# Evolution of interspecific variation in size of attachment structures in the large tapeworm genus *Acanthobothrium* (Tetraphyllidea: Onchobothriidae)

H. S. RANDHAWA* *and* R. POULIN

*Department of Zoology, University of Otago, P.O. 56, Dunedin, New Zealand, 9054*

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## SUMMARY

Parasites have evolved a myriad of attachment structures closely adapted to their hosts and sites of attachment. Here, using members of the genus *Acanthobothrium* van Beneden, 1850 (Cestoda: Tetraphyllidea: Onchobothriidae), we (i) examined the influence of host body size and phylogeny, in addition to morphological features of these tapeworms, on the size of 3 structures used in attachment (bothridia, accessory suckers and hooks) by means of general linear models and phylogenetic-independent contrasts methods, and (ii) quantified the scaling exponents of relationships between size of attachment structures and tapeworm body size. Our results indicate that there exists a positive relationship, albeit not directly proportional, between size of attachment structures and *Acanthobothrium* spp. body size, and hook size and size of bothridia and accessory suckers. These results suggest that the resource investment in whole-body growth is greater than that in attachment structures, and that a greater investment in development of bothridia and accessory suckers is required to maintain an equivalent functional efficacy to hooks. In addition, host body size also influences, though less markedly, the size of attachment structures in *Acanthobothrium* spp. independently of parasite size itself. *Acanthobothrium* species have evolved a generalized mode of attachment that is successful in maintaining their position on various intestinal mucosal topographies across a variety of hosts exploiting different food resources.

Key words: *Acanthobothrium* spp., allometric relationship, body size, Elasmobranch, general linear model, hook size, phylogenetically-independent contrasts, Tetraphyllidea.

## INTRODUCTION

Parasites have evolved a wide array of attachment structures, varying in complexity, to prevent being dislodged from their host (e.g. modified antennules, antennae, mandibles, maxillules and maxillae of parasitic copepods; hooked proboscis of acanthocephalans). Since obligate parasites depend on their hosts for survival, failure to attach would likely result in the death of the parasite. In order to maximize the parasite's odds of remaining attached, intricate attachment structures often are closely adapted to their hosts (Crompton, 1973). For instance, the width of the groove on the heads of chewing lice is generally correlated to the diameter of hair shafts of their mammalian hosts (Morand *et al.* 2000). As adults, many internal parasites live in the host's digestive tract, a dynamic habitat exerting physical forces, such as peristalsis and food movement, on its inhabitants (Hayunga, 1991). Consequently, many gastrointestinal parasites have developed attachment structures adapted to specific sites or niches within the digestive tract of their hosts (Hayunga, 1991). Several examples of morphological adaptations of tetraphyllidean tapeworms to the mucosal topography of the spiral intestine of elasmobranch fishes have been documented (e.g. Carvajal and Dailey, 1975; McVicar, 1979; McKenzie and Caira, 1998; Randhawa and Burt, 2008). Most of these studies have focused on taxa infecting a single host species. Despite the evolutionary success of gastrointestinal helminths, there remains a lack of understanding of the factors influencing interspecific variation in the size of attachment structures.

In parasites, investment of resources in different traits is rarely isometric (e.g. Keymer *et al.* 1991; Skorping *et al.* 1991; Morand, 1996; Poulin, 2007, 2009). Attaining larger sizes is beneficial for the production of more numerous offspring (e.g. Skorping *et al.* 1991; Poulin, 1996), but this benefit must be weighed against the risk of dislodgement. Consequently, natural selection could favour smaller body sizes or larger attachment structures. However, parasite body size is also influenced by other factors. For instance, host size, environmental temperature and host habitat are variables that have been found to affect tetraphyllidean tapeworm body size (Randhawa and Poulin, 2009). The synergistic effects of these variables and the relative evolutionary

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* Corresponding author: Tel: +64 3 479 4039. Fax: +64 3 479 7584. E-mail: haseeb.randhawa@otago.ac.nz

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costs of different traits will impact indirectly the size of attachment structures of gastrointestinal parasites. Indeed, Poulin (2007) reported that total hook length of acanthocephalans is positively correlated with total body volume and is also influenced by host physiology. To our knowledge, there are no studies that have examined the effects of the above-mentioned variables on interspecific variation in size of attachment structures of gastrointestinal Platyhelminthes.

Tapeworms of the genus *Acanthobothrium* van Beneden, 1850 (Cestoda: Tetraphyllidea: Onchobothriidae), in their definitive hosts, represent an ideal group to examine the variables influencing interspecific variations in size of attachment structures. First, this genus is the most speciose of the Tetraphyllidea, including 178 species or over 25% of described species of the order prior to the recognition of the recently proposed order Rhinebothriidea (Healy *et al.* 2009) (see Supplementary material in Randhawa and Poulin, 2009-Online version only). Second, species assigned to this genus are characterized by a consistent scolex morphology: 4 bothridia, each divided into 3 loculi by 2 transverse septa and bearing a pair of bifid hooks, and usually bearing a muscular pad with accessory sucker (Euzet, 1994; Campbell and Beveridge, 2002) (Fig. 1). Third, they represent the most speciose genus of tapeworms parasitizing elasmobranch fishes (Randhawa and Poulin, 2010) and infect a wide variety of elasmobranchs, including representatives of the 2 major elasmobranch lineages: batoids and sharks. Hence, among a large and diverse array of host species, host size also varies significantly. Fourth, they exhibit a remarkably high degree of host specificity (Williams, 1969; Campbell and Beveridge, 2002); therefore, the size of a parasite species generally reflects its evolution in a single or very few closely related host species. Fifth, the largest member of the genus (*A. magnum* ex *Pteroplatytrygon violacea*; 500 mm) is nearly 350 times longer than the smallest representative of the genus (*A. gnomus* ex *Himantura uarnacoides*; 1·4 mm), and there is significant variation in the size of their attachment structures (see Supplementary material S1-Online version only). Lastly, the mode of attachment of several members of this genus has been described (Rees and Williams, 1965; Williams, 1968; Twohig *et al.* 2008). The mode of attachment involves the accessory suckers, bothridia and hooks, and is generalized here, using *A. coronatum* as an example (see Rees and Williams, 1965). (1) The scolex contracts with hooks slightly withdrawn or directed backwards, (2) using its bothridia, the parasite dilates the ridge between the mucosal folds (or crypts, or space between villi in the case of species infecting hosts with different intestinal mucosal topography (see examples in Twohig *et al.* 2008 and Williams, 1968, respectively)), using its hooks to prevent slippage and (3) when the apex of the scolex reaches the base of the mucosa, the accessory suckers attach, hooks penetrate the lamina propria (Twohig *et al.* 2008) and the bothridia firmly clasp surrounding mucosa.

