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CYTOLOGICAL STUDIES OF TAIWAN FRESHWATER PULMONATE SNAILS[1,2]

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ABSTRACT

The chromosome numbers are reported for 6 species of Taiwanese basommatophoran snails. In the Lymnaeidae, 16 pairs of chromosomes were observed in *Radix ollula* and 17 pairs in *Radix auricularia swinhoei.* In the planorbidae, 18 pairs of chromosomes were found in *Gyraulus spirillus, Helicorbis umbilicalis, Hippeutis cantori* and *Segmentina hemisphaerula.*

These studies on Taiwan snails add to the information already accumulated on the conservativeness of chromosome numbers in the gastropod subclass Euthyneura. The basic chromosome number 18 is found in 40 (95%) of the 42 species of Planorbidae so far reliably investigated. In the Lymnaeidae, the number 17 is restricted to the genus *Radix*, and is found in all but 2 of its species. This constancy of chromosome numbers within the various taxa indicates that aneuploidy has been a very rare occurrence in euthyneuran snails.

Chromosome studies of freshwater pulmonate snails of the vast area of the Orient are relatively few, and they deal only with a few species from Japan and India (1-6). No studies of the chromosomes of Taiwanese snails have ever been made.

During the spring of 1962 a survey of the freshwater mollusks of Taiwan was made by members of the Museum of Zoology, University of Michigan and the U. S. Naval Medical Research Unit No. 2. The main emphasis of that survey was to study the medically important species, but during the course of the investigation an attempt was made to collect all freshwater mollusks available on the island. Some specimens of all species of freshwater pulmonate snails collected were fixed for cytological studies, and it is on these specimens that the present paper was written.

MATERIALS AND METHODS

Six species of freshwater pulmonate snails were used in this investigation. All specimens were collected from 9 localities in Taiwan during April, 1962. A list of species with locality data is

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given in TABLE I. Duplicate specimens are in the collections of the Museum of Zoology, University of Michigan.

The material examined consisted of ovotestes killed, fixed and preserved in Newcomer's fluid (7). Cells of the ovotestes were stained by the acetic-orcein squash technique (8). All observations were on cells of spermatogenesis and were made with Nikon microscopes using 100× (n.a. 1.25) oil immersion objectives and 10, 20 and 30× oculars. All drawings of chromosomes were made with the aid of a camera lucida and reproduced at a table top magnification of 4250×.

OBSERVATIONS AND DISCUSSION

Family Lymnaeidae

Previous reliable reports on the chromosomes of lymnaeid snails in the Orient are those of Natarajan (4) who studied 1 species in India and Burch *et al.* (5) who studied 4 species in Japan (TABLE II). These authors found *Radix ollula* to have 16 pairs of chromosomes, *R. luteola*, *R. japonica* and *R. onychia* to have 17 pairs of chromosomes, and the introduced European *Fossaria truncatula* to have 18 pairs of chromosomes.

Two species of the Lymnaeidae are known from Taiwan, *Radix ollula* and *Radix auricularia swinhoei*. Our observations on these species are presented below.

Radix ollula (Gould). *Fig. 1.* This is a very common snail in Taiwan and other parts of the Orient. It has often been referred to as "*Lymnaea* (*Fossaria*) *ollula pervia* (Martens)," but it seems to have closer affinities to the genus *Radix* than to *Fossaria* (5). *Limnaeus pervius* Martens 1867 is a synonym of *R. ollula*.

The 6 specimens of *Radix ollula* studied from 2 localities in Taiwan (TABLE I) all had the haploid chromosome number 16 (*Fig. 7*), the same number reported by Burch *et al.* (5) for this species from Japan. The erroneous chromosome numbers reported by Inaba and Tanaka (2) for this species and for *R. japonica* and *Gyraulus hiemantium* apparently resulted from a mixup in labels:

