

Zoology. — *On Laevicolica and Dextricolica in Tunicates.* By J. W. VAN WIJHE (Anatomical Laboratory, Groningen).

(Communicated at the meeting of March 23, 1929).

Last year, I suggested that the position of the intestinal loop and anus, morphologically either to the right or to the left side of the animal, would form a fundamental difference between the group of the Copelata (Appendicularians) and the other Tunicates (Acopa).

As terms based on positive characters I proposed *Dextricolica* (= Copelata) and *Laevicolica* (= Acopa¹).

The morphological median plane (i. e. the plane dividing the thyroid gland and the central nervous system into right and left halves) is better recognizable in the young animal (larva or bud) than in the adult. In the young animal it is generally a flat plane, but in the adult it may have got curves by the shifting of organs.

I trusted there would exist only apparent²) but no real exceptions to the rule that in the *Dextricolica* the loop morphologically lies on the right side of the animal, in the *Laevicolica* on the contrary on the left side.

Sooner than I could expect this was confirmed *in part* by such a distinguished morphologist and specialist in Tunicates as prof. GARSTANG, who had partly arrived at the same conclusion independently. He says (1928, p. 180) in his very suggestive essay, rich in facts and hypotheses: "In his latest paper (1928, p. 997) VAN WIJHE has anticipated me in drawing attention to the contrast between Appendicularians and other Tunicates in regard to the twist of the intestinal loop, but by overlooking the exceptional cases of *Doliolum* and *Anchinia* he has been unfortunately led into an untenable hypothesis³) The existence of *Doliolum*, however, with its median gut and anus prohibits the sharp division of Tunicates into 'Dextricolica' and 'Laevicolica'".

So the rule, that in my opinion is applicable to the whole group of

¹) The term "*Acopa*" is defective, being based on a negative character (absence of a tail in the adult).

²) As an *apparent* exception I mentioned that in Corellidae the intestinal loop in the adult lies on the right side of the gill-basket. But in the young larva DE SELYS LONG-CHAMPS (1900) has found that the anus opens in the left peribranchial pouch.

³) Namely the hypothesis (GARSTANG, l.c.) "that the right intestine of Appendicularians and the left intestine of other Tunicates together represent the second pair of gill-slits in *Amphioxus*, of which the left can be regarded as more potent than the right!"

I hope to show in the sequel that the hypothesis is not "untenable".

Tunicates, would become a rule of minor importance if the exception of the order of Doliolids would hold true as GARSTANG maintains.

The point in question is, whether in the young larva of *Doliolum* the intestinal loop lies either on the right or on the left side of the central nervous system.

Now GARSTANG was not able to solve the question. He says (l. c. p. 100, 101). "Unfortunately the tail of *Doliolum* is not functional as a locomotive organ, and its nerve-cord appears to atrophy at a very early stage . . . but a number of striking peculiarities in the tail and other organs corroborate the view of a close relationship with Appendicularians". He enumerates four of them (l. c. p. 101) to none of which I can attribute much force.

The second of these peculiarities might have been conclusive, as it concerns the relations between the nerve-cord and the intestine, but it runs: „The nerve-cord, as it passes backwards between the peri-branchial involutions, bends down the *right* side of the future oesophageal¹⁾ region as in *Oikopleura* (NEUMANN, Taf. II [XII] figs. 21, 22)".

This fact is rather suggestive of *Doliolum* belonging to the Laevicolica instead of to the Dextricolica (Appendicularians) and we shall see this proved in the bud of *Anchinia*, where the nerve-cord in its dextral flexure is much more developed than in the larva of *Doliolum*. In the figures of NEUMANN the nerve-cord in this flexure is very rudimentary as it shows only one cell in the transverse sections.

GARSTANG in his textfigure 5c „hypothetical larva of primitive Doliolid" (l. c. p. 97) shows the nerve-cord passing the "rectum"²⁾ at the left side, thus making it to represent one of the Dextricolica like the Appendicularians. This figure includes a *petitio principii* as it intends to demonstrate the argument he was not able to establish. The figure must be wrong, as we shall see in a moment.

