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OBSERVATIONS DURING EMBRYONIC DEVELOPMENT IN THE GENUS *DOTO* (GASTROPODA, OPISTHOBRANCHIA)

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(Plates I–III and Text-figs. 1–2)

Data on egg diameter, capsule size during embryonic development and veliger size have been compared in the three species *D. coronata*, *D. pinnatifida* and *D. fragilis*. It became clear that there are distinct specific differences in these features as well as in the time of development. Certain ratios, however – i.e. between capsule length and width at different stages, capsule volume/egg volume and the degree of egg capsule enlargement – are very similar, if not identical. The same holds true for the veliger shells, which are distinctly different in size but very similar in proportions. Some tests have been performed to check the influence of salinity, especially on the behaviour of the egg capsule. The salinity tolerance proved to be extremely limited for the embryos; the capsules, however, showed no change compared with those maintained under normal conditions if the embryo itself remained healthy.

INTRODUCTION

While working on taxonomic problems in the family Dotoidae and comparing the Mediterranean and Atlantic species (Schmekel & Kress, in the Press), it became apparent that very little embryological information was available for this group, other than that published by Alder & Hancock (1845–55), Pelseneer (1911), Miller (1958, Ph.D. Thesis, University of Liverpool) and Thompson (1967).

In the course of studies on the changes in egg-capsule volume in different nudibranch species (Kress, 1971, 1972) a fresh attempt has been made to obtain some comparative data on egg and capsule sizes and on the course and rate of embryonic development in the three species of *Doto* most commonly found in the Plymouth area, namely *D. coronata* (Gmelin, 1791), *D. pinnatifida* (Montagu, 1804) and *D. fragilis* (Forbes, 1838).

MATERIAL AND METHOD

This study is based on material collected in the Plymouth area (England) during August and September 1971 and July to September 1974.

Spawn from the three species was either brought in with the animal concerned and the hydroids on which it had been laid, or more frequently was that laid down by animals held in individual glass bowls. The spawns were kept in glass jars in the cold room (10–12 °C) or at room temperature (18–22 °C) with changes of filtered sea water every other day. Every day a portion of each spawn was examined, measurements being made of the capsules' long and short axes. The volume of the capsules was calculated according to the rotation-ellipsoid formula described in earlier papers (Kress, 1971, 1972). Differences in development within a single spawn were marked, especially during advanced development, although it should be noted that only capsules containing veligers of comparable stages were measured. The ratio between capsule volume at hatching and the egg-capsule volume itself has been entitled the 'enlargement factor'. Not all the veligers within a

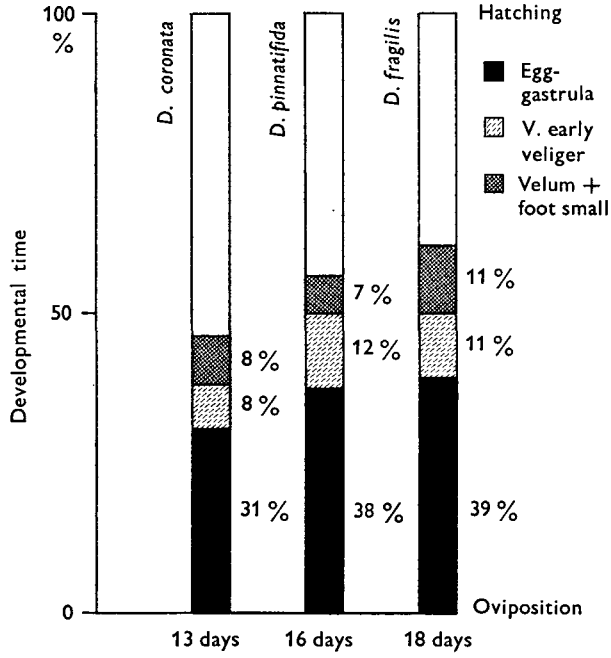
spawn hatch at the same time – this is especially noticeable in the larger spawns of *D. fragilis* and *D. pinnatifida*. Hatching was stated to have taken place on the day when the majority of veligers were observed to be free of their capsules.

RESULTS

General observations concerning the embryonic development

In all three species oviposition occurs most usually during the night or in the early morning.