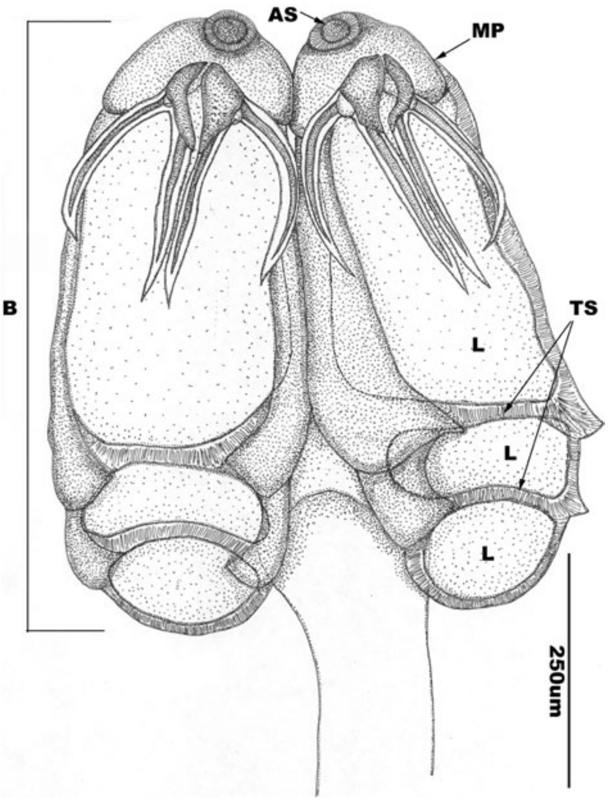

Fig. 1. Line drawing of scolex of *Acanthobothrium annapinkiense* Carvajal and Goldstein, 1971 from *Dipturus chilensis*. AS, accessory sucker; B, bothridium; L, loculus; MP, muscular pad; TS, transverse septum.

The hooks' function is analogous to that of an anchor (Bilqees and Freeman, 1969). The handle of the bifid hooks is embedded in the scolex, between the bothridia and muscular pads (Fig. 1) and generally, the axial prong is longer than the abaxial prong (Fig. 2) (see Supplementary material S1-Online version only). Within each pair, hooks are capable of independent movement controlled by 8 muscles and are assisted by movements of the scolex and bothridia (Rees and Williams, 1965). Even though the handle is generally shorter than either prong (see Supplementary material S1-Online version only), 4 of these muscles attach to the handle, 3 to the axial prong and 1 to the abaxial prong (Rees and Williams, 1965).

Commonly, multiple *Acanthobothrium* spp. are observed to not only parasitize individuals of the same host species (e.g. Campbell and Beveridge, 2002; Friggens and Brown, 2005; Fyler and Caira, 2006; Reyda and Caira, 2006), but also the same host individual (Friggens and Brown, 2005). In all likelihood, closely related host species exploiting

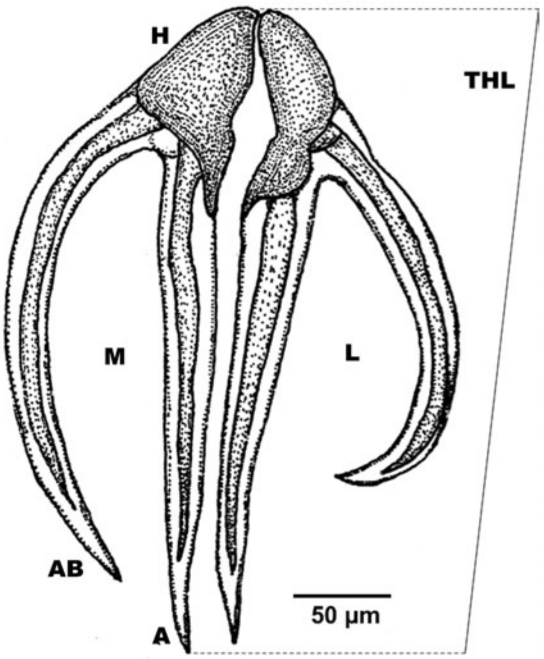

Fig. 2. Line drawing of hooks of *Acanthobothrium annapinkiense* Carvajal and Goldstein, 1971 from *Dipturus chilensis* showing hook length measured. A, axial prong; AB, abaxial prong; H, Handle; L, lateral hook; M, medial hook; THL, total hook length.

similar habitats are likely to encounter the same, or closely related, parasite faunas (Brooks, 1980). Parasites exploiting the same host, or closely related host species, may be exposed to similar selective pressures acting on their attachment structures. The objectives of this study were (i) to examine the influences of hosts (size and phylogeny) and *Acanthobothrium* parasite (length, width, and number of proglottids) on the size of structures involved in their attachment (bothridia, accessory sucker, hooks and hook handles) using general linear models (GLM) and phylogenetically independent contrasts (PIC); and (ii) to quantify the scaling exponents for the relationships between size of attachment structures and parasite size. The rationale for repeating our analyses at the level of attachment structures and hook handle is that hooks, which function as anchors (Bilqees and Freeman, 1969), are composed of 2 distinct parts (1) the prongs which penetrate the host's gut mucosa and (2) the handle embedded in the parasite's scolex. An anchor is only as strong as the chain to which it is attached, thus the relationship between hook size (i.e. anchor) and its handle (i.e. chain) is of particular interest since 4 of the 8 muscle bundles involved with hook movement attach to this latter structure (Rees and Williams, 1965). Detailed descriptions of these muscles are only available for 1 species (see Rees and Williams, 1965), but we suppose that larger muscles require larger points of attachment, thus should be associated with larger hook handles. Further, although it has been suggested that the electron-dense portion of the microtriches of tapeworms are involved in attachment (Thompson *et al.* 1980; Hayunga, 1991), these were not considered in the present study due to the paucity of microthriche measurements, or data on their density, available from the literature.

## MATERIALS AND METHODS

*Acanthobothrium* spp. measurement data were compiled from original descriptions, or re-descriptions, of adult worms (164 species) (for list of references see Supplementary material S2-Online version only). This extensive dataset therefore includes most (ca. 95%) *Acanthobothrium* species described up until May 2009. For each species, 10 variables were recorded (see Supplementary material S1-Online version only). First, host body length was recorded as the maximum length from the tip of the snout to the mid-point of the pelvic fins (as described in Randhawa and Poulin, 2009) and obtained from FishBase (Froese and Pauly, 2008) and Compagno *et al.* (2005). In rare cases where an *Acanthobothrium* sp. was recovered from more than one host species, host body length was obtained for the type host only. Second, 9 parasite measurements (maxima) were recorded: (1) length; (2) width of the strobila; (3) number of proglottids; (4) surface area of a single bothridium; (5) surface area of a single accessory sucker; (6) total medial hook length; (7) axial prong length; (8) abaxial prong length; and (9) hook handle length (see Supplementary material S1-Online version only). The first 3 represent estimates of *Acanthobothrium* sp. body size, whereas the latter 6 measurements correspond to structures used in their attachment to the gut mucosa; the latter 3 relating to different hook structures (Fig. 2). Although the bothridia and accessory suckers are 3-dimensional structures, authors generally report only their lengths and widths (or in some cases, diameter of accessory sucker); therefore, surface area (2-dimensional plane) was calculated using the formula for an ellipsoid ($\pi LW/4$), where L and W correspond to maximum length and width of these structures, respectively. Generally, *Acanthobothrium*-hooks are curved, thus the total hook length (as described by Euzet, 1959) was used as an estimate of hook length (Fig. 2). All hook measurements were taken from medial hooks.

