Respiratory Adaptations of the Pupae of Beetles of the Family Psephenidae

H. E. Hinton

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RESPIRATORY ADAPTATIONS OF THE PUPAE OF BEETLES OF THE FAMILY PSEPHENIDAE

By H. E. HINTON, F.R.S.
Department of Zoology, University of Bristol

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[Plates 3 to 6]

CONTENTS

Plastron-bearing spiracular gills have been independently evolved in two groups of the Psephenidae, the Psephenoidinae and one genus of the Eubriinae. The spiracular gills of the pupae are exclusively spiracular structures. The plastron is on the spiracle rather than on the body wall adjacent to the spiracle, as in the pupae of flies. In some species the spiracular gills are borne at the end of projections from the body wall. In one genus of Eubriinae, epidermal cells that remain in good condition are isolated in the projections from the body wall in such a way that they are completely separated by a thick wall of cuticle from the remaining tissues of the body in both the pupal stage and in the pharate adult stage.

The origin of plastron respiration in the Psephenidae is discussed. Non-aquatic pupae are found near the edges of streams where they are apt to be flooded by rises in stream level. The water/air interface of normal spiracles is too small (400 to 1100 $\mu m^2/mg$) to satisfy oxygen demands by extracting oxygen from the ambient water when they are flooded. The water/air interface of the least well-developed plastrons in insects is equivalent to about 15000 $\mu m^2/mg$ of body weight. It is suggested that every increase in the length of the spiracles has a selective advantage in that it enables the pupa to utilize atmospheric oxygen when covered by correspondingly thicker layers of water. At some stage in this process, plastron respiration through the spiracles becomes significant in satisfying oxygen demands. When this stage is reached, selective pressures begin to operate directly to increase the water/air interface of the spiracles. It is shown that if all spiracles of some forms, such as *Metaeopsephenus*, were like its longest spiracles, the linear dimensions of the spiracles would only have to be increased by a factor of 2·2 for these to have a water/air interface per mg of body weight equivalent to that of some insects with plastrons.

Spiracles that do not function in gas exchanges between the insect and the ambient environment nevertheless persist because they subserve two other functions: (*a*) when they are first formed their chambers or ecdysial tubes provide a lumen through which the old tracheae of the previous instar may be withdrawn, and (*b*) after the appearance of the new instar their chambers, now collapsed, are the means by which the tracheae of the previous instar are anchored to the cuticle that is to be shed. Spiracles that do not function in gas exchanges and have their orifices closed are known as non-functional spiracles. Once a spiracle becomes non-functional in a particular instar it remains non-functional in that instar despite the fact that it is temporarily open between the moult and the ecdysis. The loss of functional spiracles is irreversible irrespective of changes in the habits or environment of the group. Examples of irreversible losses of functional spiracles are cited that concern more than one million cases.

In some Psephenidae the spiracles of the first abdominal segment are non-functional. The spiracular atrium and the regulatory apparatus of such spiracles may nevertheless persist and be more or less identical in structure to those of functional spiracles. The evidence suggests that in the subfamily Eubriinae such non-functional structures have persisted since at least the Eocene.

Plastron-bearing spiracular gills are polyphyletic in origin. They have been independently evolved at least nine times in the Diptera and twice in the Coleoptera. In the Diptera spiracular gills are modifications of the body wall adjacent to the spiracle (e.g. Tanyderidae, Deuterophlebiidae, and Simuliidae) or of both the body wall and the spiracle (e.g. Tipulidae). In the Coleoptera they are modifications of the spiracle only although the spiracle may be borne on a long projection from the body wall (e.g. *Psephenoides volatilis* Champ). Because in each group of insects the spiracular gills are independently evolved, a phylogenetic classification of these gills is excluded, but a classification of convenience is proposed.

Introduction

Fewer than 100 species of Psephenidae have been described. The larvae live in streams. Some pupate under water, but most pupate on land close to the edges of streams. Their environment is thus an unstable one, and in this unstable environment both larvae and pupae have evolved more different types of respiratory adaptations than any other family of beetles. Indeed, this small family has almost as many types of respiratory adaptations as occur in all other beetles. No other beetles have spiracular gills, and the larvae of no other insects have brushes that sweep the surface of the spiracles clean (Hinton 1955*a*). In another respect too they are unique among insects: the larvae of some have an ecdysial line system characteristic of the Insecta but other larvae have a system resembling the Arachnida, and in the Eubrianacinae the ecdysial line system of the trunk resembles that of the Isopoda (Hinton 1963). In the Eubrianacinae and Psephenoidinae the pupae mimic their larvae, a type of mimicry that so far appears to be unique among aquatic insects (Hinton 1955*a*).

The different types of respiratory systems of the Psephenidae are described. Special attention has been paid to the structure of the plastron-bearing gills and to the apparent irreversible evolution of different types of respiratory systems.

The structures that have been called spiracular gills (Hinton 1953) are, so far as is known, confined to the pupal stage. Plastron-bearing spiracular gills occur only in the Diptera and Coleoptera. Amongst the flies they are present in all Simuliidae, Deuterophlebiidae, and Blepharoceridae and in some Tanyderidae, Tipulidae, Empididae, Dolichopodidae, and Canaceidae. Amongst beetles they occur only in the family Psephenidae.

Although spiracular gills are exclusively pupal structures, they are nevertheless the chief respiratory organs of the young adult before it has shed the pupal cuticle. The pupal–adult moult always occurs long before the pupal–adult ecdysis. During the interval between these two events, the young adult—that is, the pharate adult (Hinton 1946*a*, 1958*a*)—is only mechanically connected to the pupal cuticle that envelops it. In those species in which the larval–pupal ecdysis occurs *after* the pupal–adult moult, e.g. many Diptera, the spiracular gills of the pupa can function only in the pharate adult stage and not in the pupal stage. But even in those species in which the larval–pupal ecdysis occurs before the pupal–adult moult, the interval between these two events tends to be very short so that the duration of the non-pharate pupal stage is brief, a few hours or a day or so at most. Thus, even when there is a non-pharate pupal stage, the spiracular gills function chiefly in the pharate adult stage, which generally lasts a week or more. In some groups, the pharate adult stage may be very much longer than the non-pharate adult stage. For instance, in some Deuterophlebiidae (Kennedy 1960; Hinton 1962) the duration of the pharate adult stage in the field is about 8 or 9 days, whereas the adult lives only about 2 h after it sheds the pupal cuticle. Thus in these insects the spiracular gills of the pupa are the chief respiratory organs of the adult during most of its life.

The relations between the pupal and adult respiratory systems described in the preceding paragraph are in no way peculiar to those species that have spiracular gills. Precisely the same relations occur in insects with unspecialized or relatively unspecialized spiracles: during the whole of the life of the pharate adult gas exchanges with the ambient environment are effected chiefly through the pupal spiracles, as is evident from figure 1.

In the pharate stages the regulatory apparatus of a spiracle appears to function effectively despite the fact that the tracheae of the previous instar are enclosed within the tracheae of the new instar (figure 1). So far as is known, a regulatory apparatus is either absent in dipterous pupae or if one is present, e.g. Simuliidae, it is non-functional until after the pupal–adult moult, when it is operated by muscles within the body of the pharate adult (Hinton 1957*b*). The problem of water loss through the pupal spiracles first arises after the larval–pupal ecdysis. In the Diptera with spiracular gills, as well as in nearly all those without such gills, the pupal–adult moult occurs before or very shortly after the larval–pupal ecdysis. A non-pharate pupal stage is thus entirely suppressed or of very short duration.

A reduction in the duration of the non-pharate pupal stage almost certainly preceded the loss of the regulatory apparatus. But once the apparatus is lost, a further premium is placed upon the completion of the pupal stage before or very shortly after the larval cuticle is shed. However, in those insects in which a regulatory apparatus is lost, water loss through the spiracles can be effectively reduced by the development of a spiracular ‘felt’ chamber that reduces to insignificance the tidal flow of air through the spiracles. Such felt chambers are readily developed by insects, as is evident from their independent origin in a large number of different groups, and they also occur in dipterous pupae. For this reason, therefore, it seems that the role played by the loss of the regulatory apparatus in the further reduction or total suppression of the non-pharate pupal stage is likely to be only a minor one. In the Psephenoidinae the spiracular chamber is modified to form an unusually long felt chamber (Hinton 1947*b*). In *Psephenoides volatilis* Champ. each chamber is about one-sixth as long as the pupa, and in *P. gahani* Champ. the chambers are about one-tenth as long as the pupa. These felt chambers function to reduce water loss when the insects are exposed above water, as often happens in streams.

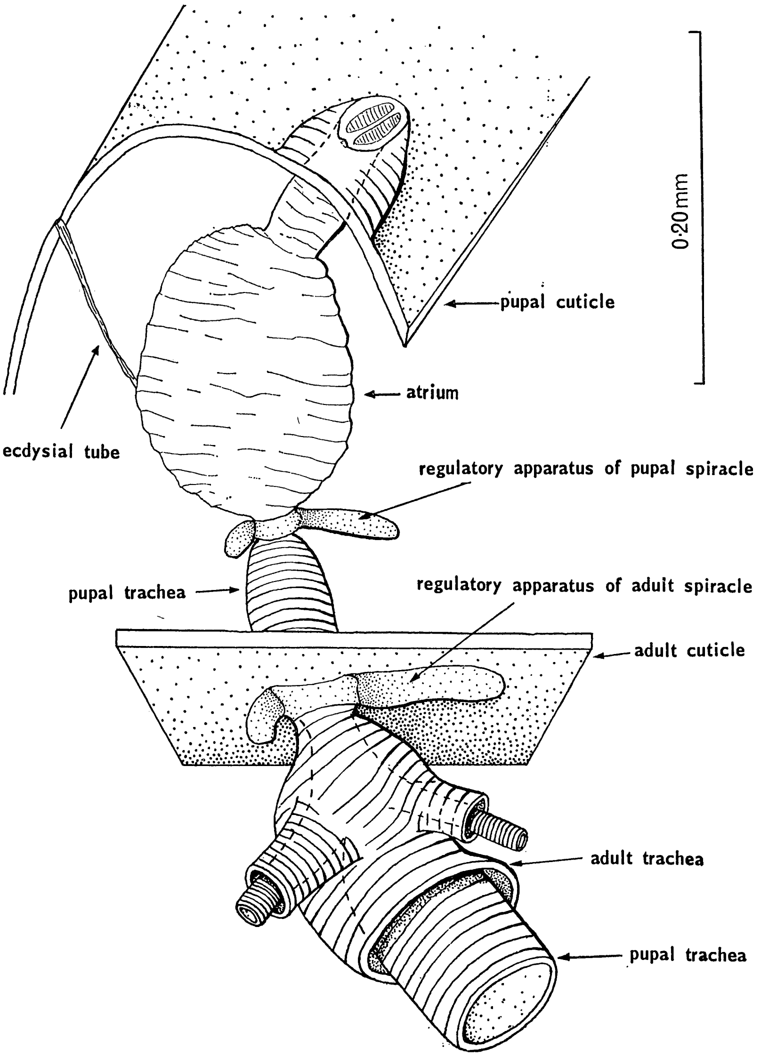

Figure 1. Pharate adult of *Sclerocyphon fuscus* Armstrong. Relation between the pupal and adult spiracles of the fifth abdominal segment. The relative positions of the pupal and adult cuticles have been slightly altered in order to bring the spiracles of the two stages into the same plane. The pupa has the specialized elateroid type of spiracle, and the adult has the primitive type of spiracle. The non-functional spiracle of the larva is on the ventral part of the pleural extension. The non-functional spiracle of the pupa is formed at the corresponding position. The functional pupal spiracle, however, is on the dorsal surface almost exactly above the external opening of the ecdysial tube through which the larval tracheae were withdrawn at the larval–pupal ecdysis.

Materials and methods

The material on which this paper is based has been accumulated during the past 30 years. Part of it was collected by myself in Canada, California, Mexico, South America, Trinidad and Australia. The remainder was obtained by exchanges or as gifts from collectors in many countries. Only about 90 species of Psephenidae have been described but pupae of 20 species and larvae of 36 species have been available for study.

The structure of the spiracles and spiracular gills was determined from whole mounts and serial sections. Phase contrast and anoptral contrast proved to be useful for some of the finer details. Confirmation of the structural detail of the spiracular gills of *Psephenoides* was obtained by means of both transmission and scanning electron microscopy.

The surface area of the spiracles was determined by drawing their outlines on paper with the aid of a camera lucida and then cutting the drawings out and weighing them. The error in the figures obtained is probably not greater than about 10%, but it could be appreciably greater without significantly affecting the conclusions. The error in the estimate of the plastron area of the new genus of Eubriinae is also probably not more than about 10%. The plastron area of the gills of the species of *Psephenoides* was very much more difficult to measure, and the error in the estimate could be twice as great.

One of the factors that necessarily determines the respiratory efficiency of a plastron is the area of its water/air interface in relation to the weight of the insect. When this is expressed as units of water/air interface per mg of body weight, a comparison can be made between the efficiency of the plastrons of insects of quite different weights. The validity of such comparisons rests upon the fact that, in general, the rate of respiration of insects is directly proportional to their total wet (live) weight.

The resistance of the plastrons of the three species of *Psephenoides* and the new genus of Eubriinae to wetting by excess pressures was not calculated. Such calculations depend upon assumptions about contact angles, which cannot be verified in specimens long preserved in alcohol. Furthermore, the equations for such calculations (e.g. Crisp 1964) make no allowance for the time that the pressure is applied and do not therefore allow for creep. Tables of the resistance to wetting by excess pressures applied for different times (e.g. Hinton 1960*a*, *b*, 1961) clearly show that figures for the resistance of plastrons to excess pressures have little meaning unless the times that the pressures were applied are also given.

Interrelation of pupal and adult respiratory systems

In the Psephenidae the pupal–adult moult occurs very shortly after the larval–pupal ecdysis so that the non-pharate pupal stage is relatively brief, and it is not yet certain that all species have a non-pharate pupal stage. The little that is known about the habits of these insects indicates that the usual period between the larval–pupal ecdysis and the pupal–adult ecdysis is 1–2 weeks. During most of this time the insect is in the pharate adult stage, but its chief respiratory organs are nevertheless the pupal spiracles.

The interrelation between the pupal and adult spiracles during the pharate adult stage are shown in figure 1. At the larval–pupal moult, the moulting process of the spiracles is of the *elateroid* kind (Hinton 1947*a*): the functional opening of the pupal spiracle is secreted by a cylinder of epidermis that is formed to one side of the cylinder of epidermis that secretes the ecdysial tube of the non-functional spiracle of the larva. The simultaneous formation of two spiracles at this time becomes a functional necessity because the tracheal linings of the larva cannot be withdrawn at the larval–pupal ecdysis through the newly formed pupal spiracle as the cuticular bars that extend across its external opening absolutely prevent this. Instead, the old tracheal lining of the larva is withdrawn through the ecdysial tube and its unoccluded external opening. After the larval–pupal ecdysis, the ecdysial tube collapses, and its external opening is more or less completely closed by centripetal contraction of the peripheral integument. All that then remains of the external opening or spiracle is a small and usually heavily sclerotized patch of cuticle often called the external scar or the stigmatic scar. At the junction of the collapsed ecdysial tube with the atrial chamber of the functional spiracle there is another scar, the internal scar (figure 1). The collapsed ecdysial tube through which the larval tracheae of the segment was withdrawn is shown in figure 1. Spiracles that have their openings obstructed like those of the Psephenidae are commonly found in the larval and pupal stages. Such spiracles cannot of course be evolved except hand in hand with ecdysial tubes that permit the withdrawal of the tracheal lining of the previous instar. The modification of a pupal spiracle to form a spiracular gill always prevents its use for the withdrawal of the tracheal lining of the last larval instar and is therefore always accompanied by the formation of an ecdysial tube.

The structure of the adult spiracle does not preclude the withdrawal of the lining of the pupal tracheae through it at the pupal–adult ecdysis. An ecdysial tube, as distinct from a functional spiracle, is thus not required and none is formed at the pupal–adult moult. The moulting process of the spiracles at this time reverts to the primitive type.

PLASTRON RESPIRATION IN INSECTS WITHOUT A PLASTRON

The spiracles of both terrestrial and aquatic insects are hydrofuge and resist the entry of water under more or less considerable hydrostatic pressures. When these are submerged in water, a water/air interface is established across the spiracular opening. The spiracles thus inevitably function as plastrons. However, their total surface area in relation to the weight of the animal is usually of the order of 500 to 1500 $\mu m^2/mg$, whereas in those insects that are known to have morphological structures that are considered to be plastrons the surface area/mg is not known to be less than 10 times greater. Most terrestrial and aquatic insects readily drown if their spiracles are not exposed to air at relatively frequent intervals. It may therefore be supposed that the amount of plastron respiration through spiracles that are normally formed, and which are adapted for atmospheric respiration, is insignificant in relation to the oxygen demands of the animals when they are submerged. Thus, because of the hydrofuge nature of normally formed spiracles of both terrestrial and aquatic insects there is, paradoxically, plastron respiration in insects without a plastron; that is, without structures especially evolved for plastron respiration.

PSEPHENINAE

The pupae of two genera of Psepheninae have been examined, *Psephenus* and *Metaeopsephenus*. In both genera functional spiracles are absent on the thorax. In *Psephenus* functional spiracles on the apices of small tubercles are present on the first seven abdominal segments (figure 2*B*). The respiratory system of *Psephenus* is thus more primitive than that of any other known pupa in the family, all of which have non-functional spiracles on the first abdominal segment except *Metaeopsephenus*, which is otherwise modified.

