Chromosomes of Some Opisthobranchiate Mollusks from Eniwetok Atoll, Western Pacific[1]

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ABSTRACT: Chromosome numbers are reported for nine species of opisthobranchiate mollusks from Eniwetok Atoll, Marshall Islands, western Pacific. In the Nudibranchia, both *Dendrodoris nigra* (Dendrodorididae) and *Herviella mietta* (Favorinidae) were found to have 13 bivalents during male meiosis. In the Anaspidea, *Dolabrifera dolabrifera* and *Stylocheilus longicauda* (Aplysiidae) both had 17 bivalents during male meiosis. In the Cephalaspidea, *Haminoea linda* and *H. musetta* (Atyidae) each had 17 pairs of chromosomes during male meiosis and *Lathophthalmus smaragdinus* and *Smaragdinella calyculata* (Smaragdinellidae) had 18 pairs. In the Soleolifera, *Onchidella evelinae* had 18 bivalents during male meiosis.

The extreme conservativeness of chromosome numbers in opisthobranchiate mollusks is demonstrated by that fact that all 18 nudibranchs from 10 families studied so far have the single haploid chromosome number 13, and that 18 of the 21 species of the orders Entomotaeniata, Anaspidea, Cephalaspidea, and Sacoglossa have 17 pairs of chromosomes. The haploid number 18 is here reported for the first time for nonsoleoliferan opisthobranchiate mollusks. The more advanced, mostly fresh-water, order Basommatophora, in which the haploid number 18 is the basic number, may well have been derived from a taxon within or related to this cephalaspid superfamily (Philinacea).

IN RECENT YEARS, detailed investigations have been made on the chromosomes of many basommatophoran and stylommatophoran snails (Burch, 1965), but relatively few species of the opisthobranchiate[4] orders have been studied, mainly because of the difficulties they present in collection and identification. Relying on the studies of various authors during the early part of the present century, Makino (1951) listed the chromosome numbers of 16 opisthobranchiate species, but recent investigations by Inaba and our present studies indicate that the earlier records are not dependable and so are obsolete. Previous reliable reports on the chromosomes of opisthobranchiate gastropods are those of Inaba and Hirota (1954, 1958), Inaba (1959*a*, 1959*b*, 1961), Natarajan (1959, 1960), Mancino and Sordi (1964*a* and *b*), and Burch (1965). These authors give the chromosome numbers of 36 species belonging to 21 families and 7 orders (Tables 1 and 2). This is a very small number when compared with the great multitude of species currently recognized in the opisthobranchiate orders. The present paper presents the chromosome numbers of 9 opisthobranchiate

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[4] Boettger (1955) considers the orders Opisthobranchiata and Pulmonata (together equivalent to the subclass Euthyneura) to be unnatural ones, and instead divides the Euthyneura into seven orders. While we do not disagree with this, it is still convenient to speak of his five lower (mainly marine) euthyneuran orders (Nudibranchia, Soleolifera, Cephalaspidea, Sacoglossa, and Anaspidea) as "opisthobranchs" as distinguished from the more advanced Basommatophora (mainly freshwater) and Stylommatophora (land inhabitants).