Scaling exponents (i.e. slopes of linear relationships in logarithmic space) were obtained from simple linear regressions. Surface area for single bothridium and accessory sucker are second power linear measurements ($\mu m^2$); therefore, scaling exponents for isometric relationships between these and other linear measurements ($\mu m$) will be 2·0 (log-log scale), whereas an isometric relationship between these two measurements, or between 2 linear measurements, will have a scaling exponent of 1·0

Table 1. Summary of measurements for all variables included in GLM analyses, including range, mean and median

(SA, surface area; AS, attachment structures; H, handle.)

| Variable | Analysis | Range | Mean | Median |
|---|---|---|---|---|
| Host body length (cm) | Size AS/H | 12·5–220·0 | 92·9 | 73·1 |
| Maximum tapeworm length (mm) | Size AS/H | 1·4–500·0 | 57·2 | 25·0 |
| Maximum tapeworm width ($\mu$m) | Size AS/H | 128–4300 | 896 | 540 |
| Maximum no. proglottids | Size AS/H | 3–1100 | 157 | 53 |
| Bothridial SA (mm$^2$) | Size AS/H | 0·01–5·88 | 0·43 | 0·13 |
| SA Accessory sucker ($\mu$m$^2$) | Size AS | 177–574 146 | 14 565 | 3473 |
| Hook handle length ($\mu$m) | Size H | 17–250 | 74 | 60 |
| Axial prong length ($\mu$m) | Size H | 20–330 | 116 | 100 |
| Abaxial prong length ($\mu$m) | Size H | 22–322 | 99 | 85 |
| Hook length ($\mu$m) | Size AS/H | 60–550 | 178 | 155 |

(log-log scale). Scaling exponents with different values than those indicated above represent allometric relationships. Since none of these measurements were normally distributed, they were log-transformed for analyses.

Several factors are likely to influence the size of attachment structures in these tapeworms. To tease out their effects, we conducted 2 separate analyses: (1) factors influencing the size of attachment structures and (2) factors influencing the size of the hook handle. We identified, *a priori*, 7 predictor variables, which may have an influence on the size of attachment structures and 8 on handle size, respectively (Table 1). Relationships between size of individual attachment structures, or hook handles, and predictor variables were assessed using General Linear Models (GLM) (normal distribution; identity link function). Prior to transformation, all *Acanthobothrium* measurements used in analyses were in $\mu$m or $\mu$m$^2$. For the first set, analyses were repeated 3 times using the same dataset, each using a different dependent variable corresponding to individual attachment structures (e.g. surface areas of individual bothridium and accessory sucker, and hook length); therefore, only 6 predictor variables were included in each set of analyses (Table 1). For the second set, using the same dataset, we repeated the analyses using handle size as the dependent variable and included 7 predictor variables (Table 1). In each case, all possible main effects models were computed. Models were ranked according to their second-order Akaike Information Criterion values ($AIC_c$). The $AIC_c$ differences ($\Delta$ $AIC_c$) and model weights ($w_i$) were computed to determine the relative importance and rank of each variable (Anderson, 2008). The latter approach provides insights into the importance of each variable, taking into account the possible multicolinearity between predictor variables and requires running all possible models so that each variable is of equal footing (Anderson, 2008). When more than 1 model was supported, the multi-model inference approach was used (Burnham and Anderson, 2002). Model-averaged parameter estimates were obtained by weighting parameter estimates according to model probabilities (see Anderson, 2008). By averaging all models from our *a priori* set, 'bad' models received a weight that tends towards '0', thus keeping model inclusion objective. Unconditional variances were obtained to calculate a 95 % confidence interval for each variable (Burnham and Anderson, 2002; Anderson, 2008). This approach provides an estimate of the independent 'slope' for each parameter (Anderson, 2008). All sets of second-degree interactions between predictor variables were computed and compared to models incorporating main effects included in the interaction (see Randhawa and Poulin, 2009). Evidence ratios between the model including the interaction term and the 'best' model from each set (based on $AIC_c$) were used to determine whether the inclusion of the interaction term improved the model significantly. The best model, excluding pretending variables (non-informative variables that do not decrease the deviance when included in a model – see Anderson, 2008), was used to predict the size of our dependent variables (1000 iterations). These were plotted against the observed data to assess the 'fit' of our model ($r^2$).

As mentioned above, many *Acanthobothrium* spp. are known to infect the same host individual or species. Although these may not represent each other's closest relative (e.g. Campbell and Beveridge, 2002; Fyler *et al.* 2009), they are exposed to similar selective pressures within their host and as such, should not be considered independent from each other in a statistical sense (Morand and Poulin, 2003; Poulin and Morand, 2004). In the absence of a suitable *Acanthobothrium* phylogeny, a host phylogeny was used to control for confounding effects of similar habitats influencing attachment structures comparably. Recognizing that onchobothriid tapeworms show little evidence of co-evolving with their elasmobranch hosts (Caira and Jensen, 2001), by no means is the host phylogeny used here as a surrogate for that of *Acanthobothrium*. Rather, we assume that similar mucosal topographies are shared among closely related host species and exert similar selective pressures on attachment structures of their gastrointestinal parasites. Hence, analyses were subsequently repeated taking into account phylogenetic relationships between host species. The phylogenetically independent contrasts method (Felsenstein, 1985) was computed using the PDAP:PDTREE programme (Midford *et al.* 2005) implemented in Mesquite version 2.5 for Mac OSX (Maddison and Maddison, 2007). Contrasts were derived from a pruned elasmobranch tree (Randhawa and Poulin, 2010; see Supplementary material S3-Online version only). Few resolved phylogenies of elasmobranch genera are available in the literature, thus preventing us from resolving most intrageneric relationships. Consequently, all unresolved nodes (polytomies) were considered 'soft polytomies' (Maddison, 1990; Purvis and Garland, 1993). Although polytomies were arbitrarily resolved by collapsing all branches within unresolved intrageneric phylogenies (Felsenstein, 1985; Purvis and Garland, 1993), the number of degrees of freedom was reduced for hypothesis testing and set to between $N-1$ and $p-1$ (N, number of independent contrasts; *p*, number of nodes in the working phylogeny) in order to limit Type I error (Purvis and Garland, 1993). An additional taxonomic level was added to that tree to reflect the multiple *Acanthobothrium* taxa infecting the same host species. This approach was preferred over averaging measurements from all *Acanthobothrium* spp. infecting a single host species, thus avoiding the loss of too many contrasts and degrees of freedom. Since no information on branch lengths for this host group was available, branch lengths were estimated assuming that characters evolved by Brownian motion (Felsenstein, 1985) and their statistical adequacy was verified (Garland *et al.* 1992). Contrasts were computed following guidelines highlighted by Garland *et al.* (1992).

## RESULTS

The majority of hosts harbouring *Acanthobothrium* spp. are smaller than 75 cm. The typical *Acanthobothrium* parasites have the following median dimensions: (1) 25 mm in length; (2) 540 $\mu$m in width; (3) 53 proglottids; (4) 0·13 mm$^2$ bothridial surface area (single bothridium) (Fig. 3a); (5) approximately 3500 $\mu$m$^2$ accessory sucker surface area (single accessory sucker) (Fig. 3b); (6) 60 $\mu$m hook handle length; (7) 100 $\mu$m axial prong length; (8) 85 $\mu$m abaxial prong length; and (9) 155 $\mu$m hook length (Fig. 3c) (see Table 1 for range and means).

