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Short Communication

Histopathology associated with haptor attachment of the ectoparasitic monogenean *Neobenedenia* sp. (Capsalidae) to barramundi, *Lates calcarifer* (Bloch)

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Capsalid monogeneans are harmful ectoparasites of ornamental and farmed fishes in tropical/subtropical marine environments (Thoney & Hargis 1991; Hirazawa *et al.* 2011; Whittington 2012). *Neobenedenia* spp. have low host specificity, a direct life cycle, high fecundity and robust eggs, which contribute to their ability to inflict mass mortalities in aquaculture (Ogawa *et al.* 1995; Deveney, Chisholm & Whittington 2001; Rückert, Palm & Klimpel 2008; Whittington 2012).

Neobenedenia spp. attach to external surfaces of their host using two attachment organs located anteriorly and one larger posterior attachment organ called the haptor. The haptor is believed to act as the principal anchoring organ of the parasite to the host (Whittington 2012). This organ has chitinous structures that provide mechanical attachment including paired anterior hamuli, accessory sclerites, posterior hamuli and peripheral hooklets (Whittington & Horton 1996). A marginal valve on the haptor allows the organ to create suction on the host. The anterior attachment organs lack accessory chitinous structures and are located directly above the pharynx, which is used to graze on epidermal and mucous cells of the fish (Whittington 2012).

The strong adhesion of the haptor, mechanical attachment of the hamuli and sclerites (Ogawa *et al.* 2006), as well as the adhesion of the anterior attachment organs when grazing, can damage the host's epidermis (Kaneko *et al.* 1988; Ogawa *et al.* 2006; Hirazawa *et al.* 2010), increasing the likelihood of secondary infections (Thoney & Hargis 1991; Leong & Colorni 2002). Lesion-level histopathology sections that exhibit the haptor–host interface are rare and examinations of the change in epidermal morphology associated with infected fish are limited. In this study, we examined epidermal damage associated with the site of haptor attachment in six separate regions of farmed barramundi. Characteristics evaluated in *Neobenedenia* sp.-infected fish included changes in epidermal thickness and in the number of mucous cells compared with similar areas in uninfected controls.

Forty hatchery reared freshwater *L. calcarifer* (mean 125 ± 25 L_T mm) were acclimated to sea water for 24 h prior to 20 fish each being infected with 20 *Neobenedenia* sp. oncomiracidia each (see Hutson *et al.* 2012; James Cook University ethics approval no. A1857). The remaining 20 fish were not infected. The *Neobenedenia* sp. used in this study is presently unidentified given the absence

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of diagnostic criteria to differentiate between geographical/host isolates and species (Whittington 2004, 2012). Parasites were accessioned in the Australian Helminth Collection (AHC), South Australian Museum Australia (SAMA: AHC 35461). Twenty-one days post-infection all 40 fish were killed with an overdose of Aqui-S aquatic anaesthetic in sea water, which did not kill parasites or cause them to detach from the host (see Sharp *et al.* 2004). Each fish was then immersed in a shallow tray containing sea water (35 g L^{-1}), and tissue samples bearing an individual live parasite were collected from infected fish using a surgical scalpel blade. Tissue samples were collected from the mandible, operculum, middle body, ventral body and caudal fins. Samples were approximately 1 cm^2 and consistently collected from equivalent locations within regions on uninfected and infected fishes. Eyes were collected whole. Samples were fixed in 1% Bouin's solution for 48 h prior to being routinely processed, paraffin embedded, and 4 μm sections stained with haematoxylin and eosin (Gibson-Kueh *et al.* 2012). Sections were made so that the interface between the haptor and the host body surface could be observed by light microscopy. Some parasites detached during the fixation and embedding process, which limited the total number of samples available for analysis (Table 1). Parasites detached from all eye preparations and were not included in the analyses.

Epidermal thickness and the number of mucous cells were quantified beneath the haptor, the epidermis adjacent to the haptor (internal control) and on equivalent epidermis from uninfected fish (Table 1). Epidermal thickness was measured from the basal epithelial layer to the external or apical layer and mucous cells were counted in four selected microscopic fields along the epidermal layer in each tissue sample at 400× magnification. In the case of infected fish, microscopic fields were selected within the haptor–host interface and haphazardly on epidermis adjacent to the haptor (internal control). Statistical analysis was performed using S-plus 8 from Spotfire®. One-way ANOVAs were used to analyse differences in epidermal morphology between uninfected and infected fish within each region. Differences between pairs of means were determined using a Tukey's HSD test. A chi-squared contingency test was used to examine differences in the number of mucous cells. Significance was accepted at $P < 0.05$.

