JØRGEN LÜTZEN

STUDIES ON PARASITIC GASTROPODS FROM ECHINODERMS

II.

ON *STILIFER* BRODERIP, WITH SPECIAL REFERENCE TO THE STRUCTURE OF THE SEXUAL APPARATUS AND THE REPRODUCTION

Det Kongelige Danske Videnskabernes Selskab
Biologiske Skrifter **19**, 6

Kommissionær: Munksgaard

København 1972

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Synopsis

New finds of species of *Stilifer*, a prosobranch gastropod endoparasitic in Indo-pacific starfishes are presented. A study of a collection of *S. linckiae* from Hawaii has revealed that this species is a consecutive hermaphrodite that functions as a male when young and as a female when older. The reproductive system of the male and the female as well as a few transitional stages are described. It is postulated that egg capsules are formed and liberated into the water. The gastropods are usually arranged in groups consisting of two or three individuals of opposite sex; it is concluded that the maintenance of such groups is dependent on the presence of the female preventing the ordinary sex transformation in the males.

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Introduction

As defined by Ivanov (1952), the genus *Stilifer* Broderip includes prosobranch gastropods that live as endoparasites in the skin of starfishes. The body is only slightly modified and bears a small, spiral shell. A cup-shaped (false) mantle or pseudopallium originates at the base of the proboscis and envelops the entire body, thus separating the gastropod from the host tissue. The elongate proboscis is a powerful structure that is introduced deeply into the body or rays of the starfish.

There has been much confusion as to the number of *Stilifer* species. Ivanov (1952) recognizes not more than three, and states that until that time, a total of 23 species have been referred to the genus; I consider even this estimate too moderate. The vague delimitation of the genus is chiefly a result of the inadequate knowledge of the species originally referred to *Stilifer*. This is most regrettable since most authors agree that the genus holds a key position in the phylogeny of the parasitic gastropods grouped in the family "Stiliferidae" (Ivanov, l.c.; Gruzov, 1965), and at the same time are near the base of an evolutionary line leading to the family Entoconchidae (Schiemenz, 1889). The necessity of a modern examination of a species of *Stilifer* has partially been alleviated by Ivanov's study on *S. ovoideus*; but since it is based on a single, immature specimen, it has left many unsolved questions and needs to be amplified.

The present account concentrates on the sexuality and reproduction of one species of *Stilifer, S. linckiae*; these subjects have been entirely ignored by earlier students. I have further taken the opportunity to present a number of new finds of various species, since a study of these have partially substantiated the findings on the reproduction.

Examination of valuable material of *Stilifer* in the British Museum (Nat. Hist.), London, was made possible by a grant from the University of Copenhagen. I am greatly indebted to Dr. R. U. Gooding (U. S. Natn. Museum, Washington) and to Mr. Bill Milisen (Pacific Biomedical Research Center, Honolulu), who assisted him, for collecting material of *S. linckiae*.

Synopsis of the Species of *Stilifer*

To support the separation of the species mentioned in the following, I have cited Habe (1951, 1952) who recognizes six species, based mainly on shell characteristics. New illustrations were deemed unnecessary for the present study, but due references are given for each species.

The known distribution of *Stilifer* is restricted to tropical and subtropical coastal regions of the Indian and Pacific Oceans, extending from Moçambique, E. Africa, to the

Galapagos Islands. The present account extends the number of genera of parasitized starfishes to six (*Ophidiaster, Linckia, Tamaria, Certonardoa,* all of the family Linckiidae; *Choriaster* of the family Oreasteridae; and *Heliaster* of the family Heliasteridae).

The institutions which have made material of *Stilifer* available to me, are abbreviated as follows: Australian Museum, Sydney: AMS; British Museum (Nat. Hist.), London: BMNH; United States National Museum, Washington, D. C.: USNM; and Zoological Museum, Copenhagen: ZMC.

1. *Stilifer astericola* Broderip 1832

The original material of this species is in the Mollusk Division and the Echinoderm Division of BMNH. The description of the soft parts given by Broderip (1832: 60, republished and illustrated in Sowerby's Genera of Recent and Fossil Shells, no. 38) is insufficient, and is probably also erroneous as to certain points (for example the structure of the so-called mantle). Since the available material is dried, substantiation of *S. astericola's* relation to the following species relies on similarity in shell structure and mode of life both of which may be dubious characters. Should new material of *S. astericola* become available, it is possible that it will prove to be less related to other species of *Stilifer* than hitherto assumed. This might have nomeclatural implications, *S. astericola* being the first species ever referred to *Stilifer.*

The material consists of (1) an infected specimen of the starfish *Heliaster* sp. from South America (?) with one gastropod, labelled 14.2.1949 and consequently not part of the original material; (2) one infected *Heliaster* (of 6) from the Galapagos Islands, with one gastropod; (3) one gastropod, labelled *S. broderipii,* without further information; (4) three gastropods of different sizes from the Galapagos Islands; and (5) one specimen of *H. cumingi* (Gray) with five gastropods in addition to five gastropods which have been removed (from the same host specimen?). As was noted by Broderip, parasitism occurs only on the oral side, preferably on the rays. Quoting Cuming, who collected the original material and made observations in the field, the gastropods are "almost hidden from sight, so deeply does the animal penetrate into the substance of the Star-fish, in which it makes a comfortable cyst for itself, wherein it most probably turns by the aid of its rudimentary foot" (Broderip, 1832).

2. *Stilifer ovoideus* H. & A. Adams 1853

The synonymy given by Habe (1952: 80) is adopted here. The species was originally incorrectly identified as *S. astericola* (by Adams & Reeve, 1850: 47), but was later given a status of its own (H. & A. Adams, 1853: 239). Habe (l.c.) identified a gastropod that had earlier been found in Japanese waters in galls in the rays of the starfish *Certonardoa semiregularis* (Müller & Troschel) as *S. ovoideus* while Hirase (1927: (8); 1932: 73), Kuroda (1934: 208), and Ivanov (l.c.: 118) considered it to be *S. celebensis* Kükenthal. The latter author studied the anatomy of an immature female; Hirase examined the histological structure of the pseudopallium (1927), and the penetration of the parasite into the body of its host (1932).

