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Identifying the developmental stages of preserved eggs of snapper, *Pagrus auratus,* from Shark Bay, Western Australia

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The primary function of the Fisheries Research Division is to provide scientific advice to government in the formulation of management policies for developing and sustaining Western Australian fisheries.

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Abstract

The characteristics used to identify 19 developmental stages of preserved eggs of snapper, Pagrus auratus*, collected from Shark Bay, Western Australia, during annual plankton surveys, are described and illustrated. A key is provided to enable the age of each egg stage to be estimated based on sea surface temperature and salinity observed during sampling. This report is intended as a reference for inexperienced staff, to ensure that between-year comparisons of snapper spawning biomass, estimated using the daily egg production method, are based on consistent egg staging and ageing methods.*

1.0 Introduction

Stocks of snapper, *Pagrus auratus* (Sparidae), inhabiting the inner gulfs of Shark Bay, Western Australia (25° 30' S, 113° 30' E), have been attracting recreational fishers to the region since the 1970s at least. Anecdotal evidence suggests a significant increase in the level and effectiveness of recreational snapper fishing through the late 1980s - early 1990s. This is thought to have culminated in serious over-fishing of some snapper spawning aggregations, particularly in the eastern gulf, in the mid-1990s. Quantitative estimates of snapper stock size, necessary to support any proposed management action were, however, unavailable at the time. Since 1997, annual stock assessment surveys using the daily egg production method (DEPM, Parker 1985) have been completed, to provide information on the status of inner gulf snapper stocks (Jackson & Cheng 2001). The DEPM is routinely used to assess sardine *Sardinops sagax* stocks in both Western and South Australia, and has been used to estimate snapper stock size in South Australia (McGlennon & Jones 1999) and New Zealand (Zeldis & Francis 1998).

The DEPM assumes that spawning biomass, *i.e.* size of the spawning (mature) stock, of a fish population can be estimated from the following parameters:

- proportion of females spawning each day (termed spawning fraction).
- average weight of a spawning female.
- ratio of females to males in the spawning population (termed sex ratio).
- average number of eggs released by a female during each spawning event (termed batch fecundity).
- daily production of eggs over the entire spawning area of the population.

The first four of these parameters are estimated from representative samples of mature snapper obtained during the peak spawning period, *i.e.* when the majority of the stock is spawning. At the same time, daily egg production, which is dependent upon the rate of daily egg mortality, is estimated from the relative numbers of snapper eggs of different ages, contained in representative plankton samples collected across the entire spawning area. This requires all collected plankton samples to be systematically sorted and any snapper eggs to be counted, staged and aged. Accurate and consistent staging of the eggs is essential to minimise bias in the estimation of daily egg production and therefore spawning biomass.

Cassie (1956) described and photographed the developmental stages of live snapper eggs obtained from spawning snapper in Hauraki Gulf, New Zealand. Crossland (1980) also described snapper egg development based on live eggs, facilitating spawning biomass estimates based on egg surveys in Hauraki Gulf. Both these published sources of information were initially used to identify and stage preserved snapper eggs collected from the inner gulfs of Shark Bay.

Because plankton surveys are conducted over as short a time-period as possible, and only small survey vessels are used in Shark Bay, real-time snapper egg counting and staging in the field is not possible. Plankton samples are therefore fixed in a preservative (buffered-formalin) at sea for subsequent sorting in the laboratory. Such preservation can produce morphological changes in fish eggs (White & Fletcher 1998). Hence, there may be

discrepancies between previous descriptions of live snapper eggs and the characteristics of preserved samples from Shark Bay. Currently, there is no published description of the developmental stages of preserved snapper eggs from Australian waters.

The identification and staging of snapper eggs collected from Shark Bay has involved a number of personnel since 1997. This has created the potential for inconsistent application of egg staging criteria among personnel and through time. It is therefore important that an accurate and usable reference is developed to assist inexperienced staff. The aim of this report is to identify and describe the developmental stages of preserved snapper eggs contained in plankton samples collected from the inner gulfs of Shark Bay. Information is also included for estimating the age of snapper egg stages. The report is intended to provide the standard reference to ensure that comparisons of snapper spawning biomass estimates between years are based on consistent egg staging and ageing methods.

2.0 Materials and methods

Plankton samples (n = 800+) were obtained from surveys conducted in the inner gulfs of Shark Bay using oblique tows of a double-bongo net (500 µm mesh) between June and August in 2000 and 2001. At sea, samples were rinsed into 250 ml plastic jars and fixed with 5% formalin and seawater buffered with approximately 1 g l^{-1} borax powder. Sea surface temperature and salinity were recorded at each tow location.

In the laboratory, each plankton sample was rinsed with tap water and sorted under a dissecting microscope. A sub-sample of all eggs (snapper and other species) was removed from some of these samples and their diameters individually measured (to nearest 1 µm) using a micrometer. All snapper eggs, identified by an experienced sorter, were removed from the sub-samples and stored separately in 5 ml glass vials. For comparison, the diameters of a sample of snapper eggs (also fixed in formalin) collected from tanks containing snapper brood stock at the Fremantle TAFE Aquaculture Centre were also measured, as were their yolk sac (to nearest 25 µm) and oil globule (to nearest 20 µm) diameters.

Individual snapper eggs from the fixed Shark Bay samples, representing the complete range of development stages, were photographed through a microscope (approximately x 40 magnification) using AS100 35 mm colour slide film. Eggs were positioned on a cavity-type glass microscope slide, immersed in a few drops of tap water, and covered with a glass slip. The eggs were manipulated to the desired orientation by sliding the cover slip back and forth. Manipulation was assisted by the addition of one or two drops of 1 molar potassium hydroxide to weaken the hydrophilic forces that can cause eggs to clump together. The colour slides were scanned using Adobe Photoshop 5.5™ software on a MacIntosh computer. For some egg development stages an illustration was created using Photoshop to draw attention to particular identifiable characteristics. The terminology used to describe egg development in this report is that of Gilbert (1988).

The period of egg development, *i.e.* time to hatching, for marine fish eggs generally decreases with increasing ambient water temperature (Pepin 1991). Cassie (1956) showed there to be a linear relationship, over a narrow temperature range, between time to hatching and water temperature for snapper eggs from Hauraki Gulf; hatching occurred after 45 hours

at 18°C and 36 hours at 21°C. Crossland (1980) estimated the relationship between snapper egg development period (H; hours) and ambient water temperature (T; °C) to be, $H = 100 - 3.06T$. More recently, the combined effect of temperature and salinity on egg development has been experimentally investigated for South Australian snapper. Results were used to develop equations that estimate time to hatching for a range of temperature and salinity conditions (D. McGlennon, unpubl.). Given the marked similarities in water temperatures and salinities between the gulfs of South Australia and Shark Bay, these equations have been used to estimate egg development time for Shark Bay snapper eggs based on the mean sea surface temperature and salinity observed during the annual plankton surveys since 1997. The age for each individual egg stage has been estimated using the egg ages reported by Crossland (1980) and assumes a linear relationship between egg development and time.

3.0 Results

3.1 Staging criteria

The distribution of egg diameters, from the sub-sample of all eggs present in the preserved Shark Bay plankton samples, was bimodal with no overlap in range. The group comprising eggs with the larger diameter was the most abundant, with a mean diameter of 864 µm (standard deviation 46.6, range 774 to 956, n = 20). This was consistent with the diameters of preserved eggs from the Fremantle TAFE snapper (mean 872, standard deviation 18.1, n = 769; yolk sac diameter mean 723 µm, standard deviation 47.2, n = 769; oil globule diameter mean 194 µm, standard deviation 11.5, n = 769; C. Wakefield, unpubl.). Results were also broadly consistent with published data of snapper egg diameters based on live eggs (Cassie 1956; Crossland 1980; Pankhurst *et al.* 1991; Battaglene & Talbot 1992). The conclusion that the eggs of larger diameter in the Shark Bay samples were derived from snapper was also consistent with the relatively high abundance of such eggs collected in the vicinity of known snapper spawning aggregation sites in Shark Bay. The second, less abundant group of eggs, mean diameter ~ 600 µm, were of unknown origin.

We distinguished 19 developmental stages for preserved snapper eggs from spawning to hatching and constructed a key to facilitate identification (Table 1). These are the same stages described by Jackson and Cheng (2001) although the notation is different, and similar to the categories reported for snapper in New Zealand by Zeldis and Francis (1998). Stages for a small number of eggs could not be determined adequately, possibly due to poor preservation and/or damage during the collection process. These indeterminate eggs, termed occluded or opaque by White and Fletcher (1998), represented only a small proportion of all snapper eggs collected during plankton surveys in Shark Bay 1998 - 2001 (range <1% - 14%, mean 6.5%), and were assumed to be unbiased with respect to stage. Photographs of all stages are presented in Figures 1 to 19, the stage and figure numbers being the same. For clarity some photographs were replicated as a labelled sketch presented at the same scale as the photograph.

Stage 1

No distinction was made between unfertilised and recently fertilized eggs that were yet to commence development. Stage 1 eggs were spherical, translucent, and had a small

perivitelline space and a single oil globule (Figure 1). Eggs were telolecithal, *i.e.* had a large yolk sac occupying most of the egg but concentrated towards one end, the vegetable pole. At the opposite (animal) pole was the single-celled germinal protoplasm.

Stage 2

The protoplasm underwent meroblastic cleavage, *i.e.* cleavage did not extend into the yolk portion of the egg. The first cleavage divided the protoplasm into two equal size blastomeres (cells), which together were approximately similar in size to the original protoplasm (Stage 2). Initially the cleavage resembled a line of bubbles across the protoplasm (Figure 2a), but developed to separate two distinct, elliptical blastomeres (Figure 2b). Note that for a number of egg stages cleavage lines may resemble a line of bubbles.

Stages 3 to 5

The second cleavage occurred simultaneously in both cells and at right angles to the first cleavage, producing 4 cells (Stage 3, Figure 3). These 4 cells divided simultaneously, the cleavage lines parallel to the first cleavage and at right angles to the second, giving rise to the 8 celled stage 4 (Figure 4). In turn, another simultaneous cell division gave rise to 16 cells in a single plane, forming a 4 x 4 array atop the yolk sac at the animal pole of the egg (Stage 5, Figure 5). Together the cells were approximately similar in size and in the same position as the original, single cell protoplasm. The yolk still occupied the majority of the egg and the oil globule remained visible, as it did until hatching.

Stages 6 and 7

Further cell division resulted in the cells becoming smaller such that their number was difficult to determine. If there were more than 16 cells that were individually visible at a magnification of x30 then the egg was classified as Stage 6 (Figure 6). Once individual cells could not be distinguished at this magnification the egg was classified as Stage 7 (Figure 7). Up to and including Stage 7, the blastoderm remained about the same size as the original germinal protoplasm from Stage 1. Thus cell division caused cell size to progressively decrease. Note that Stages 1 and 7 may be confused because the single cell protoplasm of Stage 1 was similar in appearance and size to the multicellular blastoderm of Stage 7. The latter was more opaque with a granular texture, however.

Stages 8 to 10

After Stage 7 the blastoderm increased in size by spreading over the yolk surface. Before the blastoderm reached the equator of the yolk sac the egg was classified as Stage 8 (Figure 8). When it had reached the equator it was Stage 9 (Figure 9) and when it had progressed past the equator it was determined as Stage 10 (Figure 10). At Stage 10 most of the yolk sac was enclosed, leaving a small surface area exposed, the blastopore, at the vegetable pole.

Stage 11

An embryonic streak commenced development as a thickening of the blastoderm along a line that started at the blastopore margin, and proceeded about halfway around the perimeter of the blastoderm opposite the blastopore (Figure 11). The two ends of the embryonic streak were destined to form the head and tail, but until such development had commenced the egg was classified as Stage 11.

Stage 12

The head end of the embryonic streak developed more rapidly than the tail end, which remained undifferentiated for longer. Once this difference was established, the egg was classified as Stage 12 (Figure 12). Note also that continued expansion of the blastoderm had almost closed the blastopore at this stage (Figure 12).

