Barnacle Bonding: Morphology of Attachment of Xenobalanus globicipitis to its Host Tursiops truncatus

Mark C. Pugliese, Stefanie Anne Böttger,* and Frank E. Fish

Department of Biology, West Chester University, 750 S Church Street, West Chester, PA 19383

ABSTRACT Xenobalanus globicipitis, a unique type of small pseudo-stalked barnacle occurs on the appendages of cetaceans, including the common bottlenose dolphin Tursiops truncatus. In this study, we examined attachment structures of X. globicipitis and modifications to the skin of T. truncatus in areas of attachment compared to skin nearby an attachment site. Barnacles and their six calcareous footplates were measured for their length and width. There was a positive correlation of barnacle width and length to footplate width and length. The thickness of the stratum corneum increased significantly in areas of attachment compared to skin nearby a footplate. The mitotic stratum germinativum at the base of the dermal papillae did not change significantly in areas of attachment compared to skin nearby a footplate. The stratum germinativum lining the lateral walls of the dermal papillae was significantly thicker in areas of skin nearby a footplate compared to in areas of attachment. Skin of T. truncatus nearby a footplate, displayed dermal papillae extending from the dermis and pointing roughly perpendicular to the epidermal stratum corneum. At sites of X. globicipitis attachment, the dermal papillae were forced to extend laterally, parallel to the stratum corneum, and the dermal papillae length to width ratio at an attachment site was significantly higher than on skin near an attachment site. Our results show that attachment of X. globicipitis through production of footplates organized into calcareous rings, leads to a thickened stratum corneum of the epidermis, a thinner lateral mitotic stratum germinativum and displaced structures of the upper dermis. These resulting modifications to the epidermis and dermis of the host may add to securing barnacle attachment to its host. J. Morphol. 000:000–000, 2012. © 2012 Wiley Periodicals, Inc.

INTRODUCTION

The pseudo-stalked barnacle Xenobalanus globicipitis belongs to the Cirripedia and closely resembles stalked lepomorph barnacles. Like other crustacean epizoites such as Conchoderma virgatum (rabbit-eared barnacle), Lepas pectinata and Lepas cf. Hillii (gooseneck barnacles), Syncyamus aequus (whale lice) and Pennella sp. (copepods), X. globicipitis lives attached to the smooth, hairless skin of cetaceans (Darwin, 1854; Aznar et al., 1994; di Beniditto and Ramos, 2000). Unlike species of Conchoderma that may also attach to isopods (Hastings, 1972), copepods (Benz, 1984), fishes (Jones et al., 1968; Balakrishnan, 1969; Ramos et al., 2010), and reptiles (Eckert and Eckert, 1987; Alvarez and Celis, 2004), there are no records of X. globicipitis attachment to species other than cetaceans (Rajaguru and Shantha, 1992). In addition, X. globicipitis is always attached directly to the skin of its host (Pilsbry, 1916; Barnard, 1924), while Conchoderma sp. may attach to any hardened surface including cetacean teeth (Aznar et al., 1994). These epizoites are usually located on dorsal fins, flippers and tail appendages of cetaceans (Kane et al., 2006), attached to the trailing edges, which is the region with the thinnest epidermis (Pavlov, 2003), although they have also been found on rostra and in between a host’s teeth in rare cases. Although X. globicipitis is considered a commensal organism, its presence on the fins of cetaceans disturbs the normal flow pattern over and around the cetacean body and adds to its drag, making the barnacle a likely hydrodynamic parasite. Physical and structural features of X. globicipitis are therefore likely adaptations to being dragged through the water when attached to its cetacean host (Karuppiah et al., 2004).

