Functional morphology and the adaptive radiation of the Daphniidae (Branchiopoda: Anomopoda)

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SUMMARY

Of all anomopods, daphniids have been the most successful exponents of life in open water. Many of them are completely independent of the bottom and subsist entirely on seston. A few of them are truly planktonic. Although the family has been intensively studied from many points of view, various morphological attributes have remained either inadequately known or never investigated. Some of these attributes, understanding of which is necessary if functions are to be appreciated, are considered, especially in the genus *Daphnia*, with which other genera are later compared. They include aspects of general morphology, the exoskeleton, endoskeleton and muscular system.

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How *Daphnia* swims is described, antennal movements being analysed from high-speed cine films. Locomotion is clearly derived from a naupliar mechanism, though the nauplius has long been eliminated from the anomopod life cycle. Antennal beat is more versatile than is immediately apparent and the animals are capable of far more complex manoeuvres than the simple 'hop and sink' movements in which they often indulge.

The trunk limbs are responsible for collecting and manipulating the food. Their morphology and arrangement are discussed and their armature, especially as revealed by scanning electron microscopy, is considered. The armature of limbs 3 and 4 dominates the trunk limb complex and makes up an extensive filter chamber. The mouthparts and labrum are basically the same as those already described in detail for other anomopods, but the labrum lacks a keel. A wide range of particulate foods is consumed.

A detailed account is given of the feeding mechanism, which has been studied both by direct observation and with the aid of high-speed cine-photography. Most of the basic principles involved were elucidated by Cannon, Storch and Eriksson who, however, disagreed on various points. The account now given is more detailed than any previously presented and is supported by numerous illustrations, whose lack has hitherto hindered comprehension. Parts of some of the earlier interpretations are incorrect, sometimes in ways that are not only intrinsically important, but which have led to erroneous views on such matters as the amount of energy expended in filtration.

Trunk limb movements follow a regular rhythmic cycle. Water, containing suspended particles, flows into the carapace chamber via the ventral gape to replace that driven out posteriorly by the pumping action of trunk limbs 3 and 4 and their exopodites, is drawn into the filter chamber and through the filters borne on limbs 3 and 4 into interlimb spaces, from which it is finally expelled posteriorly. Trunk limb 5, whose movements initiate both promotion (the suction and filtration phase of the cycle) and remotion (the expulsion phase), seals the posterior interlimb space posteriorly during promotion of the limbs. There is no pressing of water through the filters during remotion of the limbs. Filtration occurs during approximately half the cycle. Notwithstanding claims to the contrary, the filter plates of trunk limbs 3 and 4 are correctly designated as such and serve as filters. Material abstracted by the filter plates is cleaned off by a series of devices, seven in all, passed into the median food groove, and swept forward by mechanical means to the mouthparts. The mandibles display a high degree of both skeletal and muscular asymmetry, which improves their performance. Any excess food material collected in the food groove is discarded. From the anterior end it is removed by the ejector hooks of the first trunk limbs, then swept out by the post-abdominal claws: from the posterior end it is removed by the post-abdominal claws alone.

Errors and shortcomings in certain recent accounts that purport to explain the feeding mechanism are discussed. Trunk limbs 1 and 2 are incapable of filtration and are specialized for roles that have nothing to do with this process. The inapplicability of a model of filtration to the daphniid mechanism is noted and the importance of morphology, even in minute details, is emphasized. Contrary to recent suggestions, the function of 'bristles' cannot easily be changed without changes in morphology. The necessity of understanding a mechanism before making calculations is emphasized and examples of misleading calculations, based on erroneous data, are noted.

The habits of certain species of *Daphnia* are described. Both *D. magna* and *D. obtusa* are able to settle on their ventral carapace margins and attach themselves to surfaces, over which they can then glide forward, collecting food material by means of scraper-like spines borne distally on the second trunk limbs as they do so. *D. magna* can also lift accumulations of detritus from the bottom. Such material is then processed in the usual way.

Some species sometimes indulge in swarming behaviour, which involves remarkable coordination between individuals.

The way in which phenotypic changes in shape occur in *Daphnia* and the light this throws on phyletic changes in the genus are described, partly by the method of transformation of coordinates, which can be used to show changes in three dimensions, rather than the usual two. The influence of environmental factors is noted.

Geographical, ecological and physiological aspects of radiation are considered.

Other genera are treated more briefly. *Daphniopsis* departs little from *Daphnia* in its functional morphology and may not merit generic separation. *Simocephalus* attaches itself to a support by means of simple but effective specializations of the antennae and then remains stationary while it filters. This has enabled it to acquire a robust carapace in a way not permitted to *Daphnia* (of which a few of the more heavily built species sometimes rest on the bottom). Protection is thereby granted. Acquisition of this habit was probably assisted by the way in which *Simocephalus* swims, predominantly ventral surface uppermost. The feeding mechanism is essentially the same as that of *Daphnia*. *Scapholoberis* and *Megafenestra* have the same orientation during swimming as *Simocephalus* and have acquired the habit of hanging suspended beneath the surface film by their ventral carapace margins, for which they are highly specialized in morphology and behaviour. Here too the basic daphniid feeding mechanism is employed. *Ceriodaphnia* has specialized in small size. Although studied in less detail than *Daphnia*, it clearly has a similar feeding mechanism.

Moina and *Moinodaphnia* are now often separated from the Daphniidae as the family Moinidae, but this seems unjustified. Trunk limb structure and the feeding mechanism are essentially the same as in other daphniids. These two genera, while primitive in certain respects, have a suite of specializations related to the nourishment of eggs and embryos by secretions produced by a Nährboden, or 'placenta'. This necessitates sealing of the brood pouch, by a device involving the post-abdomen, to prevent loss of the secretion. As embryos grow during development by the accretion of material from without, rather than from stored yolk, distortion and distension of the carapace are necessary to accommodate their increasing volume.

The Daphniidae clearly arose from benthic ancestors, some indication of whose morphology and habits is given by certain extant macrothricids. Key features in the evolution of the family, which has existed since at least early Cretaceous times and probably originated even earlier than this, are listed. Of prime importance was the expansion of the gnathobasic filter plates of trunk limbs 3 and 4 at the expense of other filters.

1. INTRODUCTION

Often among the dominant animals of the plankton of freshwater lakes, anomopod 'cladocerans' of the family Daphniidae have attracted an enormous amount of attention. A vast literature on their taxonomy, numerical abundance, seasonal cycles, vertical migrations and other aspects of their biology now exists. Many papers have also been published on their feeding rates and related matters beloved by ecologists anxious to express their roles in ecosystems in quantitative terms. Some of these studies suffer from serious defects because the mechanisms whereby the daphniids concerned collect their food were not understood and investigators have not always measured what they thought they were measuring, even when using refined and elaborate techniques. The same is true of attempts to measure the energy expended in filtration. A lack of morphological understanding has also sometimes been apparent in the interpretation of observations and experiments on particle size selection, a matter that has given rise to much debate. These problems have assumed new interest since it became apparent that filter feeding in such small organisms occurs at low Reynolds number, where fluid flow is laminar, motion is dominated by viscous rather than inertial forces, and boundary layers are relatively thick. While some early students of the group appreciated that things were otherwise, there has also been a tendency to regard all daphniids as members of the plankton (even when that term has been correctly applied) and the adaptive radiation shown by the family has to a large extent been ignored.

Studies on the functional morphology and habits of the Daphniidae have been relatively few. These largely concern the genus *Daphnia* and have been concentrated largely on its feeding mechanisms, which have been the subject of considerable, and sometimes acrimonious, debate.

Not surprisingly, the earliest investigators of daphniids completely misinterpreted morphology and their accounts are instructive only in showing the difficulties that beset the pioneer microscopists who attempted to understand how these animals are constructed and operate. Swammerdam (1669) was the first to give a reasonably illustrated account of a daphniid but, while able to say something useful about its way of swimming, not surprisingly failed to appreciate how it fed and was indeed unable even to recognize the mouth. Until the work of Schaeffer (1755) subsequent investigators were no more successful. Schaeffer, however, made what for that time were amazing discoveries. His anatomical revelations, greatly in advance of anything previously recorded, enabled the structure of daphniids to be appreciated for the first time. Not only did he ascertain that they had mandibles and trunk limbs, which he illustrated in considerable detail, but he observed the taking in of food particles and their accumulation in the food groove posterior to the mandibles. Jurine's work of 1820, greatly enhanced in value by the illustrations of his daughter, added to our anatomical knowledge and provided the first real understanding of how daphniids feed. He gave an account that, while very brief, showed that he had observed and understood the essence of the feeding process in *Daphnia* far better than certain recent investigators who have employed elaborate apparatus. Baird's (1850) work, which summarizes earlier discoveries, also added to the understanding of limb structure, a matter that has received intermittent attention ever since. The work of Lilljeborg (1900), Behning (1912) and Litynski (1916) deserves special mention in this connection, but until the second and third decades of this century no serious attempt was made to elucidate the complexities of the feeding mechanisms. Cannon (1922) and Storch (1922) were the pioneers in this field, but these papers merely marked their entry into a field in which their respective claims were often contradictory. Storch's paper (on *Daphnia*) was brief; that of Cannon (on *Simocephalus*) completely erroneous, as he himself clearly came to recognize. Except in connection with the role of the labral glands and their secretions, of which he gave an excellent account, he never cited his supposed findings in his subsequent long and detailed paper on Branchiopod feeding mechanisms (Cannon 1933), where he gives a very different, and more correct, account of the processes involved in *Daphnia*. Storch (1924) was aware of the shortcomings of Cannon's first paper, which he dismissed as 'unvollständig und zum Teil unrichtig'. Storch's own later papers (1924, 1924–25, 1925), especially the first, dealt with the feeding mechanism of *Daphnia* in great detail, 'but with very unsatisfactory results', according to Cannon (1933) whose criticisms had some validity and who provided better illustrations than Storch, but who seems not always to have held the correct view on points on which he and Storch were in disagreement. Indeed, notwithstanding Cannon's strictures, Storch's work was in a real sense a pioneering investigation. Eriksson (1934) also considered the daphniid feeding mechanism. Although his account is brief and contains only a single original illustration of a daphniid, its author had the advantage of having also studied certain macrothricids (as well as, like Cannon, more distantly related branchiopods). This enabled him to view his findings from a more realistic evolutionary perspective than had hitherto been the case, though at that time insufficient information was to hand to permit an adequate analysis. He disagreed with some of the claims of Storch (Cannon's (1933) paper had not been seen when his own account was published) sometimes with justification. On the other hand, there were points on which Storch, and not Eriksson, appears to have been correct, but Eriksson's work nevertheless deserves high praise. That section of his paper which deals with the Daphniidae comprises only a small part of a wide-ranging investigation of much originality.

Subsequent to this spurt of activity, apart from a somewhat superficial comparison of the feeding mechanisms of *Daphnia* and *Ceriodaphnia* by Harnisch (1950), relatively little information on the mechanics of the process in daphniids has been produced until recently. Many papers have, however, dealt with filtering rates, the effects of food concentration and similar matters, and there has been much debate as to whether daphniids actually filter. These studies have often involved elaborate techniques. Recently, Ganf & Shiel (1985*a*, *b*) have given a new interpretation of the feeding mechanism of *Daphnia*, but this is based on erroneous conceptions of morphology and is so remote from reality that it need not concern us here. It has been dealt with elsewhere (Fryer 1987*b*). Other recent suggestions, unsupported by adequate morphological understanding, are discussed herein.

2. MATERIAL AND METHODS

Many species, whose names are recorded in appropriate places, have been studied, most of them having been seen alive. A wide range of species is available in Britain; others were collected abroad or reared from dried mud obtained from various sources. Preserved material of other species has been available for examination at the British Museum (Natural History).

Living animals were observed by whatever methods proved appropriate. These included watching them swimming freely in nature and in glass vessels, and when confined to small cells or chambers. A compressorium was used to restrain individuals without interfering with trunk limb movements. Both for cinephotography and for general observation, animals were attached by a minute blob of a 'super glue', 'Locktite', to the end of a thin needle of glass, drawn out from a haematocrit tube. The tube was pressed into a lump of 'Blu-tack' anchored at the edge of a large watch-glass, itself mounted on a turned metal base to give elevation and stability and secured with plasticine. The 'Blue-tack' provided a universal joint, enabling the glass needle to be swung and twisted so that, suitably attached by the dorsal surface of the head-shield or carapace, an animal could be viewed in almost any plane. Even delicate species such as the planktonic *Daphnia galeata* Sars remained healthy for long periods when so attached. Their antennae and trunk limbs behaved normally and the feeding mechanism could be watched by supplying suitable particles. Instead of propelling the animal forward, the antennae drove water backward. Viscous, non-toxic 'cellulose nitrate' was occasionally used to slow down limb movements. Filming of living animals was carried out at from 100 to 500 frames per second as appropriate by using a Locam 16 mm camera (Model 51) fitted with an attachment to give continuous viewing when used in conjunction with a microscope. A frame-by-frame film analyser was available.

When possible, animals to be sectioned were fixed in Zenker's fluid. A few had to be used after being fixed by other means. Sections were prepared from material embedded in low viscosity nitrocellulose, usually being stained with Mallory's triple stain. Dissections, usually in polyvinyl lactophenol, were performed as required. Most observations were carried out by ordinary light microscopy, but Nomarski optics were also used, as was scanning electron microscopy to resolve certain details.

3. AN INTRODUCTORY WORD ON RELATIONSHIPS AT VARIOUS LEVELS

(*a*) *Ctenopod: anomopod relationships: a perennial source of confusion*

In the past, attempts at understanding the phyletic relationships of anomopods have been bedevilled by the belief that they are derived from the Ctenopoda. Thus, just as Storch (1924) had earlier assumed that daphniid trunk limbs are derived from Ctenopod-like precursors, Cannon prefaced his consideration of the daphniid feeding mechanism by stating that 'I take for granted that the Daphniidae evolved from some Ctenopod-like ancestor'. This view has persisted and, often uncritically, has been accepted by many subsequent workers. Thus Goulden (1968) expressed the view that 'present evidence suggests that the anomopod Cladocera developed from a group of Ctenopod-like Cladocera' and this belief colours his subsequent discussions (§3*b*). In fact, although they share several superficial, and indeed some more deep-seated, but primitive, similarities, the Ctenopoda and Anomopoda represent two very different groups of animals, whose contrasting attributes are discussed in detail elsewhere (Fryer 1987*a*). They are sufficiently distinctive to merit assignment to different orders, which have recently been defined (Fryer 1987*c*).

Trunk limb morphology and the associated feeding mechanisms of the Ctenopoda and Anomopoda are vastly different: they developed along different evolutionary lines, and it is not possible to derive the latter from the former. Earlier investigators have been misled by the fact that both ctenopods and daphniids are filter feeders, but such similarities as are shared by their respective mechanisms have been convergently acquired. Indeed, as end points of anomopod evolution, the daphniids are among the most distantly removed of all the group from the Ctenopoda. Thus, contrary to the view of Cannon, in this paper I take for granted that the Daphniidae did not evolve from a ctenopod-like ancestor. Evolutionary relationships among the Branchiopoda will be discussed elsewhere.

(*b*) *The status of the Moinidae*

The genus *Moina* and the related *Moinodaphnia* were for long in a state of taxonomic confusion. At least 50 species were described, some inadequately, and many were of dubious status. Thanks to the work of Goulden (1968) order was brought to this chaotic state of affairs. In a thorough revision, embracing all geographical regions, he reduced the number of valid species of *Moina* to seventeen and recognized all representatives of *Moinodaphnia* as belonging to one species. This sound taxonomic base is of enormous practical convenience and gathers together information of evolutionary interest.

One outcome of Goulden's revision was his decision to separate *Moina* and *Moinodaphnia* from the Daphniidae, in which they had long resided, and assign them to a separate family, the Moinidae. This assignment has generally been followed by succeeding workers, e.g. in the authoritative work of Flössner (1972). However, its wisdom is questionable. Those who have studied a particular group of organisms are often so impressed by its attributes that they feel it should in some way receive special taxonomic recognition. This is partly the case here. The group indeed possesses distinctive features but, as will become clear, these do not appear to merit its separation as a distinct family. As Goulden himself states, 'the structure of the moinid limbs clearly suggests that they are related to the Daphniid cladocera'. This is indeed the prime, but not the sole, uniting feature, but even if it were true, as Goulden claims, that 'the evidence for retaining *Moina* and *Moinodaphnia* in the Family Daphniidae rests solely on the structure of the thoracic limbs', this evidence would be of over-riding significance. Camouflaged by the obvious 'external' differences between *Moina* and other daphniids is a fundamentally similar arrangement of complex trunk limbs on whose function an essentially similar feeding mechanism is based. Differences, such as the elongate antennules of *Moina* and of *Moinodaphnia*, which represent the retention of a primitive character, still well displayed in the Macrothricidae, are not of familial magnitude. Even the presence of a Nährboden or placenta, while an undoubted specialization, is not sufficient to merit familial separation. A similar structure has arisen independently in one ctenopod genus, *Penilia*, and, again independently, in the Onychopoda.

The trunk limbs of *Moina* and *Moinodaphnia* are typical daphniid appendages. As such, they are more similar to those of any other daphniid genus than are those of any of several pairs of macrothricid or chydorid genera to each other. Thus the trunk limb complex of such macrothricids as *Acantholeberis*, *Streblocerus*, *Lathonura* and *Iliocryptus* (see Fryer (1974) for details) is in each case vastly more different from that of the other genera than is that of *Moina* from any other daphniid. The same is true of such chydorids as *Chydorus*, *Graptoleberis*, *Leydigia* and *Pseudochydorus* (Fryer 1968) and many of these genera differ more widely from each other in other attributes than does *Moina* from other daphniids. By using the sort of criteria on which the Moinidae was erected, one would have to erect a new family for almost every macrothricid genus and for many chydorid genera. To separate *Moina* and *Moinodaphnia* from the Daphniidae gives unwarranted significance to their distinctions and obscures their relationships to other members of that family. To anticipate a conclusion, these genera are here treated as members of the Daphniidae. Their distinguishing features can conveniently, and with some advantage, be acknowledged by according them the rank of a subfamily (§8*e* (iv)).

Incidentally, these genera have distinguishing features not mentioned by Goulden. *Moina* retains a head lacking a headshield, a primitive feature, and has a flexed antennal protopod, in contrast to the straight protopod of most daphniids. These, however, are insufficient to merit familial separation. Alternative states of both characters are found in the Macrothricidae.

As noted above, Goulden's views on phylogeny were coloured by the belief that *Moina* arose from a ctenopod-like ancestor. Its true relationships are more readily apparent when this belief has been discarded.

4. PRELIMINARY REMARKS ON STRUCTURE AND HABITS

As shown herein, daphniids are in various respects the most advanced of all anomopods. Many of their structural and functional attributes are clearly derived from those still exhibited by a more primitive extant family, the Macrothricidae. Primitive features are also retained by members of the Chydoridae, but one has to use the term 'primitive' (or, more fashionably, plesiomorph) with caution when applied to a family such as this, and indeed to the Macrothricidae, both of which have put primitive attributes to specialized uses and have given rise, not only to some of the most specialized anomopods, but to some of the most mechanically complex of all arthropods. Adaptive radiation in the Chydoridae has indeed resulted in a wider range of lifestyles than has been achieved by the Daphniidae. While some chydorid attributes, such as the nature of the ephippium, are more primitive than those of the Daphniidae, both families have achieved success by using the same basic apparatus to exploit different kinds of ecological opportunities.

Complexity is not confined to exoskeletal features but, notwithstanding the primitive position of the group among the Crustacea, extends to internal anatomy, which is exceedingly complex. The haemocoelic open blood system of anomopods represents a lower grade of organization than a system employing blood vessels, but is hardly simple, and the intricate packing of many complicated organ systems within the small compass of these organisms is the antithesis of simplicity. This point deserves emphasis in view of the misleading and unsubstantiated statement in a recent textbook (Schram 1986) that internal anatomy is 'relatively simple'.

Early studies on daphniid morphology, feeding mechanisms and habits suffered from a lack of comparative information on anomopods that retained some of the habits and morphological attributes that were in various respects closer to those of ancestral forms. Much relevant information on the most primitive family, the Macrothricidae, and on the family Chydoridae, the habits and morphology of whose members give many pointers to the nature of ancestral anomopods, has been acquired in recent years (Fryer 1963, 1968, 1972, 1974; Sergeev 1970, 1971, 1972, 1973). This enables the morphology, habits and feeding mechanisms of the Daphniidae to be viewed in an evolutionary context and enables the magnitude, and limitations, of their adaptive radiation to be appreciated by comparison with that of these families.

All daphniids are essentially swimmers, but abilities differ greatly in different species. An increase in the efficiency of swimming has gone hand in hand with emancipation from reliance on substrata with which their ancestors were associated. This emancipation has been dependent on the acquisition of a feeding mechanism capable of providing adequate food by the abstraction of suspended particles alone. There is strong evidence that the ancestors of the daphniids collected food by scraping. The material thus acquired was then abstracted by filtration. Many macrothricids and chydorids collect and manipulate food in this manner today. Up to four pairs of filtering appendages are employed by such species. Improvements in the filtering device led to filtration being confined to the third and fourth pairs of trunk limbs and eventually enabled scraping to be abandoned, thus liberating the daphniids from the need to associate with substrata. As a result of specialization however, some of them have secondarily re-acquired substratum-utilizing habits, including association with the surface film. Such species, nevertheless, still collect suspended food particles and, apart from certain occasionally used specializations, all of them have abandoned crawling and scraping, two of the predominant habits of the Chydoridae and Macrothricidae.

All daphniids employ an essentially similar filtering device and the range of food-handling mechanisms is much smaller than in the Macrothricidae and Chydoridae: yet, so successful is the device acquired, that it has enabled considerable adaptive radiation to occur, albeit into a smaller range of niches than has been exploited by the chydorids in particular. The niches occupied by the daphniids are also very different from those frequented by macrothricids and chydorids and, although daphniids often coexist with members of these families in many kinds of waterbodies, they overlap to only a minor extent in the demands they make on the environment. These differences are often not appreciated by ecologists, who all too frequently refer to all anomopods (or even all small crustaceans) as constituents of the plankton, a term which they use incorrectly, and with which most of the organisms in question have nothing to do.

In sharp contrast to the situation in the Macrothricidae and Chydoridae, which have given rise to no such specialists, one end point of the adaptive radiation of the Daphniidae is life in the pelagial region of lakes, where species of several genera now form an important element of the plankton. Not all species, even of genera with planktonic representatives, are however, members of open water communities. Some have become specialized for life in shallow inshore waters and small water bodies where they display a wide range of habits and appropriate morphologies.

Although scraping has been abandoned as a primary means of food collection, some species can supplement naturally suspended material in various ways. Thus *Daphnia magna* Straus sometimes forages on suitable bottoms and brings into suspension relatively large particles that can be filtered out, and *D. obtusa* Kurz has an hitherto undescribed means of scraping that, under suitable circumstances, it uses as a supplementary food-gathering device. Under most circumstances, these species can, however, subsist easily on suspended particles alone. The ability to collect food without the need to scrape or to employ related means of handling as a preliminary to its processing, and the complete independence of the bottom thus granted (though some species use substrata for special purposes not directly concerned with feeding), has also imposed certain constraints on daphniid evolution. Intimate association with substrata has been one of the keys to the success of the Chydoridae and, to a lesser extent, of the Macrothricidae, and has led to many highly complex adaptations. The open-water, suspension feeding habits of the Daphniidae effectively exclude this kind of adaptive radiation, and the effects of this restriction are manifest in various ways. For example, the range of gross morphology, while impressive, is much less than in the Macrothricidae and Chydoridae where, especially in the latter, elaborate modifications of the carapace, especially of its ventral margins, are among the salient features of its radiation. Open-water habits also preclude many of the specializations of the post-abdomen, whose form, so diverse in the Macrothricidae and Chydoridae, is similar in general features in all daphniids.

The emphasis on swimming has led to the development in some genera, such as *Daphnia*, of large arborescent antennae armed with long natatory setae. These contrast markedly with the much shorter antennae of the benthic Chydoridae that are used as swimming organs only intermittently and for short excursions, and often serve other purposes, such as scrambling. Not all daphniid genera, however, have such long antennae. The specialized habits of species of *Simocephalus* and *Scapholeberis*, for example, call for less constant use of the antennae, and both genera, although typical daphniid filter feeders, in different ways 'rest' on suitable objects (the underside of the surface film, in the case of the latter). Here, long antennae are not merely unnecessary, they would be a liability.

Daphniid mandibles are similar to those of other anomopods, and indeed to those of most branchiopods. Like the Chydoridae and Macrothricidae they have exploited both skeletal and muscular asymmetry, and to an even greater extent.

Morphology is intimately related to habits in both gross and detailed ways. An example of the relation of gross morphology to habits is seen in the small, slender, often attenuate, bilaterally compressed, pelagic species of *Daphnia*, with their delicate carapace, compared with large, heavily built littoral and pond frequenting forms such as *D. magna*. Other ecological factors, such as water chemistry, act via physiology rather than morphology, and another aspect of the adaptive radiation has been the way that certain species have been able to colonize 'saline' waters or waters of peculiar ionic composition, though no species has conquered marine environments. Equally, like most crustaceans, daphniids have, with a few exceptions, had little success in the colonization of ion-poor, highly acidic situations.

Reproductive habits also display facets of adaptive radiation. While not a primary concern here, they are mentioned where appropriate.

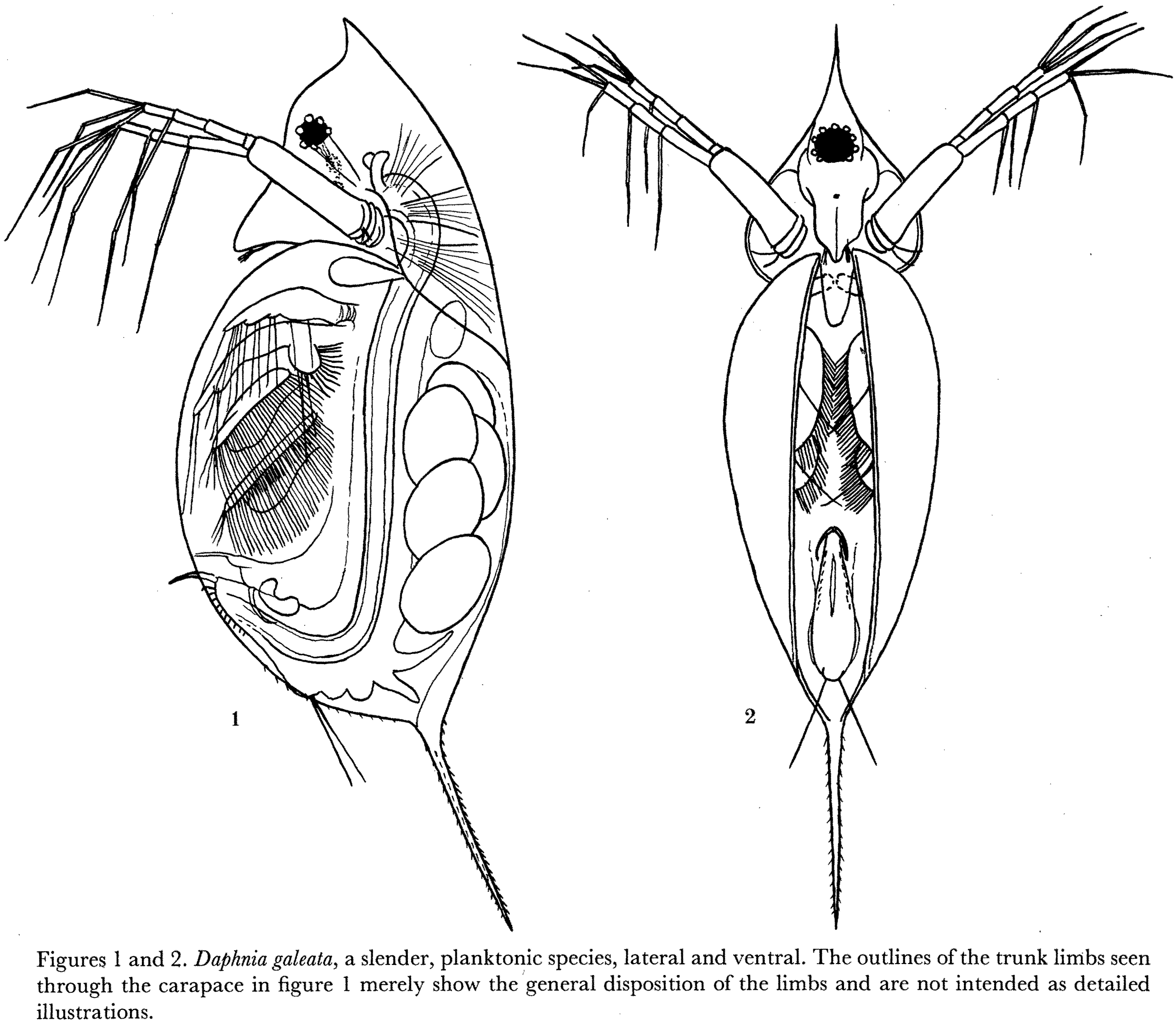
5. ASPECTS OF FUNCTIONAL MORPHOLOGY AND HABITS IN *DAPHNIA*

As the most familiar genus, *Daphnia* is dealt with first. This enables other genera to be treated more briefly and comparatively. Although *Moina* and *Moinodaphnia* are primitive in certain respects, no disadvantage accrues from relegating them to the end of the sequence.

(*a*) *General morphology*

The general morphology of *Daphnia* (figures 1 and 2) has often been referred to, and outline accounts, not always accurate, appear in several textbooks. Many aspects of gross morphology apply to all members of the genus and are well understood, but some features have never been described. The same is true of internal anatomy.

Following the pioneer work of Schaeffer (1755), Jurine (1820), Baird (1850) and Leydig (1860), Claus (1876) provided a sound basic account of the morphology of the genus supported by several good illustrations; Klotzche (1913) added further details, and Binder (1932) elucidated various aspects of the complex muscular system. Lilljeborg (1900) provided good illustrations of isolated trunk limbs, since when fine details have been revealed by scanning electron microscopy (see, for example, Watts & Petri (1981); Brendelberger (1985)). Studies such as that of Cunnington (1903) on another daphniid, *Simocephalus*, and those on numerous members of the related anomopod families Chydoridae and Macrothricidae (Franke 1925), Eriksson (1934), Fryer (1963, 1968,



Figures 1 and 2. *Daphnia galeata*, a slender, planktonic species, lateral and ventral. The outlines of the trunk limbs seen through the carapace in figure 1 merely show the general disposition of the limbs and are not intended as detailed illustrations.

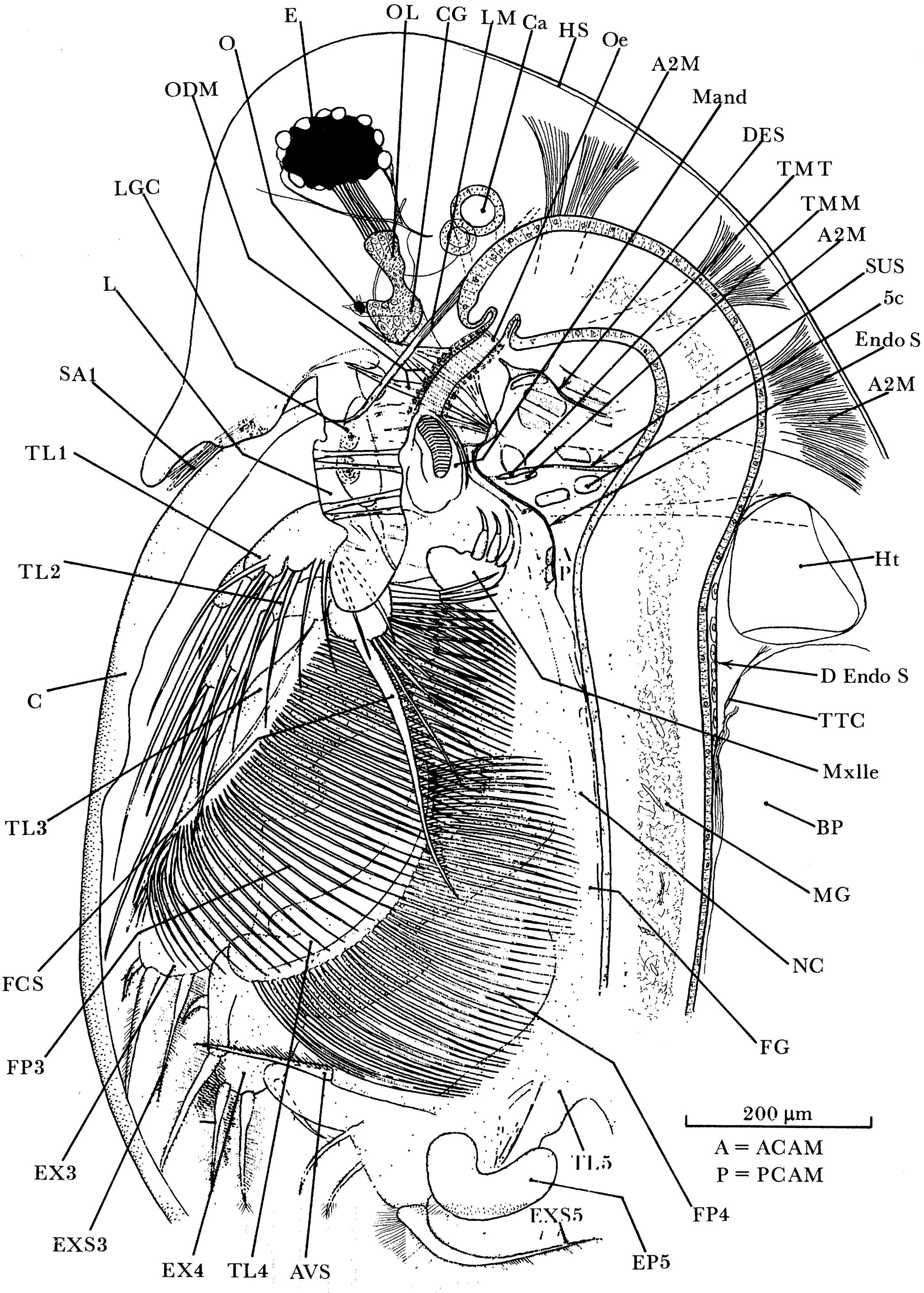


Figure 3. *Daphnia longispina*. Median view of a bisected individual, based on adjacent longitudinal slices, to show the disposition of the appendages and various aspects of general anatomy. Setules are omitted from the filter plates that dominate trunk limbs 3 and 4. The setae are shown in a simplified manner in that each is in fact emarginate from near the base, but their number and arrangement are accurately portrayed.

1974), Sergeev (1971, 1972, 1973) also facilitate understanding of anomopod organization in general, and the comparisons thereby made possible assist in the elucidation of structures and their functions and of the ways in which they may have evolved.

Various matters relevant to the feeding mechanism, such as the nature of the trunk limbs, have been accurately documented, but others remain unclear. As Cannon (1933) noted, the shape of the trunk limbs in the living animal and their relation to the food groove had not until that time been fully studied, nor had the limbs been figured as seen in the median plane. Cannon greatly increased understanding by showing the way in which the limbs arise from the trunk and, especially, by providing illustrations of all five such trunk limbs as they appear when viewed from the median plane in the living animal. Nevertheless, neither his figures nor those of Storch (1924–25, 1925) give a clear impression of the nature of the filter chamber or of the pumping device, though Storch

(1924) provided, if only in outline, illustrations of slices cut in the horizontal plane that are essential for this purpose.

To understand how the complex mechanisms employed by these organisms function, it is necessary to appreciate morphological and anatomical details hitherto inadequately explained or, in some cases, undescribed. The presentation of morphological data is therefore an integral element of this study.

Many illustrations show various species of *Daphnia* in lateral aspect (figure 1), the view most easily obtained. Appreciation of the three-dimensional nature of these animals is best achieved by considering also the ventral aspect that is seldom shown. That shown in figure 2 is of *D. galeata* Sars, a slender, transparent planktonic species of the subgenus *Daphnia* s.str. (For the diversity of gross form in *Daphnia*, see §6(*a*)).

Basic features of the morphology of a planktonic form of *D. longispina* (O. F. Müller) are shown in figure 3. This form occurs in Esthwaite Water (English Lake District) and is virtually identical in its detailed morphology to *D. galeata*, with which it coexists, though at certain times of the year, the head-shapes of the two species, which are genetically distinct (Christie 1983), are markedly different (compare figures 1 and 3). While species of the genus differ much in detail, the general arrangement of the structures shown is typical of all. Differences that diversify the external form of the many species of *Daphnia*, such as the shape of the head and carapace and the length of the carapace spine, are sometimes spectacular (see §6(*a*)), but anatomically superficial. This is particularly apparent in cyclomorphic forms in which external form sometimes differs greatly at different times of the year.

The similarities of many attributes of *Daphnia* to those of a primitive, yet morphologically complex, macrothricid, *Acantholeberis curvirostris* (O. F. Müller) are apparent when figure 3 is compared with figures 6 and 8 in Fryer (1974). Many differences, reflecting the different ways of life and feeding habits of the two animals, are also apparent. Notwithstanding many superficial differences, the heads of the two animals are clearly very similar, and indeed both display features that are found in even distantly related branchiopods of other orders. That of *Daphnia* is protected by a headshield (a specialization): that of *Acantholeberis* is not. Anteriorly, each has a large median eye (E), with 22 lenses in *Daphnia*, derived from two eyes that fuse during ontogeny, and a small, pigmented ocellus (O) with three lens-like bodies in *Daphnia* that presumably at least concentrate light. The innervation of these organs is similar in each case: from a conspicuous cerebral ganglion (CG) a large optic lobe (OL) extends towards the eye. The ocellus, closer to the cerebral ganglion in *Daphnia* than in *Acantholeberis*, has its own nerve supply. The eye muscles of *Daphnia*, three on each side, that cause the eye to 'tremble', are a specialization, as is the method of suspension. As Downing (1974) has shown, the eye is suspended, not by ligaments but by a membrane of complex shape, basically rather like a cone, that forms a liquid-tight seal between the eye and the haemocoele. It is a combination of haemocoelic pressure and muscular activity that allows the eye to rotate to a remarkable extent; up to 160° in the horizontal plane, according to Downing. Rotation in other planes is less than half this value.

Where the eye is located, the head is narrow, a matter not easy to appreciate except in horizontal sections (figure 21), being but little wider than the diameter of the eye. Thus there is little obscuring tissue between the lateral lenses and the cuticle. Anteriorly, there is nothing but the cuticle and the fluid in the chamber that contains the eye to prevent light from impinging on the anterior lenses.

In both *Daphnia* and *Acantholeberis*, there is a circumoesophageal nerve commissure with nerves to the antennules and antennae, behind which the double ventral nerve cord (NC) extends posteriorly into the trunk.

In each case a large, fleshy, median labrum (L), which houses conspicuous labral glands (LGC), extends backward, covering ventrally the masticatory region of the mandibles (Mand). It has a protective keel in the benthic *Acantholeberis*; no keel in the freely swimming *Daphnia* where such protection is not required. Anterior to the mandibles, a narrow oesophagus (Oe), provided with circular and numerous radiating dilator muscles, extends anteriorly and dorsally before opening into the wide mid gut (MG). The endoskeletal elements (Endo S) (§5(*e*)) that, among other functions, provide the sites of origin of many of the oesophageal dilator muscles, while differing in shape, are clearly homologous in the two animals.

Acantholeberis has long, conspicuous antennules: those of *Daphnia* are markedly reduced. Long antennules are primitive, but well suited to the benthic way of life of *Acantholeberis*. The antennules of *Daphnia*, no more than a mound provided with an array of sensillae (SA1), reflect its independence of the bottom, and their reduced condition is the end point of a trend displayed to differing degrees within the family.

While differing in ways that reflect the very different ways of life of the two animals, the antennae are basically similar in each (cf. figures 1 and 2 with figures 1, 2, 3 and 5 in Fryer (1974)). Those of *Daphnia* are long and slender and their armature of long elements consists entirely of natatory setae, of which there is one more than in *Acantholeberis*. The protopod is much less massive than in *Acantholeberis* and is straight and tubular, in contrast to that of the latter, which is curved in two planes (Fryer 1974). All the specializations of the antennae of *Daphnia* relate to their use exclusively as swimming appendages.

The mandibles (Mand) dominate the posterior part of the head. Skeletally, these are essentially similar to those of other anomopods (Fryer 1973, 1968, 1974) and indeed, to those of other branchiopods with rolling, crushing mandibles, and their musculature is also similar. The major features of the mandibular muscular system of *D. atkinsoni* f. *bolivari* (Richard) are shown in figures 30, 31 and 32 and certain muscles of *D. longispina* are seen in figure 3. (The basically similar systems of *Simocephalus* (figure 139) and *Moina* (figures

159 and 160) help to facilitate understanding, the principles of the mechanism being the same in all cases.) Certain details of the mandibular endoskeleton are noted in §5 (*e*) and some of the specializations of the mandibles of *Daphnia* are noted in §5 (*k*).

The similarity of the maxillules (Mxlle) of *Daphnia* to those of *Acantholeberis* is readily apparent. The maxillae, probably represented in *Acantholeberis* by papillae that bear four setae, have apparently disappeared in *Daphnia* (but see note in §9).

The most striking differences between *Acantholeberis* and *Daphnia* concern the trunk limbs. Those of *Daphnia* (TL1–5) are seen *in situ* from the median aspect in figure 3. While in both, the gnathobases of limbs 3 and 4 bear filter plates, those of *Daphnia* (possibly incorporating adjacent endites) have become enormously enlarged and dominate the filter chamber. Their development has led to a reduction of what, in *Acantholeberis*, are important elements of these limbs that play a vital role in food handling, and also to the disappearance of the gnathobasic filter plate of trunk limb 5. There have also been concomitant changes in limbs 1 and 2, perhaps most notably the loss of food-collecting scrapers by limb 2. The trunk limbs of *Daphnia* are considered in §5 (*g*).

A seldom mentioned, but important, anatomical feature is the separation of the dorsal brood pouch in which, like all anomopods, daphniids carry their eggs and embryos, from that part of the carapace chamber (here referred to simply as such) which houses the trunk limbs and post-abdomen. For reasons that will become apparent, the feeding mechanism would be rendered inoperative if water could leak between the brood pouch and carapace chamber and the two have therefore to be separated. This is achieved by means of a fibre-braced horizontal lamella (HL), reported by Storch (1924), but otherwise apparently unnoticed, that extends laterally from each side of the thorax which, by making contact with the inner wall of the carapace of its side, establishes a water-tight seal between them. The horizontal lamellae are best seen in transverse sections, e.g. figures 112 and 113. In *Daphnia*, water can enter or leave the brood pouch posteriorly, especially when, as happens from time to time, the trunk moves ventrally a little as the post-abdomen is swung, which momentarily increases the volume of the brood pouch. If well-developed embryos are present, they move actively as pressure on them is released, probably thereby assisting in the replenishment of their oxygen supply. As soon as the brood pouch is closed and pressure applied to the embryos, they cease all movement. This applies not only to most daphniids, but to anomopods of other families and can be seen easily, for example, in chydorids. It does not apply, however, to *Moina*, where a specialized system of supplying the eggs and embryos with maternal secretions necessitates sealing of the brood chamber (§8 (*e*) (ii)).

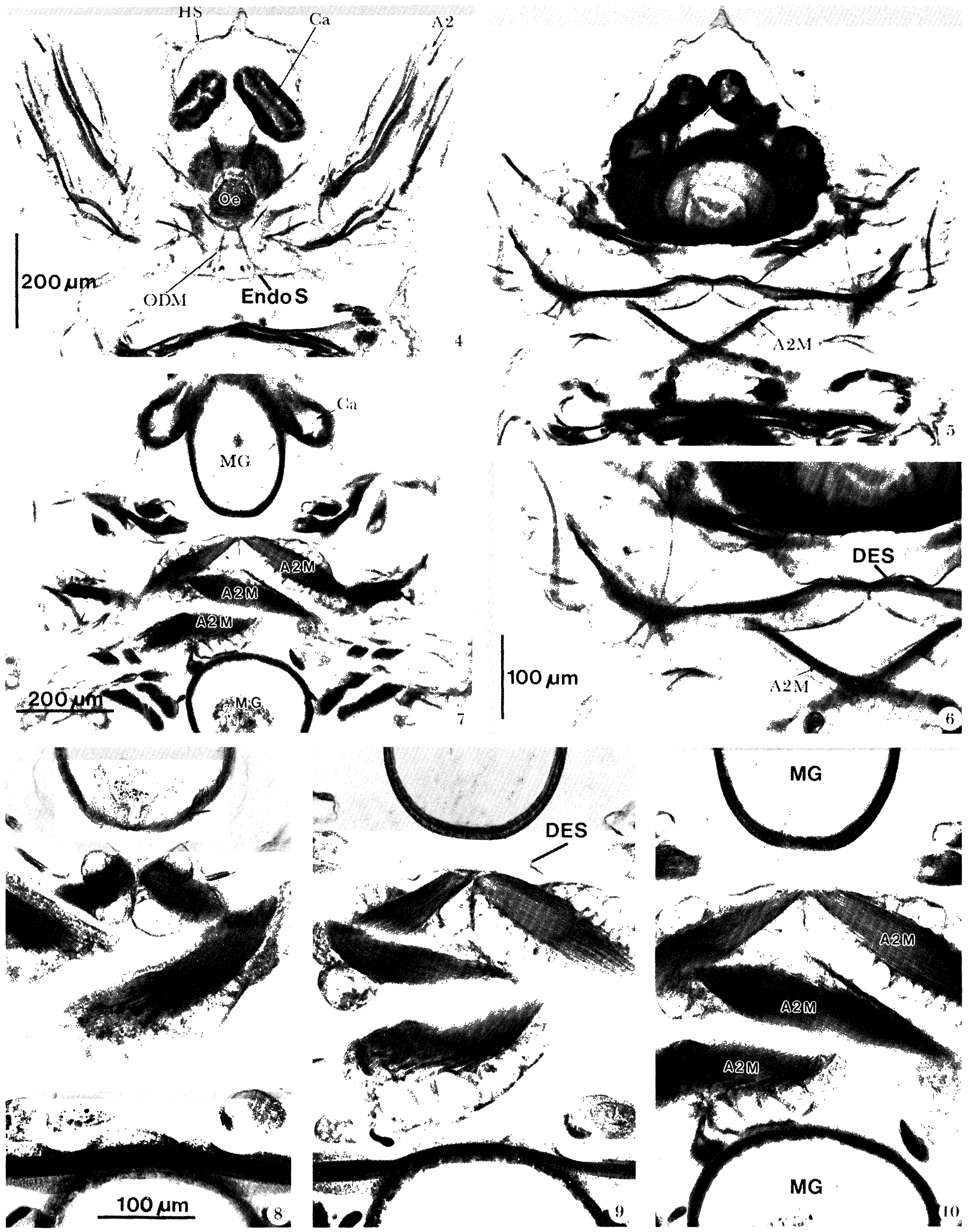
Many aspects of internal anatomy, some of which receive comment later, are inevitably revealed in various illustrations. Labelling makes some of these self-evident. Figures 28 and 29, which are horizontal slices through the ventral region of the trunk, give a good general impression of internal anatomy, especially if compared with those showing other sections, both transverse and horizontal.

(*b*) *Swimming*

Daphnia swims by using its large biramous antennae (see figures 1, 2 and 11–14) as oars. As is also true of the basic form of these appendages, antennal beat is a derivative of a naupliar mechanism such as can be seen in the nauplius of anostracans (Fryer 1983) and is a persistent survival in organisms from whose ontogeny the nauplius has long been eliminated. This is true also of *Bosmina*, a member of another anomopod family that has independently acquired free-swimming, and often planktonic, habits, though almost certainly from bottom-living ancestors different from those of the Daphniidae. Valuable information on the swimming of *Bosmina* has been provided by Zaret & Kerfoot (1980) who provide useful, if sketchy, illustrations.

The antennae of *Daphnia* are more efficient oars than their homologues in the Macrothricidae and Chydoridae that often fulfil roles additional to propulsion and whose structure is therefore a compromise between the sometimes conflicting demands of several functions. Those of *Daphnia* are specialized exclusively for rowing, if one ignores their ability as receptors via the pair of setae near the base of each (figures 1 and 2), or such secondary functions as mutual cleaning. Their natatory setae are long and elaborately armed with setules that effectively increase their surface area during the working stroke (figure 13, inset). There are no stiff spines such as those that enable certain macrothricids and chydorids to scramble or lever themselves over substrata.

The antennal muscular system of *D. magna* was described by Binder (1932) in a praiseworthy investigation, but a deeper functional analysis, not attempted here, would be rewarding. Some of the complex musculature of the protopod is seen in figures 4–10, 15 and 16. Some of the major extrinsic muscles (A2M) originate on the opposite side of the body from the appendage they serve. Crossing over can be seen in figures 5–10 and 15. This is a specialization. Appendage muscles primitively originate on the same side of the body as their appendage. As shown by the antennal muscular system of the nauplius of the anostracan *Branchinecta ferox*, crossing over is not a feature of the naupliar mechanism from which antennal propulsion was derived (Fryer 1983). Indeed, not all adult branchiopods have achieved such specialization. There is no such crossing over of antennal muscles in 'conchostracans' of the order Spinicaudata (Fryer 1987*c*). Crossing over is indeed an unusual specialization in animals. A comparable case exists, however, in the splenius capitis muscle of humming birds and swifts. Here, this muscle originates on the second cervical vertebra and, in contrast to its homologue in other birds, crosses over and inserts on the opposite side of the skull (Burton 1971; Fritsch & Schuchmann 1988). This is associated with fast head turning in these aerial specialists. In *Daphnia* and other branchiopods in which the phenomenon occurs, an



Figures 4–10. Aspects of the skeleto-musculature and nervous system of the antennae. Figure 4. *Daphnia magna*. Thick horizontal slice showing the antennal protopods and some of their muscles, ventral. The asymmetrical muscles seen at the bottom of the photograph are the transverse mandibular muscles. Note how some of the oesophogeal dilator muscles (ODM) originate on the endoskeletal sheet (Endo S). Figure 5. The same, more dorsal. Note the crossing over of two extrinsic muscles (A2M). The muscles seen here, and in figures 6–10, show how the contractile myofibril bundles of many of the muscles are surrounded by a thick sarcoplasmic cortex. Figure 6. Detail of figure 5. Note the use of the endoskeleton (DES) for the anchorage of extrinsic antennal muscles (see also figures 7–10). Figure 7. *D. atkinsoni* f. *bolivari*. Horizontal slice, ventral, to show some of the major extrinsic antennal muscles. Figures 8–10. *D. atkinsoni* f. *bolivari*. Consecutive horizontal slices, proceeding progressively dorsally, through some of the major antennal muscles, showing how some of the extrinsic muscles originate on the dorsal extension of the anterior extremity of the ventral endoskeletal sheet (DES), whose location is seen in figure 3, and how others cross over. Crossing over of the two extrinsic muscles present in the lower half of each photograph can be seen by following the portions present in consecutive slices. The mid-gut (MG), whose anterior extremity is seen in figure 8, where the last trace of the oesophagus is present, here passes dorsally before looping back, where it is cut again at the bottom of each photograph, before eventually running posteriorly.

enhanced mechanical advantage is probably the major benefit, but such muscles are longer than would be the case if they originated on the same side of the body as the appendage they serve and, as long fibres shorten faster than short, advantages may also accrue during rapid swimming.

The complexity of the antennal muscles is reflected by their elaborate nerve supply, some elements of which, having arisen from a massive branch from the ventral nerve cord of its side, can be seen in figure 16.

The antennal protopod/head joint involves rings of chitin, mostly thick but with an expanse of thin arthrodial membrane between them, that allows one ring to concertina against its neighbour, a refinement of the system that grants flexibility to the exopodite of the naupliar antenna of *Branchinecta ferox* (Fryer 1983) and doubtless other branchiopods with similar multi-ringed antennal rami. Slivers of muscle control the movements of the rings, that are supported by endoskeletal elements, as the antennal protopod swings. Some of these features are seen in figures 15 and 16, but the rings themselves are best seen in the intact appendage.