Stage 13

As the head developed the optic lobes became visible and signified Stage 13 (Figure 13). For most eggs the blastopore was closed by now although the timing of closure was variable and should not be used exclusively when staging.

Stages 14 and 15

Further development saw the segmentation of the embryo into somites that initially were more visible along the mid-region (Figure 14a). The presence of somites indicated the embryo had developed to Stage 14 at least. The anatomy of the developing tail was used to classify this and all remaining egg stages. Initially the tail margin (perimeter) was undefined (Stage 14, Figure 14b). When the tail margin became distinct (Figure 15a) but remained flush with the surface of the yolk sac (Figure 15b) the egg was classified as Stage 15.

Stages 16 and 17

Continued development of the tail saw it grow outward and rise above the surface of the yolk sac when viewed from side on (Stage 16, Figure 16), followed by the onset of separation from the yolk sac (Stage 17, Figure 17).

Stages 18 and 19

Stage 18 was reached when the tail had completely separated from the yolk sac (Figure 18a). Discrimination between Stages 18 and 19 was based on the increasing width of the tail fin relative to the width of the caudal peduncle. The tail fin width was taken from the posterior tip of the caudal peduncle to the posterior tip of the tail, defined as "x" in Figures 18b and 19b. The width of the caudal peduncle, shown as "y" in Figures 18b and 19b, was taken at a distance of "x" forward of the posterior tip of the caudal peduncle. If $x<y$ the egg was Stage 18; if $x>y$ the egg was Stage 19. Being the last stage prior to hatching, Stage 19 embryos were very well developed with a complex head structure (Figure 19c).

3.2 Egg stage development in relation to sea surface temperature and salinity

Water temperature and salinity observed in the inner gulfs of Shark Bay during winter months (June – August), *i.e.* the main snapper spawning period, did not differ significantly between the surface and mid water depth (approximately 5 - 6 m below surface) where most snapper eggs were distributed. Mean sea surface temperature in the areas where snapper eggs were collected between 1997 and 2001 ranged from 17 – 22°C and mean salinity from 35 – 45. Using the relationship between water temperature, salinity and snapper egg development derived from research in South Australia (see 2.0 above), time to hatching for snapper eggs in Shark Bay under these conditions, for these years, has been estimated at between 21 and 34 hr. Ages for each stage of egg development were estimated over the range of temperature and salinity conditions typically observed in the inner gulfs of Shark Bay (Table 2).

4.0 Discussion

This report describes 19 identifiable stages of development of formalin preserved snapper eggs collected from the inner gulfs of Shark Bay. Morphology and development were found to be consistent with descriptions of live eggs from New Zealand snapper reported by Cassie (1956) and Crossland (1980).

Although egg development has been subdivided into stages based on the most obvious characters, it should be noted that development is a continuous process. Users will find that a small number of eggs will key out to be close to the midpoint of two successive stages. In such cases users must decide on the degree of precision required and, if necessary, supplement the key with their own more precise criteria.

This report provides a future reference for staging and ageing snapper eggs for the purpose of estimating spawning biomass from DEPM surveys, and is intended to ensure that between year comparisons of snapper spawning biomass estimates are based on consistent egg staging and ageing methods.

5.0 Acknowledgements

The authors would like to thank Dave McGlennon (formerly of SARDI, South Australian Aquatic Sciences Centre) for use of his unpublished data on the effect of water temperature and salinity on snapper egg development, and Corey Wakefield for use of his unpublished data on snapper egg, yolk sac and oil globule diameters. Many staff from the Research Division of the Fisheries Department of Western Australia helped sort the Shark Bay plankton samples and identify snapper eggs 1997 – 2001, and over this period assisted in the development of the egg identification key presented here. Finally, the authors would like to thank Tim Leary and Drs Rod Lenanton, Rick Fletcher, Jill St John, and Mike Mackie for their useful comments on earlier drafts of this report.

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7.0 Tables

Table 1. Egg stage key for classifying preserved eggs of snapper *Pagrus auratus.*

Egg characteristic	Stage/Figure No.
Embryo absent	
Single celled protoplasm	1
2 cells	2
4 cells	3
8 cells	4
16 cells	5
>16 cells individually visible at magnification of 30x	6
>16 cells not individually visible at magnification of 30x	7
Blastoderm larger than original germinal disc	
Blastoderm **not** extending past equator	8
Blastoderm at the equator	9
Blastoderm extending past equator	10
Embryo present, without somites	
Embryonic streak only, head and tail undefined	11
Head distinct, tail undefined	12
Head with optic lobes	13
Somites present	
Tail margin not distinct	14
Tail margin distinct, flush with yolk sac	15
Tail raised as a bulge above yolk sac	16
Tail partly separated from yolk sac	17
Width of tail fin **less** than width of caudal peduncle	18
Width of tail fin **greater** than width of caudal peduncle	19

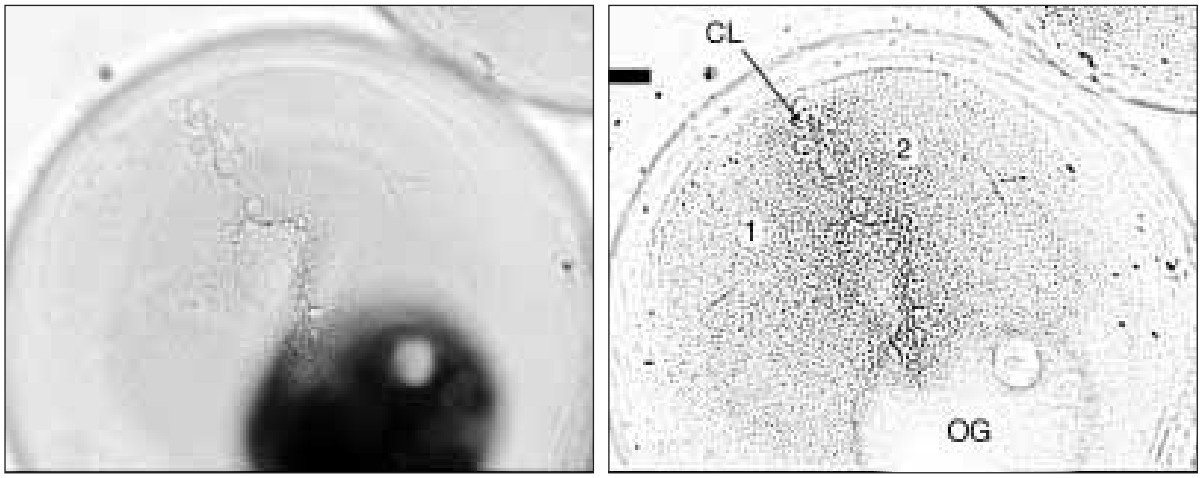
Table 2. Estimated ages (hr) of the 19 development stages of snapper eggs described, over the range of salinity and sea surface temperatures (°C) observed during Shark Bay surveys from 1997 to 2001.

Mean salinity	36				40				44			
Mean SST °C	16	18	20	22	16	18	20	22	16	18	20	22
Egg stage	Age	Age	Age	Age	Age	Age	Age	Age	Age	Age	Age	Age
1	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.3
2	1.3	1.1	0.9	0.8	1.3	1.2	1.0	0.8	1.3	1.2	1.0	0.9
3	2.2	1.8	1.4	1.3	2.2	2.0	1.6	1.3	2.1	2.0	1.6	1.4
4	3.0	2.5	2.0	1.8	3.0	2.8	2.3	1.8	3.0	2.8	2.3	2.0
5	3.9	3.2	2.6	2.3	3.9	3.5	2.9	2.3	3.9	3.5	2.9	2.6
6	4.7	3.9	3.1	2.8	4.7	4.3	3.5	2.8	4.7	4.3	3.5	3.1
7	5.6	4.6	3.7	3.3	5.6	5.1	4.2	3.3	5.6	5.1	4.2	3.7
8	7.0	5.9	4.8	4.1	7.0	6.4	5.3	4.1	7.1	6.4	5.3	4.6
9	9.0	7.8	6.3	5.3	9.0	8.3	6.8	5.3	9.3	8.3	7.0	5.8
10	11.0	9.6	7.8	6.4	11.0	10.1	8.3	6.4	11.4	10.1	8.7	6.9
11	13.0	11.3	9.2	7.7	13.0	11.8	9.8	7.7	13.7	11.9	10.3	8.2
12	15.0	13.0	10.5	9.0	15.0	13.5	11.3	9.0	16.0	13.8	11.8	9.5
13	17.0	14.7	11.8	10.3	17.0	15.2	12.8	10.3	18.3	15.6	13.3	10.8
14	19.0	16.4	13.3	11.7	19.0	16.9	14.3	11.7	20.6	17.4	14.8	12.1
15	21.0	18.3	14.8	13.0	21.0	18.8	15.8	13.0	22.8	19.3	16.3	13.3
16	23.0	20.1	16.3	14.3	23.0	20.6	17.3	14.3	24.9	21.1	17.8	14.4
17	25.5	22.4	18.0	15.9	25.5	22.9	19.1	15.9	27.6	23.4	19.8	16.0
18	28.5	25.1	20.0	17.6	28.5	25.6	21.4	17.6	30.9	26.1	22.3	18.0
19	33.0	28.8	23.5	20.3	33.0	29.5	24.8	20.3	35.3	30.3	25.8	21.0
Hatch time	36	31	26	22	36	32	27	22	38	33	28	23

8.0 Figures

Figure 1. **Egg stage 1.** Lateral view. Single celled egg with undifferentiated protoplasm. PS = perivitelline space, P = protoplasm, Y = yolk sac, OG = oil globule. Scale bar = 100 µm.

a)



b)

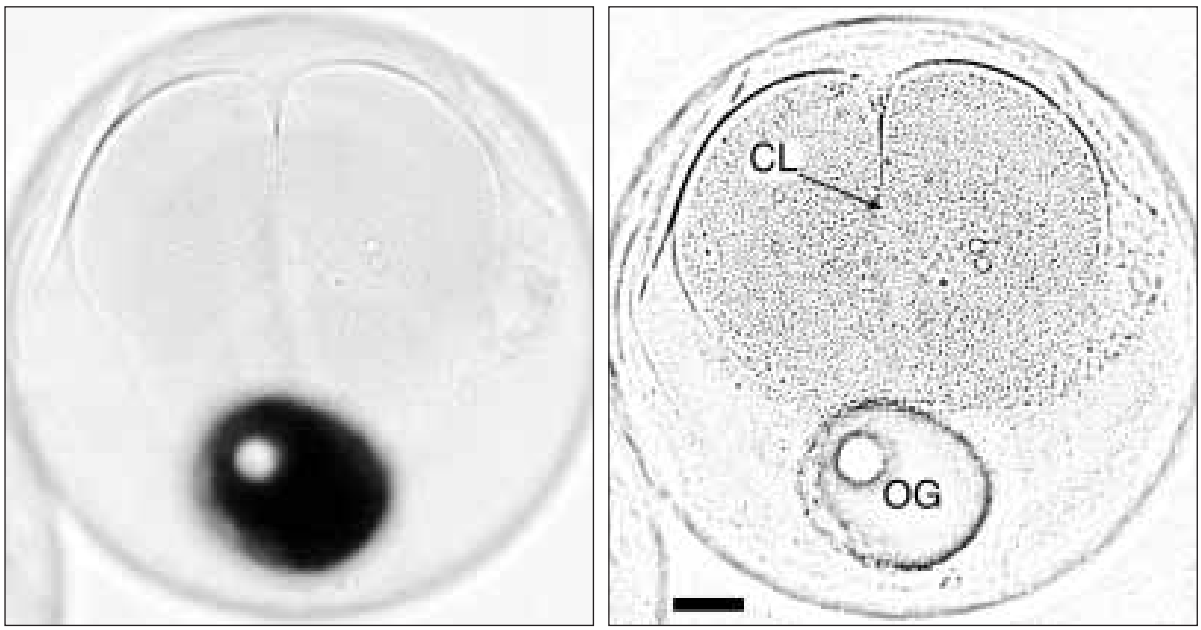


Figure 2. **Egg stage 2.** Top view. Two cells (numbered) separated by a cleavage line that initially resembles a line of bubbles (a) then forms two distinct cells (b). CL = cleavage line, OG = oil globule. Scale bar = 100 µm.

