On the Tracheal System of Collembola, with special reference to that of Sminthurus viridis, Lubb.

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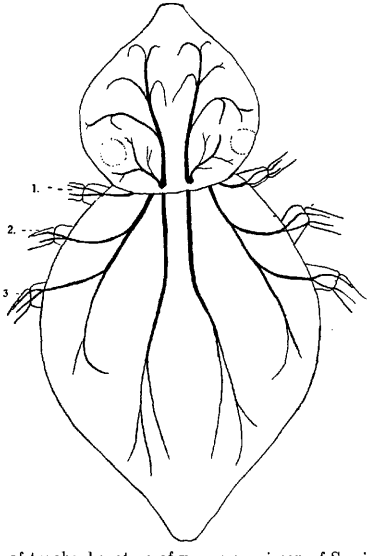
With 6 Text-figures.

1. Introductory Remarks.

The respiration in the majority of Collembola is cutaneous. Special respiratory organs have only been found in species of Sminthurus (now differentiated into the genera Sminthurus, Allacma, and Sphryotheca), Sminthurides, and in Actaletes neptuni, Giard; in these forms tracheae are present. Several writers, including Willem (**16**), Tullberg (**15**), and Haase (**4**), have given brief accounts of the tracheal system in these forms; the most comprehensive account being that of the first mentioned, where the species studied was Allacma fusca, Linne[=Sminthurus fuscus, Lubb.].

The object of this paper is to present as complete description as possible of the respiratory organs of the primitive and, morphologically, interesting group Collembola; the type

TEXT-FIG. 1.



Dorsal view of tracheal system of young specimen of Sminthurus viridis. ×80.

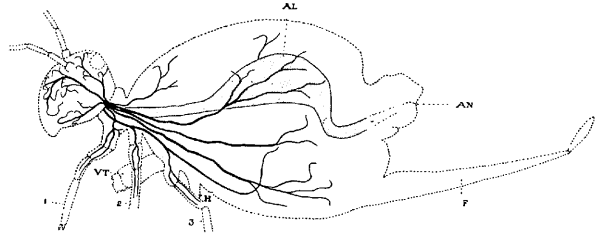
REFERENCE LETTERING TO TEXT-FIGS. 1–5.

1, prothoracic leg; 2, mesothoracic leg; 3, metathoracic leg; *a*, atrium of spiracle; *AL*, alimentary canal; *AN*, anus; *c*, cervical muscles; *ceph*, cephalic bundle of tracheae; *cl*, canal of spiracle; *cu*, cuticle; *F*, furcula; *H*, hamula; *l*, left; *mn*, mandibular muscles; *o*, spiracular opening; *r*, right; *s*, spiracle; *t*, trachea.

taken for study is Sminthurus viridis, Lubb. Quantities of this species have been swept from the grassland at Rothamsted and transferred to grass in greenhouse cages, from which

specimens have been taken as required for anatomical purposes. The main study has been carried out on adult specimens measuring 2–2·5 mm. in length. Eggs of Sminthurus viridis for the examination of the tracheal system of newly hatched specimens, were secured by transferring insects from the field to glass dishes in the laboratory. The eggs were laid usually on the damp blotting-paper lining the dish, and were transferred to damp soil and thus retained until they hatched.

TEXT-FIG. 2.



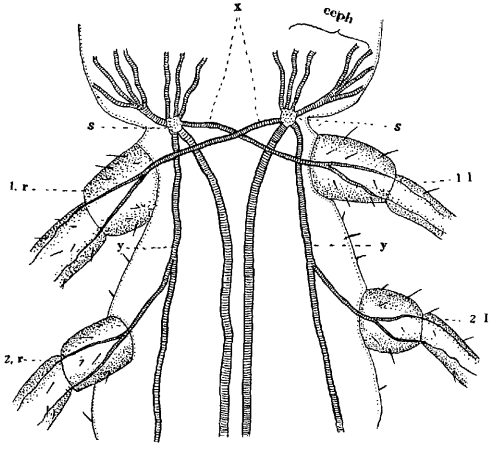
Lateral view of tracheal system of adult. × 40.

