Reproductive seasonality in the comatulid crinoid *Antedon bifida* (Pennant) from the English Channel

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SUMMARY

Genital pinnules from the northeast Atlantic comatulid crinoid *Antedon bifida* (Pennant) were sampled monthly over a five-season period, from a site at Berry Head, English Channel, U.K. *A. bifida* uniquely broods its spawned ova and early larvae, during the period May to July. Nevertheless, a high level of sexual maturity was maintained throughout the annual cycle in both males and females. Three cellular gametogenic features: facets on the mature oocyte cell wall, nutritive phagocytes in the gonad lumen and yolk nuclei in the pre-vitellogenic oocytes, were present at all times of the seasonal cycle. Development of gonads along the length of an arm was not synchronous. All specimens sampled had pinnules, sections of arm or whole arms missing, and a mean of 17% of all pinnules from the population were missing or

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regenerating. The commonest small fish in the area, *Crenilabrus melops*, was observed to nibble off the genital pinnules. A strategy is suggested involving toleration of such losses, in place of less-expendable parts of the body.

1. INTRODUCTION

(*a*) *Preliminaries*

The echinoderm Class Crinoidea includes sea-lilies (stalked crinoids) and feather-stars (free-living comatulids). Whereas sea-lilies are mainly deep-water forms, comatulids occur in shallow sub-littoral seas, but only rarely intertidally. Because extended sampling programmes for subtidal animals are not easy to sustain, the reproductive processes, in particular seasonality, have been less studied in crinoids than in other echinoderm classes.

A range of reproductive strategies has emerged from studies previously undertaken. For instance, first, the Japanese comatulid *Oxycomanthus japonicus* (Müller) shows an ultra-precise seasonal spawning pattern, in which release of gametes occurs on one fairly predictable day relative to the annual lunar cycle (Dan & Dan 1941*a*; Dan & Kubota 1960; Holland *et al.* 1975); secondly, the Caribbean *Nemaster rubiginosa* (Pourtales) (Mladenov & Brady 1987) and the Taiwanese *Comanthus parvicirrus* (Muller) and *Comatella maculata* (P. H. Carpenter) (Chang *et al.* 1990) show a more conventional seasonal cycle; and, thirdly, the Eastern Pacific *Florometra serratissima* (A. H. Clark) shows continuous reproduction throughout the year (Mladenov 1986). Other studies, such as Rutman & Fishelson (1985) on three species from the Red Sea, and Vail (1987*a*) on five species from the Great Barrier Reef, have given further examples of continuous reproduction. The present study on the northeastern Atlantic comatulid, *Antedon bifida* (Pennant), also reveals an example of reproduction involving continuous gametogenesis, but in this case it seems likely that spawning is seasonal, and that the maintenance of the gonads across the rest of the year is a response to predation.

In many animals the 'gonad index' method gives a reliable and reasonably straightforward method of defining seasonal cyclicity (Moore 1934), but the method has certain shortcomings (Nichols & Barker 1984*a*,*b*; Nichols *et al.* 1985), and should generally be accompanied by a cytological study of the gametogenic process (Giese & Pearse 1974). Crinoids generally preclude the use of the gonad index method, as the gonads of each animal are subdivided into many hundreds of small units, one to each genital pinnule, and, additionally, the arms are seldom wholly intact. So measures of both gonad and body 'size' are impracticable to undertake over an acceptable timescale and with an adequate sample size. Crinoid reproductive cycles are therefore generally studied using, for females, oocyte size–frequency plots and, for males, either the identification of spermatocyte developmental stages or measurement of the thickness of formative layers within the testis. An acceptable time span to demonstrate species-specific seasonality in temperate marine animals is usually taken as at least three calendar years. Because aspects of the results from the first three years were anomalous, the present study has utilized oocyte size frequencies and spermatocyte developmental stages over a sampling period of five seasons.

Antedon bifida is the only shallow-water comatulid crinoid recorded from British waters. It occurs from the Shetland Isles in the north, to Liberia in the south, and the Azores in the west (A. M. Clark 1972). It is particularly common in waters adjacent to the north and west coasts of the British Isles, at depths of about 5 m below chart datum to about 450 m (Mortensen 1927), but is occasionally found intertidally, particularly in southwest England, in groups of a few individuals. It is at its densest below 15 m (Clark & Clark 1967). It typically occurs on boulders and reefs at densities up to 1500 m^{-2} in areas subject to tidal currents of up to 50 $cm\ s^{-1}$ (La Touche 1978), a prerequisite for food resources adequate to sustain high densities of crinoids (Meyer & Ausich 1983). One such locality is the waters around Berry Head, South Devon, U.K. (figure 1), where it occurs from about 5 m depth to the depth at which the rock gives way to sediment, at about 10 to 20 m depth (Hunt 1884, in Clark & Clark 1967; Warner 1979). Here, it clings to the near-vertical cliff-face, to boulders and to other hard substrata.

(*b*) *Anatomy of the gonads*

The gonads lie between the radial coelomic canals and the underlying pinnular ossicles. The expansion of the gonads in season partly occludes the coelomic structures, and as each gonad expands with maturity the pinnular wall becomes very thin. Development of gonads during ontogeny starts in the more proximal pinnules of an arm, but subsequently, as maturity increases or the spawning season approaches, all gonads may maintain approximately the same stage of maturity and size (Dimelow 1958; Nichols 1991); outside the spawning season, there is variation in the developmental state of the pinnules. The gonads originate ontogenetically from a strand of tissue, the genital cord (genital rachis), which lies suspended within a haemal sinus (the so-called genital tube), which is itself suspended within a branch of the perihaemal system (the genital canal), and in arms and pinnules lies between the two adoral coelomic canals (Semper 1875; W. B. Carpenter 1876; Hamann 1889; Chubb 1926; Holland 1992). The genital cord is thin where it branches into each pinnule, then expands to form the gonad. As maturity proceeds, a

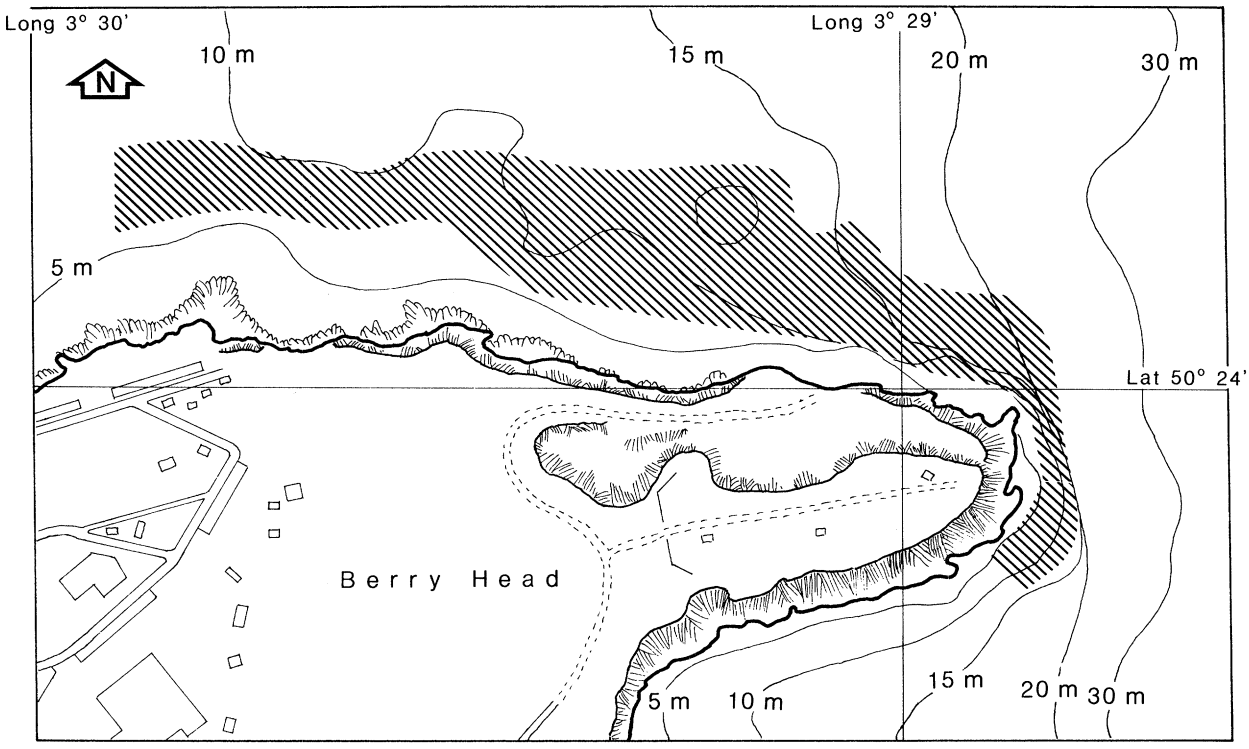


Figure 1. Map of the distribution of *Antedon bifida* (cross-hatched) at a sampling site on the north coast of Berry Head, South Devon, U.K. (latitude 3° 29′, longitude 50° 24′; O.S. reference: SX 940568).

central lumen develops in the gonad. Each gonad therefore has an outer epithelium (the wall of the haemal sinus), and, separated by a haemal space, an inner layer (the wall of the genital cord) around the central lumen. It is the inner layer of the cord that forms the germinal epithelium and from which the gametogonia originate. The inner layer may also give rise to nutritive phagocytes (accessory or nurse cells). The developing oocytes may undergo ovulation by pushing through a temporary pore in this layer to enter the gonad lumen (Mladenov 1986; Holland 1992), and the nutritive phagocytes may similarly migrate.

(*c*) *The gametogenic cycle*

The process of gametogenesis in *Antedon bifida* has been widely studied (Thomson 1865; W. B. Carpenter 1866; Barrois 1886; Perrier 1886; Cuenot 1892; Chubb 1906; A. H. Clark 1921; Harvey 1931), because, first, its eggs have provided convenient material for cytologists and developmental biologists, and, secondly, because phyletic interest was generated by the occurrence of a stalked *pentacrinule* larval stage in the life history of the animal, considered to represent recapitulation.

Except in young specimens, immature gonads are relatively uncommon. The process of gametogenesis is standard (Giese & Pearse 1974; Franzen 1987; Wourms 1987): gametogonia form by proliferation of the germinal epithelium forming the wall of the genital cord. A lumen appears early on, into which the maturing gametes are thrust as they grow. The cytological accompaniments to oogenesis have been described in detail (Chubb 1906; Harvey 1931; Cotronei & Urbani 1957), but spermatogenesis has not been followed in such detail (Holland 1992). For the developing eggs, considerable interest has been shown in the appearance of the so-called 'yolk nucleus' (Holland 1992), a usually crescentic, dark-staining body of ribosomal material which appears prior to yolk formation, then disappears towards oocyte maturity.

A. bifida is said to be fully grown after one year, and sexually mature by the summer of its second year (Thomson 1865). Where specific identification is certain, spawning has been observed in *A. bifida* only from May to July (Chadwick 1907; Clark 1921; Lahaye 1987). Oocytes may remain in the ovary at around their sheddable size of 150 μm or above for a considerable part of the year, and their cell walls become sculptured into facets (Ludwig 1880*a*; A. H. Clark 1921; Clark & Clark 1967; Holland *et al.* 1975; Holland 1977; Lahaye & Jangoux 1985).

(*d*) *Gamete shedding*

Eggs and sperm are shed through one or more temporary star-shaped gonopores on the distal side of each genital pinnule (Lahaye & Jangoux 1985). Reichensperger (1912) describes the occurrence of 'cement-secreting cells' on the ventral outer pinnular walls, close to the spot where the temporary gonopore opens, and saw these cells empty their contents over the extruded eggs after spawning. Holland & Grimmer (1975) observe that both sexes of *Oxycomanthus japonicus* secrete epidermal mucus from the pinnular wall, with females exuding substantially more than males and decreasing their secretion more rapidly after spawning, suggesting that this mucus acts as a

temporary binding for the eggs. Hendler & Meyer (1982) show photographs in which the eggs of the Indonesian comatulid *Capillaster multiradiata* (L.) are apparently bound in mucus as they are emitted, but the strands are not held by the adults. Spawning in one individual evokes others nearby to spawn (Bury 1888; Lahaye & Jangoux 1985). In *A. bifida*, shed eggs adhere in clusters to the pinnular wall adjacent to the temporary gonopore (Miller 1821; Forbes 1841; Busch 1851; Perrier 1886; Bury 1888; Chadwick 1907; Cuenot 1948; Breimer 1978; Giese & Kanatani 1987; Nichols 1991).

(*e*) *Synchrony of development along one arm*

Previous crinoid studies have suggested that developmental state is synchronous in all genital pinnules of an individual (Bury 1888; Mladenov 1986; Mladenov & Brady 1987; McClintock & Pearse 1987), whereas others (e.g. Chadwick 1907; Rutman & Fishelsen 1985; Vail 1987*a*) suggest that gonads on more proximal pinnules mature first, implying sequential development. Where such matters have been addressed, previous workers have tended to select gonads for study as representative of the reproductive state of the individual, from pinnules situated within the proximal quarter of the genital region of one arm.

(*f*) *The evolution of gonad position in crinoids*

Comatulid crinoids represent the highest grade of organization attained by the class Crinoidea (Meyer & Macurda 1977). Early fossil crinoids, from Cambrian to Triassic rocks, are exclusively stalked forms, many with a large and heavily plated theca enclosing the principal systems of the body. Lateral branches from the arms, the pinnules, probably arose polyphyletically during the Ordovician Period (Simms 1990).

In Triassic rocks, crinoids of the order Articulata appear, with greater powers of movement available to the arms. This is taken an important stage further in the Upper Triassic, when stemless forms, the Comatulida, appear, taking advantage of the flexibility of the arm bases to permit swimming by the adult. This freeing of the body from the fetters of a permanent stalk permitted such free-living forms to flourish in the Cretaceous period, and the preponderance of comatulids over the stalked forms has continued to Recent times, at least in seas of shallow to moderate depth.

The location of the gonads in fossil crinoids is not clear (Breimer 1978), although it is usually assumed that they resided within the theca (Lane & Sevastopulo 1981; Holland 1992), and that the heavy plating was an anti-predator adaptation (Lane 1984). The anal spire, a conical or elongated tube housing the distal section of the rectum and the anus, may show considerable enlargement and skeletal ornamentation in some Palaeozoic crinoids, particularly of the suborder Inadunata (Ubaghs 1978), and it has been suggested that such developments may have been associated with housing the gonad (Lane 1984). In present-day crinoids, the gonads are borne on the proximal two-thirds to four-fifths of the pinnules arising laterally on each arm. Such a position may have significance in an interpretation of the reproductive strategy adopted by *Antedon bifida*.

2. MATERIALS AND METHODS

(*a*) *Locality and sampling*

All samples were taken from a sublittoral site at Berry Head, English Channel (latitude: 3° 29′, longitude: 50° 24′; O.S. grid-reference SX 940568) (figure 1), by diving to depths of between 10 m and 15 m below c.d. The site is a headland in highly energetic water, the substratum being mainly a steep underwater cliff, to which the feather-stars cling. In places there are ledges on which boulders have come to rest, and the animals also cling to these structures (figure 2), as well as to certain macrofauna in the area, such as maiid and cancrid crabs.

Samples ($n = 10$) of individuals with length of longest arm greater than 100 mm were collected approximately monthly between July 1983 and October 1987. Because the site is very exposed, periods of adverse weather affected the sampling frequency.

To assess which genital pinnule of *Antedon bifida* should routinely be sampled for determination of reproductive cyclicity, and also to determine whether an investigation of reproductive synchrony in this animal was justified, pinnules 5 and 10 from one side of an arm from female specimens were collected in October 1982 and February 1983, that is, during the season preceding the start of monthly sampling. Specimens were examined for gonad size and processed to determine oocyte size frequencies. Frequency polygons were plotted (figure 3). An analysis of variance on the transformed data revealed no significant difference between the fifth and tenth pinnule in the October 1982 sample ($p > 0.1$), while in the February 1983 sample the two pinnules showed different development patterns ($p < 0.05$). There was, however, no difference between the tenth pinnules of the two samples ($p > 0.1$). Pinnule 10 was also in the region of greatest gonadial swelling, so this pinnule was selected as the one routinely to be processed as an indicator of gonad condition in all individuals sampled.

As an additional check during the principal sampling period, pinnules 5 and 10 from six specimens from the October 1984 sample were similarly processed, and frequency polygons plotted (figure 5). Here, a higher overall oocyte development occurred in pinnule 5 of each specimen. Means of this sample and that of October 1982 were significantly different ($p < 0.01$), suggesting that the pattern of synchrony between individual pinnules along an arm may differ over the annual cycle, and therefore deserves further study. Photomicrographs of pinnules 5 and 10 from specimen 1 of this sample are given in figure 4, and of the same two pinnules of specimen 5 in figure 6.

(*b*) *Synchrony of development along an arm*

One specimen from each of the samples taken in December 1985 and March 1986 had ovaries on

Figure 2. *Antedon*-covered boulder on underwater ledge at Berry Head, South Devon, U.K.

pinnules 3 to 35, which was the maximum extent of genital pinnules along a single arm encountered during sampling. These and three subsequent samples (May, July and September 1986) were therefore used in a study of interpinnular reproductive synchrony over one annual cycle. Every other pinnule from one arm of a single specimen with minimal pinnular predation was routinely prepared and the oocyte size frequencies plotted. For each specimen, two pinnules distal to the last one showing a recognizable gonad swelling were routinely processed in each case, to ensure that all functional gonads along a single arm had been included.

(*c*) *Treatment of monthly samples*

The tenth genital pinnule was removed from one side of one arm of each specimen on the day of collection, fixed in Bouin's fluid in seawater, cut serially at 7 μm and stained with Ehrlich's haematoxylin and eosin. Sagittal sections were taken to maximize the number of gametocytes visible in each section. The rest of the animal was preserved for subsequent studies. For females, the diameter of the first 50 nucleolar oocytes from each of the first three female specimens taken was measured by eyepiece micrometer to the nearest 10 μm and percentage size frequencies calculated in 10 μm size groups. For males, each specimen was assigned to one of five spermatogenic stages, as follows:

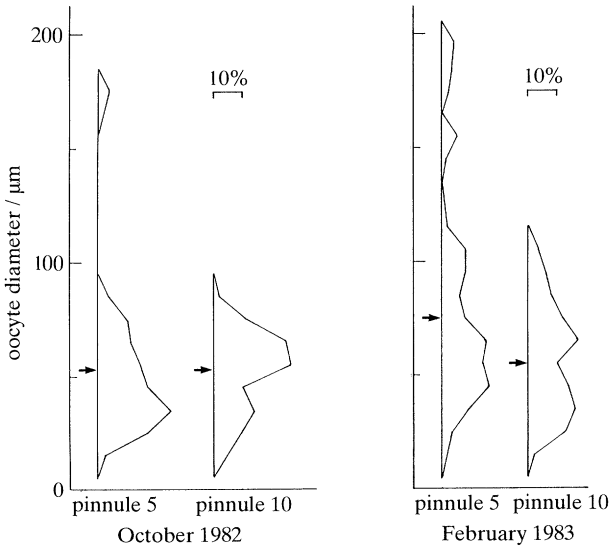


Figure 3. Oocyte size–frequency polygons ($n = 150$) of pinnules 5 and 10 from a single arm of specimens of *A. bifida* collected in October 1982 and February 1983. Mean oocyte diameters are arrowed.

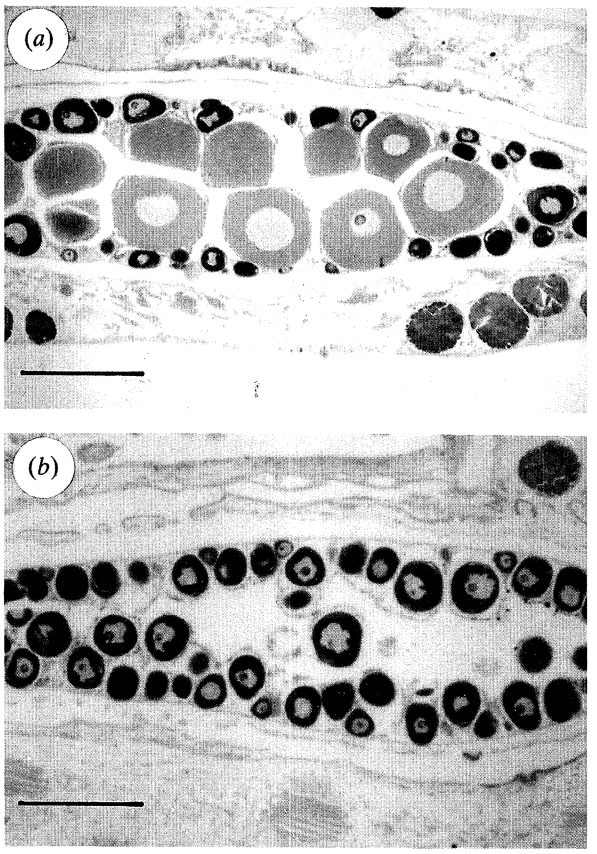


Figure 4. Comparison of ovaries from different pinnules along a single arm of specimen 1 from a sample of *A. bifida* taken in October 1982. (*a*) Pinnule 5, with vitellogenic oocytes of sheddable size in the ovarian lumen and small oogonia and oocytes adjacent to the germinal epithelium. (*b*) Pinnule 10, having only oogonia and pre-vitellogenic oocytes, some being squeezed from the ovary periphery towards its lumen. Scale bars: 200 μm.

Stage 1. Pre-developmental (figure 7). Testis is small and only slightly thicker than the genital cord, and its germinal epithelium unfolded. Very few, if any, sperm remain in the lumen, and phagocytes may be present among the residual sperm or sperm-heads in the lumen.

Stage 2. Recovery (figure 8). The testis is beginning to enlarge from the genital cord, and its germinal epithelium is thickening. The spermatogenic layer is composed mainly of spermatogonia, and is either still unfolded or the folds are just beginning to appear in places.

Stage 3. Proliferation (figure 9). Testis enlarging further, though parts of it may still not be fully expanded from the genital cord. Spermatogenic layer folded into 'pyramids', some already with sperm-tails at their apex. Some mature sperm may lie in the testicular lumen.

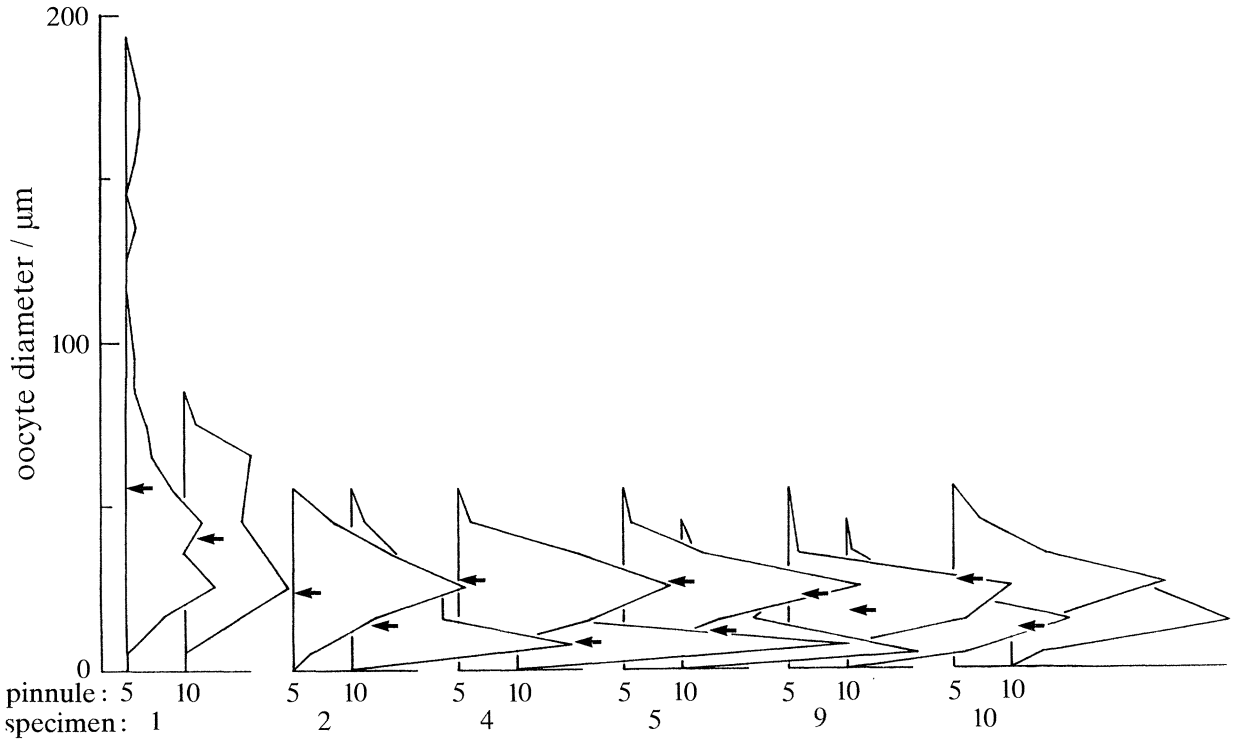


Figure 5. Oocyte size–frequency polygons ($n = 150$) of pinnules 5 and 10 from one arm of six specimens of *A. bifida* from a sample taken in October 1984. Mean oocyte diameters are arrowed.