The smooth, flexible body of X. globicipitis is characterized by its cylindrical shape that attaches to a host through individual footplates creating a shell or basal attachment plate (Rajaguru and Shantha, 1992). At its opposite end there is a reflexed hood, where two short projections or horns are present. The cirri, mouth, penis, and other associated organs are located within, and extend when in use outwards from the hood (Rajaguru and Shantha, 1992). In living specimens, the body
of *X. globicipitis* is dark-brown with a lighter colored hood; the shell is a small, thin, and irregularly star-shaped structure that is whitish-cream color (Darwin, 1854; Rajaguru and Shantha, 1992). This shell surrounds the pedicle, the central part of the pseudo-stalk or pseudo-peduncle, with six small laterally radiating footplates (Darwin, 1854), and has been referred to as a reduced version of the typical shell or opercular plates (Bourget and Crisp, 1975; Rajaguru and Shantha, 1992). The plates are created by adding concentric calcified ring segments at the base of the mural wall of the pedicle or pseudo-stalk while the old ones are shed in the upper layer of the stratum corneum (Seilacher, 2005). The newest and lowest rings are shorter than the uppermost rings, indicating that additional layers containing calcium carbonate (CaCO$_3$) crystals are added to increase ring length. Older rings have been suggested to contain more CaCO$_3$ crystals, leading to a larger and more protective upper layer of the foot plate (Seilacher, 2005).

Serving as attachment for *X. globicipitis*, cetacean skin is unique among mammals as it is non-glandular, inherently hairless and has a thick epidermis with an incompletely keratinized stratum corneum (Sokolov and Kalashnikova, 1971; Spearman, 1972; Ling, 1974; Harrison and Thurley, 1974; Brown et al., 1983). Dolphin skin grows quite rapidly and contains a large number of proliferating cells in the basal epidermal layers (Palmer and Weddel, 1964; Brown et al., 1983, Hicks et al., 1985). Rapid growth and therefore extensive sloughing of the upper layers of the epidermis will require certain adaptations to the attachments structures of the host’s epidermis for the epizoite to remain firmly attached.

This study examines the composition and microstructure of the individual footplates forming the basal attachment plate and the pedicle or pseudo-stalk of *X. globicipitis* and respective changes to the epidermis and dermis of the bottlenose dolphin *T. truncatus* at the site of that attachment. This is the first microscopic investigation of the unique attachment system used by *X. globicipitis* in the unstable environment along the trailing edge of dolphin appendages to provide a new way of understanding host-epizoite interaction.

**MATERIALS AND METHODS**

**Attachment Plate Macaroanatomy**

Twenty-five *X. globicipitis* were obtained from skin samples removed from dead, stranded *T. truncatus* collected between by the Coastal Marine Mammal Assessment Program (Wayne McFee, NOAA-NMFS, Beaufort, NC) between 1993 and 2003, fixed for 72 h in 4% formaldehyde and stored in 75% ethanol. All samples of adult (above 20 mm in size) *X. globicipitis* were preserved while remaining attached to the skin of *T. truncatus*. The physical features of the attachment structures of *X. globicipitis* were observed. Each attachment structure or shell was composed consistently of six footplates that extended laterally from the animals’ central pedicle or pseudo-peduncle (Rajaguru and Shanta, 1992). The length and width of each footplate, was measured to the nearest 0.1 mm using electronic digital calipers and a dissecting microscope (Zeiss Stemi 2000-C) and compared to the total length (measured from the bottom of pedicle which resides on top of the host’s skin to the top of the horn on the hood; Rajaguru and Shanta, 1992) of the individual and the width of the body (WB; measured at the widest part of the body; Fig. 1A). Length and width of the footplates (Fig. 1B) was correlated to length and width of the individual.

**Attachment Plate Composition**

The hardened nature of the attachment footplate of *X. globicipitis* has so far been attributed to the presence of CaCO$_3$ crystals though the deposition of silica had never been investigated. To determine whether the foot plates were composed of CaCO$_3$ or silica (H$_2$SiO$_4$) deposits, individual foot plates were removed from five randomly chosen barnacles with a sharp scalpel and treated by boiling in 1 ml of 5% sodium hypochloride for 10 min. This technique will dissolve CaCO$_3$ but will leave silica deposits unaffected.

**Microanatomy Samples**

Ten samples of *X. globicipitis* attached to their host *T. truncatus* and skin nearby attachment areas were divided into (A) *T. truncatus* skin (removed in 10–15 mm distance from the edge of a footplate) to determine integumental microstructure without the influence of commensal attachment, (B) individual *X. globicipitis* footplates on *T. truncatus* skin to observe annular composition of the foot plate (Seilacher, 2005) and determine associated changes in the integument, and (C) individual *X. globicipitis* footplates and the attached pedicles or pseudo-stalks attached to the integument of *T. truncatus* (Fig. 1C).