TABLE 1

CHROMOSOME NUMBERS PREVIOUSLY REPORTED IN THE OPISTHOBRANCHIATE ORDERS NOTASPIDEA AND NUDIBRANCHIA

SPECIES	CHROMOSOME NUMBER 2n	n	LOCALITY	REFERENCE
NOTASPIDEA				
Pleurobranchidae				
Pleurobranchaea novaezealandiae	24	12	Japan	Inaba, 1959*a*
NUDIBRANCHIA				
Dorididae				
Glossodoris festiva		13	Japan	Inaba and Hirota, 1958
Glossodoris pallescens		13	Japan	Inaba and Hirota, 1954; 1958
Rostanga arbutus	26	13	Japan	Inaba, 1959*a*
Discodoris pardalis		13	Japan	Inaba, 1959*a*
Doris verrucosa	26	13	Italy	Mancino and Sordi, 1964*a*
Dendrodorididae				
Dendrodoris miniata	26	13	Japan	Inaba and Hirota, 1958
Dendrodoris nigra	26	13	Japan	Inaba and Hirota, 1958; Inaba, 1959*b*
Triophidae				
Kaloplocamus ramosus		13	Japan	Inaba and Hirota, 1958
Plocamopherus tilesii	26	13	Japan	Inaba and Hirota, 1958
Goniodorididae				
Okenia barnardi		13	Japan	Inaba and Hirota, 1958
Fimbriidae				
Melibe papillosa	26	13	Japan	Inaba, 1959*a*
Dotonidae				
Doto bella		13	Japan	Inaba, 1961
Arminidae				
Dermatobranchus striatus	26	13	Japan	Inaba and Hirota, 1958
Cuthonidae				
Catriona pupillae		13	Japan	Inaba, 1961
Facelinidae				
Facelina ceylonica		13	Japan	Inaba and Hirota, 1958
Facelina japonica		13	Japan	Inaba and Hirota, 1958

species belonging to 6 families and 4 orders (Table 3), which were collected in shallow waters around three islands of Eniwetok Atoll in the western Pacific by the senior author and Dr. William H. Heard during early 1960.

MATERIAL AND METHODS

The species studied in this investigation and the localities where they were collected are listed below. Duplicate specimens have been deposited in the collections of the Museum of Zoology at the University of Michigan, the University of São Paulo, and the University of Hawaii.

NUDIBRANCHIA

1. *Dendrodoris nigra* (Stimpson) (Fig. 1). North end of Japtan Island, under loose pieces of dead coral. April 1, 1960.

2. *Herviella mietta* Marcus and Burch (Fig. 2). North end of Eniwetok Island on the lagoon side, in about 10 cm of water at low tide, under submerged pieces of dead coral. April 2, 1960.

ANASPIDEA

3. *Dolabrifera dolabrifera* (Rang) (Fig. 3). Under loose pieces of coral on seaward tide flat at the north end of Parry Island. March 25, 1960.

TABLE 2

CHROMOSOME NUMBERS PREVIOUSLY REPORTED IN THE OPISTHOBRANCHIATE ORDERS ENTOMOTAENIATA, CEPHALASPIDEA, ANASPIDEA, SACOGLOSSA, AND SOLEOLIFERA

SPECIES	CHROMOSOME NUMBER 2n	CHROMOSOME NUMBER n	LOCALITY	REFERENCE
ENTOMOTAENIATA				
Pyramidellidae				
Tiberia fasciata		17	Japan	Inaba, pers. comm.
ANASPIDEA				
Aplysiidae				
Petalifera punctulata	34	17	Japan	Inaba, 1959*a*
Notarchus leachii freeri		17	Japan	Inaba, 1959*a*
CEPHALASPIDEA				
Acteonidae				
Cylichnatys angusta		17	Japan	Inaba, pers. comm.
Philinidae				
Philine japonica		17	Japan	Inaba, 1959*a*
Aglajidae				
Aglaja gigliolii	34	17	Japan	Inaba, 1959*a*
SACOGLOSSA				
Elysiidae				
Elysia amakusana		17	Japan	Inaba, 1959*a*
Elysia viridis		17	Italy	Mancino and Sordi, 1964*b*
Stiligeridae				
Alderia nigra		17	Japan	Inaba, 1961
Hermaeopsis variopicta		17	Italy	Mancino and Sordi, 1964*b*
Placida dendritica	34	17	Italy	Mancino and Sordi, 1964*b*
Placida viridis	34	17	Italy	Mancino and Sordi, 1964*b*
Stiliger vesiculosus	34	17	Italy	Mancino and Sordi, 1964*b*
Juliidae				
Berthelinia limax		17	Japan	Inaba, 1961
Polybranchiidae				
Bosellia mimetica	14	7	Italy	Mancino and Sordi, 1964*b*
SOLEOLIFERA				
Veronicellidae				
Veronicella floridana		16	U.S.A.	Burch, 1965
Laevicaulis alte		17	India	Natarajan, 1960
Onchidiidae				
Oncidiella kurodai		17	Japan	Inaba, 1961
Onchidium verraculatum	36	18	India	Natarajan, 1959

4. *Stylocheilus longicauda* (Quoy and Gaimard) (Fig. 4). In tide flats of Eniwetok Island. March 4, 1960.