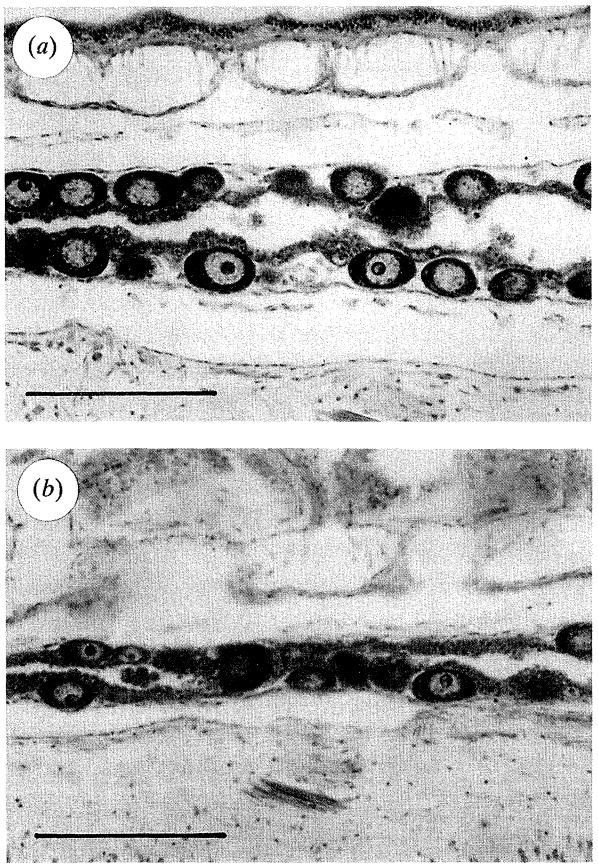


Figure 6. Comparison of ovaries from different pinnules along a single arm from specimen 5 (see figure 5) of *A. bifida* taken in October 1984. Early oocytes in pinnule 5 (*a*) larger than those from pinnule 10 (*b*). Scale bars: 100 μm.

Stage 4. Maturing (figure 10). Testis large and always fully expanded from the genital cord. Spermatogenic layer mostly folded, but with some unfolding (reduction in pyramid height) in places. Few sperm-tails attached to the pyramid apices. The testicular lumen is packed with mature sperm, usually (in fixed material) with a distinct space between the free sperm-mass and those still attached to the germinal pyramids.

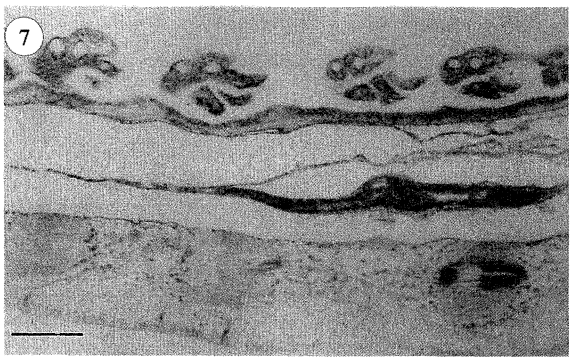
Stage 5. Fully mature (figure 11). Testis large. Folding of the spermatogenic layer reduced or non-existent, with no developing sperm attached. Lumen packed with mature sperm.

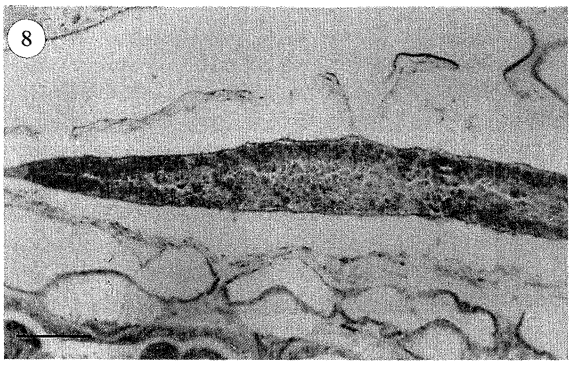
(*d*) *Facets on the egg membrane and nutritive phagocytes in the ovaries*

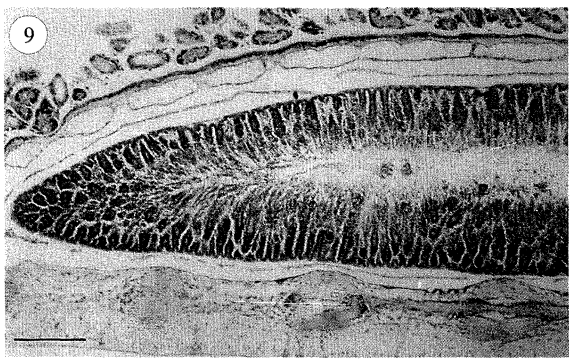
Three female specimens were examined from each monthly sample, and presence or absence of faceted oocytes and nutritive phagocytes noted.

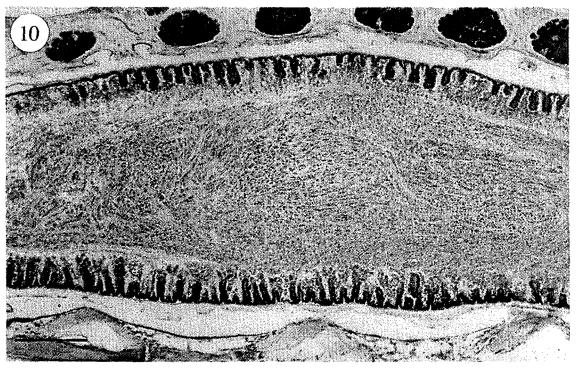
(*e*) *Predation and regeneration of pinnules*

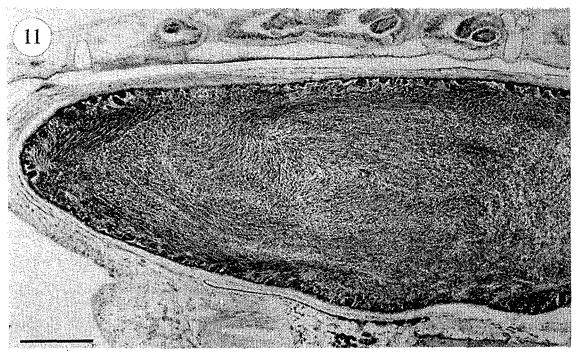
Ten individual were sampled randomly in June 1992 and the number of missing or regenerating pinnules counted for each of the ten arms. Few arms on any specimen of *Antedon* are complete: most individuals lose part of the distal region of some or all of their arms, probably as much from water movements as from predation, so calculations of pinnular predation were made on the basis of percentage pinnules lost or regenerating relative to the total number of pinnules present on each specimen. A











Figures 7–11. Micrographs of spermatogenic stages used here to assess reproductive cyclicity of *A. bifida*. Stained with Ehrlich's haematoxylin and eosin.

pinnule was regarded as 'regenerating' when its cross section was markedly less than that of pinnules adjacent to it.

(*f*) *Predation of* Antedon *by the corkwing wrasse*

Ten individuals of *Crenilabrus* (=*Symphodus*) *melops* (L.) were captured by net at the site, transferred underwater to containers with seawater and transported quickly to a closed-circuit laboratory aquarium. They were allowed to aclimatize in the tank for one week before specimens of *Antedon* were introduced.

3. RESULTS

(*a*) *Reproductive periodicity*

The ovary expands from the genital cord before the first reproductive season and also, in some individuals, following spawn-out and regression; initially, the expanded cord is solid, but early on a lumen begins as a central split (figure 12). Later, the oogonia and subsequently young oocytes, plus accessory cells, proliferate on the centrifugal side of the germinal epithelium, and increasing numbers of oocytes at this stage show crescentic yolk nuclei (figure 13). Then, oocytes enlarge and begin vitellogenesis while still enclosed in the inner ovarian epithelium (figure 14).

The number of larger, vitellogenic oocytes occupying the central part of the gonad increases (figure 15), and the oocyte's external plasma membrane may become faceted (figures 16 and 17). In the present study a process of ovulation, in which the mature oocytes break through the inner ovarian epithelium, has not been observed. Similarly, loss of the germinal vesicle in advanced oocytes before shedding has not been observed, suggesting that the final stages of meiosis take place after ovulation.

Oocyte size frequencies for the sampling period July 1983 to October 1987 are plotted in figure 18. Spawning, indicated by the appearance of brooded eggs on the external wall of the genital pinnules (figures 19 and 20), was observed only during the month of July in each of the principal sampling years; multiple spawnings, while they might have occurred,

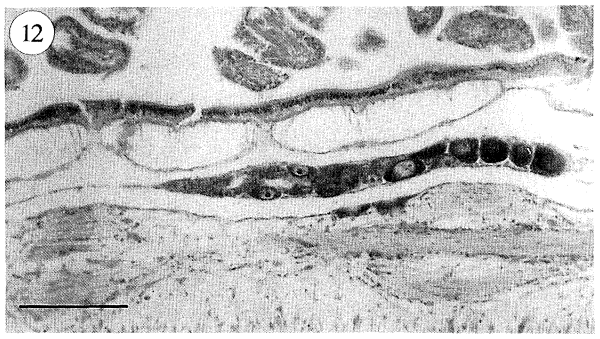
Figure 7. Stage 1. Testis small, only slightly expanded from genital cord and only partially lumenate. Scale bar: 200 μm.

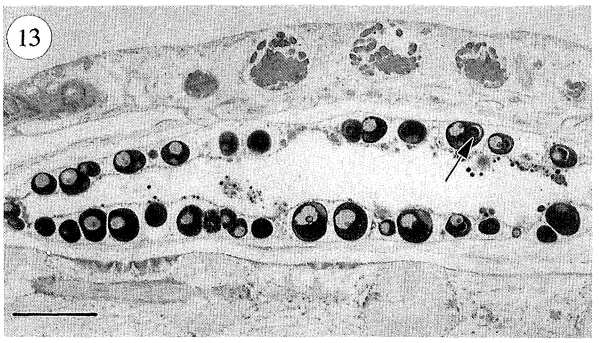
Figure 8. Stage 2. More of the genital cord has expanded and the lumen is enlarging. Germinal epithelium beginning to fold into pyramids. In this specimen, some pyramids are producing sperm. Scale bar: 100 μm.

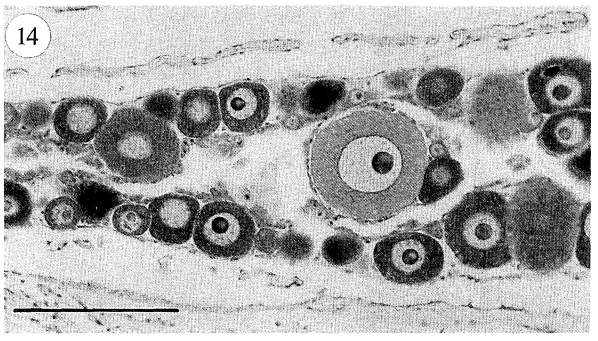
Figure 9. Stage 3. Pyramids fully formed and producing sperm, a few of which already occupy the testis lumen. Scale bar: 200 μm.

Figure 10. Stage 4. Part of testis wall at the maturing stage. Many mature sperm being proliferated at the centripetal ends of the pyramids. Scale bar: 200 μm.

Figure 11. Stage 5. Fully mature. Pyramids of germinal epithelium reduced or non-existent. Mass of mature sperm in testicular lumen. Scale bar: 200 μm.





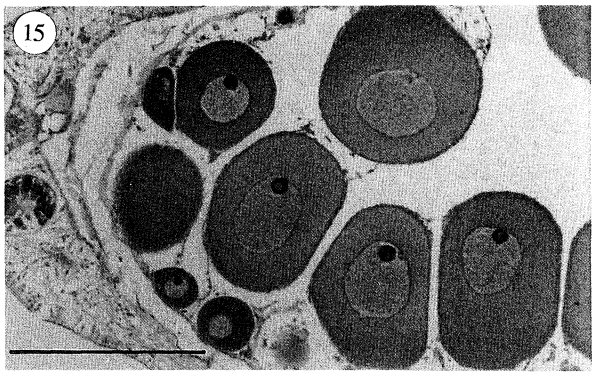


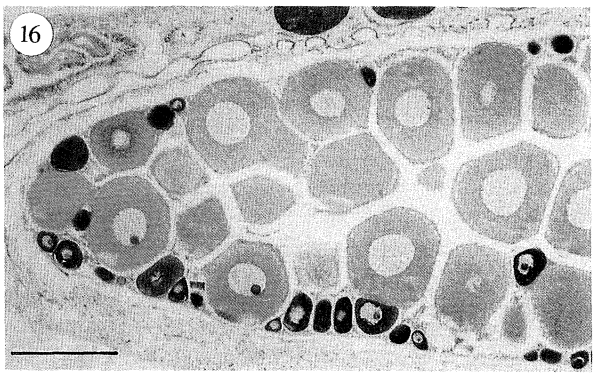
Figures 12–14. Sagittal sections of early ovarian development.

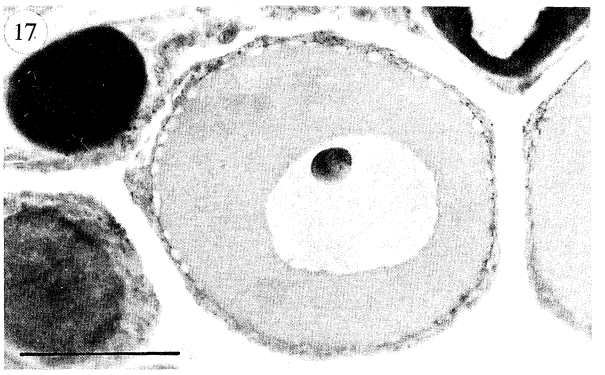
Figure 12. Genital cord (left centre) slightly expanded to form early ovary, in which some oogonia are recognizable and ovarian lumen is appearing. Scale bar: 100 μm.

Figure 13. More advanced. Oogonia and early oocytes proliferating on centrifugal side of germinal epithelium. Nutritive phagocytes present in lumen. Some oocytes already showing yolk nucleus (arrowed). Scale bar: 150 μm.

Figure 14. More advanced. Some larger oocytes incorporating yolk (lighter-coloured cytoplasm) are squeezed centripetally towards lumen, still surrounded by inner epithelium. Scale bar: 100 μm.







Figures 15–17. Advanced oocyte development.

Figure 15. Most oocytes large and yolky and still enveloped in inner epithelium of ovary; oogonia and small oocytes for next season also present. Scale bar: 200 μm.

Figure 16. Large sheddable oocytes, some with peripheral faceting. Scale bar: 200 μm.

Figure 17. Single large oocyte, showing peripheral faceting. Scale bar: 100 μm.

were not witnessed during the work. Just before and during July, the samples consistently showed the presence of oocytes above a diameter of about 150 μm, which is the diameter of the spawned eggs, and also the minimum diameter at which oocytes were seen to show peripheral faceting. But oocytes of such a size or above are also present at other times of year; indeed, only in four samples of the 31 taken (September, October and December 1984 and May 1986) were there no oocytes of sheddable size: in all other samples there was an appreciable proportion of oocytes above this size (figure 23).

Ova retained in the mucous net averaged 150 μm in diameter; they undergo cleavage to an early doliolaria larval stage (figure 21), before being released. The temporary gonopore may remain open for several months (figure 22): in the present work, female gonopores were open until August, and males until December. Mean oocyte diameters, together with mean monthly sea-temperatures and annual equinoctia, are plotted in figure 24.

For males, figure 25 shows the percentage assigned to each of the five spermatogenic stages from each sample. Only Stages 1 and 2 do not contain mature

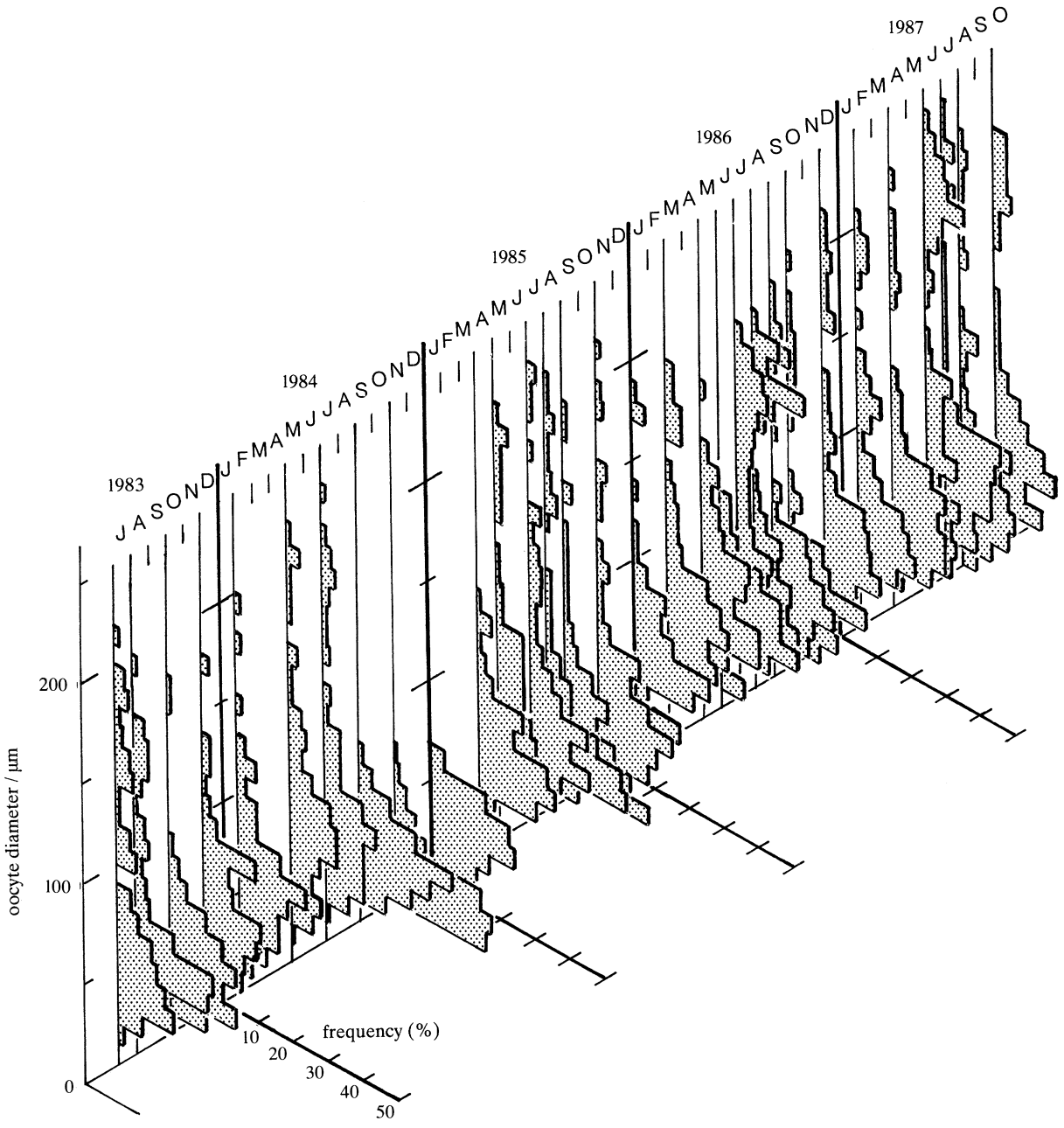


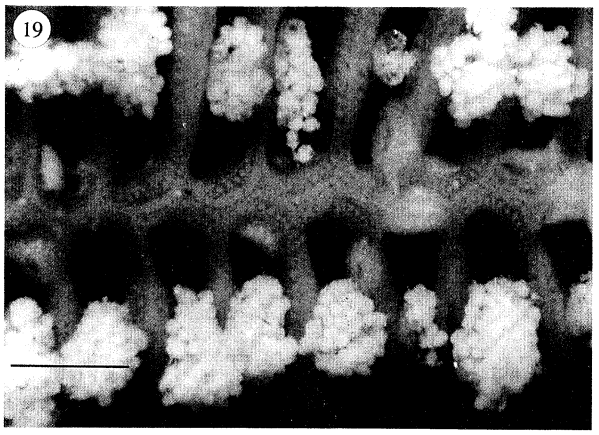
Figure 18. Isometric projection of oocyte size–frequency histograms ($n = 150$) for approximately monthly samples of *A. bifida* from Berry Head, South Devon, over five seasons.

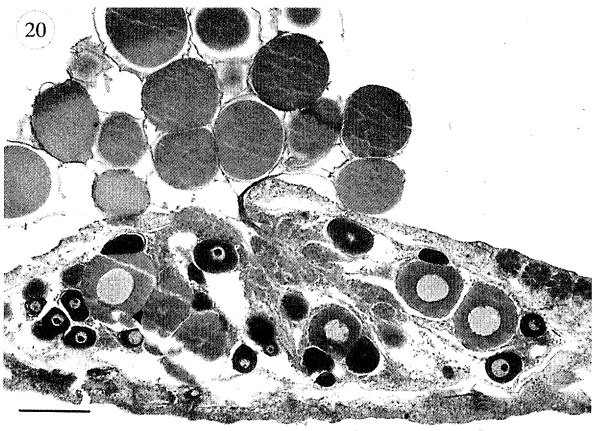
sperm, so in only seven samples (October 1983, March, February and September 1984, August and September 1986 and August 1987) were there individuals showing these immature stages. Further, in all months, except for the first sample taken, testes containing mature sperm were present. Fully mature testes were always present around July, the observed spawning time in the cycle.

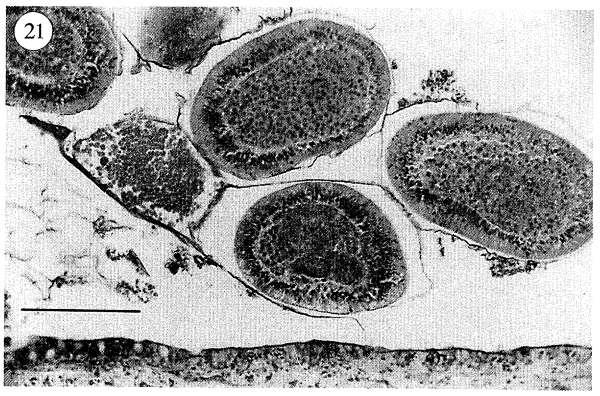
(*b*) *Synchrony of development*

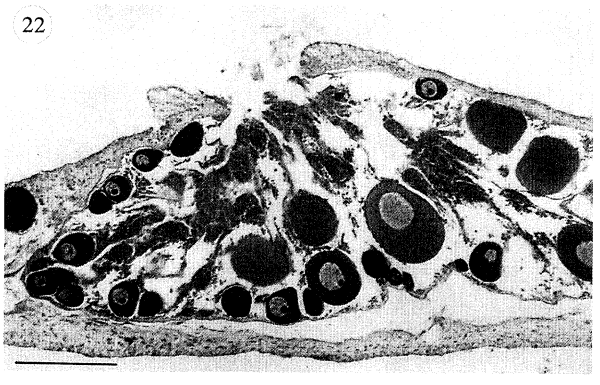
Isometric projections of oocyte size frequencies for all genital pinnules along a single arm of one specimen from samples taken roughly bi-monthly from December 1985 to September 1986 are given in figure 26*a–e*. All samples show the expected predominance of small oocytes (less than 150 μm diameter, the sheddable size), and all but the May 1986 sample show additionally the presence of larger oocytes (above sheddable size) in at least some individual gonads. The December 1985 sample has oocytes of sheddable size in pinnules 3 to 25 (with the anomalous exception of pinnule 9), but there is a decline in large oocytes distally along the arm. The March 1986 sample shows a similar pattern of oocyte occurrence, except that pinnule 35, the last one with a recognizable gonad, has most of its genital cells at the oogonia stage in the lowest size category. The May 1986 sample has no sheddable oocytes along the length of the arm, and again there are only oogonia of the two smallest recorded categories in the last genital pinnule of the arm. The July 1986 sample shows some variation in

oocyte size frequencies along the arm, while in the September 1986 sample there are oocytes of sheddable size only in pinnules 3 to 11, and by pinnule 19 the reproductive cells are mostly oogonia in the smallest size category.