Along the Japanese coast the species has been taken at Tokioka, Amakusa, Kyushu; Kushimoto, Wakayama Pref., Misaki and Hayama, Kanagawa Pref., Honshu.

The original material in BMNH (taken by the 'Samarang' at Borneo) has been examined by the author. It includes a dried shell, the one figured by Adams & Reeve (l.c., pl. XVII, fig. 5) according to the accompanying label, and two dried starfish, each with one ray parasitized; these were kindly identified by Dr. Ailsa Clark as *Ophidiaster granifer* Lütken. The gastropods seem indistinguishable from those described and illustrated by Habe from *C. semiregularis*.

A dried specimen of the starfish *Tamaria dubiosa* (Koehler) from Cocos Keeling Atoll, E. Indian Ocean (USNM, Moll. Coll. 589237) is parasitized in the basis of a ray by a small gastropod, presumably belonging to *S. ovoideus*.

3. *Stilifer linckiae* Sarasin & Sarasin 1887

The general organization of the parasite was briefly described by Sarasin & Sarasin (1886: 19; 1887: 21, pls. IV, V) who also illustrated the shell. Davis (1967: 343) showed that the tendency toward spontaneous autotomy in the host, *Linckia multifora* (Lam.), is considerably reduced in parasitized rays.

Originally described from Trincomali, Ceylon, the species has since been found at Inhaca Island, Moçambique (Macnae & Kalk, 1962: 119) and in Kaneohe Bay, Oahu, Hawaii (Davis, l.c.). A few specimens of *L. multifora* from Inhaca Island parasitized by *S. linckiae* are kept in the ZMC (one host with at least three snails) and BMNH (four hosts, of which one had two, or perhaps three infected rays). One dried *L. multifora* from Muscat, Gulf of Oman, Iranian Gulf, had a single gall (BMNH). Another dried *L. multifora* with a gall on one ray is from Lifu, the Loyalty Islands, S. W. Pacific (BMNH); two infected pieces of ray of the same species, one with five gastropods are also from these islands (BMNH). An alcohol preserved specimen of *L. multifora* with a gall derives from Solomon Atoll, Chagos Archipel, Indian Ocean (BMNH).

4. *Stilifer celebensis* Kükenthal 1897

The species is known from only one specimen, the shell of which was lost at fixation, and, consequently, will be difficult to recognize. Hirase (1918, 1927, 1932), Kuroda (1934) and Ivanov (l.c.) identified their material as *S. celebensis*, but it is more probable, as is stated by Habe (1952), that it belongs to *S. ovoideus*.

The species was found in *Choriaster*, a starfish of the family Oreasteridae, from Celebes.

5. *Stilifer utinomii* Habe 1951

A shell of this species is illustrated by Habe (1952: 80, pl. VI, fig. 22). The species is said to live in the rays of *Linckia guildingii* Gray at Shirahama, Wakayama Pref., Honshu, Japan.

In the AMS there are four dried specimens of *L. guildingii* parasitized by respectively one, one, one, and two specimens of *S. utinomii* and taken at Masthead Island,

Queensland (C 69680) and Northwest Isle, Capricorn Group, Great Barrier Reef (C 69669 & C 69678). A dried specimen of *L. laevigata* in AMS, also from Masthead Island (C 69676) is parasitized by one snail that obviously belongs to the same species.

Stilifer kochianus Sowerby (1901: 209, pl. XXII, fig. 6), of which I have seen three specimens in BMNH (two of them types, pl. I, fig. 1), seems indistinguishable from the Australian specimens of *S. utinomii*. *S. kochianus* was collected at Cebú, the Philippines. It was not claimed to be a parasitic species.

6. *Stilifer ophidiastericola* Habe 1951

The shell is illustrated by Habe (1951: 94; 1952: 80, pl. VI, fig. 19). The species parasitizes the rays of the starfishes *Ophidiaster cribrarium* Lütken and *O. lorioli* Fisher, and is known from Tomioka, Amakusa, Kyushu, and Kushimoto, Wakayama Pref., Honshu, Japan.

A specimen of *O. lorioli* from Queensland, Australia, is parasitized by this species (BMNH). The disk is swollen where two of the rays meet and has an aperture which is slightly ventrally directed. Through it, the top whorls of three snails could be seen. Two dried specimens of *O. confertus* Clark from Masthead Island, Australia (AMS, C 69681) are parasitized by what I consider to be specimens of *S. ophidiastericola*. One of the starfish has a swelling at the base of a ray containing two gastropods. The other is swollen in the mid portion of two rays where one and two gastropods respectively are found.

Stilifer spp.

Two dried specimens of *Ophidiaster* sp. from Northwest Isle, Capricorn Group, Great Barrier Reef (AMS, C 69678) are parasitized respectively on one ray and on two rays.

An alcohol preserved specimen of *Ophidiaster* (?) sp. from N. of Doe Roa, Banda Sea (Dan. Exp. to the Kei Islands, st. 39), 60 m, has swollen galls in the distal part of two rays (ZMC).

The Reproductive System of *Stilifer* with Special Reference to *S. linckiae*

Previous Investigations

Since its discovery, the sexuality of *Stilifer* has remained enigmatic. The earliest investigators (Broderip, 1832; Gray, 1939; and Adams & Reeve, 1850) failed to identify the sex in their material (*S. astericola* and *ovoideus*). Neither did Sarasin & Sarasin (1887) nor Kükenthal (1897) comment on the reproductive organs of the species that they examined (*S. linckiae* and *celebensis*). Hirase (1932) studied the genital organs of twenty specimens of *S. ovoideus*, but noted that they were all females and concluded that the males were free-living. Ivanov (l.c.) had at his disposal only a single specimen

of the same species, and this proved to be an immature female. Males are, therefore, unknown, and the genital organs of the female remains to be described. Partially as a consequence of this, the method of reproduction is also unknown. Baer (1952: 24) suggested that the species of *Stilifer* will possibly prove to be protandrous hermaphrodites, since "the only species that has been thoroughly investigated, *S. sibogae*, is an hermaphrodite with a well developed male copulatory organ." This suggestion is, however, based on the incorrect premise that "*S. sibogae*" is a true *Stilifer*, which is not the case[1].