2. GENERAL SYSTEM OF TRACHEAE.

The tracheae when filled with air present a characteristic silvery appearance; in such condition it has been possible to trace the general plan of these organs. The dorsal view (Text-fig. 1) reveals the fact that there are two entirely independent systems, one each side of the body. This absence of anastomosis presents the first of several interesting primitive features associated with the tracheal system of Collembola. Each system originates in a single spiracular opening situated on either side of the 'neck' (the exact position of these will be discussed later when the spiracles are considered). Each opening leads into a cavity, thus forming a simple spiracle from which arise the main trunks, or rather bundles, of tracheae. Passing anteriorly from each spiracle there are three such

bundles directed towards the ventral, median, and dorsal regions of the head respectively (Text-fig. 2). Along their course they branch off into finer bundles and ultimately into single tracheae. Examining the thoracic region one is surprised to find an unexpected chiasma. This, apparently overlooked by previous writers, was noted by Willem (**16**) in Sminthurus

TEXT-FIG. 3.



Ventral view of dissection of thoracic region. × *c.* 270.

fuscus. A similar condition has been observed on dissection in Sminthurus viridis, and is figured in Text-fig. 3. From each spiracle (*s*), slightly posterior to the exit of the cephalic bundles (*ceph*), a branch (*x*) leads out which takes its course across to the opposite side, with the result that a crossing of these two branches occurs mid-ventrally. These branches supply the prothoracic legs; thus the right prothoracic leg (*1r*) receives tracheae from the left spiracle, while the left prothoracic leg (*1l*) is supplied from the right spiracle.

Another small bundle (*y*) leaves each spiracle postero-ventrally and passes posteriorly along its respective side of the thorax, branching later to supply the meso- and metathoracic legs. A continuation of this bundle passes into the ventral region of the abdomen.

The anterior portion of the abdomen (Text-fig. 2) is well supplied with tracheae, but the writer has failed to discover any trace of tracheae in the anal and furcal regions. Further, it is interesting to note with regard to the function of the ventral tube that no tracheae pass to this organ.

Four main bundles of tracheae lead posteriorly from each spiracle to supply the abdomen. The smallest of these passes to the antero-dorsal portion, while the largest traverses the region of the alimentary canal. The two other bundles pass ventrally, branching into the regions of the nervous and reproductive systems.

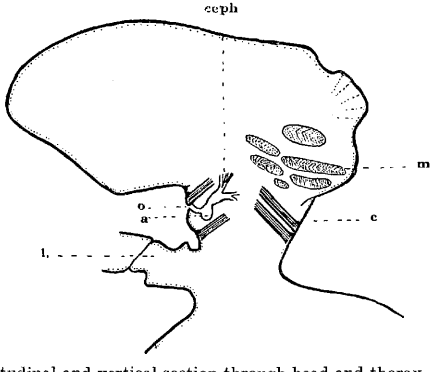
3. Spiracles.

The spiracles offer an interesting subject for special study. The presence of only a single pair of spiracles constitutes a unique condition among adult insects. The exact position of these is a point on which few writers have agreed. Lubbock (**8**) and Tullberg (**15**) have referred to the ' cephalic ' stigmata of Sminthurus, while Haase (**4**), on the other hand, states that the respiratory openings belong to the anterior border of the prothorax. Willem (**16**), further, claims that the spiracle belongs equally to both head and prothorax. In Text-fig. 4 the writer has shown the position of the spiracle as confirmed by several longitudinal and vertical sections. One spiracular opening is present on either side of the mid-ventral position. The particular spiracle drawn is that of the right side. The opening (*o*), as seen, is situated in the anterior portion of the prothoracic segment.[1] In evidence of this fact the origin of the cephalic muscles are figured. The mandibular muscles (*m*)

[1] No definite inter-segment is present between the prothorax and the head, so that it is assumed, along with other writers, that the cervicum is wanting.

at the base of the head are relatively anteriorin position to the spiracular opening, while the cervical muscles (*c*) originate in the segment containing this opening. The latter leads into a short canal which passes slightly ventrally into the cavity or atrium of the spiracle. This is more or less longitudinal in

TEXT-FIG. 4.

