STUDIES IN POST-LARVAL DEVELOPMENT AND MINUTE ANATOMY IN THE GENERA *SCALPELLUM* AND *IBLA*.

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I.—SUMMARY OF PREVIOUS LITERATURE.

1. The development of hermaphrodite and male forms of Cirripedes.
2. Anatomy of the male.
3. Question of sexual purity of the male and female forms.

1. The development of cirripedes has been dealt with chiefly by Darwin (5) and Hoek (8). Darwin (5, pp. 8 and 9) gave a summary of preceding literature (Vaughan Thompson, Burmeister, Goodsir, Spence Bate) and described the development from the nauplius, passing through the cyprid stage to the adult. In regard to that portion of the metamorphosis with which we are concerned, namely, that from the cyprid stage to the adult, he wrote: "My chief examination has been directed at this stage of development to the larvae of *Lepas australis* which are of unusual size, namely, from ·065 to even almost ·1 of an inch in length. I examined, however, the larvae of several other species of *Lepas*, of *Ibla* and of *Balanus*, with less care, but sufficiently to show that in all essential points of organisation they were identical." His descriptions are, of course, not based on the examination of sections so that some gaps still remained to be filled up in regard to the more histological aspect of the metamorphosis. He also fell into the error, which was pointed out at a later time by Krohn and Claus, of mistaking the cement cells for incipient ovaria.

Hoek (8, p. 5) again described the cyprid of *Lepas australis* and compared it with the similar larva of the male of *Scalpellum regium.* In addition to excellent descriptions he also published drawings of the two organisms and traced the evolution of the male from the cyprid.

Aurivillius (2) refers to the post-larval development of *Scalpellum erosum*, Aur., *S. obesum*, Aur., and some others, but confines himself to the external characters. He figures the cyprid larva of *S. obesum* and that of the male of *S. scorpio*, Aur. (*Vet. Akad. Handl.*, vol. 26).

My work, although it dealt with species different from those investigated by Darwin and Hoek, has given results which in general do not differ from the conclusions of these two distinguished observers. Only a brief summary of the general outlines of the development is therefore given. Indeed, my chief excuse for publishing any note on this subject lies in the fact that I have been able to obtain some excellent drawings of the different stages owing to the abundance and excellent state of preservation of the material at my disposal. These drawings will supply that which is chiefly lacking in previous accounts, since the illustrations of the metamorphosis in Darwin's work do not exhibit more than the external outlines of the larvae, and in Hoek's report in the "Challenger" series the only larvae figured are those of *Lepas australis*, Darwin, *Scalpellum regium* and *triangulare*, Hoek (8, pl. ii, figs. 1—4). This author's report on the "Siboga" expedition contains an illustration of the pupa of *S. stearnsi*, Pilsbry (9, pl. vi, fig. 10).

On the other hand, the development of the different organs—cement glands, ovaries and testes—from the cyprid to the adult has not been thoroughly traced as yet, and although Hoek (8) describes correctly the cement glands and the ovaries in both the cyprid and the adult, he does so with some doubt as to the correctness of his identification. I have been able to obtain a complete series of examples from the cyprid to the fully grown adult.

2. The anatomy of the parasitic males has also been somewhat fully dealt with. Darwin (5) described *Scalpellum vulgare*, *ornatum*, *rostratum*, *peronii*, *villosum*, and *Ibla cumingii* and *quadrivalvis*, with plates of *Ibla cumingii*, of *Scalpellum vulgare*, *peronii*, *villosum* and *rostratum*. Hoek (8) classifies the males in three groups according to their condition of degeneracy and refers to twenty-four species of *Scalpellum*. He describes minutely the male of *Scalpellum regium* (Wy. Th.), which in many respects is identical with that of *S. gruvelii*. The males of the three species described in the present paper would be referred, in the case of *S. squamuliferum*, to Hoek's class I, as showing a distinct capitulum and peduncle; in the case of *S. gruvelii*, to class III, without division to capitulum and peduncle and without rudimentary valves. *S. bengalense* should be placed in class II.

In the report on the "Siboga" expedition (9) Hoek describes the external characters of the males of *Ibla sibogae*, H., with figures, of *Scalpellum pollicipedoides*, H., *acutum*, H., *rostratum*, D., *uncus*, H., *stearnsi*, Pilsbry, *chitinosum*, H., *inflatum*, H., *javanicum*, H., *polymorphum*, H., *distinctum*, H., *pellicatum*, H., *crinitum*, H., *gracile*, H., *hexagonum*, H., *fissum*, H.

Gruvel in the *Arch. de Biol.* (6) gives a full account of both the external and internal anatomy of the male of *Scalpellum vulgare*, and in the reports on the "Travailleur" and "Talisman" collections (7) he deals with those of *Scalpellum peronii*, Gray, *villosum*, Leach, *longirostrum*, Gruvel, *gigas*, H., *hoeki*, Gru., *striatum*, Gru., *lutium*, Gru., and *velutinum*, H.

3. The cirripedia have a special interest for students of biological theory, in that they offer examples of the evolution of dioecism from hermaphroditism. The facts of the case, as is well known, were first brought to light by Darwin (5), who showed that within the group there occurred (1) true hermaphrodites—*e.g.*, *Lepas*; (2) andro-dioecious forms—such as *Scalpellum peronii*; and (3) dioecious forms—*Scalpellum ornatum* and *Ibla cumingii*.

Hoek (8, p. 18) elaborated this idea. By cutting serial sections of *Scalpellum regium* he confirmed Darwin's view as to the purely female nature of the large form of certain barnacles, while, on the other hand, he established the fact that the large form of the apparently andro-dioecious species was truly and functionally hermaphrodite (*S. vulgare*).