CEPHALASPIDEA

5. *Haminoea linda* Marcus and Burch (Fig. 5). Parry Island, in sand, in about 2 m of water, in lagoon, about 17 m from shore. March 31, 1960.

6. *Haminoea musetta* Marcus and Burch (Fig. 6). Middle part of Parry Island on seaward tide flats. April 2, 1960.

7. *Lathophthalmus smaragdinus* (Rüppel and Leuckart) (Fig. 7). Collected at the south end of Parry Island, under loose pieces of coral on seaward tide flat. March 15, 1960.

8. *Smaragdinella calyculata* (Broderip and

TABLE 3

CHROMOSOME NUMBERS OF OPISTHOBRANCHS OBSERVED IN THIS STUDY

SPECIES	CHROMOSOME NUMBER (n)	NUMBER OF SPECIMENS GIVING RESULTS
NUDIBRANCHIA		
Dendrodorididae		
Dendrodoris nigra	13	5
Favorinidae		
Herviella mietta	13 (2n = 26)	1
ANASPIDEA		
Aplysiidae		
Dolabrifera dolabrifera	17	9
Stylocheilus longicauda	17	2
CEPHALASPIDEA		
Atyidae		
Haminoea linda	17	1
Haminoea musetta	17	2
Smaragdinellidae		
Lathophthalmus smaragdinus	18	2
Smaragdinella calyculata	18	2
SOLEOLIFERA		
Onchidiidae		
Onchidella evelinae	18	2

Sowerby) (Fig. 8). In lagoon at north end of Eniwetok Island.

SOLEOLIFERA

9. *Onchidella evelinae* Marcus and Burch (Fig. 9). In cracks in coral slabs above water line (at low tide) on the lagoon side at the north end of Eniwetok Island. April 5, 1960.