Figures 19–22. Post-spawning genital pinnules of *A. bifida*.

Mean oocyte diameters for each pinnule along an arm for the five sampling dates are given in figure 27. Only in the March 1986 sample is the regression slope not significant ($r = -0.91$; $p > 0.05$); for all other samples there is a significant decline in reproductive investment in the more distal pinnules.

(*c*) *Facets on the oocyte membrane, nutritive phagocytes and yolk nuclei*

Presence or absence of facets, nutritive phagocytes and yolk nuclei from three specimens of each sample are recorded in table 1. Facets may be present in all large oocytes in a single ovary, or on just some (figures 16 and 17); they may occur in all months of the year, with little correspondence between their occurrence and the observed spawning time. Nutritive phagocytes occur to varying degrees across the year (figures 28–30), and between individuals from a single sample. Yolk nuclei are present in some developing oocytes at all times of year (figure 13), and in all specimens taken throughout the sampling period, individual exceptions being September and October 1984 and March 1987.

(*d*) *Missing and regenerating pinnules*

Counts of missing or regenerating pinnules from randomly collected specimens ($n = 10$) taken in June 1992 are given in table 2. The least-damaged specimen had 1% of pinnules from the total present in all arms either missing or regenerating, whereas the most damaged had 46% missing or regenerating. The overall mean was 17%. Figure 31 shows arms of two specimens from this sample with slight and heavy predation of genital pinnules, with regeneration.

(*e*) *Predation of* Antedon *by the corkwing wrasse*

Observation of predation on-site revealed only that the corkwing wrasse, *Crenilabrus* (= *Symphodus*) *melops*, the commonest fish seen in the area, lunged towards the pinnules of *Antedon* in rapid thrusts. In an experimental tank, after an aclimatization period, the fish were observed to take epizoics, particularly hydroid and bryozoan polyps, from the surface of rocks in the aquarium, then move towards the feather-stars and take whole pinnules by rapid darting movements towards the prey (figure 32), and predation was almost totally confined to the genital pinnules. On

Figure 19. Fertilized ova retained in a mucous net on the external pinnular wall of *A. bifida*. July 1992. Scale bar: 2 mm.

Figure 20. Sagittal section of recently spawned ovary, with post-meiotic eggs retained in mucous net. July 1986. Scale bar: 150 μm.

Figure 21. Young larvae in mucous net. July 1986. Scale bar: 100 μm.

Figure 22. Genital pinnule after hatching embryos from external brooding net, now disappeared. Temporary gonopore still present. Scale bar: 200 μm.

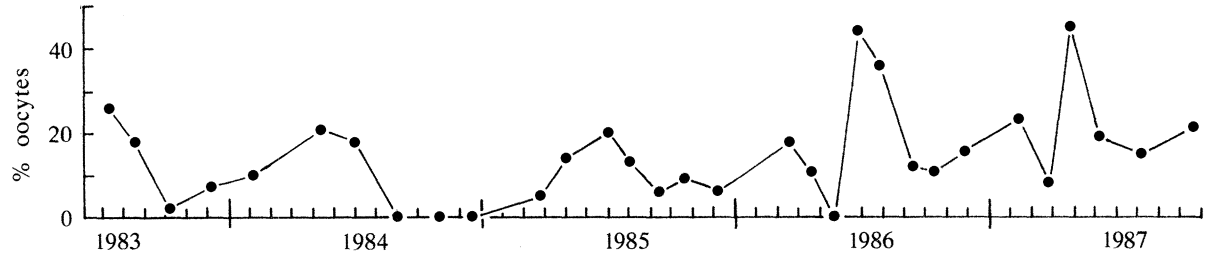


Figure 23. Percentage of oocytes ($n = 150$) above 150 μm diameter in samples ($n = 3$) of *A. bifida* taken from July 1983 to October 1987.

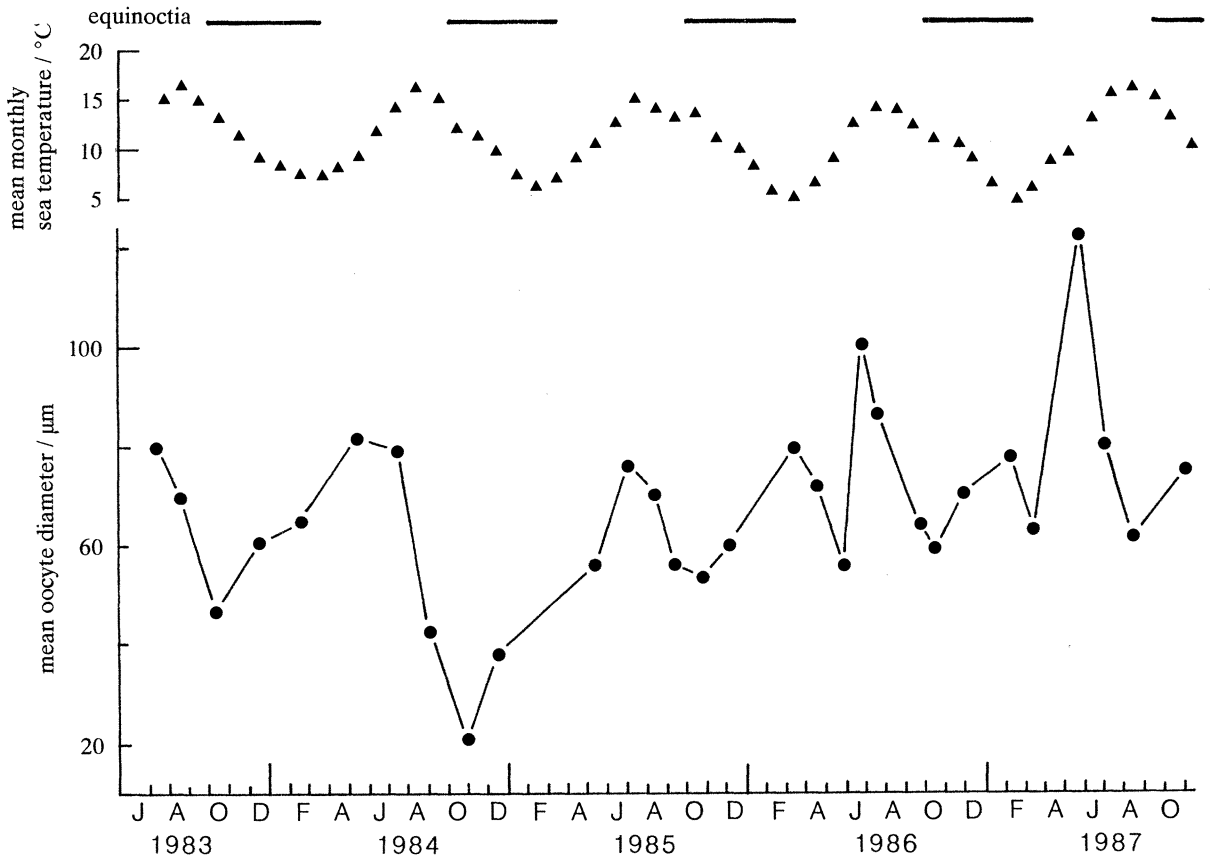


Figure 24. Mean oocyte diameters (circles) ($n = 150$) from monthly samples ($n = 3$) of *A. bifida* from Berry Head, South Devon, with mean monthly sea temperatures (triangles) from 2 m depth within Plymouth Sound, and equinoctia, from July 1983 to October 1987.

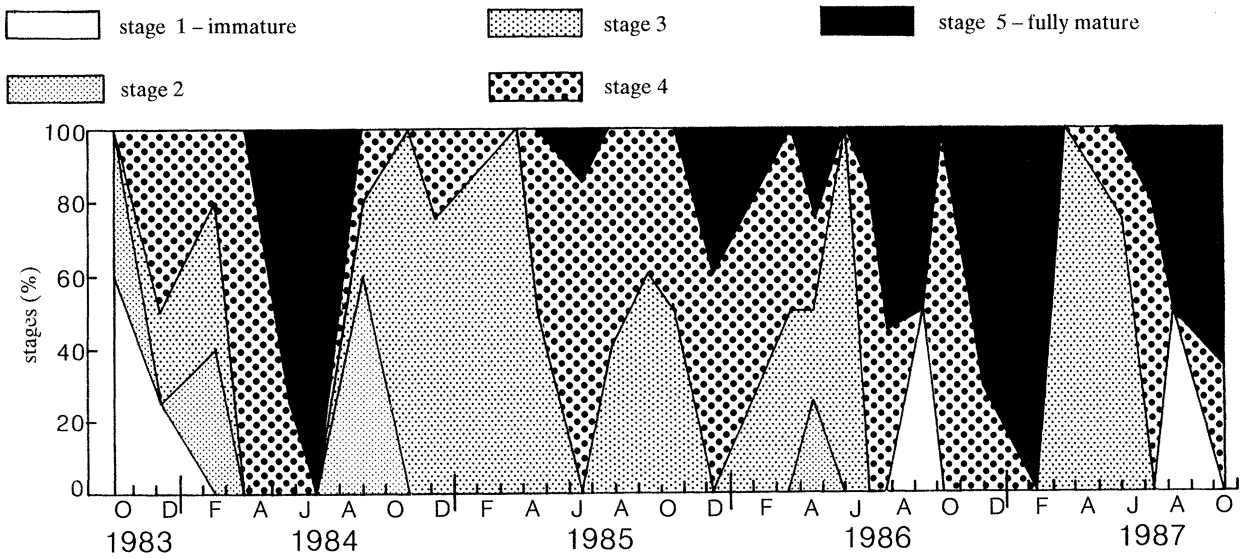


Figure 25. Percentage occurrence of spermatogenic stages from specimens ($n = 5$) of *A. bifida* sampled approximately monthly from Berry Head, South Devon.

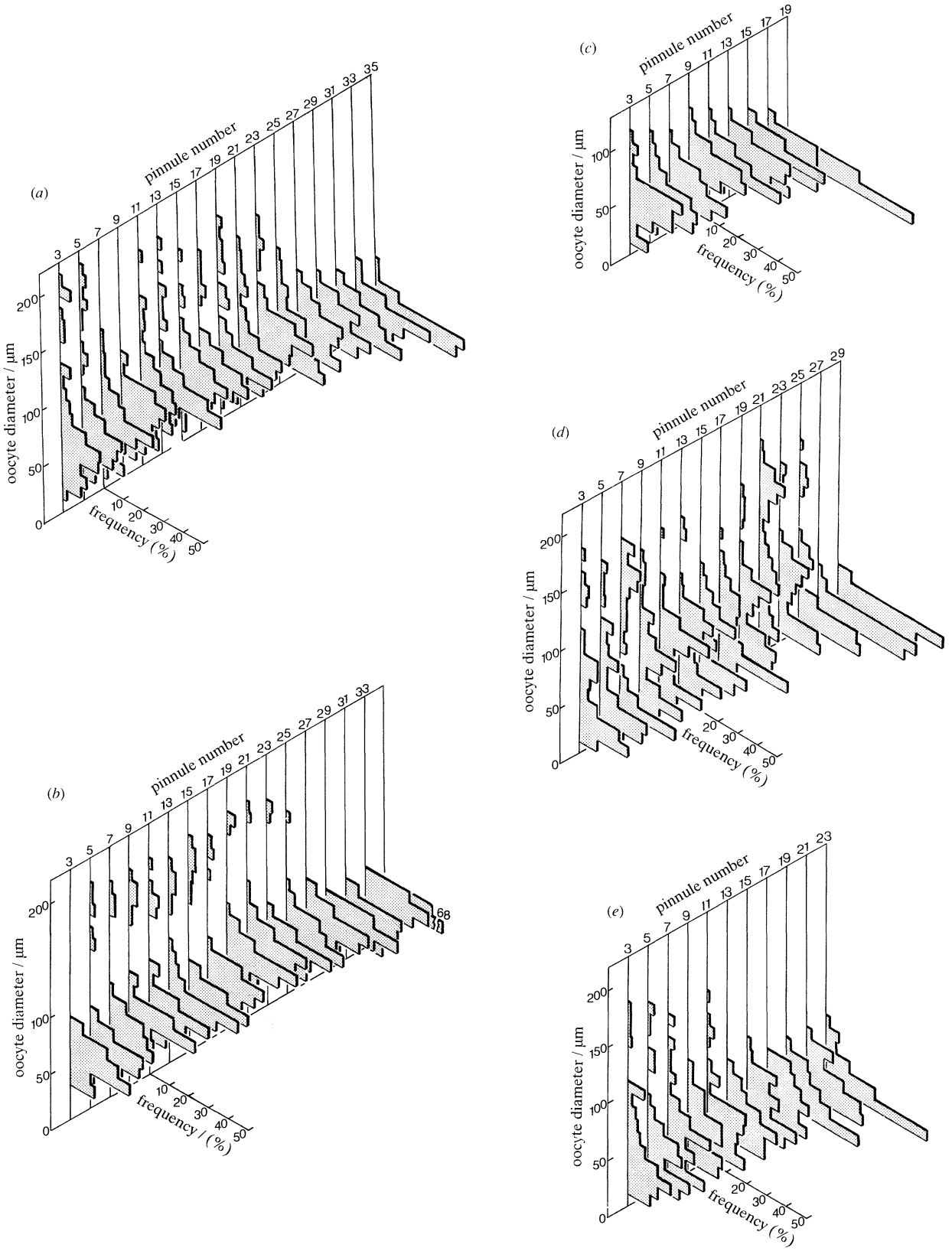


Figure 26. Isometric projections of oocyte size–frequency histograms ($n = 150$) for every other pinnule from one side of one arm of specimens of *A. bifida* from Berry Head, South Devon, over one annual cycle. (*a*) December 1985; (*b*) March 1986; (*c*) May 1986; (*d*) July 1986; (*e*) September 1986.

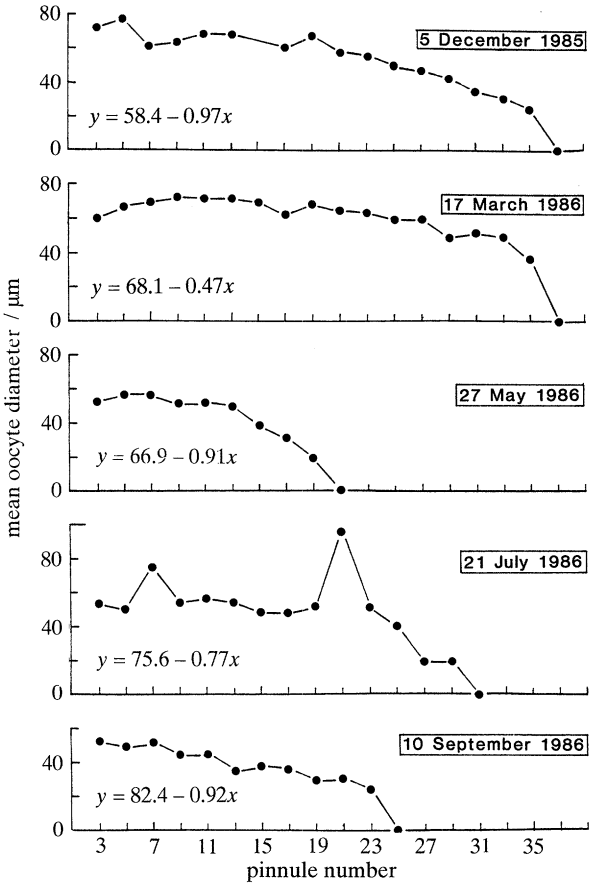


Figure 27. Mean oocyte diameters ($n = 150$) from every other pinnule from one side of one arm of specimens of *A. bifida* over one annual cycle. Size–frequency profiles for these samples are given in figure 26.

several occasions the fish were seen moving away from the *Antedon* with the ends of pinnules protruding from the mouth.

4. DISCUSSION

(*a*) *Oocyte size–frequency distribution and spermatogenic stages*

This five-season study has revealed a remarkable constancy in ovarian maturity across the seasonal cycle in *Antedon bifida*. From the start of sampling in July 1983 for a full calendar year the ovaries of some individuals possessed large oocytes. Whether these were ready for shedding is apparently immaterial for the strategy that appears to have evolved in this animal, as there may well be selective advantage in maintaining the gonads at a large size within the nutritive régime available to the animal throughout the annual cycle.

The exceptional period of the sampling programme was the winter of 1984–5, in which no individual from any of the four samples taken between September 1984 and March 1985 had mature oocytes; only one other sample (May 1986) taken over the five-season programme showed an ovary with no mature oocytes. Indeed, from June 1986 until the end of the sampling programme in October 1987 the profiles of oocyte

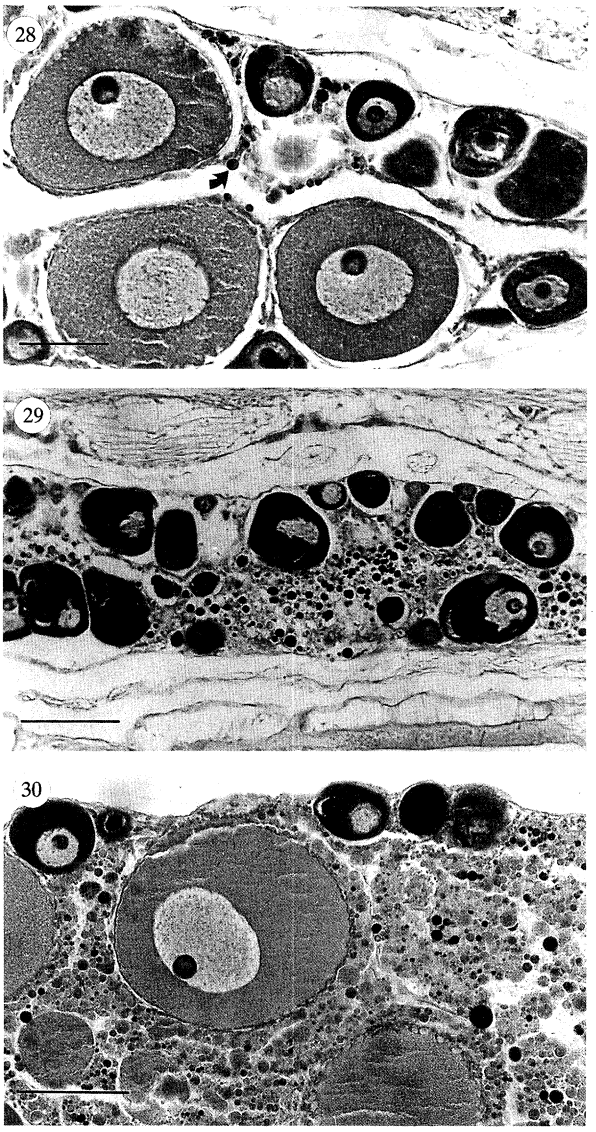


Figure 28–30. Photomicrographs of various stages in the proliferation of nutritive phagocytes (accessory or nurse cells) in the ovaryies of *A. bifida*.

Figure 28. A few nutritive cells enlarging in germinal epithelium (arrowed). May 1986. Scale bar: 50 μm.

Figure 29. Nutritive phagocytes in ovarian lumen. October 1983. Scale bar: 100 μm.

Figure 30. Nutritive phagocytes surrounding unshed advanced oocytes, one of which has a faceted periphery. March 1986. Scale bar: 100 μm.

size–frequency histograms (figure 18) are remarkably constant, with a wide range of oocyte diameters present in each sample over the cycle. This contrasts with the unique cycle in *Oxycomanthus japonicus*, in which, in a sample taken the day before the single annual spawning, all oocytes have a diameter between 160 and 230 μm (Holland 1992). The comparative constancy of developmental stages in *A. bifida*, particularly over the last three seasons of the sampling programme, is underlined by the plot of mean oocyte diameters for all samples (figure 24). As expected, troughs in this plot appear in the early autumn of each year, that is, shortly after the observed spawning

Table 1. *Occurrence of facets on the oocyte cell membrane, nutritive phagocytes in the ovarian lumen and yolk nuclei in the developing oocytes in samples (n=3) of female* Antedon bifida *over a 52 month period*

	facets	phagocytes	yolk nuclei
1983			
4 July	√	√	√ √ √
18 August		√	√ √ √
7 October		√ √ √	√ √ √
10 December		√ √ √	√ √ √
1984			
7 February		√	√ √ √
19 May	√ √ √	√ √ √	√ √ √
4 July		√ √	√ √ √
3 September		√ √	√ √
31 October		√	√
12 December		√	√ √ √
1985			
26 March		√ √	√ √ √
23 April	√	√ √	√ √ √
26 June	√ √ √		√ √ √
27 July		√ √	√ √ √
12 September	√ √		√ √ √
18 October	√ √		√ √ √
5 December	√ √ √	√	√ √ √
1986			
17 March	√ √ √	√ √ √	√ √ √
23 April		√	√ √ √
27 May	√ √ √	√ √ √	√ √ √
25 June			√ √ √
21 July	√ √ √		√ √ √
10 September	√ √		√ √ √
8 October	√ √ √		√ √ √
27 November			
1987			
12 February	√	√ √ √	√ √ √
16 March	√	√ √ √	√ √
20 May		√ √ √	√ √ √
5 July	√	√ √ √	√ √ √
5 August	√	√ √ √	√ √ √
14 October	√ √ √	√ √ √	√ √ √

Table 2. *Occurrence of missing or regenerating pinnules on the arms of* Antedon bifida *at Berry Head, South Devon, U.K.*

	numbers of pinnules per specimen			
specimen	whole	missing or regenerating	total	% missing or regenerating
1	577	23	600	4
2	223	172	410	42
3	679	20	678	3
4	642	9	651	1
5	538	79	617	13
6	377	125	502	25
7	500	65	565	12
8	385	114	499	23
9	384	7	391	2
10	244	206	450	46
				mean: 17%

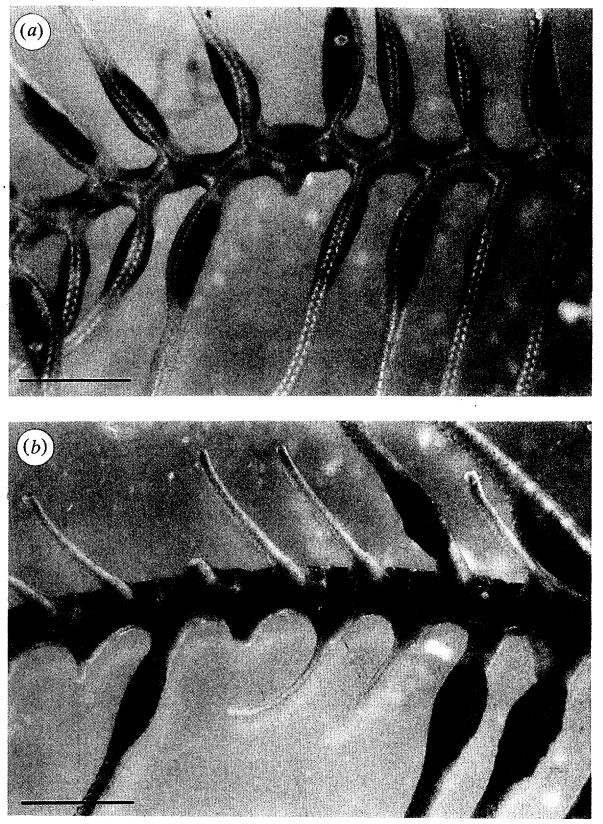


Figure 31. Part of the arm of two specimens of *A. bifida* which have suffered predation. (*a*) Slight predation, with a single genital pinnule missing. (*b*) Severe predation, with pinnules in various stages of regeneration. Scale bar: 2 mm.

season, and in only two cases (October 1984 and August 1987) were ovaries found possessing oogonia and oocytes of a diameter less than 10 μm.