Material and Treatment

Davis (1967) has provided detailed information about the occurrence of *S. linckiae* in Kaneohe Bay, Oahu, Hawaii. At my request, Dr. R. U. Gooding, who stayed at Oahu for some time, collected a number of parasitized *Linckia multifora* from a position very close to Davis' locality B8. Sixteen starfish among 40 collected were parasitized. They were taken on 17 June 1971 at a depth of 1.5 m on, in, and under rocks and coral on the reef flat just WNW of Buoy 8 (21°27′11″N, 157°47′55″W). They were placed in Bouin's fluid, which was changed once, and thereafter transferred to 70% ethyl alcohol.

When received, the material consisted of 1 whole host specimen that was parasitized on the oral side of the disk, and 17 isolated rays, each with a characteristic swelling caused by the gastropods (pl. I, fig. 2). Thirteen of the hosts had one infected ray, whereas two hosts had two rays infected.

The swellings were examined externally and photographed; two specimens were decalcified in HCl in 90% ethyl alcohol and embedded in celloidine for sectioning of the entire ray, while all gastropods were dissected out of the remaining specimens. The majority were embedded in tissuemat and cut into 6 or 8 μ thick sections and stained with H + E, tetrachrome (Weigert's iron hematoxyline, Chlorantine fast red, orange G, and Alcian blue), or Azan. Two small specimens were embedded in epon and cut into 2 μ thick sections which were stained with toluidine blue.

The Swellings or Galls

In the present species these have been illustrated earlier by Sarasin & Sarasin (1887, pl. IV, figs. 1, 2) and Davis (l.c., pl. 48). As observed by the latter author, the gall openings to the exterior occur apparently at random on the oral, lateral, or aboral surface of the rays. My material also supports the information provided by Davis that parasitism may obviously occur everywhere along the ray, from the most basal part to the very tip.

[1] Yet unpublished examinations of the original and new material of "*S. sibogae*" Schepman & Nierstrasz has convinced me that it actually consists of three species, all of which belong to the sea urchin snails, *Pelseneeria* Koehler & Vaney. These are unique in several respects and it may be seriously doubted whether they are related to any other parasitic gastropods yet described, except *Parastilifer* Ivanov. They are simultaneous, not protandrous, hermaphrodites.

On one occasion Sarasin & Sarasin (1887: 24) found two gastropods within the same swelling, and they considered the smaller to be an immature. Davis (l.c.) recorded the galls that he examined as containing from one to several (up to five) snails. Although the number of gastropods present may be estimated from the number of apertures to the exterior, it proved necessary to make a careful dissection of the galls to find all gastropods, since the apertures of the smallest individuals may often appear only as extremely minute pores or crevices in the host's skin (pl. I, figs. 2, 6). Among the 17 parasitized rays, one gall contained one snail, twelve galls contained two snails each, three galls contained three snails each, and one gall contained four snails. Three snails were present in the gall of the starfish parasitized on the disk. On an average, therefore, the galls harboured 2.3 snails each.

The apertures in *S. linckiae* may reach a considerably larger size than those of any other species of *Stilifer* (except, perhaps, *S. astericola*), but the variation is great. Through the larger apertures several of the whorls of the snail may be observed from outside, but in most of the smaller apertures this is not possible (pl. I, figs. 2–6). Each snail within a gall is independent and has its own aperture to the exterior.

The Sex in *S. linckiae*

Of a total of 41 specimens examined by sectioning, 16 were mature females. Nineteen specimens proved to be males; this is, therefore, the first time that the male sex has been demonstrated in a species of *Stilifer*. The remaining 6 specimens are particularly interesting since they show successive stages in the transition from a male to a female phase.

Only the sexual apparatus of *S. linckiae* will be described since the structure of all other organ systems are fundamentally similar to those of *S. ovoideus* as described by Ivanov (l.c.). The two sexes were found to differ only in size, females being substantially larger than males, and in the organization of the reproductive system. In the absence of a shell (which was lost at fixation) the size of any specimen is expressed as the distance separating the apex and the proboscis base (defined by the point marked by two arrows in text-fig. 1) measured on the section series. Sixteen functional females measured from 3.5 to 5.9 mm compared with 0.7 to 2.8 mm in sixteen functional males.

The Male Reproductive System (text-figs. 2 & 3A; pl. I, fig. 8)

The testis occupies the major portion of the upper coils of the visceral mass lying over the lobes of the digestive gland. The testicular duct is very long, and its posterior part is narrow and greatly coiled. More anteriorly it expands enormously, and this section acts as a vesicula seminalis, the walls of which has a very flattened squamous epithelium. The vesicula contains large quantities of sperm. On passing forward it rapidly diminishes in diameter and leads to a small, vesicular prostate gland that has a narrow entrance into the most posterior end of the mantle cavity. From the aperture of the prostate, an open glandular and ciliated seminal groove passes forward along the right side of the body to the base of the penis. In proportion to the size of the animal,

