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KARYOTYPE, MEIOSIS AND SPERM FORMATION IN THE LAND SNAIL *MACROCHLAMYS INDICA*

By

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الهيئة الكروسومية ، الانقسام الاختزالي (الميوزي) وتكوين الحيوانات المنوية في القوقع الأرضي *مكروكليمس أنديكا*

نجلاء زكي الألفي و خالد عبد الله العلي و عبد الراضي حسن عبد الرحيم

تناول البحث دراسة الهيئة الكروموسومية «الكريوتيب» والانقسام الميوزي وتكوين الحيوانات المنوية للقوقع الأرضي ***مكروكليمس انديكا*** الذي تم جمعه من بعض مشاتل نباتات الزينه بمدينه الدوحة بدولة قطر ووجد أن العدد الاحادي لكروموسومات هذه القواقع (ن) يساوي ٢٤ والعدد الزوجي (٢ ن) يساوي ٤٨ وأظهر ترتيب الكريوتيب أن هذه الكروموسومات تنتظم في مجموعتين هما : خمسة عشر زوجاً من الكروموسومات الكبيرة غير متساوية الزراعين والتي تأخذ أشكال حرف L ، وتسعة أزواج من الكروموسومات متساوية الزراعيين تأخذ أشكال حرف V . كما تضمن البحث دراسة المراحل المختلفة من الانقسام الاختزالي وتم أيضا وصف مراحل تكوين الحيوانات المنوية والتي إشتملت على ظهور أربعة خلايا يحتوى كل منها على نصف العدد الكروموسومي والتي نتجت من الانقسام الاختزالي من حويصلة منوية واحدة . وأثناء عملية تكوين الحيوانات المنوية تدخل أمهات المني في إنقسام غير مباشر (ميتوزي) سريع ومتضاعف ينتج أمهات المني الأولية والتي تتحول إلى حيوانات منوية تتجمع في حزم .

Key words: Karyotype, Meiosis, Sperm formation, *Macrochlamys indica.*

ABSTRACT

Karyotype analysis, meiosis and sperm formation of the land snail *Macrochlamys indica* collected from different farms of ornamental plants in Doha city of the State of Qatar were investigated. The chromosome number is n=24 and 2n=48. In the karyotype analysis, the chromosomes were categorized into two groups; fifteen pairs of L's shaped submetacentric and nine pairs of V's shaped metacentric. Several stages of first and second meiotic cell division were observed and described.

Differentiation of spermatids into spermatozoa were also investigated. Four haploid cells arising by meiosis from single spermatocyte. The spermatogonia which enter a phase of rapid mitotic multiplication producing the primary spermatogonia were also demonstrated. Sperm bundle maintained as a unit during sperm differentiation.

INTRODUCTION

The land snail *Macrochlamys indica* belong to the family Zonitidae, subfamily Ariophantinae, genus Macrochlamys [1]. This species was for, a long time identified with Helix vitrinoides, a shell of unknown origin and described as imperforate. *M. indica* was occur from Calcutta to Cawnpore, India. The first complete description of this species was given by Godwin-Austen and the name *M. indica* is accepted [1].

Very probably *M. indica* was introduced from India to the State of Qatar with ornamental plants. Many growers of orchards and ornamental plants in Doha are familiar with the land snails *M. indica* and *Helix aspersa* and also with the land slug *Laevicaulis alte.* While these animals have not yet been considered as serious pests in the State of Qatar, they have, however in recent years increased in abundance in certain localities and their damage has been steadily increasing. Unfortunately, in Qatar, no information is available about land snails and slugs and their role as injurious pests has not been fully assessed.

In general, great majority of gastropod species have still not yet been studied cytogenetically [2]. An attempts were made by [3] and [4] to study the karyotype of the polymorphic land snail *Cepaea nemoralis,* and by [5] on *Eobania vermiculata.* Mitosis and meiosis of *C. nemoralis* have been investigated by [6] and by [5] for *E. vermiculata.*

Variation in chiasma frequency in land gastropods play an important role in representing the cytogenetical manifestations of recombination [7]; [8]; [9] and [10] on *C. nemoralis,* [11] on the freshwater snail *Biomphalaria alexandrina* and [5] on *E. vermiculata.*