**Sample Fixation and Embedding**

All microanatomy samples were decalcified in 5% sodium ethylenediamine tetraacetic acid in sodium cacodylate buffer and dehydrated in increasing ethanol concentrations up to 100% followed by xylene. Samples were embedded in paraffin and sectioned to 5 µm by the University of Pennsylvania Veterinary School New Bolton Center’s Large Animal Pathology Laboratory. The slides were dehydrated and stained with hematoxylin and eosin. Following dehydral all slides were mounted with Permount (Fisher Scientific).

**Microscopic Measurements**

Ten samples each of *T. truncatus* skin near a footplate, skin bearing a footplate and skin bearing footplate and pedicle were analyzed using a Zeiss Axiovert 40 CFL microscope at 100× total magnification. Epidermal and dermal strata were defined according to previous studies by Ling (1974), Sokolov (1980, 1982), Brown et al. (1983), and Pfeiffer and Jones (1993). Thirty measurements per sample in micrometer were taken of the dermal papillae (length and width), the stratum germinativum (mitotic layer of the skin, 30 measurements each were taken for the stratum germinativum at the base and at the left and right lateral side of the dermal papillae), and the stratum corneum (keratinized layer) of skin with and without barnacle attachment using Axiovision (Carl Zeiss) a software that permits digital measurements to the nearest 0.01 µm.

**Statistical Analyses**

All microanatomy samples were compared by linear regression modeling of host:barnacle length and width respectively. Correlations were evaluated using JMP (version 8; SAS Institute, Cary, NC). Microanatomy measurements were compared
using a One-Way ANOVA followed by a Dunn’s pairwise comparison in SigmaStat (Systat Software, San Jose, CA). Before the statistical analyses, assessments of the assumptions of normality (Kolmogorov–Smirnov Test) and homoscedacity (Spearman–Rank Correlation) were conducted. Means were expressed as ± one standard deviation. Results were determined to be statistically significant at \( P < 0.05 \).

**RESULTS**

**Macroanatomy Measurements**

There were always six footplates composing the shell or basal attachment plate in each X. globicipitis specimen without variation (Fig. 1A, not all footplates shown). These footplates are composed of CaCO\(_3\), rather than silica. Each plate is constructed of concentric layers of rings (Fig. 1D) ranging from the newest (deepest in the integument) to the oldest (closest to the integumental surface) and increasing in length with age (Seilacher, 2005). Footplate construction occurs in the form of foliate areas of calcification (Fig. 1B, C) rather than a single sheet of calcified material constructing a ring. Each footplate radiates out from the central pedicle and together leading to the formation of what resembles a flower-shaped rather than star-shaped (Rajaguru and Shanta, 1992) attachment organ (Fig. 1A).

The average length of all adult X. globicipitis samples measured was 32.28 ± 1.0 μm and the average length of the footplate was 1.98 ± 0.1 μm. The average width of X. globicipitis was 3.87 ± 0.2 μm and the average footplate width was 1.53 ± 0.1 μm. A positive correlation of barnacle:footplate

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Fig. 1. X. globicipitis – the pseudo-stalked barnacle X. globicipitis with attachment plates, displaying (A) the entire animal (single attachment plate locations indicated by arrow) including total animal length (TL, bottom of pedicle to top of horn) and WB (widest part of the body; Rajaguru and Shantha, 1992), (B) one calcified attachment plate displaying the foliate projections and arrows indicating the location of attachment plate width (FPW measured at the widest pan of the top ring) and length (FPL, longest distance between pedicle and tip of attachment plate) measurements, (C) the bottom of an animal displaying the pedicle, two complete and two partial attachment plates and (D) a schematic drawing of the basal plate. displaying two footplates with four calcified rings each (bottom newest, top = oldest) with the stratum corneum (arrow) and its areas of measurement in the center of footplate rings (SCAP) indicated by the double pointed arrows. Other measurement locations were the edges of the footplate and pedicle (SCAPS and SCSP respectively, indicated by arrows) and the center of the pedicle (SCC, located equidistant between the newest rings of the footplate). Pictures B and C were taken using a Zeiss Stemi 2000 dissecting microscope (total magnifications ×4 and ×5) and a Zeiss Axiocam ERC 5s camera. FP = individual footplate (six present per X. globicipitis, not shown); P = pedicle or pseudo-stalk. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
length and width was observed ($P = 0.026$ and 0.026; Fig. 2A,B).

