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Notes on the cytology of Rissoacea
I. Cytotaxonomical conditions in some Hydrobiidae and Assimineidae (Gastropoda Streptoneura)

by

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INTRODUCTION

In a paper on the validity of *Vallonia excentrica* Sterki, HUBENDICK (1952) proceeding from MAYR's definition of a species concluded that the species concept is biological and not morphological. Two species should, therefore, be separated biologically, in other words by breeding experiments. This is, however, very difficult particularly in hermaphrodites where self-fertilization cannot be excluded. PARAENSE (1956) proved in a fine study, working with albino markers, that this method is very certainly feasible. In many other cases involving experimental crossings of critical species it must be kept in mind that a fertile offspring does not permit one to lump the two parental nominal species since the critical question still remains: when two "species" are able to produce fertile offspring in the laboratory, do they freely interbreed in their natural biotope? We cannot, in any case, do without morphological methods and we must consider them in relation to all other data available. We now return to HUBENDICK's advice: "If therefore a morphological method must be applied, the most certain way and undoubtedly the most correct way is to do chromosome studies". BURCH (1964) concluded from a study on chromosomes in *Oncomelania* that three genera, 19 species and 2 subspecies are in reality only one species with several geographical races.

It has always been very difficult to discriminate between *Hydrobia ulvae* (Pennant, 1777) and *Hydrobia stagnorum* (Gmelin, 1790) the latter being sometimes understood as an ecotype of the former species

(SPAINK, 1961). In this connection the validity of the Danish *Hydrobia neglecta* Muus, 1963, was doubted. From this point our interest in molluscan chromosomes was awakened and we started our research by collecting data from the literature on the cytology of the family Hydrobiidae. The literature dealing with European species is reviewed in the next pages. We thought it useful to mention data on the period of egg production and copulation as there is a better chance of finding good cell divisions in animals collected prior to that period.

Our study comprises three species in the genus *Hydrobia* mentioned above. We inspected some individuals belonging to *Potamopyrgus jenkinsi* (Smith, 1889) for the presence of sperms which were seen by KRULL (1935) and include our observations on *Bithynia tentaculata* (Linnaeus, 1758), *Bithynia leachi* (Sheppard, 1823) and on *Assiminea grayana* Fleming, 1828.

The living animals were sexed and only the males were used. The shell was gently crushed and peeled off. The testis was dissected out and rinsed in distilled water. The tissue was fixed and stained for 2-3 minutes in lacteo-orceine on a slide, squashed firmly under a cover slip and sealed off with nail varnish. The slides were studied using a Zeiss photo phase contrast microscope, 100 × oil immersion objective (n.a. 1.25), 8 × oculars, green filter and Agepann FF panchromatic film. The photographs taken were enlarged and printed on a scale of 2250 ×.

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SYSTEMATIC PART

Hydrobia aponensis von Martens, 1858

We are not familiar with *Hydrobia aponensis* von Martens. RANZOLI (1950) published a cytological study on this species. He described spermatogenesis but remained in doubt about the accurate number of chromosomes in this species. During spermatogonial mi-

tosis single chromosomes were difficult to recognize. In meisosis II he found 8 or 9 contracted, dot-like chromosomes.

Hydrobia neglecta Muus, 1963 Plate 3 fig. 1

Nothing was known about the cytology of MUUS' species. *Hydrobia neglecta* is bisexual, in shell form it is more or less intermediate between *H. ulvae* and *H. stagnorum;* there are slight differences in the radula, very clear differences in the pigmentation of tentacles and proboscis. With regard to the tolerance for chlorine ions it takes an intermediate position between *H. ulvae* and *H. stagnorum* (= *ventrosa* (Mont.), MUUS, 1963).

If *H. stagnorum* is indeed nothing more than an ecotype of *H. ulvae, H. neglecta* belongs to the same *H. ulvae* complex. It might also be a polyploid or an hybrid. We have already rejected the possibility of polyploidy for this species (BUTOT & KIAUTA, 1964).