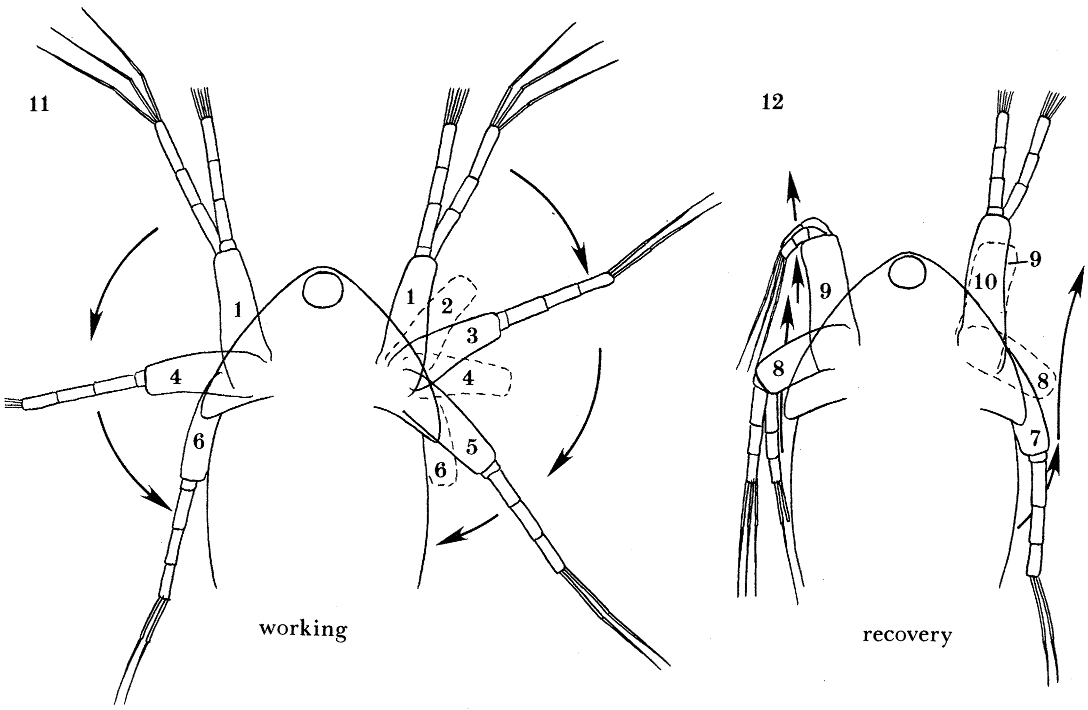
During normal swimming, the antennae operate simultaneously with their rami outstretched on the working stroke. Figure 2, which shows them more or less in the resting position, gives a good impression of outstretched antennae. Regular sweeps, usually in short bursts, punctuated by brief rests, or sometimes single sweeps, drive water somewhat obliquely backwards along the long axis of the body, there being a ventro–dorsal component to the flow. As the predominant orientation of the body is with the long axis more or less vertical, and as the antennae usually beat relatively slowly and simultaneously, progression is by a series of small increments, generally with a perceptible sinking phase during the recovery stroke. This is the same pattern as that displayed in the primitive locomotion of branchiopod nauplii (Fryer 1983) but, because of the orientation of the body, the sinking or reversal phase is here accentuated by gravity. Indeed, in adults swimming horizontally (see below), which provide a more strict comparison with naupliar locomotion, while speed drops markedly on the recovery stroke of the antennae, there is no actual reversal of motion. Such reversal can be seen in small juveniles swimming horizontally, a reflection of change in Reynolds number with increasing size. Small individuals especially, inhabit a low Reynolds number environment where viscous forces are important and where swimming is to a considerable extent a process of levering the animals through the water.

The rate of antennal beat varies much with circumstances but, to give some perspective, Fox & Mitchell (1953) found that in *D. magna*, during normal swimming at 20 °C, it ranged from about 75 to 250, and was usually between 100–200 cycles per minute. As these figures include periods of rest, they do not show the actual speed of antennal beat. *D. magna* often swims by a series of single sweeps or two or three sweeps followed by brief rests, sufficient more or less to maintain station, interspersed with periods of more sustained antennal activity as it shifts its position. Individuals glued to a needle of glass showed that at room temperature, this species can sustain continuous antennal beat at between 10 and 11 Hz over several cycles and doubtless achieves these, and possibly higher, rates when swimming rapidly. Slender, planktonic species with relatively slow sinking speeds need to beat their antennae less frequently than a bulky species such as *D. magna*, but *D. galeata* is capable of doing so with a rapidity at least equal to the maximum rate of that species when occasion demands.

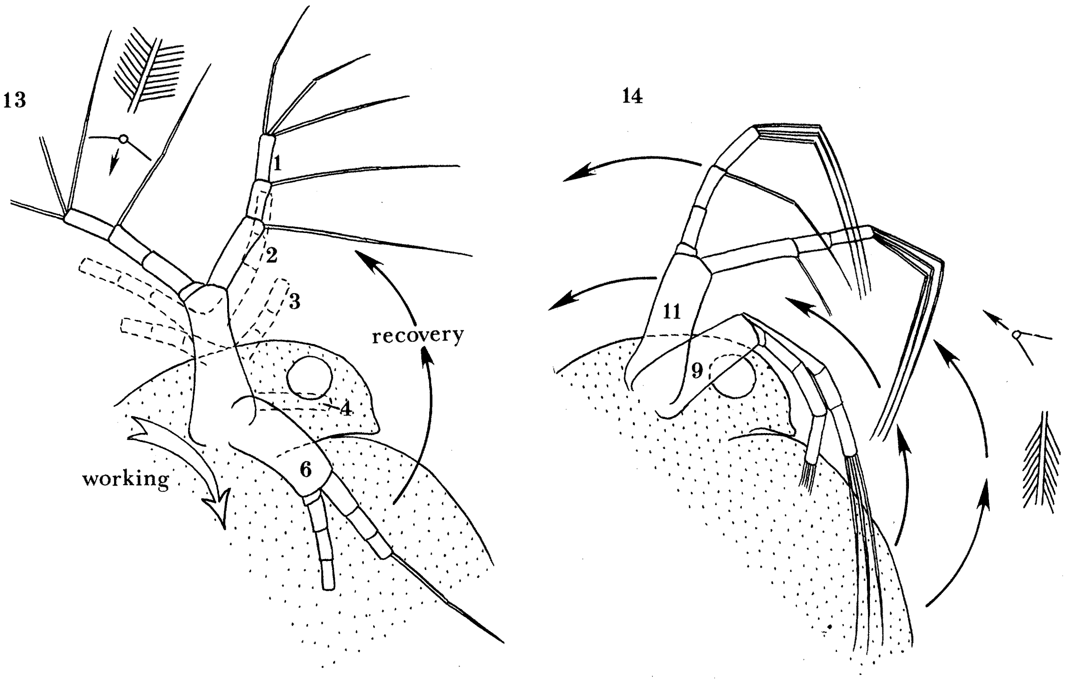
Whenever active propulsion ceases, the animal begins to sink and the antennae, which are then outstretched to more or less the position seen in figure 2, act as parachutes, slowing the speed of sinking and maintaining the correct orientation until locomotion is resumed. That, as expected, sinking speeds are reduced by the extended antennae was shown by Eydon (1923), who compared the effect of folded and outstretched antennae on the sinking speeds of narcotized *P. pulex* Leydig. Large individuals (and species) sink faster than small, as is readily seen by watching *D. magna* and smaller species, or early and later instars of the same species (see also Brooks & Hutchinson (1950)). While not negating such biological factors as predation that have recently received attention, this provides a partial explanation on purely mechanical grounds of why planktonic species tend to be smaller than those with littoral or pond-frequenting habits.

The nature of antennal beat can only be appreciated if considered in three dimensions. Also to be emphasized is that, while basically stereotyped during slow swimming, antennal movements are much more versatile than generally supposed. While a lateral view shows that the antennae swing backward through an arc during the working or power stroke (remotion), a better idea of the way in which they impart motion is obtained in an animal viewed either dorsally or ventrally. Figure 11 (position 1) shows the orientation of the antennae before the working stroke from this aspect. During remotion, the antennae pivot about their mobile insertion on the head and sweep backwards, and therefore, initially, also outwards. As they do so, they reach a point at which they are extended at right angles to the body, at which point they develop their most effective thrust, and subsequently approach the body, the angle of attack becoming less effective towards the end of the stroke, the principles being exactly the same as those displayed by anostracan nauplii (Fryer 1983) and the early larvae of notostracans (Fryer 1988). Viewed laterally (figure 13), the protopod of an antenna on the working stroke first rises towards the observer then, having passed the vertical, falls away towards the carapace. During remotion, the setules of the natatory setae are extended as a rigid palisade that increases the thrust of each seta (figure 13, inset).

As revealed by cine-photography, the return or recovery stroke (promotion) is far from being a flexed reversal of the power stroke. While during the power stroke the protopod swings backward through an arc that extends it laterally from the body, during recovery it swings ventrally and forward in a plane essentially parallel to the long axis of the body and close to it, the



Figures 11 and 12. Antennal movements in *Daphnia* as seen from the dorsal side in *D. obtusa*. Outlines are based on the position of the antennae in successive frames of a cine film run at 100 f.p.s. Except in positions 1 and 8–10, only one ramus is shown and only sufficient setae necessary to indicate events are portrayed.



Figures 13 and 14. Antennal movements in *Daphnia* as seen laterally in *D. magna*, based on successive frames from a cine film run at 100 f.p.s. The full complement of natatory setae is shown only for position 1. During the working stroke (figure 13), the antenna rises towards the observer until it lies perpendicular to the plane of the paper, then descends into the plane of the paper as it continues its backward sweep to position 6. Only two positions are shown during the recovery stroke in figure 14. Insets (semi-diagrammatic) show how the setules of the natatory setae are spread to give thrust during the working stroke, flexed to reduce drag on the recovery stroke.

two rami and their long setae 'trailing' as it does so (figures 12 and 14). The setules of the natatory setae also fold and trail during this phase of the cycle (figure 14, inset). The tip of the protopod describes an imperfect ellipse that is also distorted in the plane in which it largely swings. The ability of the protopod to return on a different course from that followed on the power stroke is a reflection of the mobility of the 'universal joint' at the junction of protopod and head and the versatility of its musculature. On the working stroke the two rami are spread so that their setae can achieve maximum thrust. By the time they reach the end of the stroke they lie adjacent to the carapace, more or less parallel to the long axis of the body, and one above the other when the animal is seen from above or below (figure 11, position 6). The course followed by the protopod on its recovery stroke is such that the endopod in effect overlies the exopod and reduces the drag imposed upon it.

During the recovery stroke, the antennal rami, that lie more or less in line with the protopod during remotion, flex markedly at their union with it (as shown by positions 8 and 9 in figure 12) and are thereby permitted to trail and reduce drag as the protopod swings forward. Their segments also flex upon each other at certain phases of promotion (figure 12, position 9). During the recovery stroke, the natatory setae flex markedly at the joint that separates each of them into proximal and distal portions (figure 14), another device for reducing drag.

Flexure of the antennal rami is achieved by contraction of robust flexor muscles. The two distal exopodite segments, and all the endopodite segments, lack extensor muscles. Extension is therefore achieved by hydrostatic pressure, possibly aided by cuticular elasticity. One suspects the latter in the extension of the natatory setae which are, of course, devoid of muscles. During remotion, the drag against which they operate is sufficient to keep both the antennal rami and their natatory setae in the fully extended position, just as it maintains the natatory setules of the latter in the 'expanded' state. While the rami and their natatory setae are capable of considerable posterior flexion, they cannot flex in the opposite direction. Figure 13 shows their appropriate anterior limits in this respect.

While no calculations of Reynolds numbers have been made, possible sources of error being sufficiently numerous to invite caution, it is clear that the setules of the natatory setae, and indeed the entire seta-setule complexes, operate at low Re values and that viscous forces are important. Considerable drag is clearly generated during the working stroke, enabling the animal to lever itself through the water, and various drag-reducing devices are operative during the recovery stroke.

As the predominant orientation of the body of species of *Daphnia* is with the long axis more or less vertical, antennal beat drives the animal upward. However, as Scourfield (1900) showed, there is a slight ventral component to motion. This is sometimes noticeable as a slight lift of the posterior end of the body as the antennae sweep backward. Persistent antennal beat of the 'normal' kind therefore tends to propel the animal along a circular course. Even if the antennae were incapable of any adjustments, this tendency would be counteracted in part by the fact that, during the recovery stroke of the antennae, the pull of gravity tends to return the body to its vertical alignment. This, however, would be insufficient to prevent deviation from a straight course in a rapidly swimming animal but, as Scourfield was the first to point out, this tendency is counteracted by the posterior carapace spine, and perhaps in some species by the anterior prolongation of the headshield crest. Actively swimming planktonic species (figures 1 and 2) tend to have the longest carapace spines and the greatest tendency to develop an anterior prolongation of the head. Juveniles of more heavily built species, such as *D. magna*, are more active than adults, and have relatively longer carapace spines. The carapace spine is clearly a more important stabilizer than is the pull of gravity during periods of rest, as its presence in fact delays the correction applied to the course by gravitational pull. Notwithstanding these facts, some species manage perfectly well without a posterior carapace spine (see below).

Scourfield's work, based on ingenious observations and experiments, revealed some of the basic aspects of swimming, as did the somewhat diffuse observations of Woltereck (1913), but this activity is more versatile and less stereotyped than hitherto suggested and the swimming behaviour of all species is not exactly the same. Even large, heavily built species, such as *D. magna*, do not merely 'hop' essentially in the vertical plane, but can swim horizontally, dive steeply and rapidly head first, pursue a meandering course that can be changed with great rapidity, swim with the body inclined to one side, and orientate and navigate with great precision. The way in which dense aggregations of such species confined in a vessel follow interweaving courses without collision, often when swimming rapidly, is one manifestation of the latter ability. Another is the occasional aggregation of pond-dwelling species into dense shoals, sometimes of thousands of individuals, that move around in a coordinated manner like a shoal of fishes or flock of birds (§5 (*o*) (iv)). *D. magna* can also take up a position for foraging on the bottom with its carapace margins resting on the substratum, a position that can also be assumed with great precision by *D. obtusa*, that can not only attach itself to, but slide over, surfaces (§5 (*o*) (i), (ii)). Such versatility is achieved by changes in the angle of attack of the antennae during the power stroke and by beating them asymmetrically.

Individuals of *D. magna* glued to a fine glass needle (which in no way impairs antennal activity: it merely results in water being driven backward instead of the animal forward) or immersed in a non-toxic viscous medium, have revealed the versatility of antennal activity. These artificial situations give an inkling of what happens in free-swimming individuals on which observations are difficult to make. Such animals have shown that one antenna can be moved more than the other, which is sufficient to explain how steering is achieved, that the speed of beat can be much increased for short bursts, that the amplitude of beat can be curtailed, that orientation during the power stroke need not always be exactly the same (the appendage may swing somewhat more dorsally than shown in figure 13), that the cycle can be reversed, that the basal articulation allows much freedom, and that one antenna can be swung across to the other side of the body. This last movement has no relevance to swimming, but crossing of the antennae permits mutual cleaning. The two rami of the antennae can also be flexed at the joints and the angle between them can be reduced so that they lie parallel to each other. Such versatility reflects the complex muscular system of these appendages and the effectiveness of the universal joint at which they articulate with the head.

With such resources at its command, *Daphnia* doubtless finds the dilemma of tending to swim in a circle more potential than real, and while the purely mechanical factors identified by Scourfield automatically help to counteract this tendency, antennal adjustments alone can doubtless do so. Thus curtailment of the backward sweep would eliminate that component of beat that contributes most to depression of the head, but recourse even to this strategy may not be necessary, and required course corrections may well be achieved by alterations in the orientation of the antennal rami. It is such versatility that explains why short-spined, or even spineless, individuals of *D. obtusa* are not constrained by purely mechanical factors. Observations on a population of a small race of this species revealed enormous agility and abilities such as those described above, yet all adult individuals had, at most, a very short carapace spine. The Australian *D. nivalis* Hebert also completely lacks a carapace spine (see Benzie (1988*a*) for good illustrations). Most species of the closely related genus *Daphniopsis* also lack a carapace spine, but are versatile swimmers (§8 (*a*) (ii)).

Rapid reversal of antennal beat may help to extricate an animal from an awkward situation or even repel a small predator. One individual, filmed while glued to a glass needle, reversed the cycle of movement several times in quick succession and appeared to be using this ability as a means of wiping the carapace surface.

(*c*) ***The exoskeleton***

Various features of the exoskeleton of *Daphnia* are familiar from published illustrations, good and bad, but the nature and significance of certain components, and some of the details, have received inadequate attention. That the carapace is folded and not hinged is well known. The ridge where the fold occurs provides a median dorsal rib of considerable rigidity. In some species, including *D. atkinsoni* f. *bolivari*, the rib (DR) is conspicuously elaborated (figures 109, 110). The ventral margins of each carapace lobe (valve) are thickened, forming rigid hoops around the margin of each. Each carapace lobe is reticulated, the reticulation forming a network that spans the space between dorsal ridge and marginal hoops. This rigid network gives form to the whole, and the rest of the outer skin of the carapace, which consists of inner and outer walls (e.g. figure 112), need then be only thin and, as it were, papers over the gap between the reticulations, the principle being the same as that which applies to aircraft wings, a skin-covered framework. Thus a light, but rigid, structure is achieved. The pattern of reticulation differs not only between species but in different parts of the carapace. In *D. magna*, for example, much of the carapace has a diamond-shaped reticulation, the long axes of whose individual components run more or less transversely and whose posterior corner is drawn out into a short spike. In the vicinity of the dorsal rib, however, the reticulations become smaller and more or less hexagonal and add to the rigidity of this region.

Where the dorsal rib meets the ventral hoop, a posteriorly directed spine is usually developed. This is often long, especially in planktonic species (figures 1 and 2). The outer wall of the carapace cuticle stains red with Mallory or, where it is thickened, has an amber colour in section, while the very thin cuticle of the inner wall stains blue, showing its soft unsclerotized nature. Details of the cuticle of the outer wall of the carapace, as revealed by transmission electron microscopy (TEM) in *D. pulex*, are given by Schultz & Kennedy (1977). Basically, there is a very thin epicuticle and two laminated layers of procuticle.

From the marginal hoops, the inner wall of the carapace follows a course for the most part parallel to the outer, but separated from it by a gap, generally of more or less uniform width (e.g. figure 112), but widening in regions where the intervening space is occupied by the tubules of the maxillary gland (figure 109). The cuticle of the inner wall is very thin, as befits its function and as it can afford to be, for here it is hidden and protected. Here, although not generally recognized as such, is surely a major seat of respiratory exchange, for blood circulates through the spaces between the inner and outer walls of the carapace lobes, spaces that maintain their separation by virtue of an abundance of fibrils that span the gap between them (e.g. figures 109 and 112) and there is a regular flow of water through the carapace chamber. While the epipodites of the limbs have often been referred to as seats of respiratory exchange, they are in fact concerned with active transport of ions, especially sodium and chloride ions, through their thick epithelium and help to maintain a constant osmolarity of the blood. They have a characteristically thick wall, as seen at low magnifications (e.g. figures 112–114). Their detailed fine structure has been revealed by Kikuchi (1982), who has also shown how chloride ions accumulate beneath their cuticle. (See also Potts & Durning (1980).)

In some species of the subgenus *Ctenodaphnia*, lateral ribs extend posteriorly along the carapace from the region of the fornix. In some forms of *D. atkinsoni* f. *bolivari* they extend only part way along the carapace: in others they continue posteriorly, but with diminishing conspicuousness, to unite with the ventral marginal hoops just before the latter meet the carapace spine at the point of union of dorsal ridge and marginal hoops. This confers increased rigidity on the carapace at scant cost in weight. In the form of *D. atkinsoni* f. *bolivari* of Palestinian provenance much used in the morphological part of this study, the ribs extend laterally like wings, being continuous anteriorly with the lateral expansions of the headshield. Transverse sections reveal them to be fibre-filled expansions of the carapace, that look like lateral horns anteriorly (figures 109 and 110). Their wing-like nature suggests a hydrodynamic, as well as a perhaps protective, function. For example, they may grant stability and prevent sinking when the animal swims in the horizontal plane. Anteriorly, and dorsal to the lateral wings, the carapace cuticle of this form is very thick (figure 109, TC) being part of the general strengthening of this region in connection with the anchoring of the powerful antennal muscles.

The carapace lobes can be drawn together by contracting the adductor muscles (ACAM, PCAM) that insert on the inner wall of each (figures 36, 109 and 110).

While the ventral part of the head is simply covered by a chitinous cuticle, strengthened like the carapace lobes by a reticulation of delicate ribs in some cases, the exoskeleton is elaborated dorsally into a headshield. This covers a lateral expansion of the otherwise narrow head with what is basically a dome-like plate, whose shape, however, differs much in different species. In *D. magna* it may be much wider than long: in *D. cucullata* Sars, considerably longer than wide. A dorsal ridge, continuous with the carapace ridge, is often present. Above the bases of the antennae, which it covers and protects (figures 2, 15 and 16), the headshield is, like the carapace, double-walled. One of its functions is protective, but it never assumes such a robust nature as is consistently the case in the Chydoridae. Another is to provide firm anchorage for the antennal muscles that originate on it. The region around the antennal articulation is also strengthened.

The headshield is elaborated in various ways. In general, it is simplest in the subgenus *Daphnia* though here, in several planktonic species, it may develop a conspicuous dorsal crest (§6(*a*)). In this subgenus, the suture between it and the carapace extends posteriorly in the mid-line, so that the head shield as it were carves out a notch in the carapace. In the subgenus *Ctenodaphnia*, it is the carapace that invades the headshield, a matter conveniently discussed in another context (§6(*a*)). In this subgenus, the headshield may also be drawn out into a plate-like crest (figures 135–137) or, in *D. lumholtzi* Sars, bear a long spine (figures 133, 134). Sometimes too, instead of being smoothly rounded, the posterior lateral corners of the headshield are drawn out into spine-like protuberances, which are especially long in *D. lumholtzi*.

The exoskeleton of course covers the trunk and all the appendages. The trunk cuticle is for the most part thin, which grants flexibility. The intricate coordination of the complex limbs during feeding, however, also demands rigidity of the trunk and this is conferred by the muscular system and associated endoskeleton, probably assisted by turgor pressure of the haemocoele. Where this is necessary, as in the walls of the food groove, the cuticle is thickened (e.g. figures 27 and 29, where the food groove cuticle can be compared with the generally flimsy trunk cuticle). There is also a local thickening of the trunk cuticle dorsally just behind the heart, where the dorsal longitudinal muscles are anchored (figure 41). Although daphniids are arthropods, they largely fail to display one of the outstanding attributes of most adult members of this taxon, and indeed the attribute from which its name is derived, namely jointed limbs. True joints are developed in the antennae, but the trunk limbs are 'turgor extremities' that lack true joints. In a limb flexing posteriorly, movement is permitted by tightening of the cuticle on the anterior face, and folding, as in the arthrodial membrane of a true joint, of the cuticle of the posterior face. Although this is a more primitive system than one employing true joints, it is exceedingly versatile and when, as in *Daphnia*, movements can be controlled with extreme precision, it can be used as the basis for some of the most intricate and complex limb movements to be seen anywhere in the Arthropoda.

(*d*) *Carapace spinules and denticles*

The spinules and denticles borne on the carapace of *Daphnia* have received scant attention. The posterior part of the dorsal carapace ridge and the posterior half or more of the ventral margins are, perhaps always, provided with at least a few spinules. Those of the dorsal ridge are in two rows, one at each side. Those of the ventral margins arise as a single row from the extreme rim of each valve and are independent of any setae that arise from its inner surface just inside its margin. Such sub-marginal setae may be simple or setose: some of the former serve as screens to prevent the ingress of unwanted material to the filter chamber, the latter are probably sensory.

The dorsal and ventral rows of spinules unite at the base of the carapace spine, which is also armed with spinules. In the subgenus *Daphnia* the spinules are often fine and short, but tend to be more robust on the carapace spine. In the subgenus *Ctenodaphnia* they are often robust and denticle-like, and in *D. lumholtzi*, where all are well-developed, those of the ventral series are longer, stouter and more widely spaced than in other species and merit designation as spines. As in other robust species of the subgenus, they probably afford protection against small predators.

In the subgenus *Ctenodaphnia*, the dorsal ridge of the carapace extends forward over the head and is enclosed by an embayment of the headshield. In *D. magna* the extension reaches at most half way along the head, but in some species, such as *D. carinata* King and *D. barbata* Weltner, where it takes the form of a simple prolongation, it is longer and extends the double row of denticles well forward along the dorsal surface of the head. In other species, after extending forward for some distance, the two rows of denticles diverge laterally to various degrees before swinging forward again to meet each other near the anterior part of the head. Thereby they enclose a plate, here called the cephalic plate, surrounded by the headshield, whose shape differs in different species, and even in different forms of a single species. In *D. atkinsoni* f. *bolivari* an area sometimes not unlike the conventional heart shape, with the apex directed anteriorly, sometimes more obviously bilobed, occupies much of the dorsal surface of the head. Its margins are fringed throughout by a row of stout denticles so that the head, as it were, bears a crown of thorns, which is undoubtedly protective. This form was originally described as having a crown made up of a double row of spinules, but Gauthier (1937) showed that only one row is present but that spinules which replace the functional series at the next moult can sometimes be seen developing beneath the cuticle of the cephalic plate.

In some members of the subgenus, including some populations of *D. atkinsoni* f. *bolivari*, the lateral rib that

Figures 15–21. *Daphnia atkinsoni* f. *bolivari*. Various anatomical details, especially of the skeleto-muscular system. Figure 15. Transverse section through the head, cutting through the protopod joint on each side. Note how some of the extrinsic antennal muscles (A2M), which originate on the head cuticle at the opposite side of the body from the appendage they serve, cross in the mid-line. Figure 16. The same, showing details of the head–antennal protopod joint. Note the rings of cuticle (RC) that grant versatility of movement, and part of the elaborate nerve supply to the antennal muscles. Figure 17. Transverse section near the anterior limits of the mandibles showing the elaboration of the endoskeletal sheet (Endo S) in the region below them. The sheet is anchored to the cuticle of the head and has three bracers ventrally on each side. Figure 18. Horizontal slice (ventral view) in the region of origin of the dorsal longitudinal muscles (DLM). Part of the heart (Ht) can just be seen between this region and the mid-gut (MG) – here cut somewhat obliquely as it curves posteriorly. Embryos (Emb), lying in various postures, have been sectioned in the brood pouch. Figure 19. Horizontal section through the mid gut region showing the anchoring fibrils (AF) of the trunk muscles. Figure 20. Longitudinal section through part of a ventral longitudinal muscle (VLM) showing an intersegmental tendon (IT) at the boundary of two segments. Dorsal side uppermost. Figure 21. Horizontal section of the head, cutting through the compound eye and the ocellus. Note the narrowness of the head in this region, so that, on each side, the eye lies just beneath the cuticle, and also the absence of any light-absorbing tissues anterior to the eye.

extends posteriorly from the fornix region on each side of the carapace sometimes partway along it, sometimes along its entire length (§5(*c*)), may also bear stout spinules. Individuals of these types, especially the latter, are sometimes particularly well-protected by spinules. Although these ridges and spinules have been illustrated in some of the better drawings in the literature, such as those by Sars (1903) of *D. triquetra* Sars, their functional significance has apparently gone unnoticed. In *D. triquetra semilunaris*, which Flössner (1987) believes to be pelagic, what are usually short, stout denticles are replaced by longer, robust spinules, both on the crown and on the dorsal ridge and lateral ribs. In the Palestinian population of *D. atkinsoni* f *bolivari* with lateral wings used in this study, the margins of the wings bore only small spinules.

In some species, there is much variation in the shape and armature of the cephalic plate. This is particularly so in *D. atkinsoni* and its close relatives. In Britain, *D. atkinsoni* has a plate of modest proportions, spatulate in shape, not expanded very much laterally and apparently always devoid of marginal denticles. Elsewhere, forms occur whose cephalic plate is widely expanded and bears a conspicuous crown of denticles. The f. *bolivari* is one such. This seems not, however, to be a simple case of geographical races. In Central Europe both forms may occur in the same area and are linked by transitional types (Hudec 1981). A similar situation probably exists in *D. triquetra*, thought by some to belong to the *D. atkinsoni* complex, but which Flössner (1987) now regards as distinct.

The invariably posteriorly directed orientation of the spinules is in keeping with the benefits of reduced drag as the animal moves forward and must serve, to however small a degree, as an anti-sinking device. Nevertheless, their primary role is clearly defensive. In other daphniids, such as *Ceriodaphnia setosa* Matile and *Scapholeberis echinulata* Sars, independently evolved spinules are scattered over much of the carapace. In *D. barbata* an arc of spinules embraces the distal part of the rostrum and is also probably defensive, but may have additional, as yet unknown, functions.

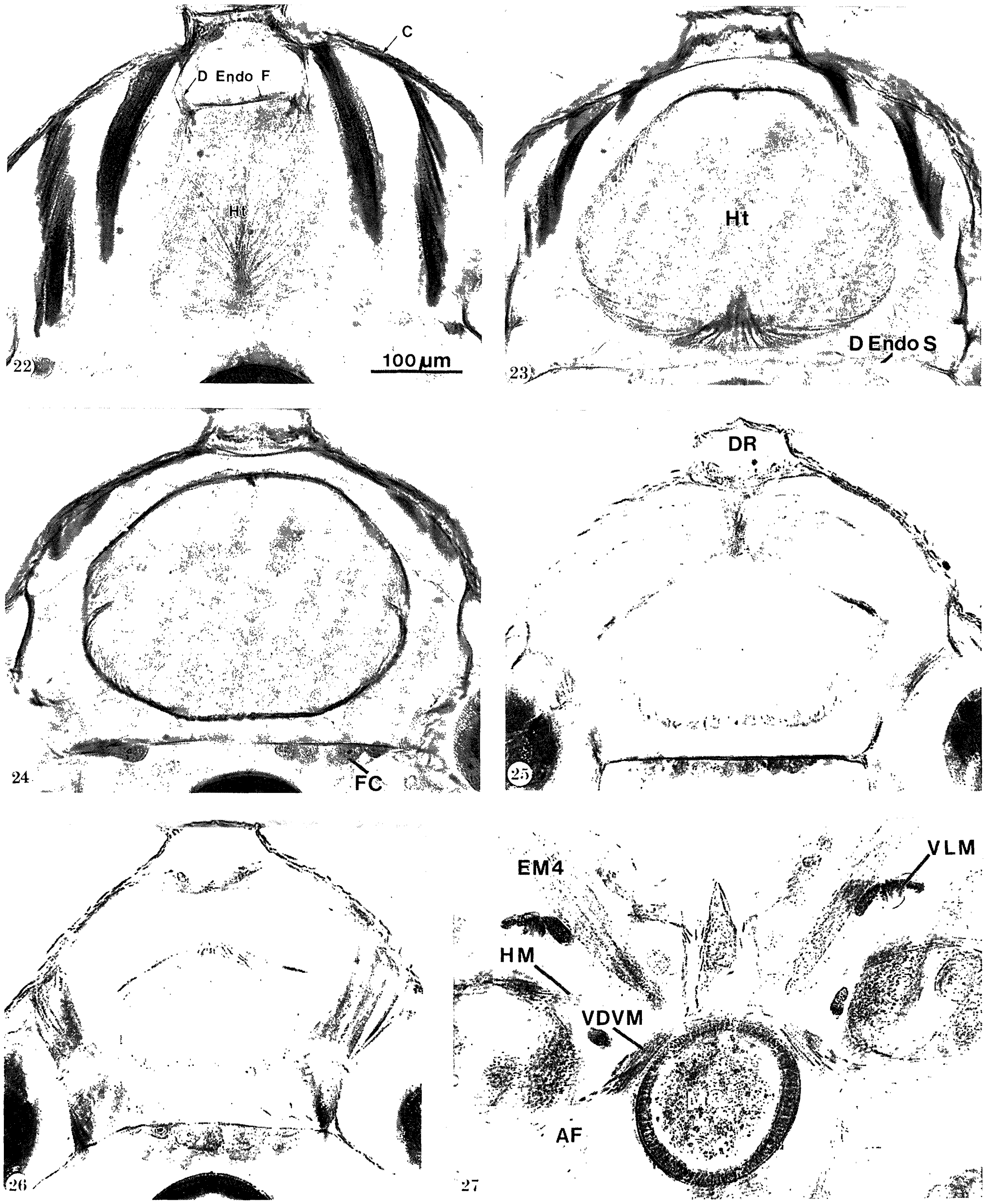
(*e*) **The endoskeleton**

The endoskeleton of *Daphnia* has received scant treatment though some features were recognized by Hérouard (1905) who concentrated on the way in which elements of it divided the body cavity into compartments and influenced the course of circulation of the blood. It is convenient to begin at the transverse mandibular tendon (TMT), which is seen in face view in figure 30 and in transverse section in figure 3, which clearly locates its position. This tendon was noted, but not fully understood, in *D. magna* by Binder (1932) and in *D. pulex* by Mahoon (1960), both of whom provided simple sketches. The transverse mandibular tendon is suspended from the headshield by two fibrous suspensors (figures 3, 31 and 109 SUS), just as it is in other anomopods (see figure 18 in Fryer (1963) for details of this in the chydorid *Eurycercus lamellatus*). As is the case in this robust chydorid, the suspensors descend from an array of load-spreading intracellular fibrils (Fib) that also provide the point of origin for the massive remotor roller muscles (4) of the mandibles (figure 32).

The transverse mandibular tendon is also anchored to a ventral endoskeletal sheet (Endo S) as can be seen in figure 3. This sheet, which runs fore and aft, was referred to as the endoskeletal plate in macrothricids (Fryer 1974), but the term sheet, used for the homologous structure in the Anostraca (Fryer 1983), seems more appropriate. Anteriorly it curves dorsally and, just anterior to where it is anchored to the roof of the atrium oris, it thickens and provides the sites of origin for the topographically dorsal, but morphologically ventral, array of oesophageal dilator muscles (figures 3 and 4). More dorsally it is utilized as the site of origin of powerful extrinsic muscles of the antennae (figures 5–10). Its still further anterior prolongation, and some of its elaboration there, is seen in part in figure 3, which also shows some of the cross links between the transverse mandibular tendon and the anterior part of the sheet.

At the level of the anterior margin of the mandibles, the ventral endoskeletal sheet is broad, spanning the full width of the here narrowing head, being anchored to the head cuticle on each side and secured by three bracers on each side (figure 17). Its posterior extension has its first simple, but important, elaboration at about the level of the maxillules. Here it thickens, is braced on each side by a long, dorsal suspensor (Sus Endo S) (figure 110), and serves as a firm point of origin for both the anterior (ACAM) and posterior (PCAM) carapace adductor muscles that extend laterally on each side to insert on the inner wall of the carapace (figures 109, 110). Their contraction enables the gap between the carapace valves to be reduced in width, or even closed, just as does contraction of the analogous adductor muscles of bivalve molluscs. Hereabouts the sheet also provides the site of origin for muscles of the maxillules (figure 109).

The suspensor (SUS Endo S) that extends dorsally and somewhat laterally on each side from the region where the carapace adductor muscles are anchored, expands at its dorsal extremity into a sheet that is itself part of a region of rigidity composed of sheets and fibrils, here referred to as the dorso-lateral fibrous complex. The nature of this region is best appreciated by reference to figures 33, 109 and 110. The tendinous sheets that make up its walls are supported and braced by a mass of fibrils that occupy the dorso-lateral region of the anterior end of the trunk and lie just below the carapace. Hereabouts the body attains its maximum width. Thus at each side of the trunk this complex makes up a region of great rigidity that serves as the site of origin for major extrinsic muscles of the anterior trunk limbs (§5(*f*)). It is here that most of the stresses imposed by the major movements of trunk limbs 1–4 are borne. The fibrils also support part of the tubule of the maxillary gland (figures 33 and 110). The use of load-spreading fibres is well shown in this region. The very slender brace, (figure 110, DLB) that extends from the same dorsal region as the suspensor to the corner of the food groove, and is here called simply the dorso-lateral brace, is partly muscular, but has a long fibrous component.



Figures 22–27. *Daphnia atkinsoni* f. *bolivari*. Aspects of the heart, endoskeleton and muscular system. Figure 22. Transverse slice through the anterior extremity of the heart (Ht). Note the dorsal endoskeletal frame (D Endo F) from which the heart is here slung. The large muscles present are the remotor rollers of the mandibles. Figure 23. The same immediately posterior. The dorsal endoskeletal sheet (D Endo S) lies beneath the heart, forming the floor of a pericardium. Figure 24. The same immediately posterior. Note the fat cells (FC) on the ventral surface of the dorsal endoskeletal sheet. Figure 25. The same immediately posterior. Note the great accumulation of fat cells beneath the dorsal endoskeletal sheet. The dark objects at each side are embryos in the brood pouch. The dorsal ridge of the carapace (DR) is well seen in this and the next figure. Figure 26. The same at the posterior limits of the heart. The anterior-most extremities of the dorsal longitudinal muscles can just be seen. Figure 27. Transverse section, ventral side uppermost, of the trunk in the vicinity of the intersegment 3/4 showing the horizontal muscles (HM), the vertical dorso-ventral muscles (VDVM) and their anchoring fibrils (AF) on each side, and some of the long extrinsic muscles of trunk limb 4 (EM 4). The ventral longitudinal muscles (VLM) are seen in transverse section. Some unidentified object is present in the food groove.

The ventral endoskeletal sheet continues posteriorly just dorsal to the food groove, with whose floor it soon becomes so closely associated that it is difficult to detect in transverse sections. At each intersegmental boundary the ventral longitudinal muscles (§5(*f*)) are separated into segmental blocks by inter-segmental tendinous sheets (figure 20, IT) in the same way as described in the anostracan *Branchinecta ferox* (Fryer 1983) and as can be seen in figure 6 of the macrothricid anomopod *Acantholeberis curvirostris* in Fryer (1974). Here are developed intersegmental endoskeletal nodes that provide anchorage for the ventral extremities of the dorso-ventral muscles of the trunk and of the horizontal muscles, and with these the ventral sheet is continuous.

At the level of the heart, sheets and fibres extend dorsally from the ventral endoskeletal sheet and are continuous dorsally with other endoskeletal elements that were noted by Hérouard (1905) who, with justification, described them as comprising a pericardium, though they are in fact part of a more extensive system. The heart, of which an adequate description was given by Claus as long ago as 1876, is suspended from the dorsal wall of the carapace by tendinous elements (see especially figure 22, D Endo F) and also anchored ventrally to a horizontal endoskeletal sheet, called the 'cloison dorsale' by Hérouard, and here called the dorsal endoskeletal sheet (D Endo S) (figures 22–26). The dorsal endoskeletal sheet can be seen in life, especially in a transparent species such as *D. galeata*, and is made conspicuous anteriorly by the way that it vibrates in sympathy with the beating heart. In sections, it is best seen in a robust species such as *D. atkinsoni* f. *bolivari* that provides the illustrations. This sheet (figures 23–26) separates the heart, and the blood flow, from the mid-gut. On its ventral side, fat cells (FC) are habitually present (figures 24–26). These cells are often associated with membranes, as noted by Jäger (1935) who studied them intensively, usually occurring on both sides. No functional explanation of why they are confined to the ventral side of the dorsal endoskeletal sheet is immediately apparent. Anteriorly they would probably interfere with heartbeat, but restriction to the ventral side holds good well posterior to the heart. In the vicinity of the heart the sheet is supported by what in effect make up the side walls of the pericardium-like arrangement, and by fibrils slung from the dorsal part of the carapace (figures 23 and 24). At about the level of the posterior limit of the heart, a short but broad bracing muscle is present on each side that, with some inclination, extends more or less dorso-ventrally between the dorsal carapace cuticle and a fibrous elaboration of the endoskeletal sheet (figure 26). The latter furnishes the anterior anchorage for the dorsal longitudinal muscles. The extreme anterior limits of the lateral bundles of these can just be seen in figure 26.

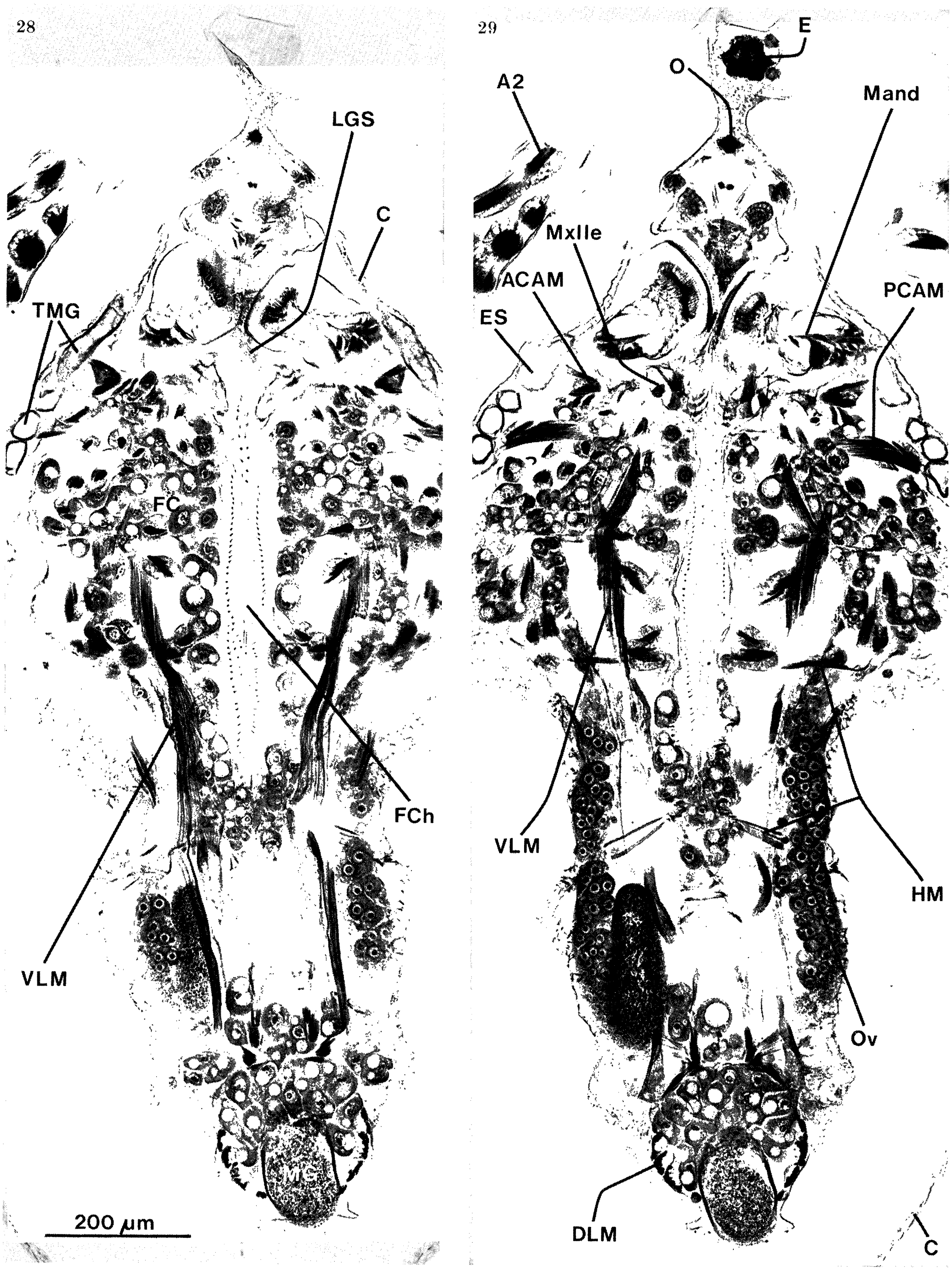
Anterior to the heart, the dorsal endoskeletal sheet thins out and reaches its anterior limits at about the level of the suspensory ligaments of the transverse mandibular tendon and the remotor roller muscles (figures 109 and 110), being supported hereabouts by the same mass of load-spreading fibrils as supports the latter structures. Posteriorly it continues, just above the mid-gut and just beneath the dorsal longitudinal muscles, to its posterior anchorage in a complex of anchoring fibrous sheets used by the post-abdominal and longitudinal trunk muscles. It is not always easy to see in longitudinal sections, but its presence is betrayed by the habitually associated fat cells of its ventral surface. Because of the proximity of the endoskeletal sheet to the mid-gut wall, these cells tend to be compressed and appear oval when seen in longitudinal section (a few can be seen in figure 3) and are often particularly difficult to see posterior to the region where the sheet has passed beneath the dorsal longitudinal muscles. In the robust *D. atkinsoni* f. *bolivari*, however, these cells can sometimes be clearly seen (figure 40). In sections, they tend to lie adjacent to the ovaries which 'camouflage' them.

At intervals along its length, the dorsal endoskeletal sheet is continuous with the intersegmental fibrous sheets and fibrils that, at each node, provide firm dorsal anchorages for the dorso-ventral trunk muscles and for certain extrinsic muscles of the trunk limbs (figure 112). It is thus an integral part of the system of struts and braces that make up the skeletomuscular system of the trunk. It serves as the analogue of a vertebral column.

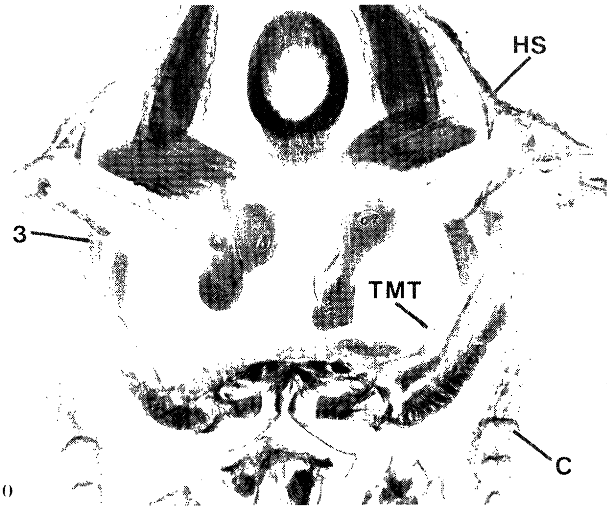
Other endoskeletal structures and fibres serves a variety of purposes in *Daphnia*. These include the suspension of the eye in a gimbal-like manner, and the provision of firm anchorage for the topographically ventral oesophageal dilator muscles (figure 3) and certain small muscles in the labrum. There is also an intricate forest of fibrils that spans the gap between the thin inner and thicker outer walls of the carapace, giving support to the former and maintaining the necessary space between two walls. Numerous fibrils that are part of this system also suspend the tubules of the maxillary gland within the space between the carapace walls (figures 109, 110).

(*f*) *Some features of the muscular system*

Binder (1932) has given a generally competent account of the muscular system of *Daphnia* based on *D. magna*, but supplementary information is necessary for the understanding of certain functional attributes. Her illustrations reveal the way in which long promotor and remotor muscles of trunk limbs 1–4 originate dorsally in a restricted region ventral to the heart (but without showing their exact origins) and how, especially those of limbs 3 and 4, inevitably incline steeply backwards as they descend to their insertions in the limbs. These muscles in fact originate on the dorsolateral fibrous complex described in §5(*e*). She also outlined the arrangement of the trunk musculature, making clear the fact that, as shown here for example in figure 112, there are four muscle bundles on each side in the dorsal longitudinal series (DLM) and three in the ventral (VLM). She correctly indicated that the muscle bundles of the ventral longitudinal series diverge anteriorly and that the median bundle, whose course is seen in figure 37 of this paper, extends far forward. It is anchored anterior to the level of the mandibles and is seen in transverse section in figures 109 and 110.



Figures 28 and 29. *D. atkinsoni* f. *bolivari*. Successive horizontal slices, viewed ventrally, to reveal various anatomical features and the relation of the median filter chamber (FCh) to the trunk and to the mouthparts. Figure 29, which lies deeper than (i.e. dorsal to) figure 28, cuts the filter chamber not far from where the tips of its component filter setae lie deep in the food groove, the thickened cuticle of whose walls can be seen. It also cuts through the maxillules (Mxlle), mandibles (Mand) and compound eye (E). (Comparison with figure 3 facilitates orientation.) Other features seen include the end sac (ES) and tubules (TMG) of the maxillary gland, housed between the inner and outer walls of the carapace (C), the carapace adductor muscles (ACAM and PCAM), the ventral longitudinal muscles (VLM), the divergence of whose bundles anteriorly is seen in figure 29, the horizontal muscles (HM), some of the complexity that prevails in the vicinity of the post-abdominal hinge, and the conspicuous ovaries (Ov). A portion of the carapace provides a point of reference posteriorly. Note the abundant fat cells (FC). Because the mid-gut curves sharply ventrally as it enters the post-abdomen (see figure 1), it is here cut more or less transversely. Mandibular asymmetry is readily apparent, and exuded labral gland secretions (LGS) are seen in figure 28.



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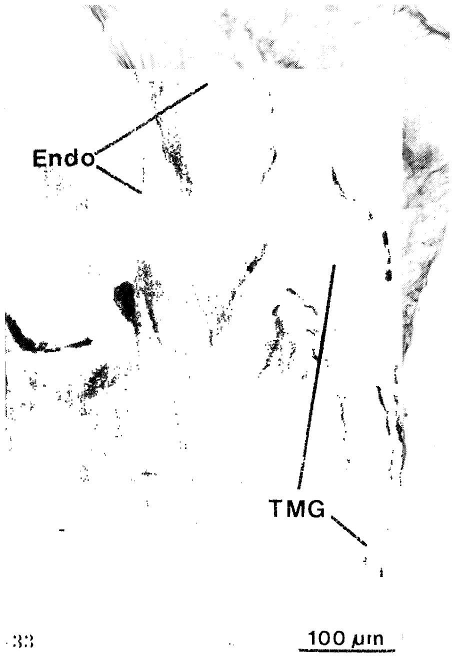

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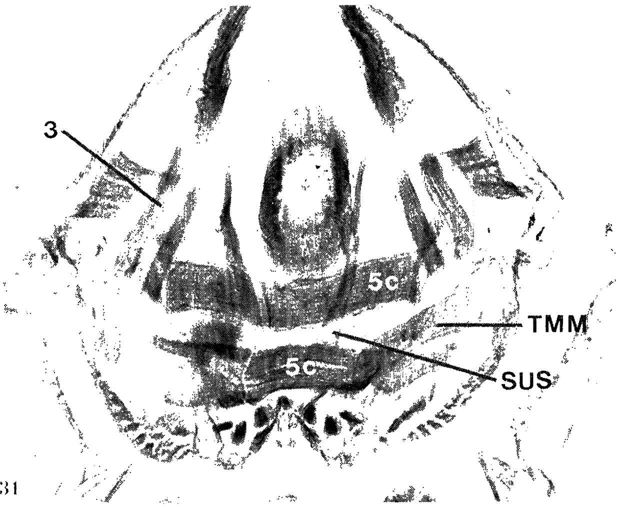

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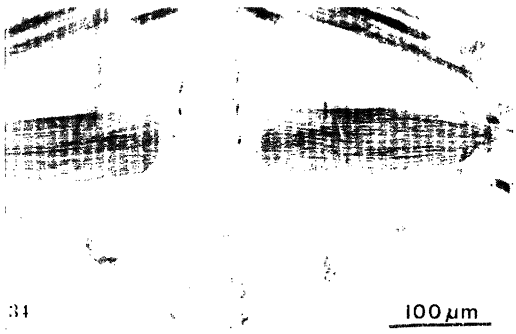

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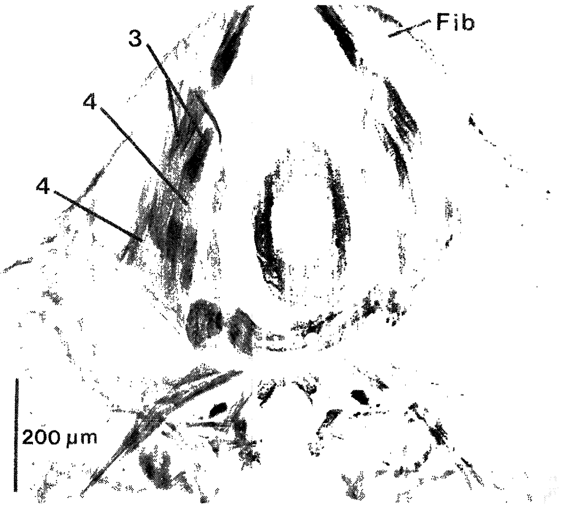

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Figures 30–35. *Daphnia atkinsoni* f. *bolivari*. Some details of skeleto-musculature. Figure 30. Transverse section at the extreme anterior limits of the mandibles. The distal extremities of the promotor roller muscles (3) are seen inserting on the anterior margins of the mandibles, and the broad face of the transverse mandibular tendon (TMT) is seen especially at the right-hand side of the section. Extrinsic muscles of the antennae, which cross over to insert on the opposite side of the head, are seen dorsally. Figure 31. The same, more posteriorly, showing many of the major mandibular muscles and the lower parts of the suspensors of the transverse mandibular tendon (SUS). The asymmetry of the major transverse muscles (TMM), long on the right (left side of the animal), short on the left, is readily apparent. Figure 32. The same, cutting through the extreme posterior region of the mandibles and through the maxillules. Note the load-spreading fibrils (Fib) dorsally, from which the suspensors of the transverse mandibular tendon (whose upper parts are seen) and the remotor roller muscles (4) descend. Figure 33. Transverse section not far behind the carapace adductor muscles, showing some of the elements of the extensive endoskeleton (Endo), here used as the source of origin of extrinsic trunk limb muscles. Tubules of the maxillary gland (TMG) are also seen.