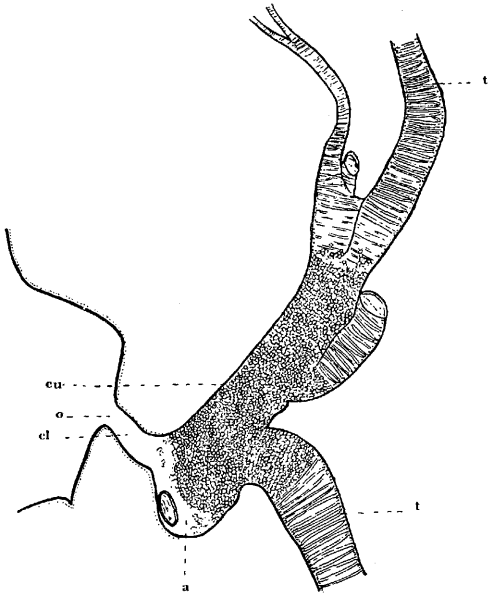


Longitudinal and vertical section through head and thorax. × 80.

position. The exits of the cephalic bundles (*ceph*) are apparently very near the posterior border of the head, but it is evident that the spiracle (excluding the extensions of the atrium) is in the prothoracic segment and not in the cephalic region. Previously Sminthurus, after statements by Lubbock (**8**) and Tullberg (**15**), had been considered the only insect possessing functional cephalic spiracles. The present work, it is observed, disagrees with these claims, and is in favour of the view that functional cephalic spiracles are not found in any insect. In reference to this subject it should be noted that Nelson (**9**) states that a pair of evanescent spiracles is present on the second maxillary segment of the honey-bee embryo; they are observed only at an early stage in its embryology and are obliterated before they function as such.

In structure the spiracle (Text-fig. 5) is very primitive, in fact it bears a close resemblance to the diagram of the spiracle of Peripatus given by Sedgwick (**12**). Its simple structure led Willem (**16**) to term it a 'crypt'. It merely consists of an

TEXT-FIG. 5.



Longitudinal and vertical section through spiracle. × 600.

invagination commencing as a canal and extending into the form of a bulb at its extremity. The shape of the bulb—the atrium—is irregular owing to its extension into the entrances of the various large 'trunks' leading from it. Free communication of the spiracle with the exterior exists, for no mechanism

for closing the spiracular opening (*o*) is present. The diameter of the latter (*o*) in an adult insect is 5 μ, the length of the canal (*cl*) 20 μ, while the total length of the spiracle is 50–60 μ. The entire spiracle is lined by an extension of the cuticle inwards, this overlies the epithelium cells and gives the wall of the spiracle a honeycomb appearance. No mechanism for closing the spiracles was found.

The number, structure, and position of the spiracles cause the respiratory system of Sminthurus to be regarded, according to Palmen's (**10**) classification, as a specialized example of the holopneustic type.

4. Tracheae.

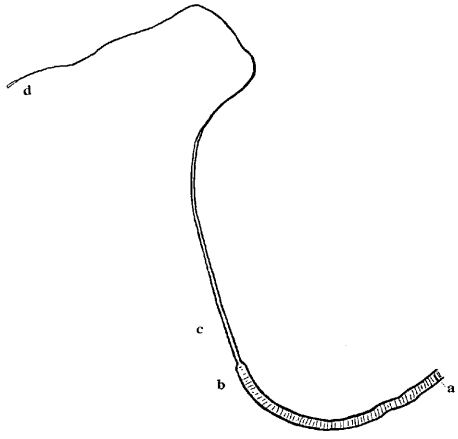
The tracheae which leave the atrium are comparatively large and measure at their origin from 10–15 μ in diameter; these proceed for varying distances, when they divide dichotomously —each branch being of equal size. The tracheae continue to branch in this manner all along their course, but do not depart from the main bundles until in the region which they will ultimately supply.