Table 1 Total number of sections examined for histopathology, epithelial thickness and mucous cell counts for each region

Region	Condition	No. fish	No. skin sections examined
Mandible	Uninfected	6	6
	Infected	4	5
Operculum	Uninfected	4	5
	Infected	6	6
Middle body	Uninfected	7	8
	Infected	5	5
Ventral body	Uninfected	5	5
	Infected	6	6
Caudal fin	Uninfected	2	2
	Infected	6	6

The haptor of *Neobenedenia* sp. caused direct mechanical damage to the fish epidermis and triggered epidermal morphological changes. Dermal inflammation (Fig. 1d) and epidermal loss (Fig. 1c, d) were observed in the majority of infected regions but was absent in uninfected controls (Fig. 1a,b). The marginal valve of the haptor caused mechanical compression of the epidermis (Fig. 2a,b,d) and may have contributed to detachment of the basal layer (Fig. 2c). Accessory sclerites ruptured the epidermal layer (Fig. 2b, inset). Rarely, was the haptor directly associated with haemorrhage or epidermal inflammation (Figs 1d & 2d, inset), and there was no evidence of ulceration in infected fish.

Infected fish had significantly lower mucous cell counts in all regions compared with uninfected fish ($P < 0.001$, Fig. 3a). Infected fish exhibited a trend for lower epidermal thickness in all regions compared with uninfected controls (Fig. 3b), which is congruent with previous studies (Hirayama, Kawano & Hirazawa 2009). Internal controls indicated that morphological changes were not limited to areas under the haptor, although the greatest impact was quantified at the site of haptor attachment (Fig. 3). This surrounding damage could be a consequence of the feeding activity of the parasite. *Neobenedenia* spp. may use the haptor as a fixed rotation point using the anterior attachment organs to aid feeding within the radius of the total body length. Furthermore, movement of capsalid monogeneans over the body surface of the host may also account for the observed surrounding damage (Whittington & Ernst 2002; Ogawa *et al.* 2006). *Neobenedenia* spp. are believed to migrate following initial recruitment on the host (Ogawa *et al.* 2006; Hirayama *et al.* 2009; Hirazawa *et al.* 2011); however, it is challenging to track migrations through

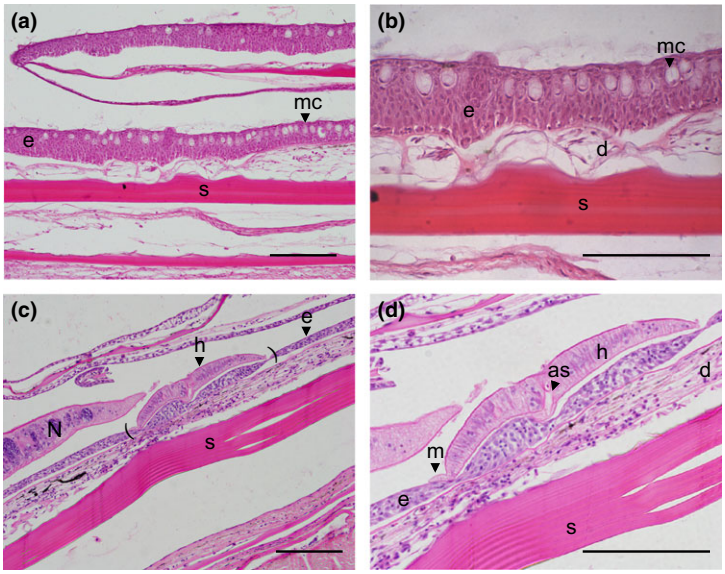

Figure 1 Histopathology of the ventral body surface in uninfected (a,b) and infected *Lates calcarifer* with *Neobenedenia* sp. (c,d). Uninfected fish had numerous mucous cells in the epidermis (a,b). The parasite's haptor compressed the epidermal layer of the host (c, brackets). Infected fish presented thinner epidermis and mucous cells were rare at the haptor–host interface (c,d). N = *Neobenedenia*; as = accessory sclerites; d = dermis; e = stratified squamous epithelial cells; h = haptor; m = marginal valve; mc = mucous cells; and s = scale. Morphological terms follow Whittington & Horton (1996) and epidermal morphology follows Takashima & Hibiya (1995). (H&E stain, a, c = ×200; b, c = ×400), scale bars = 100 μm.

