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The anatomy and attachment mechanism of the haptor of a *Capsala* sp. (Platyhelminthes: Monogenea: Capsalidae) on the blue marlin, *Makaira nigricans* (Istiophoridae)

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Abstract

The muscular anatomy and fine structure of the haptor of a monogenean, *Capsala* sp., together with an observational distribution study, are described from specimens collected from the dorsolateral and ventral surfaces of the blue marlin, *Makaira nigricans*. The haptor is composed of seven loculi, which are arranged around a central pair of accessory sclerites. Marginal hooklets and hamuli were not observed. The presence of an outline left by the haptor on the host's skin and the lack of evidence of an adhesive secretion suggested that the haptor attaches primarily by suction. Tendons originating in the extrinsic peduncle muscles insert, via a proximal notch in the accessory sclerites, into the basal tegumental lamina on the ventral surface of the haptor. On contraction the centre of the haptor is thought to be drawn upwards both directly by the extrinsic tendons in the haptoral wall but also by the accessory sclerites which are brought into a vertical position. This increases the volume beneath the haptor, consequently reducing the pressure and thus producing suction. The ribbed marginal valve prevents the inward movement of water. Intrinsic circular and radial muscles in the haptoral wall are also thought to produce suction by drawing the haptoral wall inwards, again increasing the volume beneath and causing a pressure reduction. It is suggested that interconnecting fibres observed between the intrinsic muscles may store elastic energy, allowing *Capsala* sp. to maintain suction attachment without having constantly to contract its muscles. Papillae on the inner (=ventral) surface of the haptor are suggested to aid attachment by firstly spreading the negative pressure over the inner surface of the haptor and, secondly, through resisting the shear forces encountered as the fish swims. Both functions are achieved by increasing the surface area of contact with the host's skin, and therefore the frictional forces. Theoretical estimates of the suction efficiency indicate that suction is double the maximum theoretical drag forces which would be experienced by *Capsala* sp. when *M. nigricans* is swimming at speeds of both 1 and 20 $m\,s^{-1}$ and suggests that suctorial attachment is efficient. The distribution of *Capsala* sp. is not thought to be restricted by the surface topography of the host, demonstrated by the fact that individuals were located in both the roughest and smoothest areas of the marlin's skin surface. Distribution is therefore thought to be influenced by other factors such as hydrodynamics, nutritional value of the attachment site, immunological restrictions or cross-fertilization between parasites.

Keywords: *Attachment mechanism,* Capsala, *haptor,* Makaira, *Monogenea, suction*

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Introduction

The exclusively parasitic members of the phylum Platyhelminthes, belonging to the class Monogenea, are ubiquitous on the external surfaces of both teleosts and elasmobranchs (Kearn 1994), occurring less commonly on the internal surfaces of these hosts. Much of the success of monogeneans is attributable to the morphological and structural diversity of the primary attachment organ, known as the haptor. This can attach by hooks, suction or adhesives, or a combination of these, allowing monogeneans to colonize a variety of attachment sites (Kearn 1994).

Some of the largest known monogeneans belong to the Capsalidae, a group of fish ectoparasites comprising around 45 genera (Whittington et al. 2004), which includes a number of important pathogens of cultured fish (Ogawa et al. 1995; Whittington 1996). Capsalids attach predominantly by suction generated by the haptor, but also by hooks, which together provide secure attachment to the scaled, mucus-covered external surfaces of their fish hosts, some of which are the fastest fish in the ocean and therefore subject to large hydrodynamic forces of drag. For example, *Capsala* species are commonly found on the blue marlin, *Makaira nigricans* (Lacépède, 1802), which generally cruises at around $1\,m\,s^{-1}$ in the top 10 m of the ocean. However, these fish are also thought to be capable of reaching speeds of around $20\,m\,s^{-1}$ during short 10–30 s swimming bursts either at the surface or occasionally when diving down to 200 m (Block et al. 1992).

Haptoral attachment is employed alternately with the anterior attachment areas, which can be temporarily glued to the host's surface, to facilitate leech-like movement. This reflects the mobility requirements of monogeneans, moving to new areas when the epidermis on which they feed may become depleted, also allowing cross-fertilization and possibly avoiding stimulation of a host immune response (Kearn 1999).

Much of our current understanding of how the Capsalidae attach has originated from studies of *Entobdella soleae* (van Beneden and Hesse, 1864) (see Kearn 1964). However, suctorial attachment in other capsalids, with different haptoral structures, is not well understood. This lack of understanding has been compounded by the phenetic classification of the Capsalidae to date (see Whittington et al. 2004), and has probably also been hindered by problems associated with sample collection from their wide-ranging hosts. The variable environmental conditions encountered by individual capsalids are also likely to complicate identification, since there may be significant intraspecific variation in body size and morphology (I. D. Whittington, personal communication). In view of this gap in our understanding of capsalid attachment mechanisms, the aims of the current study were to examine the anatomy of the haptor in a *Capsala* sp. and, based on this, to provide an explanation of the likely mechanism by which the haptor produces suction. The theoretical efficiency of the suctorial attachment is also estimated in relation to the drag forces experienced on the surface of the host.

Suckers generally require a relatively smooth surface in order to form an air- or water-tight seal (Nachtigall 1974; Vogel 1988). The haptor of capsalids is not thought to be an exception to this rule. However, preliminary observations of *Capsala* sp. have shown that they attach to a wide variety of host sites which appear to vary in their surface topography. This may indicate that the distribution of *Capsala* is not restricted to smooth surfaces. The second aim of the study was therefore to determine if this is indeed the case, by conducting a distribution study of a *Capsala* sp. on the blue marlin, *M. nigricans*, and comparing the results with the host's skin topography.

Materials and methods

An observational distribution study of a *Capsala* sp. on *Makaira nigricans* was made from 15 recently killed fish (168 ± 74 kg) collected at the Port Stephens Game Fishing Tournament (New South Wales, Australia) in February and March 2002. The presence/absence of capsalids was noted on the dorsolateral and ventral regions of each fish that was brought into the weigh station. The exact number of capsalids was not recorded because there was insufficient time to do so. Instead, an observational study was conducted as the best method of rapidly and reliably assessing parasite distribution.