Spermiogenesis and sperm morphology in molluscs have been investigated in the prosobranchial *Chorus giganteus* by [12] in mussel by [13] and in pulmonata by [14]. The main aims of the present investigation are to study the karyotype, meiosis, sperm formation of the introduced land snail *M. indica.*

MATERIALS AND METHODS

Individuals of the land snail *M. indica* were collected from different sites of ornamental farms in Doha city. They were dissected out of their shells in saline solution. Their hermaphrodite glands which embedded in the digestive gland, were isolated and cleaned of digestive tissues. A small pieces of the hermaphrodite glands were cut off and fixed in Carnoy fixative (3 parts of absolute ethanol and 1 part of glacial acetic acid) for at least 24 hours in a refrigerated incubator at 5+1°C. They were stained in aceto-orcein (2% orcein in 45% acetic acid) for about 7-10 minutes. After being stained, small pieces of hermaphrodite gland were quickly rinsed in 45% aqueous acetic acid to remove excess stain, and then transferred to a clean microscopic slide, covered gently with a cover slip, and squashed by manual pressure.

Suitable preparations were made permanent by soaking off cover slip in the acetic acid-ethanol mixture, rinsing quickly in two changes of absolute ethanol, and remounting in depex. Observations were made using light microscopy. Different stages of meiosis were then photographed using black and white film. All photographs were taken at 100 X.

RESULTS

Description of the karyotype

In the primary spermatocytes of the land snail *M. indica* the basic chromosome complement showed 24 bivalents. Typical diplotene cells showed that there are fifteen pairs of submetacentric and nine pairs are metacentric. Chromosomal morphologies were distinguishable in terms of centromeric location and length (Figure 1).

The autosomal pairs from 1-10 are submetacentric and arranged in decreasing order of length. Pair one is quite easy to identify because of its obvious length in comparison with other members. The length of the short arm is about half of the long arm. However, pairs from 11-14 are metacentric chromosomes. Also, the last 5 pairs from 20 to 24 are metacentric. In addition, pairs 15-19 are submetacentric. All autosomes showed a gradation in length which permits recognition of the individuals pairs except the last five autosomal chromosomes which are uniform in size and it is difficult to distinguish between them (Figure 1).

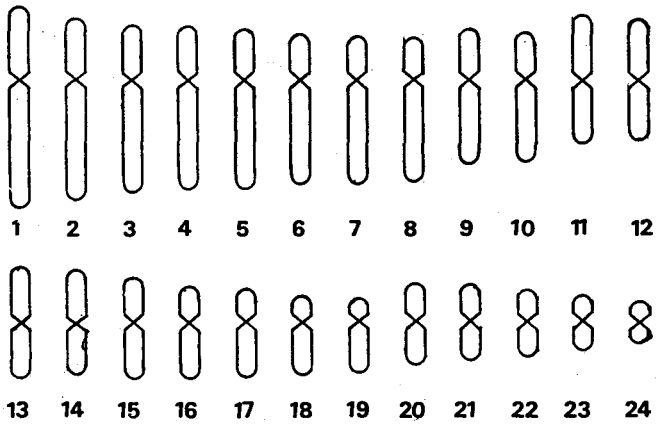

Fig. 1: Diagrammatic representation of the karyotype of *M. indica.*

Meiosis

Meiotic stages in *M. indica* were also examined Figures 2-14. The nucleus enlarges to form the first meiotic prophase nucleus. The pre-leptotene nucleus contain granular chromatin formed into chromatin strands and pear-shaped in appearance. It is difficult to follow each single chromosome throughout its entire length (Figure 2). In late leptotene, the free ends of the chromosomes are polarized i.e. associated at one side of the nucleus (Figure 3). The active pairing of homologous chromosomes into association takes place in zygotene (Figure 4). These chromosomes pair's length wise with each other, with pairing being initiated at one or more sites along the length of the chromosomes, forming the "bouquet stage" (Figure 4). The chromosomes being to synapse at the polarized end (see arrow in Figure 4) while the remainder of the strands are yet unpaired in "bouquet" formation. The zygotene chromosomes appear to be contracted and darkly stained than the leptotene chromosomes. Pairing of homologous strands is completed in zygotene forming

pachytene chromosomes of double thickness and haploid in number.

