Benedenia rohdei n. sp. (Monogenea: Capsalidae) from the gills of *Lutjanus* carponotatus (Perciformes: Lutjanidae) from the Great Barrier Reef, Queensland, Australia, with a description of the oncomiracidium

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Abstract

A benedeniine monogenean, *Benedenia rohdei* n. sp., is reported from the gills of the stripey *Lutjanus carponotatus* (Richardson, 1842) (Lutjanidae) from Heron Island and Lizard Island, Queensland, Australia. The oncomiracidium of the new species is also illustrated. *B. rohdei* n. sp. differs from all known species of the genus in the possession of a sclerite at the tip of the penis. Examination of type-specimens of *B. jaliscana* Bravo-Hollis, 1952 has shown that the three penis 'hooks' described by Bravo-Hollis are unsclerotised conical papillae.

Introduction

Examination of the gills of stripey, Lutjanus carponotatus (Richardson, 1842) from Heron Island, Great Barrier Reef, Queensland, Australia in April 1989 by I.D.W. and M.B.-B. and in February 1991 by I.D.W. and G.C.K. revealed specimens of a benedeniine (capsalid) monogenean. In July 1992, I.D.W. also found benedeniines on the gills of L. carponotatus from Lizard Island, Great Barrier Reef. An undescribed capsalid monogenean from the gills of the same species of host was listed by Lester & Sewell (1989) in a checklist of parasites from Heron Island. This entry was ascribed to Professor Klaus Rohde. A preliminary comparison of Rohde's specimens with those collected by the present authors indicated that all the specimens belonged to a single, previously undescribed species and it was decided to make a detailed study of the parasite. Since many living parasites were available, eggs were collected and the oncomiracidia were hatched and studied.

Materials and methods

Live specimens of *Lutjanus carponotatus* were caught by hand-line at the Heron Island Research Station of The University of Queensland at the southern end of the Great Barrier Reef and at the Lizard Island Research Station of The Australian Museum in the northern waters of the Great Barrier Reef. Fish were identified from Grant (1987) and Randall *et al.* (1990). The fish were killed by severing the spinal cord. The gills were removed as quickly as possible, immersed in filtered seawater (FSW) and examined immediately with a stereomicroscope using incident and/or transmit-

ted light. Monogeneans were removed and transferred to a dish containing clean FSW. The anatomy of some living specimens, under slight coverslip pressure, was studied using bright field and phase contrast microscopy. Other specimens were either flattened and preserved beneath a coverslip in 10% buffered neutral formalin (BNF) at room temperature or fixed unflattened in boiling 10% BNF. Some of these parasites were stained with Ehrlich's haematoxylin, acetocarmine or Grenacher's carmine alum, but others were left unstained. All specimens were dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted in Canada balsam. Preserved adult parasites viewed with a compound microscope equipped with a camera lucida were measured using a digitising tablet linked to an IBM-compatible personal computer using a system similar to that described by Roff & Hopcroft (1986). The lobes of the marginal valve were best counted in live specimens or in preserved specimens with the haptor detached from the body because lobes at the anterior end of the haptor were often obscured by the body proper.

Eggs were harvested, pooled, exposed to natural lighting and maintained at a temperature in the range 24-26 °C. Each batch of eggs was transferred to fresh FSW at intervals of 2 or 3 days and the eggs were examined for signs of hatching at similar intervals. When hatching had occurred, living larvae were lightly compressed with a coverslip and examined using bright field, phase contrast and oil immersion apparatus. The description of the larva is based on at least 5 living larvae, 3 of which were measured using a calibrated ocular micrometer. Body length was measured on living larvae subjected to sufficient coverslip pressure to prevent them from swimming; haptoral sclerite lengths were measured after specimens were completely flattened.

All measurements are given in micrometres as the range with the mean in parentheses: where measurements are presented in paired sets separated by a multiplication sign, the first figure is length, the second width. Terminology for haptoral sclerites of capsalids follows that proposed by Kearn (1963). The marginal hooklets are numbered in a posterior-anterior succession as suggested by Llewellyn (1963) and as adopted at the Fourth International Congress of Parasitology (see Euzet & Prost, 1981).

Sources from which specimens were borrowed and museums in which specimens have been deposited are indicated in the text as follows: HWML, University of Nebraska State Museum, H.W. Manter Laboratory, 529 W. Nebraska Hall, Lincoln, Nebraska, USA 68588-5014; IBUNAM, Instituto do Biología Universidad Nacional Autónoma de México, Departamento de Zoología, México; KR, personal collection of Klaus Rohde, University of New England, Armidale, NSW, Australia; NHML, The Natural History Museum, London, UK; QM, Queensland Museum, Brisbane, Australia; USNMHC, United States National Museum Helminthological Collection, Beltsville, Maryland, USA.

Classification of the host is that of Nelson (1984).