Specimens of a *Capsala* sp. were collected together with skin from around their attachment sites on *M. nigricans* and all samples were fixed in 70% ethanol. The sclerotized attachment structures of two capsalids were isolated to aid identification, adapting the methodology described by Shinn (1996). This involved dissolving the surrounding tissue by enzymatic digestion for a reduced period of 24 h—a time sufficient to release the structures from the haptor in this study.

Five unattached specimens and one attached specimen, together with host skin samples from the attachment sites, were dehydrated for scanning electron microscopy (Philips 515 SEM), through a graded ethanol series to acetone, critical-point dried, mounted on metal stubs, coated in 20 nm of gold, and viewed at 5 kV.

One attached capsalid was prepared for histological sectioning, first by removing the scales from the fish's skin using forceps, preventing these from reducing the ease with which the samples are cut. The sample was then dehydrated through a graded ethanol series to Histoclear™, embedded in paraffin wax, sectioned at 7 μm, and stained with Massons trichrome.

Two monogeneans were dehydrated through a graded ethanol series to 100% ethanol, cleared in methyl salicylate, and mounted on a microscope slide in a mixture of 1.6 g of Canada balsam in 1 ml of methyl salicylate for differential interference contrast (DIC) analysis.

Attempts were made to gain reliable estimates of the suction force of a *Capsala* sp. during specimen collection, to compare with the theoretical drag forces experienced when *M. nigricans* is cruising and during bursts of speed. This would have given a measure of the suction efficiency. However, all *M. nigricans* had been captured and out of the water for at least 6–8 h and consequently most of the parasites examined were either dead or weakened, which reduced the attachment strength. Therefore an estimate of the suction force was made by calculating the theoretical drag forces on the haptor.

Results

All of the capsalids studied were identified as belonging to *Capsala* using generic characters (pharynx with a constriction; testes usually, if not always, extending laterally to intestinal crura) (Sproston 1946; Chauhan 1953) and previous host records (Vigeras 1935; Dollfus 1949; Chauhan 1951; Dyer et al. 1992). However, the species was unknown due to problems with the group's current taxonomic status (Whittington et al. 2004). Despite this, all specimens examined possessed the same features and were therefore treated as the same species.

Specimens were approximately oval and had a mean total length of 6.9 ± 1 mm ($n=5$) and width 5.1 ± 0.5 mm ($n=5$) (Figure 1a). Dorsal marginal spines were not observed. Ventrally, the body surface was covered in papillae and there was a posterior haptor and a

pair of circular anterior attachment areas. These anterior organs were of mean diameter 1.6 ± 0.2 mm ($n=5$) and their ventral surface was covered in numerous papillae.

Anatomy of the haptor

The posterior haptor was circular with a mean diameter of 3.2 ± 0.5 mm ($n=5$) (Figure 1b).

It was connected via a stalk (=the peduncle), to the ventral body surface. Ventrally, the haptor was divided into seven radial loculi surrounding a central region. The surface of each loculus was covered in papillae with a mean height of 0.03 ± 0.01 mm and width 0.04 ± 0.01 mm ($n=5$) (Figure 1c).

The density of papillae increased from zero at the central-most septa of the loculi, to almost complete coverage of the ventral haptoral surface at the outermost edge of the loculi. The central loculus of the haptor was devoid of papillae. The haptoral margin was composed of a valve with transverse divisions at 0.09 ± 0.01 mm ($n=5$) (Figure 1d).

Hamuli and marginal hooks were not observed. However, a pair of accessory sclerites was present (Figure 1b, e).

The tips projected 0.4 ± 0.02 mm ($n=5$) from sheaths and terminated in rounded, anteriorly directed points. The proximal end of the accessory sclerite was notched (Figure 1f).

The total length of the accessory sclerites, freed by enzymatic digestion, was 0.8 ± 0.02 mm ($n=5$) (Figure 1f). Beginning just anterior to the tip, longitudinal surface ridges extended 0.35 ± 0.01 mm ($n=5$) posteriorly down the length of the accessory sclerite, around the complete circumference. They were spaced regularly at 2–7 μm intervals (Figure 1g), depending on their position on the tapered accessory sclerite.

Examination of the haptoral musculature with DIC revealed a pair of intrinsic muscles, each originating at the proximal end of the accessory sclerites, that passed anteriorly over the haptor and inserted on the opposite side at the base of the peduncle. This arrangement formed a cross-shape towards the anterior of the haptor (Figure 2a, b).

Two large extrinsic peduncular tendons passed via the proximal notches on the accessory sclerites and inserted via two lateral tendons in the ventral haptoral wall (Figure 2b). Intrinsic circular and radial muscles were observed throughout the haptoral wall, interspersed with other connective tissue, the nature of which was uncertain (Figure 2b). There was no evidence of muscles within the septa of the loculi or the marginal valve.

Attachment site of the haptor

Histological sections of a parasite attached to marlin skin showed that the haptor was affixed to the host's epidermis (Figure 3a). It was not possible to see whether the accessory sclerites were embedded in the skin because they were too hard to cut and the sections tore in these areas due to the hard nature of the sclerotized material.

Scanning electron micrographs of the host's skin surface after the parasite was removed revealed a mucus outline beneath the anterior attachment areas (Figure 3b)—the origin of which was unclear. Posteriorly, there was an impression of where the haptor had been attached, although no mucus was evident here (Figure 3c). An impression of the marginal valve was also observed, together with more faint indents where the septa were. No puncture marks were found that corresponded to the location of the accessory sclerites.