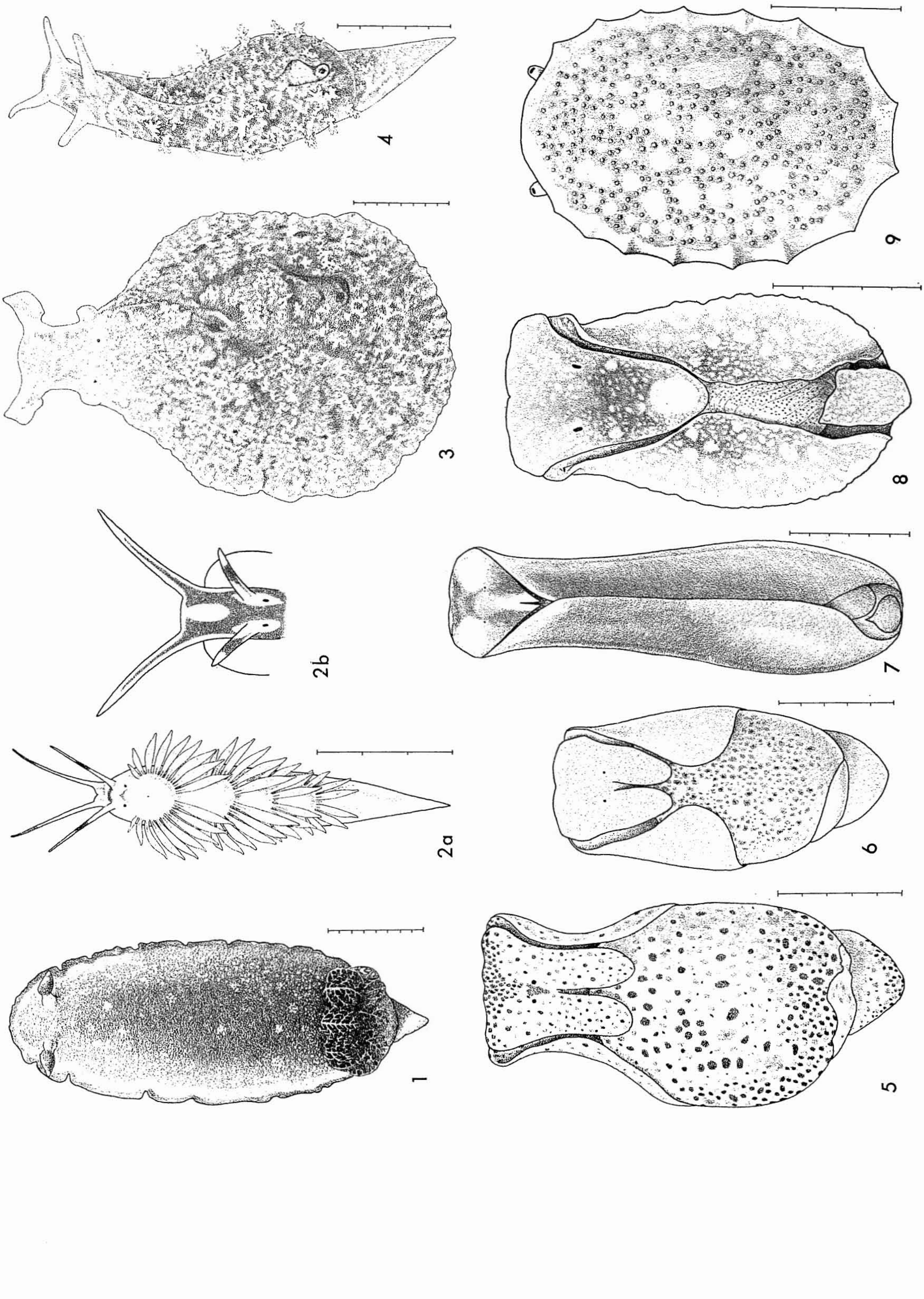
The materials examined consisted of ovotestes fixed in either Newcomer's (1953) or Carnoy's (1887) fluids, or the fixative of Sanfelice (1918). The material fixed in Newcomer's or Carnoy's fluids was stained by the acetic-orcein squash technique (La Cour, 1941), and reproductive tissues fixed in Sanfelice's fluid were sectioned at either 8 or 10 micra and stained with Newton's (1926) crystal violet. All observations were made on meiotic cells of spermatogenesis (except in *Herviella mietta*, where spermatogonial cells were also studied) with a Nikon (Nippon Kogaku) microscope equipped with a 100× (n.a. 1.25) oil immersion objective and 10×, 20×, and 30× oculars. Drawings were made with the aid of a camera lucida and reproduced at a table-top magnification of 4260×.

OBSERVATIONS

1. *Dendrodoris nigra* (Fig. 10). The five individuals of this species on which we were able to obtain satisfactory observations all had 13 bivalents during prophase of the first meiotic division.

2. *Herviella mietta* (Fig. 11). Only one specimen of this species gave satisfactory results. It had 13 bivalents during diakinesis and 26 chromosomes during metaphase in spermatogonial cells.

3. *Dolabrifera dolabrifera* (Figs. 12 and 13). Nine specimens had meiotic cells that were satisfactory for chromosome number determinations. All had 17 bivalents during Prophase I and Metaphase I. The chromosomes of a cell during diakinesis from an acetic-orcein squash preparation are shown in Figure 12. Metaphase I bivalents from a cell of paraffin sectioned material are shown in Figure 13.