At the beginning of diplotene, the homologous begin to "repel" one another, causing the chromosomes to separate. The separation is generally not complete, however, the paired chromosomes are held together at one or more points along their length. The longer the paired chromosomes, the greater, in general, is the frequency of contact points (points of crossing over see arrows Figure 5). The distinction between diplotene and diakinesis is not a clear one (Figures 5 and 6). Terminalization of chiasmata begins and contraction continues forming the rings and crosses (see arrows Figure 5). Diakinesis is characterized by a more contracted state of the bivalent (Figure 6). Metaphase I is a period of relatively little movement of chromosomes, the paired homologous are still joined by the previously formed chiasmata. Bivalents are highly condense with deep stain (Figure 7. side view). The chromosomes begin their poleward movement, the chiasmata lose their relative influence and free the separating homologous. In telophase I, the chromatids of each homologous are contracted, free of each other and tend to flare apart widely. The chromosomes are widely separated from each other to the opposite poles of the cells. Each pole receives one-half the original chromosome number of the meiocyte (one set of chromosomes in the case of diploids) (Figure 8). Interkinesis is observed between the first and second meiotic divisions. During interkinesis, the rest of the cell is beginning to divide. The cell membrane pinches inward and the cytoplasm is dividing into two masses (see arrows in Figure 9). As the cell approaches to division into two cells, the individual chromosomes become less distinct and partly uncoiled.

When half bivalents reappear at second prophase (Figure 10), the two chromatids of each chromosome are to be loosely coiled, but they appeared contract to the metaphase II (Figure 11). The chromosomes of metaphase II are highly contracted and deeply stained (Figure 12. side view). The chromatids rapidly separate to opposite poles at anaphase II. In the telophase II, the chromosomes reach their respective poles, grouped together and cytokinesis begins to takes place (Figure 13). The meiotic cycle is completed when the four haploid nuclei were formed (Figure 14), giving rise to four cells. All four cells usually develop into gametes.

Sperm formation

The male portion of the hermaphrodite gland contain spermatogonia which enter into a phase of rapid mitotic multiplication producing the primary spermatocyte. The primary spermatocytes with unreduced chromosome number gives rise, by the first meiotic division, to two secondary spermatocytes and these in turn, at the second meiotic division to four haploid spermatids with reduced chromosome number (Figure 15). Each spermatid differentiate into the mature male gametes (the spermatozoon) without further nuclear division (Figure 16). Any of the sperm mother cells derived from spermatogonia give rise to a "spermatid bundle" (Figure 15). It is a group of spermatids of common origin maintained as a unit during spermatid differentiation. Each spermatogonia consists of a head and tail (see arrows Figure 16). The tail is about 6 times of the head length.

DISCUSSION

In pulmonate molluscs, the chromosome numbers vary from 5 pairs, found in the land snail *Catinella rotundata* (Goud) of Hawaii, to the 60 pairs recorded in the European. *Ancylus fluviatilis* (Mull) and the 72 pairs detected in the Ethiopian freshwater Planorbid *Bulinus octoploidus* [15]. To our knowledge, no information is available concerning the chromosome numbers of the land snail *M. indica.* Its number was determined in the present work; the haploid number begin 24 chromosomes, therefore the diploid is 48 chromosomes. Karyotype analysis of *M. indica* chromosomes has demonstrated that these chromosomes are mostly elongate submetacentric and metacentric. The larger chromosomes of the present snail have revealed that they exhibit L's and V's shapes; and due to the crowding on the spindle, their arms extend peripherally away from their centromeres, which are attached to the spindle. These nomenclatures were similarly proposed in the polymorphic land snails by [7] in *Cepaea nemoralis* and [5] in *Eobania vermiculata.*

In the present investigation, various meiotic stages were also observed and described. These were early-leptotene and late leptotene, zygotene, diplotene, diakinesis, metaphase I, telophase I and interkinesis. There is no recognizable anaphase I owing to its short duration. Second meiotic division stages were also observed, prophase II, metaphase II and telophase II. They all follow a normal course of meiosis of land snail *C. nemoralis* [6] and *E. vermiulata* [5].

It was also recognized that in the present snail, bivalents were held together by chiasmata. These, usually, serve as a genetic function as they represent the cytogenetical manifestations of recombination. They also have a mechanical role in that they maintain the association of homologous chromosomes after the intimate pairing of early prophase had lapsed [11].

The present investigation demonstrated that the spermatozoon is a very long, uniflagellate composed of a conical head. This similar to the structure of spermatozoon of the snail *Siphonaria algesirae* [14].