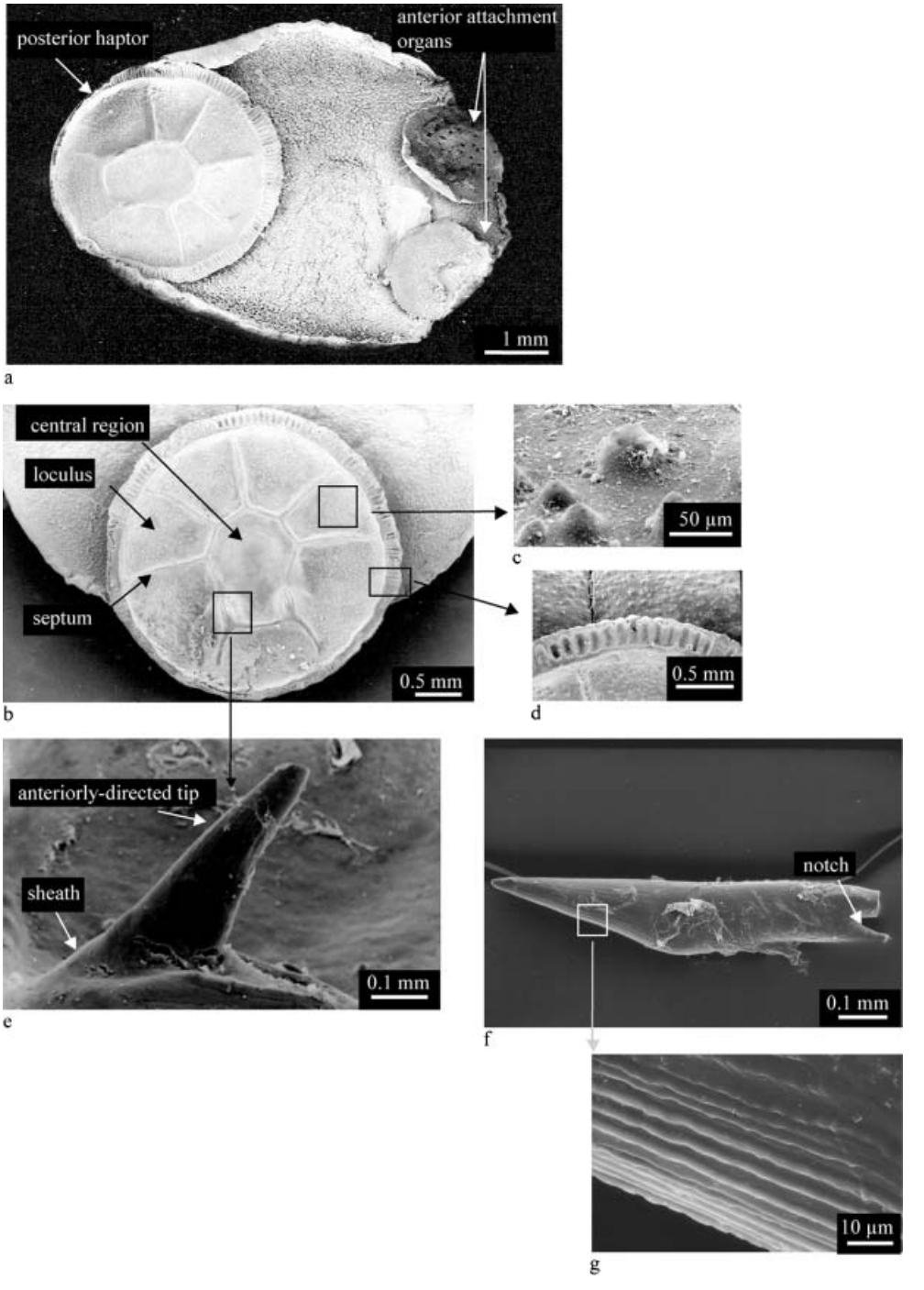

Figure 1. Scanning electron micrographs of the attachment structures of a *Capsala* sp. from the skin of *Makaira nigricans*. (a) Ventral surface showing the papillate body surface and the location of the posterior haptor and anterior attachment organs; (b) the posterior haptor showing details of the loculi; (c) the haptoral papillae; (d) the haptoral marginal valve; (e) lateral view of an accessory sclerite showing the tegumental sheath; (f) digested accessory sclerite indicating proximal notch; (g) longitudinal terminal surface ridging on the accessory sclerite.

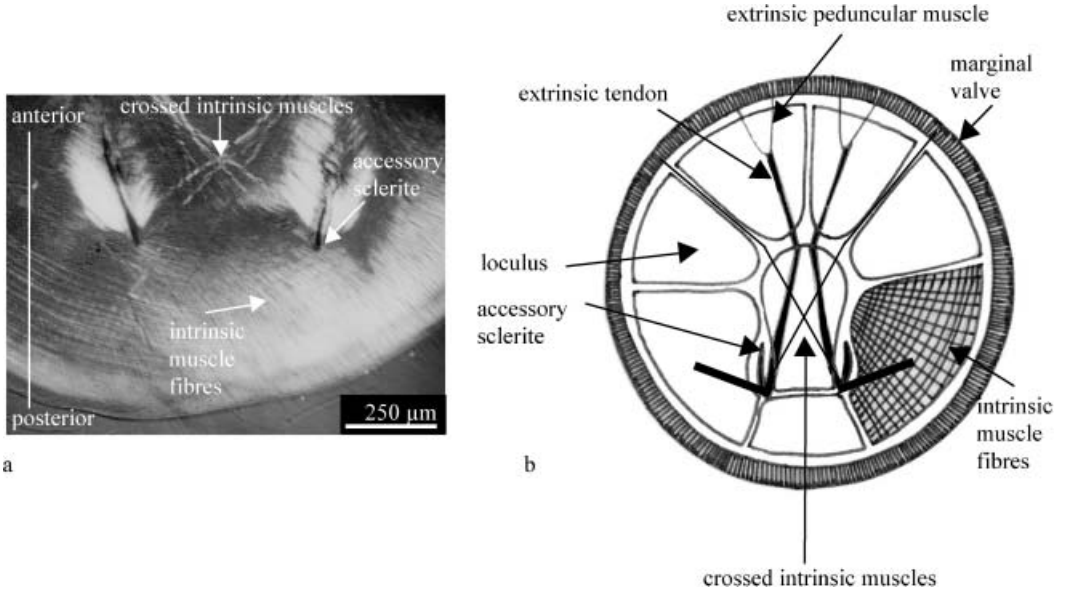

Figure 2. The haptoral anatomy of a *Capsala* sp. (a) Differential interference contrast photograph of the posterior haptor; (b) schematic representation of the anatomy of the haptor—in the same orientation as indicated in (a).

