DWARF MALES IN THE BARNACLE *ALEPAS PACIFICA* PILSBRY, 1907 (THORACICA, LEPADIDAE), A SYMBIONT OF JELLYFISH

BY

Y. YUSA[1,3], S. YAMATO[2], M. KAWAMURA[2] AND S. KUBOTA[2]

[1] Nara Women's University, Kitauoya-nishi, Nara 630-8506, Japan
[2] Seto Marine Biological Laboratory, Kyoto University, Shirahama, Wakayama 630-2211, Japan

[3] Corresponding author; e-mail: yusa@cc.nara-wu.ac.jp

Running head: Y. YUSA ET AL.
DWARF MALES IN *ALEPAS*

ABSTRACT

In the pedunculate barnacle *Alepas pacifica* Pilsbry, 1907 a symbiont of jellyfish, several small individuals were found attached to conspecifics rather than directly to the host. We investigated whether these individuals act as dwarf males, as is known in some other barnacle species. The conspecific-attached individuals had longer penes than juvenile hermaphrodites of similar sizes attached directly to jellyfish, although there was no other morphological difference between these two types of individuals. Only the largest conspecific-attached individual was ovigerous. We conclude that the conspecific-attached individuals are dwarf males, which develop male function at a smaller size than hermaphrodites do, with a small possibility of becoming hermaphroditic. This is the first report of dwarf males, and hence the coexistence of males and hermaphrodites (androdioecy), in the family Lepadidae. In addition, the record of *A. pacifica* attached to *Nemopilema nomurai* Kishinouye, 1922 is new to science.

INTRODUCTION

Barnacles have an exceptionally diverse array of sexual systems and therefore can be used as model systems for the evolution of sexual systems in animals (Darwin, 1852; Charnov, 1987; Høeg, 1995; Yusa et al., 2012). Most barnacles are self-infertile simultaneous hermaphrodites, for example, the intertidal acorn barnacle *Semibalanus balanoides* (Linnaeus, 1767) (cf. Yuen & Hoch, 2010) and the neustonic pedunculate *Lepas anserifera* Linnaeus, 1767 (cf. Inatsuchi et al., 2010). However, in many deep-sea or symbiotic species, pure males coexist with hermaphrodites (called androdioecy) as is known in *Scalpellum scalpellum* (Linnaeus, 1767) (cf. Buhl-Mortensen & Høeg, 2006) and *Koleolepas avis* (Hiro, 1931) (cf. Yusa et al., 2001); males may coexist with females (dioecy) as in *Scalpellum stearnsii* Pilsbry, 1890 (cf. Ozaki et al., 2008) and *Verum brachiumcancri* (Weltner, 1922) (cf. Buhl-Mortensen & Høeg, 2013). Male barnacles are attached on conspecific hermaphrodites or females and are always much smaller than the conspecifics (Darwin, 1852). In this paper, the males that are less than half the length of conspecific hermaphrodites or females are defined as dwarf males, even if a small proportion of the males may later become hermaphrodites, following the preceding literature (Crisp, 1983; Buhl-Mortensen & Høeg, 2006; Yusa et al., 2010, 2012; Spremberg et al., 2012).

Barnacles are sessile organisms, and mating occurs primarily within reach of their penes. Under such conditions, the sex allocation theory predicts that mating group size is the major cause of sexual system variation (Charnov, 1982, 1987; Yamaguchi et al., 2008, 2012). In a confined but relatively large group, as is often observed in intertidal or neustonic habitats, the mating success of males is limited by the number of eggs produced within the group. Therefore, male fitness does not increase linearly with the resource input, and individuals should allocate some resources to egg production as well as male function to maximise fitness; thus, they should become hermaphrodites. When deep-sea or symbiotic habitats limit the size of mating groups, sperm competition among hermaphrodites is less intense, and dwarf males with limited resources can be competitive with male-acting hermaphrodites due to the advantages of having better access to eggs for fertilisation and higher rates of survival to maturity; thus, androdioecy evolves. In further smaller groups, large individuals may give up male function to become pure females because they have almost no mates to inseminate; thus, dioecy evolves. Limited space for attachment and short longevity of the substratum further facilitate the evolution of dwarf males due to their small size and short time to maturity, respectively (Yamaguchi et al., 2013a, b). These theoretical predictions are fairly well supported empirically (Kelly & Sanford, 2010; Yusa et al., 2012). Although at least three species of barnacles broadcast sperm (Barazandeh et al., 2013, 2014), this factor does not appear to affect the general trend of sexual system variation according to mating group size.