While Binder showed the course of the dorso-ventral muscles, she did not mention the horizontal muscles (HM) that, while very difficult to see in the whole animal viewed laterally, and not easy to see even in longitudinal sections (figure 39), are of vital importance. They are best seen in horizontal and transverse slices (figures 29, 36 and 112), but again may easily escape detection in the former as they become enveloped by the ovaries. Three pairs of such horizontal muscles are present, their arrangement being similar to that in the anostracan *Branchinecta* (Fryer 1983). Each is anchored at an intersegmental node and all pass laterally to the body wall in the horizontal plane, the two anterior pairs at right angles to the long axis of the trunk, the last pair inclined somewhat posteriorly. The muscles present are those of intersegments 2/3, 3/4 and 4/5 of the trunk. As figures 29 and 36 show, the series gives the impression of being continued anteriorly by the posterior carapace adductor muscles and it is highly likely that, by a shift of their insertions, the horizontal muscles of intersegment 1/2, which are otherwise not represented, were transformed to carapace adductors when the carapace was evolved. It is assumed that the carapace, although an ancient possession, is a derived and not a primitive feature of the ancient forms from which the anomopods are ultimately descended. The anterior carapace adductor muscles (figures 36 and 109) which arise well anterior to the posterior adductors and are inclined ventrally and anteriorly as they pass laterally, conceivably represent the horizontal muscles of the maxilla–trunk segment 1 intersegment. Binder (1932) says that both pairs of muscles belong to the 'Maxillarsegment', but this is improbable. Although less massive than their posterior partners, the anterior pair operates at a better mechanical advantage. These muscles are able to pull the opposed margins of the anterior end of the carapace together and can completely occlude the gape anteriorly, but not posteriorly in the vicinity of the post-abdomen. It is in this posterior region that defensive spinules are developed on the carapace margin (§5(*d*)). Adjustments of gape width can easily be observed in an individual glued by its dorsal region to a fine needle of glass. As these movements can be made almost instantaneously, one wonders how reliable are measurements of gape width of live animals that have been related to phytoplankton density (Gliwicz 1980; Gliwicz & Seidlar 1980), though it is not denied that *Daphnia* may use changes in gape width to control the inflow of particles, or to exclude them completely on occasion.

Various aspects of the trunk muscles, and to some extent those of the limbs, are evident from several illustrations and their captions (especially figures 28, 29, 33, 35–39, 109, 110 and 112). These, supplementing the sketches of Binder (1932), show how the skeleto-muscular system of the trunk is arranged. The importance of endoskeletal fibres in the anchorage of various muscles is also apparent in several illustrations and can be seen, for example, in figures 19 and 27.

Binder also gave a pioneering account of the mandibular muscles, but certain corrections are called for and her functional interpretations are now known to be erroneous. Salient features of the mandibular musculature are seen in figures 30–32 (especially 31) (see also figure 109).

(*g*) *Trunk limb morphology and arrangement*

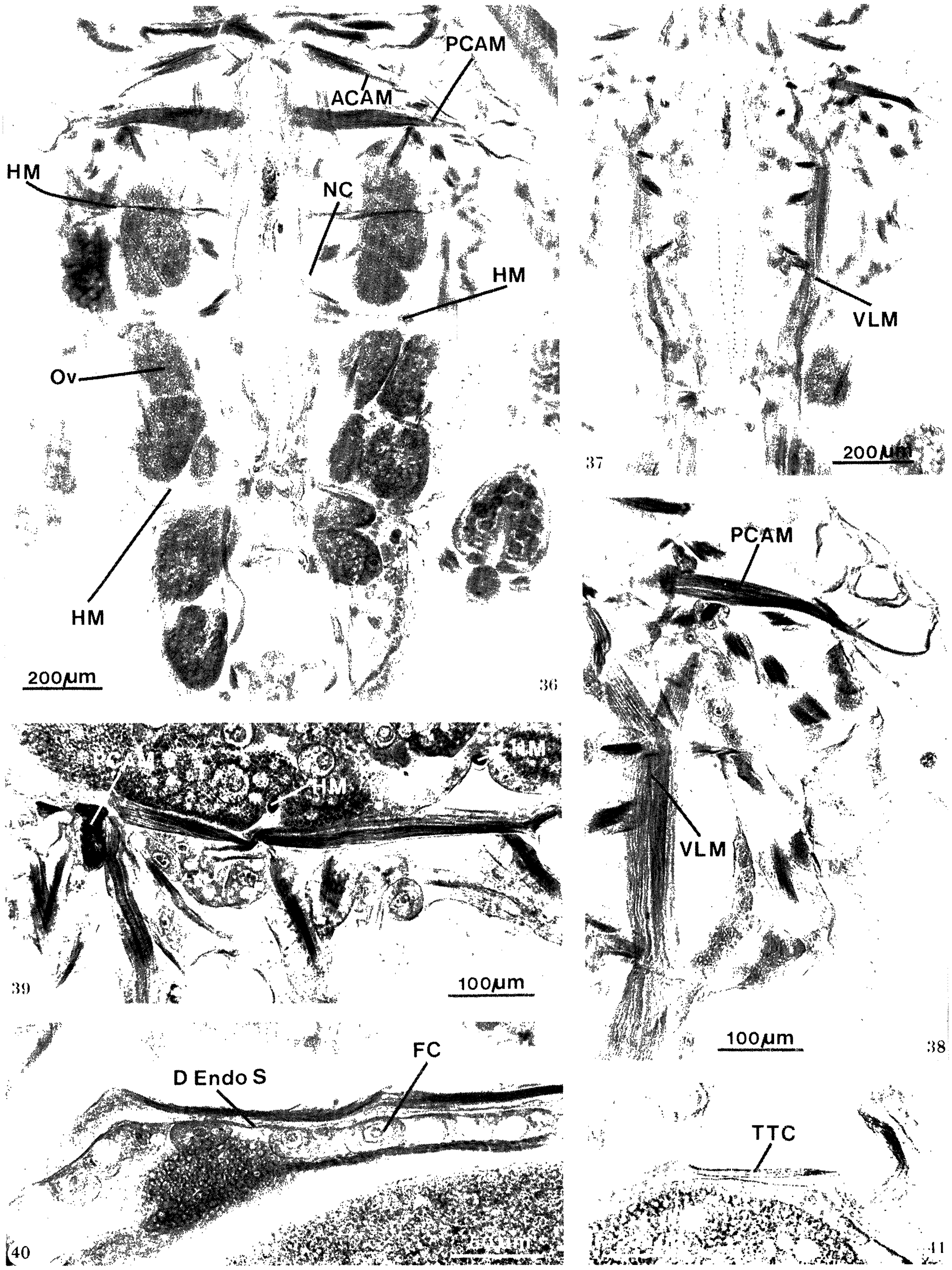
Cannon's (1933) illustrations of the trunk limbs of *D. magna* as seen in the median view (reproduced here as figures 42–46) provide an excellent starting point for understanding the disposition of individual limbs in life. Although of a different species, they facilitate understanding of the spatial inter-relations of the limbs seen *in situ* in figure 3, and in transverse sections in figures 108–118.

Cannon's figures, supplemented by those of Lilljeborg (1900) which, while of flattened appendages, are very helpful, and details revealed by scanning electron microscopy (SEM) (Watts & Petri 1981) provide much of the information required to understand trunk limb structure, but some additional details are necessary. Further relevant information is presented when the arrangement of the limbs is considered, and details of the fine structure of limb armature, especially that of the filter plates, are given in §5(*h*).

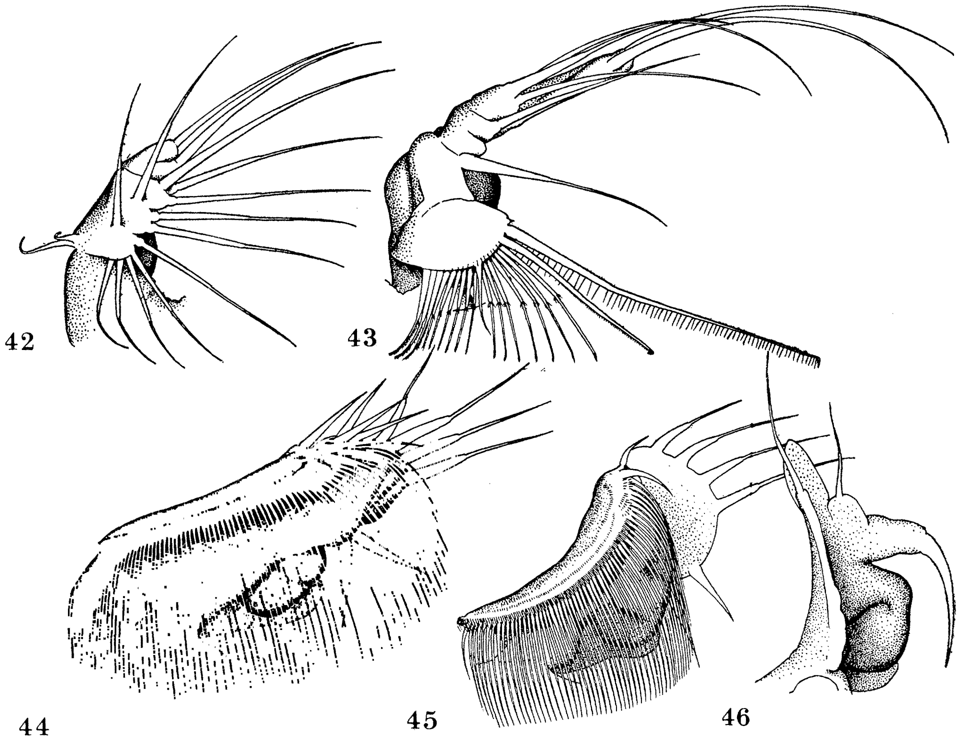
The general form of the first trunk limb is shown in figures 3 and 42. The long, mostly backwardly directed setae of its endites are provided with setules that were deliberately omitted by Cannon, as they are also in figure 3. These setules are arranged in two uniseriate rows on opposite sides of each seta (figure 47). Although not all in the same plane, the tips of the setules of adjacent setae lie close together so that the distal and median ensemble of setae makes up the walls of a funnel leading posteriorly into the filter chamber. This is readily apparent in transverse sections (figures 108–110). These setae are henceforth referred to as funnel setae. Structural details, as revealed by SEM, are given in figure 86. Two specialized spines, the ejector hooks (figures 42 and 68), arise near the base of the limb, just as they do in chydorids and macrothricids. These, which are directed anteriorly in the resting limb, have been illustrated by SEM photographs of *D. magna* by Watts & Petri (1981).

The nature of the distal portion of the second limb is evident from figures 3 and 43. Figure 48 shows the armature of its distal endites in *D. magna*, but without giving details of the medial spine of the distal endite. This spine is modified into a scraper in *D. magna*, but

Figure 34. Horizontal slice through the carapace adductor muscles. Ventral aspect. The massive posterior muscles occupy the centre of the photograph. Parts of the more obliquely inclined anterior muscles are seen. The fibrous anchorage of other muscles to the food groove walls is also seen. Figure 35. Horizontal section through the extreme posterior end of the trunk which has here flexed ventrally to form the post-abdomen. The mid-gut is therefore cut transversely. Note the detrital nature of its contents. The continuation of the dorsal longitudinal trunk muscles (DLM) into the post-abdomen is readily seen. All four bundles retain their integrity.



Figures 36–41. *Daphnia atkinsoni* f. *bolivari*. Aspects of the skeletomusculature. Figure 36. Horizontal slice, ventral, at a level just ventral to the floor of the food groove and therefore just dorsal to the ventral longitudinal muscles that, save for a trace anteriorly, are not seen. The section shows the carapace adductor muscles (ACAM and PCAM), the horizontal muscles (HM) and portions of the dorso-ventral muscles of the trunk. Elements of the transverse muscles of the mandibles can also be made out. The ventral nerve cords (NC) and the bulky ovaries (Ov) are seen at each side of the food groove. Embryos in the brood pouch, cut in various planes, can be seen at each side of the trunk. Figure 37. Horizontal slice showing the topographic relations of the ventral longitudinal muscles (VLM). Figure 38. Horizontal slice showing details of a ventral longitudinal muscle (VLM), how its bundles separate anteriorly, and the arrangement of the intersegments. The anchorage of the posterior carapace adductor muscle (PCAM) is also seen. Figure 39. Longitudinal section through a ventral longitudinal muscle. This also cuts the posterior carapace adductor (PCAM) and horizontal muscles (HM) transversely. Note how the latter tend to be enveloped by the ovaries. Traces of the extrinsic musculature of the anterior trunk limbs are seen. Figure 40. Longitudinal slice showing how the presence of the dorsal endoskeletal sheet (D Endo S) is easily located by the presence of fat cells (FC) associated with its ventral surface, and here sandwiched between the dorsal endoskeletal sheet and the mid-gut. Figure 41. Longitudinal section through a local thickening of the trunk cuticle (TTC) in the region where the dorsal longitudinal muscles originate. A trace of one of these is just seen, out of focus, to the left. The anteriorly located bracing muscle runs to the carapace at the anterior end of the brood pouch.



Figures 42–46. Trunk limbs 1–5, respectively of *Daphnia magna*, seen in median view (from Cannon 1933). Trunk limb 5, in fact, has three vertical seta (see figure 54).

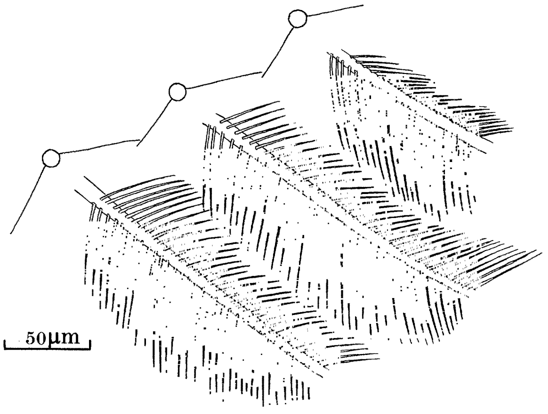
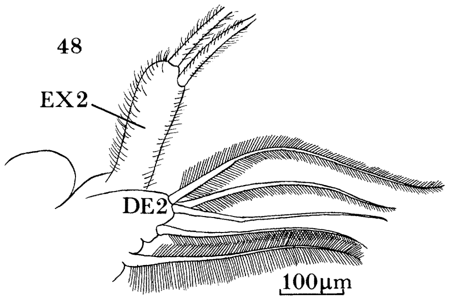


Figure 47. *Daphnia atkinsoni* f. *bolivari*. Funnel setae of trunk limb 1 viewed from the median side. The arrangement of these setae and setules as seen in transverse section is also indicated diagrammatically.



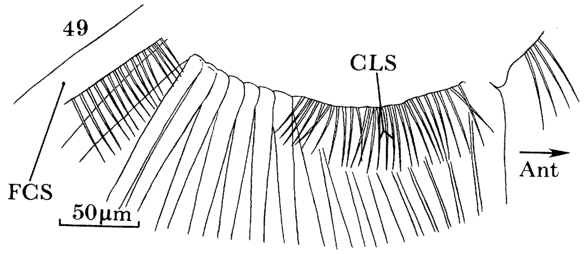


Figure 48. *D. magna*. Distal endites and exopodite (EX2) of trunk limb 2. The armature of the median spine of the distal endite is omitted. For details of this spine see figure 126.
Figure 49. *D. magna*. Arrangement of the basal portions of the gnathobasic spines and adjacent spinules (CLS) of trunk limb 2 as seen from the outer side. The spinules of the gnathobase lie *behind* the spines whose median face is seen in figure 3 and are therefore directed towards the filter plate of trunk limb 3, as are the long spinules of the long filter-cleaning spine (FCS).

not in many other species where, however, the arrangement of the armature is the same. Details of its armature in *D. magna* are given in §5(*o*) (i) (figure 126), where its function is described. Figure 48 also shows the exopodite of this limb which, in essence, is a much reduced version of that of the two succeeding limbs. Its outer margin is fringed with fine setules and its two long terminal setae are armed with a scattered array of soft setules that contrast with the stiff, slender setules of the endite setae that are arranged in regularly spaced uniseriate rows.

The gnathobase of trunk limb 2 is seen in figures 3 and 43. Its armature, which differs somewhat from species to species, has been described and shown by Lilljeborg (1900), Storch (1924) and Cannon (1933) and, with SEM photographs, by Watts & Petri (1981). Notwithstanding interspecific differences, the principles are similar in all. Posteriorly, and directed backwards, is a long spine, here called the filter-cleaning spine (FCS) that bears a uniseriate row of long, widely spaced spinules whose orientation is evident from the figures. These give it a comb- or rake-like appearance. By comparison with *Moina*, Cannon (1933) believed that it was not primitively part of the gnathobase but was derived from the second endite. Adjacent and anterior to it, and similarly directed, is a shorter spine, but nevertheless the second longest of the armature. Over its distal two thirds this is armed on its dorsal side with two rows of short, stout, blunt-tipped spinules. Some idea of the relationship of these two posterior spines to the filter plates of the third trunk limbs can be obtained from figure 3, but it should be noted that the relative positions of these structures change much during the regular beating of the limbs.

Anterior to these spines is a uniseriate row of spines (see §5 (*h*)) whose orientation is seen in figure 3. Apart from the anterior three, the rest, the median spines, are all similar in structure. There are 6 median spines in *D. longiremis* Sars, about 8 in *D. longispina* and its allies, about 10 or 11 in *D. pulex* and *D. obtusa*, about 12 in *D. atkinsoni* f. *bolivari* (figure 69), as many as 16 in *D. magna* and 18 in *D. nivalis* and *D. carinata*, but the number is somewhat variable, especially in species with most spines. Watts & Petri (1981) report between 12 and 16 in *D. magna*, and Benzie (1988*a*) between 9 and 18 in *D. carinata*. Irrespective of number, the general form and arrangement of these spines is always essentially the same, though there are small differences even within a series. Proximally they are straight. About half-way or rather more towards the tip, at a distinct junction, they begin to incline, and often to curve somewhat anteriorly, this being most evident in the more posterior members of the series. This position is clearly marked by an array of long spinules that radiates outwards in the manner of the ribs of an umbrella, as Watts & Petri (1981), who give SEM photographs of those of *D. magna*, so effectively describe. These are confined to the median side of the spine, so the two arrays face each other (figures 87 and 88). Only a few sparse setules are present in the proximal region of these spines but, distal to the umbrella-like spinules, they are profusely provided with setules. The way in which these are directed outwards, towards the adjacent filter setae of trunk limb 3, which they clean, is clearly revealed in SEM photographs (figures 87 and 88) of *D. atkinsoni* f. *bolivari*, which show this limb *in situ* overlying the filter setae of trunk limb 3. The orientation of the setules can also be seen in transverse sections of the setae (horizontal sections of the animal) in figures 73–75. As Watts & Petri (1981) have shown in *D. magna*, the tips of most of these spines (the three or four most posterior excepted) are drawn out into a tuft of brush-like setules. The same is true of other species (figures 87 and 88). Arising from the gnathobase near the middle of the median row, which it overlies when viewed medially, is a simple, elongate, tapering spine.

In view of recent discussions on the feeding mechanism of *Daphnia* it is necessary to emphasize that the spines of the median row are not filtering structures. Their form and armature are very different from those of filter setae and their function is not that of filtration. Like Storch (1924) before him, Cannon (1933) was aware of this and was explicit concerning their non-filtering nature, noting that 'they are armed with setules in such a way that they cannot act as filters' (p. 306).

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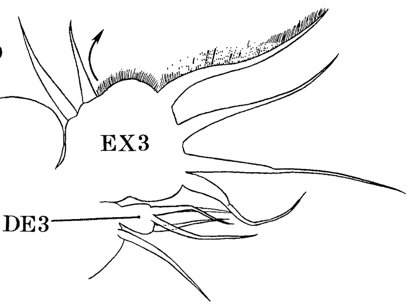

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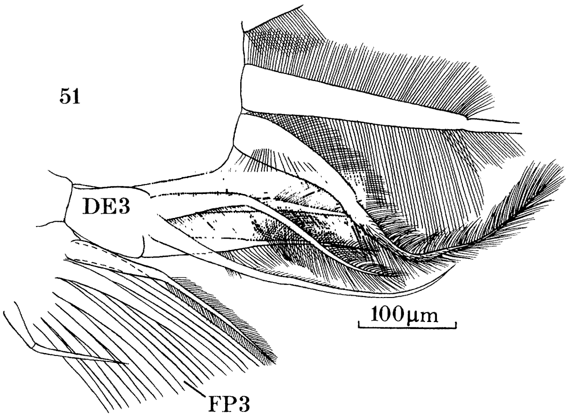


Figures 50 and 51. Aspects of the structure of trunk limb 3 in *Daphnia atkinsoni* f. *bolivari*. Figure 50. The distal part of the corm and the exopodite, here somewhat flattened. In life, the two lateral exopodite setae curve dorsally (into the plane of the paper) as indicated schematically by the arrow. An indication of the fringing armature of setules is given only for one region. All the exopodite setae are so provided. Some details are shown in figure 51. Figure 51. Details of some of the armature of the exopodite and of the flattened distal endite (DE3). Filtratory setules are omitted from the basal portions of the filter setae of the filter plate (FP3).

Irrespective of the number of spines in the median row, there are always three spines located anterior to them that are of a different kind. These are stout and their distal portion, which curves forward somewhat, is armed with numerous close-set, robust spinules whose proximity to the maxillules is evident in figure 3.

A feature not mentioned by previous investigators is the presence on the dorsal outer margin of the gnathobase adjacent to the bases of the gnathobasic spines, of a uniseriate row of long spinules (figure 49, CLS). These are confined to approximately the anterior half of the region occupied by the spine bases. Their orientation with respect to the filter setae of trunk limb 3 is seen in figure 110. Their role in cleaning these setae is noted in §5 (*k*).

The third trunk limb, effectively shown by Cannon (1933) (figure 44 here) is dominated by the enormous filter borne on its gnathobase. Cannon (1933) discussed the possibility that two more distal endites are incorporated at the tip of the gnathobase, but even if this is so, the structure is essentially gnathobasic in origin and is functionally a single unit. The way in which, distally, the filter setae arise in two rows was

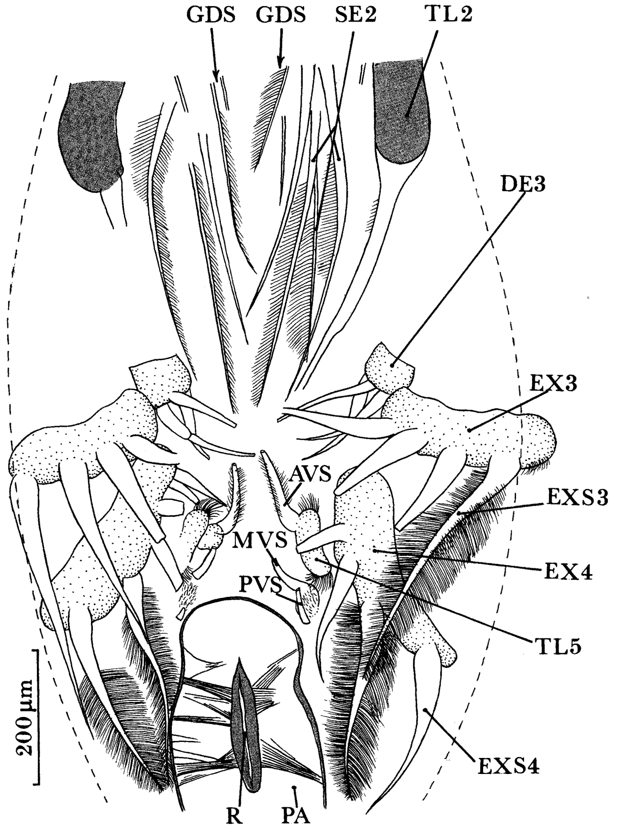


Figure 52. *Daphnia atkinsoni* f. *bolivari*. Horizontal slice, based on adjacent sections, at the level of the distal portion of the exopodites of trunk limbs 3 and 4, to show how their effective size is greatly increased by their armature of soft setae, each of which is provided with two uniseriate rows of densely arranged setules aligned in the same plane as the exopodite itself. The exopodites here lie in a partially remoted position. The relation of the exopodites to the distal extremity of trunk limb 5 and to the post-abdomen is also seen. The dashed lines indicate the cut edge of the carapace. Setules are shown for only a representative sample of the exopodite setae.

noted by Cannon and is shown in figures 3 and 51. Figure 3 shows only the setae, and not the setules, of the filter plate. For simplicity, it also shows the setae simply as tapering structures, though each is in fact emarginate. Details of the nature of the setae and their setules, as revealed by scanning electron microscopy, are given in §5 (*h*).

Along the length of the gnathobase, not far from the base of the row of filter setae, is a uniseriate row of spinules (figures 3 and 44).

The disto-lateral portion of the limb forms a conspicuous broad paddle (EX3), only part of which can be seen in figure 3. Basically flat, it is somewhat curved from side to side, with its convex face ventral. It is shown in outline, somewhat flattened, in figure 50. Considered in isolation its homology would be obscure. Cannon (1933) for example, thought it may represent three distal endites Comparisons with the Chydoridae and Macrothricidae, however, leave no doubt that it is the exopodite, as which it was indeed recognized by Storch (1924, 1925).

Little attention has been paid to the exopodites of the posterior limbs. Some details of exopodite 3 are given in figures 50 and 51 and parts of these structures are seen *in situ* in figures 52 and 53. Transverse sections reveal further details of structure and location (figures 112–114, 116 and 117). Not only is each exopodite a flattened paddle, but its effective area is greatly increased by the long setae with which it is provided. These are soft, pliable and of wide diameter proximally. Each is armed with long, soft setules that, in most cases, arise in a close-set row on each side of the seta, lie in the same plane as the exopodite, and link up with the setules of adjacent setae so as effectively to close the spaces between them (figures 51 and 52). On certain setae the setules, at least distally, arise in a bottle-brush-like array, as in the most median seta seen in figure 51. As in the similar setae of ***Eurycercus*** (Fryer

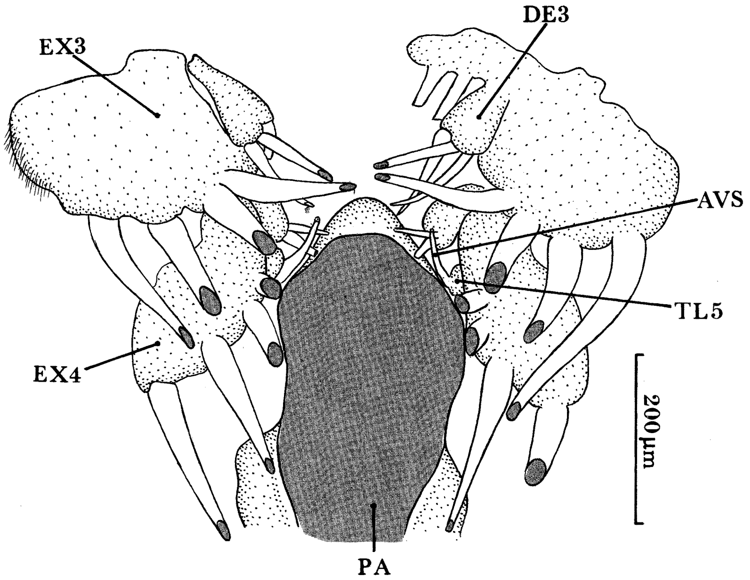


Figure 53. *Daphnia atkinsoni* f. *bolivari*. Horizontal slice showing the distal parts of the exopodites of trunk limbs 3 and 4 in a more remoted position than they occupy in figure 52. All setules of the exopodite setae omitted. From this figure and figure 52, it is easy to see how, during promotion, the expanding inter-limb spaces 3/4 and 4/5 are sealed ventrally by exopodites 3 and 4 and their setae so that suction through the filter plates inevitably ensues, and how, during remotion, water is squeezed from the interlimb spaces and driven posteriorly by exopodites 3 and 4.

1963) these setules appear to be 'sticky' and stain rapidly with alcian blue.

Although somewhat curved in life, each exopodite (EX3) lies more or less in the horizontal plane (figures 52 and 53) with its four distal setae directed posteriorly. Of the two lateral setae, the proximal curves dorsally, lies adjacent to the carapace wall and helps to seal the gap between it and the corm of the limb, while the distal extends more posteriorly (figures 112, 113, 119, EXLS3). Between them and the outermost of the distal setae, the margin of the exopodite is provided with a fringe of long setules similar to those of the setae, so that there is a seal between the exopodite and carapace wall irrespective of the position in the cycle of movement of the former.

Medially the distal endite takes the form of a small lobe that bears four setae, whose form and armature are seen in figures 50 and 51. It extends the seal of the exopodite medially (figures 52 and 53) and ensures that interlimb space 3/4 is sealed in that region during promotion of the limb. Adjacent to this lobe are two setose setae, borne at the extreme distal end of the gnathobase. Only one of these is seen in figures 50 and 51: the other, almost completely hidden by it, is omitted.

As well as a bulbous epipodite, the lateral side of the corm of the limb bears proximally a thin lobe, perhaps most easily seen in the dissected limb (e.g. see figure 1, plate 11, in Lilljeborg (1900)), but well shown in a more life-like position by Cannon (1933), whose figure is reproduced here as figure 44. Lilljeborg succinctly described it as 'einen grossen, sehr dunnen, am Ende abgerundeten und in der Rändern gefiederten Zipfel'. Although easily overlooked, this lobe plays an important part in rendering watertight the anterior wall of interlimb space 3/4.

The fourth limb (figure 45) is also dominated by a filter-bearing gnathobase, similar to, but shorter than, that of the third limb. Adjacent to the base of the filter is a band of fine spinules, not a single row as on the third limb, but a band. These are seen in, for example, figures 65, 76, 77, CLS, and 112 of *D. atkinsoni* f. *bolivari*. Details, as revealed by SEM, are shown in figures 102 and 103. Here they are smooth. As shown by Watts & Petri (1981, figure 4*d*), their homologues in *D. magna* have minute side spinules.

This limb too has a large, paddle-like exopodite (figures 52, 53, EX4), whose area is effectively increased by four wide-diameter setae (EXS4), each of which is fringed on each side by long soft setules (some of which, in *D. carinata*, are seen in figure 4, in Fryer (1987)). It lies in a similar plane to that of trunk limb 3, by which it is partially overlain anteriorly (figures 52 and 53). As on the third limb, there are two lateral setae with similar roles (see figure 114, EXLS4). The corm bears proximally a lateral lobe.

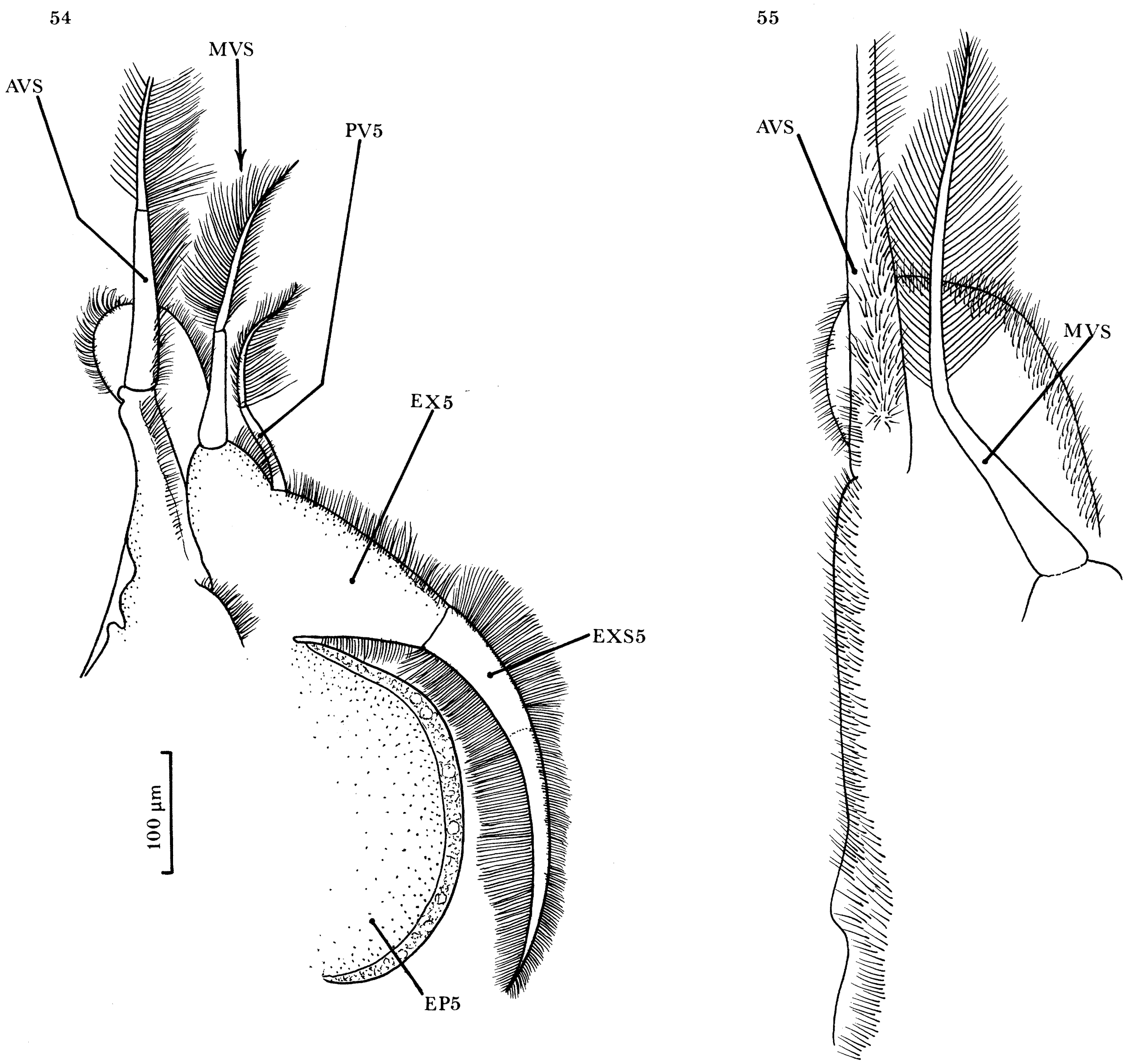
The similarity of exopodites 3 and 4 and their setae to those of the posterior limbs of such a chydorid as *Eurycercus*, where limb 5 also has a large paddle, is self-evident (see Fryer 1963, figures 35, 38 and 39). In *Eurycercus*, however, the exopodites lie more or less in the vertical plane: in *Daphnia*, they lie more nearly horizontal.

The fifth trunk limb (figures 3, 46, 54 and 55) is also most readily interpreted by comparison with that of such a chydorid as *Eurycercus*. (Its arrangement is best appreciated by consideration of sections.) Like Behning (1912) before him, Storch (1924) attempted to homologize the parts of this limb with those of the fourth. In this he was largely successful but comparisons with *Eurycercus* reveal certain discrepancies. In *Eurycercus*, a primitive chydorid, it displays considerable similarity to the fourth limb, having a filter plate and a large, flap-like exopodite that forms part of the pumping mechanism (Fryer 1963, figure 38). In *Daphnia*, it has lost the filter plate and, while still an important part of the pump, no longer helps to produce the current. It has become specialized as a valve that is closed during the time that water is being sucked through the filters, and opened to allow it to be expelled from the posterior interlimb chamber (§5 (*k*)). It also serves to clean some of the posterior filter setae of trunk limb 4.

As in *Eurycercus*, and indeed in other chydorids and in many macrothricids, there is a vertical-standing corm that bears a long, also vertically standing, seta, here called the anterior vertical seta (AVS) (figure 54). Storch (1924) attempted to homologize this seta with those of the filter plate of the preceding limb. This would have been dubious even had comparisons with other species not been possible. Comparison with *Eurycercus* reveals its true homology. The appearance of the corm *in situ* as seen in a transverse slice is seen in figures 114–117. The exopodite has been greatly reduced from the large flap-like paddle present in *Eurycercus*. Ventrally, where Cannon (1933) shows only a single seta, it bears two vertically directed, softly setose setae, here called the median (MVS) and posterior (PVS) vertical setae. With the seta of the corm these make up a closely associated trio. The dorsal part of the exopodite and its large scimitar-like seta (EXS 5) make up a single functional unit fringed on both sides with long, soft, sealing setules. As Storch was the first to appreciate, this unit is so shaped that, when swung forward during the appropriate phase of a cycle of movement (§5 (*k*)), its outer margin fits neatly against the carapace wall, to whose curvature its own is matched, and the inner corresponds to the shape of the adjacent epipodite, thus effectively sealing the posterior limits of interlimb chamber 4/5.

The corm and its seta are armed with long cleaning setules (figure 55), whose presence and function have not been reported. These clean the posteriormost filter setae of trunk limb 4.

To appreciate the nature of the filter chamber, the relations of the trunk limbs to each other and to the carapace and post-abdomen, and the manner in which the trunk limbs operate, it is necessary to consider the trunk limb complex in the horizontal, as well as in the longitudinal and transverse planes. Only Storch (1924) has considered trunk limb arrangement as revealed by horizontal sections, but little attention has been paid to his findings, probably because the subsequent results presented by Cannon (1933) have been thought, at least by English-speaking readers, to supersede them. Storch's illustrations, while only outlines, are helpful in

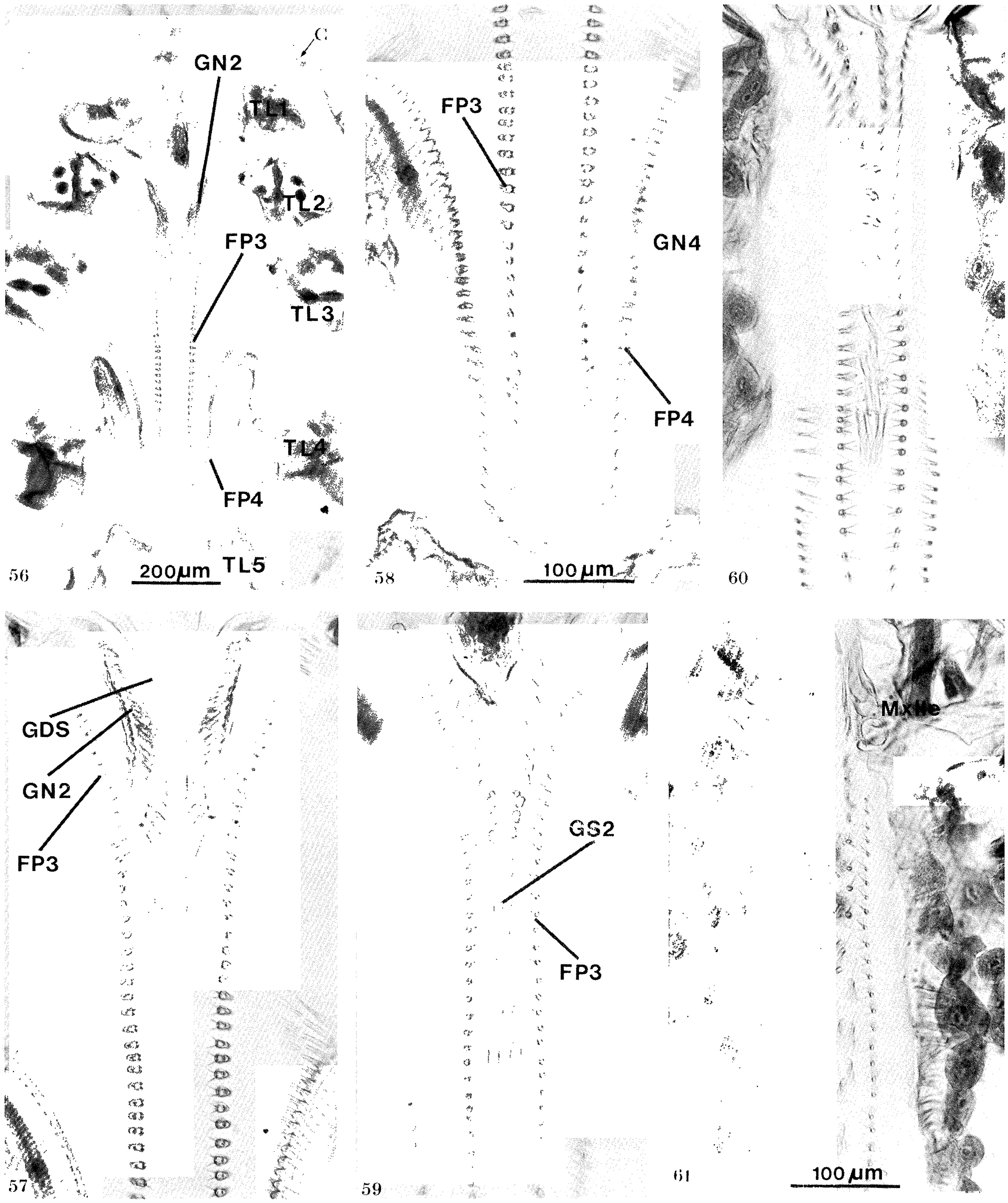


Figures 54 and 55. Trunk limb 5, *Daphnia atkinsoni* f. *bolivari*. Figure 54. The median–posterior face. It is the highly setose exopodite seta (EXS5), whose curvature matches that of the adjacent carapace, that plays a major role in sealing the carapace chamber posteriorly during the filtration phase of feeding. Figure 55. Part of the limb, twisted to reveal some of the setules of the corm and anterior vertical seta.

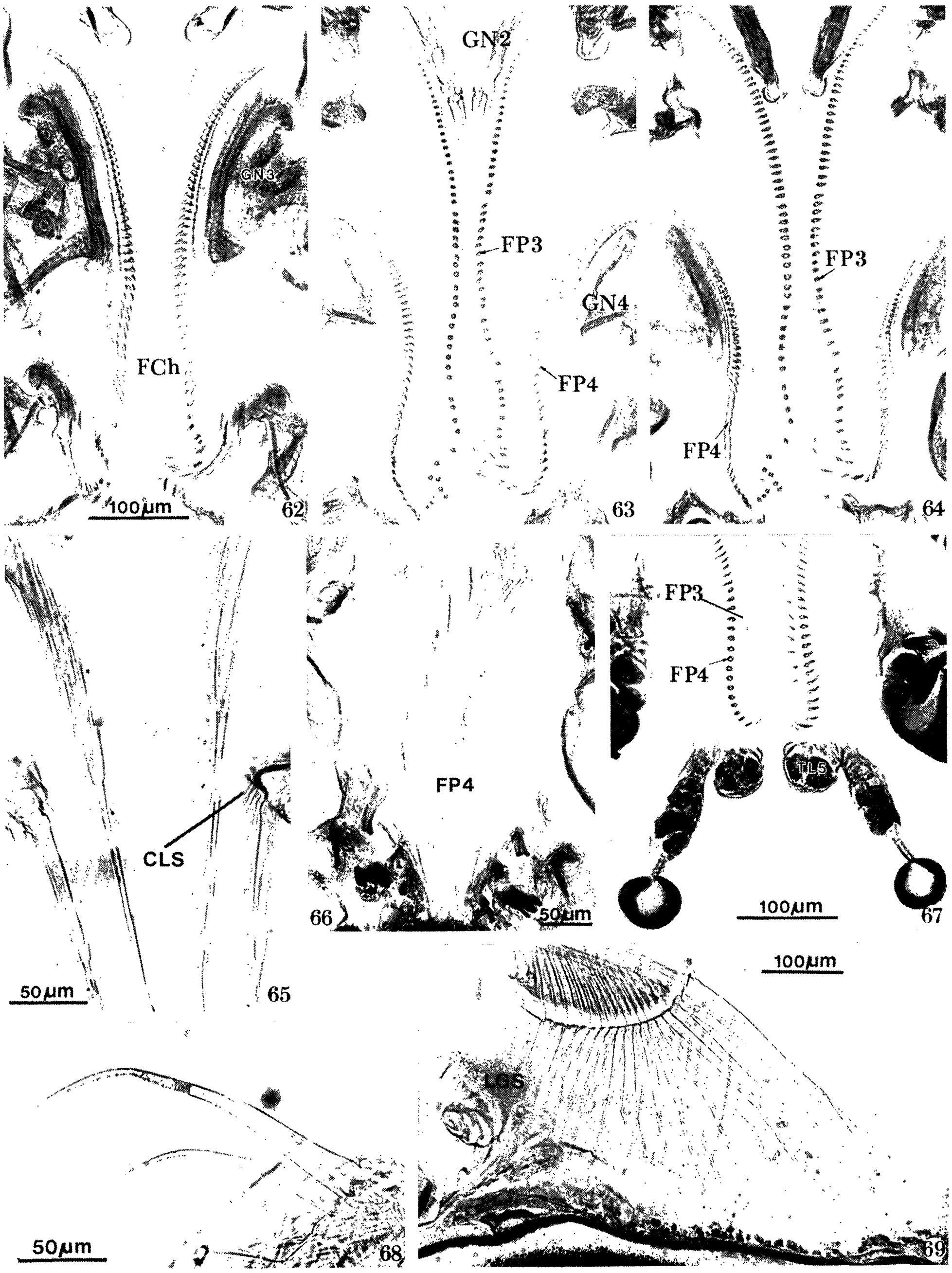
revealing the general location of the filter chamber but are erroneous in one respect. According to his account, sections cutting through the distal portions of the trunk limbs show these to be arranged in sequence, one pair behind the other from front to back, which is true (figure 56). Where they arise from the trunk, however, he says that, anteriorly, the appendages lie so close together that the insertion of the first limb has been pushed laterally and that this limb arises to the outside of trunk limb 2. Such an arrangement is shown in his sketch. This is incorrect. Trunk limb 1 arises anterior to trunk limb 2 and not lateral to it, as can easily be seen by simple inspection of an intact *Daphnia* and as is evident from figure 56. This region can be confusing in sections and Storch clearly misinterpreted limb boundaries. It is curious that he made this mistake. Such an arrangement would greatly interfere with the movements of trunk limb 1 and probably trunk limb 2 also.

Notwithstanding recent contrary claims, trunk limbs 3 and 4 are components of a filter chamber. This name would be inappropriate were the filters of limbs 3 and 4 to function, as some have suggested, not as such but as paddles. As described in §5(*k*), filtration is carried out by the grids of setule-bearing setae borne by these limbs and the term filter chamber is therefore employed as an accurate description, based on function, of one component of the trunk limb complex.

Figure 3 shows the relation of the filter plates of trunk limbs 3 and 4 to each other and how that of trunk limb 3 overlies the filter-plate-bearing gnathobase of trunk limb 4. A horizontal slice (figure 56), which cuts through the dorsal part of the gnathobase of trunk limb 2, shows not only the positions of the corms of the various limbs but how the filters of the third trunk limbs (FP3) dominate the median space between the limbs. That two different species are concerned is



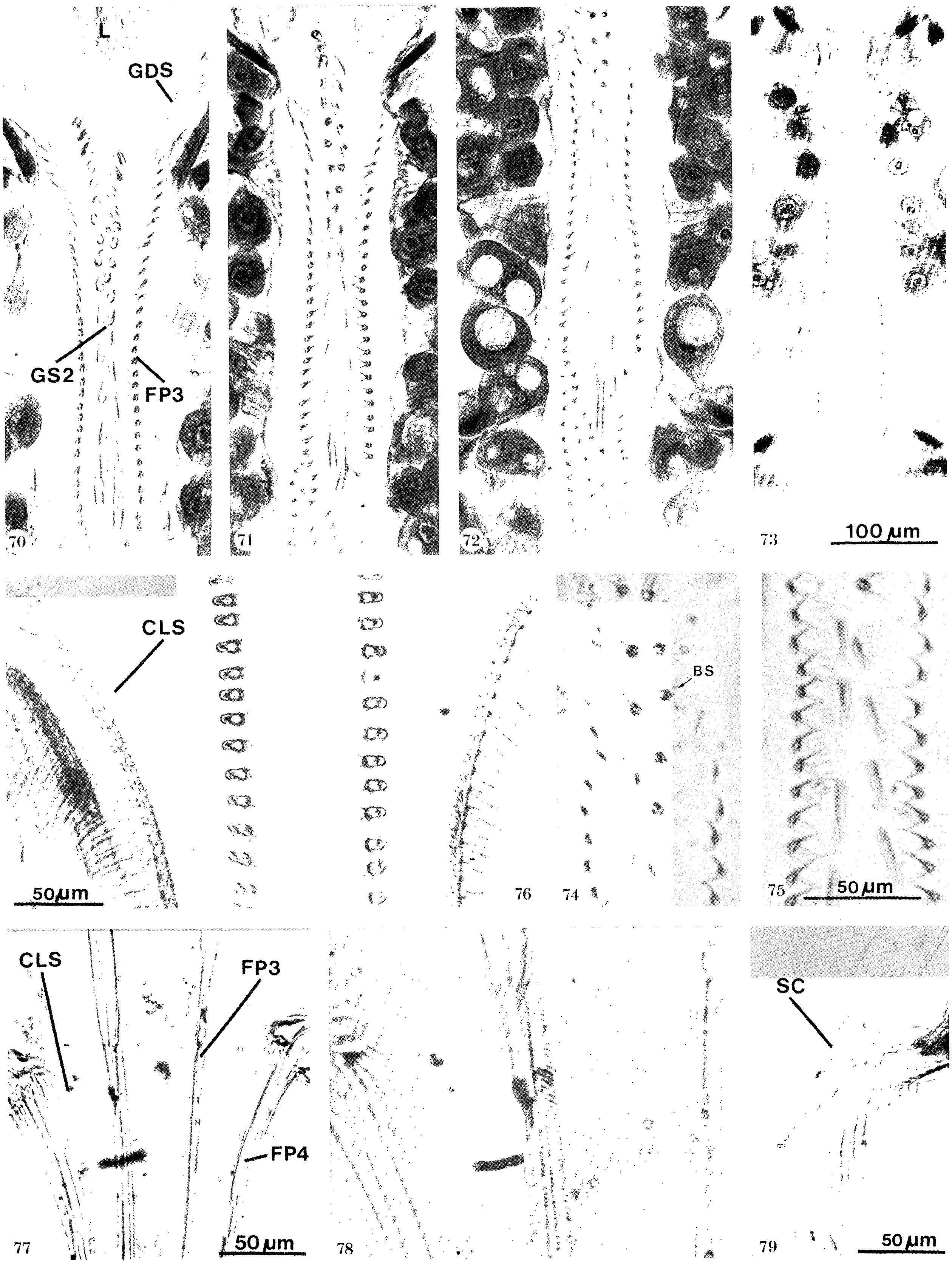
Figures 56–61. *Daphnia atkinsoni* f. *bolivari*. Figure 56. Horizontal slice through the trunk limbs (TL1–5) showing the location and composition of the filter chamber. Ventral. Figure 57. The same, showing the anterior ends of the filters of trunk limb 3 (FP3) with the gnathobases of trunk limb 2 (GN2) between them anteriorly, and one of the guide setae (GDS) of trunk limb 1 anterior to that. Parts of the gnathobases of trunk limb 4 are seen posteriorly. Figure 58. The same, showing the posterior ends of the filters of trunk limb 3 (FP3) and, exterior to them, those of trunk limb 4 (FP4). Note how the latter curve medially at their posterior extremities to fence off the filter chamber posteriorly immediately in front of limb 5. Figure 59. The same, more dorsally at the anterior end of the filter chamber, cutting the filters of trunk limb 3 (FP3) and the gnathobasic setae of trunk limb 2 (GS2). Figure 60. The same, more dorsally. Figure 61. The same, more dorsally. The food-handling setae are here seen in the food groove and the section cuts near the tips of the gnathobasic setae of trunk limb 2 between which are seen particles of food. The maxillules (Mxlle) are also seen in section.



Figures 62–69. Elements of trunk limb arrangement and armature. Figures 62–64. *Daphnia pulex.* Progressively more dorsal horizontal slices through the filter chamber (FCh) (viewed ventrally) to show its essentially similar composition to that of *D. atkinsoni* f. *bolivari* and other species. Figure 65. *D. atkinsoni* f. *bolivari.* Transverse section through the filter plates of trunk limbs 3 and 4. Note the cleaning setules (CLS) borne on the gnathobase of trunk limb 4. Figure 66. *D. pulex.* Transverse section at the posterior end of the filter chamber, showing how the filter setae of trunk limb 4 curve round and seal its posterior end. Figure 67. *Simocephalus vetulus.* Horizontal slice encompassing the posterior end of the filter chamber and showing the relation of the posterior fence of filter setae of the fourth pair of trunk limbs (FP4) to the adjacent fifth pair. Note the similarity of the arrangement to that of *Daphnia.* Figure 68. *D. atkinsoni* f. *bolivari.* Ejector hooks of the first trunk limb. Figure 69. *D. atkinsoni* f. *bolivari.* Longitudinal section showing the gnathobase of trunk limb 2 *in situ.* Some of the more anterior setae have been severed. Note that none of the elements of the armature of this gnathobase are filter setae. Coagulated and stained secretions of the labral glands (LGS) appear as a dark smear anteriorly.

irrelevant: the arrangement is essentially the same in all, as shown by comparison with *D. pulex* (figures 62–64), and as indeed it is in all daphniids, e.g. *Simocephalus vetulus* (figure 67).