For males, there were small testes present in only the first sample taken (October 1983); thereafter, some individuals in each sample possessed well-grown testes, usually with mature spermatozoa in the lumen. In more than 45% of the male samples taken over the five seasons there were testes present at Stage V (fully mature).

A feature of the ovarian cycle over the sampling period has been the occasional lack of correspondence between the same months in different years, despite

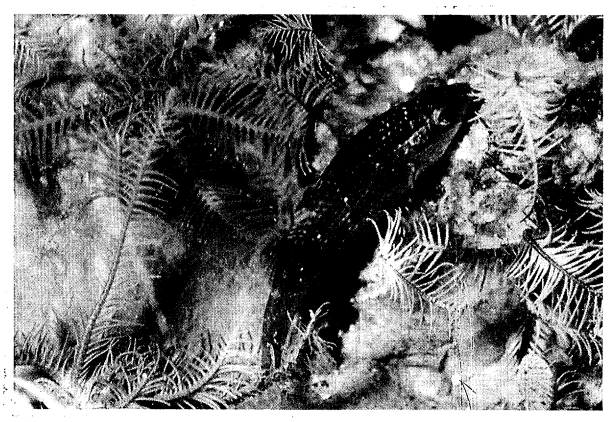


Figure 32. Corkwing wrasse, *Crenilabrus* (=*Symphodus*) *melops*, attacking genital pinnules of *A. bifida* in an experimental tank.

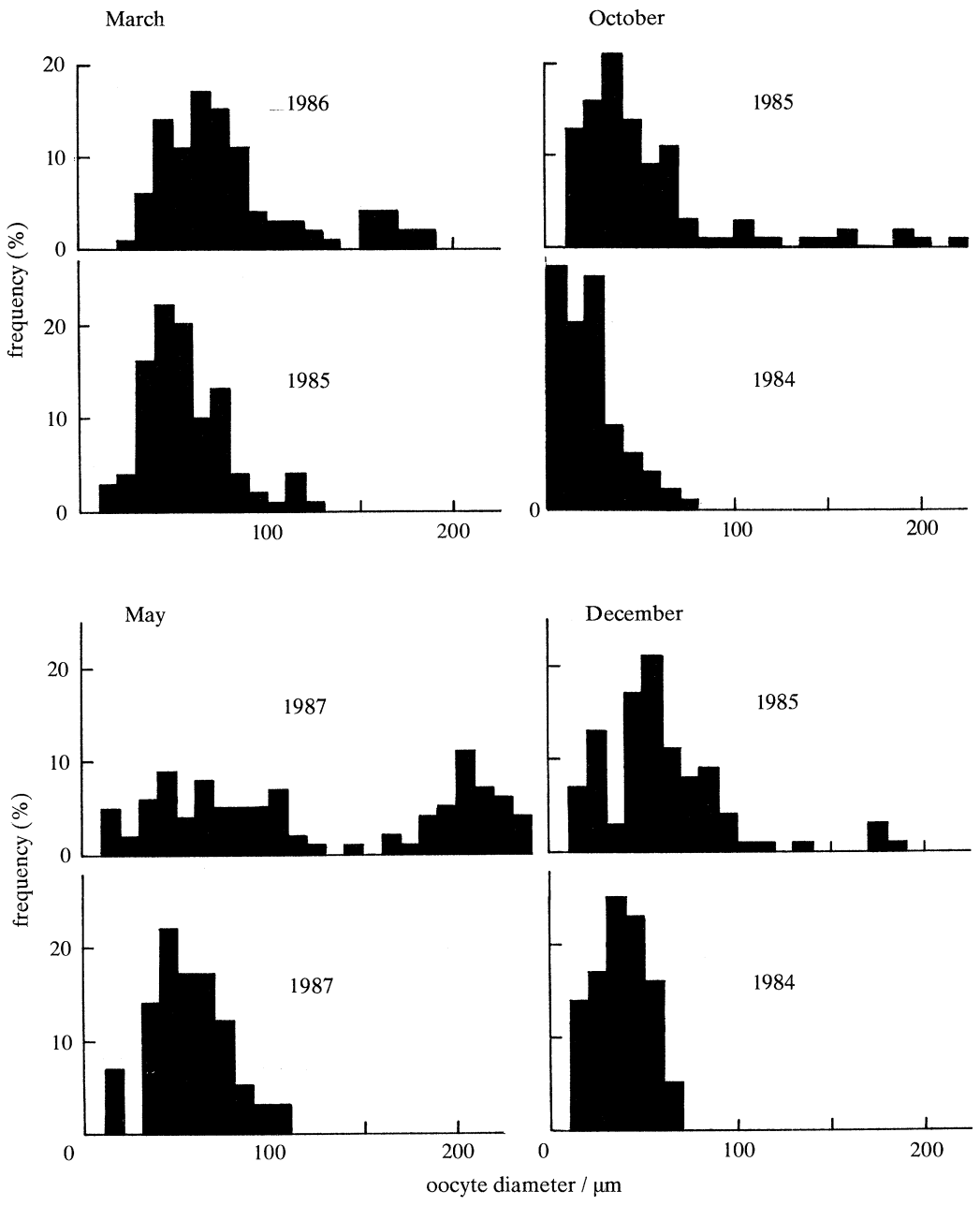


Figure 33. Oocyte size–frequency histograms ($n = 150$) from specimens ($n = 3$) of *A. bifida* in the same months in consecutive years.

the usually wide spectrum of oocyte size frequencies present. Figure 33 shows examples from four months in consecutive years over a 30 month span of the sampling period. In each case the month in one year lacks oocytes of diameter above about 130 μm, while the same month in the following year has an appreciable population of larger oocytes. Despite such annual differences in oocyte size frequency, however, the occurrence of nutritive phagocytes (table 1) in the ovaries in similar months shows remarkable constancy, suggesting that there is a turn-over of genital material across the season whatever the profile of oocyte size frequencies. Presumably, the nutritive spherules serve as in some other crinoids, to transfer stored food material, some undoubtedly obtained by phagocytosis of previous non-spawned oocytes, to the new generation of oocytes (Holland 1992).

The technique used in this study of allocating each sampled male to a reproductive stage does not permit comparisons of similar months in consecutive years. Unlike the seasonal events in the female cycle, such as a high state of development each year around spawning time, the males show a preponderance of advanced stages at periods in the year other than those in which spawning is known to take place, such as the entire period December 1985 to February 1987, and again from July to October 1987. In males, therefore, the apparent drop in spermatogenic maturity from about

May to July is probably because the sampled individuals have spawned, perhaps several times during this period.

(*b*) *Continuous gametogenesis*

Continuous reproduction is common in tropical crinoids, where cyclical exogenous stimuli are not well-defined. Indeed, Vail (1987*a*) shows that in the Great Barrier Reef crinoids *Himerometra bartschi* (A. H. Clark), *H. robustipinna* (P. H. Carpenter), *Cenometra bella* (Hartlaub) and *Colobometra perspinosa* (P. H. Carpenter), there is a high level of continuous reproductive activity, whereas in *Oligometra serripinna* (P. H. Carpenter) there is at most only a hint of bimodal reproductive cyclicity. In temperate species, however, a unimodal annual cycle is more usual, and where there is little sign of such seasonality, it has been assumed that such species may spawn at any time of the calendar year. Thus, for *Florometra serratissima*, a temperate species, Mladenov (1986) suggests that a female may spawn at least nine times a year, and males will be in a state to fertilize. Mladenov draws attention to several brief periods of fluctuation in the production of gametes, and suggests that this might be due to perturbations in the supply of seston, on which the animals feed. The same may well be true for *Antedon*: the period of low oocyte productivity from September to December 1984 may be ascribed to a similar cause. Further, Mladenov comments that in *Florometra* the large pool of small oocytes in a female never disappeared, so that, following lower winter breeding, the females were able to respond quickly to more favourable circumstances by producing large, mature oocytes from this pool within two months.

Antedon is apparently unique among comatulids, in that the spawned eggs are retained in the pinnular mucous net, and therefore the shedding period can be delimited. No example of shedding was observed outside the period May to July over ten years of observations during the present work, so it is unlikely that shedding in this species is adventitious at other times of year, unless eggs shed and fertilized outside the principal spawning time are broadcast directly and not retained in a mucous net.

The results of this study underline the need for an extended sampling period if uncharacteristic features in any one year are not to suggest an erroneous interpretation. In the present study, plots of both oocyte size frequencies (figure 18) and mean oocyte diameters for females (figure 24) and spermatogenic stages for males (figure 25) taken over the first two seasons (July 1983 to July 1985) could have been interpreted as indicating a cyclical reproductive strategy similar to those of other echinoderms from the same general locality of the English Channel (Barker & Nichols 1983; Nichols & Barker 1984*a*,*b*; Nichols *et al.* 1985). In the first two years of the present study there is a pronounced cyclicity which is absent from the results for the period July 1985 to October 1987. In particular, in the late summer of both the 1983 and 1984 seasons there is a marked decline in gametocyte production which does not occur in samples from the subsequent three years.

(*c*) *Synchrony of development within individuals*

Reports from previous studies suggest that in some comatulids gametogenesis is synchronous in pinnules along the length of a single arm. McClintock & Pearse (1987) show that pinnules from the 'basal, middle and terminal' portions of an arm of *Promachocrinus kergeulensis* P. H. Carpenter are remarkably synchronized in their oocyte development; Mladenov & Brady (1987) suggest that similar synchrony occurs in *Nemaster rubiginosa*; and Holland *et al.* (1975) state that all gonads of an individual *Oxycomanthus japonicus* are at about the same condition, except for the markedly smaller distal gonads.

The present work shows that appreciable synchrony is exhibited in *Antedon bifida* only just before spawning. Figures 26*b* and 27 show that a female specimen sampled in March 1986 had at least its first 27 reproductive pinnules at a very similar level of oocyte development, whereas samples taken in May, July, September and December had proximal pinnules significantly more advanced than distal ones. An unusual situation occurred in July 1986 (figure 27), with two anomalous ovaries (pinnules 7 and 21) having significantly higher mean oocyte diameters than their adjacent gonads.

(*d*) *Facets on the oocyte membrane*

Ludwig (1880*a*) first described and illustrated the appearance of facets below the chorion, that is, on the oocyte surface, in *Antedon rosacea* (= *bifida*) and its gradual smoothing-out once spawned and in contact with seawater. More recent authors (Dan & Dan (1941*a*) and Holland *et al.* (1975) on *Oxycomanthus japonicus*; Mladenov (1981) on *Florometra serratissima*; Mladenov & Brady (1987) on *Nemaster rubiginosa*; and Vail (1987*a*) on five species of Great Barrier Reef comatulids) describe a similar phenomenon, usually referred to as 'dented' or 'wrinkled' oocytes, on the vitelline membrane surrounding the advanced oocyte. The New Zealand comatulid *Oxycomanthus plectrophorum* also shows the same phenomenon (Mladenov & Nichols, in preparation). Both Mortensen (1920) and A. H. Clark (1921) identified structures on the fertilization membrane of shed eggs of *Antedon petasus* as 'spines'.

Holland & Jespersen (1973) have made a detailed study of this transient structure and figure scanning and transmission electron micrographs of the external membrane of the recently fertilized egg of the Pacific comatulid *Oxycomanthus japonicus*, which does not brood its ova. They show that the fertilization membrane forms during the last 10 days before spawning, from the vitelline membrane external to the oocyte surface, together with cortical granules that have migrated centrifugally to the exterior ovum surface. Holland (1977) shows that the external oocyte topography moulds the pattern of the jelly layer on the surface of the unfertilized egg, and this, in turn, acts as a second template to shape the pattern of ridges on

the fertilization membrane; the 'spines' of Mortensen (1920) and A. H. Clark (1921) are therefore most probably a visual illusion.

No previous author has made a study of facet occurrence throughout the reproductive cycle. The present study shows that faceted oocytes, although not present continuously, have been observed in some specimens in all months of the year in *Antedon bifida* (table 2). These results suggest that faceting is not necessarily a phenomenon associated with the onset of spawning, but might occur when oocytes attain a certain size (above about 150 μm diameter), whether or not they are destined to remain within the ovary for an extended period. The present study confirms (albeit by light microscopy only) that the recently shed ova of *Antedon bifida* lose the ornamental facets on the fertilization membrane as they are shed and gathered into the mucous net on the external pinnular wall (figure 20).

(*e*) *Nutritive phagocytes in the ovary*

It is known that the occurrence of these accessory cells fluctuates during the course of the annual cycle (Raven 1961; Holland & Kubota 1975; Wourms 1987). Harvey (1931) remarks that such cells can be identified in the ovarian germinal epithelium of *Antedon* at an early stage, by the formation within them of large osmiophilic droplets to one side of the nucleus. These cells remain small and accumulate lipids until they are nearly filled by fat droplets. They then move into the ovarian lumen, between the developing oocytes. Holland & Kubota (1975) suggest that in *Oxycomanthus* these accessory cells store nutrients during most of the year, then donate this material to the oocytes as they grow prior to spawning. By contrast, Mladenov (1986) reports that such cells are absent from the ovaries of *Florometra*. The present study has revealed that in *Antedon* (table 2) such cells are present in some individuals throughout the year, but are more often absent during the summer and autumn months, that is, after the spawning time, and are usually present during the winter and spring build-up of mature oocytes. A detailed study of the fluctuations in these accessory cells is beyond the scope of this study, but the results reported here suggest that phagocytosis of relict oocytes takes place during most winters, presumably to absorb yolk and other materials from unshed oocytes for re-use by those developing from the germinal epithelium for the succeeding season. What initiates phagocytosis in some individuals to remove relict oocytes, while others apparently store mature oocytes at non-spawning times of year, requires further study.

(*f*) *The 'yolk nucleus' in* Antedon

The appearance during oogenesis of the so-called 'yolk nucleus' (figure 13), possibly uniquely in the genus *Antedon* (Holland 1992), represents a transient phenomenon. Urbani (1955) shows that these bodies contain much RNA, together with aromatic amino acids and lipoproteins. Holland (1976) has studied these structures by electron microscopy, and suggests that dense perinuclear granules in young oocytes migrate to an eccentric position within the cell where the crescent-shaped body arises, and to which peripheral mitochondria may also migrate. The bodies possibly represent an aggregation of the protein-rich perinuclear dense material seen as granules in the oocytes of many other animals, and may contribute to the supply of free ribosomes, though they do not contribute to the synthesis of yolk granules (Nørrevang 1968). Their appearance as a dense body in *Antedon* may be because of an unusually slow dissolution of ribosomal material during oocyte maturation. Table 2 shows that they occur at all times of year, which is not unexpected, as there is a population of previtellogenic oocytes present throughout the cycle. The three individuals lacking yolk nuclei were sampled during the anomalous period of September and October 1984, when the ovaries of all sampled specimens were poorly developed and their oocytes of small average size, that is, before the onset of vitellogenesis.

(*g*) *Control of the gametogenic cycle*

Only circumstantial evidence has been advanced in the identification of factors controlling gametogenic activity in crinoids (Holland 1992). For instance, a falling ambient sea temperature and reducing day length coincides with the onset of formation of gonial cells in *Oxycomanthus japonicus* (Holland 1981). In *Antedon bifida*, the present work reveals relatively minor gametogenic cyclicity over a season, yet there are strong indications that the same exogenous factors may be involved in the initiation of gonial cell formation. For instance, the highest proportion of oogonial cells occurs in September and October in each of the seasons studied (figure 18), which might reflect an external stimulus for the start of oogenesis as occurring a few weeks before this. It may therefore be significant that the annual drop in sea temperatures and decreasing day length both begin in about August (figure 24).

(*h*) *Crinoid predation*

From the early days of their study, crinoids were regarded as virtually immune from predation, a view apparently initiated by H. L. Clark (1917), who showed that crinoids, allowed to free-fall to the seabed, were avoided by fish. This observation coloured opinion for many years (Mortensen 1927; A. H. Clark 1921; Fell 1966; Breimer 1978), until Brun (1972) reported the remains of *Antedon bifida* in the stomachs of the predatory starfish *Luidia ciliaris* (Philippi), which feeds preferentially on other echinoderms by ingesting the animals whole. Randall (in Meyer & Macurda 1977) reports finding crinoid remains in fish stomachs.

Observations of crinoids in the field have yielded few instances of their predation, though Meyer & Ausich (1983) suggest that, despite the lack of actual observation, predation must be considered a possibility, particularly by arthropods and fish. Vasserot

(1965) cites a case in which a lobster, *Panulirus vulgaris* (Latreille) attacked and devoured a specimen of *Antedon bifida* in an aquarium. Meyer & Ausich (1983) report that specimens of the Great Barrier Reef snapperfish, *Chrysophrys auratus*, had fresh crinoid fragments in their stomachs, and Walker (1975) reports similar observations for the groupers *Lethrinus chrystostomus* (L.) and *L. nebulosus*. Vail (1987*b*) reports that on three occasions a trigger-fish, *Ballistoides conspicillum* (Block & Schneider), was seen to attack the arms of crinoids, and, more significantly, Fishelsen (1974) observed the Red Sea clingfish *Lepadichthys lineatus* (Briggs) feeding on the actual pinnules of *Lamprometra klunzingeri* (Hartlaub). Conan *et al.* (1981) give photographs of oreostomatid fish feeding on the pinnules of a deep-water stalked crinoid, *Diplocrinus* (*Annacrinus*) *wyvillethomsoni*. Lastly, Mladenov (1983) indicates that a starfish, *Pycnopodia helianthoides* (Brandt), and a crab, *Oregonia gracilis* (Dana), are likely predators of the eastern Pacific comatulid *Florometra serratissima*.

Comatulid crinoids in the region of the Great Barrier Reef have been observed with sub-lethal damage to their bodies that may be the result of predation (Meyer & Macurda 1977; Meyer & Ausich 1983; Meyer 1985), and Meyer *et al.* (1984) give photographs of a saddled coralfish, *Chaetodon ephippium* Cuvier & Valenc., with the arm of the comatulid *Himerometra robustispina* (P. H. Carpenter) protruding from its mouth.

P. H. Carpenter (1879) notes that the gonads on the shorter posterior arms of asymmetrical crinoids are larger and more numerous than those on the longer anterior arms, and this is confirmed by Vail (1987*b*), who further shows that the four asymmetrical species *Comanthus parvicirrus* (Müller), *C. gisleni* Rowe, *C. wahlbergi* (Müller) and *Oxycomanthus exilis* Rowe normally live with their longer arms in crevices, extruding only the tips of the shorter arms into open water while keeping the gonad-bearing proximal portions hidden. Vail adds: 'If pinnular gonads are potential food of predators, then their concealment would be effective predator-avoidance behaviour.'

It is interesting to note that the fossil record is yielding increasing evidence of durophagy, probably by teleosts (Meyer & Macurda 1977; Aronson 1991). Donovan (1985) remarks that predation on crinoids, as manifested by the presence of regenerating arms, dates back at least to the Middle Ordovician.

(*i*) *The position of the gonads during crinoid evolution*

The location of the gonad(s) in fossil crinoids is uncertain (Holland 1992; M. J. Simms, personal communication). There is, for instance, no feature of stereom microstructure of crinoid brachial or pinnular ossicles that might indicate that the gonads were borne on the arms or pinnules. Further, Lane & Sevastopoulo (1981) suggest that in fossil crinoids that are preserved adequately enough to show such features, there appears to be no confluence of thecal and arm coelomic systems, so that a nutrient route using such coelomic spaces would not have been possible, and they conclude that the gonads in these early forms are unlikely to have been outside the theca. However, evidence from ultrastructural studies of Recent forms shows that a peritoneum surrounds the gonadial coelom of the genital pinnules and so this cavity probably lacks direct connexion with the perivisceral coelom (Holland 1971; Smiley 1990), an indication that a confluent nutrient route from theca to genital pinnules may not be present in modern crinoids either.

A further reason for uncertainty as to the gonad position in forms ancestral to the comatulids is that an undisputed gonopore has yet to be identified in any fossil crinoid, even though such structures have been confirmed in other thecate echinoderms, such as cystoids (Paul & Smith 1984). The question of gonad location in ancestral crinoids must therefore remain open pending better-preserved fossil material or a technique that might indicate the proximity of gonads to fossilized ossicles.

The considerable modification to the anal spire that has been recorded for some fossil crinoids lends support to suggestions that this structure houses organs additional to the terminal run of the animal's rectum. In disparid inadunate crinoids the spire may take the form of a highly elongated tube, as in *Aesiocrinus* Miller & Gurley from the Pennsylvanian of Missouri (Lane 1975) and *Dystactocrinus* (Hall) from the Upper Ordivician of Ohio (Ulrich 1925), or a distended sac, as in *Daedalocrinus* Ulrich from the Ordovician of Ontario (Ulrich 1925). In *Tholocrinus* (Wachsmuth & Springer) from the Mississipian of Kentucky and *Botryocrinus* Kesling from the Middle Devonian of Michigan the spire may have spine-like ornamentation (Springer 1926; Kesling 1973), suggesting a protective function.

But it is in the cladid inadunates that the organ reaches its greatest diversity in size and shape. In particular, the position of the anal pore in this group suggests a function for the spire additional to that of containing the distal part of the gut. Thus, in *Eratocrinus* (Miller) from the Mississipian of Missouri the anus opens at the base of the spire; in *Scytalocrinus* Wachsmuth & Springer from the Mississipian of Indiana it opens about half-way up; and in *Aulocrinus* Wachsmuth & Springer from the Mississipian of Indiana it opens at the end of a lateral branch (Springer 1926).

In the case of *Aesiocrinus* from the Pennsylvanian of Missouri, Lane (1975) states that nine specimens he examined from the Gurley Collection show anal spires which were broken or injured during life and subsequently regenerated. Regeneration in parts of such fossils is identified by stereom differences in such structures. In a figure of one such specimen (Lane 1875, Plate 1, fig. 1) the distal part of the spire is narrower, and hence most probably regenerated, and the external surface plating of this structure is smoother than in undamaged spires of the same species; in other specimens, the entire spire is narrower and has smooth plating, indicating that in such cases the entire spire was damaged and subsequently regenerated. Lane (1985) concludes that the enlarged anal

spires of dendrocrinoid and poteriocrinoid inadunates housed the gonads, that such separation of gonads from other vital organs was an advantageous response to predation, coinciding as it did with the extensive deployment of jawed predatory fish, and that the articulate crinoids (including comatulids) carried the separation of gonads from the viscera a stage further, placing them on the arms or pinnules.

(*j*) *Conclusions: a possible reproductive strategy for* Antedon bifida

The results of a five-season study of gametogenesis show that: (i) *A. bifida* maintains a high degree of sexual maturity over the entire annual cycle; (ii) the animal spawns only during a single month period in the cycle; (iii) the corkwing wrasse feeds on the swollen genital pinnules; and (iv) regeneration of lost pinnules occurs.

In present-day crinoids the gonads are always extra-thecal, mostly on the pinnules. In *A. bifida* predation by fish has been observed, with consequent heavy loss of gonads and subsequent regeneration, suggesting that this animal has adopted a strategy of tolerating predation of the pinnules to protect the more vital 'central' parts of the body located within the theca. Such a strategy would be justified only if the loss and regeneration at the observed level could be equated in energetic terms. Grahame & Branch (1986) have remarked that the cost of reproduction, either direct in terms of resources used, or indirect in terms of additional risks taken, is a central feature of life-history strategies, risks that represent an optimal solution, not a maximal one; if the energy budget of an animal is not taken into account, then data on reproductive effort can be misleading. Calow (1979) points out that reproduction will have an adverse effect on the parents when the process uses energy that may be otherwise needed for the metabolic well-being of the parent; usually, developing gonads compete for energy with other metabolic processes, and diversion of resources to reproduction and away from maintenance activities in times of nutritional impoverishment has been demonstrated in many examples from the animal kingdom.