Recently, sexual expression of barnacles is suggested to be more plastic than previously considered (Yusa et al., 2013). For example, in the acorn barnacle *Chelonibia testudinaria* (Linnaeus, 1758), small individuals who are attached to conspecific hermaphrodites develop a penis and act as dwarf males, although some of them may later become hermaphrodites (Crisp, 1983; Zardus et al., 2014). Similar examples have been reported in the pedunculate barnacles *Octolasmis warwickii* Gray, 1825 (cf. Yusa et al., 2010) and *O. unguisiformis* Kobayashi & Kato, 2003 (cf. Sawada et al., in press).

Irrespective of the recent findings of dwarf males in otherwise hermaphroditic barnacles (Yusa et al., 2010, 2012), no dwarf males have been reported in the family Lepadidae. This may chiefly be due to their tendency to form large groups on floating objects (wood, sea algae, plastics, etc.; Thiel & Gutow, 2005). However, *Alepas pacifica* (Lepadidae) is symbiotic with jellyfish, and the number of individuals on the same host is relatively small (normally 1 - 3; although sometimes 'numerous' or 'more than 100' per host were recorded in the literature; Pagès, 2000).

We found that small individuals were attached to the capitulum and peduncle of conspecifics in *A. pacifica* (fig. 1). Although most dwarf males are attached to the capitulum in other species, Sawada et al. (in press) found that individuals attached to the peduncle also act as dwarf males in *Octolasmis unguisiformis*. Therefore, we hypothesised that these small individuals of *A. pacifica* were dwarf males. However, simply being attached to conspecifics is not a prerequisite for dwarf males, as similar conspecific-attached individuals are not dwarf males but juvenile hermaphrodites in the pedunculate *Poecilasma kaempferi* Darwin, 1852 (cf. Yamaguchi et al., 2014). The purpose of this study was to investigate whether these conspecific-attached individuals in *A. pacifica* are dwarf males or juvenile hermaphrodites by inspecting their male and female sexual status.

MATERIAL AND METHODS

Fourteen individuals of jellyfish with *Alepas pacifica* were collected aboard or on shore along the western coast of Japan between 2005 and 2009 (table I). All barnacles were fixed in 5 – 10% seawater formalin until further analyses. The exact size of the host is unknown in most cases because only part of the host was cut and preserved due to the large size of the host jellyfish (up to 2 m in diameter in the case of *Nemopilema nomurai* Kishinouye, 1922; cf. Uye, 2008).

The barnacles were detached from the host jellyfish, and the total length, capitulum length, and capitulum width were measured for all individuals using a Vernier calliper to the nearest 0.1 mm. Next, each individual was dissected, and the presence of an egg mass in the mantle cavity was checked under a binocular microscope; the penis length was measured to the nearest 0.025 mm using a micrometre. There were high correlations between total length and capitulum length (N = 118, $r = 0.99$, $P < 0.001$, both $\log_{10}$-transformed after adding 0.5) and between capitulum length and width ($r = 0.98$, $P < 0.001$). The capitulum length was used as a measure of body size because it is the most accurate measurement.

Parametric tests, including Pearson's correlation, analysis of covariance (ANCOVA) and logistic regression, were primarily used as statistical tests. Before conducting ANCOVA, the lack of the significant interaction between the explanatory variable (attachment site) and the covariate (capitulum length) was checked. All data were $\log_{10}$-transformed after adding 0.5, but when the assumption of normality was violated even after transformation, non-parametric tests (Kendall's τ or Mann-Whitney's U) were used.

RESULTS

A total of 91 individuals of *Alepas pacifica* were found attached on 14 host jellyfish (11 *Nemopilema nomurai* and three *Cyanea nozakii* Kishinouye, 1891; Table I). The record of *A. pacifica* attached to *N. nomurai* is new to science. The number of individuals per host varied greatly, from 1 to 38 (mean ± SD = 6.5 ± 10.5). Even when there were two or more individuals on the same host, they were distantly distributed in most cases (fig. 1A). In addition, 62 individuals (27 individuals after metamorphosis and 35 individuals still inside the cyprid shell) were found attached to conspecifics. The capitulum lengths of conspecific-attached individuals (mean ± SD = 1.59 ± 0.95 mm, N = 27) were much smaller than those directly attached to the host (9.02 ± 6.65 mm, N = 91).