In regard to the sexual purity of the apparently male dwarf forms, Darwin (5, p. 289) wrote: "I was not able to discover a vestige of ova or ovaria in the two male Iblas (*quadrivalvis* and *cumingii*); and I can venture to affirm positively that the parasites of *Scalpellum peronii* and *villosum* are not female." Hoek also appears to have no doubt as to the purely male characters of these little forms. Gruvel, on the other hand, claims to have found rudimentary female organs in the male of *Scalpellum peronii* (7, p. 121): "Or j'ai rencontré, dans des coupes transversales du pédoncule, quelques cellules arrondies, à noyau assez gros et nucléole très brillant, ne ressemblent nullement à des cellules cémentaires et que je crois être quelques cellules ovariennes non développées, reste, évidemment, de la forme hermaphrodite ancestrale." This is the only species in which he has found cells of this nature.

Such a discovery would be of very great theoretical importance, since it would suggest that the evolution of the unisexual from the hermaphrodite form occurred gradually by the slow progressive diminution of one set of sexual organs. Such a change would in all probability be due to either (1) a disuse atrophy, the progressive diminution of activity being primary and the diminution in structural development secondary; or (2) in the present case the semiparasitic habit of the incipient male might have caused it to diminish in size with a consequent diminution in the structural development of the ovaries. (See also G. Smith, 11, p. 35.)

It therefore appeared desirable that the matter should be further investigated, and, in order to arrive at a conclusion as to the nature of the various cells found, it was necessary (1) to trace the development of the ovary in a hermaphrodite or female and thus to become acquainted with the various appearances of the ova in all their successive stages from the earliest point at which they can be recognized; having done this, to examine the different cells to be found in the male and its larval stages, and to compare them with the developing ova: (2) to trace the development of the testis in a hermaphrodite and to ascertain if anything similar could be found in a "female."

To obtain the best results, it was also clearly desirable to examine males and females, which differed in the least degree possible from the hermaphrodite type.

For this purpose the male of *Scalpellum squamuliferum*, Welt., and the female of *Ibla cumingii*, Darwin, were chosen. The former differs from its hermaphrodite chiefly in size and in the absence of peduncular plates and latera. The separation of the capitulum from the peduncle is quite as marked as in *Scalpellum peronii*, and the six chief capitular valves are well developed. The latter, *Ibla cumingii*, has a close ally in *I. quadrivalvis*. Darwin (5), in defining the latter species, wrote: "All the external parts (of the hermaphrodite) so closely resemble those of *S. cumingii* that it would be superfluous to describe more than the few points of difference" (p. 204). Indeed, the chief point of distinction of the two species lies in the fact that the large form of *Scalpellum quadrivalvis* is hermaphrodite and possesses a penis, whereas the large form of *S. cumingii* has no penis and appears to be devoid of any male organs. We have then in these two forms a male and a female, which have evolved from the hermaphrodite condition only comparatively recently, and in which, if anywhere, we would expect to find some trace of the lost female and male organs.

A large number of excellently preserved specimens of *Scalpellum squamuliferum* bearing numerous males was stored in the "Investigator" collections in the Indian Museum, and I obtained a considerable number of *Ibla cumingii* on the reefs of Diamond Island off the coast of Burma during the winter of 1909. I was also so fortunate as to obtain an excellent specimen of the male of *Scalpellum peronii* for comparison with those of *S. squamuliferum.*

The development of the ovaries and testes of the hermaphrodite was followed out in *S. squamuliferum.*

The determination of the species of the specimens used is in all cases the work of my friend Dr. Annandale, to whom my thanks are due for much assistance in this investigation. For the definition of the species this author's work should be consulted (1).

II.—Post-larval development of *Scalpellum squamuliferum*, Welt., and anatomy of the male.

- A.—General outlines of the development.
  - (i) The hermaphrodite—
    - (1) The pupa.
    - (2) The young adult.[1]
  - (ii) The male—
    - (1) The pupa.
    - (2) The young adult.
    - (3) The adult.
- B.—Histology and development of the organs of the peduncle.
  - (1) The vesicular spaces of the peduncle of the pupa.
  - (2) Rostral duct and lymph spaces of the adult.
  - (3) The connective tissue cells and fibres.
  - (4) The yolk.
  - (5) The cement cells and ducts.
  - (6) The ovaries.
  - (6a) Absence of rudimentary ovaries in the male.
  - (7) The cells of unknown function.

III.—Absence of rudimentary ovaries in the male of *Scalpellum peronii.*

IV.—Anatomy of the male of *Scalpellum bengalense.*

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[1] The term adult is used for all the stages after the casting off of the pupal coverings and the extension of the capitulum on the peduncle. This is, of course, not pedantically correct since in the earlier of these stages the animal is not truly adult, it has, however, assumed the outward form of the adult.

V.—Post-larval development and anatomy of the male of *Scalpellum gruvelii.*
(1) The cyprid larva.
(2) The pupa.
(3) The adult.

VI.—*Ibla cumingii.*
A.—Anatomy of the male.
B.—Absence of rudimentary testes in the female.

VII.—Sensory hairs of *Scalpellum squamuliferum, bengalense* and *gruvelii*, and of *Ibla cumingii.*

II.—POST-LARVAL DEVELOPMENT OF *SCALPELLUM SQUAMULIFERUM,* WELT., AND ANATOMY OF THE MALE.

The expression post-larval development is intended to include the various stages from the fixation of the cyprid larva to the attainment of adult form and complete sexual maturity.

A.—GENERAL OUTLINES OF THE DEVELOPMENT.

(i) The Hermaphrodite.

(1) THE PUPA.

The general outlines of the development are so well known as to call for little reference.[1] Plate iv, fig. 1, represents the cyprid during the course of the first ecdysis. A younger cuticle bearing the hairs characteristic of the later stages has actually been formed under the bivalve shell of the cyprid, but is only visible in sections. The two larval eyes (*e.*) are being cast off. The antennae are embedded in a mass of cement. The alimentary canal (*oes.* and *st.*) is already patent, the stomach filled with a mass of excretory matter. The rudimentary testes can be distinguished in sections as two minute cell-masses, one on either side of the stomach. The nervous system consists of the cerebral ganglion (*ce.g.*) and the five ganglia of the ventral chain (*vn.c.*). The larval integuments are not furnished with sensory hairs. The outlines of the terga, scuta, carina and rostrum can already be distinguished, but are not represented in the figure.