TABLE I

Taiwanese snails studied with collection sites

Species	No. specimens from which chromosome counts were made	Collection sites*
Lymnaeidae		
Radix ollula	6	(1), (3)
Radix auricularia swinhoei	8	(2), (6), (8)
Planorbidae		
Gyraulus spirillus	24	(1), (6)
Helicorcis umbilicalis	1	(5)
Hippeutis cantori	7	(2), (6)
Segmentina hemisphaerula	12	(1), (4), (6), (7), (9)

* The collection sites were as follows:
(1) Kueishan, Taoyuan Prefecture;
(2) Chungli, Taoyuan Prefecture;
(3) Alilao, Taipei Prefecture;
(4) Toucheng, Ilan Prefecture;
(5) Chiaochi, Ilan Prefecture;
(6) Puyen, Changhua Prefecture;
(7) Puyen, Changhua Prefecture;
(8) Kentin, Pingtung Prefecture;
(9) Kangshan, Kaohsiung Prefecture.

Inaba and Tanaka (1953) (2)
Gyraulus hiemantium (=*spirillus*), n=16
Fossaria (=*Radix*) *ollula pervia*, n=17
Radix japonica, n=18

Burch *et al.* (1964) (5)
G. spirillus, n=18
R. ollula (=*pervia*), n=16
R. japonica, n=17

Radix auricularia swinhoei (Adams). *Fig. 2.* The type locality of this race of the widely distributed *R. auricularia* is Takow, Taiwan. Hubendick (9) does not consider this subspecies restricted to Taiwan, but to occur also from the eastern part of mainland China (Shantung in the north to Hainan) to Tonkin, northern Annam, the non-peninsular part of Thailand and eastern Burma in the west. Our Taiwan specimens came from 3 localities (TABLE I). The 8 specimens investigated all had 17 pairs of chromosomes (*Fig. 8*).

The genus *Radix* has an especial cytological interest, since it is the only group in the higher limnic Basommatophora (outside of the aberrant Ancylidae) in which a haploid chromosome number less than 18 has been found. Indeed, only 17 pairs of chromosomes seem to be characteristic of this genus, with the exception of the Oriental *R. ollula* (n=16) and European *R. limosa* (n=18)*. In addition to *R. japonica* and *R. onychia* (Japan), and *R. luteola* (India), the following members of the genus have also been found to have 17 pairs of chromosomes: *R. auricularia* (Europe), *R. ovata* (Europe), *R. pereger* (Europe and Turkey), *R.* sp. (Italy), and *R. hovarum* (Madagascar) (6, 10).

Family Planorbidae

The chromosome numbers of 5 species of Oriental planorbids have been determined previously (4, 5). All 5 of these species, *Camptoceras hirasei*, *Gyraulus spirillus* (=*G. hiemantium*), *G. perstriatulus*, *G. tokyoensis* and *Indoplanorbis exustus*, had 18 pairs of chromosomes. Two specimens of *G. tokyoensis*, in addition, had supernumary chromosomes, forming an extra bivalent (n=19), or a bivalent plus a univalent (n=19+1).

Gyraulus spirillus (Gould). *Fig. 6.* This species is a rather common small planorbid snail in Japan, and the chromosomes of Japanese specimens have been studied by Burch *et al.* (5). We are basing our identification of the Taiwan specimens on the similarity of their shells to the specimens previously identified as *G. spirillus* by Dr. Habe from Japan. Dr. Habe (11) now considers *G. spirillus* to be a synonym of *G. chinensis convexiusculus* (Hutton). However, because of the lack of detailed comparative anatomical studies of Oriental planorbid snails, we hesitate to continue changing the names of our specimens until further morphological studies are made. According to Abbott (12) and Habe (11), *G. convexiusculus* has a wide distribution (and large synonymy) in the Orient, being found in India, Indo-China, the East Indies, the China mainland, Japan, and the Philippines.

The 24 specimens of *Gyraulus spirillus* from Taiwan from which we were able to have accurate counts all had 18 bivalents present during the first meiotic division of spermatogenesis (*Fig. 9*) and 36 chromosomes in spermatogonial cells (*Fig. 13*). These are the same numbers reported in this species from Japan by Burch *et al.* (5). [Note: *Fig. 33* of the previous report on Oriental freshwater Basommatophora (5) is a drawing of Metaphase I chromosomes of *G. tokyoensis* (n=19) and not *G. spirillus* (n=18) as mistakenly labelled.]

