

# Trunk spines in cystacanths and adults of *Corynosoma* spp. (Acanthocephala): *Corynosoma cetaceum* as an exceptional case of phenotypic variability

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**Abstract** Adults of the acanthocephalan *Corynosoma cetaceum* deeply attach to the stomach of dolphins using the proboscis and its spiny foretrunk as a disk while the spiny hindtrunk bends to also embed its ventral spines. During deep attachment, two ventral folds of tegument, anterior and posterior, are created. Spine growth is inhibited to a variable degree in folds, generating an extraordinary phenotypic variability, with most individuals, especially females, having folds partially or totally devoid of spines. Little is known on how this variability is generated and why it is not apparently found in other *Corynosoma* spp. In this paper, we examined the trunk armature of 77 and 388 cystacanth larvae of *C. cetaceum* and *C. australe*, respectively, from teleosts, and over 8800 adult specimens of *C. australe*, *C. bullosum*, *C. cetaceum*, *C. strumosum*, *C. villosum* and *C. wegneri* from marine mammals. Cystacanths and adults of *C. cetaceum* exhibited the same range of fold spine reduction and variability, suggesting that they are generated prior to the adult stage

(i.e., before spines are functional) and do not result from phenotypic plasticity. The other *Corynosoma* species analyzed created only the anterior fold during deep attachment, but it was always spined. Females of *C. cetaceum* had significantly larger foretrunk and hindtrunk spines than the other species and likely suffer stronger fold compression during deep attachment. The exceptional colonization of a harsh microhabitat, the stomach, could have generated a trade-off in *C. cetaceum*, which must bend the trunk to attach (as other *Corynosoma* spp.) but must also produce large spines that, in the folds, presumably are maladaptive and must be reduced.

**Keywords** *Corynosoma* · Acanthocephalan · Phenotypic variability · Attachment · Spine

## Introduction

Parasites have evolved a wide array of holdfast mechanisms that maximize the likelihood of successful attachment upon recruitment to their hosts. Acanthocephalans, in particular, live attached to the intestine, rarely the stomach, of their definitive hosts and have developed a proboscis armed with hooks as a primary anchoring device (Taraschewski 2000; Herlyn and Ehlers 2001; Poulin 2007; Heckmann et al. 2012a, b). However, many species of the classes Palaeacanthocephala and Eoacanthocephala also have spines on the trunk that engage on the gut surface and may play a significant role in attachment (Van Cleave 1952; Aznar et al. 2006; Dezfuli et al. 2008; Amin et al. 2011; Silva et al. 2014). Interestingly, the fine structure of trunk spines is similar to that of hooks, i.e., both are mineralized outgrowths of the tegument covered by epidermis (Crompton and Lee 1965; Taraschewski 2000; Herlyn

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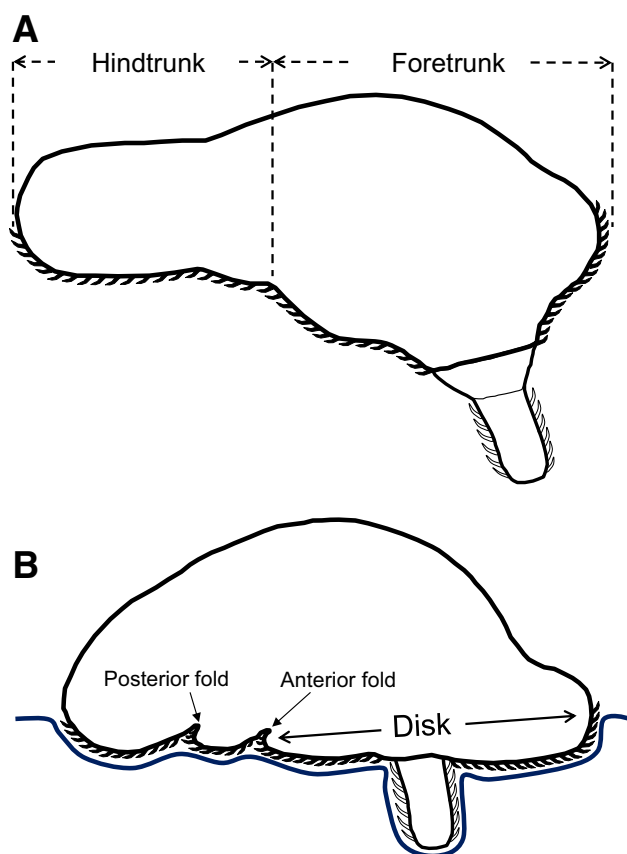
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and Ehlers 2001; Brázová et al. 2014) and share a common elemental composition that includes Ca, P and S (Heckmann et al. 2012a, b; Brázová et al. 2014).

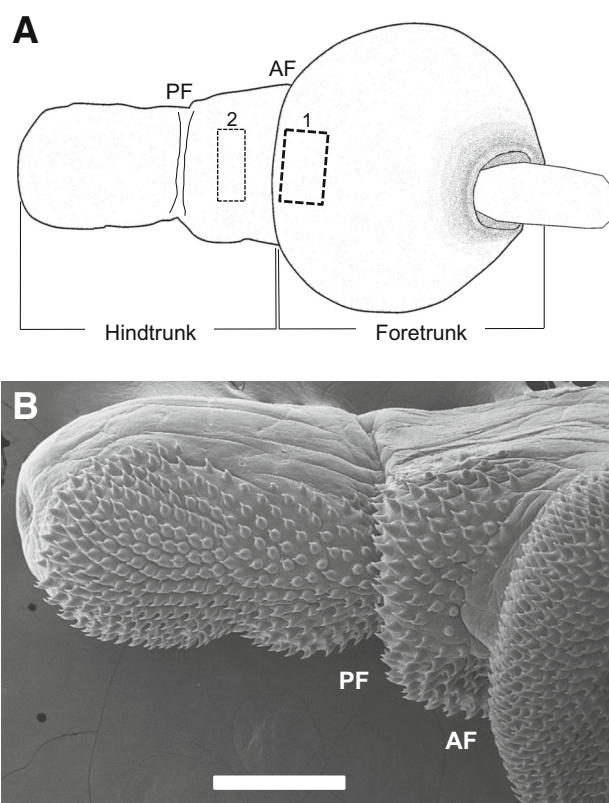
Species of *Corynosoma* (Palaeacanthocephala, fam. Polymorphidae) have a pipe-shaped body with the foretrunk, and a variable portion of the ventral hindtrunk, covered with spines (Aznar et al. 2006; Amin et al. 2011; Fig. 1a). Worms exhibit different degrees of attachment to the gut wall (Amin et al. 2011; Silva et al. 2014). In superficial attachment, only the proboscis is attached to the intervilli spaces of the intestine; in intermediate attachment, the whole foretrunk is also embedded in the intestinal wall, and in deep attachment, the hindtrunk is also partially embedded in the intestine (Silva et al. 2014; Fig. 1b). During deep attachment, the tip of the inflated foretrunk is flattened and forms a spiny disk which greatly enhances the attachment function of the proboscis, whereas

the hindtrunk bends downwards to put also its spines into contact with the substratum (Fig. 1b; see Aznar et al. 1999a, 2002, 2006, for details). In intermediate and deeply attached individuals, trunk spines are fundamental for mucosal embedding and even produce a concavity at the attachment site (Van Cleave 1952; Silva et al. 2014; Aznar unpub. obs.).