We may now pass to *Anchinia* where to the observant reader the case is clear, provided that the description³⁾ of BARROIS (1885) be compared with his figures.

In clinging to the text, without paying much attention to the figures,

¹⁾ If we compare the larva before cutting (NEUMANN, 1905, Taf. XI, fig. 5) with more advanced stages (Taf. XIII, figs. 1, 2) I believe that the sections (Taf. XII, figs. 21, 22) as they pass by the hinder part of the external cloacal slit, will not show the oesophageal but the stomachal region of the intestinal primordium. This difference, however, does not influence the considerations in the text.

²⁾ The term "rectum" is generally used to denote the whole distal limb of the intestinal loop (the proximal limb being formed by oesophagus and stomach). In various Tunicates the distal limb is microscopically and macroscopically differentiated in two different parts.

In these cases it is desirable to restrict the term "rectum" to the part at the anus. The other part, beginning at the stomach, may be termed "colon".

³⁾ This description, founded on pretty plenty material, is a revision and considerable extension of the short paper, founded on rather scanty material, by KOWALEVSKY and BARROIS (1883).

one might get the impression that the bud of *Anchinia* would pass by an Appendicularian-stage. BARROIS was so much under this impression by the striking similarities he had discovered, that he tried in any way to see the distal limb of the intestinal loop in his bud on the right side of the body. His preparations, however, clearly show that its situation is on the morphologically left side.

At the time that the anus acquires its external opening he remarks (l. c. p. 233) "Il existe donc un stade dans lequel l'anús débouche par le fait à la surface de la peau (fig. 20¹) entre les ouvertures des deux poches cloacales [peribranchial pouches] et à droite du tube nerveux".

The figure here quoted is, however, not fit to demonstrate the position of the anus at the right side of the neural cord. We shall see below that, according to other figures, almost the whole of the distal limb ("rectum") of the intestinal loop remains at the left side of the cord and that only its extreme end with the anus crosses the *dorsal* side of the cord to open a little to the right of the *topographical* median plane.

Speaking on the distal limb of the intestinal loop he says on the same page: "elle n'occupe pas une position rigoureusement médiane mais se trouve légèrement infléchiée vers la droite (fig. 23 A, 23 B)".

But the hind part of the nerve-cord (and *pari passu* this part of the morphologically median plane) is also very markedly inflected to the right in fig. 23 B²).

On our plate are copied four figures of BARROIS (succession of the stages see footnote¹). They show two successive buds³) in dorsal aspect (fig. 19 A and 20 C), a frontal section through a bud of the next stage (fig. 21 B), and a more advanced bud, seen from the right side (fig. 24).

Fig. 19 A shows the nerve-cord still straight. Swollen at both ends and thinner in the middle it displays an astonishing resemblance to the primordium of the nerve-cord of a mammal at the time of the appearance of the first mesodermic somites. Of course this resemblance is only a curiosity.

The external openings of the peribranchial sacs are still separate. The stomach lies not quite symmetrically, but already more in the left than in the right half of the body. The "rectum" is developing as a little outgrowth directed dorsalwards from the stomach (not visible in this figure). It has not yet attained the ectoderm.

¹) The succession of the numbers of the figures in BARROIS' work (e.g. 19, 20, 21, 22, 23) indicate at the same time the succession of the stages. Different aspects of the same stage are represented by addition of a type (e.g. 23, 23 A, 23 B).

²) In the figure quoted (Pl. XI, fig. 23 B) it is not to be seen whether the anus crosses the dorsal or the ventral side of the cord, but that it must be the dorsal side is obvious in various figures of Pl. X e.g. figs. 20, 24, 25, 26, 27.

³) BARROIS describes three different series of buds, two of them remaining sterile, only the third developing genital glands to maturity. As in the first series the neural cord is best developed, I shall confine myself especially to this series.

