the bottom of the container, in what will be called a "resting" attitude, with the body straight and undulating rhythmically, sometimes with the discs on the head region in close contact with the glass. This attitude is occasionally interrupted by "searching" movements during which the head region swings from side to side, sometimes accompanied by elongation of the body. "Searching" movements may or may not be followed by locomotion. Other common behavioural events include protrusion of the pharynx and its attachment to the glass in an attempt to feed and egg laying.

Mating was observed on several occasions when two parasites were pushed together so that they were attached side by side and orientated in the same direction. A striking change in the posture of one or both parasites signalled imminent mating. This behavioural change involved the lateral bending of the body in a horizontal plane in the direction of the partner, accompanied by protrusion of the penis. A similar response by the partner usually culminated in mating (Text-fig. 2) but if the partner failed to respond, the posturing individual then straightened the body, withdrew the penis and resumed the "resting" attitude.

The pre-mating posturing was not observed when two adjacent parasites were "resting", even when the edges of their bodies were overlapping and making repeated contact as a result of body undulations. However, contact between a "searching" parasite and its neighbour frequently evoked posturing. Since "searching" often took place when parasites were pushed across the bottom of the dish by the experimenter, posturing frequently occurred when such a parasite made contact with another. Posturing did not occur when the edge of the body of an isolated "resting" individual was touched with a needle.

As noted above, the genital opening through which the penis protrudes and the vaginal opening are close together near the left-hand margin of the body, the penis opening ventrally and the vagina opening dorsally. In the situation where two "resting" parasites lie next to each other, with their bodies parallel and their heads pointing in the same direction, considerable body contortion, especially of the in-
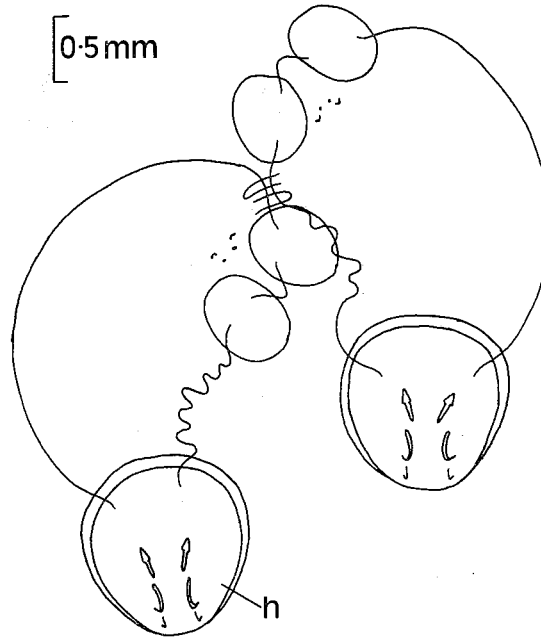
Fig. 2. Mating in *Benedenia seriolae*. h, Haptor.

dividual on the left, is necessary to bring together the genital regions of the co-copu-
lants. This is achieved within a few seconds by the left-hand individual rotating the
anterior region of the body in the horizontal plane to the right, matched by a cor-
responding movement of the other individual to the left. The mutual insemination
event itself is a very brief encounter, lasting less than one second, during which the
strongly-curved penis of each individual enters and leaves the large distal vestibule
of the co-copulant's vagina. The parasites then separate. After separation of mat-
ed individuals in *Entobdella soleae*, each co-copulant carries a spermatophore, at-
tached externally to the ventral surface (Kearn, 1970), but in *B. seriolae* no sperma-
tophore was observed. However, a small mass of dark material was observed in the
distal vestibular region of the vagina of some mated individuals. This material se-
ems likely to be spermatozoa perhaps mixed with the accessory gland secretion from
the reservoir in the penis sac, but when attempts were made to confirm this by trans-
ferring the newly-mated parasite to a compound microscope, the material in the
distal chamber was no longer visible. In *E. soleae* strong contractions of the vaginal
region of the body occur after the separation of mating partners (see Kearn, 1970)
but no such contractions were observed after separation of mating individuals of *B.
seriolae*.

It was found that mating between two individuals did not prevent mating oc-
curring again, sometimes within a few minutes, between the same two individuals.
On one occasion a pair of parasites mated for a second time when pushed together only
5 min after their first mating and less than one hour later again adopted the pre-mating

posture but failed to complete this mating event.

The relative positions of the penis and vaginal openings in *B. seriolae* are such that self-insemination is physically possible, provided that the penis is sufficiently flexible, but on no occasion was self-insemination observed to take place.

Discussion

There is a general similarity in the arrangement of the reproductive organs and ducts in *Benedenia seriolae* and *Entobdella soleae*. Minor differences include, in *B. seriolae*, the absence of glands of Goto and seminal receptacles and the presence of a seminal vesicle at the proximal end of the vas deferens between the testes and the germarium. A more fundamental difference between the two parasites concerns the structure of the vagina and the position of the vaginal opening and this seems to be related to a difference in sperm transfer, *B. seriolae* apparently depositing sperm in the vagina by intromission and *E. soleae* attaching spermatophores externally to its partner.