Members of the genus *Doto* belong to the development-Type I, a species with small eggs and planktotrophic larvae (Thompson, 1967). Certain features of embryonic development can be followed in the intact capsule. Some stages have been defined according to changes of the exterior morphology and used as pointers for grouping. The



Text-fig. 1. Comparison of time intervals necessary to complete gastrulation and early stages of veliger growth.

sequence of observable embryonic events appears similar in each of the three species investigated, only the time-course of the events proving somewhat variable (Text-fig. 1). If the number of days between oviposition and hatching is taken as 100%, *Doto coronata* needs 4 days (=31% of the whole development) to complete gastrulation, while *D. pinnatifida* takes 6 days (38%) and *D. fragilis* 7 days (39%).

After gastrulation the embryo assumes a more ovoid shape and the apical area becomes ciliated (Pl. IIH and I). Later the first signs of foot and velum appear. This stage was called 'very early veliger' (Pl. IIIA) and the period it lasted varied somewhat in the three species. When foot and velum are more distinct, the embryo is classified as 'small'

(Pl. III B). Different time intervals appear to be necessary within the three species before this shape is attained. At this stage the embryo starts to rotate. The typical veliger form is then rapidly assumed. It would be arbitrary to separate more definite stages within the development on the basis of one daily measurement only.

The capsule membrane becomes softened at hatching time. The veliger distorts the capsule by the beating movement of its cilia along the wall.

Doto coronata

The spawning animals were *Doto coronata* with no red marks at the base of the inner side of the cerata (cf. Schmekel & Kress, in the Press). They were between 6 and 11 mm long (Pl. I A). The spawn was found mostly on *Nemertesia antennularia*, a few on *Obelia geniculata*. It consists of a gelatinous colourless ribbon folded in a zig-zag manner, measuring 1–3 mm in width and up to 10 mm in length (Pl. II A); the eggs themselves are white. In all, 18 spawns were used to obtain measurements. Three were measured from oviposition to hatching and another 15 from blastula stage to hatching or to intermediate stages of development. The egg capsules have a smooth surface and contain one egg per capsule. Twins could occasionally be detected in some of the spawns. The average egg diameter is 72·5 μm (62·4–81·6).

As a rule 1–3 polar bodies were visible, oval or round in shape and with a diameter of 4·8–7·2 μm. These could be seen until very shortly before hatching. At 10–12 °C the developmental period was 13 days, at room temperature (18–22 °C) 7–8 days.

Changes in capsular size and volume (Table 1)

The ratio between the egg-capsule volume and the egg volume itself was 1·9 (1·6–3·0). The capsule volume showed no increase until the earliest signs of veliger development, and the first appearances of minute velar cilia. Differences in volume measurements up to that stage are due to differences within the spawn itself. As the veliger grows there is an increase in capsule volume. When the veliger shows its first distinct characteristics the capsule still fits closely to it, but from this point on the capsule obviously increases faster than the veliger and this allows more space for a free and active rotation of the veliger.

The ratio between the hatching volume of the capsule and the egg-capsule volume was entitled the 'enlargement factor', the average for *D. coronata* being 3·5 (3·1–4·0). The ratio between the capsule length and width at the onset of development was 1·3 at hatching 1·1, the capsules becoming more spherical in shape (Pl. III D). The newly hatched veliger is type I (Thompson, 1961) the shell measuring 116 μm (100·8–124·8) in length, 95 μm (86·4–100·8) in width and 95 μm (86·4–100·8) in height (Pl. III E).

Comment

The spawn of *Doto coronata* has been pictured by Alder & Hancock (1854–5) and by Larsen (1925), and described by Løyning (1927). Our measurements of egg diameter (68–73 μm) accord with Miller's data (1958) and with those of Thompson (1967) (61–67 μm). One of the spawns examined contained eggs of an exceptionally large diameter, 76–81·6 μm, which therefore increased the average measurement. On the other hand, Schmekel gives 75 μm as a mean value for the Mediterranean *D. coronata*

TABLE 1. *DOTO CORONATA*: MEAN VALUES OF EGG AND CAPSULE SIZES AND VOLUMES

Stage	Mean value (μm)	Standard deviation	Ratio between
Egg diameter	72·5	6·8	
Capsule size			
Egg to gastrula stage			
Length	103	6·3	Length and width: 1·3
Width	82	6·7	
Hatching			
Length	142	8·2	Length and width: 1·1
Width	131	9·5	
Veliger shell			
Length	116	4·6	Veliger shell length and
Width	95	4·2	egg diameter: 1·6
Height	95	4·2	
	Volume ($\mu m^3 \times 10^3$)		
Egg	198	58·6	Egg-capsule volume and egg volume: 1·9
Capsule containing Egg, Blastulae, Gastrulae	379	72·5	
Capsule containing veliger			
Very early	379	50·4	
Foot and velum small	451	64·1	
Foot and velum medium	532	52·9	
Foot and velum distinct	647	91·5	
Foot and velum distinct	753	187·9	
1 day before hatching	814	156·6	
2 days before hatching	1109	189·7	
Hatching	1312	270·8	Capsule volume at hatching and egg-capsule volume: 3·5