Family Capsalidae Baird, 1853 sensu Yamaguti (1963)

Subfamily Benedeniinae Johnston, 1931 sensu Whittington & Kearn (1993)

Genus Benedenia Diesing, 1858 sensu Whittington & Kearn (1993)

Benedenia rohdei n. sp. (Figs 1-3)

Host: Lutjanus carponotatus (Richardson, 1842) (Lutjanidae); "stripey" or "Spanish flag".

Site on host: Gills.

Type-locality: Heron Island, Great Barrier Reef, Queensland, Australia (23°27' S, 151°55' E).

Other localities: Lizard Island, Great Barrier Reef, Queensland, Australia (14°40' S, 145°28' E).

Infection details: At Heron Island: prevalence 94% (n = 17 fish; total length range, 22.5–35 cm); intensity 3–18 (8.7) (intensity calculated from n = 7 fish examined within 3 days of capture; total length range, 29–35 cm). At Lizard Island: prevalence 50% (n = 2 fish; total length range, 24.5–25 cm); the parasitised fish carried 3 specimens.

Specimens studied: Numerous living specimens and 24 whole-mounts (8 unflattened and 16 flattened specimens) of preserved, sexually mature parasites from Heron Island. Type series comprises 16 flattened specimens collected from Heron Island in February 1991: holotype (one slide) (no. GL18312) and 4 paratypes (4 slides) (nos. GL18313-16) deposited in QM; 4 paratypes (4 slides) deposited in NHML (no. 1992.9.7.1-4); 3 paratypes (3 slides) deposited in USNMHC (no. 82577); 2 paratypes (2 slides) in collection of IDW and 2 paratypes (2 slides) in collection of GCK. Eight voucher specimens of parasites from Heron Island collected in April 1989 deposited in HWML (nos. HWML35213). Three voucher specimens (3 slides) of parasites from Lizard Island, collected after the description below had been completed, in collection of IDW.

Material examined for comparative purposes: KR, 4 unflattened, mounted specimens (2 slides) of a Benedenia sp. from the gills of L. carponotatus at Heron Island collected in October 1973; IBU-NAM, No. 24-5 (paratypes), 7 specimens (7 slides) and No. 219-6, 6 specimens (6 slides) of B. jaliscana Bravo-Hollis, 1952.

Etymology: Named for Professor Klaus Rohde of the Department of Zoology, University of New England, Armidale, New South Wales, Australia, who first found this parasite on the gills of L. carponotatus in 1973 at Heron Island.

Description of the adult

All 16 specimens of type-series measured, including all their sclerites, unless stated otherwise. Total length including haptor 1,344–2,575 (1,826); maximum breadth 578–1,046 (822) at a level posterior to testes (Fig. 1A).

Haptor elliptical, noticeably longer than wide, 578–972 (782) \times 342–657 (552). Haptor extrinsic muscles large and conspicuous; onginate near posterior border of testes and displace vitelline follicles from posterior median region of body. Accessory sclerites (modified central marginal hooklets I?; see Kearn, 1963; Llewellyn, 1963) 95–133 (110) long (Fig. 1A,B). Anterior hamuli 100–152 (124) long, slender; distal tip rounded with sharp subterminal spike-like protrusion at 90° to long axis of hamulus (Fig. 1C). Posterior hamuli slender with curved distal tip 60-85 (70) long (Fig. 1D). Fourteen peripheral marginal hooklets (pairs II-VIII) each 7-11 (9) long (n =42 marginal hooklets) (Fig. 1E). Ventral haptor surface without papillae. Marginal valve scalloped, with reasonably consistent and characteristic number of lobes as follows (from n = 3 live specimens): one large lobe between each of marginal hooklet pair II on posterior border of haptor; 2 large lobes between marginal hooklets II and III; one large lobe between marginal hooklets III and IV, and between pairs IV and V; 4-5 (4) small lobes between marginal hooklets V and VI; 5-6 (5) small lobes between marginal hooklets VI and VII; 9-11 (10) small lobes between marginal hooklets VII and VIII; 10-13 (11) lobes between each of marginal hooklet pair VIII on anterior border of haptor (Fig. 1A).

Anterior attachment disks elliptical, 156-267 (197) \times 93-191 (144). Eyes 2 pairs, pigment shielded, dorsal, immediately anterior to pharynx; remnants of lenses sometimes present. Mouth ventral, median, at level of eyes. Pharynx 122-227 (163) \times 147-294 (224). Branched gut caeca, not united posteriorly.