The pupa of *Metaeopsephenus japonicus* Mats. has only five pairs of functional spiracles with a total surface area of 76 285 μm^2. These are on the first three abdominal segments and on segments six and seven. The spiracles of the fourth and fifth segments are apparently non-functional (figure 3*D*). The spiracles of the sixth and seventh segments are greatly enlarged (figures 3*A*, 4*A*). The second and third pairs of spiracles are not only proportionally about as large as the functional spiracles of some other Psephenidae that do not have spiracular gills, but they are also about as large as those of many other insect pupae of the size of *Metaeopsephenus*. If all seven pairs were the size of the second and third pairs, their total surface area would be 62443 μm^2. There has thus been no significant gain in total surface area as a result of the changes undergone in the structure of the spiracles. The interstices between the struts across the openings of the spiracles occupy a little less than a third of the surface area (figure 4*B*). If a third is allowed in order to account for the bulging of the menisci between the struts, the total water/air interface—the effective plastron area—is only about 25000 μm^2. The wet weight of a pupa is 60 mg, and there are therefore about 400 μm^2 of plastron/mg, a spiracle area/weight relation normal to terrestrial pupae. A Tanyderid pupa, *Eutanyderus wilsoni* Alex., has an effective plastron area of 0·398 mm^2 (Hinton 1966*b*). In this insect the ratio of plastron area to body weight is the

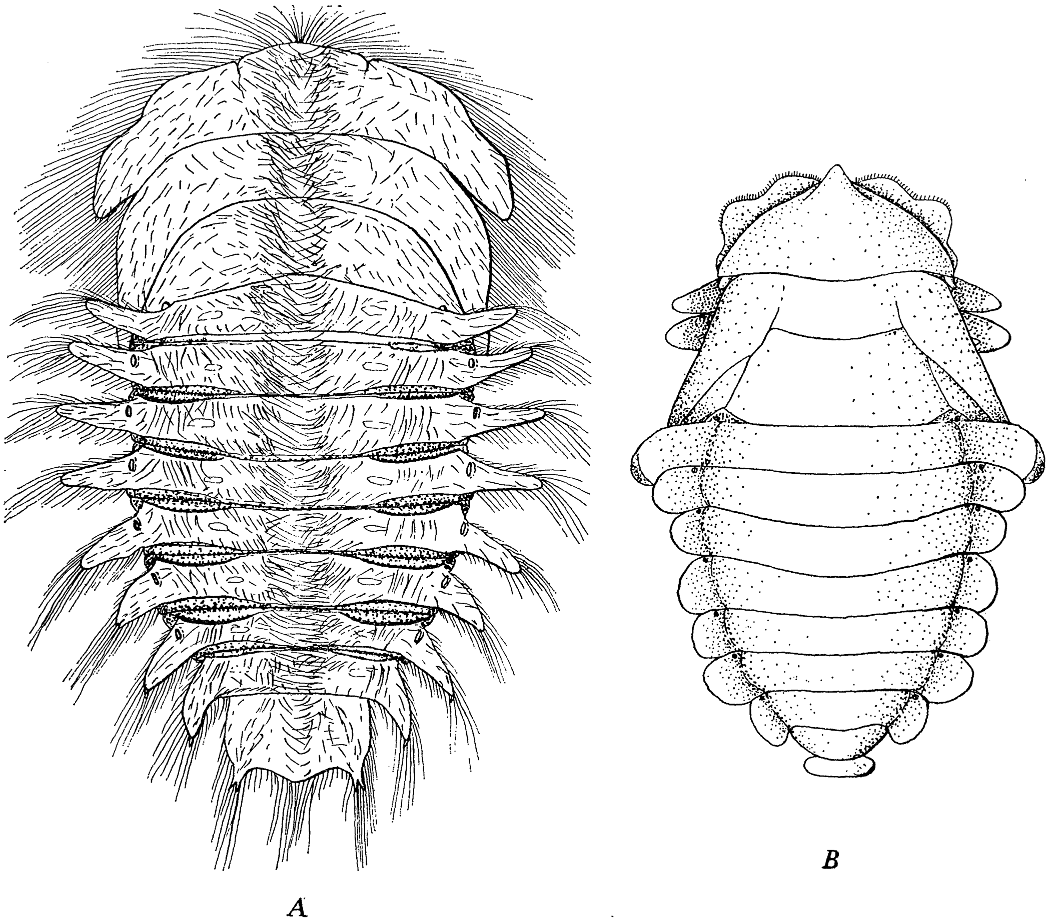

FIGURE 2. (*A*) Dorsal view of a pupa of *Sclerocyphon* sp. from Tasmania. Well-developed gin-traps (see Hinton 1946*b*) are present on either side between abdominal segments 2–3, 3–4, 4–5, 5–6, and 6–7. (*B*) Dorsal view of the pupa of *Psephenus palpalis* Champ.

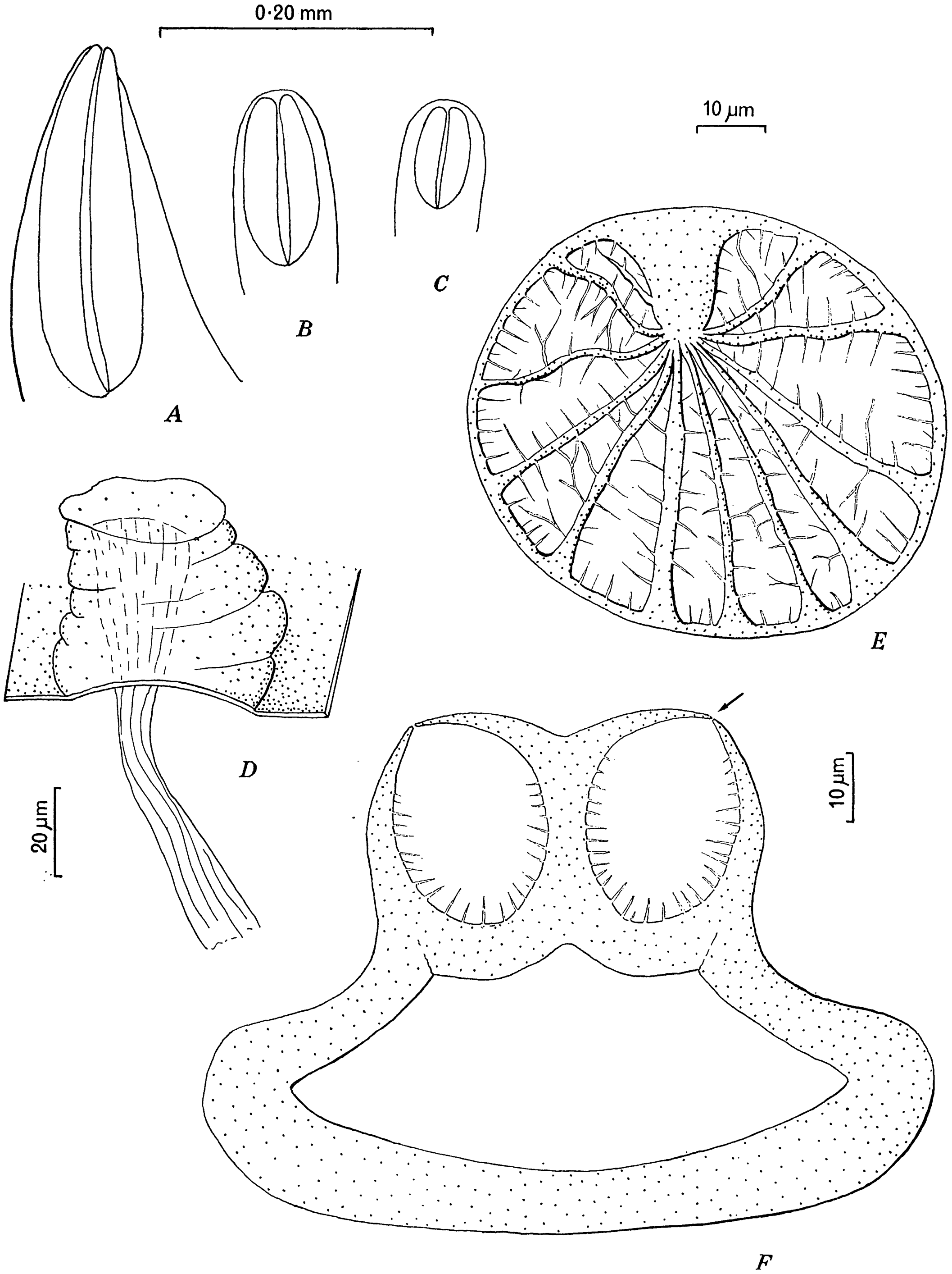

Figure 3. Pupa of *Metaeopsephenus japonicus* Mats. (*A*) Outline of spiracle of seventh abdominal segment. (*B*) Same of third segment. (*C*) Same of first segment. (*D*) Non-functional spiracle of fourth abdominal segment; the regulatory apparatus is not shown in the drawing. (*E*) Transverse section through the stem of the atrium of the spiracle of the seventh abdominal segment. (*F*) Transverse section of the spiracle of the seventh abdominal segment about 50 μm from the apex. The arrow indicates the position of a cleft present in all of the spiracles examined of either the sixth or seventh segments. The edges of the cleft are irregular, and there seems to be little doubt that it is an artifact despite the regularity of its position.

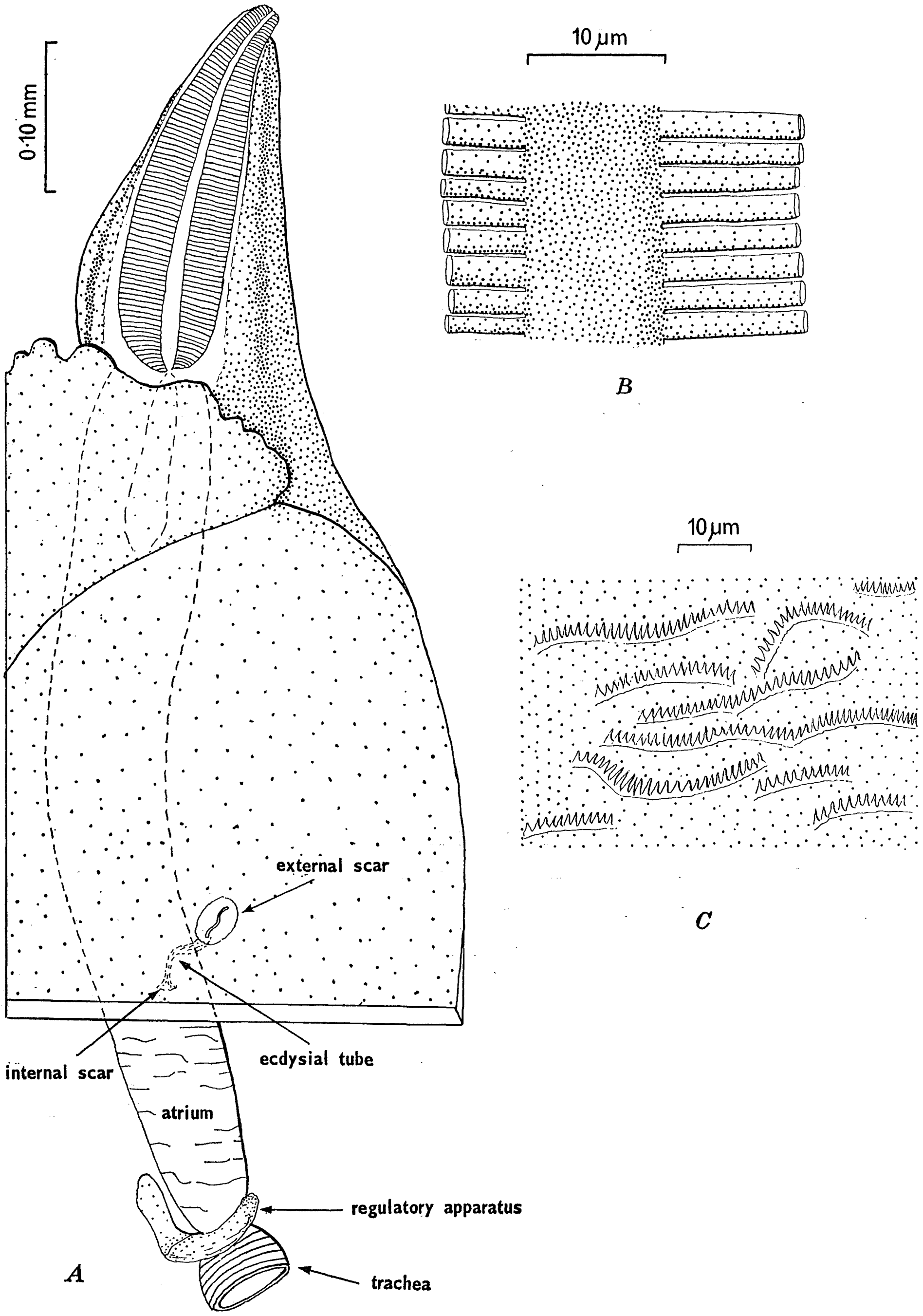

FIGURE 4. Pupa of *Metaeopsephenus japonicus* Mats. (*A*) Spiracle of seventh abdominal segment. (*B*) Surface view of a small section of the middle of the same spiracle. (*C*) Inner view of surface of the spiracular atrium near the regulatory apparatus.

most unfavourable one known, but it is nevertheless 15918 μm^2/mg or 40 times better than in *Metaeopsephenus*.

The selective advantage of the great enlargement of the spiracles of the sixth and seventh abdominal segments must be concerned with some function other than plastron respiration. The lengthened spiracles would enable the pupa to utilize atmospheric oxygen even when it was covered by a thin layer of water, a circumstance frequently encountered by insects that pupate close to the edges of streams.

The condition of the spiracles of *Metaeopsephenus* makes it possible to visualize some of the early stages in the evolution of spiracular gills in the Psephenidae. Periodic flooding of the pupa as a result of rises in stream level select out forms with lengthened spiracles that are able to utilize atmospheric oxygen even when covered by a layer of water. Every increase in length has a selective advantage in that it enables the pupa to utilize atmospheric oxygen when covered by correspondingly thicker layers of water. At some stage in this process plastron respiration becomes significant in satisfying oxygen demands, and, when this stage is reached, selective pressure for an increase in plastron area as such begins to operate for the first time.

In the Psephenidae with spiracular gills all of the modified spiracles are more or less equally developed. If all of the spiracles of the first seven abdominal segments of *Metaeopsephenus* were as large as those of the sixth and seventh segments, and if the struts were subdivided so that the effective plastron area was equal to the whole of the spiracular area because of the bulging of the minisci, the ratio would be 3158 μm^2/mg or a fifth of that of *Eutanyderus*. Thus the plastron area/mg of body weight could be equal to that of *Eutanyderus* by increasing the linear dimensions of the spiracles by a factor of only 2·23, but plastron respiration would probably be significant well before the linear dimensions of the spiracles in the example considered were doubled.

EUBRIANACINAE

This subfamily includes only the genus *Eubrianax*. Pupae of the following species have been examined: *E. edwardsi* Lec. of California, *E. granicollis* Lewis of Japan, *E. vittaticollis* Pic of Rhodesia, *E. scotti* Pic of Abyssinia, and an unidentified species from Uganda. The species normally pupate on damp stones just above the water line or on the sides of stones up to a few metres from the stream. According to Essig (1926), *E. edwardsi* pupates beneath the surface of the water. The species that quit the water before pupation nevertheless pupate in places where they are liable to be submerged by relatively slight rises in the level of the stream. The pupae retain the cuticle of the last larval instar. The longitudinal ecdysial lines that are present on the sides of the head and the ventro-lateral sides of the thorax and first six abdominal segments are joined by a transverse ecdysial line between the tergites of the sixth and seventh abdominal segments (Hinton 1963). The ecdysial line system of the larva is thus reminiscent of that of the Isopoda and quite unlike that of any other known insect. At the larval–pupal ecdysis the ventral part of the head and trunk up to the seventh abdominal segment is shed together with all of the abdomen behind the sixth segment. Thus the anterior part of the pupa is covered by the larval cuticle but segments seven to nine of the abdomen are exposed. The exposed abdominal segments are so modified that they closely resemble the missing end of the larval cuticle, and even the external scars of the non-functional spiracles of the eighth abdominal segment are enlarged and resemble the functional post-abdominal spiracles of the larva. The possible significance of this mimicry of the larva by the pupa has been discussed by Hinton (1955*a*, p. 560).

The respiratory systems of the pupae of the different species of *Eubrianax* are similar in all essentials and are unlike those of any other beetle pupa or, so far as I am aware, any other endopterygote pupa. All spiracles are non-functional except those of the seventh abdominal segment. The pupae have previously been thought to be apneustic, chiefly because the openings of the spiracles are widely scattered on small tubercles and do not resemble the spiracles of any other beetle pupa nor do they closely resemble those of any other known insect.

The external structure of the spiracles of the seventh abdominal segment is shown in figure 5. In *E. edwardsi* each spiracle has from 41 to 61 separate biforous openings (figure 5*C*), each on the top of a small tubercle. Each opening communicates with the large atrial chamber by means of a short tube (figure 5*D*). The number of biforous openings of the three other species examined varied from 50 to 70. In all species not only do the numbers vary intraspecifically, but the numbers on the right and left sides of the same individual sometimes differed by as much as 5%.

Eubrianax is the only known insect pupa with a metapneustic respiratory system although the functional spiracles are on the seventh rather than on the eighth abdominal segment (figure 6). Beetle pupae as a primitive feature lack functional spiracles on the eighth abdominal segment. The significance of this is not yet understood, but, as is well known (e.g. Keilin 1944; Hinton 1947*a*), spiracles that become non-functional in a particular instar remain non-functional in that instar irrespective of subsequent changes in the habits of the instar. That is, the loss of functional spiracles is irreversible, and no exceptions to this rule are known. Thus *Eubrianax*, like all other recent beetle pupae, is evolved from ancestors that had lost functional spiracles on the eighth abdominal segment, and the nearest approach that can be made to a metapneustic system is therefore to restrict functional spiracles to the seventh abdominal segment.

The pupa, unlike the larva of many Psephenidae, has no means of keeping the spiracular openings free of silt or other debris. Many separate openings over a large area seems to be the next best solution to the problem of preventing the spiracles from becoming totally blocked by debris.

In terrestrial pupae the combined surface area of the spiracles is usually 500 $\mu m^2/mg$ to 1000 $\mu m^2/mg$. The wet weight of a small pupa of *E. edwardsi* is 6 mg, and the total area of its spiracles is 4201 μm^2 or 700 $\mu m^2/mg$. The multiplication of the spiracular openings thus not only serves to prevent total blocking by debris but also increases the surface area to an amount quite comparable to that of terrestrial pupae with 7 to 8 pairs of functional spiracles.

When the pupa is immersed in water the effective water/air interface of the spiracles is 700 $\mu m^2/mg$, whereas in the Psephenidae with plastrons it is 70000 $\mu m^2/mg$ or more. It seems reasonable to assume that a plastron is not evolved until for one reason or another the surface area of the spiracles has become, for reasons other than plastron respiration, sufficiently large for plastron respiration to assume some significance. Not until this stage is reached does selective pressure for plastron respiration begin to operate directly further to increase the surface area of the spiracles. It is not yet possible to specify such a stage accurately; it has certainly not been reached by *Eubrianax*, quite apart from the fact that its spiracle area/weight relations are no better than those of the average fully terrestrial pupa.

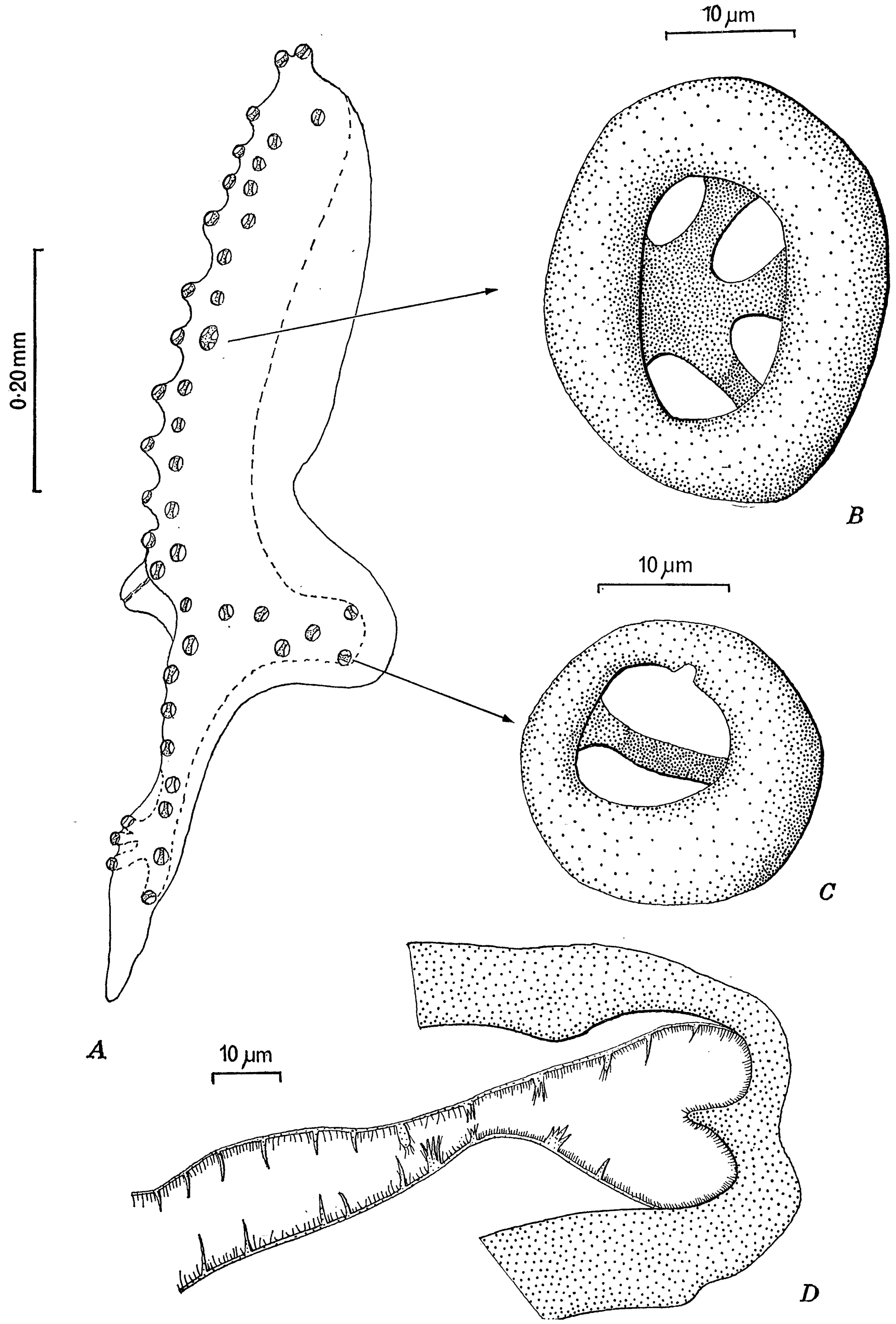

Figure 5. Pupa of *Eubrianax edwardsi* Lec. (*A*) External view of left spiracle of seventh abdominal segment. (*B*) One of the spiracular openings that is more divided than usual. (*C*) A normal biforous spiracular opening. (*D*) Section through a biforous opening and the tube that extends from it to the enlarged spiracular chamber.