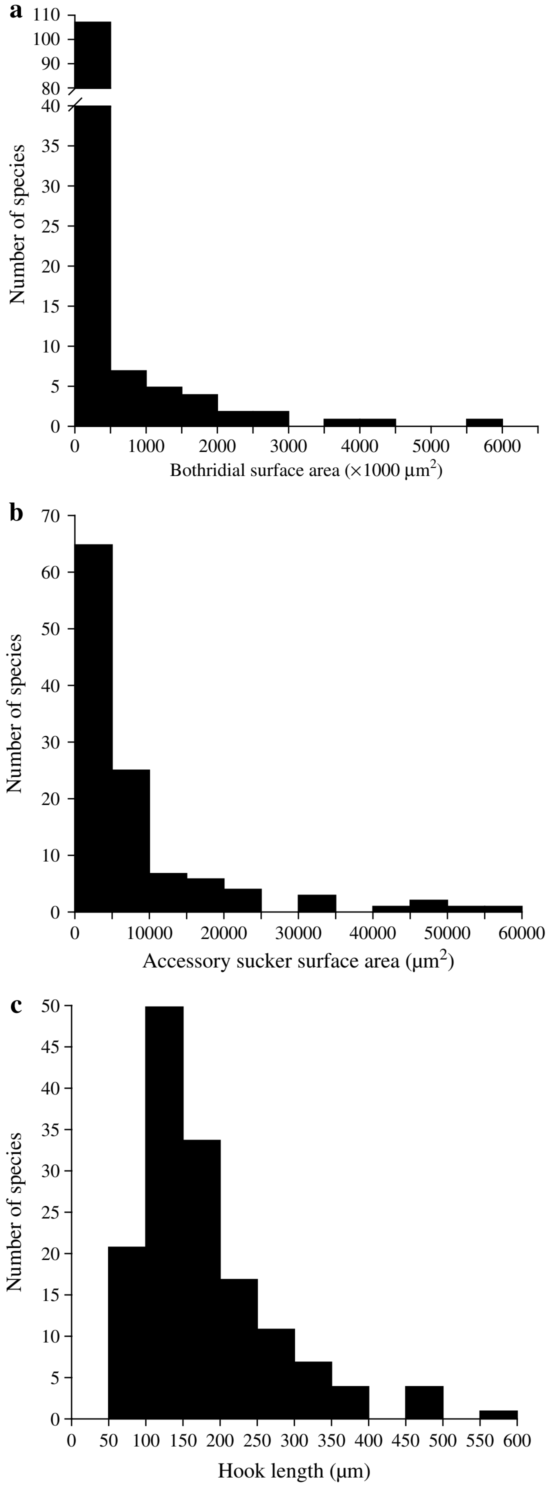

Fig. 3. Frequency distribution of (a) bothridial surface area, (b) surface area of accessory sucker, and (c) hook length ($n = 130$, 118, and 149, respectively).

### *General linear models* (*GLM*)

*Factors influencing size of attachment structures.* The GLM analyses included 82 species for which data were available for all dependent and predictor variables. The best model explaining the interspecific variation in bothridial surface area ($AIC_c = -209{\cdot}49$) included all variables. All 10 'best' models included accessory sucker surface area and a combination of maximum number of proglottids ($n = 9$), hook length ($n=8$), host length ($n=7$), maximum length ($n=6$), and maximum width ($n=5$) (Table 2). The relative importance of each variable mirrors that of the frequency of inclusion (descending order) in the 10 'best' models, except that hook length is identified as being less important than host length (Table 3). Accessory sucker surface area, hook length, maximum number of proglottids, and host length have 95% confidence intervals bound away from '0', whereas those for maximum length and width are not; therefore, the latter 2 are pretending variables (variables that do not significantly improve the likelihood of a model by their inclusion – see Anderson, 2008). Additionally, main effects models were not improved significantly by the inclusion of their respective interaction terms. The model including accessory sucker surface area, hook length, maximum number of proglottids, and host length was used to predict bothridial surface area (quadratic equation; $r^2=0{\cdot}86$; Fig. 4a). No outliers were identified using the Robust Regression and Oultier Removal method in Prism5.

The best model explaining the interspecific variation in accessory sucker surface area ($\text{AIC}_\text{c}=-176{\cdot}42$) included host length, maximum length, maximum number of proglottids, and bothridial surface area. All 10 'best' models included bothridial surface area and a combination of maximum length ($n=7$), host length ($n=7$), maximum number of proglottids ($n=4$), maximum width ($n=3$), and hook length ($n=2$) (Table 2). The relative importance of each variable mirrors that of the frequency of inclusion in the 10 'best' models (Table 3). Only bothridial surface area has a 95% confidence interval bound away from '0'. Additionally, main effects models were not improved significantly by the inclusion of their respective interaction terms.

The best main effects model explaining the interspecific variation in hook length ($\text{AIC}_\text{c}=-315{\cdot}54$) included only bothridial surface area. All 10 'best' models included bothridial surface area and a combination of host length ($n=4$), maximum length ($n=3$), maximum number of proglottids ($n=2$), accessory sucker surface area ($n=2$), and maximum width ($n=2$) (Table 2). The relative importance of each variable mirrors that of the frequency of inclusion in the 10 'best' models (Table 3). Only bothridial surface area has a 95% confidence interval bound away from '0'. Additionally, main effects models including bothridial surface area in combination with either maximum length, width and number of proglottids, and accessory sucker surface area, were improved significantly by the inclusion of their respective interaction terms (Table 4). The best model explaining the variation in hook length ($\text{AIC}_\text{c}=-335{\cdot}53$) included maximum length, bothridial surface area and their interaction term was used to predict hook length (cubic equation; $r^2=0{\cdot}45$; Fig. 4b). No outliers were identified using

Table 2. Summary of the top 10 models for *Acanthobothrium* sp. attachment structure size (surface areas [SA] for bothridium and accessory sucker, and hook length) and hook handle based on AIC

(Models are shown including the number of parameters ($K$), Log Likelihood (Log ($L$)), $\Delta$-$\text{AIC}_\text{c}$, and Akaike weights ($w_i$) of each model given the data.)

SA of bothridium

| Model* | $K$ | Log ($L$) | $\text{AIC}_\text{c}$ | $\Delta$-$\text{AIC}_\text{c}$ | $w_i$ |
|---|---|---|---|---|---|
| {123456} | 8 | 113·73 | −209·49 | — | 0·289 |
| {1456} | 6 | 111·01 | −208·91 | 0·58 | 0·216 |
| {12456} | 7 | 112·09 | −208·66 | 0·83 | 0·191 |
| {13456} | 7 | 111·75 | −207·99 | 1·50 | 0·136 |
| {12345} | 7 | 110·58 | −205·65 | 3·84 | 0·042 |
| {456} | 5 | 107·46 | −204·14 | 5·35 | 0·020 |
| {1245} | 6 | 108·55 | −203·98 | 5·51 | 0·018 |
| {2456} | 6 | 108·43 | −203·74 | 5·75 | 0·016 |
| {1356} | 6 | 108·28 | −203·43 | 6·06 | 0·014 |
| {23456} | 7 | 109·41 | −203·31 | 6·18 | 0·013 |

* Predictor variables: 1, Host length; 2, Max length; 3, Max width; 4, Max no. proglottids; 5, SA accessory sucker; 6, Hook length.

SA of accessory sucker

| | | | | | |
|---|---|---|---|---|---|
| {1245} | 6 | 94·77 | −176·42 | — | 0·165 |
| {125} | 5 | 92·98 | −175·18 | 1·24 | 0·089 |
| {245} | 5 | 92·75 | −174·71 | 1·71 | 0·070 |
| {15} | 4 | 91·57 | −174·61 | 1·81 | 0·067 |
| {25} | 4 | 91·42 | −174·31 | 2·11 | 0·057 |
| {12456} | 7 | 94·86 | −174·20 | 2·22 | 0·054 |
| {12345} | 7 | 94·83 | −174·15 | 2·27 | 0·053 |
| {135} | 5 | 92·27 | −173·75 | 2·67 | 0·043 |
| {1256} | 6 | 93·17 | −173·22 | 3·20 | 0·033 |
| {35} | 4 | 90·86 | −173·19 | 3·23 | 0·033 |

* Predictor variables: 1, Host length; 2, Max length; 3, Max width; 4, Max no. proglottids; 5, SA bothridium; 6, Hook length.

