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Reproductive strategy of two deep-sea scalpellid barnacles (Crustacea: Cirripedia: Thoracica) associated with decapods and pycnogonids and the first description of a penis in scalpellid dwarf males

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Abstract We investigated the sexual system in two pedunculate barnacles of the family Scalpellidae. Both inhabit deep water and are attached to mobile arthropod hosts. *Verum brachiumcancri* was attached to the majid crab *Rochinia hertwigi,* and *Weltnerium nymphocola* to the pycnogonidan sea spider *Boreonymphon rubrum*. Both barnacles have separate sexes and females almost always carry two dwarf males that are almost fully embedded in a pair of symmetrically situated receptacles inside the rim of the mantle cavity. The dwarf males of *V. brachiumcancri* have a complex penile structure extending into the female mantle cavity. This is the first time a copulatory structure has been described in detail for a dwarf male of a scalpellid barnacle. Both species lack free nauplii and their larvae are released as cyprids; the brood size is small. This is probably an adaptation for settling close to the parent population. We compare reproductive strategies among scalpellids and suggest that the present males are highly specialized and that the females are allocating resources to few offspring.

Keywords Cyprid · Dwarf male · Penis · Reproductive strategy · Settlement · Larval biology

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Introduction

All scalpellids are pedunculate suspension-feeding barnacles with the upper part of the body (the capitulum) covered with shell plates. They range extensively in size, are found from shallow water to the deep sea, and can attach to a variety of substrata, from rock surfaces to various sorts of plants and animals. In addition to a wide range in size and habitat, members of this species-rich family (~281 species) also exhibit a range of sexual systems, rendering them eminently suited for ecological and evolutionary studies (Svane 1986; Buhl-Mortensen and Høeg 2006; Spremberg et al. 2012). In the Scalpellidae, larval development can comprise the usual cirripede series of nauplii followed by a cyprid, or larvae can be released at the cypris stage, thus abbreviating life in the plankton and limiting capacity for dispersal. The sexual system of the Scalpellidae ranges from pure hermaphroditism through androdioecy (hermaphrodites and males) to dioecy (separate sexes) (Buhl-Mortensen and Høeg 2006; Kelly and Sanford 2010; Yusa et al. 2011) but, wherever found, the males are always minute dwarf males that are permanently attached to their partner. Most previous studies on scalpellids have been either taxonomic, describing the female/hermaphrodite, or concerned with the external morphology of the dwarf males (Krüger 1920; Broch 1922; Nilsson-Cantell 1921, 1931; Klepal 1987; Klepal and Nemeschkal 1995). Despite being highlighted by Darwin (1851), there has been a surprising lack of information on most other aspects of their reproductive biology (Krüger 1920; Svane 1986; Buhl-Mortensen and Høeg 2006; Ozaki et al. 2008; Yusa et al. 2011; Spremberg et al. 2012).

Buhl-Mortensen and Høeg (2006) performed the first comparative study on the sexual systems in the Scalpellidae, and documented interesting differences between the androdioecious shallow-water species *Scalpellum scalpellum* (Linnaeus 1767), the small-sized shelf-inhabiting and dioecious *Ornatoscalpellum stroemii* (M. Sars 1859), and the large deep-water and dioecious *Arcoscalpellum michelottianum* (Seguenza 1876). Both the shallow-water *S. scalpellum* and the deep-sea *A. michellottianum* have pelagic nauplii enabling long-distance dispersal whereas *O. stroemii* releases its larvae as cyprids. In all three species the males were located in two symmetrically situated receptacles on the paired scutal plates, but distinct differences were observed. Both *S. scalpellum* and *A. michellottianum* can carry many males, but they are relatively poorly protected. In contrast, females of *O. stroemii* carry only two males each, but these are very well protected in a deep, pouch-like pocket inside the rim of the scutal plates. The systematic comparison of scalpellid reproductive strategies revealed reproductive strategies ranging from many but superficially attached males to a highly evolved situation with the two profoundly protected males (Buhl-Mortensen and Høeg 2006). However, the process by which the dwarf males fertilize the eggs in the mantle cavity of the partner organism, and how this relates to their location, remain completely unknown.

Buhl-Mortensen and Høeg (2006) showed that a comparison of scalpellid species from different depths and substrata will increase our understanding of evolutionary adaptations that lead to different reproductive strategies. This variation encompasses (1) the sexual system; (2) the number, size, position, and morphology of the dwarf males; (3) the brood size; and (4) the size and type of development of the larvae. In the quest to understand this variation, we have studied the reproductive biology if two little-known deep water scalpellids: *Verum brachiumcancri* (Weltner, 1922) and *Weltnerium nymphocola* (Hoek, 1883). Both species are attached to mobile bottom-dwelling arthropods (Zevina 1981), and this represents a challenge to both reproduction and dispersal. *V. brachiumcancri* is attached to the deep-sea crab *Rochinia hertwigi* (Doflein, 1904), and *W. nymphocola* to the pycnogonidan sea spider *Boreonymphon robustum* (Bell, 1855). We focus on both the possible advantages to inhabit such a live, mobile substratum and on the larval development, dwarf males, receptacles and other aspects of the reproductive biology in these two species. In particular, we provide the first detailed report of a very remarkable penile structure used by the dwarf males of *V. brachiumcancri* during copulation with their partner.

Material and methods

Verum brachiumcancri

We examined 51 specimens *V. brachiumcancri* obtained from the Cirripedia collection in the Museum für Naturkunde, Berlin (nos. 22257, 22258, and 22261). Of these, 46 specimens were attached to 13 individuals of the majid crab *Rochinia hertwigi* (crabs labelled as *Scyramathia hertwigi* Doflein, the original generic combination). Another five specimens of *V. brachiumcancri* were already detached from their host(s); one additional *R. hertwigi* crab hosted no scalpellids at all, and it may have been the source of these loose specimens. The crabs examined were sampled by the "Deutsche Tiefsee-Expedition" from stn. 103 in the South Atlantic (35°10.5′S and 23°2′E) at 500 m depth and a bottom temperature of 7.8 ° C (Weltner 1922). The same museum also houses an additional five specimens of *Scyramathia hertwigi* (so-labelled) carrying a total of 24 *V. brachiumcancri*. These specimens, left intact on their crabs, do not form part of our tabulated material, but some information on males was gleaned from one of the loose specimens.

