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Reproduction and Sex in the Androdioecious Barnacle *Scalpellum scalpellum*: Darwin's Model for Studying Reproductive System Evolution

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Based on recent studies using both field surveys and laboratory experiments, we review a number of central reproductive traits of the androdioecious barnacle *Scalpellum scalpellum*. For the first time in any scalpellid species, development has been followed from cypris settlement until a mature dwarf male and compared with the early ontogeny of hermaphrodites. Cyprids settled in preformed receptacles on mature hermaphrodites started to deviate structurally from hermaphrodites almost immediately after attachment. After 14 days they had matured into adult dwarf males that do not resemble any stage in hermaphrodite ontogeny. Therefore, *S. scalpellum* males are not hermaphrodites arrested in development but the result of a much more profound evolutionary history. Settlement experiments showed that all cyprids are capable of settling and developing into hermaphrodites. Development into males happens only in cyprids attached in receptacles on adult hermaphrodites and must therefore be governed by environmental sex determination (ESD), presumably induced by some chemical factor(s) present only in the receptacle area.

We observed mating between hermaphrodites and between a hermaphrodite and its dwarf males. Hermaphrodite to hermaphrodite mating resembles that seen in balanomorphan barnacles, except that adjacent specimens often check their environment with their cirri for the presence of a mating partner. Dwarf male mating is by means of a unique penis structure, made almost exclusively of cuticle and extending from inside the male and bending down into the brood chamber of its partner. This male penis is much larger (relatively to body size) than the structurally very different penis of the hermaphrodite individuals. The hermaphrodite recognizes when the dwarf male extends its penis and arrests its cirral motion so as not to damage or disturb its tiny partner.

For the adult hermaphrodites we showed that their allocation of resources to male and female functions is in agreement with predictions from sex allocation theory. As predicted, solitary hermaphrodites allocated fewer resources to male function than those settled gregariously, where one or several hermaphrodite partners are within mating distance. The solitary individuals had both a shorter penis and less developed testes than the gregarious ones. As also predicted, solitary hermaphrodites were more likely to carry males than the gregarious ones.

Keywords: Reproductive systems, Dwarf male, Receptacle, Mating system, Resource allocation, Sex determination

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BACKGROUND

Darwin (1851) was the first to emphasize the importance of variation in reproductive systems for evolutionary theory. He chose barnacles (cirripedes) as some of his first "model organisms" for testing his ideas on evolution in general and in particular with respect to the evolution of reproductive systems. Organisms, including animals, can reproduce in a variety of ways, including various types of asexual and sexual reproduction. Understanding these systems and how and when a species or lineage switches between reproductive modes is obviously of prime importance when studying the process of evolution itself. This is best done by focusing on a taxon that comprises a variety of such systems, and where the phylogeny and general biology are well known, so that character evolution can be traced and analyzed. It was obvious to Darwin that barnacles offer this possibility, although at his time questions could be posed but rarely answered.

Today, this situation has changed dramatically. The Cirripedia (barnacles sensu stricto) are now recognized as an assuredly monophyletic taxon for which the phylogeny is known in great detail (Chan et al. 2021a), and a very considerable amount of data exists on their life cycles and reproductive systems (Figs. 1, 2). Most cirripedes are permanently sessile as adults, although exceptions exist such as the lepadid *Dosima* and some turtle barnacles (Chan et al. 2021b). Comprising ca. 2,200 species, they have evolved an astonishing variety of morphological types and life forms (Anderson 1994; Chan et al. 2021a). The taxon comprises three major taxa, Thoracica, Rhizocephala and Acrothoracica (Fig. 3). Thoracica are, with few exceptions, suspension-feeders, and comprise pedunculated barnacles (Calanticomorpha, Pollicipedomorpha and Scalpellomorpha), asymmetric barnacles (Verrucomorpha) and acorn barnacles (Balanomorpha). Most of thoracicans have the body wholly or in part clad in an armor of shell plates. But some species almost or completely lack hard shell plates, including the rather few parasitic forms scattered within the taxon. The Rhizocephala are all parasitic on various crustaceans, principally decapods. Their morphology is extremely reduced, without shell plates, appendages, segmentation or any other organs than the reproductive ones (Høeg 1995; Høeg and Lützen 1995). The Acrothoracica (burrowing barnacles) similarly lack shell plates, but are morphologically somewhat comparable to the Thoracica (Anderson 1994; Kolbasov 2009). They live in burrows in lime stone, and most are epibiotic in *e.g.*, corals or the gastropod shell of hermit crabs, but unlike thoracicans and rhizocephalans, their reproductive biology is little studied (Turquier 1972; Nielsen et al. 2016).

The only morphological feature common to all

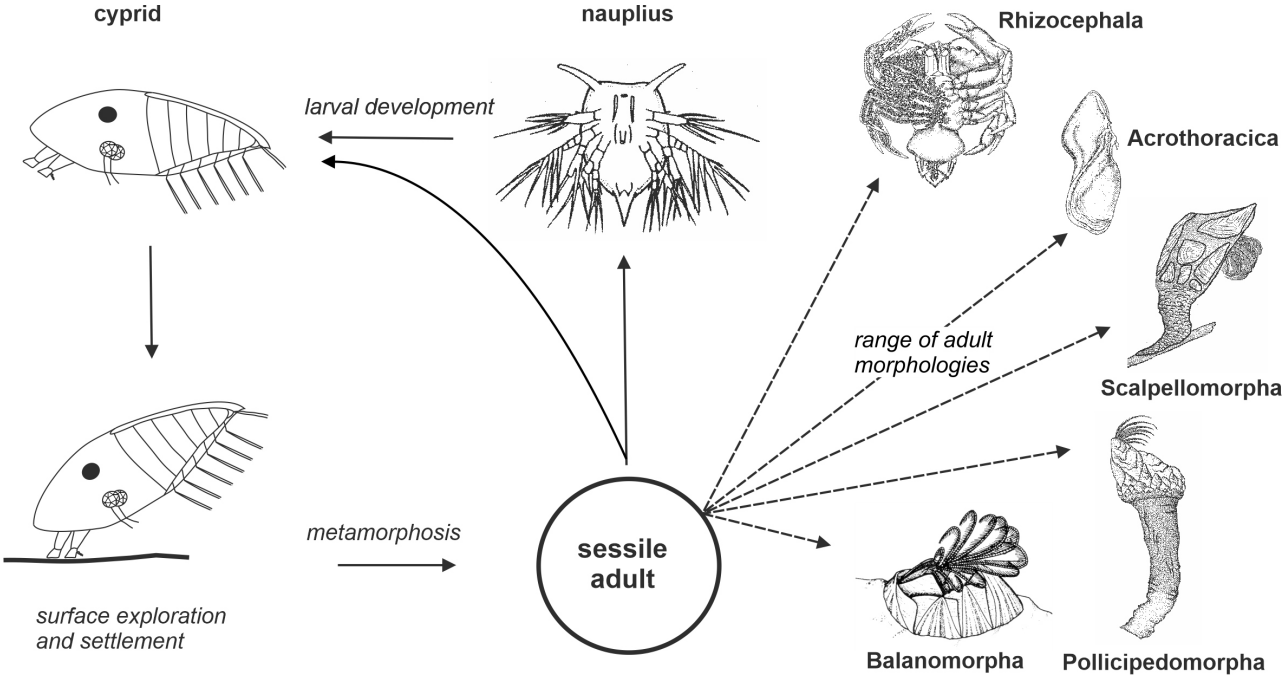

Fig. 1. Generalized cirripede life cycle. Larvae are released from the sessile adult, either as nauplii or in some species directly as cyprids. After exploring surfaces for a suitable settlement site, the cyprid cements itself irreversibly and proceeds with metamorphosis. The resulting adults have an extreme range in structure and life style; see text. Individual figures are at different scales.

barnacles is a rather stereotyped larval development (Fig. 1). It normally starts with a series of naupliar stages, but the terminal larva is always the highly specialized cyprid that locates a suitable substratum for settlement, attaches and commences the metamorphosis into the juvenile and adult phases (Høeg and Møller 2006; Martin et al. 2014). Thus, it is only from cypris settlement that the various cirripede types begin to

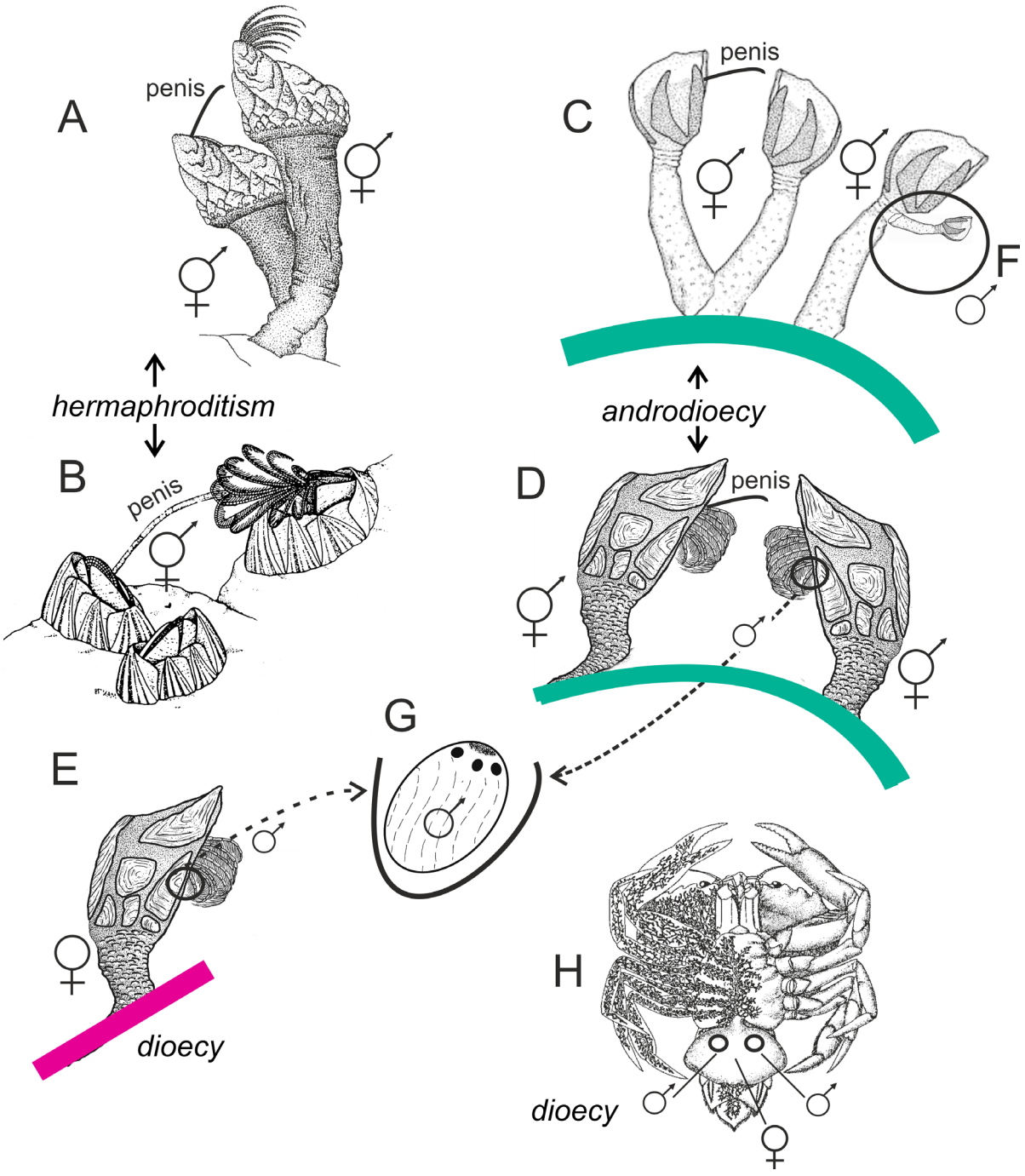

Fig. 2. Overview of sexual systems in the Cirripedia. Purely hermaphroditic species such as *Pollicipes pollicipes* (A) or balanomorphan acorn barnacles (B) use a penis for mating with a nearby partner. In species with androdioecy, *e.g.*, some species of *Octolasmis* (C) and *Scalpellum scalpellum* (D), hermaphrodites can use their penis to mate with a nearby hermaphrodite or with a dwarf male attached to their body. Scalpellid barnacles with dioecy, *e.g.*, *Ornatoscalpellum stroemi* (E), can only reproduce if carrying a dwarf male. Dwarf males range from resembling small-sized hermaphrodites (F) to being morphologically reduced, non-feeding and situated in special receptacle pockets (G). The extremely reduced rhizocephalan males are hosted and nourished within the female body (H).

diverge from each other in both structure and life style. This highlights early ontogeny as a research focus in cirripedes (Høeg et al. 2012).