The vagina of *B. seriolae* has a large dorsally situated aperture leading to a relatively spacious vestibule whereas in *E. soleae* the corresponding aperture is very small, ventrally located and leads to an extremely narrow tube. These differences reflect the fact that during mating in *B. seriolae* the vaginal vestibule accommodates the relatively large penis, whereas in *E. soleae* the equally large penis does not appear to enter the vagina but deposits a spermatophore externally in the region of the vaginal aperture (Kearn, 1970). The uptake of spermatozoa from the spermatophore of *E. soleae* takes place after the co-copulants separate and is accompanied by vigorous contractions of the vaginal region of the body; similar contractions were not observed after mating in *B. seriolae*. The proximal region of the vagina in both *B. seriolae* and *E. soleae* is inflated and provides a storage depot for spermatozoa. In *B. seriolae* this storage region is isolated proximally and distally by lengths of constricted ducting; the distal constricted duct is surrounded by what appears to be circular muscle. Presumably these constrictions have a valvular function permitting movements of spermatozoa in a proximal direction but resisting sperm movements distally. Since there are no seminal receptacles communicating with the ovo-vitelline duct in *B. seriolae* as there are in *E. soleae*, spermatozoa must be transported directly to the central chamber of the germarium via the vitelline reservoir. In this chamber spermatozoa mix freely with mature oocytes and it seems likely that fertilization occurs here as it does in the corresponding chamber in *E. soleae* according to Tappenden (1989). In *Epibdella* (=*Benedenia*) *melleni*, Jahn & Kuhn (1932) described a rather more elaborate intra-ovarian fertilization chamber which they regarded as the proximal region of the oviduct with a communicating seminal receptacle.

The different modes of sperm exchange in *B. seriolae* and *E. soleae* seem well-suited to their microenvironments. The strong water currents generated by the activity of the yellowtail, the fast-swimming, round-bodied host of *B. seriolae*, would threaten

to dislodge externally attached spermatophores, especially during the process of transfer between parasites. In this situation, intromission with internal deposition of spermatozoa is a safer procedure. Adult specimens of *E. soleae*, on the other hand, inhabit the lower surface of the teleost flatfish *Solea solea*, which is known to spend most of the day-time resting on the bottom and at night-time swims relatively slowly (see Kearn, 1971a). *E. soleae* is rarely disturbed by strong currents and spermatophore transfer is probably not a hazardous procedure, but the advantages of spermatophore exchange rather than direct intromission in these circumstances are not apparent.

An important component of the spermatophore of *E. soleae* is a secretion which is stored in a capacious reservoir within the penis sac (see Kearn, 1970); a similar two-chambered reservoir and the extensive gland responsible for the secretion stored in the reservoir, were described by Llewellyn & Euzet (1964) in the related spermatophore-producing monogenean *E. diadema*. Since *Benedenia* spp. and other capsalids have a similar reservoir in the penis sac, it has, in the past, been tempting to regard this feature as indicative of spermatophore production, but the observations on mating in *B. seriolae* reported in the present paper introduce a note of caution here, although they throw no light on the function of this accessory secretion in *B. seriolae*. It is worth noting also that, although Kearn (1971b) did not observe mating behaviour between specimens of *Trochopus pini*, a gill parasite regarded by Kearn as related to *Benedenia*, he commented on the failure of living parasites to deposit spermatophores and noted that the similarity in the sizes of the penis and the vagina were consistent with intromission taking place. Apart from these observations, information on the reproductive biology of capsalids is lacking and it remains to be seen whether intromission and spermatophore transfer are fundamental features of *Benedenia* spp. and *Entobdella* spp. respectively.

If it is assumed that the interaction described in this paper between two adult specimens of *B. seriolae* attached to glass is the natural mating process, and there are no abnormalities of behaviour which would lead one to think otherwise, then intromission is exceedingly brief, lasting less than a second, the quantity of sperm transferred is small and parasites are ready to mate for a second time within a few minutes. Although spermatophores are exchanged rapidly in *E. soleae*, the volume of sperm transferred seems to be greater than in *B. seriolae* and second matings of the same individual were not observed. These differences may reflect differences between the two parasites in mating opportunities, which are governed by factors such as population size, activity of parasites and ability to locate a partner. *B. seriolae* may have more opportunities to mate than *E. soleae* and may be able to take advantage of these opportunities to increase the genetic diversity of its offspring by limiting the volume of sperm it receives at each mating and continually maintaining its readiness to accept a partner. However, although *B. seriolae* is abundant on farmed yellowtail, information on natural population levels is almost entirely lacking. The only record of the parasite from the natural environment seems to be that of Yamaguti (1934) who recorded four parasites on a specimen of *Seriola aureovittata* of unknown

size from the Inland Sea of Japan.

It is worth noting that adoption of the pre-mating posture in *B. seriolae* appears to be associated with body contact during which one or both of the parasites is indulging in "searching" behaviour. Pairs of adjacent "resting" parasites were not observed to adopt the pre-mating attitude, in spite of the fact that their bodies made repeated contact as a result of performing undulating movements, and mechanical stimulation of an isolated individual with a needle likewise failed to induce a sexual response. It is conceivable that "searching" parasites release a chemical substance that stimulates sexual behaviour when contact is made with a potential partner. Small, probably unicellular, glands, similar to those recorded in *E. soleae* by El-Naggar & Kearn (1983) open on the ventral body surface of *B. seriolae* (Kearn, previously unrecorded observation) and may be the source of a chemical stimulant.

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