Hook length

| | | | | | |
|---|---|---|---|---|---|
| {5} | 3 | 160·92 | −315·54 | — | 0·181 |
| {15} | 4 | 161·44 | −314·35 | 1·19 | 0·100 |
| {56} | 4 | 161·02 | −313·53 | 2·01 | 0·066 |
| {25} | 4 | 160·99 | −313·46 | 2·08 | 0·064 |
| {35} | 4 | 160·97 | −313·41 | 2·13 | 0·063 |
| {45} | 4 | 160·96 | −313·40 | 2·14 | 0·062 |
| {156} | 5 | 161·68 | −312·57 | 2·97 | 0·041 |
| {125} | 5 | 161·58 | −312·38 | 3·16 | 0·037 |
| {245} | 5 | 161·56 | −312·34 | 3·20 | 0·037 |
| {135} | 5 | 161·44 | −312·09 | 3·45 | 0·032 |

* Predictor variables: 1, Host length; 2, Max length; 3, Max width; 4, Max no. proglottids; 5, SA bothridium; 6, SA accessory sucker.

Handle length

| | | | | | |
|---|---|---|---|---|---|
| {23468} | 7 | 244·29 | −473·24 | — | 0·103 |
| {3468} | 6 | 242·50 | −472·01 | 1·23 | 0·056 |
| {234678} | 8 | 244·87 | −472·00 | 1·24 | 0·056 |
| {468} | 5 | 241·33 | −471·95 | 1·29 | 0·054 |
| {234568} | 8 | 244·69 | −471·65 | 1·59 | 0·047 |
| {4568} | 6 | 242·31 | −471·64 | 1·60 | 0·047 |
| {24568} | 7 | 243·41 | −471·49 | 1·75 | 0·043 |
| {2468} | 6 | 242·23 | −471·47 | 1·77 | 0·043 |
| {4678} | 6 | 242·08 | −471·18 | 2·06 | 0·037 |
| {123468} | 8 | 244·38 | −471·02 | 2·22 | 0·034 |

* Predictor variables: 1, Host length; 2, Max length; 3, Max width; 4, Max no. proglottids; 5, SA bothridium; 6, Axial prong length; 7, Abaxial prong length; 8, Total hook length.

Table 3. Predictor variable relative importance weights [$w_{+}(i)$], ranks, weighted model average parameter estimates, and 95% confidence interval

(Parameter estimates in bold indicate those bounded away from '0'.)

SA of bothridium

| Predictor variable | $w_{+}(i)$ | Rank | Parameter estimate | Confidence interval |
|---|---|---|---|---|
| **Host length** | **0·9323** | **3** | **−0·238** | **−0·403 to −0·073** |
| Max tapeworm length | 0·5813 | 5 | −0·133 | −0·290 to 0·024 |
| Max tapeworm width | 0·5225 | 6 | 0·138 | −0·040 to 0·316 |
| **Max no. proglottids** | **0·9786** | **2** | **0·382** | **0·167 to 0·597** |
| **SA accessory sucker** | **1·0000** | **1** | **0·539** | **0·410 to 0·668** |
| **Hook length** | **0·9113** | **4** | **0·487** | **0·134 to 0·841** |
| SA of accessory sucker | | | | |
| Host length | 0·6570 | 3 | 0·145 | −0·010 to 0·299 |
| Max length | 0·6818 | 2 | 0·191 | −0·006 to 0·388 |
| Max width | 0·3141 | 5 | 0·044 | −0·077 to 0·164 |
| Max no. proglottids | 0·5030 | 4 | −0·125 | −0·306 to 0·055 |
| **SA bothridium** | **1·0000** | **1** | **0·805** | **0·624 to 0·985** |
| Hook length | 0·2614 | 6 | −0·033 | −0·169 to 0·103 |
| Hook length | | | | |
| Host length | 0·3587 | 2 | 0·018 | −0·017 to 0·053 |
| Max length | 0·3049 | 3 | −0·012 | −0·042 to 0·018 |
| Max width | 0·2673 | 6 | 0·008 | −0·030 to 0·046 |
| Max no. proglottids | 0·2865 | 4 | 0·010 | −0·021 to 0·040 |
| **SA bothridium** | **1·0000** | **1** | **0·143** | **0·067 to 0·218** |
| SA accessory sucker | 0·2807 | 5 | −0·006 | −0·031 to 0·020 |
| Handle length | | | | |
| Host length | 0·2736 | 8 | 0·004 | −0·009 to 0·018 |
| Max length | 0·5225 | 5 | −0·029 | −0·064 to 0·007 |
| Max width | 0·5701 | 4 | 0·040 | −0·004 to 0·085 |
| **Max no. proglottids** | **0·9135** | **3** | **0·079** | **0·027 to 0·131** |
| SA bothridium | 0·3929 | 6 | 0·011 | −0·006 to 0·028 |
| **Axial prong length** | **0·9657** | **2** | **−0·496** | **−0·808 to −0·185** |
| Abaxial prong length | 0·3714 | 7 | −0·037 | −0·103 to 0·028 |
| **Total hook length** | **1·0000** | **1** | **1·364** | **1·018 to 1·709** |

the Robust Regression and Oultier Removal method in Prism5.

*Factors influencing hook handle size.* The GLM analyses included 92 species for which data were available for all dependent and predictor variables. The best model explaining the interspecific variation in handle length ($\text{AIC}_c = -473{\cdot}24$) included all 3 surrogates for body size, axial prong length and total hook length. All 10 'best' models included maximum number of proglottids, axial prong length and total hook length and a combination of maximum length ($n = 6$), maximum width ($n = 5$), bothridial surface area ($n = 3$), abaxial prong length ($n = 2$), and host length ($n = 1$) (Table 2). The relative importance of each variable mirrors that of the frequency of inclusion (descending order) in the 10 'best' models (Table 3). Total hook length, axial prong length and maximum number of proglottids have 95% confidence intervals bound away from '0'. Additionally, none of the main effects models were improved significantly by the inclusion of their respective interaction terms. The model including maximum number of proglottids, axial prong length, and hook length was used to predict handle length (cubic equation; $r^2 = 0{\cdot}85$; Fig. 4c). No outliers were identified using the Robust Regression and Oultier Removal method in Prism5.

### *Phylogenetic independent contrasts* (*PIC*)

Prior to using the PIC method, the adequacy of our branch lengths was verified (Garland *et al.* 1992) and an exponential transformation of branch lengths, following their estimation using the method described by Grafen (1989), was deemed necessary for all PIC analyses. Additionally, the number of degrees of freedom for all PIC analyses was reduced by 79 since our tree included 79 polytomies.