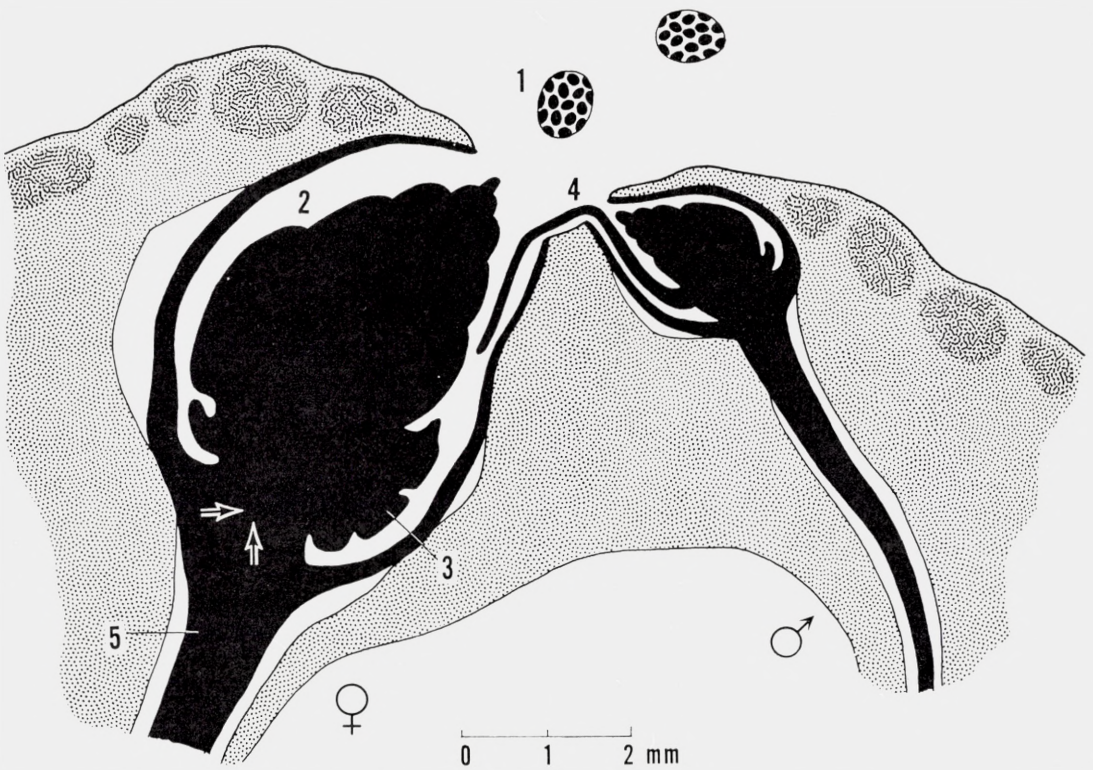

Fig. 1. Diagram showing the position of two specimens, a male and a female, of *Stilifer linckiae* in a gall in the ray of the starfish *Linckia multifora*. Expulsion of egg capsules (1) from the pseudopallial cavity (2) as indicated has actually never been observed. 3, foot; 4, penis; 5, proboscis.

the latter is a large structure, arising from behind the head. When at rest, it lies in a single spiral, folded up in the exterior part of the mantle cavity which is, however, far too small to accomodate it. The penis is entirely ciliated. In transversal section it appears to be flattened, and has a deep seminal groove along its entire length. While the proximal part is predominantly muscular with only a few blood lacunae, vascular spaces are numerous throughout the thickness of its apical half. When extruded and filled with blood, the penis undoubtedly extends considerably, as is indicated by the abundant and closely placed transversal folds seen on its surface.

The Female Reproductive System (pl. I, fig. 7)

The ovary occupies the central and right part of all spire whorls. Dorsally and to the left it is separated from the body surface by a continuous layer of the digestive gland. Numerous haemocoelic spaces issue from the ascending posterior aorta and interpenetrate the ovary lobules. The oviduct emerges far back in the body whorl from the ventral aspect of the ovary, and runs forward along the right side of the main sinus of the digestive gland. Its walls are slightly folded and composed of tall, ciliated cells with

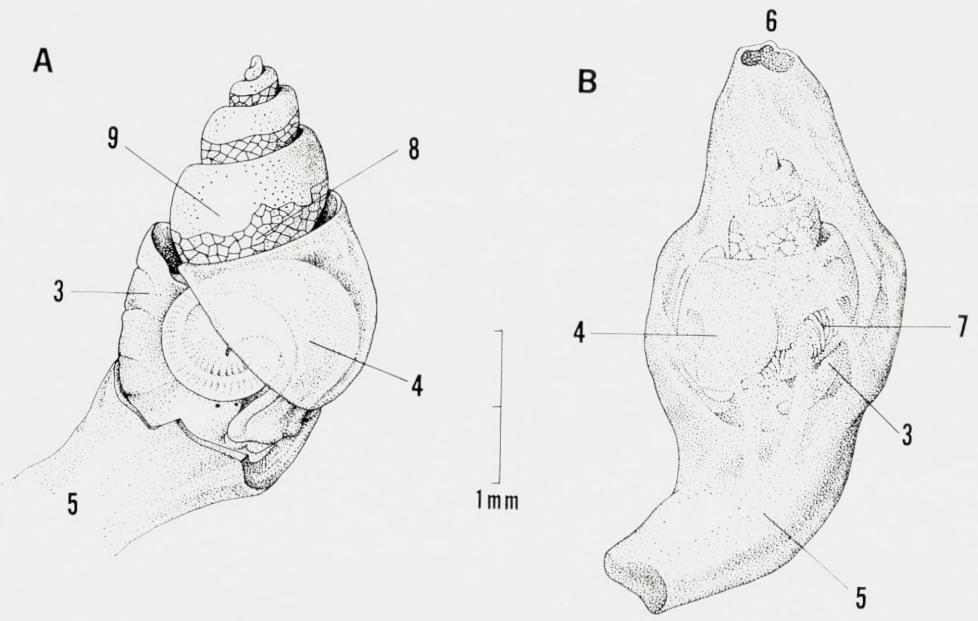

Fig. 2. Two male *Stilifer linckiae*. The pseudopallium has been removed in A to show animal. Note the open seminal groove on the surface of the penis. The right and, less distinctly, the left eye are seen in front of the penis in A. 6, aperture of pseudopallial cavity; 7, ctenidium; 8, digestive gland; 9, testis. Other symbols as in Fig. 1.

basal nuclei and a plasma that is left almost unstained. Two oviducts were present in two specimens. Anteriorly the oviduct narrows and debouches into the albumen gland. This is a rather conspicuous and subdivided organ that lies to the right of the oviduct and along the surface of the whorl. The gland cells are of two types: large, globular cells with basal nuclei and a plasma that stains intensively with alcian blue, and among them small, slender cells with centrally placed nuclei and short cilia are found. The walls of the next section of the female duct are deeply and heavily folded. This section is far back in the thickened right side of the mantle wall next to the albumen gland on one side and the receptacular pouch and capsule gland on the other. The epithelial cells are small, ciliated, and have relatively large nuclei; much larger goblet cells that stain with alcian blue occur among them, either singly or in groups. Since non-orientated sperm were frequently observed here, this section may be regarded as a bursa copulatrix. It has a narrow connection with the receptaculum seminis, a rather capacious, irregularly shaped sac in which large amounts of sperm are stored; the sperm here are closely packed and orientated with the tips of their heads embedded in the wall cells and with their tails directed into the lumen.