Dr. MUUS has put some individuals of his species at our disposal, collected at Isefjord, Denmark, October, 1964. We studied the testes and found 18 bivalents present in meiosis I and 18 chromosomes in spermatocytes II. The species differs cytotaxonomically from *H. stagnorum* in having only one small element the bivalent nature of which could not be discerned. In meiotic metaphase I chromosomes are of decreasing magnitude ranging from 3.8 μ to 0.9 μ in nine cells measured (Table I). The largest chromosomes range from 2.3 μ to 3.8 μ; the smallest chromosomes from 0.9 μ to 1.8 μ. The mean ratio is 2.2 (2.9 : 1.3)

Hydrobia stagnorum (Gmelin, 1790) Plate 3 fig. 2

Nothing was known about the cytology of this species which is bisexual. Eggs are produced in the months from May until August (VAN BENTHEM JUTTING, 1923). Our material was collected at Veere, Zeeland, Netherlands, in September and November 1964 and at Petten, Noord-Holland, Netherlands, December 9th 1964. The animals were kept alive in the laboratory and reproduced although not much attention was paid to them. Among 13 preparations made from 13 individuals in November, 1964, only three individuals showed several unclear cell divisions. Of seven preparations made from seven individuals of the Petten March 7th, 1965, series we obtained four slides (four individuals) with clear metaphase figures.

The chromosome set of this species contains two small elements the bivalent structure of which is not visible, while the other 16 bivalents do clearly show their bivalent nature. Bivalents of metaphase I are of decreasing magnitude, their length ranging within limits of

Table I

Species	Length in micron		ratio
	largest chromosome	smallest chromosome	
Hydrobia			
neglecta	2.8	1.2	
	2.8	1.2	
	2.6	1.4	
	2.6	1.1	
	3.8	1.8	
	3.0	1.6	
	2.8	0.9	
	2.3	1.0	
	2.8	1.3	
	3.0	1.8	
mean	2.9	1.3	2.2
stagnorum	4.2	1.3	3.2
ulvae	2.5	1.0	
	3.0	1.4	
	3.2	1.2	
mean	2.9	1.2	2.4

4.2 μ and 1.7 μ. The two smallest elements of oval shape measure only 1.3 μ. There is a ratio of 3.3 between the longest and shortest chromosome (4.2 : 1.3) this being by far the largest ratio in the three species examined. We regret that only one photograph permitted accurate and comparable measuring (Table I).

Hydrobia ulvae (Pennant, 1777) Plate 3 fig. 3

Hydrobia ulvae is bisexual. Egg capsules are found from the end of May and larvae are found from July 14th to the beginning of August in the neighbourhood of the island of Sylt (HENKING, 1894).

There are 18 tetrades in the germinal vesicle; two maturation divisions occur of which the first is reductional. Smears and serial sections of male germinal tissue reveal 18 chromosomes in the spermatocytes (SANDERSON, 1940).

We can confirm SANDERSON's counts. In spermatocyte metaphase II we could observe 18 chromosomes. Our material was collected in the north of Wieringen island, Noord-Holland, Netherlands, June 13th, 1964 and at Termunten, Groningen, Netherlands, May 4th, 1965. In all eleven males from Wieringen and in eleven out of 15 males from Termunten we found active testes. In material originating from Yerseke, Zeeland, Netherlands, and kindly put at our disposal by Drs. C. BIERSTEKER, Delta-Institute, The Netherlands, where a colony is kept at the laboratory, we found stages of spermatocyte metaphase I clearly showing 18 bivalents of regularly decreasing magnitude. In about 20 cells photographed no particularly small chromosomes occur. In three cells measured (Table I), the largest bivalents reach a size of 2.5, 3.0 and 3.1 μ. The smallest bivalents measure 1.0, 1.4 and 1.2 μ. The measurements give a mean ratio of 2.4 (2.9 : 1.2).