Helicorbis umbilicalis (Benson). *Fig. 3.* This small planorbid snail is also common in the Orient. According to Abbott (12) it not only occurs in Taiwan, but also on

* Forcart (1950, *Arch. Molluskenk.*, **79**: 73-75) considers *Helix limosa* Linnaeus 1758 to belong to the prosobranch genus *Bulimus* (=*Bythinia*). It is uncertain just what lymnaeid spceics Le Calvez and Certain (1950, *C. R. Acad. Sci.*, Paris, **231**: 794-795) studied when they reported "*Lymnaea limosa*" to have a haploid number of 18.

TABLE II
Chromosome numbers of Oriental Lymnaeidae and Planorbidae

Species	Haploid no.	Diploid no.	Source	Reference
Lymnaeidae				
Radix				
R. ollula	16	—	Taiwan	This report
	16	32	Japan	Burch *et al.*, 1964
R. auricularia swinhoei	17	—	Taiwan	This report
R. japonica	17	34	Japan	Burch *et al.*, 1964
R. onychia	17	34	Japan	Burch *et al.*, 1964
R. luteola	17	34	India	Natarajan, 1960
Fossaria				
F. truncatula	18	36	Japan	Burch *et al.*, 1964
Planorbidae				
Gyraulus				
G. spirillus	18	36	Taiwan	This report
	18	36	Japan	Burch *et al.*, 1964
G. perstriatulus	18	—	Japan	Burch *et al.*, 1964
G. tokyoensis	18, 18+	36	Japan	Burch *et al.*, 1964
Helicorbis				
H. umbilicalis	18	—	Taiwan	This report
Hippeutis				
H. cantori	18	—	Taiwan	This report
Segmentina				
S. hemisphaerula	18	—	Taiwan	This report
	18	36	Japan	Burch *et al.*, 1964
Camptoceras				
C. hiraesi	—	36	Japan	Burch *et al.*, 1964
Indoplanorbis				
I. exustus	18	36	India	Natarajan, 1960