Within barnacles is found a variety of sexual systems (Fig. 1; Table 1). They comprise pure hermaphroditism, dioecy (separate sexes) and the rare but evolutionarily important system of androdioecy, where both hermaphrodites and males co-occur in the same population (Charnov 1987; Kelly and Sandford 2010; Weeks et al. 2000 2006; Weeks 2012; Yusa et al. 2012). Where males occur, they are always permanently attached to a female or hermaphrodite and are small or even minute compared to their partner (Anderson 1994; Høeg 1995; Klepal 1987; Urano et al. 2009). Their morphology ranges from feeding forms resembling small hermaphrodites to highly reduced, non-feeding

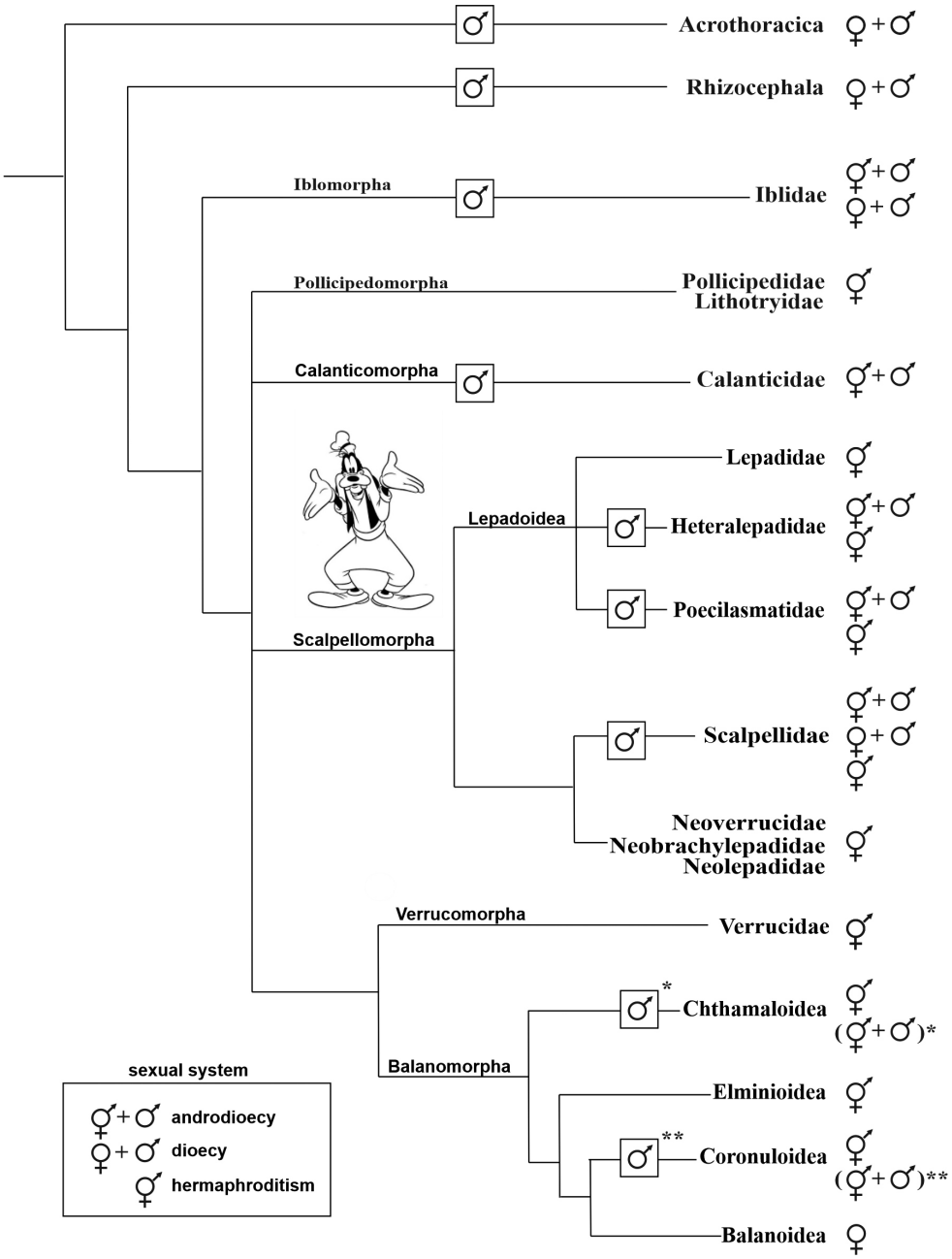

Fig. 3. Sexual systems and cirripede phylogeny. Distribution of sexual systems within the phylogeny of the Cirripedia. Males are always in dwarf form, but they differ extensively in both morphology and position on their female or hermaphrodite partner; they have most likely evolved convergently many times. In Balanomorpha males are rare, but occur in a few species of Chthamaloidea (*) and Coronuloidea (**) (Table 1). Based on Chan et al. (2021a).

males (Fig. 2). The presence of such dwarf males in many species of barnacles poses several questions: How much do dwarf males deviate from females/hermaphrodites in morphology and how early can this be detected during ontogeny? What factors are involved in sex determination? How do tiny and morphologically reduced males mate with their much larger partner? And what factors have favoured the evolution of such males (Darwin 1873; Lin et al. 2015; Yusa 2019; Yusa et al. 2012 2013; Yamaguchi et al. 2012 2013a b c).

First found by Darwin (1851), androdioecy is a rare sexual system among animals, but attracts special attention, because it is believed to be an evolutionary pathway between hermaphroditism and dioecy (Weeks et al. 2000 2006; Weeks 2012; Yamaguchi et al. 2012 2013a b c 2014; Yusa et al. 2012; Lin et al. 2015; Pannell 2002; Dreyer et al. 2018a). In barnacles, groups at relatively low taxonomic levels, such as the ca. 250 species of Scalpellidae, contain forms with all the three sexual systems (Fig. 3). Moreover, recent analyses indicate that transitions between sexual systems occurred multiple times and in multiple lineages within barnacles (Yusa et al. 2012; Lin et al. 2015). As explained in Appendix 1 and summarized in figure 4, the theory for allocation of sexual resources predicts that in populations with androdioecy, hermaphrodites should allocate fewer resources to their male function, when an individual experiences a situation where opportunity for mating with other hermaphrodites is low or non-existent (*i.e.*, low Mating Group Size) (Charnov

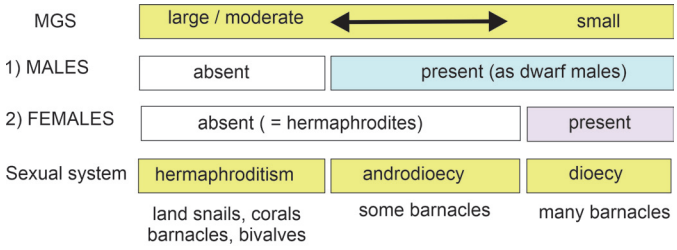

Fig. 4. Effect of Mating Group Size (MGS) on sexual systems in species that have limited mobility or are sessile and rely on direct contact with a partner for mating. Large to moderate MGS favours hermaphroditism; towards very low MGS dioecy with dwarf males is the optimal system; in a "window" in between hermaphroditism and dioecy, androdioecy can exists as a an evolutionary stable system.

Table 1. Sexual systems and sex determination in the Cirripedia

Taxon	sexual system	females/hermaphrodites	males	sex determination
Acrothoracica	dioecy	females	yes	ESD? (Turquier 1972)
Rhizocephala "kentrogonid type"	dioecy	females	yes	GSD (Høeg 1984 1995; Glenner et al. 1989; Walker 1985)
Rhizocephala "akentrogonid type"	dioecy	females	yes	ESD (Høeg 1995)
Iblomorpha	dieocy androdioecy	females hermaphrodites	yes	unknown
Pollicipedomorpha	hermaphroditism	hermaphrodites	no	not applicable
Poecilasmatidae	hermaphroditism androdioecy	hermaphrodites	yes	ESD (Yusa et al. 2010 2012)
Calanticidae	androdioecy	hermaphrodites	yes	ESD?
Heteralepadidae	androdioecy hermaphroditism	hermaphrodites	yes	ESD?
Lepadidae	hermaphroditism	hermaphrodites	no	not applicable
Scalpellidae	hermaphroditism androdioecy dieocy	hermaphrodites females	yes	ESD (+ GSD?) (Svane 1986; Høeg et al. 2016)
Neolepadoidea	hermaphrodites	hermaphrodites	no	not applicable
Verrucomorpha	hermaphrodites	hermaphrodites	no	not applicable
most Balanomorpha	hermaphroditism	hermaphrodites	no	not applicable
a few Balanomorpha*	androdioecy*	hermaphrodites	yes	ESD, GSD? (Gomez 1975)

* Within Balanomorpha very few species have dwarf males attached to hermaphrodites, *e.g.*, *Conopea galeata*, *Chionelasmus darwini*, *Bathylasma hirsutum*, and the turtle barnacle *Chelonibia patula* (Gomez 1975; Hui and Moyse 1984; Crisp 1983; Neuhaus 2025). GSD was claimed to operate in *C. galeata*.

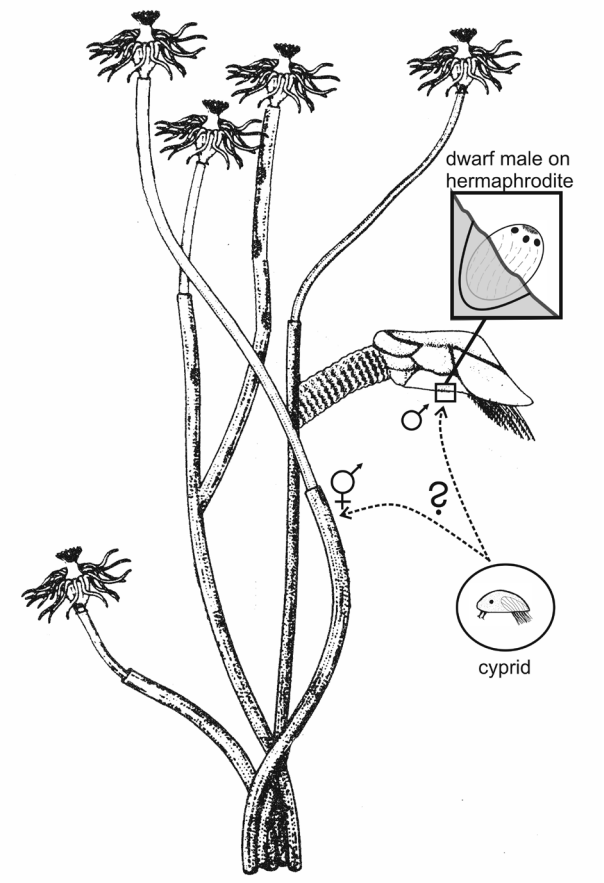

Fig. 5. Settlement in *Scalpellum scalpellum*. The cypris larva can settle on, *e.g.*, a hydroid colony and develop into a hermaphrodite. Alternatively, it can settle in the preformed receptacle on an adult hermaphrodite and develop into a dwarf male.

1982 1987; Weeks 2012; Yamaguchi et al. 2007 2008 2012 2013a b c; Tamechika et al. 2020). However, this important point in sexual systems evolution has rarely been tested in natural populations.

We have chosen the androdioecious barnacle *Scalpellum scalpellum* as a model for studying the above-mentioned questions in reproductive biology (Figs. 5, 6). In a recent series of studies we have used this species to focus on early ontogeny, sex determination, dwarf male mating and allocation of sexual resources (Buhl-Mortensen and Høeg 2006; Spremberg et al. 2012; Høeg et al. 2016; Dreyer et al. 2018a b c d). Here we review these results and use them to discuss general aspects of reproductive biology evolution. In addition, we present new results on the morphology of the male settlement sites (receptacles) in *S. scalpellum*.

General biology of *Scalpellum scalpellum*

Darwin (1851) was the first to describe both androdioecy and dwarf males in the animal kingdom. He found this situation in the pedunculated barnacle *Scalpellum scalpellum* (Scalpellidae) and was "infinitely curious" as to what had favored the evolution of such a system. *Scalpellum scalpellum* is a pedunculated barnacle belonging to the monophyletic family Scalpellidae, which includes more than 200 mostly deep-water species (Buhl-Mortensen and Høeg 2006; Gale 2016; Chan et al. 2021a). Most scalpellids have separate sexes (dioecy), consisting of large feeding hermaphrodites carrying minute non-feeding

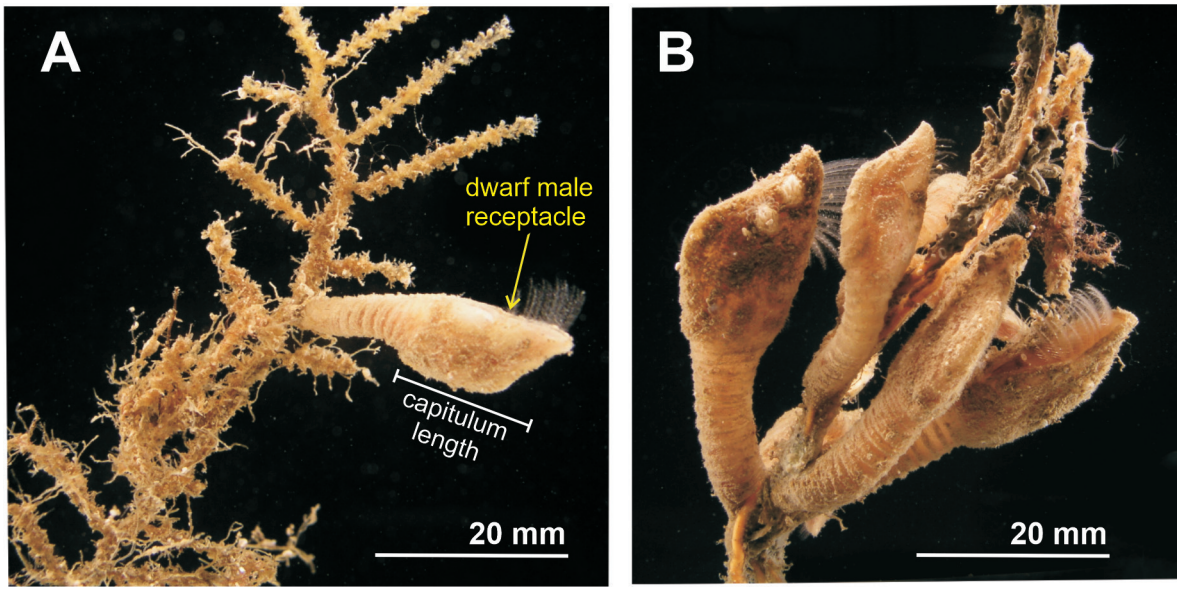

Fig. 6. Live specimens of *Scalpellum scalpellum* photographed in an aquarium. A, A solitary hermaphrodite; the site of the dwarf male receptacles is indicated. B, A group of five hermaphrodites that can all mate with each other (Mating Group Size = 5).

dwarf males. A minority of the species, including *S. scalpellum*, have androdioecy, where the large feeding individuals are hermaphrodites (Fig. 5). *Scalpellum scalpellum* is excellently suited to study androdioecy and reproductive biology in general. It occurs in relatively shallow waters along the coast of Western Europe and can thus easily be sampled alive. Specimens can be maintained for observation and experiments in the laboratory, and the larvae can be raised and subjected to experiments on settlement and ontogeny (Kaufmann 1965; Svane 1986; Spremberg et al. 2012; Høeg et al. 2016; Dreyer et al. 2018d). *Scalpellum scalpellum* seems not to be substratum-specific but utilizes whatever is available for settlement. In the field we have found them attached in large numbers to lost nylon net fishing gear, and in the lab, they will settle on the nylon net bottom of culture dishes (Spremberg et al. 2012; Høeg et al. 2016). The hermaphrodites reach sexual maturity at a capitulum length of 7 mm at which size we estimate them as being about a year old, with most adult hermaphrodites having a capitulum length of 10–20 mm although occasionally reaching about 50 mm (Buhl-Mortensen and Høeg 2006; Spremberg et al. 2012; Dreyer et al. 2018a).