Fig. 20 C, the next stage, is important for our purpose as it shows the curvature of the hind part of the neural cord to *the right side* of the body, so that now the cavity of the stomach lies almost quite to the left side of the cord. This curvature must have been caused by the development of the "rectum" growing dorsalwards from the stomach. We see here the dextral flexure of the cord better developed than in the larva of *Doliolum*. In this larva the cord is very rudimentary at the flexure and the swollen hind end is absent. It is also lacking or rudimentary in both the other series of buds of *Anchinia*.

The external openings of the peri-branchial sacs are coalescing on the dorsal side of the neural cord and here the anus appears (not shown in the figure) at this stage.

Fig. 21 B represents a very interesting frontal section. Both thickenings (the rostral and the caudal, not the thinner middle part) of the neural cord are cut. The stomach lies as clear as possible *to the left* of the caudal thickening. The explanation of the figure only says that it is a "coupe horizontale" but a comparison with fig. 20 C is convincing to see that the left side of the figure corresponds to the left of the animal¹⁾.

Fig. 24 represents a more advanced bud, seen from the right side. It shows clearly that the intestinal loop lies to the left of the neural cord. The posterior thickening of the cord (three large cells lying on it in the figure) lies to the right of the spot where the stomach is continued in the distal limb of the intestine. BARROIS says (l.c. p. 235)²⁾ "La portion postérieure du tube nerveux . . . s'infléchit vers la droite, et le renflement arrondi qui la termine vient se placer sur l'intestin [i.e. *to the right* of the intestine, *not dorsal* to it, the figure showing the right side of the bud] au point où ce dernier s'unit avec l'estomac".

To resume: *Anchinia* belongs to the *Laevicolica*, its bud having the intestinal loop at the left side of the morphologically median plane.

The same must obtain in the whole group of *Doliolids*, as in the larva of *Doliolum* the neural cord shows a rudiment of the dextral flexure (the primordium of the intestine lying at its left side) so well developed in the first series of buds of *Anchinia*.

¹⁾ BARROIS draws the attention to quite another point in which this section is highly remarkable. It shows the walls of the right and left peribranchial sacs continuous with the wall of the pharynx but the opening does not fall in this section. In another section of the same stage, however, the opening is seen.

BARROIS (l.c. p. 260), probably correctly, compares it to the gill-opening of *Appendicularians*, but he is not sure whether it may close later on or be the same as a narrower one in a little later stage (l.c. p. 237, fig. 24 A) "qui est certainement la première fente branchiale".

²⁾ The peripheral nervous system, already visible in this bud is very simple.

After BARROIS (l.c. p. 239) "Cela conduit à donner un schéma du système nerveux périphérique beaucoup plus simple que celui de notre première mémoire" (KOWALEVSKY et BARROIS, 1883).

Besides the unpaired neural cord there are only two paired peripheral nerves, the one supplying the region of the mouth, the other the region of the cloacal opening.

The exception claimed by GARSTANG for the order of Doliolids¹⁾ does not exist; they belong to the Laevicolica as well as the allied order of Salps and there is no exception to the rule that in Appendicularians the intestinal loop lies at the right of the morphologically median plane, in all other Tunicates at the left.

On the Position of the Anus in Tunicates.

The loop lying lateral, one still might regard the anus as a median formation. If, however, we consider the position of the anal opening in the group of Tunicates apart, free from every prejudice about this opening in other groups, and ask whether the anus is a median or a lateral organ, the weight of evidence is on the side of a lateral, for:

1. In the Laevicolica the *dorsal* anus lies in all orders except one on the left side; only in Doliolids it is found in the topographically median plane²⁾ or shifted a little to the right, but Anchinia proves that morphologically, it belongs to the left side (cf. the figures of BARROIS, quoted p. 274 footnote).

2. In the Dextricolica (Appendicularians) the *ventral* anus lies in all groups³⁾ except one on the right side, only in Oikopleurinae in the topographically median plane. But as this group is probably the least primitive of Appendicularians it is not probable that the position of their anus would be primitive, the more as its forward extension between the gill-slits is clearly secondary, as GARSTANG already has pointed out.