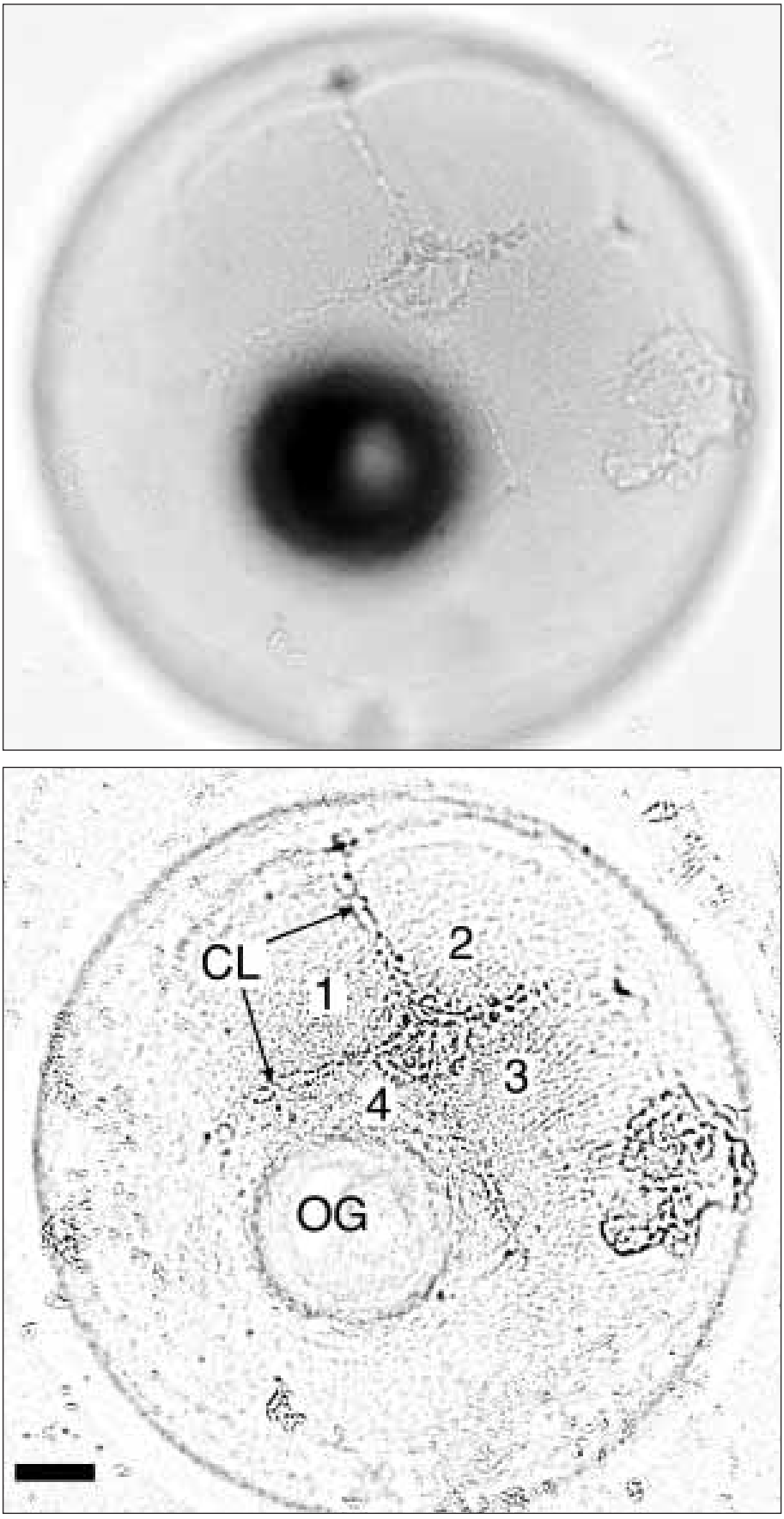


Figure 3. **Stage 3.** Top view. Four cells present (numbered). CL = cleavage line, OG = oil globule. Scale bar = 100 µm.

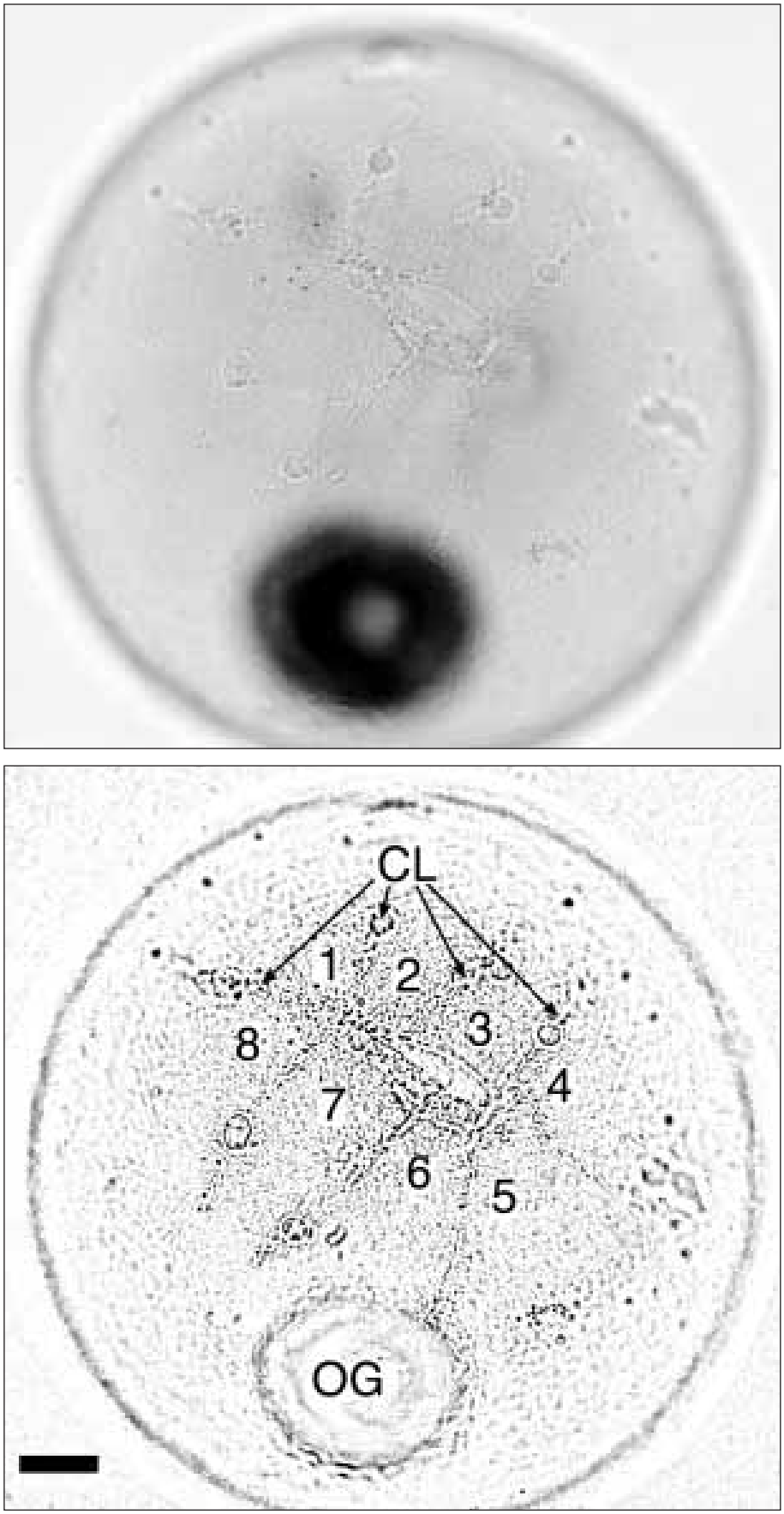


Figure 4. **Stage 4.** Top view. Eight cells present (numbered). CL = cleavage line, OG = oil globule. Scale bar = 100 µm.

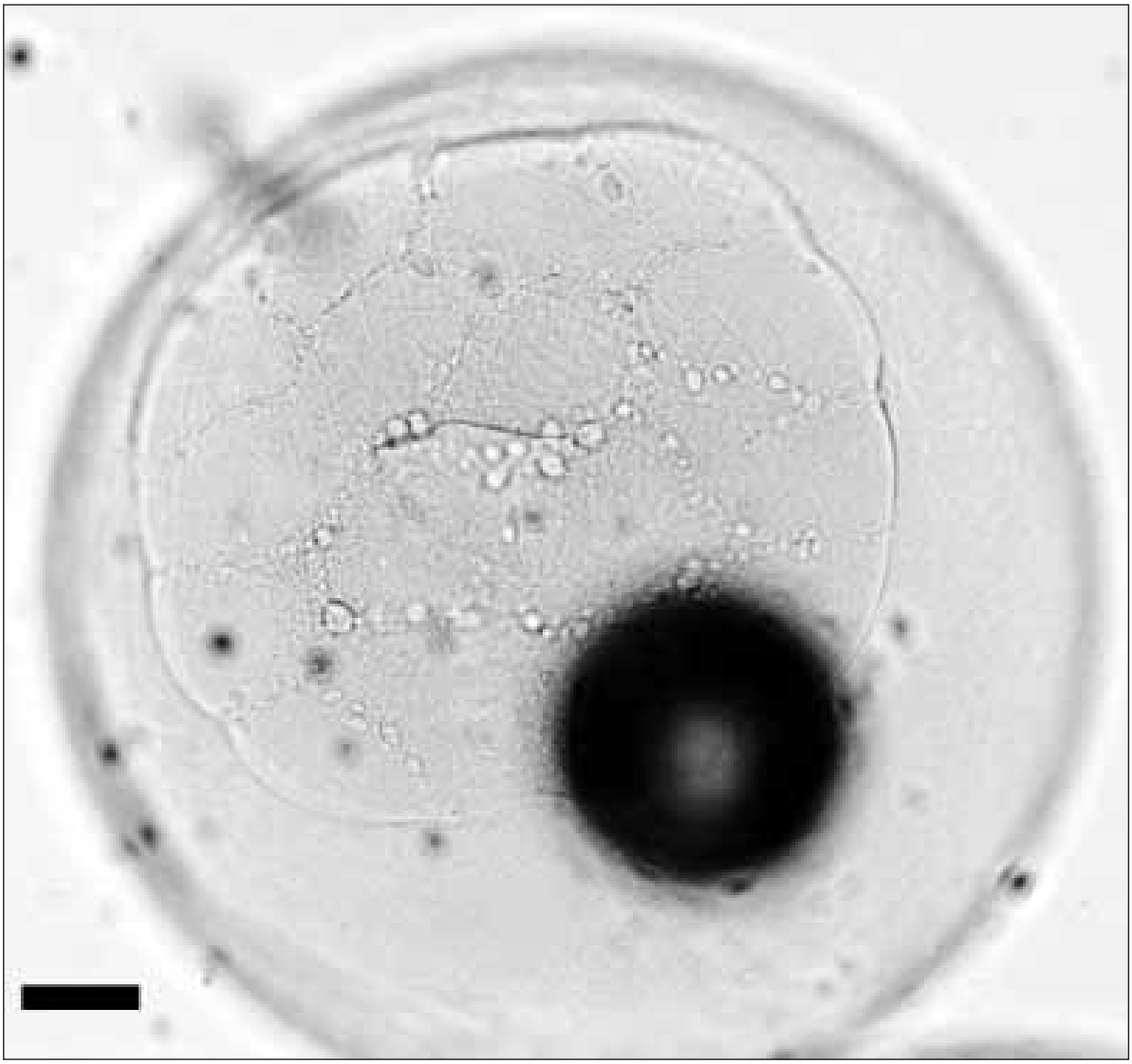


Figure 5. **Stage 5.** Top view. Sixteen cells in single plane forming a 4 x 4 array. Scale bar = 100 µm.