The African, Oriental, and American species of *Eubrianax* are extremely similar and clearly the peculiar structure of their spiracles is not polyphyletic. The distribution of *Eubrianax* suggests that the genus in much its present form is pre-Eocene, and a larva, *Eubrianax vandeli* Bertrand (1963 *b*), is known from the middle Eocene of France. If the water/air interface of the spiracles of *Eubrianax* were sufficiently large for plastron respiration to begin to be significant, that is for selective pressure to operate in favour of the plastron, it might be supposed that by now the surface area of the spiracles would have been increased by at least an order of magnitude.

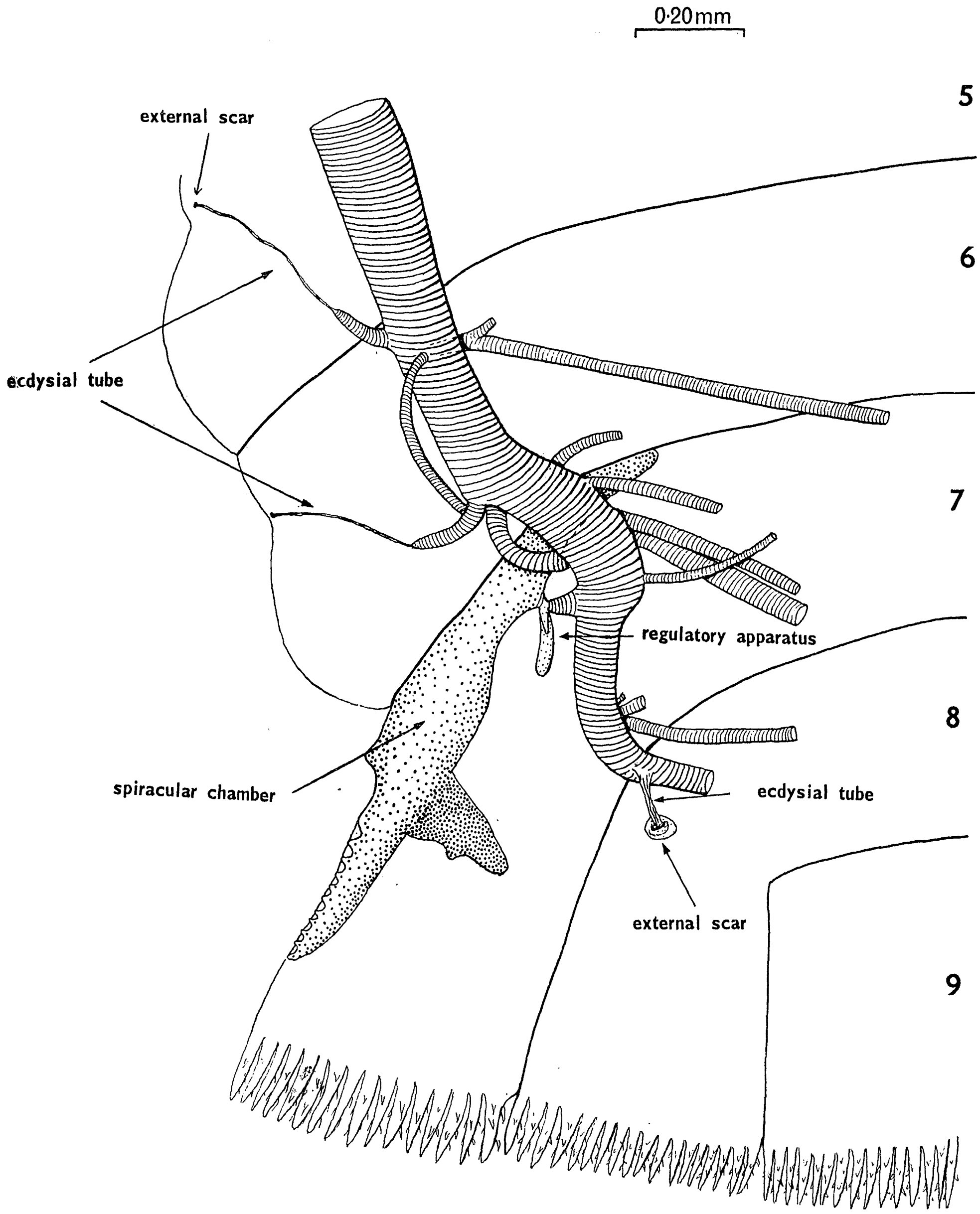

Figure 6. Pupa of *Eubrianax edwardsi* Lec. Ventral view of respiratory system of the fifth to eighth abdominal segments. Only the major branches of the tracheae are indicated. The external scar of the non-functional spiracle of the eighth abdominal segment is very large as compared with the scars of the non-functional spiracles of the fifth and sixth abdominal segments. This is a secondary enlargement: its role in the mimicry of the larva by the pupa is discussed by Hinton (1955*a*).

Eubriinae

This subfamily contains more species and genera than any other in the family. In all genera functional spiracles are confined to the second to seventh abdominal segments. Although the spiracles of the first abdominal segment are non-functional, they have a well-developed atrial chamber and regulatory apparatus (see p. 241). One genus of Eubriinae has evolved plastron-bearing spiracular gills.

Eubria, Afroeubria, and Sclerocyphon

The species of *Afroeubria* pupate beneath the surface of the water (*A. flava* Vill., *A. monodi* Vill.) or (*A. bertrandi* Vill., *A. hygropetrica* Vill.) on wet stones or over those on which

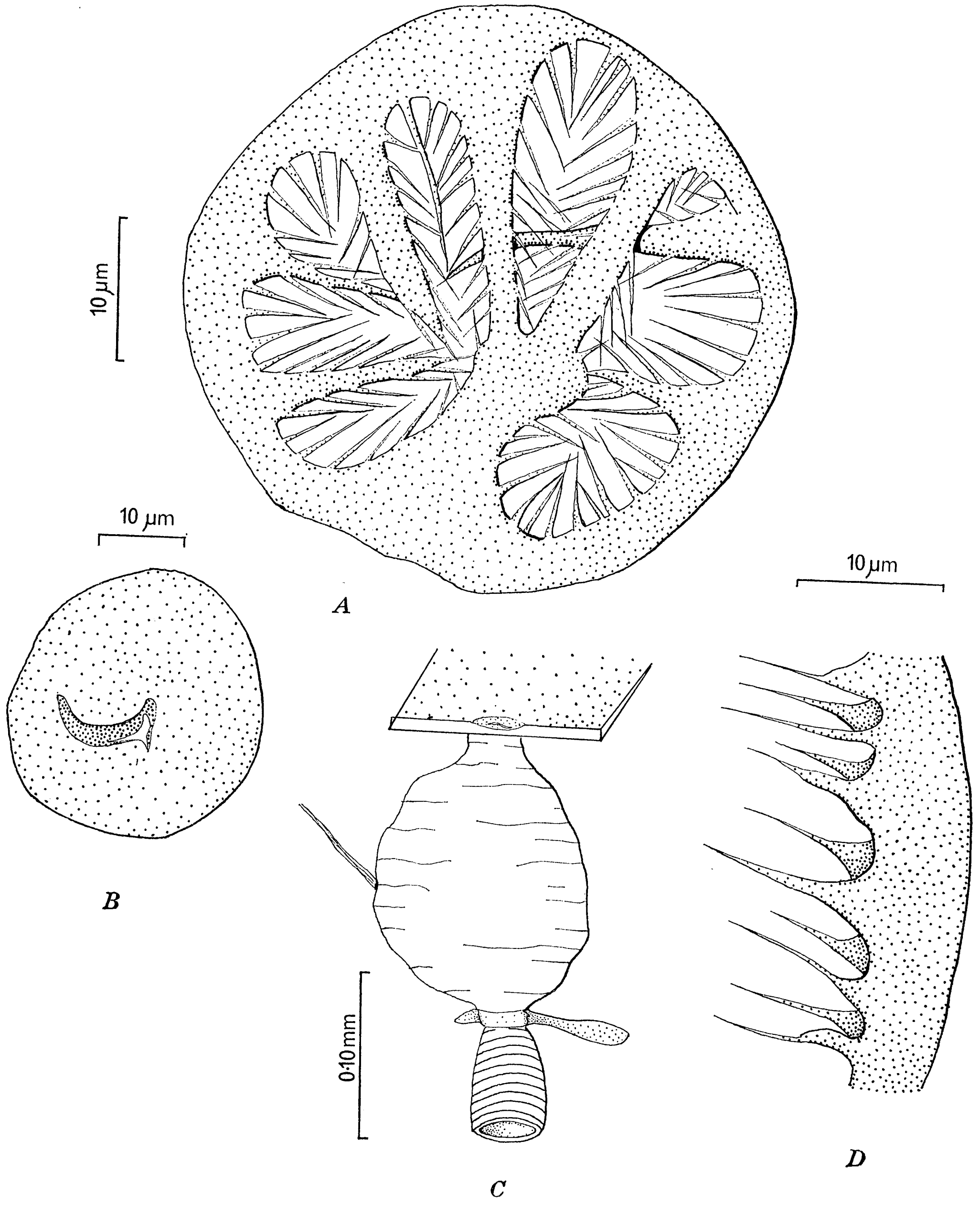

FIGURE 7. The non-functional spiracle of the first abdominal segment of *Sclerocyphon fuscus* Armstrong. (*A*) Transverse section through the stem of the spiracular atrium. (*B*) Surface view of external scar. (*C*) Lateral view of spiracle, showing the regulatory apparatus and the ecdysial tube through which the larval tracheae were withdrawn. (*D*) Section through the wall of the spiracular chamber at the level of the ecdysial tube to show the cuticular projections that line the lumen.

a film of water is moving (Bertrand 1963*a*). *Eubria palustris* Germ. pupates on wet stones close to the edge of the stream. *Sclerocyphon* pupates in cracks in stones that protrude just above the water line (C. Watts, personal communication).

The functional spiracles open on the apices of conical or cylindrical tubercles. These may be long as in *Eubria* (figure 8), many of the species of *Afroeubria* (Bertrand 1961, 1963*a*), and some unidentified Oriental genera. In some of the species of *Sclerocyphon* they are proportionally shorter (figure 2*A*), and in *S. maculatus* Blackburn they are very short. In many species of *Afroeubria*, e.g. *A. bertrandi* Villers, the spiracles become progressively smaller from the second to the seventh segments. The surface area of the spiracles of all genera examined is similar. In *Eubria palustris* (figure 8*B*) it is 3086 μm^2 or 1143 $\mu m^2/mg$, which is about 15 times less than the surface area required to satisfy oxygen demands by means of plastron respiration when it is submerged. This suggests that pupation normally occurs above water level. Records of pupae found submerged in the stream may be due to the fact that the stream level rose slightly after the insect pupated. The fact that respiration through the spiracles when they are submerged is inadequate to satisfy demand, together with the fact that pupation occurs in places that must be frequently submerged, suggests that oxygen requirements can be largely satisfied by cutaneous respiration. The functional

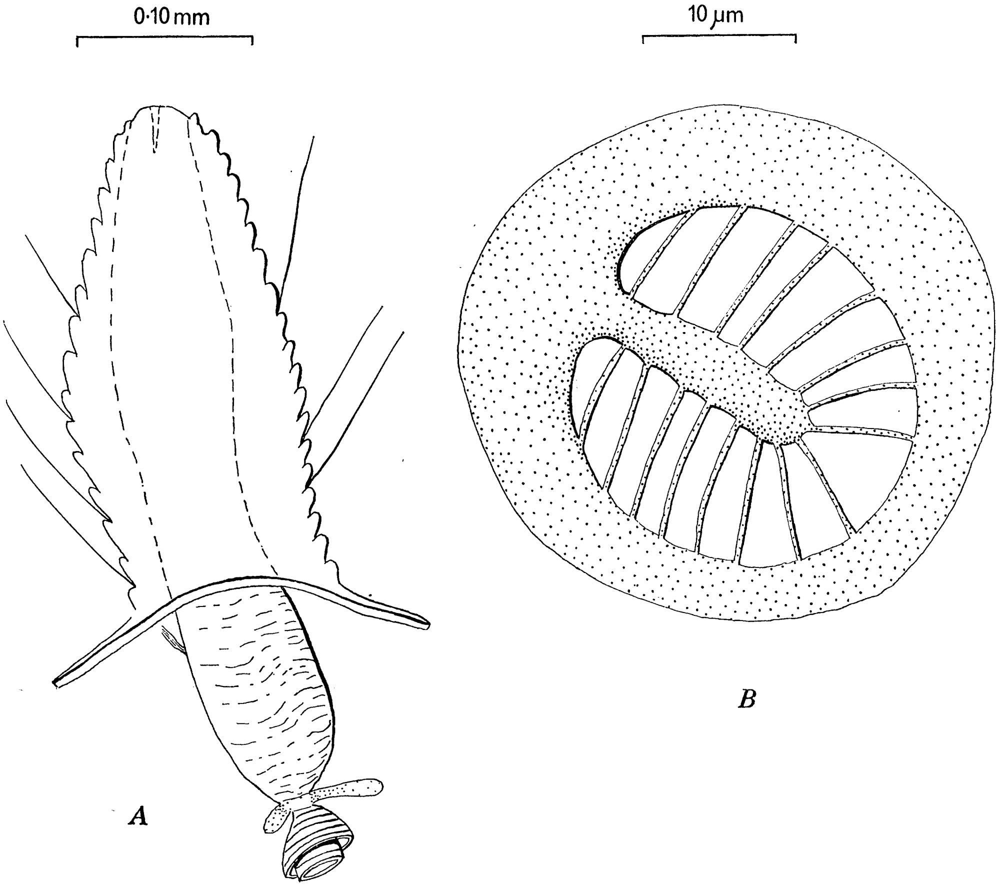

FIGURE 8. Pupa of *Eubria palustris* Germ. (*A*) Spiracle of fifth abdominal segment. (*B*) Surface view of same.

significance of the spiracular tubercles is to be seen in the fact that they enable the pupa or pharate adult to utilize atmospheric oxygen even when covered by a thin film of water.

Eubriinae, new genus

Two pupae are available of a species of Eubriinae that appears to belong to an undescribed genus. These were found in a stream in South Africa. The pupa has functional spiracles on abdominal segments 2 to 7 (figure 9). All functional spiracles are similar, and each is modified to form a plastron-bearing spiracular gill.

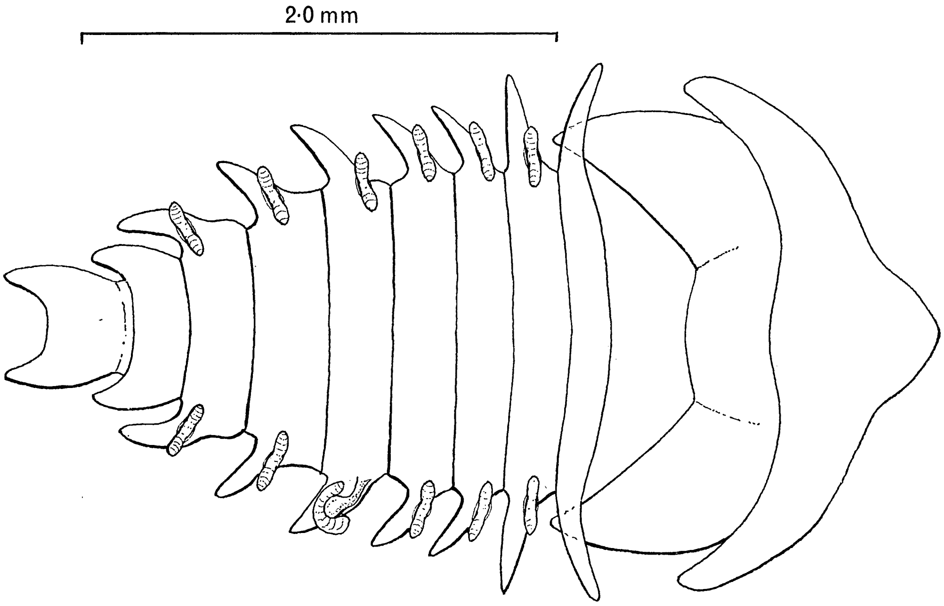

FIGURE 9. Pupa of a new genus of Eubriinae from South Africa. The spiracular gill of the right side of the fifth abdominal segment is bent down to show its general shape.

Each spiracle is at the end of a more or less vertical projection of the body wall that is about 0·4 mm long. The proximal part of the projection is a hollow cylinder through which extends the atrial chamber of the spiracle (figure 10*A*). Except for a large semi-spherical bulb near the apex, the opposed walls of the distal two-fifths of the projection are flattened and cemented together, presumably by a cuticularization of the epidermis between them. The semi-spherical apical bulb, however, is hollow, and within it are numerous epidermal cells with nuclei about 10 μm long (figure 11). When due allowance is made for the poor state of preservation of the specimens, it seems evident that the epidermis in the sphere has not undergone any appreciable degeneration although it has been completely isolated from any other tissues in the animal. The isolation of the tissue occurs in the pupal stage, and it of course remains isolated in the succeeding pharate adult stage. The fate of the two layers of epidermis in the basal three-fifths of the body wall projection could not be determined with any certainty for lack of material, but it appears that these epidermal cells are retracted rather than discarded.