Background for the review

For the results reviewed here, details of sampling and experimental methods were given in our underlying articles (Buhl-Mortensen and Høeg 2006; Spremberg et al. 2012; Dreyer et al. 2018a b c d) and are only briefly summarized here. The present account draws on data from a population on the west coast of Sweden, where it was dredged on rocky slopes at 40–60 m (Spremberg et al. 2012; Dreyer et al. 2018a). At this locality the hermaphrodite specimens were attached mostly to hydroid polyps (*Tubularia indivisa* and *Nemertesia antennina*).

Experiments with larvae

The larvae are released as nauplii and pass through six non-feeding instars before they moult into the final cypris stage that accomplishes settlement (Kaufmann 1965; Svane 1986). For experiments on settlement and metamorphosis, laboratory-reared cyprids were allowed to attach either in empty culture dishes, on hydroid colonies or on mature hermaphrodites (Spremberg et al. 2012; Høeg et al. 2016; Dreyer et al. 2018d). On hermaphrodites the dwarf males are situated in a pair of receptacles (Figs. 3, 7). These are small pockets, located symmetrically on the hermaphrodite body on either side of the opening into the brood chamber, also called mantle cavity (Spremberg et al. 2012; Dreyer et al. 2018c). In *S. scalpellum*, each receptacle can accommodate only a limited number of males (Spremberg et al. 2012; Dreyer et al. 2018a c). As in Svane (1986), laboratory-reared cypris larvae were offered adult hermaphrodites with the receptacles devoid of any previous males. This made it easy to determine when settlement took place and thus enabled us to accurately estimate the age of the developing males. The dwarf males are non-feeding (lecithotrophic) and therefore never grow beyond the size of the settled cyprid. They reach sexual maturity within 2–3 weeks after settlement (Spremberg et al. 2012; Dreyer et al. 2018d). Settled cyprids could be removed from either hydroid tubes or from within receptacles and incubated in vitro in small vessels in filtered seawater. Comparison with events in specimens left on their natural substratum showed that such transplanted specimens would develop normally. This procedure was used in the experiments on sex determination.

Studies on mating

Hermaphrodites were kept under close observation in aquaria. This allowed observations of normal behavior, mating between adjacent hermaphrodites and mating between dwarf males and their hermaphrodite partner. Hermaphrodite to hermaphrodite mating was documented visually and by video with a macro-lens. Male mating was documented by microphotography and video in the dissection microscope, and selected specimens were preserved for light and scanning electron microscopy during the process. Details of the procedures are given in Dreyer et al. (2018b).

Population studies. The capitulum lengths of the sexually mature hermaphrodites (capitulum length ≥ 7mm) were measured. For these mature specimens the length of their penis and the number of live males in both receptacles were also recorded. Finally, the number of mature hermaphrodites within mating distance of each other was noted in order to estimate the Mating Group Size (MGS; see procedure in Dreyer et al. 2018a). A sub-set of hermaphrodites were subjected to paraffin sectioning in order to estimate the relative size and stage of development of the testis.

Receptacle structure and male numbers in *S. scalpellum*

In scalpellid barnacles, cyprids settling as dwarf males attach on their partner animal in a pair of preformed recepttacles, located on either mantle rim inside the shell scutal plate. We investigated the receptacle area in *S. scalpellum* using both light microscopy, SEM and epoxy based sections (Fig. 8).

The receptacle is a broadened area on the mantle rim, comparatively free of setae. Within the area, the mantle cuticle is infolded as several (~5) narrow and closely spaced pockets. After attachment and using its antennules, the cyprid pulls itself downward into a pocket thereby expanding it. In the receptacle area there is a pronounced depression (cavity) in the scutal plate, serving to afford space for the newly settled cyprids as they penetrate down into the pockets, thus expanding them. After a few hours, the cyprid is deeply buried and starts the metamorphopsis into a dwarf male. When the cyprid carapace has been shed, only the upper part

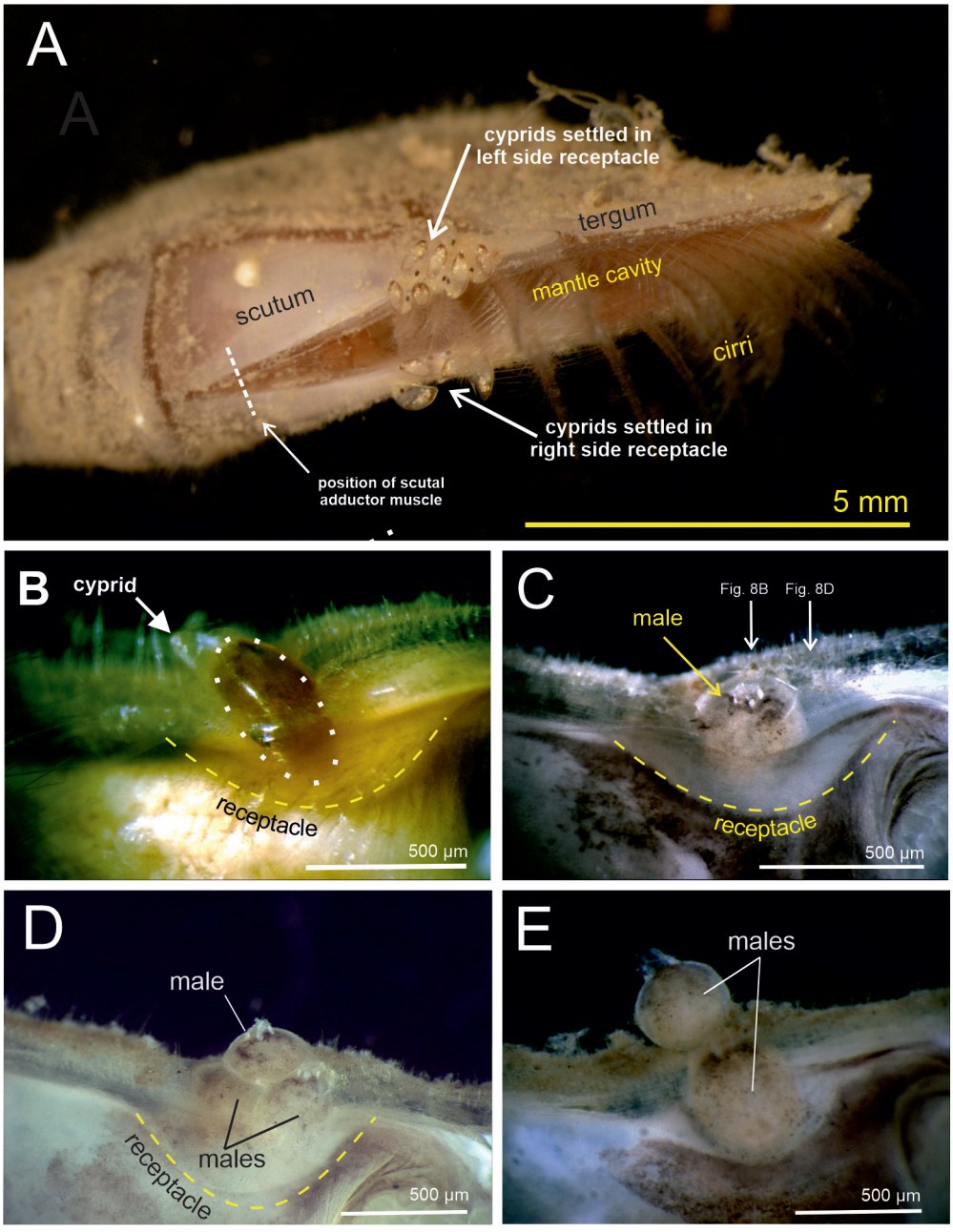

Fig. 7. Dwarf male settlement in *Scalpellum scalpellum*. A, A hermaphrodite exposed to cyprids in the laboratory; many have settled as potential dwarf males in the left and right receptacles situated symmetrically on either side of the mantle aperture (brood chamber). B, Cyprid halfway penetrated into the receptacle, 6–12 hours after settlement. C, mature dwarf males situated deep in a cuticular pocket in the receptacle area. Note the four white and diminutive shell plates at its apex; arrows indicate the sectional planes of figures 8B and 8D. D, Receptacle with three dwarf males: one situated superficially and two other more deeply buried. Receptacle pocket in C and C indicated by dashed lines. E, Unusual situation, where a dwarf male sits directly above another deeply buried male; the upper-most male almost fully exposed.

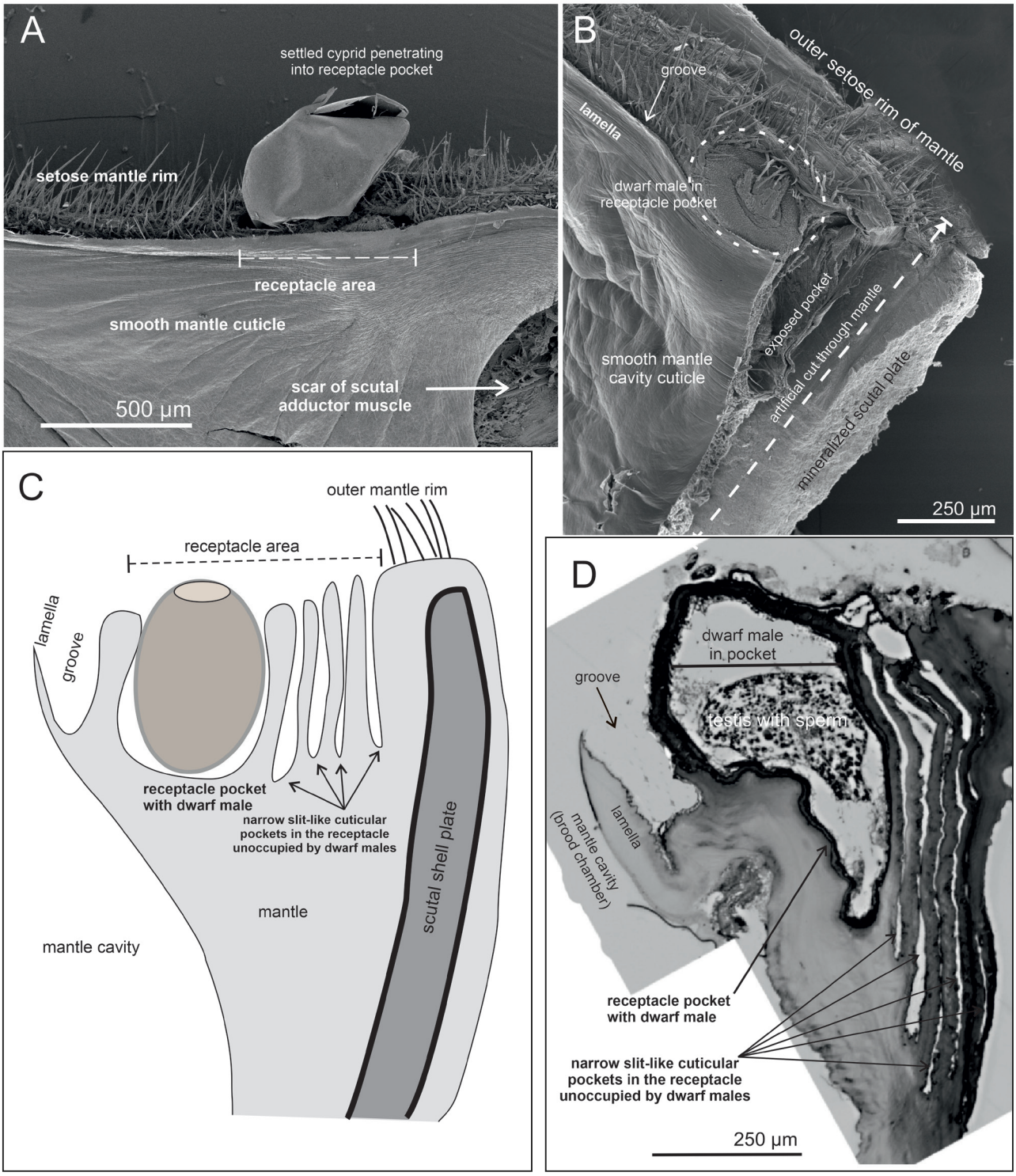

Fig. 8. Receptacle structure in *Scalpellum scalpellum*. A, SEM micrograph of a cyprid 5–10 hours after settlement and now partially penetrated into one of the cuticular pockets in the receptacle area shown in B–D; setae line the general mantle rim while the cuticle facing the mantle cavity is smooth. B, SEM micrograph a specimen cut through the receptacle with a scalpel (adapted from Spremberg et al. 2012); this reveals the dwarf male deeply buried in its cuticular pocket. C, Schematic drawing of a cross section of the mantle wall in the receptacle area located inside the scutal plate; in the receptacle the mantle cuticle is down folded into several slit-shaped pockets, preformed before the arrival of any males; one pocket has received a male and is therefore greatly expanded. D, Epoxy plastic section through a receptacle as in C; the innermost cuticular pocket close to the mantle cavity contains a dwarf male; five adjacent pockets are still narrow and empty slits; note sperm cells inside the dwarf male that principally consists of a large testis (see Dreyer et al. 2018c).

of the dwarf males is visible from the pocket. In older hermaphrodites estimated to be 1–2 years old, pockets could contain empty shrouds of dwarf males that had perished due to having exhausted their limited energy supply.