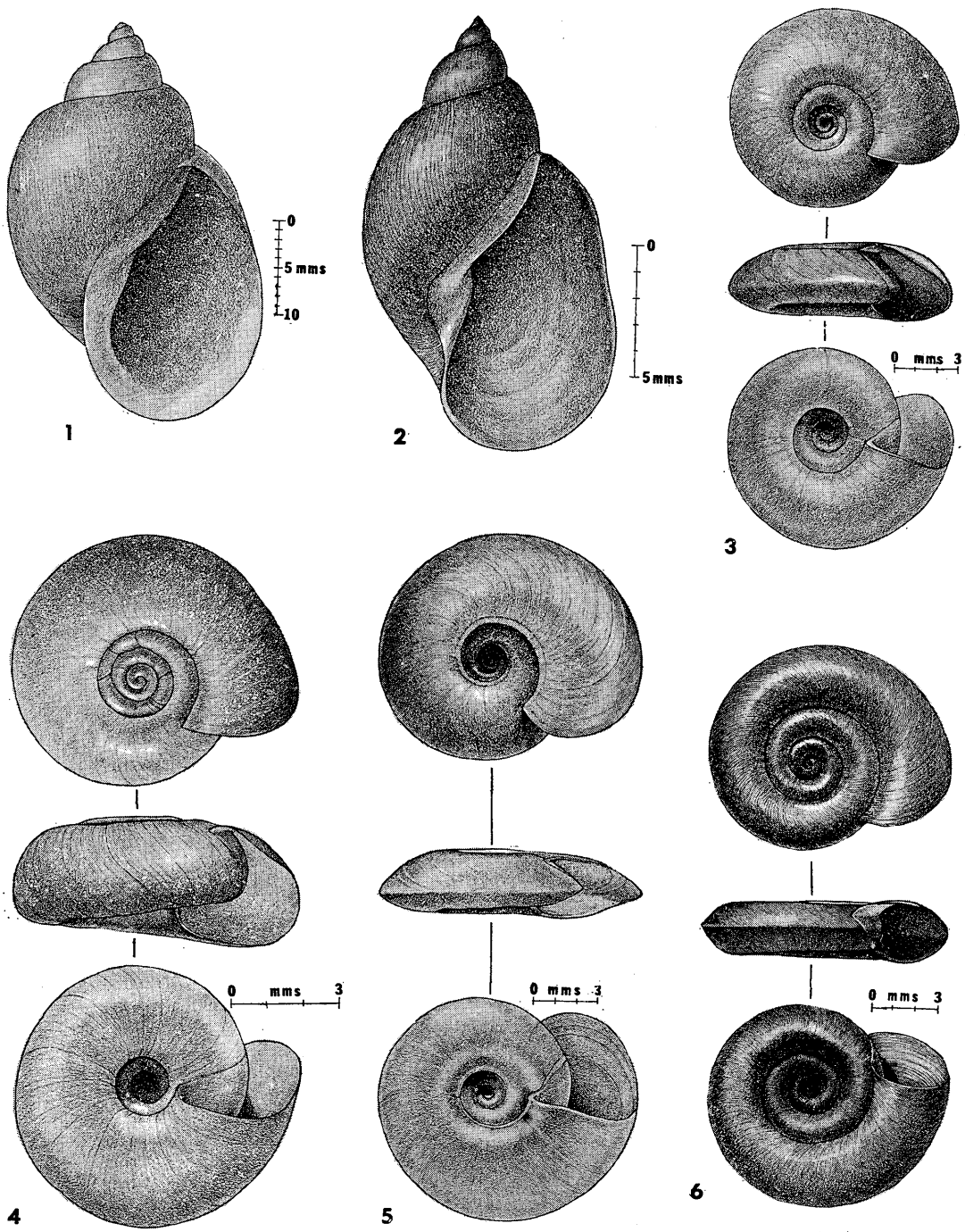
the southern China mainland, in Indo-China and India. *H. umbilicalis* is possibly an intermediate host of the lung fluke, *Paragonimus westermani.*

We were able to study the chromosomes in only 1 specimen, in which 18 bivalents were clearly present at male diakinesis (*Fig. 12*). This is the first time a member of the genus *Helicorbis* has been studied cytologically.

Hippeutis cantori (Benson). *Fig. 5.* This species is common also on the China mainland, and it is the first intermediate host of the intestinal fluke, *Fasciolopsis buski.*