(personal communication). The occasional occurrence of twins is mentioned in Pelseneer (1911) and Miller (1958) and this also accords with our observations. The colour of the spawn observed was white in every instance but Miller (1958) records some of a pink colour. The developmental time given by Thompson (1967) is 16 days at 10 °C. In our case all three species were kept in a cold room with slightly varying temperature between 10 and 12 °C, a fact which would account for the somewhat faster development (13 days). Hadfield (1963) mentioned 7 days at 10 °C, and this makes it very doubtful that he really dealt with *D. coronata* at all.

Doto pinnatifida

All the animals concerned correspond to the description given by Schmekel & Kress (in the Press) (Pl. 1B). The spawn was obtained from large animals, from 17 to 22 mm in length. Spawns used were found in the Plymouth area on *Nemertesia antennularia* together with the animals concerned, or were those laid down by animals held in glass jars in the laboratory. The spawn consists of a strongly undulated ribbon measuring up

to 35 mm in length (unstraightened), the ribbon itself being 3–5 mm in width (Pl. II B). The eggs are pink or white. The same animal is capable of producing a spawn of either colour.

TABLE 2. *DOTO PINNATIFIDA*: MEAN VALUES OF EGG AND CAPSULE SIZES AND VOLUMES

Stage	Mean value (μm)	Standard deviation	Ratio between
Egg diameter	95·5	4·2	
Capsule size			
Egg to gastrula stage			
Length	140	11·20	Length and width: 1·3
Width	113	5·8	
Hatching			
Length	192	18·5	Length and width: 1·1
Width	175	10·6	
Veliger shell			
Length	160	10·8	Veliger shell length and
Width	124	6·6	egg diameter: 1·7
Height	123	8·9	
	Volume ($\mu m^6 \times 10^3$)		
Egg	455	62·4	Egg-capsule volume and egg volume: 2·1
Capsules containing Eggs / Blastulae / Gastrulae	937	140·9	
Capsules containing veligers			
Very early	938	147·0	
Foot and velum small	1047	92·6	
Foot and velum medium	1178	70·9	
Foot and velum distinct	1466	138·6	
Foot and velum distinct	1758	140·3	
2 days before hatching	2494	239·4	
1 day before hatching	2742	323·8	
Hatching	3202	724·1	Capsule volume at hatching and egg-capsule volume: 3·4

In all twelve spawns were measured, two from oviposition to hatching, the remaining ten for different lengths of time during the developmental process. The egg capsules have a smooth surface and normally contain one egg per capsule, although pairs of twins can now and again be observed. A few empty cases of variable shape are mostly found at each end of the ribbon. The average egg diameter is 95·5 μm (91–108). Usually 1–3 polar bodies were noted and these when oval measured *ca.* $4{\cdot}8 \times 12$ μm or when round $9{\cdot}6 \times 9{\cdot}6$ μm in diameter. Again they could be seen until a late stage in development. At 10–12 °C the developmental period was about 16 days, at room temperature (18–22 °C) 9–10 days.

Changes in capsular size and volume (*Table 2*)

The ratio between the egg-capsule volume and the egg volume itself was 2·1 (1·8–2·4). The capsule volume showed no increase until the earliest signs of veliger development

and the first appearances of minute velar cilias. As in the other two species, there is a certain variation of capsule size within each spawn. As the veliger grows and starts to rotate there is a marked increase in capsule volume which is maintained until the hatching stage is reached. The ratio between the capsule volume at hatching and that of the original egg-capsule volume (enlargement factor) was 3·4 (2·9–4·1). The ratio between the capsule length and width at the beginning of development was 1·3 and at the time of hatching 1·1, and again the shape of the capsule became more spherical. The newly hatched veliger is type I (Thompson, 1961), the average shell measuring 160 μm [134–172] in length, 124 μm [110–137] in width and 123 μm [100·8–134] in height.

Comment

Miller (1958) describes the egg colour as white or pink, as found in our case. No further data are available on egg diameter or time of development.

Doto fragilis

The spawning animals measured between 18 and 28 mm (Pl. Ic). Spawns used were found in the Plymouth area on *Nemertesia antennularia* only, together with the animal concerned, or were those laid down by animals after they had been brought into the laboratory. The spawn consists of a band of tightly packed windings, measuring up to 3·5 cm in length (unstraightened), the band itself being up to 5 mm in width (Pl. IIc). Again, at the end of the band empty capsules could be found. The eggs themselves were white. In all nine spawns were measured, four from oviposition to hatching, the remaining five for different lengths of time during development.