Table 2 surveys the dwarf male numbers among 126 adult hermaphrodites from the west coast of Sweden (Dreyer et al. 2018a). The mean number of dwarf males was 1.35 in solitary specimens and 0.83 in gregarious ones. This is less than 1 per receptacle, entailing that there is normally ample space and empty pockets for consecutive recruitment of new males as older ones perish. On the other hand, the range in numbers of dwarf males carried is considerable and we have (Spremberg et al. 2012) found up to 13 live males per hermaphrodite, in such specimens the receptacle becomes very crowded with dwarf males and some are forced to sit more exposed on the general receptacle cuticle, rather than being protected in a pocket (Fig. 7D).

Comparison of male and hermaphrodite ontogeny

Based on settlement experiments in the laboratory, Dreyer et al. (2018c) studied the earliest ontogeny of males and hermaphrodites in *S. scalpellum*. The purpose was to decide how early dwarf males can be distinguished from hermaphrodites and to decide whether or not the males could be seen as hermaphrodites arrested at some stage of development as is known from *e.g.*, poecilasmatid barnacles (Fig. 2). These studies also provided the set of criteria to separate *S. scalpellum* dwarf males from hermaphrodites very early in ontogeny, thus enabling the studies on the mechanism of sex determination.

The early ontogeny was studied by two methods. Firstly, we allowed laboratory-reared cyprids to attach on their natural substrata, which were hydroids or empty receptacles in adult hermaphrodites. At various time intervals after attachment, specimens were also removed for detailed microscopical examination. Clear-cut differences between hermaphrodite and male ontogeny was apparent already 24 hours after settlement (Fig. 9). Hermaphrodites would begin to develop an elongated primordial capitulum and a primordial peduncle. Dorsally there appeared an elongated carinal primordial. In addition, there appeared the paired primordial scuta and terga alongside a slit-shaped mantle opening, and these four opercular plates had a distinct angular-polygonal outline.

At the same age, dwarf males assumed an ovoid shape and never developed any peduncle. The mantle aperture was minute and circular and surrounded by paired scuta and terga, which were oval, not polygonal in outline. No carina developed at any stage. Judging from external morphology, males reached their final morphology about 7–14 days after settlement. At this age, hermaphrodites were still very small, but after ca. 3 weeks they began to develop the full complement of shell plates found in the adult and commence cirral feeding.

Sex determination in *S. scalpellum*

We first subjected a large number of cyprids from many different broods to a morphological analysis by both light and scanning electron microscopy to asses whether they exhibited sexual dimorphism (Høeg et al. 2016). We found no such dimorphism in neither size nor in any morphological aspect. This suggests that, unlike the well-known situation in rhizocephalan larvae (Glenner et al. 1989), those of *S. scalpellum* are not predestined to any particular sexual development. Next, we used laboratory-raised cypris larvae in a series of experiments to determine the effect of the settlement substratum on their development following attachment (Høeg et al. 2016). The results are illustrated

Table 2. Distribution and mean number of dwarf males on 126 adult hermaphrodites of *Scalpellum scalpellum*. Adapted from Dreyer et al. (2018a) where statistical test showed that numbers of hermaphrodites with and without dwarf males differ significantly between solitary and gregarious specimens, while total numbers of males did not differ significantly (see text)

Hermaphrodite category	Solitary	Gregarious
hermaphrodites investigated	60 (100%)	66 (100%)
without dwarf males	19 (32%)	34 (52%)
with dwarf males	41 (68%)	32 (48%)
Total dwarf male number	81	55
Mean dwarf male number per hermaphrodite	1.35	0.83
Mean dwarf male number per hermaphrodite with males	1.98	1.72

in figure 10, and they can be summarized as follows.

No natural substratum

Cyprids kept in their naked culture vessels would eventually settle on the nylon net bottom, and all of several thousand larvae would develop into hermaphrodites as determined by the criteria given above.

Hydroids

Cyprids exposed to hydroid colonies would settle on these and all developed into hermaphrodites.

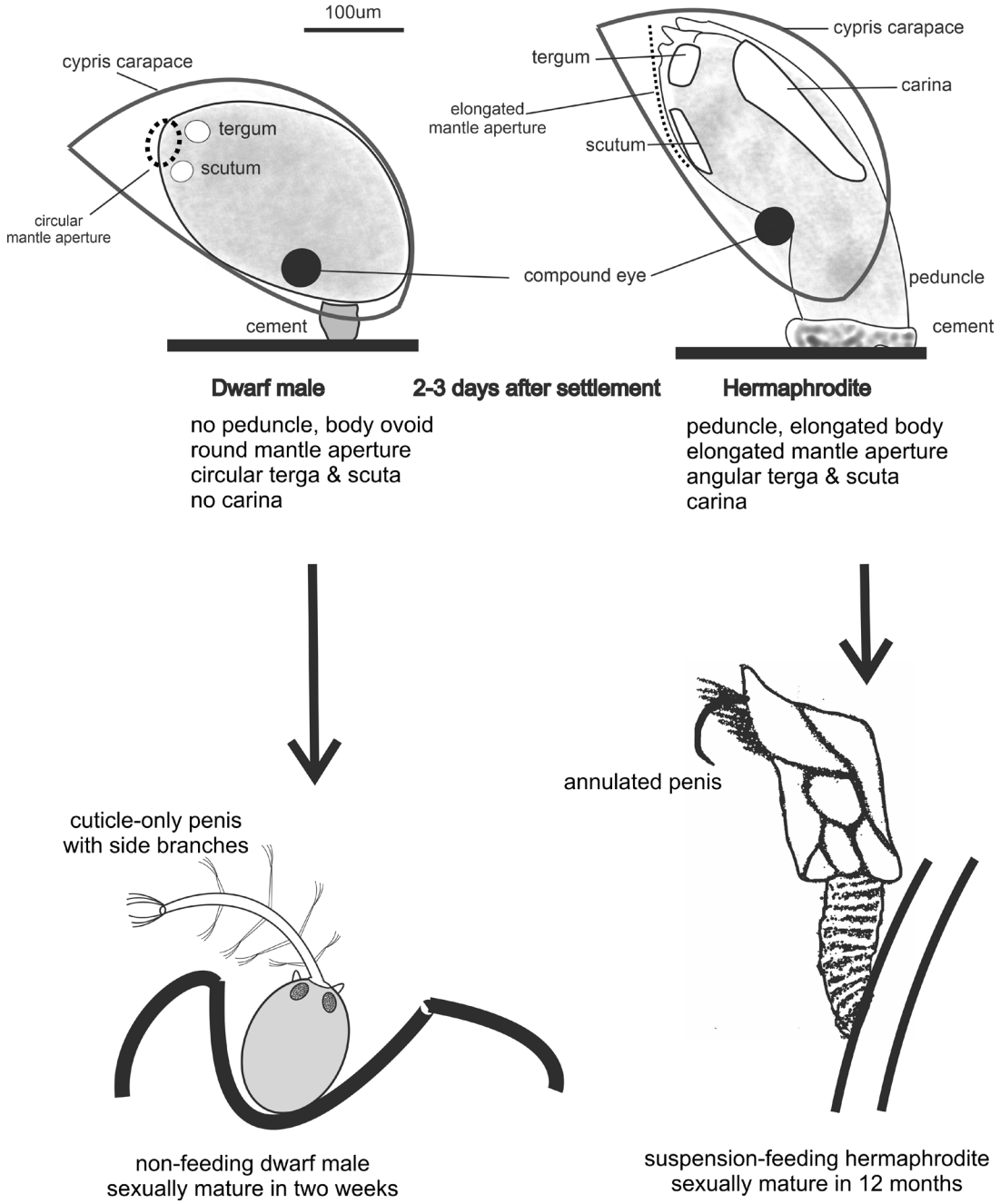

Fig. 9. Early ontogeny in *Scalpellum scalpellum*. Male and hermaphrodites begin to deviate in several distinct features already 2–3 days following attachment. The shape of the mantle apertures is indicated but not seen in these lateral views. The adult dwarf male is ovoid and has a tubular penis structure that can be extended and retracted. The adult hermaphrodite is armed with shell plates, carries cirri for suspension feeding and has a typical cirripede penis.

Adult hermaphrodites

When exposed to adult hermaphrodites without males, many would settle in their empty receptacles and would invariably develop into dwarf males. Interestingly, a few cyprids could also attach either to the adult peduncles or even to the side of their capitulum. But as already noted by Svane (1986), such cyprids settled away from the receptacles always became hermaphrodites. Thus, development into dwarf males definitely occurred only in the receptacle-settled larvae.

Transplanted cyprids

This experiment served to assess whether cyprids settling in receptacles could also become hermaphrodites. Receptacle-settled cyprids were dissected free without any accompanying hermaphrodite tissue. They were then transferred to small culture vessels with clean seawater and monitored regularly through the next 6–7 days. The removal and transplantation took place at various time intervals (3–24 hours) after intial attachment in the hermaphrodite's receptacles. Cyprids that had been attached in the receptacles for longer than 3 hours invariably became males. But among those that were removed within the first 3 hours, a large fraction developed into hermaphrodites with an ontogeny following the exact pattern as those settled on hydroids or in naked vessels. The reason not all transplanted cyprids became hermaphrodites is due to experimental limitations, as the larvae were exposed to the hermaphrodites for a minimum of 3 hours to allow for any settlement to take

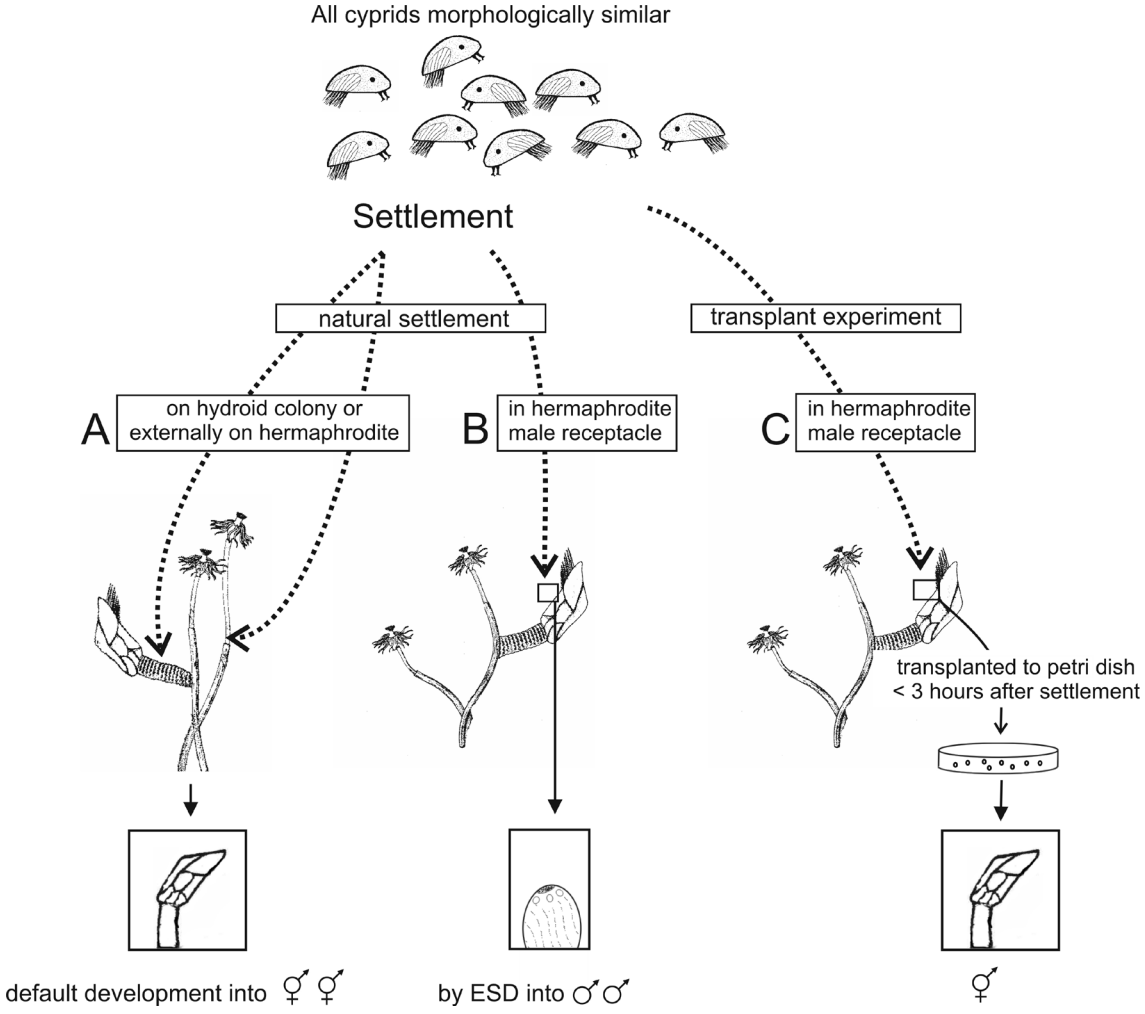

Fig. 10. Sex determination in *Scalpellum scalpellum*. All cyprids are morphologically similar. Development into hermaphrodites occurs if settlement occurs anywhere else than in a receptacle on a hermaphrodite. All cyprids that settle and remain in a receptacle become dwarf males. Cyprids settled in a receptacle in the laboratory, but removed within less than 3 hours after attachment and incubated in vitro, will develop into hermaphrodites. Further explanation in text.

place. Thus, the transplanted cyprids could have been in the receptacle anywhere from 0–3 hours, and those that became males had probably settled close to the upper time limit.