The isolated tissue appears to have no function. In those insects in which a stage with long appendages is succeeded by a stage without such appendages, the epidermis that secreted the appendages is frequently discarded in the lumen of the appendages (summary in Hinton 1965). The tissue discarded within the appendages of the previous stage ceases to have a function and therefore a selective value for the new stage. It would seem that the cost of such a loss of tissue to the insect is in the long run less than the cost of retrieving it either by retracting it or cytolizing and absorbing it. To retrieve the tissue in either of

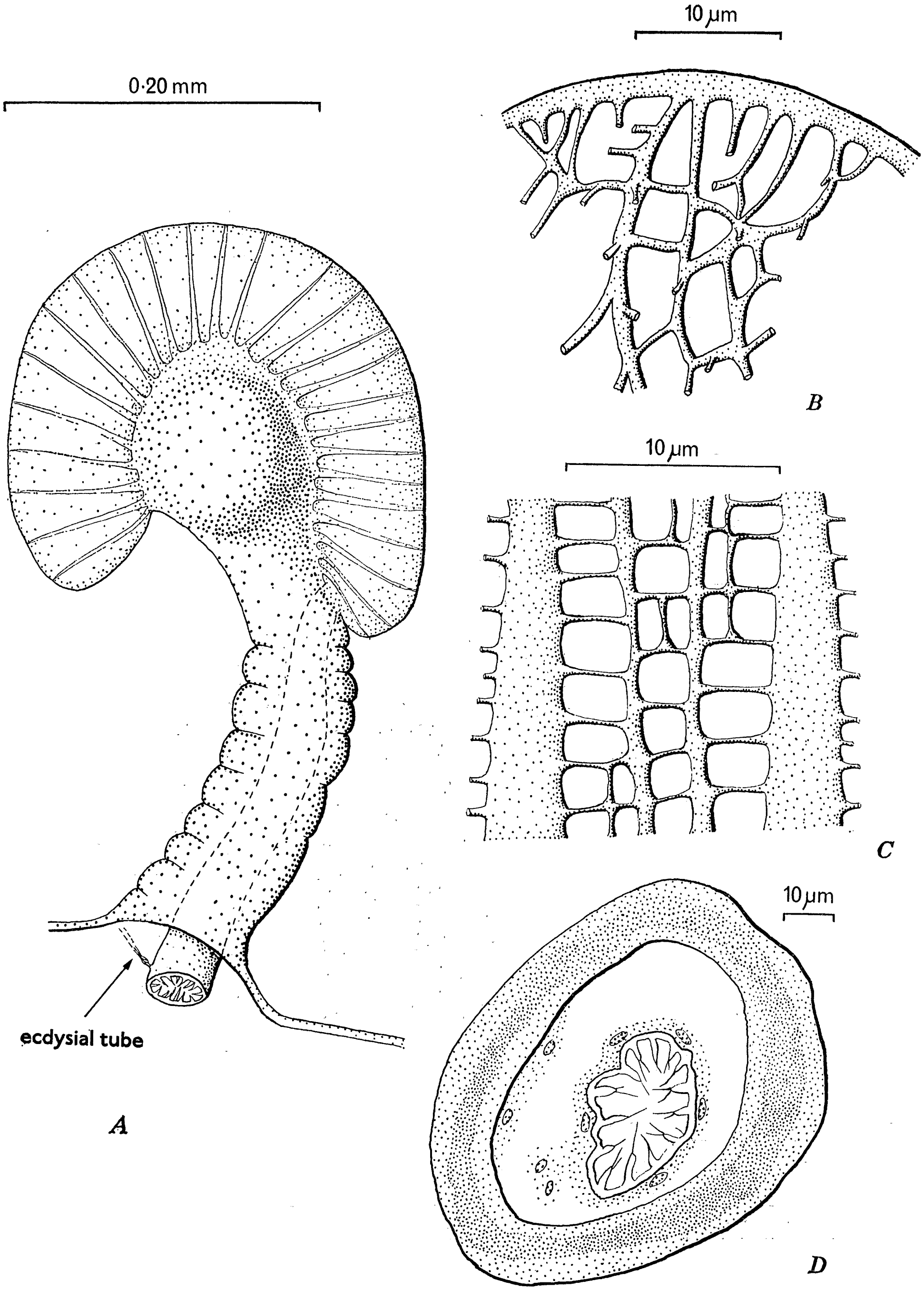

Figure 10. Pupa of a new genus of Eubriinae from South Africa. (*A*) Lateral view of one of the pair of spiracular gills of the fifth abdominal segment. (*B*) Meshwork supporting the plastron. (*C*) Plastron network between two supporting cuticular ribs; in most areas of the gill the network is much less regular than in the drawing. (*D*) Transverse section near the base of the projection from the body wall that bears the spiracle at its apex.

these ways requires a prolongation of the moulting period, a period when the insect is particularly vulnerable. It has been suggested (Hinton 1965) that in some insects tissue is discarded in certain appendages of the previous stage because, on balance, the loss it represents as a reserve of food is less than the cost that would be incurred in other ways by its absorption or retraction into the body of the new stage.

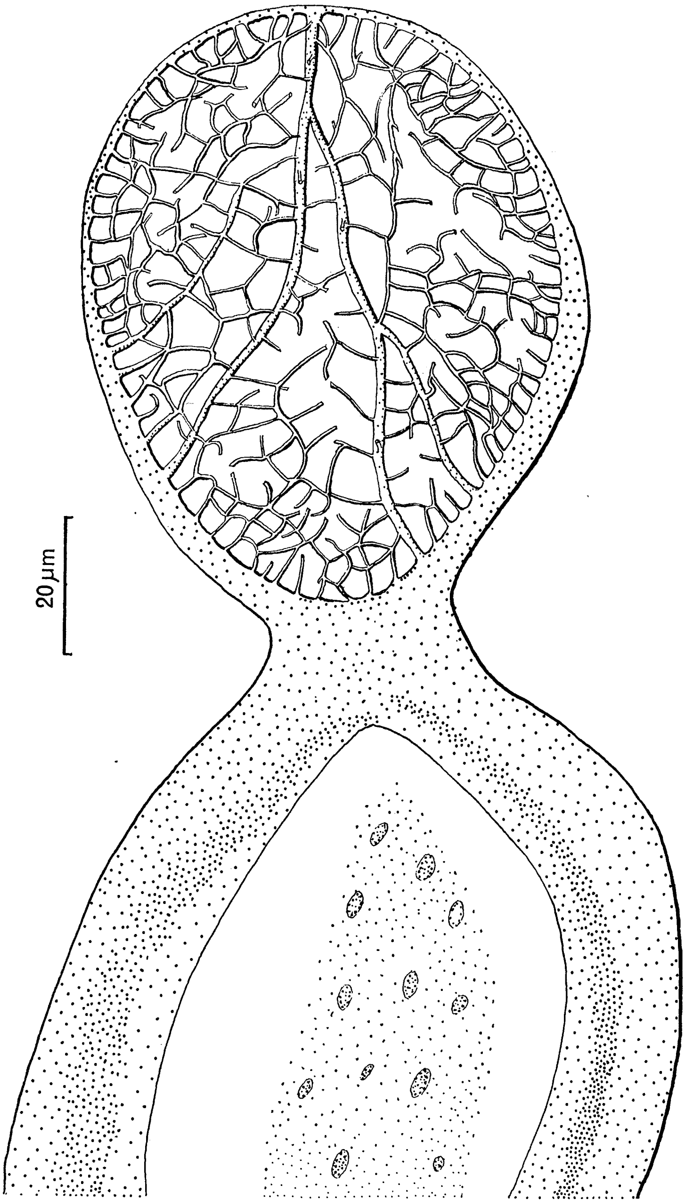

Figure 11. Pupa of a new genus of Eubriinae from South Africa. (*A*) Transverse section through the spiracular gill and the semi-spherical swelling near the apex of the projection from the body wall. The tissue shown in the semi-spherical swelling is completely isolated from the remaining tissues of the animal by a thick wall of cuticle.

Some of the insects that have evolved the habit of discarding tissue in the appendages of the previous stage exploit the attributes of the discarded tissue, which then comes to have a selective value it did not originally possess. For instance, in some flies of the family

Tipulidae, e.g. *Taphrophila* (Hinton 1957*a*) and *Orimargula* (Hinton 1965), the isolated tissue maintains the positive internal pressure necessary to avoid crumpling of the surface of the gill, and it seals breaks in the gill wall with a plug of sclerotin. In the Simuliidae (Hinton 1957*b*, 1964) the isolated tissue serves two very important functions at different times: (1) during the pharate pupal period it prevents the expansion of the gill between the larval and pupal cuticles because the solute concentration of the isolated tissue is less than that of the moulting fluid; and (2) after the larval–pupal ecdysis when the gill first comes into contact with the ambient water, absorption of water by the isolated tissue expands the gill.

The external form of the spiracle is shown in figures 9 and 10*A*. About 26 (figure 10*A*) to 30 stout cuticular struts extend around the spiracle. Between the stout struts there is a fine and open hydrofuge network that provides a large water/air interface when the gill is submerged. This network is occasionally reticulate, as shown in figure 10*C*, but is more often considerably less regular. Beneath the plastron network there is a dense supporting meshwork of cuticular struts, as shown in figures 10*B* and 11. The supporting meshwork is also hydrofuge. Thus, if pieces are accidentally broken off the gill, its respiratory function is in no way affected except that the effective plastron area is reduced. The total water/air interface of the twelve gills is $1 \cdot 6 \times 10^6$ μm or 3×10^5 μm/mg, which compares favourably with the related *Psephenoides* and is considerably better than the plastron-bearing gills of some other insects, e.g. the flies *Orimargula* and *Eutanyderus*.

PSEPHENOIDINAE

Psephenoides is the only known genus in this subfamily. It is widely distributed in the Oriental region, and one species, *P. marlieri* (Basilewsky), occurs in the littoral of Lake Tanganyika in Africa.* All of the species pupate on stones beneath the surface of the water in streams or the littoral of large lakes. The pupae have functional spiracles only on the second to seventh abdominal segments. Each spiracle is a branched plastron-bearing spiracular gill. The structure of the gills of the Indian species, *P. volatilis* Champ. and *P. gahani* Champ., was described by Hinton (1947*b*), but the structure of the plastron network, previously called the gill membrane, was omitted.

The general appearance of one of the spiracular gills of *P. volatilis* is shown in figure 12*B*, and its connexion with the tracheal system is shown in figure 13*C*. There are two lateral longitudinal tracheal trunks (figure 13*C*) that extend most of the length of the body. Marlier (1960, p. 13) described the tracheal system of *P. marlieri* as follows: 'La trachée s'enfonce dans l'intérieur du segment, montre vers l'arrière une courte ramification en corne, avengule, puis se dirige vers l'avant dans le segment antérieur où elle se divise en deux branches presque parallèles qui se terminent librement dans l'hémocèle sans rejoindre la trachée du segment antérieur.' A pupa of *P. marlieri* has been examined. Its tracheal system is similar in all essential details to that of both *P. volatilis* and *P. gahani*.

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* A new genus, *Afropsephenoides*, was erected for the African species by Basilewsky (1959). He claimed that it differed from *Psephenoides* chiefly in that the female had antennal segments 3 to 7 flabellate instead of only slightly serrate. However, the female of the Indian *P. volatilis* Champion has the antennae flabellate like the African species. Furthermore, the structure of the larvae and the pupae of the African species is typical of *Psephenoides*. *Afropsephenoides* Basilewsky (1959) must therefore be sunk as a synonym of *Psephenoides* Gahan (1914).

Marlier's account appears to be based on a shed pupal cuticle in which the connexions of the longitudinal trunks of one segment with those of the segments before and behind have been ruptured. No pterygote insect is known with a tracheal system like that described by Marlier for *Psephenoides*.

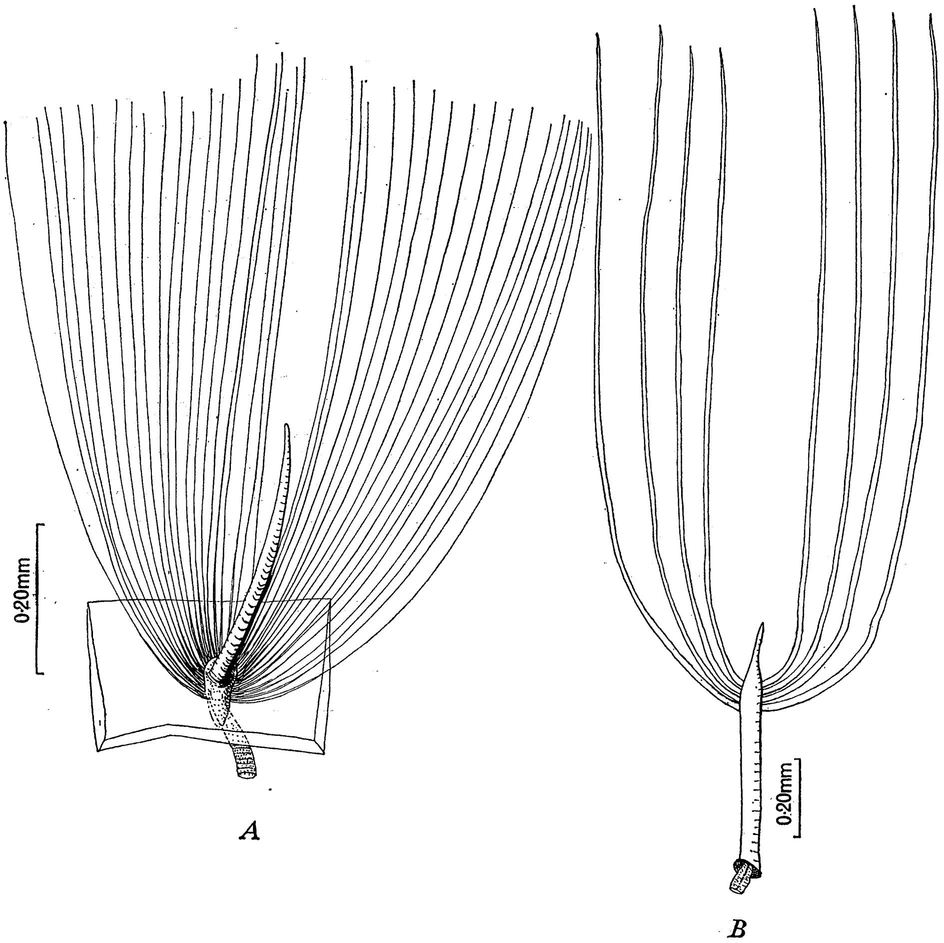

Figure 12. (*A*) Spiracular gill of right side of third abdominal segment of the pupa of *Psephenoides gahani* Champ. (*B*) Spiracular gill of right side of third abdominal segment of the pupa of *Psephenoides volatilis* Champ.

In five pupae of *P. volatilis* the number of gill branches varied as follows: second segment, 8 to 10; third, 8 to 10; fourth, 8 to 10; fifth, 8 to 10; sixth, 6 to 10; and seventh, 4 to 6. The number of branches was not always the same on both sides of a segment, and some segments had an odd number of gills. The structure of a gill branch is shown in figure 13 *A*. Each branch is a tube that is narrowly elliptical proximally but becomes more and more circular in section distally, and many branches are more or less circular in their distal half. Two of the opposed walls are very thick and strongly sclerotized. These thick walls are connected by stout cuticular struts that are somewhat irregularly distributed. These cross-struts maintain the integrity of the lumen of the branch and at the same time prevent crumpling of the two thin sides of the branch. The thin sides of the branch are sometimes connected by transverse struts (figure 19*A*, *B*, plate 3). The thin sides of the gill branch consist of longitudinal and more or less parallel struts. Between each pair of struts is a row

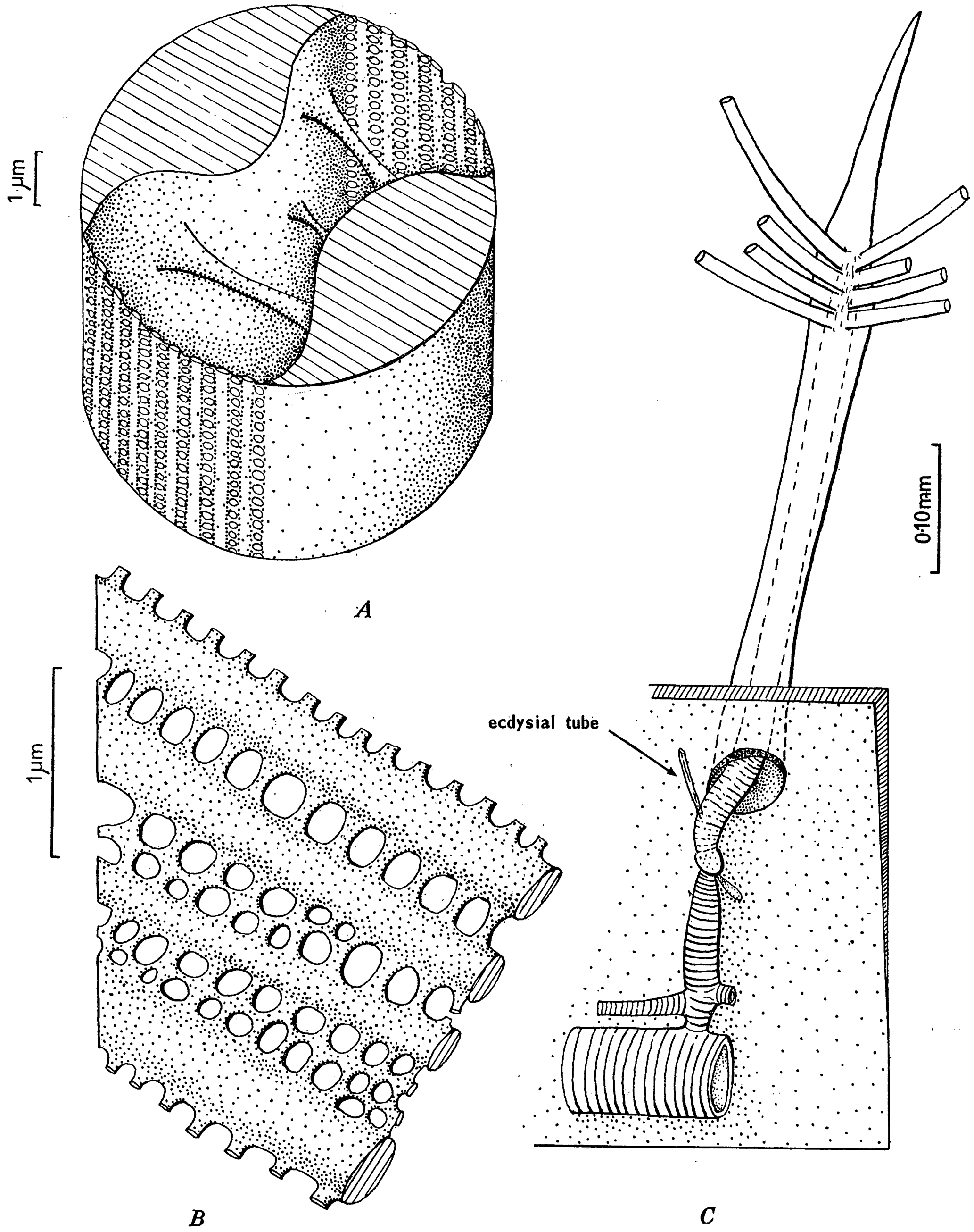

FIGURE 13. Pupa of *Psephenoides volatilis* Champ. (*A*) Structure of one of the branches of a spiracular gill. (*B*) Plastron network of a gill branch near its base. Compare with stereoscan electron micrographs shown in figure 20, plate 4. (*C*) Connexions of the spiracular gill with the tracheal system.

of holes that open directly into the lumen of the branch. Over much of the length of the branch, single rows of holes alternate regularly with longitudinal struts. Sometimes, especially near the base of the branch, there may be several rows of holes between two struts (figure 20*C*, *D*; plate 4; figure 13*B*). The holes in the two thin sides of each gill branch provide the water/air interface or the effective plastron area. They are round or broadly oval and usually 0·1 to 0·25 µm broad. The effective plastron area of a pupa with eighty-four gill branches was estimated to be approximately $2{\cdot}8 \times 10^5\,\mu m^2$ or $7 \times 10^4\,\mu m^2/mg$.