In *Corynosoma cetaceum*, a species that lives as adult in the stomach of dolphins, deep attachment results in the creation of two ventral folds of its tegument (Aznar et al. 1999a, 2002; Figs. 1b, 2). The anterior fold is created by necessity when both the tip of the foretrunk (i.e., the disk) and the hindtrunk simultaneously attach (Aznar et al. 2002, 2006; Fig. 1b). The posterior fold seems to be generated by the forward traction of the tegument in the precise line of insertion of the muscles that bend the hindtrunk (Aznar et al. 1999a, 2002). In adults of *C. cetaceum*, especially the females, Aznar et al. (2002) reported an extraordinary variability in the size and distribution of spines in the folds. In some individuals, folds were covered with small spines,



**Fig. 1** Morphology of the acanthocephalan *Corynosoma cetaceum*. **a** Lateral view of a relaxed female. The anterior inflated tip of the trunk and the proboscis are bent ventrally, resulting in a pipe-shaped body. Spines cover both the foretrunk and the ventral side of the hindtrunk. **b** Female deeply attached to the host's stomach. The foretrunk flattens and becomes a disk that expands laterally, exerting both a ventral and lateral wedge force against the substratum. The spiny hindtrunk bends and also contacts the substratum. During bending, two folds are invariably formed. See Aznar et al. (1999a, b, 2002, 2006) for details



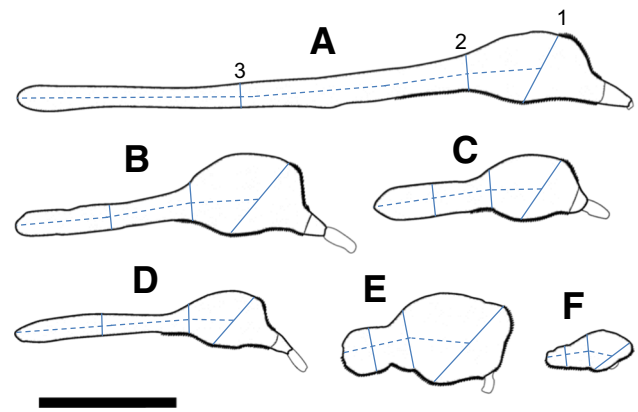
**Fig. 2** Body regions in species of *Corynosoma*. **a** Ventral diagrammatic view of a female of *Corynosoma cetaceum*. *AF* anterior fold, *PF* posterior fold. The boxes 1 and 2 indicate the areas where spine measurements were made (see also Fig. 4 and the text for details). **b** Latero-ventral view of a female cystacanth of *C. cetaceum* collected from the teleost *Xystreuris rasile*. The anterior and posterior folds are clearly visible. Note the wide bare area in the anterior fold (see also Fig. 4). Scale bar: 300  $\mu$ m

but in most individuals, folds were partially or completely devoid of them.

Regarding the proximate causes of this striking phenotypic variability, Aznar et al. (2002) found no signs of spine breakage in folds, but did find evidence that spine growth had been inhibited to a variable degree. Inhibition could have resulted from genotypic variability in fold spine growth, phenotypically plastic inhibition during spine development, or both (Aznar et al. 2002). Regarding the ultimate causes of variability, Aznar et al. (2002) suggested that fold spines might have become non-functional because they cannot contact the substratum. Relaxation of stabilizing selection, or even selection against using resources for useless spines, could have generated vestigialization. Alternatively, there could be directional selection against fold spines if they hamper deep attachment (Aznar et al. 2002).

In this paper, we provide new evidence about the proximate and ultimate causes of the exceptional fold spine variability found in *C. cetaceum* using a comparative approach. The life cycle of *C. cetaceum* involves invertebrates (likely amphipods) as intermediate hosts in which an acanthor larva passes through an acanthella stage to become an encapsulated cystacanth; teleosts act as paratenic (transport) hosts in which the parasite does not experience ontogenetic changes, and dolphins act as definitive hosts in which cystacanths become adults and reproduce (Aznar et al. 2012 and references therein). We made a thorough examination of cystacanth larvae of *C. cetaceum* obtained from teleosts using both stereomicroscopy and scanning electron microscopy (SEM) to shed light on when, and how, fold spine variability is created during ontogeny.

To investigate ultimate causes, we analyzed the trunk armature in adults of six species of *Corynosoma*, including *C. cetaceum*. Our first aim was to determine whether trunk spine variability also occurred in species other than *C. cetaceum*. There are ca. 30 species currently included in the genus *Corynosoma*, and all have a comparable trunk morphology and armature (Aznar et al. 2006 and references therein; Fig. 3). If attachment performance were similar in all species (see, e.g., Silva et al. 2014), one would expect to find folds and, possibly, similar processes to remove spines in folds, in other species. Although bare areas have not been described in the trunk armature in species of *Corynosoma* other than *C. cetaceum* (references in Aznar et al. 2006), the available data are not necessarily reliable. For instance, the four available descriptions of *C. cetaceum* prior to Aznar et al.'s (2002) study overlooked description of fold spine variability in this species (see Aznar et al. 1999b and references therein). Our second aim was to make an interspecific comparison of three factors that could hamper hindtrunk attachment, i.e., hindtrunk diameter, spine density, and spine size, to shed light on the hypothesis that fold spines are maladaptive.



**Fig. 3** Lateral diagrammatic view of adult females of the six *Corynosoma* spp. examined in this work: **a** *Corynosoma bullosum* from the southern elephant seal, *Mirounga leonina*. **b** *Corynosoma wegeneri* from the ringed seal, *Phoca hispida*. **c** *Corynosoma villosum* from Steller's sea lion, *Eumetopias jubatus*. **d** *Corynosoma strumosum* from the ringed seal, *Phoca hispida*. **e** *Corynosoma cetaceum* from the Franciscana dolphin, *Pontoporia blainvillei*. **f** *Corynosoma australe* from the South America sea lion, *Otaria flavescens*. Note that the proboscis and the neck have a variable degree of evagination. Solid lines indicate the three points where trunk width was measured; broken lines indicate how trunk length was measured. Scale bar: 4 mm

## Materials and methods

### Study of cystacanths

Cystacanths of *C. cetaceum* were collected alive from four teleost species, i.e., *Xystreuris rasile*, *Pinguipes brasiliensis*, *Mullus argentinae*, and *Pseudoperca semifasciata*. Additional material was obtained from individuals of *Paralichthys isosceles* and *Prionotus nudigula* that had been frozen prior to analysis (Table 1). For morphological comparison, cystacanths of *Corynosoma australe* were also collected from *P. isosceles* (Table 1).