In the transverse plane the filter setae, particularly of trunk limb 3 but to some extent also of limb 4, are for the most part gently curved, the convex face of the curve facing that of the setae on the opposing limb. More distally, however, they are often almost straight (figures 65, 110–113). The distal portions of the filters of the third pair of trunk limbs lie not very far apart, there being only a narrow gap between them (e.g. figures 59–61, 112). The width of this gap varies somewhat during a cycle of movement, but is never



Figures 70–78. *Daphnia atkinsoni* f. *bolivari*. Figure 79, *D. obtusa*. Figure 70. Horizontal section (ventral) slicing the proximal region of the gnathobasic setae of the second trunk limbs (GS2) and the adjacent filter setae of the gnathobases of the third trunk limbs (FP3). The tip of the labrum (L) is just visible as a landmark anteriorly and some of the guide setae (GDS) of the first pair of trunk limbs are also seen. Figure 71. The same, more dorsal, that is deeper into the food groove. Figure 72. The same, more dorsal. Note the dense aggregation of fat cells at each side of the food groove. Figure 73. The same, more dorsal, near the level of the tips of the anteriormost gnathobasic setae of the second trunk limb. The filter setae of the third trunk limb are cut progressively nearer their tips towards the anterior end of the region shown and do not extend into the anterior extremity of the food groove. The maxillules

wide. Thus the filter plates of these limbs make up a cage, elongate along the anterior–posterior axis of the body, and funnel-like in transverse section, the mouth of the funnel being morphologically ventral. As is easy to see in figures 56 and 58, the filters of the fourth pair of trunk limbs (FP4) curve towards the mid line at their posterior end and close the filter chamber posteriorly. The way these posterior setae fence off the chamber can be seen in transverse sections of *D. pulex* (figure 66) and *D. atkinsoni* f. *bolivari* (figure 114). Other species are the same. Behind this posterior palisade is the vertically standing corm of trunk limb 5 (figures 114–116). The walls of the cage are made up of uniseriate rows of close-set setae with consistently uniform gaps between adjacent setae. These gaps are spanned by regularly arranged filtering setules, whose nature and arrangement are discussed in §5(*h*) and which, like the gaps between the setae, can be seen in transverse section in figure 76.

Because of movements of limbs 3 and 4, the size of the cage is not constant. Figure 3 shows it at more or less its maximum size. As is best appreciated from a transverse section, such as figure 112, the volume of the filter chamber makes up a relatively small, but variable, proportion of that of the carapace chamber. The filters of the third limbs arch over those of the fourth and, at certain phases of a cycle of movement, pass between them much as the blade of a penknife passes into its protective case. This is evident from several of the illustrations. These movements are dealt with in §5(*k*).

The filter chamber is separated from the brood chamber by the horizontal lamella on each side (§5(*a*)). In collaboration with trunk limb 5, the corms of limbs 3 and 4, their thin lateral lobes, and their exopodites, make up the moving parts of a pump that draws water into the interlimb spaces that develop between them during promotion. This water can enter by only one route; through the filters of limbs 3 and 4. On remotion, limbs 3 and 4 come together and drive out this water posteriorly (§5(*k*)).

(*h*) *Trunk limb armature and the nature of the filter plates*

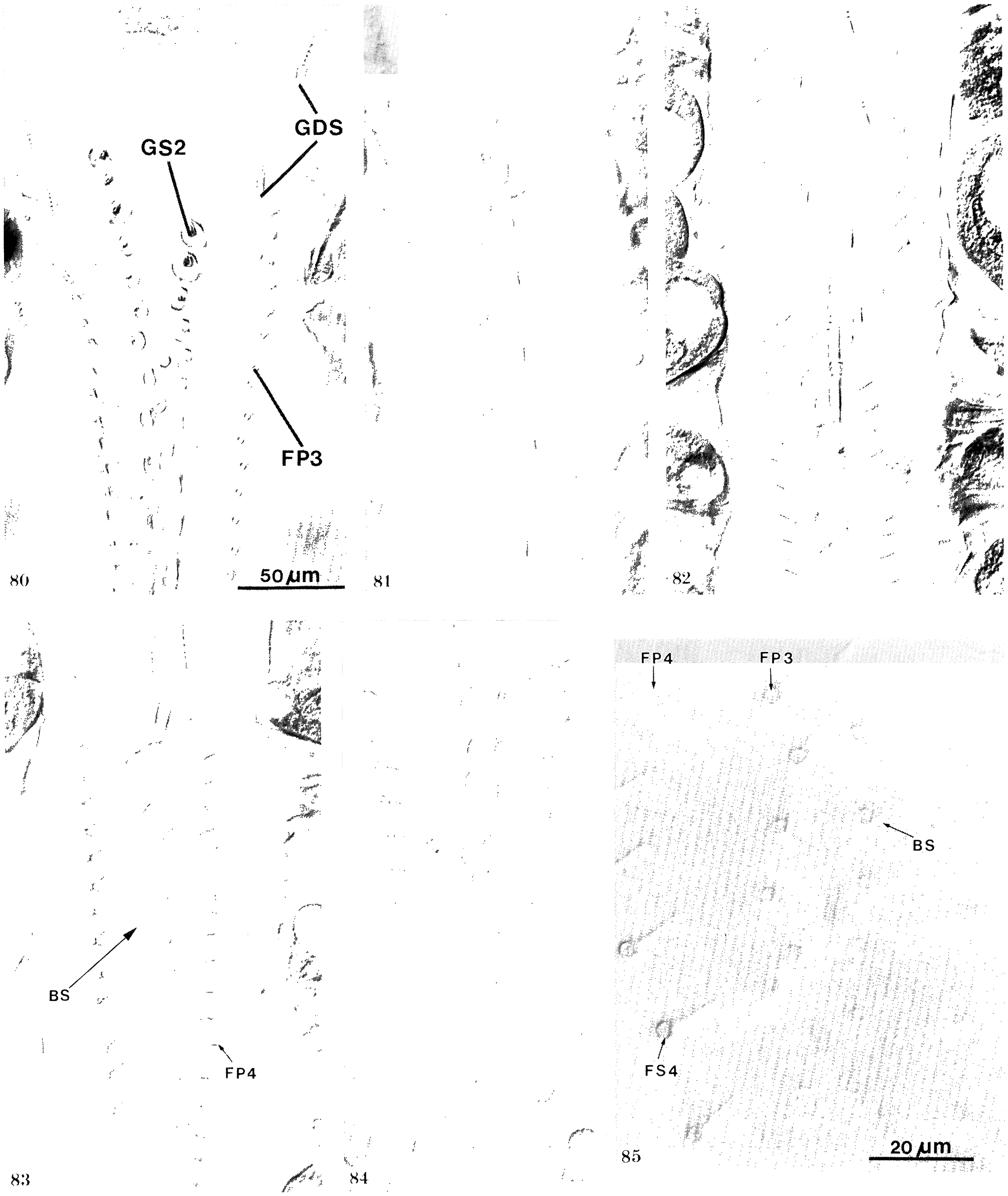
The availability of scanning electron microscopy has led to the publication of various photographs of trunk limb setae of *Daphnia*, especially the filter setae. Some of these are excellent and informative; others extremely bad. SEM photographs seldom show the appendages *in situ*, so the often intimate relation of the setae and setules of one appendage to those of another cannot be seen. When they do, they sometimes reveal gross distortion and show setae twisted into positions they could never occupy in life.

The following details, presented largely pictorially, are based mostly on SEM studies on Palestinian material of *D. atkinsoni* f. *bolivari*, the species much used for other morphological aspects of this investigation. No such details have hitherto been presented for this species. The animals used were *ca.* 4 mm in length, exclusive of the posterior carapace spine. For convenience, the SEM photographs of aspects of the armature of trunk limbs 1 and 2 are included in this section, though the descriptions have been presented in §5(*g*).

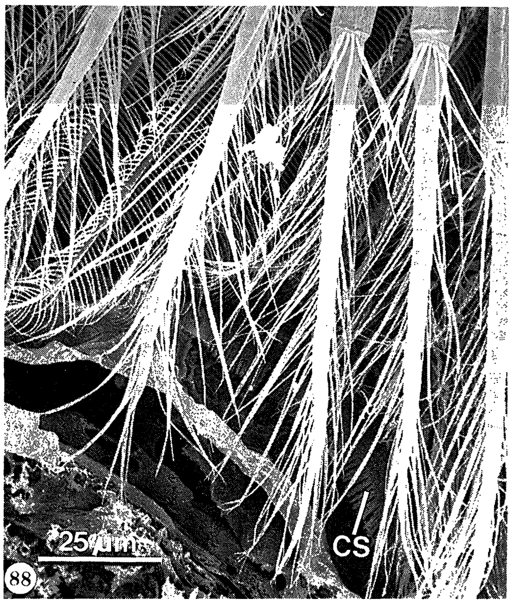
Of reports on the fine structure of the filter plates of trunk limbs 3 and 4, those of Brendelberger (1985) and Watts & Petri (1981) are particularly noteworthy. Not all investigators have appreciated that the arrangement is not uniform throughout. For example, Ganf & Shiel (1985*b*) say that the setal combs are of 'uniform morphology' in limbs 3 and 4. In fact, while a uniform pattern prevails over large areas, not only does the arrangement of the setules differ considerably in different parts of a filter seta, but it is not identical in all setae and, in some species at least, the arrangement of the filters, and the mesh size of the major filtering regions, are not the same in limbs 3 and 4. These facts have important consequences and reveal the proneness to error of calculations of Reynolds number, boundary layers and related matters, errors that are sometimes compounded by other false assumptions and by simplifications (§5(*l*)).

Neither Brendelberger (1985) nor Watts & Petri (1981) specify from which limb the meshwork arrangement that they illustrate was obtained. So far as the principles involved are concerned, this is of no consequence, but it obscures the important point that in some species, such as *D. atkinsoni* f. *bolivari*, the meshwork of trunk limb 3 is coarser than that of limb 4. Brendelberger notes the importance of ascertaining the mesh size of both limbs and comments that meshes are not everywhere the same in one animal, as indeed Korínek & Machácek (1980) and Geller & Müller

are seen in section at the anterior limits of the food groove. Note the thickened cuticle of the food groove walls. Figures 74 and 75. As figure 72, more highly magnified, to show the brush-like armature (BS) of the distal portions of the median row of gnathobasic setae of the second trunk limbs and its orientation. The regions shown follow each other in the two figures, with a little overlap to aid location. Note how the soft, brush-like setules are directed outwards towards the filter setae of the adjacent trunk limb 3, which they sweep and clean. There are also spinules on the food groove wall that assist in cleaning the tips of the filter setae from the outside. The nature and location of these is most clearly revealed by SEM (see figure 101). Figure 76. Horizontal slice, ventral, through some of the filter setae of the third trunk limbs to show their arrangement and that of their filtering setules, and the array of fine setules (CLS) on the adjoining gnathobases of the fourth trunk limbs that scour filtered particles from them. Figure 77. Transverse section through the filter plates of the third (FP3) and fourth trunk limbs (FP4) to show their topographic relations and the array of fine setules (CLS) borne on the gnathobases of the fourth trunk limbs that scour filtered particles from the filter setae of the third trunk limbs. Immediately before fixation, the animal had been removing minute particles from suspension and these appear as a haze in the filter chamber between the opposed filters of the third trunk limbs, whose filtering efficiency is clearly revealed. Figure 78. The same, more highly magnified. Figure 79. *D. obtusa*. Part of the scraper (SC) of trunk limb 2. The proximal portions of some of the filter setae of trunk limb 3 are also seen.



Figures 80–85. *Daphnia atkinsoni* f. *bolivari*. Details of the filter setae of trunk limbs 3 and 4 and of the gnathobasic armature trunk limb 2 as seen in transverse section (horizontal section of the animal) and revealed by Nomarski optics. Ventral. Figure 80. Gnathobasic spines of trunk limb 2 (GS2) and anterior filter setae of the filter plate (FP3) of trunk limb 3. Parts of the guide setae of trunk limb 1 (GDS) are also seen. Figures 81–84. The same, progressively more posteriorly and at different levels towards the distal ends of the filter setae. The filter plates of trunk limb 4 appear in figure 82. Note in figure 83, and particularly 84, which are, respectively near the extreme tips and very close to the tips, of the filter setae of trunk limb 3, the brush-like setules (BS) to the *outside* of the filter setae of trunk limb 3 that help to clean material from the filters of trunk limb 4. In figure 83, the distal extremities of some of the filter setae of trunk limb 3 have been displaced during fixation by the long, posteriorly directed cleaning spines of the gnathobases of the second trunk limbs, but the arrangement is otherwise perfectly clear. Figure 85. As figure 84, but more highly magnified (oil immersion objective) to show the brush-like setules of the distal extremities of trunk limb 3.

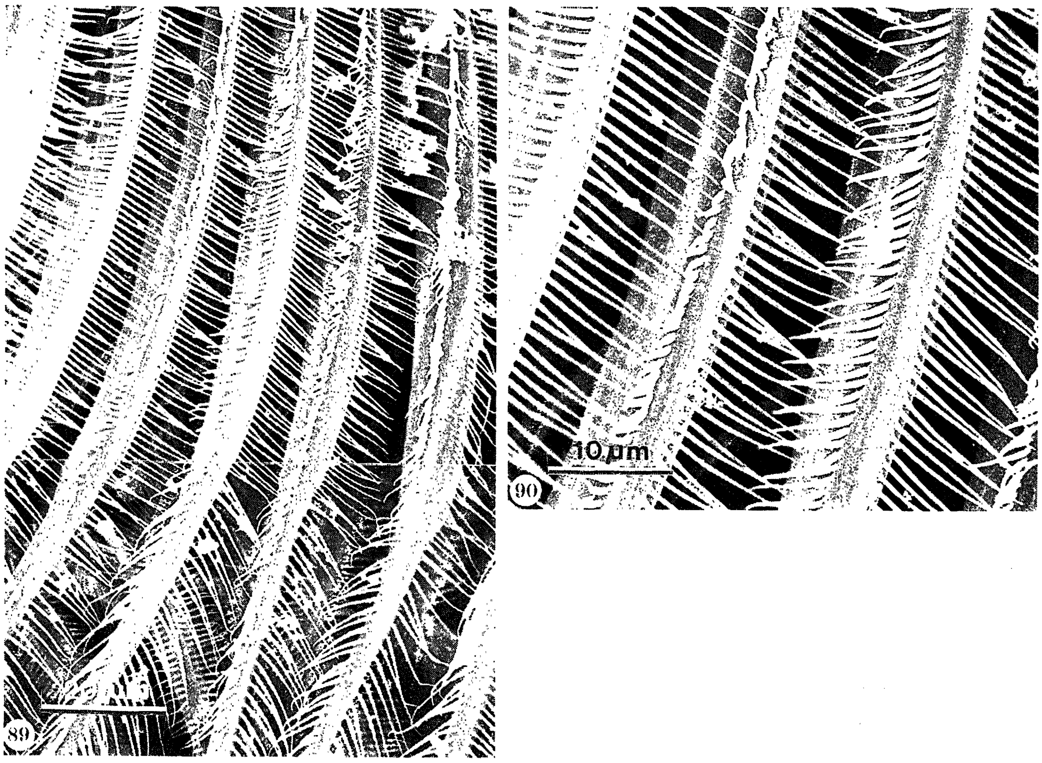


Figures 86–88. *Daphnia atkinsoni* f. *bolivari*. Figure 86. Guide setae of trunk limb 1. Figure 87. Gnathobasic spines of the median series of trunk limb 2 *in situ*. Right hand side of photograph is anterior. Note the brush-like nature of these spines and the way that their setules are directed laterally so as to be able to sweep material from the more laterally located (underlying) filter setae of trunk limb 3, which are clearly seen behind them. The median spine, here appearing white, serves as a marker to identify the sequence of gnathobasic sweeping spines. Note the umbrella stay-like array of spinules at the base of the armed distal region of the sweeping spines (cf. figures 3 and 111). Figure 88. The same, more highly magnified. The brush-like tips of the gnathobasic spines are clearly shown, as are the filter setae of trunk limb 3. Note the row of cleaning setules (CS) on the food groove wall that can just be seen near the tip of the sweeping spines.

(1981) had already observed in *Daphnia pulicaria* Forbes and in several other species, respectively. However, while he expresses the dimensions of the filtering meshwork as a cumulative frequency, he evidently pooled measurements for both limbs and does not comment on any difference between them. In *D. atkinsoni* f. *bolivari* the difference is striking, as can be seen, for example, by comparing figures 89 and 102.

As the dimensions of the setules differ in different regions of the setae, and as intersetular distances not only differ according to the size of the animal, but do so in different populations according to the concentration of available food particles (Korínek & Machácek 1980; Brendelberger & Geller 1985; Korínek *et al.* 1986), few measurements are cited here for *D. atkinsoni* f. *bolivari* other than those that can be obtained from the illustrations. To give some perspective, however, it may be noted that while the setules of this species are more robust than in some smaller planktonic species, their diameter, even near the base, as in figures 95 and 96 which are based on an original magnification of ×40000, is only about 0.5 µm.

The arrangement of the filter setae and their setules in the mid proximal region of the filter plate of trunk limb 3 is shown in the upper part of figure 89, details being shown at a greater magnification in figure 90. The arrangement of the filtering setules is here very uniform. However, as the distal region of the setae is approached, there is a marked change in both their nature and arrangement, as is clearly seen in the lower part of the photograph. Here the setules, essentially straight more proximally, become curved, and are noticeably more robust, longer and more widely spaced. As the tips of the setae are approached, even greater changes take place, but consideration of these is best deferred until other attributes of the filter plate have been considered. The meshwork shown in figure 90 is regular (where setules have been deflected out of line, their real position is obvious) and it is almost certain that in life, the tip of each setule makes contact with that of a setule on the adjacent seta, as first



Figures 89 and 90. *Daphnia atkinsoni* f. *bolivari*. Figure 89. Part of the filter plate of trunk limb 3. Note how the armature of the filter setae changes towards their distal ends. The setules become more robust, larger, more widely spaced, more curved, and independent of their fellows on adjacent setae. A filter seta of trunk limb 4 can just be seen beneath the filter plate in the lower part of the photograph. Figure 90. Details of the filter setae of trunk limb 3, proximal region.

described for *D. magna* by Watts & Petri (1981), to establish a hook-like linkage. Such is detectable in the photographs by Brendel-Berger (1985) and the same author in Lampert (1987). (See also limb 4 below.) Contact between the tips of setules of adjacent filter setae can indeed be seen by light microscopy in well-fixed sectioned material, where the regular arrangement of rows of many consecutive setae makes it certain that they are not disturbed and where the regularity of the arrangement of setules confirms the fact. This is seen, for example, in figure 71, and can be deduced from figure 76, where the exceedingly fine tips of the setules are generally not visible. Confirmation is provided by observation using Nomarski optics, as in the right-hand row of setae (left side of animal) in figure 81.

Figure 90 shows the regular arrangement of setules in the region portrayed. That the setules of one side appear thicker than those of the other is an artefact imposed by the limitations of SEM. It is the whitish, thicker and shorter setules that are distorted. The rows of setules arise on either side of a ridge that runs along the median side of each seta (figure 94). From this, each setule is directed obliquely medially, an acute angle being subtended between them (see below).

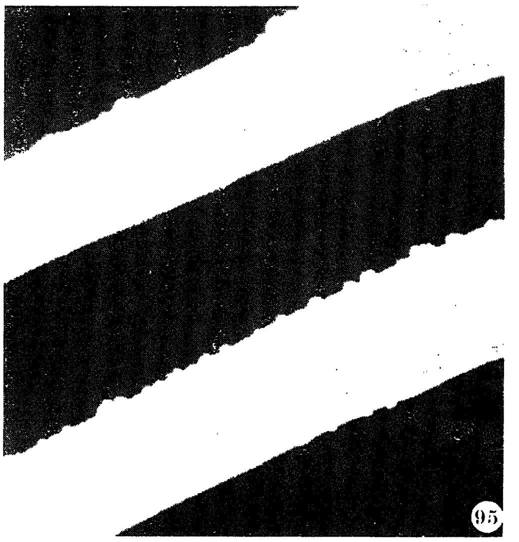
The way in which setules are inserted on a seta are shown in figures 94 and 96. The spaces between the setules in this region (which determine the mesh size of the filter) are regular but, as the setules taper gradually towards their tips, the gaps inevitably become wider there, so it is not possible to give a precise measurement of mesh size, though this has been attempted for several species by others. The gaps are, however, about 0.6 µm wide not far from the base of the setules, but this dimension, which is broadly in line with those given for other species (see Lampert (1987) for a list from various sources) is an inadequate basis for calculations such as have been made by various workers. The validity of some of the measurements given with such confidence in the literature is indeed sometimes questionable, quite apart from the problem of widening gaps (see Fryer 1987*b*). Even the best work can be confusing in this respect. Thus the dimensions of the filter of *D. magna* shown by the scale on the excellent photograph of Brendelberger (1985) differ greatly from those cited in the text, a discrepancy that is repeated without question by Lampert (1987).

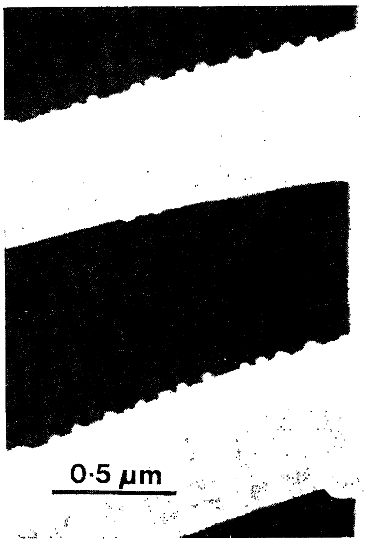
That the rows of setules of a filter seta are arranged at an acute angle to each other was well known to both Cannon and Storch. That they never mentioned that this increased the apparent area of the filter was probably because they regarded this as self-evident. However, some of their less well-informed successors, intent on quantifying a process which they did not understand, have taken the superficial area of a filter plate to represent the area of the filter, an error pointed out by Brendelberger & Geller (1985). The area of the filtering surface is much greater than the superficial area of the filtering portion of the filter plate but, because the arrangement differs in different parts of the filter, it is exceedingly difficult to obtain precise figures. In *D. atkinsoni* f. *bolivari*, in that part of the filter plate of trunk limb 3 seen in section in figure 76, the angle between the rows of setules is about 50°. Their

Figures 91–94. *Daphnia atkinsoni* f. *bolivari*. Figure 91. Setae of the filter plate of trunk limb 3, approaching their distal ends. Filter setae of trunk limb 4 can just be seen behind them. Figure 92. Details of a single seta. Figure 93. A single seta. Although the setules have been dislodged and somewhat distorted in fixation, the way they interlock with those of the adjoining seta can be appreciated. Figure 94. Details of a seta at high magnification, showing how the setules insert on it.

length is such that the filtering area in this region is at least 2.6 times as great as would be the case if they merely spanned the gap between adjacent setae. More distally, at the level seen in figure 70, the rows of setules are set at a more acute angle to each other, usually about 35°, and are so long that here the filtering area is about 4.7 times as great as the superficial area. Still more distally (figure 71) they are less acute, again about 50°, and here the filtering area is about three times that of the superficial area. That the actual filtering area is larger than the apparent area is of great significance in relation to the energy required to draw a given volume of water through the filter in a set period of time.

On the more posterior setae of trunk limb 3 the setules take on a slightly different arrangement, as can be seen in figures 91–94. The posteriormost of the long setae of the filter plate of trunk limb 3 (figure 97) is not a filter seta. Its setules are much longer and more widely spaced than those of the adjoining filter setae with which they can be compared in the photograph. Its role is probably to retain coarse particles that drift to the posterior end of the filter chamber. Modifications of its homologues are frequent in the Chydoridae. Sometimes, as in *Alonopsis elongata* Sars, the modification is no more than a lengthening of the proximal setules (Fryer 1968, figure 7); in *Peracantha truncata* (O. F. Müller) the seta is more coarsely setose than its companions, which are all filter setae, and is probably non-filtratory (Fryer 1968, figure 47); and the modification is dramatic in *Disparalona rostrata* (Koch) where this seta forms a conspicuous brush (Fryer 1968, figures 60 and 61). Modifications more conspicuous than those of *A. elongata* but less striking than those of *D. rostrata* have been observed in several other chydorids by Dr R. George Michael (personal





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Figures 95–97. *Daphnia atkinsoni* f. *bolivari*. Figure 95. Trunk limb 3. Details of the filtering setules as seen at very high magnification. The 'pustules' may be artifacts. Figure 96. The same, showing the insertion of the setules on the seta. Figure 97. Posteriormost long seta of the filter plate of trunk limb 3. This is not a filtering seta. Note how its setules are much longer and more widely spaced than those of the adjacent filter setae. Filter setae of trunk limb 4 can be seen beneath it.

communication) and are clearly a regular feature in this family.

That the tips of the filter setae are much modified was noted above. As food is passed forward by these tips, it is convenient to begin with the posterior setae, some of which are seen in figures 98 and 99. Here, each tip takes the form of a brush made up of the soft terminal part of the seta itself, and by long setules, clearly homologous with, but very different from, the more proximal filter setules. As Cannon (1933) noted on the basis of light microscopy, these brushes are directed laterally towards the filter setae of limb 4, from which they help to sweep collected particles (§5(*k*)).

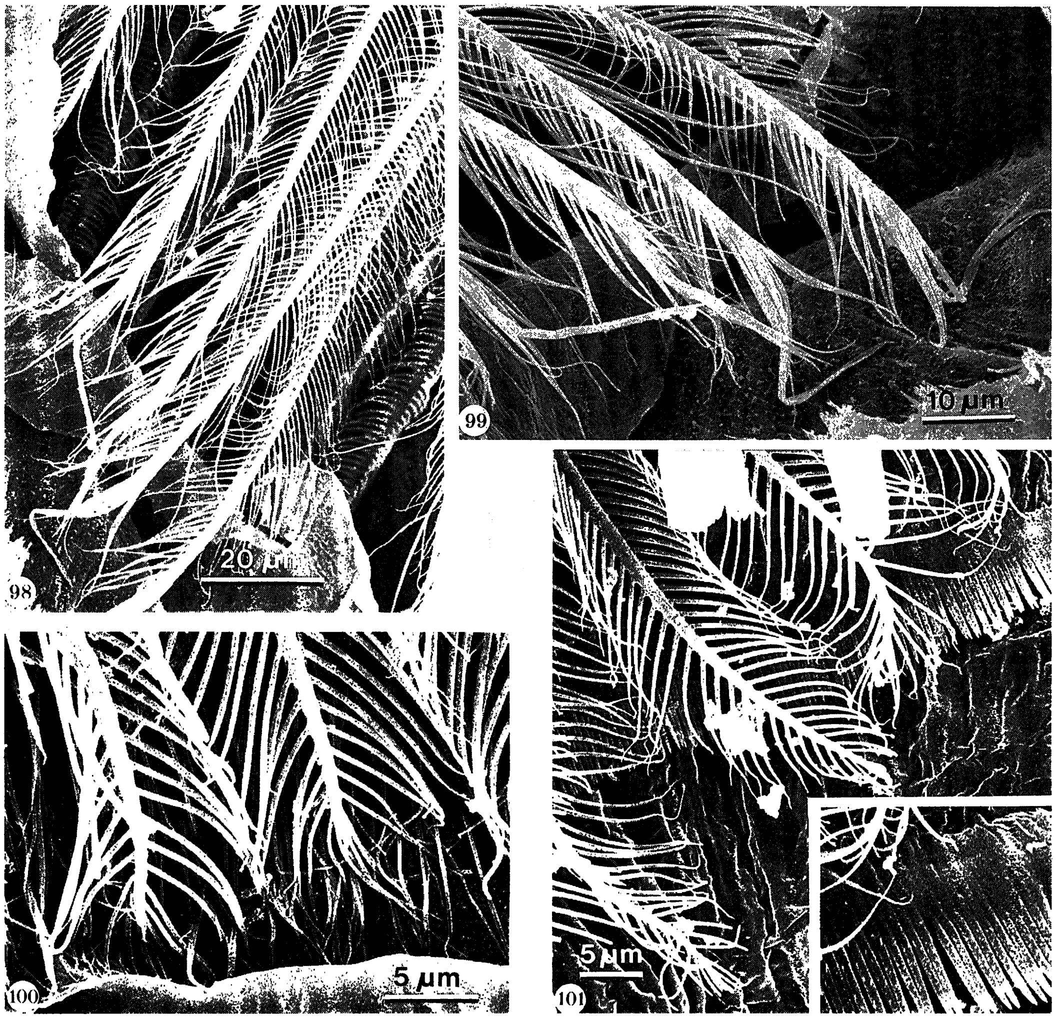
At the other end of the series the anterior setae also have brush-like tips, but the arrangement, seen in figure 100, is different, the setules being more robust. Posterior to these, the brushes are slightly different, as seen in figure 101.

Part of the filter of trunk limb 4 is seen in figure 102, which also shows the conspicuous fringe of setules (CLS) that arises from a ridge running parallel to the line of insertion of the row of filter setae. Its function is to clean the filter setae of trunk limb 3 (§5(*k*)). The filter setae of this limb, while similar in form to those of limb 4, are slightly different and have a finer mesh, only about 0.45 µm, with the reservations expressed earlier. (For details, see figures 104–107.) Figure 103 shows part of a filter plate of trunk limb 4 *in situ*, with the distal portions of setae of the filter plate of limb 3, whose tips have been broken off to reveal limb 4, lying above them. The difference is striking and reflects the different functions that are performed by the various parts of the filter setae of limbs 3 and 4 during the abstraction and subsequent handling of food particles.

Other details of these setae are noted in the description of the feeding mechanism (§5*k*)) where the relation of structure to function is most easily appreciated.

(*i*) *The mouthparts and labrum*

The mouthparts require only brief mention. The mandibles, their suspension and musculature, and their mode of operation, are similar to those described for other anomopods (Fryer 1968, 1974 and especially 1963, which gives a detailed account of *Eurycercus*). Salient features of their musculature are seen in figures 30 to 32, 109 and 110 and reference to their endoskeletal elements is made in §5(*e*). Ocioszynska-Bankierowa (1933) was the first to give a reasonable

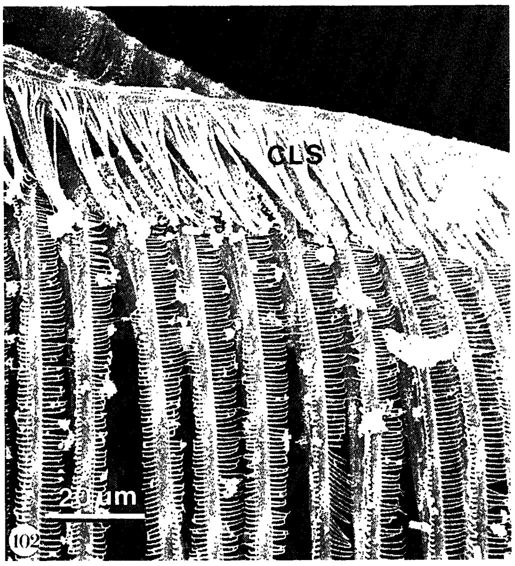


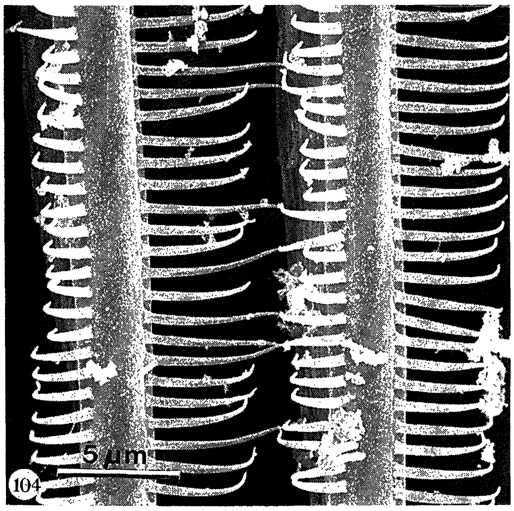
Figures 98–101. *Daphnia atkinsoni* f. *bolivari*. Figure 98. Distal portion of some of the more posterior filter setae of the filter plate of trunk limb 3, showing the brush-like nature of their tips. Filter setae of trunk limb 4 lie beneath them. Figure 99. Details of the brush-like tips of posterior filter setae, trunk limb 3. Figure 100. Distal portion of anterior filter setae of trunk limb 3, showing their modified tips. Figure 101. Tips of filter setae of trunk limb 3 that lie anteriorly, but posterior to those of figure 100. Note the row of stout spinules on the food groove wall, shown at higher magnification in the inset.

account of the masticatory surface of the mandibles of *Daphnia*. She showed that the right mandible of *D. magna* has a more or less flat masticatory surface provided with numerous ridges, while the left is drawn out ventrally into a series of rakes. This enables the mandibles to work in concert as described for *Eurycercus* (Fryer 1963). More recently Edwards (1980) has provided details of the masticatory surface of the mandibles of 18 species of *Daphnia* based on SEM observations. Unfortunately, for 10 of these she describes only the right mandible, and has nothing to say about function, but the results are nevertheless revealing. While the basic principles are the same throughout, Edwards showed that the masticatory surfaces display well-marked specific differences and that the three representatives of the subgenus *Ctenodaphnia* formed a distinctive group, whose right mandible has a masticatory surface whose length:width ratio is greater than in the 15 studied species of *Daphnia* s. str. Within the latter subgenus, a considerable diversity of armature was revealed. While minor variations of the ridges and spines of the masticatory surfaces might be expected to be equally efficient, the differences revealed by Edwards are sometimes striking and can scarcely be other than adaptive. They are presumably related to differences in the predominant foods of different species, that reflect different ways of life. For example, the right mandible of the planktonic *D. galeata mendotae* Birge has three large crushing teeth postero-dorsally, which perhaps serve to crack the skeletons of the diatoms that must often be eaten by this species.

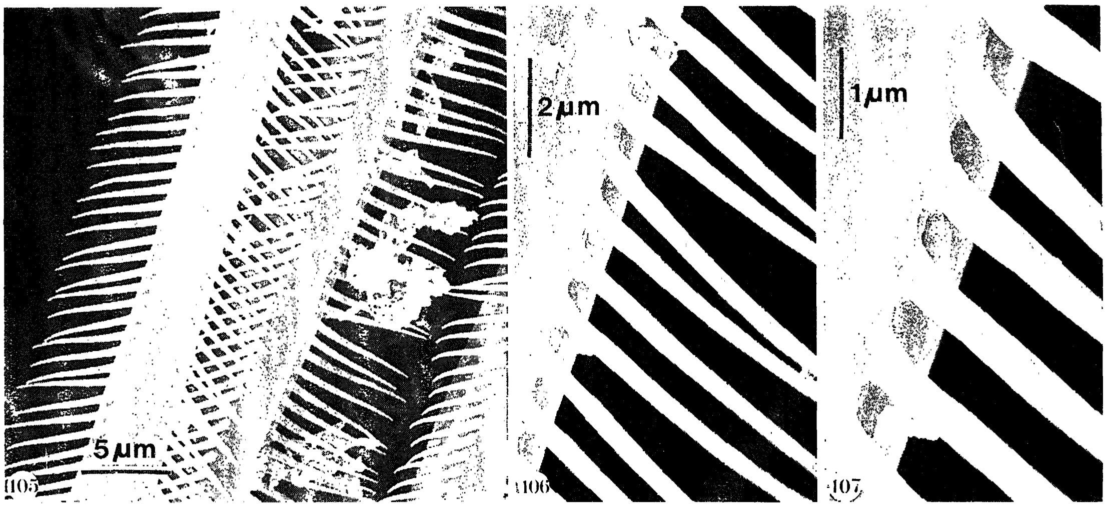
The maxillules (figures 3 and 109, Mxlle) are similar to those of other anomopods.

The fleshy labrum (L) extends posteriorly and covers the mouthparts ventrally (figures 3, 108 and 109). Its system of gland cells was well described by Cannon (1922) and further details were given by Sterba (1957) who showed that, by endomitosis, their large nuclei have become highly polyploid. At 2048-ploid they have perhaps the highest ploidy of any known cells. They produce copious secretions that are discharged by well defined exit ducts and entangle food particles (§5(*k*)). Zaffagnini (1964) suggested that the





Figures 102–104. *Daphnia atkinsoni* f. *bolivari*. Figure 102. Part of the filter plate of trunk limb 4. Note the difference between the setae of this limb and limb 3. The fringe of cleaning setules (CLS) that cleans the filter of trunk limb 3 is clearly displayed. Figure 103. Part of the filter plate of trunk limb 4, with some of the overlying filter setae of trunk limb 3, whose distal, brush-like tips have been cut off to reveal the underlying filter. Note the fringe of cleaning setules (CLS) and the changing arrangement of the filter setules near the insertion of the setae. Figure 104. Details of filter setae of trunk limb 4.



Figures 105–107. *Daphnia atkinsoni* f. *bolivari*. Figure 105. Filter setae of trunk limb 4. Although two adjacent setae have been pulled together during fixation, their setule-by-setule correspondence is well seen and the intermeshing of their tips in life is easy to visualize. Figure 106. Details of the insertion of the setules on a filter seta of trunk limb 4. Figure 107. The same, more highly magnified.

labral glands may have endocrine functions and Zaffagnini & Zeni (1987) carried out ultrastructural investigations with this in mind. They showed that the gland cells are not syncytial as previously believed, but have complete plasma membranes, and believe that the cells at the base of the head may indeed have an endocrine function and may take part in the secretion of cuticle. They also made the strange claim that the

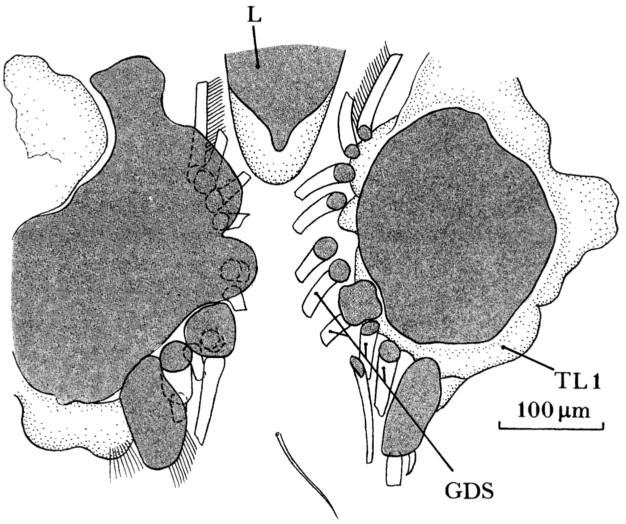


Figure 108. *Daphnia atkinsoni* f. *bolivari*. Thick transverse section, seen from in front, through the distal regions of the first trunk limbs, showing how the guide setae (GDS) line the sides of a narrowing channel that leads posteriorly into the filter chamber.

function of the duct cell is unknown and that 'it would appear to be unable to excrete all the secretion' of the gland cells, and did not themselves see the passage of secretions into it. Nevertheless, they discuss the possibility that the secretions may contain proteolytic enzymes! In fact, copious amounts of exuding secretions have been seen, photographed (Fryer 1962) and drawn (Fryer 1963, 1968) in various anomopods, thus confirming the view of Cannon, who was able to shown the presence of secretions in various branchiopods, but only to infer their source. Discharged secretions of *Daphnia* are seen in figures 28 and 69 (LGS). More recently, Zeni & Zaffagnini (1988), and Zeni & Franchini (1990) acknowledged that secretions are discharged and may serve to entangle food particles. Zeni & Franchini show that the secretions are probably mostly glycoproteins, though neutral polysaccharides are also present, and that they do not seem to be of an enzymatic nature.

Details of the innervation of the labral glands, first reported for *Simocephalus* by Cannon (1922), have now been provided by electron microscopy (Zeni & Zaffagnini 1988). Other than in daphniids only two other cases of innervation of epidermal glands in crustaceans are known. Zeni & Zaffagnini suggest that the secretory activity of these glands is under the direct control of the cerebral ganglion and that if their secretion products serve to entangle particles (as they do), the rate of ingestion might be regulated by that of secretion, which is in turn controlled by the nervous system.

(*j*) *The food*

Species of *Daphnia* ingest particulate matter of diverse origins, living and dead. Algae and, probably to a lesser extent, bacteria, constitute the major element of the live material. These items, especially algae, are more important to planktonic species than to those of small water bodies where large amounts of organic detritus are often consumed. The importance of detritus tends to be underestimated as studies have concentrated on planktonic species, to which, however, it is also sometimes important.

There has been much discussion about the ability or otherwise of *Daphnia* to select its food. What is available certainly determines to a large extent what is eaten. Species such as *D. pulex* and *D. magna* sometimes occur in foul water bodies where there are few algae. Their guts may then contain a mass of material, black to the naked eye or with low-power microscopy, in which algal remains are scarce or non-existent. In other situations large amounts of flocculent organic matter, usually brownish in colour, are collected. Under yet other circumstances these species flourish largely on algae of various kinds, the gut then being conspicuously green. They can be reared on ostensibly monospecific cultures of many different algae, though contamination by bacteria, which may supplement the diet, is generally inevitable.

Daphnia can cope with particles of diverse shapes and sizes. Such versatility is necessary in planktonic species. In temperate lakes, seasonal succession in the phytoplankton often involves rapid changes in species composition and the spectrum of available algae at times changes almost from day to day as different species 'bloom'. Likewise the composition of the phytoplankton differs from lake to lake. The range of species ingested is great, from unicellular µ-flagellates, to colonial diatoms such as *Melosira*, and includes green algae protected by a thick gelatinous sheath, and such branched colonial forms as *Dinobryon*, which is sometimes consumed in large quantities by the planktonic *D. galeata* (G. Fryer, personal observations). Filaments of the centric diatom *Melosira* more than 200 µm in length are collected, sometimes in large numbers as seen in photographs in Infante & Edmondson (1985), but are broken into individual cells before ingestion. Elongate filamentous algae sometimes enter the filter chamber, but are generally ejected. Species such as *D. magna*, that forage at times on the bottom, sometimes collect and ingest relatively large lumps of detritus.

By contrast, observations and experiments have shown that *Daphnia* can feed on bacteria, though it is generally thought that in nature these minute particles are usually a supplementary, rather than a major element, in their diet. As well as bacteria filtered from suspension, many others adhering to organic detritus are inevitably ingested and are at times perhaps important in the diet of certain species. They are doubtless more easily digested than the sometimes intractable material to which they adhere, which must yield scant nourishment during a passage through the gut that often takes only a few minutes.

That the food spectrum is wide does not preclude inter-specific differences in food preferences or filtering efficiency. The different habits of coexisting species in even a small water body are often sufficient to ensure that competition is minimal (see, for example, Fryer (1985)), a point sometimes ignored by ecologists. On the other hand, in the plankton or in other situations

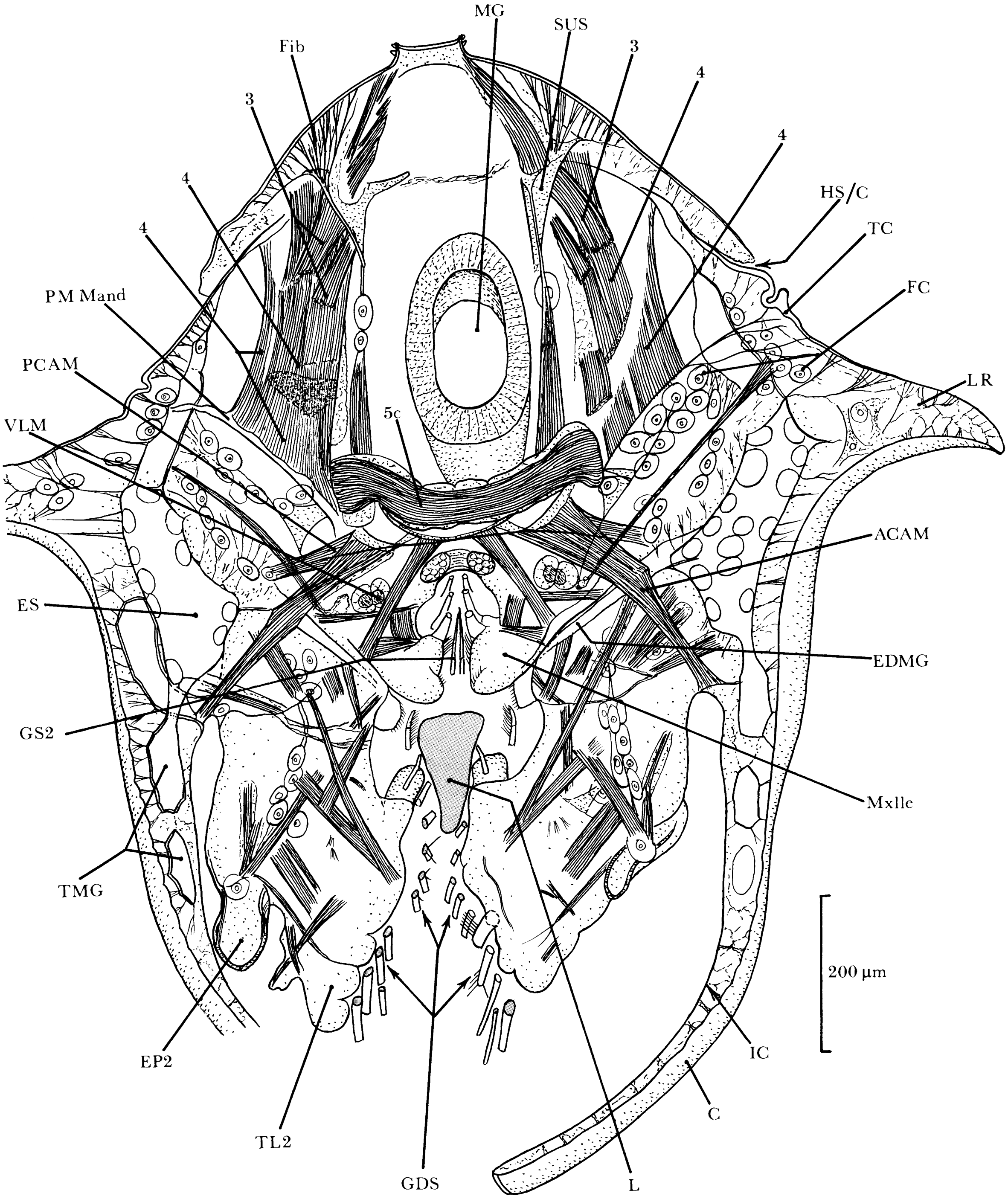


Figure 109. *Daphnia atkinsoni* f. *bolivari*. Thick transverse section, seen from in front, whose anterior limits lie at the level of the posterior margins of the mandibles, and which cuts through parts of trunk limbs 1 and 2 and the maxillules. The section includes the lower of the 5c muscles, and parts of both the promotor (3) and remotor (4) roller muscles of the mandibles, the insertion of the latter on the posterior margin of the mandible (P M Mand) being seen on the left. Parts of the suspensory ligaments (SUS) of the mandibles are also seen. Other features include the anterior carapace adductor muscles (ACAM), the end sac (ES), exit duct (EDMG) and some of the tubules (TMG) of the maxillary glands, the latter lying within the folds of the carapace (C), various endoskeletal elements, and some of the complex musculature of trunk limb 2. The lateral ridges of the carapace (LR), so characteristic of this form of *D. atkinsoni*, are a striking feature of transverse sections of the anterior end of the carapace. The complicated corona of the dorsal ridge of the carapace is also seen, but is seen even better in figure 110. The numerous ovoid bodies, often associated with membranes, are fat cells (FC).

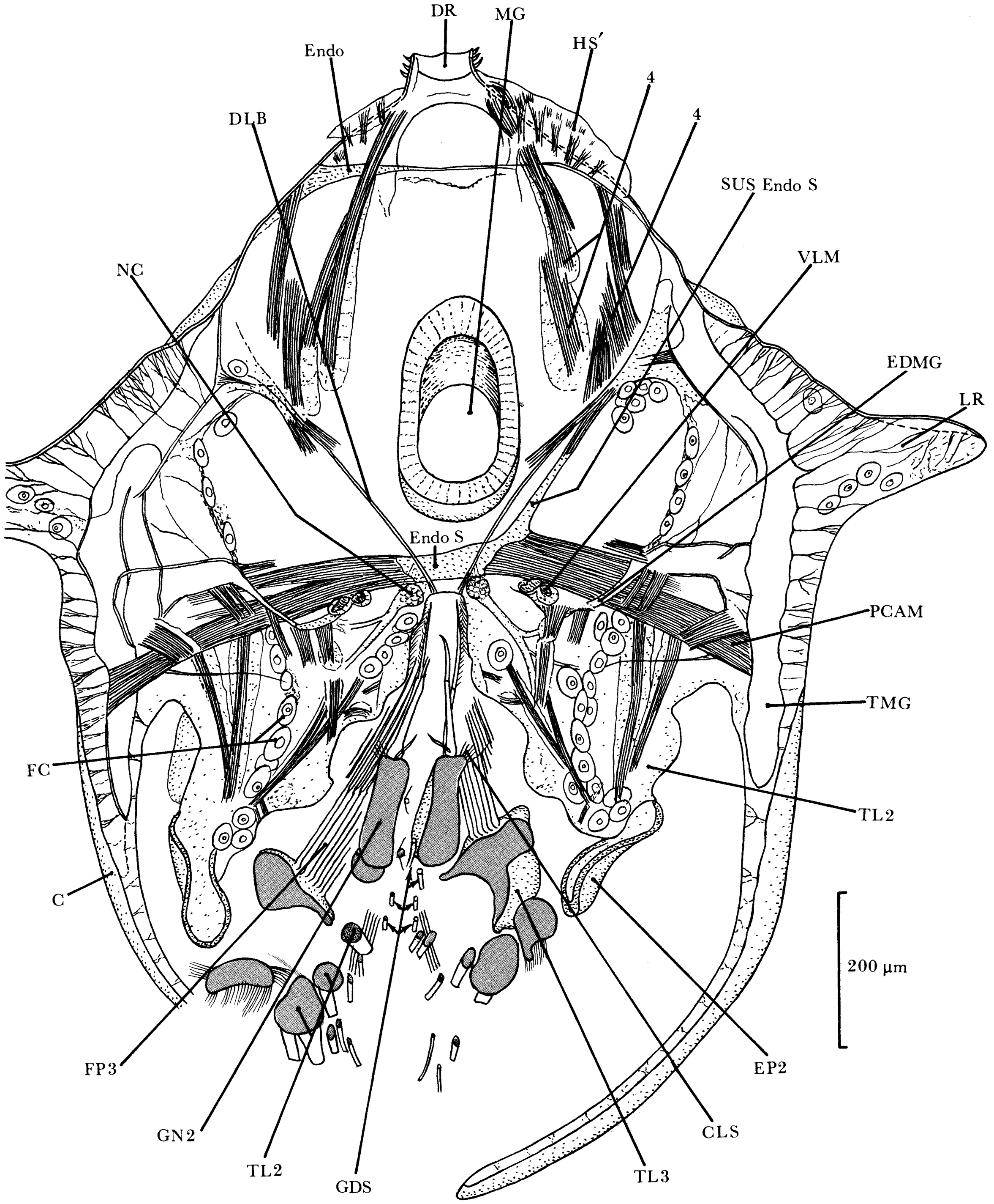


Figure 110. *Daphnia atkinsoni* f. *bolivari*. Thick slice immediately behind that shown in figure 109. The posterior carapace adductor muscles (PCAM) and their associated endoskeleton (Endo S) are well shown. The section cuts through the gnathobases of the second (GN2) and some of the setae of the filter plates of the third, trunk limbs (FP3). Parts of the exit ducts of the maxillary glands (EDMG), which pass anteriorly, are also seen. The nature of the dorsal ridge of the carapace (DR) and its armature of spines are more readily apparent in sections than in the entire animal.

where closely related species coexist, similarities in the gut contents are sometimes striking. While this probably applies mostly at times of superabundance, it sometimes appears to reflect availability rather than specific preferences. When different food 'preferences' can be shown, they seem often to be based on differences in overall size and in the dimensions of the filters. A recent paper dealing with such comparisons in eight species of *Daphnia* is that of Brendelberger (1985). These matters are complicated by an ability, that differs from species to species, to react to changes in the concentration of the food by altering the mesh size of the filters at ecdysis (Koza & Korínek 1985; Korínek *et al.* 1986).