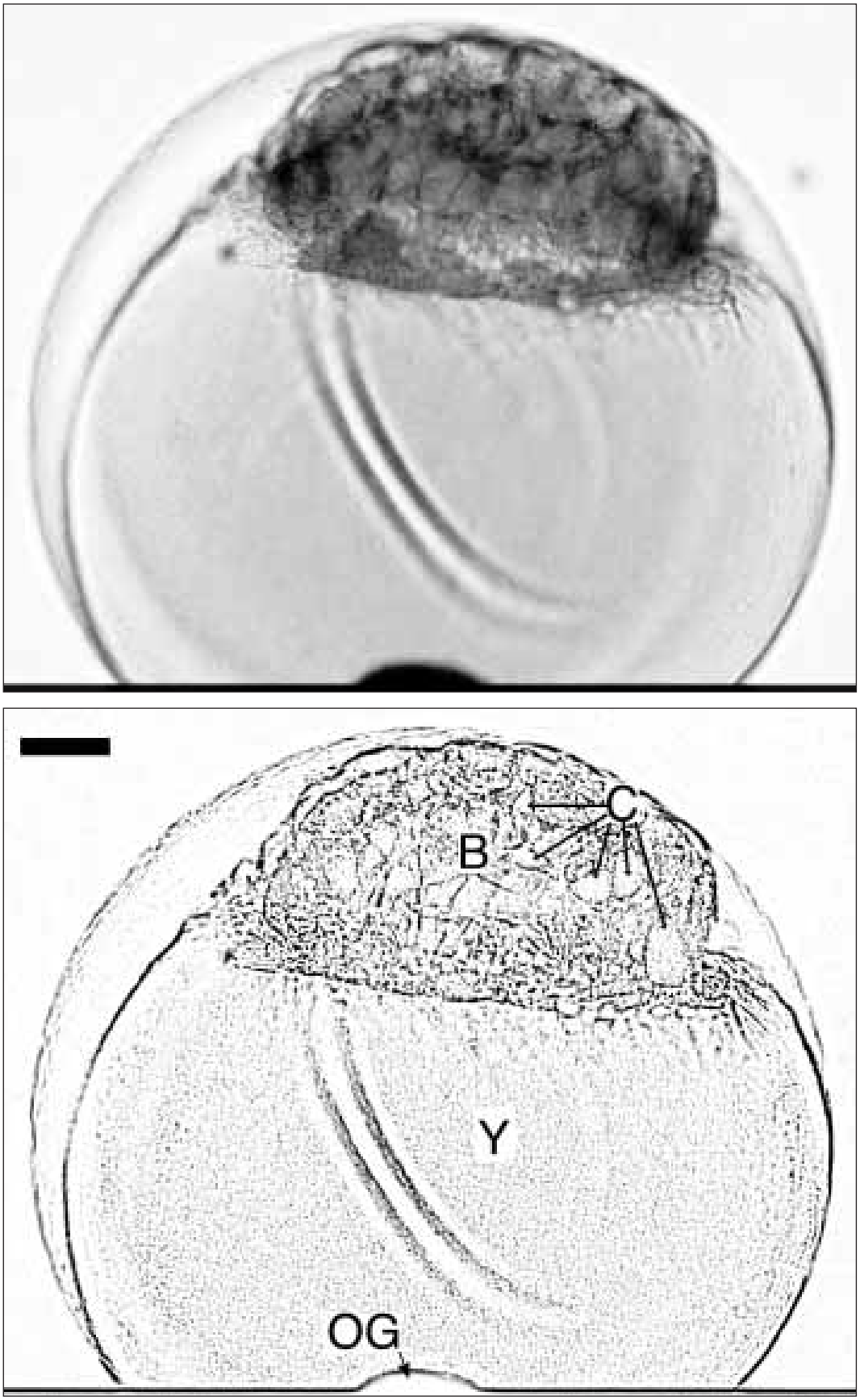


Figure 6. **Stage 6.** Lateral view. Blastoderm with individually visible cells when viewed at a magnification of 30x. B = blastoderm, C = individual cells, Y = yolk sac, OG = oil globule. Scale bar = 100 µm.

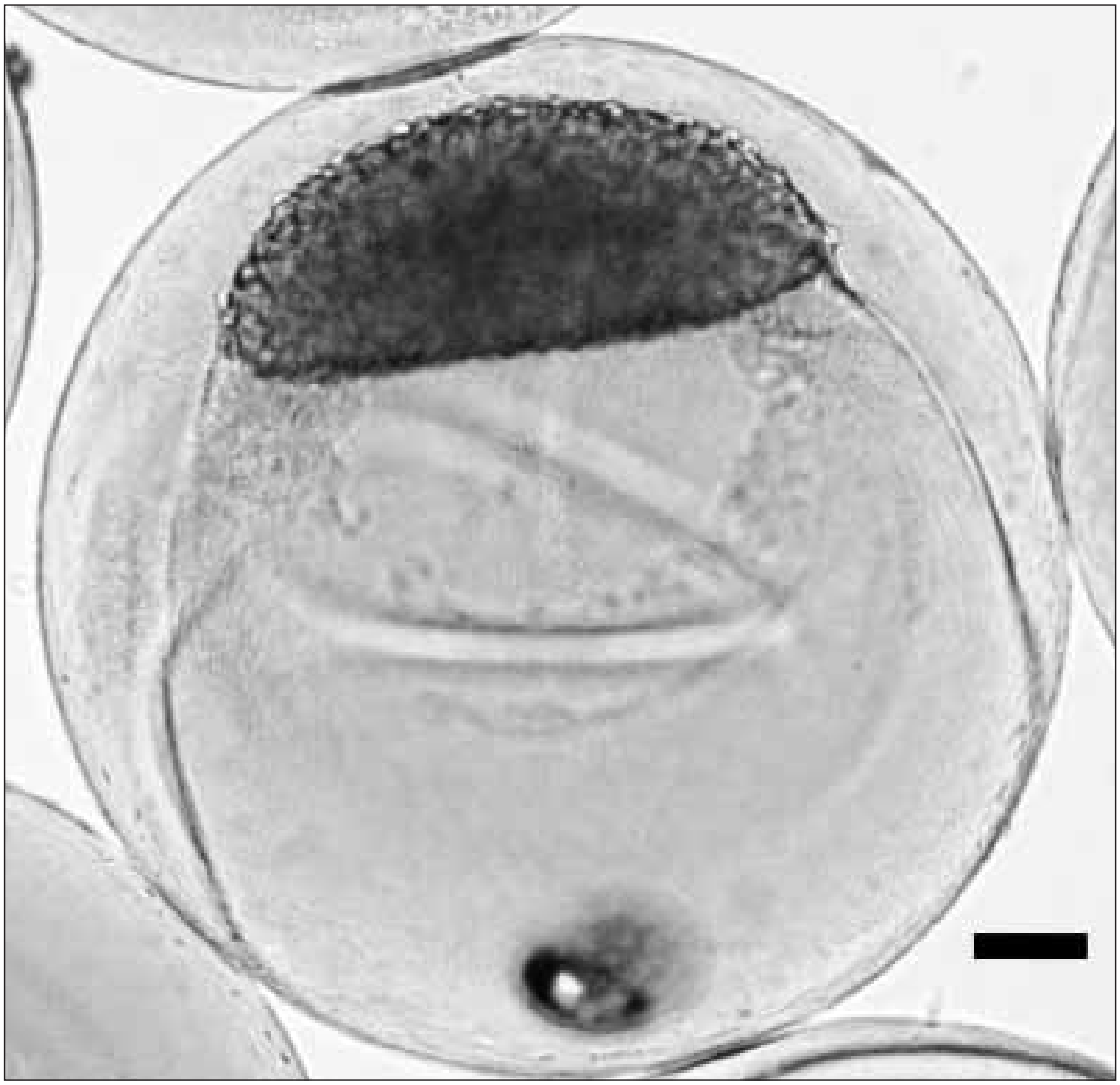


Figure 7. **Stage 7.** Lateral view. Blastoderm with individual cells not visible when viewed at a magnification of 30x. The oil globule is at the bottom. Scale bar = 100 µm.

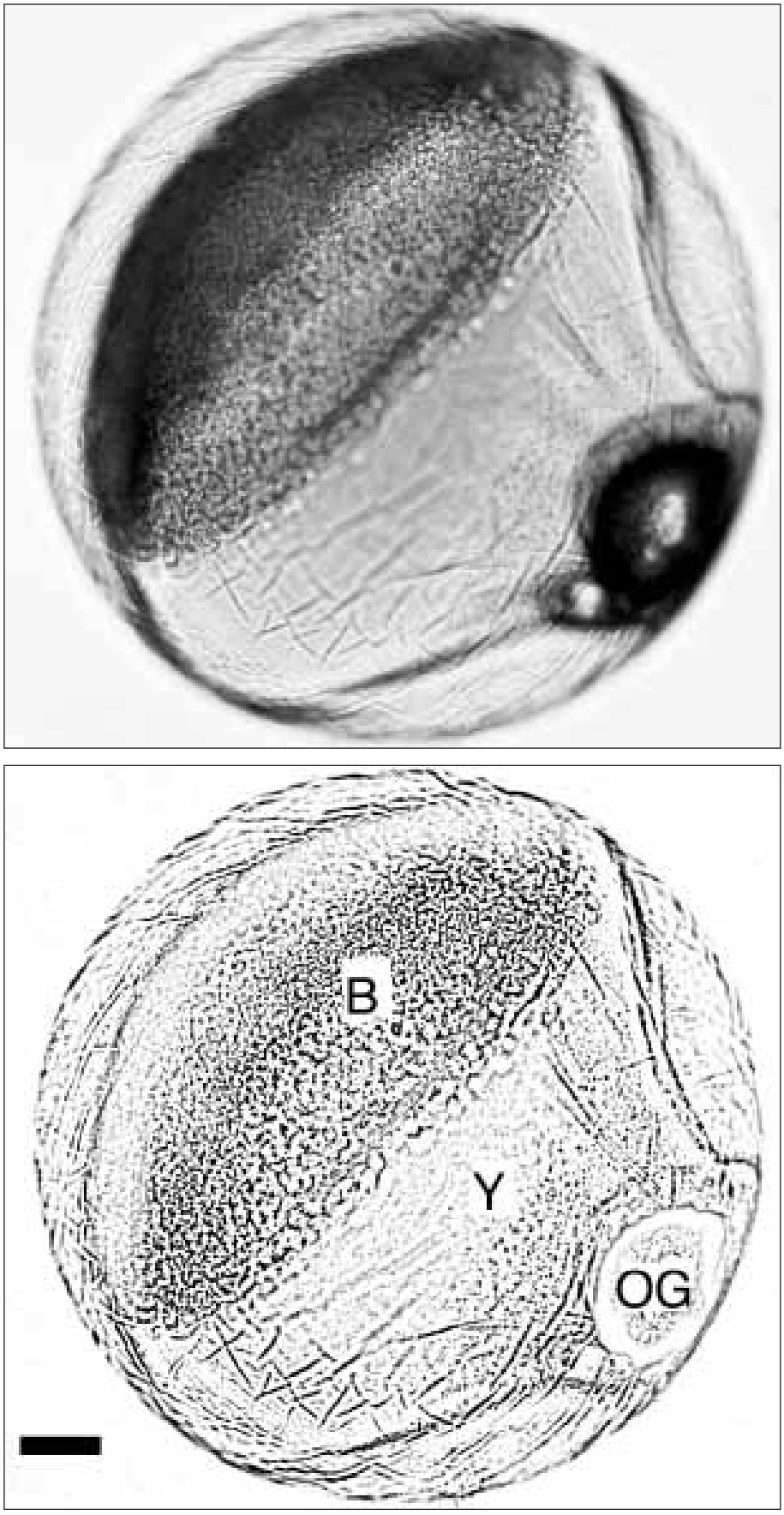


Figure 8. **Stage 8.** Lateral view. Blastoderm spreading across the surface of the yolk sac but has not yet reached the equator. B = blastoderm, Y = yolk sac, OG = oil globule. Scale bar = 100 µm.