It is a pity that our knowledge of the embryology of Appendicularians is limited to that of Oikopleura dioica, in its generative organs the most specialized of Tunicates.

Accordingly we come to the conclusion that the anus in Tunicates belongs to the same side as the intestinal loop.

It is a problem how the dorsal anus of Laevicolica may become the ventral anus of Appendicularians (or *vice versa*) and BARROIS in his admirable memoir has tried to solve the question in the case of Anchinia.

After enumerating three important points (1885, p. 260) in which developmental stages of Anchinia resemble the Appendicularians, he says that the chief point of difference lies in the position of the anus, which in Anchinia is placed "entre le cordon nerveux et l'un [the right one] des tubes cloacaux" and he continues (l.c. p. 261) "cependant . . . on

¹⁾ Dolchinia mirabilis (KOROTNEFF) is found by NEUMANN (1913) to belong to the genus Doliolum and FEDELE (1923) could identify it with the species Doliolum Chuni, NEUMANN, so that it should be named Doliolum mirabile (KOROTN.) FEDELE 1923.

²⁾ If we knew the origin of the neural cord in the larva of Anchinia, where it (lying in the dorsal median line) in all probability will be a derivative of the ectoderm, we might say that it is *not possible* to consider the anus as a median dorsal organ.

³⁾ E.g. the genera Fritillaria, Appendicularia and Kowalevskia, which may be considered as representatives of different groups.

peut supposer que cette inflexion du côté droit se soit exagérée chez l'Anchinie jusqu'à refouler l'anus du côté dorsal".

On this supposition, however, BARROIS would not get an Appendicularian, for, apart from the fact that the chief mass of the intestinal loop would remain on the left side of the bud, only the extreme anal part bending down at the right side, the gut would cross the neural cord *dorsally*, a phenomenon not seen in any Appendicularian.

The same holds true for Laevicolica in general. Their left-sided anus cannot become an Appendicularian one by crossing *over* the neural cord and running ventralwards at the right side of the animal¹⁾.

Crossing *under* the nerve-cord is phylogenitically excluded, as the anus must always have retained its external opening.

On the other hand it is likewise excluded that the anus of an Appendicularian by moving dorsalwards at the right side and crossing over the neural cord would become an anus of a Doliolid.

Theoretically there is a possibility of avoiding the difficulty with the neural cord, by supposing that the anus has wandered not along the right but along the left side of the animal, crossing the ventral median line.

There is, however, no Appendicularian with the anus on the left side, as might be expected.

This objection holds as well when one tries with BARROIS to derive the anus of Appendicularians from that of Anchinia (or of Laevicolica in general) as when one derives *vice versa* — as may be the general opinion — the anus of Anchinia from that of Appendicularians.

Comparison with Amphioxus.

On account of the striking affinities in the early development of Ascidi-ans and Amphioxus it is generally granted that Amphioxus is the nearest ally of Tunicates.

GARSTANG (1928, p. 155) says „Hitherto the Ascidian tail has been generally regarded as equivalent to the postanal region of Amphioxus, specialized for larval life by degeneration of the myotomes”.²⁾

This may be true for the majority of workers on Ascidi-ans, but there is a minority, which, on the example of VAN BENEDEN and JULIN (1886) believe that the musculature of the “tail” of the Ascidian larva is represented, not in the postanal, but in the preanal musculature of Amphioxus. They cannot believe, that in Ascidian larvae the whole of the musculature and the corresponding part of the notochord of the trunk of Amphioxus would have disappeared without leaving a trace. They

¹⁾ Of course the neural cord must have existed still at the time when the anus was supposed to cross the dorsal median line. If it had disappeared already at that time, one would get no Appendicularian at all.

²⁾ In the opinion of GARSTANG only the end of the tail in the region of the larval fin of Amphioxus would be the homologue of the Ascidian tail.

neither believe that the intestine would have remained, only curving forwards, without showing this motion in ontogeny. On the contrary, one sees the intestinal loop developing in the body of the Ascidian whilst the entoderm in the tail still forms a straight row of cells.