Suction force of the haptoral attachment

If *Capsala* sp. is assumed stationary on the host, the balance of forces on its body must be acting in equal and opposite directions according to Newton's Third Law (Figure 4).

The drag force tends to pull the haptor in the direction of the flow but in equilibrium this is equally opposed by friction, resisting such movement. This frictional force between the surfaces of the parasite and the host is dependent on the normal reactionary force opposing the suction force:

$$F_F = \mu F_N \tag{1}$$

where F_F is the frictional force between the surfaces of the parasite and the host, opposing drag ($\mathrm{N\,m^{-2}}$); μ is a coefficient (approximately 0.5); and F_N is the frictional force normal to the suction force generated by the parasite ($\mathrm{N\,m^{-2}}$).

The magnitude of the frictional force is therefore dependent on the force acting normal to the interface, i.e. the suction force.

$$F_F = \mu F_S \tag{2}$$

where F_F is the frictional force between the surfaces of the parasite and the host, opposing drag ($\mathrm{N\,m^{-2}}$); μ is a coefficient (approximately 0.5); and F_S is the suction force generated by the parasite ($\mathrm{N\,m^{-2}}$).

If the frictional force is equal and opposite to the drag force, then the drag force depends on the suction force:

$$F_D = F_F = \mu F_S \tag{3}$$

Transposing Equation 3:

$$F_D = \mu F_S \tag{4}$$

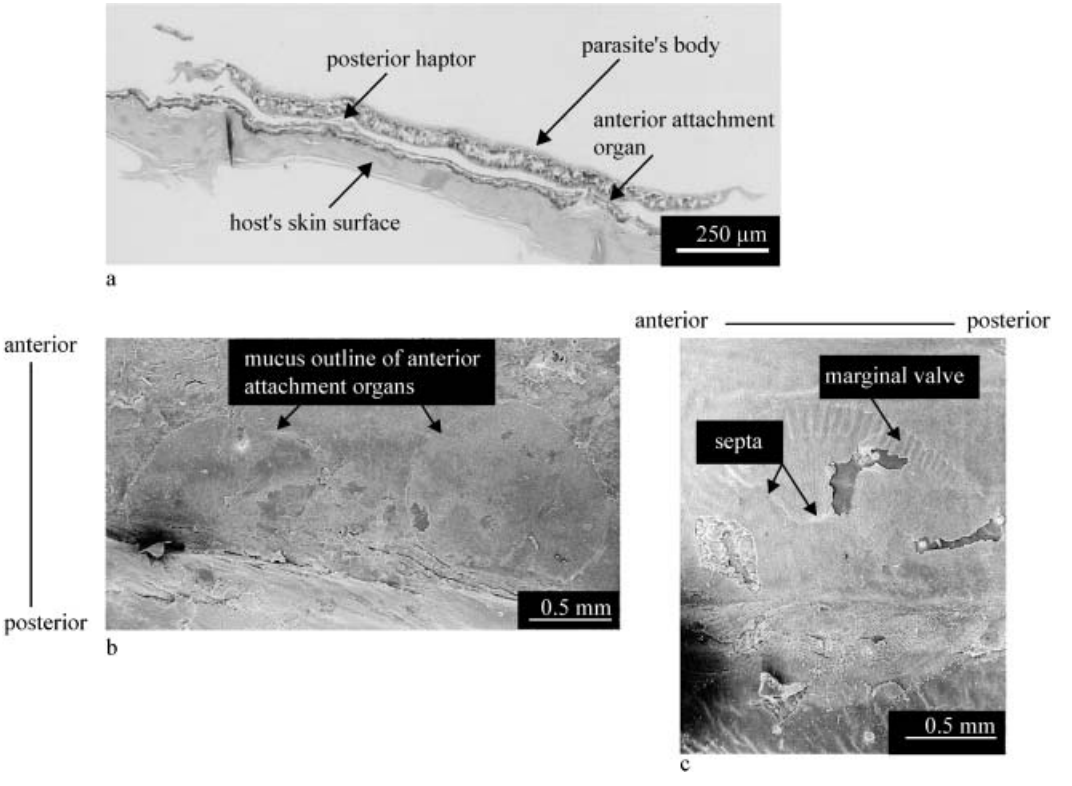

Figure 3. The attachment to the host surface by a *Capsala* sp. (a) Longitudinal section of an individual attached to the surface of *Makaira nigricans*, showing the location of the haptor and anterior attachment organs; (b, c) scanning electron micrographs showing the outlines left by (b) the anterior attachment organs, and (c) the haptor showing the impression of the septa and marginal valve.

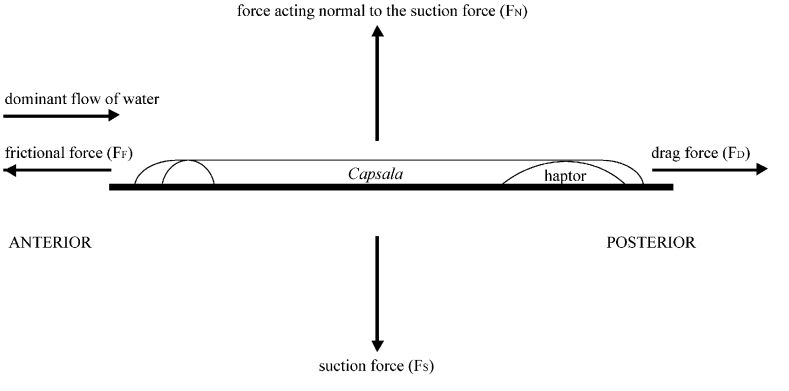

Figure 4. Diagrammatic representation of the balanced forces acting on the body of *Capsala* sp. while it is attached to the outer surface of *Makaira nigricans* (arrows indicate the direction in which the force is acting).

where F_D is the drag force ($\mathrm{N\,m^{-2}}$); F_F is the frictional force between the surfaces of the parasite and the host, opposing drag ($\mathrm{N\,m^{-2}}$); μ is a coefficient (approximately 0.5); and F_S is the suction force ($\mathrm{N\,m^{-2}}$).