All cystacanths were collected from the body cavity and washed in saline. Live cystacanths were fixed in hot 70 % ethanol or 4 % formaldehyde and preserved in 70 % ethanol. Cystacanths obtained from frozen hosts were fixed and preserved in 70 % ethanol at room temperature. Fixation of live cystacanths in warm 70 % ethanol or 4 % formaldehyde avoided shrinkage or wrinkling of the specimens. Cystacanths obtained from frozen hosts also exhibited a smooth surface with neither signs of contraction nor wrinkling after fixation.

Assessment of fold spine coverage was carried out with a dissecting microscope (at 80–100 $\times$ ) according to Aznar et al. (2002) criterion: The anterior fold (AF) or the posterior fold (PF) was considered spiny (+) if visible spines covered >90 % of their surface; partly spined (p) if spines covered between 10 and 90 %, and bare (–) if spines covered <10 % of the fold. Individuals were thus assigned

**Table 1** Samples of cystacanths of *Corynosoma cetaceum* and *C. australe* collected from six teleost species from the Argentinean coast

Host	Locality	N	<i>C. cetaceum</i>		<i>C. australe</i>	
			♀	♂	♀	♂
<i>Xystreuris rasile</i>	Golfo Nuevo (43°34'S, 62°23'W)	2	9	5	–	–
<i>Xystreuris rasile</i>	Off Península Valdés (42°52'S, 62°06'W)	2	–	3	–	–
<i>Pinguipes brasilianus</i>	Mar del Plata (38°08'S, 57°32'W)	1	–	1	–	–
<i>Pseudoperca semifasciata</i>	Off Península Valdés (42°00'S, 42°45'W)	2	33	19	–	–
<i>Mullus argentinae</i>	Mar del Plata (38°08'S, 57°32'W)	1	2	1	–	–
<i>Paralichthys isosceles</i>	Off Península Valdés (42°52'S, 62°06'W)	1	1	–	190	198
<i>Prionotus nudigula</i>	Off Península Valdés (42°52'S, 62°06'W)	3	–	4	–	–

'N' is the number of individual hosts from which samples of cystacanths were obtained

**Table 2** Collection data of adult specimens of *Corynosoma* spp. examined for trunk armature under dissecting microscope and scanning electron microscope

Species	Host	N	Locality	No. worms examined	
				Dissecting microscope	SEM (♀)
<i>C. australe</i>	<i>Otaria flavescens</i>	10	Puerto Madryn (Argentina)	525	2
<i>C. cetaceum</i>	<i>Pontoporia blainvillei</i>	10	Necochea (Argentina)	8263	2
<i>C. bullosum</i>	<i>Mirounga leonina</i>	1	Antarctica	10	2
<i>C. strumosum</i>	<i>Phoca hispida</i>	1	Russia	30	2
<i>C. wegneri</i>	<i>Phoca hispida</i>	1	Russia	30	2
<i>C. villosum</i>	<i>Eumetopias jubatus</i>	1	Bering Sea	30	2

'N' is the number of individual hosts from which acanthocephalan samples were obtained

to 1 of 9 possible coverage morphotypes (AF/PF): (+/+), (+/p), (+/-), (p/+), (p/p), (p/-), (-/+), (-/p) and (-/-) (Aznar et al. 2002). Distribution of phenotypes was compared, for each sex, between cystacanths and the adults examined by Aznar et al. (2002) (Table 2) using Chi-square tests.

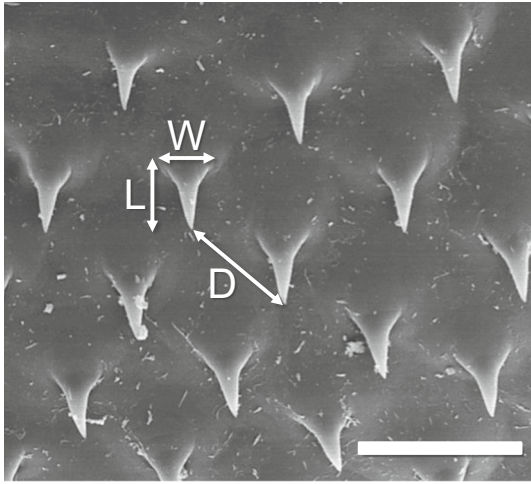
Spines on the foretrunk, hindtrunk and fold areas were also examined using scanning electron microscopy (SEM) for cystacanths of both *C. cetaceum* and *C. australe* (4 females and 3 males for each species). Specimens were dehydrated through an ethanol series, critical-point-dried, and coated with a gold–palladium alloy to a thickness of 250 nm. Specimens were then examined with a Hitachi 4100 FE scanning electron microscope operating at 20 kV.

### Study of adults

Adult individuals of six species of *Corynosoma* were obtained from five marine mammal species, i.e., South American sea lion, *Otaria flavescens*; Franciscana dolphin, *Pontoporia blainvillei*; southern elephant seal, *Mirounga leonina*; ringed seal, *Phoca hispida*, and Steller's sea lion, *Eumetopias jubatus* (Table 2). Worms were collected based on opportunist sampling of hosts that were found stranded or by-caught. Worms were found dead, removed from the intestine or the stomach, washed in saline, and

fixed in 70 % ethanol at room temperature. The relative size of the six species of *Corynosoma* and the extension of trunk armature are depicted in Fig. 3.

Fold spine coverage was assessed in all individuals as described above. We also examined the hypothesis that reduction or removal of spines in folds should increase with the degree of fold compression, and the size and density of trunk spines. Fold compression is primarily related with width of the trunk: the hindtrunk essentially behaves as a cantilever when it bends (Aznar et al. 1999a, 2002, 2006), and thus the trunk should suffer a higher amount of dorsal tension and ventral compression as it is wider (Vogel 1988). In 5–10 relaxed female specimens of each species, depending on availability, we measured trunk length, and trunk diameter at three points as indicated in Fig. 3. Body spines on the foretrunk, hindtrunk, and fold areas (Figs. 1a, 2a) were examined with SEM using two female specimens of each *Corynosoma* species (Table 2). The analysis was focused on females because it is the sex that experiences stronger pressures to develop holdfast structures (Hernández-Orts et al. 2012) and is the one with higher fold spine variability in *C. cetaceum* (Aznar et al. 2002). For each specimen, we randomly selected five spines at the ventral edge of the foretrunk and in the middle of the spiny hindtrunk (i.e., the 'interfold' region) (Fig. 2a). Then we measured (at ×1000) (1) spine width at its base,



**Fig. 4** Frontal view of the ventral foretrunk edge (disk) of an adult male of *Corynosoma australe* indicating the spine measurements that were taken. *D* distance to nearest neighboring spine, *L* spine length in frontal view, *W* width at the base of spine. Scale bar: 30  $\mu$ m

(2) spine length in frontal view, and (3) distance to the closest neighboring spine (Fig. 4). The distance between neighboring spines was considered as an index of spine density. Note that frontal SEM pictures ignore the slight curvature that spines have (see, e.g., Fig. 2b). However, no clear differences in curvature were observed between spines of different species, and all selected spines laid parallel to the substratum, and thus we assumed that measurement error was similar among species.