Daphnia (like other daphniids) frequently ingests indigestible material. Inorganic particles are taken in by *D. magna* that forages on the bottom, and sometimes even by planktonic species. Toth *et al.* (1987) show

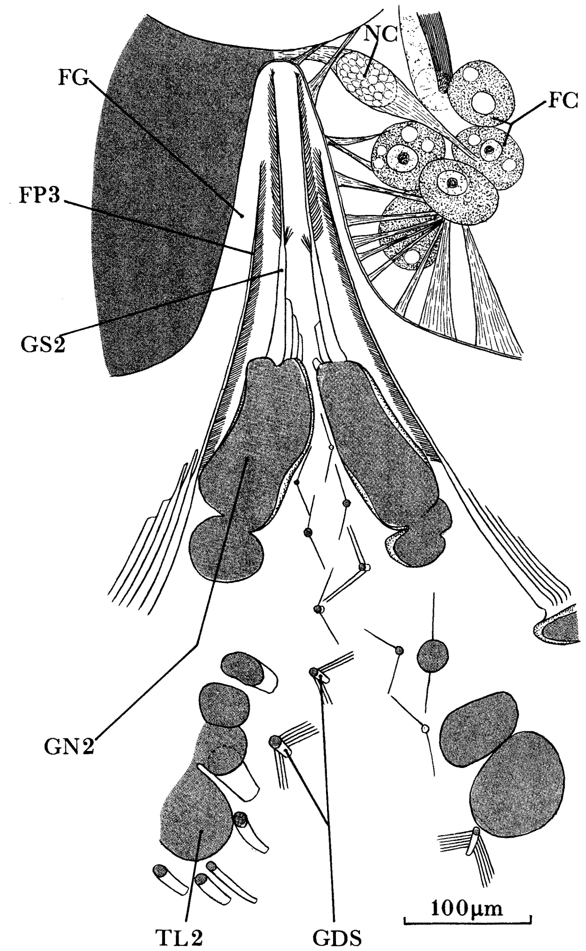


Figure 111. *Daphnia atkinsoni* f. *bolivari*. Transverse section, seen from in front, through the gnathobases (GN2) and adjacent parts of the second trunk limbs. Adjoining filter setae of the filter plate of the third trunk limb (FP3) are also seen. Note how the brush-like armature of the gnathobasic setae of the second trunk limbs is directed outwards and towards the filtratory setules of the filter setae of the third trunk limbs, which are cleaned by it, and how the gnathobasic setae extend deeply into the food groove (FG).

that, in the shallow Lake Balaton where much such material is evidently in suspension, the gut contents of the planktonic *D. galeata* and *D. cucullata* may consist largely of mineral particles, and give SEM photographs showing the guts packed with these. Adhering films of organic matter may provide some nutriment even from these inert items. Many algae also pass through the gut undigested. These include spherical green species whose cellulose cuticle is undamaged as it passes between the mandibles, and species whose cell or cells are covered by a thick gelatinous layer. Some nutrient materials may be obtained from the gelatinous material of the latter. Many such algae can be grown after passing through the gut of *Daphnia* and indeed some have been found to display enhanced growth after such transit! (Porter 1976). Some of the many studies on feeding rates, assimilation and related matters are reviewed by Lampert (1987).

(*k*) *The feeding mechanism*

The feeding process in *Daphnia* involves the abstraction of particles from water drawn through filters borne on the third and fourth pairs of trunk limbs. Although they held divergent views on various points, this was agreed by Cannon (1933), Eriksson (1934) and in part by Storch (1922, 1924, 1924–25, 1925), the three principal investigators of this mechanism. Storch though that the fourth pair formed a protective fence around the third, and Eriksson believed that limbs 1 and 2 were also involved in filtration. Most recent students also accept that limbs 3 and 4 are responsible for filtration. Relevant publications are those of Arruda (1983), Brendelberger (1985); Brendelberger & Geller (1985); Brendelberger *et al.* (1986); Crittenden (1981); DeMott (1985); Geller & Müller (1981); Geller & Knisely (1988); Gophen & Geller (1984); Koza & Korínek (1985); Korínek *et al.* (1986); Lampert (1987); Mangalo (1987), for *Simocephalus*, and Watts & Petri (1981).

On the other hand, Gerritsen & Porter (1982) claimed that, because low Reynolds number (viscous flow) regimes prevail, the boundary layer around the setules of the filter setae may exceed their inter-setular distances and that little or no flow takes place between these setules. Such beliefs appear to receive support from observations made on copepods (Koehl & Strickler 1981) but, as shown below, this situation differs in a fundamental respect from that which prevails in daphniids. Porter *et al.* (1983) then had to postulate that the first and second pairs of trunk limbs may be the primary food collectors, a suggestion tacitly abandoned by Gerritsen *et al.* (1988). Porter *et al.* also tentatively suggested that the filter plates of the third and fourth trunk limbs 'acted more as pumps', a suggestion that Ganf & Shiel (1985*a*, *b*) attempted to substantiate. The way in which the latter authors completely misinterpreted the morphology of the animal, and therefore, inevitably, the mechanism in question, has been shown elsewhere (Fryer 1987*b*) and their erroneous account will only be mentioned when certain aspects of it are contrasted with the situation that actually prevails. Some of the points raised by Gerritsen *et al.* (1988) are discussed in §5(*l*).

Those who seek to disprove what they call 'the sieving hypothesis' fail for one simple reason. They have not understood the structure, arrangement and modes of action of the appendages involved (see, for example, Fryer (1987*b*) and §5(*l*)). Indeed, some of them have made no real attempt to do so and evidently believe that they can 'explain' the mechanism without understanding its component parts, an approach akin to that of someone who, unaware of the role of cylinders and pistons, attempts to explain the workings of an internal combustion engine by lifting a car bonnet and making deductions from what little is thereby revealed. As a result, some accounts can only be described as grotesque. Usually, no use is made of sectioned material, which is essential for understanding, and some of the few recent illustrations are extremely crude. Scanning electron microscopy provides a further

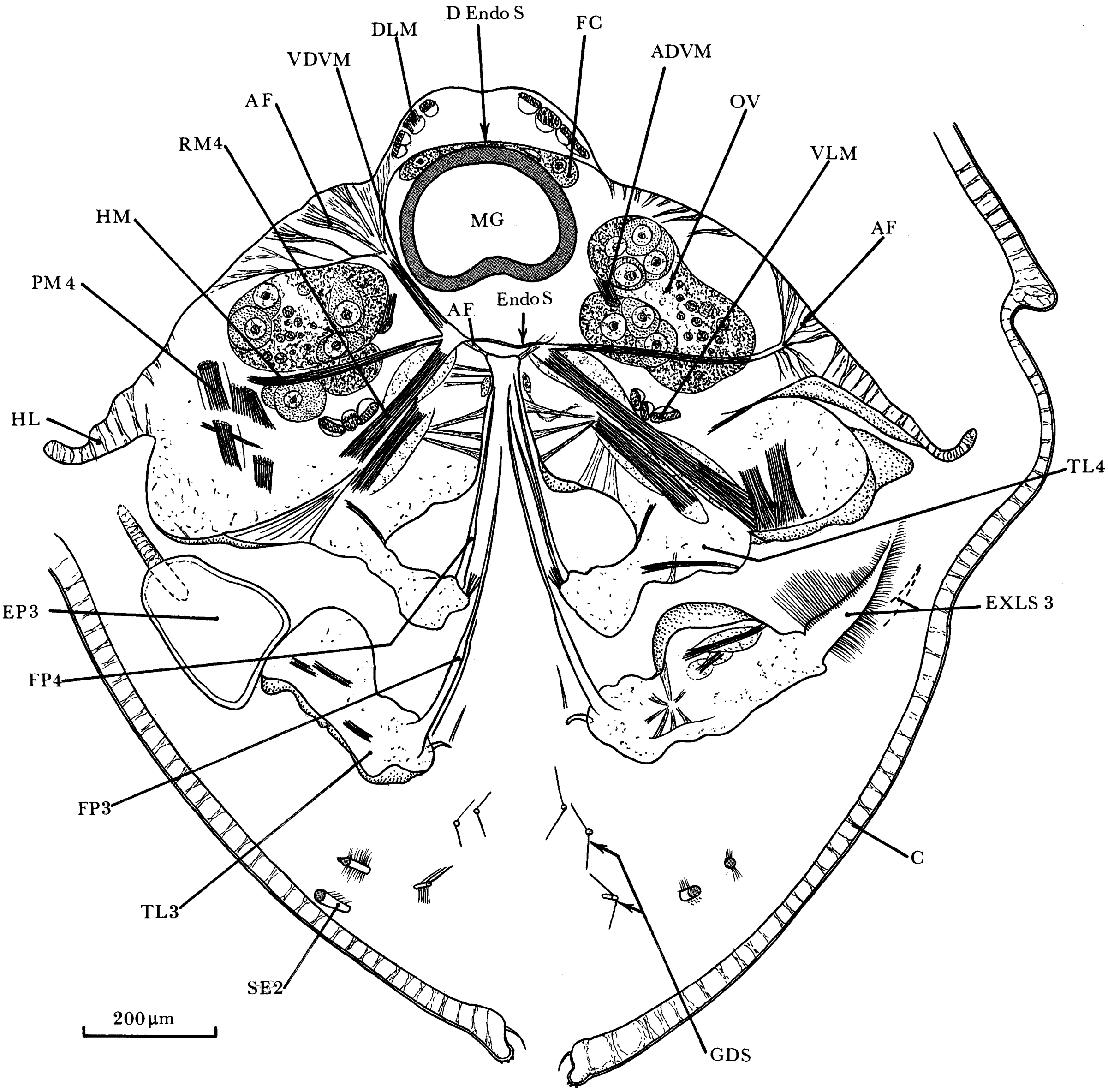


Figure 112. *Daphnia atkinsoni* f. *bolivari*. Transverse section, seen from in front, through the trunk at a level that shows the contribution of the filter plates of trunk limbs 3 (FP3) and 4 (FP4) to the filter chamber. Some of the major trunk muscles, including the horizontal muscles (HM) that happen to be revealed by this slice, and some of the extrinsic muscles of trunk limb 4 are also seen, RM4 being remotors. Note the horizontal lamella (HL) which, in life, serves as a water-tight seal that separates the dorsal brood pouch from the ventral carapace chamber on each side. It has become distorted in fixation. Apart from the ovaries (Ov), most internal structures other than the skeleto-muscular system are omitted. Only those fat cells (FC) associated with the dorsal endoskeletal sheet (D Endo S) are shown. The ventral indentation of the mid-gut (MG) is an artefact of fixation.

pitfall. It is easy to obtain SEM photographs but if, as is sometimes the case, the structures portrayed are grossly distorted, such photographs are virtually worthless.

Daphnia is a filter feeder. Filtration can only be practised if a particle-bearing current is drawn or pressed through a filter. This demands a rhythmically operating pump. The pump of *Daphnia* is derived from a device of the kind used today by those of its essentially bottom-frequenting relatives in the Macrothricidae and Chydoridae that also filter. Except in exceptional circumstances, however, chydorids and macrothricids collect most of the food by means of scrapers of various kinds and pass it into the filter chamber. A prime evolutionary feat of the daphniids has been to develop the pump and filtering device to such an extent that sufficient food particles can be drawn into the filter chamber without the use of scrapers. This has enabled them not only to dispense with these structures, but has freed them from dependence on substrata.† These developments have involved restriction of filtration to the two pairs of limbs directly concerned with pumping, an increase in the size of their filter plates, and concomitant changes in the pumping device. In chydorids and macrothricids that scrape as well as filter, scraping is an intermittent

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† Two cases where specialized scraping is sometimes employed are described in §5 (*o*) (i), (ii), but these species can subsist solely by filtering suspended particles.

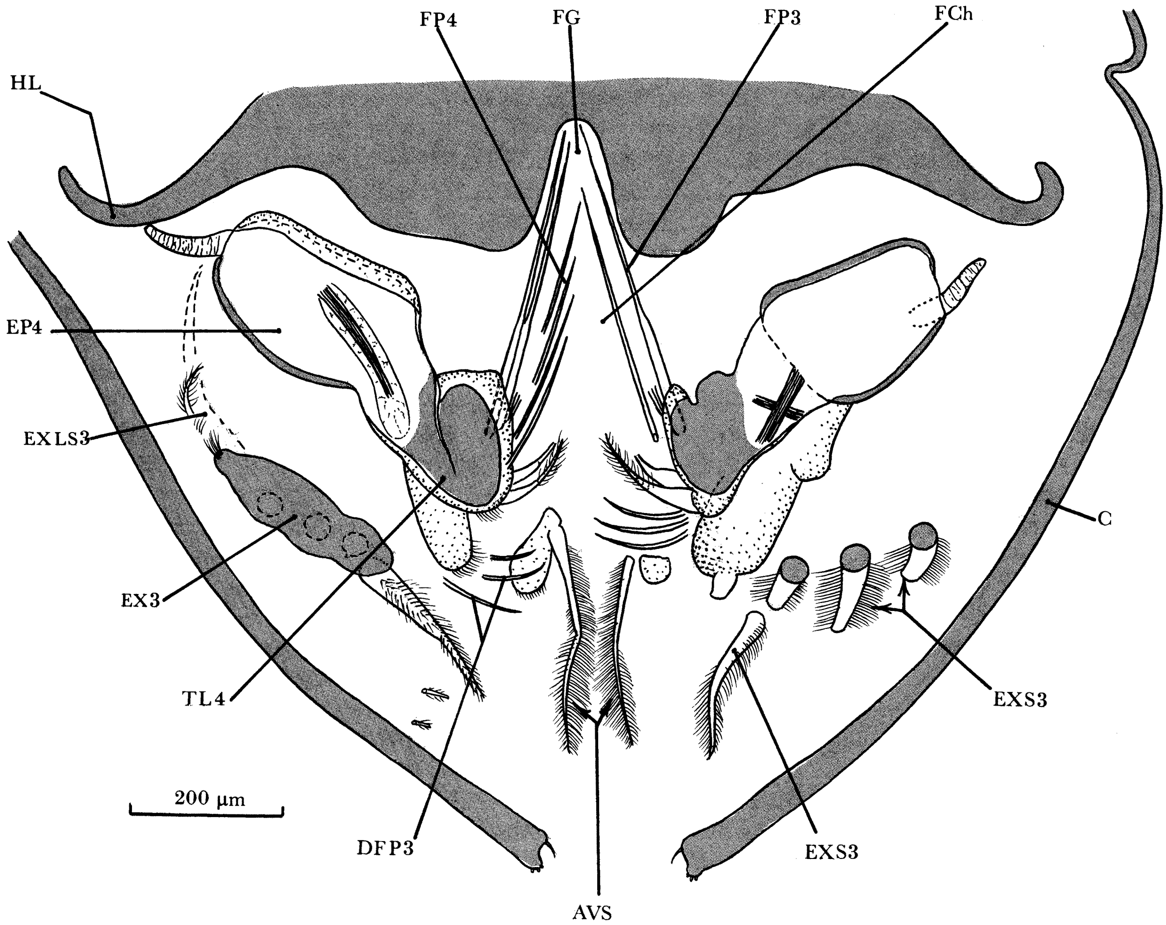

Figure 113. *Daphnia atkinsoni* f. *bolivari*. Transverse section, seen from in front, near the posterior end of the filter chamber (F Ch) showing the relation of the filter plates of trunk limbs 3 (FP3) and 4 (FP4) to each other. The exopodite of trunk limb 3 (EX3) and its posterior setae (EXS 3) are seen to the left and right, respectively. The extreme distal parts of trunk limb 5, whose long anterior vertical setae (AVS) are conspicuous, are just coming into view.

activity that is varied according to circumstances. Just as does its precursors, *Daphnia* pumps rhythmically, but there is no intermittent scraping.† The process involves the regular repetition of a fixed cycle of events, though this cycle is at times interrupted to allow excess food particles to be ejected or limbs to be cleaned, and pumping may occasionally cease for brief periods. Regular rhythmical beating of the trunk limbs is, however, a characteristic attribute of daphniids. This sets up currents that presumably serve also for respiration, though as it is scarcely possible to slow down or stop limb beat without depressing metabolism, experimental proof is lacking and the respiratory role of these currents is debatable. Some macrothricids and chydorids manage without them. Their role in feeding in daphniids is indispensable.

Because the beating of the trunk limbs of *Daphnia* is rhythmic and repetitive, its rate can be measured without necessarily studying the mechanism involved, and data on beat frequency, which are useful in the study of feeding rates, exist. Limb-beat frequency was measured manually by McMahon & Rigler (1963) by punching a telegraphist's key linked to a recorder. This method has limitations, particularly at high speeds. Burns (1968) provided more detailed and more accurate data by measuring beat frequency photometrically and recording the results on the chart of a potentiometric recorder. By focusing on a single trunk limb, or on the mandibles, she was able to ascertain both the frequency of beat of the former, and therefore of the entire trunk limb mechanism, and the rate of mandibular roll. While Burns' aim was to ascertain some of the factors that influence feeding rates, her results are valuable in a functional context and provide data on the rates at which *Daphnia* can carry out its vital activities. By using similar principles, Porter *et al.* (1982) provided information for four species of *Daphnia* and a species of *Ceriodaphnia*.

In *D. rosea* Sars (a species closely related to *D. longispina*) Burns (1968) recorded limb beat frequencies of between about 5 and almost 13 Hz at 20 °C, depending on food concentration. The highest rates were in the smallest individuals. At the same temperature, McMahon & Rigler (1963) recorded rates of about 4 Hz, occasionally more, and often rather less, in *D. magna* and found that individuals established a rate of only about 2.5 Hz when high concentrations of the yeast *Saccharomyces cerivisiae* and the alga *Chlorella vulgaris* were available as food. Unlike Burns, Porter *et al.* (1982) found that beat rate was independent of

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† Two cases where specialized scraping is sometimes employed are described in §5(*o*) (i), (ii), but these species can subsist solely by filtering suspended particles.

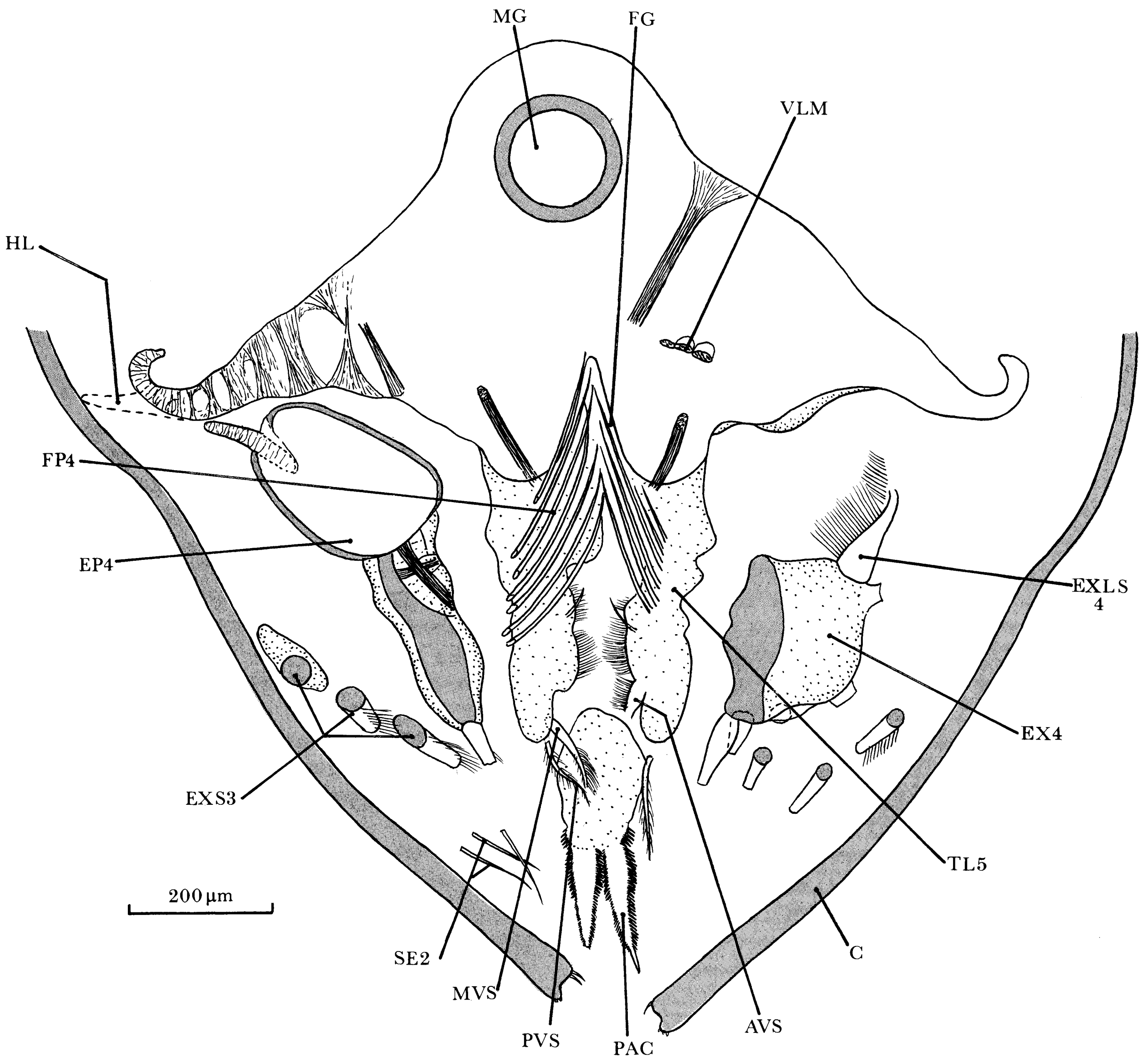

Figure 114. *Daphnia atkinsoni* f. *bolivari*. Transverse section some way posterior to that shown in figure 113. Note how the posterior-most filter setae of the filter plates of the fourth trunk limbs (FP4) form a fence closing the filter chamber posteriorly in concert with the fifth trunk limbs (TL5).

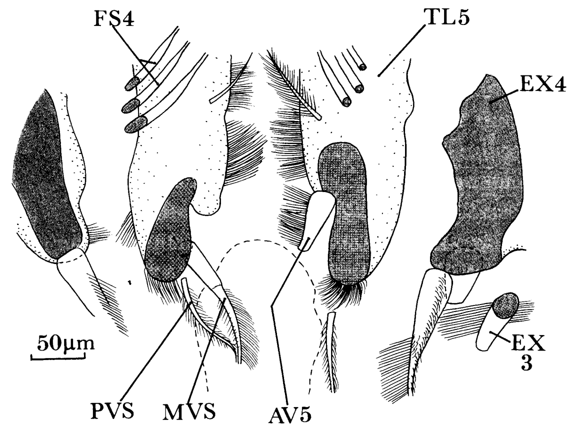

Figure 115. *Daphnia atkinsoni* f. *bolivari*. As figure 114, to a larger scale showing details of the fifth trunk limbs.

size within a species, but that it increased with decreasing size between species. The fastest rates, up to *ca.* 16 Hz, were recorded in the small *D. parvula* Fordyce. At room temperature, individuals of *D. obtusa* filmed in the present study generally operated at about 5 Hz or a little faster, *D. galeata* at about 6 Hz or a little less and *D. magna* at about 3.5 Hz or a little less, but as many variables influence the rate of limb beat, these are no more than general indicators of the sort of rates at which these species operate. For example, *D. galeata* has been seen operating at only about 2.6 Hz, while *D. pulex* regularly beats its limbs at a higher, but unmeasured, frequency than any of these species. Because low Re values are always involved (see below) these differences in limb velocity have little effect on the steepness of the shear gradients around them (Cheer & Koehl 1987).

The terms promotion and remotion are unambiguous and are used here because motion of some of the limbs cannot be divided into working and recovery phases. They work in both directions. Abduction and adduction are inappropriate terms for some of the movements involved and, although employed by Storch, are eschewed here. The account given here is generalized and is based on observations made on several species and on films made of *D. magna*, *D. obtusa*

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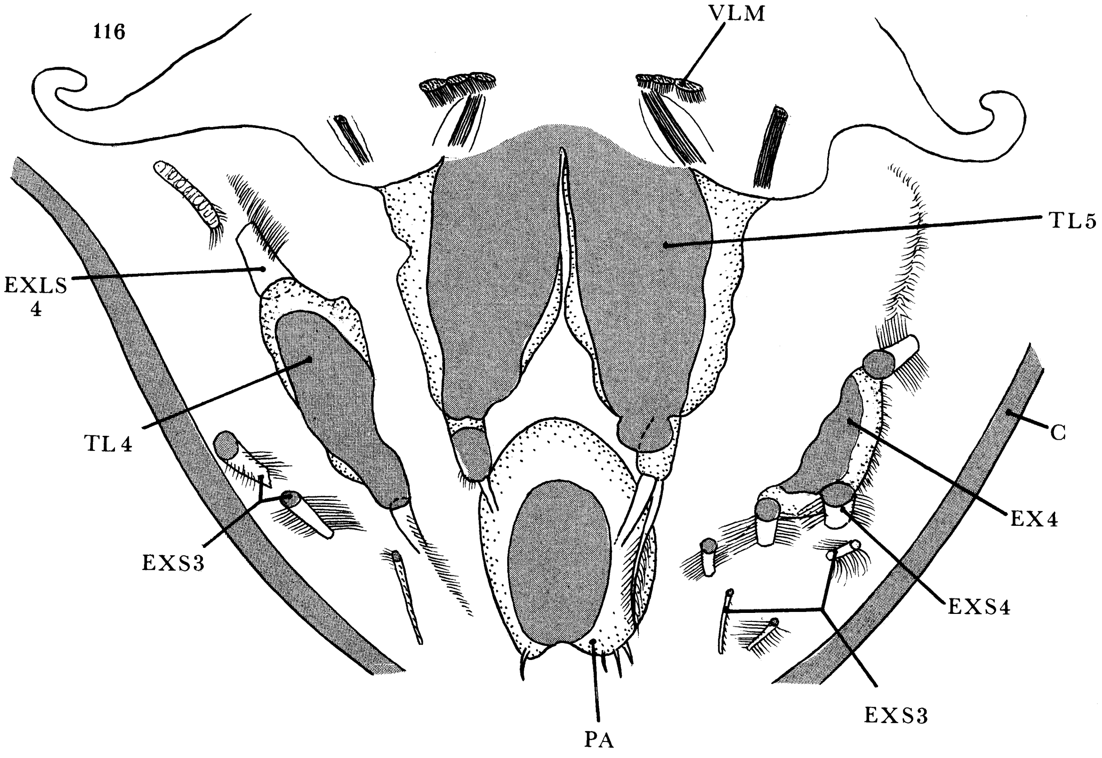

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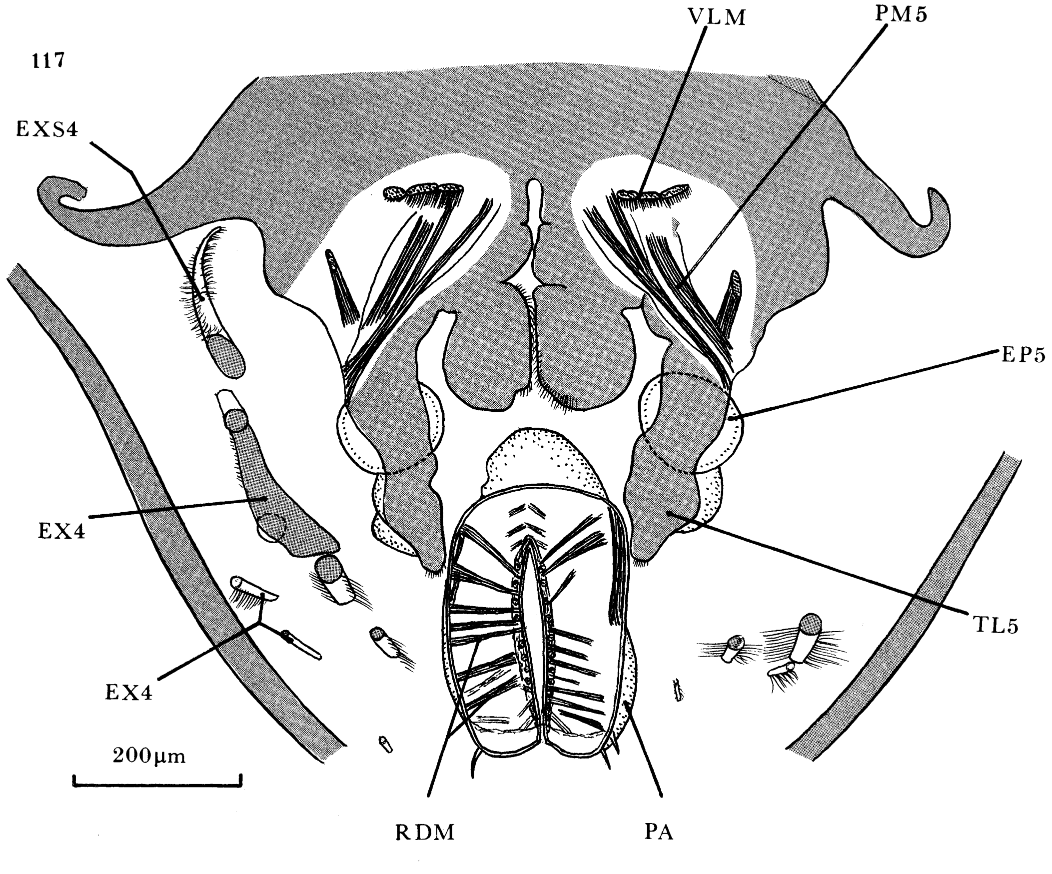

Figures 116 and 117. *Daphnia atkinsoni* f. *bolivari*. Figure 116. Transverse section immediately behind that shown in figure 114 and cutting through the fifth trunk limbs. Figure 117. Transverse section immediately behind that shown in figure 116 showing the muscles of the fifth trunk limbs that rotate the sealing seta of the exopodite into position on each side.

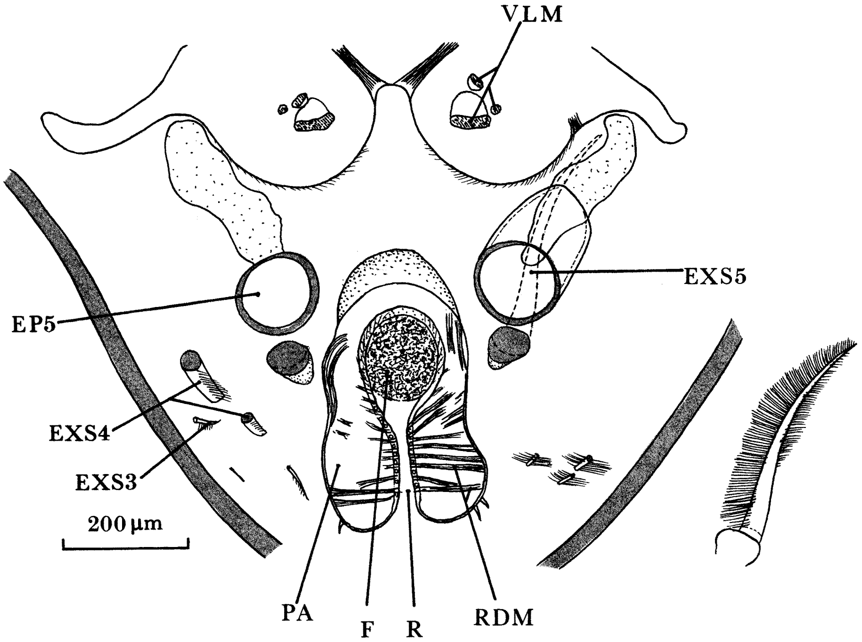

Figure 118. *Daphnia atkinsoni* f. *bolivari*. Transverse section immediately behind that seen in figure 117 to which, from more posterior slices, the sealing seta of the exopodite of trunk limb 5 (EXS5), indicated by dashed lines, has been added. The inset shows a complete reconstruction of the sealing seta from successive sections.

and *D. galeata*. Reference is made to individual species when necessary. Figures 121–125 facilitate appreciation of the complex movements involved.

A cycle of limb beats can best be described by beginning at the point where the fifth trunk limbs are in the position occupied at the end of remotion and the fourth trunk limbs, which follow quickly, are almost in this position (figure 121, *i* and *a*, where the limb has just begun promotion). At this time the flattened corm of each fifth trunk limb lies parallel, and adjacent, to the lateral face of the postabdomen, i.e. parallel to the long axis of the body. Its free margin, which bears the curved sealing seta, is directed backwards. The corm of the fourth trunk limb is inclined almost as far back as the passing of its filter plate into the food groove will allow and lies at a very acute angle to, and in some species, such as *D. galeata*, almost parallel to, the long axis of the body. The third limb, also in remotion, is inclined backward and overlies the fourth. Both lie close together so that interlimb spaces 3/4 and 4/5 are obliterated (figure 121*a*) or almost so. Trunk limb 2 lies close to trunk limb 3 and the long posterior filter-cleaning spine of its gnathobase lies not far from the food groove towards which it is swinging at this stage.

It is during remotion of limbs 3 and 4 that water is driven out of the carapace chamber posteriorly on each side through the gaps left by the remoted fifth trunk limbs, and ventral to them. Details are given below. To replace this water more is inevitably drawn into the carapace chamber via the narrow ventral aperture. This water carries suspended particles that enter the carapace chamber medially. All except the very finest are prevented from passing laterally by the funnel-like array of guide setae of the first trunk limbs whose arrangement, but not armature of setules, is seen in figure 3, and in transverse section in figures 108 and 109. Particles are therefore confined medially as they pass into the filter chamber. As remotion ends, so does the flow of exhalent water from the carapace and, inevitably, the influx that replaces it.

Promotion is initiated by trunk limb 5, which swings forward as Cannon (1933) graphically described, 'just as a door swings about its hinges', (figures 121 *a–d* and, diagrammatically, 125 *a–f*). It does so through an angle that differs somewhat in different species. In *D. magna* it is about 50°; in *D. galeata* it approaches a right angle. By this action its corm comes to lie transversely across the gap between the trunk and carapace wall (figure 122) and seals the posterior exit channels from interlimb spaces 4/5, via which much of the filtered water from these spaces leaves the carapace chamber at each side of the post-abdomen. More so than did Cannon, Storch (1924) described the way in which sealing was effected by trunk limb 5. At the end of promotion the curved sealing seta lies adjacent to the wall of the carapace, to whose contour its curvature is adjusted. Its setules ensure that there is no gap between carapace and seta through which water can escape, so an effective water-tight barrier is placed across the exit channel on each side. Promotion of the fifth trunk limb takes place rapidly. In individuals of *D. obtusa* with limbs beating at slightly less than 6 Hz, most of the swing is accomplished in about 30 ms, though the final 'sealing' in position adds a little to this time. In *D. obtusa* and in *D. galeata* limb 5 begins to swing forward just before limb 4 has completed remotion (figure 121 *a*). Even before trunk limb 5 is in the fully promoted position, trunk limb 4 begins the promotion phase of its

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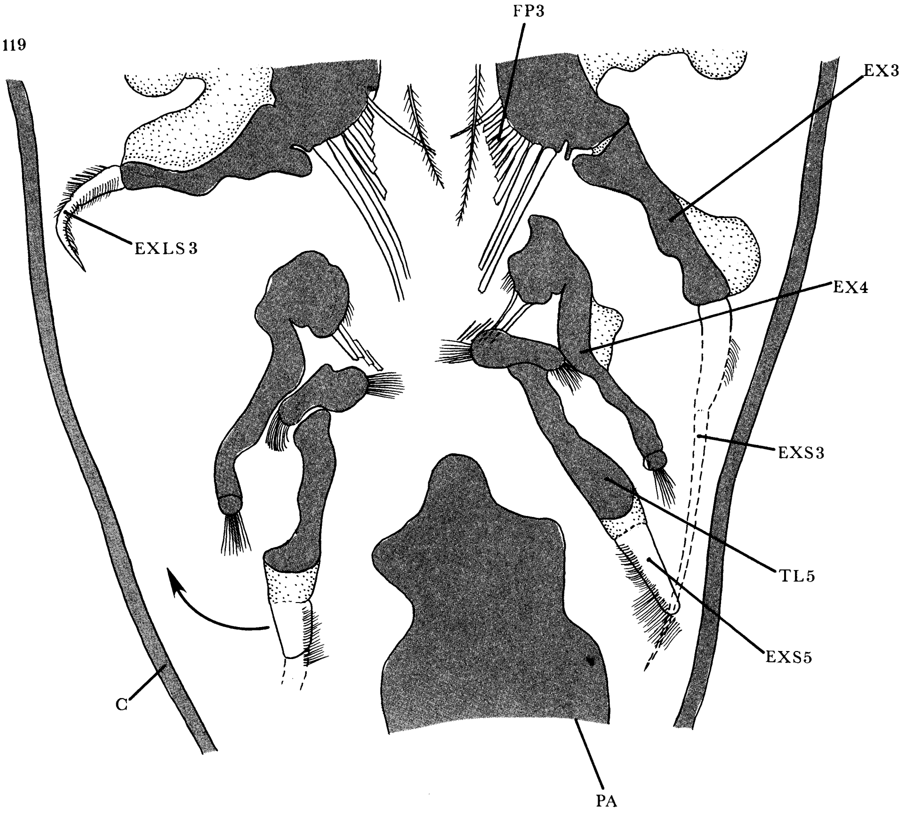

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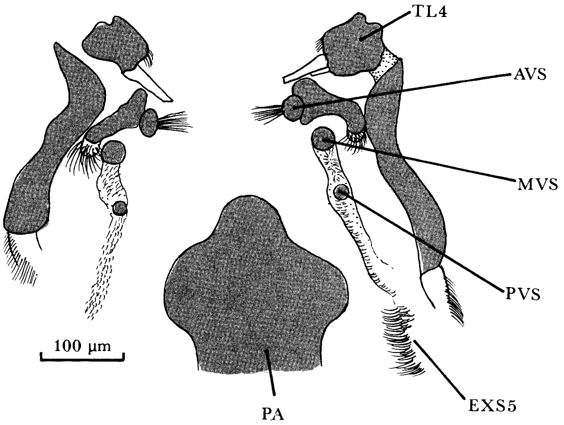


Figures 119 and 120. *Daphnia atkinsoni* f. *bolivari*. Figure 119. Horizontal slice, ventral aspect, cutting the distal parts of trunk limbs 3, 4 and 5. The posterior lateral exopodite seta of trunk limb 3 (EXLS3) is visible on the left: its anterior companion, not visible, dives more steeply dorsally. Only the basal region of the outermost of the posterior exopodite setae of trunk limb 3 (EXS3) is visible on the right, but its full extent, ascertained from adjacent sections, is shown by dashed lines. The arrow shows the direction of swing of the exopodite of trunk limb 5 and its sealing seta during promotion. On that side, it is here seen fully remoted. Figure 120. The same more ventrally, that is, nearer the distal ends of trunk limbs 4 and 5. The locations of the vertical setae (AVS, MVS, PVS) of trunk limb 5 are readily seen.

cycle (figure 121 *c*). This must momentarily reduce the effectiveness of promotion of limb 4 in drawing water through the filter (see below), but it doubtless assists promotion of the delicate trunk limb 5. The final, minute, movements of limb 5, shown by dots in figure 124, are no more than a sealing of the valve, and the seal is probably effective throughout most, or all, of this phase.

Trunk limb 3 begins promotion almost immediately after limb 4 (figure 121 *c*, *d*), and of necessity or the action of limb 4 would be prevented. Promotion of limb 4 is dominated by a forward swing of the corm, but there is also a small lateral component (see below). The filter plate, which stands more or less vertical to the food groove, swings essentially in the vertical plane as the limb promotes, its proximal region leading and its distal portion following. Save to a minor extent, it does not move face-on to the water. The same orientation and movement applies to the filter plate of limb 3. Forward swing of the corm drags the exopodite in its wake, its lateral margin adjusting itself to the shape of the adjacent carapace. As promotion of limb

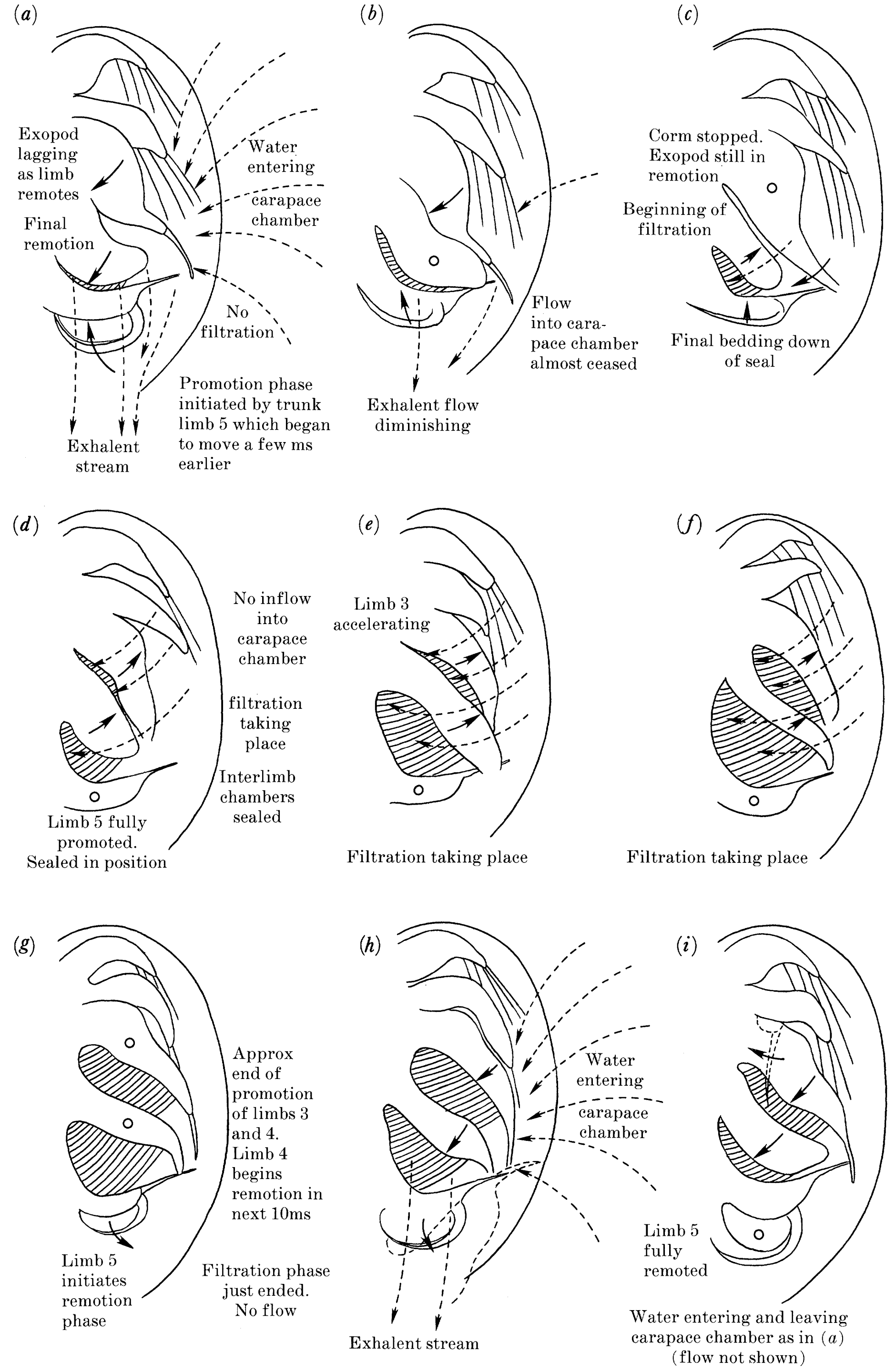


Figure 121. Outlines of the trunk limbs of *Daphnia* at different stages of a cycle of motion, based on a cine film of *D. obtusa* whose limbs completed a cycle in *ca.* 180 ms. There is an interval of 20 ms between each position shown, including positions I and A. No attempt is made to show the complex arrangement of the exopodites of limbs 3 and 4 that make up an important element in the pump. Some idea of how these lie as seen laterally and ventrally can be obtained from figures 3, 52 and 53, respectively. The position of the rapidly swinging, long posterior filter-cleaning spine of the gnathobase of trunk limb 2, shown at I, is only approximate. Solid arrows shown limb movements, dashed arrows those of water (and of the particles they bring into the filter chamber). Open circles indicate a limb at rest. No arrows are shown on limbs 1 and 2 whose movements, which can be deduced from the illustrations, are small and not related in the setting up of currents. Flows into the interlimb spaces are indicated as if seen from the mid line. The postabdomen, against which limb 5 presses at the end of remotion, is indicated by a dashed outline in H. For detailed comments, see text.

4 proceeds, a widening gap appears between it and trunk limb 5 (figure 121*c*–*f*). That is, interlimb space 4/5, obliterated at the end of remotion, begins to reform. As trunk limb 4 promotes, the setule fringed margin of its exopodite and its setae soon effectively prevent the entry of water at the ventral margin of the interlimb space. Because this space is also sealed posteriorly by trunk limb 5, anteriorly by its corm and

the thin lateral lobe, laterally by the carapace, and dorsally by the trunk and its horizontal lamella, there is only one entrance to it and that is through the filter plate of the limb. Water therefore passes from the filter chamber, through the filter plate, into the lateral interlimb space. Suction will inevitably draw the tips of the filter plate against the food groove wall or, in the case of trunk limb 3, against the filter plate of limb 4, ensuring the sealing of this region.

Because suction begins while the corm of trunk limb 4 is still directed posteriorly and the entrance to interlimb space 4/5 is located dorsally (figure 121, *c*), water is sucked from the filter chamber somewhat dorsally, that is in the vicinity of the food groove, at this phase of the cycle, as it is shortly afterwards in the case of interlimb space 3/4, which partly explains why some particles are drawn deeply into the filter chamber without apparently touching its walls. Because the interlimb spaces, especially 4/5, are located posteriorly, water, and the particles it contains, are drawn predominantly towards the posterior end of the filter chamber, especially during the early stages of promotion. This is readily seen in a feeding *Daphnia* and was indeed noted as long ago as 1820 by Jurine, whose accurate statement is quoted elsewhere (Fryer 1987*b*). It can be shown by supplying an individual with *Lycopodium* spores (Fryer 1987*b*) or other particles, or with flocculent material that is drawn in en masse. The route taken by the latter is easy to follow and has been recorded on film.

Trunk limb 4 continues promotion (figure 121 *d–g*) until, viewed laterally, it makes an angle of about 50° or a little more (almost 60° has been measured in *D. magna*) to the long axis of the trunk. Initially trunk limb 3 moves with limb 4, but as its speed of movement is greater, a gap, interlimb space 3/4, quickly develops between these limbs as they both promote (figure 121, *d–g*). Furthermore, limb 3 approaches the anterior limits of promotion after limb 4, thus widening the gap still more.

As trunk limb 3 promotes (swings forward), its exopodite is pulled across what would otherwise be the ventral gap between this and the succeeding limb and drawn in by suction so that it, its marginal sealing setae, and the small distal endite and its sealing setae, seal off the ventral region of interlimb space 3/4. (This can be appreciated from figures 52, 53, 114 and 119.) This point was explained better by Storch (1924) than by Cannon (1933), though both appreciated the sealing of the interlimb spaces. Sealing is as for interlimb space 4/5, save that the posterior wall of the chamber is provided by trunk limb 4. The amplitude of beat of trunk limb 3 is greater than that of limb 4 and the volume of interlimb space 3/4 is greater than that of space 4/5, just as the filter plate of the third trunk limb is larger than that of the fourth.

Thus two laterally located suction chambers, interlimb spaces 3/4 and 4/5, gradually enlarge, drawing water from the filter chamber through the filter plates of trunk limbs 3 and 4.

There is no escape route for particles from the posterior end of the filter chamber which is fenced off by the posteriormost filter setae of trunk limb 4 that are arranged in an arc for this purpose (figures 56, 58 and 114). In *D. atkinsoni* f. *bolivari* (and perhaps other species) the posteriormost seta of the filter plate of trunk limb 3 is not a true filter seta but takes the form of a coarser sieve (figure 97). It presumably helps to capture any coarser particles that are sucked towards the posterior end of the filter chamber.

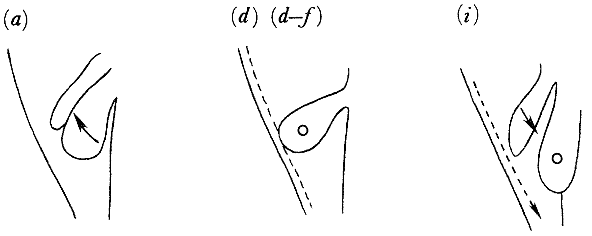


Figure 122. Ventral view of trunk limb 5 (and in part limb 4) from a film of *D. magna* (the action in *D. obtusa* is the same) showing the approximate positions of this limb at certain phases of the cycle shown in figure 121. Letters show the position occupied in similarly lettered diagrams in figure 121.

Although Storch (1924, 1925) and Cannon (1933) differ in their interpretation of events, and although their accounts differ from that given here, they too both agree that particle-bearing currents are drawn directly into the posterior part of the filter chamber, an inevitable consequence of the posterior location of the pumping device. Contrary to the suggestion of Ganf & Shiel (1985*a*, *b*) there is little flow in the vicinity of trunk limb 2. A few particles are doubtless filtered by the anterior portion of the filter plate of trunk limb 3, but direct observation shows that the vast majority are abstracted towards the posterior end of the filter chamber. It is towards here that water is drawn.

As limbs 3 and 4 promote and the volume of the posterior interlimb spaces increases, compensatory changes in volume elsewhere are necessary because during this phase of the cycle no water is entering or leaving the carapace chamber. Only internal movements of a fixed volume of water take place: from the ventral region, into the filter chamber, thence to the interlimb spaces (figure 121*c–f*).

As limbs 3 and 4 promote, their filter plates swing in their own plane, passing through the water like the blade of a knife in the reverse direction to its cutting action. In addition, the corms tilt a little. This gives a small but distinct lateral component to the movement of the filter plates, especially those of trunk limb 3, so that opposed filter plates move apart somewhat. They can do so to only a limited extent distally, because of the constraints imposed by the narrowness of the food groove. It is at this time that the filter plates of trunk limb 3, which swing faster than those of limb 4, are cleaned by the cleaning setae or spinules near the base of the latter and when the distal portions of the setae of limb 4 in particular are cleaned by the setae or spinules of the food groove walls. Before the end of promotion the lateral movement of the filter plates is reversed and opposed filter plates begin to move towards each other a little. This movement is such that, in the transverse plane, the base of a single seta follows a course like a narrow curved ellipse during a cycle of movement, moving ventrally and somewhat laterally during much of promotion, then medially before its dorsal movement during remotion.

As noted in §5 (*h*), the gap between the setules of the filter setae of trunk limb 3 become wider towards their tips. It is therefore possible that, at the very end of promotion, an escape route via coarser meshes than those of most of the filtering surface of the filter plate sometimes becomes available. Should such an 'escape route' be used, it would contribute to the sucking of particles deeply into the filter chamber. It is also possible that mechanical constraints are involved and that, if intersetular gaps did not widen towards the tips of the filter setae, the load on the distal parts of the setae may become unsustainable towards the end of promotion. Because of their distance from the base, and because they taper distally, the tips of the setae become progressively less able to bear loads without bending. Wider gaps ease this problem.

That viscous forces are important in the mechanism thus far described does not, as some have supposed, prevent water from passing through the filters. That it does so can be shown by the use of coloured fluids as Cannon (1933) noted long ago, as well as by observing the course taken by particles as they pass into the filter chamber. The filter chamber is a closed system during the suction phase and, as Koehl & Strickler (1981) note, 'water can, of course, be forced between very narrow gaps when given no other escape route'. Comments on calculations of the energy involved in filtration are given in §5 (*l*).

By the time trunk limb 3 has reached the end of promotion, at which time its corm, viewed laterally, lies more or less parallel to that of limb 4 (figure 121 *g*), a considerable proportion of the water originally contained in the carapace chamber has been drawn through the filters of these two limbs. In view of prevailing misconceptions it is worth reiterating that the filtering phase of the cycle just described is a purely 'internal' activity. No water is drawn into, or expelled from, the carapace chamber. During filtration it is also irrelevant whether the animal is stationary or moving through the water, nor is its orientation significant.

That there is no flow into the carapace chamber during the filtration phase of the cycle is usually evident in films. Particles approaching the ventral aperture of the carapace chamber stop during this phase and begin to be drawn in again as soon as remotion of limbs 3 and 4 begins. On one occasion some flow was observed during the filtration phase in *D. obtusa*. One possible explanation was an increase in the volume of the carapace chamber caused by relaxation of the adductor muscles, but there was no indication of this on the film. One therefore suspects that some water was squeezed out posteriorly by the exopodites as they moved to seal off the interlimb spaces, which seems not usually to be the case.