In *Antedon*, however, a different situation may obtain. The crinoid gonads are enveloped in an absorptive epithelium, part of the active external membrane that envelopes the entire echinoderm body (Bickell *et al.* 1980; Lawrence 1987). Further, Holland & Nealson (1978) and Grimmer & Holland (1990) have described gram-negative bacteria within or below the crinoid cuticle which are most probably symbiotic. It seems likely, therefore, that nutrients supplying the developing gametes may be obtained, wholly or partly, by uptake of dissolved organic material. Such a nutritional pathway has yet to be demonstrated in this particular case, as has the presence of suitable and sufficient dissolved organic material in the ambient seawater across the year. If subsequently shown to exist, the pathway could indicate that the pinnule-borne gonads of the crinoid are maintained by a parenteric process that has minimal effect on the energy needs of the rest of the body, and that the maintenance of reproductive tissue at a high level throughout the year may thus be energetically economical.

In an initial study of the utilization of dissolved substances by a crinoid, West (1978) showed that active uptake of glucose and amino acids by *Leptometra phalangium* (J. Müller) was higher in the arms than in the theca, suggesting that these animals use dissolved substances to supplement their conventional nutrient supply, particularly to the highly active tube-foot system of the arms and pinnules (Nichols 1960). Unfortunately, the 'conventional' transport physiology of crinoids is poorly known, so the contribution made by food obtained through both enteric and parenteric agencies cannot yet be adequately assessed.

This study has underlined the need for more detailed work on the life history of extant crinoids. Calow (1984) has pointed to the influence of natural selection on resource utilization, and the need for animals to allocate resources between often-conflicting processes. The 'economization principle' suggests that organisms invest in a way that ensures the retention of vigour through the normal expectation of life. Fisher (1930) sees the need to know the mechanism by which a 'just apportionment of nutrients between the gonads and the rest of the body' are made, and the circumstances in the life history of an organism that renders it profitable to divert a greater or lesser share of available resources to the production of gametes. Post-traumatic evisceration of gonads, followed by full regeneration, is a fairly common feature in other echinoderm classes, particularly holothuroids (Pawson 1966), which involves a relatively large sacrifice of organic material in the interests of saving the future reproductive potential of an iteroparous organism. In the case of *Antedon bifida*, the conclusion seems inescapable that a high level of sexual maturity is maintained across the year, so that, despite losses by predation, the overall result is an optimal maintenance of reproductive potential.

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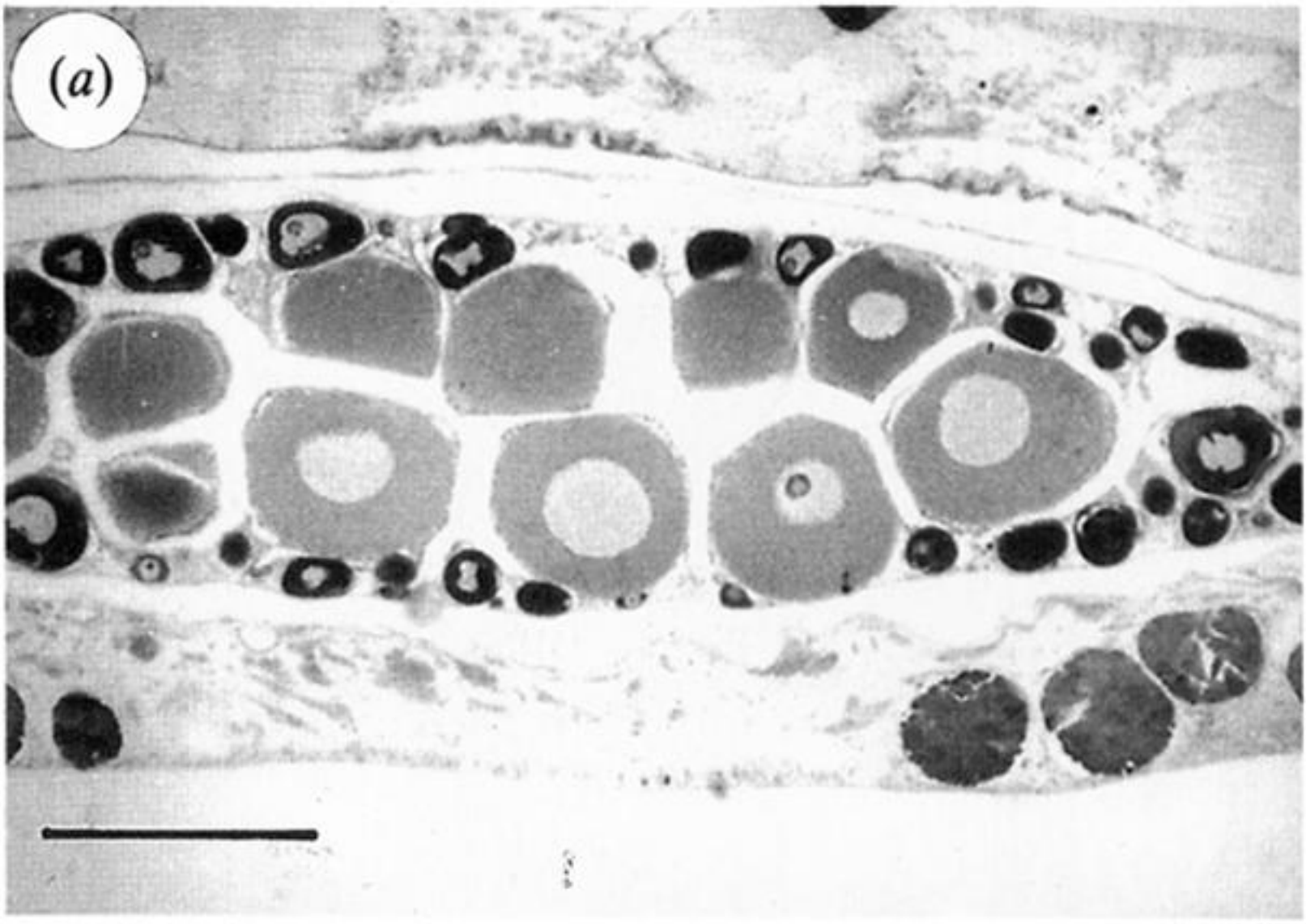
Figure 2. *Antedon*-covered boulder on underwater ledge at Berry Head, South Devon, U.K.



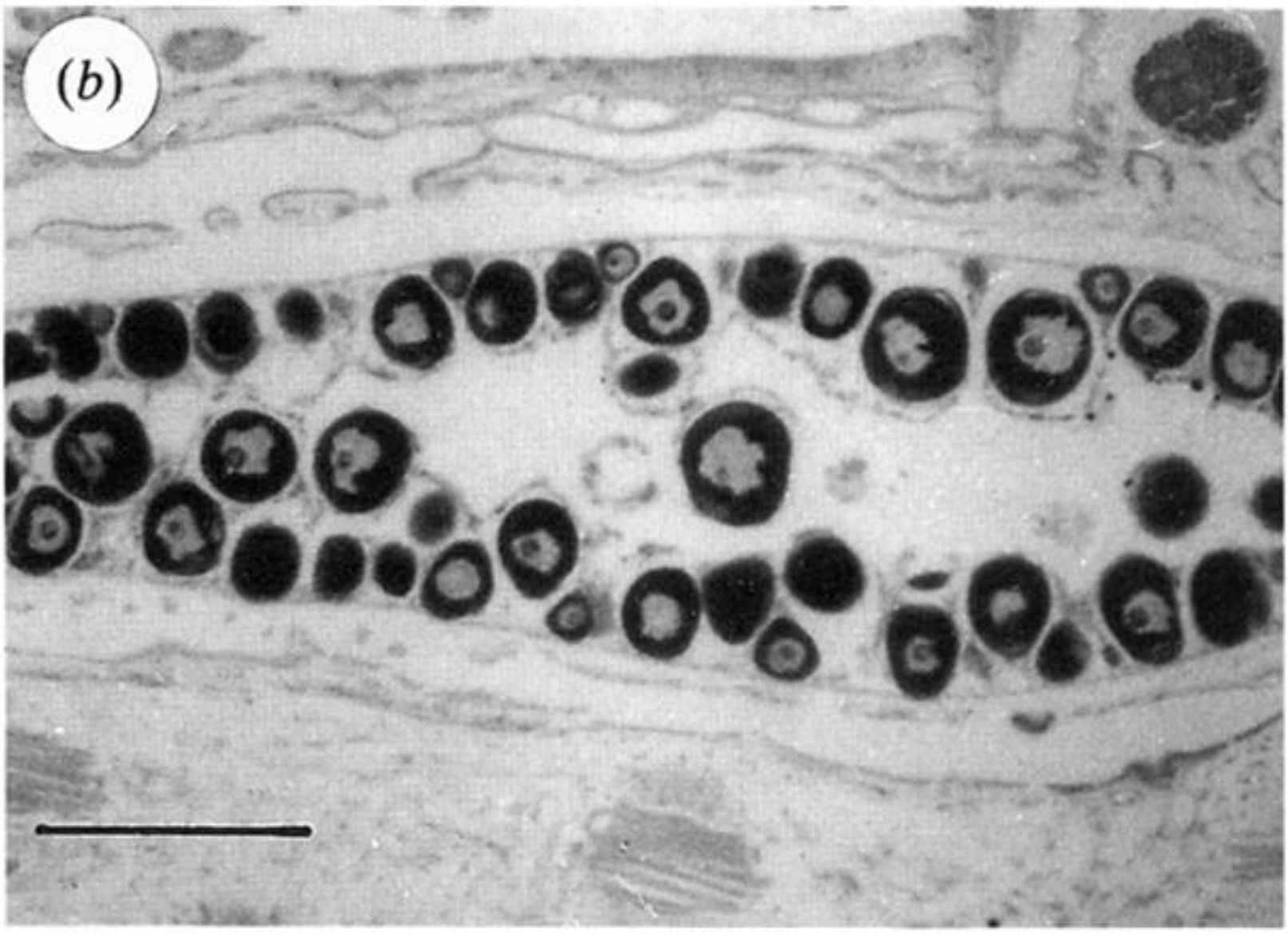
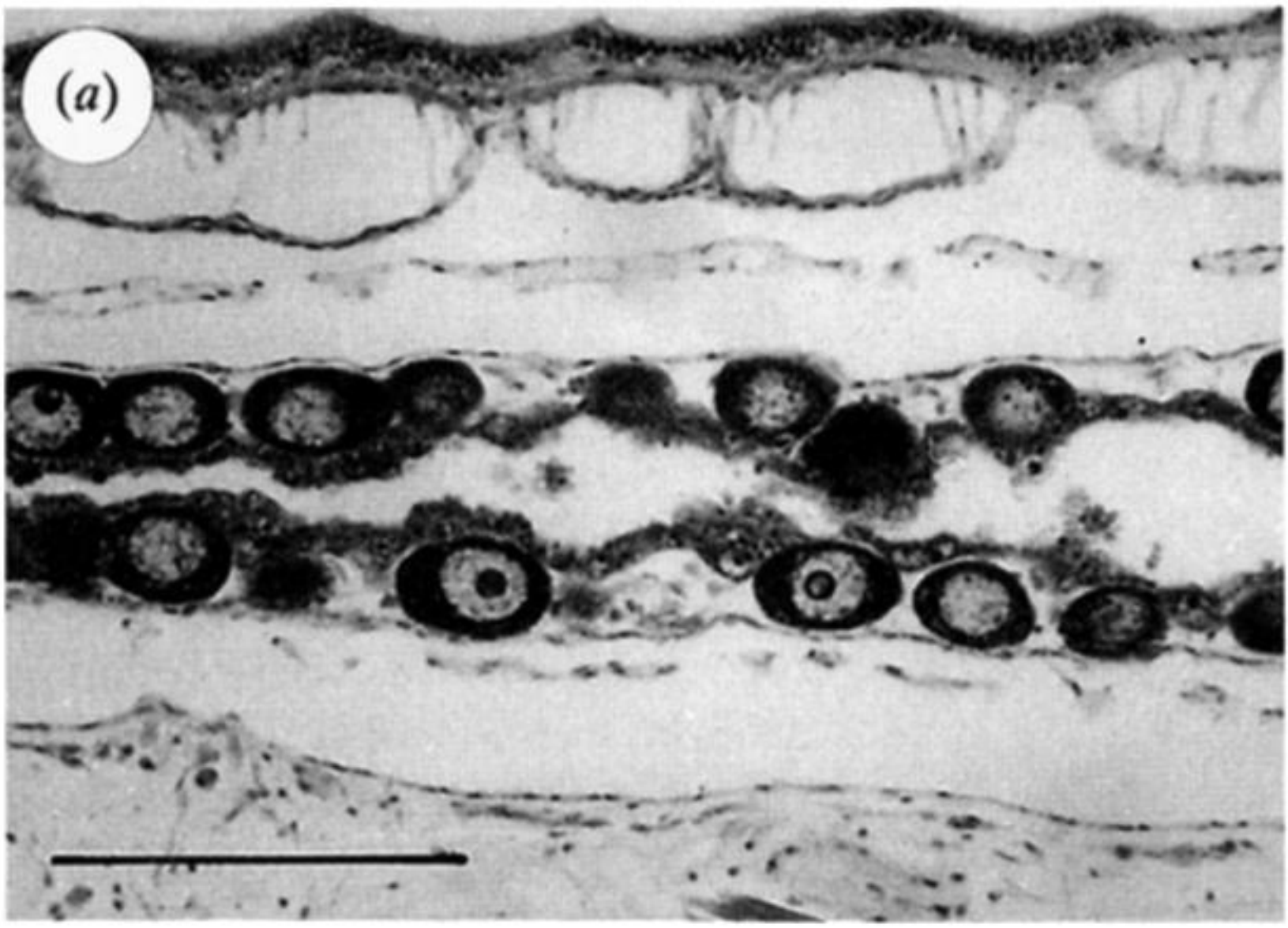


Figure 4. Comparison of ovaries from different pinnules along a single arm of specimen 1 from a sample of *A. bifida* taken in October 1982. (*a*) Pinnule 5, with vitellogenic oocytes of sheddable size in the ovarian lumen and small oogonia and oocytes adjacent to the germinal epithelium. (*b*) Pinnule 10, having only oogonia and pre-vitellogenic oocytes, some being squeezed from the ovary periphery towards its lumen. Scale bars: 200 μm.



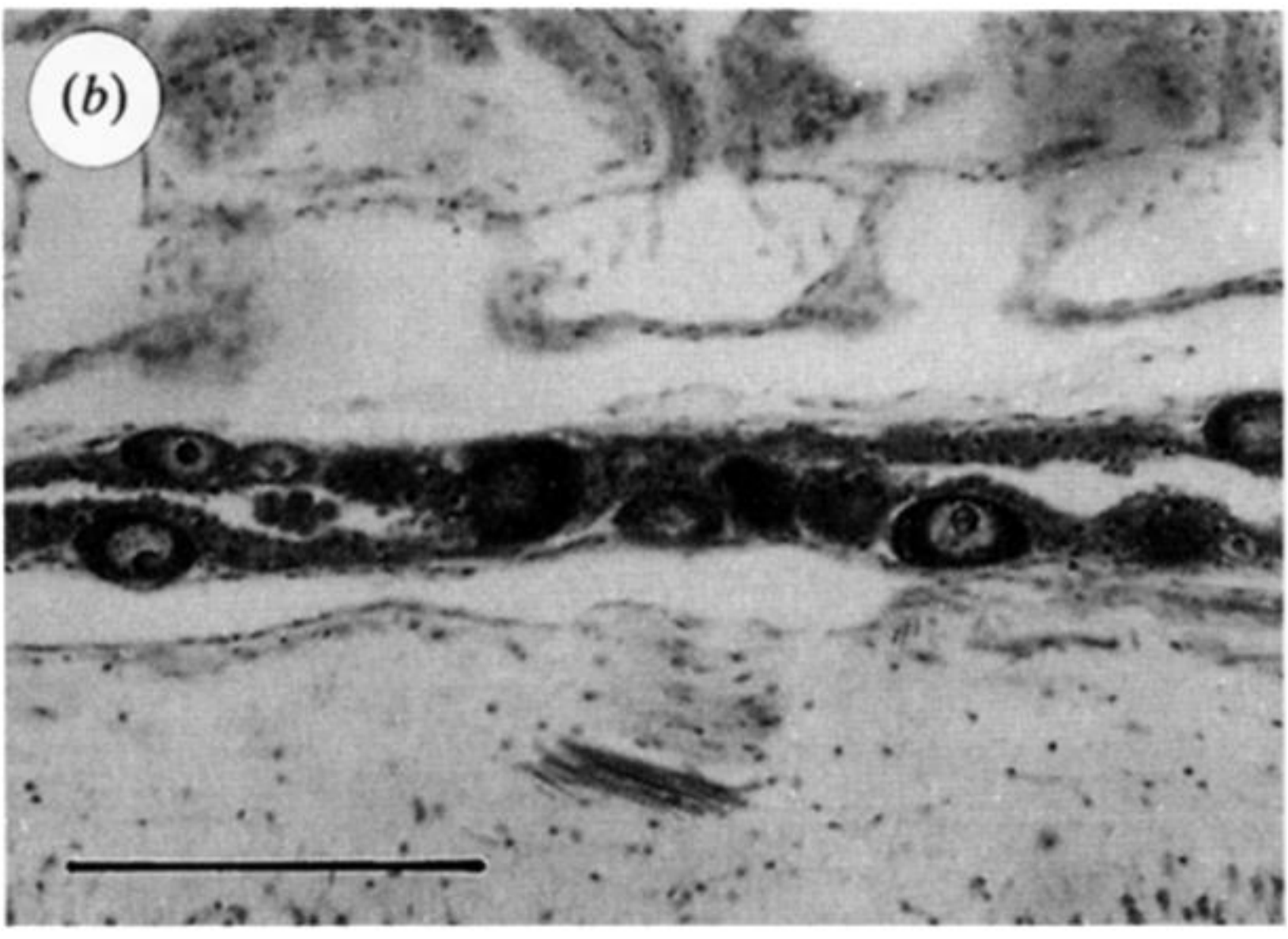
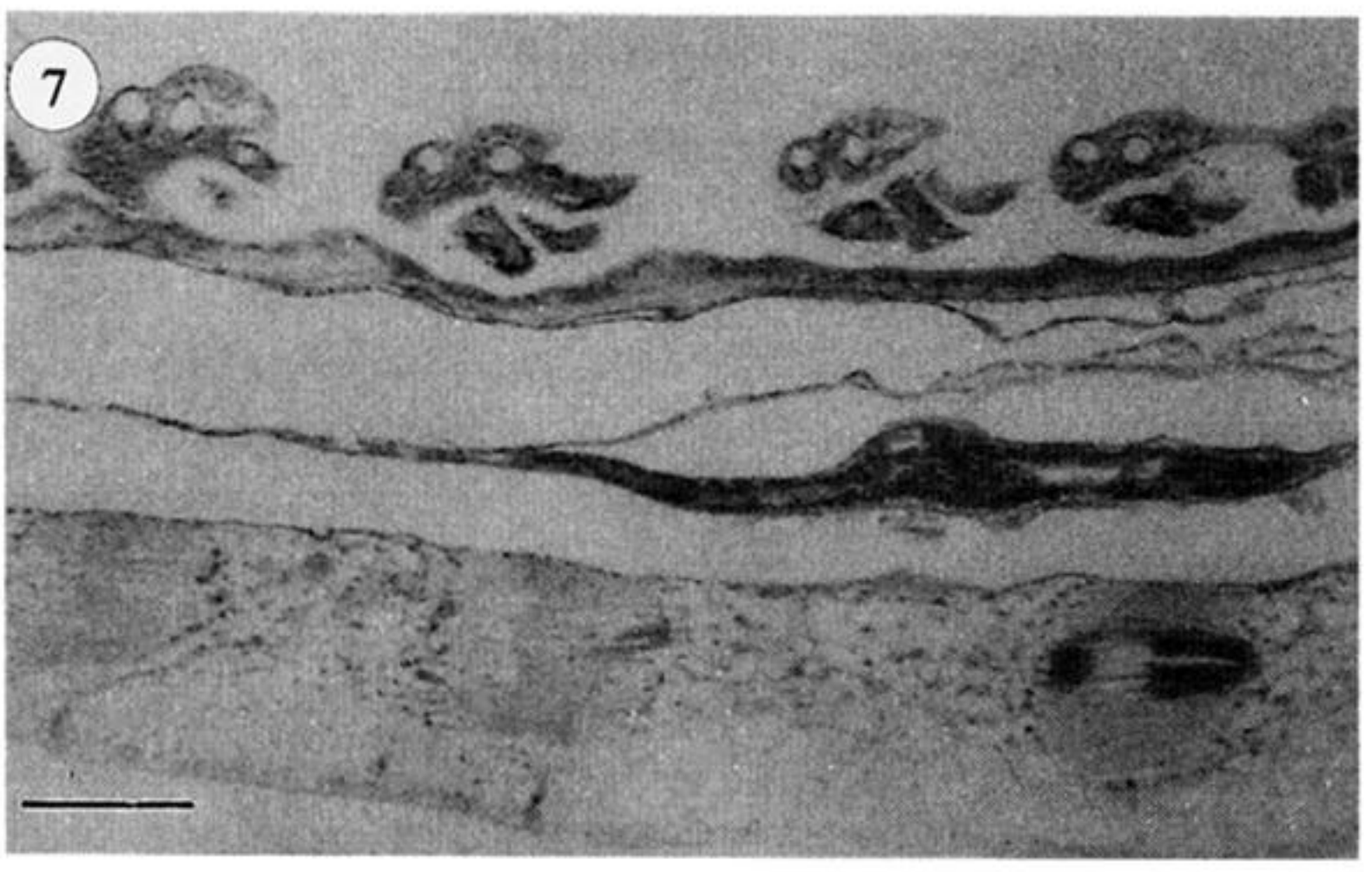
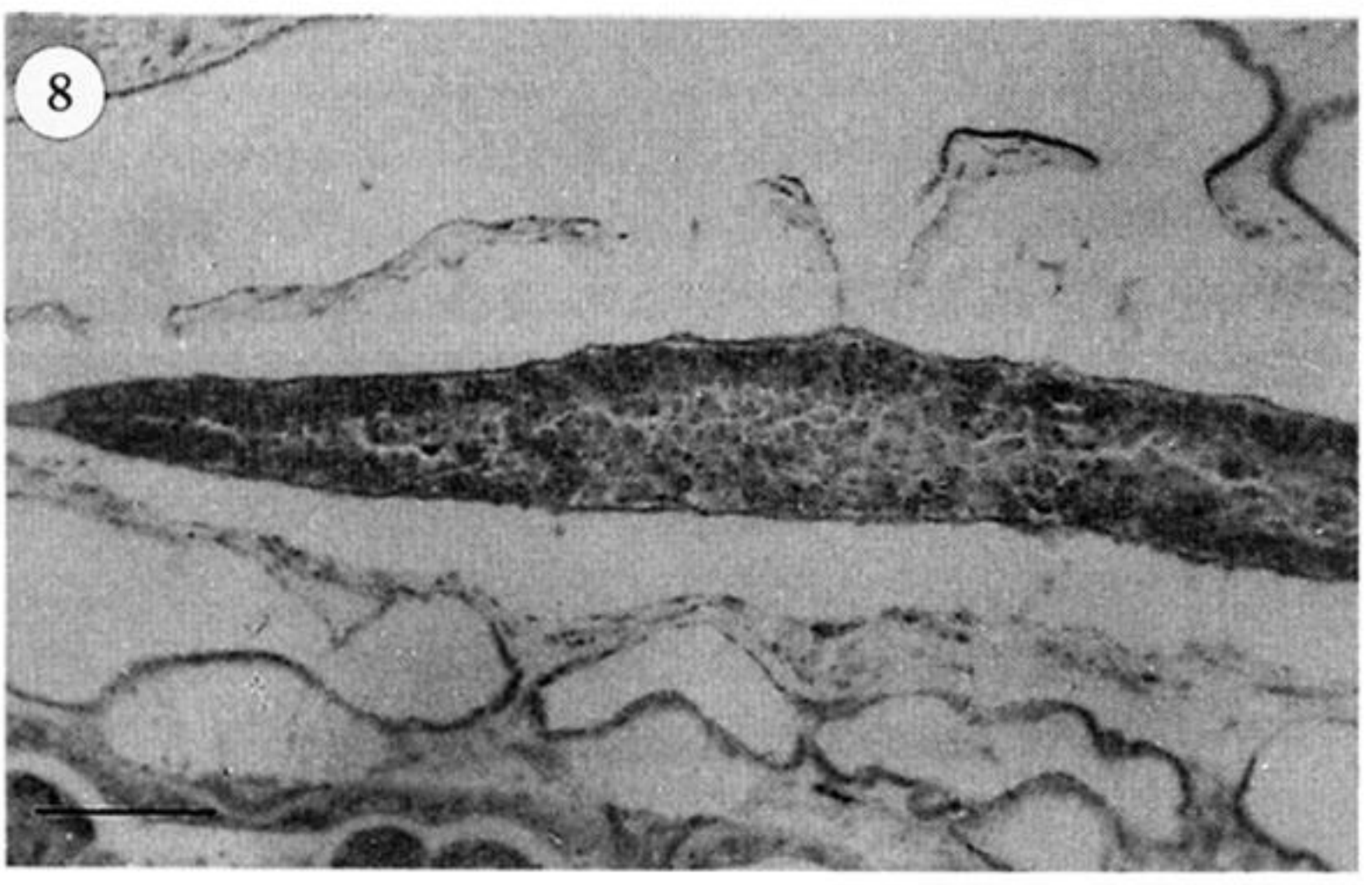
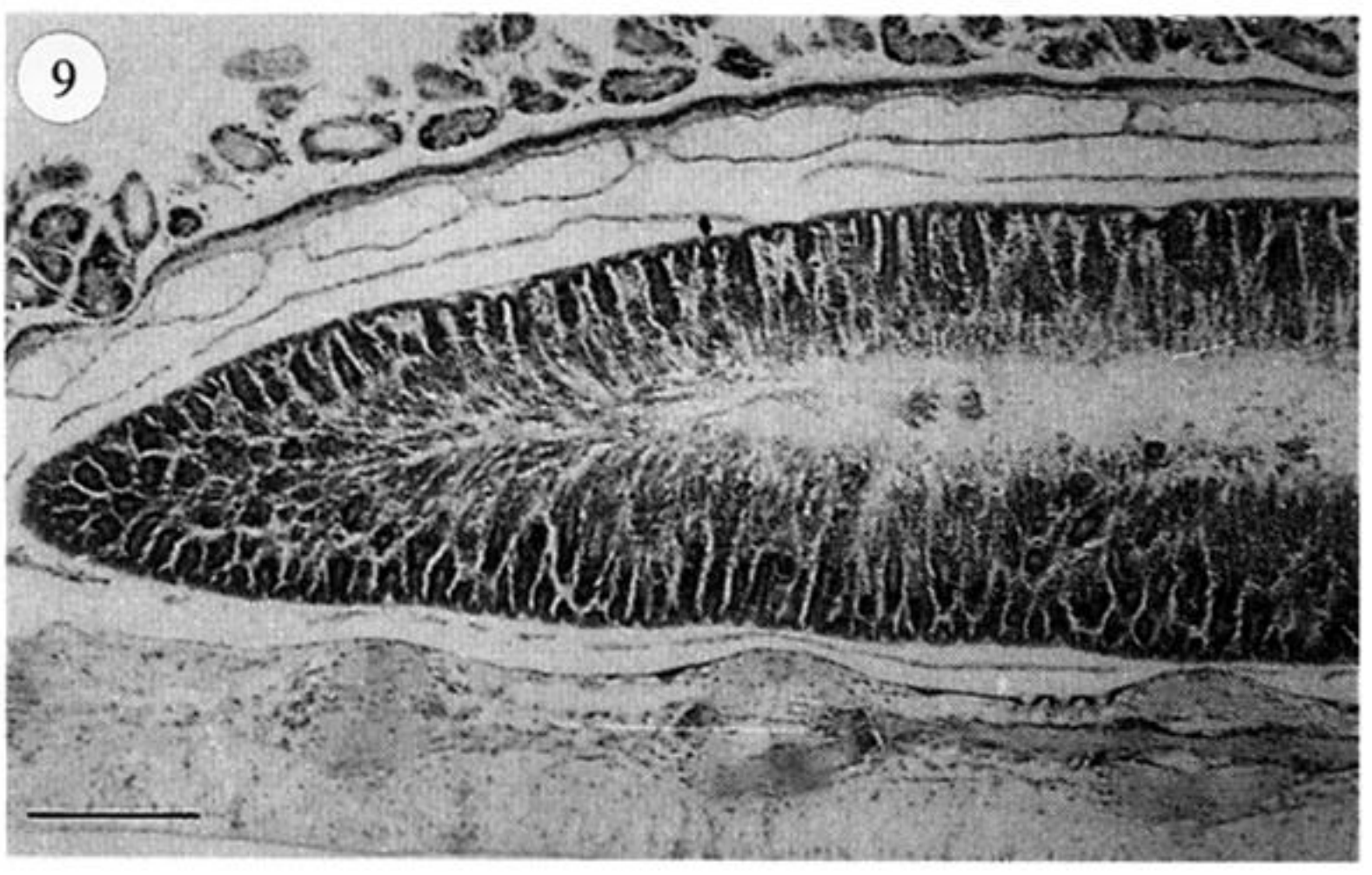
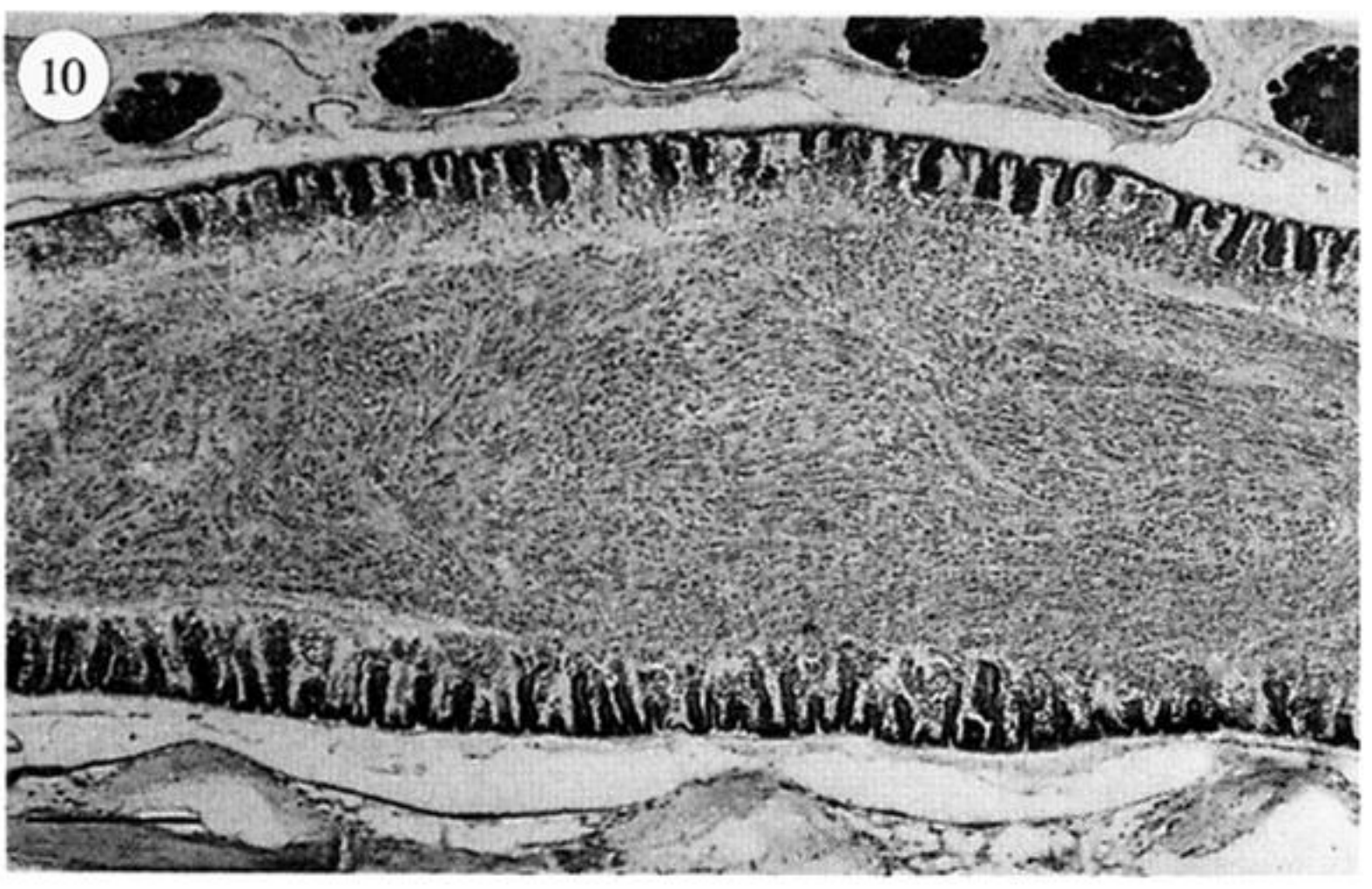