When highly magnified a trachea presents a very characteristic striated appearance, which is due to the fact that the chitinous lining or intima is specially thickened at regular intervals to form closely arranged thread-like ridges—the taenidia. In Sminthurus viridis their form suggests annuli rather than a spiral as found in the majority of insects. Taenidia are present in the large tracheae near the spiracles, and are visible in all branches to the diameter of 1·5 μ (Text-fig. 6, *b*). At this point (*b*) the trachea has a distinct constriction and proceeds afterwards as a finer branch—the tracheole (*c*) of 0·7 μ in diameter. No taenidia are observed in the tracheole, which continues to diminish in diameter until at its ultimate end (*d*) it measures 0·25 μ. A small portion at its extremity appears transparent instead of silvery, which suggests the presence of fluid rather than air.

Examination for the presence of ' transition ' or ' end-cells ', by all the methods of technique given later, has yielded negative

results. Further, the flagellum-like terminations of the tracheoles, without union or anastomosis, leads one to infer that ' transition cells ' do not exist.

TEXT-FIG. 6.



Termination of a trachea and tracheole. ×670. Special lettering: *a*, diameter 2 μ; *b*, diameter 1·5 μ; *c*, diameter of tracheole 7 μ; *d*, diameter of tracheole, 0·25 μ.

5. THE APPEARANCE OF GAS IN THE TRACHEAE OF NEWLY HATCHED COLLEMBOLA.

The eggs of Sminthurus viridis prior to hatching split equatorially, the young springtail then pushing the anterior cap forwards and thus emerging. Specimens at this stage (i. e. just prior to hatching) were examined by the technique described later, and the tracheae appeared transparent owing to the presence of fluid in their lumena. The outlines of the main trunks were traceable, but they contained no air. Newly hatched specimens were later examined, and it was seen that air had not yet entered the tracheae.

Bearing in mind the generalization [1] made by Keilin (**6**) that ' it is well known that the tracheae of insects before, and often for a short period after, their hatching from the egg, are filled with a fluid which suddenly disappears and is replaced by gas ', specimens of Sminthurus viridis were examined at different periods after hatching, but not until after half an hour's duration from the time of hatching was air observed in the tracheae. At this period it was present only in the entrances of the main tracheal trunks near the two spiracles. Specimens two hours old were next examined, and the air had, by that time, traversed slightly farther along the main trunks of the cephalic system and a short distance along the two main abdominal trunks. Specimens a day old were then treated and a considerable portion of the two main abdominal trunks, as well as the main and subsidiary cephalic trunks, contained air. Examination of these and older specimens of Sminthurus viridis revealed two important facts: (1) that the tracheal system becomes initially filled with air which enters at the spiracles, (2) that the process is a gradual one, for in specimens a week old only a few of the main tracheal trunks found in the adult are filled with air; it thus differs from the sudden exchange found in other insects.

An explanation of these phenomena appears to rest on the physiological processes associated with the entrance of gas into the tracheae. As summarized by Keilin these are threefold, viz. (1) the secretory theory of Frankenberg (**3**) and Pause (**11**), (2) Tillyard's (**13** and **14**) ' diffusion theory ', and finally (**3**) the ' absorption theory ' of Keilin.

In the present observations the first two offer no explanation for the phenomena observed. Firstly, the secretion of gas within the tracheae would undoubtedly result in its appearance at different points along the tracheal system and, further, the

[1] Calvert (**2**) first described the appearance of air in the tracheal system of young dragon-fly larvae (Sympetrum vicinum). The writer has also observed the sudden initial entrance in the case of larvae of the grain beetle (Gnathocerus cornutus), eggs of which were kindly given by Professor R. N. Chapman.

passage of fluid would be in the reverse direction to that observed.