Among the 118 individuals excluding the cyprids (91 jellyfish-attached and 27 conspecific-attached individuals), 10 (five from each attachment site) were broken or empty and apparently died before collection; these individuals were excluded from further analyses. Among the 108 intact individuals, the relationship between capitulum length and penis length was studied for the individuals smaller than 6 mm in capitulum length (N = 49) to match the size between jellyfish-attached and conspecific-attached individuals (fig. 2). There was a positive relationship between capitulum length and penis length (F = 204.20, P < 0.001, ANCOVA). Notably, the conspecific-attached individuals had larger penes than did the jellyfish-attached individuals (F = 5.29, P = 0.026, ANCOVA), although the largest ovigerous conspecific-attached individual had a penis length similar to that of those attached to jellyfish (fig. 2). Otherwise, there was no difference in morphology between the individuals with different attachment sites.

Seven out of the 108 individuals (6.5%) had egg masses in the mantle cavity. The smallest ovigerous individual directly attached to the jellyfish had a capitulum length of 9.1 mm. In addition, the largest conspecific-attached individual (5.9 mm capitulum length) had an egg mass. There was a positive relationship between capitulum length and the presence of egg masses (N = 108, likelihood χ^2 = 10.51, P < 0.01, multiple logistic regressions), but this relationship was not different between the attachment sites (likelihood χ^2 = 3.00, P = 0.08).

There was a positive relationship between the capitulum length of jellyfish-attached individuals and the number of conspecific-attached individuals (including cyprids) (N = 91, τ = 0.39, P < 0.001, Kendall's rank correlation). The ovigerous hermaphrodites also had more conspecific-attached individuals than non-ovigerous hermaphrodites (U = 69, P < 0.001, Mann-Whitney's U test).

DISCUSSION

Individuals of *Alepas pacifica* attached to the conspecifics were on average much smaller than individuals attached to the jellyfish. However, the conspecific-attached individuals had relatively longer penes for their body sizes than those directly attached to the host jellyfish. These results coincide with results in *Chelonibia testudinaria* (Crisp, 1983) and *Octolasmis warwickii* (Yusa et al., 2010), indicating that the conspecific-attached individuals act as dwarf males. This is the first report of dwarf males in the family Lepadidae.

There was a tendency that larger or ovigerous hermaphrodites had more dwarf males. A similar tendency has been reported in *Koleolepas avis* (Yusa et al., 2001) and *Octolasmis warwickii* (Yusa et al., 2010). Two general explanations are possible for these relationships. First, dwarf males may be attracted to larger and/or ovigerous hermaphrodites because a greater fertilisation success is expected (Yusa et al., 2010). In barnacles, large individuals tend to lay more eggs (Zann & Harker, 1978; Yusa et al., 2001, 2010; Ozaki et al., 2008), and currently ovigerous individuals are expected to lay eggs more likely than non-ovigerous ones in a near future simply because they are mature. Second, larger (or ovigerous) individuals tend to be old and may have accumulated dwarf males, even if there is no preference by dwarf males for larger individuals. However, the high proportion of dwarf males still inside the cyprid shell (56%; 35/62) suggests that most dwarf males were still very young, presumably just after settlement, rendering the second explanation (accumulation of dwarf males over time) unlikely. Thus, although the mechanism is unknown, the data suggest that dwarf males preferentially settle on larger/ovigerous hermaphrodites.

