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NOTES ON THE DIDEMNIDAE (ASCIDIACEA)

II. THE NUMBER OF ROWS OF STIGMATA IN *DIDEMNUM GELATINOSUM* MILNE EDWARDS AND IN *DIDEMNUM MACULOSUM* (MILNE EDWARDS)

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Milne Edwards (1841) established the genus *Leptoclinum* for those Didemnidae which possessed a largely expanded common cloacal system. His type of the genus was *L. maculosum* which is now placed in the genus *Didemnum* Savigny (1816). Other species were *L. asperum* and *L. durum* which are now regarded as mere forms of *Didemnum maculosum* (e.g. Harant & Vernières, 1933); *Leptoclinum fulgens* which is closely related (see Harant & Vernières), if not a mere colour form of *Didemnum maculosum*; *Leptoclinum listerianum* which has been transferred to the genus *Diplosoma* Macdonald; and *Leptoclinum gelatinosum*. This last has often been considered synonymous with *Diplosoma listerianum*, but my reasons for disagreeing with this assumption are elsewhere expressed (Carlisle, 1953). Milne Edwards did not of course describe the course of the sperm duct by which *Diplosoma* is distinguished from *Didemnum*. In the absence of a description of this diagnostic character we are reduced to a consideration of numerous details of the anatomy of *Leptoclinum gelatinosum* in order to decide where to place it in the classificatory scheme. When such a point-by-point comparison of shape and curvature of the gut, position of anus, form of atrial and buccal apertures, nature of test, etc., is made, it appears that the one point in which the drawings and descriptions given by Milne Edwards differ between *L. gelatinosum* and *Didemnum gelatinosum* Milne Edwards (1841) is the presence of a common cloacal system in the former and its absence in the latter. But the presence or absence of a common cloacal system is no longer regarded as a generic distinction (see, for example, van Name, 1945). Berrill (1950), on the basis of one specimen, and presumably with Milne Edwards's authority, states that *D. gelatinosum* has no common cloacal system. I am unable to agree with this statement. Large colonies of *D. gelatinosum* collected and identified by myself, and other large colonies collected and determined by Miss P. Kott (and in the type collection of this laboratory), frequently contain a large common cloacal system stuffed with faecal pellets, whereas small colonies are without such cavities. It seems to me then, that we have no clear grounds for separating *Leptoclinum gelatinosum* Milne Edwards from *Didemnum gelatinosum* Milne Edwards (1841) and, so far as it

is possible to recognize the species from his descriptions, these are most probably synonymous, particularly as his drawings and descriptions of *D. gelatinosum* are based on a small specimen with only a small number of zooids, while his description and drawings of *Leptoclinum gelatinosum* are evidently based on a much larger colony. The identification of the one with the other can of course never be certain. Certainly, however, the description of *L. gelatinosum* is unlike that of any other species of didemnid found in the English Channel.

One thing stands out as strange in Milne Edwards's drawings and descriptions—he states that in *L. gelatinosum*, 'Le sac branchial est garni de cinq rangées de fentes stigmatiformes' (1841, p. 300), and he illustrates it so. Similarly, he states of *Didemnum gelatinosum*, 'Le thorax est gros et n'offre que cinq rangées transversales de stigmates branchiaux' (p. 296). He figures it with sometimes three, sometimes four, and sometimes five rows of stigmata (his plate 7, figs. 5*a–e*), so that his 'n'offre que cinq rangées' must mean 'has no more than five rows,' rather than 'has only five rows'. How is all this to be squared with the usual statement that the Didemnidae possess either three or four rows of stigmata—e.g. van Name's statement (1945, p. 78) 'There are (apparently always) either three or four rows of stigmata'—or with the diagnostic difference usually offered between *Didemnum* and *Tridemnum* that the former has four rows and the latter only three rows of stigmata? Clearly, since the species *Didemnum gelatinosum* is ascribed to Milne Edwards this matter requires a fresh investigation. If the species that we now call *D. gelatinosum* Milne Edwards always possesses four rows of stigmata then it cannot truly go by this name in view of Milne Edwards's deliberate statements. At the same time it would be as well to examine other species ascribed to Milne Edwards to determine the situation there.