Glands of Goto present; each contains 8 nuclei. Vasa efferentia short. Vas deferens expands to form median seminal vesicle at level of anterior margin of testes, then follows course shown in Fig. 2A. Wall of vas deferens swells immediately before entering penis-sac; swelling characteristically contains 5 nuclei (Fig. 2A). Vas deferens enters penis-sac dorsally near mid-line, follows winding course along length of penis to join duct from accessory gland reservoir near distal tip of penis. Wall of penis-sac weakly muscular, leads into common genital duct passing laterally and anteriorly to open ventrally via common genital aperture on left side of body at level of anterior end of pharynx (Figs 1A, 2A). Accessory gland reservoir with thick wall, occupies proximal end of penis-sac; receives 2 bundles of fine ducts from accessory glands, one bundle enters proximal end of sac, other bundle runs parallel with vas deferens and enters sac more distally (Fig. 2A). Duct



Fig. 1. Benedenia rohdei n. sp. A. Entire animal, ventral view. The intestine and the branches of the vitellarium other than those joining the vitelline reservoir are not shown for the sake of clarity. B. Accessory sclerite. C. Anterior hamulus. D. Posterior hamulus. E. Marginal hooklet. F. Egg; full length of appendage not shown. *Abbreviations*: a, accessory sclerite; aa, anterior attachment organ; ah, anterior hamulus; b, excretory bladder; c, common genital aperture; g, germarium; gG, glands of Goto; h, haptor; he, haptor extrinsic muscles; m, marginal valve; mh, marginal hooklet; o, ootype; p, pharynx; pe, penis; ph, posterior hamulus; s, penis sclerite; t, testis; te, tendon; v, vagina; vf, vitelline follicles. Scale-bars: A, 500 μ m, B, C, D, 50 μ m; E, 10 μ m; F, 100 μ m.



Fig. 2. Benedenia rohdei n. sp. A. Reproductive system, ventral view. Vitellarium (except vitelline reservoir and associated ducts), bladders and intestine omitted. B. Penis sclerite in side view. X denotes measurement that was made. C. Penis sclerite viewed from distal (serrated) edge. *Abbreviations*: ag, accessory gland duct; agr, accessory gland reservoir; ic, internal (fertilisation?) chamber of germarium; M, ducts of Mehlis' gland; ov, ovovitelline duct; ps, penis sac; sp, spike of penis sclerite; sv, seminal vesicle; sw, swelling of wall of vas deferens; u, uterus; vd, vas deferens; vp, vaginal pore; vr, vitelline reservoir. Other lettering as in Fig. 1. *Scale-bars*: A, 250 μ m; B, 10 μ m; C, 5 μ m.

from accessory gland reservoir joins vas deferens near distal tip of penis. Penis protrusible via common genital duct and common genital aperture. Distal tip of penis armed with sclerite 12-14 (13) wide (n = 4 sclerites, measures as shown in Fig. 2B); sclerite gutter-shaped along its longitudinal axis (Fig. 2C); distal edge with serrated appearance due to teeth and single, long spike.

Germarium with relatively large internal fertilisation (?) chamber containing ripe oöcytes and some spermatozoa. Uterus short (Fig. 2A). Vaginal opening on dorsal surface, posterior to common genital aperture, at level of middle of pharynx (Fig. 2A). Distal region of vagina narrow, 13-26 (19) long (n = 2), then abruptly expands and travels posteriorly to enlarged proximal chamber communicating with vitelline reservoir by short, narrow duct, apparently surrounded by circular muscles. Spermatozoa observed in proximal chamber and wide tubular regions of vagina. Vitelline follicles extend anterior to eyes and posteriorly to haptor peduncle, but, posterior to testes, follicles are displaced from median region by extrinsic muscles of haptor.

Eggs tetrahedral (Fig. 1F), sides 77–120 (106) long (n = 4 free eggs); appendage slender 759– 1,734 (1,216) long (n = 5 eggs) from one of 3 nonoperculate poles.

Description of the oncomiracidium

The eggs of *B. rohdei* hatched after 4–6 days at 24–26 °C. The oncomiracidium of *B. rohdei* n. sp. (Fig. 3) is about 240 long (n = 3) and its anatomy is shown in Fig. 3. The lengths of the haptor sclerites (n = 3 larvae) are as follows: accessory sclerites 17–18 (17); anterior hamuli 29–32 (30); posterior hamuli 22–24 (23); marginal hooklets 9–10 (9). Further description is unnecessary because of the similarity with the oncomiracidium of *B. lutjani*, which has been described by Whittington & Kearn (1993).

Differential diagnosis for B. rohdei n. sp.

B. rohdei differs from other species of the genus in possessing a sclerite at the tip of the penis. It is a parasite of the gills of *Lutjanus carponotatus*.



Fig. 3. The anatomy of the oncomiracidium of *Benedenia* rohdei n. sp. Entire animal, ventral view. Abbreviations: am, anterior median head gland; bg, body gland; ci, cilia; d, domus; du, duct of haptor gland(?) (haptor glands not observed); e, pigment-shielded eye; f, flame cell; gG, gland of Goto(?); i, intestine; l, lateral head glands; oe, oesophageal gland duct; pm, posterior median head glands. Other lettering as in Fig. 1. Scale-bar: 100 μ m.

