한국패류학회지 : 제 4 권 1호
Korean J. Malacol.
Vol. 4(1): 30-40, 1988

A Study on Sexual Maturation of *Mactra veneriformis* Reeve

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＝국문요약＝

동죽, *Mactra veneriformis* Reeve의 性成熟에 關한 硏究

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1986年 3月부터 1987年 2月까지 1年間에 걸쳐 우리나라 西海岸의 전라북도 내초도 조간대에서 채집된 동죽, *Mactra veneriformis* Reeve를 대상으로 生殖巢發達過程, 生殖年周期, 그리고 群成熟度를 組織學的으로 조사하였다.

동죽은 雌雄異體이며, 生殖巢는 內臟囊의 肝中腸腺下部와 足部의 網狀結締組織사이에 위치하고 있다. 卵巢는 數많은 卵巢小囊으로 構成되어있고 精巢는 여러개의 精巢小葉으로 構成되어 있다. 不分化間充組織과 好酸性 顆粒細胞들은 初期 生殖細胞의 形成 및 發達에 營養細胞로 관여하고 있다.

産卵期는 6월 初旬에서 9月까지 이었고, 主産卵은 水溫이 23℃以上인 7月과 8月사이에 일어나고 있다.

本 種의 生殖年周期는 連續的 5段階인 分裂增殖期(1-3月), 成長期(3-5月), 成熟期(4-8月), 放出期(6-9月), 退化 및 休止期(9-2月)로 區分할 수 있었다.

肥滿度의 月別變化는 生殖年周期와 相互 密接한 關係를 가지고 變化하였다.

群成熟度調査에서 殼長 2.1-2.5 cm인 個體들에서는 암·수共히 50%를 넘었으며, 殼長 2.6 cm이상인 個體들은 再生産에 100% 참여하였다.

INTRODUCTION

Mactra veneriformis Reeve has been mainly produced in the southern and western coast of Korea. It is one of the important commercial food bivalves. However, in connection with the recent sharp reduction in the standing stock, this clam has been noted as a possible organism for commercial aquaculture. To solve practical problems in culture, it is important to understand the biological characteristics on the reproduction of this species.

Up to now, some works have been done on the artificial discharge of reproductive substances (Iwata, 1948), classification (Lee, 1956) and the ecological aspect (Choi, 1969) of this species, while we could not find out any reports on sexual maturation and the biological characteristics of the reproduction for propagation of this clam.

Therefore, the main purpose of this study is to know the gonadal development, the annual reproductive cycle, the fatness coefficient, and the

Recieved September 27, 1988

first sexual maturity of the clam based on histological examination and some morphometric data.

MATERIALS AND METHODS

Specimens of *Mactra veneriformis* were monthly collected at the intertidal zone of Naecho-do, Chollabuk-do, Korea, for one year from March 1986 to February 1987 (Fig. 1).

A total of 472 clams were used for the study. The clams collected were transported alive to the laboratory where several measurements (shell length, shell height, total weight, meat weight) were recorded for each clam. The measurement of shell length and shell height were made to the nearest 0.01 cm, and total weight and meat weight to the nearest 0.0005 g.

A piece of the gonad near the visceral cavity of each specimen was prepared for histological examination by fixation in Bouin's solution and dehydrated by ethanol. The tissues of each gonad embedded with paraffin were cut into 5-6 μm in thickness, and stained with Hansen's haematoxylin-0.5% eosin, Mallory's triple stain and PAS stain.

The fatness coefficient was calculated by the formula of meat weight x 100/(shell length)^3.

To investigate monthly relative frequency distributions of egg diameters, some one thousand eggs a month which nuclei were centrally cut were monthly measured, and then we expressed them by the frequency curve method of Pears (1965).

The percentage of the first sexual maturity was calculated from March (before spawning) to late september (after spawning) by the method of Chung *et al.* (1987).

