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Development and metamorphosis of two psolid sea cucumbers, *Psolus chitonoides* and *Psolidium bullatum*, with a review of reproductive patterns in the family Psolidae (Holothuroidea: Echinodermata) *

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Abstract. Reproduction and development of two psolid sea cucumbers in waters of the San Juan Archipelago, Washington, were studied from April 1981 through August 1984. Spawning in *Psolus chitonoides* Clark and *Psolidium bullatum* Ohshima in the laboratory and in the field waters of the San Juan Archipelago, Washington, occurred from February through may, with heaviest spawning in April and May. The egg of *Psolus chitonoides* was bright red in color and 627 μm in diameter; *Psolidium bullatum* released eggs that were golden yellow in color and 330 μm in diameter. Maximum measured fecundties (spawned eggs) for *Psolus chitonoides* and *Psolidium bullatum* were 34 700 and 3 074, respectively. Developmental schedules were similar in both species. Early cleavages were equal, radial, and holoblastic; slightly subequal cleavage was common in *Psolus chitonoides*. The gastrula elongated to form a uniformly ciliated larva and the vestibule first appeared as a ventral crescent-shaped depression, after which the larva decreased in length and formed three posterior ridges that encircled the early doliolaria. Just prior to settlement, there was loss of cilia between the three ridges and on the posterior end of the early doliolaria, leaving three ciliary rings that are characteristic of the dendrochirote doliolaria larva. Vertical swimming ensued, and five primary tentacles protruded through the vestibule, with occasional attachment to the substratum using the primary tentacles. Upon settlement, two primary podia emerged and all external ciliation was lost except among tentacular papillae and on the end of tube feet. Timing of appearance and of loss of ciliary rings suggest that the rings function in vertical swimming and substratum selection behaviors. Metamorphosis involved the following simultaneous events: (1) resorption of the preoral lobe, (2) formation of large, dorsal, calcareous ossicles, (3) flattening of the trivium, and (4) proliferation of ventral tube feet on Ambulacral Radii B and E to form the sole. Reproduction in the family Psolidae is reviewed. The large proportion of brooders in this family appears to be related to small size, the possession of protective armor and sole, and geographic distribution (mainly in Antarctica). Lecithotrophic doliolaria larvae with three ciliary rings are produced by large northern psolids.

Introduction

Psolid holothuroids comprise one of six families of sea cucumbers in the order Dendrochirotida (Pawson 1982) and are among the most derived forms of echinoderms, being characterized by a flattened sole for adhesion to hard substrata and by an armored dorsum of large calcareous plates. Aside from taxonomic descriptions, published accounts on the biology, particularly the reproduction and development, of the Psolidae are few (McEuen 1986). Brief descriptions of various brooding adaptations among the Psolidae are found in Thomson (1878a, b), Theel (1886), Ludwig (1897b, 1898), Clark (1901), Vaney (1906, 1907, 1914, 1925), Ekman (1925) and Miller (1983, 1985). A more complete but unpublished study on the brooding and embryology of *Lissothuria* (formerly *Thyonepsolus*) *nutriens* was carried out by Wootton (1949). Partial descriptions of pelagic larval development are known for *Psolus phantapus* (Runnström and Runnström 1919, Thorson 1946) and an unidentified *Psolus* species (Smith 1977).

Psolus chitonoides and *Psolidium bullatum*, the subjects of this study, are the only abundant shallow-water psolids encountered among the San Juan Islands. *Psolus chitonoides*, the larger of the two psolids, is usually reddish orange in color with bright red tentacles, reaches a length of 7.5 cm, and may occasionally be seen intertidally at very low tides in crevices, under rocks, or underneath overhanging ledges. It is found in Japan and on the west coast of North America from the Bering Sea to central California (Austin 1985, p. 174). In the San Juan Islands, this species is primarily subtidal in distribution,

and frequently occupies all of the primary substratum on rock walls, large boulders and cobble; it is most abundant at 25 to 35 m depths in high-current areas (Young and Chia 1982 and own personal observations).

Psolidium bullatum is found in greatest densities (10/0.1 m) in San Juan Channel at depths of 13 to 35 m, in areas of low-current speeds, on the upper surfaces of large boulders covered with a thin layer (2 to 7 mm) of detritus and silt, leaving only the tentacles visible. This species is also common in some habitats alongside *Psolus chitonoides* at depths of 25 to 30 m on vertical rock walls and on top of, but never underneath, rocks and large boulders. *Psolidium bullatum* is only occasionally seen living at subtidal depths shallower than 11 m and has not been collected from the intertidal. Hadfield (1961) found this sea cucumber on mussel clumps (*Modiolus modiolus*), small rocks, and empty pecten shells dredged from 46 to 111 m. This psolid is 2.6 cm in maximum body length, colored pink to light lavender, and very low in profile, lying flat against the rock surface and preferring small depressions and corners. The geographic range of *Psolidium bullatum* extends from central California to the Aleutian Islands, Alaska (Austin 1985, p. 174). Both species are essentially sessile, but rare errant individuals of *Psolus chitonoides* have been sighted crawling over rocks and hydroids.

Early embryos and larvae of *Psolus chitonoides* are one of the most obvious elements in the spring plankton of the San Juan Islands (Johnson 1931, Johnson and Johnson 1950, and own personal observations). Fernald (1959) first mentioned the facility with which this species can be cultured in the laboratory, and Jones (1960) studied the histologic details of some early developmental stages. Chia and Spaulding (1972) induced metamorphosis with tubes of an unidentified species of *Phyllochaetopterus*. In their study of the effects of larval settling and post-settling behavior on the spatial distribution of *Psolus chitonoides*, Young and Chia (1982) presented a brief summary of development and an analysis of the behavior of the vitellaria and pentactula stages. Aside from a mention by Hadfield (1961) of "a period of little or no sexual activity" in the late fall and early winter, and of finding ripe individuals in late spring months, there is no information on the reproductive biology of *Psolidium bullatum*.

This paper presents information for the first time on the development of *Psolidium bullatum*. As well, a more complete picture of the development and metamorphosis of *Psolus chitonoides* is provided. Moreover, with so little known of psolid reproduction, a review of the literature on the reproduction of the entire family would seem important to help clarify reproductive trends and adaptations in a taxon that is so highly specialized in body form (Pawson 1966).

Materials and methods

Individuals of *Psolidium bullatum* Ohshima were initially dredged at Iceberg Point, Lopez Island, Washington, USA, in April 1981. Collections and observations of both species using SCUBA were made from August 1981 through August 1984 in San Juan Channel at

depths of 25 to 30 m; specimens were kept in flow-through seawater tables at the University of Washington Friday Harbor Laboratories. Observations of egg abundance for *Psolus chitonoides* Clark in the surface plankton were made from the laboratory dock and during SCUBA dives.