Discussion

With the proposal of *Benedenia rohdei* n. sp., the total number of known species in the genus *Benedenia* is 25. The gill parasite *B. rohdei* is readily distinguished from *B. lutjani* Whittington & Kearn, 1993 from the fins and body skin of the same host, Lutjanus carponotatus. B. rohdei is larger, has a longitudinally elongate haptor and a penis sclerite, and the vitelline follicles are displaced from the posterior median region of the body posterior to the testes by the large extrinsic muscles of the haptor. In fact, the possession of a penis sclerite has been reported in only one species of the genus, namely B. jaliscana Bravo-Hollis, 1952. Bravo-Hollis (1952) reported that the distal end of the large penis of B. jaliscana possessed three stout hooks of which two are clearly depicted in her figure 1 and are distinctly curved. Buhrnheim et al. (1973) accepted Bravo-Hollis' description and described the copulatory organ of B. jaliscana as an 'armed cirrus'. Our examination of specimens of B. jaliscana has confirmed that the penis bears three projecting structures, one being close to the aperture at the tip and the other two in more proximal positions. However, the projections were not sclerotised or hook-like and would be better described as elongate papillae with conical tips. Thus, the possession of a penis sclerite by B. rohdei appears to be a unique feature not present in any other known member of the genus.

Sclerotised structures associated with the male copulatory organs of monogeneans may serve one or more of the following functions: a tubular sclerotised ejaculatory duct or an accessory rod-shaped sclerite may provide rigidity during intromission; sclerites may provide a firm basis for muscle insertion; hooks or opposable jaws may serve to hold parasites together during copulation (Llewellyn, 1960); and hooks or tubular sclerites may be involved in hypodermic impregnation (Macdonald & Caley, 1975; Llewellyn, 1983). The penis sclerite of B. rohdei bears little resemblance to any of the structures considered above and, consequently, it is difficult to ascribe a function. The sclerite is not a development of the penis duct but is situated on the outer surface of the organ close to the penis opening. It is a single structure, longitudinally shaped like a gutter, but with projecting teeth at its distal edge, one of which is spike-like and longer than the others. These teeth are mostly rather blunt and even the longer spike has no recurved point and clearly could not act as a hook. The long spike might seem suitable for perforating the tegument, as a first step towards hypodermic impregnation, but there is no evidence that this takes place. In fact, Kearn & Whittington (1992) observed specimens of B. rohdei (reported as Benedenia sp. 1) indulging in mutual copulation in which the penis of one individual was protruded and inserted in the vagina of the co-copulant for about one minute. Since the penis is relatively large this means that the narrow distal region of the vagina must expand considerably to accommodate it, but there is no corresponding sclerite within the vagina that might serve to interlock with the penis sclerite and hold the two individuals together. Nevertheless, if appropriately orientated, the penis sclerite might resist premature withdrawal of the penis and disengagement of the co-copulants. Two mating parasites are held together only by the interlocking penises and vaginae, and the strong and continual gill-ventilating current of the host is likely to place severe strain on this connection, threatening to separate mating partners and interrupt insemination.

Another distinctive feature of *B. rohdei* is the shape of the haptor which is longitudinally elongate. This distinctive shape may be related to the adhesive attitude of the parasites. Whittington & Kearn (1991) showed that *B. rohdei* (cited as *Benedenia* sp. 1) attaches itself to the inner edge of the primary gill lamella of *L. carponotatus* by folding the haptor longitudinally. Since the haptor is narrow, interference by this large parasite with the secondary gill lamellae and with the respiratory functions of the gills will be minimal, and this may be important for host survival and hence for the survival of the parasite.

The oncomiracidium of *B. rohdei* has been examined and is closely similar to those of *B. lutjani* (see Whittington & Kearn, 1993) and *B. seriolae* (Yamaguti, 1934) (see Kearn *et al.*, 1992). The larvae of *B. rohdei* and *B. lutjani* from the same species of host are indistinguishable apart from a difference in the lengths of the haptor sclerites, those of *B. rohdei* n. sp. being slightly larger than those of *B. lutjani*, which are as follows: accessory sclerites 15; anterior hamuli 22– 24 (23); posterior hamuli 18–20 (20); marginal hooklets 7–8 (8). The larvae of each of these species have the same number and distribution of flame cells, but the oncomiracidium of *Neobenedenia* (= *Epibdella*) *melleni* (MacCallum, 1927) is reported to have more flame cells in the body and in the haptor (Jahn & Kuhn, 1932). If this difference is confirmed, it may be a distinction at the generic level between *Benedenia* Diesing, 1858 and *Neobenedenia* Yamaguti, 1963.

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