Trunk dimensions, spine width and length, and distance between neighboring spines, were compared between *C. cetaceum* and each of the five other *Corynosoma* species using mixed models. ‘Species’ was considered as a fixed factor, and ‘individual specimen’ as a random factor. Specific contrasts of *C. cetaceum* versus other *Corynosoma* spp. were made with *t* tests by setting parameters for *C. cetaceum* to zero. Parameters were estimated using restricted maximum likelihood methods.

All statistical analyses were carried out with SPSS v. 22. Statistical significance was set at  $p < 0.05$ . In multiple comparisons, nominal probability values were corrected by the sequential Bonferroni procedure (Rice 1989).

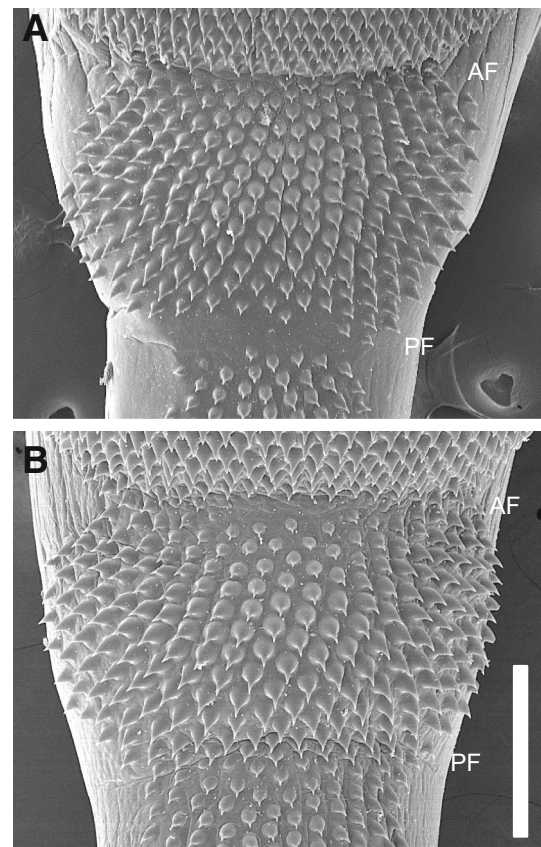
## Results

### Cystacanths

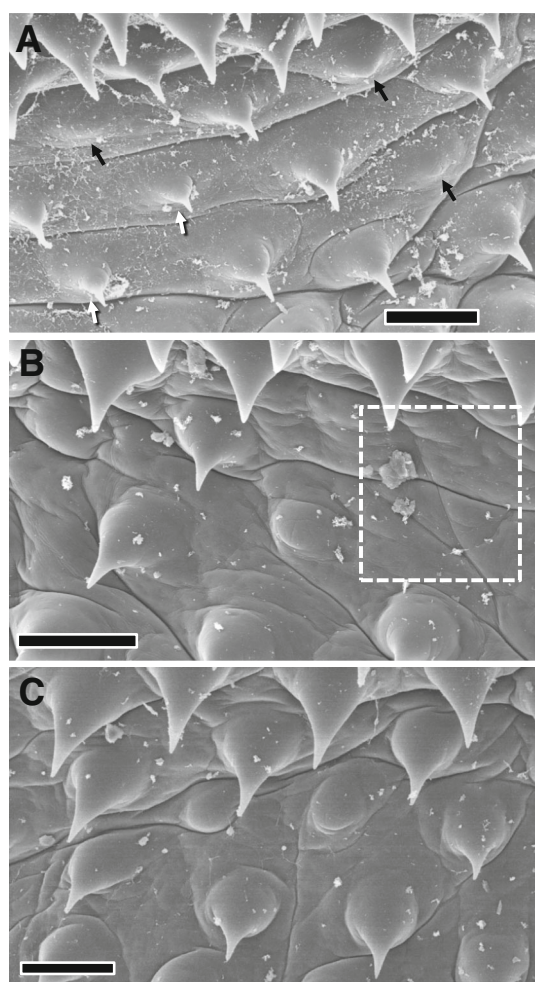
Similarly as described in adults, the foretrunk and the hindtrunk of all cystacanths of *C. cetaceum* examined were ventrally covered with spines, except the anterior and posterior folds, in which the degree of spine coverage was very variable, i.e., folds were totally or partially covered by

spines, or devoid of them (Fig. 5). Spines in folds followed the lattice arrangement observed in neighboring spines, but fold spines were observed to be at different stages of development; relative inhibition of growth between adjacent spines often followed an apparent random distribution (Fig. 6a). A clear sequence of development could be reconstructed: (1) smooth body wall without external traces of spines in places where they should be developed according to the lattice arrangement (Fig. 6b); (2) “bumps” on the surface created by developing spines (Fig. 6a, b, c); (3) “bumps” with short, conical tip spines rising at the top (Fig. 6a, b, c), and (4) longer, funnel-shaped spines of variable length (Fig. 6a, b, c). No broken or distorted spines were observed in fold areas.

Similarly as reported in adults, female cystacanths of *C. cetaceum* exhibited spine variability in both the anterior and posterior folds, whereas males presented variability only in the anterior fold (Table 3). In addition, a lower proportion of females (only 7 out of 45) had a spined (+) anterior fold compared with males (16 out of 33); the difference was highly significant (Yates’ Chi-square,



**Fig. 5** Two examples of variability in fold spine coverage in cystacanth specimens of *Corynosoma cetaceum* collected from *Xystreuryx rasile* in Patagonia. **a** Ventral view of a female with a (+/–) morphotype, **b** Ventral view of a female with a (p/+) morphotype. Scale bar: 300  $\mu$ m



**Fig. 6** Variability in fold spine development in cystacanths of *C. cetaceum*. **a** Detail of an anterior fold of a male. Although the patterning is conserved, neighboring spines exhibit a variable degree of development, from spine primordia (black arrows) to small spines (white arrows). **b** Detail of spines in an anterior fold of a female. Note spine primordium (“bump”) surrounded by both sharp-pointed spines and an area devoid of spines which, according to patterning, should have spines (box). **c** Detail of spines in a posterior fold of a female at different degrees of development. Note two spine primordia surrounded by sharp-pointed spines. Scale bar: 30  $\mu$ m

$\chi^2 = 8.41$ , 1 *df*,  $p = 0.0003$ ). To statistically compare morphotype distribution between cystacanths and adults, we pooled morphotypes for which expected frequency values in cystacanths were  $<5$ . In females, we pooled (+/+), (+/p) and (+/-) on one hand, and (p/p) and (p/-), on the other hand; in males, no pooling was necessary (Table 3). The morphotype distribution was not significantly different between cystacanths and adults in either sex (females: Chi-square test,  $\chi^2 = 10.34$ , 5 *df*,  $p = 0.066$ ; males:  $\chi^2 = 0.75$ , 2 *df*,  $p = 0.689$ ).