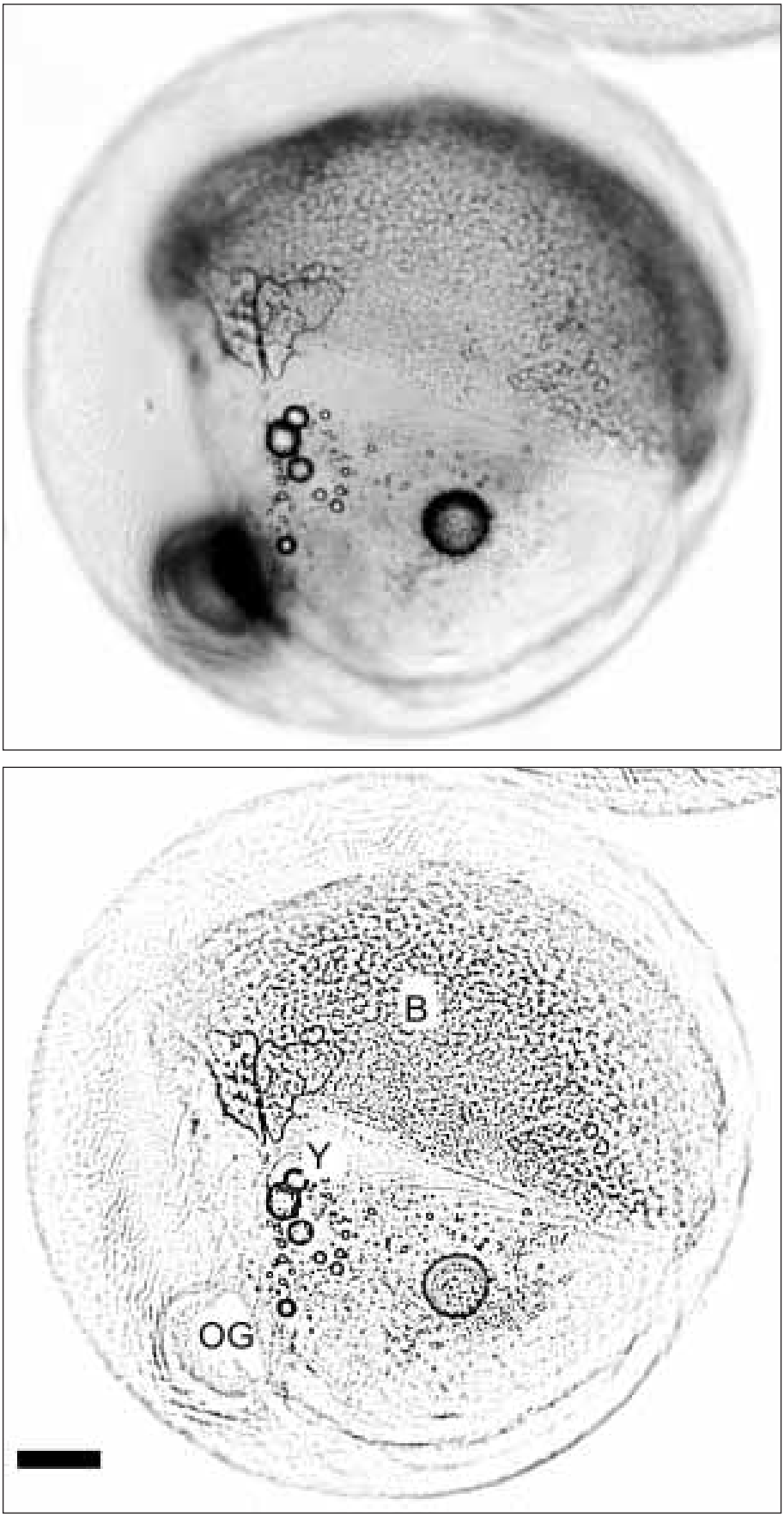


Figure 9. **Stage 9.** Lateral view. Blastoderm has spread across the surface of the yolk sac to the equator. B = blastoderm, Y = yolk sac, OG = oil globule. Scale bar = 100 µm.

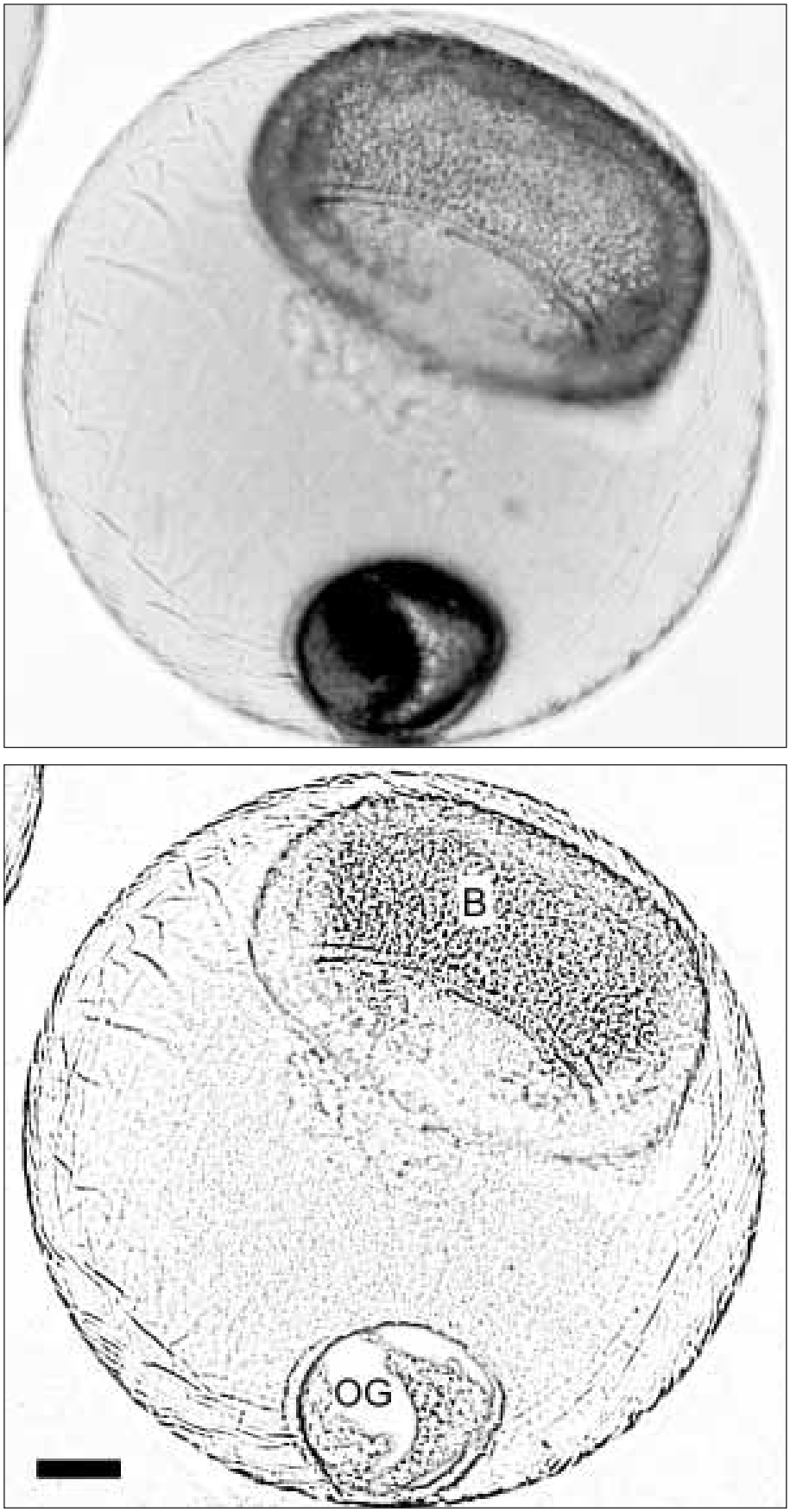


Figure 10. **Stage 10.** Lateral view. Blastoderm has spread across the surface of the yolk sac past the equator. Note that this egg has a comparatively small yolk sac. B = blastoderm, OG = oil globule. Scale bar = 100 µm.

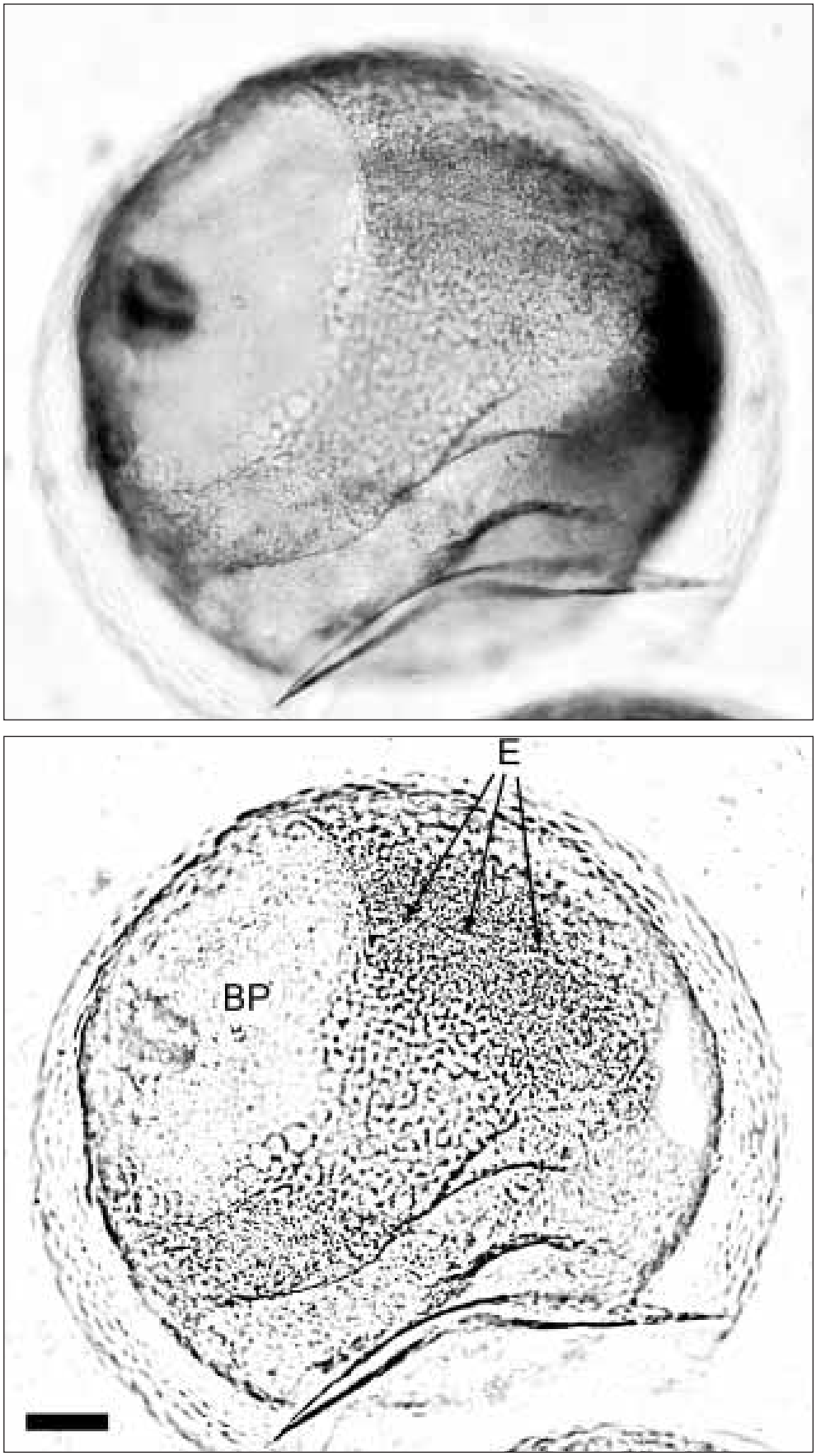


Figure 11. **Stage 11.** Oblique view. Embryonic streak visible. E = embryonic streak, BP = blastopore. Scale bar = 100 µm.