Weltnerium nymphocola

We examined a total of ten specimens *W. nymphocola*. Eight of them are in the Museum für Naturkunde, Berlin (nos. 6570 and 22251) attached to two individuals of the North Atlantic pycnogonidan sea spider *Boreonymphon robustum*. One host with three *W. nymphocola* is shown in Fig. 3a–b, and these specimens were left intact. Six *W. nymphocola* attached to the other host were detached for detailed examination. This material was also collected by the Deutsche Tiefsee-Expedition, from stn. 7 near Bergen, Norway, (60°37′N, 5°42′W) at 588 m depth and a temperature of 0.8 °C. Two additional specimens, both detached from their host, were kindly lent to us from the Zoological Museum in Bergen, Norway (ZMBN-32774 and ZMBN-15890), from the "Michael Sars" Expedition (66° 42′N, 26° 40′W), taken at 550 m depth, and the "Armauer Hansen" 1914 Expedition (62°15′N 0°15′E), taken at 800 m depth, respectively. Specimens in situ on host crabs were macro-photographed using the macro-optics of an Olympus C5050 camera. Individual barnacle specimens were photographed with a Leica MZ dissection microscope, using either an Evolution® camera or the same Olympus C5050 fitted with an ocular tube adapter. Macro-photography was done using specimens immersed in 70 % alcohol. Cyprids and dwarf males were dissected free and photographed under a dissecting microscope. Specimens used for scanning electron microscopy (SEM) were dehydrated in alcohol and acetone and critical-point dried from liquid CO_2. They were then mounted on stubs with double adhesive tape, sputter-coated with palladium, and examined in a JEOL JSM-6335 F scanning electron microscope. Photo plates were constructed using CorelDraw® or Adobe Photoshop®.

We recorded the numbers of scalpellid specimens attached to a particular host, and the position, orientation, and length of the capitulum of each. By opening the specimens to expose the mantle cavity, we also recorded the number and detailed position of the dwarf males. We also checked all these specimens for the presence of a penis to ascertain whether they were hermaphrodites or females. In ovigerous individuals we measured the brood size and the size range of embryos or brooded larvae.

Results

Morphology of *V. brachiumcancri*

Both the original description of *V. brachiumcancri* by Weltner (1922) and that offered by Zevina (1981) are very brief. Here we provide some additional information on the morphology of the female. The capitulum is oval and length is 1.5 times the width. The carina margin is rounded and the occludent margin slightly convex. Plates are striated and with thin rowth lines and covered with a thin cuticle (Fig. 1). Cirri with almost equal length of inner and outer rami. Number of segments on the inner and outer rami of cirri CI to CVI are: CI 6 and 7; CII 11 and 10; CIII 12 and 13; CIV 13 and 14; CV 14 and 15; and for CVI 14 and 15.

Position and occurrence on host

V. brachiumcancri

The total material collected by the Deutsche Tiefsee-Expedition comprised 70 *V. brachiumcancri* specimens attached to 21 *Rochinia hertwigi*, which carried 1–11 (mean 3.3) barnacles per crab (Weltner 1922). One *V. brachiumcancri* was found attached to *Parapagurus pilosimanus* Smith, 1879, and five specimens were detached from their host. The 13 crabs examined by us carried 1–8 specimens of *V. brachiumcancri* (mean=3.4; *N*=46), with three crabs having only one barnacle each. The size distribution of *V. brachiumcancri* ranged from 1.0 mm to 10.5 mm in capitulum length (Table 1). Juvenile specimens were relatively few compared to mature specimens (Figs. 2d and 3). All specimens were attached to the dorsal surface of the carapace with no clear pattern in preferred position. There was however a slight tendency of more specimens being attached to the left side of the host, and very few specimens were attached close to the rear end of carapax. The occludent margin of the capitulum, with the mantle aperture, could be directed both anteriorly and posteriorly relative to the host (Fig. 2; Table 1).

W. nymphocola

The number of *W. nymphocola* was three to six specimens per host. The *Boreonymphon robustum* host with six speciments of *W. nymphocoloa* carried additionally a single specimen of the scalpellid *Tarasovium cornutum* (G. Sars, 1879). The size range of *W. nymphocola* was 2.3–8.3 mm (Table 2). Adult and juvenile specimens occurred together. The specimens could be attached to either the legs or the central part of the body (Fig. 4).

Sexual systems

The sexual systems of *V. brachiumcancri* and *W. nymphocola* are almost identical and therefore they are described together. Both species have separate sexes (dioecy), since all specimens attached to the crab or pycnogonidan hosts were females,

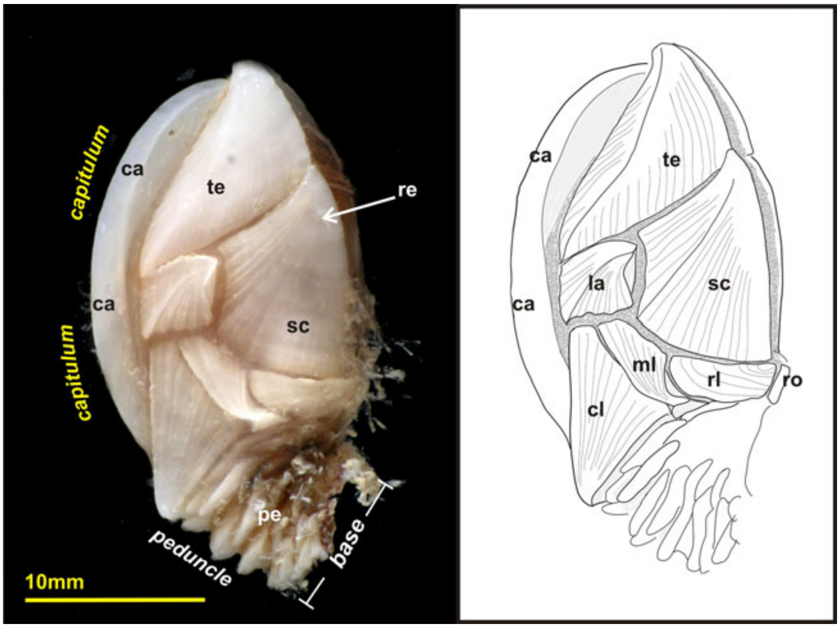

Fig. 1 *Verum brachiumcancri*, photo and drawing of adult female, with arrow pointing to site of male receptacle (*re*) on inside of scutal plate (*sc*). *ca* Carina, *cl* carino-latus, *la* latus, *ml* median latus, *pe* peduncle, *rl* rostro-latus, *ro* rostrum, *te* tergum

Table 1 *Verum brachiumcancri* on *Rochinia hertwigi*. Number, size, position and orientation of females; number and size of dwarf male; and reproductive data on eggs and larvae. *Post* Occludent margin of capitulum oriented towards the posterior end of host, *Ant* occludent margin oriented towards the anterior end of host