During the period when trunk limb 3 is in promotion (figure 121 *d–f*) and suction into interlimb space 3/4 is taking place, the gnathobase of trunk limb 2 also undergoes promotion so that its long posterior filter-cleaning spine swings ventrally away from the food groove (figure 124: see legend). In a rhythmically beating series of trunk limbs, the corm of limb 2 often moves scarcely at all, but in *D. obtusa* the proximal portion undergoes some promotion during the early part of that period in which the gnathobase is in promotion, though at the same time, its distal portion actually moves backward, as in remotion. As Cannon (1933) noted, limb 2 seems to be pushed forward slightly by limb 3 at the end of the latter's promotion. I cannot, however, confirm his statement that its movement is 'mainly lateral', for which there is no scope, and his remarks concerning the phase difference between it and limb 1 seem inappropriate. In some species limb 1 scarcely moves during normal rhythmic limb beating. In others, there is a slight back and forth movement as shown for completeness for *D. obtusa* in figure 124 (see also *Daphniopsis* and *Simocephalus*) though this is insufficient to establish significant currents. In *D. galeata* limb 1 generally oscillates with small amplitude, but I have seen this species in action with limbs 1 and 2 virtually stationary, except for the active swinging of the gnathobase of the latter (see below). In *D. magna* limb 1 is often essentially stationary but sometimes

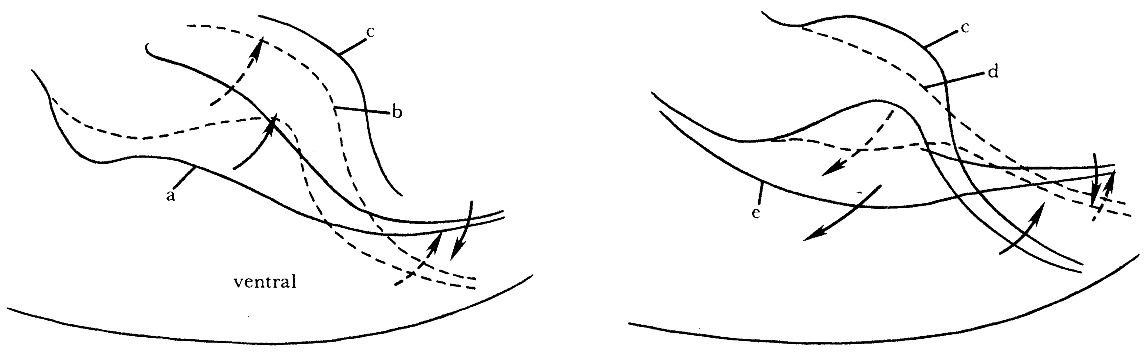


Figure 123. *Daphnia galeata*. Outlines derived from cine film (300 frames s^{-1}) of the movements of the corm and exopodite of trunk limb 3 during the latter part of remotion and the initial phase of promotion. Lateral. For clarity successive positions are indicated in two sets of outlines. The time intervals between a and b, b and c, c and d, and d and e are 14, 7, 21 and 14 frames, respectively. At a, the limb is in remotion. As its corm swings, the exopodite lags and pressure of the water against which it pushes deflects it forward. At b, the corm is near the end of remotion and its exopodite is also swinging towards the trunk. At c, the corm is virtually at the end of remotion and has obliterated interlimb space 3/4. Its exopodite continues its swing. From c to just before d, the corm is stationary but has begun promotion at d. As the corm promotes, its exopodite is sucked back to seal the enlarging interlimb space. Promotion continues at e. Both the corm and its exopodite are swinging forward as trunk limb 4 does likewise.

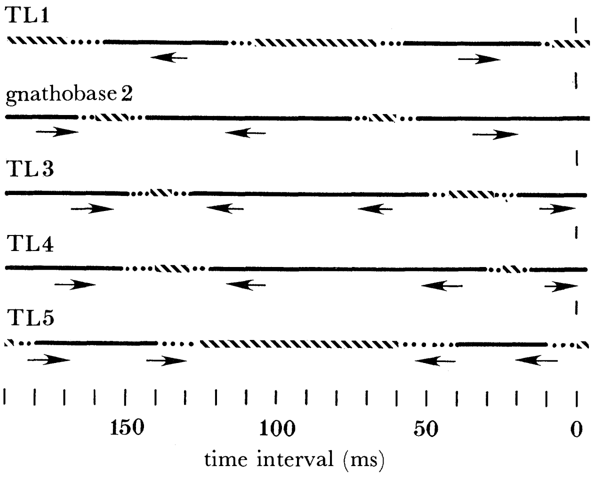


Figure 124. Analysis of the trunk limb movements of *Daphnia obtusa* derived from a cine film run at 100 f.p.s. and showing a complete cycle of activity achieved in *ca*. 190 ms. The sequence can best be understood if it be assumed that the animal is lying ventral surface uppermost so that arrows to the left indicate promotion; to the right, remotion. Periods of most rapid motion are indicated by solid lines, slower motion, towards the end of, or at the beginning of, an active phase, by dots. Diagonal striping indicates periods when a limb is at rest. It is not always possible to ascertain the *exact* moment when a limb stops or begins to move, but the diagram is a close approximation. In the case of trunk limb 2, it is only the movement of the gnathobase, based on that of the long, posterior filter-cleaning spine, that is shown. It is important to appreciate that this spine has a wide amplitude of swing and therefore moves considerably faster than the filter plate of trunk limb 3, which it cleans. There is a brief period towards the end of promotion and at the beginning of remotion where, because of congestion of the limbs, this spine cannot always be seen, but in some cycles it has been clearly visible. The movements of trunk limb 1 are slight and in no way comparable with those of other limbs. In some species, this limb remains virtually stationary during feeding, but as a rudimentary cycle is detectable in *D. obtusa*, this limb is included for completeness. The sequence is most easily followed by beginning with the movements of trunk limb 5.

swings rhythmically with small amplitude. Cannon's curves showing a modified form of metachronal rhythm are somewhat idealized so far as limbs 1 and 2 are concerned, though the three posterior limbs certainly operate in a sequence that merits the term metachronal, though this is generally applied to a longer series.

So far, it is the forward swing of the filter bearing limbs that has been emphasized. That the filter plates also have a lateral component has been noted, but additional complexity is introduced by the fact that, particularly at the point of extreme remotion, the filter plate of limb 3 overlies that of limb 4. Storch (1924) and Cannon (1933) were, of course, aware of this overlap and Storch referred to it in connection with the forward transport of food along the food groove, while Cannon noted its significance in relation to the cleaning of filtered material from the filter plates. These matters are discussed below. They did not, however, consider apparent implications for filtration. The overlap is seen, for example, in figures 56, 58 and 112. As is often the case in fixed material, trunk limbs 3 and 4 have been pulled into more or less the positions occupied at the end of remotion, which is when maximum overlap of the filters occurs. These sections make it easy to appreciate how, during promotion, the filter plates of trunk limb 3 pass over those of limb 4.

The figures suggest that, over a considerable part of it, the filter chamber is double walled and that currents must pass through two filters. For the most part this is not so. A little water may pass through two filters at the beginning of the suction phase but, as promotion proceeds, the filters of the third limbs overlap those of the fourth to an ever diminishing extent, as can easily be appreciated from figure 3, which shows limbs 3 and 4 in positions that they occupy near the end of promotion. Observations on living animals, and films, show that, while slight overlap occurs, filtration is carried out essentially by the filters of the third trunk limb anteriorly and the fourth posteriorly.

Figure 3 shows limbs 3 and 4 in more or less the positions occupied at the end of promotion. Remotion is initiated posteriorly by trunk limb 5 (figure 121*g* and 125 *g*) which, in an action that is the reverse of its promotion, swings backward so as eventually to lie adpressed to the post-abdomen (figures 121*g–i* and 125 *g–i*). This breaks the seal of interlimb space 4/5. Trunk limb 5 may begin to swing back just before trunk limb 3 has completed promotion but is very close to having done so. Trunk limb 4 begins remotion as limb 5 swings back and water is forced backward on either side of the post-abdomen (figure 121*h*) and leaves the carapace chamber posteriorly. Trunk limb 3 follows immediately after limb 4, gradually reducing the space between them. The exopodites of limbs 3 and 4 are initially pushed away by the pressure exerted as the limbs undergo remotion (figure 121*a*), but as this process continues the exopodites help to push out water posteriorly (figure 123). Nothing hinders the backflow of water from the erstwhile large interlimb chambers. Much of that from interlimb spaces 4/5 passes through the gaps left by the remoting (and remoted) fifth trunk limbs, and some of it ventral to these limbs: all that from interlimb spaces 3/4 is pushed ventral to the fifth trunk limbs. Figure 123 shows in outline some of the movements of the exopodite of trunk limb 3 during remotion. During remotion, the filter plates cut the water like the blade of a knife.

The filter plates move somewhat medially during remotion. Just as lateral movement is constrained by the narrowness of the food groove, the filter plates cannot approach each other too closely during remotion because of the presence between them of the gnathobases of the second trunk limbs whose armature, including the long posterior cleaning spines, is in action during this phase of the cycle. At the very end of remotion, the tips of the posteriomost filter setae of trunk limb 4 sweep into the bottom of the food groove and the tips of opposed setae appear actually to touch momentarily, at least in some species. They do not do so more anteriorly. It is towards the very end of remotion that the gap between the posterior portions of the filter plates of the fourth pair of trunk limbs narrows most rapidly. As trunk limb 3 swings back

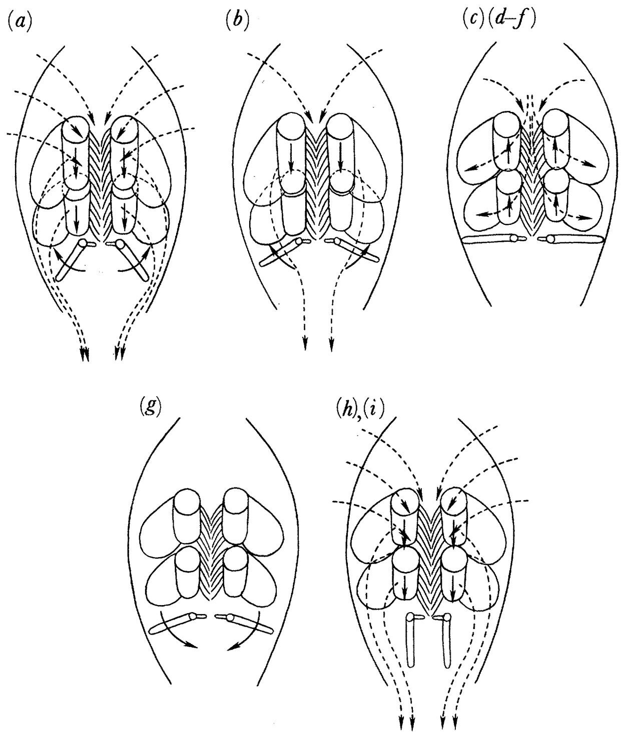


Figure 125. Diagrammatic representation of a cycle of movements of trunk limbs 3 to 5 of *Daphnia*, viewed ventrally. While it is impossible to show in two-dimensional sketches, events that take place in three dimensions, and while the figures are purely schematic, they show the essence of the processes of food collection and filtration. They are labelled to correspond approximately with the outlines, based on a cine film, shown in figure 121, with which comparison is helpful. Solid arrows show movements of limbs; dashed arrows, currents. Limbs bearing no arrows are stationary at that phase. Trunk limb/body 'articulations' are shown by circles. The exopodites of limbs 3 and 4 are indicated by arbitrary shaped flaps. For simplicity, changes in volume of the filter chamber are not shown. At (*a*), limbs 3 and 4 are approaching the end of remotion, water is being expelled from inter-limb spaces 3/4 and 4/5 and is leaving the carapace chamber posteriorly as trunk limb 5 begins promotion that will eventually seal interlimb space 4/5 posteriorly. Much of the water from interlimb space 3/4 passes ventral to trunk limb 5 (as shown at (*b*)), but to avoid the impression of crossing flows, this flow is omitted. Water is entering the carapace chamber antero-ventrally to replace that driven out. The process continues with diminished effect at (*b*), where limb 4 has reached the end of remotion. Limb 3 has almost done so and is driving out the last of the water to be expelled. Promotion of limbs 3 and 4 takes place from about (*c*) and throughout (*d*)–(*f*), involving expansion of the sealed interlimb spaces 3/4 and 4/5 and drawing water into them through the filter plates of limbs 3 and 4. No water enters or leaves the carapace chamber during this phase. At (*g*), limbs 3 and 4 have reached the end of promotion and are neither drawing water into their interlimb spaces nor driving it out. Limb 5 is just beginning remotion. At (*h*, *i*) limb 5 is fully remoted so that interlimb space 4/5 is open posteriorly as well as ventrally. Water is driven posteriorly by limbs 3 and 4, now actively remoting. A volume of water equal to that expelled enters the carapace chamber antero-ventrally.

faster than limb 4, the distal brushes of its filter setae are able to clean the filter of limb 4 during remotion.

As water exits posteriorly from the carapace chamber, it is replaced by inflow through the gap between the ventral carapace margins (figures 121*h*, *i*, *a*, which last follows *i* in the cycle, and 125*h*, *i*, *a*). This is the current that bears suspended food particles. Although it is drawn into the carapace chamber, it enters as the inevitable consequence of water being expelled by a force pump and not by the direct action of a suction pump. Limbs 3 and 4 act as a suction pump during promotion (the filtering phase of the cycle); as a force pump during remotion.

In several species of *Daphnia* there is a short row of setose setae towards the middle region of the carapace margin. These, which extend across the ventral gape, help to prevent the entry of unwieldy items, but may also have other functions. Scourfield (1942) lists those species in which he found such setae to be present and those that lack them. The latter are predominantly open water forms where such a screen is presumably unnecessary (and where other roles for the setae are precluded). Species in which these setae occur belong mostly to the sub-genus *Ctenodaphnia*, and include *D. magna* and *D. atkinsoni* f. *bolivari*, much used in the morphological part of this investigation. However, *D. lumholtzi*, a planktonic member of this sub-genus, has no such setae. The occurrence of the setae also cuts across taxonomic groupings in the subgenus *Daphnia*, which suggests a relation to function rather than to phylogeny. While there are no such setae in *D. pulex*, they are present in *D. obtusa* (Scourfield 1942).

The distal setae of the corm of trunk limb 2 also evidently prevent some large particles from entering the filter chamber. Those so screened out can be removed by the post-abdomen (p. 60). Eriksson (1934) mistakenly believed that these setae, and the guide setae of trunk limb 1, are filter setae and that, like the filters of limbs 3 and 4, they contributed to the collection of food particles. (See *Simocephalus*, §8(*b*).) He criticized Storch for ignoring their importance in filtration, though in fact the latter was correct, for they are not filter setae.

In the main, inflow of water ventral to the filtering system is a straightforward process. Its entry is easy to observe whenever suspended particles reveal its course, and the intermittent nature of its flow, so difficult to detect by simple observation as the intervals are so short, is readily apparent in high-speed cine films. A note of complication is introduced by the fact that the filter-bearing gnathobases, especially of trunk limb 3, do not simply swing backward during remotion, but also approach their partners on the opposite side, thereby narrowing the chamber and slightly reducing its volume. Even if there were no other complicating factors, it would not be easy to follow the fate of a particle located within the filter chamber as its walls converged. Dimensions are small and viscous forces important, but some degree of turbulence might be anticipated. However, as limbs 3 and 4 undergo remotion, the long backwardly directed posterior filter cleaning spine of the gnathobase of trunk limb 2 (best seen in figure 3, FCS) swings towards the food groove, sweeping the surface of the more slowly moving filter plate of trunk limb 3 of its side as it does so (figure 124, and shown in a simplified way in figure 121*i*). Furthermore, the median row of spines of this gnathobase also sweep the adjacent parts of trunk limb 3. Details of these operations are given below, but it is easy to visualize their effect by reference to figures 3, 56, 59–61, 69–75, 80–85, 87, 88 and 111. Apart from cleaning the adjacent filter plate, their major role, these spines will inevitably frustrate the escape of most particles from the filter chamber during this phase of activity.

As remotion of limbs 3 and 4 comes to an end, the tips of their filter setae approach the food groove. The curvature of these setae (convex posteriorly in the relevant plane: figure 3) is such that, even during a simple swing, their tips would give a small anterior component to any particles pushed into the food groove. This tendency is, however, accentuated because, towards the end of remotion, the gnathobase, and therefore its filter plate, are pulled forward somewhat as they swing, so the tips of the filter setae not only push material into the food groove but sweep, or even rake, it forward, and sometimes compact it. This subtle 'lunging' movement, which adds yet a further element of complexity to the cycle of motion, is another example of the versatility conferred by the 'jointless' nature of these limbs. Trunk limbs 3 and 4 do not simply swing during pro- and remotion, but move laterally and medially and, viewed laterally, the distal end of each gnathobase follows a somewhat elliptical course.

A complete cycle of limb movements in an individual of *D. obtusa* feeding at room temperature is shown diagrammatically in figure 124. This records the time sequence of movements for each limb. These times are more precise than it is possible to show in figure 121, *a–i*. A complete cycle was achieved in *ca.* 190 ms (180 ms in figure 121). Promotion of limbs 3 and 4 spanned a period of about 100 ms if their brief periods of acceleration and deceleration are ignored. This is the phase of the cycle during which the interlimb spaces are being enlarged and water is being sucked through the filters. If, during the brief period that trunk limb 5 is not fully sealed in position at the end of its promotion, suction through the filter of limb 4 is not very effective, then, as a close approximation, filtration is effective for about half the cycle. To know the time spent in filtration is important: the longer the period taken to filter a fixed volume of water, as in this case, the slower the velocity of flow and the more energy-efficient the process. In their calculations of the energetics of filtration, Brendelberger *et al.* (1986) and Geller & Knisely (1988) assumed that there is flow through the filter, not only by suction during promotion, but also as a result of pressure during remotion of the limb, which is not the case, and that there is a pressure drop across the filter throughout the cycle. In fact, this condition obtains for only about half the pumping time. On the other hand, Gerritsen *et al.* (1988), who seriously misinterpreted the feeding mechanism, thought that 'the time available for sieving may be as much as 0.25 of the entire cycle, but is probably less', which is a gross underestimate.

The time spent expelling water posteriorly, a process that is energetically much cheaper than sucking it through a close-meshed filter, is about 60 ms plus a very short period when the limbs are accelerating or decelerating. Thus the time taken to expel the filtered water is roughly 60% of that devoted to its filtration, or a trifle more if filtration time is lost as limb 5 is sealed firmly into position.

Although flow is intermittent during the feeding sequence, the pauses that separate phases of limb activity are so brief, a total of no more than *ca.* 30 ms for both pauses in an individual operating at *ca.* 5 Hz, that it appears to be virtually continuous. That it is indeed intermittent is, however, readily shown by use of coloured fluids. The expulsion from the carapace chamber of water previously sucked into the interlimb spaces can then easily be seen to take place in a series of rapid pulses. In a stationary individual, these appear as near-concentric 'ripples' of colour of which, because of the viscous forces involved, several persist at any one time before those at the periphery enlarge and eventually lose their identity. The intermittent nature of the flow is of course readily revealed by high-speed cine-photography. Because of the small dimensions involved, viscous forces are important and there is no risk of particles being lost from the filter chamber during the brief pauses, even when the animal is swimming.

Before the further fate of collected particles is discussed, several points on which the account of the process described thus far differs from those of Storch (1924, 1925) and Cannon (1933) require mention. Both authors agree that the major flow of water into and through the filter chamber takes place posteriorly, as is indeed the case. The two major, unbroken, arrows on Cannon's figure 20 (showing currents 1 and 4) are however somewhat misleading, suggesting as they do an uninterrupted flow from outside the carapace chamber, into the latter, into the filter chamber and through the filters, whereas the flow is intermittent. Nevertheless, the general picture is as described here. The minor current indicated by Cannon in the vicinity of trunk limb 1 is not caused by that limb (which in some species moves scarcely at all during rhythmic limb beating), but is a peripheral consequence of the pumping that takes place posteriorly.

Cannon believed that particles collected by the filters were ultimately propelled along the food groove by currents (see below). The main source of the alleged current arises, he believed, as an outcome of the remotion of limbs 3 and 4. He believed that, as these limbs near the end of remotion, each interlimb space is divided into an inner median and an outer lateral compartment by the corm of the limb. He claimed that while water from the outer compartment is ejected as described in this account, that from the small median compartment is squeezed out as a jet into the food groove and forward, and that this provides the means of transporting food along the groove. I have not been able to confirm the existence of such a current, and certainly disagree with the idea that food is so transported in the food groove. There are various reasons why such transport would be impossible. A mechanical means whereby this is achieved is described below. Storch also describes mechanical transport of the food particles.

In connection with the alleged anteriorly directed current in the food groove, Cannon made another suggestion that I believe to be incorrect. He believed that an important contribution to this current is made by the fifth trunk limbs, during promotion. According to his account, just as the fourth limb is finishing its backstroke (remotion), 'the lateral parts of the fifth limb swing forward as a door pressing on the water in the interlimb space' from the median compartment of which he claimed the water has only one way out and that 'it must be directly pushed forwards by the moving fifth limb'. Apart from doubting the existence of the median compartment, I do not believe that the fifth limb ever pushes water anywhere. Its function is that of a seal, as Cannon was aware, and it has no role in propulsion. Its swing during promotion is not such as to propel water. It begins to swing as trunk limb 4 is completing, or has completed, remotion. Any water still being pushed back by limb 4 escapes around it. As soon as limb 4 begins promotion, the sealing setae of its exopodite fall into place to seal the interlimb space ventrally and the limb is ready to set up suction as limb 5 completes it promotion. It is the suction set up in interlimb space 4/5 that locks limb 5 into position. Until limb 5 is in the fully promoted position, any pressure in the relevant interlimb space leads, not to water being pushed forward, a role that limb 5 is not equipped to perform, but to its escape posteriorly from the space. The feeding mechanism of *Daphnia* is a complicated process, but this alleged refinement has no part in it.

An important way in which this account, like that of Cannon, differs from that of Storch (1924, 1925), concerns the remotion phase of the cycle of trunk limb movements. This is the phase during which filtered water is expelled posteriorly and, simultaneously, as an inevitable consequence of this process, though completely separate from it, water and its contained particles are drawn into the carapace chamber ventrally. According to Storch, filtration is a continuous process. He describes how, during remotion (which he refers to as the 'Adduktionsphase'), the filter plates of limbs 3 and 4 approach their partners on the opposite side, which is true. However, he goes on to say that the region of the gnathobase of limb 3 from which the row of filter setae arises, meets the same region of the limb of the opposite side, and closes the filter chamber ventrally. The filters of opposite sides are then said to be brought closer together and, as the filter chamber is allegedly closed, water is said to be squeezed through the filter plates. Because the filter plates are so curved that their convex faces oppose each other (e.g. figures 65 and 111–113), their coming together in the manner suggested by Storch would inevitably necessitate flexure of their setae in the opposite direction, so that the normally convex face of the filter plates became concave. This would be a complex, and I believe impossible, operation. The setae are curved in two planes (see figure 3 for their anterior–posterior curvature), and change from convexity to concavity would involve passing through a straight phase when their effective length increased. Such a change would also interfere with the arrangement of the filtratory setules. Nevertheless, diagrammatic representations of this alleged process are given by Storch (1924) figure 20 and (1925) figure 6. Although it also provides him with an explanation of his 'vertikaler Transportström' (see below), the process that he describes does not conform with my observations, nor with those of Cannon (1933) or Eriksson (1934).

I have been unable to observe, either by direct viewing or in cine film, contact between the region of the gnathobase of trunk limb 3 that lies alongside the bases of the filter setae and the same region on the opposite limb; nor could Cannon and it does not seem possible for a seal to be established here. Anteriorly, setae of trunk limbs 1 and 2 prevent these regions from making contact. If a seal were to be effective it would have to be established along the entire length of the gnathobase. The filter chamber would also have to be sealed anteriorly, which is impossible.

To observe the positions of the filter plates at the relevant point in the cycle it has been found helpful to employ reflected light in the examination of animals glued to a needle of glass. Animals so illuminated can be viewed so that the filter plates can be seen from the posterior end of the body, or obliquely so, as well as from other advantageous angles. Reflected light causes the filter plates to shine and appear as solid silvery walls. So illuminated, from some angles they produce irridescence, the structural colours changing as they swing. At low magnifications, the whole filter plate can be seen as it goes through a cycle of movement. Except possibly at their tips, which is quite different from what is alleged to take place by Storch, no contact between opposed filter plates has been seen. Furthermore by virtue of the curvature of the filter setae (e.g. figure 112), their proximal parts never approach each other as closely as do their more distal portions. Only the improbable flexure in the opposite direction shown in Storch's diagram could accommodate such contact.

There is a further fundamental reason why the mechanism suggested by Storch could not possibly operate. It is curious that, while he fully understood the role of trunk limb 5 in sealing the system during the suction (filtration) phase, he did not appreciate that his alleged squeezing of water from the filter chamber during remotion of the limbs implies its passage into the interlimb spaces during the period in which these spaces are *diminishing* in volume, and are eventually obliterated. Even if water could be forced through the filters there is nowhere for it to go at this phase of the cycle. Apart from the improbable flexing of the filter setae and sealing of the filter chamber, this alone rules out the operation of his suggested mechanism.

Cannon (1933), who did not elaborate on the mechanical improbability of the process, objected to the likelihood of water being squeezed into the interlimb spaces at a time when pressure there is high. It is in fact the diminishing volume of the spaces that rules out any such influx, even if a squeezing device had been available, which is not the case.

The conclusion is inescapable: there is no closure of the filter chamber during remotion, and no pressing of water between the filter setae during this phase of the cycle. Filtration takes place only during the phase in which water is being sucked through the filters. This point is of more than intrinsic interest. The most widely accepted calculations of the energy expended in filtration have been based on the assumption that the process is continuous, which is not the case.

Cannon (1933) in particular has explained to a considerable extent how material collected on the filter plates is removed from them and transferred to the food groove prior to its forward transport to the mouth. His account is here amplified and to some extent modified.

The filter plate of trunk limb 3 is cleaned in several ways. Cannon noted the band of setules, in robust species they are more spinule-like, that runs along the gnathobase of trunk limb 4 adjacent to where the row of filter setae of this limb arise, and explained how these clean material from the posterior part of the filter of trunk limb 3 as the latter swings forward relative to limb 4. The nature of this band is *D. atkinsoni* f. *bolivari* as revealed by SEM is seen in figures 102 and 103 (CLS). The relation of this band of setules and spinules to the overlying filter of trunk limb 3 is clearly seen in figure 76, and in transverse section in figures 77, 78 and 112. If one makes a dissection of limbs 3 and 4 in which these limbs are left attached to the trunk and mounts the ensemble in an ample volume of polyvinyl lactophenol so that the limbs lie more or less as in life, it can be seen how these spinules can pass between the filter setae and remove material from them. Where adjacent rows of filtering setules are hooked together (Watts & Petri 1981), the hooks will 'unzip' as the spinules part the adjacent rows, and re-hook as the setules come together.

More anteriorly the filter plate of trunk limb 3 is cleaned from the inside by elements of the armature of the gnathobase of trunk limb 2 as both Storch (1924) and Cannon (1933) record. The long posterior filter-cleaning spine swings as described above. Its amplitude of swing is large and it swings much more rapidly than the filter setae. Although it has to be viewed through the carapace, and for part of its cycle through adjacent trunk limbs, and although it is usually momentarily lost to view at the end of promotion or beginning of remotion, its general beating towards and away from the food groove can often be clearly seen in lateral view. Generally it sweeps the entire length of the filter setae, but occasionally it has been seen, in *D. galeata*, to cease promotion before reaching the end of the swing and therefore to sweep over only the distal portions of the setae. In the same species it has also been seen to swing so far on remotion that its tip touched the food groove and actually bent slightly. Thus although motion of this spine is usually regular and rhythmic, it is not completely stereotyped and can be adjusted, presumably according to circumstances. In *D. obtusa* the long gnathobasic spine of one limb has occasionally been seen to be slightly out of phase with its partner, so that both could be seen in lateral view. This spine is assisted by its adjacent, smaller, companion.

That one function of the row of median gnathobasic spines anterior to the long filter-cleaning spines of trunk limb 2 is to brush material from the adjacent surface of the filter plate of trunk limb 3 was appreciated by both Storch and by Cannon and is readily confirmed. Figures 59–61, 70–75, 81, 82, 87, 88 and 111 reveal the exact relations of these spines to the filters that they clean. Figures 71–75, in which the relevant spines of gnathobase 2 have been cut more or less transversely, show the brush-like nature of their armature particularly clearly and reveal how intimate is the relation of these brushes to the filter setae that they clean. The limits of that anterior portion of the filter plate of trunk limb 3 which is cleaned by these setae can readily be appreciated by reference to figure 3.

The gnathobase of trunk limb 2 is provided with a further means of removing material from the filter plate of trunk limb 3 that has not previously been described. This is the uniseriate row of spinules (CLS) that lies adjacent to the insertions of the gnathobasic spines shown in figures 49 and 110. This inevitably sweeps material towards the tips of the adjoining filter setae as limbs 2 and 3 swing relative to each other. The relation of the spinules to the filter plate of trunk limb 3 is readily appreciated from figure 110.

There is yet a further cleaning device of the filters of the third trunk limb. This is a strip of setules or spinules on the wall of the food groove that combs the tips of the filter setae. In some species that strip is made up of fine setules: in the robust *D. atkinsoni* f. *bolivari* of more robust spinules. Such a device is widely employed in filter-feeding branchiopods. As Cannon (1933) noted, these setules are very difficult to see by ordinary microscopy. They are, however, more clearly revealed by the use of Nomarski optics and the row of spinules of *D. atkinsoni* f. *bolivari* is well shown by SEM (figure 101 and inset).

Cannon (1933) noted that Storch (1924) mentioned these setules and went on to say that, 'for some reason not stated, suggests that they represent the missing maxilla'. Thus baldly stated, Storch's suggestion would appear to be ridiculous. In fact Storch is less emphatic than Cannon implies, saying that, in his opinion, the setules most probably represent a rudiment of the maxilla, and in his figure 15 he labels them 'Dornen Rudiment der 2 Maxille?' His figure is a transverse section in the region of the gnathobases of the second trunk limbs, and therefore cut in the vicinity where one might expect to find maxillae if they were present. Storch was presumably unaware that these setules extended along the entire food groove, so the reason for his suggestion is understandable, though he was completely misled and his tentative deduction was erroneous.

Cannon says that 'the residue on the fourth limb is combed off entirely by the third limb', but this is not so. Although it extends posteriorly sufficiently far to overlie most of the filter plate of the fourth limb, the filter plate of trunk limb 3 cannot reach its extreme posterior region, which is cleaned by means not reported by Cannon. Much of the filter plate of trunk limb 4 is indeed cleaned by devices on the filter setae of limb 3. Cannon showed that the tips of the posterior filter setae of limb 3, that is those that overlie the filter plate of limb 4, have modified tips. As he noted, details are very difficult to make out by ordinary optical microscopy, but involve a reversal of the setules to form a brush-like arrangement (figures 74 and 75, BS). This is well seen by the use of Nomarski optics (figures 84 and 85) and SEM (figures 98 and 99).

The few posteriormost filter setae of trunk limb 4 are cleaned by an array of setules borne on the adjacent part of trunk limb 5. These are difficult to see in sections, but some are seen in figures 114 and 115, and on a dissected limb in figure 55.

A small contribution to the cleaning of the tips of the fourth trunk limb is also doubtless made by the setules or spinules of the food groove wall.

Thus every part of the filtering surface is provided with the necessary means of removing from it material that it has filtered out. The result is the transfer of material from the filters to the food groove. As Eriksson (1934) was aware, there is no need for any 'vertikale Transportströmung' that, according to the account of Storch (1924, 1925), originates as a consequence of the closing off and 'squeezing' of the filter chamber, and carries particles towards the food groove. As noted above, I have been unable to confirm the sealing off of the filter chamber, without which neither filtration by squeezing nor the establishment of a 'vertical transport stream' is possible.

Water expelled posteriorly follows the route of least resistance. It is possible that there is a slight leakage through the filter plates, in the opposite direction to that followed during filtration, as the interlimb spaces diminish in size, but this would be extremely difficult to demonstrate. The slight medial movement of the filter plates during remotion would not affect this. Any such leakage would help to dislodge filtered particles from the setules and would facilitate the operation of the various mechanical cleaning devices.

As a result of the processes thus far described, filtered material accumulates in the food groove, much of it towards its posterior end. There has been disagreement as to how it is passed forward to the mouthparts. Cannon (1933) maintained that it was swept forward by currents emanating from interlimb spaces 4/5 and 3/4. This was in keeping with his belief that orally directed currents flow in the food groove of other branchiopods (Anostraca, Ctenopoda). While I am unable to confirm the existence of such currents, other than those inevitably set up by the forward component of swing during remotion of the filter plates of limbs 3 and 4, there is one way in which particles are sometimes moved forward by non-mechanical means (see below). The situation is in keeping with the results of observations made not only on other anomopods of the families Macrothricidae and Chydoridae (Fryer 1963, 1968, 1974) whose feeding mechanisms are, I believe, more similar to those of the ancestral anomopod stock than are those of the Daphniidae, but also with those made on the Anostraca (Fryer 1966, 1983). In all cases, food is moved forward by mechanical means. Even if, as is conceivably the case, spurts of water were to enter the food groove from the inter-limb spaces, and even if these tended to flow anteriorly, they would often be ineffective in passing material forward. As is even more the case in the Chydoridae (Fryer 1963, 1968), where scraping often brings in large accumulations of material, particles are sometimes collected and accumulated by *Daphnia* faster than they can be ingested. They therefore pile up in the food groove in which they are compacted, posteriorly by the action of the tips of the filter setae, more anteriorly by the more posterior armature of the gnathobase of trunk limb 2, thereby blocking the food groove. It is indeed a frequent occurrence for material, compacted into what Storch (1924) aptly referred to as a 'sausage', to accumulate posterior to the mouthparts which deal only with small amounts at each cycle of movement. Such accumulations completely block the food groove so that it would be impossible for currents to flow forward along it. Were the forward transport system to depend on such flow, the entire feeding mechanism would be rendered inoperative. Likewise, large items are collected at times, not only by such bottom-grubbers as *D. magna*, but by slender planktonic species such as *D. galeata* that sometimes accumulates diatoms and other algae in the anterior part of the food groove. Such accumulations bespeak of other than a hydraulic transport system. The contents of the food groove are readily seen in the transparent *D. galeata*. When this species is amply supplied with carmine particles, it sometimes fills the food groove from end to end, a state of affairs that is incompatible with the flow of water there.

There is one way in which currents move particles forward. Throughout much of the cycle the dorsal (deep) portion of the filter chamber is wider at its posterior end than it is anteriorly. At the very end of remotion, however, this region narrows. As water is being expelled from the diminishing interlimb spaces, that which is located in this region of the filter chamber cannot pass through the filters and is squeezed forward. This was seen particularly well in *Simocephalus* viewed ventrally and supplied with *Lycopodium* spores, but the situation in *Daphnia* is the same. Such 'flow' is not a jet along the food groove, but may sometimes assist the forward transfer of particles. It certainly cannot convey material along the food groove in the manner of Cannon's alleged currents. It may have been the movements described here that he saw. Similar movement of particles has been seen in *D. magna* immersed in a syrupy solution of 'cellulose nitrate', where some particles find their way to the food groove, showing incidentally that some filtration takes place even in this viscous medium.

These observations also confirm that there is no squeezing of water through the filters at this phase of the cycle.

I therefore agree with Storch (1924, 1925) that food particles are swept forward along the food groove by mechanical means. As Storch (1924) noted, the tips of the filter setae, especially those located posteriorly, are curved anteriorly and can push material forward. This they do towards the end of each phase of remotion, at which point their action is accentuated by the 'lunging' previously described. Not only are the tips of the setae, other than those of limb 3 that clean the filter plate of limb 4, suited to this but, towards the extreme tip of the seta, the setules that, more proximally, form the filtering meshwork are slightly stouter and diverge slightly from each other towards their tips. They here serve as brushes and not as filters. Such tips, of setae towards the anterior end of the filter plate of trunk limb 3, are shown in figures 100 and 101.

The work of passing material forward is facilitated by the action of the long posterior filter-cleaning spines of the gnathobase of trunk limb 2 that sweep it into the food groove where it is conveniently placed for the tips of the filter setae of trunk limb 3 to act upon it. Once within reach of the rest of the armature of gnathobase 2 its progress towards the mouthparts is self-evident. As Storch (1924) noted long ago, the median row of setae help to force it into the food groove and forward. It is essential that food is here forced deeply into the food groove so that it can be pushed to the maxillules. The umbrella-stay spines and the brushes that clean the adjacent filter plate do this (figure 111). The brush-like tips (see SEM photographs in Watts & Petri (1981) for *D. magna* and figures 87 and 88 for *D. atkinsoni* f. *bolivari*) facilitate sweeping forward. Food in this region is seen in figures 36, 37, 61 and 69. Cannon (1933) agrees that these setae force food into the food groove, but does not mention their role in sweeping it forward. He maintains that the food groove here is very deep and that water streaming forward along it rises to pass into inter-limb space 1/2 whereby it escapes laterally. Even if a current were to flow here as suggested by Cannon, it would have to contend with the blocking array of spines of the gnathobase of trunk limb 2, that lie adjacent to the food groove at that phase of the cycle when the alleged spurt occurs, and with the viscid labral gland secretions that, as Cannon himself notes, are swept towards the food groove by the gnathobasic armature of trunk limb 2. These secretions help to bind particles together and facilitate later handling. If a current were to flow as suggested by Cannon, it would tend to carry these secretions away from the scene of operations.

The employment of entangling labral gland secretions was first noted, in *Simocephalus*, by Cannon (1922) and this role has since been amply shown in many anomopods (Fryer 1962, 1963, 1968, 1974) and is employed by other branchiopods. Sterba (1957) considered whether these secretions contain digestive enzymes but found that sequestered gland cells of *D. magna* had no influence on starch and glycogen and Zeni & Franchini (1990) confirm the absence of α-amylase. As yet there is nothing to support the suggestion of Schram (1986) that they may serve as surfactants or 'wetting agents', but roles other than entangling should not be dogmatically ruled out. Schram is impressed by the sometimes large storage capacity for entangling secretions which he thinks might suggest a function other than agglutination. Feeding in anomopods is, however, generally an almost continuous process and a continuous supply of entangling secretions is therefore necessary. The amounts stored by *Daphnia* are less than in some benthic, scraping anomopods, some of which produce them from trunk limb as well as labral glands. Such species sometimes collect large amounts of food in a short time. Labral gland secretions (LGS) are seen in figures 28 and 69.

Storch (1924, 1925) and Cannon (1933) are agreed that the three anterior spines of gnathobase 2 pass food to the maxillules. Storch notes how they are suited to slicing pieces from the 'sausage' of compacted food and passing them forward. The maxillules continue the process, passing material to the mandibles.

The asymmetry of those major transverse mandibular muscles that originate on the transverse mandibular tendon (figure 31) has the same effect as described in detail for the mandibles of *Eurycercus* (Fryer 1963): the right mandible swings more than the left. In *D. magna* its swing is greater than in *Eurycercus*. Furthermore it swings more persistently than in *Eurycercus*. Usually the mandibles swing together, but sometimes the left remains stationary as the right swings. This confirms the observation of Ocioszynska-Bankierowa (1933) who was apparently the first to record that 'Zuweil die eine Mandibel unbeweglich bleibt, wahrend die andere ihr Kauflache auf ihr in Reibung setzt'. Mahoon (1960) also noticed that in *D. pulex* the left mandible is also almost stationary, a fact for which he was unable to provide an explanation.

The exploitation by *Daphnia* of both muscular and skeletal asymmetry to increase the efficiency of crushing food and passing it forward, represents the culmination of a trend that can be traced in various branchiopods. In the Anostraca the transverse mandibular muscles are symmetrical and both mandibles swing actively: many anomopods have acquired an asymmetrical system in which the left mandible swings much less than the right: in *Daphnia* it is at times almost stationary.

As in other branchiopods with rolling, crushing mandibles, before the commencement of the remotor roll, there is posteriorly a gap between the masticatory surfaces, triangular in section in the horizontal plane, into which food can be pushed. In *D. magna* the gap between the mandibles can be widened by a greater, but still small, amount of abduction than has been seen in, for example *Eurycercus*. In rolling mandibles of this kind there are no direct abductor and adductor muscles, but some small degree of abduction can be granted by contraction of muscles of the 5d series located towards the articulation of the mandible, and corresponding relaxation of muscles of the 5g series that are located nearer to the masticatory regions (see figure 18 in Fryer (1963) and Manton (1964) for the simpler system in the Anostraca for the principles involved). True biting is, however, far from being achieved.

Rates of mandibular roll vary much according to the amount of food with which they have to deal, so casual measurements are not particularly helpful. In *D. rosae* Burns (1968) recorded rates of up to about 190 rolls per minute though this was exceptional and at lower food concentrations the rate was often less. At 18–21 °C the rate of roll increased with the concentration of yeast cells available until it achieved about 150 rolls per minute, a rate maintained thereafter over a several-fold increase in food concentration. Similar results were obtained by McMahon & Rigler (1963) for *D. magna*, but the rate of roll settled down at roughly 125 rolls per minute. The mandibles often remain at rest, sometimes for many seconds.

At the end of the remotor roll, the gap which existed posteriorly between the opposed masticatory surfaces has, as it were, been carried forward and a similar gap now exists anteriorly. This can be seen in figure 29 where the mandibles lie in a more or less full remoted position. Food particles that enter the posterior gap are rolled forward and expelled anteriorly in the vicinity of the atrium oris, whence they are sucked up the oesophagus by peristalsis.

If excess material accumulates at the anterior end of the food groove, it is raked out by the conspicuous ejector hooks, figure 68, just as in chydorids and macrothricids (Fryer 1963, 1968, 1974), so that it can be swept out of both the filter chamber and carapace chamber by the post-abdominal claws. Trunk limb 1 is extremely mobile and the ejector hooks can easily reach into the anterior end of the food groove. Their shape and armature are well suited to their task. Watts & Petri (1981) report that they also clean the inside of the carapace and perhaps the labrum.

It is not only material dislodged by the ejector hooks that is removed by the post-abdominal claws. Often large accumulations of material enter the filter chamber posteriorly and, before these have had time to be fully processed and passed forward along the food groove, the post-abdomen swings, and its claws, seen in figure 114 (PAC), remove them. To clear excess material from the filter chamber the post-abdomen is first drawn towards the trunk by contraction of the post-abdominal adductor muscles that are located on its morphologically ventral side. As it approaches the trunk, making a hairpin bend against it, the trunk itself begins to flex ventrally and therefore to carry the post-abdomen forward as the ventral longitudinal trunk muscles contract. This inevitably increases the capacity of the brood pouch. The post-abdominal processes that retain the eggs or embryos, also swing forward with the post-abdomen so that retention of the brood pouch contents depends momentarily on pressure from the carapace and the narrowness of the aperture postero-ventrally. Reversal of the process is initiated as the trunk begins to straighten again. As it does so, the post-abdomen is swung rapidly backward; that is the angle between it and the trunk increases from narrowly acute to obtuse, and has passed 90° before the trunk has swung back more than a small amount. The angle made by the post-abdomen and trunk continues to increase as the former swings outside the carapace chamber, its claws carrying away unwanted material, and the trunk straightens. The post-abdomen then swings forward again to the 'resting' position.

The post-abdomen can reach so far forward that its claws emerge from the carapace chamber just behind the head. These claws can therefore scour the entire filter chamber.

Watts & Petri (1981) make the important observation that the post-abdominal claws serve also to clean the filter setae, especially those of trunk limb 3, an action which they liken to feather preening in birds. Setae are separated from their neighbours by 'unzipping' the rows of interlocking setules, freeing particles that have lodged there. As the setae spring back into place, the hook-like tips of their setules re-link and the integrity of the filter is restored. It is in this aspect of cleaning that the role of the row of fine denticles present on the claws of all species of *Daphnia* (figure 114), irrespective of whether or not they bear a basal comb, receives an explanation.

Accounts which claim that the filters behave as 'solid walls' and function as paddles, and that filtration is affected by the first and second pairs of trunk limbs leave many matters unexplained. For example, they do not explain how filtration can be carried out by appendages not equipped to filter, or how the alleged paddles work, or give an account of the course of the currents they are presumed to establish, nor do they provide an explanation of the role of such structures as the exopodites of trunk limbs 3 and 4 or the various filter-cleaning devices.

If what are in fact filters were paddles, one would anticipate a different kind of structure from that which is obtained. A meshwork of regularly spaced setules borne on a uniseriate row of setae can indeed form a paddle. A good example is the pretarsal fan of the surface dwelling veliid bug *Rhagovelia*, of which an excellent illustration is provided by Andersen (1982). But the details of this are very different from those of a daphniid filter. The arrangement is fan-like and the individual setules overlap each other, especially near the base of the fan, thereby reducing permeability and presumably giving mutual support. The arrangement is one that offers maximum resistance to the water, as required of a propulsive fan, and is different from that of a filter. The filter plates of *Daphnia* are clearly not paddles. If they were impermeable and behaved as 'solid walls', the movements that they make would be impossible. Expansion of the interlimb chambers is possible only because water can enter via the filters as suction proceeds.

The description provided here explains the function of every limb, accounts for the way that suspended particles are brought into the carapace chamber, how water is drawn into the filter chamber, through its walls into the interlimb spaces, and forced out as the exhalent stream; how particles are filtered; how every part of the filter can be scoured of filtered particles, seven separate devices being involved, and how the particles are passed to the food groove and thence forward to the mouthparts. The whole process forms a complex integrated mechanism. If any one part of the process did not operate as described, the whole system would be rendered inoperative. What is more, many aspects of the process are easy to observe. The comparative approach also enables one to understand how this mechanism has been derived from one employed by the sort of benthic ancestors from which the daphniids were probably derived. It makes phylogenetic sense.

In one important respect the mechanism in *Daphnia*, and other anomopods, differs from that of filter feeding copepods. In copepods, the filters are not part of a closed system, but afford a stream of water the opportunity of flowing around them. This is not so in filtering anomopods. As Koehl & Strickler (1981) have noted, notwithstanding the viscous forces involved, water can be forced between very narrow gaps when given no other escape route. Anomopods exemplify this principle to perfection.

Much discussion has been devoted to the question of how much selection can be practised by a feeding *Daphnia*. Several selective devices operate. Large particles can be excluded by restricting the width of the ventral gape of the carapace. In some species the ventral carapace margins are provided with setae, whose roles inevitably include that of a protective screen. As noted earlier, gape width can be adjusted rapidly by the carapace adductor muscles and, as a temporary expedient, the valves can be closed completely if necessary. Such selection is aimed at the exclusion of large particles.

The mesh size of the filters inevitably determines to some extent what is captured, selection being aimed at allowing the smallest particles to pass through. Different mesh sizes are most efficient for particles of different sizes (see, for example, Brendelberger (1985); DeMott (1985); Hessen (1985)), and various observers have shown that, in some species at least, mesh size changes, not only with increase in size, but in ways that appear to be correlated with environmental conditions, especially the amount of food available (see, for example, Geller (1985); Korínek & Machácek (1980); Koza & Korínek (1985); Korínek *et al.* (1986)), and, in *Simocephalus*, Mangalo (1987). These changes can take place at successive moults.

Different species have different mesh sizes and therefore inevitably tend to abstract particles of different sizes (see, for example, Geller & Müller (1981)), a potential means whereby different food niches can be exploited by coexisting species. Lampert (1987) gives a table of mesh sizes derived from various authors (but note the inaccuracies in some of the original papers).

Nevertheless, what is filtered depends to a large extent on what is available and both suitable and unsuitable material is abstracted. Much that is indigestible or intractable is ingested, which presents few problems to an animal that, when particles are abundant, passes material through the gut in a few minutes. Other unsuitable material collected in the filter chamber is rejected. It is removed by the post-abdominal claws, either after having been forked out from the anterior end of the food groove by the ejector hooks of the first trunk limb, or directly from the posterior end when large masses accumulate there. As noted elsewhere (Fryer 1987*b*) selection can be rigorous. Objects like *Lycopodium* spores can be abstracted and passed to the anterior end of the food groove in hundreds, or even thousands, without a single one being ingested, whereas carmine particles are readily swallowed.

It is impossible for filters to be opened or closed like a fan, an action which Crittenden (1981) suggested might alter their 'pore distribution' but which, not surprisingly, he never saw. There is no means of moving the setae, and any movement would destroy the filter. The impossibility of adjusting the mesh size of filters of this sort by fan-like expansion of their setae was dealt with when rebutting the suggestion of Ganf & Shiel (1985*a*, *b*) that the gnathobasic armature of trunk limb 2, which is neither a filter nor capable of such movements, serves as a filter whose mesh size can be regulated in this way (Fryer 1987*b*).

(*l*) *A critique of some recent views on the feeding mechanism*

While it is generally agreed that the abstraction of particles is carried out by trunk limbs 3 and 4 (see references on p. 44) Ganf & Shiel (1985*a*, *b*) gave accounts of the feeding mechanism of *Daphnia* in which it was claimed that filtration is carried out by trunk limbs 1 and 2 and that the filters of limbs 3 and 4 act as paddles. As shown elsewhere (Fryer 1987*b*), these accounts completely misinterpreted morphology, identified cuticle-secreting strands of tissue as muscles, whose alleged action would be impossible even if they were such, attributed a filtering role to structures incapable of filtration, and provided a completely erroneous, and indeed bizarre, description of the feeding mechanism. It would have been unnecessary to refer to these papers again had not Shiel, Ganf and Gormley presented a paper at a conference in 1987 in which the results of cine photography were described and in which they persisted in their views, reporting that 'all particles were handled by limbs I and II, with no evidence of a sieving function by III and IV'. This is particularly curious in view of the fact that, although they seek to deny that the filters of trunk limbs 3 and 4 function as such, Shiel & Ganf (1987) published another paper that is based largely on the assumption that they do! This deals with the dimensions of the filtering setules and inter-setular gaps in three species of *Daphnia* (and other daphniids), relates spacing to body length and seasonal changes, and uses such expressions as 'the filtering area of limbs III and IV', 'filter dimensions' and 'each filtering limb' (implying limbs 3 and 4). Only at the end of the paper do they say that 'an analysis of flow patterns in the vicinity of the third limb' showed that Re was very small. In fact no such analysis is reported, no observations of flow anywhere in the system are recorded, and indeed no observations on living animals are described. Because a small Re implies laminar flow and relatively large boundary layers, they suggest that the animals 'are *likely* (my italics) to have flow patterns across, not through, the third and fourth limbs'. Observations on a living animal immediately show this supposition to be incorrect. There is nowhere to which water can go if it flowed merely 'across' the filters. Unfortunately only a brief abstract of the subsequent conference paper was published (Shiel *et al.* 1988) though this promises a more comprehensive account elsewhere. As yet, this has not appeared and the authors kindly inform me that the paper is still incomplete. As described here; as was well known to Cannon and Storch half a century ago; and indeed to Jurine in

1820; as is accepted by most recent investigators; and as can be seen by spending a few minutes watching a living *Daphnia*; it is the filters of trunk limbs 3 and 4 that abstract particles from the current, drawn by the pump described in this paper, through the cage which they comprise. Trunk limbs 1 and 2 have nothing to do with particle abstraction, of which they are incapable, lacking as they do any means of filtration and being specialized for quite other roles. Furthermore, they are so located that, even if they could filter, only a small fraction of the particle-bearing current flows in their vicinity. A detailed critique of some of the major shortcomings of the two earlier papers is given elsewhere (Fryer 1987*b*).

Gerritsen *et al.* (1988) have also given an account of certain aspects of the feeding mechanism of *Daphnia*. They traced the course taken by fluorescent dyes and particles as revealed by epifluorescent video image analysis and high speed cine-photography, and also the course followed by a stream of Indian ink as it passed through the filter chamber. The observations on the course taken by particles are shown by five crude line drawings that give no indication of the complex morphology involved. The first two show the course usually followed and, while sufficient to refute the claims of Shiel *et al.* (1988), merely confirm what Jurine described as long ago as 1820. The other three show the routes taken by particles that failed to enter the filter chamber or which became temporarily stuck to a limb, and are of no relevance to the feeding process. Gerritsen *et al.* claim to have seen particles captured by limbs 2–5, but say that 'the majority of particle retention occurs on appendages 3 and 4' as is indeed the case, and that 'most captures were on or near the ends of the filtering setae of appendages 3 and 4'. While an occasional particle may stick briefly to limb 2 or be retained by the vertical setae of limb 5, these limbs play no direct role in particle abstraction. It is also reported that much of the flow observed is tangential to the surface of the filter plates and not through them, but this does not preclude water from passing 'perpendicularly' through the intersetular spaces at the moment of filtration.