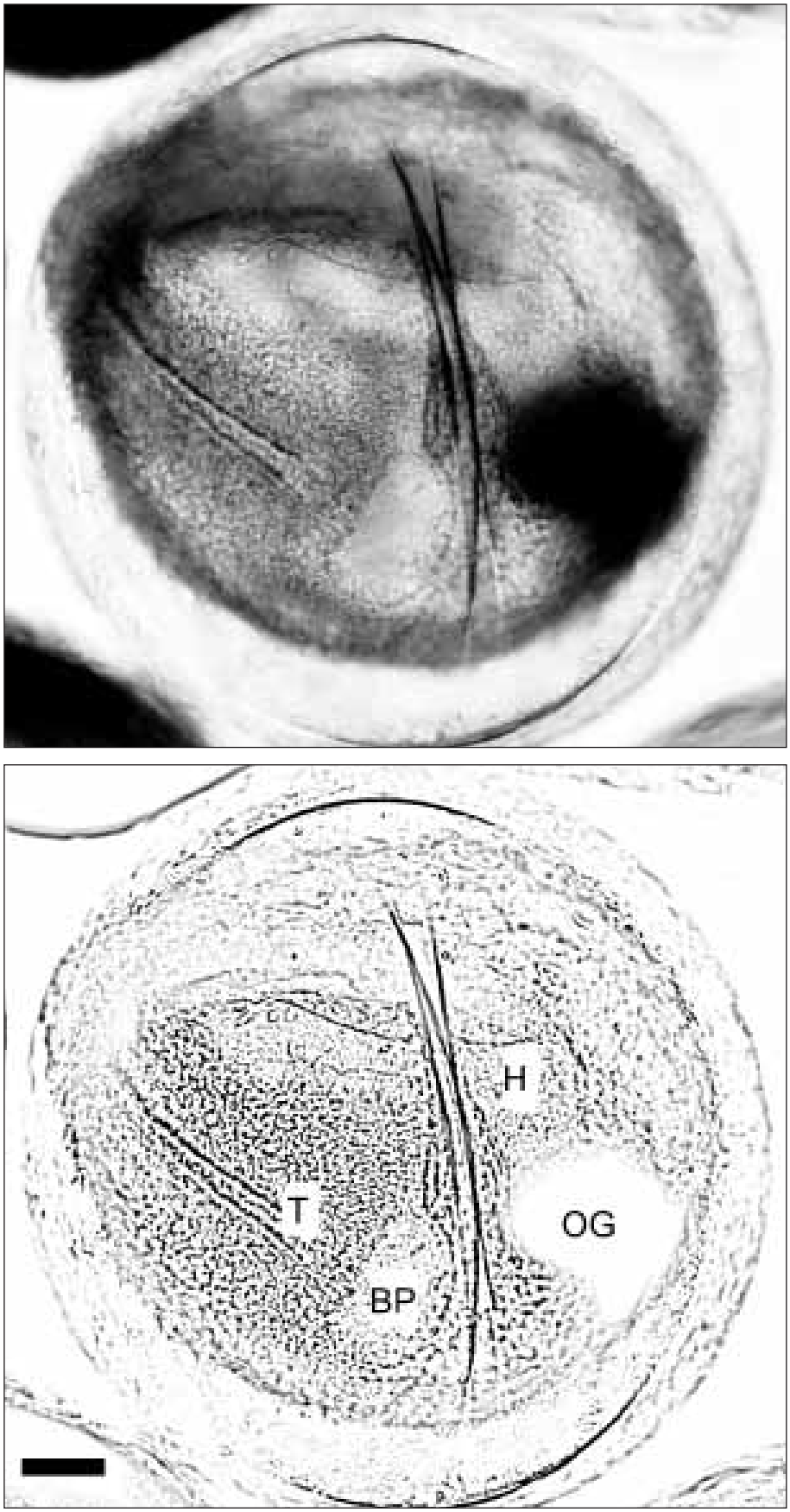


Figure 12. **Stage 12.** Oblique view. The tail is undifferentiated relative to the head, which is out of focus at the far side of the egg. Blastopore almost closed. H = head, T = tail, OG = oil globule, BP = blastopore. Scale bar = 100 µm.

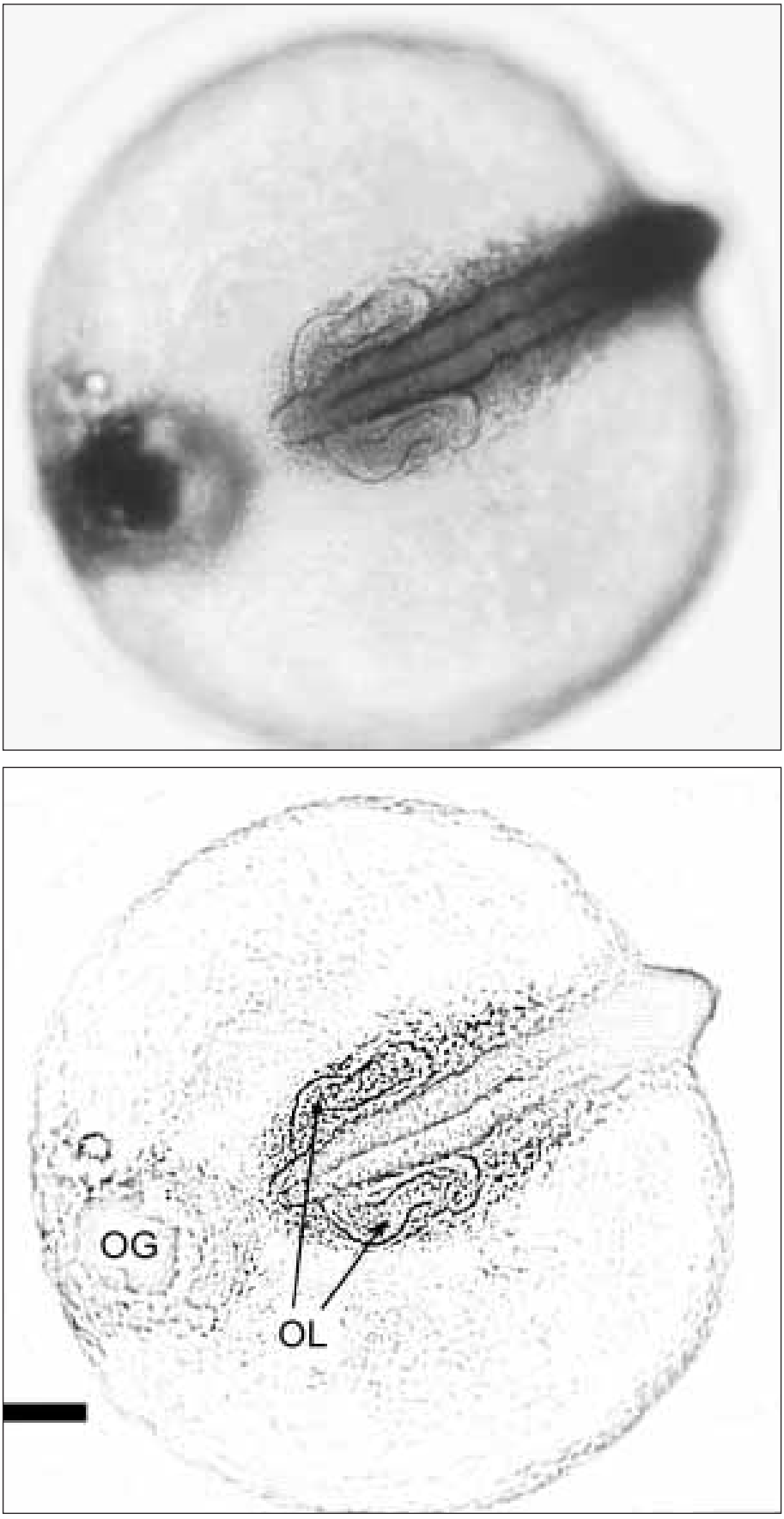
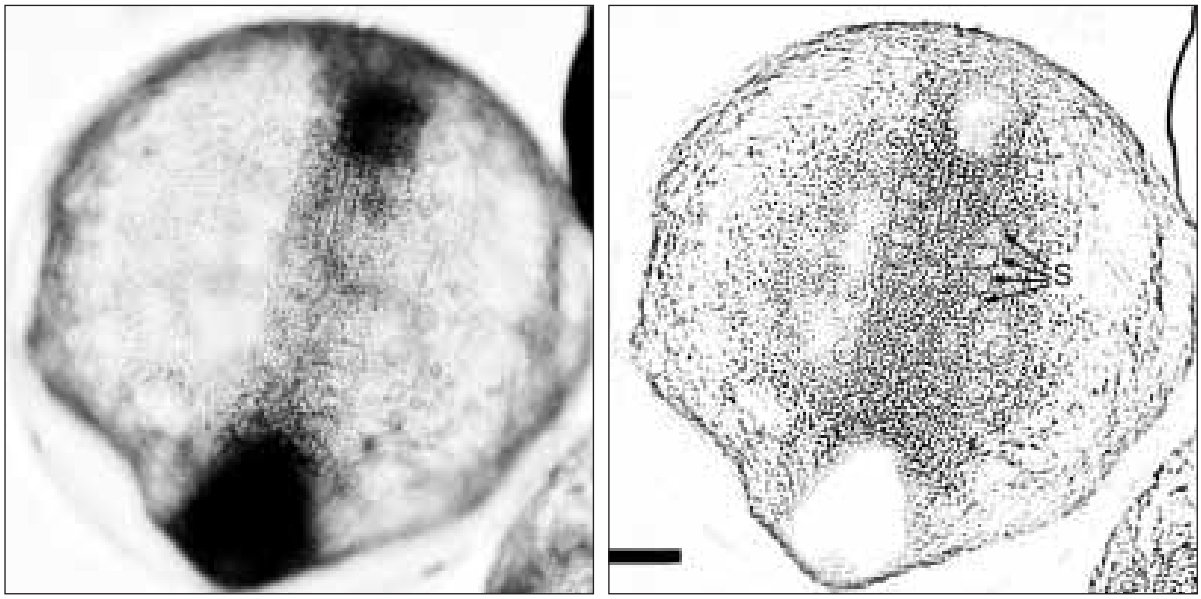


Figure 13. **Stage 13.** Oblique view. Head with optic lobes present. OG = oil globule, OL = optic lobes. Scale bar = 100 µm.

a)



b)