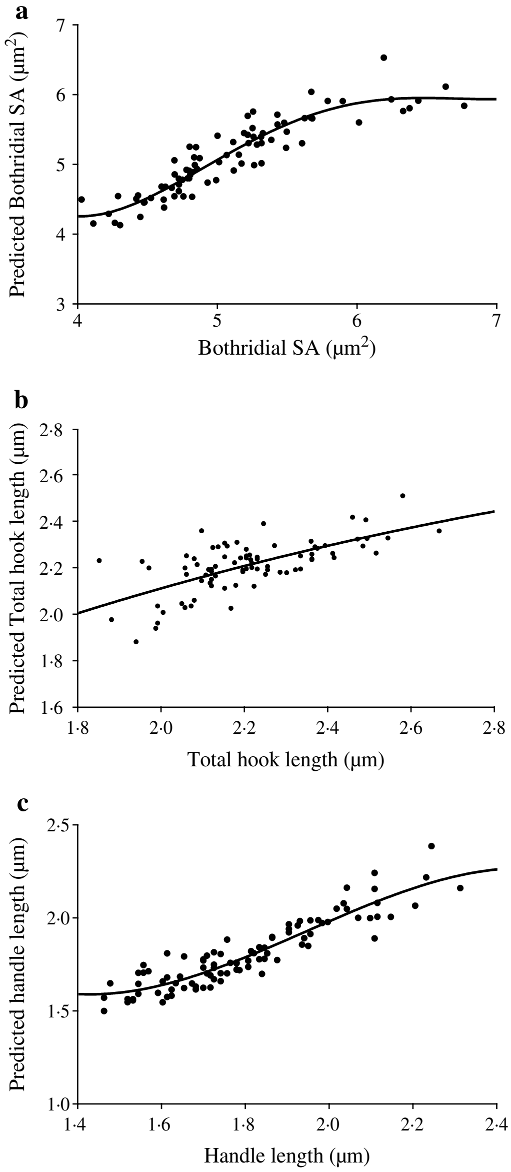

Fig. 4. Relationships between predicted values from models and observed data for (a) bothridial surface area (BothSA) [quadratic equation: accessory sucker surface area + hook length + maximum number of proglottids + host length; $\mathrm{AIC_c} = -208{\cdot}91$; $r^2 = 0{\cdot}86$]; (b) hook length [cubic equation: maximum length + BothSA + (maximum length*BothSA); $\mathrm{AIC_c} = -335{\cdot}53$; $r^2 = 0{\cdot}45$]; and (c) handle length [cubic equation: maximum number of proglottides + axial prong length + total hook length; $\mathrm{AIC_c} = -471{\cdot}95$; $r^2 = 0{\cdot}85$]. Note the logarithmic scale on both axes.

*Factors influencing size of attachment structures.* Bothridial surface area, accessory sucker surface area, and hook length were all positively correlated ($P < 0{\cdot}0001$) with maximum length, width and number of proglottids and both predictor variables corresponding to size of attachment structures, respectively. Accessory sucker surface area and hook length were both positively correlated with host length ($P < 0{\cdot}05$) (Supplementary Material S4-Online version only).

*Factors influencing hook handle size.* Correcting for host phylogeny, handle length was positively correlated ($P < 0{\cdot}0001$) with all 7 predictor variables.

### *Scaling exponents*

*Factors influencing size of attachment structures.* The scaling exponents for relationships between bothridial and accessory sucker surface areas versus parasite length (slope = 0·667; 95% CI = 0·517–0·823 and slope = 0·660; 95% CI = 0·495–0·824, respectively) are below 2·0 ($P < 0{\cdot}0001$), an indication of hypo-allometry (Fig. 5a). The same applies for hook length (slope = 0·088; 95% CI = 0·032–0·144; $P < 0{\cdot}0001$) (Fig. 5b). Additionally, the scaling exponent between hook length and surface area of a single bothridium and accessory sucker reveals hypo-allometric (< 0·5) relationships (slope = 0·141; 95% CI = 0·088–0·195; $P < 0{\cdot}0001$ and 0·108; 95% CI = 0·053–0·163; $P < 0{\cdot}0001$, respectively) (Supplementary material S5a-Online version only), whereas that between bothridial and accessory sucker surface areas is nearly isometric (slope = 0·859) (Supplementary Material S5b-Online version only).

*Factors influencing hook handle size.* The scaling exponents between handle length and total hook length (slope = 1·046; 95% CI = 0·920–1·171; $P = 0{\cdot}47$) and axial prong length (slope = 0·8684; 95% CI = 0·6844–1·052; $P = 0{\cdot}16$) reveal isometric relationships (see Supplementary Material S6-Online version only). Additionally, the scaling exponent for relationships between handle length and bothridial surface area was below 0·5 (slope = 0·2258; 95% CI = 0·1729–0·2787; $P < 0{\cdot}0001$), an indication of hypo-allometry (Supplementary material S7a-Online version only). The same applies for the maximum number of proglottids (slope = 0·2208; 95% CI = 0·1712–0·2703; $P < 0{\cdot}0001$) (Supplementary material S7b-Online version only).

## DISCUSSION

It is obvious from our results that the size of the bothridia, accessory suckers and hooks of *Acanthobothrium* spp. is correlated with size of the host, size of the parasite itself, and that of other attachment structures. First, bothridial surface area is positively correlated with (1) surface area of the accessory sucker (Supplementary material S5b), (2) number of proglottids, (3) host length (Fig. 6) and (4) hook length (Supplementary material S5a). Second, handle size is positively correlated with (1) total hook length (Supplementary material S6a), (2) axial prong length (Supplementary material S6b), and (3) number of proglottides (Supplementary material S7b). Lastly, when correcting the size of the attachment

Table 4. Summary of the interactions between predictor variables that significantly improved the main effects models for variation in hook length

(Models are shown including their $AIC_c$, Akaike weights ($w_i$) and evidence ratio (ER).)

| Interaction | Model* | $AIC_c$ | $w_i$ | ER |
|---|---|---|---|---|
| MaxL ∗ BothSA | MaxL | −300·49 | 0·000 | 40 661 602·69 |
| | BothSA | −315·54 | 0·000 | 21 907·16 |
| | MaxL + BothSA | −313·46 | 0·000 | 62 137·16 |
| | MaxL + BothSA + (MaxL ∗ BothSA) | −335·53 | 1·000 | 1·00 |
| MaxW ∗ BothSA | MaxW | −305·18 | 0·000 | 16 150·40 |
| | BothSA | −315·54 | 0·011 | 90·73 |
| | MaxW + BothSA | −313·41 | 0·004 | 262·61 |
| | MaxW + BothSA + (MaxW ∗ BothSA) | −324·56 | 0·985 | 1·00 |
| NoP ∗ BothSA | NoP | −305·58 | 0·000 | 21 367·68 |
| | BothSA | −315·54 | 0·007 | 147·14 |
| | NoP + BothSA | −313·40 | 0·002 | 428·06 |
| | NoP + BothSA + (NoP ∗ BothSA) | −325·52 | 0·991 | 1·00 |
| AcSA ∗ BothSA | AcSA | −305·27 | 0·000 | 48 887·70 |
| | BothSA | −315·54 | 0·004 | 288·46 |
| | AcSA + BothSA | −313·53 | 0·001 | 789·62 |
| | AcSA + BothSA + (AcSA ∗ BothSA) | −326·87 | 0·995 | 1·00 |

* AcSA, surface area of accessory sucker; BothSA, surface area of bothridium; MaxL, maximum Length; MaxW, maximum width; NoP, maximum number of proglottids.

structures and hook handles for shared evolutionary histories of hosts, consistent trends emerge: (1) larger *Acanthobothrium* spp. have larger attachment structures and hook handles; (2) species with larger bothridia have larger accessory suckers and bear larger hooks with longer handles. Models, generated from our study, predicting bothridial surface area and handle length provide excellent 'fits' to the actual measurements for these variables. Strikingly, our model predicts a maximum size for bothridia. This result suggests that the functional performance of bothridia plateaus from 1·75 $mm^2$, at which point, the cost of growing larger bothridia at the expense of other life-history traits may outweigh the benefits. Considering the ancient nature of the relationship between *Acanthobothrium* and its elasmobranch hosts, these parasites may have maximized efficient resource allocation. Evidence presented herein suggests that the amount of resources is, for any given size, less than directly proportional to total body size.