Secondly, if the diffusion theory be accepted it would follow that the air would diffuse uniformly throughout the entire system, which is not the case. The present observations, however, are strongly in favour of the 'absorption theory', i.e. that the tracheal fluid is absorbed by the cells of various tissues from the intracellular capillary tracheoles. A simple experiment with newly hatched specimens appears to confirm this. The specimen was mounted in water under a coverslip and examined microscopically. A bubble of air was seen to envelop the creature. The water was drawn off with a piece of blotting-paper and the tracheae in the region of the spiracle critically observed. No air was present in the tracheal trunks at this stage. Eventually pressure caused the contents of the alimentary canal to be discharged anally with the accompanying removal of internal pressure. Examination again revealed the tracheae devoid of air. Later, however, various tissues in the vicinity of the wall of the alimentary canal were discharged, and presumably the ends of the tracheoles associated therewith were broken and the tracheal fluid withdrawn. Immediately air was observed entering the main tracheal trunks near the spiracle, and a condition comparable to that of a two-hour old specimen was assumed. It would appear, then, that the natural process of entrance of air into the tracheal system had thus been brought about experimentally, that is the tracheal fluid is removed from the ultimate ends of the tracheal system and air automatically follows in its course.

It seems highly probable also that in young specimens the absence of air in several of the main tracheal trunks observed in the adult stage is due to the delay in the absorption of tracheal fluid in these regions.

It yet remains to explain why, in Collembola, the process of filling the tracheae with air is gradual and not sudden as in other insects. An explanation of this appears to lie in the difference in the number of spiracles possessed by Collembola and that found in other insects. The tracheal system of

Collembola—bearing one spiracle in each half of the two independent systems—can be compared to a capillary tube having one entrance and numerous exits, the entrance representing the spiracle and the exits the region of absorption near the tracheoles. The whole tube is filled with fluid. The removal of the fluid at the exits is accompanied by the inflow of air at the entrance; this air will slowly pass along the main stem and eventually reach the point of removal of the fluid. If, on the other hand, we consider the case of other insects possessing several spiracles, we must compare the system to a capillary tube with several entrances of uniform diameter and numerous exits. Again, the tube is filled with fluid, and in this case when the fluid is withdrawn at the exits air passes in at the entrances, which, being several in number, will cause the air quickly to fill the main tube and thence along to the exits.

Hence in the tracheal system of Collembola, where only one spiracle exists in each half system, it will be impossible for a sudden filling of the tracheal system to occur.

6. Observations on the Tracheal System of other Species.

The presence of tracheae is confined, at least in British species,[1] to the sub-globular species of the group Symphypleona, Borner. All species of this group that have been collected during the period of this study have been examined, and, as the presence or absence of tracheae is a criterion for classification in certain species, it is deemed desirable to publish the observations made.

Species of the family Neelidae, Folsom, have been examined, and Willem's (**16**) statement regarding the absence of tracheae in Megalothorax minimus, Will., has been confirmed. The species Neelus murinus, Fols., has also been observed not to possess tracheae.

Linnaniemi (Axelson) (**7**, p. 246) states that tracheae are

[1] Willem (**17**) states that tracheae are present in the head-region of Actaletes neptuni, Giard. This species is not found in the British Isles.

present in members of the sub-family Sminthuridinae, Born., while Borner (**1**), referring to this sub-family, states ' tracheae (always ?) present '. Willem (**17**) claims that a cephalic tracheal system is present in Sminthurides aquaticus, Bourl. Three species of this sub-family have been examined by the writer, and in each case no trace of tracheae could be found. The species examined were: Sminthurides malmgreni, Tullb., var. elegantula, Reut.; Sminthurinus aurea, Lubb., f. principalis, Krausb., and var. ornata, Krausb.; and Sminthurinus fenestratus, Born.