The pupa measures 1·1 mm. in length.

(2) THE YOUNG ADULT.

Figure 1 should be compared with pl. iv, fig. 2, taken from a young hermaphrodite measuring 2·5 mm. in length: *ant.* are again the embedded antennae. The oesophagus now has a strong coat of circular muscles. The two caeca which arise from the anterior end of the stomach were not visible in the preparation. All the

[1] Compare description of cypris of *Lepas australis*, Hoek (8, p. 6).

capitular valves are represented as well as some of the peduncular plates. The young hermaphrodite can thus be readily distinguished from the male, which only possesses terga, scuta, the carina and rostrum. Figure 3 is from a section through a similar specimen, showing the stomach (*al.c.*), testes (*t.*), ventral nerve cord and the sensory hairs on the outer surface of the capitulum (*h.*). The letters *r.d.* indicate the rostral duct—the main blood or lymph channel from the prosoma to the peduncle, which is again shown in pl. v, fig. 5, from a transverse section through the peduncle of a specimen of similar size.

Development of the testes in the hermaphrodite.—The testes can be clearly distinguished in sections of the pupa as two small syncytial bodies lying at the sides of the stomach. Plate iv, fig. 3, represents a transverse section through the prosoma of an adult measuring 2·5 mm., in which *t.* indicates the testes. It will be seen that they lie somewhat on the ventral side of the stomach. Each testis is clothed by a fine membrane. There are no cell outlines in their protoplasm. The nuclei contain either one or two nucleoli and numerous minute chromatin granules.

(ii) The Male.

(1) THE PUPA.

I was sufficiently fortunate to obtain a specimen of a male pupa in the same stage as the hermaphrodite figured in pl. iv, fig. 1; that is, in the course of the first ecdysis after fixation. Plate iv, fig. 4, is taken from this specimen. On comparing it with the hermaphrodite it will be observed to be much shorter in comparison to its height, and the peduncular region is proportionately greater in comparison with the capitular region.[1] It measures ·75 mm. in length.

The stomach is not clearly visible, as it is overlaid by the left testis (*t.*). The testes are, even in this early stage, much larger than in the hermaphrodite. The mouth is open, but there is as yet no anus, the intestine being closed posteriorly. The stomach contains matter which is apparently excretory. The condition of the valves in this stage is shown in pl. iv, fig. 5.

(2) THE YOUNG ADULT.

Plate iv, fig. 6, represents a later stage, after the completion of the ecdysis. The only remaining portion of the larval integument is to be seen at the base of the antennae. The capitulum has become extended on the peduncle into the adult attitude.

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[1] In connection with the views of Geoffrey Smith (11, p. 37) as to the nature of the parasitic males, it is important to note that the male differs from the hermaphrodite before fixation (compare the proportional measurements of the cyprid shell in the male with those of the hermaphrodite). Maleness is therefore not the result of the position of attachment.

It is hardly necessary to bring forward this proof, since in *S. squamuliferum* hermaphrodites occur attached to all parts of other hermaphrodites, including the margin of the pallial aperture. That is to say, they occur in that position which Smith considers to be the cause of the non-development of the hermaphrodite character in the "males."

(3) THE ADULT.

The anatomy of the male of Scalpellum squamuliferum.

The shape of these animals is sufficiently indicated by pl. v, fig. 1. Mounted specimens measure from 1 mm. to 1·4 mm. in length, with a maximum horizontal measurement of ·7 mm.

The outer surface is covered with hairs, which will be fully described later, together with those of the hermaphrodite.

The alimentary canal is a tube of uniformly narrow calibre. The oesophagus is endowed with a sheath of circular muscular fibres. The stomach is narrow, not dilated, as in the pupa. Throughout its course the canal lies dorsal to and between the testes, seminal vesicles and ducti. It opens at the end of the body immediately dorsal to the base of the penis.

Nothing in the nature of food, faecal or excretory matter, was to be found in any part of the intestine. The caeca, two simple epithelial tubes, arise from the anterior part of the stomach and run for a short distance forward along the oesophagus.

The reproductive system consists of two simple tubular organs, uniting close to their external aperture at the tip of the penis. The different regions—testes, seminal vesicles and ducti—are distinguished only by the nature of their contents, the wall consisting of a fine layer of endothelium throughout, except close to the external aperture, where there is a sphincter.

B.—HISTOLOGY AND DEVELOPMENT OF THE ORGANS OF THE PEDUNCLE IN THE HERMAPHRODITE AND MALE.

It will be necessary to discuss this matter in some detail since the construction of the tissues, especially in the pupa, is somewhat complex, and since it is not easy to follow out the development of the separate elements owing to the fact that they change their appearance considerably in the successive stages.

Darwin (5, p. 20) was the first to give an account of the development of these organs, taking *Lepas australis* as the chief object of his study. He, however, regarded the cement glands as the incipient ovaria. The true relations of the cement glands and the ovaries were first defined by Krohn and Claus.

Hoek (8) gives an excellent account of the condition of these organs in the cypris of *Lepas australis*, with a figure which clearly distinguishes the cement apparatus, the young ovaries, the yolk masses, and the vesicular spaces of the peduncle. He also describes the cyprid larva of the male of *Scalpellum regium*, but confounds the testis with a yolk mass.