The egg capsules have a smooth surface and contain one, sometimes two eggs per capsule (Pl. IIIa). Some spawns show no twins at all, others have up to 50%. Sections of a spawn may show a prevalence of twin ova. The average egg diameter is 83 μm (76·8–86·4). As a rule 1–3 polar bodies were noted, oval or round in shape and of an average diameter 7·2 × 9·6 μm. They were still recognizable shortly before hatching. At 10–12 °C the developmental period was about 18 days, at room temperature (18–22 °) 9–10 days.

Changes in capsular size and volume (*Table 3*)

The ratio between the egg-capsule volume and the egg volume itself is 2·4 (2·0–2·6). There is no change in capsule volume up to the gastrula stage, then a slight increase can be noticed until the first signs of veliger development. The marked increase of capsule volume, however, starts at the same early veliger stage as in the other two species described. The enlargement factor (ratio between volume of the capsule at hatching time and egg-capsule volume) is 3·8 (3·4–4·3). The ratio between the capsule length and width after oviposition is 1·3, at hatching time 1·1, i.e. the capsules show the same tendency to become more spherical as in the other two species described. The newly hatched veliger is type I. The average shell measuring 145 μm [134–158] in length, 111 μm [106–120] in width and 113 μm [106–120] in height (Pl. IIIf, g).

TABLE 3. *DOTO FRAGILIS*: MEAN VALUES OF EGG AND CAPSULE SIZES AND VOLUMES

Stage	Mean value (μm)	Standard deviation	Ratio between
Egg diameter	83	2·2	
Capsule size			
Egg to gastrula stage			
Length	131	8·2	Length and width: 1·3
Width	101	4·8	
Hatching			
Length	186	10·6	Length and width: 1·1
Width	164	7·9	
Veliger shell			
Length	145	6·3	Veliger shell length and egg diameter: 1·7
Width	111	5·2	
Height	113	4·4	
	Volume ($\mu m^3 \times 10^3$)		
Egg	300	33·1	Egg-capsule volume and egg volume: 2·4
Capsules containing Eggs / Blastulae / Gastrulae	698	87·4	
Capsules containing veligers			
Very early	744	78·1	
Foot and velum small	853	113·2	
Foot and velum medium	1057	106·1	
Foot and velum distinct	1403	190·4	
Foot and velum distinct	1977	361·4	
1 day before hatching	2101	296·7	
2 days before hatching	2243	351·0	
Hatching	2666	365·6	Capsule volume at hatching and egg-capsule volume: 3·8

Comment

One typical way of laying down their spawn is shown in Alder & Hancock (1845–55). Our measurements of egg diameters are within the range of those measured by Miller (1958) of 70–87 μm. He observed pink-coloured eggs as well as white ones. We have never found a pink *D. fragilis* spawn.

Salinity tests

The observed changes in capsule volume in *Doto* as well as in other nudibranchs (Kress, 1971, 1972), and interest in possible factors responsible for this phenomenon, led us to study the capsule behaviour in different salinities. Only a few preliminary tests have been performed to narrow the possible field for further experiments, but nevertheless some interesting results emerged. The sea-water salinity measured for rearing the veligers under normal conditions was 35·0‰.

Blastula stages put into jars with differently diluted sea water for further development showed some striking differences between various species of opisthobranchs. Both the species tested, *D. coronata* and *D. fragilis*, reacted in the same way. Even small changes

proved damaging (Table 4). *Placida dendritica* developed normally and without delay even in sea water of only 26·25‰ salinity. In no case was any increase in capsule volume found which exceeded the normally measured volume as shown in Hagerman's paper (1970) for *Elysia viridis*.