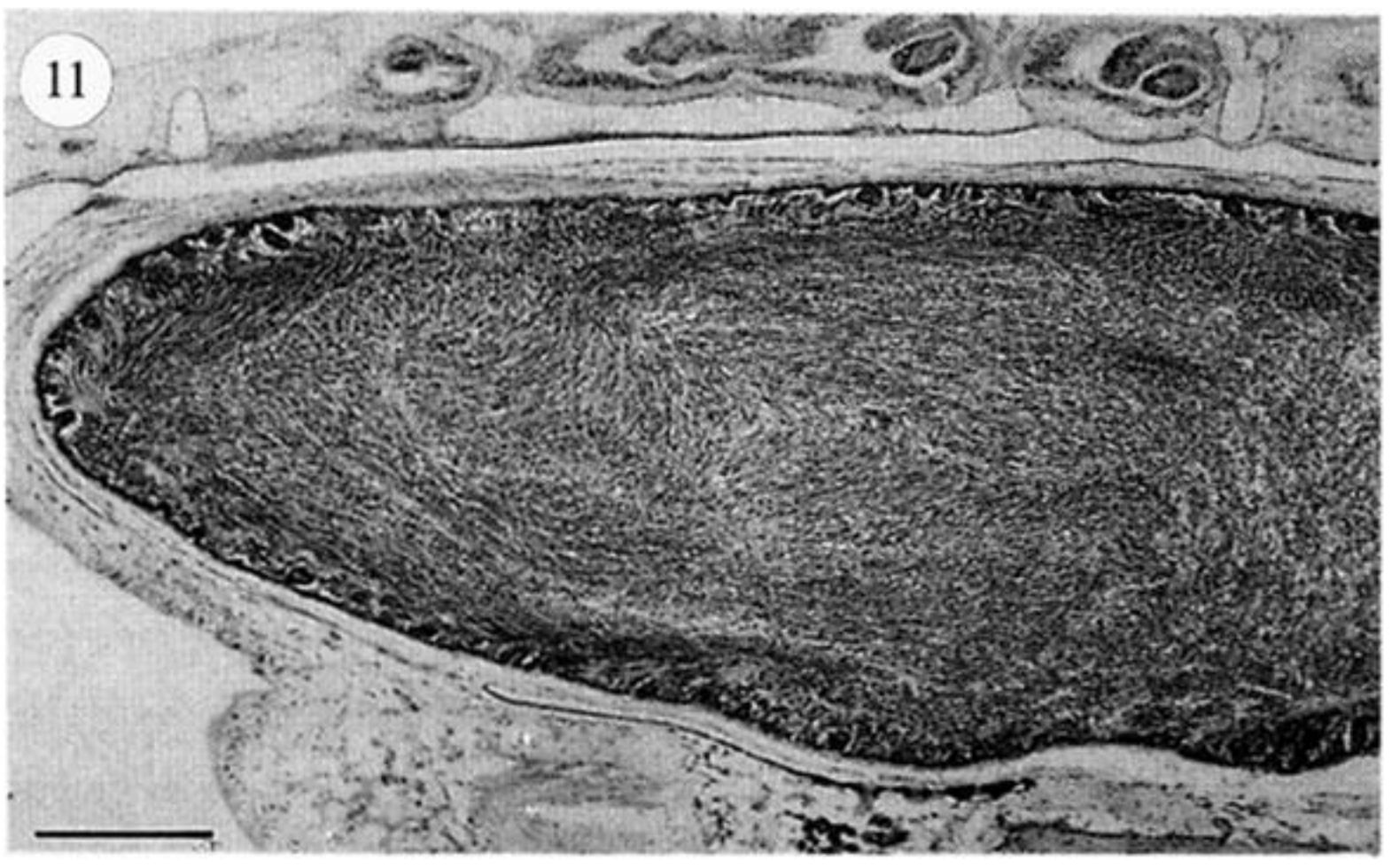
Figure 6. Comparison of ovaries from different pinnules along a single arm from specimen 5 (see figure 5) of *A. bifida* taken in October 1984. Early oocytes in pinnule 5 (*a*) larger than those from pinnule 10 (*b*). Scale bars: 100 μm.











Figures 7–11. Micrographs of spermatogenic stages used here to assess reproductive cyclicity of *A. bifida*. Stained with Ehrlich's haematoxylin and eosin.

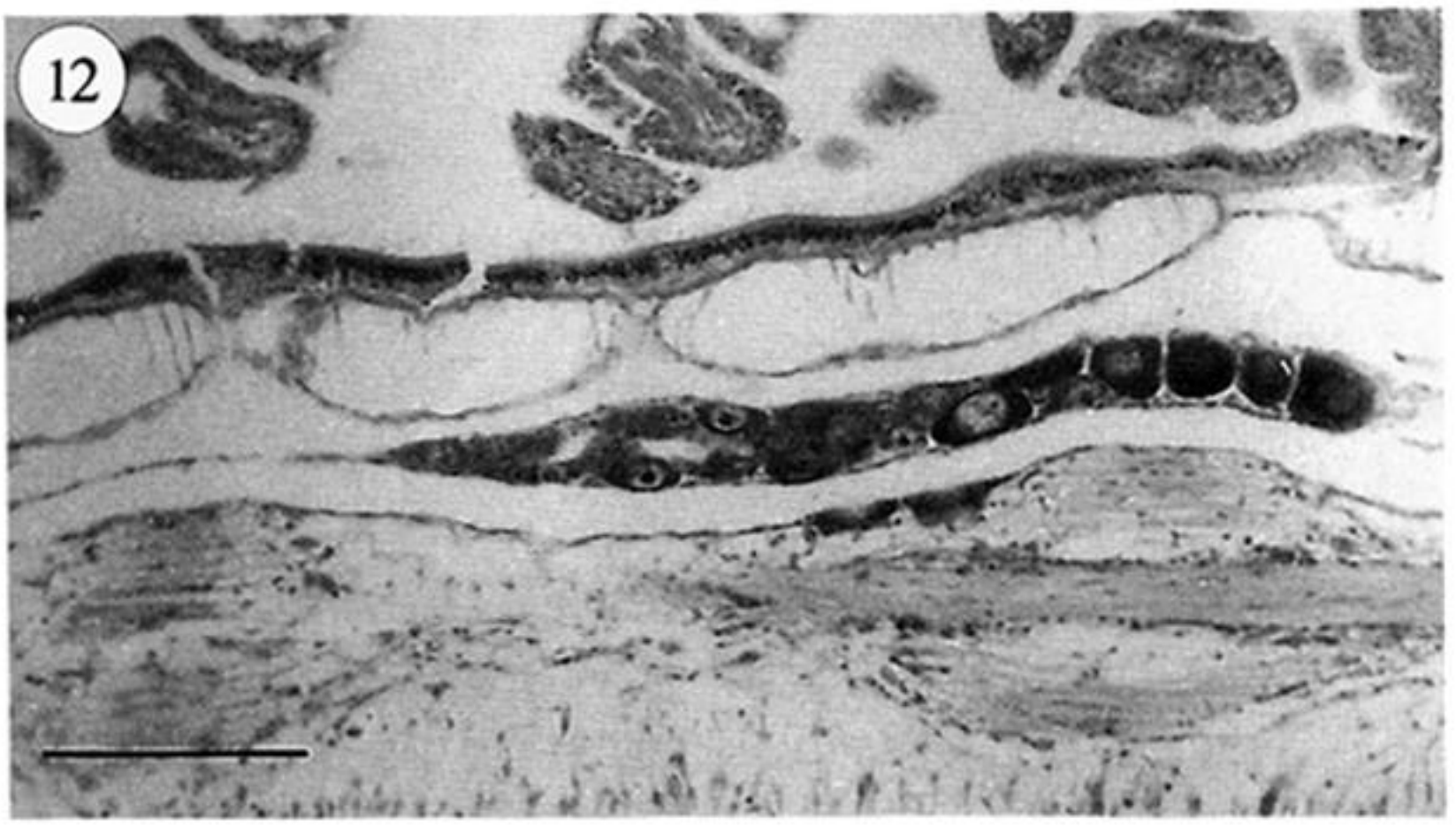
Figure 7. Stage 1. Testis small, only slightly expanded from genital cord and only partially lumenate. Scale bar: 200 μm.

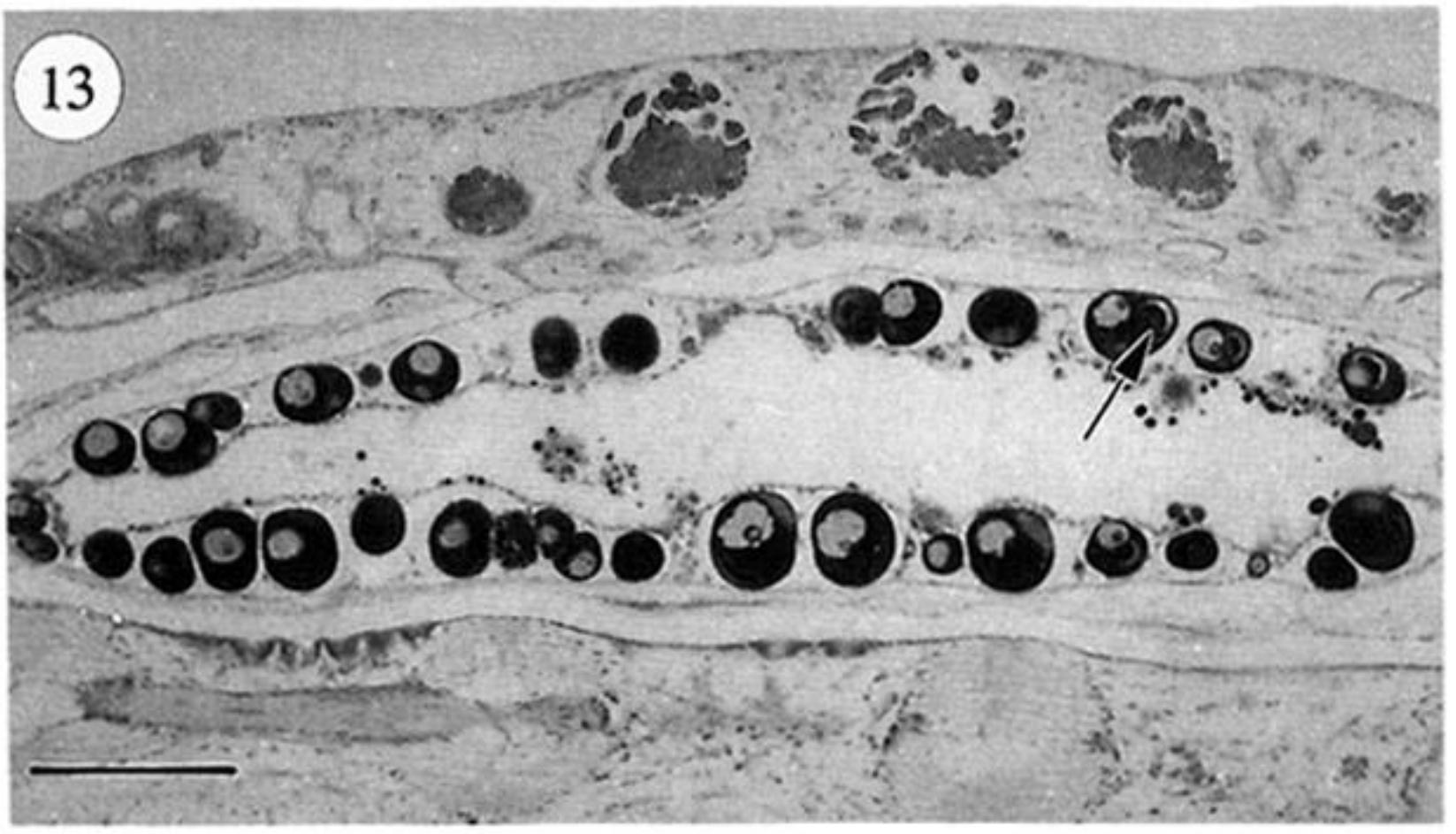
Figure 8. Stage 2. More of the genital cord has expanded and the lumen is enlarging. Germinal epithelium beginning to fold into pyramids. In this specimen, some pyramids are producing sperm. Scale bar: 100 μm.

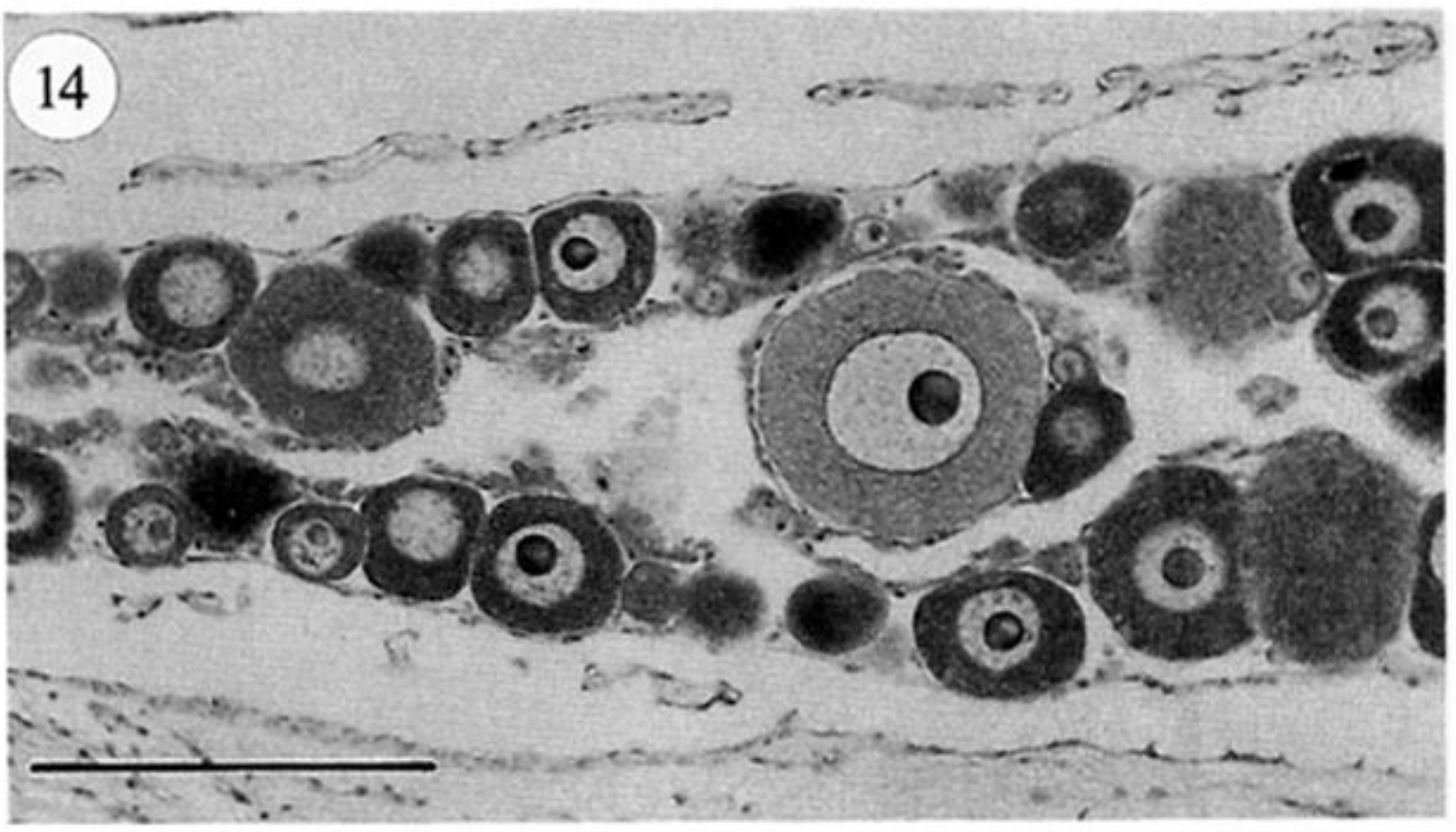
Figure 9. Stage 3. Pyramids fully formed and producing sperm, a few of which already occupy the testis lumen. Scale bar: 200 μm.