The occurrence of dwarf males in this species is congruent with the sex allocation theory in that dwarf males evolve when mating groups are small (Charnov 1982, 1987; Yamaguchi et al., 2012). In the family Lepadidae, species of neustonic *Lepas* generally form large groups (Inatsuchi et al., 2010; Yusa et al., 2012), and although smaller (consisting of 2 – 7 individuals), *Dosima fascicularis* (Ellis & Solander, 1786) that form their own floats also live in groups (Zheden et al. 2015). *Alepas pacifica* most likely form the smallest mating groups in the family: 67% of the individuals in Pagès (2000) lived solitarily on the host. In the present study, only 9% (8/91) of the individuals lived solitarily, but the great majority of individuals appeared to be too distant from each other to mate (fig. 1A), even if they lived on the same host individual (that can reach 2 m in diameter; Uye, 2008). This barnacle is known to feed on the host tissue including the gonads (Pagès, 2000), and it may be disadvantageous for the barnacles to live close to each other for feeding purposes. Thus, small group size due to low density per host and host feeding appear to be the factors for the evolution of dwarf males in this species. The short lifespan of the host jellyfish (less than 1 year; Uye, 2008) may also facilitate the evolution of dwarf males (Yamaguchi et al., 2013a). A similar condition in response to the parasitic or commensal life applies to several androdioecious barnacles (e.g., species of *Chelonibia*, *Octolasmis warwickii*, *O. unguisiformis*, and *Koleolepas avis*; Crisp, 1983; Yusa et al., 2001, 2010; Zardus et al., 2014; Sawada et al. in press).

Dwarf males in *A. pacifica* represent an example of 'conditional' dwarf males (Yusa et al., 2013) in that they have no substantial morphological differences from the hermaphrodites and a small proportion of them actually become hermaphroditic. Similar examples have been documented in *Chelonibia testudinaria* (Crisp, 1983; Zardus et al. 2014), *Octolasmis warwickii* (Yusa et al., 2010), and *O. unguisiformis* (Sawada et al., in press). Because simultaneous hermaphrodites in many barnacles tend to develop male function first (i.e., protandric simultaneous hermaphrodites; Inatsuchi et al., 2010), dwarf males in these species can be regarded as potential hermaphrodites that mature earlier as males and arrest growth in response to the attachment to conspecifics. These contrast with dwarf males in the scalpellids, such as *Scalpellum scalpellum*, where the males are morphologically distinct from hermaphrodites (Spremberg et al., 2012).

The presence of such 'conditional' dwarf males in otherwise hermaphroditic species renders the distinction between hermaphroditism and androdioecy unclear (Yusa et al., 2013). Nevertheless, the 'hermaphrodite' and 'dwarf male' routes are not interchangeable once individuals choose whether to settle on a usual subtratum or a conspecific. Because androdioecy is a highly rare phenomenon among animals (Weeks et al., 2006), further research is needed on various 'conditional' dwarf males, which represent an incipient condition of androdioecy.

ACKNOWLEDGEMENTS

We thank Dr. Shunshiro Ueno of National Fisheries University who provided much of the material and information on the jellyfish attached with barnacles.

REFERENCES

BARAZANDEH, M., C. S. DAVIS, C. J. NEUFELD1, D. W. COLTMAN & A. R. PALMER, 2013. Something Darwin didn't know about barnacles: spermcast mating in a common stalked species. Proceedings of the Royal Society B: Biological Sciences, **280**: 20122919.

------, C.S. DAVIS & A.R. PALMER, 2014. Where even a long penis can't help: Evidence of long-distance spermcast mating in two acorn barnacles. Journal of Experimental Marine Biology and Ecology, **454**: 49–54.

BUHL-MORTENSEN, L. & J.T. HØEG, 2006. Reproduction and larval development in three scalpellid barnacles, *Scalpellum scalpellum* (Linnaeus 1767), *Ornatoscalpellum stroemii* (M. Sars 1859) and *Arcoscalpellum michelottianum* (Seguenza 1876), (Crustacea: Cirripedia:Thoracica): implications for reproduction and dispersal in the deep sea. Marine Biology, **149**: 829–844.

------ & ------, 2013. 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. Organisms Diversity and Evolution, **13**: 545–557.

CHARNOV, E.L., 1982. The theory of sex allocation. (Princeton University Press, Princeton, NJ).

------, 1987. Sexuality and hermaphroditism in barnacles, a natural selection approach. In: A. J. SOUTHWARD (ed.), Barnacle biology: 89–104. (A. A. Balkema, Rotterdam, The Netherlands).

CRISP, D.J., 1983. *Chelonibia patula* (Ranzani), a pointer to the evolution of the complemental male. Marine Biology Letter, **4**: 281–294.