The absence of tracheae in the sub-family Dicyrtominae, Born. (= Papiriidae, Lubb.), is usually accepted, though Borner (**1**) queries this generalization. Willem (**16** and **17**), however, while admitting the absence of a tracheal system, states that ' vestiges, extremely reduced ', have been observed in Papirius fuscus, Lubb. (= Dicyrtoma fusca, Lubb.).

The presence of tracheae appears to be a constant characteristic throughout the sub-family Sminthurinae, Born., though again Borner (**1**) queries the point. Sminthurus viridis, Lubb., as observed, has a well-developed system. The general tracheal system of Allacma fusca, Linne (= Sminthurus fuscus, Linne), also has been described by Willem (**16**). The writer has examined two species of the genus Bourletiella, viz. B. hortensis, Fitch (= pruinosa, Tullb.), and B. lutea, Lubb., both of which have been seen to possess well-developed systems. In the case of the latter the system is even more extensive than in Sminthurus viridis. The abdominal branches extend farther into the anal, furcal, and reproductive regions. It is interesting to note also that no ' chiasma ' is found in the thoracic region, but that the first thoracic legs are fed by tracheae leading from the main trunks of their respective sides.

All the elongate species belonging to the group Arthropleona examined for the tracheal system yielded negative results.

7. Technique.

Specimens of Sminthurus (also other leaping forms of Collembola) are best collected alive by means of a fine glass tube with a suitable bulb blown at about 1–2 mm. from the end of the tube, into which the Collembola jump and are retained in its base.

Freshly killed specimens are required for the study of tracheae. It is essential, in order to examine the general tracheal system, that the tracheae contain air. Specimens are killed on the slide with chloroform or acetic acid vapour; they are then treated with 10 per cent. potassium hydrate. The use of 'wax feet' under the coverslip proves very useful in the case of Symphyleona forms. The process of dissolving the soft parts is observed under the microscope, and a stage is reached when the chitinous tracheae, filled with air, are easily visible. Later the tracheae become transparent owing to the removal of air.

In the case of young specimens and species where the chitinous body-wall is transparent, excellent results have been secured by merely mounting the live specimens in water under a coverslip and then gradually withdrawing the water with a piece of blotting-paper. The body is thus flattened, and in certain instances the contents of the alimentary canal discharged, so that the entire system is easily visible. The preservation of such specimens is satisfactorily obtained by mounting in Keilin's fluid.

The technique which gave best results for histological work is as follows: specimens were killed by immersing in boiling water and then further fixed with picrosulphuric acid. The clearing agent used was cedar-wood oil. All staining was done on the slide, and the best results were obtained with Mann's eosin methyl blue, Heidenhain's haemotoxylin superposed with eosin, methylene blue, and the Golgi bichromate and silver method.

This work was carried out at the suggestion of Dr. A. D. Imms, whose valuable advice is gratefully acknowledged. The

writer is also indebted to Mr. J. M. Brown for confirming the identification of certain species involved.

Summary.

1. No extensive study of the tracheal system of Collembola has previously been made.

2. The general plan of the tracheae has been worked out on Sminthurus viridis; two independent systems exist.

3. The presence of only a single pair of spiracles constitutes a unique condition among adult insects. The position of these is in the anterior region of the prothorax, and not in the head or cervicum as previously believed. The structure of the spiracles is extremely primitive and they possess no closing apparatus.

4. The tracheae branch dichotomously, but no anastomosis exists between the systems of the two sides of the body. Taenidia are present, but no 'transition cells' have been observed and the fine tracheae terminate in unbranched tracheoles.

5. The initial entrance of air into the tracheae is through the spiracles; the displacement of fluid is very slow and the whole system is not completely filled with air until about fourteen days after emergence from the egg.

6. Tracheae are found to be a constant feature in all members of the Sminthurinae examined.

7. Various methods of technique adopted are given.

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