TABLE 4. EFFECT OF SALINITY ON EMBRYONIC DEVELOPMENT

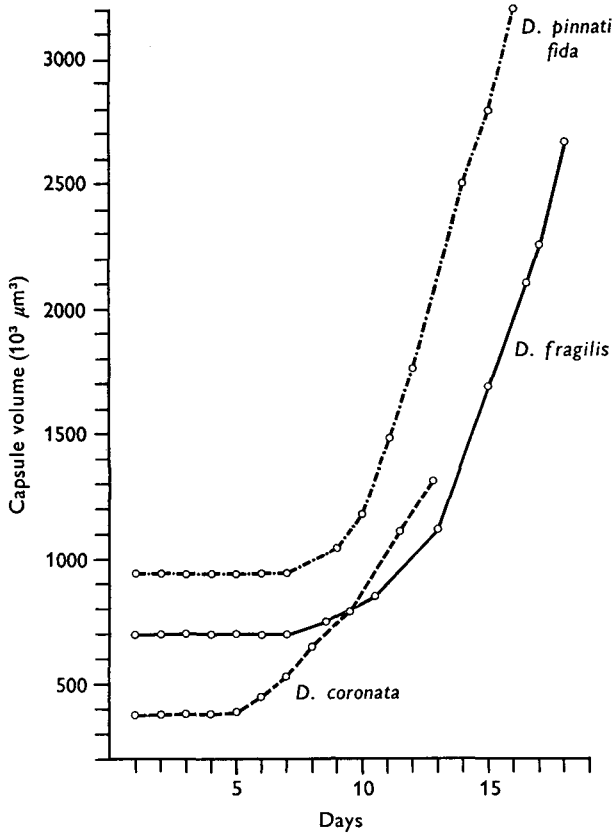
Salinity	Results
24·5 % (diluted 33 %)	All embryos dead within 48 h
26·25 % (diluted 25 %)	A few embryos reach a very early veliger stage at the time when normally kept animals hatch
31·5 % (diluted 10 %)	Two out of 10 embryos hatched with 2 days delay compared with normally kept animals. The others are retarded or died at different stages of development
33·25 % (diluted 5 %)	Seven out of 10 embryos hatched with a delay of 2 days

TABLE 5. COMPARATIVE EMBRYOLOGICAL DATA OF *D. CORONATA*, *D. PINNATIFIDA* AND *D. FRAGILIS*

Mean values of diameters, volumes and different ratios	*Doto coronata*	*Doto pinnatifida*	*Doto fragilis*
Egg diameter (μm)	72·5 μm	95·5 μm	83 μm
Egg volume ($\mu m^3 \times ^3$)	198	455	300
Ratio: egg-capsule volume/egg volume	1·9 (1·6–3·0)	2·1 (1·8–2·4)	2·4 (2·0–2·6)
Ratio: capsule length/width (egg-capsule)	1·3	1·3	1·3
Ratio: capsule length/width (hatching stage)	1·1	1·1	1·1
Ratio: length capsule (hatching stage)/length egg capsule	1·4	1·4	1·4
Ratio: volume capsule at hatching/egg-capsule volume = enlargement factor	3·5 (3·1–4·0)	3·4 (2·9–4·1)	3·8 (3·4–4·3)
Capsule volume at hatching ($\mu m^3 \times 10^3$)	1312	3202	2666
Veliger shell (μm)			
Length	116	160	145
Width	95	124	111
Height	95	123	113
Ratio: veliger shell width/height	1	1	1
Ratio: veliger shell length/width or height	1·2	1·3	1·3
Ratio: length of capsule at hatching/veliger shell length	1·2	1·2	1·3
Ratio: veliger shell length/egg diameter	1·6	1·7	1·7
Development time (10–12 °C) in days	13	16	18

DISCUSSION

The summarized facts of egg-capsule changes during development of the embryo in Text-fig. 2 exhibit distinct differences within the three species after oviposition as well as at hatching time, thus the specific increase characteristics are almost the same. Table 5 on the other hand shows that in spite of the above-mentioned differences in size and volume of capsule and egg itself, the ratio of some of the features is very similar if not identical in all three species. This holds true for the ratio capsule length/capsule at hatching stage, or the ratio length of the capsule after oviposition/length at hatching. The ratio egg-capsule volume/egg volume appears different in the given mean size, but there is considerable overlapping. The enlargement factor itself is very similar and varies



Text-fig. 2. Mean values of capsule volumes during development of the three *Doto* species.

practically within the same range for all three species. The shell sizes of the newly hatched veligers are, however, distinctly different. The shell sizes display the expected relation to the egg diameter. The species with the smallest egg diameter, *D. coronata*, produces the smallest veliger, *D. pinnatifida* the largest, the ratio between veliger shell length and egg diameter being in all three cases 1·6 or 1·7 respectively. The proportions of the veliger shells themselves are practically identical. The sizes of capsules are more uniform and the development is much more synchronous at the beginning of embryogenesis and show much greater differences nearer to the time of hatching, in the embryo itself as well as in capsule volume. The latter is clearly expressed in the greater standard deviation given in Tables 1, 2 and 3.