Mating in *S. scalpellum* males

Already Krüger (1920) had observed what he believed to be a penis in *S. scalpellum* dwarf males, but the first detailed description of the structure and the copulation between a dwarf male and a hermaphrodite partner was provided by Dreyer et al. (2018b). On hermaphrodites maintained in aquaria some males had suddenly extended a long, transparent tube from the small mantle aperture (Fig. 11). The tube was slowly extended from the male until it reached a length four times that of the male body itself. When fully extended, it began a waving action, while still being more or less straight. Subsequently, it bent into a U-shape with the tip extending into the brood chamber of the hermaphrodite,

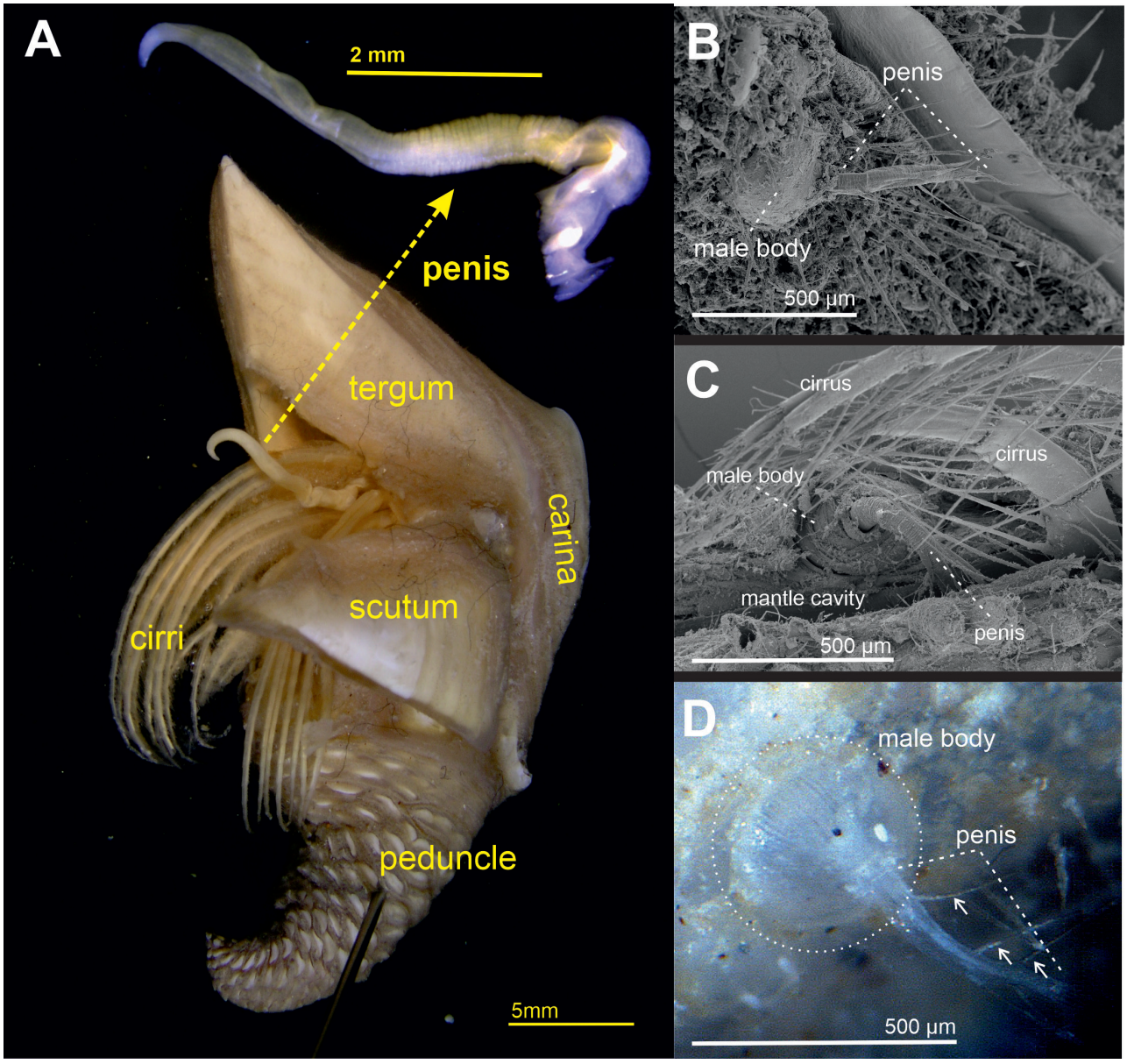

Fig. 11. Mating in *Scalpellum scalpellum*. A, Mature hermaphrodite of *Scalpellum scalpellum*, cut partially open in the left side to show the penis. Note the distinct bend on the penis. B, Dwarf male extending its penis towards the mantle aperture of its hermaphrodite partner; the penis consist of cuticle, only, but carries a series of side branches beset with setae. C, Dwarf male having bent the penis into a u-shape so its reaches into the mantle cavity of the hermaphrodite. In this situation, the hermaphrodite cirri, interlocked with the penis, have stopped feeding and perform small rocking motions. D, Light microscopy of a live dwarf male with penis extended; note the side branches (arrows) and the near transparency of the structure.

thus being ready for the release of sperm.

By using sections of epoxy-embedded specimens, we demonstrated that the tube is connected to the testis of the male. The tube, or "penis" itself, is transparent and seems to consist almost entirely of cuticle, and running through its whole length is a narrow sperm canal. A cluster of several sensory setae was present around the apical tip, and along the length there extended around four pairs of side branches that also carried sensory setae. Unlike the hermaphrodite penis (Fig. 11A), there were no muscles visible inside the extended male penis, so its motility must depends on muscles attached at its base hidden inside the dwarf male body (Fig. 11B–D).

The most fascinating behavioral aspect was the intimate interaction between the male and the hermaphrodite. Normally, the hermaphrodite performs regular feeding movement with its cirri, passing close by the receptacle. Therefore, if the male penis was extended and bent into the brood chamber it would be directly in the pathway of the cirri and could easily be damaged if they kept sweeping. But immediately on touching the male "penis", the hermaphrodite would stop moving the cirri and engage the nearest cirrus intimately with the "penis" so that their respective setae became closely interlocked (Fig. 11C). This was followed by small rocking motions suggesting that both parties were sensing each other. This behaviour, video documented in detail in Dreyer et al. (2018b), contrasts to what happened if the penis was touched by foreign object. Unlike staying extended when engaging the hermaphrodite cirri, the penis tube would, if touched by a fine needle or plastic cord, immediately retract at least partially into the male body, probably triggered by input from its sensory setae. But after a short while it would extend again. Eventually, the U-bending penis reaches into the brood chamber ready for sperm release. The videos in Dreyer et al. (2018b) showed that the whole process of dwarf male copulation lasts at least 30 minutes.

Hermaphrodite reproduction and male longevity

S. scalpellum hermaphrodites can live for at least three years, but reproduces only during the summer season (Svane 1986, unpublished observations). Our observations from long time lab maintenance of *S. scalpellum* hermaphrodites show that during the summer season broods are released about once every month (Spremberg et al. 2012). Dwarf males of scalpellids lack an alimentary canal and any other means of obtaining food (Klepal 1987). They therefore depend entirely on the resources originally laid down in the egg by their mother animals and thus live on limited resources. Often, the receptacles of a hermaphrodite contain both recently settled males, old ones partially fouled by dirt and epifauna and even empty cuticular shrouds of dead males. This all indicates that new males are constantly recruited by the hermaphrodites. This is in contrast to the less specialized males of *e.g.*, poecilasmatid barnacles, which retain their food collecting cirri and a functional gut. At the other extreme stands the males of the Rhizocephala. In these parasitic cirripedes, the males sit in highly specialized receptacles, where they obtain nourisment form their female partner (Høeg 1995; Høeg and Lützen 1995) and thus stay associated for the lifetime of these parasites. Although scalpellid males can sit deeply buried in their receptacles (Fig. 8), they are completely isolated from their large partner both by their own body cuticle and that of the hermaphrodite receptacle (Dreyer et al. 2018c).

Hermaphrodite to hermaphrodite copulation

Dreyer et al. (2018b) also studied mating between hermaphrodites by using two kinds of specimens: The first were specimens that had been sampled in a gregarious situation, meaning that two or more mature specimens were attached within copulation range. The second type were hermaphrodites that had been sampled as solitary but then were placed into an artificially paired situation in the laboratory.

The normal behavior in the gregarious specimens was to perform suspension-feeding while also regularly turning towards each other and using the cirri to strike the partner, and then turning away and resuming feeding. The artificially paired specimens would begin to similarly engage in touching each other within minutes after being placed together. For the actual copulation, the two hermaphrodites turn towards each other on their peduncles. The male active specimen extends its penis and slowly inserts it into the brood chamber of the other hermaphrodite. The entire process lasted only a few minutes, compared to the 30+ minutes used for dwarf male copulation.

Mating group size and sexual systems

The theory of sex allocation predicts that solitary hermaphrodites should allocate fewer resources to male function than gregarious ones, since they have no option to copulate with any hermaphrodite partner but must rely entirely on the presence of dwarf males. In addition, dwarf males should also be more frequent on solitary individuals than on gregarious ones, where they must compete with male function in adjacent hermaphrodites (Charnov 1982 1987; West 2009; Yamaguchi et al. 2008 2012 2013c; Yusa 2019). We examined the population

of *S. scalpellum* from the west coast of Sweden in terms of both male presence and total allocation to male function in solitary and gregarious (MGS ≥ 2) hermaphrodites. We statistically tested (Table 3) the following four theoretical predictions. 1) The penis was predicted to be relatively longer in gregarious hermaphrodites 2) the degree of development of the testis based on histological examination was similarly predicted to be more advanced in gregarious specimens (Fig. 12). 3) Solitary hermaphrodites were predicted to more frequently carry males than gregarious ones (*i.e.*, any number of males: 1+ versus none). 4) Finally, the total number of dwarf males was predicted to be greater among the solitary hermaphrodites. We found significant statistical support for predictions 1–3 (Table 3, test details in Dreyer et al. 2018a)). Relative penis length (1) proved to be significantly shorter among solitary individuals. By classifying the testes into three stages of development, we could also show (2) that solitary individuals had their testes significantly less developed than gregarious ones. Both results agree with sex allocation theory in indicating that fewer resources are used for male function in solitary *S. scalpellum* hermaphrodites. We also showed (3) that solitary individuals are more likely to carry males (any number) than gregarious ones. In the solitary population, 68% of the adult hermaphrodites carried one or more males opposed to only 48% in the gregarious population (Table 2). But, contrary to prediction (4), the total number of males did not differ significantly between the two classes of hermaphrodites.

DISCUSSION

Male ontogeny

In all cirripedes the settled cyprid must undergo a metamorphosis into the juvenile and adult shape (Walley 1969; Walker 1992; Chan and Høeg 2015; Høeg et al. 2012 2015). In purely hermaphroditic species, such as most acorn barnacles and many pedunculated forms, all individuals follow the same ontogenetic path. In contrast, species with dwarf males have two divergent ontogenetic pathways, viz. male or female/hermaphrodite. In *S. scalpellum* we have shown that males begin to diverge from hermaphrodites already one day after attachment (Fig. 9). Moreover, the mature male has a morphology unlike any stage passed through in hermaphrodite ontogeny. Clearly therefore, in *S. scalpellum* the dwarf males are not hermaphrodites arrested in development but represent a different and highly specialized individual. Since all scalpellids have males with an extensively simplified structure, it seems safe to assume that their ontogeny generally follows pathways similar to those found in *S. scalpellum* males and is the result of a long evolutionary history (Klepal 1987; Buhl-Mortensen and Høeg 2006 2013). This is also the case for the similarly very specialized dwarf males found in the Acrothoracica, Rhizocephala and pedunculated barnacles of the genus *Ibla* (Høeg and Lützen 1995; Klepal 1985 1987; Nielsen et al. 2016). In cirripedes, the Poecilasmatidae may well illustrate an earlier phase in dwarf male evolution than present in the Scalpellidae (Lin et al. 2015; Sawada et al. 2015; Yamaguchi et al. 2014; Yusa et al. 2010 2012). Poecilasmatid dwarf males, such as in some species of *Octolasmis*, resemble small-sized hermaphrodites with a peduncle, shell plates and cirri that can be used in suspension feeding (Fig. 2). They must therefore have an ontogeny almost similar to the hermaphrodites. The only difference between dwarf males and similar-sized hermaphrodites is an assumedly precocious development of the male organs, since they can be sexually active at a size when hermaphrodites are still immature (Yusa et al. 2010). Moreover, if sexually mature dwarf males of *Octolamis* are separated from their hermaphrodite partner and glued to a neutral substratum they will resume growth into normal-sized hermaphrodites. A still earlier stage in dwarf male evolution seems to exist in the so-called apertural males of the balanomorphan turtle barnacle *Chelonibia* (Crisp 1983; Chan and Høeg 2015). This is in stark contrast to *S. scalpellum*, where specimens settled in

Table 3. Differences between solitary and gregarious hermaphrodites in 60 solitary and 66 gregarious hermaphrodites. Tests used were General Linear Mixed Model 8GLMM) and Cumulative Link Model (CLM). For test details see Dreyer et al. (2018a); * = significant result

Response variable	Test	Explanatory variable	Coefficient	SE	Wald χ^2	*P*
Relative penis length	GLMM	group status	-0.705	0.181	15.17	< 0.001*
Testis development	CLM	group status	2.531	0.705	8.77	< 0.001*
Male presence/absence	GLMM	group status	0.755	0.377	4.02	0.045*
Number of males		group status	0.346	0.261	1.76	0.185

a hermaphrodite receptacle are committed to dwarf male ontogeny, unless they are experimentally removed within the very short time window of 1–3 hours after attachment. Therefore, dwarf males of Scalpellidae are at a much more advanced stage of specialization in terms of both morphology (Klepal 1987; Dreyer et al. 2018a) and sex determination (Høeg et al. 2016) than those found in Poecilasmatidae and Balanomorpha, only to be superseded by the dwarf males found in the parasitic Rhizocephala (Høeg and Lützen 1995; Høeg et al. 2015).