**Plate Composition and Structure**

Absence of rigidity in the footplates (Seilacher, 2005) following boiling in 5% sodium chloride determined that the rings of the footplates were composed from CaCO$_3$. The CaCO$_3$ appears to be sequestered in defined layers (Fig. 3A) within the rings of the footplate (Seilacher, 2005) that are arranged according to age, the oldest ring in the shallowest part of the stratum corneum of the epidermis. Older rings in each attachment structure are longer than the rings before with thickened layers of the epidermal stratum corneum connecting each ring (Fig. 3B).

**Microanatomy Measurements**

Microscopic analysis of the integument of *T. truncatus*, revealed that the outermost layer of the epidermis, the stratum corneum, displayed an average thickness of 71.10 ± 2.8 µm in areas between 10 and 15 mm distance from the footplates (Fig. 4A,C). In areas of barnacle footplate attachment a significantly thicker stratum corneum ($P < 0.001$) of 154.09 ± 23.4 µm was observed (Fig. 4B,D). In areas of attachment, the stratum corneum of *T. truncatus* was also observed to be significantly thicker ($P = 0.005$) underneath the center of the pedicle (Fig. 1D) and footplates (137.0 ± 0.5 and 283.37 ± 10.0 µm) compared to their edges (Fig. 1D: 84.05 ± 6.7 and 91.74 ± 5.5µm; Fig. 4C). There was no significant difference between the measurements of the edge of the footplates and pedicle as compared to the host skin near footplates.

The mitotic stratum germinativum or basal layer of cells in the epidermis was measured at the base and at equal lateral views to the left and right side of the dermal papillae (17.19 ± 8.3 µm at mid-height; Fig. 5). There were no significant differences in the thickness of the basal stratum germinativum at (10.92 ± 5.8 µm), or away from

![Fig. 2. Footplate sizes – Ratios of animal/footplate (A) length (black) and (B) width (grey). All *X. globicipitis* available in this study were above 20 mm in length.](image)

![Fig. 3. Footplate microanatomy – microscopic structure of the attachment plates displaying (A) the distinct layers of CaCO$_3$ inside a single ring of a footplate (400× total magnification, as found in R1 = newest ring of a footplate, arrow indication orientation toward the top of the footplate/host’s skin) and (B) showing several rings (R 1–3, displaying the oldest to newest rings, respectively) of a footplate with the lowest one being the newest (most recently laid down) and each sequential ring (1–3) being further secured by the host’s stratum corneum (SCAP, stratum corneum between two rings of the footplate). The oldest rings are the longest and will become sloughed off first (Seilacher, 2005).](image)
(17.19 ± 8.3 μm), a barnacle attachment site (Fig. 5B). The average thickness of the stratum germinativum at both lateral locations of dermal papillae in the host lacking attachment was 8.36 ± 0.5 μm. In areas of attachment, the lateral stratum germinativum of the dermal papillae was significantly thinner (P < 0.001; 5.13 ± 0.4 μm).

The dermal papillae displayed an average length:width ratio (Fig 6A) in the skin of T. truncatus near a footplate of 13.22 ± 1.0. The average dermal papillary length:width ratio of a host bearing barnacle footplates had significantly increased (P < 0.001) to 33.06 ± 3.0, indicating the presence of longer and thinner dermal papillae. Papillae also showed displacement directly underneath the barnacle pedicle and the attachment plates compared to areas lacking footplates (Fig. 6B,C)

**DISCUSSION**

This study provides new microscopic detail regarding the nature of attachment of the barnacle X. globicipitis, focusing on the modifications to the epidermis and dermis of the skin of its host T. truncatus. The structure of the footplates (six per basal attachment plate or shell) and the mechanism of adding new calcified ring segments at the base of the mural wall while the old ones are shed in the upper layer have previously been described by Seilacher (2005). Our observations in combination with those made by Seilacher (2005) indicate that the successful attachment of X. globicipitis appears to be a combination of epizoite structures and the defense mechanism of the hosts’ integument.