The fore- and hindtrunk of cystacanths of *C. australe* were covered with spines with the same arrangement observed in adults. No variability in the degree of development of spines in the anterior fold was found in any individual (Fig. 6), although some spines appeared to be slightly reduced in some specimens. The posterior fold was not detected in many individuals, especially males (Fig. 7).

### Adults

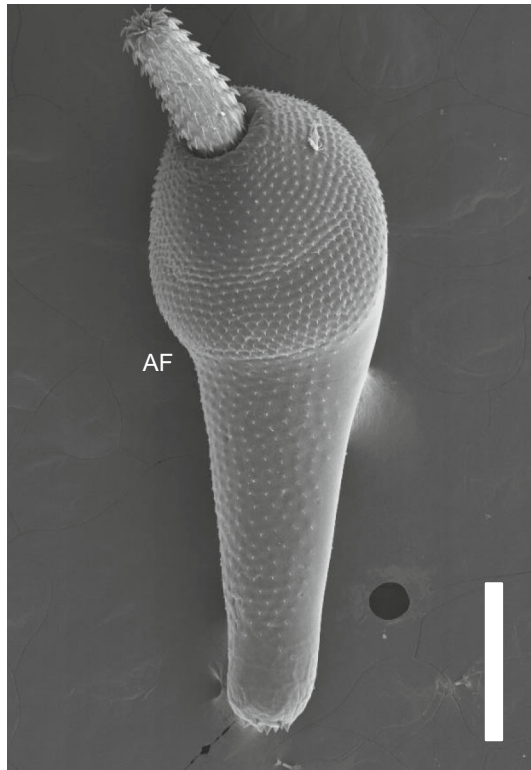
An anterior fold was observed in all specimens from the six *Corynosoma* species. However, the posterior fold was clearly visible only in females, and ca. 85 % of males, of *C. cetaceum*. Variability in trunk armature was observed only in *C. cetaceum* (Table 3); in the remaining species, a similar coverage and degree of development was observed in all specimens of each sex, with no obvious reduction of spines even in the anterior fold.

Data from body dimensions of the six *Corynosoma* species are shown in Table 4. Females of *C. cetaceum* had the largest ratio between trunk length/width of all *Corynosoma* species (Fig. 3; Table 4). Also, its foretrunk and hindtrunk spines were at least twice the size of the corresponding spines from other *Corynosoma* spp. (Table 5; Figs. 8, 9), and this difference was highly significant (Table 6). However, the distance between neighboring spines did not significantly differ, at any location, between *C. cetaceum* and the other *Corynosoma* species (Tables 5, 6).

**Table 3** Frequency of morphotypes of fold spine coverage in female and male cystacanths and adults of *Corynosoma cetaceum* from teleosts and Franciscana dolphins, *Pontoporia blainvillei*, respectively, collected in Argentinean waters

	Morphotype (AF/PF)								
	+/+	+/p	+/-	p/+	p/p	p/-	-/+	-/p	-/-
<i>Cystacanths</i>									
Female	2	3	2	9	3	2	7	8	9
Male	16	–	–	10	–	–	7	–	–
<i>Adults</i>									
Female	633	99	212	418	199	236	1054	835	1526
Male	1648	–	–	732	–	–	681	–	–

Data from adults were obtained from Aznar et al. (2002). Anterior fold (AF); Posterior fold (PF); spined fold (+); partly spined fold (p), non-spined fold (–)



**Fig. 7** Latero-ventral view of a male cystacanth of *Corynosoma australe*. A continuous field of foretrunk and hindtrunk spines can be observed. The anterior fold (AF), but not the posterior fold, is visible. Scale bar: 400  $\mu\text{m}$

## Discussion

At least in palaeoacanthocephalans, proboscis hooks and trunk spines appear to share a basic morphology, fine structure, and composition. They are sharp-pointed structures curved toward the posterior part of the worm; so when they contact the host's tissue, they can exert a reaction force against flow-related dislodgment forces. To perform this function, both structures need to exhibit rigidity to withstand flow yet also be flexible to avoid breakage (see Heckmann et al. 2012a). This is achieved by a combination of architectural and compositional features. Available information suggests that hooks and spines appear to originate from different areas, i.e., subtegumental connective tissue versus the feltwork layer of the tegument, respectively (Crompton and Lee 1965; Taraschewski 2000; Brázová et al. 2014). However, they are both mineralized structures that are made up of three basic elements, namely, Ca P and S, which appear to form a rigid calcium phosphate apatite with disulfide bonds (Heckmann et al. 2012a, b; Brázová et al. 2014). The higher concentration of S toward the tip of both hooks and spines suggests that the base of both hooks and spines is more flexible and the tip more rigid (see Heckmann et al. 2012a, b; Brázová et al. 2014).

Results from this study confirm that, in cystacanths of *C. cetaceum*, the growth of spines in folds is inhibited to

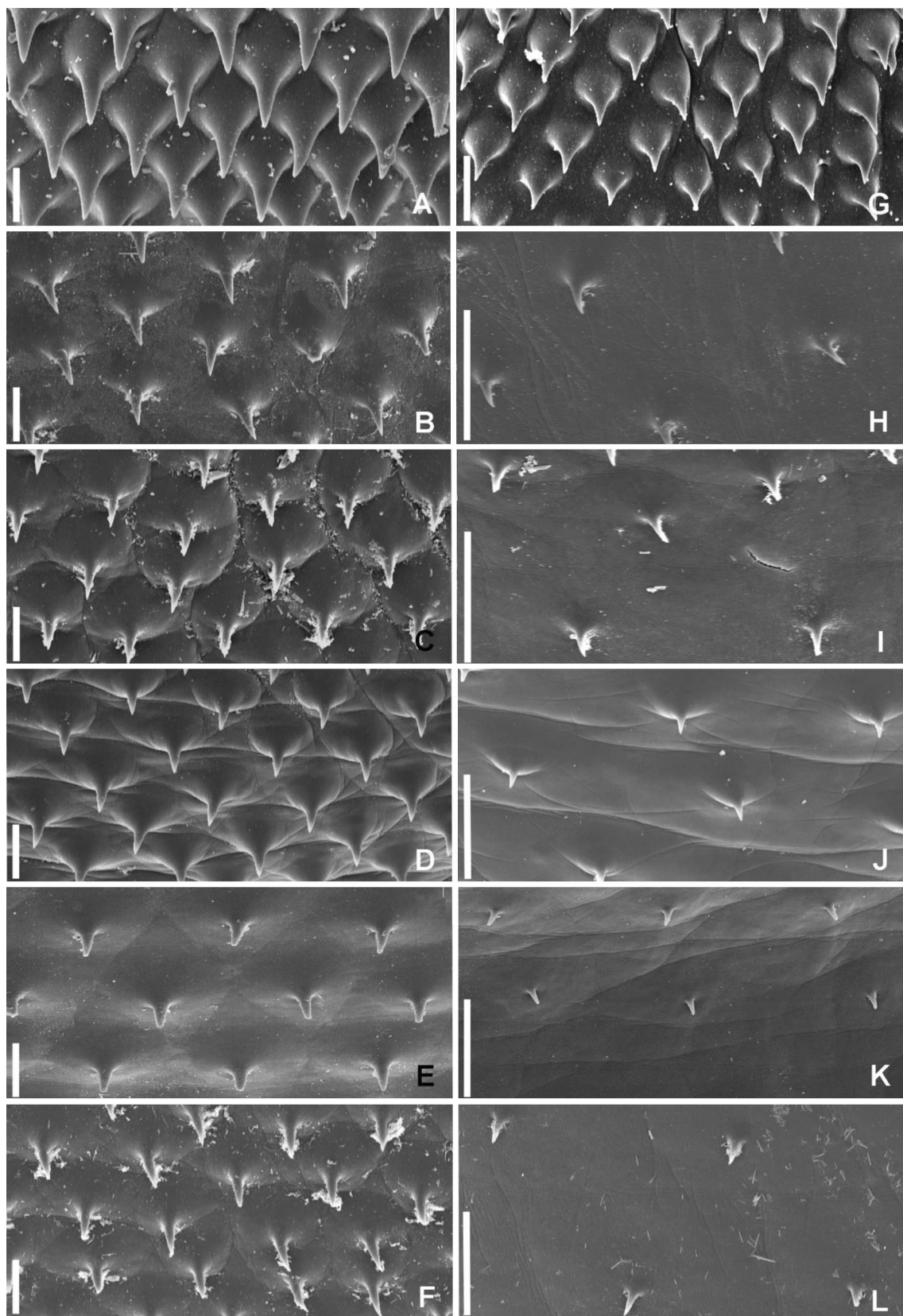
**Table 4** Mean values in mm (with SD in parentheses) of trunk length, and width measured at three points (see Fig. 3) of adult females of six species of *Corynosoma*