Cirripede dwarf males

Cirripede males are always dwarfish and permanently attached to a much larger female or hermaphrodite partner. Darwin (1851 1873) used "dwarf males" for those sitting on females and "complemental males" for those on hermaphrodites. However, "dwarf male" denotes a structure while "complemental male" denotes a function, and those on females and hermaphrodites can be structurally very similar despite their very different biological situations, such as is the case in species of the Scalpellidae (Buhl-Mortensen and Høeg 2006 2013). We therefore prefer to use "dwarf males" for both types of males and define them as males having the following characteristics. (1) much smaller (less than half in length) than the female or hermaphrodite partner to which they are permanently attached; (2) capable of fertilizing the broods spawned by their partner; (3) at least to some extent, arrested in growth; (4) they use the energy saved from growth for male reproductive function, entailing (5) that they have relatively larger male organs than hermaphrodites of the same size.

Within barnacles dwarf males have evolved repeatedly (Fig. 2; Table 1), and they display a range of stages from types that apparently differ little from their larger partner, as in the Poecilasmatidae, to the extremely specialized and morphologically advanced forms found in the Scalpellidae, Acrothoracica and Rhizocephala (Klepal 1987; Anderson 1994; Sawada et al. 2015; Yamaguchi et al. 2014; Yusa et al. 2010; Lopez-Greco 2013; Chan and Høeg 2015; Lin et al. 2015; Nielsen et al. 2016). What ecological conditions have favored the evolution of such males represents a very interesting question in evolutionary reproductive biology and will be discussed in more detail below.

Sex determination

Sex is determined either by genetic factors (genetic sex determination, GSD) or by environmental factors (environmental sex determination, ESD). In GSD the sex is set either in the unfertilized egg or at fertilization (Beukeboom and Perrin 2014; Wahl 2025). In ESD it is determined later in ontogeny by physical, chemical or biological factors in the environment (Lopez-Greco 2013; Benvenuto and Weeks 2020; Dunn et al. 2020; Vogt 2020). In *S. scalpellum* development into males is clearly governed by ESD, probably by some chemical factor limited to the receptacle pockets (Høeg et al. 2016). All cyprids have the potential to settle and metamorphose into hermaphrodites, and this is the default ontogeny if they attach anywhere but inside a hermaphrodite receptacle. Transplant experiments confirmed that the receptacle-settling cyprids were potential hermaphrodites (Fig. 10). It is possible that *S. scalpellum* may prefer settlement in receptacles. Becoming a dwarf male entails that it can reproduce within a few weeks after settlement and mortality may be much lower than if settled as a hermaphrodite, since the male sits protected in a much larger partner. The ESD mechanism then means that larvae failing to find a receptacle can alternatively settle as hermaphrodites. Yet, the situation may be more complicated if the hypothesis of Svane (1986) can be verified. He found that only up to 50% of all cyprids would ever settle as males, and this was supported by the data in Spremberg et al. (2012), although not offering a true test. If so, a combined ESD/GSD mechanism could be at play, preventing many larvae from settling as males. Normally, *S. scalpellum* receptacles carry only one or two males, but given the chance, as in the confined space of laboratory trials, they will indeed crowd together in these sites (Fig. 7).

Our results on sex determination in *S. scalpellum* cannot be uncritically extended to the other 200+ scalpellid species. Most of these have separate sexes and it is possible that some could rely on GSD. Within cirripedes GSD is clearly proven for Rhizocephala of the kentrogonid type (Yanagimachi 1961; Ritchie and Høeg 1981; Høeg 1995; Høeg and Lützen 1995; Kajimoto et al. 2024). In these rhizocephalans the cyprids are sexually dimorphic, coupled to the very divergent male and female metamorphosis in kentrogonid type rhizocephalans (Glenner et al. 1989; Høeg 1984 1987; Walker 1985; Glenner et al. 1989). Gomez (1975) claimed that GSD operates in the androdioecious balanomorphan *Conopea galeata*, but the experimental evidence was based on artificial induction of metamorphosis and is in need of verification.

In purely hermaphroditic cirripedes, there is obviously no need for any mechanism of sex determination, but they mostly occur in groups (gregariously), and this is triggered by species-specific, chemical factors that induce cyprids to settle on or near conspecifics (Knight-Jones 1953; Walker 1995; Aldred

and Clare 2009). This gregarious settlement behaviour ensures that they will mostly attach within mating distance of a conspecific. Thus, it is not surprising if this evolved into a mechanism used in settlement as dwarf males on a female or hermaphrodite partner. In species where dwarf males resemble juvenile hermaphrodites, such as in *Octolasmis*, it is doubtful whether there is a true mechanism of "sex determination". Rather, cyprids that attach on a mature hermaphrodite will just be chemically induced to arrest growth and allocate most or all resources to male function. In contrast, barnacles with specialized dwarf males such as scalpellids, rhizocephalans and acrothoracicans must have mechanisms that determine which of two very divergent ontogenetic pathways is taken. It is now clear that this can be both by ESD as in *S. scalpellum* or GSD as in most rhizocephalans (Høeg et al. 2016; Yamaguchi et al. 2014).

Sexual system and sex determination are obviously linked, but they can evolve somewhat independently. As an example, in tetrapod vertebrates, virtually all species have dioecy, but the mechanism of sex determination has changed through evolution, with two different mechanisms of GSD by sex chromosomes in birds (female heterogamety, or ZW sex determination) and mammals (male heterogamety, or XY), while many reptiles rely on an ESD system. It is likely that such changes in the mechanism of sex determination have also occurred in barnacle evolution, especially because dioecy and androdioecy have evolved multiple times (Fig. 3; Yusa et al. 2012; Lin et al. 2015; Benvenoto and Weeks 2020;). For decapod crustaceans, there has been recent progress in understanding the molecular level of sex determination (Vogt 2020; Wahl et al. 2025). For *S. scalpellum* we are still in the dark concerning the molecular process driving receptacle stimulus to male development. Nevertheless, a likely candidate could be some variety of the settlement-inducing protein complexes (SIPC) now known to enable gregarious settlement in hermaphrodite barnacles (Aldred and Clare 2009; Clare 2010).

Mating between hermaphrodites

Being sessile and incapable of broadcast spawning, barnacles rely on mating by means of a long penis. The only exception seems to be the recently demonstrated mechanism of sperm casting, where packages of sperm released into the water can be caught by the cirral apparatus of another barnacle (Barazandeh et al. 2013). Yet, for this mechanism to be successful, the individuals must live at a high density if such sperm packages are to be caught by another individual. We do not suspect this mechanism to be significant under the low MGS values where androdioecy and dioecy are relevant.

The copulatory act documented between hermaphrodites of *S. scalpellum* resembles what is known from acorn barnacles (Balanomorpha). The flexible penis located at the posterior end of the thorax enables hermaphrodites to mate with individuals even if located some distance away. This increases the number of mating partners (MGS) and enables reproduction when individuals sit in a scattered situation. The penis in acorn barnacles is famed to be the longest such organ relative to body length in the animal kingdom (Neufeld and Palmer 2008; Vogt 2016). The penis found in *S. scalpellum* and other pedunculated barnacles is somewhat shorter, but the individual can compensate for this, since their flexible peduncle enables them to extend in almost any direction, as is seen in videos by Dreyer et al. (2018b). Dwarf males in poecilasmatid barnacles such as *Octolasmis warwickii* similarly use their peduncle and penis so that they can introduce sperm into the brood chamber of the partner to which they have attached (personal observations).

Because *S. scalpellum* generally lives at low population densities (MGS < 10 individuals), it makes sense that individuals spend considerable time using their cirri to search for and touch nearby mating partners. A nearby partner may suddenly be lost or a hermaphrodite previously without a mating partner may find that an adjacent individual has now grown into sexual maturity (Fig. 12). Both cases change the mating group size of the individual, and the hermaphrodite should expectedly react by changing its behavior and allocation of sexual resources.

Dwarf male mating and male numbers

Dwarf males of *S. scalpellum* possess a copulatory organ very different from that in hermaphrodites (Fig. 11). In structural terms the male penis represents a much larger part of its body size than in hermaphrodites, and its length and motility ensure that the minute male can securely deposit its tiny amount of sperm inside the brood chamber of its partner. The success of mating is obviously also assisted by the very intimate interaction we observed between the male and hermaphrodite partner. Our observations on the behaviour of dwarf males confirm that a similar structure, found in museum material of *Verum brachiumcancri* and several other scalpellid species, is indeed a penis structure (Buhl-Mortensen and Høeg 2013), and we therefore expect such a male penis to be present in all scalpellid dwarf males. The scalpellid penis seems to emulate an extendable, telescopic structure. The presence of a series of paired side branches on the penis could

indicate that it represents an entire and highly modified thorax, with the branches being extensively specialized cirri. But clearly the structure. motility and action of the dwarf male penis and comparison with its counterpart in hermaphrodites is in need of additional studies.

Important questions remain concerning copulation between scalpellid dwarf males and their partner. *Scalpellum scalpellum* hermaphrodites can occasionally carry up to 13 dwarf males, entailing male competition for fertilizing a brood (Spremberg et al. 2012). For gregarious specimens the males must also compete with the much larger sperm volume contained in nearby hermaphrodites, but their position may entail that they have an advantage in detecting when their partner is ready for mating. These questions could be pursued by analyzing the parentage of a brood of larvae. This would involve comparing the genetic (*i.e.*, microsatellite) profiles of larvae, the dwarf males carried by the mother hermaphrodite and that of any other hermaphrodites within the same mating group (Kobayashi et al. 2020; Tamechika et al. 2025). Another important issue is why dwarf male numbers carried by a female or hermaphrodite varies significantly among species of Scalpellidae. In many large and deep-sea inhabiting scalpellids, the males can be present in high double-digit numbers (Dreyer et al. 2018c). Conversely, some usually small-sized scalpellids never carry more than a single male in each of the two receptacles, but they also release small broods and thus have no need for large sperm volumes (Buhl-Mortensen and Høeg 2006 2013; Dreyer et al. 2018c; Hashizoe et al. 2026). Having no option for growth, the scalpellid males always have a body size similar to that of the originally settled cyprid, measuring less than 1,000 µm, and they can therefore only contain a small amount of sperm. A large-sized partner animal may therefore require several males to ensure that all eggs of their large broods are fertilized. Moreover, the non-feeding males must necessarily