Host	Female size	Number and size of males, eggs and larvae						Position on host	
	Capitulum (mm)	No. of dwarf males	Male (mm)	No. of eggs	Eggs (mm)	No. of cypris	Cypris (mm)	*L* Left, *R* right, *C* center, *r* rear	Direction of capitulum relative to host
Detached	9.7	2		25	0.5–0.55			?	?
	10.1	2	0.7	60	0.55–0.65			?	?
	7.9	2		Mass				?	?
	7.6	2		Mass				?	?
	10.2[a]	4[a]						?	?
	5.5	2						?	?
crab-1a	8.7	2		Mass				R	Post
	2.4	0						C	Ant
	1.4	0						C	Ant
crab-1b	8.8	2		Mass				R	Post
	9.2	2		Mass				L	Post
	4.7	2						R	Ant
	3.8	0						L	Post
crab-2a	9.1	2		150	0.6			L	Ant
	7	2						L	Post
	1.5	0						L	Post
	1	0						?	?
crab-2b	10	2		70				C	Post
	10	2		Mass				R	Post
	8.9	2		Mass				L	Ant
	8.1	2		Few				R	Post
	6.5	2	0.775	26	0.55–0.65			L	Ant
	5.7	2						L	Ant
	5.1	2						?	?
	1.5	0						?	?
crab-3	8.6	2				1	0.85	L	Ant
	7.3	3						L	Ant
	3.3	0						L	Ant
	2.2	0						L	Ant
crab-4	2.7	0						?	?
	8.3	2						L	Post
	9.2	2		12	0.5–0.65			C	Post
	9.5	1		235	0.6–0.65			R	Post
crab-5	6.9	2						R	?
	3.3	0						R	?
	0.9	0						?	?
	1.1	0						?	?
	0.85	0						?	?
crab-6	10.5	2		95	0.55–0.6	67	0.83–0.86	rL	Post
crab-9	10	2		227	0.5–0.55			rR	Post
	2.4	0						?	?
	9	2		220	0.55–0.65			?	Post
	5.7	2						?	Ant
	2.4	0						?	?
	1.8	0						?	?
	1.5	0						?	?
crab-11	7.5	2						C	Post
crab-15	7.3	2				158	0.85	rL	Post
crab-16	7	1						rC	Post
	0.6	0						C	Post
crab-17	8.2	2		86	0.6–0.65			rL	Side
	1.25	0						?	?
Sum observations	52	67	0.7–0.775	230	0.5–6.5	158	0.83–0.86	*L/R/C/r:* 16/9/7/5	P/A: 19/12

[a] Normally the dwarf males are embedded in the female receptacle tissue (Fig. 6); an exception was the observation of four males observed in the receptacle area

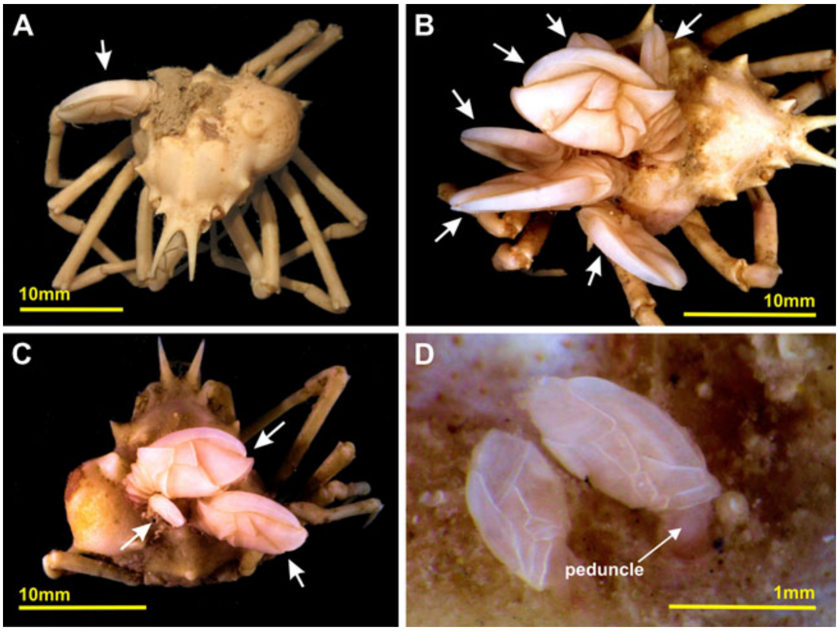

Fig. 2 **a**–**d** *V. brachiumcancri* on its host, the majid crab *Rochinia hertwigi*. **a** Crab with one barnacle (*arrow*). **b** Crab with six variously sized barnacles (*arrows*). **c** Crab with two large and one juvenile barnacles (*arrows*). **d** Close-up of two small juveniles on host, demonstrating presence of peduncle even in such small specimens, little exceeding a cypris larva in size

without even a trace of a penis. Based on the presence of males or brooded embryos/larvae or both (Table 1), the size of sexual maturity for females was estimated to be 4.0–4.5 mm in capitulum length for *V. brachiumcancri* and 4.0–4.7 mm for *W. nymphocola* (Table 2). For *V. brachiumcancri*, the brood size was 150–235 and the size range of embryos and cyprids in the brood chamber were 500–650 µm and 830–860 µm respectively. For *W. nymphocola* the brood size is smaller, 26–78, and the size of embryos and cyprids are 500–560 µm and 880–1,000 µm. One of the specimens was observed with only seven embryos and the brood chamber seemed to have been partly damaged and embryos were probably lost.

Cypris larva

In both species the embryos hatch as cypris larvae. Two females of *V. brachiumcancri* contained mature cyprids in the mantle cavity (Table 1, Fig. 5). The cyprids had a pair of compound eyes and a length range of 831–862 µm (mean=849 µm, N=50). One adult female of *W. nymphocola* contained mature cypris larvae with a length range of 880–1,000 µm (mean=950 µm, N=7).