Species	n	Length	Width		
			1	2	3
<i>C. cetaceum</i>	10	3.38 (0.22)	2.47 (0.15)	1.58 (0.07)	1.16 (0.08)
<i>C. australe</i>	10	2.32 (0.23)	1.37 (0.09)	0.92 (0.14)	0.60 (0.09)
<i>C. bullosum</i>	5	15.20 (0.60)	2.87 (0.15)	2.12 (0.33)	1.29 (0.25)
<i>C. strumosum</i>	10	6.95 (0.99)	1.79 (0.09)	1.17 (0.14)	0.57 (0.06)
<i>C. villosum</i>	10	6.43 (0.57)	2.62 (0.26)	1.49 (0.20)	0.96 (0.08)
<i>C. wegneri</i>	10	7.21 (0.45)	2.69 (0.23)	1.50 (0.13)	0.85 (0.12)

**Table 5** Mean values ( $\mu\text{m}$ ) of distance between spines, and spine width and length, for five spines selected on the edge of foretrunk and the middle hindtrunk of each of two specimens of six species *Corynosoma* (see also Fig. 4)

Species	Foretrunk spines			Hindtrunk spines		
	Distance	Width	Length	Distance	Width	Length
<i>C. cetaceum</i>	32.6 (4.2)	32.7 (2.9)	37.7 (2.1)	49.2 (6.5)	30.4 (2.3)	32.4 (2.3)
<i>C. australe</i>	24.6 (2.5)	16.7 (1.0)	18.0 (1.4)	32.9 (2.0)	12.0 (1.1)	11.6 (1.0)
<i>C. bullosum</i>	30.6 (6.3)	18.0 (2.8)	19.2 (1.4)	48.3 (14.1)	11.5 (1.5)	12.1 (0.9)
<i>C. strumosum</i>	37.2 (6.4)	11.7 (4.2)	17.1 (3.1)	65.8 (8.1)	6.0 (0.9)	13.9 (1.0)
<i>C. villosum</i>	32.7 (2.4)	14.5 (2.8)	15.6 (1.8)	60.5 (12.8)	9.8 (1.8)	9.3 (0.7)
<i>C. wegneri</i>	26.5 (4.7)	9.6 (1.7)	15.3 (1.7)	62.9 (6.2)	7.7 (1.0)	12.3 (8.0)

Standard deviation is in parentheses





**Fig. 8** Spine arrangement at the disk edge and the middle hindtrunk in six species of *Corynosoma*: **a** Disk of *C. cetaceum*. **b** Disk of *C. bullosum*. **c** Disk of *C. strumosum*. **d** Disk of *C. australe*. **e** Disk of *C. villosum*. **f** Disk of *C. wegneri*. **g** Hindtrunk of *C. cetaceum*. **h** Hindtrunk of *C. bullosum*. **i** Hindtrunk of *C. strumosum*. **j** Hindtrunk of *C. australe*. **k** Hindtrunk of *C. villosum*. **l** Hindtrunk of *C. wegneri*. Scale bar: 50  $\mu\text{m}$

variable degree, generating an extraordinary phenotypic variability. Similarly as in the case of adults (Aznar et al. 2002), (1) variability was observed in both folds of female cystacaths, but only in the anterior fold of male cystacaths, and (2) the tendency to exhibit full inhibition of spine growth (e.g., a spineless anterior fold) was more pronounced in females. In addition, the relative frequency of each morphotype did not significantly differ between cystacaths and adults. It is worth noting that most cystacaths examined in this study were sampled in Patagonia, whereas adults were collected in Buenos Aires Province, i.e., ca. 600 km apart. In teleosts sampled in Buenos Aires Province, Sardella et al. (2005) also reported cystacaths of *C. cetaceum* having folds with and without spines. Overall, these observations strongly suggest that (1) the morphotype variability in *C. cetaceum* is a geographically widespread phenomenon, and (2) it is generated before the adult development. The latter conclusion conforms to the hypothesis that the trunk armature of acanthocephalans is already developed in the intermediate host, i.e., prior to being used for attachment in the definitive host (Van Cleave 1952; Petrochenko 1956). Nevertheless, it is interesting to note that, in females of *C. cetaceum*, spines seem to experience also slight growth (10–20 % depending on body area) after recruitment to the definitive host (Hernández-Orts et al. 2012). This fine-tune of the final size of spines in the definitive host seems to be an adaptive response to stronger selective pressures on females to ensure longer attachment in a highly disturbed microhabitat (Hernández-Orts et al. 2012).