The general appearance of one of the spiracular gills of *P. gahani* is shown in figure 19*A*, *B*, plate 3 and figure 12*A*. Each gill usually has from 36 to 40 branches. The shape and structure of a gill branch is shown in figure 14. Thick and heavily sclerotized struts that extend the full length of the branch maintain its shape. The opposed walls of each pair of longitudinal struts are so shaped that a semi-elliptical cavity is formed between them. The longitudinal struts are partly held in position by cuticular bars that extend across the cavities they enclose. On each outer wall between each pair of longitudinal struts there is usually a row of holes about $0{\cdot}12\,\mu m$ wide that open directly into the spaces between the struts. Near the base of a branch there are usually eight or nine longitudinal struts. Towards the apex of the branch the longitudinal struts become much more slender and fuse with one another so that the extreme distal end of a branch may have only a single longitudinal strut. The thin wall on each side of the gill consists of a semi-reticulate network, the interstices of which are about $0{\cdot}2$ to $0{\cdot}5\,\mu m$ wide. This network forms the greater part of the effective plastron area of the branch, the total plastron area of the holes between the longitudinal struts amounting to very little in comparison. The total effective plastron area of a pupa with 432 gill branches is about $2 \times 10^6\,\mu m^2$ or $5{\cdot}4 \times 10^5\,\mu m^2/mg$.

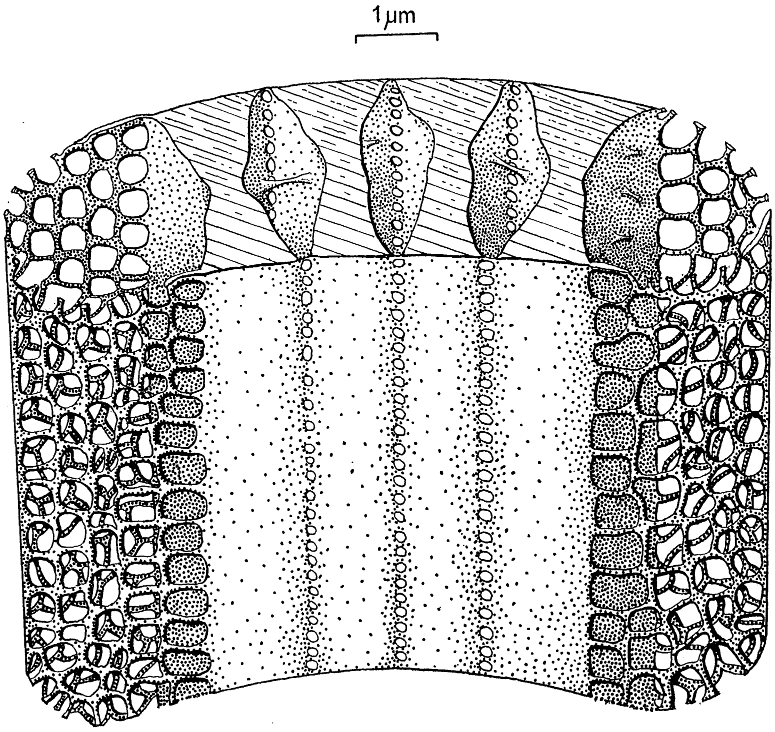

FIGURE 14. Structure of a branch of a spiracular gill of *Psephenoides gahani* Champ. The plastron network on either side is supported by slender struts arising from the outer face of the outer longitudinal strut. The number and distribution of these slender struts was not determined.

The spiracular gills of the African *P. marlieri* resemble those of *P. volatilis* rather than those of *P. gahani*. The gill branches arise near the apex of a hollow projection from the body wall, as they do in *P. volatilis* (figure 12*B*), but the projection is proportionally shorter than in the latter species. Each gill usually has fourteen branches. The shape and structure of a gill branch is shown in figure 15*A*. There was much variation in different parts of the same branch in the degree to which the two thick longitudinal struts were connected to each other, but figure 15*A* represents the usual appearance at about the middle of the length of a branch. In some gills the two thick longitudinal struts are completely fused for part of their length, and in such parts of the gill there is no communication between the lumen on either side. The plastron area is proportionally more extensive than that of *P. volatilis*. Its shape is maintained by fine struts extending across the lumen (figure 15*A*). The structure of the plastron (figure 15*B*) is very similar to that of *P. volatilis* and quite unlike that of *P. gahani* (figure 14). The total effective plastron area of a pupa with 168 gill branches is approximately $3{\cdot}4\times10^6$ μm^2 or $6{\cdot}2\times10^5$ $\mu m^2/mg$.

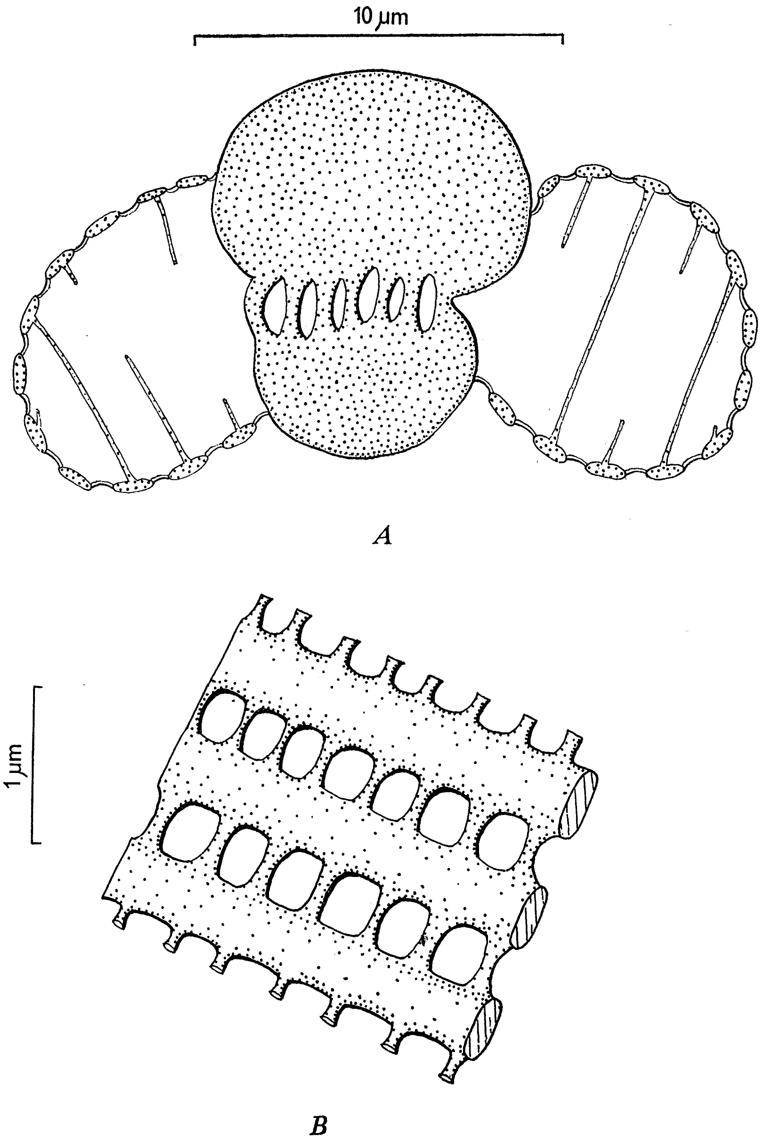

FIGURE 15. Pupa of *Psephenoides marlieri* (Basilewsky). (*A*) Transverse section of the branch of a spiracular gill at about the middle of its length. (*B*) Plastron network at about the middle of a gill branch.

Drop in pressure along the plastron

The value of $\sqrt{(i_o x_1^2/Dh)}$ determines the shape of the curve of distribution of partial pressures within the plastron (i.e. the efficiency of the plastron as a respiratory structure for a given mean drop in partial pressure between the outside medium and the spiracular atrium) where i_o is the invasion coefficient of oxygen, x_1 the greatest distance of the plastron from the spiracular atrium, D the diffusion constant of oxygen in the plastron space, and h the thickness of the plastron (Crisp 1964). The efficiency of the plastron so far as concerns the extent to which oxygen is drawn uniformly over its whole area depends only upon the function $\sqrt{(i_o x_1^2/Dh)}$. As may be seen from the curves given by Crisp (1964), the value of the function $\sqrt{(i_o x_1^2/Dh)}$ should not exceed 1·0 if oxygen is to be drawn uniformly over the whole area of the plastron; when the value exceeds 3·0 only the half of the plastron nearest to the spiracle is being effectively used.

The values for the three species of *Psephenoides* were found to be:

	$\sqrt{(i_o x_1^2/Dh)}$	$\sqrt{(2i_o x_1^2/DR)}$
P. gahani	0·30	—
P. volatilis	0·28	0·56
P. marlieri	0·45	0·42

The value of i_o ($4 \cdot 8 \times 10^{-4}$ ml. cm^{-2} s^{-1}) was not altered for *P. gahani* because it is probable that the bulging of the menisci between the horizontal struts of the plastron network (figure 14) compensates for the loss of water/air interface due to the struts themselves. In the other two species the value of i_o was reduced to account for the loss of water/air interface due to the supporting structures (figures 13*B* and 15*B*). The diffusion constant of oxygen (0·18 ml. cm^{-2} s^{-1}) requires no correction because the space within the plastron of all three species so greatly exceeds the mean free path of the oxygen molecule (0·098 μm at 18 °C). The plastrons of both *P. volatilis* and *P. marlieri* can be considered as cylindrical. If the cylinder is assumed to be of uniform radius of cross-section R, h must be replaced by $R/2$. The values of $\sqrt{(2i_o x_1^2/DR)}$ are therefore also given for both *P. volatilis* and *P. marlieri*. Using either value, however, it is clear that in all three species the whole length of the plastron of each gill branch functions efficiently. The values known for the plastrons of other spiracular gills are 0·29 to 0·33 for the tipulid, *Taphrophila vitripennis* Meig. (Hinton 1957*a*) and 0·97 for the simuliid *Simulium ornatum* Meig. (Hinton 1964). The values for the plastrons of a number of adult insects range from 0·09 to 0·75 (Thorpe & Crisp 1949).

Discussion

Summary of respiratory systems of the Psephenidae

The respiratory systems of the pupae of the Psephenidae are summarized in table 1, which also includes a summary of those of the larvae. It has previously been reported that the first abdominal spiracles of a Tasmanian pupa were functional (Hinton 1955*a*, p. 558). It is now known that this pupa was a species of *Sclerocyphon*, and the spiracles of the first abdominal segment are non-functional. Bertrand (1935) has described and illustrated the larva of a Sumatran species of Eubriinae in which he claims that functional spiracles are present on the first seven abdominal segments. It appears from his illustration that he has contrived to draw a pupa to which he has added larval legs. The functional spiracles of the

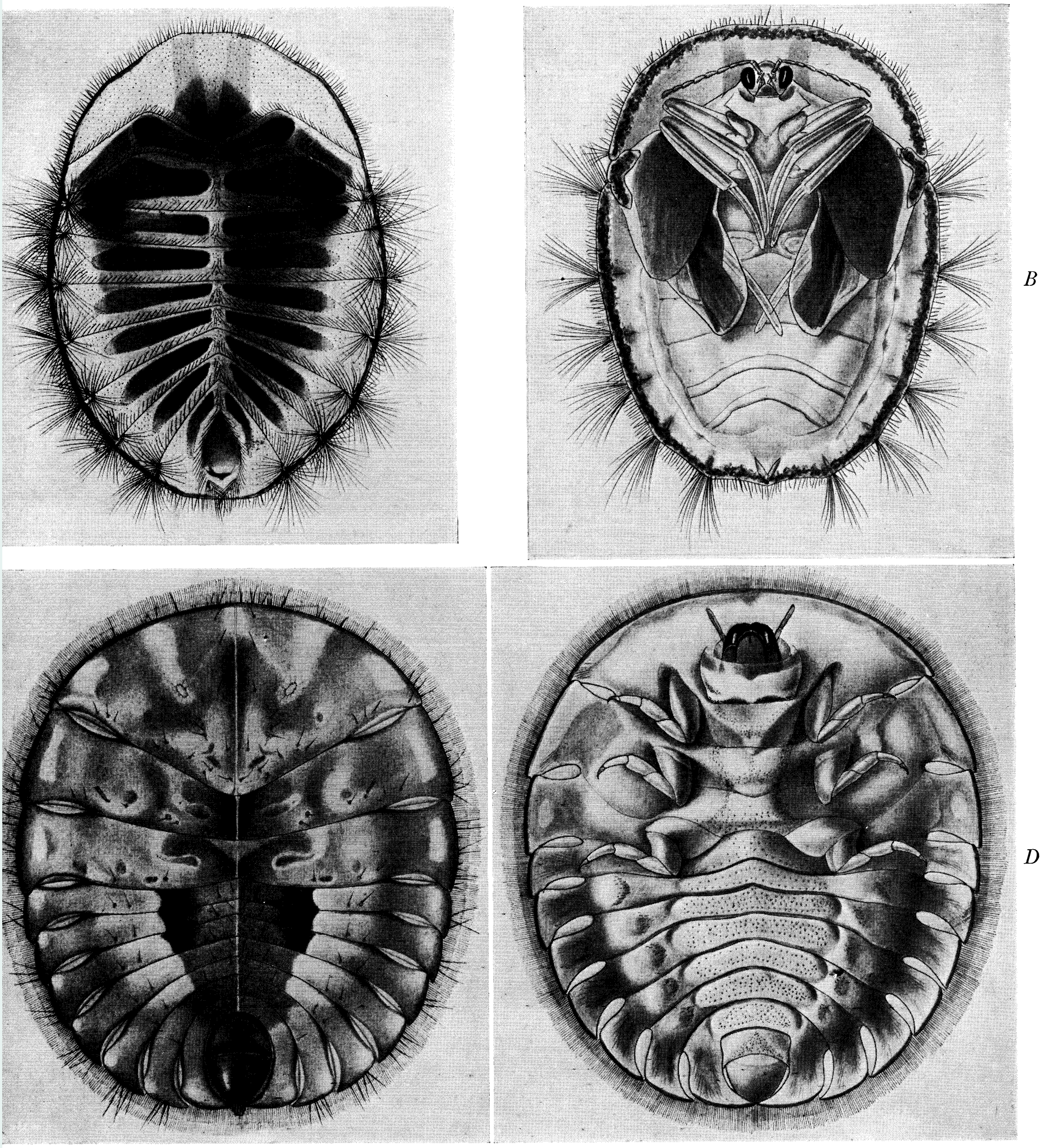

FIGURE 19. (*A*) Dorsal view of pupa of *Psephenoides gahani* Champ. (*B*) Ventral view of same. (*C*) Dorsal view of a larva of *Psephenoides* sp. (*D*) Ventral view of same.

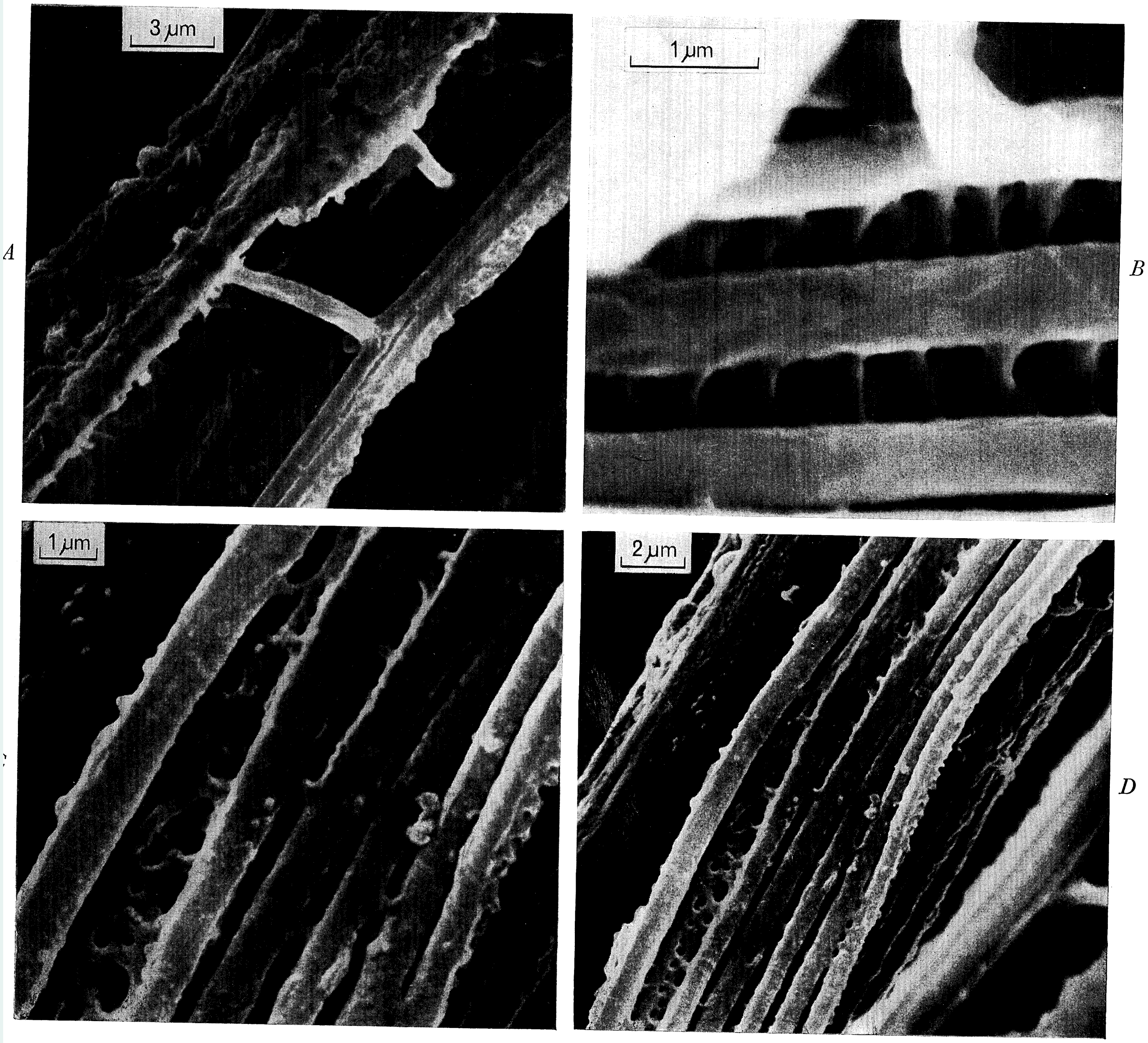

FIGURE 20. Spiracular gill of the pupa of *Psephenoides volatilis* Champ. taken with a stereoscan electron microscope. Scanning time approximately 100 sec. (*A*) Internal view of a gill branch showing that the thin walls are sometimes supported by transverse struts that arise on the thin longitudinal struts. (*B* to *D*) Different views of the thin longitudinal struts of a gill branch in about the basal third of its length.