In conclusion, the present investigation provide the first attempt to describe the karyotype and meiosis in the land snail *M. indica.* Further work is needed to study these chromosomes in more details using banding and molecular techniques.

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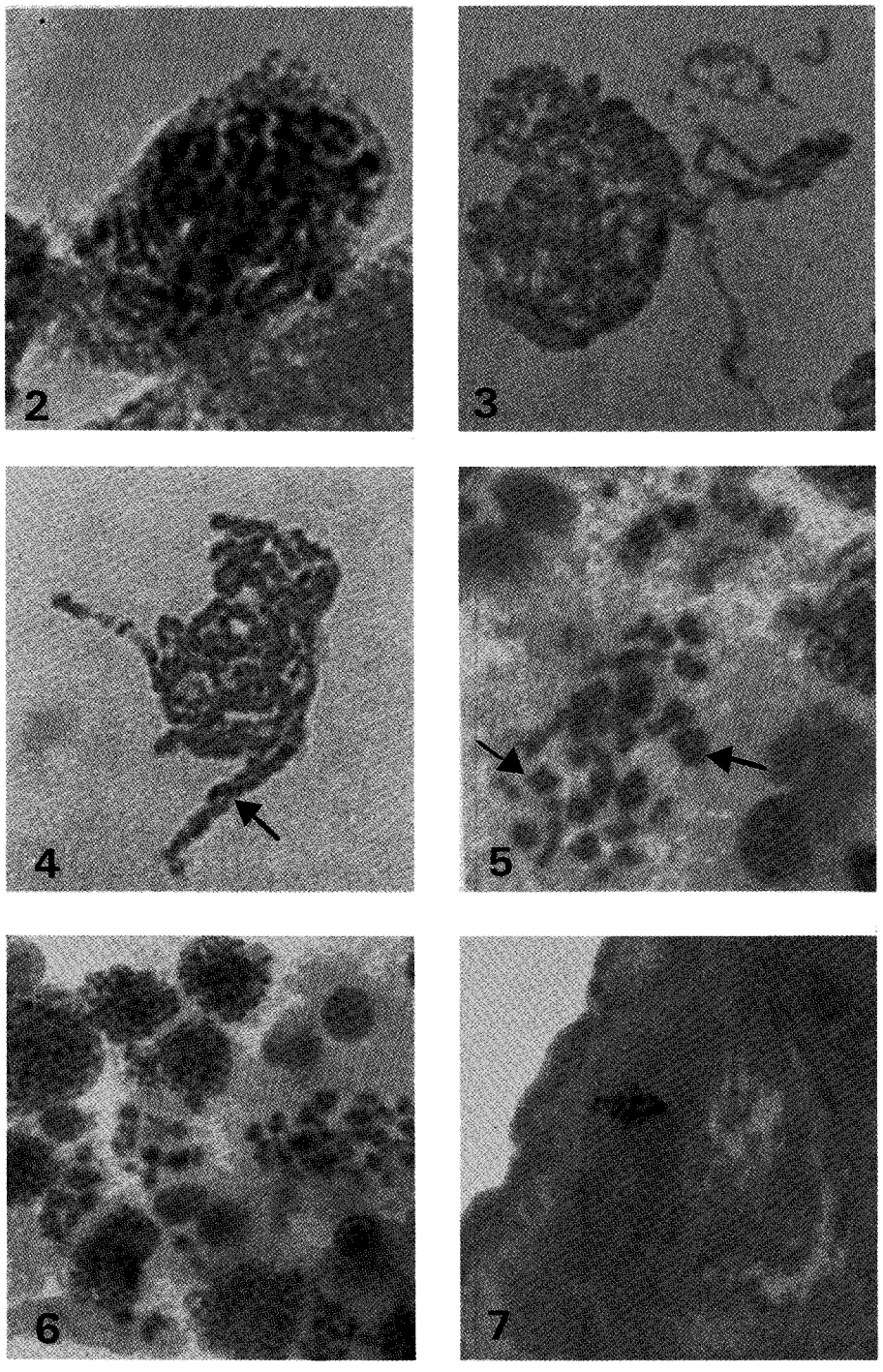


Fig. 2: Pre-leptotene (X= 1000).

Fig. 3: Late leptotene (X= 5800).