He describes the cement glands and ovaries of the adult from the study of a *Lepas*, of *Scalpellum vulgare* and *regium*, of *Conchoderma virgatum* and of a *Balanus*. Gruvel (7a, p. 448) also gives a short account of the cement glands and ovary of the cyprid of *Lepas australis*; he appears, however, to regard the vesicular spaces of the peduncle as giant cells—"cellules jaunes de la pedoncule." The claim of this investigator to have found ovarian cells in the "male" of *Scalpellum peronii* (7, p. 121) has been referred to above and will be considered again later.

Surveying all the stages of development in both the male and the hermaphrodite, the various elements which are found in the peduncle are as follows :—

(1) The vesicular spaces of the pupa.
(2) The rostral duct and the lymph-spaces of the adult.
(3) The connective tissue cells and fibres.
(4) The yolk.
(5) The cement cells and ducts.
(6) The ovaries.
(7) The coarsely granular cells of unknown function.

Before proceeding to consider these elements separately, it would be well to glance at pl. v, fig. 4, which gives a general view of the peduncular region of the pupa of a male. The vesicular spaces (*sp.*) occur throughout the region. The connective tissue cells are marked *f.g.* Yolk is marked *y.* and *f.g.* The cement cells (*r.c.*) are chiefly aggregated just above the larval eyes, and the coarsely granular cells (*x*) occur in the dorsal midline.

(1) *The vesicular spaces of the pupa* are always spherical, and it is hardly possible to doubt that they have been distended by some fluid. Their walls are formed by fine membrane or by any of the cellular elements. It is not clear whether they develop into the lymph-spaces of the adolescent and adult or not.

(2) *The rostral duct and the lymph-spaces of the post-pupal stages.*—The rostral duct is found in the hermaphrodite from the pupa onward (pl. vi, figs. 1, 2, 3) and in a less developed condition in the adult male. In the adult hermaphrodite it extends from the root of the prosoma to the base of the peduncle in the rostral midline and lies immediately internal to the muscular wall. At the base of the peduncle it curves inward to the centre of the peduncle and opens out into the network of the lymph-spaces. Small branches also pass off from it throughout the whole of its course. In the young 2·5 mm. adult its wall is identical in character with that of the lymph-spaces, being formed of delicate membrane with fine connective tissue nuclei. In the adult it acquires a thickened sheath of connective tissue.

In the male the rostral duct is shorter, reaching only from the root of the prosoma to the upper quarter of the peduncle.

This system of spaces forms an erectile tissue, by means of which the animal sways to and fro and elongates or shortens its peduncle.

(3) *The connective tissue cells and fibres.*—In the pupa, especially in the pupa of the male, the connective tissue cells are often of large size with a considerable mass of protoplasm and a rounded nucleus. In this condition they may also be loaded with yolk-granules, and it is not easy to distinguish them from the cement cells. Their protoplasm, however, does not stain at all with carmine, while that of the cement cells does take up this stain faintly. The more common form of connective tissue cell and

the only form in the later stages is the usual connective tissue nucleus with scanty protoplasm, occurring among the fibres and membranes.

(4) *The yolk* is the chief cause of confusion in separating the cellular elements from one another. It is most abundant in the pupa of the male ; somewhat less abundant in that of the hermaphrodite. Some yolk masses are still to be found in the adult male. It is not entirely confined to the peduncle, as it occurs around the stomach and ventral nerve cord in the prosoma. It is of a pale yellow colour and stains intensely with iron haematoxylin or eosin—not at all with basophil colours or carmine. It occurs chiefly in the connective tissue, which surrounds the vesicular spaces in the peduncle of the pupa. In its most dense form it stains intensely and uniformly with iron haematoxylin (*y.*, pl. v, fig. 4): after it has been partly absorbed, it has a finely granular appearance (*f.g.*, pl. v, fig. 4). When it completely surrounds a vesicular space, the latter has somewhat the appearance of a yellow cell—hence the "cellules jaunes de la pedoncule" of Gruvel (7a, p. 448).

The large eosinophil granules which occur in the cement cells of both the male and hermaphrodite pupa and in all but the most completely ripe males are also probably yolk-granules.

(5) *The cement glands and ducts.*—The glands are two groups of cells placed one on either side of the midline in the posterior (in the adult—upper) part of the peduncle. In the pupa of both hermaphrodite and male (pl. iv, figs. 4 [1] and 6) and in the adult male (pl. v, fig. 3) they are more or less spherical cells with abundant protoplasm and a spherical nucleus. A certain number contain in their protoplasm large granules of irregular shape, whose staining reaction is identical with that of the yolk (pl. v, figs. 3, 4). (See Hoek, 8, pl. ii, fig. 5, and p. 28.) The abundance of the granules varies in a manner parallel to the abundance of the yolk (compare Hoek, 8, p. 7); they are most numerous in the pupa of the male (pl. v, fig. 4), less in the pupa of the hermaphrodite, only disappearing in the male when it has reached complete maturity (pl. v, fig. 3), but not found in the adult of the hermaphrodite even at an early stage (pl. v, fig. 5). That is to say, they are gradually absorbed or extruded with increasing age. They are probably yolk-granules which may or may not be in process of conversion to form cement. In unstained preparations or in preparations stained with carmine the cells containing these granules have a yellow colour (pl. iv, figs. 5 and 6, *c.g.*) and may be mistaken for the "cellules jaunes de la pedoncule" referred to above.

In the young adult of the hermaphrodite (specimens measuring about 2·5 mm. in length, pl. v, fig. 5) the cement cells resemble the non-granular cement cells of the pupa and of the male, but on account of their size it can now be seen that there are certain large granules in the centre of the nucleus which stain more intensely and rapidly with iron haematoxylin than do the ordinary chromatin bodies. In the larger hermaphrodites (16 mm. and upward) the cells (pl. v, fig. 6) are very large (·3 mm.), the protoplasm finely granular. The nucleus (·12 to ·2 mm.) is polymorphic and

[1] In pl. iv, fig. 4, the letters *c.g.* point by mistake to the spaces of the peduncle instead of to the cement glands. The latter structures can be seen as a group of small but sharp nuclei lying above the eye.

contains five or six of the large darkly-staining granules which are almost certainly cement-substance. The protoplasm is finely granular, and contains in many cases small rounded vesicular nuclei, each containing a single chromatin granule. These minute nuclei appear to be identical with the wandering nuclei of the ova, and are doubtless supplying nourishment to the cement cells.