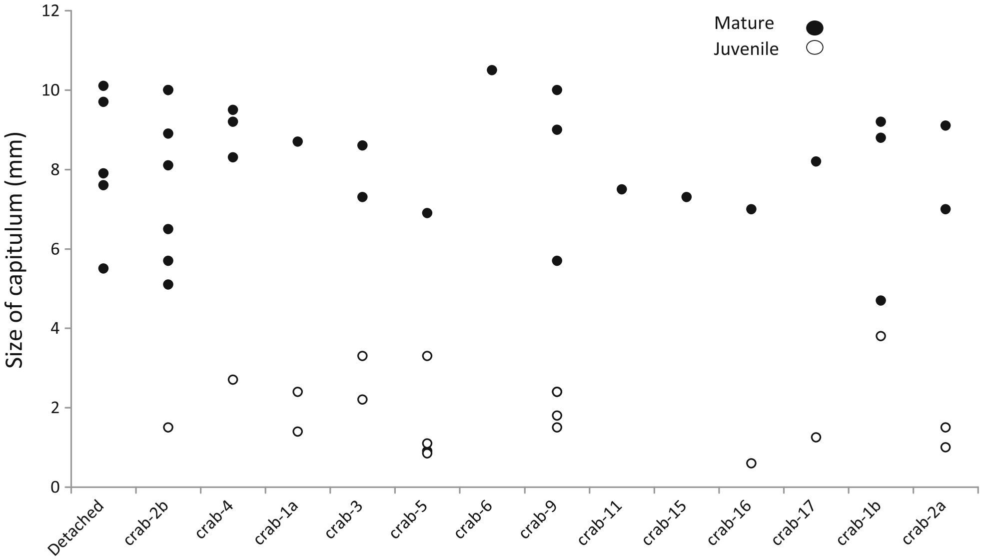

Fig. 3 Size distribution of *V. brachiumcancri* on host *R. hertwigi*, host id corresponds to Table 1

Table 2 *Weltnerium nymphocola* on *Boreonymphon rubrum*: number, size, position and orientation of females; number and size of dwarf male; and reproductive data on eggs and larvae. The sum of all observations is listed at the bottom of the table

Female size	Number and size of males, eggs and larvae					
Capitulum length (mm)	No. of dwarf males	Male (mm)	No. of eggs	Eggs (mm)	No. of cyprid	Cyprid (mm)
Two host specimens from Museum für Naturkunde, Berlin[a]						
7	2	0.7	7	0.5–0.55		
6.7	2				33	0.88–1.00
2.3	0					
4	0					
4.7	0				2	0.95
Detached specimens from Zoological Museum, Bergen						
8.3	2	0.74–0.76	78	0.56		
7.4	1		26	0.52		
7	7	0.7–0.76	78	0.5–0.56		0.88–1.00

[a] Three species, not included in the table, were photographed only and left on the host at the museum

Male position and receptacle

Almost without exception, the females of both species carried two dwarf males each (Tables 1 and 2). The males were located one in each of two symmetrically placed receptacles situated just inside the margin of the scutal plates (Figs. 6 and 7). The receptacle is a deep pocket in the female tissue, within which the elongate male is almost totally embedded. Only the apical part of the male with the aperture and plates is exposed (Figs. 6c, d, and 7a). The receptacle is located within a well-defined depression in the calcified part of the scutum (Fig. 7b), and in *W. nymphocola* the plate is thinner and almost transparent in this area (Fig. 4d). Only on one occasion did we encounter more than a single male on each side. A large female of *V. brachiumcancri* carried four males close together in the receptacle area on one side; none of the males was embedded in the female receptacle tissue but they had all completed metamorphosis.

Dwarf male morphology

In *V. brachiumcancri*, the dwarf male is sac-like and 700–770 µm long (Fig. 8a). The apical part of the male (capitulum pole) carries four reduced shell plates armed with long setae, but there are no cirri (Fig. 8b). Following Nilsson-Cantell (1931), this classifies them as males of type G2. In specimens without a visible penis (see below), the apertural (or mantle) opening is seen between the two lower apertural plates as a narrow slit bordered by a raised lip (Fig. 8b). When dissected free from its receptacle, the external cuticle

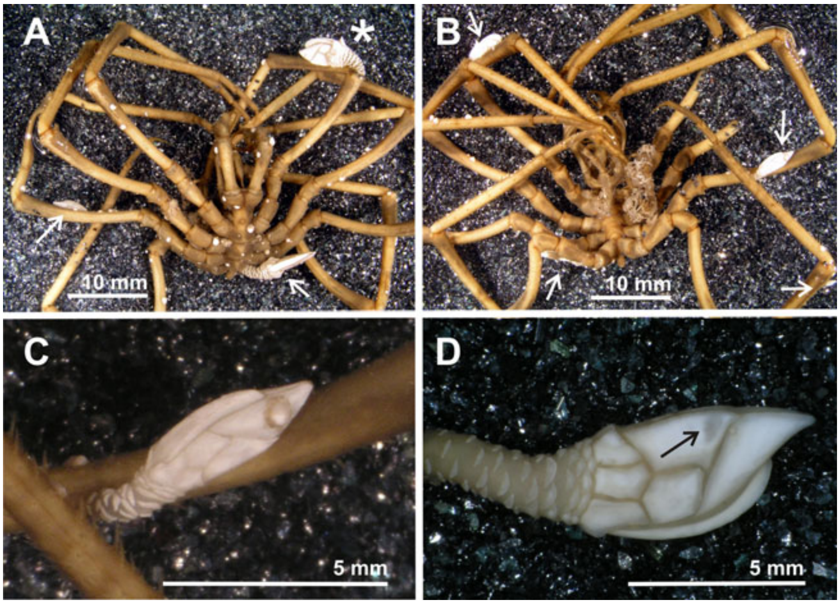

Fig. 4 **a**–**d** *Weltnerium nymphocola*. **a**, **b** Dorsal and ventral views of a *Boreonymphon robustum* hosting three *W. nymphocola* (*arrows*) and a single *Tarasovium cornutum* (*asterisk*); specimens situated both on legs and main body of host. **c** Detail of a *W. nymphocola* in situ. **d** Detached adult *W. nymphocola*, with *arrow* pointing to transparent area of scutal plate, corresponding to location of male receptacle on inner wall (see text)

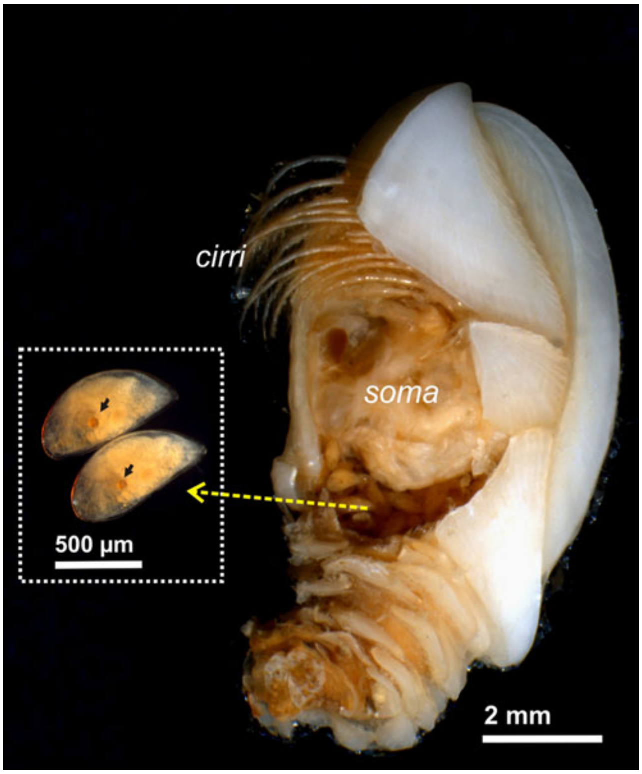

Fig. 5 *Verum brachiumcancri*. Female opened to show partially extended cirri and a brood of cypris larvae in lower part of mantle cavity (brood chamber). *Inset* Two cypris larvae extracted to show their shape and presence of compound eyes (*arrows*)

of the male body is seen to be armed with closely spaced and apically directed cuticular combs (Fig. 8c). The dwarf male of *W. nymphocola* is 740–760 µm long (Fig. 7c). Apically it carries an aperture and very rudimentary plates, visible only as small, transparent lenses. Its surface is also armed with apically directed cuticular combs (Fig. 7d, e).