1
2a
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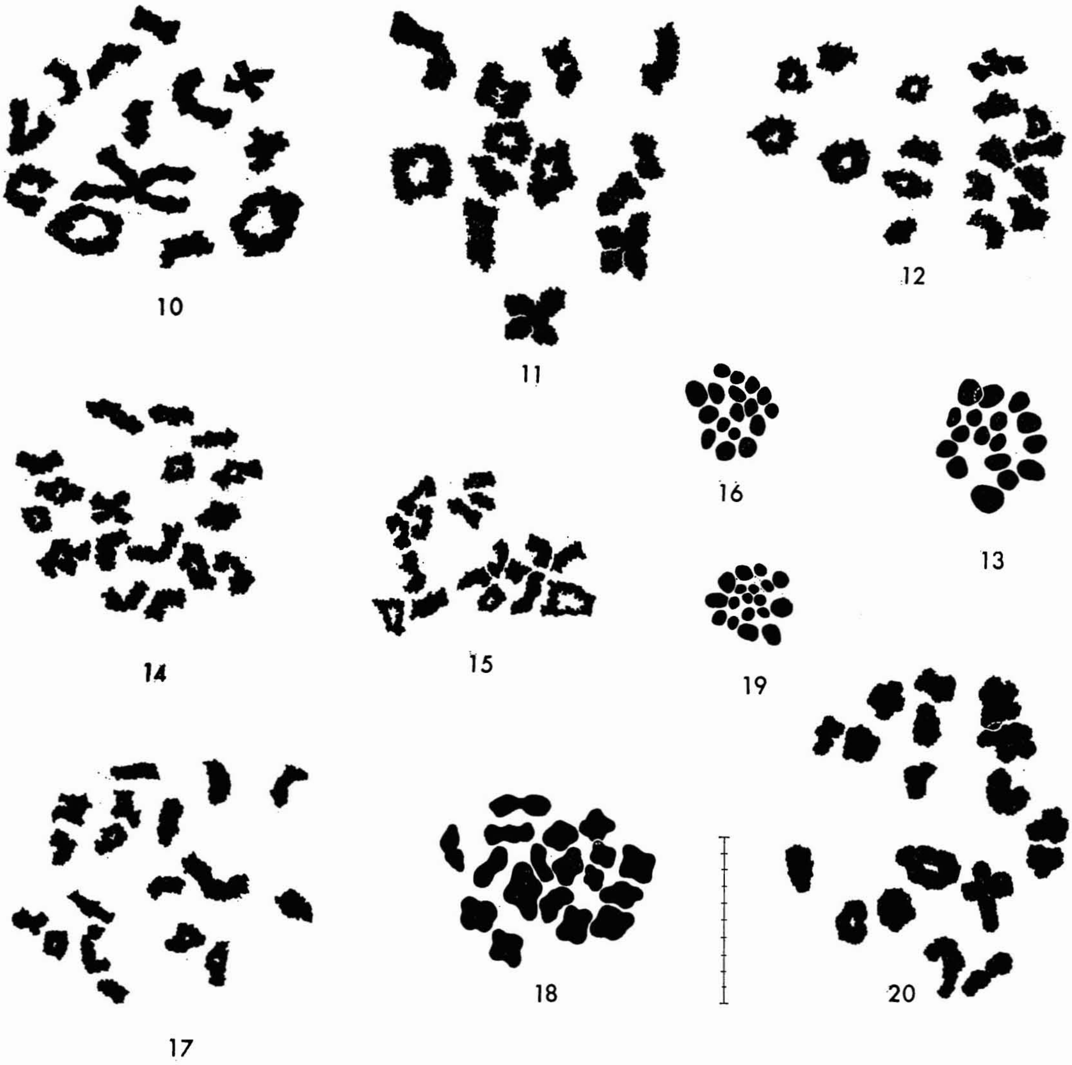

FIGS. 10–20. Chromosomes of Eniwetok opisthobranchs. *10, Dendrodoris nigra. 11, Herviella mietta. 12* and *13, Dolabrifera dolabrifera. 14, Stylocheilus longicauda. 15, Haminoea musetta. 16, H. linda. 17, Lathophthalmus smaragdinus. 18, Smaragdinella calyculata. 19* and *20, Onchidella evelinae.*

Figs. *10–12, 14, 15, 17,* and *20* are of male diakinesis bivalents; *13, 16, 18,* and *19* are of male Metaphase I bivalents. Figs. *10–12, 14, 15, 17, 18,* and 20 are from acetic-orcein squash preparations; *13, 16,* and *19* are from sectioned material stained with crystal violet. Measurement line divided into micra.

FIGS. 1–9. Eniwetok opisthobranchs used in this study. *1, Dendrodoris nigra. 2, Herviella mietta. 3, Dolabrifera dolabrifera. 4, Stylocheilus longicauda. 5, Haminoea linda. 6, H. musetta. 7, Lathophthalmus smaragdinus. 8, Smaragdinella calyculata. 9, Onchidella evelinae.* Measurement lines are divided into millimeters.