The course of a blob of Indian ink through the filter chamber is shown by prints from six successive frames of a cine film in which a complete cycle was enacted in eight frames. While the progress of the ink can be followed, the photographs are so lacking in detail that, even for one familiar with the animal, it is scarcely possible to recognize any limb, let alone details, and it is details that are required. The description of limb movements is also inadequate and I would interpret what can be seen in a different way from Gerritsen *et al.* (1988). The first two frames are said to show promotion (which they call the forestroke), the second to show the end of the process. Here the ink blob, said to be just inside the carapace chamber in the first frame, can be seen to be drawn in, incidentally from a more anterior position than is usually the case with food particles, and must be passing obliquely posteriorly and towards the food groove. These two frames do indeed show promotion, which is probably in an early stage in the first. The third frame is said to show the 'instroke' where the filter plates are said to come together and to constrict the blob. In fact, the limbs are still clearly in promotion and, far from being constricted, the distal extremity of the ink blob, which lies deepest in the filter chamber, is beginning to be *drawn* between the filter plates of each side, a process that continues in the fourth frame where, on each side, the ink can be seen passing into the interlimb spaces, even if the limits of the latter cannot be discerned. The location of the ink streams, one only on each side, suggests that they reveal only interlimb spaces 3/4. No ink lies in a position to show the more posterior interlimb spaces. This frame is said to show the end of the 'instroke' with ink jets spurting out laterally. No such spurting occurs, nor is it mechanically possible. Water under pressure, as implied here, would follow the route of least resistance: it would not spurt through the filters. Water is sucked into the expanding interlimb spaces via the filters as the two streams of ink clearly show. This is one thing that the photographs unambiguously reveal. Even though the filter plates have now moved towards each other, as they do towards the end of promotion, they do not, and cannot, squeeze water through their meshes. It is sucked through, as is readily seen from the photographs. Promotion has ended and remotion has just begun in the fifth frame, which is in agreement with the statement that this and the last frame show the 'backstroke'. In the fifth frame the two streams of water have passed laterally and can be seen leaving the interlimb spaces in the last frame, where they are moving ventrally (towards the observer) as well as posteriorly. The filter plates, while not revealed clearly, are here close to their partners of the opposite side. The two missing frames must show remotion.

Thus, while the film lacks detail, it clearly shows the sucking of a stream of water into the interlimb spaces on each side during promotion. The ink blob is certainly not constricted so that jets squirt out laterally from it on each side. Squeezing, of which there is no indication in the photographs, could never produce such jets, but suction, which is what takes place during promotion of the limbs, can draw out such streams.

Gerritsen *et al.* (1988) are dubious about whether filtration occurs and say that, if it does, it could happen only during the period shown in the fourth frame of their series (when suction and filtration are indeed taking place) which represents only about an eighth of the cycle, but go on to say that 'there may be more motion in the time between successive frames so that the time available for sieving may be as much as 0.25 of the entire cycle', which, had they been correct, would have been a generous estimate. In fact, they are not correct. Promotion and filtration are in progress in the first four frames. It is only the remoteness of the ink blob from the filters in the first two that prevent it from being seen, and it is readily seen in frames three and four. As shown in the present account (see figures 121–125), filtration occupies at least half the cycle, as it can be seen to do in this film.

Gerritsen *et al.* (1988) also separate 'intake', filtration and expulsion as separate processes and say that flow stops between each of them. While basically true, this tends to obscure the fact that 'intake' and expulsion are parts of a common process. During remotion, as water is driven out posteriorly from the interlimb spaces, more inevitably enters the carapace chamber via the ventral aperture to replace it.

Assuming other aspects of the calculations to be valid, the claim that *Daphnia* 'must use 20–60 % of its total metabolism on filtering' (20–45 % is the range given in the abstract) is therefore greatly in error. Because the fraction of the cycle devoted to filtration is much greater than Gerritsen *et al.* believe, the energy expended to filter a given volume is much less than their estimate.

That the mechanism has been misinterpreted is evident from the statement, otherwise amplified only by reference to particles that never properly entered the system, that 'a major flow of incurrent water is expelled from the carapace [chamber] on the fore stroke of the appendages and is therefore not processed to remove particles'. This is not so. Apart from a minor loss noted below, whatever the mechanism, water can only be expelled during remotion of the limbs. It is impossible for it to be expelled during promotion. During this phase of the cycle, there is no flow either into or out of the system: purely internal movements of water take place. Further, during a normal cycle of limb beats, there is only one way through the system, into the filter chamber, through the filters into the interlimb spaces, and out posteriorly. There is sometimes a minor escape from the inhalent current postero-ventrally during remotion, which is generally sucked back into the inhalent stream. Otherwise no part of the inflow is 'not processed'. The 'near misses' reported by Gerritsen *et al.* (1988) are not elements of the normal feeding process. Currents may be disrupted if the animal is observed under abnormal conditions. For example, if an individual lies on its side on a slide in a thin layer of water, its limbs may beat more or less normally but the inflow may be disrupted, probably by surface tension effects, and some water may swirl posteriorly without entering the carapace chamber.

These authors also note that, unlike what they call 'efficient' filter feeders that filter continuously, *Daphnia* does so only intermittently, and imply that it is not an efficient siever, noting that the metabolic cost, here shown to be incorrect, is substantial 'when more efficient mechanisms are available'. Even if the value had been correct, this is to take a curious view of one of the most successful of all filter-feeding animals that, in appropriate conditions, can not only maintain itself and grow rapidly but display enormous fecundity.

The powering elements of most continuous filter feeders that produce their own currents, essentially cilia or flagella, of course have to undergo a recovery stroke. As Dr Q. Bone reminds me, salps are exceptions. They feed by muscular action, jet propulsion used in locomotion producing continuous flow through a filter. Otherwise continuity is maintained only because at any given time, some elements are undertaking the working stroke. As recovery has to take place against a current, it might be argued that it is these systems that are 'inefficient'. The limb exopodite pumps of *Daphnia* never operate against a flow, and work in both directions for much of the cycle. Filter feeding can of course be efficient whether it is continuous, as in *Amphioxus* and many bivalve molluscs, or intermittent, as in certain fishes and in *Daphnia*.

Gerritsen *et al.* are not even convinced that filtration occurs, or that if it does, *Daphnia* is exclusively a filter feeder. They suggest that, if filtration occurred, clouds of diffuse dye would be seen where coloured streams pass through the filters but that they have not seen such. That expectations are not fulfilled sometimes means that they are unfounded. In fact, the passage of a stream of Indian ink through the filter, as seen in their cine photographs, shows some diffusion. One would not expect diffuse clouds.

Gerritsen *et al.* however, are not convinced that all the water passes through the filters. They suggest, as Porter *et al.* (1983) had previously done, that 'the flow may pass under the flexible tips of the setae of the filtering comb'. While it would appear obvious that, were this the case, suspended particles would do likewise, they suggest that the tips of the filter setae 'act as valves to allow the water in the median space to escape into the lateral spaces during the instroke of the appendages, entraining any remaining particles in their boundary layers as they pass'. Terminological problems apart, this is entirely unrealistic and is supported by no positive evidence.

The same authors recalculate the energetic costs of filtration provided by Brendelberger *et al.* (1986). They are correct in criticizing the latter for assuming that there is a permanent pressure drop across the filters. Brendelberger *et al.* accepted the view of Storch that 'pressure' during remotion forces water through the filters, as indeed do Gerritsen *et al.*, but this is not so. If the other elements of their calculations are correct, the cost of filtration, estimated as about 5 % of total metabolic requirements by the methods in question, is therefore too low, but the real figure will still be much less than those of Gerritsen *et al.* which for some size classes are about an order of magnitude greater than theirs. The figures of Gerritsen *et al.* are based on the erroneous belief that filtration takes place during about an eighth, or a possible maximum of about a quarter, of the cycle, when in fact it does so during at least half of it. Their re-calculations are set out in a table of questionable validity which, for example, cites flow rates in centimetres per hour when seconds are intended.

Brendelberger *et al.* (1986) used two models which gave rather similar results. Geller & Knisley (1988) have subsequently calculated the energetic costs of feeding in *Daphnia* by a third method which estimates drag forces on the basis of the theory of slender bodies in Stokes flow as developed by Batchelor and by Cox, whose papers they cite. The results are in good correspondence with the first two models but, like them, assume continuous filtration. As filtration occupies only about half the cycle, the energetic costs will be greater than estimated, but still much less than those calculated by Gerritsen *et al.*

Why the filters, which dominate the trunk limb complex (figure 3), should ever be thought to be anything other than what they so obviously are, and as which they can be seen to operate, is difficult to appreciate. If calculations involving boundary layers, flow regimes and Reynolds numbers suggested that filtration is unlikely, it should have been the calculations, and the data on which they were based, and not the reality of what can easily be observed to take place, that should have been questioned. Many filter-feeding anomopods have such structures that make up a cage into which particle-bearing water is drawn, and from which there is no escape for the water except by passing through the filters. Always these filters, of which there may be up to four pairs, have a similar structure to those of *Daphnia*. Details are given for several chydorids and macrothricids by Fryer (1963, 1968, 1974). There are, however, other species which employ other feeding mechanisms based on the same basic machinery, but which do not involve currents and therefore do not filter (Fryer 1968, 1974). As noted elsewhere (Fryer 1987*b*), in every such case the homologues of the filter setae of filter feeders lack filtratory setules. The filters of *Daphnia* are among the largest and most specialized of all those found in the Anomopoda and it is their employment that has permitted the conquest of open waters where there is completely dependence on their ability to filter suspended particles with great efficiency.

(*m*) *Comments on a model of filtration and on the significance of morphology*

Cheer & Koehl (1987) have given a useful account of aspects of flow through what they refer to as the 'bristled appendages of small organisms', but their model is in several respects inapplicable to the daphniid mechanism. In particular, in daphniids (and indeed in all filtering anomopods and probably in all filtering branchiopods) water is not 'free to move around the sides of the setae ... as well as to move through the spaces between neighbouring setae'. As this account makes clear, all of it is sucked through the filter, the system being essentially that of 'an infinite row of cylinders between which all the fluid is forced to move', which, as Cheer & Koehl emphasize, is one for which their model is inappropriate.

The system they model is very different from that which prevails in *Daphnia*. Thus they note that if shear gradients around the setae of a bristled appendage are thick, the setae cannot 'reach out and grab a food particle because the particle is pushed away by the water moving with the setae'. No such movements occur in *Daphnia* where the filter setae never move into the flow, which is caused by suction from behind the filters. Movements of the filter plates during the suction phase are across the flow. Likewise in the system modelled by Cheer and Koehl, the distal ends of setae 'travel at a greater velocity than do their basal ends', which is not applicable to *Daphnia* (or to other branchiopods) whose filter plates are not swung in an arc against the flow, but essentially cut the water as a knife cuts an object. All parts of the setae move at the same speed. Cheer & Koehl then go on to make deductions of functional significance. In their model, the distal ends have the potential of processing a greater volume of water per unit time than have the proximal regions. In *Daphnia* the reverse is true. Suction begins when only the proximal ends of the setae are available and continues in this region throughout the suction phase, but is effective for progressively shorter periods of time towards the distal ends. It is therefore the proximal regions that process the greatest volume of water. (Possible complicating events in the filter chamber are here ignored, as are important differences in the armature in different regions.)

Cheer & Koehl refer to mechanisms that 'increase leakiness'. These are relevant to the daphniid mechanism. Especially, they acknowledge that if filters are bordered by structures that inhibit fluid movement around their perimeters, as they are in *Daphnia*, their performance can be changed. They also note that 'leakiness' can be enhanced by drawing fluid through the filter 'by moving another structure away from the downstream side', which is exactly the role of the exopodite pumps and limb corms of *Daphnia*.

One conclusion of Cheer & Koehl invites caution. They say that their results suggest that, under some circumstances, a simple change in the size or speed of movement of a bristled appendage can lead to a novel mode of functioning, whereas in other situations, differences in morphology or behaviour have little effect on performance. It is true that, as an organism, or appendage, increases in size, its Re increases unless its velocity is commensurately reduced. However, while statements that 'structures physically constrained to be paddles at small size acquire the potential to be strainers at larger size' and that changes in size or velocity afford means whereby novel functions can arise without drastic changes in morphology, may be theoretically attractive, they ignore the detailed morphological specializations required of spines and setae for such functions as locomotion, filtering, sweeping and scraping. These functions are not changed by simple changes in size or speed. Filter setae are immediately recognizable as such, whether they occur in a branchiopod, a copepod, an ostracod or a malacostracan, and are morphologically distinct from setae used in propulsion, whether on the antennae of a branchiopod such as *Daphnia*, the trunk limb paddles of other branchiopods such as anostracans, the thoracic limb paddles of copepods, the pleopods or thoracic exopodites of swimming malacostracans, or even the setae used as paddles in insects such as those of the pretarsal fan of *Rhagovelia* shown by Andersen (1982) and referred to earlier. Furthermore, setae that drive water in one direction (or push against it), such as those used in locomotion, have to be of a fundamentally different design from those that, like the exopodite paddle setae of anomopods, drive water as they move in one direction and also draw it towards themselves as they move in the other. A simple change in size or speed of movement could never change the function of a filter seta to propulsion nor of a natatory seta to that of filtration. Detailed morphological changes would be necessary. While the evolutionary potential of changes in size and velocity may indeed be important, they are not independent of morphology: indeed it is doubtful whether significant changes in function are ever independent of changes in morphology. The intricate specialization of the multitude of different spines and setae of *Daphnia* (and other small crustaceans) for particular functions, long apparent and now revealed in more intimate detail by scanning electron microscopy, emphasizes the reliance of their complex machinery on morphological specializations in the minutest details.

(*n*) *Pitfalls in the theoretical quantitative approach to filtering*

Many calculations that have, or are reputed to have, a bearing on aspects of the feeding mechanism of *Daphnia*, or which aim to throw doubt on the utility of the filters to perform this office, have been made, of Reynolds numbers, boundary layers, 'flow regimes', filtering rates and the like. These often leave much to be desired, or are sometimes meaningless because the mechanism to which they have been applied has been misunderstood and the figures used are derived from the wrong events or are less accurate than is assumed. Basic measurements are sometimes at fault. Dimensions of the filters have often been given. Frequently it is assumed that the filter is uniform throughout, which is not so. Even in relation to regions of the filters where relatively uniform conditions prevail, simple measurements are sometimes questionable. Attention has already been drawn to serious discrepancies in the measurements given by Ganf and Shiel (Fryer 1987*b*), but this is not the only case. For example, for *D. magna*, Porter *et al.* (1983) quote intersetular distances (gap widths) as 1.00+0.02 μm and give two SEM photographs with scale lines where this can be checked. A glance at the first photograph shows that the gaps are not uniform: indeed, the widest in the region shown are > 25% wider than the narrowest and neither corresponds to the scale line. In the second photograph, according to the scale line, the gaps are only about half as wide as in the first. Erroneous as they are, these figures are at least attempts to measure the correct thing. This cannot be said of calculations of Re in the same paper. This involves measurement of the speed of the setules involved (or the speed of flow past the setules) and was calculated on the basis of previous measurements of 'maximum average beat rates' under certain conditions and 'the maximum distance moved during a cycle'. The latter was calculated 'assuming that the leading distal edge of the appendage completed a cycle through an arc of 60°, or one third the circumference of a circle with a diameter equal to the length of the filtering comb'. These movements, so imprecisely determined, are not those required. They are only indirectly, and in a complex way, related to filtering rates. Because the filters of *Daphnia* cut the water like the blade of a knife, these measurements relate to movements across the flow and, even had they been the figures required, would be meaningless. Because the limbs swing from the base, the distal filter setae, and their setules, swing further and faster than the proximal, so it is impossible to calculate a speed of setule movement in the manner attempted. Even if it were, the figure required is not this but the rate at which water flows over the setules as it is sucked into the interlimb spaces, more or less at right angles to the direction followed by the setules in the meaningless calculation. The relevant calculation is complicated by the fact that the area of the filter plate through which filtering takes place, changes throughout the promotion (suction) phase of the cycle and by the lack of uniformity of the dimensions of the filter plates.

Misleading measurements and calculations bedevil understanding of these complex events. It is in fact very difficult to obtain accurate data for such things as flow rates over setules, and therefore Re values, and calculations that begin with inadequate data run the risk of compounding errors as they proceed and are sometimes very misleading. It is also essential to understand the mechanism involved before meaningful calculations can be performed.

(*o*) *The habits of certain individual species and some related morphological features*

Members of the genus *Daphnia* live in a wide range of habitats, from small, stagnant water bodies to the pelagial region of large lakes. Different situations call for different morphologies and sometimes for special habits. While often ignored by, or unknown to, those concentrating on highly technical approaches, these habits may be of fundamental significance to the animals concerned. Ignorance of them can give an erroneous impression of the niches they occupy.

(i) *Daphnia magna*

The heavily built *D. magna* often occurs not far above the bottom. In very shallow ponds this is inevitable. While able to live independent of it, it is still able to exploit some of the opportunities offered by its ancestral habitat. Some surfaces provide places on which to rest and large females carrying many eggs or embryos sometimes use them. They assume an oblique posture, repose on the lateral part of the carapace, and prop themselves up on one antenna. If additional support is available, they also sometimes rest on the posterior carapace spine.

In standing waters, organic particles that settle on the bottom, and the algae and microorganisms that flourish there, are a rich source of food for animals equipped to deal with them. *D. magna* often deliberately swims into flocculent bottom deposits. Sometimes it merely touches them with the ventral carapace margins before rising, an action that enables light particles to be drawn into the filter chamber. At other times, it deliberately lifts even bulky loads of flocculent material from the bottom. This has been seen many times and is clearly an energetically efficient means of obtaining food. In a culture, *D. magna* was several times seen to collect a faecal ribbon of the snail *Limnaea peregra*, sometimes considerably longer than itself, from the bottom and swim away with it. It was held between the carapace valves, presumably by the specialized spines of the second trunk limbs described below. Such actions enable large accumulations of food to be acquired with small effort.

Individuals sometimes swim over, and persistently grovel in, bottom deposits, an activity noted also by Horton *et al.* (1979) and referred to by them as browsing. Grovelling, which brings material into suspension, sometimes continues for periods in excess of a minute. Similar behaviour has been noted by Horton *et al.* (1979) in what they identified as the North American form of *D. pulex*, but which was conceivably *D. obtusa*. *D. magna* also has a more specialized, apparently unrecorded, means of association with the bottom. In glass vessels that have accumulated a layer of detritus or algae, it often swims obliquely towards the bottom, with which it makes contact, settles, and aligns itself precisely, resting on its ventral carapace margins as do many chydorids and macrothricids. The antennae are extended in the normal resting position or somewhat posterior to it. It then either moves forward slowly with the carapace margins in contact with the surface without using the antennae, or does so assisting progress by an occasional antennal sweep, or sometimes a couple of sweeps. Much probably depends on the nature of the substratum, but individuals sometimes move forward for several centimetres without using the antennae, though distances of up to a centimetre are more usual. Such activity can be very persistent. In a dish with about 5 cm of water overlying sand on which filamentous algae had developed, more than 100 *D. magna* were present. When deliberately unfed so that there was almost no obvious particulate matter in suspension, these habitually fed on the bottom, moving over it while resting on the carapace margins in a manner probably akin to that of primitive anomopods. At any given time, all save literally two or three individuals would be diligently working the bottom in this manner. Excursions into open water were few. This behaviour is completely different from that with which one generally associates this species. Individual animals remained on the bottom for minutes on end. Their gut contents revealed that they were unable to obtain the algae that were attached to the sand grains and that they were collecting fine detritus.

Individuals intermittently frequenting the algal-bestrewn bottom of a glass vessel, observed with an inverted microscope of necessity at low magnifications and in imperfect viewing conditions, showed that the animals slid forward on the carapace margins as the limbs followed what appeared to be a typical rhythm of activity. Often the course was adjusted by a single sweep of one antenna. While the first trunk limbs may help to lever the animal forward, no special means of so doing was observed nor do these appendages show any obvious adaptations for this, though some of their distal setae are provided with fine spinules that can conceivably grip the substratum. The general impression was that normal trunk limb activity, which involves driving water posteriorly, is sufficient to propel the animal forward. Rates of progression were variable, but often about 1 body length per second. Much doubtless depends on the nature of the substratum. Smooth glass is probably near to ideal for such gliding. Considering the curvature of the ventral carapace margins, balance is remarkably effective. Both large and small individuals indulge in this practice.

D. magna is equipped to collect material from surfaces in a way that, while less specialized, recalls the mechanically elaborate, but probably phyletically primitive, food collecting device of both macrothricids and chydorids. The medial element of the armature of the distal endite of trunk limb 2 is a long spine (figure 48) provided with a row a minute spinules (figure 126) that can gather particles as the distal parts of the limb swing towards the mid line. These particles are then inevitably sucked into the filter chamber with the feeding current.

So far as I am aware, an adequate illustration of this spine has been given only by Glagolev (1983) in a paper on the taxonomic significance of limb structure in daphniids that appeared in an obscure Russian publication, but Watts & Petri (1981) give an SEM photograph of part of it. The latter authors were unable to specify a function for this spine, but suggested that it may serve to clean the filter plate of trunk limb 3 and other structures. While it conceivably has a secondary function as a cleaner, this is unproven, and seems unlikely as in several other species its homologue is smooth (see below). Proximally, the spine bears many close-set, relatively fine spinules. More distally the spinules are more robust and more widely separated so that near the tip, where the spine is more slender, they are widely spaced to form a rake-like row. The arrangement is more suited to raking loose material than to scraping in the manner of the scrapers of chydorids and macrothricids, and the sharp-pointed, distal spines are clearly able to grip and drag the sort of flocculent material among which *D. magna* often forages. They are also well suited to lifting material from the bottom, as described above.

The specialized nature of this spine is revealed by comparisons. In *D. atkinsoni* f. *bolivari*, a member of the same sub-genus (*Ctenodaphnia*) as *D. magna*, its homologue is very similar in form, but is completely smooth. Clearly this species is not equipped for collecting material from the bottom in the manner regularly practised by *D. magna* nor, during the limited observations made on living animals, was it ever seen to do so.

When using the inverted microscope it was necessary to illuminate the vessel from above. This led to an increase in the percentage of individuals gliding over the bottom. As overhead illumination is the rule in nature, it may be that bottom foraging is more important than observations in glass vessels would suggest. Certainly *D. magna* is much more able to utilize substrata than hitherto suspected.

D. magna also possesses the ability, shown to a greater degree by *D. obtusa* (§5 (*o*) (ii)), to settle with its ventral carapace margins against even a vertical surface, to remain there, and even to move forward slightly while so attached. Adults are too heavy to perform this trick very successfully and generally beat the antennae intermittently while attached, presumably to maintain position. Nevertheless, individuals have been seen to rest in this way for 5 s without aid

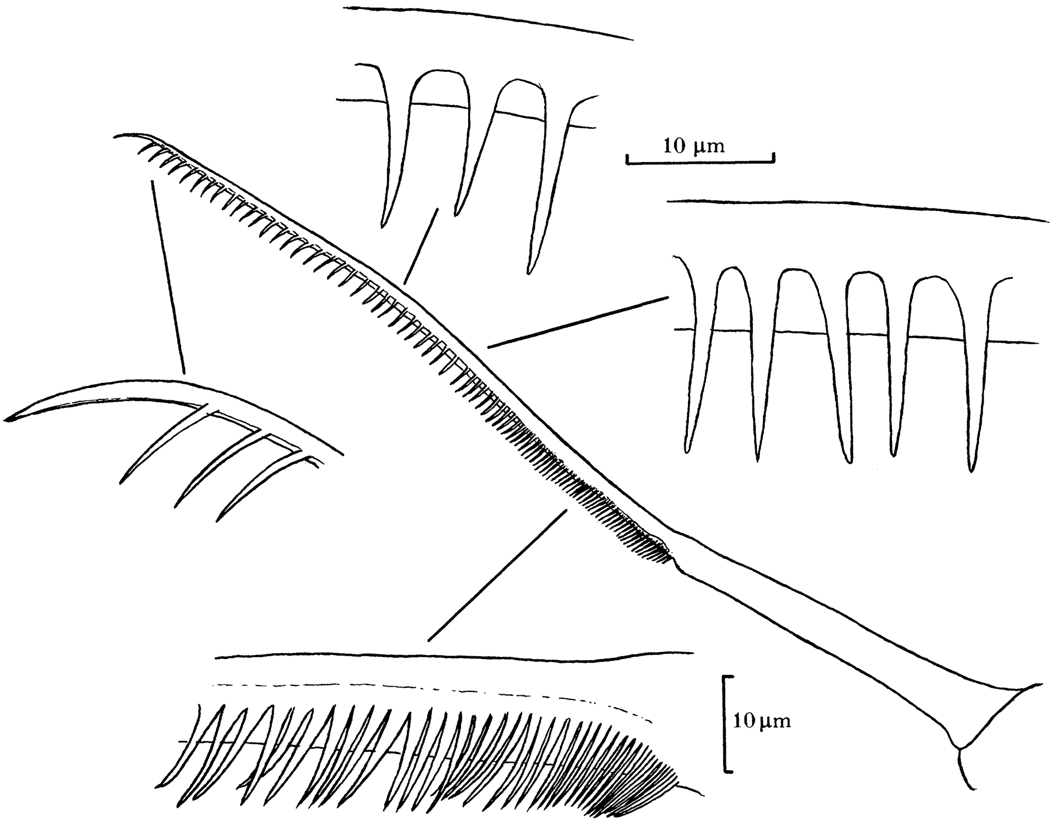


Figure 126. Medial spine of distal endite of trunk limb 2 of *Daphnia magna*, showing its specialization as a scraper. The insets give details, revealed by scanning electron microscopy (SEM), of its armature in different regions. For the topographic location of this spine, see figure 48.

from the antennae, and for up to 13 s aided by 15 sweeps of the antennae. Juveniles, however, being less dense, are more adept at this and have been seen attached for up to 63 s, moving forward about 2 cm in the process. When attached they tend to use the antennae frequently and to swim over the surface, but may remain for several seconds without using these appendages. It is presumed that, during attachment, food collection is at times supplemented by sweeping.

The robust build of *D. magna* fits it better for foraging among bottom deposits than does the light-weight construction of planktonic species. Its bulk necessitates powerful antennal muscles, for which in turn a firm anchorage is needed. Hence the stout headshield which, in contrast to that of planktonic species, is broad. A broad headshield is suitable for bottom grovelling: the narrow headshield of planktonic species, often with a crest, is not. The carapace of *D. magna* is also better able to withstand buffettings and abrasion than its delicate counterpart in planktonic forms.

At least in cultures, there is often a depth gradation of size classes. While individuals of all sizes occur at all depths and while, for obvious reasons, large females burdened with large clutches or broods are more prone to rest briefly on the bottom, early instars tend to occur predominantly in open water. Juveniles are not only smaller but have relatively longer carapace spines than adults, growth of this spine being allometric. Their habits perhaps show that progenesis was an element in the evolution of planktonic species. Juveniles of *D. magna* display both the morphological and behavioural precursors that, if selected, would give rise to characteristic attributes of planktonic forms.

Notwithstanding its propensity for bottom foraging, *D. magna* swims well and, given deep water and a plentiful supply of particulate food, often remains persistently in suspension. It collects a wide size range of particles, including those larger than most of its congeners. Thus, of six species of *Daphnia* supplied with plastic beads of various diameters (up to 80 μm) it ingested much larger beads than its congeners (Burns 1968). Some of these may have been recovered from the bottom. In large containers, for reasons unknown, individuals near the surface often swam rapidly and almost vertically downward to the bottom, while others undertook the opposite journey. As the route was sometimes oblique, and never perfectly straight, measurements underestimate swimming speeds, but 24 measurements over vertical distances of 10 cm or more (max. 25 cm) totalling *ca.* 416 cm gave an average diving speed of *ca.* 1.41 cm s^{-1}. Much the fastest dive measured, over a distance of 25 cm, was at *ca.* 2.27 cm s^{-1}, but another dive, over a vertical distance of 11 cm at *ca.* 1.83 cm s^{-1}, was markedly oblique, so swimming must have exceeded 2 cm s^{-1}.

Twenty five ascents over vertical distances of 11 cm or more (max 23 cm) and totalling *ca.* 413 cm gave an average climbing speed of *ca.* 1.21 cm s^{-1}, not much slower than diving speeds, but the fastest speed was *ca.* 1.63 cm s^{-1} achieved over 13 cm. Vertical ascents of 23, 22 and 19 cm were also achieved at rates of *ca.* 1.48, 1.47 and 1.46 cm s^{-1}, respectively, so actual swimming speeds must have been in excess of 1.5 cm s^{-1}. It was not feasible to measure the individuals involved but few of them exceeded about 3 mm in length. Very large individuals did not feature in these activities.

Such versatile behaviour, like its ecological tolerance, is reflected in the wide geographical distribution of *D. magna* and its ability to build up dense populations when the opportunity occurs. It suffers, however, from a physiological limitation that prevents it from colonizing acidic and ionically dilute waters such as those of moorlands. Under such conditions, it is unable either to obtain, or retain, its essential sodium requirements (Potts & Fryer 1979).

(ii) *Daphnia obtusa*

That *D. obtusa* may rest with its ventral carapace margins against a surface was noticed by Woltereck (1913) and Scourfield (1942) but that it can glide over such surfaces seems not to have been recorded, though it has been seen independently by Mr D. S. Gibbons, who described his observations to me some years ago. Horton *et al.* (1979) also reported that *D. pulex* settled likewise and 'usually remained fixed... on the bottom or moved slowly across it'. This may refer to *D. obtusa* that had not been recognized in North America at that time. This habit is essentially the same as that of *D. magna* but of the two, *D. obtusa* is the more skilful exponent and, unlike *D. magna*, frequently indulges in it on vertical surfaces. Here, with its antennae outstretched, it has often been seen to glide for periods in excess of 1 min, (max timed 1 min 41 s). When so gliding, it seldom follows a straight course and may curve markedly, making it difficult to estimate speeds and distances, but 1 cm is often covered in 14–20 s. It moves slowly when gliding upwards, more rapidly when gliding down. It has been seen to glide down the vertical wall of a glass vessel and continue along its floor. Individuals remain much longer on horizontal surfaces. On the bottom of a slightly dirty vessel in which a culture had been maintained for some days, they often did so for several minutes (max. timed periods 6 min 10 s and 5 min 54 s).

Like *D. magna*, *D. obtusa* has a row of setae inside the carapace margins (see Scourfield (1942) for illustrations) that may assist in attachment as Scourfield noted, but details have not been elucidated. It is also provided with a scraper-like spine on the second trunk limb (figure 79) by means of which material may be whisked into the feeding current as it is in many chydorids and macrothricids. This scraper, again apparently noticed previously only by Glagolev (1983) who gives a simple sketch, is perhaps more effective at whisking and scraping than that of *D. magna*, which seems more suited to scratching, raking or dragging.

As these two species belong to different subgenera of *Daphnia*, they must either have independently evolved this scraping device or retained it in some form from early ancestors. Latent scraper-determining genes, active in ancestral anomopods, were perhaps available for reactivation when the necessity for such structures arose in daphniids. This seems feasible in the light of the demonstration by Beeman (1987) that apparently homologous clusters of homoeotic genes are present in *Tribolium* (Coleoptera) and *Drosophila* (Diptera), two orders of insects more remotely related than are the anomopod families involved here.

It may be that other species of *Daphnia* can scrape, and some can probably lift particles from the bottom. Adequate illustrations in the literature are few, but indicate that *D. middendorffiana* Fischer has a finely serrate spine (Glagolev 1983), that *D. nivalis* has a serrate, and some forms of *D. carinata* and *D. cephalata* a finely serrate, spine (Benzie 1988*a*). In *D. occidentalis* Benzie, this spine is short, curved and somewhat serrate (Benzie 1988*a*) and may have some as yet unknown specialized function. In other species, it is smooth. A survey relating the structure of this spin to habits may prove rewarding.

D. obtusa can also hang momentarily from the surface film – a habit that foreshadows the specializations of *Scapholeberis* and *Megafenestra* (§8 (*c*) (ii), (iii)). No difficulty is evidently experienced in making and breaking contact. Periods in contact are generally short: the longest measured was 46 s but this is certainly exceeded at times. Sixteen measured periods (range 10–46 s) averaged just over 21 s. Periods of contact of < 10 s have often been seen. A settled individual moves forward slowly beneath the surface film without using its antennae.

(iii) *Daphnia galeata*, *D. cucullata* and other planktonic species

Of these open-water planktonic species, *D. galeata* (figures 1 and 2) is a common inhabitant of the plankton of lakes in Northern Europe. Compared with heavily built species such as *D. magna* it is slender, bilaterally compressed and has a longer carapace spine. It is also smaller. Particularly in summer, it develops a conspicuous headshield crest. Its surface area: volume ratio is greater than that of *D. magna* and both the protopod and the segments of the rami of the antennae are longer in relation to their diameter.

Bilateral compression, and the lack of any need to synthesize haemoglobin in well-aerated waters, combined with the thin cuticle permitted by the slender proportions, have enabled it to become hyaline, which offers protection against predators that hunt by sight. Much has been written about the eye as the structure that most conspicuously reveals the presence of hyaline species of *Daphnia*, but often, at least to the human observer, it is not the eye, but eggs or embryos in the brood chamber that are the most conspicuous feature in *D. galeata*. At certain stages of development, the contents of the brood chamber are grey-green in colour and, while less intense than the black-pigmented eye, are much larger, the size depending on the number of eggs or embryos present, and these render the animal more conspicuous than does the eye. Even two eggs in the brood chamber sometimes appear to be more conspicuous than the eye. Viewed from certain angles, the eye cannot be seen in females carrying eggs or embryos. Furthermore, in recent years a form of *D. galeata* with a patch of melanic pigment on the postero-ventral parts of the carapace and on the post-abdomen has become abundant in the plankton of Windermere (G. Fryer, unpublished observations). According to theory, such a patch should render its possessor conspicuous and be selectively disadvantageous.

Notwithstanding differences in shape, *D. galeata*, like more rotund species, is adept at rapid horizontal swimming and, like them, can dive more or less vertically when occasion demands. As in pond-frequenting species, each forward movement is accompanied by a dorsal swing of the posterior end.

D. galeata is subject to an environmental hazard from which pond-dwelling forms such as *D. magna* seem to be immune. Like many chydorids and the bosminids, it is prone to entrapment in the surface film. Invariably seen in plankton collections, this also happens in nature. In summer in Windermere, for reasons unknown, vast numbers become trapped in the surface film. Some patches must contain millions of individuals. On calm days, they produce a noticeable sheen on the surface, and are sufficiently abundant to merit skimming off by mallards, *Anas platyrhynchos*. Unless some of them escape by moulting, individuals thus trapped seem to be doomed: escape from the forces of surface tension is evidently impossible.

Apart from the problem of how these animals are brought to the surface, the different susceptibilities of *D. galeata* and *D. magna* to capture by the surface film demand an explanation. Clearly the cuticle of the former is less wettable than that of the latter. As an adaptation to an open water life, *D. galeata* has reduced the thickness of its cuticle. This presumably makes easier the osmotic uptake of water. One means of reducing this uptake may be to render the cuticle water-repellent. Under normal circumstances, this presents no problems, but should contact be made with the surface film, it is a lethal hazard.

As Wesenberg-Lund (1926) suggested, of the European species of *Daphnia*, *D. cucullata* is probably the most specialized for a planktonic mode of life. It occurs predominantly in lakes: seldom in smaller water bodies. It is usually smaller than *D. galeata*, very slender, much compressed bilaterally, and extremely hyaline. It has a very small eye and, as Wesenberg-Lund notes, seldom carries more than 2 or 3 eggs in summer, during which season members of some populations develop a high headshield crest. In all these respects, it takes the morphological trends seen in *D. galeata* even further. As Hrbáček & Hrbáčková-Esslova (1960) have observed, thanks to its small size and transparency, it survives better in the presence of crustacean-eating fishes than do larger species of the genus.

Complete emancipation from the bottom, as in such planktonic species as the Old World *D. hyalina*, *D. galeata* and *D. cucullata* and their New World equivalents, restricts the range of possible habits, but has led to some that are very striking, such as diurnal vertical migration. It also permits the evolution of preferences for particular strata, and these may have a morphological basis. In lakes in which two or more species occur, it is common for a crest-bearing ('helmeted') species to frequent the more superficial epilimnetic waters and for a rounded headed ('non-helmeted') species to live at greater depths. In Europe *D. cucullata* and a member of the *longispina/hyalina* complex may fulfil these respective roles (see, for example, Findenegg (1943)): in North America *D. retrocurva* Forbes and *D. galeata mendotae* are often the occupants of the epilimnion, *D. longiremis* or a form of *D. pulex* (of which European races are predominantly pond-frequenting) inhabiting the hypolimnion (Juday (1904), Woltereck (1932), and subsequent investigators). Whatever the factors involved, the differences include gross morphology whose precise significance is still debatable.

Planktonic species have arisen independently in the subgenus *Ctenodaphnia*, the most successful exponents of this way of life probably being *D. barbata* and *D. lumholtzi*. These display convergent similarities to planktonic members of *Daphnia* s. str.

(iv) *A note of shoaling behaviour*

As Baird (1850) was the first to report precisely, some pond-frequenting species of *Daphnia* at times congregate in dense shoals of thousands, or even millions, of individuals that move around in a coordinated manner. Since then, this phenomenon, which occurs in certain other daphniids, and which is to be distinguished from the less spectacular 'patchiness' of planktonic organisms, has received scant attention. Aggregations of *D. magna* and of *Moina*, have been reported in nature by Young (1978) and Ratzlaff (1974), respectively. In containers, Santharam *et al.* (1977) and Brandl & Fernando (1971) reported them in *D. carinata* and *Ceriodaphnia* respectively. In all these cases aggregation was believed to be related to sexual reproduction. Young also infers that the shoals of *D. magna* reported by Berg (1931) were related to reproductive activities, but this is by no means certain. These reports led to tests for the existence of sexual pheromones by Crease & Hebert (1983), with negative results. In other cases (see §8 (*e*) (i) for *Moina*) sexual reproduction may not have been involved (see also below). While aggregations may at times perhaps be associated with mating, some shoals may consist entirely of parthenogenetic females, but this would be difficult to prove. Certainly, however, the most striking attribute of shoals is not mere association but the fact that they move in a coordinated manner, like a school of fishes or flock of birds. Neither sexual attraction nor ecological factors provide an explanation of such remarkable social behaviour.

Shoals differ greatly in size and shape. Sometimes they take the form of bands up to several metres in length, whose shape changes as they move. In a narrow moat I watched several shoals of intensely red, and therefore very conspicuous, *D. pulex* that were almost circular in plan, which rotated slowly in a clockwise manner. Some, but not all, of these had a central 'nucleus' of a small clump of floating *Lemna*. At the same time, other shoals formed elongate straggling bands. In all, millions of individuals must have been involved. Some divided shoals at least, quickly re-unite.

Whatever the explanation or advantages of shoaling, much remains unknown. Neither the mechanism of aggregation nor the means of coordination are understood. A shoal acts like a 'super-organism' and coordination presumably involves some form of communication and an ability to react in a specific manner to the presence and actions of other individuals. In clupeid fishes, for example, shoaling is believed to be controlled by an integrated system involving not only the lateral line, but the swim bladder, inner ear and gas-filled pro-otic or pterotic bullae. Daphniids possess nothing remotely like such a system and it is difficult to believe that vision alone is adequate to explain the coordinated movements of a shoal, though it is doubtless involved when, as sometimes happens, its members react in unison to the casting of a shadow. Chemical communication appears insufficiently precise to act as a means of control. Whether *Daphnia* has as yet undiscovered pressure or vibration detectors that are involved in the phenomenon remains, like almost everything related to shoaling, to be investigated.

Shoals demand attention if only because some of the suggested advantages of the analogous schools of fishes or flocks of birds are clearly inapplicable here. Even if the confusion effect acts as an anti-predator device, this is unlikely to be important as shoals often occur in fish-free habitats. It is curious that such a spectacular phenomenon has been so seldom reported.

6. THE EVOLUTION OF EXTERNAL FORM IN DAPHNIA

(*a*) *Shape transformations: ephemeral and phyletic*

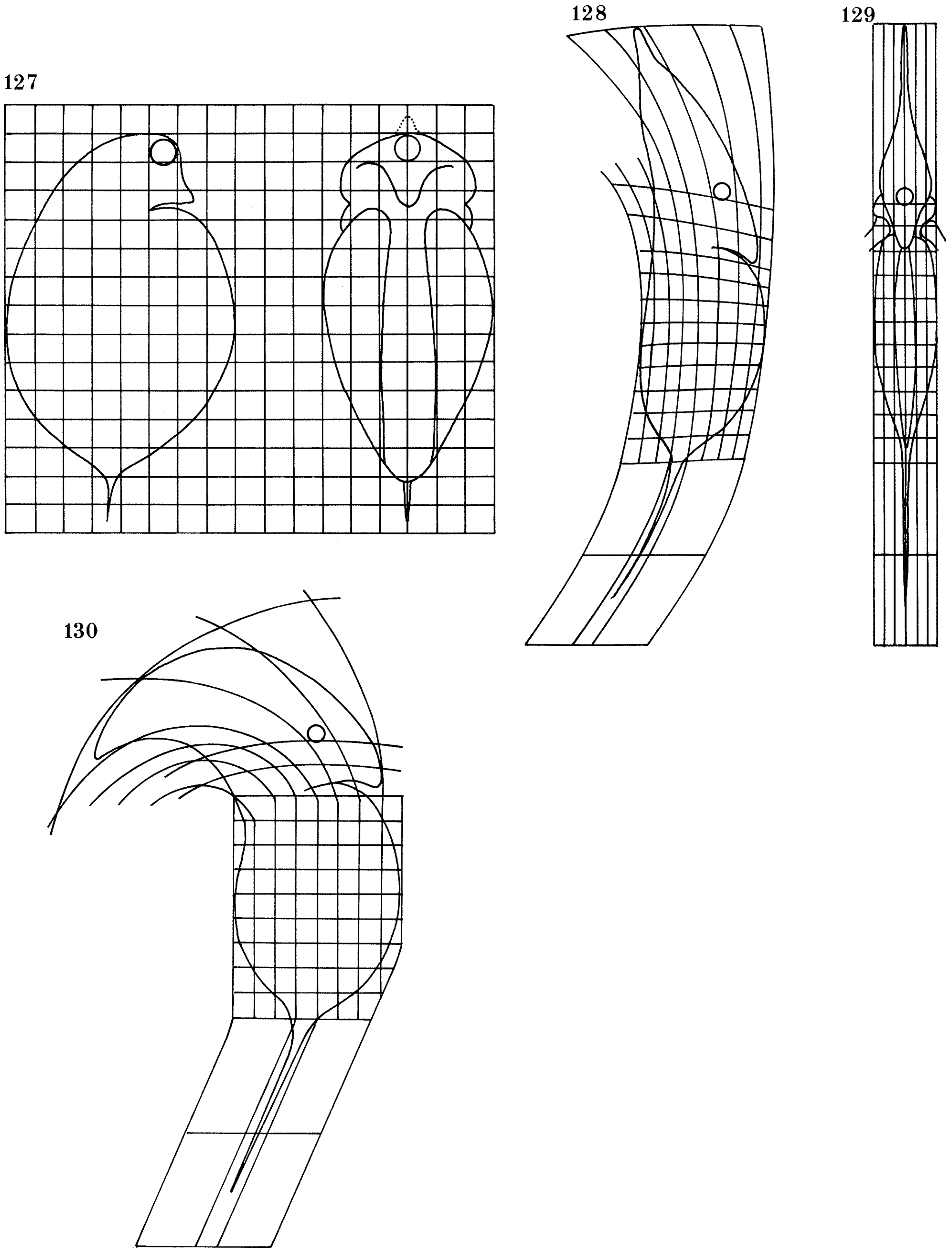
Apart from the problem of *Daphniopsis* (§8 (*a*)), the assignment of a given animal to the genus *Daphnia* is never in doubt. Nevertheless, the range of shapes is considerable. Furthermore, not only does shape change at successive moults but, in species that undergo cyclomorphosis, does so during the course of an annual cycle of overlapping generations. Other changes are responses to predators. Nevertheless, because the exoskeleton of those parts that contribute to gross form consists of only two pieces, carapace and headshield, whose shapes are relatively simple, differences in form between (or sometimes within) species, and ontogenetic and cyclical changes, are also simple. These shapes are mathematically less complicated than those that delineate outer form in many organisms. Furthermore, comparisons, or the analysis of changes, are scarcely complicated by variants of independent parts, and when such variants are involved, they concern such simple structures as the posterior carapace spine, and sometimes the headshield crest or lateral extensions of the fornices.

The simplicity of external form stands in marked contrast to the awesome complexity of internal organization and of the trunk limb complex. Likewise the marked differences of external form, sometimes even between representatives of the same species at different seasons, in different localities and habitats, or in the presence or absence of predators, contrast strikingly with the constancy of form of 'deep seated' structures. The complexity of the latter prohibits great changes: the simplicity of the former permits them. As shown dramatically by the non-cyclical variability of such a species as *D. cephalata*, and by extreme cases of cyclomorphosis, external form is 'plastic' to a remarkable degree in some species of *Daphnia*.

Changes in external form were probably easily evolved, but have played an important part in adaptive radiation. Just as cyclomorphic species respond quickly to environmental stimuli, analogous long-term changes reflect persistent directional selection. Nevertheless, changes in external form are subject to mechanical constraints. If, as abundant evidence indicates, daphniids are derived from bottom-frequenting ancestors, the assumption of persistent swimming would lead to selection for a lightly built exoskeleton. Chydorids, whose specializations are predominantly related to benthic habits, have adopted the alternative strategy and have exploited thicker, stouter carapaces and headshields (Fryer 1963, 1968). Light construction is desirable in a swimmer that begins to sink as soon as active propulsion ceases. Heavily built species such as *D. magna* have to maintain almost continuous antennal activity merely to maintain station. Certain requirements inherent in the daphniid, and indeed in the basic anomopod, body plan, also impose constraints on changes in external form. For example, rigid support for the antennae and firm anchorages for their extrinsic muscles have to be provided, and adequate space is needed for a dorsal brood pouch. Furthermore, during evolution from a benthic ancestor, adjustments of external form were necessary to accommodate changes in other structures. For example, space had to be provided for the much enlarged filter plates of trunk limbs 3 and 4. In *Daphnia*, this involved elongation, and often ventral extension, of the carapace.

On the other hand, open-water habits have freed daphniids from some of the constraints imposed on benthic forms. One manifestation of this is the self-evident fact, but one whose mechanical implications have been inadequately explored, that it is only planktonic, and not benthic, anomopods that undergo significant cyclomorphosis. Open water habits enable width to be increased or decreased without reference to some of the restrictions imposed on such changes in certain benthic species and also remove major restraints imposed by elaborations of the ventral carapace margins that have seen so important in chydorids.

The simplicity of gross shape in *Daphnia* lends itself to easy use of the transformation of coordinates, as expounded by Thompson (1915), but seldom exploited. It is particularly appropriate here: closely related (congeneric) species are involved, between which there is complete morphological correspondence in the parts compared. It can also be applied to temporal changes, or geographical variations in form, of a single species. As Woodger (1945) pointed out, Thompsonian transformations may have been shunned because, as hitherto applied, they are not transformations in the sense that they belong to the beginnings and ends of actual processes. As considered here, however, at the interspecific level, they may be just that. Medawar (1958) noted that such transformations are over-simplifications: in reality one adult does not change into another, but related embryos turn into related adults. Yet in *Daphnia* there is a real sense in which one adult can be transformed into another in that it can give birth by parthenogenesis to offspring



Figures 127–130. Shape transformations of the headshield and carapace of *Daphnia*. Figure 127. Outlines of *D. pulex*, a species of 'generalized' shape, viewed laterally and ventrally against isometric coordinates. Figure 128. A high-helmeted summer form of *D. cucullata*, viewed laterally against coordinates corresponding to those used for *D. pulex*. Note the enormous anterior extension of the headshield. Figure 129. *D. cucullata*, viewed ventrally against coordinates corresponding to those used for *D. pulex*. Figure 130. An extreme form of *D. retrocurva*, viewed laterally against coordinates corresponding to those used for *D. pulex*.

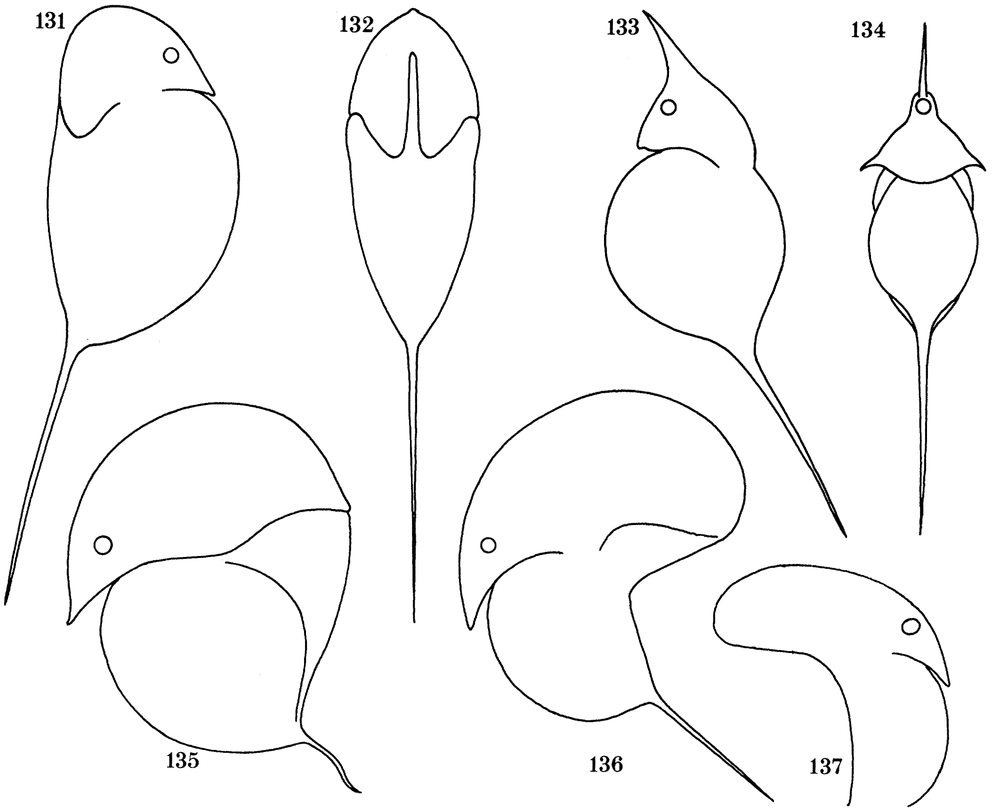
genetically identical to itself and to each other, and that these offspring can differ in form from their mother, which can also give birth to successive broods of genetically identical offspring that differ from each other both at birth and as adults (see, for example, Brooks (1946)). Notwithstanding his remarks, Medawar was impressed by the way that superimposed grids instantly give the sense and trend of transformations and show that numerous explanations of the many differences between two forms are not always necessary, but that a single system of 'morphogenetic forces' may perhaps account for them all. Thompson evidently viewed such transformations in an evolutionary context and, in spite of their limitations, they have a bearing on the evolution of closely related species.

While the shape of the ancestral *Daphnia* is not known, it was probably more akin to that of such species as *D. pulex* and *D. magna* than to that of any of the extreme forms that exist today. By simple deformation, of such a 'generalized' shape, the whole gamut of external form of extant species can easily be derived. Consideration of the animals in different planes makes it possible to consider transformations in three dimensions, not merely the usual two. Comparisons reveal the direction and magnitude of transformations and perhaps even hint at the nature of the forces that affect them. The geometrical differences involved are often small, simple and frequently symmetrical: they reflect in part what is, and what is not, mechanically feasible.

The most striking differences both between species and between the extreme forms assumed by cyclomorphic species concern the crest or 'helmet' of the head. Figure 127 shows the generalized body form of the largely pond-frequenting *D. pulex*. The carapace of a high-helmeted summer form of the planktonic *D. cucullata*, while somewhat more attenuate in lateral aspect, differs little in shape from that of *D. pulex*, but is bilaterally much compressed (figures 128 and 129). The heads of the two species reveal a very different state of affairs. In *D. cucullata* there is some attenuation or narrowing posteriorly, but anteriorly, from about the level of the posterior margin of the eye, an enormous crest is developed whose geometrical relations to the equivalent region in *D. pulex* are apparent from the coordinates. A similar situation prevails in an extreme form of the planktonic, North American *D. retrocurva* (figure 130). The carapace shows even less deformation than that of *D. cucullata* and the posterior part of the headshield, while somewhat distorted by events that have occurred more anteriorly, is not greatly enlarged save some extent ventrally. Anteriorly, however, it is much extended and dramatically recurved. Simple planimetry of figures 127, 128 and 130 shows that, in lateral aspect, the ratio of the area of the head to the carapace in *D. pulex* is about 1:5, in *D. cucullata* about 1:1.35 and in *D. retrocurva* about 1:1.1, which quantifies approximately the enormous expansion of the helmet in the two planktonic species. While taking no account of curvature, these figures permit meaningful comparisons. In an evolutionary context, the head of *D. retrocurva* has shown a several-fold increase in surface area over that of generalized forms such as *D. pulex*.