Potamopyrgus jenkinsi (Smith, 1889)

The species was described from the Thames estuary where it was found in 1883. It was introduced and invaded Europe within a relatively short period in modern times. In appears first in brackish water localities and from there the species invades freshwater (BONDESEN & KAISER, 1949). It propagates by parthenogenesis (BOYCOTT, 1919, confirmed by QUICK, 1920 and ROBSON, 1923). Males were unknown until 1958 when PATIL published the occurrence of a male individual. KRULL (1935) denied the parthenogenetic propagation of this species and thought it to be a hermaphrodite prosobranch since he had observed sperms in the gonad. THORSON (1946) believed that hermaphrodites exist as well as parthenogenetic individuals, evidence, however, is lacking. JAECKEL (1955) studied animals of different age and never found sperms. We studied three individuals taken at Arnemuiden, Zeeland, Netherlands, June the 6th, 1965, but could not find dividing cells in which chromosomes presented good counts. The animals carried embryos. Sperms were not found. In Scotland the breeding season is at its height in August and September (SANDERSON, 1940).

In dividing cells of the embryos 2n = 20 and 22 were counted. The species showed a diploid parthenogenesis (RHEIN, 1935). In Scottish forms reductional divisions did not occur. In individuals studied by SANDERSON (1940) she found several good metaphase plates of 36 and about an equal number showing 44 chromosomes. With insufficient evidence i.e. RHEIN's investigations only, she suggested parthenogenetic tetraploidy as valid for British populations and parthenogenetic diploidy as valid for continental populations. VANDEL (1939) had defined geographical parthenogenesis. Two races or two closely related species of which one is bisexual and the other parthenogenetic often occupy different geographical areas. The parthenogenetic race is generally also polyploid. The extension of parthenogenetic and polyploid forms towards the north (where they occupy more unfavourable sites) is linked more to their polyploid state than to their parthenogenetic reproduction.

BOETTGER (1951) recognized *Potamopyrgus badius* (Gould, 1848), a New Zealand Hydrobiid, as conspecific with *P. jenkinsi* (Smith, 1889). The tetraploid Scottish form was named by him *Potamopyrgus jenkinsi septentrionalis*. The holotype resp. a holotypic slide awaits selection. The locus typicus is without doubt Barry Burn, Carnoustie, Angus, Scotland.

For practical reasons only BOETTGER did not use the specific name *badia* Gould, 1848, although it clearly has priority over *jenkinsi* Smith, 1889. KUIPER (1956) and BERNER (1963) discuss the possibility of including *Amnicola lanceolata* Paladilhe, 1869, in the synonymy. VAN BRUGGEN (1956) proposed to make *jenkinsi* a nomen conservandum in order to prevent *badia* Gould taking priority. This proposal is premature, because BOETTGER's identification is very doubtful as BERNER (1963) rightly pointed out. There is still a parthenogenetic genus *Austropyrgus* Cotton, 1943, which should be compared and some other *Potamopyrgus* species make a chance of being the source from where *P. jenkinsi* has arisen.

The definition of the species concept is clearly in terms of sexual reproduction. Species are groups of populations the gene exchange between which is limited or prevented in nature by one or by a combination of several reproductive isolating mechanisms (DOBZHANSKI, 1951). Also MAYR's definition cited by HUBENDICK (1952) supposes sexual reproduction. Therefore we should not apply this definition to populations of individuals which are solely propagating by means of parthenogenesis. It would be absurd to claim full species rank for every population or individual reproducing parthenogenetically just because parthenogenesis is an isolating mechanism.

However, parthenogenetic forms may be considered incipient species, connected with bisexual forms through a stage in which parthenogenetic individuals occur incidentally in bisexual populations. At any stage a change to polyploidy is theoretically possible. As soon as parthenogenesis is no more reversible and no more incidental, we think that subspecific status can be given to the population(s) in which this phenomenon has been established. This is, of course, only possible when the parental species is known; as long as it is unknown the parthenogenetic form will stand as a full species. We are inclined to consider polyploid forms as specifically distinct from the parental diploid species.