To estimate the suction force, we therefore first need to calculate the drag force as follows:

$$F_D = {}^1/_2 C_D A \rho \upsilon^2 \tag{5}$$

where F_D is the drag force ($\mathrm{N\,m^{-2}}$) at $1\,\mathrm{m\,s^{-1}}$; C_D is the drag coefficient (assume approximately 0.1); A is the cross-sectional area of the parasite perpendicular to the flow ($3.2 \times 10^{-6}\,\mathrm{m^2}$); ρ is the density of the medium ($1000\,\mathrm{kg\,m^{-3}}$); and υ is the velocity of the host's body when cruising, relative to the medium ($1\,\mathrm{m\,s^{-1}}$).

The drag force acting on *Capsala* sp. is therefore equal to $0.02 \times 10^{-2}\,\mathrm{N\,m^{-2}}$ when *M. nigricans* is cruising at $1\,\mathrm{m\,s^{-1}}$. Transposing Equation 5 to find the suction force:

$$F_S = F_D / \mu \tag{6}$$

$$F_S = 0.02 \times 10^{-2} / 0.5 \tag{7}$$

The suction force required by *Capsala* sp. to generate sufficient friction to overcome drag at $1\,\mathrm{m\,s^{-1}}$ is approximately double the drag force at this speed or $0.04 \times 10^{-2}\,\mathrm{N\,m^{-2}}$.

When *M. nigricans* swims faster, the drag force can be calculated by substituting $20\,\mathrm{m\,s^{-1}}$ as the velocity in Equation 5. Thus, the drag force is now $8 \times 10^{-2}\,\mathrm{N\,m^{-2}}$. Inserting this figure into Equation 7:

$$F_S = 8 \times 10^{-2} / 0.5 \tag{8}$$

The suction force required to oppose drag and allow *Capsala* sp. to remain stationary is again double the drag force or $0.16\,\mathrm{N\,m^{-2}}$. This means that *Capsala* sp. must increase the suction force by three orders of magnitude, or around 400 times, when *M. nigricans* increases its swimming speed from 1 to $20\,\mathrm{m\,s^{-1}}$.

It is possible to go further and estimate the pressure reduction which must be generated beneath the sucker to produce a sufficient pressure differential for suction, with the ambient water pressure at 10 m and *M. nigricans* cruising at $1\,\mathrm{m\,s^{-1}}$. This can be calculated from:

$$P_r = P_a - F_S / A_C \tag{9}$$

where P_r is the theoretical reduced pressure beneath the haptor at 10 m depth; P_a is the theoretical ambient pressure at 10 m depth (calculated from ρ (density of the medium (1000 kg $\mathrm{m^{-3}}$)) $\times g$ (gravity ~ 10) $\times h$ (depth 10 m)+P_o (atmospheric pressure 100,000 Pa)=approximately 200,000 Pa); F_S is the suction force ($0.04 \times 10^{-2}\,\mathrm{N\,m^{-2}}$); and A_c is the cross-sectional area of the animal (approximately πr^2 or $3.14 \times (3.2 \times 10^{-6}\,\mathrm{m^2})$.

To produce sufficient suction to maintain the position of *Capsala* sp. when *M. nigricans* is swimming at $1\,\mathrm{m\,s^{-1}}$ at 10 m, the pressure beneath the haptor is around 199,960 Pa, which is 40 Pa below ambient pressure. This figure will vary according to the velocity at which *M. nigricans* swims at 10 m because, as shown in Equation 8, the suction force varies with velocity. To calculate the pressure beneath the haptor when *M. nigricans* swims at $20\,\mathrm{m\,s^{-1}}$ at 10 m, the suction force at this speed is substituted into Equation 9. The pressure is around 184,000 Pa, which is 16,000 Pa below ambient pressure and 400 times lower than the pressure at $1\,\mathrm{m\ s^{-1}}$, which creates the 400-fold increase in suction force calculated above.

When *M. nigricans* dives from 10 to 200 m at $20\,\mathrm{m\,s^{-1}}$, substituting depth in Equation 9, the theoretical ambient pressure increases by 10 times, to 2,001,000 Pa, and the pressure

beneath the haptor is 1,985,000 Pa. The pressure differential between the inner and outer surfaces of the haptor will increase with increasing depth, if the speed is constant. This is because the ambient pressure increases with increasing depth but the pressure beneath the haptor will not correspondingly increase as the water-filled cavity is almost incompressible and the volume of the haptor is not therefore reduced by increasing ambient pressure.

Parasite distribution

Parasites appeared to be distributed in large clumps, the members of which were generally orientated with the haptor facing into the oncoming water, flowing over the host. Dorsally, parasites were located around the tip of the bill, the lateral region of the dorsal fin, and the caudal keels (Figure 5a, b). Ventrally, they were observed beneath the head, between the pectoral fins, and around the first and second anal fins (Figure 6a–d).

The corresponding surface topography of these areas differed (Figures 5, 6). The dorsal surface of the bill and head were covered in numerous irregularly spaced projections of between 0.02 and 0.06 mm in height (Figure 5a). By contrast, the area around the dorsal fin was relatively smooth with scales just breaking the surface of the skin and a few widely spaced projections in between the scales of between 0.01 and 0.03 mm in height (Figure 5b). Ventrally, the area beneath the head was also relatively smooth (Figure 6a) but between the pelvic fins there were many scales which broke the skin surface (Figure 6b). Around the first anal fins, the skin surface was punctuated with ovoid openings of around 0.25 mm long by 0.2 mm wide (Figure 6c). The surface of the skin around the second anal fin was uneven due to grooves that produced a criss-crossed pattern (Figure 6d).