Figure 10. Stage 4. Part of testis wall at the maturing stage. Many mature sperm being proliferated at the centripetal ends of the pyramids. Scale bar: 200 μm.

Figure 11. Stage 5. Fully mature. Pyramids of germinal epithelium reduced or non-existent. Mass of mature sperm in testicular lumen. Scale bar: 200 μm.





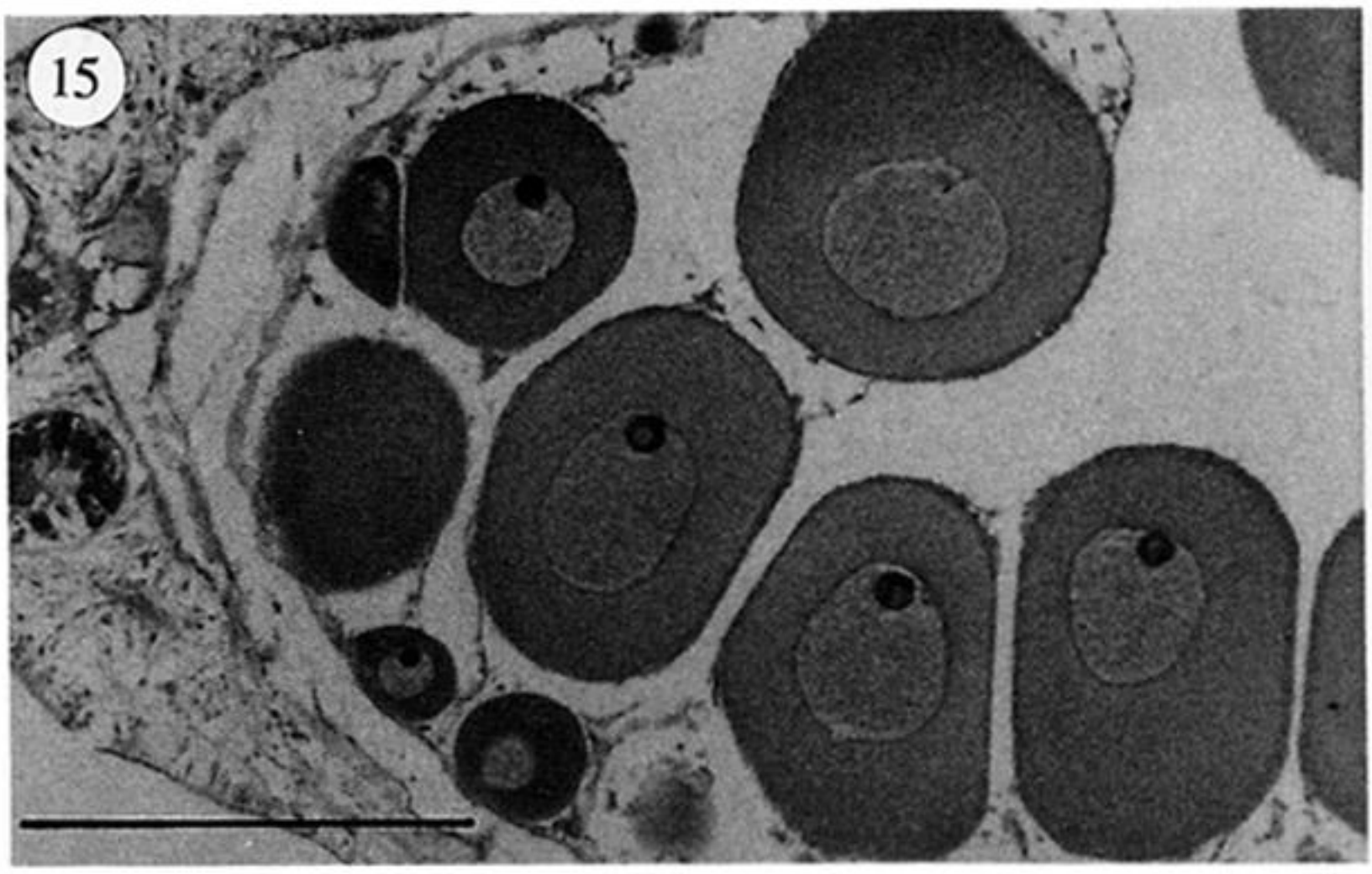


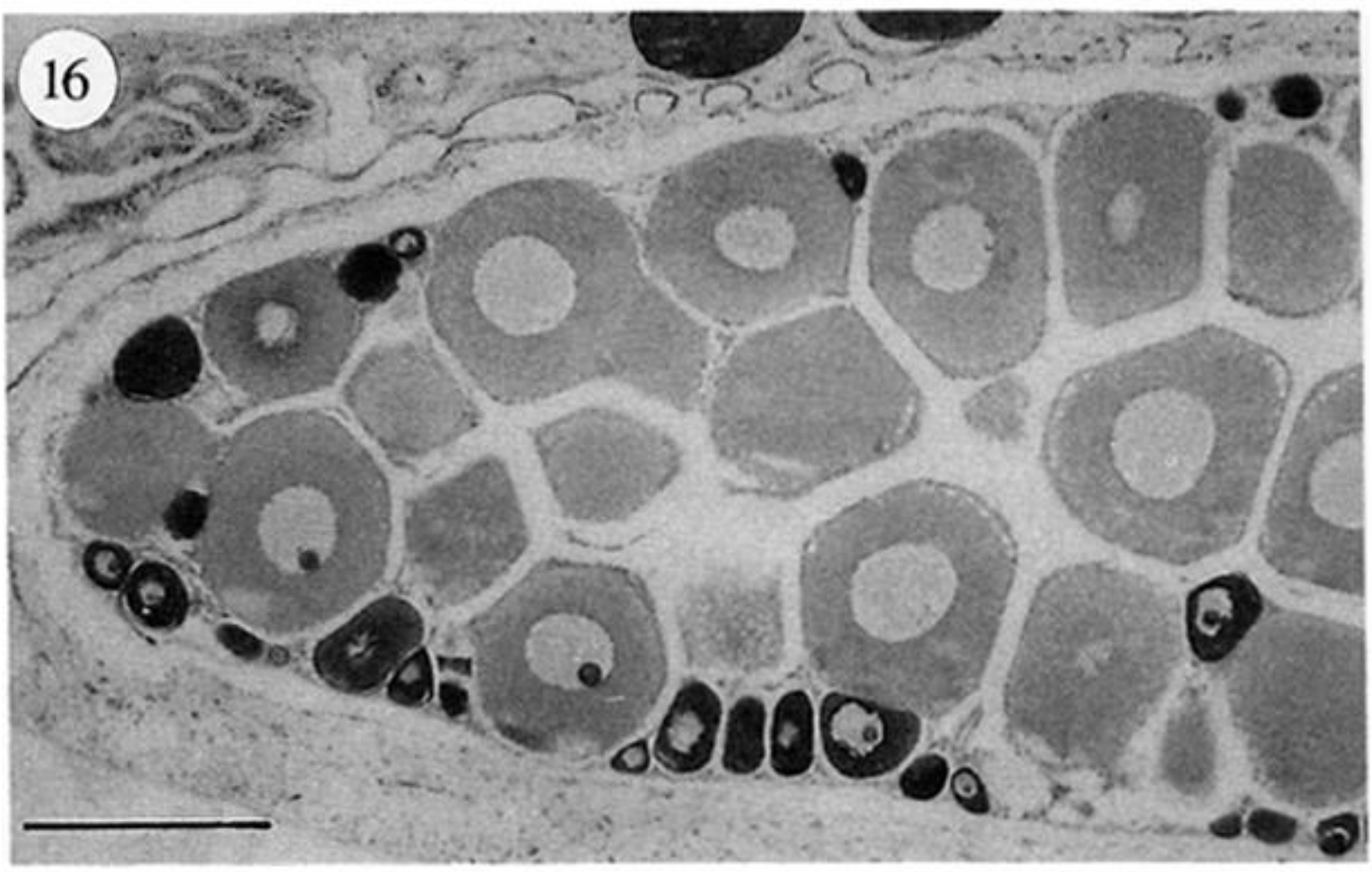
Figures 12–14. Sagittal sections of early ovarian development.

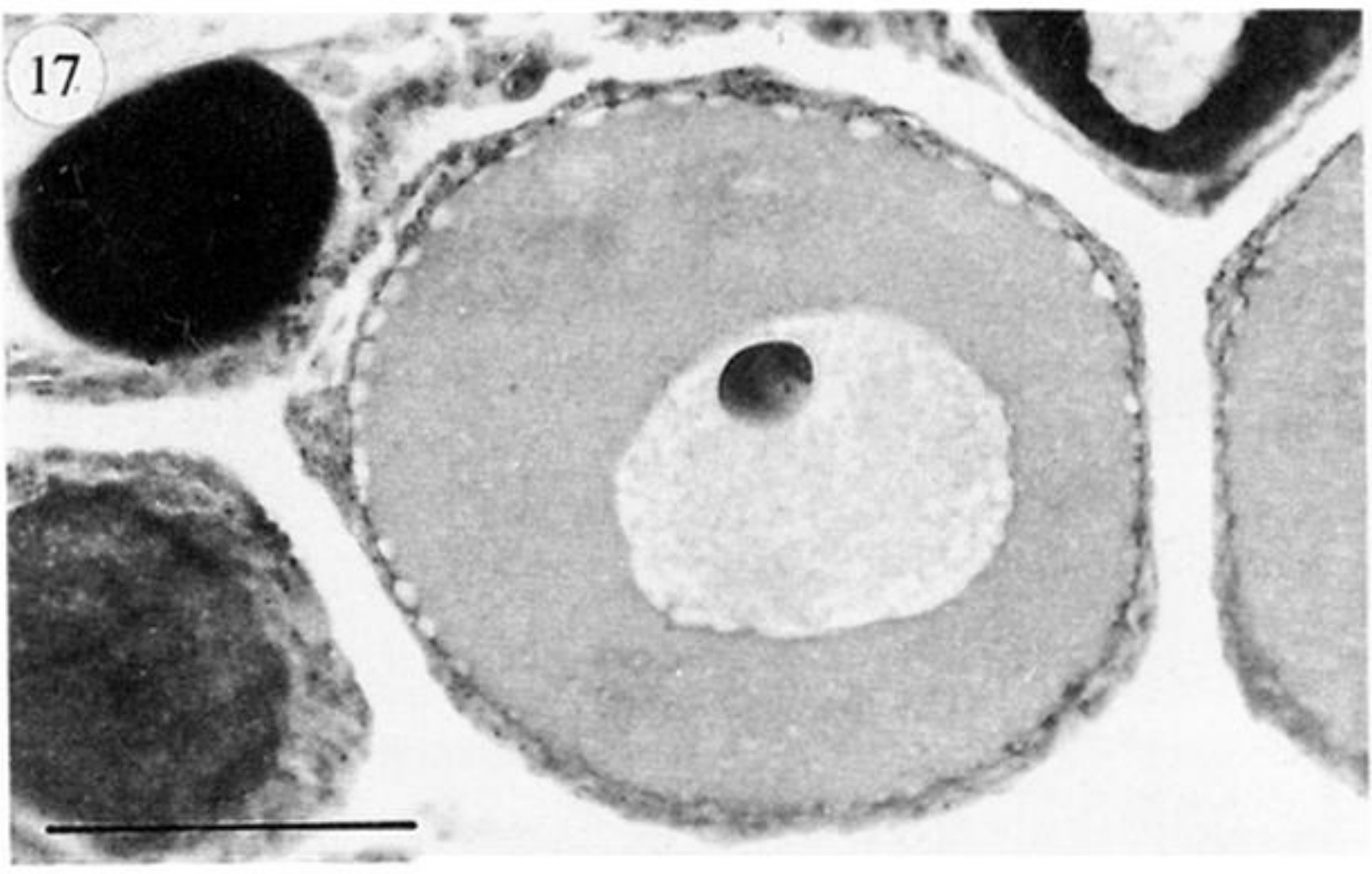
Figure 12. Genital cord (left centre) slightly expanded to form early ovary, in which some oogonia are recognizable and ovarian lumen is appearing. Scale bar: 100 µm.

Figure 13. More advanced. Oogonia and early oocytes proliferating on centrifugal side of germinal epithelium. Nutritive phagocytes present in lumen. Some oocytes already showing yolk nucleus (arrowed). Scale bar: 150 µm.

Figure 14. More advanced. Some larger oocytes incorporating yolk (lighter-coloured cytoplasm) are squeezed centripetally towards lumen, still surrounded by inner epithelium. Scale bar: 100 µm.





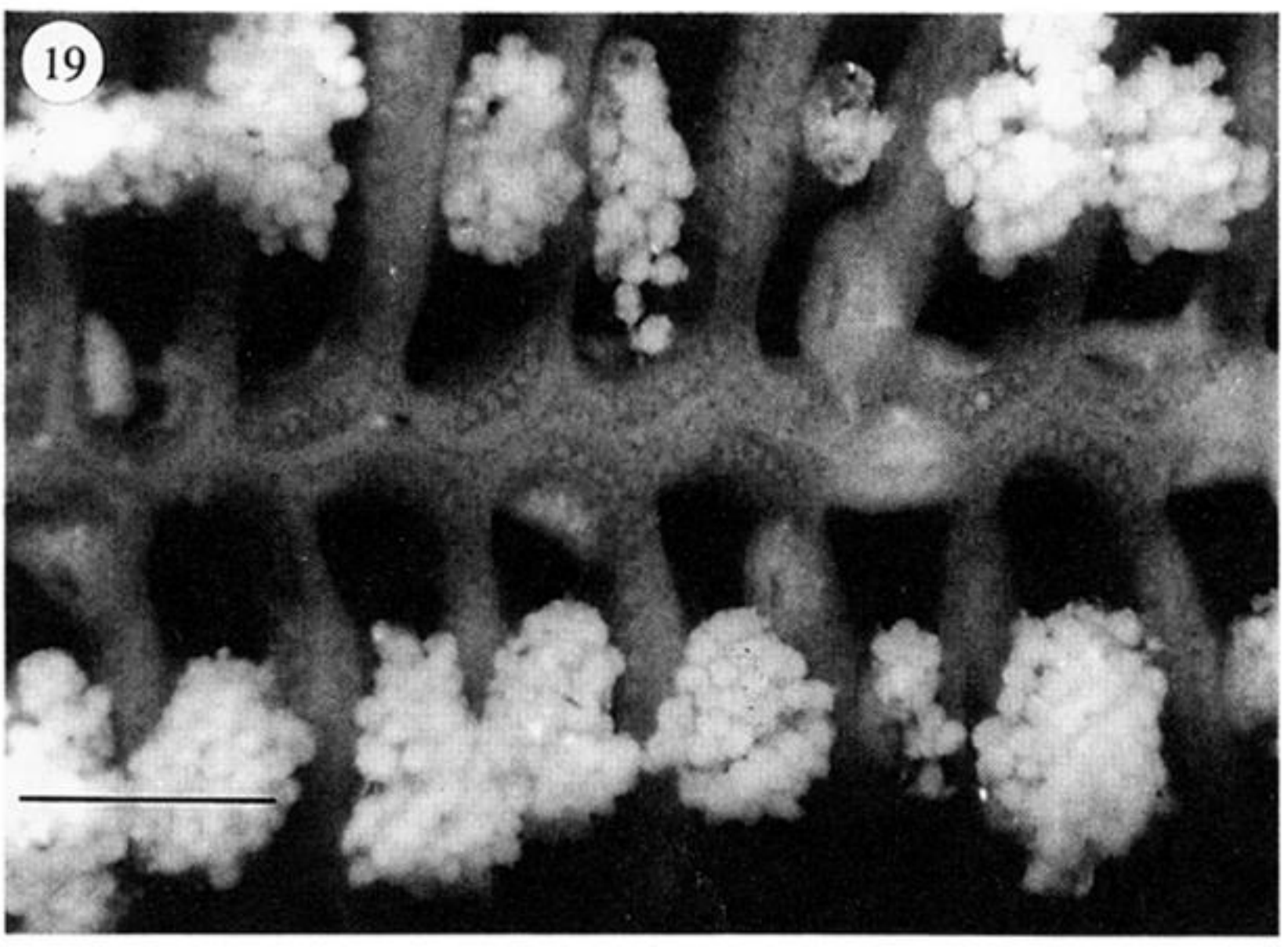


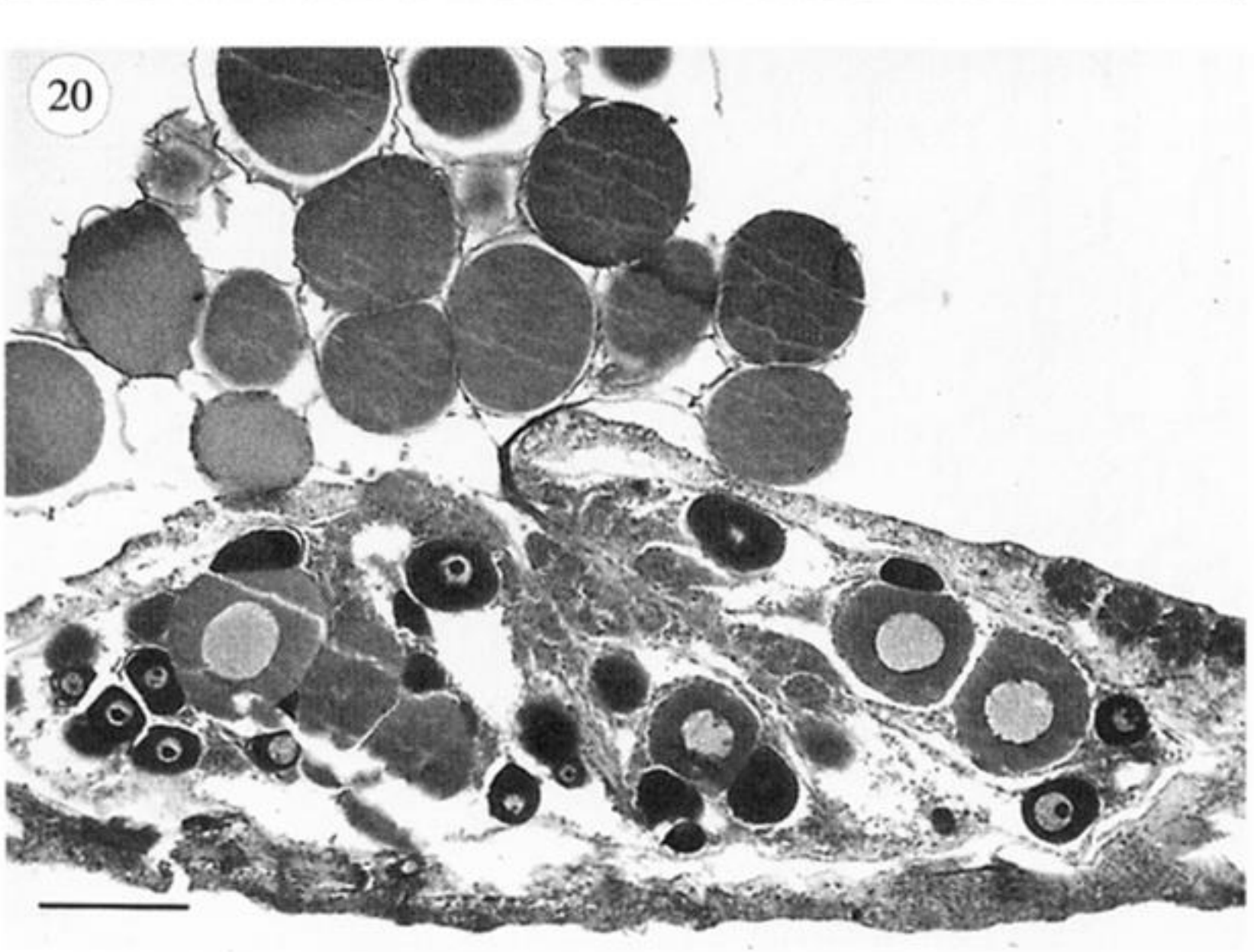
Figures 15–17. Advanced oocyte development.

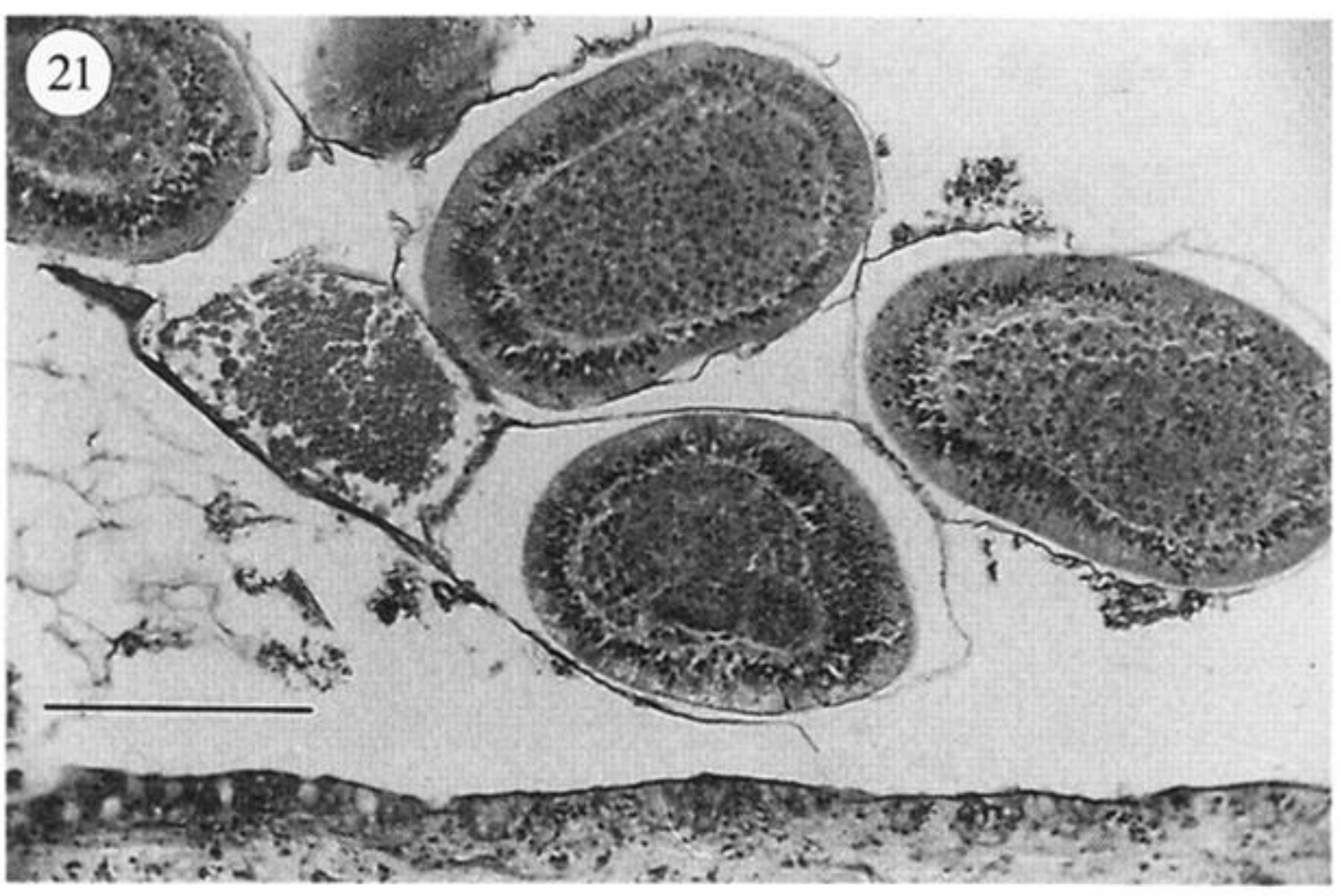
Figure 15. Most oocytes large and yolky and still enveloped in inner epithelium of ovary; oogonia and small oocytes for next season also present. Scale bar: 200 μm.