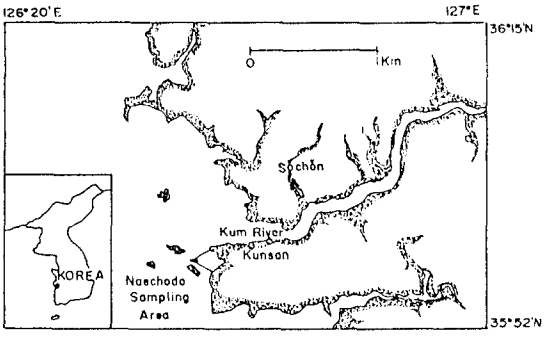

Fig. 1. Map showing the area where the specimens for the study were collected.

RESULTS

1. Position and structure of the gonads

The clam is dioecious, the gonads are located between the subregion of mid-intestinal glands in the visceral cavity and the reticular connective tissues of the foot. The ovary is composed of a number of ovarian sacs, and the testis comprise several testicular lobules (Fig. 2).

Associated with the progress of maturation, external feature of the ovary shows light brown and that of the mature testis yellowish-white in colour.

At this time, if they are slight scratched, ripe eggs and milky-white sperms flow out readily. Therefore, their sex can be distinguishable easily by external features. But after spawning, the gonads are degenerated and they became difficult to distinguish their sex.

2. Monthly changes in relative frequency distributions of the egg diameters

Monthly changes in relative frequency distributions of the ovarian egg diameters from March 1986 to February 1987 are shown in Fig. 3.

In January and February 1986, relative frequencies ranging from 10 μm to 20 μm in diameter were over 90%. In March, those of a large group of egg diameter began to increase, and attained to 27% in those over 30-40 μm, and 9% in those over 40-50 μm (mature oocyte) in April. Percentages of frequency over 50-60 μm (ripe oocyte) in diameter were about 35 % in May. In June when the spawning period began, ripe oocytes measuring about 60 μm began to decrease considerably in number because of their

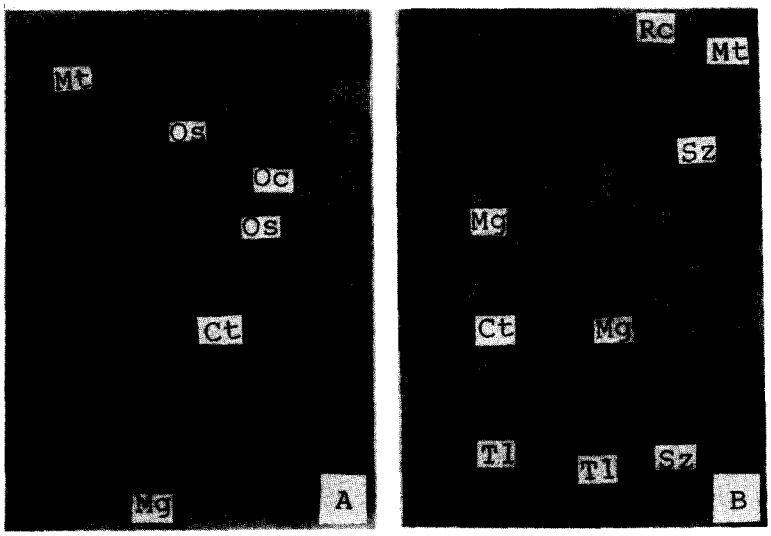

Fig. 2. Positions and structures of the gonads.
A. Growing ovary, B. Mature testis

Ct	:	Connective tissue	Mg	:	Mid-intestinal gland
Mt	:	Muscular tissue	Oc	:	Oocyte
Os	:	Ovarian sac	Rc	:	Reticular connective tissue
Sz	:	Spermatozoa	T1	:	Testicular lobule

discharge. And a few of large remaining oocytes were degenerated after July. From September to February it could be observed that a few of oogonia and oocytes measuring 10-20 μm in diameters remained in the ovarian sac.

3. Annual reproductive cycle

Based on morphological and histological observation of the germ cells and tissue cells around them, the gonadal phases could be classified into five successive stages (Fig. 4). The criteria used in defining the catagories are as follows:

1) Multiplicative stage

Oogenesis occurred in the germinal epithelia of the ovarian sacs. Oogonia propagated on germinal epithelium and every oogonium measuring about 10 μm in diameter had a distinct nucleus, while the cytoplasm was very poor (Pl. I Fig. 1). In this stage, a great number of eosinophilic granular cells and undifferentiated mesenchymal tissues were seen along the germinal epithelium.