DARWIN, C., 1852. A monograph of the subclass cirripedia with figures of all species. The Lepadidae, or, pedunculated cirripedes. (The Ray Society, London).

HØEG, J.T., 1995. Sex and the single cirripede: a phylogenetic perspective. In: R.F. SCHRAM & J. T. HØEG (eds.), New frontiers in barnacle evolution: 195–207. (A. A. Balkema, Rotterdam, The Netherlands).

INATSUCHI, A., S. YAMATO & Y. YUSA, 2010. Effects of temperature and food availability on growth and reproduction in the neustonic pedunculate barnacle *Lepas anserifera*. Marine Biology, **157**: 899–905.

KELLY, M W. & E. SANFORD, 2010. The evolution of mating systems in barnacles. Journal of Experimental Marine Biology and Ecology, **392**: 37–45.

OZAKI, Y., Y. YUSA, S. YAMATO & T. IMAOKA, 2008. Reproductive ecology of the pedunculate barnacle *Scalpellum stearnsii* (Cirripedia, Lepadomorpha: Scalpellidae). Journal of the Marine Biological Association of the U.K., **88**: 77–83.

PAGÈS, F., 2000. Biological associations between barnacles and jellyfish with emphasis on the ectoparasitism of *Alepas pacifica* (Lepadomorpha) on *Diplulmaris malayensis* (Scyphozoa). Journal of Natural History, **34**: 2045–2056.

SAWADA, K., R. YOSHIDA, K. YASUDA, S. YAMAGUCHI & Y. YUSA, 2015. Dwarf males in an epizoic barnacle, *Octolasmis unguisiformis*, and their implication for sexual system evolution. Invertebrate Biology, in press.

SPREMBERG, U., J.T. HØEG, L. BUHL-MORTENSEN & Y. YUSA, 2012. Cypris settlement and dwarf male formation in the barnacle *Scalpellum scalpellum*: A model for an androdioecious reproductive system. Journal of Experimental Marine Biology and Ecology, **422–423**: 39–47.

THIEL, M. & L. GUTOW, 2005. The ecology of rafting in the marine environment. II. The rafting organisms and community. Oceanography and Marine Biology: An Annual Review, **43**: 279–418.

UYE, S., 2008. Blooms of the giant jellyfish *Nemopilema nomurai*: a threat to the fisheries sustainability of the East Asian Marginal Seas. Plankton and Benthos Research, **3** (Supplement): 125–131.

WEEKS, S.C., C. BENVENUTO & S. K. REED, 2006. When males and hermaphrodites coexist: a review of androdioecy in animals. Integrative and Comparative Biology, **46**: 449–464.

YAMAGUCHI, S., E.L. CHARNOV, K. SAWADA & Y. YUSA, 2012. Sexual systems and life history of barnacles: A theoretical perspective. Integrative and Comparative Biology, **52**: 356–365.

------, K. SAWADA, Y. YUSA & Y. IWASA, 2013a. Dwarf males, large hermaphrodites and females in marine species: a dynamic optimization model of sex allocation and growth. Theoretical Population Biology, **85**: 49–57.

------, K. SAWADA, Y. YUSA & Y. IWASA, 2013b. Dwarf males and hermaphrodites can coexist in marine sedentary species if the opportunity to become a dwarf male is limited. Journal of Theoretical Biology, **334**: 101–108.

------, S. YOSHIDA, A. KANEKO, K. SAWADA, K. YASUDA & Y. YUSA, 2014. Sexual system of a symbiotic pedunculate barnacle *Poecilasma kaempferi* (Cirripedia: Thoracica). Marine Biology Research, **10**: 635–640.

------, Y. YUSA, S. YAMATO, S. URANO & S. TAKAHASHI, 2008. Mating group size and evolutionarily stable pattern of sexuality in barnacles. Journal of Theoretical Biology, **253**: 61–73.

YUEN, B. & J.M. HOCH, 2010. Factors influencing mating success in the acorn barnacle, *Semibalanus balanoides*. Journal of Crustacean Biology, **30**: 373–376.

YUSA, Y., M. TAKEMURA, K. MIYAZAKI, T. WATANABE & S. YAMATO, 2010. Dwarf males of *Octolasmis warwickii* (Cirripedia: Thoracica): The first example of coexistence of males and hermaphrodites in the suborder Lepadomorpha. Biological Bulletin, **218**: 259–265.