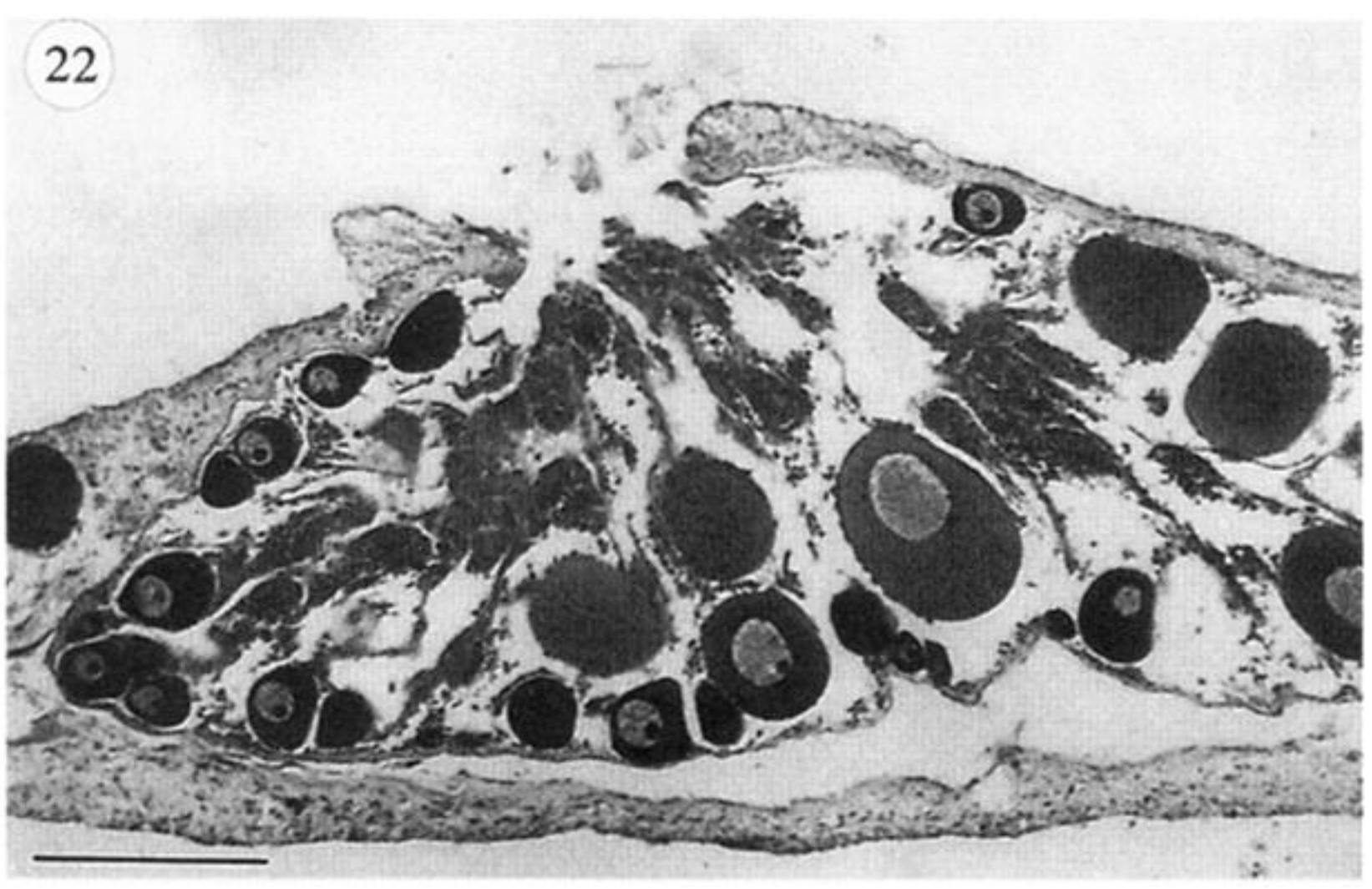
Figure 16. Large sheddable oocytes, some with peripheral faceting. Scale bar: 200 μm.

Figure 17. Single large oocyte, showing peripheral faceting. Scale bar: 100 μm.









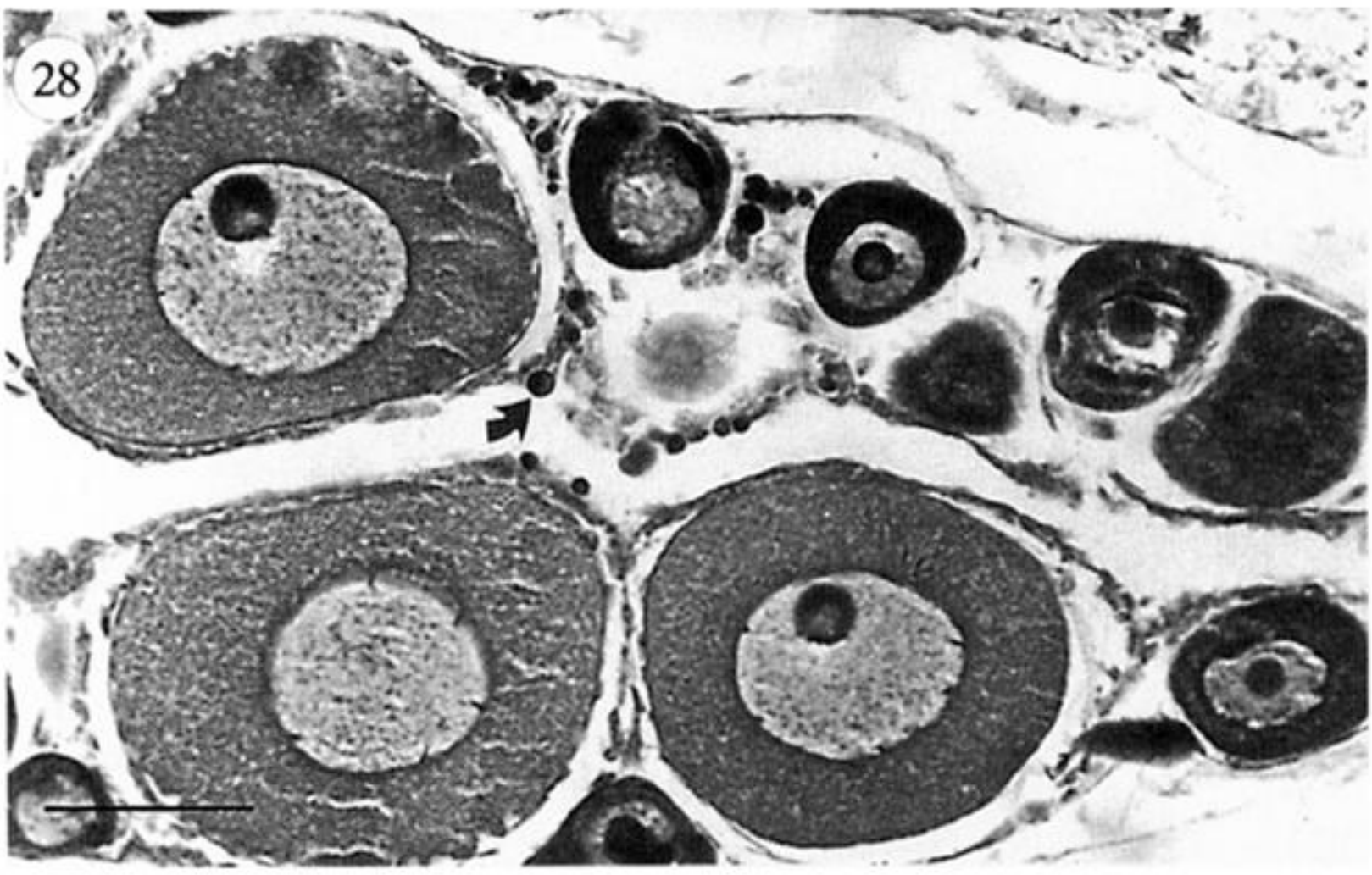
Figures 19–22. Post-spawning genital pinnules of *A. bifida*.

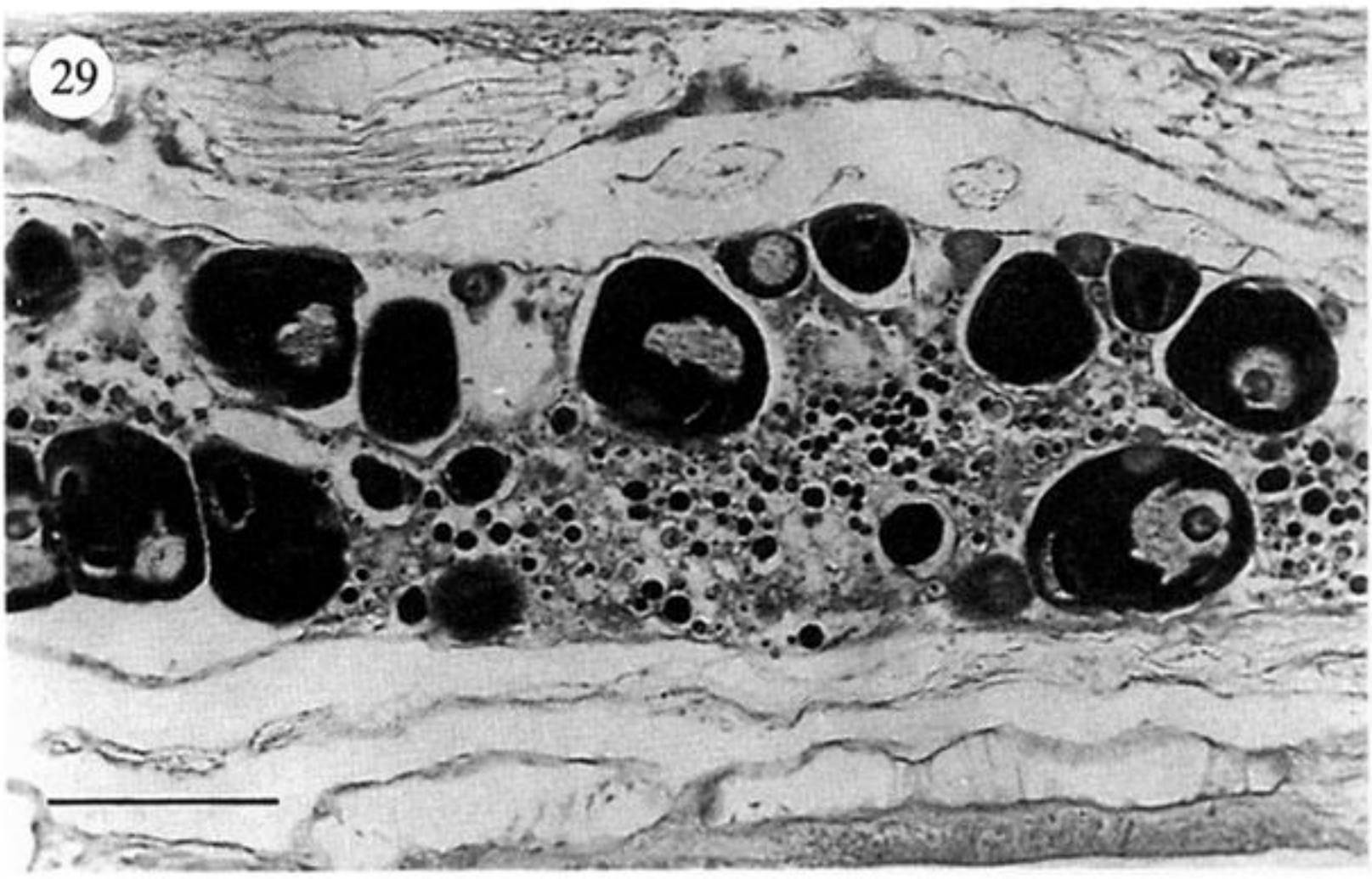
Figure 19. Fertilized ova retained in a mucous net on the external pinnular wall of *A. bifida*. July 1992. Scale bar: 2 mm.

Figure 20. Sagittal section of recently spawned ovary, with post-meiotic eggs retained in mucous net. July 1986. Scale bar: 150 μm.

Figure 21. Young larvae in mucous net. July 1986. Scale bar: 100 μm.

Figure 22. Genital pinnule after hatching embryos from external brooding net, now disappeared. Temporary gonopore still present. Scale bar: 200 μm.





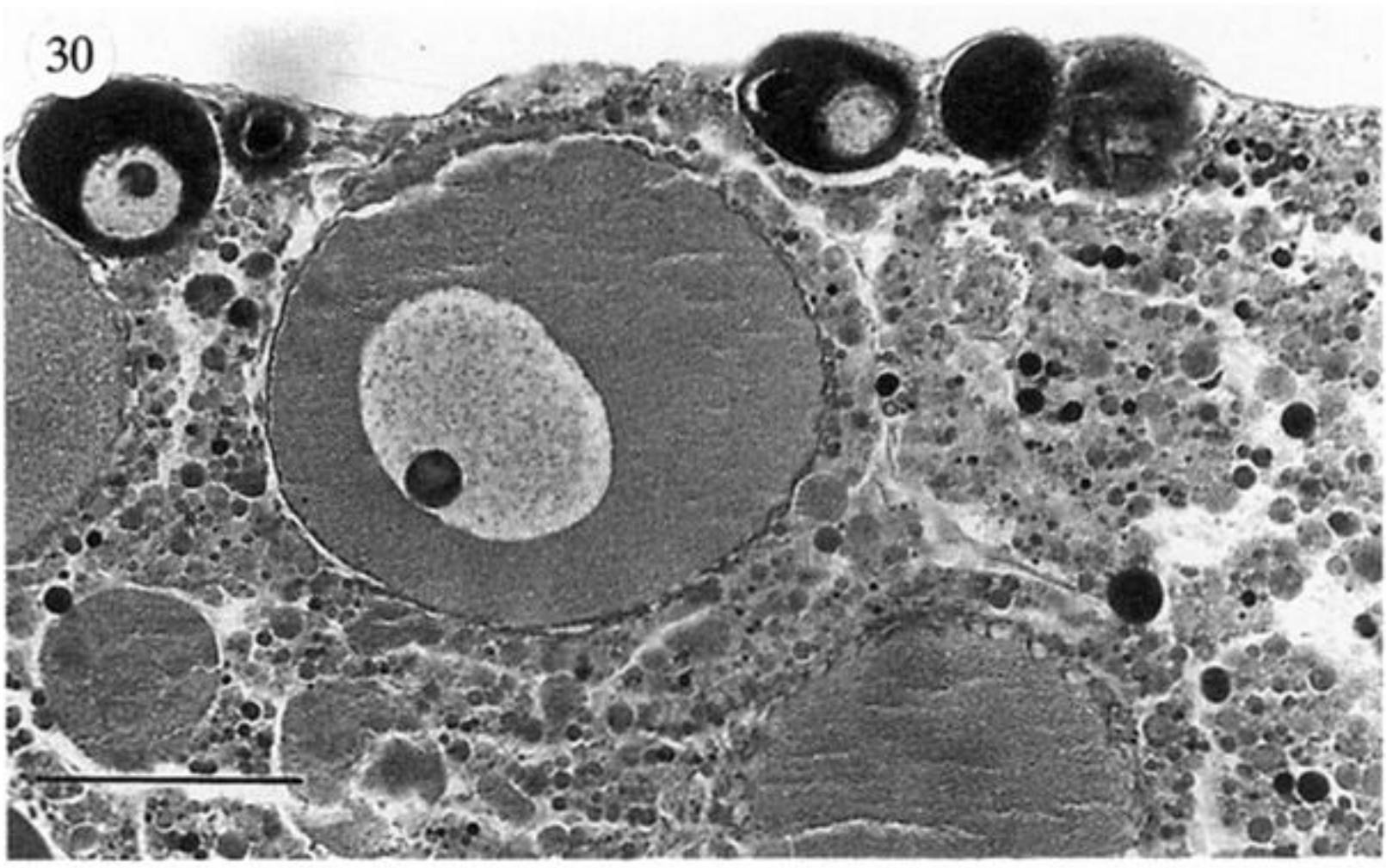


Figure 28–30. Photomicrographs of various stages in the proliferation of nutritive phagocytes (accessory or nurse cells) in the ovaryies of *A. bifida*.

Figure 28. A few nutritive cells enlarging in germinal epithelium (arrowed). May 1986. Scale bar: 50 µm.

Figure 29. Nutritive phagocytes in ovarian lumen. October 1983. Scale bar: 100 µm.

Figure 30. Nutritive phagocytes surrounding unshed advanced oocytes, one of which has a faceted periphery. March 1986. Scale bar: 100 µm.

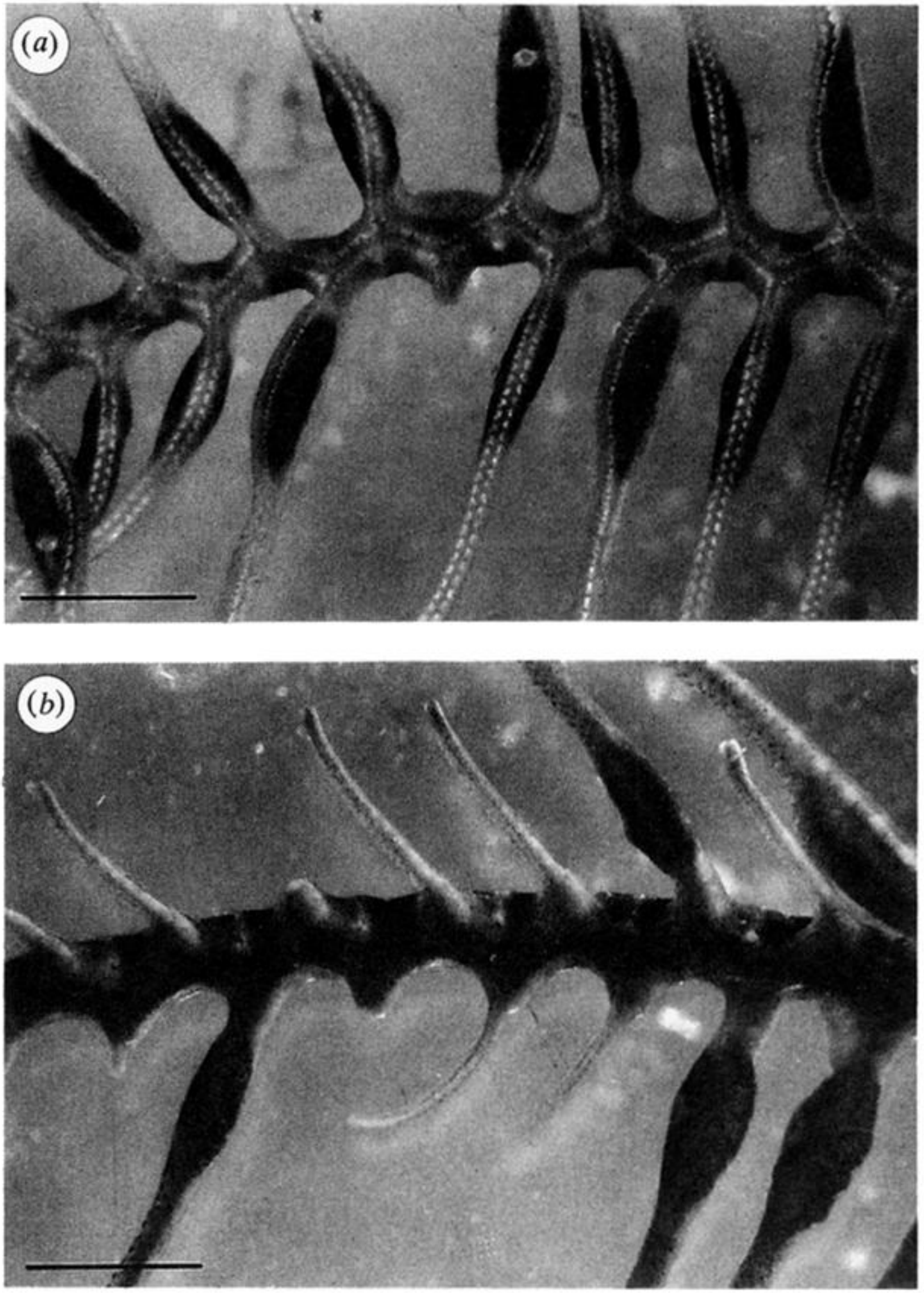


Figure 31. Part of the arm of two specimens of *A. bifida* which have suffered predation. (*a*) Slight predation, with a single genital pinnule missing. (*b*) Severe predation, with pinnules in various stages of regeneration. Scale bar: 2 mm.

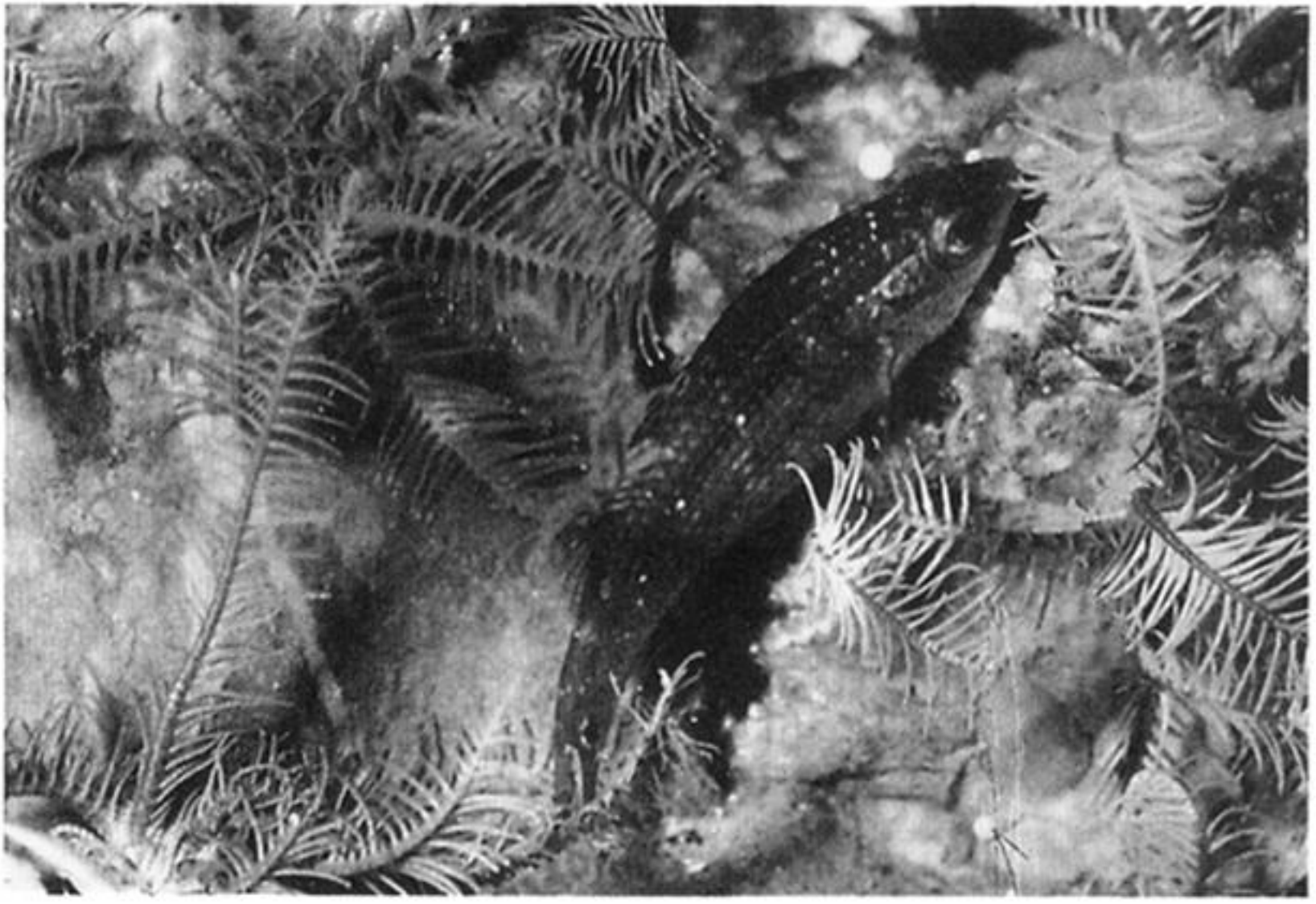


Figure 32. Corkwing wrasse, *Crenilabrus* (=*Symphodus*) *melops*, attacking genital pinnules of *A. bifida* in an experimental tank.