Spermatogenesis occurred in the germinal epithelia of the testicular lobules. A large number of spermatogonia were actively proliferating among the eosinophilic cells and the mesenchymal tissues on the germinal epithelia. Spermatogonia are about 5-8 μm in diameter and attached to the germinal epithelium (Pl. II. Fig. 9)

The individuals in this stage appeared from January to March.

2) Growing stage

In female, the early growing oocytes had a round nucleus containing a nucleolus. The nucleus had a diameter of 8-15 μm, and was still rather larger than the cytoplasm (Pl. I. Fig. 2)

When the oocytes grew to 30-50 μm in diameter, each of them made an egg stalk connected to the germinal epithelium, and its nucleus enlarged to be a germinal vesicle (Pl. I. Fig. 3). When oocytes were stained with PAS stain, positive materials stained by

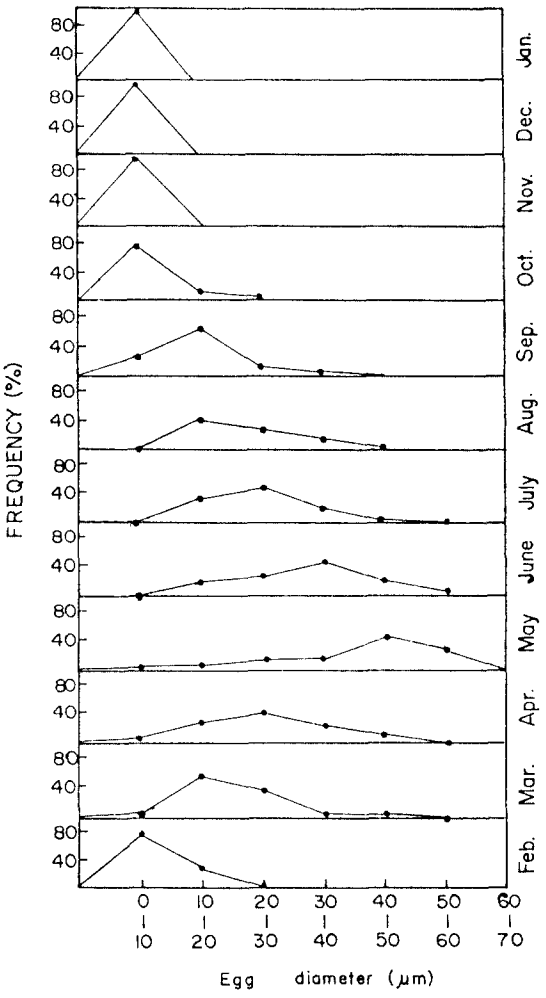

Fig. 3. Relative frequency distribution of the ovarian egg diameter through the reproductive cycle.

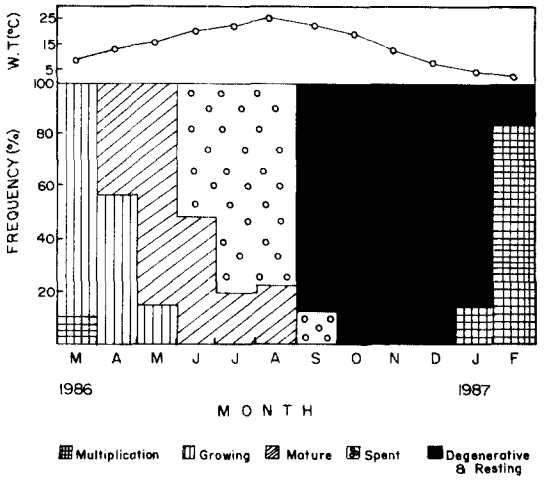

Fig. 4. Frequency of gonadal phases of the *Mactra veneriformis* through the reproductive cycle and monthly changes of mean sea-water temperatures from March 1986 to February 1987.