The bursa copulatrix has a fairly wide connection anteriorly with the capsule gland. This is a very large structure which occupies the entire dorsal and right side of the mantle skirt, and opens to the mantle cavity by a ventral slit along the greater part of its length.

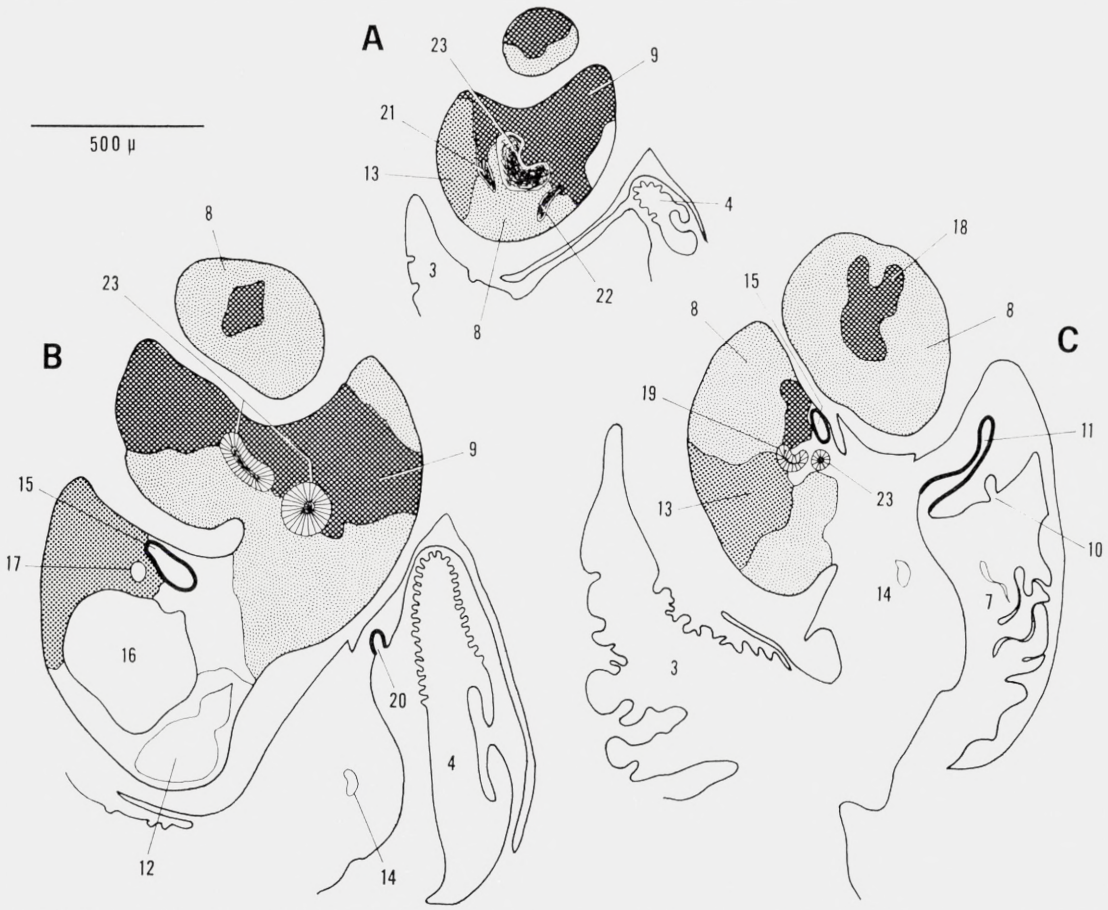

Fig. 3. *Stilifer linckiae*. Sections through A: male phase (length 1.6 mm, gonad index 68%); B: early transitional stage (length 2.3 mm, gonad index 38%); and C: late transitional stage (young female, length 2.1 mm, gonad index 20%). 10, anus; 11, capsule gland being formed (from seminal groove); 12, heart; 13, kidney; 14, oesophagus; 15, posterior part of seminal groove; 16, posterior part of mantle cavity; 17, rectum; 18, rudimentary ovary; 19, rudiment of oviduct; 20, seminal groove; 21, start of testicular duct; 22, testicular duct; 23, vesicula seminalis, in B and C containing only a few sperm cells and with a tall epithelium. Other symbols as in Figs. 1 and 2.

Transitional Stages from Male to Female Phase (text-fig. 3 B & C)

Functional males and females only occurred in 14 galls (text-fig. 4, nos. 1–14), of which eleven contained one male and one female each (nos. 1–11); in three other there were one female and two males (no. 12), two females and one male (no. 13), and one female and three males (no. 14). Six of the nine individuals in the remaining four galls (nos. 15–18) were clearly undergoing sex transformation. Three were in an early phase, and three were in later phases of the transition from male to female.

The masculine character of the former three specimens (length as earlier defined: 2.1–2.3 mm) is unmistakable, but the testis has atrophied considerably, its volume being 31, 37, and 38% of the combined volume of gonad and digestive gland (calculated

from the section series) as compared with 59, 68, and 83% in three functional males (chosen at random). Rather few testis lobes showed spermiogenesis; the vesicula seminalis was packed with sperm cells in one specimen, but was largely empty in the other two and had a minute lumen throughout. The epithelial lining of the vesicula differed from that found in the functional males in that the cells were cubical, or even tall and cylindrical. In two specimens sperm cells were noted within the cells of the epithelial lining, and were presumably being digested. The open seminal groove of the specimen with sperm in the vesicle was of normal construction, while the walls had hypertrophied in the other two specimens, especially posteriorly resulting in a deepening of the groove. The penis of all three specimens was of usual size.