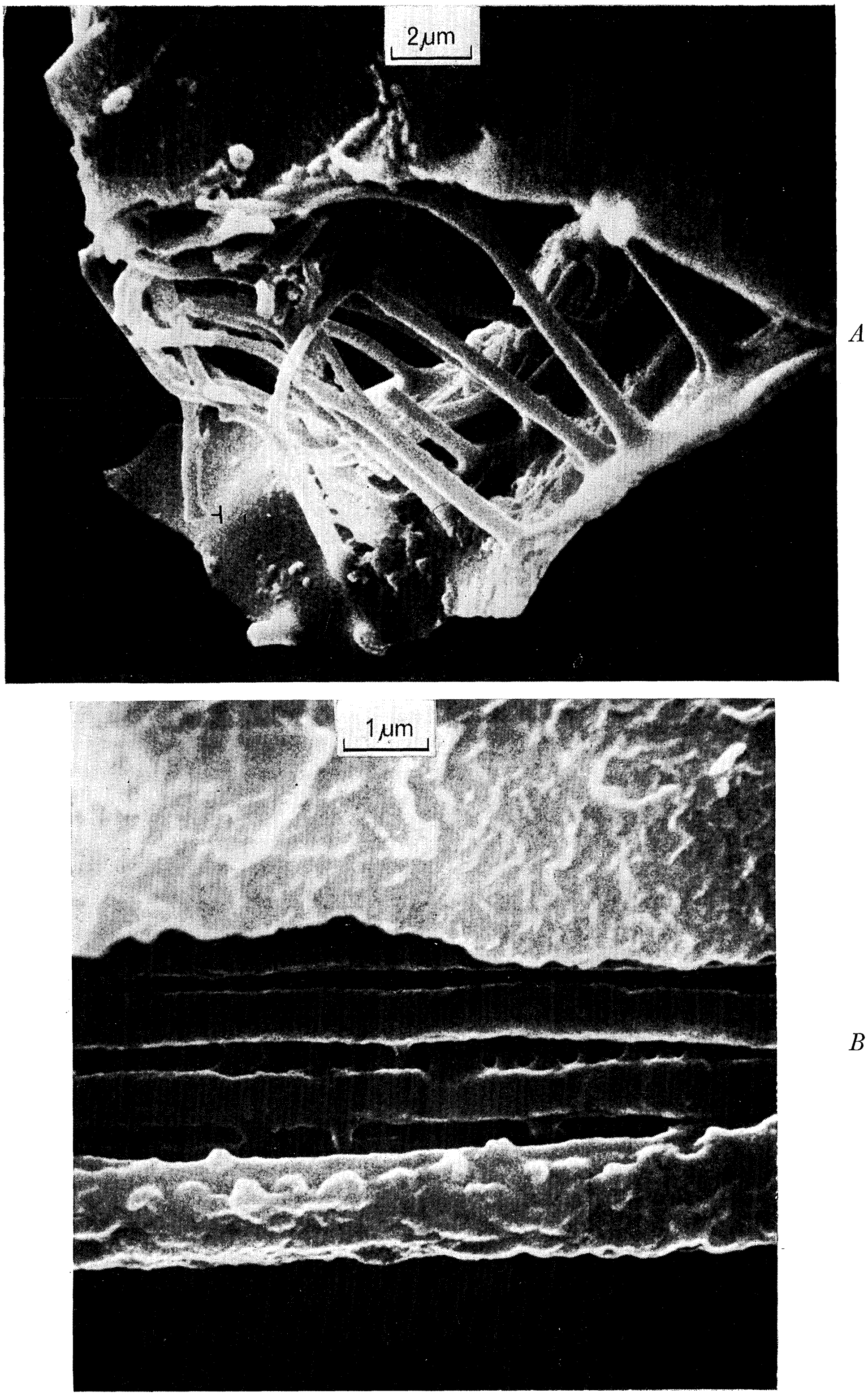

FIGURE 21. Spiracular gill of the pupa of *Psephenoides volatilis* Champ. (*A*) Internal view of a gill branch near its junction with the spiracular atrium. The plastron network of the surface is almost entirely clogged by the gold–palladium alloy. (*B*) Internal view of the thin wall of a branch near the middle of its length. The opposed thin wall has been removed.

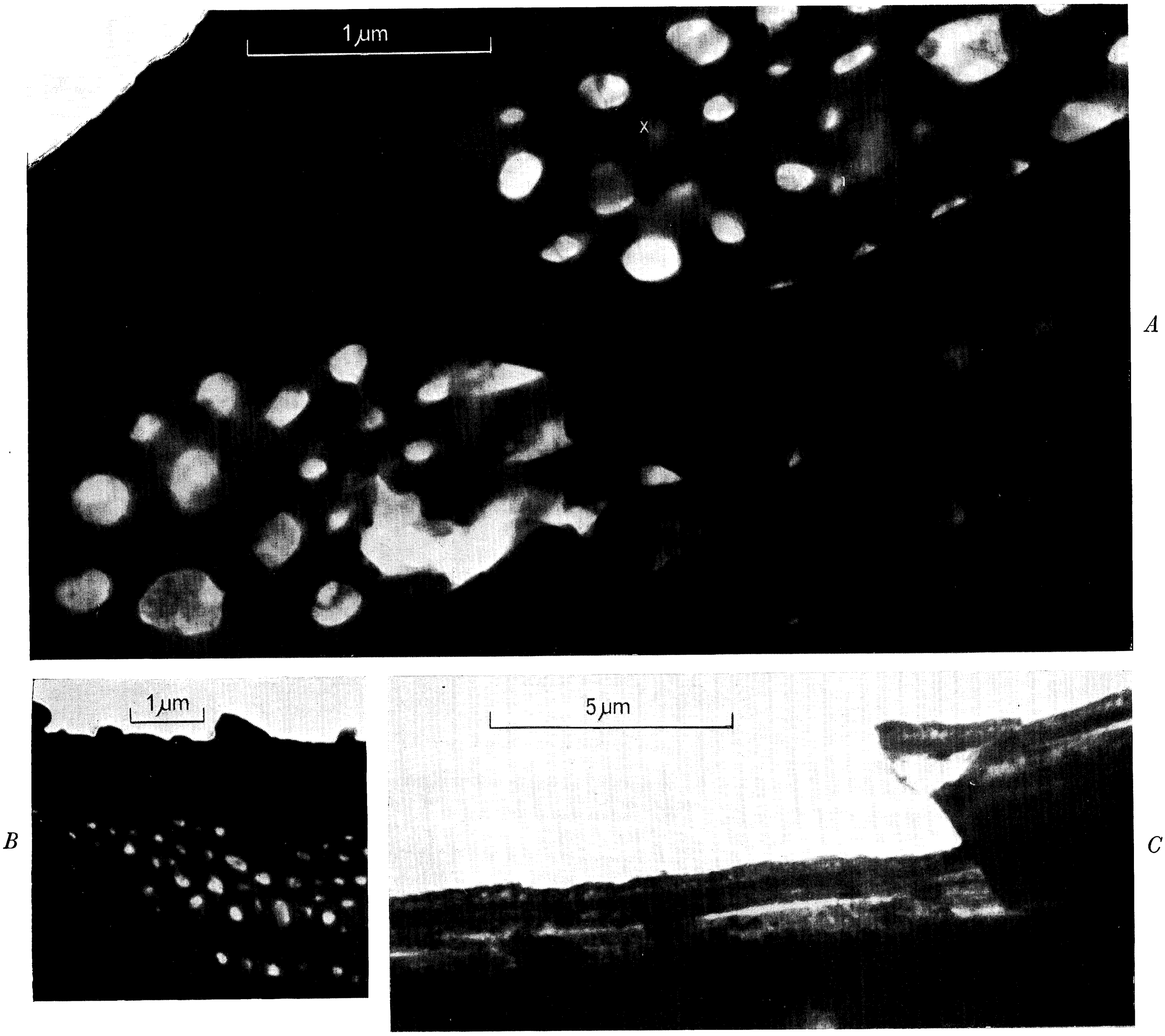

Figure 22. Spiracular gill of the pupa of *Psephenoides volatilis* Champ. taken with the transmission electron microscope. (*A*, *B*) Thin wall of a gill branch near basal third where the plastron network often consists of several rows of holes between the thin longitudinal struts. (*C*) A chip from a gill branch near the middle of its length. It shows a single row of holes between each of the thin longitudinal struts.

pupa are probably confined to abdominal segments two to seven, those of the first segment being large but non-functional, as they are in the species of *Sclerocyphon*.

The irreversibility of reductions in respiratory systems makes it possible to construct a partial phylogeny for the family (figure 16). The distribution of each subfamily fixes the date of origin of each as pre-Eocene, which is amply confirmed by the discovery of a larva of *Eubrianax* in the middle Eocene of France. The structure of this larva is not essentially different from that of recent forms (Bertrand 1963*b*). The respiratory system of the pupa of the Eubrianacinae (S$_7$) prevents the derivation of any other group in the family from *Eubrianax*-like ancestors.

Table 1. Respiratory systems of the larvae and pupae of the Psephenidae

There are always ten pairs of spiracles. Open circles indicate functional spiracles.

	thorax		abdomen								
	II	III	1	2	3	4	5	6	7	8	gills
Psepheninae											
Psephenus											
larva, all instars	o	.	.	.	.	.	.	.	.	o	ventral
pupa	.	.	o	o	o	o	o	o	o	.	—
Metaeopsephenus											
larva, all instars	o	.	.	.	.	.	.	.	.	o	ventral
pupa	.	.	o	o	o	?	?	o	o	.	—
Eubrianacinae											
Eubrianax											
larva, all instars	o	.	.	.	.	.	.	.	.	o	ventral
pupa	.	.	.	.	.	.	.	.	o	.	—
Eubriinae											
Eubria											
larva, all instars	.	.	.	.	.	.	.	.	.	o	anal
pupa	.	.	.	o	o	o	o	o	o	.	—
Indian genus											
larva, all instars	.	.	.	.	.	.	.	.	.	o	anal
pupa	.	.	.	o	o	o	o	o	o	.	—
Sclerocyphon											
larva, early instars	.	.	.	.	.	.	.	.	.	.	anal
larva, final instar	.	.	.	.	.	.	.	.	.	o	anal
pupa	.	.	.	o	o	o	o	o	o	.	—
Afroeubria											
larva, all instars	.	.	.	.	.	.	.	.	.	o	anal
pupa	.	.	.	o	o	o	o	o	o	.	—
S. African genus											
larva, all instars	.	.	.	.	.	.	.	.	.	o	anal
pupa	.	.	.	o	o	o	o	o	o	.	spiracular
ʼsephenoidinae											
Psephenoides											
larva, all instars	.	.	.	.	.	.	.	.	.	.	anal
pupa	.	.	.	o	o	o	o	o	o	.	spiracular

The least reduced respiratory systems are those of the Psepheninae (larva, S$_{\text{II},8}$; pupa, S$_{1-7}$), and the respiratory systems of all other subfamilies can be derived from a respiratory system like that of the Psepheninae.

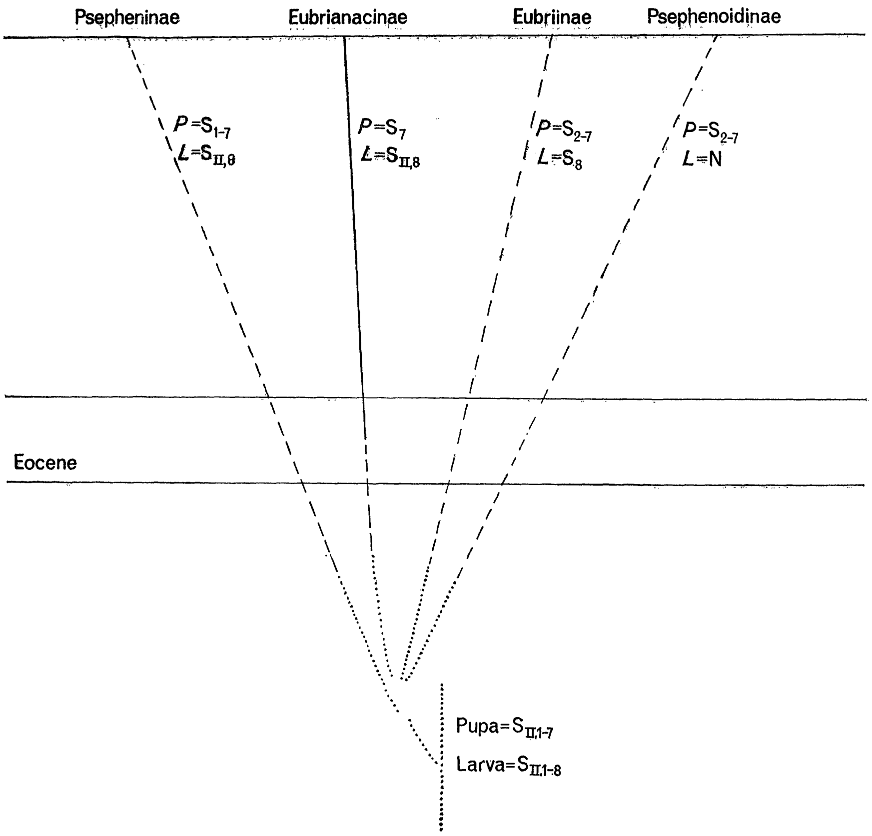

Figure 16. Diagram of the relationships of the subfamilies of Psephenidae. The solid line is based on fossil evidence. The broken lines are necessary conclusions from both distribution and comparative morphology as well as the discovery of *Eubrianax* in the middle Eocene. The dotted lines are purely speculative. The respiratory systems of the pupae (P) and larvae (L) are included.

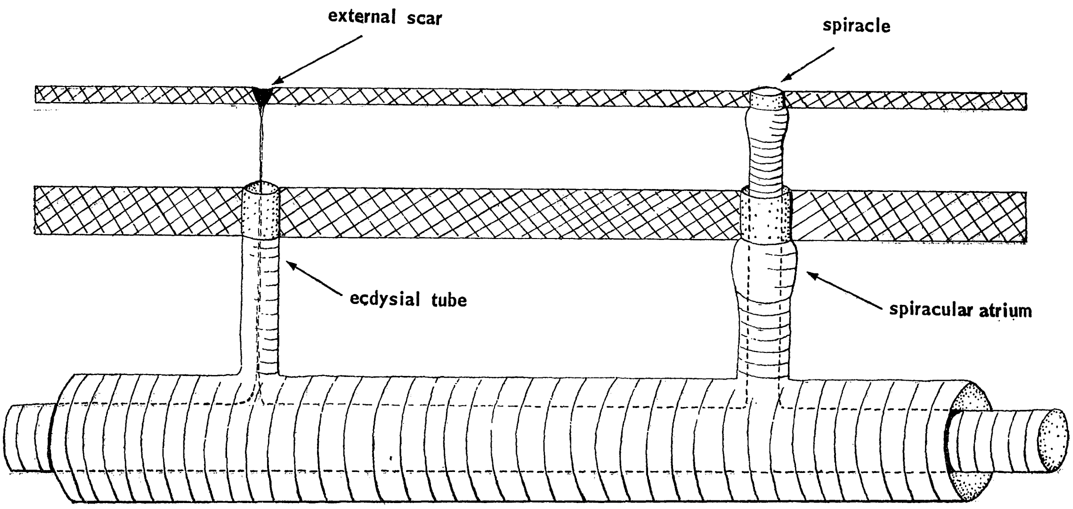

Figure 17. A pharate third-instar larva. Diagram of the moulting process of the two posterior tracheo-spiracular metameres of a species that is metapneustic in all instars.

Although it seems very unlikely that the retractile anal gills of the larvae of the Eubriinae and Psephenoidinae are independently evolved, it must be noted that there are at least three independent origins for such gills in beetle larvae, the two others being in the families Elminthidae and Limnichidae (Hinton 1939). The Eubriinae cannot be derived from a *Psephenoides*-like ancestor because *Psephenoides* has a larva that is apneustic in all instars. However, the reverse derivation is possible. Thus, if *Psephenoides* was derived from a common ancestor like the Eubriinae, the derivation may have occurred after the reduction of the spiracles of the first abdominal segment. However, in view of the large size of these reduced spiracles in the Eubriinae, and the fact that the regulatory apparatus is still present, it would seem that it is necessary to postulate an independent reduction of the first abdominal spiracles in the two subfamilies.

Irreversible evolution of respiratory systems

Dollo's (1893, 1909) generalization that evolution is irreversible is very well illustrated by the evolution of respiratory systems in insects. The importance of Dollo's law in phylogenetic studies lies in the fact that it makes it possible to distinguish between permissible and impermissible derivations. At first sight Dollo's law would appear to be no more than a statement of probability, but, as shown below, the nature of the irreversibility of respiratory systems suggests that the question of probability is not a crucial one. No exception to the law is known amongst insects with respect to any structure. Examples of irreversible losses of functional spiracles are cited that concern more than a million cases. In order fully to appreciate the instances of irreversibility cited, the primitive or basic form of the respiratory system must first be described.

In the Lepismatidae and pterygote insects the tracheal system is formed from ectodermal invaginations in the last two segments of the thorax and the first eight of the abdomen. There are thus ten pairs of spiracles, and no insect is known that has more than two pairs of thoracic or more than eight pairs of abdominal spiracles. Some or all of these may be open only at the moults.

The most anterior pair of thoracic spiracles is sometimes not on the mesothorax but on the prothorax, e.g. all larvae of the superorder Panorpoidea. This has given rise to the belief that the most anterior tracheo-spiracular metamere is prothoracic and that the mesothorax has not its own tracheal system but is tracheated from the segments before and behind. This view has been maintained by Keilin (1944 and earlier papers), but it is incorrect. In the embryo the most anterior pair of spiracles always arises on the mesothorax. For instance, in the embryo of *Diacrisia virginica* F. the external orifice of the tracheal invagination on the anterior margin of the mesothorax does not migrate on to the prothorax until after the anterior and posterior tracheal branches are recognizable, that is, the migration occurs after the 65th and before the 77th hour after the egg is laid (Johannsen & Butt 1941). Functional prothoracic and mesothoracic spiracles exclude each other (Palmén 1877; Krancher 1881); and from the distribution of the anterior thoracic spiracle in various pterygote orders it seems clear that its presence on the prothorax is secondary and not primitive (Hinton 1947*a*).

It has been said that the spiracles primitively originate in the intersegmental regions (Keilin 1944, p. 24), but there are no intersegmental (intermetameric) regions in insects.

The line between two adjacent segments is like a Euclidean point: it has position but no magnitude. Keilin's belief in the intersegmental origin of spiracles is partly based on his assumption that dipterous larvae have only eight abdominal segments: 'It is well known that the maximum number of segments (not counting the head) in dipterous larvae is eleven.' Many dipterous larvae have thirteen segments behind the head, e.g. Chironomidae (Hinton 1955*b*). Even if all dipterous larvae had only eight abdominal segments, evidence of the origin of spiracles is better based on more primitive orders, most of which have at least nine or ten well-differentiated abdominal segments.

In primitive insects, and even in some highly specialized ones, all ten pairs of spiracles remain open and function in respiration. In very many insects, however, one or more pairs of spiracles cease to function in respiration and are then described as non-functional. The non-functional spiracles persist. Thus the number of functional plus non-functional spiracles is always ten. The persistence of non-functional spiracles has been discussed by Palmén (1877), Keilin (1944), and Hinton (1947*a*). Although the non-functional spiracles are not concerned in gas exchanges between the insect and the ambient environment, their persistence is attributable to the fact that they function both at the beginning and at the end of each instar (Hinton 1947*a*, p. 457): '... (1) when they are first formed their chambers (ecdysial tubes) provide a lumen through which the old tracheae may be withdrawn: and (2) after the appearance of the new instar their chambers, now collapsed, are the means by which the tracheae are anchored to the cuticle that is to be shed'. Thus non-functional spiracles are only non-functional in the sense that they are not used in gas exchanges. Without them the insect would be quite unable to expel the old tracheae at ecdysis. It is for this function—the mechanical expulsion of the old tracheae—that they persist in, for instance, larvae that are apneustic in all instars.