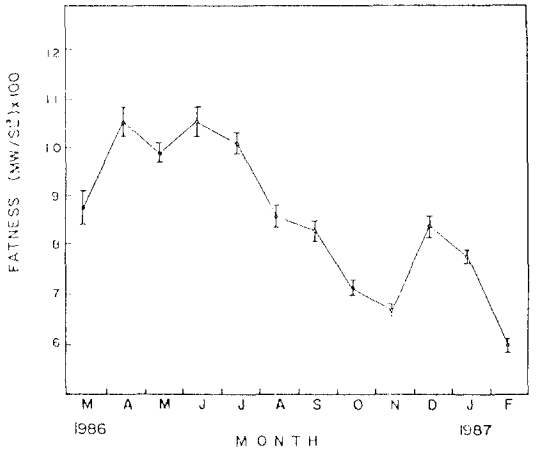

Fig. 5. Monthly changes of fatness in *Mactra veneriformis* Reeve.

PAS reaction which appeared in the base part of the egg stalk connected to the germinal epithelium diffused into the oocyte in this stage.

In male, spermatogonia grew to spermatocytes. They moved toward the center of the lumen, these spermatocytes measuring 3-5 μm in diameter showed duplicative arrangement (Pl. II Fig. 10). With the further development of the testis advanced, the testicular lobules were composed of spermatogonia, spermatocytes, spermatids in groups, and they were arranged in stratified layers (Pl. II Fig. 11). The individuals in the growing stage were found from March to May.

3) Mature stage

In female, the majority of oocytes grown to 40-50 μm in diameter became round or oval, each ovarian sac was filled with the mature oocytes in the center of the lumen. However, the germinal epithelium became very thin and the undifferentiated mesen-

chymal tissues and eosinophilic granular cells were very few (Pl. I Fig. 4). Each ripe oocyte measuring 50 -60 μm in diameter was surrounded by the gelatinous membrane and its cytoplasm contained a large number of yolk granules (Pl. I Fig. 5).

In male, a few of spermatids formed by meiosis began to undergo transformation into differentiated spermatozoa in the center of the lumen. Thereafter, spermatozoa occupied the center of the lumen. At this time, ripe testes are characterized by the formation of streams of spermatozoa in their testicular lobules (Pl. II Fig. 12). The individuals in the mature stage appeared from April to August.

4) Spent stage

In femals, since about 80% of oocytes in an ovarian sac are discharged, the lumen became considerably empty. However, there remained a few of ripe oocytes undischarged as well as young oocytes (Pl. I Fig. 6). In male, a large number of spermatozoa in the lumen of the testicular lobule were discharged into the surrounding water, and the lumen became considerably empty also. But, a number of undischarged spermatozoa remained in the lobules (Pl. II Fig. 13, 14).

The spawning period appeared from early June to September, and the main spawning occurred between July and August.

5) Degenerative and resting stage

After spawning, the undischarged oocytes in the lumen underwent cytolysis and each ovarian sac was contracted and degenerated (Pl.I. Fig. 7).

After degeneration, the rearrangement of the newly formed ovarian sacs occurred in the resting stage(Pl.I. Fig. 8). A few of remaining spermatozoa were scattered in the lumen of the testicular lobule after spawning, but they began to degenerate (Pl. II Fig. 15). After degeneration, the rearrangement of the newly formed testicular lobules occurred in the resting stage also (Pl. II Fig. 16). The individuals in this stage were found from September to February.

4. Relation between the reproductive cycle and environmental sea water temperature

Relation between the gonadal phase and monthly changes of the mean sea-water temperatures are shown in Fig. 4. The individuals in the multiplicative stage appeared from January to March when the sea water temperatures gradually rose in the growing period of the gonads from March to May. The individuals in the mature stage appeared from April to August when the mean water temperatures were high (14.9—26.2℃). The spawning began in early June, and lasted till September. The main spawning occurred between July and August when the water temperatures reached above 24℃. And then, the individuals in the degenerative and resting stage were found from September to February when their temperatures gradually decreased. Therefore, it seems that the gonadal phase is closely related to the environmental sea water temperature.

5. Monthly changes of the fatness coefficient

Investigation of the fatness coefficient is to certify relation between the gonadal development and the changes of body weight.