When trying to apply this theory to European *Potamopyrgus*, nomenclatorial difficulties arise. We know that a tetraploid form occurs in Scotland and a diploid on the continent, but we ignore if the Thames estuary population after which *P. jenkinsi* was described is diploid or tetraploid. So *septentrionalis* Boettger may be a synonym of *jenkinsi* (Smith) and in that case the continental form should have another name. Possibly the name *lanceolata* (Paladilhe) could be revived for it, but cytological study of topotypes would be required to settle that question. On the other hand, if the Thames estuary population will appear to be diploid, the name *jenkinsi* (Smith) may be preoccupied by *lanceolata* (Pal.). Consequently for reaching a stable nomenclature cytological study of topotypes of both PALADILHE's and SMITH's forms will be necessary. The fact that the parental species is unknown is another complication. The diploid European form will prove to be fully identical with some exotic species only when the latter shows the same chromosome complement and is reproducing parthenogenetically.

Bithynia leachi (Sheppard, 1823) Plate 3 fig. 4

KEYL (1955) studied the spermatogenesis but did not publish the results. According to his statement (l.c. p. 416) the prophase is disturbed in the same manner as it is in *B. tentaculata.* Our material was collected by Mr. L. W. G. HIGLER at Kortenhoef, Noord-Holland, Netherlands, March 27th, 1966. Preparations were made the next day. We had less difficulties in determining the chromosome number than we had in *B. tentaculata.* We found 17 bivalents in spermatocytes I and $2n = 34$ in male mitotic divisions. Eupyrene cells in *B. leachi* seem to be more numerous than in *B. tentaculata.* In view of heterotypic karyotypes spermatogenesis of the two *Bithynia* species is in need of statistical studies. The "atypic" cells are clearly recognizable by essentially higher or lower chromosome complement. In

the hyperpyrene cells the determination of the chromosome complement is hardly or not possible as they do not spread due to agglomeration. The species is bisexual.

Bithynia tentaculata (Linnaeus, 1758) Plate 3 fig. 5

VON KEMNITZ (1914) was the first to produce cytological data on this species in a paper on sperm dimorphism. According to later studies he misrepresented the facts, probably because of the application of faulty fixing and staining techniques. He is right in the discovery of two kinds of sperms which were called "typical" and "atypical". His counts in meiotic divisions n = 24-28 and in mitotic divisions 2n = 24-28 are incorrect. ANKEL (1924) clearly finds n = 17 and gives a good photograph (ANKEL, 1933, Fig. 17, p. 184). In spermatogonial mitoses more than 30 chromosomes are present. TUZET (1930) counts 17 bivalents. In mitotic divisions she determined the existence of 34 chromosomes. Other results of her research are incompatabile with ANKEL, 1924 (ANKEL, 1933) and also KEYL (1955) could not agree with her views. In three karyotypes of chromosomes in diakinesis KEYL pictures 17 bivalents. He also mentioned that the different phases of spermatogenesis described in his paper can be found in mature males from March till September. Although TUZET's counts of chromosomes in several prosobranchs are much critisized, her counts in *Bithynia tentaculata* appear to be correct. The species is bisexual.

We studied individuals collected at Kortenhoef, Noord-Holland, Netherlands, April 27th, 1966, by Mr L. W. G. HIGLER, hydrobiologist at the State Institute of Nature Conservation Research. Preparations were made the next day. Although the study of spermatogenesis of this species seems to be difficult because of frequent and pronounced variation of the karyotype resulting in the formation of oligopyrene, eupyrene and hyperpyrene cells, we can agree with ANKEL (1924), TUZET (1930) and KEYL (1955) who determined the haploid chromosome number of *Bithynia tentaculata* as n = 17.

Assiminea grayana Fleming, 1828 Plate 3 fig. 6

Nothing was known about the cytology of this bisexual species. In one individual from a sample collected at Holwerd, Friesland, Netherlands, June 22nd, 1965, we found three spermatocyte metaphases and could determine the chromosome number n = 12. The animals copulate in April (ADAM, 1960) and the advanced season might be the reason that we did not find optimal active testes in this sample. March 12th, 1966, a good sample of the species was collected

at Nieuwendijk, Zuid-Holland, Netherlands, and preparations were made March 13th and 18th. Another sample was collected at Bierum, Groningen, Netherlands, by Mr A. K. SCHUITEMA March 19th, and from this sample preparations were made March 22nd. In both series we found dividing cells in abundance and obtained many good photographs of all stages of spermatogenesis. The chromosome number was easily determined n = 12 and 2n = 24. *Assiminea grayana* will probably prove to become another good example for the study of spermatogenesis provided that the animals are collected prior to the beginning of copulation, as spermatogenesis seems to be restricted to that period.