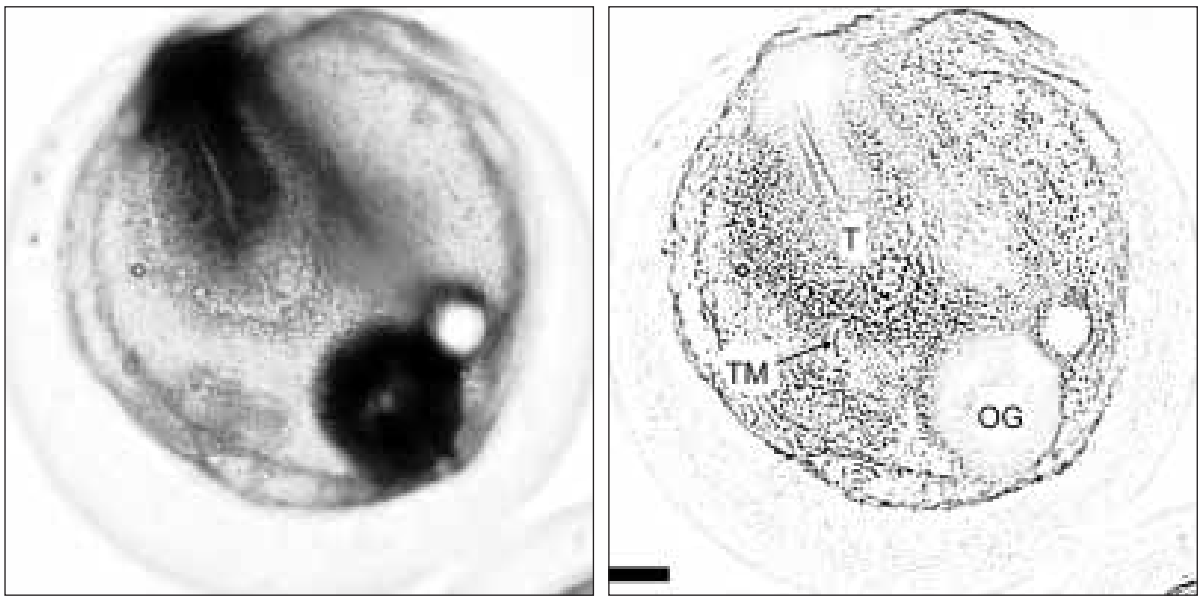
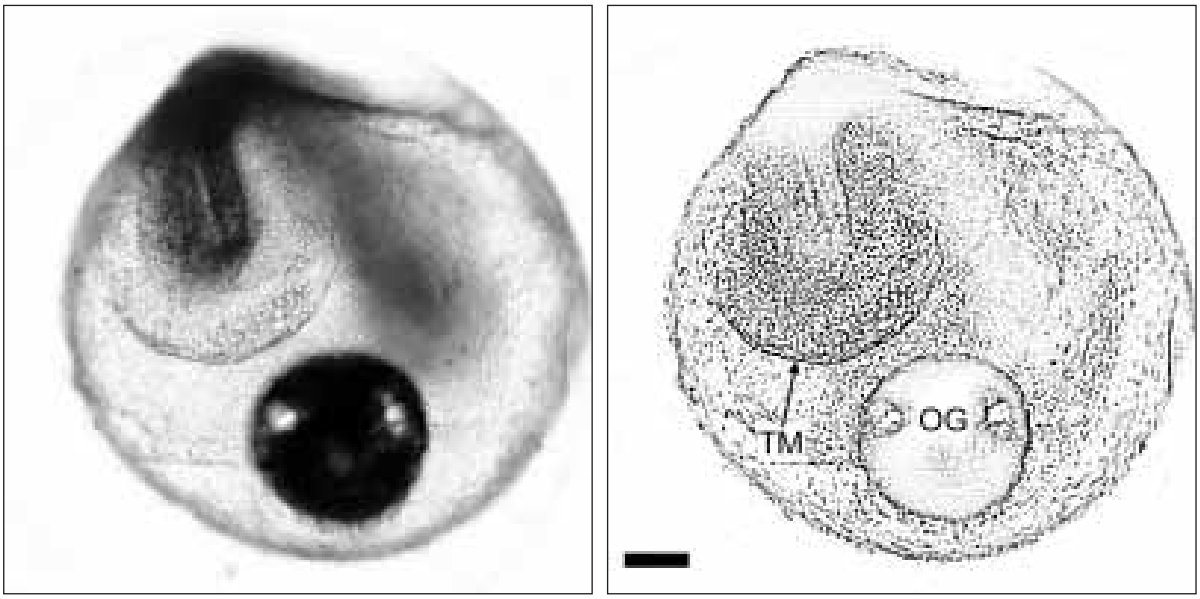


Figure 14. **Stage 14.** Somites (segments) are present along the mid-region of the embryo (a) and the tail margin is not distinct (b). S = somites, E = embryo, T = tail, TM = tail margin, OG = oil globule. Scale bars = 100 µm.

a)



b)

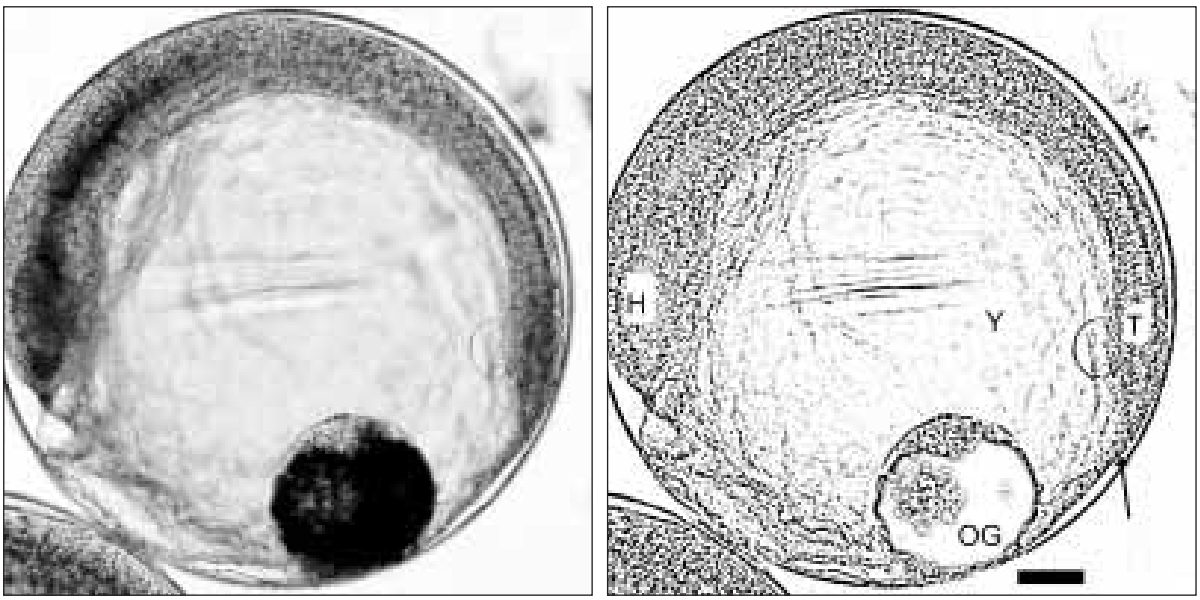


Figure 15. Stage 15. Oblique view showing distinct tail margin (a) and lateral view of tail, which is flush with the surface of the yolk sac (arrowed) (b). TM = tail margin. OG = oil globule, H = head, Y = yolk sac, T =tail. Scale bars = 100 µm.

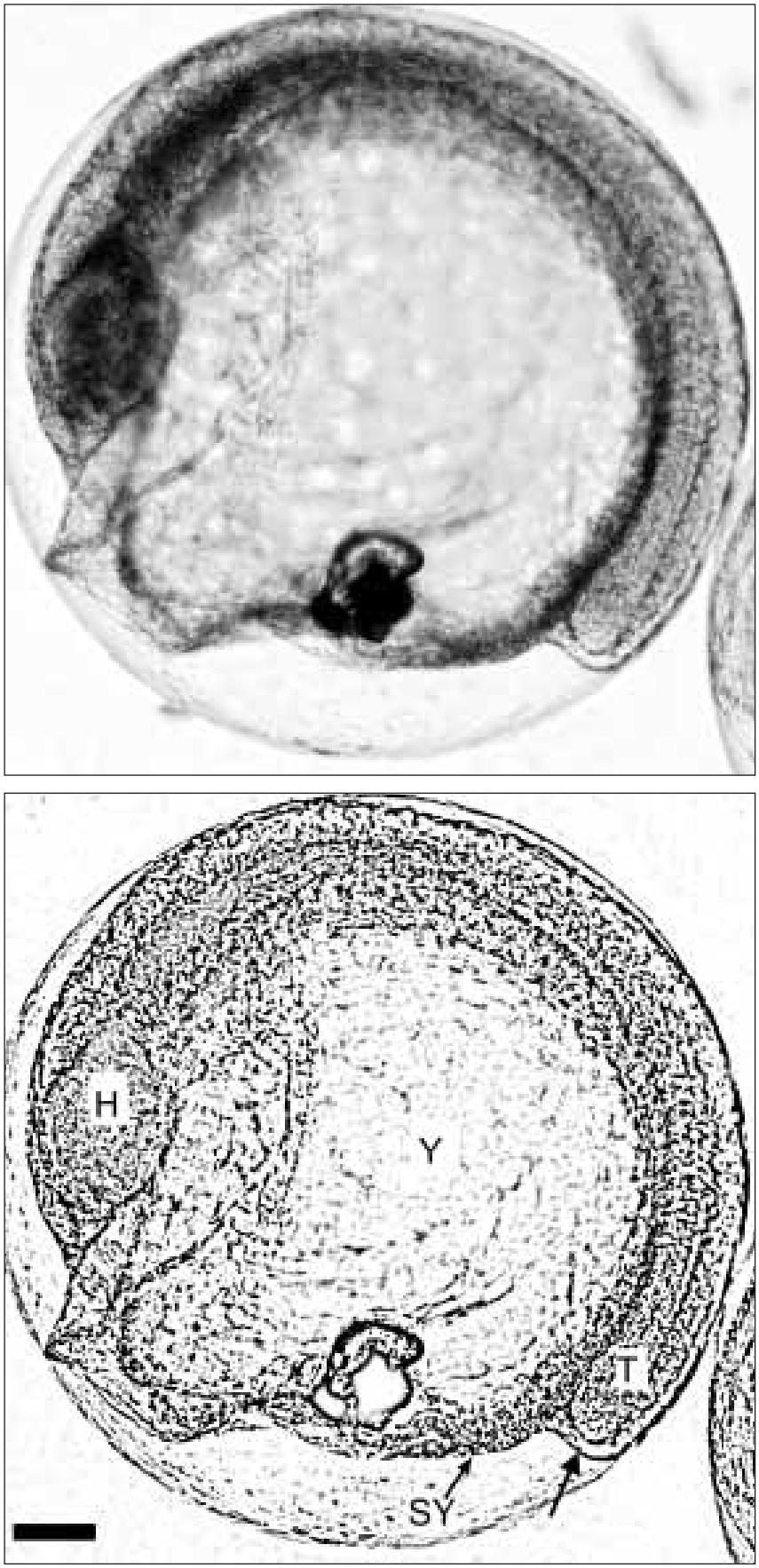


Figure 16. **Stage 16.** Lateral view of tail raised as a bulge (arrowed) above the surface of the yolk sac. H = head, Y =yolk sac, T = tail, SY = surface of yolk sac. Scale bar = 100 µm.