With regard to proximate causes of reduction and variability of fold spines, Aznar et al. (2002) suggested two non-exclusive hypotheses. First, there could be intraspecific genetic variation of spine growth that would result in partial or total removal of fold spines in many individuals. Second, partial to total inhibition of fold spine growth could just result from individual variability in the degree of fold compression during spine development. This epigenetic hypothesis would explain differences of growth between neighboring spines (Fig. 6) by random topology of contact: spines that abut other spines would be most inhibited in growth, while those that fall between the spines on the opposite side of the fold would be less inhibited (Aznar et al. 2002). However, results from this study clearly indicate that patterns of reduction and variability are already determined at the cystacanth stage. Since larval

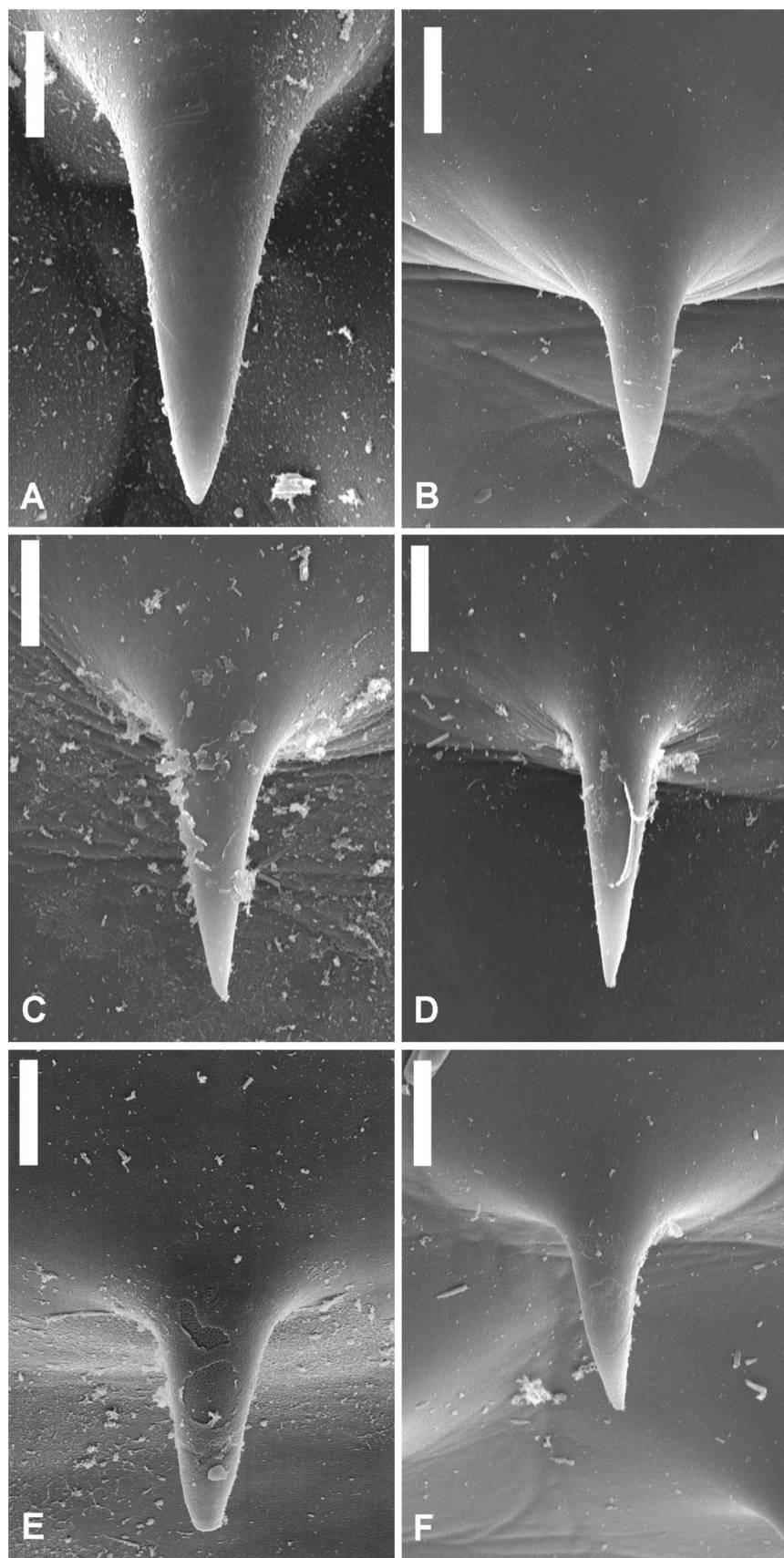
stages of polymorphids develop, and remain encapsulated, in the hemocoel of crustaceans (e.g., Denny 1969; Podesta and Holmes 1970), it is very unlikely that a pure epigenetic mechanism may generate the observed patterns of growth inhibition of spines in folds. There are no obvious reasons for larvae to bend the hindtrunk, thus forming and compressing folds, nor are reasons for this potential behavior to differ between females and males, or even among individuals.

With regard to ultimate causes, Aznar et al. (2002) suggested that the reduction and variability of fold spines could simply result from relaxation of functional demands for these spines since, in deep attachment, fold spines can hardly contact the substratum. Alternatively, or additionally, there could be selection against spines in folds because they hamper deep attachment. *Corynosoma cetaceum* lives in a microhabitat with maximal physical disturbance, i.e., the stomach of dolphins (Aznar et al. 2001). Therefore, optimal attachment performance could be critical to avoid worms' dislodgment. Aznar et al. (2002) showed two lines of evidence supporting the 'maladaptation' hypothesis. First, there was a significant correlation between the degree of deep attachment and the amount of spines in folds. Second, spine removal should be more beneficial when fold walls are compressed more intensely and, in fact, the degree of spine reduction in the anterior versus the posterior fold, and in females versus males, correlated with predicted differences of fold compression (see Aznar et al. 2002 for details).

The comparative analysis presented in this study also lends support to the 'maladaptation' hypothesis. Variability of trunk armature was not observed in cystacaths of *C. australe* or in adults of five species of *Corynosoma*. Given that the posterior fold was rarely observed in these species, one could postulate that they are able to deeply attach without producing the posterior fold; therefore, there would be no need to remove spines on this area. Interestingly, females of *C. cetaceum* tend to have a short, thick hindtrunk, and it is the only species that reduces body diameter in the precise line where the hindtrunk is bent and the posterior fold is formed (Fig. 3; see also pictures in Aznar et al. 1999a). This strongly suggests that the diameter at this line is reduced to facilitate hindtrunk bending, similarly as for any cantilever (Vogel 1988). Thus, the posterior fold would be generated in *C. cetaceum* as a side effect.