A report on the spermatogenesis of this species is in preparation.

DISCUSSION

In three species of the genus *Hydrobia* we could determine the chromosome number n = 18. One bivalent is always considerably larger than all other elements in the meiotic metaphase plate. A comparison of the chromosome complements of *H. neglecta* and *H. stagnorum* clearly shows the existence of two small elements in *H. stagnorum* whereas in *H. neglecta* only one element is clearly of inferior size. *Hydrobia ulvae* does not show outstanding small chromosomes; the chromosomes in this species regularly diminish in size. As this phenomenon occurs throughout our material we consider the difference as of specific character. We are of the opinion that for this reason we may exclude the possibility of *H. neglecta* being no more than a hybrid between *H. ulvae* and *H. stagnorum* or an ecotype of *H. ulvae*. We agree with MUUS, 1963, that *H. neglecta* may claim full species rank. It will, however, be of interest to start chromosome studies on individuals of known hybrid origin if one could raise them (cf. BURCH, 1964).

The chromosome number recorded by RANZOLI (1950) for *H. aponensis* is very low compared with the numbers now known in other Hydrobiidae. PAULUCCI (1878) placed the species in *Thermhydrobia* Paulucci, 1878 and suggested collecting all thermal Hydrobiids into this genus. This proposal is—correctly—not accepted. RANZOLI advocated leaving the species in *Hydrobia*, the genus in which it was originally described by VON MARTENS. Until the anatomy of this species is known its systematic position will remain uncertain. Cytotaxonomically the possibility of the fusion of chromosomes might be an explanation for its low chromosome number. In the photographs offered by RANZOLI chromosomes are not individually recognizable or measurable.

Table II

Classification		2 n	sex-det. mech.	n	Locality	Reference
Wenz, 1934	Thiele, 1929					
Hydrobiidae	Hydrobiidae					
Hydrobiinae	Hydrobiinae Hydrobieae					
Hydrobia aponensis				8-9	Italy	Ranzoli, 1950
neglecta				18	Denmark	this study
stagnorum				18	Netherlands	this study
ulvae				18	Scotland	Sanderson, 1940
					Netherlands	this study
Littoridininae	Littoridinae					
Potamopyrgus jenkinsi		20-22		—	Germany	Rhein, 1935
		36-44		—	Scotland	Sanderson, 1940
Truncatellidae	Truncatellinae					
Pomatiasinae						
Pomatiopsis cincinnatiensis		32♂	XY	17	U.S.A.	Burch, 1960; Patterson, 1963
Pomatiopsis lapidaria		33♂	XO	17	U.S.A.	Burch, 1960; Patterson, 1963
Tomichiinae						
Oncomelania formosana		34♂	XY	17		Burch, 1960; Patterson, 1963
hupensis				17		Burch, 1960
nosophora		34♀		17		Burch, 1960; Patterson, 1963
quadrasi		34♀		17		Burch, 1960; Patterson, 1963
Bithyniidae	Bithyniinae					
Bithynia leachi		34♂		17	Netherlands	this study
Bithynia tentaculata		34♂		17	Germany	Ankel, 1924; 1933; Keyl, 1955
					France	Tuzet, 1930
					Netherlands	this study
Assimineidae	Assimineidae					
Assiminea grayana		24♂		12	Netherlands	this study

The species considered in this study have been classified by THIELE (1929) into two families: Hydrobiidae (including the subfamilies Hydrobiinae, Truncatellinae and Bithyniinae) and Assimineidae. *Potamopyrgus* has been classified in the tribe Littoridinineae within the subfamily Hydrobiinae. WENZ (1934) recognized four families: Hydrobiidae (with subfamilies Hydrobiinae and Littoridininae), Truncatellidae, Bithyniidae and Assimineidae.