The glands in the fully grown specimens (compare Hoek, 8, p. 30) consist of these large cells scattered separately throughout the upper half of the peduncle, often lying among the ovarian tubes, but never in any way connected with these. Each cell is enclosed in a capsule of fibrous tissue, which is perforated at one point by the ductule (*d.*). This ductule, still retaining its fine nucleated wall, enters the body of the cement cell, approaches the nucleus, and branches freely around this latter structure (*dl.*) After leaving the capsule of the cell, neighbouring ductules unite together, and the ducts so formed enter certain curious little spherical bodies composed of fibrous tissue. Here the ducts are thrown into convolutions, the convolutions being bound together by the fibrous tissue of the nodules. On leaving the nodules they acquire an internal lining of cuticle. They unite with each other until finally only two main ducts are left.

The nodules are the bodies which Darwin regarded as the cement glands.

It appears then that, in the younger stages, many of the cement cells contain a considerable quantity of yolk from which the cement may be manufactured, and that in the older forms the nucleus is the portion of the gland cell which produces this secretion.

(6) *Development of the ovaries.*[1]—The youngest specimens in which it is possible to recognize the ovaries are adults measuring 2·5 mm. in length (pl. vi, fig. 2). In these two small aggregations of nuclei can be found embedded one on either side in the wall of the rostral duct in the uppermost portion of the peduncle (*ov.*).

The nuclei, of which not more than three appear in a section, lie in a thin cylindrical protoplasmic mass, measuring approximately ·3 mm. in length. This syncytium is surrounded by a delicate membrane continuous with the connective tissue of the wall of the rostral duct. The nuclei are minute (·005 mm. in diameter), generally oval, with a delicate nuclear membrane, very fine chromatin granules and a minute nucleolus. They cannot be distinguished from the ordinary connective tissue nuclei, except by their position and grouping together. There is no trace of a duct leading to the prosoma, and to attain the adult functional state the glands must grow both upward and downward.

In the pupa (pl. vi, fig. 1) at the corresponding points there are more nuclei than in other portions of the rostral duct. They are not, however, shut off, and, although some of them are doubtless the developing ovarian nuclei, it is not possible to say which are. They are generally about the same size as the ovarian nuclei in 2·5 mm. specimens. There is some likelihood from the appearances found in these sections that the two ovarian cylinders arise from the epithelium lining the rostral duct

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[1] Compare Hoek, 8, p. 7, and pl. ii, figs. 1 and 2.

by the formation of two grooves, and their separation from the wall of the duct, in the same way as the notochord is formed from the gut of vertebrate embryos.

In an adult measuring 5 mm. the young ovaries resembled those of the 2·5 mm. specimens, but were larger and more clearly marked off. In specimens measuring 10 mm. they were still longer, but were still fine unbranched solid cylinders.

In a specimen measuring 12 mm. (pl. vi, fig. 3) the tubes are also still single. They run through the upper quarter of the peduncle, and in the lower portion of their course are no longer embedded in the wall of the rostral duct. The distinction between ovary and oviduct has also appeared. In the lower ovarian portion the cylinder is definitely solid, and the nuclei are central: in the upper oviducal portion there is a rudimentary lumen, and the nuclei are peripheral.

The tube measures ·035 mm. in diameter, the nuclei ·005—·01 mm.

In a 16 mm. specimen they have practically reached the adult form, an oviduct runs along either side of the rostral duct, and ovarian tubes branching through the peduncle below the cement glands. At the fundus of an ovarian tube (pl. vi, fig. 4) inside the outer enveloping membrane (*gc.n.*) there is still the syncytium. The nuclei lie somewhat peripherally, almost identical in character with those of the 2·5 mm. specimen, but with larger nucleoli. Young ova (·04 mm. diameter) with abundant protoplasm, large vesicular nuclei (·015 mm.) with a single large spherical chromatin mass (·005 mm.), are found close to the fundi of the tubes (pl. vi, fig. 5).

In the mature hermaphrodite the ova can be classed in two stages: (1) without yolk,—the protoplasm vacuolated, the nucleus as in the 16 mm. specimens, except that it often contains a second smaller chromatin mass; minute nuclei are found in the protoplasm of the cell identical with those of the epithelium of the tubes;[1] (2) large ova crowded with yolk (pl. vi, fig. 6).

It will be noticed that in the course of its development the rudimentary ovary grows both upward toward the prosoma to form the oviduct and downward and inward into the peduncle to form the branching ovarian tubes.

(6*a*) In consequence of Gruvel's statement that he had found a rudimentary ovary in the male of *Scalpellum peronii* I carefully examined several dwarf specimens (males) of *Scalpellum squamuliferum* in various stages of development to ascertain if any trace of a rudimentary ovary could be discovered in this species. The specimens were very well preserved, and the sections were extremely good and complete, but no trace of such an organ could be found. It might be expected that, if such an organ did occur in the male, it would resemble the ovary of the hermaphrodite in one of its earlier stages of development, and would occur in the same situation. For this reason particular attention was given to the walls of the rostral duct, but not even the slight accumulation of nuclei at the two sides of the duct which occurs in the pupa of the hermaphrodite could be found. Nor could I find any cells of the character described by Gruvel (7, p. 121) which did not clearly belong to the cement glands.

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[1] Compare Gruvel (7a, p. 439). He regards these nuclei as belonging to unsuccessful ova, which are being consumed by the large successful ones.

(7) *The large coarsely granular cells of unknown function* occur in the peduncle in the young stages of the male. They are spherical, and are filled with large spherical granules or more properly vesicles. I have not been able to distinguish the nucleus. They have a degenerate appearance.