Consistent with the trend observed for all tetraphyllidean tapeworms, longer *Acanthobothrium* worms were wider and possessed more numerous proglottids (see Randhawa and Poulin, 2009). Larger worms tended to have larger bothridia, accessory suckers and hooks. However, the hypo-allometric relationship between size of all 3 attachment structures and parasite length indicate that investment in attachment structures is not directly proportional to investment of resources in growth. For example, a 10-fold increase in length translates into an 8·62 ×-increase in number of proglottids, 4·67 ×-increase in bothridial surface area, a 4·57 ×-increase in surface area of the accessory sucker, 1·57 ×-increase in handle length, a 1·11 ×-increase in axial prong length and a 1·28 ×-increase in hook length. The rate of growth required for functional efficacy of hooks in *Acanthobothrium* is less than that for bothridia or accessory suckers. Furthermore, the handle, axial prong and total hook length are influenced equally by *Acanthobothrium* size, but bothridial size increases at a greater rate than can be explained by increasing hook musculature alone. Alternatively, the relative difference in bothridial/accessory sucker size and hook size may be determined by ontogenic constraints. The foundations of the tetraphyllidean scolex are laid down early in development (Williams, 1966). Rudimentary bothridia and accessory suckers are present in onchobothriid metacestodes, whereas hook production is initiated in the definitive host (Hamilton and Byram, 1974; Caira and Ruhnke, 1991; Chambers *et al.* 2000; Holland *et al.* 2009). Although little is known about hook development in onchobothriid tapeworms, studies on cyclophyllidean tapeworms have demonstrated that hooks develop at a slower rate than other scolex structures, including the suckers (e.g Crusz, 1947; Bilqees and Freeman, 1969; Mount, 1970). Another possible explanation is that the efficiency of attachment structure in preventing dislodgement is not scaled with body size (see Poulin, 2009). The absence of studies investigating the efficiency of hooks as anchoring structures prevents us from testing these hypotheses. Future studies investigating the relationship between different life-history traits and body size in this group of tapeworms, such as the one conducted by Zamparo *et al.* (1999), may provide insights into resource allocation in tapeworms.

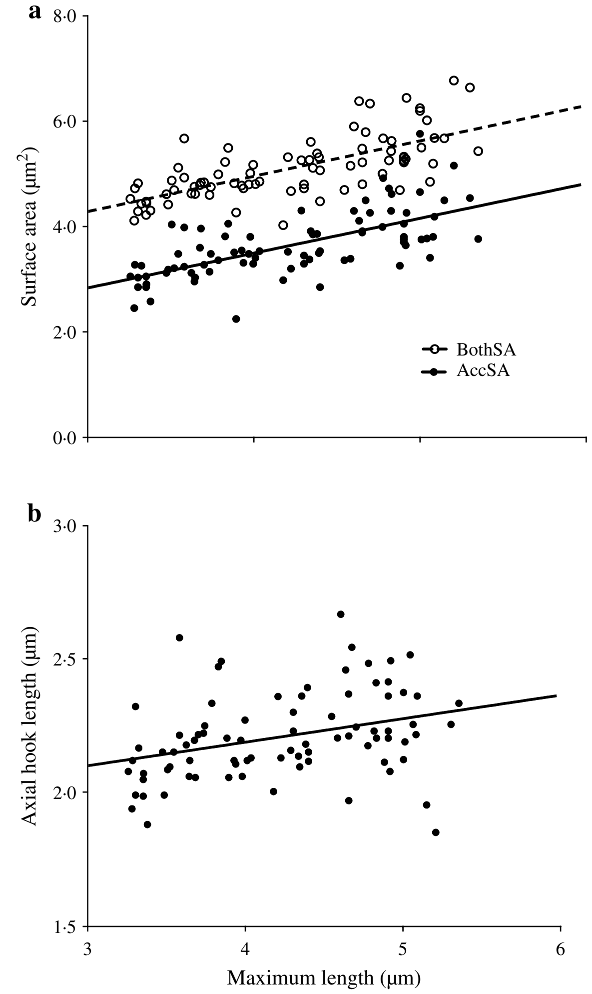

Fig. 5. Hypo-allometric relationships between attachment structures and *Acanthobothrium* spp. ($n = 82$) maximum tapeworm length: (a) bothridial surface area (BothSA) (open circles, dashed line) and surface area of accessory sucker (AccSA) (black circles, full lines) and (b) hook length (HL) (black circles, full lines). The lines represent the best fit from simple linear regression: (a) Log (BothSA) = 2·2764266 + (0·6696627 × Log[Maximum tapeworm length]); r = 0·698; $P < 0·0001$ and Log (AccSA) = 0·8579910 + (0·6593419 × Log[Maximum tapeworm length]); r = 0·666; $P < 0·0001$; (b) Log(HL) = 1·8367304 + (0·0881335 × Log[Maximum tapeworm length]); r = 0·330; $P = 0·0024$. Note the logarithmic scale on both axes.

Additionally, a similar pattern emerges when considering Pearson product-moment correlation coefficients: maximum tapeworm length is better at explaining the variance in bothridial surface area and that of accessory sucker than the variance in hook length (r = 0·698, 0·666 and 0·330, respectively). These coefficients are similar even when considering host phylogenetic history (see Supplementary material S4), implying that *Acanthobothrium* spp. have evolved similar resource investment strategies regardless of the hosts they parasitize. Total hook length is better at explaining the variance in handle size than axial prong length and number of proglottids (r = 0·866, 0·704 and 0·682, respectively). These coefficients are similar even when considering host phylogenetic history, implying that *Acanthobothrium* spp. with larger ‘anchors’ require longer ‘chains’.

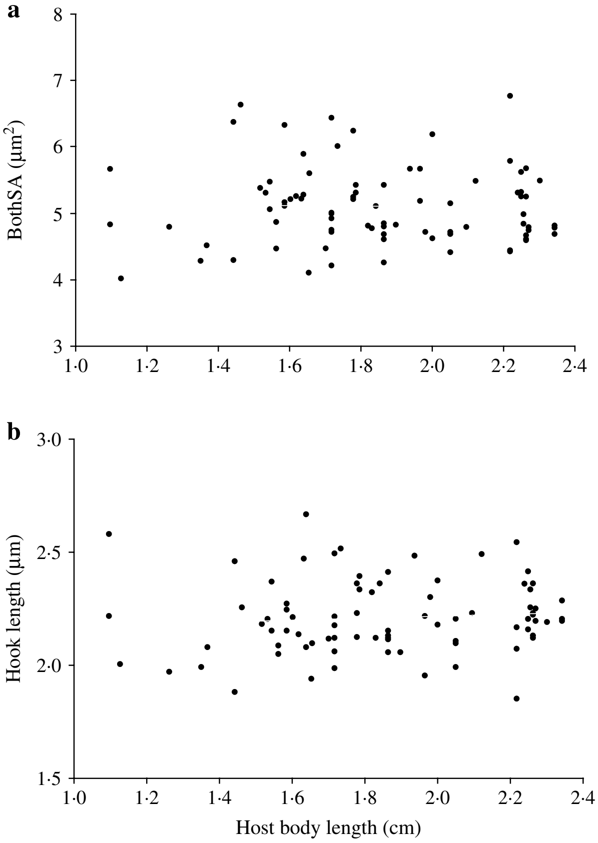

Fig. 6. Relationships between host body length and: (a) bothridial surface area (r = 0·02) and (b) total hook length (r = 0·09) for 82 *Acanthobothrium* species. Note the logarithmic scale on both axes.