Individuals were placed in aquaria and in separate culture bowls in seawater tables to examine spawning behavior and to collect gametes. Eggs of *Psolus chitonoides* began to extrude from the follicular layer 30 to 60 min after being stripped from ripe ovaries (Fig. 1 A). Attempts to fertilize these stripped eggs were unsuccessful. Spawning of *P. chitonoides* occurred frequently in the water tables and could occasionally be induced by either turning off water circulation in the seawater table (McEuen 1987, 1988) or by removing bowls containing sea cucumbers from the seawater table and allowing them to warm to temperatures of 15° to 17 °C (from 9° to 11 °C) for 1 to 2 h. Fertilized eggs were obtained for both species from natural spawnings in seawater tables and from artificial fertilizations. For artificial fertilization, 0.5 ml of dilute sperm (1 drop "dry" sperm from spawning males or from ripe testes, diluted with 50 ml Millipore-filtered seawater) was added to every 50 ml of seawater with 150 to 200 spawned eggs; after 10 min, eggs were rinsed several times with filtered seawater. Fertilized eggs (100 to 200) were cultured in glass bowls with 300 ml of filtered seawater and maintained in an incubator at 11 °C ± 0.5 C°, with a 12 h photoperiod, using fluorescent light for uniform temperature and light conditions. Additional cultures were also kept in seawater tables. Incubated embryos from at least two culture bowls from each of 3 to 4 females of each species were monitored for development schedules. Timing of developmental stages was determined when approximately 50% of the incubated embryos or larvae had reached a particular stage.

Materials and procedures used to prepare embryos and larvae for scanning electron-microscope study were the same as outlined in McEuen and Chia (1985).

Results

Gonads, gametes and spawning

Gonads of *Psolus chitonoides* and *Psolidium bullatum* are tufts of tubules lying slightly left of the anterior-posterior axis, and occupy a substantial portion of the coelomic cavity when ripe. Ripe ovarian tubules of *Psolus chitonoides* were dark red, sometimes a reddish brown, and the ripe testis light pink or white; the coloration of mature ovaries and testes of *Psolidium bullatum* was tan and white, respectively. Sexes could often be distinguished in

Fig. 1. *Psolus chitonoides.* (A) Ovulation of oocyte removed from ripe ovary, showing oocyte escaping from layer of follicle cells; (B) first polar body and protuberance on oocyte surface; (C) appearance of second polar body at 10 to 50 min after fertilization; (D) 48 h gastrula; (E) elongate, uniformly ciliated larva (65 h); (F) early ciliated larva showing first signs of vestibule (v) and podial pit (p) formation; (G) early doliolaria larva with enlarging vestibule (v) and a podial pit (p) just posterior to vestibule; (H) light micrograph of early doliolaria – arrows indicate the 3 epidermal ridges where the 3 ciliary rings will later form – darkest opaque region is the yolky preoral lobe; (I) dorsal view of 7 d doliolaria with 3 distinct ciliary rings and evenly ciliated preoral lobe – hydropore is also visible between second and third rings; (J) emergence of the 5 tentacles from ventral vestibule in 7 d doliolaria – the 2 podial pits of primary podia are between second and third ciliary rings. Elevated ciliary rings on raised epidermal ridges (as in H) are not evident in (I) and (J) due to shrinkage during fixation. Scale bar in (D) = 50 μm, all other scale bars = 100 μm
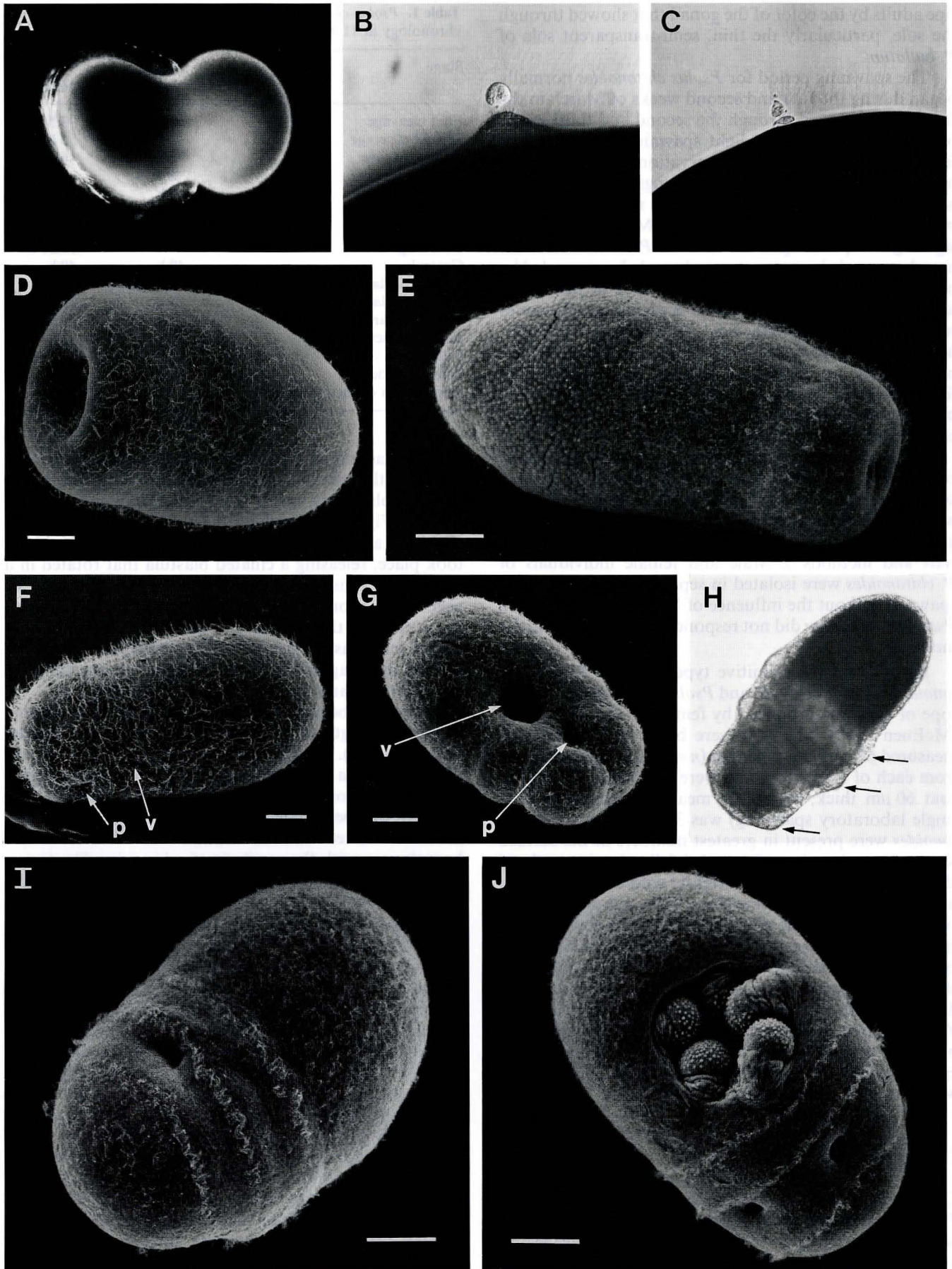
ripe adults by the color of the gonad that showed through the sole, particularly the thin, semi-transparent sole of *P. bullatum*.

The spawning period for *Psolus chitonoides* normally began during the first and second weeks of March in the field and continued through the second and third weeks of May, with occasional light spawning preceding and following this main period. Observations throughout 3 yr indicated that extensive field spawnings of *P. chitonoides* were frequently accompanied by mass release of gametes by individuals held in the laboratory (McEuen 1986). No sightings of spawning in the field for *Psolidium bullatum* have been made by us nor, to our knowledge, recorded by other divers. If the timing and frequency of uninduced laboratory reproductive behavior is a reliable indicator of reproductive activity in the field, as demonstrated by *Psolus chitonoides*, we would predict the most intense interval of spawning for *Psolidium bullatum* to last from the fourth week of March through the first week of May, based upon the recorded spawnings of adults of this species in the laboratory (McEuen 1988).