III.—ABSENCE OF A RUDIMENTARY OVARY IN THE MALE OF *SCALPELLUM PERONII*, GRAY.

Through the kindness of Dr. Calman and of the authorities of the British Museum I was able to examine a specimen of the male of *S. peronii*. This specimen was most excellently preserved, and after examining it entire in cedar-wood oil I obtained a complete set of sections.

These sections were first of all examined for the cells which Gruvel regards as the rudimentary ovaries, but none were found. The only cells with large nuclei and distinct nucleoli were the cells of the cement glands. I could not find any cells differing from them in appearance in the situation defined by Gruvel—namely, above the cement glands.

The rostral duct in *S. peronii* resembles very closely that of the male of *S. squamuliferum*. As in the latter species, the walls of this structure were examined in vain for any trace of a rudimentary ovary.

Cement glands of the male of *S. peronii*.—Gruvel (7) states that the cement glands are entirely contained in the peduncle—"acineuses très lobées." His figure (pl. iii, fig. 4) represents them as occupying the upper half of the peduncle.

In my specimen they did not extend below the upper eighth of the peduncle, but they extended well into the lower portion of the capitulum. In fact they formed a cup, the hollow of which was the lowest portion of the pallial cavity. They were compact, and not lobate. The cells are entirely uniform, and are identical in appearance with the non-granular cement cells of *S. squamuliferum*. I was able to trace one cement duct into their substance.

IV. THE ANATOMY OF THE MALE OF *SCALPELLUM BENGALENSE*, ANNANDALE.

Plate vi, fig. 7, represents the general outline of the male of *S. bengalense*. There is no marked outward boundary between the peduncle and capitulum. The appendages, etc., have been described by Dr. Annandale (1).

The mouth cavity is small in comparison with the size of the capitulum; since the wall of the capitulum is relatively thick, the true body is also of course small.

The adductor scutorum muscle persists in its usual situation, although in the majority of specimens there are no scuta. It may be able to narrow the opening of the pallial cavity.

Alimentary system.—The alimentary canal has the usual V-shape. Both mouth and anus are open. The stomach is a fairly large sac, but it, as well as the other portions of the canal, are empty. The two caeca arising from the anterior end of the canal are present.

Nervous system.—There is a pair of large cerebral ganglia and a massive ventral nerve cord. The hairs which cover the outer surface doubtless fulfil the usual sensory function—*vide infra*, p. 16.

Reproductive system.—The testes occupy the same position as in *Scalpellum squamuliferum*, that is, one on either side of the stomach. At the root of the prosoma there is a somewhat indefinite space, which may possibly be the equivalent of the rostral duct. There are no cells which it is possible to recognize as ovarian.

The cement glands are two groups of cells, identical with those of *S. squamuliferum*, which are to be found in the second quarter of the length of the peduncle counting from above. As in *S. squamuliferum* many of them contain the large irregular yolk-like granules which stain intensely with haematoxylin, while a smaller number are without these. Ducts were not found.

The yolk also appears in the peduncle just as in *S. squamuliferum*. It, however, tends to lie more above than below the cement glands. In some cases the yolk masses appear to be lying actually within cells,—vesicular cells with flattened nuclei,—the yolk mass filling up the vesicle.

It is a noteworthy fact that two species in which the hermaphrodite forms resemble each other so closely as *S. squamuliferum* and *bengalense* should possess males which differ to such a degree.

V.—POST-LARVAL DEVELOPMENT OF THE MALE OF *SCALPELLUM GRUVELII*, ANNANDALE.

(1) THE CYPRID LARVA.

It closely resembles in its structure the cyprid of *S. regium* described by Hoek.[1]

Plate vi, fig. 8, represents a cyprid after attachment but before the first post-larval ecdysis. It measures 1 mm. in length, ·6 mm. in height. The prosoma bears six pairs of jointed legs. In comparing it with the slightly older stage of *S. squamuliferum* in which the ecdysis is in progress, various differences due to the increased degeneracy and specialisation of the male adult will be noticed. The alimentary canal is reduced to a closed oesophagus (pl. vii, fig. 2, *oes.*) and a small sac-like stomach (pl. vi, fig. 8, *st.*) with two blunt forward prolongations representing the caeca (pl. vii, fig. 2, *st.*). There is no hind gut or anus.

The nervous system is still well developed, but the segmentation of the ventral nerve cord (*v.n.c.*, pl. vi, fig. 8, and pl. vii, fig. 2), if present, is not perceptible. The nerve cord appears twice in the transverse section represented in pl. vii, fig. 2, owing to the curvature of the prosoma. I have not found larval eyes in the cyprid.

The testis (pl. vi, fig. 8, and pl. vii, figs. 1 and 2, *t.*) is relatively much greater. It occupies the space given up to the stomach and intestines in *S. squamuliferum*. It

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[1] Compare Hoek, 8, p. 7.

is surrounded by a layer of mesodermal parenchyma cells (*m.p.*) continuous with the parenchyma which forms the mass of the prosoma behind the testis and nerve cord. The gonoduct, at this stage represented by a solid rod of cells passing from the anterior pole of the testis for a short distance upward and backward, can be seen to be developed from the parenchymatous covering, and not from the testis itself.

Hoek (8) does not describe a testis in the cyprid of *S. regium*. He refers, however, to a "dark-coloured mass consisting for the greater part of yolk fragments," which "makes up a great deal of the true body." From his figure it appears probable that this mass is the rudimentary testis.

The cement glands (pl. vi, fig. 8, *c.gl.*) occupy a different position from those of *S. squamuliferum* and *S. regium*, being confined to the capitular instead of the peduncular half of the body. They also occur in this situation in *S. triangulare* among others. They are made up of large cells crowded with spherical granules, whose staining reactions are the same as those of the granules of the cement cells in *S. squamuliferum*. They are, however, smaller and more regularly spherical.