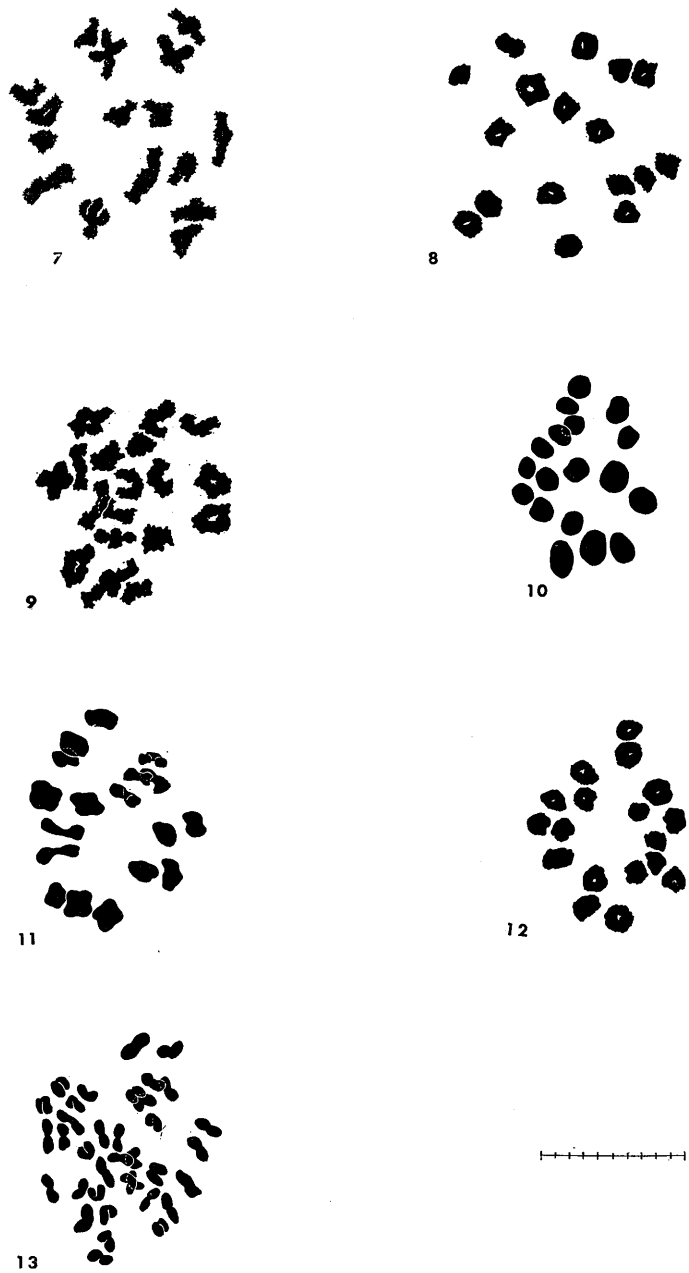
The 7 specimens of *Hippeutis cantori* successfully studied from 2 different localities all had 18 bivalents present during prophase and metaphase (*Fig. 10*) of spermatogenesis. This is the first cytological examination of a member of this genus.

Segmentina hemisphaerula (Benson). *Fig. 4.* This species is widely distributed throughout Taiwan, the China mainland and Japan. Like *Hippeutis cantori*, it also serves as the first intermediate host of *Fasciolopsis buski.* According to Abbott (12), *S. swinhoei* Adams is a synonym of *S. hemisphaerula.* The chromosomes of specimens of *S. hemisphaerula* from Japan were previously studied by Burch *et al.* (5).



Figs. 1-6. Shells of Taiwan freshwater pulmonate snails.

Fig. 1. Radix ollula (Gould). *Fig. 2. Radix auricularia swinhoei* (Adams). *Fig. 3. Helicorbis umbilicalis* (Benson). *Fig. 4. Segmentina hemisphaerula* (Benson). *Fig. 5. Hippeutis cantori* (Benson). *Fig. 6. Gyraulus spirillus* (Gould).



Figs. 7-13. Chromosomes of Taiwan freshwater pulmonate snails.

Fig. 7. Radix ollula, male diakinesis bivalents. *Fig. 8. Radix auricularia swinhoei,* male diakinesis bivalents. *Fig. 9. Gyraulus spirillus,* male diakinesis bivalents. *Fig. 10. Hippeutis cantori,* male Metaphase I bivalents. *Fig. 11. Segmentina hemisphaerula,* male Metaphase I bivalents. *Fig. 12. Helicorbis umbilicalis,* male diakinesis bivalents. *Fig. 13. Gyraulus spirillus,* spermatogonial metaphase chromosomes. Length of measurement line, 10 micra.

Twelve specimens of *Segmentina hemisphaerula* from 5 different localities in Taiwan had satisfactory cells for chromosome counts. All of these specimens had 18 bivalents in meiotic cells of the first division of spermatogenesis (*Fig. 11*). This is the same haploid chromosome number reported for this species from Japan (5).

It is interesting to note the conservatism in regard to chromosome numbers in the Planorbidae. Forty-two species and subspecies have now been reliably investigated; 34 (81%) of these have 18 pairs of chromosomes, and another 6 species or subspecies have a haploid chromosome number that is a multiple of 18 (6, 10, 13 and this report). Only 2 species (5%) have a number other than 18 (*i.e.*, n=19) or a multiple of 18 (*i.e.*, n=36; n=72). This constancy in the chromosome number of the Planorbidae indicates that aneuploidy has been a very rare occurrence, and can hardly be of much significance in speciation and the evolution of higher taxa.

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