Discussion

The mechanism of suction attachment

The lack of evidence for an adhesive secretion, absence of puncture marks, and the presence of an impression of where the haptor had been attached indicated that the primary means of haptoral attachment in the *Capsala* sp. studied was by suction. The extrinsic and intrinsic muscles observed in the peduncular and haptoral wall, respectively, are suggested to generate this suction in the following way. When *Capsala* sp. places the haptor on the host's skin, the extrinsic and intrinsic muscles are relaxed, allowing the haptor to spread out and cover the largest possible surface area of skin. The marginal valve also spreads out, and by virtue of its flexibility and ribbing, covers any minor irregularities to form close skin contact. The extrinsic peduncular muscles are then contracted, drawing the centre of the haptoral wall upwards directly. The marginal valve is also drawn inwards and its close contact with the host prevents the inward flow of water to fill the new increase in volume beneath the haptor. Water, like all liquids, resists tension and does not therefore readily spread out with the volume increase. However, the relatively small increase in the volume of the water-filled haptor produces a sufficient reduction in the pressure to create suction.

The lack of evidence of puncture marks corresponding to the accessory sclerites suggests that they are not normally employed in piercing the skin. They are instead thought to be jammed against the skin surface irregularities, facilitated by their terminal ridging, thereby acting as props. This may prevent the haptor from sliding off when the host travels at high speed. The accessory sclerites will also indirectly generate suction because when upright, they will raise the haptoral roof, increasing its volume and reducing the pressure beneath.

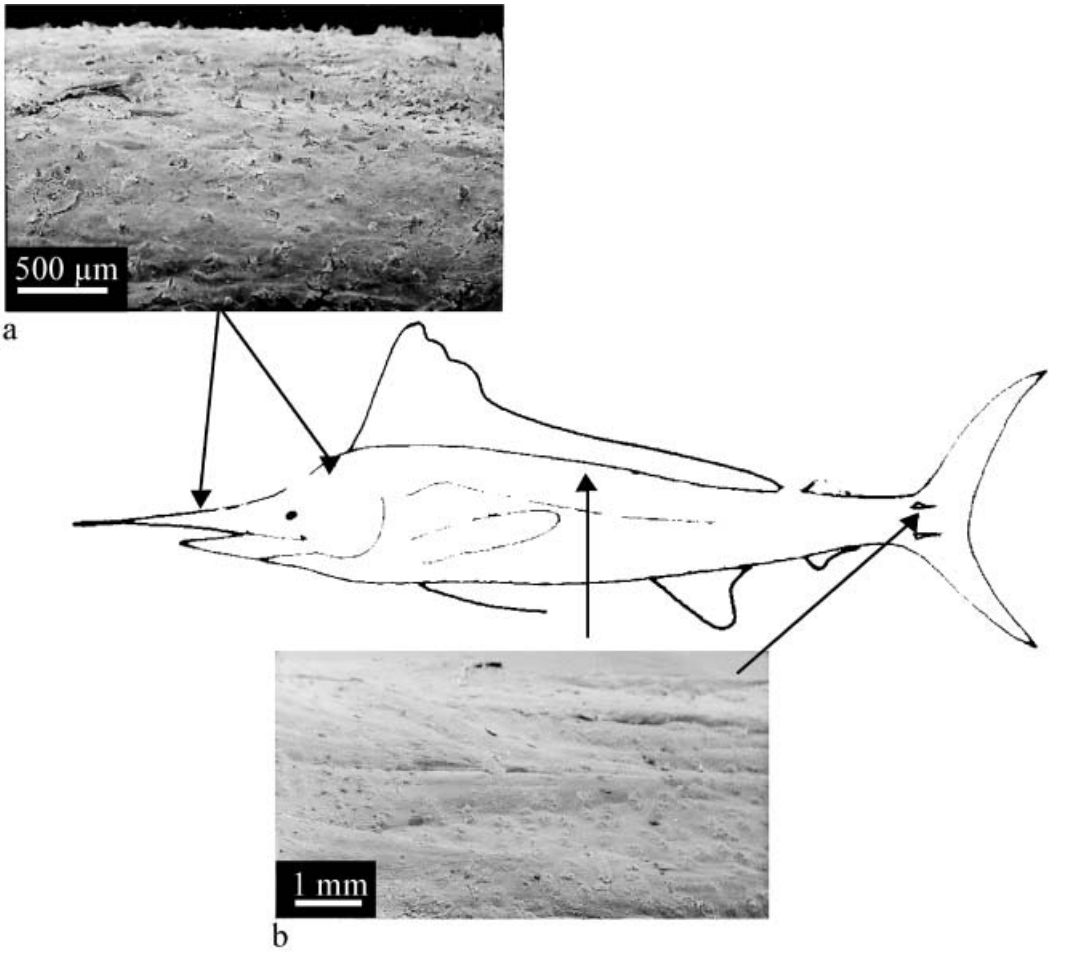

Figure 5. Diagrammatic view of a marlin, showing scanning electron micrographs of the dorsal skin of *Makaira nigricans* at two different locations, where a *Capsala* sp. was observed. (a) The bill and on top of the head; (b) region lateral to the dorsal and caudal fins.

Suction is not only thought to be generated extrinsically but also intrinsically by the circular and radial muscles which are present throughout the haptoral wall. The combined contraction of these muscles causes the haptor to be drawn upwards into the centre, again increasing the volume beneath the haptor, causing a decrease in the pressure. This method of generating suction is also thought to have been employed by *Entobdella soleae* (see Kearn 1994). Specimens of *E. soleae* were observed to attach to the hard, smooth surfaces of a glass aquarium into which the hamuli, employed to raise the roof of the haptor to create suction, were unable to penetrate.