I have not been able to distinguish any definite cement ducts in the cyprid.

(2) THE PUPA.

In the pupa after the first post-larval ecdysis considerable changes have taken place. The testis has greatly enlarged itself, growing forward to occupy the greater part of the peduncular half of the body and carrying the pallial cavity with it. The pallial cavity has become reduced in breadth, and associated with this change is the reduction and degradation of the prosoma.

Hairs have been developed on the surface under the loosened larval integuments.

The alimentary canal has rather advanced than retrograded, since the oesophagus is now open. The stomach is filled with a mass of granules and globules, apparently excretory, and doubtless the opening of the oesophagus is due to the necessity of discharging this mass of excrement.

The position of the cerebral ganglia and ventral nerve cord has been affected by the general torsion consequent on the growth of the testis and the reduction of the prosoma. They both now lie outside the prosoma. They have also undergone considerable reduction in size.

The cement glands are also somewhat reduced, and two cement ducts can be distinguished running from the glands to the antennae.

Plate vii, figs. 3 and 4, represent transverse sections through a pupa, fig. 3 passing through the stomach (*st.*), testis (*t.*), the anterior poles of the cement glands (*c.gl.*) and of the ventral nerve cord (*v.n.c.*), *ps.* indicating the prosoma lying surrounded by the pallial cavity; fig. 4 is somewhat further back, and shows the oesophagus between the cerebral ganglia and the ventral nerve cord. It also gives a good representation of the structure of the cement glands.

This animal in this stage appears to closely resemble in outer form the adult male of *S. velutinum*, Hoek (Gruvel, 7, pl. iv, fig. 11).

(3) THE ADULT MALE.

The adult of the male is a pear-shaped little body, the anterior half being thicker than the posterior. It measures 1 mm. in length, ·5 in maximum breadth.

Plate vii, fig. 5, represents a specimen slightly flattened out by mounting. The antennae are in the position characteristic of the larva, and not of the ordinary adult, since this form does not undergo the unfolding and straightening out which is characteristic of the development of the ordinary adult from the pupa

The sensory hairs are to be seen in pl. vii, fig. 5. They are supplied with nerves in the same way as the hairs of *S. squamuliferum*. The muscle fibres running principally from the anterior to the posterior pole are clearly indicated.

The pallial cavity and prosoma are much reduced, as in the case of *S. regium*, described by Hoek, but I cannot agree with this author's statement that the pallial cavity is lined by a layer of condensed connective tissue—this layer is clearly a fine epidermis. Neither the cavity (*p.c.*) nor the prosoma is clearly shown in fig. 5, but they both extend through the posterior three-quarters of the body.

The alimentary tract is represented by a small hollow ball of cells (*st.*) containing some cuticular and excretory matter.

The nervous system is also much reduced. The cerebral ganglion (*d.n.g.*) in fig. 5 has been somewhat shifted from its proper place, which is on the opposite side of the stomach from the reduced ventral nerve cord.

The testis (*t.*) is heart-shaped; the seminal vesicles being empty were not visible in the incompletely mature specimen from which fig. 5 was taken. The ejaculatory mechanism apparently acts in the following manner. On the receipt of some stimulus —very probably conveyed by the sensory hairs and passed through the reduced central nervous system—the longitudinal muscles contract, the prosoma carrying the ejaculatory duct is thrust through the opening of the pallial cavity, and at the same time the tension in the little body is so raised that the seminal vesicles evacuate themselves.

The cement glands (*c.gl.*) consist of two or three small spherical masses of cells lying posterior to the testis. Some of the cells contain the same granules as in the cyprid. Two long fine ducts lead to the antennae.

VI.—*IBLA CUMINGII*, DARWIN.

A.—ANATOMY OF THE MALE (pl. iv, fig. 7).[1]

The body is vermiform. The prosoma is only partly marked off from the peduncle. The mantle takes the form of a hollow cup, from the base and dorsal side of which the prosoma arises.

Black pigment is distributed around the mouth and on the legs. It is noteworthy that in the prosoma of the female pigment is also confined to these two regions.

[1] Compare Darwin, 5, p 189.

The external anatomy will be fully dealt with by Dr. Annandale in a later publication.

The oesophagus is thick and muscular, the stomach rounded and filled with small yellow globules. The anus is open and lies between the last pair of appendages. The alimentary tract has all the appearance of being actively functional.

Nervous system.—The nervous system consists of two closely approximated oval cerebral ganglia, bearing the large eye at their junction, and of a large ventral ganglion representing the ventral nerve cord. These two are connected by the usual commissures.

There is no cerebral ganglion close to the mouth as represented by Gruvel in the case of *Ibla quadrivalvis*. In this place it is true there is a small cellular body which might be mistaken for a ganglion, but it is in reality a small salivary gland, and is not connected with the nervous system.

The eye consists of an almost closed retinal vesicle of nerve cells. This vesicle contains pigment granules arranged in symmetrical masses like the segments of an orange inside the peel. At the pole of the eye nearest to the surface the retinal layer is absent and the pigment comes to the surface of the eye.

The reproductive system consists of two many-lobed testicles, two muscular vasa, two seminal vesicles and a terminal ductus. There is no penis.

The cement glands are two masses of cells lying below the testicle. They are entirely free from the granules which occur in *Scalpellum squamuliferum, bengalense* and *gruvelii*.

There are none of the yolk masses such as occur in *Scalpellum*, but the tissue spaces of the peduncle are filled with minute fine granules, which are doubtless of the same nature.

B.—ABSENCE OF RUDIMENTARY TESTES IN THE FEMALE OF *IBLA CUMINGII*.

The entire prosoma of a specimen was cut into serial sections and carefully examined for the rudiments of a testis, the developing testis of the hermaphrodite of *Scalpellum squamuliferum* (*vide supra*) being examined for comparison. No vestige of such an organ was found.