Monthly changes of the fatness coefficient are shown in Fig. 5. In April, the mean value of the fatness coefficient was 11.60, the highest value during the entire period of experiment. But thereafter, it rapidly decreased in the spawning period, reached to the lowest value (6.75) in November. Therefore, it seems that the fatness coefficient is closely related to the gonadal phase.

6. The first sexual maturity

The first sexual maturities of a total of 244 (113 females and 131 males) individuals of *Mactra veneriformis* were investigated histologically in order to certify these shell length participated in reproduction from March (before spawning) to late September (after spawning).

Table 1. The shell length of the fifst sexual maturity of *M. veneriformis*

Shell length (cm)	Female		Male	
	Number	Mature (%)	Number	Mature (%)
1.5 – 2.0	8	12.5	9	33.3
2.1 – 2.5	12	58.0	11	63.6
2.6 – 3.0	30	100	42	100
3.1 – 3.5	39	100	36	100
3.6 – 4.0	20	100	24	100
4.1 – 4.5	4	100	9	100
Total	114		131	

As shown in Table 1, percentages of the first sexual maturity of female and male clams were over 50% among those individuals ranging from 2.1 to 2.5cm, and 100% in those over 2.6cm in shell length.

DISCUSSION

Most of marine invertebrates belong to external fertilization species, and their breeding season is different from the seasonal changes. (Lee, 1974). Boolootian *et al.* (1962) described that the breeding habits of molluscs in general fall into three large categories, (1) year-round breeders, (2) winter breeders which spawn between the end of autumn and the begining of spring, (3) summer breeders which spawn between the end of spring and the beginning of autumn. By the result of present histological observation, it was found that *Mactra veneriformis* belong to summer breeders.

Regarding the nutritive materials concerning the gonadal development, Loosanoff (1937 a,b) described that phagocytic nutritive cells could be regarded as nutritive materials concerning the gonadal development in *Venus mercenaria*. However, several authors (Lee, 1972; Lee and Chung, 1980; Chang and Lee, 1982; Chung *et al.*, 1986, 1987) described that the undifferentiated mesenchymal tissue and eosinophilic cells were abundant on the germinal epithelium in the multiplicative stage and reduced gradually with the maturity of the gonad tubules, therefore, they could be considered as a kind of nutritive materials. Such results as above mentioned showed in this species, therefore, we consider them the nutritive cells in the formation and development of the germ cell in the early stage.

Medcof (1939) demonstrated that once oyster are ripe, spawning occurs with rising temperatures which may or may not reach 20℃, the critical spawning temperature recorded by Nelson (1928). And Galtsoff (1938) stated that temperatures of 18.6℃ to 20.5℃ are the lowest reguisite for induction of spawning. In this study, the water temperature rises from April to August in the mature stage, and shows over 20℃ during the spawning period. This watter temperature in the spent stage closely agrees with the spawning temperature (20℃) induced by above mentioned authors.

Iwata (1948) described that the spawning period of *M. veneriformis* occurred twice a year from April to early July and from September to early October in Tokyo Bay. But in this study, the spawning period of this species in the western coast of Korea was once a year from early June to early September. Some local variations of the spawning period might be related to the geographical differences in water temperature, time of the food production and some other environmental factors.

The fatness coefficients of this species rapidly increased in the spring season, and reached the peak in April, while they were very low in September when spawning was completed. *Fulvia mutica* were also low after spawning (Inoue, 1955; Matsuoka *et al.*, 1968), and were increased along the growth and the maturity of the gonad (Chang and Lee, 1982). Therefore, monthly changes of the fatness coefficient is well correlated with the reproductive cycle showed by the histological observation of the gonad.

In the first sexual maturity, percentages of the

frist sexual maturity were 58.0% in female, and 63.6 % in male among those individuals ranging from 2.1 cm to 2.5 cm in shell length. Comparing the first sexual maturity of male with that of female, the phenomenone of male sexual prematurity appeared in this clam as that occurred in the other species occasionally.

SUMMARY

The gonadal development, the annual reproductive cycle and the first sexual maturity of surf clam, *Mactra veneriformis* Reeve were studied histologically. Speciemens were monthly collected at the intertidal zone of Naechodo, Chŏllabuk-do, Korea, for one year from March 1986 to February 1987.