After hatching the larvae swim upwards (first obligatory phase) and get caught in the water-surface film as described by Thompson (1958). Experiments aimed at obtaining larval settlement have so far proved unsuccessful and nothing is known about the duration of planktonic life. Because it was not possible to follow the entire life cycle, examination of any differences in egg or capsule sizes of first or later spawns laid by the same animal could not be carried out. Data from other species of the suborder Dendronotoidea are so scarce that no comparison within this group is at present possible.

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EXPLANATION OF PLATES

PLATE I

A, *Doto coronata*, 10 mm; B, *Doto pinnatifida*, 20 mm; C, *Doto fragilis*, 22 mm.

PLATE II

A. *Doto coronata*, spawn, length 10 mm.

B. *Doto pinnatifida*, spawn, length 22 mm.

C. *Doto fragilis*, spawn, length 28 mm.

D. Egg in egg-capsule immediate lyafter oviposition and prior to extrusion of polar body (*D. fragilis*). (× 167)

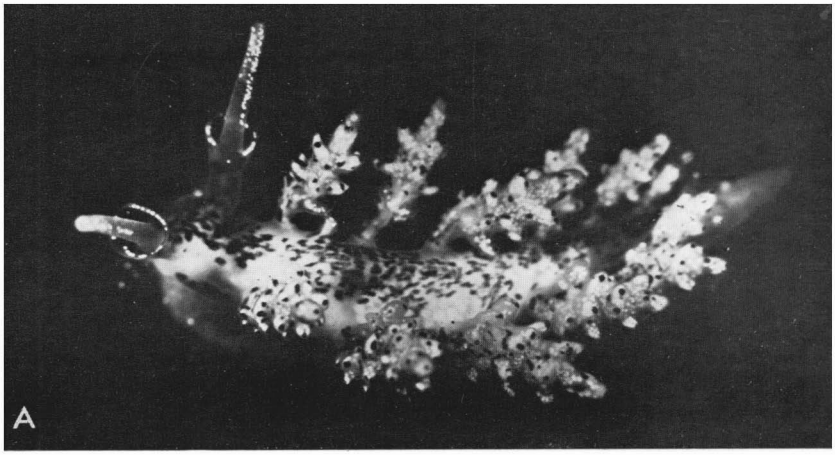
E. Egg during formation of the first polar body (*D. fragilis*). (× 167)

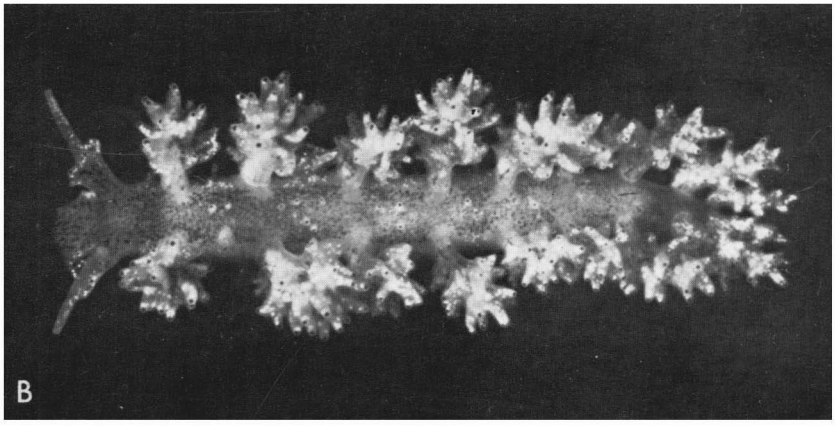
F. Blastula with two polar bodies (*D. fragilis*). (× 167)

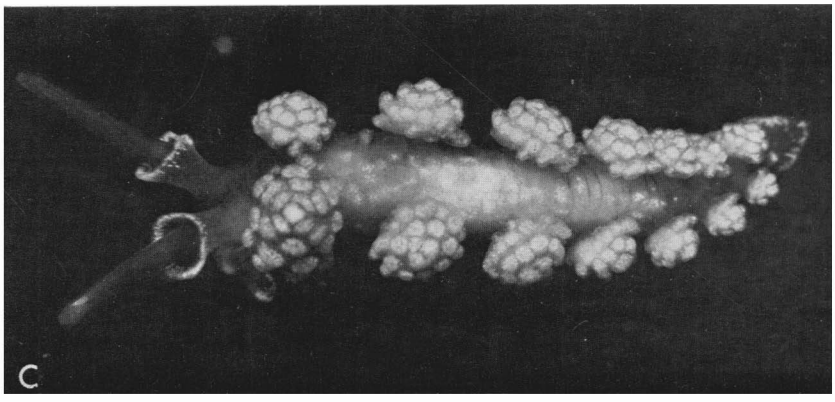
G. Gastrula (*D. pinnatifida*). (× 167)