4. *Stylocheilus longicauda* (Fig. 14). Satisfactory results were obtained from two specimens. Both had 17 bivalents during diakinesis.

5. *Haminoea linda* (Fig. 16). We were able to obtain cells that were satisfactory for study from only one specimen. These cells had 17 bivalents during Metaphase I.

6. *Haminoea musetta* (Fig. 15). The two specimens studied both had 17 bivalents in cells at the diakinesis stage. The diakinesis bivalents of one such cell are shown in Figure 15.

7. *Lathophthalmus smaragdinus* (Fig. 17). Two specimens had meiotic cells that were satisfactory for chromosome number determinations. All dividing cells from which accurate counts could be made had 18 bivalents.

8. *Smaragdinella calyculata* (Fig. 18). Satisfactory results were obtained from two specimens. Both had 18 bivalents during diakinesis and Metaphase I.

9. *Onchidella evelinae* (Figs. 19 and 20). The two individuals on which we were able to obtain satisfactory observations both had 18 bivalents during diakinesis and Metaphase I.

DISCUSSION

The chromosome numbers of the eight genera of opisthobranchiate mollusks presented here add to the information previously obtained by reliable authors. Of the species studied five belong to three families not studied in past reports, i.e., the Favorinidae (Nudibranchia), and the Atyidae and Smaragdinellidae (Cephalaspidea).

Dendrodoris nigra (Nudibranchia, Dendrodorididae) was studied previously by Inaba and Hirota (1958). We found the same number of chromosomes (n=13) for this species from Eniwetok as they reported it to have from Japan. *Herviella mietta* (Nudibranchia, Favorinidae) from Eniwetok also had a haploid number of 13, which adds to the growing information regarding the extreme conservativeness of chromosome numbers of most opisthobranchs. All 16 species of nudibranchs (belonging to nine different families) studied so far have this same haploid number, n=13.

Among the Cephalaspidea three species have been studied previously (Inaba, 1959*a* and personal communication), each belonging to a different family. Each of these three species had a haploid number of 17. In the present investigation two species from each of two additional families were studied. *Haminoea linda* and *H. musetta* (Bullacea, Atyidae) had a haploid chromosome number of 17, but *Smaragdinella calyculata* and *Lathophthalmus smaragdinus* (Philinacea, Smaragdinellidae) each had the haploid number 18. It will be interesting to see if species of the other families of the Philinacea (the Philinidae, Scaphandridae, Aglajidae, Gastropteridae, and Runcinidae) also have 18 pairs of chromosomes. If so, this would separate this superfamily from all other cephalaspideids and, additionally, from all other cytologically known entomotaenids and anaspideids and from most of the sacoglossans. The haploid number 18 in this group may have another significance in that it seems to strengthen Pelseneer's (1893) and Boettger's (1955) views regarding the origin of the Basommatophora from the Cephalaspidea, since the haploid number 18 is basic for the Basommatophora.

Dolabrifera dolabrifera and *Stylocheilus longicauda* (Anaspidea, Aplysiidae) both had the haploid number 17, as did the two species studied from this family by Inaba (1959*a*).

Onchidella evelinae (Soleolifera, Onchidiidae) had a haploid number of 18, which is one bivalent more than that reported by Inaba (1961*a*) for *O. kurodai* of the same genus from Japan, but n=18 is the same number reported by Natarajan (1959) for *Onchidium verraculatum* from India. Much more cytological information is desirable for the various species belonging to this aberrant order, which is sometimes placed with the "pulmonates" (e.g., Baker, 1955).

The conservativeness of chromosome number in the opisthobranchs indicates that these mollusks are extremely resistant to changes in chromosome numbers, regardless of major evolved morphological diversities within the various groups, and that certain major divisions (i.e., the Nudibranchia and the orders with n=17 and higher chromosome numbers) have probably been separated for an extremely long geological time. In this regard, *Bosellia mimetica* is either an extremely aberrant species, or its cytological evolution has been much more rapid than has been the evolution of its gross morphology in respect to all other opisthobranchs so far studied.

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