The three late transitional stages (length: 1.9–2.6 mm) show further advancements toward a female condition. The volume of the gonad is further reduced since it shows an index of 20, 16, and 9%. The gonad is solid, restricted to the extreme right side of the whorls, and contains oogonia. From the gonad of one of the specimens (gonad index: 20%) two narrow ducts of subequal diameter arise independently (text-fig. 3 C, 19 & 23). One of them is short and curved, whereas the other is rather long, sinuous, and contains some sperm cells throughout; the latter is consequently considered to represent the reduced testis duct including the vesicula seminalis, and its content accordingly autosperm that has escaped digestion; the former is presumably the rudimentary oviduct. The two ducts unite and open by way of a common, narrow aperture into the most posterior part of the open pallial groove, which is unusually deep throughout its length in this specimen. A penis is present, although it is reduced to a vestige. In the two other specimens, an oviduct is clearly distinguishable, but no testis duct could be traced. The pallial groove is very deep, and its posterior part is separated from the mantle cavity by fusion of its walls. The oviduct opens into this portion, which presumably later differentiates into the albumen gland, the bursa copulatrix and the receptacle. A vestigial penis is present in one specimen, but is absent in the other.

The Type of Sexuality in *S. linckiae*

The findings related above suggest that *S. linckiae* is a consecutive hermaphrodite that functions as a male when young and as a female when older. Cessation of spermiogenesis and resorption of the spermatic tissue initiate the transitory period. At the same time, the cells lining the vesicula seminalis become cubical or cylindrical and start to digest the superfluous sperm cells stored in the vesicle. The testis duct atrophies completely and a new gonadial duct is established at a later stage. In the remainder of the gonad, oogonia appear (by transformation of spermatogonia?) and the gonad resumes growth. It seems fairly certain that the albumen gland, the bursa, and the receptacle are formed by elaboration of the posterior part of the open pallial groove of the male that is partitioned off from the mantle cavity, whereas the section anterior to this area becomes the capsule gland through incomplete fusion of its walls. The penis is reduced, but it may persist as a vestige even in adult females; it was found in two young and two func-

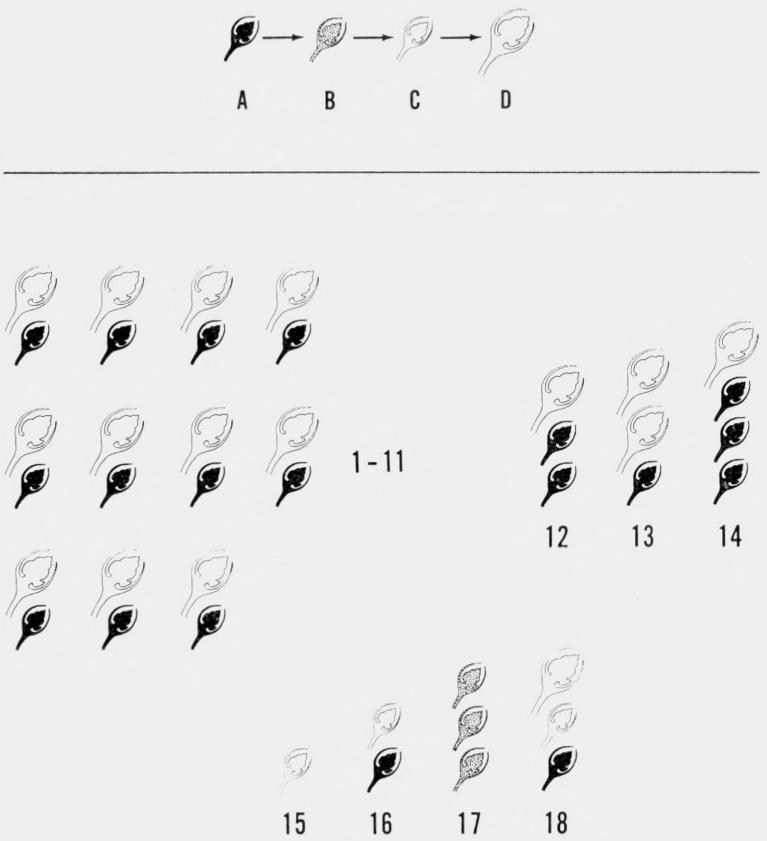

Fig. 4. *Stilifer linckiae*. Diagram showing the sequence of stages found in male phase (A), early and late transitional phases (B and C) and female phase (D), and the distribution of these in the 18 galls examined.

tional females. The fact that more than one oviduct occurred in a few females may indicate that the testis duct is occasionally preserved and transformed into an accessory oviduct.

Although the males of *S. linckiae* possess the ability to undergo sex transformation, it is very interesting to note that this potential is obviously suppressed by the proximity of the female sex. This is apparent upon consideration of the composition of the gall inhabitants (text-fig. 4): With one exception (no. 18), all six gastropods in transitory phases come from the only three galls in which functional females were absent (nos. 15–17); and none of 17 males in 14 galls with functional females (nos. 1–14) had initiated a sex change. Unless two or more gastropods settle on a host at about the same time, this mechanism will ultimately assure the establishment of pairs, trios, or quartets, etc. of gastropods of opposite sex, since those settled first will develop into females (through a male phase) and these will maintain the maleness of individuals settling at a later date. This mechanism could presumably be experimentally tested by studying the effect of extirpation of their female partners on the males.

Consecutive hermaphroditism has been recorded among the Mesogastropoda in the families Ianthinidae (Ankel, 1926), the Scalidae (Ankel, 1936), the Capulidae (Graham, 1954), and the Calyptraeidae (Orton, 1909); the evidence of protandry in the Hipponicidae is not conclusive as is pointed out by Laws (1970). In *Clathrus clathrus* (L.) of the family Scalidae, a sex change is undergone several times throughout life, in species of *Ianthina*, probably only once. In the Capulidae and in *Calyptraea chinensis* (L.), sex change always occurs at a particular stage in the life cycle and appears to be unaffected by external stimuli. Extensive literature on sex reversal in various species of the slipper limpets, *Crepidula* (family Calyptraeidae), summarized by Fretter & Graham (1964), suggests that the sex transformation here is the result of an interaction between the various members of the social group that forms the well known chain. Gould (1919) has proposed that the lower members of the chain, the functional females, secrete a substance into the surrounding water that effects maintenance of the masculinity of the males. It is likely that the sex of the young *Stilifer* may prove to be controlled in a similar way.

Copulation and Oviposition

The *S. linckiae* material examined permits us to draw some conclusions as to how reproduction takes place.

Sperm is stored in the male in the vesicula seminalis. It is then discharged into the final section of the testicular duct and, further on, into the prostate. Here it mingles with the prostatic secretion to form the seminal fluid which is conveyed along the open seminal groove to the tip of the penis.