Two species of *Didemnum* are to be found in the Plymouth area in abundance, *D. gelatinosum* and *D. maculosum* auctt. (?Milne Edwards). An examination of a large number of colonies of these two species has shown that the majority of zooids possess four rows of stigmata apiece. Before the breeding season commences, however, when the colonies are growing actively by vegetative means, the zooids around the edges of the colonies are much smaller. These zooids have only three rows of stigmata each, and usually one or two less than usual in each row. During the period of sexual reproduction the number of these smaller zooids is progressively less, and successive tracing of the outline of colonies at Salcombe (Devon) at monthly intervals has shown that during this season growth in area of the colonies is almost at a standstill. At any season of the year in colonies with more than about forty zooids one can usually find one or a few thoraces with five rows of stigmata in both species. Sometimes, when a colony, from the abundance of the faecal pellets and from the general appearance, seems particularly well fed, quite a high proportion of the zooids possess five rows of stigmata, even as high a proportion as 15%

when the animals are not in the breeding season. Towards the end of the breeding season, when the larvae are fully developed, the thoraces of the zooids are resorbed and only abdomina, larvae, and faecal pellets are to be seen in the colonies.

The zooids around the edges of the colony are always smaller than those nearer the centre. The difference of size is more pronounced in the thorax (which, in a zooid from the centre of the colony, may be $2\frac{1}{2}$ times as large [linear measure] as in one from the periphery) than in the abdomen (which is rarely more than $1\frac{1}{2}$ times as big). The difference in size of the abdomina is reflected in that of all the organs. In particular, the spermduct makes fewer turns around the testis in the smaller zooids, and the mature ovum is smaller. Correspondingly, the larva developed from a smaller ovum, derived from a smaller peripheral zooid, is smaller than one produced from a larger central zooid, and such a smaller larva is furnished with only three rows of stigmata instead of the usual number of four which is found in the larvae in the centre of the colony. Larvae from the centre of the colony of *D. gelatinosum* have five pairs of anterior ectodermal ampullae, while the smaller peripheral ones have only four pairs; in *D. maculosum*, which has a smaller larva, the central larvae have four pairs of ampullae, or rarely five, while those from the periphery have only three (see also Kott, 1952). Further differences are to be seen in the anatomy of the zooids from the centre and from the periphery of the colony. The course of the gut is less circuitous in the smaller peripheral zooids. Thus in *D. maculosum* the rectum in the larger central zooids makes two sharp bends to form a shallow S (see Milne Edwards, 1841; Millar, 1949); in the smaller zooids from the periphery the course of the rectum is almost straight. This may perhaps be correlated with the larger amount of food that the larger thoraces may supply to the central zooids, which requires a longer gut for its digestion. The mid-intestine is relatively rather larger in the smaller zooids, though absolutely slightly smaller.

Evidently Milne Edwards was correct in his statement that *D. gelatinosum*, 'n'offre que cinq rangées transversales de stigmates branchiaux' (1841, p. 296), and that in *Leptoclinum maculosum* the structure of the zooids 'est essentiellement la même' (p. 298). His descriptions and figures suffer simply from the general fault of those of his period—he always worked from the largest and best developed, never from the typical or average specimen. This is true not merely of his descriptions and figures of didemnids but also of other ascidian species. More recently the emphasis has been on examining average specimens; in determining the species of a colony of a didemnid it is usual practice to cut off and dissect a 'typical portion', neither too thick nor too thin, nor too near the edge. Such a portion would contain almost entirely zooids with the usual number of rows of stigmata for the colony—four in *Didemnum*. It is notoriously difficult to count the number of rows of