Spawning was induced in both sexes of ripe *Psolus chitonoides*, sometimes one month before the normal spawning season, by turning off water circulation in seawater tables or by placing adults in bowls of seawater and allowing them to warm to room temperature (see "Materials and methods"). Male and female individuals of *P. chitonoides* were isolated in separate bowls, and both spawned without the influence of "egg water" or sperm. *Psolidium bullatum* did not respond as readily to artificial methods.

Sperm is of the primitive type for both *Psolus chitonoides* (Chia et al. 1975) and *Psolidium bullatum*. A long rope of eggs was produced by female *Psolus chitonoides* (McEuen 1988). The eggs were bright red in sunlight, measured 627 ± 5 μm in length ($n = 50$, 10 eggs measured from each of 5 females), and were surrounded by a jelly coat 60 μm thick; maximum measured fecundity (in a single laboratory spawning) was 34 700. Eggs of *P. chitonoides* were present in greatest numbers in the surface waters for a duration of 1 to 3 d following intervals of clear, sunny weather; these brightly colored eggs are one of the most conspicuous members of the spring plankton. Infrequently, a female would also release globular material with the eggs in the rope, of the same color as the eggs. In dissected individuals, the normally cylindrical-shaped ripe ovaries were on occasion seen abnormally distended with this globular material. Females of *Psolidium bullatum* discharged up to 3074 golden yellow to brownish orange eggs of 330 ± 4 μm length ($n = 20$, 10 eggs measured from each of 2 females). A loose mucus was normally spawned with the eggs.

Development of *Psolus chitonoides*

Cleavage type and pattern for the large opaque eggs of *Psolus chitonoides* were equal, holoblastic and radial, with the first two divisions along the animal-vegetal axis and the third cleavage horizontal, with subsequent cleavages alternating between the two cleavage planes. Many

Table 1. *Psolus chitonoides* and *Psolidium bullatum*. Development chronology at $11\,°C \pm 0.5\,C°$ (after fertilization). –: no data

Stage	Psolus chitonoides	Psolidium bullatum
First cleavage	2 h	3.5 h
Second cleavage	3.75 h	5 h
Third cleavage	5 h	6.5 h
Fourth cleavage	6.25 h	8 h
Fifth cleavage	8 h	9 h
Sixth cleavage	10.25 h	10.25 h
Blastula	18 h	19.75 h
Hatching	–	28.50 h
Gastrula	40 h	40 h
Late gastrula	60–70 h	55 h
Early doliolaria	75 h	70 h
Late doliolaria	7 d	4.75 d
Early pentactula	9 d	7.25 d
Settlement	12 d	8.25 d
Sole formation (completion of metamorphosis)	28 d	?

cultures had a high percentage of embryos (~ 50 to 75%) with slightly subequal cleavage. Time of extrusion of the second polar body varied from 10 to 50 min after fertilization (Fig. 1C). Wrinkled blastulae appeared at 18 h (Table 1) and began smoothing out by 24 h; hatching took place, releasing a ciliated blastula that rotated in a counterclockwise direction. The first external signs of vegetal plate formation became apparent with a lightening in color of the periphery of the vegetal half, reaching a light yellow as the vegetal half flattened; the blastopore formed by invagination at the center of the vegetal plate (Fig. 1D) as early as 44 h in some embryos.

Gastrulae began to elongate at 60 h, sometimes to a maximum length of 1 125 μm, into a uniformly ciliated larva (Fig. 1E). The blastopore commenced to close over (Fig. 1E) and a small, ventral, crescent-shaped, ectodermal invagination, slightly to the right of the central axis, marked the beginning of the vestibule (Fig. 1F). (It should be noted that the holothuroid vestibule is not homologous with the vestibule of echinoids.) The region of the larva anterior to the vestibule is the preoral lobe. Also accompanying the appearance of the vestibule was a small posterior indentation, the podial pit of the future left primary podium (Fig. 1F, G).

Reduction in length (to 925 μm) along with an increase in width began the transformation of the uniformly ciliated larva into a doliolaria. On the early doliolaria, three posterior ridges formed at 4 d to give the appearance of there being three ciliary rings, but ciliation was still uniform over the entire larval surface (Fig. 1H). The vestibular pit, now positioned just anterior to the first ciliary ridge, continued to enlarge.

Shortly thereafter, another podial pit became visible in the posterior third of the larva; it was also positioned between the second and third ciliary rings, but laterally and 90° to the right of the podial pit that is directly posterior to the vestibule. Cilia between the three ridges and on the posterior end of the larva were then lost, leaving three distinct rings of cilia and a completely ciliated preoral lobe (Fig. 1I, J). These distinct rings charac-

terize the typical dendrochirotid larval form, the dolio-
laria. During the early and late doliolaria stages, it was
difficult to determine with the light microscope whether
the larvae still retained or had lost the cilia between the
rings. The vestibule continued to enlarge and the five
primary tentacles became visible and began to protrude
from the vestibule (Fig. 1 J). The mouth, however, had
not yet formed. In the doliolaria, the podial pit on the
right side gradually assumed a ventral position alongside
the other pit (Fig. 1 J). With formation of the three ciliary
rings, the doliolaria began vertical swimming to the bot-
tom of the culture bowl and back to the surface. In this
phase of vertical swimming, the preoral lobe was reddish
orange and the region posterior to the vestibule was yel-
low. The preoral lobe was always maintained upward
(probably due to a large store of lipid) while swimming,
and the spinning direction of almost all larvae remained
clockwise (viewed from the apical end). Faint glints of the
first ossicles appeared in the late doliolaria towards the
end of the vertical swimming stage.

During the vertical swimming phase, the five primary
tentacles began to elongate and readily adhered to sur-
faces with which they came into contact; the larva at this
time frequently attached with the tentacles to the bottom
of the bowl for varying lengths of time. The dorsal hydro-
pore had made its appearance by this time. The mean
swimming rate for advanced doliolaria larvae swimming
upward (rising) was 0.142 ± 0.027 cm s^{-1} ($n = 10$); down-
ward swimming rate was more than twice as slow at
0.067 ± 0.016 cm s^{-1} ($n = 10$). After the larva settled, cilia
disappeared and two primary podia emerged from the
podial pits and elongated.

The individual with five primary tentacles is termed
the pentactula (Semon 1888), and becomes permanently
benthic after loss of the cilia. Occasional larvae were
noted to protrude the primary podia before settlement.
Hyaline papillae and short cilia (Fig. 2 A, B) covered the
knobbed ends of the primary tentacles and were present
from the time the tentacles came to view within the
vestibule. Higher magnification with SEM revealed nu-
merous micropapillae covering each papilla (Fig. 2 B, C).
Also on the tentacle surface among the papillae were
numerous elaborate microvilli that appear as strands and
small globules (Fig. 2 B, C). Short cilia were also found
on the flat-bottom surface of the primary podia. The
negatively phototactic pentactula (Young and Chia 1982,
and own personal observations) walks over the hard sub-
stratum, using the "sticky" tentacles and podia to attach
to surfaces. The first pair of secondary podia emerged in
the late pentactula stage just prior to formation of the
sole (Fig. 2 E), and the dorsal hydropore reduced in size
(Fig. 2 F). From the time of emergence, the tentacles dis-
played the normal dendrochirotid behavioral rhythm of
alternately stuffing the tentacles into the region of the
future mouth even though the mouth had not formed at
this time (Fig. 3 A).