As seen from table II the type number sensu WHITE (1954) is clearly 18 within the subfamily Hydrobiinae. In THIELE's family Hydrobiidae the only other number appears to be 17. In WHITE (1954) we read: "When we find that a particular species has a chromosome number which deviates very widely from those of all its relatives there must be a strong presumption that its chromosome number has been derived from a more normal one unless there is any reason to suspect on morphological grounds that the species occupies a particularly isolated or primitive position in regard to the rest of the group."

Potamopyrgus jenkinsi of SANDERSON ($2n = 36$-44) and *P. jenkinsi* of RHEIN ($2n = 20$-22) might stand rightly in the Hydrobiidae series with 18 as basic chromosome number. In order to understand the chromosome number renewed study of the cytology of *Potamopyrgus jenkinsi* and other *Potamopyrgus* species will be necessary.

Nothing is known of the sex determining mechanism in *Hydrobia* and *Bithynia*. In *Pomatiopsis lapidaria* PATTERSON (1963) found the sex determining mechanism to be of the XO type ($2n = 33$ in males) while in *P. cincinnatiensis* this mechanism appeared to be of the XY type ($2n = 32$ in males). The XY type is also sex determining in all *Oncomelania* "species" ($2n = 34$). The sex determining mechanism need not therefore be constant in a given genus. The difference in this mechanism shown by PATTERSON to exist in the two *Pomatiopsis* species in the subfamily Truncatellinae might be only very slight as this phenomenon could easily be explained by the fusion of the original X-element thus forming a neo-XY system.

The chromosome number of the only cytologically examined member of the family Assimineidae ($n = 12$) cannot be easily derived from the type number in Hydrobiidae sensu THIELE ($n = 17$ or 18). Therefore cytological evidence is more in favour of THIELE's classification separating two families which could also be distinguished cytologically. The classification of WENZ does not express the close relation which exists between his families Truncatellidae, Bithyniidae and Hydrobiidae.