To reach the female at copulation, the erected penis has to be extended through the male aperture and introduced in the nearest female opening (text-fig. 1). The depth to which the penis penetrates to discharge its contents is open to guesses. It is possible, although hardly likely, that the seminal fluid is simply poured into the pseudopallial cavity, and from there transferred to the female genital opening by the inhalant water current. The penis is more probably introduced into the mantle cavity and the sperm deposited somewhere in the open pallial duct; assuming this, the length to which the penis can extend may be estimated as follows: the greatest distance separating a male and a female pseudopallial aperture was 2.0 mm; the distance from the penis base to the margin of the male aperture was 1.8 mm; and the distance from the margin of the female aperture to the mantle opening, 3.5 mm. Accordingly, the penis would have had to extend at least 7.3 mm should copulation occur; the fact that sperm were found in the receptacle pouch of the female in question proved the penis' ability to do so.

Unorientated sperm were observed in the bursa (and occasionally in the albumen gland), and the eggs are presumably fertilized here after they have been embedded in albumen. As they pass anteriorly they become covered by a secretion from the capsule gland. None of the females examined by sectioning had been preserved at the moment of formation of egg capsules. But since the length of the ventral opening of the capsule

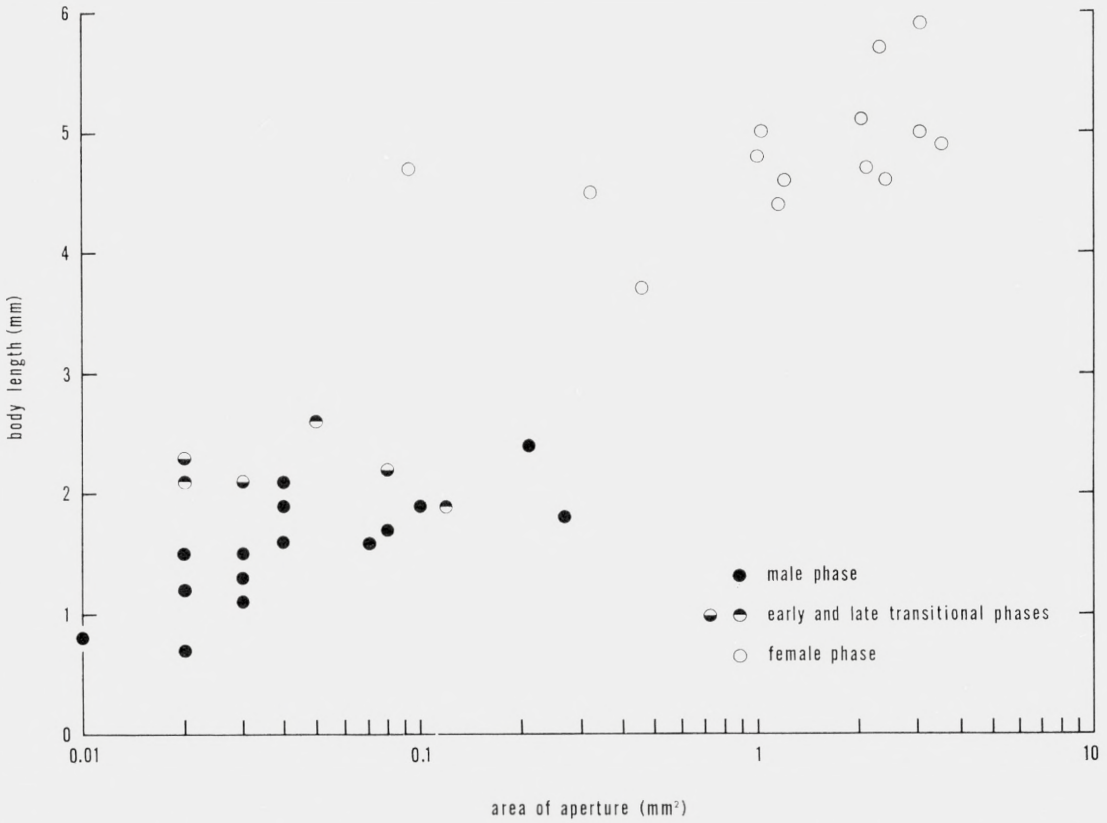

Fig. 5. Relation between body length (as defined in the text), area of pseudopallial aperture, and sexual phase in *Stilifer linckiae* (35 individuals).

gland is undoubtedly correlated with the size of these capsules, we may estimate them to be relative large and to contain several eggs.

Spawning has never been observed in any species of *Stilifer*. The egg capsules of parasitic gastropods are frequently attached to a substrate, as, for example, the skin of the host or the shell of the gastropods; species with a pseudopallial cavity may brood the eggs or capsules. None of the 16 females of *S. linckiae* with sperm in their receptacle were found to contain egg capsules, and Davis (l.c.) did not report any from the many galls which he inspected either. Presumably, therefore, the capsules are expelled directly into the surrounding water through the pseudopallial aperture immediately upon their formation as suggested in text-fig. 1. This would seem advantageous since brooding of the capsules in the incapacious pseudopallial cavity is likely to restrict the number produced at a given time, and further, to function as an obstacle to the respiratory water currents. Hirase (1932: 74) suggests that the foot (in *S. ovoideus*) acts as a type of piston, which together with contraction and expansion of the pseudopallial wall may flush water

in and out through the aperture; Cheng (1968: 679) maintains that the sex products are discharged at the same time. It is unclear whether the latter statement is founded on observations or not. It is apparent from text-fig. 5 that the size of the apertures (measured from close-ups of photographs) increases with the dimensions of the gastropods; as a consequence of this, they are larger in the females (usually 1 to 4 mm^2) than in the males. This allows for expulsion of quite large egg capsules.

Comparison with other Species of *Stilifer*

The salient feature of the galls of *S. linckiae* is that they usually contain two or more gastropods, and that these are most frequently of two different types: small specimens (males or transitional phases) and large specimens (females). The *Stilifer* material at my disposal other than *S. linckiae* has been mainly dried, ill-preserved museum specimens, the sex of which can no longer be determined. The number and size of the gastropods may, however, still be examined.