Penis of dwarf male

Among a total of 63 dwarf males studied in *V. brachiumcancri,* we observed 2 attached to the same female, both of which had a "penis-like" structure extending from it (Figs. 6a and 9a–c). The penis is 2 mm long, about three times the body length of the dwarf male itself (Fig. 9a). It clearly emerges from the interior of the male, leaving only a narrow slit between itself and the rim of the now wide-open aperture (Fig. 9b, c). From here the penis extends far downward into the mantle cavity of the female (Fig. 9a), which in all cirripedes serves as the site of fertilization and as a brood chamber for the embryos (Anderson 1994). The penis carried five conspicuous pairs of symmetrically arranged side branches (Fig. 9a). Each side branch is basally articulated to the penis stem and distally armed with two setae (Fig. 9a). An additional four, much shorter branches are located two by two at the base of the free part of the penis (Fig. 10d, e). A sperm canal traversed the length of the penis which exits with a distinct opening near the distal end (Figs. 9a and 10a–c). More than 20 setae are located at the tip of the penis and around the opening of the sperm canal, sitting directly on the penis stem (Fig. 10a–c).

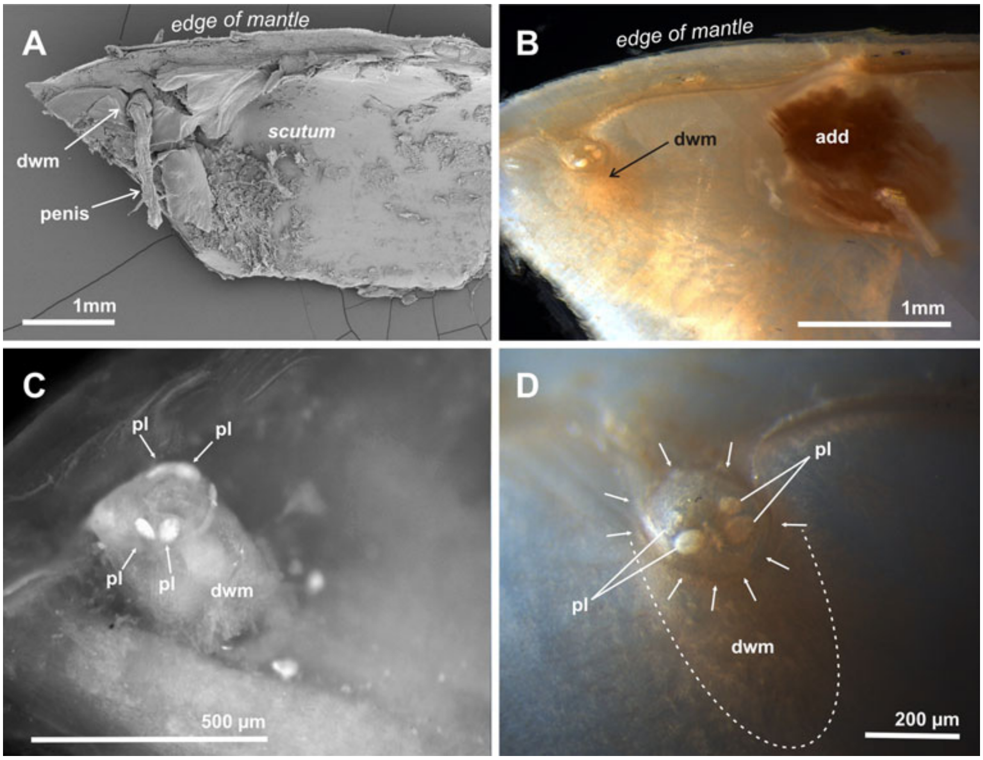

Fig. 6 **a**–**d** *Verum brachiumcancri*. Dwarf males in situ on inside of scutal plate. **a** Scanning electron micrograph (SEM) of dwarf male position with penis extended, apical end broken off during preparation. **b** Light microscope (LM) view of dwarf male in receptacle. **c** LM view of dwarf male, with four minute shell plates, embedded deeply in receptacle. **d** LM view of dwarf male with dotted line indicating its position inside receptacle; only parts outlined by *arrows* exposed. *add* Adductor muscle, *dwm* dwarf male, *pl* shell plate