Fig. 4: Zygotene (arrow indicate the position of synapse) (X= 9800).

Fig. 5: Diplotene stage (arrow indicate the position of chiasmata and different shapes of bivalent; ring and cross) (X= 8000).

Fig. 6: Diakinesis stage (X= 5000).

Fig. 7: Metaphase 1 (side view) (X= 10000).

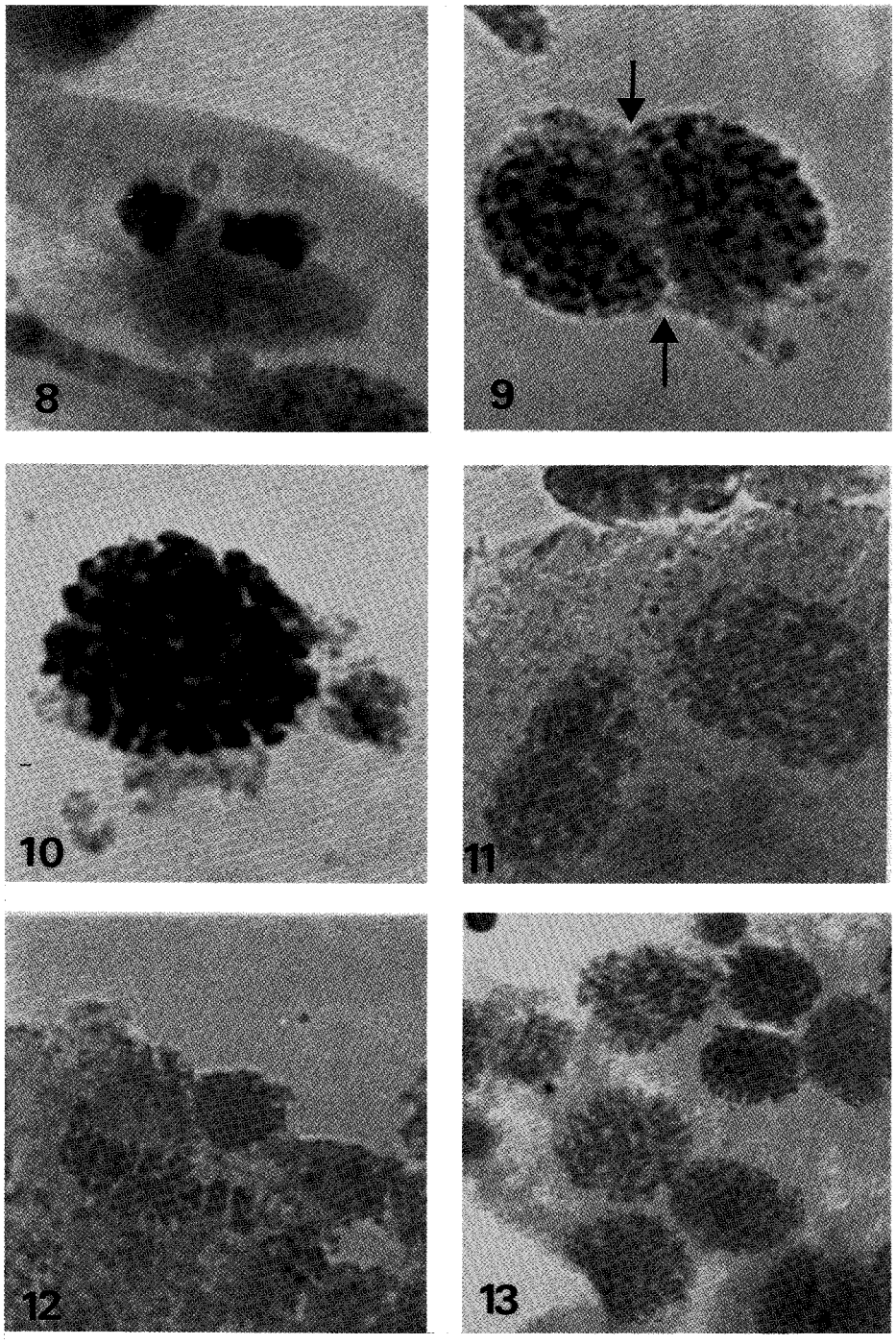


Fig. 8: Telophase 1 (X= 10000).