Recently settled larvae were examined to determine
whether they were positively or negatively buoyant. If the
larvae displayed no swimming behavior when detached
from the bottom of the bowl and then pipetted into the
water column, they were classed as permanently settled.

Of these permanently settled larvae, 26 in a bowl were
warmed over a hot plate for 1 min and then the adhesive
tentacles and podia of the dead larvae were loosened
from their attachment to the dish bottom. All but three
individuals floated to the surface, apical end (reddish
orange in color) up; the larvae were examined and those
not floating were discovered to have large ossicles.

Soon after settlement, there was rapid formation of
ossicles simultaneous with resorption of the preoral lobe.
No ossicles formed within the preoral lobe. As the pre-
oral lobe receded, the primary tentacles moved from their
ventral position to the anterior position seen in adults.
The most obvious events during this final phase of psolid
metamorphosis were the formation of large calcareous
plates (Fig. 3 B) that covered the dorsal bivium (Ambu-
lacral Radii C and D; Fig. 3 C) and the flattening of the
ventral trivium (Ambulacral Radii A, B and E) to form
the sole. The settled pentactula adhered initially to the
substratum with the five primary tentacles and two pri-
mary podia. At 29 d, there was still no branching of the
tentacles nor growth of new or secondary tentacles. The
first bifurcations of the tips of the three dorsal tentacles
occurred at 30 to 33 d, while the two ventral tentacles
retained their pad-like appearance (Fig. 3 D). By 20 d, a
few pentactulae possessed large ossicles and were just
beginning formation of the sole. With deposition of the
dorsal calcareous plates and the dorso-ventral flattening,
the first pair of secondary podia formed by 29 d anterior
to the two primary podia and slowly moved outwards,
remaining on the edge of the expanding sole. A fifth
podium became visible at 31 to 32 d on the anterior end
of the sole, slightly to the left of the midline (Ambulacral
Radius A). The usual appearance of podia, thereafter,
resumed in pairs with one podium on either side of the
midline opposite the other on Ambulacral Radii B and E.
Fig. 3 E depicts the position and order of appearance of
the 14 podia present at 54 d. A circular zone of differen-
tiated epidermis and scattered short cilia were visible on
the bottom surface of tube feet of pentactulae and early
juveniles (Fig. 3 F, G). Viewed ventrally, new tube feet
could be seen forming internally (before they emerged) as
clear areas in the orange yolk ringing the interior edge of
the sole. If these juveniles were dislodged and turned
over, they immediately attached the tentacles to the sub-
stratum and flipped themselves over, regaining their orig-
inal stance. Length of the 54 d juvenile ranged from 232
to 1 309 μm ($n = 5$), measured from the posterior edge of
the sole to the anteriormost oral plate.

Larvae settled readily on nearly all hard surfaces;
some attached their primary tentacles to the culture-wa-
ter surface tension and completed a normal metamorpho-
sis upside down. In additional bowls under crowded con-
ditions (300 to 400 individuals), instances of larvae coa-
lescing (fusing) were noted, where most went through an
extended metamorphosis that, in some cases, lasted up to
8 mo before the dorsal armor and sole were complete. In
10 to 12 individuals, ossicles grew to a large size but, after
a period of 1 yr, no flat sole had yet been established. In
cultures supplied with algae and fine detritus, juveniles
obtained their food by deposit-feeding on the bottom of
the dish. Fecal boluses were first noticed at 50 to 52 d.

Juveniles kept in cultures with Millipore-filtered seawater and no unicellular algae added for food were still alive at the end of 1 yr, were no longer than 1.2 mm, and had not added new tentacles to the original five primary ones. Ossicles were larger and more numerous, and the looped gut was visible through the light orange semi-transparent body wall.

Development of *Psolidium bullatum*

Development of this species was very similar to that of *Psolus chitonoides*, with some differences in morphology, behavior and timing of developmental stages (Table 1).

The embryo of *Psolidium bullatum* was smaller (330 to 430 μm) and less opaque than the embryo of *Psolus chitonoides* (627 to 1 125 μm), facilitating observations of early developmental events. The schedule of early cleavages was close to that of *P. chitonoides*. In the late coeloblastula (25 h), there was a thickening of the cells at the vegetal pole; these cells were easily distinguished by their light yellow color in contrast to the more opaque light tan-orange color of the rest of the embryo. Once the egg membrane was broken, the spinning blastula took about 1.5 min to leave the surrounding membrane and jelly layer (Fig. 4A). By 35 h, the embryo had elongated to 400 μm and was egg-shaped; the early gastrula formed 7 h later with continued thickening and flattening of the vegetal plate, giving the embryo a rounded cone shape. The posterior of the gastrula became less opaque and whitish in color by 51 h at a length of 410 μm. Larvae congregated side by side in groups of 3 to 50 individuals, in a vertical position, with the orange-pigmented (apical) end up (similar to *P. chitonoides*: Young and Chia 1982); some swam along the dish bottom in a horizontal position, spinning as they went. Of 5 larvae, the fastest spinning rate measured was 1.0 r/s. Further lengthening to 430 μm changed the larva into a long cylindrical shape that had a slight flattening of the venter; the first sign of the developing vestibule was barely visible as a slightly differentiated circle bordering the lower ventral edge of the pigmented region. The spinning increased in speed to 2 r/0.82 s when the larva reached a length of 485 μm; the larva reversed its direction of spinning (to clockwise) at 70 h and swam at the surface.

At 76 h, 80% of the larvae were swimming on the dish bottom; a few hours later, the three ridges appeared that would bear the ciliary rings. The vestibule opened by 89 h and the early doliolaria measured 600 μm long. A circular depression presaging a primary podium was evident at

4 d, directly posterior to the vestibule and between the second and third ciliary rings. Small brown splotches and streaks of various shapes could be seen in the posterior third of the body. The primary podia protruded from the vestibule (Fig. 4B) and swimming behavior was up and down in a vertical position similar to *Psolus chitonoides*, with some larvae swimming horizontal to the bottom, apical end forward. The older doliolaria decreased in length to 550 to 560 μm, with some doliolarias as small as 475 μm. This shortening also resulted in a wider larval body.

Details of settlement were similar to those for *Psolus chitonoides*, with larvae occasionally attaching to the bottom in the typical posture shown in Fig. 4C. The primary tentacles of *Psolidium bullatum*, although proportionately much shorter than tentacles of pentactulae of *Psolus chitonoides*, were very prominent at settlement and occupied a substantial area of the ventral surface of the 7 d-old, 445 μm-long larva (Fig. 4D). Settling and settled pentactulae of both psolids maintained in cultures in seawater tables were subjected to bright sunlight entering the windows on the south side of the laboratory, and aggregated in the bottom of the bowls opposite these windows. Incubated cultures showed no such behavior, probably owing to the even illumination of the incubator. Larval swimming behavior appeared more consistent for incubated larvae than for larvae kept in seawater tables; culture bowls of the latter were only partially submerged and were subject to temperature gradients and fluctuations, particularly during warm days.