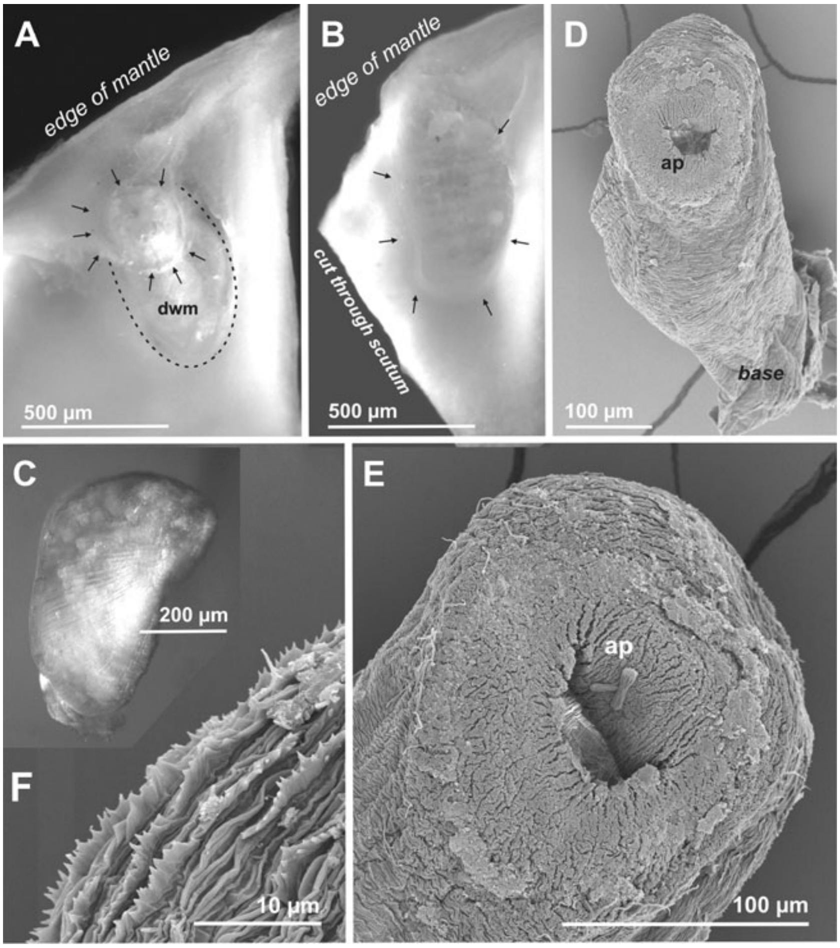

Fig. 7 **a**–**f** *Weltnerium nymphocola*. **a** Dwarf male (*dwm*) in receptacle on inner side of scutal plate; *dotted line* body part embedded in receptacle; only *arrowed* part exposed (cf. Fig. 6d). **b** Inner surface of scutal plate with receptacle tissue removed, revealing depression (*arrows*) below (see also exterior view in Fig. 4d). **c**, **d** Dwarf males dissected free from receptacles (SEM and LM respectively). **e** Detail of aperture (*ap*) in **d**. **f** Surface of male with apically directed cuticular combs

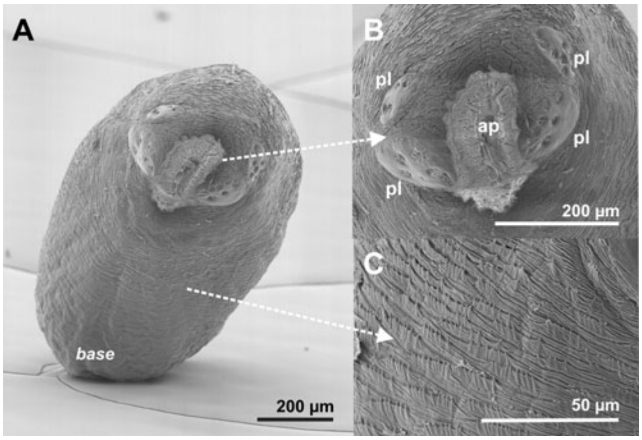

Fig. 8 **a**–**c** V*erum brachiumcancri*, SEM of dwarf male dissected free from receptacle. **a** Whole view. **b** Detail showing four reduced plates (*pl*) with long setae near apical end (capitulum pole) and slit-shaped aperture (*ap*) situated between two lower plates. **c** Detail of surface with bands of cuticular combs directed toward apical end

Discussion

Larval development and settlement

Verum brachiumcancri and *Weltnerium nymphocola* resemble *Ornatoscalpellum stroemii* (Buhl-Mortensen and Høeg 2006) in lacking a naupliar phase and releasing their larvae as cyprids (Table 3). Conversely, both *Scalpellum scalpellum* and *Arcoscalpellum michellottianum* develop first as free-swimming, lecithotrophic nauplii followed by cyprids (Kaufmann 1965; Buhl-Mortensen and Høeg 2006). Unfortunately, there are very few other records on the larval biology for the Scalpellidae, so the degree to which an abbreviated larval phase with no free nauplii occurs within this family is currently unknown. Abbreviated or even direct development is found in many deep-sea organisms, making their recruitment independent of feeding and transport as plankton. While also capable of swimming, the cirripede cypris represents a larval state specifically adapted for locating and settling on a suitable substratum (Høeg et al. 2004). They are always non-feeding (Høeg and Møller 2006) and must

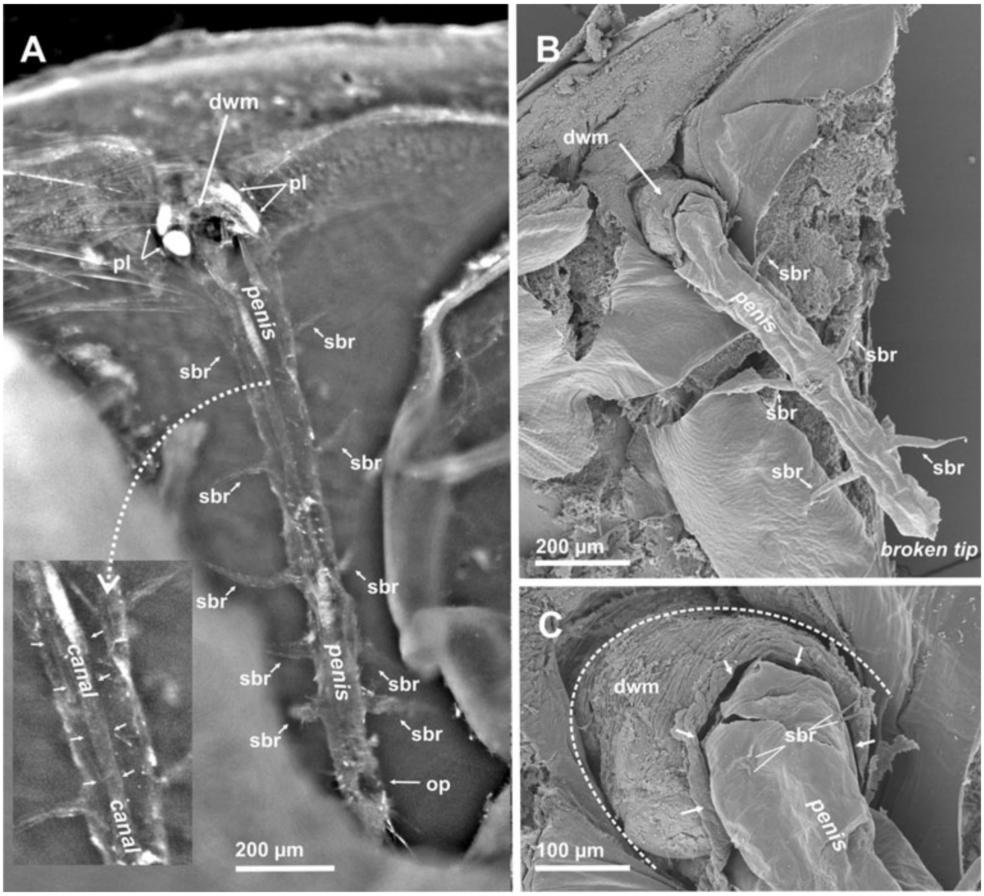

Fig. 9 **a**–**c** *Verum brachiumcancri*, dwarf males in situ with penis. **a** LM view of male with penis extending into mantle cavity (brood chamber) of female, with enlarged inset showing central sperm canal in penis; five pairs of branches extend from sides of penis, and sperm will be expelled from distal opening. **b** Detailed SEM view of penis (also in Fig. 6a). **c** Detail of **b**, penis emerging through wide open aperture of dwarf male, leaving only a narrow slot (*arrows*); dotted line indicating exposed apical part of male, rest of body being protected within female receptacle. *dwm* Dwarf male, *op* distal opening of penis, *pl* shell plate, *sbr* side branch