The irreversible reduction of functional spiracles could hardly be a more critical test of Dollo's law of irreversibility because each non-functional spiracle is in fact open at each moult and does not close again until after the ecdysis following the moult. It would seem to be a relatively slight matter to stabilize the spiracle and the atrial chamber in the open condition at ecdysis: tanning of the cuticle of the spiracle, and often also of the atrial chamber (ecdysial tube), occurs in any event just after ecdysis. At this time there is a more or less centripetal contraction of the peritreme of the spiracle until the orifice is closed. Closure of the non-functional spiracle is preceded or accompanied by a collapse of its atrial chamber or ecdysial tube. But notwithstanding the heavy selective pressure that must exist against closure of the spiracle in some groups, there is no known exception to the rule that once a spiracle has become non-functional in a particular stage it always remains non-functional in that stage although it may become functional in a later ontogenetic stage. The relation between moulting and ecdysis of functional and non-functional spiracles is shown in figure 17.

(1) *Eighth abdominal spiracles of adult beetles*

From an examination of a few primitive forms of Cucujiformia (suborder Polyphaga), a group of about 150000 species, it was predicted that all would lack functional spiracles on the eighth abdominal segment. Subsequent examination of large numbers of species showed that this was indeed an irreversible loss (Crowson 1965).

(2) *Metathoracic spiracles of endopterygote pupae*

The metathoracic spiracles are non-functional in primitive Hymenopteroidea, Neuropteroidea and Panorpoidea. It therefore seems certain that they are non-functional in all endopterygote pupae. They are non-functional in all of the more specialized forms that have been examined, although they are sometimes unusually large, e.g. beetles of the family Lycidae. The cause of the reduction of this pair of spiracles is not known. It is sometimes said to be due to the fact that the appropriate area of the body wall is covered by the wings. In species with more or less wingless pupae the metathoracic spiracles remain non-functional. The metathoracic spiracles may have become non-functional in the common ancestor of the endopterygotes or independently in the ancestors of all three superorders. In either event, the loss is irreversible and concerns nearly 600000 species.

(3) *Eighth abdominal spiracles of endopterygote pupae*

(*a*) *Neuropteroidea*. The condition of the eighth abdominal spiracle is not known in the suborder Archostemata, but it is non-functional in the other suborders of the Coleoptera. The irreversible loss of a functional spiracle in this order thus concerns some 250000 to 300000 species. The loss of a functional spiracle on the eighth abdominal segment is irreversible irrespective of the relations between the pupa and its environment. For instance, when selective pressures are such that only the most posterior spiracles are functional, e.g. *Eubrianax*, it is the spiracles of the seventh and not those of the eighth that are functional. The loss of a functional spiracle on the eighth abdominal segment has occurred several times independently within the Neuropteroidea. In the Megaloptera it is functional in the Raphidiidae but not in the Sialidae, and it is functional in many Neuroptera, e.g. Osmylidae, Chrysopidae, and Mantispidae.

(*b*) *Panorpoidea*. The eighth abdominal spiracles are functional in the Zeugloptera, Mecoptera, and Boreidae, all of which are peripneustic ($S_{\text{II},1-8}$). The Trichoptera are apneustic or only have the mesothoracic spiracles functional (Rhyacophilidae). The Limnophilidae are apneustic. The pupa of the secondarily terrestrial Limnophilid, *Enoicyla*, has not been examined, but it is taking no risk to predict that it too will be apneustic because it is derived from apneustic ancestors.

In the Lepidoptera the primitive Monotrysia and Ditrysia lack functional spiracles on the eighth abdominal segment as do all the more specialized forms of both suborders that have been examined. The loss of functional spiracles on this segment is irreversible and concerns over 100000 species. In the most primitive suborder of the Lepidoptera, Dacnonypha, the spiracles of the eighth abdominal segment are non-functional in *Eriocrania*. It therefore seems that they may be non-functional in all lepidopterous pupae, although functional spiracles are said to be present on the eighth abdominal segment of *Agathiphaga* (Eriocraniidae), a genus I have not been able to examine.

The Diptera include some 80000 species. Primitive forms have lost the functional spiracles of the eighth abdominal segment. This loss is not known to be reversed in any species of the order in spite of the remarkable diversity of their habits.

(4) *Ontogenetic sequences of functional spiracles*

From a study of the distribution of functional spiracles in successive instars of aquatic larvae, it was possible to formulate a number of generalizations (Hinton 1947*a*). Two of these are relevant to the question of irreversibility:

(1) Whenever there is a difference in the number of functional spiracles between two larval instars of the same species, the number of functional spiracles of the earlier instar is less than that of the later instar.

(2) Whenever the respiratory system is apneustic in one larval instar it is apneustic in all previous larval instars. This is a necessary corollary of the first generalization.

The direct dependence upon atmospheric oxygen of all aquatic larvae remains the same or increases, but never decreases, throughout larval life. It is this biological fact that makes it possible to generalize about the developmental sequences of functional spiracles.

Since the formulation of these two generalizations in 1947, a much wider range of forms has been examined, and no exceptions have been found. These generalizations are also valid for groups that are now terrestrial but are derived from animals that lived in water or in a liquid medium. This would not be expected but for the fact that spiracles that become non-functional in a particular instar in one group remain non-functional in the corresponding instar of any groups subsequently evolved from that group. A particularly striking example of this concerns the Diptera. Keilin (1944) has shown that the first instar of dipterous larvae never have more than one pair of functional spiracles. They are either apneustic or metapneustic irrespective of whether they are aquatic or terrestrial. This is the clearest kind of evidence that the larva of the common ancestor of recent Diptera lived in a liquid. That the liquid could not have been water is indicated by the fact that all recent dipterous larvae lack legs: no instance is known of an insect losing legs after invading water, but, on the contrary, invasion of water by larvae without legs usually results in the evolution of prolegs (Hinton 1955*b*). The common ancestor of recent Diptera almost certainly lived in liquid or semi-liquid organic matter, an environment in which both functional spiracles and legs are lost (Hinton 1947*a*).

(5) *Secondarily terrestrial apneustic species*

Although insects as a class are primitively terrestrial in all stages, the larvae of some recent orders and families are primitively aquatic and have become apneustic. Some of these apneustic larvae leave the water and become secondarily adapted to life on land, e.g. *Enoicyla* (Trichoptera) and many Chironomidae and Ceratopogonidae. They remain apneustic on land. This is of particular interest because there must be heavy selective pressures to return to an open tracheal system which would permit them to waterproof the body wall cuticle and dispense with cutaneous respiration and its attendant disadvantages on land.

Persistence of non-functional structures

When the relations between the organism and its environment change so that some structure or other characteristic of the organism ceases to have a selective value, that structure or characteristic is sooner or later lost. The time required for its loss will vary according to a great number of factors: whether it is biologically neutral (apart from the energy required for its production) or actively selected against, the magnitude of the alterations necessarily involved in its loss, and so forth.

The non-functional first abdominal spiracles of the Eubriinae still retain a normal atrial chamber with the usual cuticular projections that prevent large particles of debris from entering the trachea (figure 7*A*). Of even more interest is the fact that they retain a well-developed regulatory apparatus (figure 7*C*) that does not differ in any important particular from that of the functional spiracles.

The genera of Eubriinae with non-functional first abdominal spiracles that have a well-developed atrial chamber and regulatory apparatus occur in Europe, Africa, Asia and Australia. A distribution of this kind for a group with rigid environmental requirements, and therefore unlikely to be accidentally transported from one continent to another, is evidence of a pre-Eocene origin. A pre-Eocene origin for the Eubriinae can also be established on other grounds, as noted on p. 235. We are thus left with two possibilities: (1) the first pair of abdominal spiracles has become non-functional independently in each genus of the subfamily, or (2) the common ancestor of the subfamily lacked functional spiracles on the first abdominal segment. The second alternative seems the more likely, but it does imply the persistence of non-functional structures since the pre-Tertiary.

A regulatory apparatus is present on the apparently non-functional spiracles of the fourth and fifth abdominal segments of *Metaeopsephenus japonicus* Mats. In this instance, however, it is not possible to give an approximate estimate of the time when the spiracles became non-functional.

Polyphyletic origin of spiracular gills

The plastron-bearing spiracular gills of insect pupae provide a very good example of the polyphyletic origin of a complex structure that always subserves the same function. They have been independently evolved in at least nine different groups of Diptera and two groups of Coleoptera, as follows:

Diptera (Nematocera)

(1) Tanyderidae (*Eutanyderus*) (Hinton 1966*b*)
(2) Tipulidae: some Limoniini (Hinton 1957*a*, 1965)
(3) Tipulidae: some Eriopterini (Hinton 1955*c*)
(4) Simuliidae (Hinton 1957*b*, 1964)
(5) Blepharoceridae (Hinton 1958*b*, 1962)
(6) Deuterophlebiidae (Hinton 1962)

Diptera (Brachycera-Orthorrhapha)

(7) Empididae: some Hemerodrominae (Hinton 1958*b*)
(8) Dolichopodidae (*Aphrosylus*) (Hinton 1966*a*)

Diptera (Brachycera-Cyclorrhapha)

(9) Canaceidae (*Canace*) (Hinton 1966*a*)

Coleoptera

(10) Psephenidae: one genus of Eubriinae
(11) Psephenidae: all Psephenoidinae

The evidence for their independent origin in each group of Diptera has been cited recently (Hinton 1962, 1965) and need not be repeated here. That the spiracular gills are of independent origin in the two subfamilies of the Psephenidae seems evident. All known Psephenoidinae have spiracular gills; whereas such gills are uncommon in the Eubriinae, all but one of the genera so far described (*Eubria*, *Afroeubria*, *Sclerocyphon*, and a number of unidentified genera) have biforous spiracles. If the gills of the two subfamilies are not of independent origin they must be derived from a common ancestor with gills, which in turn involves the unlikely supposition that the vast majority of the Eubriinae have lost spiracular gills. The very great differences in structure between the gills of the two subfamilies also tells against a common origin.

Classification of spiracular gills

A natural classification of spiracular gills is precluded because they have been independently evolved in each group of insects, but the classification of convenience proposed by Hinton (1958*b*) may now be amended as follows:

A. Spiracular gills without a plastron
    Chironomidae

B. Spiracular gills with a plastron

1. Spiracular gills are modifications of the spiracle. The plastron is always on the spiracle and never on the adjacent body wall. Epidermis and blood are never isolated in the gill itself. A spiracular regulatory apparatus is always present
    Psephenidae

2. Spiracular gills are modification of the body wall adjoining the spiracle (figure 18*C*) or of both the body wall and the spiracle (figure 18*D*). The plastron is always on the modified body wall and never on the spiracle. Epidermis and blood are always isolated in the spiracular gill. A spiracular regulatory apparatus is absent except in the Simuliidae

    a. Spiracular gills formed entirely by body wall adjacent to the spiracle. The spiracle extends little or not at all beyond the general level of the body wall (figure 18*C*)

    Tanyderidae

    Simuliidae

    Blepharoceridae. In some species of *Edwardsina* the gills are borne on a projection of the body wall into which the spiracular atrium extends, although, as in other Blepharoceridae, it does not extend into the gill leaflets

    Deuterophlebiidae

    Empididae

b. Spiracular gills formed by the body wall adjacent to the spiracle and by the spiracle itself, the atrium of the spiracle extending into the gill (figure 18*D*)

Tipulidae

Dolichopodidae

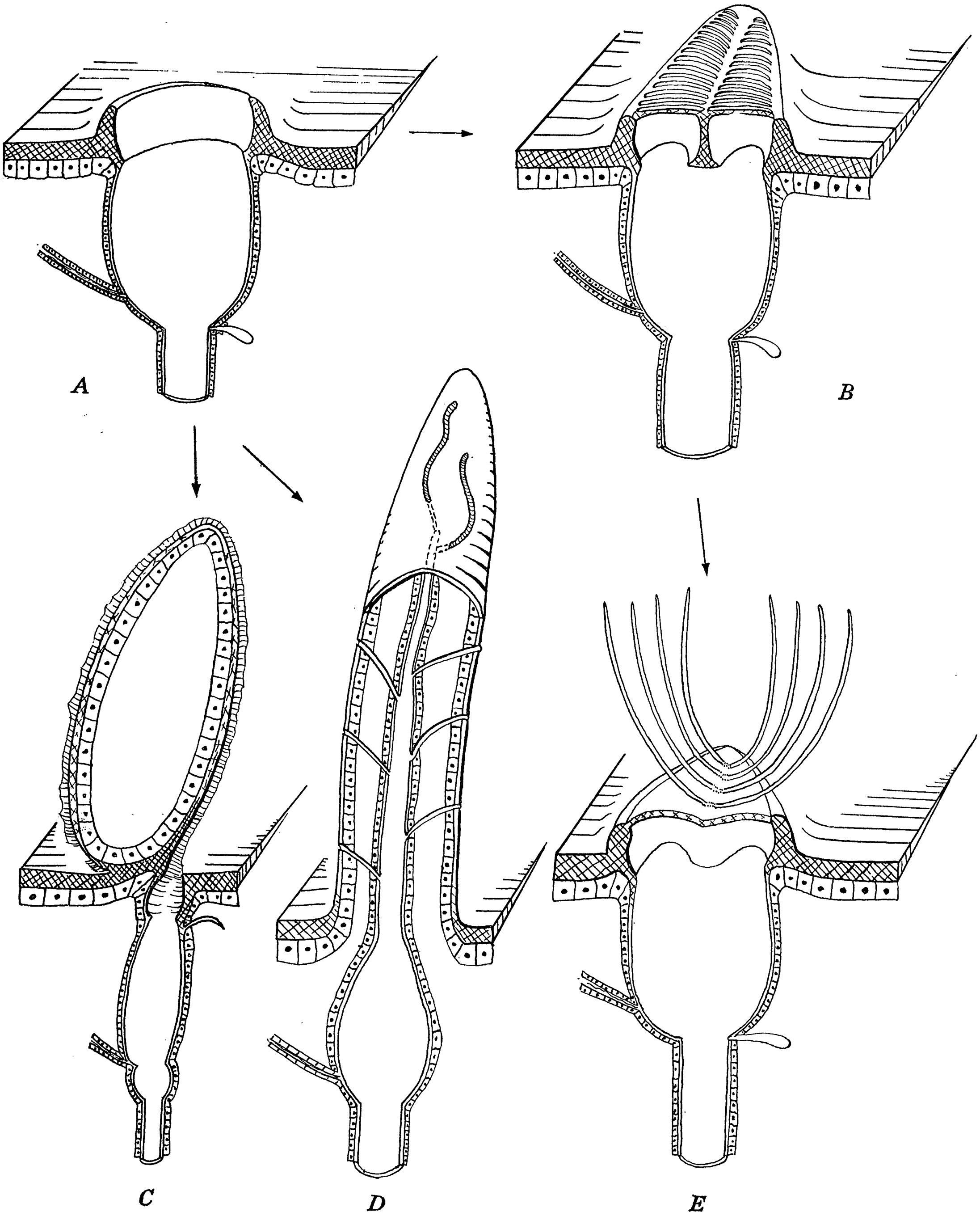

FIGURE 18. Origin of different types of spiracular gills from or near a spiracle like that shown in (*A*). (*B*) Spiracle similar to the type found in *Metaeopsephenus japonicus* Mats. (*C*) Spiracular gill of the type found in the Simuliidae. (*D*) Spiracular gill of the type found in the Tipulidae. (*E*) Presumed stage in the origin of a spiracular gill of the type found in *Psephenoides* from a stage represented in (*B*).

Further study of the structure of the plastron-bearing spiracular gills of the Canaceidae is required before their position in the classification given here can be determined.

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REFERENCES

Basilewsky, P. 1959 Description d'un genre nouveau de Pséphénides du Congo Belge (Coleoptera, Psephenidae). *Rev. Zool. Bot. afr.* **59**, 29–32.

Bertrand, H. 1935 Larves de Coléoptères aquatiques de l'expedition limnologique allemande en Insulinde. *Arch. Hydrobiol.* **14** (Suppl.), 193–285.

Bertrand, H. 1961 Contribution à l'étude des premiers états des Coléoptères aquatiques de la région éthiopienne. *Bull. Inst. fr. Afr. noire* (A) **23**, 716–738.

Bertrand, H. 1963*a* Les Eubriides (Col.) d'Afrique. *Entomologiste* **19**, 11–20.

Bertrand, H. 1963*b* Une larve 'Pséphénoide' du genre *Eubrianax* Kiesenw. (Coléoptères, Eubrianacidae) dans l'Éocène lacustre de l'Aude. *Bull. Soc. Hist. nat. Toulouse* **98**, 232–241.

Crisp, D. J. 1964 Plastron respiration. *Recent Prog. Surface Sci.* 2, 377–425.

Crowson, R. A. 1965 Classification, statistics and phylogeny. *Syst. Zool.* **14**, 144–148.

Dollo, L. 1893 Les lois de l'évolution. *Bull. Soc. belge Geol.* **7**, 164–166.

Dollo, L. 1909 La paléontologie éthologique. *Bull. Soc. belge Geol.* **23**, 377–421.

Essig, E. O. 1926 *Insects of western North America.* New York: Macmillan & Co.

Gahan, C. J. 1914 A new genus of Coleoptera of the family Psephenidae. *Entomologist* **47**, 188–189.

Hinton, H. E. 1939 An inquiry into the natural classification of the Dryopoidea, based partly on a study of their internal anatomy (Col.). *Trans. R. ent. Soc. Lond.* **89**, 133–184.

Hinton, H. E. 1946*a* Concealed phases in the metamorphosis of insects. *Nature, Lond.* **157**, 552–553.

Hinton, H. E. 1946*b* The 'gin-traps' of some beetle pupae; a protective device which appears to be unknown. *Trans. R. ent. Soc. Lond.* **97**, 473–496.

Hinton, H. E. 1947*a* On the reduction of functional spiracles in the aquatic larvae of the Holometabola, with notes on the moulting process of spiracles. *Trans. R. ent. Soc. Lond.* **98**, 449–473.

Hinton, H. E. 1947*b* The gills of some aquatic beetle pupae (Coleoptera, Psephenidae). *Proc. R. ent. Soc. Lond.* (A) **22**, 52–60.

Hinton, H. E. 1953 Some adaptations of insects to environments that are alternately dry and flooded, with some notes on the habits of the Stratiomyidae. *Trans. Soc. Brit. Ent.* **11**, 209–227.

Hinton, H. E. 1955*a* On the respiratory adaptations, biology, and taxonomy of the Psephenidae, with notes on some related families (Coleoptera). *Proc. zool. Soc. Lond.* **125**, 543–568.

Hinton, H. E. 1955*b* On the structure, function, and distribution of the prolegs of the Panorpoidea, with a criticism of the Berlese–Imms theory. *Trans. R. ent. Soc. Lond.* **106**, 455–545.

Hinton, H. E. 1955*c* The structure of the spiracular gill of the genus *Lipsothrix* (Tipulidae), with some observations on the living epithelium isolated in the gill at the pupa–adult moult. *Proc. R. ent. Soc. Lond.* (A) **30**, 1–14.