------, M. TAKEMURA, K. SAWADA & S. YAMAGUCHI, 2013. Diverse, continuous, and plastic sexual systems in barnacles. Integrative and Comparative Biology, **53**: 701–712.

------, S. YAMATO & M. MARUMURA, 2001. Ecology of a parasitic barnacle, *Koleolepas avis*, Relationship to the hosts, distribution, left–right asymmetry and reproduction. Journal of the Marine Biological Association of the U.K., **81**: 781–788.

------, M. YOSHIKAWA, J. KITAURA, M. KAWANE, Y. OZAKI, S. YAMATO & J.T. HØEG, 2012. Adaptive evolution of sexual systems in pedunculate barnacles. Proceedings of the Royal Society B: Biological Sciences, **279**: 959–966.

ZANN, L.P. & B.M. HARKER, 1978. Egg production of the barnacles *Platylepas ophiophilus* Lanchester, *Platylepas hexastylos* (O. Fabricius), *Octolasmis warwickii* Gray and *Lepas anatifera* Linnaeus. Crustaceana, **35**: 206–214.

ZARDUS, J.D., D.T. LAKE, M.G. FRICK & P.D. RAWSON, 2014. Deconstructing an assemblage of "turtle" barnacles: species assignments and fickle fidelity in *Chelonibia*. Marine Biology, **161**: 45–59.

ZHEDEN, V., W. KLEPAL, S.N. GORB & A. KOVALEV, 2015. Mechanical properties of the cement of the stalked barnacle *Dosima fascicularis* (Cirripedia, Crustacea). Interface Focus, **5**: 20140049.

FIGURE LEGENDS

Fig. 1. (A) Part of the jellyfish *Nemopilema nomurai* Kishinouye, 1922 (host ID = 1) showing a distant distribution of the barnacle *Alepas pacifica* Pilsbry, 1907 (attachment site is indicated by the arrowhead). (B) The largest individual of *A. pacifica* in (A), removed from the jellyfish, with 14 small individuals attached to it. The largest conspecific-attached individual was ovigerous.

Fig. 2. *Alepas pacifica* Pilsbry, 1907. Relationship between capitulum length (mm) and penis length (mm) for conspecific- (open circles) and jellyfish-attached (closed circles) individuals with a capitulum length of less than 6 mm.

Table I

Jellyfish hosts of *Alepas pacifica* Pilsbry, 1907 in this study.

Date of collection	Species	Host ID	No. of barnacles (excluding dwarf males)
2009/10/30	*Nemopilema nomurai*	1	38
2005/7/15	*Nemopilema nomurai*	2	20
2005/11/4	*Nemopilema nomurai*	3	8
2006/11/21	*Nemopilema nomurai*	4	1
2006/12/12	*Nemopilema nomurai*	5	1
2006/12/12	*Nemopilema nomurai*	6	1
2009/7/22	*Nemopilema nomurai*	7	1
2009/7/23	*Nemopilema nomurai*	8	1
2009/7/3	*Nemopilema nomurai*	9	1
2009/7/4	*Nemopilema nomurai*	10	1
2009/7/4	*Nemopilema nomurai*	11	1
2005/7/10	*Cyanea nozakii*	12	7
2009/11/5	*Cyanea nozakii*	13	7
?	*Cyanea nozakii*	14	3