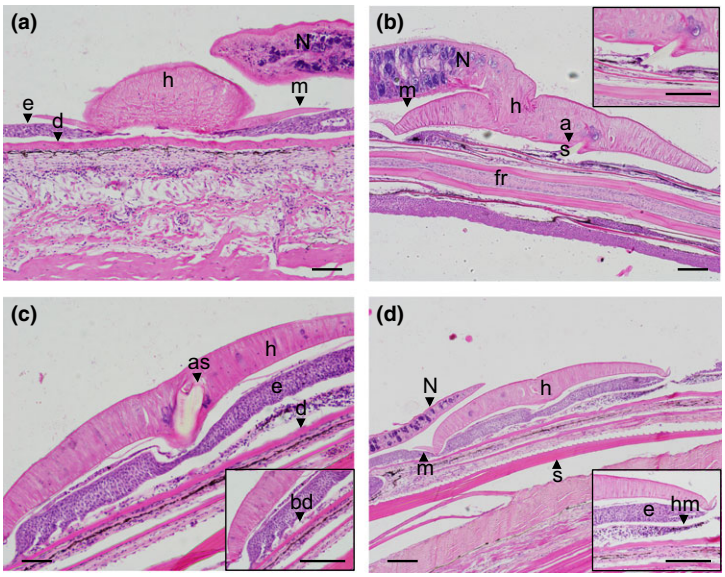

Figure 2 Histopathology associated with haptor attachment of *Neobenedenia* sp. to *Lates calcarifer* on the middle body (a,d), caudal fin (b) and ventral body surface (c) of infected fish. Epithelial loss was observed in the majority of samples at the haptor–host interface (AD). Accessory sclerites caused epidermal damage (b, b inset, c) and the marginal valve compressed the epidermis of the host (a, b, d). N = *Neobenedenia*; as = accessory sclerite; bd = epithelial basal layer (detached); d = dermis; e = stratified squamous epithelial cells; fr = fin ray; h = haptor; hm = haemorrhage; m = marginal valve; and s = scale. Parasite terminology follows Whittington & Horton (1996) and epidermal morphology follows Takashima & Hibiya (1995). (H&E stain, ×100, insets = ×400), scale bars = 100 μm.

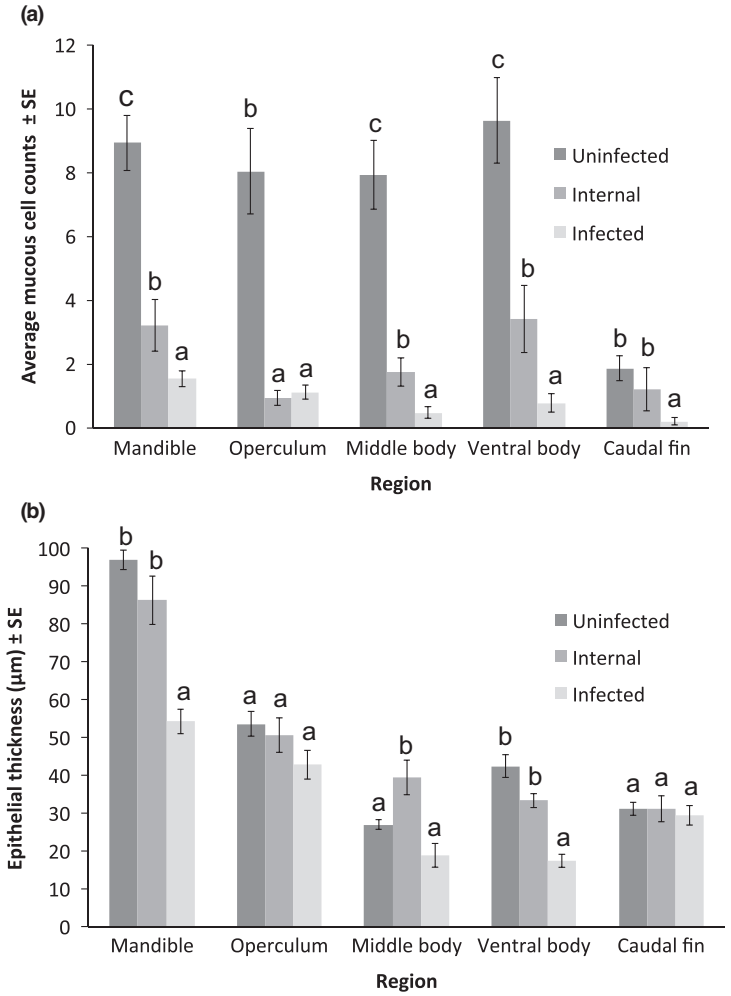

Figure 3 Average mucous cell count (a) and epidermal thickness (b) of sampled regions on infected and uninfected *Lates calcarifer*. Internal controls were taken from comparable locations adjacent to the haptor–host interface. 'a', 'b' and 'c' = differences between pairs of means determined using Tukey's HSD test in (a) and differences in count proportions using a chi-squared contingency test in (b).

time due to their small size and cryptic nature (*Neobenedenia* spp. have transparent bodies; Whittington 1996). The extent to which monogeneans remained anchored to a single location on the host is unclear; however, low standard error observed within infected regions (Fig. 3) indicates that consistent damage occurred over the experimental period.

Epidermal damage can impair the host's immune response to external pathogens. Cutaneous mucus, secreted by mucous cells present in the epidermis, is an important component of teleost immune responses and is considered the first line of defence against infection through skin epidermis (Zhao, Findly & Dickerson 2008). A significant drop in the number of cutaneous mucous cells (Fig. 3a) could affect the fish's ability to withstand other opportunistic pathogens (Bonga 1997; Subramanian, MacKinnon & Ross 2007; Zhao *et al.* 2008). Teleost epidermis is a metabolically active tissue; significant epidermal damage can affect ion and thermal regulation, sensory perception and locomotion (Elliott 2000). Reduced epidermal thickness and diminished numbers of mucous cells, associated with parasite attachment, could impair the host's metabolic and regulatory processes and expose the host to other opportunistic pathogens.

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