Shortly after settlement, the two primary podia increased in length and attached to the substratum. Cilia were first lost from the ciliary rings and then from the preoral lobe. Ossicles became visible posterior to the preoral lobe at 8.5 d; the whitish opaque posterior two-thirds of the body later became more transparent and the preoral lobe a light yellowish orange, with individuals possessing many large ossicles posteriorly at 14.5 d. By this time, numerous ossicles had formed in the primary podia, but only a few in the tentacles. Resorption of the preoral lobe was a gradual process that continued until approximately 22 d, when individuals (630 to 755 μm length, $n = 5$) were well armored with a coat of ossicles and had very little preoral lobe remaining.

No secondary podia, additional tentacles, nor branching of the primary tentacles had occurred at the end of 36 d. Branching of the three dorsal primary tentacles began at 5.5 mo.

Pentactulae in bowls held in seawater tables were provided rock, conspecific adults, adult *Psolus chitonoides*, and tubes of spionids and *Phyllochaetopterus* sp., but there was no complete metamorphosis to the flattened juvenile form as in *Psolus chitonoides*, even in cultures maintained for 1 yr. Although embryo cultures from 3 to 5 females of *Psolidium bullatum* were obtained each breeding season for four consecutive years (1981–1984) and the larvae reared through settlement, no success was achieved with attempts to induce the final stages of metamorphosis. Individuals kept for 1 yr in bowls of Millipore-filtered seawater and with no unicellular algae added for food, were still alive and had the tentacles,