Hinton, H. E. 1957*a* The structure and function of the spiracular gill of the fly *Taphrophila vitripennis*. *Proc. Roy. Soc.* B **247**, 90–120.

Hinton, H. E. 1957*b* Some little known respiratory adaptations. *Sci. Progr.* **45**, 692–700.

Hinton, H. E. 1958*a* Concealed phases in the metamorphosis of insects. *Sci. Progr.* **46**, 260–275.

Hinton, H. E. 1958*b* The spiracular gills of insects. *Proc. 10th int. Congr. Ent.* **1**, 543–548.

Hinton, H. E. 1960*a* The structure and function of the respiratory horns of the eggs of some flies. *Phil. Trans.* B **243**, 45–73.

Hinton, H. E. 1960*b* The chorionic plastron and its role in the eggs of the Muscinae (Diptera). *Quart. J. Micr. Sci.* **101**, 313–332.

Hinton, H. E. 1961 The structure and function of the egg-shell in the Nepidae (Hemiptera). *J. Insect Physiol.* **7**, 224–257.

Hinton, H. E. 1962 The structure and function of the spiracular gills of *Deuterophlebia* (Deuterophlebiidae) in relation to those of other Diptera. *Proc. zool. Soc. Lond.* **138**, 111–122.

Hinton, H. E. 1963 The ventral ecdysial lines of the head of endopterygote larvae. *Trans. R. ent. Soc. Lond.* **115**, 39–61.

Hinton, H. E. 1964 The respiratory efficiency of the spiracular gill of *Simulium*. *J. Insect Physiol.* **10**, 73–80.

Hinton, H. E. 1965 The spiracular gill of the fly *Orimargula australiensis* and its relation to those of other insects. *Aust. J. Zool.* **13**, 783–800.

Hinton, H. E. 1966*a* Plastron respiration in marine insects. *Nature, Lond.* **209**, 220–221.

Hinton, H. E. 1966*b* The spiracular gill of the fly *Eutanyderus* (Tanyderidae). *Aust. J. Zool.* (in the Press).

Johannsen, O. A. & Butt, F. H. 1941 *The embryology of insects and myriapods*. New York: McGraw-Hill.

Keilin, D. 1944 Respiratory systems and respiratory adaptations in larvae and pupae of Diptera. *Parasitology* **36**, 1–65.

Kennedy, H. D. 1960 *Deuterophlebia inyoensis*, a new species of mountain midge from the alpine zone of the Sierra Nevada Range California (Diptera: Deuterophlebiidae). *Trans. Amer. Micr. Soc.* **79**, 191–210.

Krancher, O. 1881 Der Bau der Stigmen bei den Insekten. *Z. wiss. Zool.* **35**, 505–574.

Marlier, G. 1960 La morphologie et la biologie de la larve de l'*Afropsephenoides* (Coléoptère Pséphénoide). *Revue Zool. Bot. afr.* **61**, 1–14.

Palmén, J. A. 1877 *Zur Morphologie des Tracheensystems*. Leipzig.

Thorpe, W. H. & Crisp, D. J. 1949 Studies on plastron respiration in the Coleoptera. *J. exp. Biol.* **26**, 219–260.

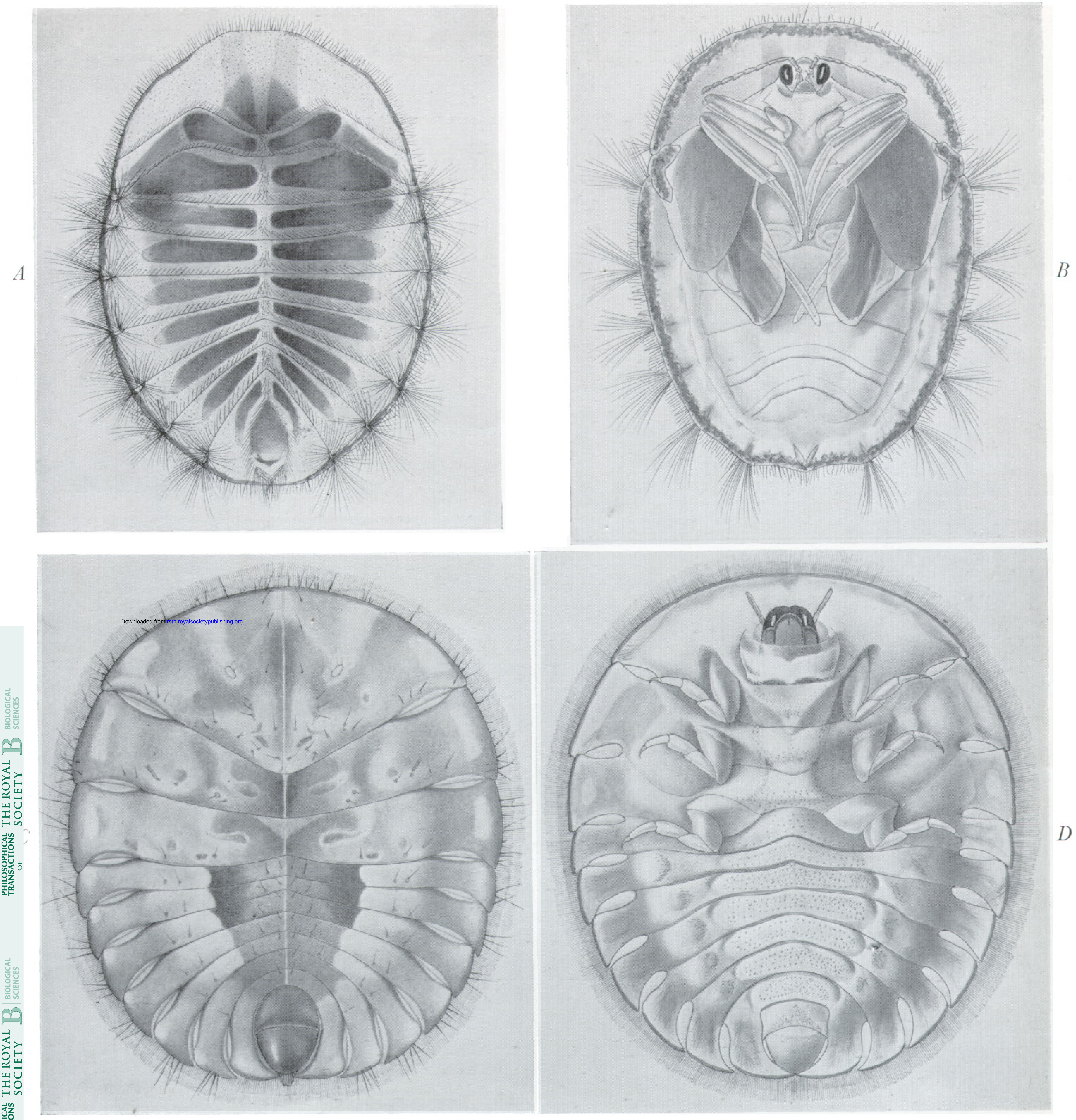

FIGURE 19. (*A*) Dorsal view of pupa of *Psephenoides gahani* Champ. (*B*) Ventral view of same. (*C*) Dorsal view of a larva of *Psephenoides* sp. (*D*) Ventral view of same.

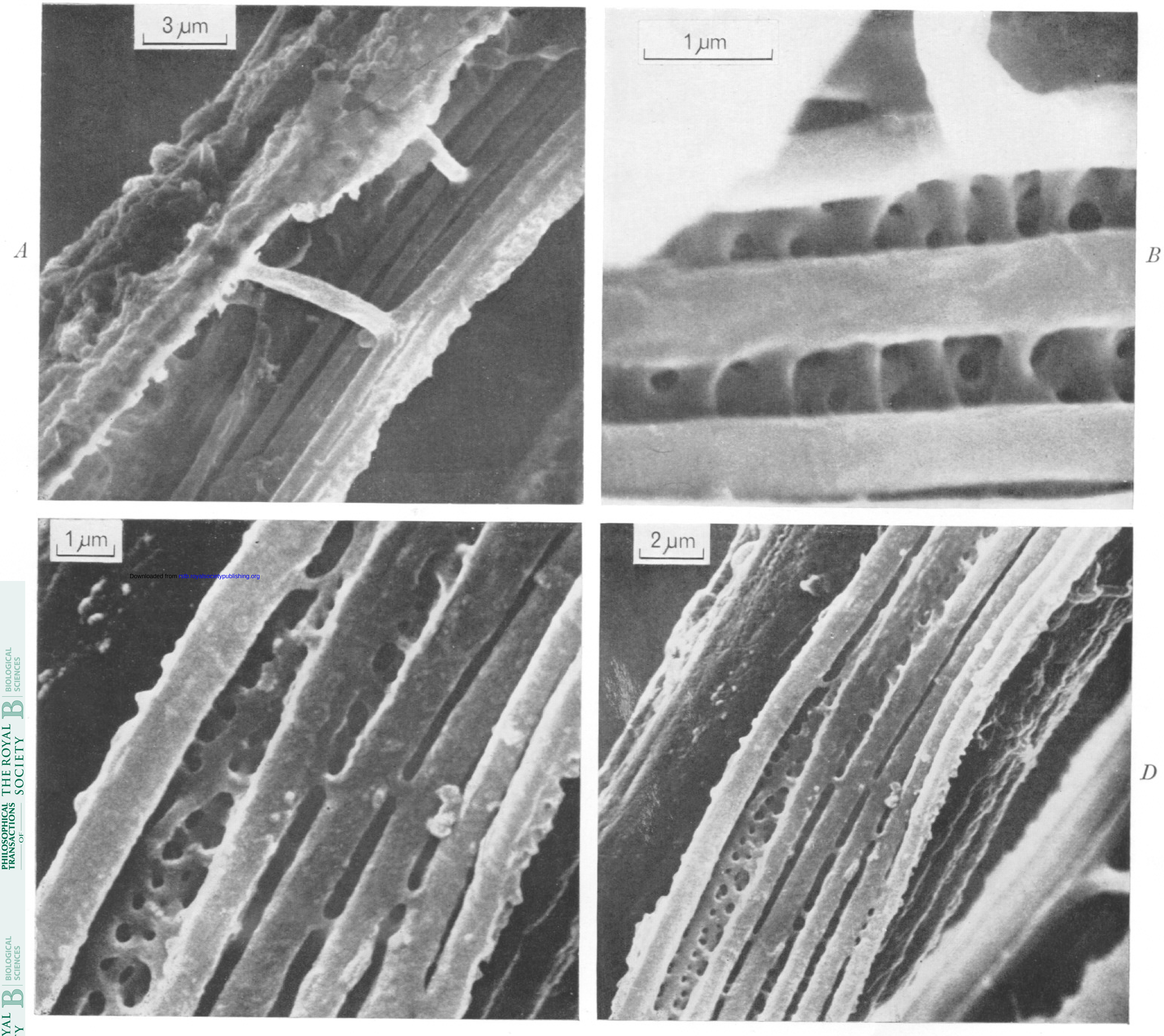

FIGURE 20. Spiracular gill of the pupa of *Psephenoides volatilis* Champ. taken with a stereoscan electron microscope. Scanning time approximately 100 sec. (*A*) Internal view of a gill branch showing that the thin walls are sometimes supported by transverse struts that arise on the thin longitudinal struts. (*B* to *D*) Different views of the thin longitudinal struts of a gill branch in about the basal third of its length.

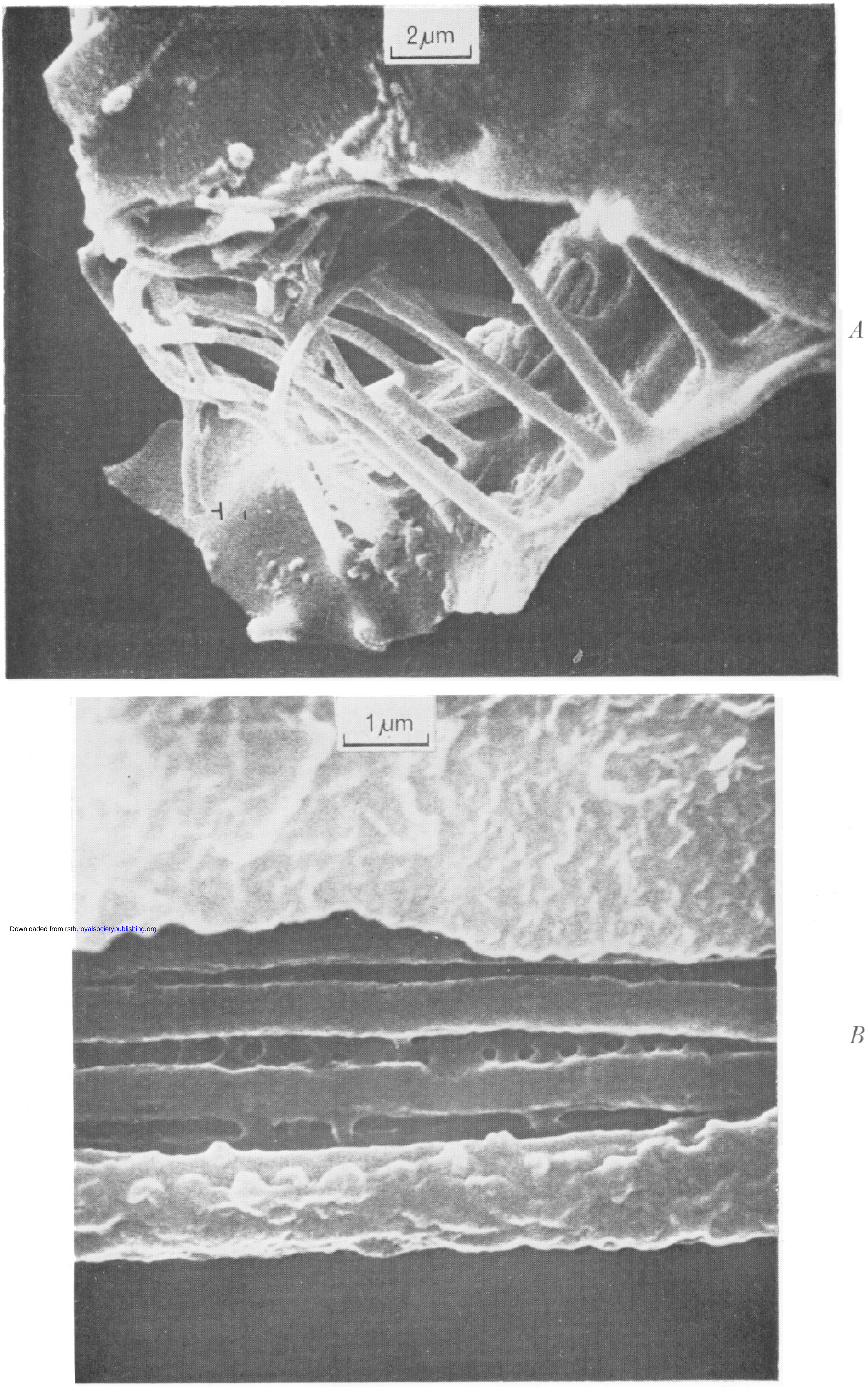

FIGURE 21. Spiracular gill of the pupa of *Psephenoides volatilis* Champ. (*A*) Internal view of a gill branch near its junction with the spiracular atrium. The plastron network of the surface is almost entirely clogged by the gold–palladium alloy. (*B*) Internal view of the thin wall of a branch near the middle of its length. The opposed thin wall has been removed.

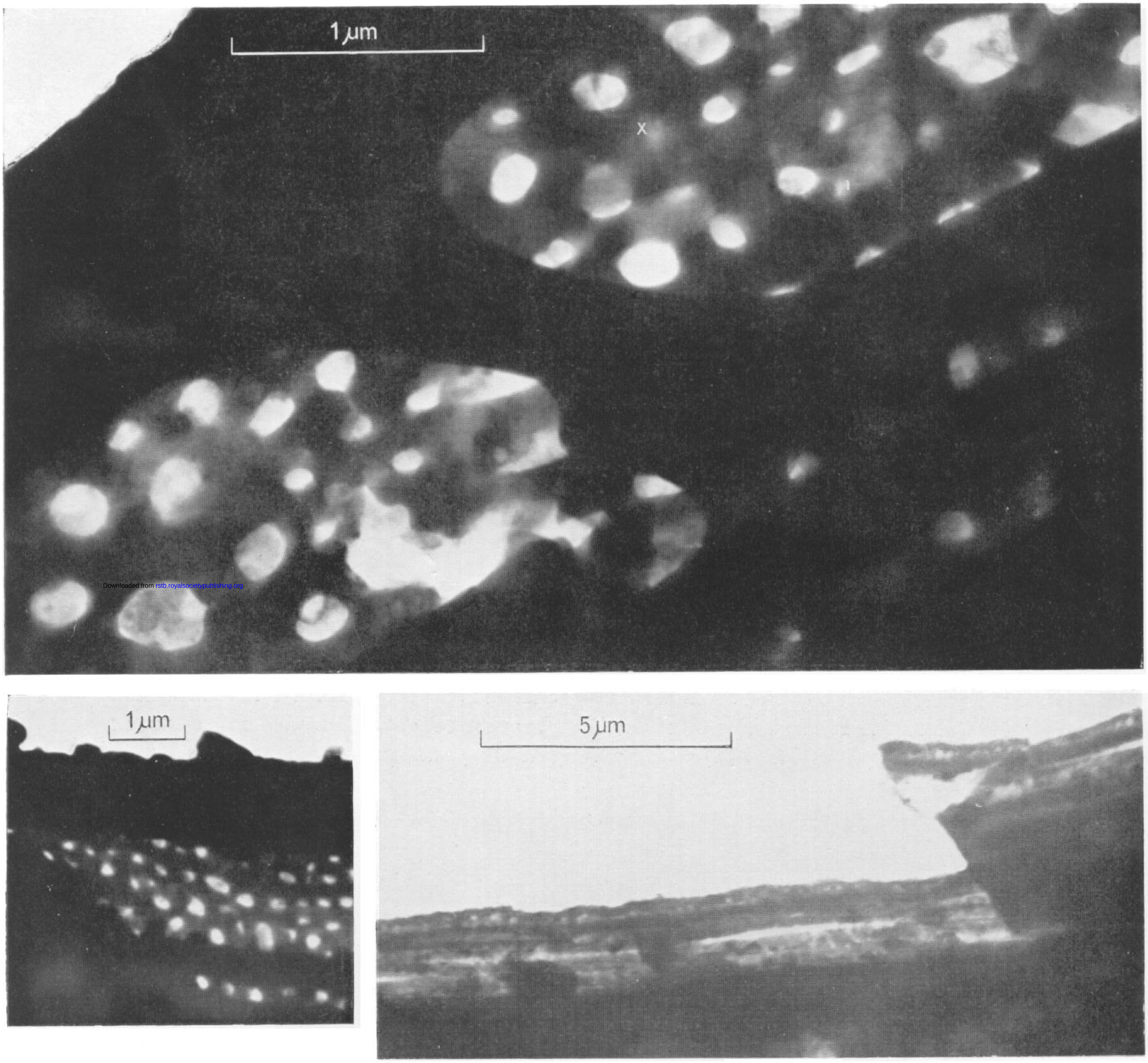

FIGURE 22. Spiracular gill of the pupa of *Psephenoides volatilis* Champ. taken with the transmission electron microscope. (*A*, *B*) Thin wall of a gill branch near basal third where the plastron network often consists of several rows of holes between the thin longitudinal struts. (*C*) A chip from a gill branch near the middle of its length. It shows a single row of holes between each of the thin longitudinal struts.