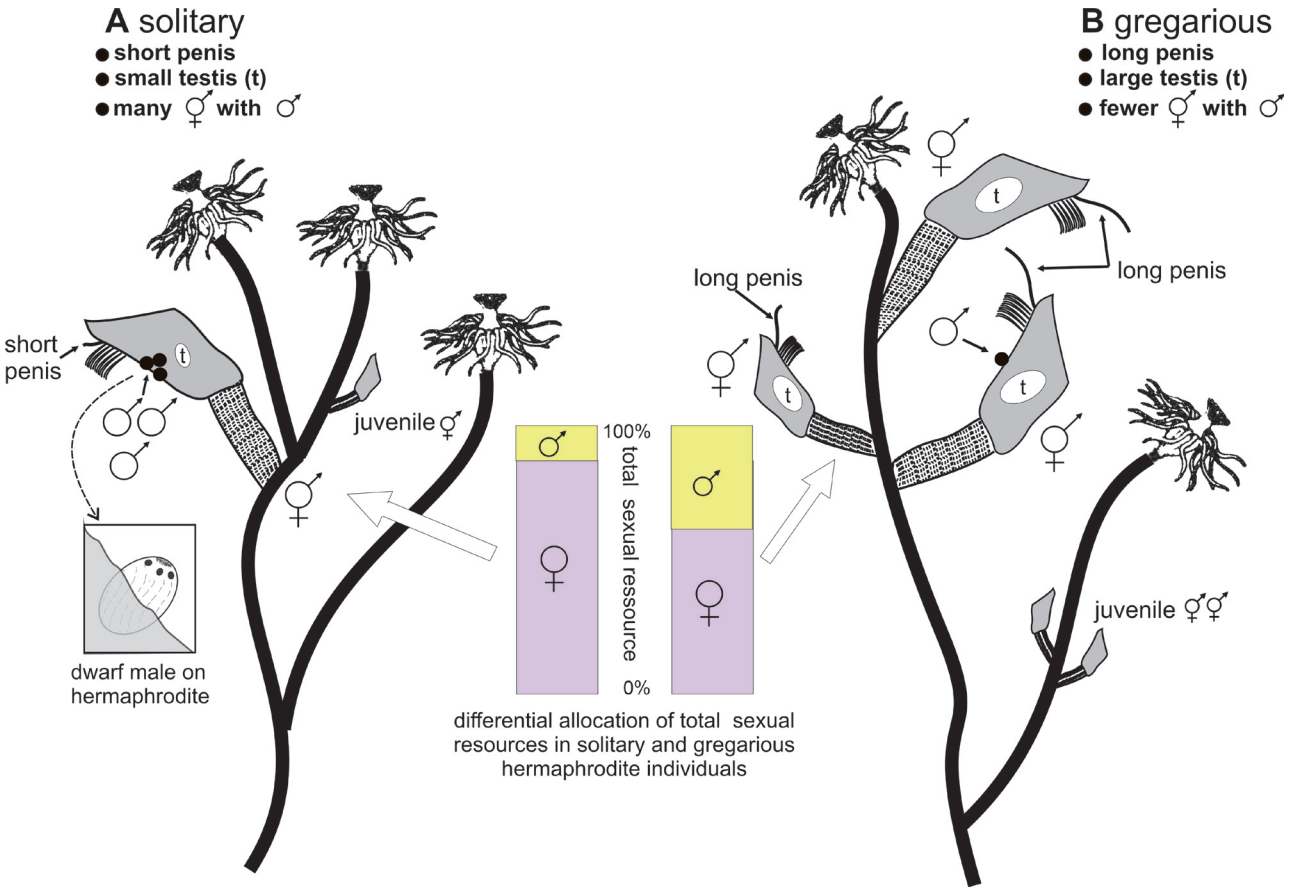

Fig. 12. Allocation of sexual resources in *Scalpellum scalpellum*. A, Few sexual resources are allocated to male function in solitary hermaphrodites; they have a short penis and a small testis (t), but are more likely to carry dwarf males than gregarious ones. B, Gregarious hermaphrodites allocate more resources to male function; they have a longer penis and a larger testis but are less likely to carry males. In (A) the solitary hermaphrodite carries three dwarf males. In (B) all three hermaphrodites can copulate with each other (Mating Group Size = 3, see text); one of them also carries a single dwarf male. The juvenile hermaphrodites do not count into the mating group size, but (A) will have MGS = 2 if the juvenile becomes sexually mature while the present adult hermaphrodite remains alive.

have a limited lifetime, so a long-lived partner almost certainly needs to constantly recruit males throughout its assumedly years-long lifetime (Yusa et al. 2018). In agreement, we have in receptacles of both *S. scalpellum* and other species seen carcasses of dead males together with healthy ones and recently settled cyprids (Dreyer et al. 2018c).

Allocation of sexual resources

MGS is the number of partners with which an individual can potentially reproduce (+ itself), and MGS is considered to be a principal factor in deciding which sexual system will be an evolutionarily stable state (Charnov 1982 1987; Yamaguchi et al. 2008 2012 2013c; Yusa 2019; Yusa et al. 2013; Weeks 2012; Appendix 1). Most barnacles live in small mating groups where hermaphroditism is favoured, because the length of the penis limits the number of possible mating partners (Fig. 12). This puts a premium on all individuals being able to act as both males and females. In a population with a very low mean MGS, many individuals exist in solitary without a potential partner with which to mate. This will favor dioecy, in the special form where large females carry one or several dwarf males. Most members of the Scalpellidae inhabit the deep sea, live in very scattered populations and they have this form of dioecy (Buhl-Mortensen and Høeg 2006 2013). In a narrow window around and below MGS = 2, androdioecy is predicted to be a stable sexual system. This narrow window of ecological conditions probably explains why androdioecy is rare among animals, with only somewhat more than 120 species, many of which are barnacles (Charnov 1982 1987; Weeks 2012; Weeks et al. 2000 2006; Yamaguchi et al. 2012 2013a b; Dreyer et al. 2018a). In species with androdioecy, such as *S. scalpellum*, there will usually be a variation in the MGS value experienced by individual hermaphrodites. Some may sit in solitary while other individuals sit gregariously, having one or more potential mating partners (MGS ≥ 2). In such a population, theory predicts that solitary individuals should downgrade resources to the male function, which they cannot use. In turn, they should upgrade resources to female function. It is also predicted that the number of hermaphrodites carrying any number of males exceed that among gregarious ones and that the total number of males on solitary ones exceed that on the gregarious ones. For *S. scalpellum*, we were able to confirm three of our four predictions from the theory of sex allocation (Dreyer et al. 2018a). Solitary hermaphrodites had (1) smaller, less mature testes and (2) a relatively shorter penis. Recently, the same was shown by Wijayanti et al. (2024) for the androdioecious poecilasmatid *Octolasmis unguisiformis*. We could also confirm (3) that solitary hermaphrodites carry more males than gregarious ones. The reason for the prediction is that males on gregarious individuals will face serious competition from the male function of the much larger, nearby hermaphrodite partner(s). Theory also predicts that the total number of males in the solitary hermaphrodites should exceed that in gregarious ones and also this was recently verified for *Octolasmis unguisiformis* (Wijayanti et al. 2024). Our failure to support this latter prediction for *S. scalpellum* could well be due to insufficient numbers of specimens in our dataset.

CONCLUSIONS

In some cirripedes, such as the Poecilasmatidae, the dwarf males seem to be little more than hermaphrodites arrested in development and they use a similar type of penis for copulation. Being able to feed, poecilasmatids males can also serve in fertilizing several successive broods of their partner hermaphrodite. In contrast, the males in *S. scalpellum* and probably all other scalpellids are structurally highly reduced, without any means of feeding or receive nutrient from their partner. Presumably, they can therefore only survive on their partner for a short time, and it is possible that they can only reproduce once, whence the partner animal must continuously acquire new males. Scalpellid males are also specialized in possessing a copulatory organ very unlike that found in the hermaphrodites. This testifies to a long evolutionary history for scalpellid males. Within cirripeds, the evolutionary pinnacle is found in the Rhizocephala. Unlike scalpellids, their dwarf males are not separated by cuticles as in scalpellids, but lie in direct cellular contact with their partner female, from which they therefore can receive nutrients so the partnership can persist for the life time of the parasite. It is intriguing to speculate that the rhizocephalans may ancestrally have passed through stages comparable to extant poecilasmatids and scalpellids.

In *S. scalpellum*, development into males is by an ESD mechanism, triggered by an unknown factor present only in the receptacles of adult hermaphrodites. Settlement on any other substratum resulted in hermaphrodites. However, Svane (1986) suggested that while all cyprids can settle as hermaphrodites, only up to 50% have the capability to settle as males. If so, a GSD factor may also be present in the sex determination system of *S. scalpellum*, but this is in need of further investigation. Our analyses provided evidence that allocation of sexual resources in *S. scalpellum* hermaphrodites is in accordance with

theoretical predictions. The same was in part true for the numbers of dwarf males present on the hermaphrodites. Structurally, *S. scalpellum* larvae showed no sexual dimorphism, but hermaphrodites and dwarf males begin to diverge in morphology already within a couple of days after cypris settlement and mature males have no resemblance to any stage in hermaphrodite ontogeny.

We conclude that our study species, *S. scalpellum*, has proven to be exceptionally suited to study aspects of reproductive biology. Future research could focus on long-term maintenance in the laboratory, following specimens from settlement into the adult phase. This would allow even more detailed studies on ontogenetic and reproductive biological parameters.

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REFERENCES

Aldred N, Clare AS. 2009. Mechanisms and principles underlying temporary adhesion, surface exploration and settlement site selection by barnacle cyprids: A short review. *In*: Gorb SN (ed) Functional Surfaces in Biology: Adhesion Related Phenomena, Vol. 2, Springer, pp. 43–65.

Anderson DT. 1994. Barnacles: structure, function, development and evolution. Chapman & Hall, London Glasgow New York Tokyo Melbourne Madras.

Barazandeh M, Davis CS, Neufeld CJ, Coltman DW, Palmer AR. 2013. Something Darwin didn't know about barnacles: spermcast mating in a common stalked species. Proc R Soc B: Biol Sci **280:**20122919. doi:10.1098/rspb.2012.2919.

Benvenuto C, Weeks S. 2020. Hermaphroditism and gonochorism. *In*: Cothran R, Thiel M (ed) The natural history of the Crustacea, Vol. 6: Reproductive biology, Oxford University Press, pp. 197–241.

Beukeboom LW, Perrin N. 2014. The evolution of sex determination. Oxford University Press.

Buhl-Mortensen L, Høeg JT. 2006. Reproduction and larval development in three scalpellid barnacles (*Scalpellum scalpellum* (Linnaeus 1767), *Ornatoscalpellum stroemii* (M. Sars 1859) and *Arcoscalpellum michelottianum* (Sequenza 1876), Crustacea: Cirripedia: Thoracica): implications for reproduction and dispersal in the deep sea. Mar Biol **149:**829–844. doi:10.1007/s00227-006-0263-y.

Buhl-Mortensen L, Høeg JT. 2013. Reproductive strategy of two deep sea scalpellid barnacles (Crustacea: Cirripedia: Thoracica) associated with decapods and pycnogonids and first description of a penis in the dwarf males. Org Divers Evol **252:**522–535. doi:10.1007/s13127-013-0137-3.

Chan BKK, Høeg JT. 2015. Diversity of lifestyles, sexual systems, and larval development patterns in sessile crustaceans. *In*: Thiel M, Watling L (eds) Natural History of Crustacea Vol. 2, Lifestyles and Feeding Biology. Oxford University Press, pp. 14–34.

Chan BKK, Dreyer N, Gale AS, Glenner H, Ewers-Saucedo C et al. 2021a. The evolutionary diversity of barnacles, with an updated classification of fossil and living forms. Zool J Linn Soc Lond **193:**789–846. doi:10.1093/zoolinnean/zlaa160.

Chan BKK, Wong Y-Him, Robinson N, Lin Chi, Yu S-P et al. 2021b. 500 million years to mobility: Directed locomotion and its ecological function in a turtle barnacle. Proc R Soc B: **1960:**20211620. doi:10.1098/rspb.2021.1620.

Charnov EL. 1982. The theory of sex allocation. Princeton University Press.

Charnov EL. 1987. Sexuality and hermaphroditism in barnacles: a natural selection approach. *In*: Southward AJ (ed) Barnacle biology. Crustacean issues 5, A.A. Belkema, Rotterdam, pp. 89–103.

Clare AS. 2010. Toward a characterization of the chemical cue to barnacle gregariousness. *In*: Breithaupt T, Thiel M (eds) Chemical communication in crustaceans, Springer, New York. pp. doi:10.1007/978-0-387-77101-4_22.

Crisp DJ. 1983. *Chelonobia patula* (Ranzani), a pointer to the evolution of the complemental male. Mar Biol Lett **4:**281–294.

Darwin C. 1851. Living Cirripedia, A monograph of the sub-class Cirripedia, with figures of all the species. The lepadidæ; or, the pedunculated cirripedes. London, the Ray Society.

Darwin C. 1873. On the males and complemental males of certain cirripedes, and on rudimentary structures. Nature **8:**431–432. doi:10.1038/008431B0.

Dreyer N, Sørensen S, Yusa Y, Sawada K, Svennevig N et al. 2018a. Sex allocation and maintenance of androdioecy in the pedunculated barnacle *Scalpellum scalpellum* (Crustacea: Cirripedia: Thoracica). Biol J Linn Soc Lond **124:**776–788. doi:10.1093/biolinnean/bly081.

Dreyer D, Høeg JT, Hess M, Sørensen S, Spremberg U et al. 2018b. When dwarf males and hermaphrodites copulate: First record of mating behaviour in a dwarf male using the androdioecious barnacle *Scalpellum scalpellum* (Crustacea: Cirripedia: Thoracica). Org Divers Evol **18:**115–123. doi:10.1007/s13127-017-0349-z.

Dreyer N, Yusa Y, Gale A, Melzer RR, Yamato S et al. 2018c. In the

footsteps of Darwin: dwarf male attachment sites in scalpellid barnacles (Crustacea: Cirripedia: Thoracica) – implications for phylogeny and the evolution of sexual systems. Zool J Linn Soc Lond **184:**999–1023. doi:10.1093/zoolinnean/zly018.

Dreyer N, Olesen J, Dahl RB, Chan BKK, Høeg JT. 2018d. Sex-specific metamorphosis of cypris larvae in the androdioecious barnacle *Scalpellum scalpellum* (Crustacea: Cirripedia: Thoracica) and its implication for the adaptive evolution of dwarf males. PLoS ONE **13:**e0191963. doi:10.1371/journal.pone.0191963.

Dunn AM, Rigaud T, Ford AT. 2020. Environmental influences on crustacean sex determination and reproduction: environmental sex determination, parasitism and pollution. *In*: Cothran RD, Thiel M (eds) Reproductive Biology. New York: Oxford University Press, pp. 394–428.

Gale AS. 2016. Phylogeny of the deep-sea cirripede family Scalpellidae (Crustacea, Thoracica) based on shell capitular plate morphology. Zool J Linn Soc Lond **176:**266–304. doi:10.1111/zoj.12321.

Glenner H, Høeg JT, Klysner A, Brodin Larsen B. 1989. Cypris ultrastructure, metamorphosis and sex in seven families of parasitic barnacles (Crustacea: Cirripedia: Rhizocephala). Acta Zool (Stockholm) **70:**229–242. doi:10.1111/j.1463-6395.1989.tb00936.x.

Gomez ED. 1975. Sex determination in *Balanus* (*Conopea*) *galeatus* (L.) (Cirripedia Thoracica). Crustaceana **28:**105–107. Available at: https://www.jstor.org/stable/20102181.

Hashizoe N, Høeg JT, Kano Y, Yusa Y. 2026. Comparison of the reproductive characters in of six species of scalpellid barnacles. Zool Stud **65:**09. doi:10.6620/ZS.2026.65-09.