By my study on the development of *Amphioxus* I am induced to adhere to the minority and believe that the preanal myotomes of the young larva of *Amphioxus* with one gill-slit are represented in the body and the tail of the Ascidian larvae.¹⁾

The rudimentary myotomes (about ten in number) of the Appendicularian tail must also be represented by as many preanal myotomes in the young larva of *Amphioxus*. In this cryptometameric stage of the larva it is very difficult to observe the segmentation of the myotomes (see my "Verslag" etc. 1928, p. 632, 633) — no wonder that in Appendicularians the rudimentary segmentation is denied altogether by many distinguished observers.

So the majority (with GARSTANG) and the minority have a very different standpoint and the minority cannot assume, that the Tunicate intestine would be homologous with that of *Amphioxus* (cf. GARSTANG, p. 180).

They must assume that, phylogenetically, the intestine in the tail of Ascidiaceans has disappeared as does its rudiment in ontogeny, the tail only developing the muscular function, and that another part of the gut developed in the meantime the intestinal loop of the Ascidian.

In my opinion this part of the gut has been a second gill-pouch of which in *Amphioxus*²⁾ only the left antimere (moving to the right side of the body) is preserved in the larva. It is in this larva the first in the later row of functional pouches, but disappears in the period of metamorphosis.

In other words (cf. my paper, 1914, p. 71—74) the ancestor of Tunicates was an animal with two pairs of gill-pouches, an antimere of the latter pair being metamorphosed in the intestinal loop, the other antimere disappearing as only one loop is necessary.

In *Laevicolica* it was the left antimere that developed in this way and it is not impossible that it was so too in Appendicularians. In the case of the Appendicularians, however, we would have the difficulty to assume that the loop of the left side must have wandered to the right side, crossing the ventral median line as the gill-pouch does in *Amphioxus*.

We saw, however, above (p. 277) that the probability is against such a wandering.

Since the sharp division of Tunicates in *Laevicolica* and *Dextricolica* became clear to me, I prefer the simpler supposition that in Appen-

¹⁾ The preoral ("premandibular") myotome is lost in Tunicates.

²⁾ The first pair in *Amphioxus* being represented by the mouth and the club-shaped gland. GARSTANG regards this gland as a homologue of the epicardial pouch in Tunicates.

dicularians it has been the right pouch, in the other Tunicates the left one, that developed into the intestinal loop¹⁾).

Appendix.

As the reader probably will find it difficult to believe that the single gill-slit in the young larva of *Amphioxus* may perform the function of an anus, an observation may have a place here, which, though not conclusive, at least shows the possibility.

Last summer, at the Zoological Station of Naples, I tried to feed these young larvae with microscopically fine carmine grains, which more advanced larvae (with 13—15 gill-slits at Heligoland) took so eagerly.

The young larvae, however, refused them generally.

After having tried in vain to feed larvae of an earlier date, I placed a lot of them on the fifth day after fertilization in a watch-glass with the carmine grains. After an hour I saw that in four larvae the ilio-colonring was full with a lump of the red grains, lying there quiet, without rotation.

Watching them to see how the carmine might leave the body, as the end of the rectum still seemed to be without lumen, I saw one of them taking up in the intestine a second lump of carmine grains, which stopped a little way behind the gill-slit. After remaining quiet there for about half an hour, I saw it moving forwards and being expelled by the gill-slit.

After isolating the four larvae in glasses without carmine and watching them until the evening, I did not see any motion in the ilio-colonring. The next morning the carmine had disappeared from the intestine.

I regret not to have watched these larvae during the night, as afterwards I did not succeed in my feeding experiments, so that only the single observation with the second lump of carmine may demonstrate the possibility of the gill-slit performing the function of an anus.

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¹⁾ I suggest this explanation in the opinion that the anus and loop are lateral organs in Tunicates.