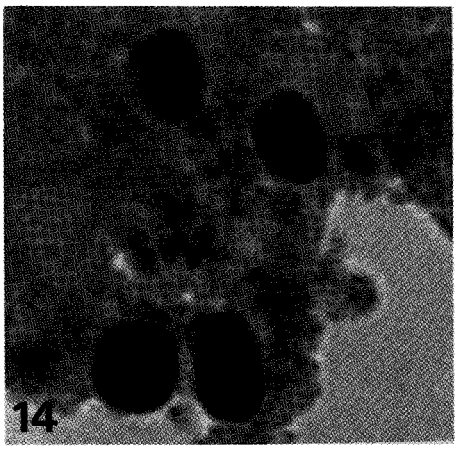
Fig. 9: Interkinesis (arrows showing cell membrane pinches inwards) (X= 5800).

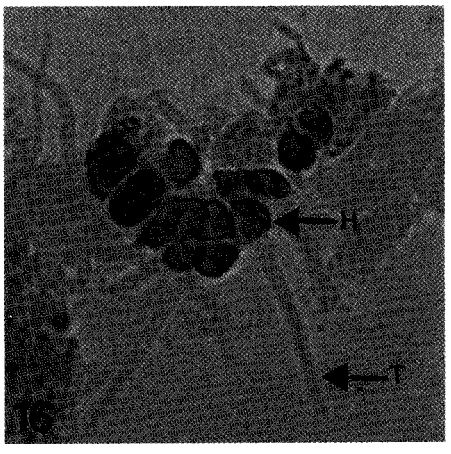
Fig. 10: Prophase II (X= 5800).

Fig. 11: Early metaphase II (X= 7750).

Fig. 12: Metaphase II (side view) (X= 6500).

Fig. 13: Telophase II (X= 7000).





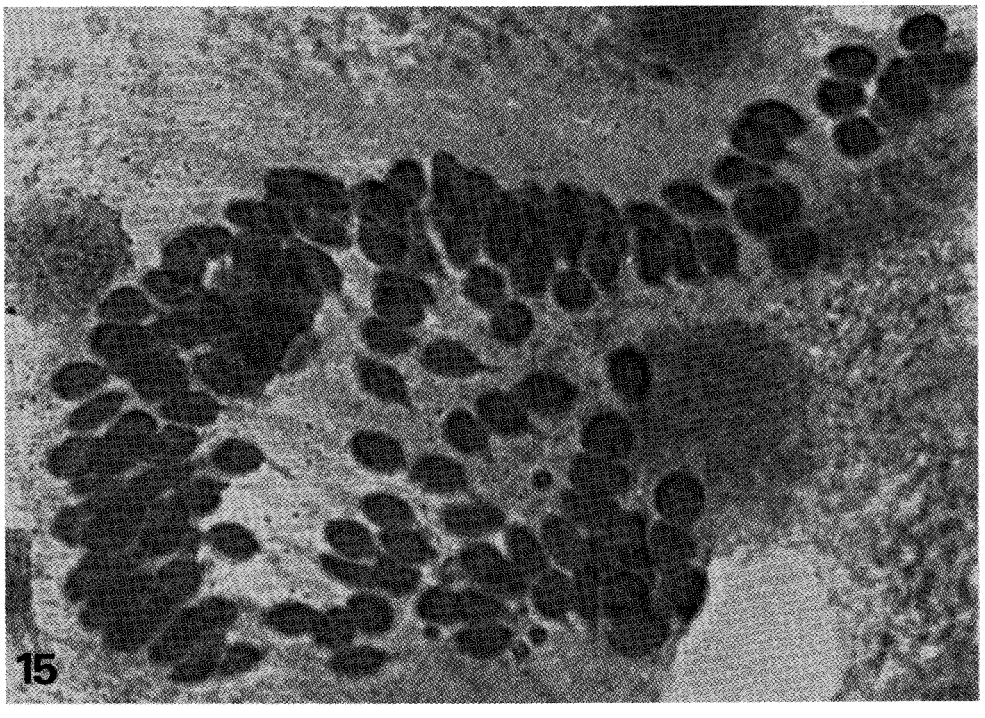


Fig. 14: The four nuclei of the gametes (X= 9000).

Fig. 15: "Spermatid bundles" (X= 7500).

Fig. 16: Mature male gametes (spermatozoa) (arrows pointed to sperm head (H) and tail (T)) (X= 5000).

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