therefore settle within 3–4 days before they deplete their finite amount of stored reserves (Lucas et al. 1979). Hatching as cyprids therefore enables *V. brachiumcancri*, *W. nymphocola*, and *O. stroemii* to search for a settlement site in the home habitat while avoiding any long-distance dispersal. This developmental strategy is likely to be advantageous in deep-sea organisms that depend on rare and patchy habitats (Table 4). It is even possible that cyprids of *V. brachiumcancri* and *W.*

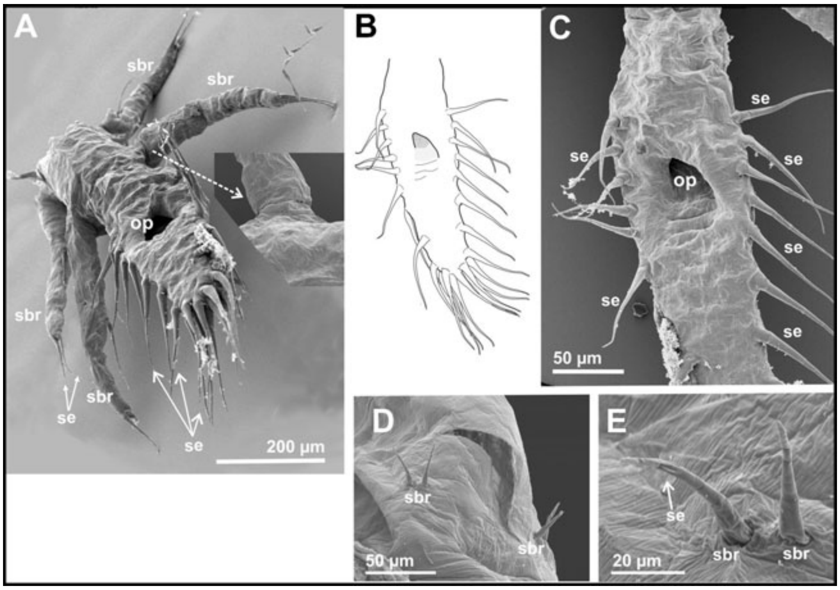

Fig. 10 **a**–**e** *Verum brachiumcancri*, details of male penis. **a** Oblique distal view of penis with side branches and distally placed setae near opening (aperture) of sperm canal; inset shows basal articulation of side branch. **b**, **c** Drawing and SEM of distal end of penis with aperture of sperm canal and numerous setae. **d** Basal part of penis in Fig. 9c, showing four small side branches grouped in pairs; the rip is an artifact. **e** Detail of **d** showing pair of apical setae on side branch. *op* Opening of sperm canal, *se* seta, *sbr* side branch

Table 3 Comparison of adult size, reproductive data, and larval development of five scalpellid species. Data from present paper and Buhl-Mortensen and Høeg (2006)

	Sex	Capitulum maximum (cm)	Egg size (μm)	Egg no.	Capitulum/ egg ratio	Dispersal stage	Cypris size (μm)	Male size (μm)	Male no./ recept	Time plankton
S. scalpellum	*Herm.*	2.2	300–500	100–250	40	Naupl. & cypr.	694	300–550	2–5	>7 days
A. michelottianum	*Sep.*	4.3	1,000–1,200	1,600	40	Naupl.& cypr.	1,340	1,600	6–12	Weeks
O. stroemii	*Sep.*	1	500–600	100–150	17	Cypris	1,000	800–900	1	Days
V. branchiumcancri	*Sep.*	1.05	500–650	150–235	17	Cypris	831–862	700–770	1	Days
W. nymphocola	*Sep.*	0.83	500–560	26–78	14.8	Cypris	888–1,000	700–760	1	Days

nymphocola may settle on the crab or sea spider carrying their mother individual, thus obviating the need to search for another suitable host.

The presence of compound eyes in a deep-water species such as *V. brachiumcancri* is somewhat surprising. The few ommatidia present in the cypris compound eye (Hallberg and Elofsson 1983) severely limits image quality, but the eyes should easily be able to detect contrast and movement and may perhaps act in predator avoidance or in locating a motile host animal.

Host specificity

V. brachiumcancri appears to be a near-obligate associate of *Rochinia hertwigi*, since it has only very exceptionally been recorded from other substrata (Weltner 1922). The situation is less clear for *W. nymphocola*, which has been recorded from bryozoans and stones (Zevina 1981) as well as the present species of sea spider. *W. nymphocola* is also rather unspecific with respect to the body part on which it attaches.

Crabs of the family Majidae, such as *R. hertwigi*, have a terminal moult at maturity (Hartnoll 1985). Cyprids settling on a sexually mature crab are thus not at risk by moulting of their host. Although information is scarce, some pycnogonidan species seem to cease moulting as adults (Tomaschko et al. 1997; Lovely 2005), and this might also be the case for *Boreonymphon robustum*. Scalpellids are very frequently epibiotic on other organisms (Broch 1924; Zevina 1981), but *V. brachiumcancri* seems to be exclusively attached to mobile arthropods (present data; Weltner 1922). As discussed by Reiss et al. (2003) for epifauna associated with hermit crabs, a mobile substratum to some degree protects them against predation. The activities of the crab will also ensure that the scalpellids are always exposed to the surrounding water, and the confinement of *V. brachiumcancri* specimens to the dorsal part of the carapace is in line with this explanation. This position facilitates feeding and ensures that the barnacles are never plunged into the bottom sediment. Perhaps most importantly, “messy feeding” on the part of the crab may significantly increase the suspended food available to its attached barnacles in the otherwise nutrient-poor deep sea.