Each loculus in the haptor is likely to function as a sealed unit and therefore as an individual sucker. Such an attachment would be advantageous for three reasons. If the marginal valve of one of the sucker's units is for some reason dislodged, for example, by irregularities in the host's surface, the other units will remain intact because they are sealed and the sucker should not fail. Secondly, the septum of each loculus could have a supportive function like the spokes of a bicycle wheel, each supporting a fraction of the

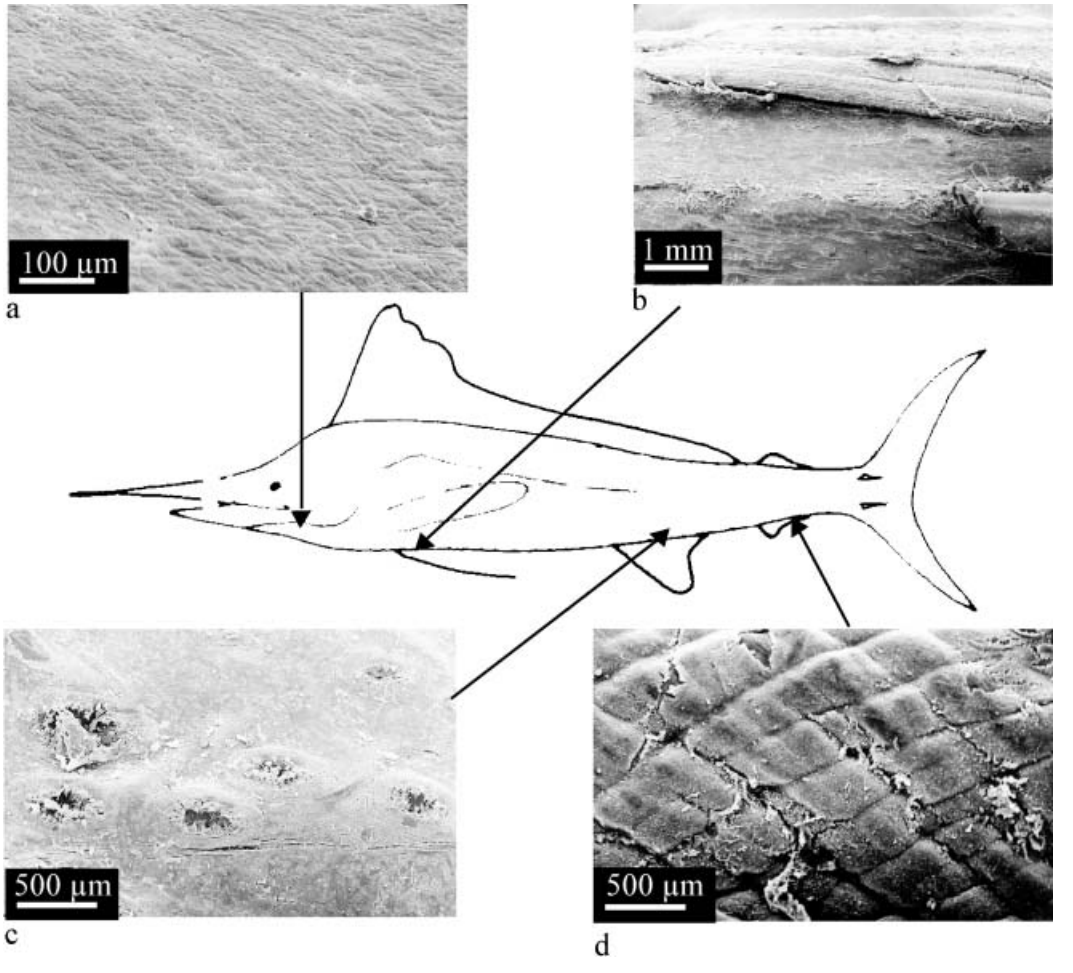

Figure 6. Diagrammatic view of a marlin, showing scanning electron micrographs of *Makaira nigricans* at four different locations ventrally, where a *Capsala* sp. was observed. (a) Beneath the head; (b) between the pectoral fins; (c) region lateral to the first anal fin; (d) region lateral to the second anal fin.

sucker under the suction force, thus reducing the likelihood of it collapsing. This is likely to be the case because it is usually the monogeneans with the largest haptors, which are most vulnerable to collapse and therefore require the greatest support, that possess loculi (Kearn 1994). Thirdly, the septa will increase the contact area with the host's skin and therefore the area over which intermolecular bonds can form to produce adhesive forces. This will increase the strength of the haptor's attachment.

The selective advantages of loculate suckers are evident by their presence elsewhere in the Monogenea. For example, the Monocotylidae, which exclusively parasitize the inner and outer surfaces of their chondrichthyan fish hosts (Chisholm and Whittington 1998), possess septate haptors. Loculate suckers are also found elsewhere in the Platyhelminthes. For example, in the Trematoda or flukes, by members of the Aspidogastrea (see Rohde 1972) and the Notocotylidae (see Yastrebov 1997), and in the Cestoda or tapeworms, where the scolices of tetraphyllids (Tetraphyllidae) possess leaf-like, multiloculate bothridial suckers (Caira and Ruhnke 1990).

Loculate suckers have also evolved convergently in other taxa, where their dividing walls are thought to function supportively and not as independent suctorial units. For example, supporting struts are seen in the water beetle, *Dytiscus* (Dytiscidae), in which the suckers can attain relatively large diameters of around 1.5 mm (Nachtigall 1974).

In both extrinsic and intrinsic muscular-generated suction, *Capsala* sp. will expend energy. Since it must remain securely attached to the host at all times, it will therefore be contracting its muscles almost constantly. This is energetically expensive and it is likely that *Capsala* sp. and other similarly attached monogeneans have evolved a way around this. One way in which this may be achieved is by storing elastic energy in the interconnecting fibres between the circular and radial muscles. When these muscles contract, the interconnective fibres will be contracted simultaneously. If the fibres are composed of a resilient material like collagen which can store strain energy, then when the circular and radial muscles relax, releasing the stored energy, this will act to spread out the wall of the haptor and increase the volume beneath, producing a suction force. This energy-efficient method of generating suction is also thought to be employed by cephalopods (Kier and Smith 2002). An alternative suggestion is that the haptoral muscles are similar to the specialized adductor or catch muscles of bivalves (Mollusca), which enable the valves to be rapidly closed and kept tightly shut by sustained energy-efficient contraction (Smyth and Halton 1982).

The function of the papillae, present on the ventral surface of the haptor, has been suggested to be sensory and to aid attachment by assisting in resisting shear drag forces encountered when the fish host is manoeuvring (Lyons 1973). The papillate ventral body surface of *Capsala* sp. is also suggested to increase overall friction and resist drag forces; its role in sensory perception has not been investigated. Comparison with cephalopod suckers (Kier and Smith 1990, 2002), which also have papillate inner surfaces, suggested another mechanism by which the haptoral papillae may aid attachment, by transmitting the reduced pressure over the inner surface of the haptor. By forming interconnecting, water-filled channels through which the pressure can be transmitted to the margin, the papillae could help generate a suction force over the entire haptoral surface.