H. After gastrulation, beginning of the formation of a very early veliger (*D. fragilis*). (× 167)

I. Very early veliger, first signs of cilia. (× 167)







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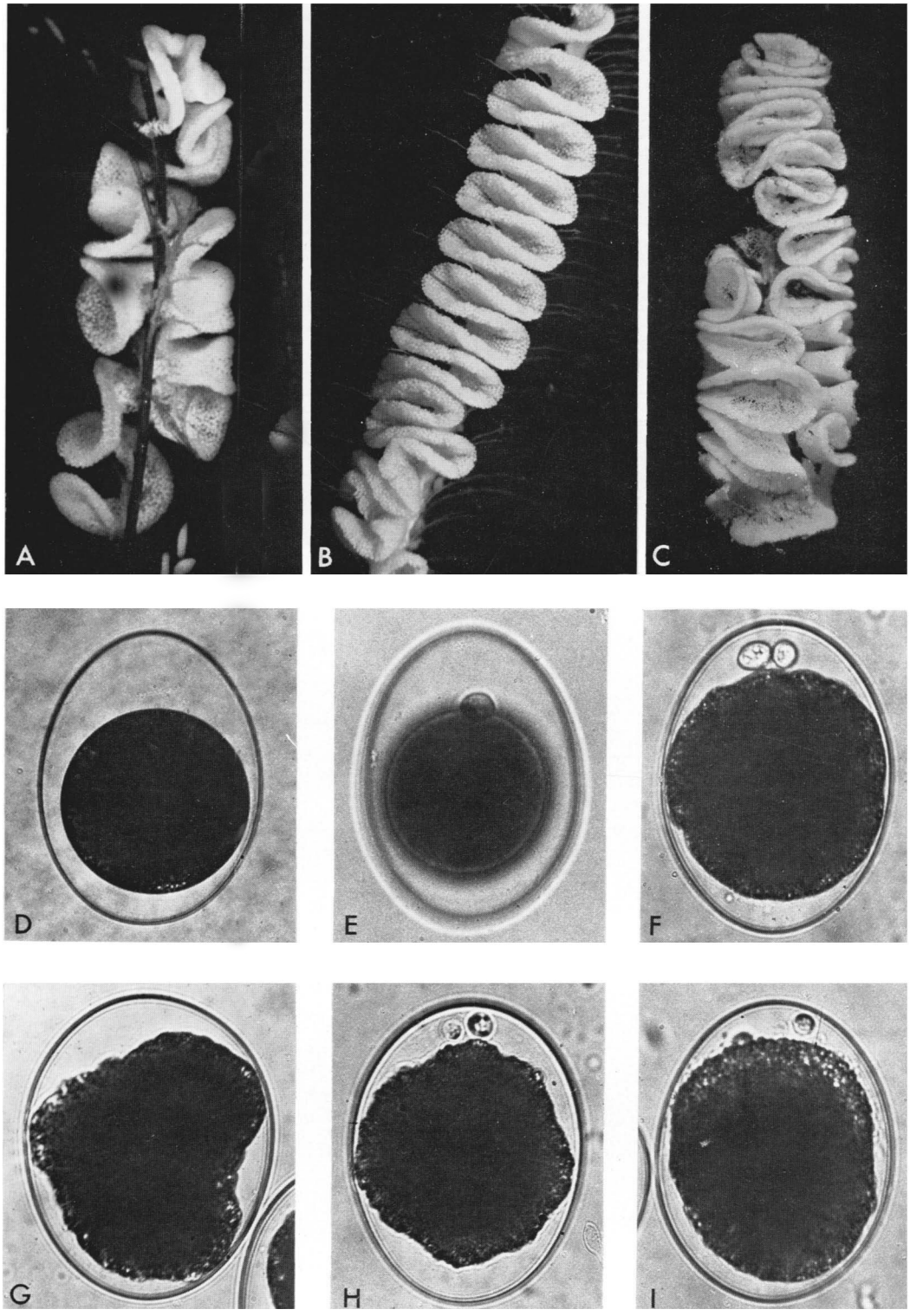