Dwarf males and receptacles

The cuticular bands on the surface of males of *V. brachiumcancri* and *W. nymphocola* resemble the ornamentation described from males of *O. stroemii* by Buhl-Mortensen and Høeg (2006). Klepal and Nemeschkal (1995) described the variation in cuticular surface structures among males of some scalpellid species and used these data in a taxonomic analysis, but they offered no functional explanation. We suggest that these apically directed ornaments ensure that a dwarf male remains safely anchored within the narrow receptacle pocket in the female tissue. Furthermore, we only very rarely observed more than a single male in a female receptacle, so it is possible that these comb rows may also impede other males from penetrating into an

Table 4 Comparison of distribution and habitat of five species of Scalpellidae. Data from Nilsson-Cantell (1978), Zevina (1981), Young (2001), Morato and Pauly (2004), Buhl-Mortensen and Høeg (2006), and own observations

Species	Bathymetric distribution (m)	Geographic distribution	Substratum
Scalpellum scalpellum	30–200; 50–100[a]	NE Atlantic Oceans littoral zone	Not “substratum-specific”
Ornatoscalpellum stroemii	100–1,600; >100[a]	N Atlantic Oceans shelf/slope	Gorgonians/Hydroids
Arcoscalpellum michelottianum	64–5,190; >500[a]	Atlantic, Pacific, and Indian Ocean Sea mounts and ridges	Gorgonians
V. branchiumcancri	500	South Africa, one location	Decapods
Weltnerium nymphocola	28–1,358	Arctic Ocean	Pycnogonids

[a] Main depth range

already occupied receptacle and thus diminish male–male competition. If so, this would be a parallel to the one-male-per-receptacle situation that is prevalent in rhizocephalan barnacles (Høeg 1987).

Male penis and fertilization

The penile structure observed here in *V. brachiumcancri* is a new discovery and highly relevant for the understanding of the role of the dwarf males in the sexual system. The only previous observation of such a structure is in the neglected paper by Krüger (1920). In a hermaphrodite of *S. scalpellum*, he observed a dwarf male carrying a shorter (same length as the male) and less complex structure, which he called a penis (Fig. 11). The penis of *V. brachiumcancri* penis described here (Figs. 9 and 10) is much longer, three times the length of the male's body, and it carries more and larger side branches than are seen in males of *S. scalpellum*. The morphology of dwarf males has been rather extensively studied in scalpellid barnacles (Klepal 1987), and only one of these former observations indicates that the penis is present, or at least visible, for a very short time interval. On this background we suggest that it is extended only when the female spawns eggs into the brood chamber (mantle cavity). Its fate following fertilization remains unknown, although we find it unlikely that such a large and complex structure could be retracted and reused. The setae situated on the side branches and at the tip are most likely sensory in nature just like those present on the penis in other hermaphrodite barnacles (Munn et al. 1974). The side branches may possibly also assist in anchoring the penis securely in the female brood chamber during the release of sperm.

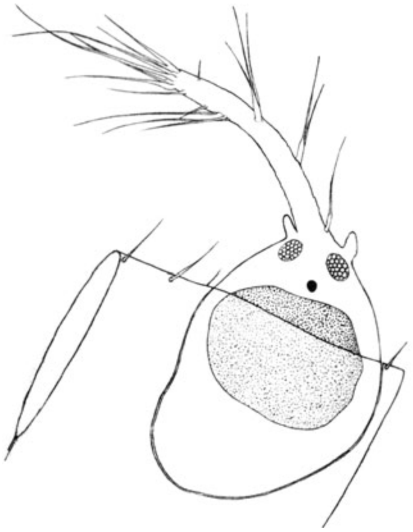

Fig. 11 *Scalpellum scalpellum* dwarf male with penis extended (redrawn from Krüger 1920: fig. 7); cf. longer and more complex penis of *Verum brachiumcancri* (Fig. 10)

Reproductive strategies in scalpellid barnacles

Almost all scalpellid barnacles have dwarf males that are minute compared to their partner, which can be either a hermaphrodite (androedioecy) or a female (dioecy) (Buhl-Mortensen and Høeg 2006). In androdioecious species, such as *S. scalpellum*, the dwarf males are "complementary" (Darwin 1851) insofar as the large hermaphrodites, if situated gregariously, can be fertilized by the penis of a nearby hermaphrodite (Spremberg et al. 2012). In dioecious scalpellids the females must rely entirely on their dwarf males, and the situation is exacerbated if the number of hosted males is small, as in *V. brachiumcancri* and *W. nymphocola*. The sexual systems of *V. brachiumcancri* and *W. nymphocola* are almost identical to what we previously described for *O. stroemii* (Table 3). In all three species the larvae are released as cyprids, ensuring settlement success in the local environment. The number of males is restricted to (normally) two per female, but located in well-defined receptacles on the inside of the mantle wall and firmly protected by being deeply immersed in the female tissue. At least in *V. brachiumcancri*, the male also invests heavily by developing a long and complex penis that appears capable of releasing sperm near the egg mass inside the female's mantle cavity. Both the position and structure of the female receptacle and the male penis seem likely to act in concert to ensure successful fertilization. Obviously, this is of paramount importance for a species in which two dwarf males are the only possible source of sperm. The sexual system in these three species differs from those seen in the dioecious *Arcoscalpellum michellotianum* and in the androdioecious *Scalpellum scalpellum* (Table 3). These two species have large receptacles that can host several males but offer much less protection, being located on the edge of the mantle valves and thus constantly exposing their occupants to the exterior environment. Furthermore, the penis in *S. scalpellum* is much shorter and far less complex than the one present in *V. brachiumcancri*. This comparison indicates that females of *V. brachiumcancri*, *W. nymphocola*, and *O. stroemii* invest much more in the care and protection of their limited number of males. The limited number of males for these species compared to some other scalpellids (Buhl-Mortensen and Høeg 2006) or the small size of the dwarf male is probably not limiting the brood size. Comparison of female or hermaphrodite size with brood size (Table 3) indicates that the body size dictates the capacity for large brood size rather than the slight difference in dwarf male size. This agrees well with the general patterns documented by Strathmann and Strathmann (1982). Strathmann and Strathmann (1982) reviewed the relationship between adult size and brooding in marine invertebrates and found that both within and between species larger individuals usually have larger clutches. They also observed that brooding to provide protection to more advanced stages is generally more common for smaller adults. This agrees with our observations of larval release as cyprids of the smaller sized scalpellids *V. brachiumcancri*, *W. nymphocola*, and *O. stroemii*.

Compared to the information available from other scalpellids (Tables 3 and 4) we conclude that *V. brachiumcancri*, *W. nymphocola*, and *O. stroemii* ensure reproductive success in rare and patchy habitats by a set of unique adaptations in larval development, receptacle structure, and male morphology. We predict that further comparative studies on scalpellid species concerning the morphology, position, and number of dwarf males will increase our understanding of the benefits gained from the diverse reproductive strategies found in these barnacles and unravel new and interesting evolutionary information.

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