Comparison of the mechanism of attachment with other capsalids

The *Capsala* sp. studied generates predominantly extrinsically derived suction via peduncular muscles inserting into the haptoral wall, but also suction generated intrinsically. This places this capsalid somewhere between those members of the Capsalidae which generate suction entirely by extrinsic muscles and those which generate suction entirely by intrinsic muscles.

The gradual loss of the hamuli and accessory sclerites in some capsalids is thought to be responsible for the transition from extrinsic to intrinsically generated suction (Kearn 1994). For example, in *Entobdella soleae*, the tendons from the extrinsic muscles pass posteriorly via a proximal notch on the accessory sclerites and insert on the bases of the anterior hamuli (Kearn 1964). Suction is generated by the contraction of the extrinsic muscles, which draw the accessory sclerites into an upright position, propping up the centre of the haptor. The volume of the haptor is thus increased and the pressure drops to create suction. The anterior and posterior hamuli are simultaneously drawn anteriorly and dig into the host's skin, fixing the haptor in place. Marginal hooks positioned around the circumference of the haptor prevent the sucker from collapsing inwards when it is drawn away from the host's surface. By contrast, the *Capsala* sp. studied has no hamuli on which to insert the extrinsic tendons and therefore they insert via the accessory sclerites into the ventral haptoral wall,

where upon contraction they draw the centre of the haptor upwards to create suction. This localization of the suction force is thought to have led to the partitioning of the haptor into loculi as shown in many *Capsala* (see Kearn 1994) and *Trochopus* species (see Whittington and Kearn 1991). The uncoupling of the extrinsic tendons from the hamuli has led to the evolution of intrinsic muscular control over haptoral suction, the end-point of which being the loss of the median accessory sclerites altogether and the total dependence on intrinsically generated suction, for example, in *C. martinieri* (Logan and Odense, 1974) (see Kearn 1994).

Suction force of the haptoral attachment

Assuming that *Capsala* sp. remains stationary whilst *M. nigricans* cruises at $1\,m\,s^{-1}$ at 10 m depth, the theoretical suction force required to generate sufficient friction to counteract drag is approximately double the drag force. When, as it is thought, *M. nigricans* increases its speed to $20\,m\,s^{-1}$, the suction force remains twice that of drag. Haptoral suction is therefore suggested to be an efficient mode of attachment in *Capsala* sp.

When *M. nigricans* speeds up from 1 to $20\,m\,s^{-1}$, the suction force of *Capsala* sp. increases by a factor of 400 to balance the same increase in the drag force, allowing the parasite to remain attached. This increased suction is thought to be generated by the combined action of the water flowing dorsally over *Capsala* sp., which tends to push the haptor down on to the host, and by the contraction of the extrinsic and intrinsic muscles and the action of the accessory sclerites, which are pushed against the host's skin as it swims faster. This brings the accessory sclerites into an upright position, raising the haptoral roof, which increases the volume of the haptor and reduces the pressure beneath. Increased suction force will bring the inner surface of the haptor into closer contact with the host's skin, thereby increasing the friction resisting drag.

When *M. nigricans* dives from 10 to 200 m at $20\,m\,s^{-1}$, *Capsala* sp. does not need to generate more suction if the swimming speed is constant because the drag force will also be constant. However, there is an increase in suction force simply because the pressure differential between the ambient pressure and that beneath the haptor increases.

Parasite distribution

The results of the distribution study suggest that haptoral attachment by *Capsala* sp. is not restricted by the surface topography of the host's skin. Individuals were located dorsally on areas with relatively large and closely packed projections, which would theoretically disrupt the marginal valve of the haptor. Parasites could also attach to relatively smoother scaled ventral surfaces, which would present no such problems for the valve. The ability to attach to a variety of topographies is most likely due to the loculi, which function as individual suckers, thereby preventing suction from being entirely disrupted. In addition, the papillae may span the areas between the surface projections or they may interdigitate with the projections, thereby assisting attachment.

The clumped, non-random distribution of *Capsala* sp. may be a result of other factors which differ over the host, for example, hydrodynamic forces. The drag force on an object tends to pull it in the direction of the flow, which varies with the pressure over the body of a fish, depending on the fish's anterior incline (Videler 1993). Pressure normally decreases up to the widest cross-sectional area of the fish, where it is around zero. Beyond this point it increases posteriorly. This pressure difference means that the water follows the shape of the

fish closely at the anterior in laminar flow, continuing to the widest cross-sectional area of the fish. Beyond this point, however, the narrowing of the fish's body causes the pressure to increase and the water separates from the body, causing turbulence and drag. Hence, if hydrodynamic drag were a controlling factor in the distribution of *Capsala* sp., individuals would be expected to be predominantly anterior of the widest cross-sectional area on *M. nigricans*, where the drag is lowest. This was generally found to be the case, however, there were also clumps of parasites located posteriorly, suggesting that these may have been a different species or that there are additional factors affecting distribution. For example, the nutritional value of the skin could affect distribution and, more specifically, the accessibility of the epidermis on which monogeneans graze. The density and morphology of scales covering a marlin's surface are not uniform and scales are, for example, smaller, less numerous, and more sparsely arranged posteriorly, making the epidermis more accessible in this region (La Monte 1955; Davie 1990). The thickness of a marlin's epidermal layer also varies, and ventrally it is relatively thinner, suggesting that there is less on which to graze in this region (A. L. Ingram, unpublished data). There may also be immunological factors which contribute to the distribution of *Capsala* sp. For example, monogenean epidermal grazing, which produces a wound on the host, can stimulate a host immune response (Kearn 1999). Monogeneans avoid this by harvesting the epidermal cells but continually move to new areas to allow wounds to heal before returning to re-graze them (Kearn 1999). Finally, there is strong evidence to suggest that site specificity, which produces clumping, may be to increase the chances of mating and cross-fertilization (Rohde 1994).

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