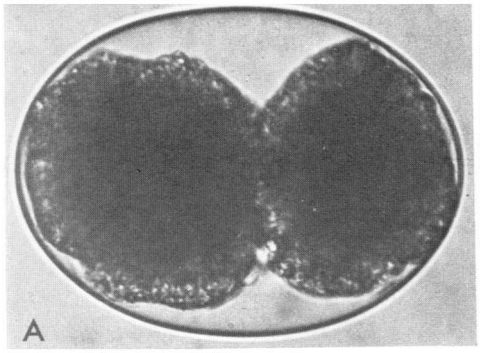

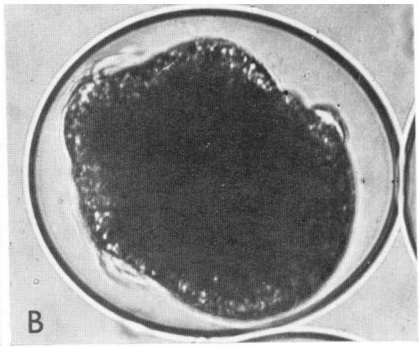

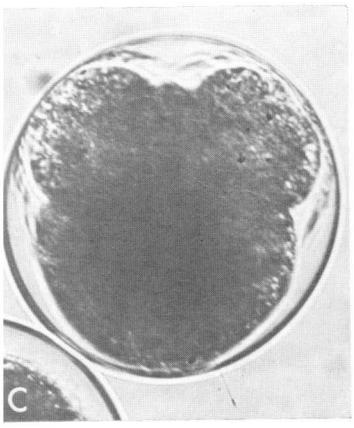

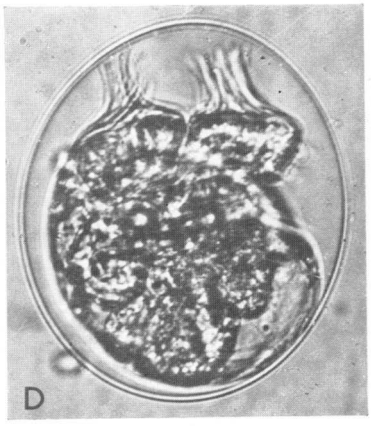

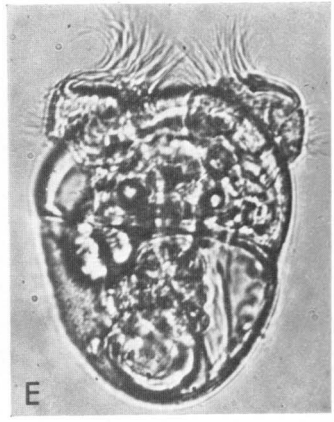

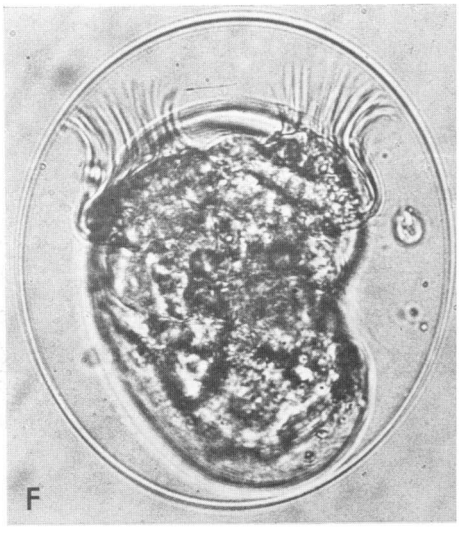

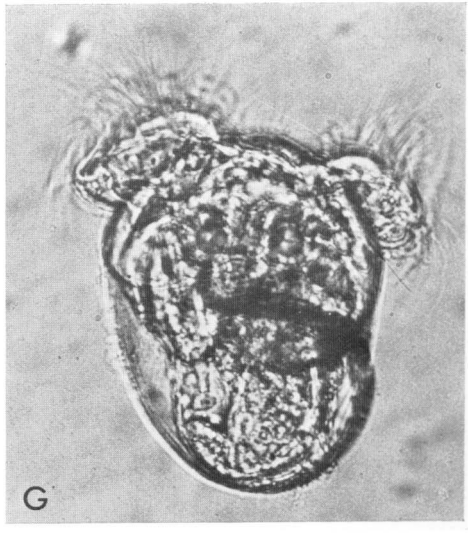

PLATE III

(Magnification × 167)

A. Twin-formation, very early veliger (*D. fragilis*).

B. Small veliger, first signs of foot and velum (*D. pinnatifida*).

C. Young veliger (medium) (*D. pinnatifida*).

D. Veliger shortly before hatching (*D. coronata*).

E. Newly hatched veliger (*D. coronata*).

F. Veliger shortly before hatching (*D. fragilis*).

G. Newly hatched veliger (*D. fragilis*).