Sexuality of the clam is dioecious. The gonads were located between the subregion of mid-intestinal gland in the visceral cavity and the reticular connective tissues of the foot. The ovary is composed of a number of ovarian sacs, and the testis comprise several testicular lobules. The undifferentiated mesenchymal tissues and eosinophilic granular cells function as nutritive cells in the formation and development of the germ cells in the early stage. The ripe eggs were about 50-60 μm in diameter, and they were surrounded by the gelatinous membranes.

The spawing period was from early June to September, the main spawning occurred between July and August when the water temperature reached above 24℃.

The annual reproductive cycle of this species could be classified into five successive stages: multiplicative (January to March), growing (March to May), mature (April to August), spent (June to September), degenerative and resting (September to February).

The monthly changes of fatness coefficient closely correlated with the annual reproductive cycle. Percentages of the first sexual maturity of female and male clams were over 50% among those individuals ranging from 2.1 to 2.5 cm, and 100% in those over 2.6 cm in shell length.

ACKNOWLEDGMENTS

The authors are grateful to the members of Embryology Laboratory, Department of Marine Bilolgy, National Fisheries University of Pusan for their assistance in preparation of the photo-micrographs, and to Miss In-Sun Choi, Research Institute of Marine Development, Kunsan National University for her assistance in preparation of the manuscript.

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Explanation of Abbreviations

Ct: Comective tissue
Dd: Digestive diverticulum
Doc: Degenerating oocyte
Dsz: Degenerating spermatozoa
Eg: Eosinophilic granular cell
Es: Eggstalk
Ge: Germinal epithelium
Gv: Germinal vesicle
Ig: Intestinal gland
Lu: Lumen
Ml: Muscle layer
Mt: Mesenchymal tissue
N: Nucleus
No: Nucleolus
Oc: Oocyte
Og: Oogonia
Rs: Residual substance
Sc: Spermatocyte
Sg: Spermatogonia
St: Spermatid
Sz: Spermatozoa
Yg: Yolk granule

Explanation of Plates

PLATE I

Fig. 1. Transverse section of ovarian sacs in the multiplicative stage. ×400.
Note proliferation of small oogonia along the germinal epithelium, the undifferentiated mesenchymal tissue and granular cells.

Fig. 2. Section of ovarian sac in the growing stage. ×400.
Note the oogonia and the growing oocytes along the germinal epithelium.

Fig. 3. Section of a growing ovary. ×200.
Note an egg-stalk of the growing oocyte connected to the ovarian sac wall.

Fig. 4. Transverse section of ovarian sacs in the mature stage. ×100. The lumen was filled with the mature oocytes.

Fig. 5. Section of ovarian sac in the same stage above mentioned. ×600.
Note fully ripe oocytes in the ovarian sac. A large number of yolk granules are found in the cytoplasm, and the nucleolus is found in the large germinal vesicle.

Fig. 6. Section of ovarian sacs in the spent stage. ×100.
Note the presence a few of undischarged oocytes and residual substances which are in the ovarians sacs after spawning.

Fig. 7. Section of ovarian sacs in the degenerative and resting stage. ×200.
Note degenerating oocytes in the ovarian sacs.

Fig. 8. Section of ovarian sacs in the same stage above menttioned.
Ovarian sacs became withering and a few of degenerating oocytes and residual substances remained in the ovarian sacs.

PLATE II

Fig. 9. Section of the testicular lobules in the multiplicative stage. ×200.
Note a several spermatogonia and undifferentiated mesenchymal tissue appeared along the germinal epithelium.

Fig. 10. Section of the testicular lobules in the early growing stage. ×100.
Note a number of spermatogonia and spermatocytes on the germinal epithelium in the testicular iobules.

Fig. 11. Transverse section of late growing testis. ×200.
Note the layers composed of spermatocytes and spermatids.

Fig. 12. Section of testis in the mature stage. ×200.
Note a large number of spermatozoa with the tails in the testicular lobules.

Fig. 13. Transverse section of the spent testis. ×200.
Note a few number of undischarged spermatozoa remain in the lobules.