Fig. 2. *Psolus chitonoides.* (A) Primary tentacle of pentactula with knobbed end covered with papillae (scale bar = 25 μm); (B) papillae on primary tentacles are composed of numerous micropapillae – short cilia are also present between papillae and usually occur in pairs (scale bar = 2 μm); (C) subsurface of primary tentacle with microvillar strands and globules strung out over and among papillae (scale bar = 2.5 μm); (D) settled pentactula after loss of cilia; (E) receding preoral lobe (pl) and newly forming secondary podia (arrowed) on pentactula (scale bar = 225 μm); (F) dorsal view of pentactula with diminishing hydropore (arrowed)
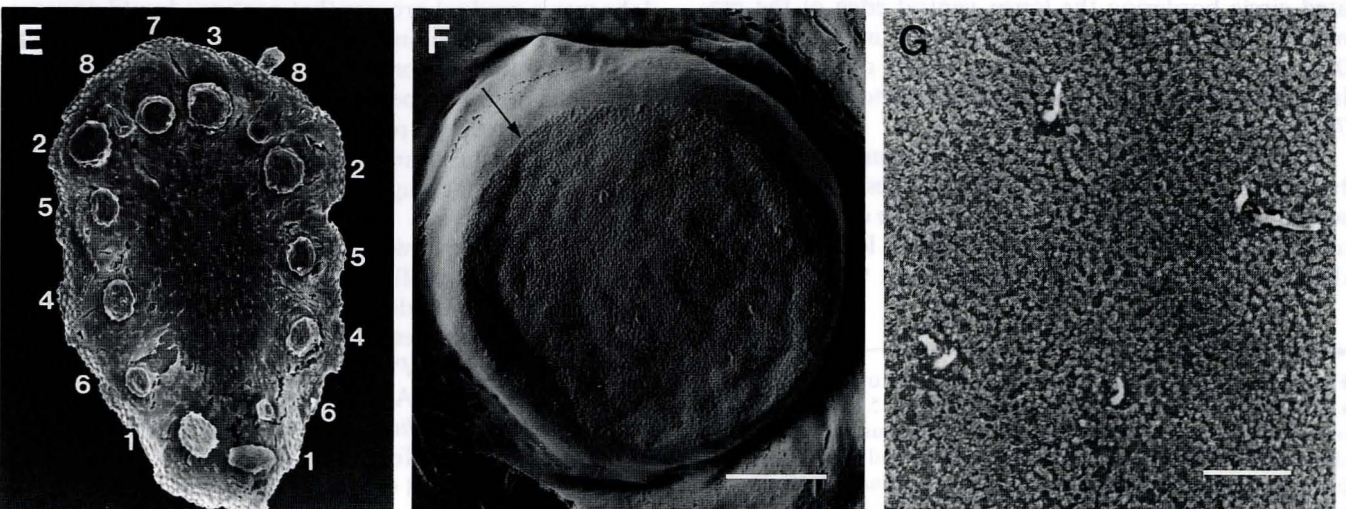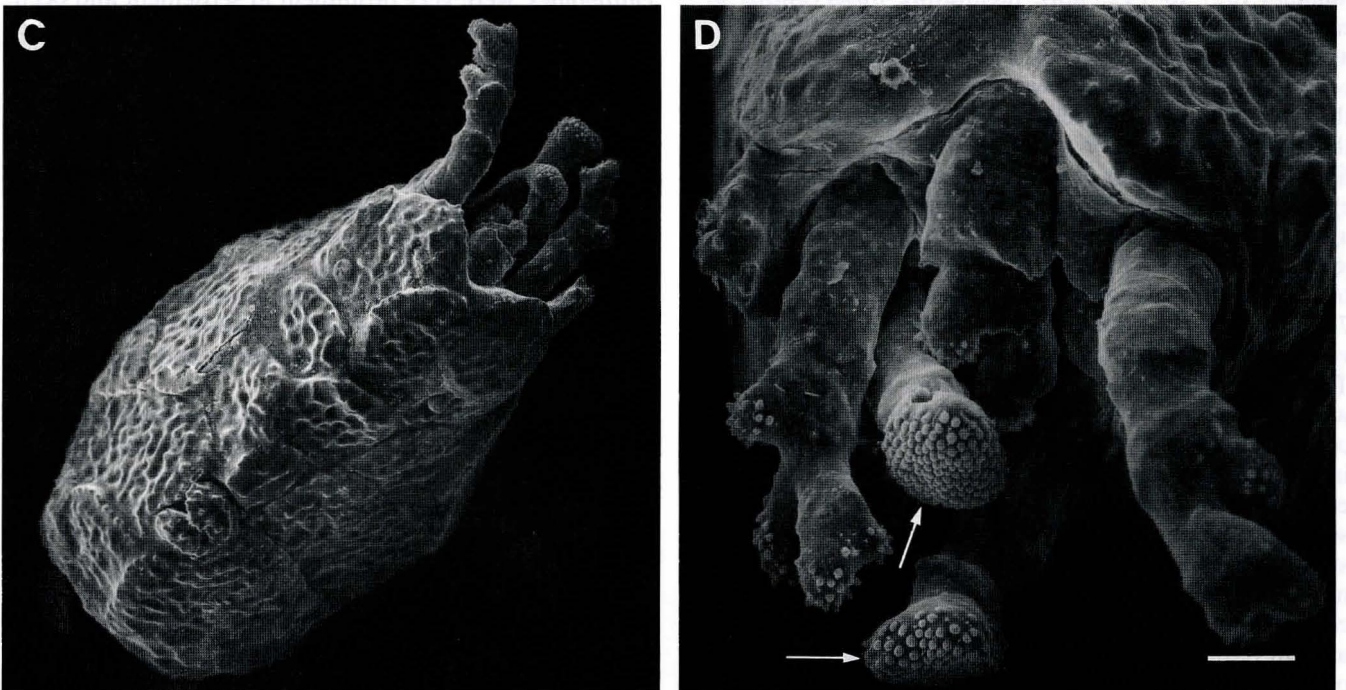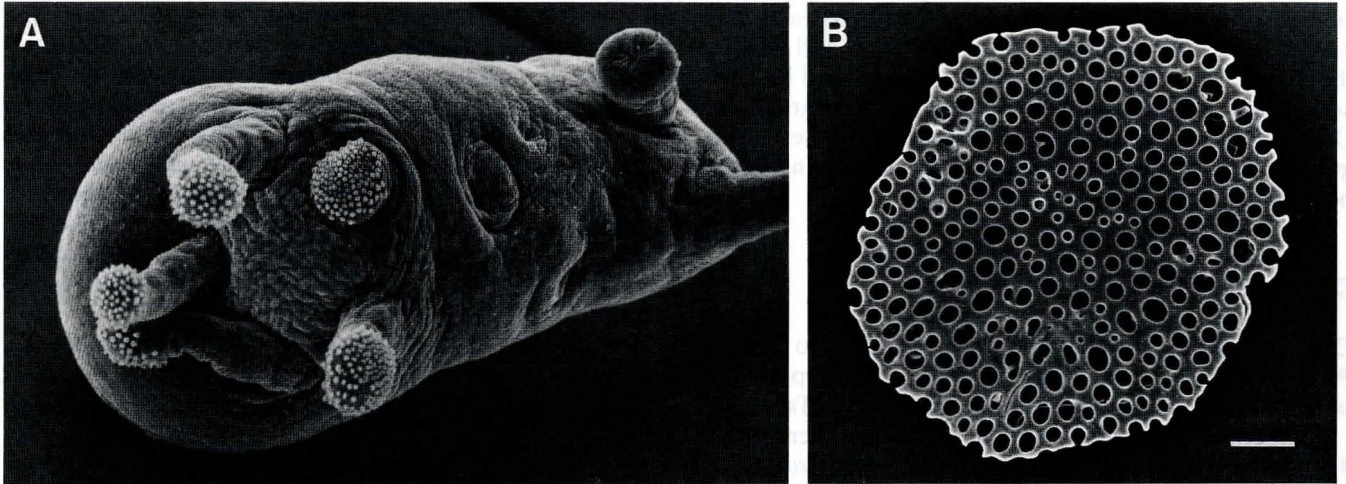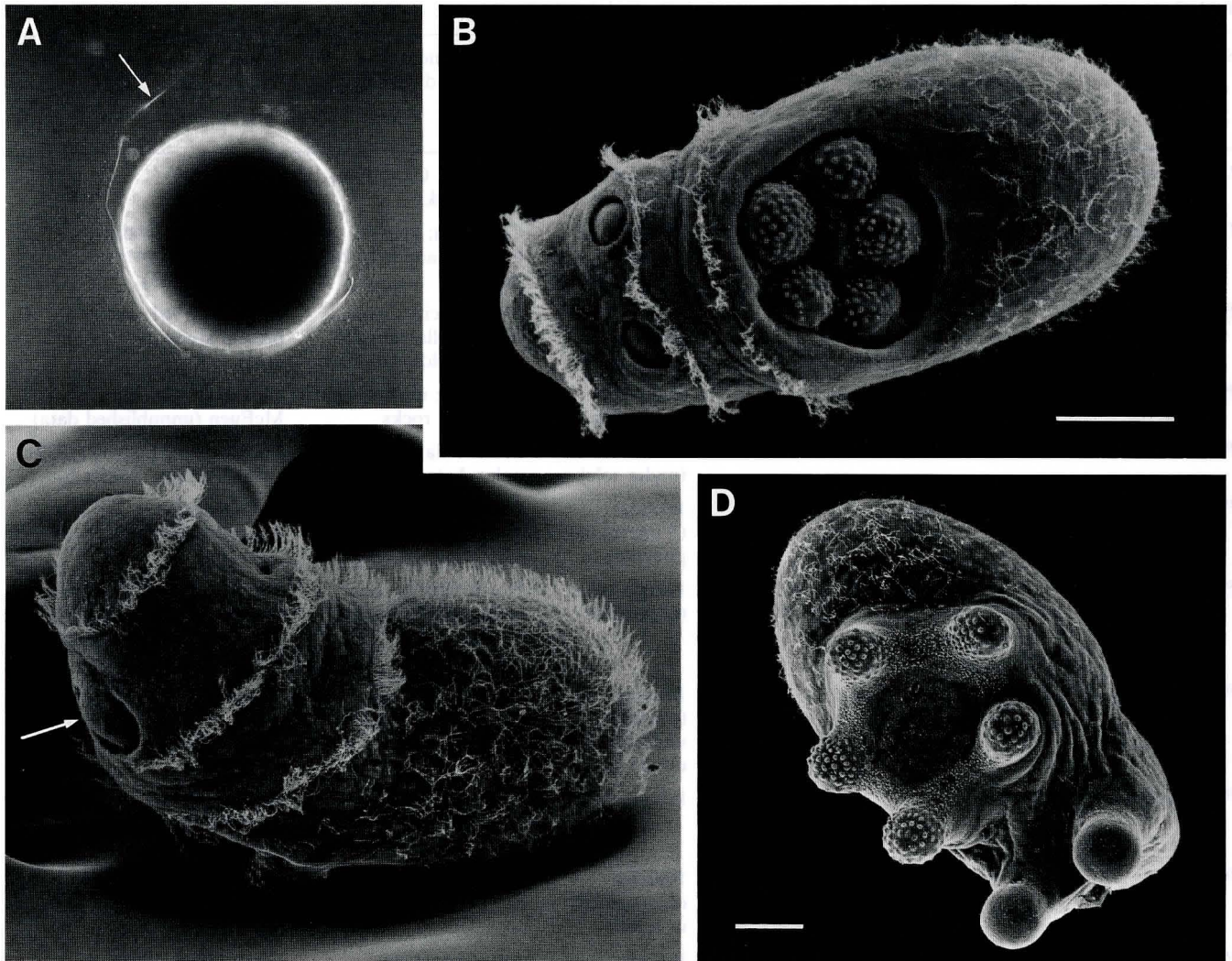
Fig. 4. *Psolidium bullatum*. (A) Hatching blastula at 28.5 h, showing detached membrane (arrowed); (B) 3-ringed doliolaria with emerging 5 primary podia and 2 posterior podial pits (scale bar = 75 μm); (C) normal position of larva at settlement, with a primary podium beginning to push out (arrowed) – opposite ventral podium and just posterior to second ring of cilia is the small dorsal hydropore; (D) ventral view of recently settled pentactula with some cilia still remaining on preoral lobe (scale bar = 60 μm)

podia and body completely covered with ossicles. In addition to the original 2 primary podia, six individuals had grown 3 to 4 dorsal podia and one individual had 8 tentacles with only simple bifurcations. Those provided with polychaete tubes fed on the detritus and microflora on the tubes and defecated boluses at 8 to 9 wk of age.