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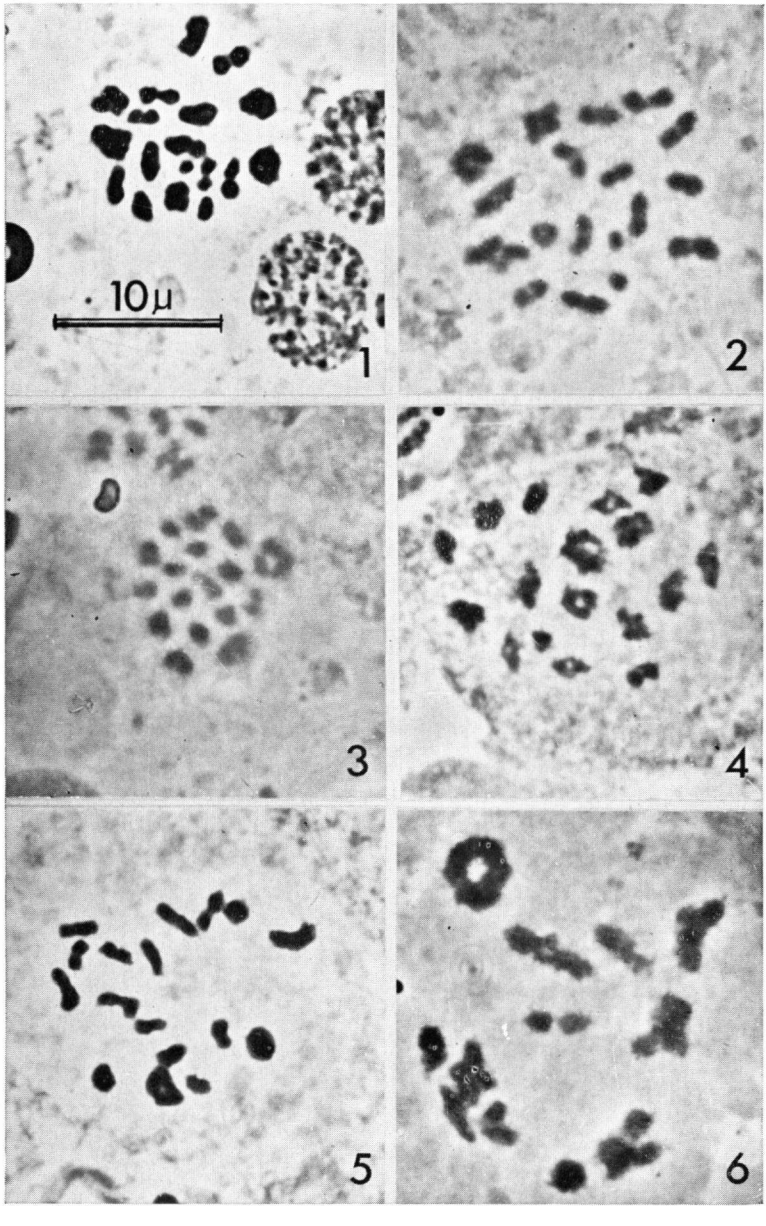
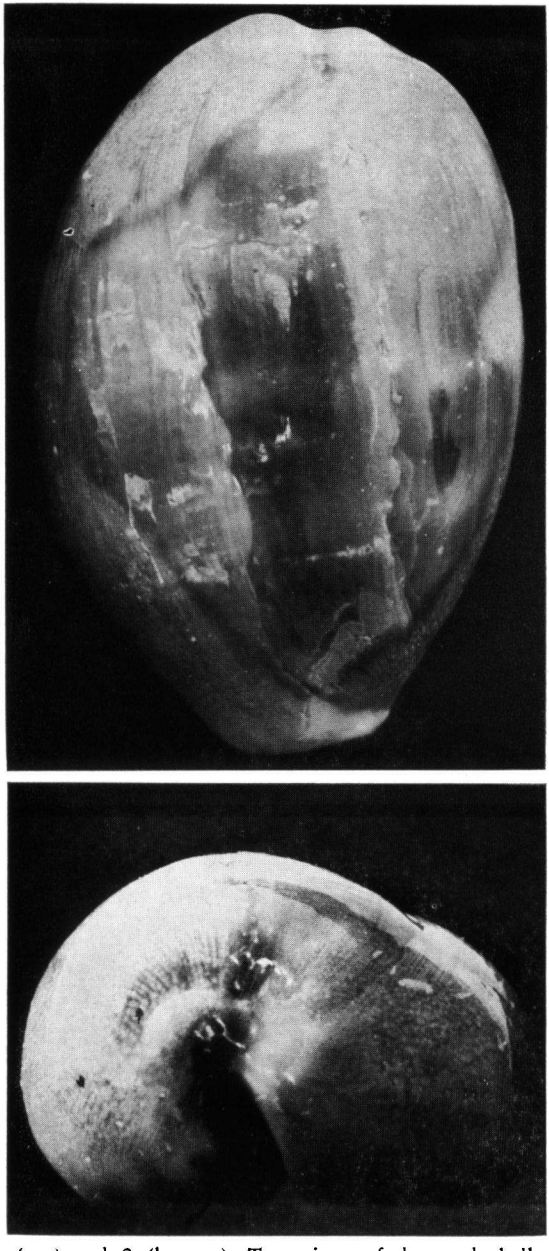
Plate 1. Polar views of primary spermatocyte metaphase (× 2250).

Fig. 1. *Hydrobia neglecta* Muus, 1963, Isefjord, Denmark; Fig. 2. *H. stagnorum* (Gmelin, 1790), Petten, Noord-Holland, Netherlands; Fig. 3. *H. ulvae* (Pennant, 1777), Yerseke, Zeeland, Netherlands; Fig. 4. *Bithynia leachi* (Sheppard, 1823), Kortenhoef, Noord-Holland, Netherlands; Fig. 5. *Bithynia tentaculata* (Linnaeus, 1758), Kortenhoef, Noord-Holland, Netherlands; Fig. 6. *Assiminea grayana* (Fleming, 1828), Nieuwendijk, Zuid-Holland, Netherlands.

PLAAT 1
(PLATE 1)





Figs. 1 (top) and 2 (bottom). Two views of damaged shell of *Cypraea carneola* L. from Pennington, Natal. Actual length 42.4 mm. Photographs Laura M. Kelsall, Natal Museum.