S. astericola. Of three specimens of *Heliaster* with gastropods still *in situ*, two had one parasite each, and one harboured five. There were probably more parasites at one time in the latter specimen since two or three cystiform deepenings in the starfish skin were now empty, but had probably accomodated some smaller snails.

S. ovoideus. A gall caused by this species in *Ophidiaster granifer* contained three gastropods: one was comparatively large (length of shell: 5.7 mm) and had been dissected out; the remaining two were still attached to the inner wall of the gall, each located in a shrivelled pseudopallial cover and with the shells, both 1.8 mm long, pointing toward the gall aperture. Through the opening of another gall (in *O. granifer*), which was not dissected, one snail could be seen. The parasitized specimen of *Tamaria dubiosa* contained a single gastropod.

S. utinomii. Of the five galls examined, four contained one snail each; the fifth, two snails measuring 4.0 and 5.5 mm in shell length.

S. ophidiastericola. As related above, a gall in the ray of an *O. lorioli* accomodated three gastropods; since the gall was not opened, however, they could not be measured. Five gastropods in three galls in *O. confertus* measured as follows: 1) ca. 4 mm; 2) 2.8 and 1.9 mm; 3) ca. 5 mm and 1.5 mm.

Stilifer sp. from *Ophidiaster* sp. (AMS). One gall was opened from inside, thus exposing one large and one tiny gastropod (length 5.2 and 1.2 mm resp.) placed close together and apparently opening separately to the exterior. Two other galls were left unopened, but from the outside each could be seen to contain one very large and one minute gastropod; the apertures of the latter opened at the margin of the larger's aperture or 1.2 mm from it.

Stilifer sp. from *Ophidiaster* (?) sp. (ZMC). The two galls on the infected starfish accomodated two and five subequally large gastropods.

We may, therefore, conclude that two or more specimens occur together in a gall more frequently than only a single specimen in other species of *Stilifer* too. The sex of

these could only be determined in one case, *viz.* in the *Ophidiaster* (?) gall taken by the Kei Island Expedition. Although very badly preserved, section series of the five gastropods within a single gall showed that there were one female and four slightly smaller males. It is to be expected, therefore, that the type of sexuality found in *S. linckiae* characterizes all members of the genus.

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PLATE

PLATE I

Fig. 1. *Stilifer kochianus* Sowerby, type specimen (cleaned shell), BMNH. Scale 2 mm.

Figs. 2–8. *Stilifer linckiae* Sarasin & Sarasin.

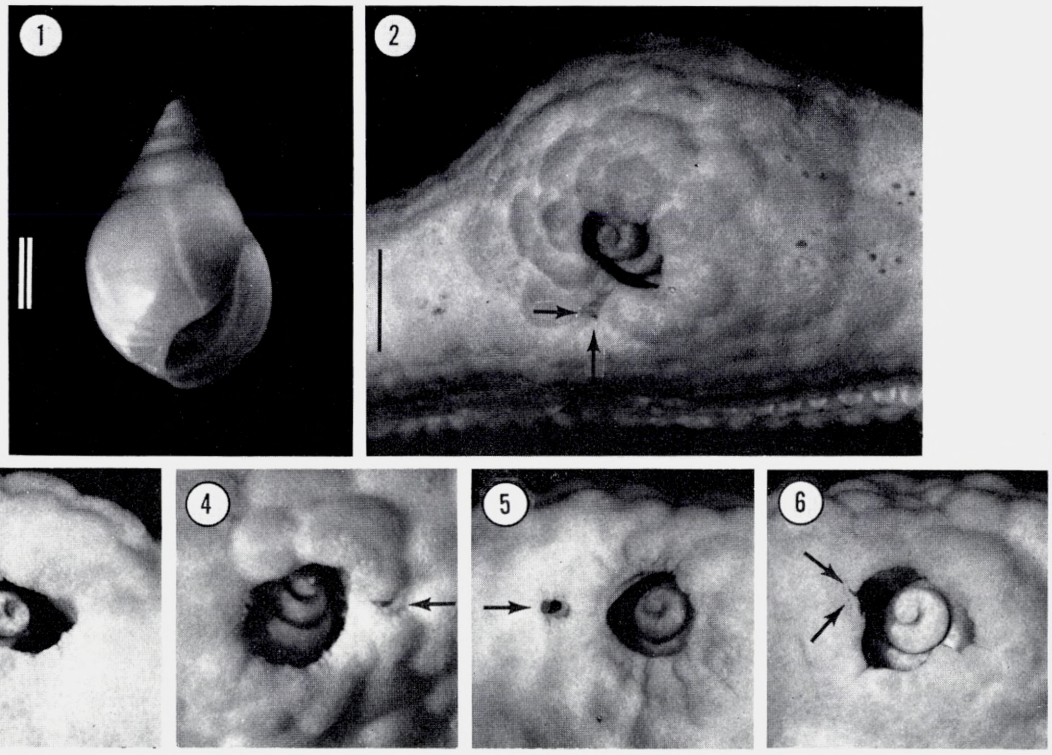
Figs. 2–6. Position in *Linckia multifora* as viewed from outside; the arrows indicate the apertures of the male's pseudopallial cavity. Scales represent 2 mm (Fig. 2) or 1 mm (Figs. 3–6).

Fig. 7. Celloidine section through ray of infected *L. multifora* (the specimen shown in Fig. 2) containing two gastropods in female and male phase. 20μ, tetrachrome. Scale 1 mm.

Fig. 8. Longitudinal section through male. The arrows indicate open seminal groove. Tissuemat, 8μ, tetrachrome. Scale 200μ.

Symbols

1, albumen gland
2, ambulacral groove
3, ambulacral piece
4, atrium
5, capsule gland
6, coelomic cavity of starfish
7, columellar muscle
8, digestive gland
9, foot
10, kidney
11, oesophagus
12, opening of prostate into mantle cavity
13, ovary
14, pedal gland (anterior)
15, penis, basal part
16, penis, middle section
17, penis, apical section
18, proboscis
19, pseudopallial cavity
20, pseudopallial aperture
21, pseudopallium
22, radial water canal
23, skeletal ossicles
24, strand of connective tissue separating pseudopallium and coelom of host
25, testis
26, testicular duct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