Høeg JT. 1984. Size and settling behaviour in male and female cypris larvae of theparasitic barnacle *Sacculina carcini* Thompson (Crustacea: Cirripedia: Rhizocephala). J Exp Mar Biol Ecol **76:**145–156. doi:10.1016/0022-0981(84)90062-5.

Høeg JT. 1987. The relation between cypris ultrastructure and metamorphosis in male and female *Sacculina carcini* (Crustacea, Cirripedia). Zoomorphology **107:**299–311. doi:10.1007/BF00312176.

Høeg JT. 1995. The biology and lifecycle of the Rhizocephala (Cirripedia). J Mar Biol Assoc UK **75:**517–555. doi:10.1017/S0025315400038996.

Høeg JT, Deutsch J, Chan BKK, Semmler Le H. 2015. "CRUSTACEA": CIRRIPEDIA. *In*: Wanninger A (ed) Evolutionary Developmental Biology of Invertebrates Vol. 4. Springer, Wien, New York, pp. 153–181.

Høeg JT, Lützen J. 1995. Life cycle and reproduction in the Cirripedia Rhizocephala. Oceanogr Mar Biol Annu Rev **33:**427–485. doi: doi:10.1017/S0025315400038996.

Høeg JT, Maruzzo D, Okano K, Glenner H, Chan BKK. 2012. Metamorphosis in balanomorphan, pedunculated, and parasitic barnacles: a video-based analysis. Integr Comp Biol **52:**337–347. doi:10.1093/icb/ics053.

Høeg JT, Møller OS. 2006. When similar beginnings lead to different ends: Constraints and diversity in cirripede larval development. Invertebr Reprod Dev **49:**125–142. doi:10.1080/07924259.2006.9652204.

Høeg JT. Yusa Y, Dreyer N. 2016. Sex determination in the androdioecious barnacle *Scalpellum scalpellum* (Crustacea Cirripedia). Biol J Linn Soc Lond Lond **118:**359–368. doi:10.1111/bij.12735.

Hui E, Moyse J. 1984. Complemental male in the primitive balanomorph arnacle, *Chionelasmus Darwini*. J Mar Biol Assoc UK **64:**91–97. doi:10.1017/S0025315400059658.

Kajimoto A, Yanagimachi R, Takahashi T, Yusa Y. 2024. Sex determination by a univalent chromosome in the rhizocephalan *Peltogasterella gracilis* (Cirripedia: Rhizocephala: Peltogasterellidae). Biol Bull **246:**98–107. doi:10.1086/734711.

Kaufmann R. 1965. Zur embryonal und Larvenentwicklung von *Scalpellum scalpellum* L. (Crust. Cirr.) mit einem Beitrag zur Autökologie dieser Art. Zoomorphology **55:**161–232. doi:10.1007/BF00399512.

Kelly MW, Sanford E. 2010. The evolution of mating systems in barnacles. J Exp Mar Biol Ecol **392:**37–45. doi:10.1016/j.jembe.2010.04.009.

Klepal W. 1985. *Ibla cumingi* (Crustacea, Cirripedia)—a gonochoristic species (anatomy, dwarfing and systematic implications). Mar Ecol **6:**47–119. doi:10.1111/j.1439-0485.1985.tb00320.x.

Klepal W. 1987. A review of the comparative anatomy of the males in cirripedes. Oceanogr Mar Biol Annu Rev **25:**285–351.

Kobayashi M, Yusa Y, Sekino M. 2020. Microsatellite DNA markers applicable to paternity inference in the androdioecious gooseneck barnacle *Octolasmis warwickii* (Lepadiformes: Poecilasmatidae). Molec Biol Rep **47:**4885–4890. doi:10.21203/rs.3.rs-7024057/v1.

Kolbasov GA. 2009. Acrothoracica, burrowing Crustaceans. KMK Scientific Press, Moscow.

KnightJones EW. 1953. Laboratory experiments on gregariousness during settling in *Balanus balanoides* and other barnacles. J Exp Biol **30:**584–598. doi:10.1242/jeb.01628.

Krüger P. 1920. Studien an Cirripedien. Zeitschr f indukt Abstamm u Vererbungslehre **24:**105–156. doi:10.1007/BF01845834.

López-Greco LS. 2013. Functional anatomy of the reproductive system. *In*: Watling L, Thiel M (eds) Functional Morphology and Diversity: the Natural History of the Crustacea, Volume 1, Oxford University Press, New York, pp. 413–450.

Lin HC, Høeg JT, Yusa Y, Chan BKK. 2015. The origins and evolution of dwarf males and habitat use in thoracican barnacles. Mol Phylogenet Evol **91:**1–11. doi:10.1016/j.ympev.2015.04.026.

Martin JW, Olesen J, Høeg JT. 2014. Atlas of Crustacean Larvae. Johns Hopkins University Press, Baltimore. doi:10.1353/book.31448.

Nielsen SK, Høeg JT, Yusa Y. 2016. Host relation, size and reproduction in the burrowing barnacle *Trypetesa lampas* (Hancock) (Crustacea Cirripedia Acrothoracica). Zool Stud **55:**14. doi:10.6620/zs.2016.55-14.

Neufeld CJ, Palmer AR. 2008. Precisely proportioned: intertidal barnacles alter penis form to suit coastal wave action. Proc R Soc B Biol Sci **275:**1081–1087. doi:10.1098/rspb.2007.1760.

Neuhaus J. 2025. On dwarf males in the deep-sea acorn barnacle *Bathylasma hirsutum* (Thoracica: Bathylasmatidae). Mar Biodivers **55:**24. doi:10.1007/s12526-025-01509-0.

Pannell JR. 2002. The evolution and maintenance of androdioecy. Ann Rev Ecol Syst **33:**397–425. doi:10.1146/annurev.ecolsys.33.010802.150419.

Ritchie LE, Høeg JT. 1981. The life history of *Lernaeodiscus porcellanae* (Cirripedia Rhizocephala)and coevolution with its porcellanid host. J Crustacean Biol **1:**334–347. doi:10.2307/1547966.

Sawada K, Yoshida R, Yasuda K, Yamaguchi S, Yusa Y. 2015. Dwarf males in the epizoic barnacle *Octolasmis unguisiformis* and their implications for sexual system evolution. Invertebr Biol **134:**162–167. doi:10.1111/ivb.12083.

Spremberg U, Buhl-Mortensen L, Yusa Y, Høeg JT. 2012. Cypris settlement and dwarf male formation in the barnacle *Scalpellum scalpellum*: a model for an androdioecious reproductive system. J Exp Mar Biol Ecol **422-423:**39–47. doi:10.1016/j.jembe.2012.04.004.

Svane I. 1986. Sex determination in *Scalpellum scalpellum* (Cirripedia Thoracica Lepadomorpha), a hermaphroditic goose barnacle with dwarf males. Mar Biol **90:**249–253. doi:10.1007/BF00569135.

Tamechika MM, Matsuno K, Wada S, Yusa Y. 2020. Different effects of mating group size as male and as female on sex allocation in a simultaneous hermaphrodite. Ecol Evol **10:**2492–2498. doi:10.1002/ece3.6075.

Tamechika MM, Yusa Y, Akita S, Sekino M. 2025. Microsatellite DNA markers for paternity identification in the hermaphroditic barnacle *Chthamalus challengeri* Hoek. Molec Biol Rep **52:**853. doi:10.1007/s11033-025-10957-7.

Turquier Y. 1972. Contribution à la connaisance des Cirripèdes Acrothoraciques. Archs Zool exp gén **113:**499–551.

Urano S, Yamaguchi S, Yamato S, Takahashi S, Yusa Y. 2009. Evolution of dwarf males and a variety of sexual modes in barnacles: an ESS approach. Evol Ecol Res **11:**713–729.

Vogt G. 2016. Structural specialties, curiosities, and record-breaking features of crustacean reproduction. J Morphol **277:**1399–1422. doi:10.1002/jmor.20582.

Vogt G. 2020. An overview of sexual systems. *In*: Cothran R, Thiel M (eds) The natural history of the Crustacea, Vol. 6, Reproductive biology, Oxford University Press, New York, pp. 145–176.

Wahl M. 2025. From validation of an all-female biotechnology in aquaculture to insights into ovarian development regulation and the link between sex determination and differentiation in a decapod crustacean. Phd Thesis, Ben Gurion University of the Negev, Beer-Sheva, Israel.

Wahl M, Borojovich Y, Levy T, Asculai N, Manor R et al. 2025. Identifying sex-chromosome-linked determinant of sexual differemtiation in a ZW sex inheritance crustacean. Biol Reprod **113:**838–855. doi:10.1093/biolre/ioaf138.

Walker G. 1985. The cypris larvae of *Sacculina carcini* Thompson (Crustacea: Cirripedia: Rhizocephala). J Exp Mar Biol Ecol **93:**131–145. doi:10.1016/0022-0981(85)90154-6.

Walker G. 1992. Cirripedia. *In*: Harrison FW, Humes AG (eds) Microscopic anatomy of invertebrates, Vol. 9. Crustacea, WileyLiss Inc., New York, pp. 249–311.

Walley LJ. 1969. Studies on the larval structure and metamorphosis of *Balanus balanoides* (L.). Phil Trans R Soc Lond **1969:**237–280. doi:10.1098/rstb.1969.0042.

Weeks SC. 2012. The role of androdioecy and gynodioecy in mediating evolutionary transitions between dioecy and hermaphroditism in the Animalia. Evolution **66:**3670–3686. doi:10.1111/j.1558-5646.2012.01714.x.

Weeks SC, Crosser BR, Bennett R, Gray M, Zucker N. 2000. Maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana*: Estimates of inbreeding depression in two populations. Evolution **54:**878–887. doi:10.1111/j.0014-3820.2000.tb00088.x.

Weeks SC, Benvenuto C, Reed SK. 2006. When males and hermaphrodites coexist: a review of androdioecy in animals. Integr Comp Biol **46:**449–464. doi:10.1093/icb/icj048.

West SA. 2009. Sex allocation, Princeton University Press, Princeton NJ.

Wijayanti H, Sawada K, Yasuda K, Yusa Y. 2024. Labile sex allocation and sex ratio in the androdioecious barnacle *Octolasmis unguisiformis*. Biol J Linn Soc Lond **143:**blae083. doi:10.1093/biolinnean/blae083.

Yamaguchi S, Charnov EL, Sawada K, Yusa Y. 2012. Sexual systems and life history of barnacles: a theoretical perspective. Integr Comp Biol **52:**356–365. doi:10.1093/icb/ics046.

Yamaguchi S, Ozaki Y, Yusa Y, Takahashi S. 2007. Do tiny males grow up? Sperm competition and optimal resource allocation schedule of dwarf males of barnacles. J Theor Biol **245:**319–328. doi:10.1016/j.jtbi.2006.10.009.

Yamaguchi S, Sawada K, Yusa Y, Iwasa Y. 2013a. Dwarf males, large hermaphrodites and females in marine species: a dynamic optimization model of sex allocation and growth. Theor Popul Biol **85:**49–57.

Yamaguchi S, Sawada K, Yusa Y, Iwasa Y. 2013b. Dwarf males and hermaphrodites can coexist in marine sedentary species if the opportunity to become a dwarf male is limited. J Theor Biol **334:**101–108. doi:10.1016/j.jtbi.2013.05.027.

Yamaguchi S, Yusa Y, Sawada K, Takahashi S. 2013c. Sexual systems and dwarf males in barnacles: integrating life history and sex allocation theories. J Theor Biol **320:**1–9. doi:10.1016/j.jtbi.2012.12.001.

Yamaguchi S, Yusa Y, Yamato S, Urano S, Takahashi S. 2008. Mating group size and evolutionarily stable pattern of sexuality in barnacles. J Theor Biol **253:**61–73. doi:10.1016/j.jtbi.2008.01.025.

Yamaguchi S, Yoshida S, Kaneko A, Sawada K, Yasuda et al. 2014. Sexual system of a symbiotic pedunculate barnacle *Poecilasma kaempferi* (Cirripedia: Thoracica). Mar Biol Res **10:**635–640. doi:10.1080/17451000.2013.841943.

Yanagimachi R. 1961. Studies on the sexual organization of the Rhizocephala. III. The mode of sex-determination in Peltogasterella. Biol Bull **120:**272–283.

Yusa Y. 2019. Hermaphrodites, Dwarf Males, and Females: Evolutionary Transitions of Sexual Systems in Barnacles. *In*: Leonard J (eds) Transitions Between Sexual Systems, Springer, Cham. doi:10.1007/978-3-319-94139-4_8.

Yusa Y, Takemura M, Miyazaki K, Watanabe T, Yamato S. 2010. Dwarf males of *Octolasmis warwickii* (Cirripedia: Thoracica): the first example of coexistence of males and hermaphrodites in the suborder Lepadomorpha. Biol Bull **218:**259–265. doi:10.1086/BBLv218n3p259.

Yusa Y, Takemura M, Sawada K, Yamaguchi S. 2013. Diverse, continuous, and plastic sexual systems in barnacles. Integr Comp Bio **53:**701–712. doi:10.1093/icb/ict016.

Yusa Y, Yoshikawa Y, Kitaura J, Kawane M, Ozaki Y et al. 2012. Adaptive evolution of sexual systems in pedunculate barnacles. Proc R Soc Lond B **279:**959–966. doi:10.1098/rspb.2011.1554.

Yusa Y, Yasuda N, Yamamoto T, Watanabe H, Higashiji T et al. 2018. Direct growth measurements of two deep-sea scalpellid barnacles, *Scalpellum stearnsii* and *Graviscalpellum pedunculatum*. Zool Stud **57:**26. doi:10.6620/ZS.2018.57-29.

Supplementary materials

Appendix. Mating group size and sexual system. (download)