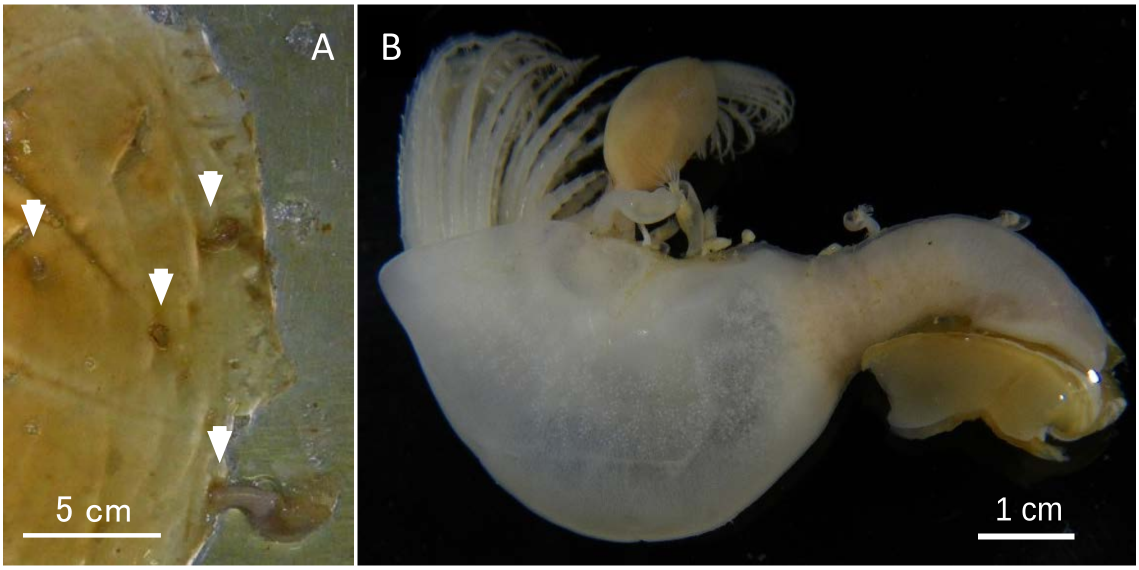
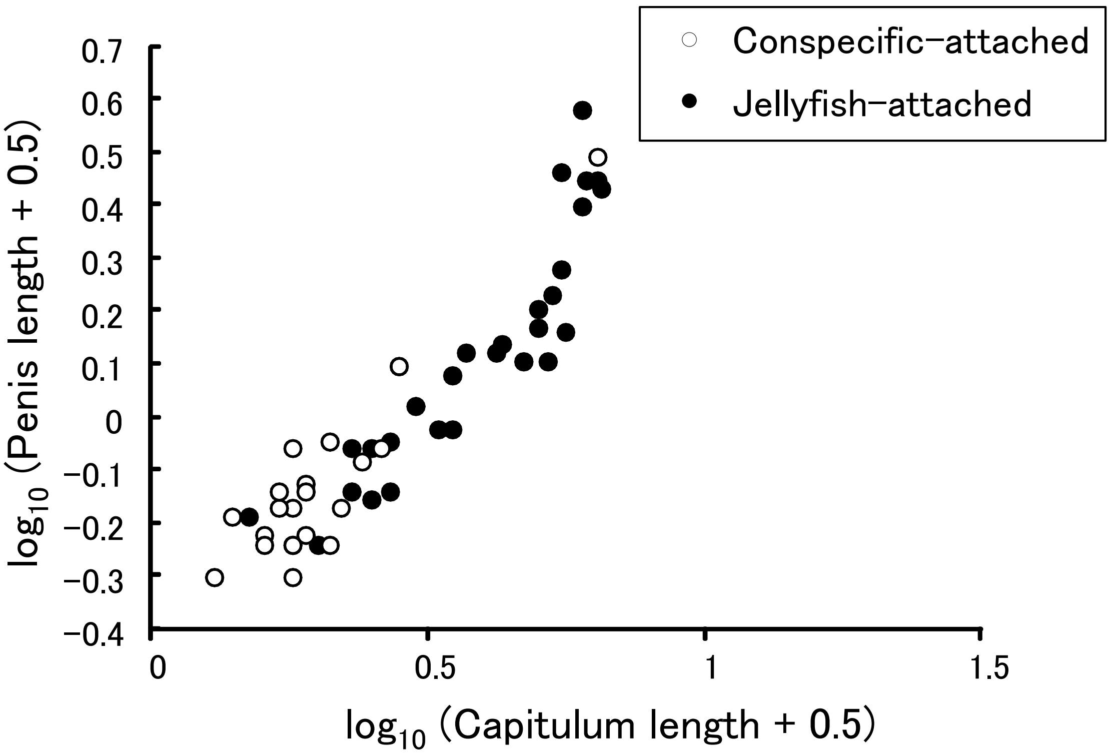


Fig. 1.



Conspecific-attached
Jellyfish-attached
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
−0.1
−0.2
−0.3
−0.4
0
0.5
1
1.5
$\log_{10}$ (Penis length + 0.5)
$\log_{10}$ (Capitulum length + 0.5)

Fig. 2.