The anterior fold was observed in all *Corynosoma* species, which is unsurprising because, for deep attachment, these parasites must bend the hindtrunk. Yet, the anterior fold was covered by spines in all species examined except *C. cetaceum*. Interestingly, all these species had significantly smaller spines than *C. cetaceum*, and this should alleviate the potential physical problems associated to

**Fig. 9** Spines at the edge of the foretrunk (disk) in *Corynosoma* spp: **a** *Corynosoma cetaceum*. **b** *Corynosoma australe*. **c** *Corynosoma bullosum*. **d** *Corynosoma strumosum*. **e** *Corynosoma villosum*. **f** *Corynosoma wegeneri*. Scale bar: 6  $\mu$ m



**Table 6** Parameter estimation for differences between females of *Corynosoma cetaceum* and those from each of five species of *Corynosoma*. Parameters for *C. cetaceum* were set to zero

Species	Comparison	Parameter (SE)	df	t	P
<i>C. australe</i>	Trunk diameter 1	-1.101 (0.078)	49	-14.13	<b>&lt;0.001</b>
<i>C. bullosum</i>		0.405 (0.095)	49	4.25	<b>&lt;0.001</b>
<i>C. strumosum</i>		-0.677 (0.078)	49	-8.69	<b>&lt;0.001</b>
<i>C. villosum</i>		0.149 (0.078)	49	1.91	0.062
<i>C. wegeneri</i>		0.224 (0.078)	49	2.87	<b>0.006</b>
<i>C. australe</i>	Trunk diameter 2	-0.655 (0.073)	49	-8.94	<b>&lt;0.001</b>
<i>C. bullosum</i>		0.540 (0.089)	49	6.03	<b>&lt;0.001</b>
<i>C. strumosum</i>		-0.409 (0.073)	49	-5.58	<b>&lt;0.001</b>
<i>C. villosum</i>		-0.083 (0.073)	49	-1.14	0.261
<i>C. wegeneri</i>		-0.074 (0.073)	49	-1.008	0.318
<i>C. australe</i>	Trunk diameter 3	-0.561 (0.050)	49	-11.21	<b>&lt;0.001</b>
<i>C. bullosum</i>		0.127 (0.061)	49	2.07	0.044
<i>C. strumosum</i>		-0.593 (0.050)	49	-11.84	<b>&lt;0.001</b>
<i>C. villosum</i>		-0.196 (0.050)	49	-3.91	<b>&lt;0.001</b>
<i>C. wegeneri</i>		-0.304 (0.050)	49	-6.08	<b>&lt;0.001</b>
<i>C. australe</i>	Spine distance (foretrunk)	-8.020 (3.527)	6	-2.27	0.063
<i>C. bullosum</i>		-1.942 (3.527)	6	-0.55	0.602
<i>C. strumosum</i>		0.073 (3.527)	6	0.02	0.984
<i>C. villosum</i>		4.624 (3.527)	6	1.31	0.238
<i>C. wegeneri</i>		-6.110 (3.527)	6	-1.73	0.134
<i>C. australe</i>	Spine width (foretrunk)	-16.026 (2.978)	6	-5.38	<b>0.002</b>
<i>C. bullosum</i>		-14.698 (2.978)	6	-4.94	<b>0.003</b>
<i>C. strumosum</i>		-20.968 (2.978)	6	-7.04	<b>&lt;0.001</b>
<i>C. villosum</i>		-18.154 (2.978)	6	-6.10	<b>0.001</b>
<i>C. wegeneri</i>		-23.085 (2.978)	6	-7.75	<b>&lt;0.001</b>
<i>C. australe</i>	Spine length (foretrunk)	-19.663 (2.070)	6	-9.50	<b>&lt;0.001</b>
<i>C. bullosum</i>		-18.458 (2.070)	6	-8.92	<b>&lt;0.001</b>
<i>C. strumosum</i>		-20.528 (2.070)	6	-9.92	<b>&lt;0.001</b>
<i>C. villosum</i>		-22.059 (2.070)	6	-10.66	<b>&lt;0.001</b>
<i>C. wegeneri</i>		-22.357 (2.070)	6	-10.80	<b>&lt;0.001</b>
<i>C. australe</i>	Spine distance (hindtrunk)	-16.289 (7.536)	6	-2.16	0.074
<i>C. bullosum</i>		-0.840 (7.536)	6	-0.11	0.915
<i>C. strumosum</i>		16.620 (7.536)	6	2.21	0.070
<i>C. villosum</i>		11.296 (7.536)	6	1.50	0.185
<i>C. wegeneri</i>		13.689 (7.536)	6	1.82	0.119
<i>C. australe</i>	Spine width (hindtrunk)	-18.420 (1.001)	6	-18.41	<b>&lt;0.001</b>
<i>C. bullosum</i>		-18.971 (1.001)	6	-18.95	<b>&lt;0.001</b>
<i>C. strumosum</i>		-20.588 (1.001)	6	-20.56	<b>&lt;0.001</b>
<i>C. villosum</i>		-24.402 (1.001)	6	-24.37	<b>&lt;0.001</b>
<i>C. wegeneri</i>		-22.753 (1.001)	6	-22.72	<b>&lt;0.001</b>
<i>C. australe</i>	Spine length (hindtrunk)	-20.760 (1.112)	6	-18.67	<b>&lt;0.001</b>
<i>C. bullosum</i>		-20.245 (1.112)	6	-18.21	<b>&lt;0.001</b>
<i>C. strumosum</i>		-18.488 (1.112)	6	-16.63	<b>&lt;0.001</b>
<i>C. villosum</i>		-23.110 (1.112)	6	-20.78	<b>&lt;0.001</b>
<i>C. wegeneri</i>		-20.138 (1.112)	6	-18.11	<b>&lt;0.001</b>

Differences that were significant after the sequential Bonferroni correction are in bold

accommodate small spines in the anterior fold, particularly if fold compression is also reduced. As noted above, ventral compression decreases when a deflected cantilever is

thinner (Vogel 1988), and except *C. bullosum*, females of all the *Corynosoma* species analyzed have thinner bodies than *C. cetaceum*.

*Corynosoma cetaceum* is the only acanthocephalan species infecting birds or mammals that lives in the stomach (Aznar et al. 2001), and the stomach of dolphins generates a strong and unpredictable flow of digesta (Hernández-Orts et al. 2012). This might explain why *C. cetaceum* likely has the largest trunk spines, not only of the species included in this study, but possibly of all described species of *Corynosoma* (see references in Aznar et al. 2006). Thus, it could be hypothesized that *C. cetaceum* is experiencing an ongoing trade-off between using a spiny bent trunk as a key attachment device and producing large trunk spines to live in the hosts' stomach.

The present study raises three interesting issues that should be addressed in the future. First, extensive sampling on coastal benthic crustaceans, especially amphipods, should be carried out in Argentina to detect and collect larvae of *C. cetaceum* from its crustacean intermediate hosts. Hopefully, a thorough analysis of the earliest development stages of this species could shed more light on the way fold spine variability is produced. Second, the trunk armature should be examined in other species of *Corynosoma*. If the hypothesis developed in this study is correct, species with short and wide trunks and/or long spines, e.g., *Corynosoma validum*, *C. hamanni* or *C. pseudohamanni* (see Van Cleave 1953, Zdzitowiecki 1984) should be more prone to reduce spines, at least in the anterior fold. Third, the reason(s) why this variability is maintained in *C. cetaceum* is an intriguing question. Functional trade-off, ongoing directional selection to reduce spines, and/or developmental constraint are candidate explanations which will, however, be difficult to investigate empirically. In any event, given the great conservatism in holdfast structures among acanthocephalans, the proximate and ultimate causes of the exceptional phenotypic variability found in *C. cetaceum* deserve further attention.

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