Fig. 3. *Psolus chitonoides*. (A) Antero-ventral view of pentactula; no mouth has yet formed. (B) One of the large ossicle plates covering newly metamorphosed juvenile (scale bar = 50 μm); (C) young juvenile (1.2 mm long) with dorsal plates, 3 plates surround anus; (D) early branching of the 3 dorsal tentacles in early juvenile, arrows indicate the 2 ventral tentacles that remain pad-like in shape long after the other 3 primary tentacles have undergone extensive growth and branching (scale bar = 50 μm); (E) sole of young juvenile with 14 podia on Ambulacral Radii B and E, numbers represent order of appearance of podia, podia with the same number emerge simultaneously – the first 2 primary podia denote posterior end; (F) view of bottom surface of tube foot of a juvenile, edge of zone of differentiated epidermis is arrowed (scale bar = 20 μm); (G) magnification of bottom of tube foot of juvenile, showing presence of short cilia (scales bar = 5 μm)

Discussion

Sexual reproduction predominates among psolid holothuroids, with brooding and pelagic lecithotrophic larvae being the recognized modes of development (Table 2). No instance of planktotrophy is known among psolid larvae. The only report of asexual reproduction in this family is transverse fission in *Psolus valvatus*, where each half of the split sea cucumber regenerates the lacking parts (Mortensen 1927).

Previous descriptions of psolid development for species with pelagic larvae are brief and incomplete; some general similarities and differences with our findings are discussed here. The details of development for the two psolids, *Psolus chitonoides* and *Psolidium bullatum*, presented in this study generally agree with the stages of psolid development depicted and described by Thorson (1946) and Young and Chia (1982). Similar to our observations, Runnström and Runnström (1919) and Jones (1960) noted embryos of *Psolus* species with slightly subequal cleavage. The blastula of *P. chitonoides* is com-

Table 2. Summary of information on reproduction in the holothuroid family Psolidae. –: no data

Species	Max. adult size (mm)	Breeding season	Egg size (μm)	Reproductive pattern	Geographic distribution and habitat	Source
Lissothuria antillensis	15	Continuous	400	Brooder (coelomic)	Bermuda, Caribbean; under rocks	Miller (1983)
L. (Thyonepsolus) nutrients	26	Feb.–Dec.	580	Brooder (dorsal depressions)	California; kelp holdfasts and kelp bed, rock	Clark (1901, 1902), Deichmann (1941), Wootton (1949)
Psolidium bullatum	26	Mar.–May	330	Pelagic larva (doliolaria)	North Pacific; rock walls, shells, mussels, cobble fields, boulders	Hadfield (1961) and present study
P. incubans	12	Continuous (?)	640 (young?)	Brooder (folds of sole)	Antarctic; undersides of littoral rocks	Ekman (1925), McEuen (unpublished data)
Psolus antarcticus	55	–	–	Brooder (under sole)	South Atlantic, circumpolar-Antarctic; rocks	Theel (1886), Ludwig (1897b, 1898), Pawson (1969a, b)
P. charcoti	85	Jan.	650	Brooder (5 interradial pouches)	South Georgia, Antarctic Peninsula; sand, clay, gravel	Ekman (1925), Arnaud (1974)
P. chitonoides	75	Mar.–June	625	Pelagic larva (doliolaria)	North Pacific; rock walls, cobble fields, boulders	Johnson (1931), Johnson and Johnson (1950), Fernald (1959), Jones (1960), Young and Chia (1982), present study
P. ephippifer	40	Jan.–Feb.	–	Brooder (chambers underneath dorsal plates)	Antarctic; coarse gravel, sand, mud	Thomson (1878a, b), Theel (1986), Ludwig (1897a, 1898), Pawson (1969b)
P. (Cuvieria) fabricii	100	–	–	Pelagic larva	North Atlantic; under stones	Agassiz (1864), Agassiz and Agassiz (1865), Deichmann (1930)
P. figulus	9	–	640	Brooder (depressions in sole)	Antarctic; sand	Ekman (1925)
P. granulosus	22	–	500	Brooder (folds of sole)	Antarctic; shore	Vaney (1906, 1907)
P. koehleri	65	–	–	Brooder (interradial pouches surrounding tentacular crown)	Antarctic; rock, gravel, mud	Vaney (1914, 1925), Pawson (1969b)
P. patagonicus	21	Feb.	–	Brooder (under sole)	Argentina; bottom of stones, *Macrocystis* sp. fronds and holdfasts	Bernasconi (1941), Hernández (1981)
P. phantapus	150	Feb. to midsummer	590	Pelagic larva	North Atlantic; stones, shells	Mortensen (1901, 1927), Mortensen and Lieberkind (1928), Nordgaard (1912), Runnström and Runnström (1919), Deichmann (1930) Thorson (1946)
P. punctatus	14	–	360–640	Brooder (depressions in sole)	Antarctic; gravel, sand, clay	Ekman (1925), Pawson (1969b)

posed of a single layer of cells surrounding a blastocoel (Jones 1960); time of hatching, though not recorded by us, is supposed to occur at 55 h (at 12 °C) at the gastrula stage (Jones 1960). Jones' histological examination of embryonic stages also revealed that, subsequent to vegetal plate formation, mesenchyme cells are released from the vegetal plate as well as from the inner surface of the ectodermal wall.

In the study of Jones (1960), three lobes of the hydrocoel in the early larva of *Psolus chitonoides* lengthen and push out to form primary tentacles before the other two lobes, but our scanning electron micrographs seem to

indicate that appearance of the five primary tentacles may be simultaneous (Figs. 1 J, 4 B). Jones also reported 3 to 5 rings of cilia on the doliolaria larvae, but we never saw more nor less than 3. Although not described as bearing three ciliary rings, illustrations of the larvae and early pentactula of *P. phantapus* (Thorson 1946) and of the early pentactula of *P. fabricii* (Agassiz 1864, Agassiz and Agassiz 1865) show three ridges present posterior to the preoral lobe. We interpret these to indicate that the larvae of these two species have three ciliary rings, as we found for *P. chitonoides* and *Psolidium bullatum*. A postero-dorsal "anal opening" in illustrations of the "floating-crawling stage" (settling pentactula) of *Psolus phantapus* was described by Thorson (1946); no such opening has been seen in *P. chitonoides* nor in other dendrochirotids (McEuen 1986) until post-settlement, when juveniles are covered with ossicle plates (Fig. 3 C).

Our current knowledge of the actual functional roles of the ciliary rings of marine invertebrate larvae is limited (Chia et al. 1984). Observations on the larvae of *Psolus chitonoides* show that the three ciliary rings appear just before the onset of vertical swimming behavior, during which time the larvae occasionally attach to the bottom and resume swimming. Larvae are still positively buoyant at settlement, indicating that descent through the water column is by active swimming using the ciliary rings. The rate measured for upward "swimming" was more than twice as fast as the downward swimming rate; the former rate may be more a result of passive rising than active swimming. Comparative work has shown that larvae with ciliary rings generally swim faster than larvae that are evenly ciliated or that have convoluted ciliary bands (Lee 1984, 1985). In field and laboratory experiments, Young and Chia (1982) demonstrated that the pentactula of *P. chitonoides* settles gregariously on adults and that late larvae and early juveniles are strongly photonegative. Ciliary rings may provide improved maneuverability, enabling larvae to select shaded habitats, which Young and Chia have demonstrated are preferred by pentactulae and juveniles. This is further corroborated by the fact that as the larva settles and protrudes the two primary podia, the ciliary rings are quickly lost. It settlement is postponed due to lack of suitable substratum, the larva may become negatively buoyant, with resorption of the preoral lobe and an increase in size and number of ossicles; in this situation, the ciliary rings may also function to keep the larva in suspension.