The majority of authors, however, believing that these organs must be considered as morphologically median, must assume that the anus (ventral as in *Amphioxus*) and the loop have migrated in *Appendicularians* to the right (only in *Oikopleurinae* the anus remaining median) in all other *Tunicates* migrating dorsalwards to the left.

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EXPLANATION OF PLATE. FIGURES FROM BARROIS (1885).

- a* Anus.
- cl* Poches ou tubes cloacaux.
- cm* Portion moyenne (comm.) du cloaque.
- e* Endostyle.
- g* Cellules disséminées.
- i* Intestin.
- m* Bandes musculaires.
- n* Masse nerveuse.
- oe* Oesophage.
- p* Cavité du péricarde.
- pd* Pédoncule.
- ph* Sac pharyngien.
- s* Corpuscules du sang?
- st* Sac stomacal, estomac.

Fig. 19A. Stade vu de dos, montrant le tube nerveux s'étendant d'un bout à l'autre du corps. Grossissem. 310 diam.

Fig. 20C. Face dorsale. Grossissem. 330 diam.

Fig. 21B. Coupe horizontale. Grossissem. 430 diam.

Fig. 24. Stade plus avancé. Grossissem. 260 diam.

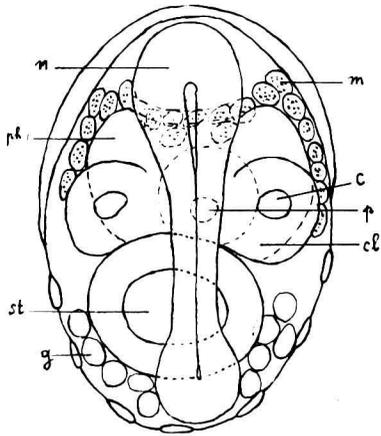


Fig. 19 A.

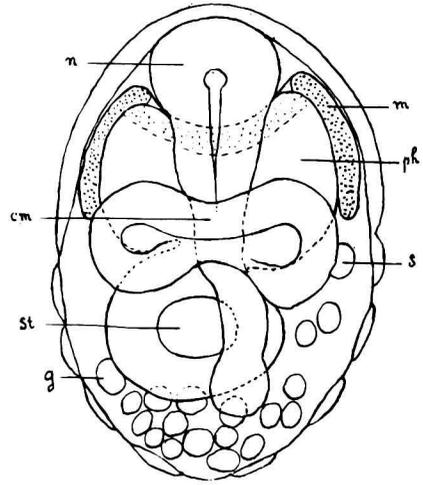


Fig. 20 C.

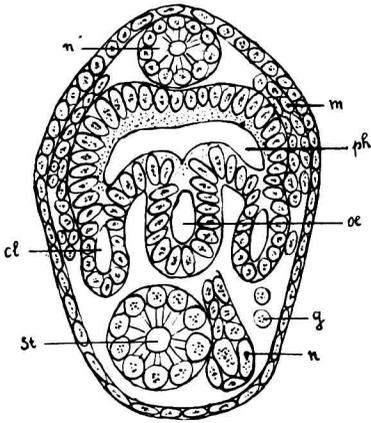


Fig. 21 B.

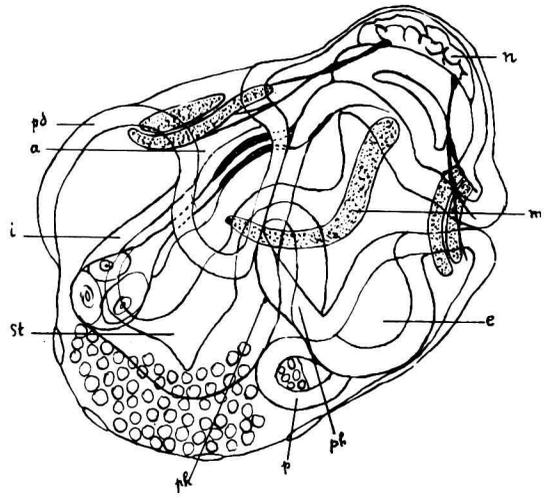


Fig. 24.