Fig. 14. Section of testicular lobule in the spent stage. ×200. The lumen became considerably empty.

Fig. 15. Section of testis in the degenerative stage. ×200.
Testicular lobules become withering and a few of undischarged spermatozoa and residual substances remain in the testicular lobules.

Fig. 16. Section of testicular lobules in the same stage above mentioned. ×200.
After degeneration, the rearrangement of the newly formed testicular lobules occur in the resting stage.

PLATE 1

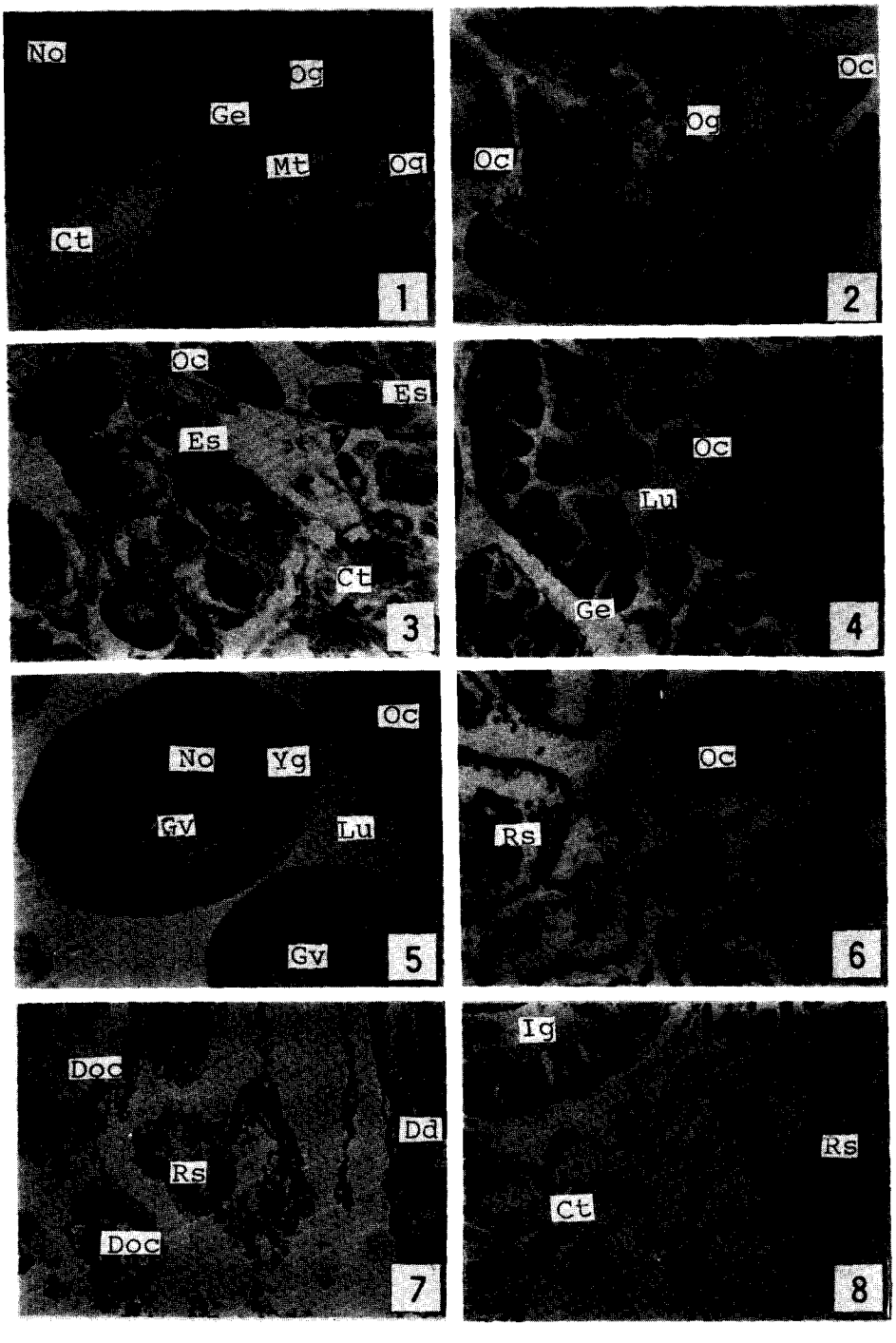
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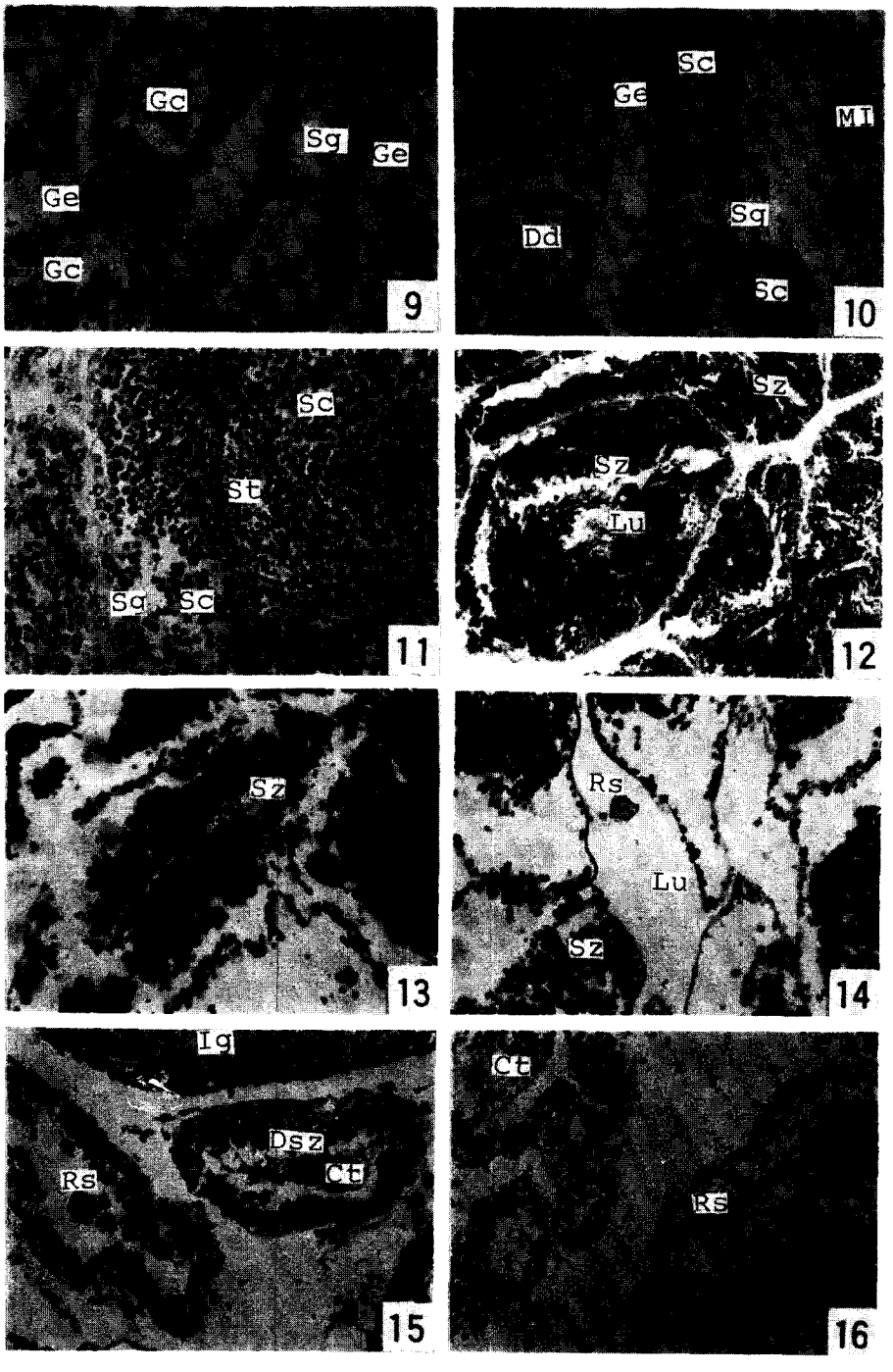
PLATE 2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