Hoek (8. p. 19) made the same examination of the large form of *Scalpellum regium*, H., and did not find any trace of a testis.

VII.—SENSORY HAIRS IN *SCALPELLUM SQUAMULIFERUM, BENGALENSE* AND *GRUVELII*, AND IN *IBLA CUMINGII*.

(Pl. vi, figs. 9 and 10.)

The entire surface of both capitulum and peduncle in the above three species of *Scalpellum* is covered with minute hairs both in the dwarf and large forms. The peduncle of *Ibla cumingii* is also covered with hairs, but they are of a different nature from those of *Scalpellum*, while the male is bare.

Genus *Scalpellum*.—The distribution of the hairs is bilateral; they are parted in the carinal and rostral midlines, all the hairs of each side being directed towards the rostral line. They are fairly uniformly distributed, but in the male of *S. bengalense* there is a broad band of larger hairs on either side of the carinal midline.

The hairs appear to be specialised outgrowths of the outermost layer of the cuticle. They consist of an outer cortical portion which (in contrast with the cuticle) stains intensely with iron haematoxylin and a core which does not stain.

In the hermaphrodite of *S. squamuliferum* and *bengalense* the hairs are large, spike-like and compound. The main shaft arises from a bell-like base, which rests on a papilla of cuticle. Small simple branches arise from this base from the lower portion of the shaft. The base of the hairs is broader in *S. bengalense* than in *S. squamuliferum*, and the branches approximate more nearly in size to the main shaft.

In the male of *S. squamuliferum* the hairs are perfectly simple, but in that of *S. bengalense* the larger hairs have a bifid tip. In both forms of *S. gruvelii* the hairs are simple, and not so rigid as in the other two species.

The hairs in all three species are supplied with nerves (fig. 9). Fine tortuous fibrils can be seen traversing the cuticle or the valves from the epiderm to the bases of the hairs. These fibrils consist of a strongly staining cortex and an unstained medulla. Arrived at the base of a hair the cortex becomes somewhat thickened and ceases, while the medulla enters that of the hair.

In the male of *S. bengalense* I found a small ganglion in the outer wall of the pallial cavity in the carinal midline (fig. 10). It consists of a single row of cells, which are continuous with the epidermis, but rise slightly above the general level. A thin layer of intensely staining nervous matter spreads out from the nuclei on either side under the bands of larger hairs, to which I referred above. On entering the bodies of the ganglion cells this nervous matter forms a series of thickened rings around the nuclei. It is not clear whether the nervous matter consists of a series of fibrils or of a continuous membrane-like expansion.

The cuticle is either entirely absent or very much thinned over the ganglion.

In the hermaphrodite of *S. squamuliferum* the cuticle is invaginated inward between the contiguous valves at the base of the capitulum, narrow finger-like canals being formed, which are open at their outer and upper ends, and pass downwards and inward through the substance of the cuticle to come in contact with the epiderm. They are thickly lined with hairs and presumably are especially sensitive to vibration of the water.

Genus *Ibla*.—In *Ibla* the hairs are stouter and more hollow. The outer layer of the hair arises from the innermost layer of the cuticle, while the cortex of the hair, although not cellular, is continuous with the epidermis. The entire hair thus passes through the thickness of the cuticle.

As regards the question of theory referred to on p. 34, our results (*vide supra*, pp. 43, 44 and 48) are negative. There are no traces of female organs in the males of

Scalpellum squamuliferum or *peronii* or of male organs in the female of *Ibla cumingii*. There is therefore no proof that, in the change from the hermaphrodite to the unisexual condition, the loss of one set of sexual organs has been a gradual change due either to disuse atrophy, or to the semiparasitic habit of the male (*vide supra*, p. 35).

It appears justifiable to emphasize one point, although it has been known to science since the time of Darwin, namely, that the only distinction between the species *Ibla quadrivalvis* and *cumingii* lies in the fact that the large form is hermaphrodite in the former and female in the latter. Experimental work on Mendelian lines has suggested that the distinction between the two sexes of one species is of the same nature as that between the varieties of one species. The distinction between the two Iblas appears closely to resemble that between a "negative variety" of De Vries and the parent species. The negative variety differs from the parent species in having lost one character, generally that of colour, and in *Ibla cumingii* the large form has lost the single character of possessing male sexual organs.

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EXPLANATION OF LETTERS USED IN PLATES.

ad.m., adductor scutorum muscle; al.c., alimentary canal; ant., antennae; a.p.c., aperture of pallial cavity; b.sh., bivalve shell; ca., carina; caec., caecum; ce.g. and cer.g., cerebral ganglion; c.g. and c.gl., cement gland cell; cu. and cut., cuticle; d.c.g., dorsal cerebral ganglion; d.e., ductus ejaculatorius; e., eye; e.c., cuticle of cyprid surrounding pupa; ect.n., ectodermal nucleus; ep., epidermis; ep. 1, epidermis lining pallial cavity; ep. 2, epidermis of true body; f.g., finely granular yolk mass; gang., ganglion; g.c.n., gonocoel nucleus; h., hair; i.c., cuticle of pupa; int., intestine; la., laterum; l.m., longitudinal muscle (in pl. v, fig. 2, points to mass in stomach); m., mouth; m.p., mesodermal parenchyma; n., nerve; oes., oesophagus; o.d., wall of ovarian tube; ov., ovary (ov. in pl. iv, fig. 5, indicates ovum); ov.n., ovarian nucleus; p., penis; p.ap., pallial aperture; p.c., pallial cavity; p.s., prosoma; r., rostrum; r.c., red cells = cement cells; r.d., rostral duct; sc., scutum; s.h., sensory hair; sp., spaces of the peduncle; st., stomach; t., testis; te., tergum; ves.sem., vesiculae seminales; v.n.c., ventral nerve cord; x, cell of unknown function; y., yolk; y.c., yolk cell.