With the positive buoyancy of the settled larva, the sticky tentacles and primary podia serve to retain the metamorphosing individual on the selected substratum until more podia and ossicles are produced and the yolk material is resorbed, thereby rendering the young psolid negatively buoyant. The numerous minute microvillary strands and globules (Figs. 2 C, 4 B) covering the tips of primary tentacles were assigned an adhesive function by McEuen (1986). In the recent excellent work by McKenzie (1987), however, it has been shown that these microvilli are support-cell microvilli that underlie surface coats (designated as lower cuticle, upper cuticle, and "fuzzy coat" by McKenzie). These surface coats were not preserved by our fixation methods.

A similar arrangement of papillae and cilia has been found on primary tentacles of the pentactula of *Leptopentacta* (formerly *Cucumaria*) *elongata* by Chia and Buchanan (1969) who also demonstrated that these papillae contain acid mucopolysaccharides that may be involved in feeding. Cilia interspersed among the hyaline papillae and the micropapillae on the hyaline papillae (Fig. 2 B), as well as cilia on the tube feet (Fig. 3 F, G), presumably function for mechanoreception or chemoreception in substratum selection and for feeding in the young juvenile. Cilia and papillae are also present on adult tentacles of *Psolus chitonoides* (Fankboner 1978) and other dendrochirotid holothuroids (Smith 1983, McKenzie 1985, 1987) and have been proposed by these workers to serve as mechanoreceptors and to entrap food particles.

The failure of settled larvae of *Psolidium bullatum* to complete metamorphosis in laboratory cultures could be due to one or more factors, including (1) lack of a specific settling cue, (2) a long period required for metamorphosis, or (3) the need for settled young to feed to obtain additional nutrition for completion of metamorphosis. In light of our knowledge of settlement and metamorphosis of *Psolus chitonoides*, the first alternative seems the most probable. *Psolidium bullatum* is also abundant on detritus-covered rocks in quiet inlets and fjords (P. Lambert personal communication). Although not used in our studies, the milieu of detritus and silt on the tops of rocks in the adult habitat may be the stimulus for metamorphosis and an important food source for juveniles.

Brooding and patterns of reproduction

Brooding for five species of psolids was discussed by Ludwig (1904) and Vaney (1925) in their early reviews of holothuroid broodcare. More recently, Hyman (1955) and Boolootian (1966) have briefly summarized breeding seasons and brooding for 9 to 10 psolid species. Table 2 summarizes the available information from published literature on sexual reproduction in the family Psolidae. Not included in the table of information on psolid species with known development are egg sizes of 0.8 mm for *Ceto cuvieria* (Pawson 1971 b), 200 μm for *Psolus neozelanicus* (Mortensen 1925), "less than 500 μm" for *Lissothuria mortenseni* (Pawson 1967), 0.2 mm for *Ekkentropelma brychia* (Pawson 1971 a), and a mention of "large eggs" for *P. tuberculosus* (Miller and Pawson 1984).

Of the approximately 85 species contained in the family Psolidae (Pawson 1982), there now exists at least partial information on the development of 15 species, or 18% of all psolids; approximately 73% (11 species) of the 15 species listed (13% of all psolids) brood (Table 2). Although most species for which we have information on reproduction are brooders, this may simply be a result of the fact that external brooding with such unique forms as psolids having a hard skeleton can be easily detected. Eight of the 11 instances of brooding listed in Table 2 are known primarily by brief descriptions from early antarctic collecting-expeditions.

Four different modes of brooding are found: (1) on the dorsum, (2) on the ventral sole, (3) around the crown

of tentacles, and (4) internally. Dorsal tube feet of *Lissothuria* (formerly *Thyonepsolus*) *nutriens* retain developing embryos in external dorsal depressions (Wootton 1949) and *Psolus ephippifer* possesses brood chambers beneath the dorsal calcareous plates (Thomson 1878a, b, Theel 1886). Large interradial pouches surrounding the tentacular crown house the broods of *P. koehleri* (Vaney 1914) and *P. charcoti* (Ekman 1925, Arnaud 1974). Young can be found attached to the ventral sole, as in *P. antarcticus* (Ludwig 1897b, 1898) and *P. patagonicus* (Bernasconi 1941, Hernández 1981), or with the embryos in pockets, folds, or swellings of the sole as is known for *Psolidium incubans* (Ekman 1925), *Psolus figulus* (Ekman 1925), *P. granulosus* (Vaney 1907), *P. punctatus* (Ekman 1925). Miller (1983, 1985) recently discovered coelomic brooding in *L. antillensis*, a form that apparently releases 1.0 to 1.5 mm-long juveniles, having well-developed dorsal plates, through the anus. Any particular adaptive significance associated with each of the above forms of brooding remains unknown.

The high percentage of brooding members in this family may also partly be due to the protective advantages afforded by the armored dorsum and the adhesive sole, manifest in the numerous ways in which these external body parts are utilized to incubate embryos and young. Brooding the young until they are covered with a coat of imbricated calcareous plates would be a further safeguard, especially against benthic predators in the Antarctic where, in some habitats, epibenthic forms dominate and carnivorous asteroids are abundant (Dayton et al. 1974). At least one antarctic psolid, *Psolus antarcticus* (Ludwig 1897b, 1898), broods young under the sole until the coat of ossicles and the flat sole have formed. Even with the coelomic brooder *Lissothuria antillensis*, young remain within the female and are not expelled through the anus until they have a well-developed skeleton of imbricating plates (Miller 1983, 1985). The coat of calcareous plates laid down by newly settled young of *P. chitonoides* does serve as an effective shield against invertebrate predators (McEuen 1984). In laboratory experiments (McEuen unpublished data), juveniles covered with a dorsal armor of calcareous ossicle plates experienced significantly less mortality than eggs and larvae when fed to the benthic carnivorous amphipod *Parapleustes pugettensis*. When armored juveniles were loosened from their hold on the substratum, amphipods attacked them through the thin membranous sole, hollowing them out and leaving the dorsal shell of ossicle plates.

Another trend that appears to be evident from Table 2 is the occurrence of pelagic larvae (or broadcasters) in the larger psolids (*Psolus chitonoides*, *P. fabricii*, and *P. phantapus*) of 75 to 150 mm length, although a smaller form (*Psolidium bullatum*, 26 mm) also has a pelagic doliolaria larva. However, even within the genus *Psolidium*, the one brooder (*P. incubans*) is still smaller than the broadcasting *P. bullatum*. Most of the smaller species (*Lissothuria antillensis*, *L. nutriens*, *Psolidium incubans*, *Psolus antarcticus*, *P. charcot*, *P. ephippifer*, *P. figulus*, *P. granulosus*, *P. koehleri*, *P. patagonicus*, and *P. punctatus*), with body lengths of 9 to 85 mm, are brooders and appear to fit the generally observed trend

among most marine invertebrate taxa of smaller adult size being associated with broodcare. Large size of psolid eggs (330 to 800 μm) with the attendant brooding and doliolaria larval form is also common in other echinoderm taxa (Hyman 1955, Hendler 1975, McEuen 1986), but no range of egg sizes characteristic for either mode of psolid development is yet discernible.

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