stigmata, especially in dead and preserved specimens, and indeed neither Michaelsen (1923) nor Hartmeyer (1924) was able to determine the number of rows in the species which the former named *D. helgolandicum*. Accordingly, it is usual to examine a number of zooids and discount as impossible to observe properly any which seem to show an unusual number of rows of gill-slits. Thus any zooids in a *Didemnum* colony with other than four rows of gill-slits would be unlikely to be taken for examination and if examined would most probably be discounted. It is only when one examines living zooids of *Didemnum* species that the number of rows of gill-slits is seen, with practice, clearly enough for one to be unable to mislead oneself with a belief that unusual zooids are actually ones which are difficult to observe correctly and not really unusual at all. It comes as rather a shock to see five rows of gill-slits in a living zooid of *D. maculosum*, with each slit clearly outlined by a ring of beating cilia, and to realize that preserved ones, previously discarded as poorly preserved and misleading, really did show five rows after all. It may be noted that Milne Edwards (1841, p. 219), unlike most of his successors, examined his specimens alive.

The account given by Salfi (1933, 1950) of budding in the Didemnidae gives some explanation of the way in which the number of rows of stigmata may increase with the age of the zooid. He describes budding in these forms as proceeding by the formation of half-buds. The oesophageal region of a zooid buds off separate thorax and abdomen, either together or at different times. Either of these half-buds can complete itself by the formation of the other half, or two halves formed simultaneously and near together may combine. But more thoracic buds are formed than abdominal buds and not all of these go to form fresh zooids. Some of them are used by the parent zooid to replace its own thorax which is worn out. The old thorax is resorbed and the newly budded one takes its place, or rather the other way round, for the new one begins to function before the old thorax is resorbed. This may apparently happen several times in the life of a single zooid. This I can confirm from my own observations. At a time when the colony of *D. maculosum* is growing most actively by vegetative means, most new zooids are formed from abdominal buds which then form their own thoraces. Such buds are small, chiefly around the edge of the colony and most have only three rows of stigmata. As they grow larger a new thoracic bud is formed, by each such zooid, which takes over the function of the old thorax which is now too small and worn out. This new thorax has the typical four rows of stigmata. This may happen several times as the zooid grows larger until when it is very large indeed a new thorax may be large enough to have five rows. The limiting factor for the number of rows of stigmata seems to be the size of the thorax at the time when it begins to take over its functions. Once it is functioning there seems to be no further development of fresh rows of stigmata, although there may occasionally be an increase in the number in one row. When the

breeding season starts most of the energy of the colony goes to the production of embryos. New budding and growth of the colony stops and vegetative growth seems confined to necessary repair work: zooids may replace their thoraces but not form entirely new buds. This may account for the decrease in number of zooids with three rows of stigmata during the breeding season. Small zooids naturally produce small eggs and the larger, older zooids produce larger eggs. Again the size of the thorax of the larva seems to determine how many rows of stigmata it shall have—as many rows develop as there is room for. Small larvae have only three rows, larger ones four. It is probable that in the past larvae with only three rows of stigmata from *D. maculosum* have been interpreted as not fully developed, but this is not so, for free-swimming, small larvae may possess only three rows up to metamorphosis, beyond which I have not observed them further. Such differences in the structure of larger and smaller larvae may be compared with similar differences which I have observed in such oviparous species as *Phallusia mammillata* (Cuvier), between larvae obtained by artificial fertilization of eggs from the oviduct and of eggs from the ovary, which are always slightly smaller and produce larvae with a simplified sensory system and other simplifications and reductions in their anatomy.

The situation with respect to the number of gill-slits is similar in *Didemnum* (*Leptoclinides*) *faeröense* (Bjerkan) as described in part I of these notes (Carlisle & Carlisle, 1954). In *Diplosoma* spp., however, I have never seen other than four rows of stigmata in any zooid.

Summary

The number of rows of stigmata in three species of *Didemnum* varies from zooid to zooid within the colony. Peripheral, newly formed, small zooids have only three rows, most zooids have four rows, while a few very large ones have five rows. This is probably a result of the replacement of the thorax by partial budding during the life of the zooid, accompanied by a steady increase in size. The number of rows of stigmata seems to be always four in *Diplosoma* species. The importance of examining living specimens is stressed.

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