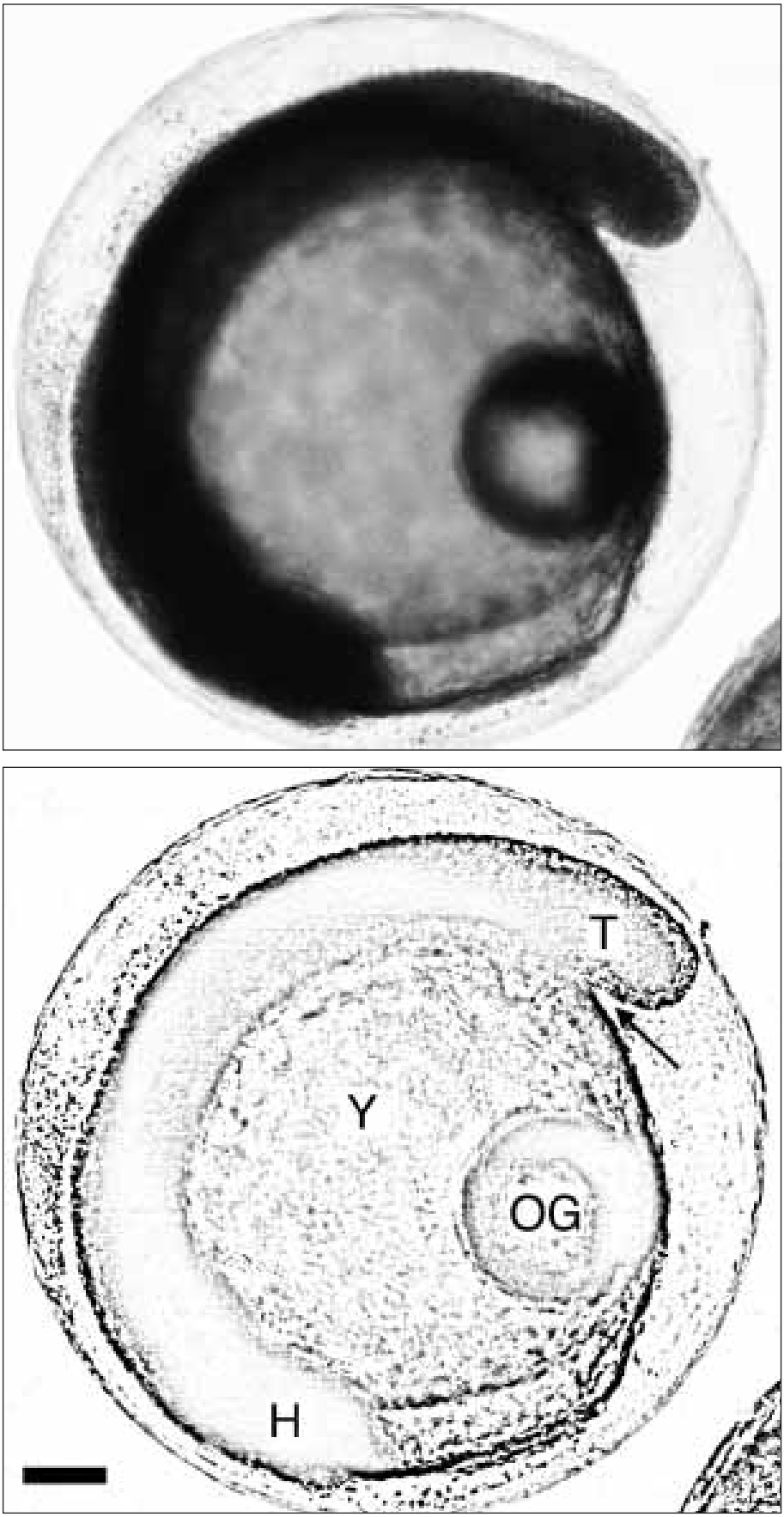
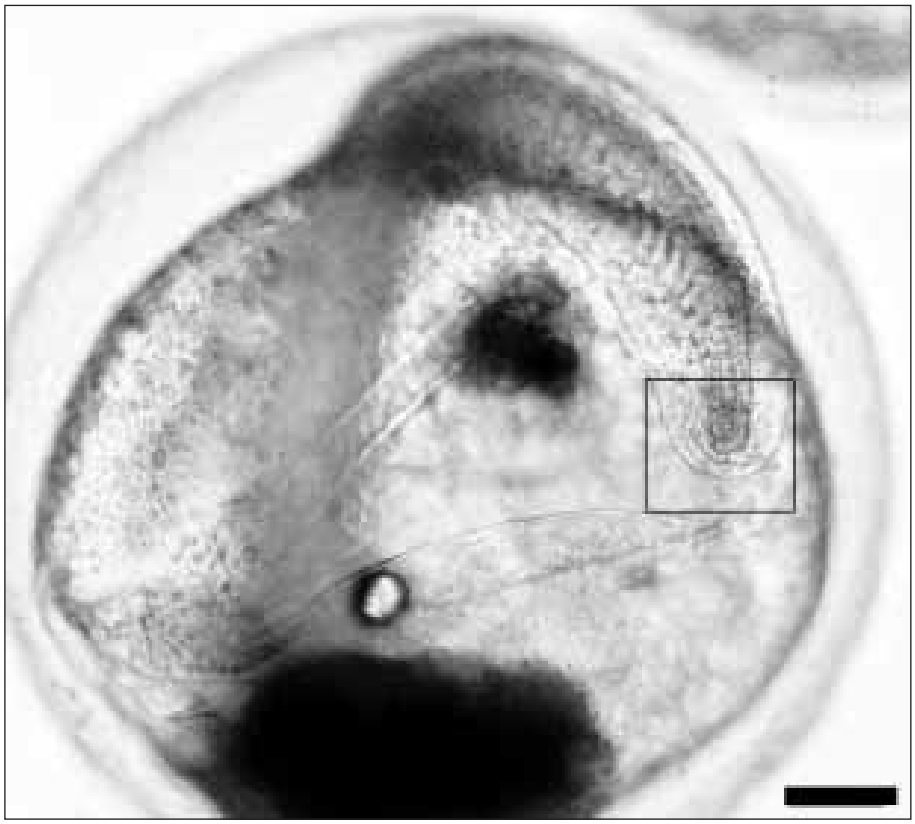


Figure 17. **Stage 17.** Lateral view of tail partially separated from the surface of the yolk sac (arrowed). T = tail, Y = yolk sac, OG = oil globule, H = head. Scale bar = 100 µm.

a)



b)

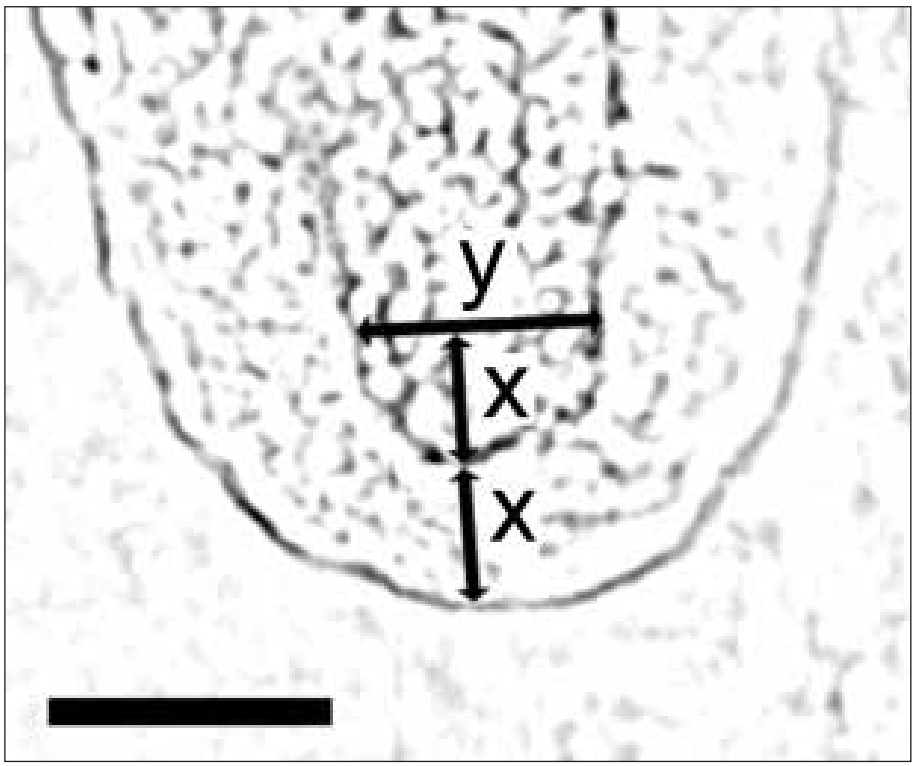


Figure 18. **Stage 18.** Tail has fully separated from yolk sac (a). The insert in a is magnified and sketched in b, showing the width of the tail fin from the posterior tip of the caudal peduncle to the posterior tip of the tail (x) is less than the width of the caudal peduncle (y) measured at a distance of x forward of the posterior tip of the caudal peduncle. Scale bars = 100 µm (a); 50 µm (b).

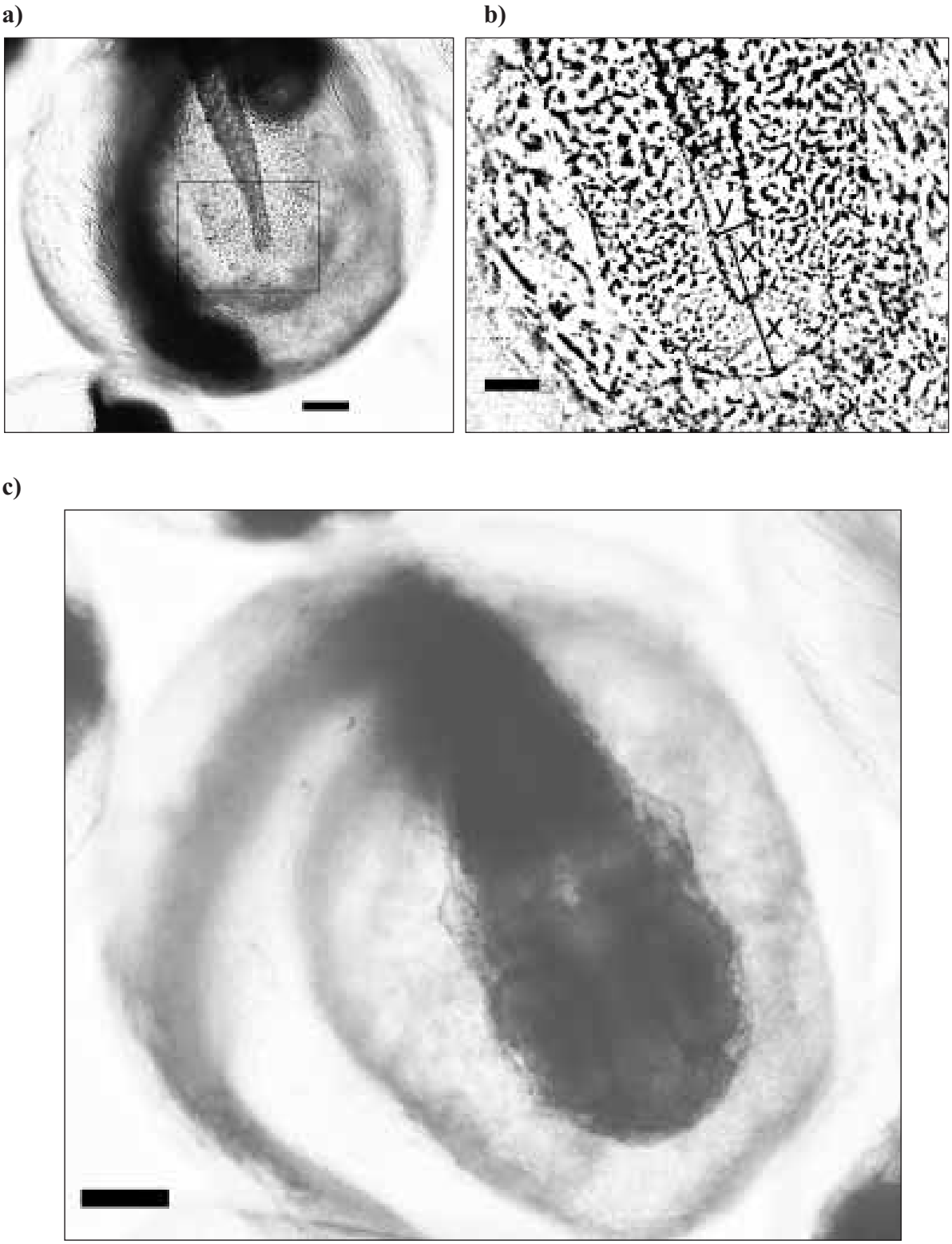


Figure 19. **Stage 19.** Tail with wide fin (a). The insert in a is magnified and sketched in b, showing the width of the tail fin from the posterior tip of the caudal peduncle to the posterior tip of the tail (x) is greater than the width of the caudal peduncle (y) measured at a distance of x forward of the posterior tip of the caudal peduncle. c, well developed head structure viewed from above. Scale bars = 100 µm (a, c); 50 µm (b).

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