The outer stratum corneum of the epidermis of the host T. truncatus is considerably thicker in areas directly below the pedicle and the attachment structures particularly between the different rings of the attachment plates. In areas between rings (Seilacher, 2005), it reaches 329.41 μm in a central location directly between two rings. Increased epidermal sloughing rates in cetaceans combined with high rates of epidermal production (Hicks et al., 1985; Palmer and Weddel, 1964) necessitate the continuous production of rings and are likely the reason for the increased stratum corneum thickness.
neum between the rings. Dolphins have a high rate of skin sloughing, with the outermost cell layers of *T. truncatus* being sloughed on average 12 times per day (Hicks et al., 1985) and epidermal cell production being 250–290 times that of humans (Palmer and Weddel, 1964). Therefore, the thickened stratum corneum between the rings could indicate the inability of the top epidermal layer of the skin to slough except in areas where it is exposed (in attachment plates only at the top ring). The defense mechanism displayed by having a covering stratum corneum combined with the high turnover of epidermal layers in dolphins therefore appears to positively affect and even secure barnacle attachment.

The dermal papillae, the epidermal layer associated with the papillae, and the stratum germinativum are absent underneath the barnacle pedicle and attachment plates, with only the layers of keratinized stratum corneum remaining. The dermal papillae in attachment areas show displacement toward the edge of the attachment structures and are more closely distributed, thinner and taller. The thinner stratum germinativum along the lateral sides is most likely a result of the elongated appearance of these dermal papillae. This pattern appears to be dictated by the growth of the attachment structures of *X. globicipitis*. As the barnacle grows, a portion of the epidermis and part of the underlying dermis are displaced laterally, which accounts for displacement of the dermal papillae along the pedicle and footplates. It is therefore likely that the larger the barnacle and its footplates grow, the more displaced and thinner the dermal papillae and the lateral stratum germinativum will become. The central portion underneath the pedicle displays layers of keratinization of the stratum corneum only, which most likely had been produced prior to barnacle attachment and could never be sloughed off due to the epizoite/host attachment.

*X. globicipitis* is usually attached to the trailing edges of the host’s appendages, where the water separates from the appendage producing eddies (Seilacher, 2005). These eddies provide areas of low water velocity, where barnacle larvae are more likely to settle (McDougall, 1943). This approach of attaching to the appendages of a host allows for the changes observed in skin of *T. truncatus* during animal attachment. Following settlement, metamorphosis and production of early attachment plates, the plates likely increase in size relative to barnacle growth. This is demonstrated by the correlation of barnacle:footplate height and width ratios. Larger individual *X. globicipitis* will gener-

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**Fig. 5.** Stratum germinativum – the stratum germinativum (A) in comparison at basal and lateral locations of the dermal papillae in areas of footplate attachment and areas nearby footplates. Abbreviations mark the following locations: SGUB – stratum germinativum of *T. truncatus* lacking footplate attachment, SGA – stratum germinativum in an area of *X. globicipitis* footplate attachment. (B) The stratum germinativum in dermal papillae of *T. truncatus* skin only, lacking attachments. (i) Basal measurement location, (ii) lateral measurement location (measurements were conducted at half height on the left and right lateral location of the dermal papillae). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**Fig. 6.** Dermal papillae – comparison of the dermal papillae (A) length:width ratios of areas of *T. truncatus* skin nearby and with *X. globicipitis* attachment. Dermal papillae in skin of *T. truncatus* (B) nearby barnacle attachment and (C) in an area of *X. globicipitis* attachment, displaying displacement of the dermal papillae directly underneath the barnacle footplate. Abbreviations of different epidermal and dermal structures: SG – stratum germinativum, DP – dermal papillae, SC – stratum corneum, R – ring of footplate. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
ate increased drag due to the host’s movement. Larger attachment features are therefore required (Eckman et al., 1990). The attachment structures displayed in X. globicipitis appear to be uniquely adapted to attach to the pliable, hairless skin of cetaceans rather than hardened surfaces that serve as attachment sites for many lepomorph and balanomorph barnacles. Our data suggest that attachment structures in adult X. globicipitis may be a collaboration between host and barnacle as Darwin (1854) proposed. Barnacle attachment and production of the rings of the footplates would serve as an irritant to the dolphin skin and could result in increased production of the keratinized stratum corneum. This in association with reduced sloughing of the stratum corneum due to the protection provided by the rings of the footplates could serve as an additional anchoring mechanism for the shell. In this case, the defensive mechanism of the host’s skin may further strengthen the attachment of the epizoite.

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