Host body size is positively correlated with tetraphyllidean tapeworm body size (Randhawa and Poulin, 2009) and with overall tapeworm species richness in elasmobranch fishes (Randhawa and Poulin, 2010). Our results imply that host body size also influences the size of attachment structures in *Acanthobothrium* spp. independently of parasite size itself. Host body length is considered an important predictor variable in our GLM analyses for bothridial surface area. This influence is not significant in the absence of other predictor variables, nor when correcting for effects of host phylogeny, yet this latter approach does reveal a weak significant effect of host length on surface area of accessory sucker and hook length (r = 0·233 and 0·201, respectively). The absence of a relationship between host body length and bothridial surface when correcting for host phylogeny can likely be explained by hosts harbouring multiple *Acanthobothrium* spp. which vary in size. For instance, *Myliobatis australis* is host to 2 species measuring 1·9 mm and 250 mm (*A. martini* and *A. pichelinae*, respectively). Other examples of host species harbouring *Acanthobothrium* spp. with maximum length ratios >40 : 1 include: *Rhynchobatus djiddensis* (76 : 1), *Gymnura micrura* (46 : 1), *Trigonorrhina fasciata* (43 : 1) and *Aetobatus narinari* (43 : 1) (see Supplementary material S1). Furthermore, the measure of host body size correlated with tetraphyllidean body size is host weight, not host length (Randhawa and Poulin, 2009). Although host weight may be a better proxy of host size, too few data for host weight were available to be included in these analyses. Repeating these analyses to include host weight, when and if these data become available, as a predictor variable may reveal an increased importance of host size in the evolution of size of attachment structures in *Acanthobothrium* spp.

Not surprisingly, tapeworm size and other morphological features are influenced by multiple variables. For instance, tetraphyllidean body size is influenced by host size, environmental temperature and host habitat (Randhawa and Poulin, 2009). In fact, models presented herein demonstrate that bothridial surface area, hook length, and handle length are influenced by multiple variables (4, 3 and 3, respectively). The distribution of bothridial surface area measurements is predicted to follow a sigmoid curve based on a model including 4 predicting variables (accessory sucker surface area, hook length, maximum number of proglottids, and host length). The lag phase of the curve comprises small *Acanthobothrium* species infecting mostly myliobatiform hosts and suggests that below a certain body size, minimal investment in bothridial development is necessary to ensure functional efficiency. The plateau phase of the curve includes mostly large *Acanthobothrium* species, suggesting that beyond a certain size, investment in bothridial development/growth does not increase the functional efficiency of this structure. Additionally, congeners infecting the same host species often differ markedly in size and can be found at both extremes of the sigmoid curve, i.e. smaller species in the lag phase of the distribution and larger species in the plateau portion of the distribution (e.g. *A. mooreae* and *A. robertsoni* ex *Trigonorrhina fasciata*; *A. lasti* and *A. xiamenensis* ex *Rhynchobatus djiddensis*). Although there is no comprehensive or well-resolved phylogeny of the genus, attempts using both morphological features and molecules show that congeners infecting the same host species are not each other's closest relative (see Campbell and Beveridge, 2002; Fyler *et al.* 2009, respectively). Furthermore, a morphology-based phylogenetic analysis of 34 Australian species of *Acanthobothrium* identified a large clade comprising 'small' species and a basal group comprising the 'large' species of this genus (Campbell and Beveridge, 2002). Global and comprehensive molecular phylogenies of *Acanthobothrium*, and of their hosts, are necessary to test hypotheses of coevolution between these parasites and their hosts. However, comparisons of *Acanthobothrium* fauna from the eastern Pacific/southern Caribbean and western Pacific/Indian Ocean reveal general similarities among 'large' and 'small' species (Marques *et al.* 1997; Cambpell and Beveridge, 2002). Future comparisons using robust analytical tools to compare tree topologies between hosts and parasites may reveal similar co-speciation patterns between clades of 'small' and 'large' species.

Although the predicted hook length distribution shows an almost linear increase in length, the predicted distribution of handle lengths is similar to that of bothridial surface area. Most species with larger hooks than predicted were those small species that were also found in the lag portion of the bothridial size distribution, indicating that in small *Acanthobothrium* species, the lack of increase in size of bothridia is compensated by larger hooks, thus maintaining the functional efficiency of attachment structures. Furthermore, species with smaller hooks than predicted also possessed large bothridia for their size, indicating that the bothridia may compensate for the small size of the hooks to ensure functional efficiency in attachment. The handle anchors 4 of the 8 muscle bundles associated with hooks (Rees and Williams, 1965), thus suggesting that handle length is limited by size of the bothridia (Supplementary material S7a). Again, the lag in the sigmoid distribution of handle lengths comprised small species infecting myliobatiform hosts; many of which also comprised the lag in the distribution of bothridial surface area. Moreover, the species composition of the plateaus of both the handle and the bothridial distributions was similar.

In summary, (1) larger *Acanthobothrium* spp. have larger attachment structures, although the investment in development of attachment structures is not directly proportional to body size, (2) species with larger bothridia have larger accessory suckers and bear larger hooks, the latter with longer handles, albeit hook size does not increase in direct proportion to that of bothridia and accessory suckers, (3) although indirectly, host length influences the size of attachment structures, (4) several smaller *Acanthobothrium* species bear larger hooks than predicted by our models, which compensates for the low rate of increase in bothridial size and (5) several large *Acanthobothrium* species possess larger bothridia than expected for their body size, which compensates for bearing smaller hooks than predicted by our models. *Acanthobothrium* species have evolved a generalized mode of attachment involving at least 3 different structures that is successful in maintaining their position on various intestinal mucosal topographies across a wide variety of hosts exploiting different food resources. This consistent scolex morphology varies little in ratios of different attachment structures and has enabled *Acanthobothrium* to exploit hosts with mucosal topographies dominated by villi (e.g. *A. annapinkiense* ex *Dipturus chilensis*) or crypts and ridges (e.g. *A. marymichaelorum* ex *Himantura walga*), thus explaining its ability to exploit a wide range of unrelated hosts. However, the diversity of *Acanthobothrium* is limited in shark taxa. The onchobothriid fauna in sharks is dominated by several genera usually not found in batoid fishes, e.g. *Calliobothrium* van Beneden, 1850, *Phoreiobothrium* Linton, 1889, *Platybothrium* Linton, 1890 and *Triloculatum* Caira & Jensen, 2009 in Carcharhiniformes; *Pedibothrium* Linton, 1909, *Spiniloculus* Southwell, 1925 and *Yorkeria* Southwell, 1927 in Orectolobiformes; several of which possess armed bothridia with accessory sucker(s). The generic diversity of onchobothriids in sharks and the species diversity of *Acanthobothrium* in batoids may be explained by ecological segregation rather than differences in host mucosal topographies or phylogenetic influences. The radiation of *Acanthobothrium* in batoid hosts is impressive. Furthermore, not unlike the trypanorhynch genera *Grillotia* and *Pterobothrium* (see Olson *et al.* 2010) *Acanthobothrium* species parasitizing sharks are restricted primarily to species associated with benthic habitats not dissimilar to those exploited by batoids (e.g. heterodontiform and orectolobiform sharks). Unfortunately the paucity of data on onchobothriid life cycles limits further discussion on the coevolution of *Acanthobothrium* and their hosts. A comparison of factors influencing size of attachment structures in more specialized genera of related tetraphyllideans with similar attachment structures, such as *Calliobothrium* or *Pedibothrium* (Caira *et al.* 1999, 2001), and hooked tapeworms from different orders (e.g. Diphyllidea and Trypanorhyncha), would help establish whether patterns observed herein are shared amongst the different groups of hooked tapeworms infecting elasmobranch fishes.

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