D. pulex has a modest spine: *D. cucullata* and *D. retrocurva* are long-spined, a frequent attribute of planktonic species. At the other extreme, *D. obtusa* is sometimes spineless. Some indication of the range of external form of species of *Daphnia* is indicated by these figures and by figures 131–137.

As confirmed by studies on cyclomorphic species (see, for example, Brooks (1946); Jacobs (1961)), these differences reflect allometric growth of the headshield and carapace. While there are sometimes complicating factors (Brooks 1946), in cyclomorphic species there is often little difference in the growth rates of these parts in cool water, but during the warmth of summer, the headshield grows faster than the carapace. It is the anterior (topographically upper) part of the headshield that displays most of the accelerated growth. The underlying body wall often forms a ridge that secretes a plate of chitin which extends in the vertical plane as a crest, or, in *D. lumholtzi*, a spike. While often of modest proportions (e.g. figures 1 and 2), the crest sometimes develops to a remarkable degree, completely transforming overall shape, as seen in some forms of *D. cucullata* and *D. retrocurva*. Clearly some factor that affects growth in a specific area is involved, and is probably hormonal (Jacobs 1965). If such changes can be so easily achieved within the compass of a few generations, or to some extent within the life span of an



Figures 131–137. Examples of differences in gross shape within the genus *Daphnia*. Figures 131 and 132. *D. similis*. Figures 133 and 134. *D. lumholtzi*. Figures 135–137. *D. cephalata*.

individual, it is easy to see how evolutionary changes in shape based on allometric growth could have taken place.

These cyclomorphic forms show that even the most extreme changes in the headshield are superficial and scarcely affect the basic anatomy of the head. Some changes in carapace shape are similarly independent of the trunk, but bilateral compression, or expansion, involves corresponding adjustments in the exopodite pumps and seals of the posterior trunk limbs, though these are easily accommodated and do not affect the operation of the filtering apparatus.

An enormous helmet is sometimes developed in the Australian *D. cephalata* and assumes many strange forms. In some, the keel-like helmet is continuous with an expanded dorsal ridge of the carapace, the whole lamella sometimes being referred to as a carina. While this greatly modifies the shape of the carapace, as the illustrations of Sars (1914) show, that of the carapace chamber is scarcely affected. (Sars described these as forms of *D. carinata*, another species in which a dorsal carina, that may greatly modify the shape of the carapace, is developed in some populations.) 'Plasticity' in outward form is well illustrated by *D. cephalata*, extremes of which look like very different species, but which Sars (1914) decided could not be so distinguished, a conclusion supported by electrophoretic studies (Benzie 1988*b*).

Of the other species shown, *D. similis* Claus does not always have such a long carapace spine as the individual figured. *D. lumholtzi* is also variable. Some individuals, and populations, develop a long cephalic spine, as shown: others do not.

(*b*) *Environmental influences*

Various environmental forces operate selectively on *Daphnia*. If the habits and habitats of the species involved are known, it is possible to appreciate how these have affected the form of different parts. Thus in the progressive advance into open water and the ultimate conquest of the pelagial zone of lakes the trend has been towards a reduction in size, attenuation of the body, bilateral compression and the acquisition of a long posterior carapace spine. A long cephalic crest is also frequently acquired and its extension and elaboration during the warm part of the year in temperate lakes is sometimes the most striking, if superficial, feature of cyclomorphosis.

Planktonic species of *Daphnia* are often relatively small and hyaline, which has led to the suggestion, for which there is observational and experimental support, that predation has selected for these attributes. However, what now undoubtedly serve as anti-predator devices (small size, transparency, a long carapace spine, and perhaps even a small eye) may have been acquired fortuitously as adaptations to life in open water. Small size, bilateral compression, a thin cuticle, a long carapace spine and perhaps a headshield crest, all help planktonic species to remain in suspension with minimum effort. Of two animals of the same shape but different sizes, the smaller has the greater surface area in relation to volume and offers the greater frictional resistance to sinking. Transparency is perhaps also an 'accident' insofar as inconspicuousness is concerned. Large, pond-frequenting species are generally broad-bodied and have thicker carapace and headshield cuticles than open water species, which renders them relatively opaque. Moreover, they often frequent habitats where they need to synthesize haemoglobin, which renders them conspicuous. Less bulky planktonic species have less difficulty in getting oxygen to deep-lying tissues, expend less energy on swimming, and therefore have a lower oxygen demand. They also live in what are usually well-aerated environments where haemoglobin is not required and are therefore transparent animals, as would doubtless be the case whether they suffered predation or not, though transparency, like small size, is certainly an advantage insofar as certain (but not all) predators are concerned. Planktonic daphniids (which include species of *Ceriodaphnia* and *Moina* as well as *Daphnia*) are therefore perhaps pre-adapted in this respect. Indeed such forms, precursors of present-day species, perhaps evolved before the advent of plankton-eating fishes, which could hardly acquire such habits until a source of food was available. Long carapace spines undoubtedly frustrate such predators as certain larval fishes. Likewise, the headshield and fornix spines of *D. lumholtzi* offer some protection against plankton-eating fishes (Green 1967). Such predation should theoretically reinforce selection for the development and retention of spines, but need not always be the prime explanation of their presence. Furthermore, while reduction in size may reduce predation by fishes, it may bring the animal within the size range vulnerable to such predators as cyclopoid copepods which, because abundant, may be more important than fishes. They are also probably adversaries of longer standing. That purely mechanical factors have been important in the evolution of planktonic habits is easy to appreciate. Thus while *D. magna* is a swimmer that often moves into open water, its shape is unsuited to a planktonic lifestyle, even if it were never subjected to predation. Cyclomorphosis, basically a phenotypic phenomenon, and a sometimes dramatic example of the influence of environment on form, is beyond the scope of this discussion, as is the induction of 'neck teeth' in some clones of *D. pulex* by water-soluble substances released by invertebrate predators.

7. GEOGRAPHICAL, ECOLOGICAL AND PHYSIOLOGICAL ASPECTS OF ADAPTIVE RADIATION

Adaptive radiation of *Daphnia*, here considered largely on the basis of functional morphology and related habits, also involves a geographical element. Ecological, physiological and genetic traits have also contributed to diversification and the filling of a variety of niches. A brief survey of these aspects of radiation emphasizes the complex interplay of factors involved in the process.

Although daphniids are advanced anomopods, *Daphnia* is an old genus, as attested by fossil ephippia of Oligocene age from Europe (Heydon 1862) and others of similar and Late Eocene age from China (Lai & Li 1987). On this evidence alone, it has had about 40 million years in which to undergo differentiation and dispersal, and may be considerably older. Australian species include representatives of three distinct groups. According to Benzie (1987), the geographical distribution of each group is consistent with a Gondwanic or pre-Gondwanic origin. This implies that they have been distinct since at least Late Cretaceous times, i.e. for some 70 million years, and implies an even earlier origin of the subgenera *Daphnia* and *Ctenodaphnia*, and a yet earlier origin for the genus itself. Such antiquity is in accord with the existence of fossil ephippia from the early Cretaceous (age *ca.* 120 Ma) that can be assigned to the extant daphniid genus *Simocephalus* (Fryer 1991).

Some 50 species are recognized by classical criteria. These occur throughout the world, save in Antarctica (where the closely related, if not congeneric, *Daphniopsis* occurs) and the equatorial forest zone. Some have wide geographical ranges, but none is cosmopolitan: others are markedly restricted. Allopatric speciation has clearly occurred.

The facile explanation of extensive ranges is the possession of resistant resting eggs enclosed in a modified part of the carapace and constituting the ephippium, of which daphniids produce the most elaborate and specialized examples of all anomopods, and of which the two-egged structure of *Daphnia* is among the most specialized of all. Traditionally, such ephippia are supposed to promote dispersal and undoubtedly do so at times. Paradoxically, however, while water bodies are rapidly colonized by locally occurring species, presumably by ephippia, electrophoretic studies have revealed differences between local populations (see, for example, Hebert (1974)). Individuals of *D. magna* from ponds only a few metres apart showed fixed gene differences at certain loci, which indicates little or no gene flow between sites. Adjacent populations may also differ in physiological traits, such as rate of sodium uptake (Potts & Fryer 1979). Founder effects are probably involved. They also often differ in such aspects of morphology as carapace shape, length of the posterior spine, and size, but such differences may sometimes be phenotypic, as may the different degrees to which planktonic species display cyclomorphosis in different lakes. A further paradox is that, notwithstanding the high degree of differentiation in adjacent ponds, regional differentiation is ill-defined. This was found to be so in *D. magna* in East Anglia (Hebert 1974) and, in a much wider survey, in *D. carinata* in Australia (Benzie 1986). As expected, mean genetic distances between populations increased with geographical distance, but regional or subcontinental separation was small relative to that among local populations; no clinal patterns were observed, nor was variation associated with such things as river basins. (See map of geographical variation in gene frequencies of a particular locus in Benzie (1986).) A further complication is that the extensive inter-population differentiation shown in small water bodies does not seem to apply to populations of at least *D. cucullata* and *D. galeata mendotae* in large lakes (Mort & Wolf 1985).

The role of ephippia in dispersal is debatable. In several anomopods of the families Macrothricidae and Chydoridae, far from promoting dispersal the ephippia serve specifically to frustrate it, some of them being firmly attached to substrata, others entangled in material that is unlikely to be dispersed (Bretschko 1969; Fryer 1972; Frenzel 1983). Some daphniid ephippia seem better suited to dispersal, but the 'hooks' in species such as *D. magna* may impede rather than promote it. Much remains to be learned about dispersal.

Such puzzling matters are offset by more readily appreciated facts. Several species have restricted distributions, which suggest evolution in isolation by orthodox means. Likewise electrophoretic comparisons between conspecific European and North American stocks of several species reveal pronounced divergence (Hebert 1987) which, like the similar situation in Australia, is in keeping with expectation and sets the scene for race formation and incipient allopatric speciation. Orthodox taxonomy is complicated by the plasticity of external form and the difficulty of differentiating between genetically distinct geographical races and ecomorphs. For example, Flössner (1987) describes a distinctive form of *D. triquetra* but believes it to be only an ecomorph. Similar problems concern the *bolivari* form of *D. atkinsoni*.

Ecologically, species of *Daphnia* cover a wide spectrum. Habitats range from small, sometimes ephemeral water bodies to large lakes. Many niches are exploited. Notwithstanding their free-swimming lifestyle, several species retain at least a tenuous connection with the bottom: others are successful exponents of the planktonic way of life. Morphological adaptations to these diverse ways of life have been noted in §5 (*o*) and §6. Ecological preferences and physiological specializations are inextricably related. For example, tolerance of low levels of dissolved oxygen by pond and swamp frequenting species is related to their ability to synthesize haemoglobin at need, and lose it when not required, a matter dealt with extensively by Fox and his co-workers (see, for example, Fox (1948, 1955)). Compared with individuals with little haemoglobin, or in which this pigment has been rendered ineffective by carbon monoxide, the possessors of extra haemoglobin survive better in poorly aerated water, swim more vigorously and collect more food. They are also more fecund, probably because they feed more efficiently. Haemoglobin also passes into parthenogenetic eggs and increases the rate of development. Widely separated populations may differ in their ability to synthesize haemoglobin (Green 1956).

Inter-specific differences in thermal preferences have also played a part in radiation. *D. middendorffiana*'s ability to cope with low temperatures has enabled it to penetrate Arctic regions, and *D. longiremis* and *D. cristata*, also adapted to cool conditions, cannot tolerate high temperatures (Freidenfelt 1913). Others, such as *D. lumholtzi*, *D. carinata* and *D. barbata* are restricted to regions that are permanently warm or have hot summers. More subtle adaptations have also been evolved. For example, clones of a single daphniid species may differ physiologically (Obreshkove & Banta 1930; Banta 1939). In a single population of *D. magna*, Carvalho (1988) found several electrophoretically recognizable clones that replace each other seasonally. Each is adapted to temperatures prevailing at a particular season, at which it survives best and is most fecund. Other seasonal changes in gene frequency, detectable by electrophoresis (see, for example, Hebert (1974)) also probably reflect natural selection.

Acid-water tolerance is a physiological achievement in which *Daphnia* has been less successful than certain other anomopods. Many species are largely restricted to alkaline waters of relatively high ionic content: only a few are able to live in moderately acidic, ion-poor waters, which constitute 'difficult' environments for most freshwater animals. Of the British species, *D. obtusa* is much the most successful in this respect: its physiological abilities enable it to live in moorland and heathy situations unfrequented by its congeners (Fryer 1985). By contrast, highly mineralized waters are often tolerated. Thus *D. magna* frequents organically enriched ponds and waters of high salinity and diverse ionic compositions. Other species also occur in inland saline waters, sometimes at salinities exceeding that of seawater.

Daphnia also displays versatility in reproductive physiology and behaviour. As in other anomopods, cyclical parthenogenesis has been much exploited. By this strategem rapid parthenogenetic reproduction, by means of what are sometimes called subitaneous eggs, that are afforded parental protection, enables populations to be built up quickly when conditions are favourable, while the alternating production of sexually produced resting eggs tides the population over winter or other adverse events. That the parthenogenetic phase may involve a succession of seasonal clones adds an element of complexity to what formerly seemed to be a simple sequence and poses unanswered questions.

Short generation times during the parthenogenetic phase have been important. Such tactics are particularly appropriate in situations that do not permit year-round activity. When it is, as in the plankton of large lakes, emphasis is often shifted towards more persistent parthenogenesis. For example, the largely planktonic *D. longiremis* reproduces almost entirely by means of parthenogenetic subitaneous eggs. Males are extremely rare.

Some races of some species have become obligate parthenogens (Hebert 1981). This has been achieved by producing resting eggs mitotically. Fertilization is not then required. The production of viable ephippial eggs without fertilization was first inferred by Olofsson (1918) in Spitzbergen, who found abundant ephippial females of what is now usually called *D. middendorffiana*, but which may be only a pigmented form of *D. pulex* (Haney & Buchanan 1987), but never saw a male. It was demonstrated in a clone of what was designated as *D. pulex* by Banta (1926) and Schrader (1926), but the full significance of these 'pseudosexual eggs' was not then appreciated. Some obligate parthenogens still produce males. This is because meiosis suppression and the suppression of male production are under separate genetic control (Hebert 1978, 1981, 1983). As males of asexual clones still produce sperms and are capable of fertilizing females, they can act as carriers of the gene for asexuality. This has been shown by actual crosses (Innes & Hebert 1988) in which males transmitted the meiosis-suppressing genes to the offspring of mothers capable of sexual reproduction, some of whose daughters are obligate parthenogens. Because meiosis suppressors are superimposed on animals possessed of the ancestral genotypic diversity, the latter property is not lost. Furthermore, there are indications that introgression has introduced the meiosis suppressor into the gene pools of closely related species, converting them to obligate asexuality. Complete asexuality facilitates the establishment of polyploid clones. Tetraploid clones of *D. pulex* and *D. middendorffiana* and hexaploid clones of the latter have been recorded (Hebert 1987) but have been little studied.

Many, but not all, Arctic and high altitude populations of *D. middendorffiana* appear to have eliminated males from the life cycle (Olofsson 1918; Edmondson 1955; Ferrari 1967, 1970). Where pools are unfrozen for three months or less in summer, it is advantageous if every individual is potentially capable of producing eggs. Some orthodox parthenogenesis by subitaneous eggs occurs at the beginning of the season, but most augmenting of the population, as well as overwintering, is by means of unfertilized ephippial eggs.

Interspecific hybridization seems also to have played at least some part in adaptive radiation. Suggestions of hybridization in *Daphnia* have often been made on the basis of apparent 'intermediates' between species and several cases have now been proved by electrophoretic methods. Indeed, related species of *Daphnia* appear to have a relatively high level of genetic compatibility. As the predominant mode of reproduction, by parthenogenetic subitaneous eggs, involves only mitosis, hybrids might be expected to be capable of asexual reproduction even if sexually sterile. A single interspecific cross could theoretically give rise to a dominant, long-persistent clone in the plankton of a large lake where adults persist throughout the year, though unless viable asexual ephippial eggs are produced, opportunities for dispersal would be small, and such populations seem ultimately to be evolutionary dead-ends.

All these adaptations and strategems require an efficient vehicle. This is provided by the complex morphological machinery that makes *Daphnia* such an efficient exploiter of a variety of freshwater habitats. While *Daphnia* is an old genus, there are indications of a recent spate of evolutionary activity. Many north-temperate regions occupied today were ice-covered during the Pleistocene glaciations and have been colonized in the last 10–15000 years, or even less in some places. Spread was doubtless facilitated by the habit of parthenogenesis. A single resting egg is potentially capable of establishing a new population. Many founder events must have occurred during this extension of range, with all that this implies for the establishment of genetically distinct populations. Among pond-dwelling species especially, vast numbers of isolated populations exist in recently glaciated areas, providing much scope for the development of genetically distinct populations. Whether new species have evolved during this period is unproven, but Benzie (1987) has suggested a post-Pleistocene origin for the Australian *D. nivalis* that is endemic to glacial lakes less than 20000 years old in the Snowy Mountains. Differentiation, however, may have begun elsewhere before these lakes were colonized.

8. FUNCTIONAL MORPHOLOGY AND HABITS OF OTHER DAPHNIIDS

(*a*) *Daphniopsis*

(i) *Geographical and ecological attributes*

The validity of this genus is upheld by some, disputed by others. It was formerly believed to be represented by very few, inadequately known, species, but recent discoveries have added to their number. A major problem in deciding affinities is that the type species, *Daphniopsis tibetana* Sars, whose males and ephippial females are undescribed, stands somewhat isolated from all other described species. Hrbáček (1987) believes that this species at least could be accommodated in the subgenus *Ctenodaphnia* of *Daphnia* and that the other species of *Daphniopsis* could perhaps be assigned to another sub-genus, for which the name *Daphniopsis* would not be available. Such treatment would imply that the currently recognized genus lacks phyletic integrity.

Apart from *Daphniopsis tibetana* to which, however, Crawford (1974) has attempted to extend similar recognition, the genus had until recently an apparently Gondwanic distribution. It is widely distributed in Australia, including Tasmania, where it is represented by several species (some of them undescribed), Antarctica, Kerguelen and several sub-antarctic islands (one species), India (one species), and South America, where the recently described *D. chilensis* Hann occurs in the highest known body of fresh water in the world (5883 m) in the Chilean Andes (Hann 1986). If the generic assignment of the recently described *D. ephemeralis* Schwartz and Hebert is correct, however, its occurrence in North America disrupts the otherwise convincing Gondwanic distribution of those species that show the greatest morphological similarities.

Sars (1903) thought that *Daphniopsis* (to use this name for convenience) was intermediate between *Daphnia* and *Simocephalus*. However, Rühe (1914) showed convincingly that *D. studeri* Rühe stood close to *Daphnia*, a conclusion amply supported by recent work (Hann 1986; Sergeev & Williams 1983, 1985; Sergeev 1990, 1991) and by observations noted here. There is little to support the statement of Schwartz & Hebert (1985) that the 'morphological intermediacy' of *Daphniopsis* suggests that it is ancestral to *Daphnia* and *Simocephalus*.

D. tibetana apart, the characteristics of *Daphniopsis* are readily apparent. The lack of a carapace spine in adult females is an obvious, if superficial, example. A distinction from the otherwise *Ctenodaphnia*-like ephippium noted by Hann (1986) was the absence of any chitinous projections from the antero-dorsal or antero-posterior margins. However, Kokkin & Williams (1987) have obtained ephippia from various populations of *Daphniopsis* which they can assign to six different morphotypes, two of them being *D. pusilla* Serventy and *D. australis* Sergeev and Williams, four of them unattributed. Two of the latter category of ephippia have projections (part of the dorsal ridge of the carapace) from at least one end so this distinction from the subgenus *Ctenodaphnia* does not hold good in some species. However, the ephippia of at least two species (*D. pusilla* and an undescribed form) differ from those of all species of *Daphnia* in containing only a single egg. (For an illustration of the ephippial female of *D. pusilla*, see Sergeev & Williams (1983); for SEM photographs of the ephippium of this and the undescribed species, see Kokkin & Williams (1987).) The way in which the ephippium is carried seems also to differ from that in *Daphnia*, but a detailed study is needed. In the production by different species of an ephippium that contains either one or two eggs, *Daphniopsis* resembles *Moina* and differs from *Daphnia*, in which the number is fixed at two (save for abnormalities), and *Simocephalus*, in which it is fixed at one.

Most, but not all, species appear to differ from *Daphnia* by lacking spinules on the dorsal ridge and ventral margins of the carapace. This is perhaps correlated with the ecology of the animals and not necessarily an indicator of affinities. Species of *Daphniopsis* seem generally to occur in habitats with few or no predators (or competitors) where protection from spinules is not required. The Antarctic and sub-antarctic island localities provide examples, as does the world's highest water body. The North American *D. ephemeralis* appears only for short periods in early spring and autumn, when temperatures are low and predators (and competitors) few. Schwartz & Hebert (1985) note how it was replaced by *Daphnia pulex* within six to eight weeks of appearing in spring. The Australian species are proving to be an important element in the fauna of the saline waters so prevalent in that predominantly arid continent, where predators and competitors are again few. The highest salinity thus far recorded, for a species originally attributed to *D. pusilla* but whose identity is uncertain, is 70‰, about twice that of 'average' oceanic water. Individuals positively identified as *D. pusilla* have been collected at a salinity of 58.1‰ (Sergeev & Williams 1983). Apart from the alleged occurrence of *Daphnia similis* in Canada at a salinity of 106‰, and records of *Moina salina* Daday at *ca.* 92 and 104.7‰, these are far higher salinities than those generally tolerated by other anomopods. Physiologically the difference between these saline habitats and those colonized in places like Antarctica is enormous. The pattern displayed is reminiscent of that shown by defenceless anostracans.

Schwartz & Hebert (1985) interpreted the then available ecological data as showing that species of *Daphniopsis* are relict taxa confined to marginal habitats in which they are spatially or temporally separated from other daphniids. Additional information confirms the frequenting of predator- and competitor-poor habitats, but the physiological specializations required for life at salinities greater than those tolerated by most species of *Daphnia* should not be overlooked. The occupation of, and radiation within, saline habitats in Australia by *Daphniopsis* seems as indicative of the exploitation of opportunities as of refuge seeking.

Until the placement of the species assigned to *Daphniopsis* is clarified, it is convenient to retain the use of this name, bearing in mind that the type species at least may be phyletically distinct from the rest. These animals, while certainly close to *Daphnia*, share at least some distinctive attributes of morphology, as well as of ecology and physiology, and to a large extent constitute a geographical entity.

(ii) *Observations on D. pusilla*

I have been able to study live material of *D. pusilla*, hatched from dried bottom debris collected in the Coorang area, South Australia, from which a flourishing culture was reared. *D. pusilla* (see Sergeev & Williams (1983, 1985), for illustrations) looks like a *Daphnia* without a posterior carapace spine. Notwithstanding this lack, it swims in the manner of *Daphnia* (§5(*b*)) and not like *Simocephalus* (§8(*b*) (i)). The evolution of a *Daphnia*-like spineless daphniid is a clear demonstration of the fact that, while the carapace spine of *Daphnia* is no doubt helpful in the way what Scourfield (1900) suggested long ago, daphniids of this general body from can steer accurately without it and that, by appropriate adjustments as the antennae sweep, can readily compensate for any deleterious effects engendered by its lack. Indeed juveniles (and males) of at least some species of *Daphniopsis* have a short *Daphnia*-like posterior spine, which probably indicates descent from a spine-bearing ancestor.

D. pusilla is darkly pigmented dorsally, especially on the head and, to a somewhat lesser extent, in the dorsal region of the carapace. Such pigmentation, which it shares with several other members of the genus, may afford protection against ultra-violet radiation and can be afforded in predator-free environments where conspicuousness is not a liability. The orientation of its body is *Daphnia*-like and the 'hop and sink' behaviour so often practised by *Daphnia* is followed. Like *Daphnia*, it can also swim in the horizontal or near-horizontal plane and does so often and effectively. It can also dive steeply downward just as does *Daphnia*. As the antennae make their working stroke, there is a well marked dorsal lift of the posterior dorsal region. This presumably reflects the lack of a posterior spine, but is clearly no impediment to effective locomotion.

The antennae are rather short. Their rate of beat varies much according to circumstances. At room temperature, 'cruising' individuals swept their antennae at rates as low as 1.5–1.8 cycles s^{-1}, but rates of 2–3 cycles s^{-1} were often observed and one individual following the 'hop and sink' procedure made 64 sweeps in 20 s (3.2 cycles s^{-1}). Faster beating is doubtless employed during diving or escape movements that cannot be followed without special equipment.

Individuals in culture often swam more or less horizontally just over the bottom which, from time to time they touched momentarily, sometimes making a short, but perceptible, pause. Gut contents revealed that they collected material from this source, but even without any actual whisking, their often close association with the bottom will allow the sucking in of flocculent material when this is available.

As Rühe (1914) showed, the trunk limbs of *Daphniopsis* are very similar to those of *Daphnia*. Likewise, the feeding mechanism of *D. pusilla* is the same as that of *Daphnia*. The three posterior pairs of trunk limbs operate in a typical *Daphnia*-like manner and particles are drawn into the posterior end of the filter chamber, whence they proceed forward along the food groove exactly as in that genus. Trunk limb 2 moves more or less in time with limb 3, but with much smaller amplitude. Its corm appears to be pushed by limb 3 on promotion and to move with it on remotion. Its action seems to be in part related to the swing of the gnathobase, whose long posterior filter-cleaning spine is easy to see and which swings as in *Daphnia*. Remotion of this limb involves a laterally directed component, as is inevitable if the gnathobasic armature is to clean the filter plates of limb 3. Limb 2 is clearly not concerned with suction or filtration. Limb 1 remains essentially stationary during feeding or displays a slight oscillation.

(*b*) *Simocephalus*

(i) *General morphology and habits*

The readily apparent morphological differences between *Simocephalus* (figure 138) and *Daphnia* obscure many fundamental similarities. They are nevertheless sufficient to permit the exploitation of very different lifestyles, and members of these two genera, while employing an essentially similar feeding mechanism, occupy a very different array of niches. They probably compete very little. Some two dozen species of *Simocephalus* have been described though not all may be valid. On the other hand, genetic work suggests that some apparently specific entities may consist of more than one reproductively isolated assemblage (Hann & Hebert 1986), and it is not certain that European and North American animals currently given the same name are indeed always conspecific (Hann & Hebert 1986; Hann 1987).

The genus has given rise to several successful inhabitants of weedy situations where they live among both submerged and emergent vegetation in ponds, swamps, the littoral region of lakes, and slow flowing waters. Here they sometimes occur in prodigious numbers. They are less frequently encountered in areas where there is little or no vegetation. Ecological success is matched by occurrence in a wide range of climates in all continents save Antarctica. Species of *Simocephalus* are among the most frequently encountered daphniids. Nevertheless, several species appear to share some of the physiological limitations of many species of *Daphnia*. While some of them are able to withstand an admixture

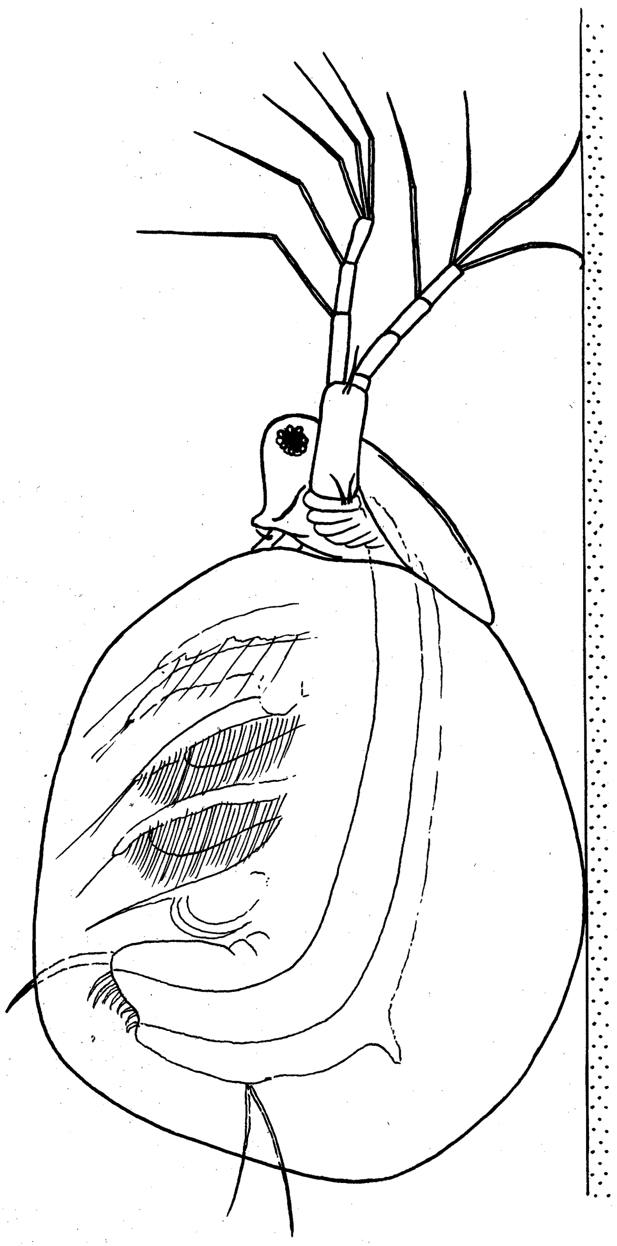


Figure 138. *Simocephalus vetulus*, length about 2.5 mm, suspended by its antennal hooks from a vertical surface. The outlines of the limbs are intended to do no more than show their position.

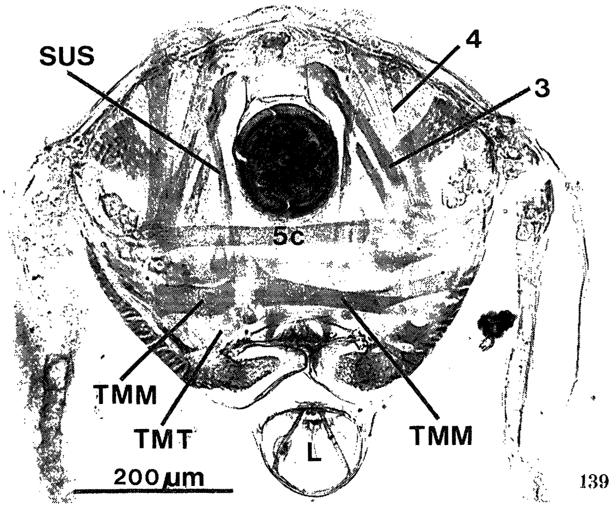
of brackish water, they are intolerant of acidic waters of low ionic content and are therefore excluded from moorland situations. Even the extremely common and ecologically euryvalent *S. vetulus* (O. F. Müller) can tolerate only weakly acidic conditions, and the common *S. exspinosus* (Koch) appears to be only slightly more tolerant. On the other hand, *S. serrulatus* (Koch), which I have not found in Britain, evidently colonizes acidic waters on moorlands and heaths, as well as those frequented by *S. vetulus* and *S. exspinosus*.

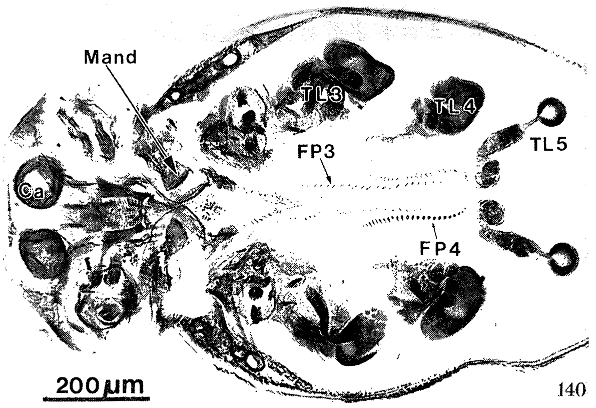
S. vetulus (figure 138) can be taken as representative of the genus. Other species display variants of this basic shape. A noteworthy feature is the lack of a posterior carapace spine. In some species, such as *S. serrulatus*, there is a blunt posterior protuberance, and in species such as *S. lusaticus* Herr, *S. latirostris* Stingelin and *S. iheringi* Sars, a similar protuberance is sometimes drawn out almost to a point, but there is never a *Daphnia*-like true spine.

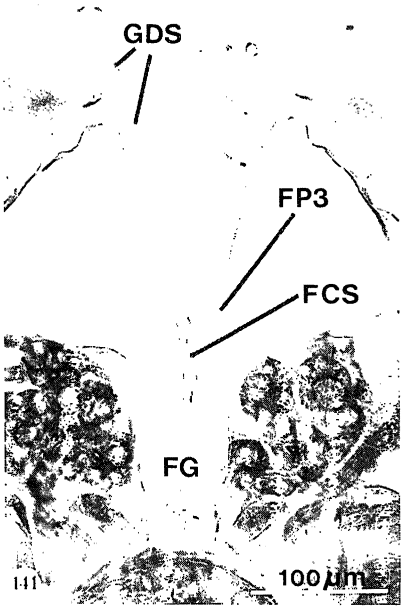
A striking difference between *Simocephalus* and *Daphnia*, observed by Schaeffer as long ago as 1755, is the way in which they swim. Both have similar antennae, but those of *Simocephalus* are relatively smaller than those of *Daphnia* and have a specialization not developed in that genus. While, except when diving, *Daphnia* always swims with the body aligned either vertically or obliquely forward with the dorsal surface uppermost, *Simocephalus* swims with the ventral surface uppermost, the anterior end of the long axis being higher than the posterior. This difference, and its significance, were explored by Scourfield (1900). *Simocephalus* is also a much less persistent swimmer than *Daphnia*. It is indeed essentially a sedentary organism several of whose structural adaptations are related to sedentary and not, as in *Daphnia*, to swimming, habits. An undisturbed individual swims little.

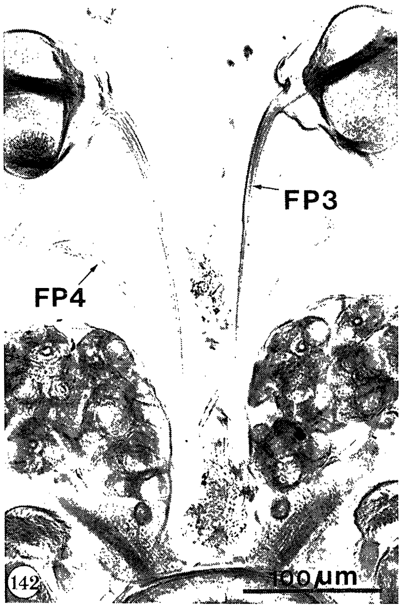
Notwithstanding the different orientation of the body during swimming, the direction of antennal beat is essentially the same as in *Daphnia* and this sets up currents somewhat oblique to the long axis of the body, there being a slight postero-dorsal inclination to the flow. As Scourfield (1900) showed, the centre of gravity of *Simocephalus* is located dorsal to the long axis, which explains why individuals sink lying obliquely, dorsal surface down, and why they swim in a somewhat similar position. Like *Daphnia*, *Simocephalus* is theoretically prone to assuming a circular path, but manages without a compensating spine. The fact that it is not a persistent swimmer may help here, but, like *Daphnia* and the spineless *Daphniopsis*, it can doubtless make all necessary adjustments by modifying the cycle of antennal beat.

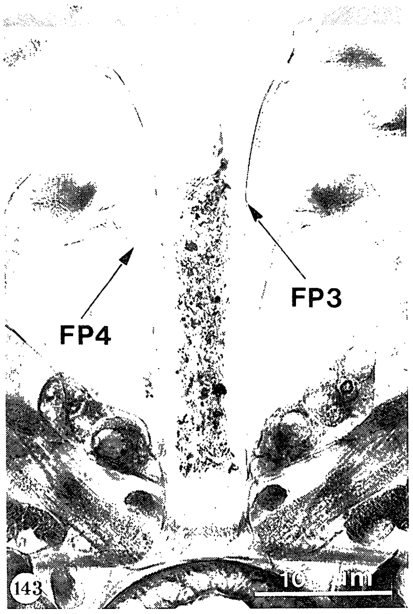
The habit of *Simocephalus*, reported by Schaeffer in 1755 but apparently still not always appreciated, is to attach itself to same suitable object. This is does by means of the outermost seta of the distal segment of each antennal exopodite, whose tips are curved into minute hooks for this purpose. When circumstances permit, the adjacent natatory seta may be pressed against the object of attachment, perhaps giving stability. From such objects the animal hangs, often with the dorsal surface of the carapace serving as the other point, in a three point arrangement (figure 138). The hooks of the antennae are so minute that many 'smooth' surfaces such as the side of a glass vessel that has developed a film of algae or microorganisms, furnish sufficient irregularities for this purpose. The difference in density between the animal and the surrounding water is very small, so the load is likewise. Attachment is purely mechanical. The impression given by some writers that the 'dorsal' or 'nuchal' organ is involved, as it is in the ctenopod *Sida* where it forms a sucker, is erroneous. *Simocephalus* indeed sometimes hangs by the antennae alone, or even by a single antenna. When masses of filamentous algae are available, its hooking setae can arch over these and provide the sole means of suspension as, on rare occasions, can the surface film. Individuals are not averse to resting at times without use of the antennae.

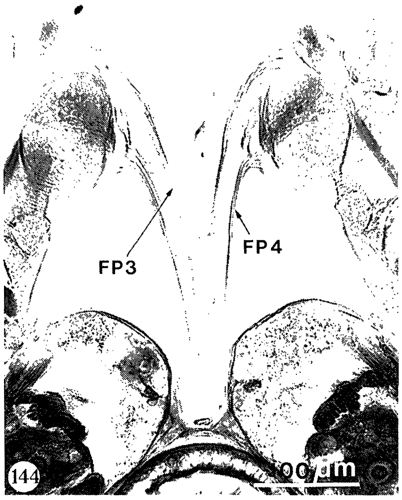












Figures 139–146. *Simocephalus vetulus*. Figure 139. Transverse slice through the posterior region of the mandibles, seen from in front, revealing the suspensors (SUS) of the transverse mandibular tendon (TMT) and most of the major muscles. Note the striking asymmetry of the transverse muscles (TMM) that originate on the transverse mandibular tendon. The muscles of the left mandible (right-hand side of figure) are much longer than their homologues of the right mandible. The relation of the labrum (L) to the mandibles is well shown. Figure 140. Horizontal slice at about the level of the masticatory region of the mandibles to show the topographic relations of the corms of the trunk limbs and the size and location of the filter chamber. Figure 141. Transverse section, ventral surface uppermost, through trunk limb 3, anterior to the level at which the filter plate of trunk limb 4 is also cut, showing the shape of the filter

For example, *S. exspinosus* sometimes rests its carapace on a supporting mass of filamentous algae.

Such sedentary habits have a profound effect on the morphology and way of life of *Simocephalus*. They have permitted the development of a more robust, and therefore more protective, carapace than that of *Daphnia*. The effort expended in swimming a given distance, by the use of smaller antennae than those of *Daphnia*, especially after the sacrifice of a natatory seta on each appendage, transformed into the anchoring device, is therefore clearly greater than in that genus but, as swimming is only intermittent, can be afforded. Energy expenditure on swimming is probably much less than in *Daphnia*.

Inverted swimming was probably the feature that enabled anchorage by the antennal setae to be achieved during the evolution of the genus. It places an alighting individual in an appropriate position. The orientation of a swimming, or even a passively sinking, *Daphnia* is not conducive to the acquisition of such an anchoring device.

(ii) *Trunk limb morphology and the feeding mechanism*

The basic similarity of the trunk limbs of *Simocephalus* to those of *Daphnia* is immediately obvious. Their arrangement is the same and they comprise an operationally similar mechanism. Of the differences in details, few call for comment in a functional account such as that given here.

The guide setae of trunk limb 1 are arranged in an arc, as they are in *Daphnia*, and the filter plates of trunk limbs 3 and 4 make up a filter chamber, just as they do in that genus (figure 140). As in *Daphnia*, the posterior setae of the filters of the fourth limbs curve towards the mid line and fence off the posterior end of the filter chamber immediately anterior to trunk limb 5 (figures 67, 140). Figure 141 shows how the gnathobase of trunk limb 2 lies medial to the filter of limb 3 and how its long, filter-cleaning spine (FCS) extends posteriorly, exactly as in *Daphnia*.

Figures 141–146 not only show the arrangement of the filter chamber as seen in transverse section, but throw graphic light on the nature of the feeding mechanism as a result of the fortunate way in which fixation has retained food particles within the filter chamber. Beginning at the posterior end of the trunk limb series, figure 146 shows the corms of the fifth trunk limbs (TL5) which make up part of the posterior wall of interlimb chambers 4/5 and serve also to clean the posteriormost filter setae of limb 4. On each side can be seen part of the exopodite of limb 4 (EX4). Immediately anterior to this limb is the curved transverse fence (figure 145) made up by the posteriormost filter setae of trunk limb 4 (FP4). More anteriorly (figures 143, 142) the walls of the filter chamber are clearly shown. Figure 143 is a section approximately half way along the filter plate of limb 4, and therefore towards the posterior end of the filter chamber. Here, within the narrow confines of the chamber, a mass of food particles is seen. It is not claimed that these particles were fixed exactly in the position they occupied in the living animal, where their movement is rapid, but certainly this is the region into which flocculent masses of food are often drawn, not merely in *Simocephalus* but in *Daphnia* and other daphniids, and the slice gives an excellent impression of how food passes deeply into the filter chamber in its posterior reaches.

Figure 142 is of a more anteriorly located slice, the anterior extremities of the filter plates of the fourth limb (FP4) can just be seen. Here the food particles lie deeper in the filter chamber, many of them in the depths of the food groove. Figure 141 is of a still more anteriorly located slice. Here the long, posteriorly directed cleaning setae (FCS) of the ganthobases of trunk limb 2 can be seen. These have swept towards the food groove any material that lay in their path as they swung dorsally. Note that no particles lie ventral to them. The only particles here lie deep in the food groove. This series gives a good impression of the progress of food particles into the posterior end of the filter chamber, whence they pass into the food groove and forward. In figures 141 and 142, the guide setae of trunk limb 1 (GDS) can be seen. Their arrangement and role are as in *Daphnia*.

At room temperature, adults of *S. exspinosus* operated at almost exactly 5 Hz. Juveniles operate their limbs at higher speeds: approximately 10 Hz was recorded in a juvenile *S. vetulus*. This is consistent with maintaining similar Re values as size changes, but other factors may be involved. Because *Simocephalus* readily lies with the

plates of trunk limb 3 (FP3) and their relations to each other and to the food groove. The section cuts through the long posteriorly-directed filter-cleaning spines (FCS) of trunk limb 2. Note also the guide setae of trunk limb 1 (GDS) and the particles of food in the depth of the food groove (FG) that have been swept there by the long gnathobasic filter-cleaning spines of trunk limb 2. Figure 142. The same, more posteriorly, cutting at the level at which the first trace of trunk limb 4 and its filter plate (FP4) is appearing on each side. Note the mass of filtered particles that has been collected in the filter chamber. Much of this material, which lies beyond the reach of the filter-cleaning spines of trunk limb 2, is still en route to the food groove. Figure 143. The same, more posteriorly. More of the filter plates of the fourth trunk limbs (FP4) are now visible. Note how these lie lateral to those of trunk limb 3 (FP3). This section shows particularly well a mass of food particles collected in the filter chamber, whose narrowness towards the end of the remotion phase of the cycle is made apparent. Compare the distribution of these particles with those in figure 142, at which level they have been pushed deep into the food groove. Figure 144. The same, more posteriorly, approaching the posterior limits of the food groove. The filter setae of the third trunk limbs are some of the most posterior elements of these filter plates. Figure 145. The same, more posteriorly, showing how the posteriormost filter setae of trunk limb 4 fence off the posterior end of the filter chamber (cf. figure 140). No filter setae of trunk limb 3 lie so far back. Note how the food groove is here narrowing and becoming shallow as it peters out. Figure 146. The same, more posteriorly, showing the 'gates' of the fifth trunk limbs (TL5) that lie immediately posterior to the fence of setae of the fourth trunk limbs seen in figure 145. Part of the exopodite of trunk limb 4 (EX4), which helps to seal interlimb space 4/5, can be seen on each side.

ventral gape of its carapace uppermost, even unrestrained individuals can be watched, or filmed, in this position, allowing one to look directly into the filter chamber. Such observations confirm that the gluing of *Daphnia* to a needle of glass has no effect on limb movements.

The pattern of limb movements is as in *Daphnia* but trunk limb 5 is minutely ahead of the sequence outlined for that genus. It begins its remotion before limb 3 has completed promotion. This does not mean that the seal between limb 5 and the carapace is broken at this stage, interlimb space 4/5 would hardly begin to discharge its contents while space 3/4 was still filling, and this precocious action on the part of limb 5 probably reflects the relevant curvature of the carapace, which evidently allows the seal to persist over several degrees of swing.

The exopodite of trunk limb 2 is armed with two long distal setae whose setules resemble those of the exopodites of limbs 3 and 4. Limb 2 has a regular cycle of movement, but of small amplitude. This conceivably makes a trivial contribution to the posterior flow of water during its remotion. Its very small swing during promotion can have no direct effect on the feeding process. Interlimb space 3/4 is sealed at that time so limb 2 can in no way assist inflow into it.

Eriksson (1934) believed that the distal setae of the corm of trunk limb 2 of daphniids (and of trunk limb 1) served as filters and he shows those of *S. vetulus*, his only illustration of a daphniid. As is now readily apparent, and can indeed be deduced from Eriksson's figure which shows the way in which the setules of these setae overlap each other, these are not filter setae, and this limb, like the first, is not involved in the abstraction of particles in *Simocephalus*, or in any daphniid.

One difference between *Simocephalus* and *Daphnia* requires particular emphasis in view of certain suggestions that have been made about the daphniid feeding mechanism. Whereas in the various species of *Daphnia* the number of brushing setae in the median row of the gnathobase of trunk limb 2 ranges from at least six to as many as eighteen, in *Simocephalus* there are only four such setae, at least in the two species available. It is clear that, as in *Daphnia* where Cannon (1933) appreciated long ago that their homologues do not remotely resemble filter setae, these are not equipped to filter and have a very different role. In both *S. vetulus* and *S. exspinosus* they are widely separated and their brush-like setules occupy less than half their length. Nevertheless, it was claimed by Ganf & Shiel (1985*a*, *b*) that their homologues in *Daphnia* served as filters. As noted elsewhere (Fryer 1987*b*), even if these had been filter setae, their number in *Simocephalus* would be inadequate to allow them to abstract the food. They serve not to filter but, as in *Daphnia*, to brush material from the adjacent filters of trunk limb 3, to ensure that food is forced into the food groove, and to pass it anteriorly.

Material collected by the trunk limbs is passed forward along the food groove as in *Daphnia* and proceeds, via the maxillules, to the mandibles. Like the mandibles of *Daphnia*, those of *Simocephalus* display well marked skeletal and muscular asymmetry. This is very clearly seen in figure 139, which shows most of the major muscles, and the suspensors of the transverse mandibular tendon. The mandibular mechanism is as in other anomopods.

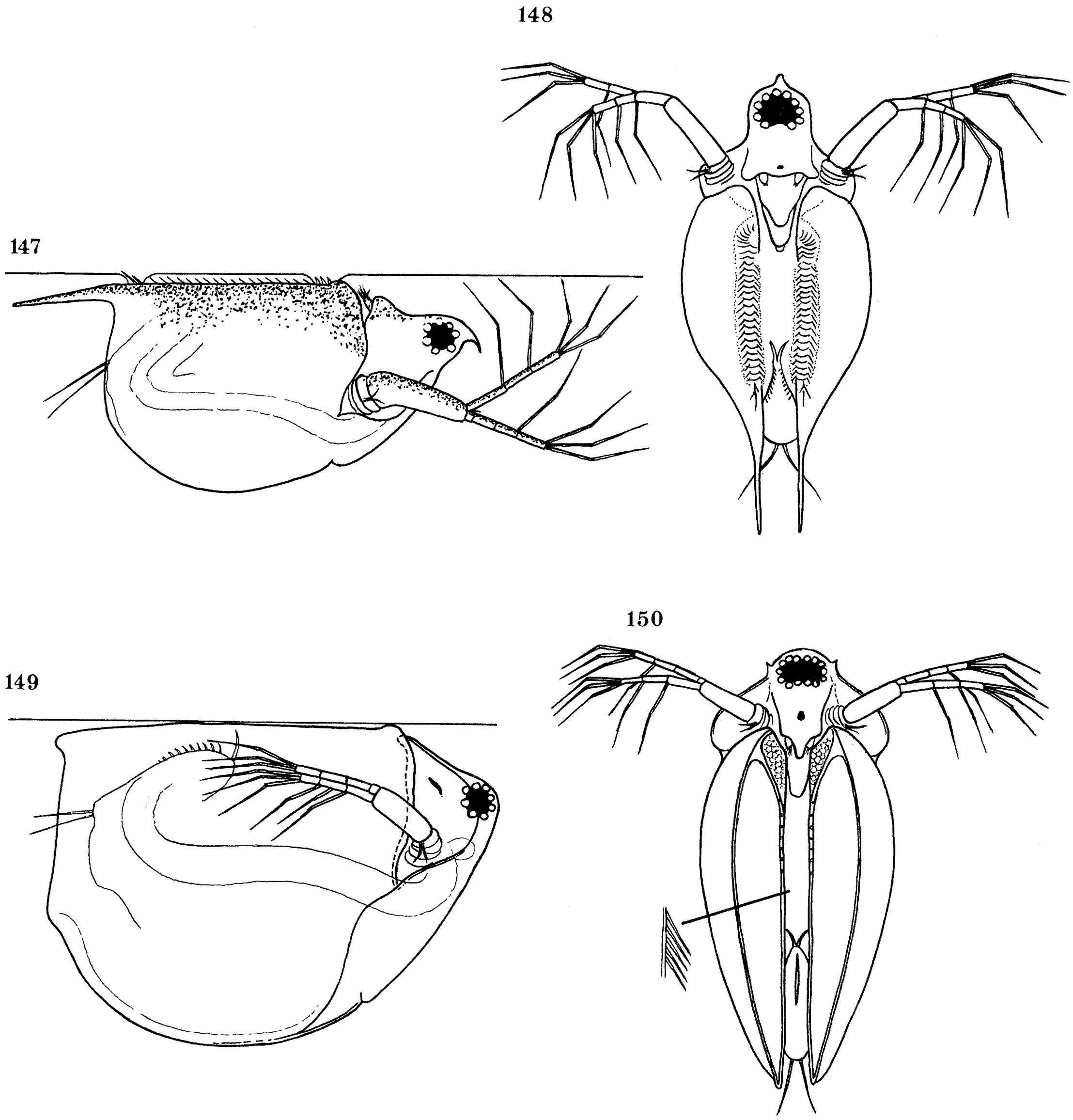
While the feeding mechanisms of *Simocephalus* and *Daphnia* are basically the same, there are differences in the armature of trunk limb 2 (as well as those in gnathobasic armature) that probably reflect the different habits of the two and their long separation. In *Simocephalus*, only three long endite elements are well developed compared with five in *Daphnia*. Two are small and there are also two additional small spines. The distal endite bears two setae, similar to but shorter than, those of *Daphnia*, and the spine of the latter is represented by a minute structure not shown even by the meticulous Lilljeborg (1900), but shown by Glagolev (1983). In some species of *Daphnia*, this spine is serrated and, in *D. magna* and *D. obtusa* at least, can sweep or grasp material in the ancestral manner. Its persistence as a smooth structure, apparently not used for this purpose, in other species may reflect relatively recent use. *Simocephalus* has been distinct from *Daphnia* since at least the Eocene (Lai & Li 1987), and almost certainly since the Cretaceous (Fryer 1991). Its feeding habits, feeding while attached to an object, precludes the use of such a spine, which has been virtually lost during its long ancestry. The reduction of the gnathobasic spines may be related to the fact that species with scrapers can sweep or lift large accumulations of material which call for an array of sweeping and cleaning spines, whereas *Simocephalus* is restricted to taking in only finely suspended particles.

(*c*) *Scapholeberis* and *Megafenestra*

(i) *Introduction and taxonomy*

The long-established genus *Scapholeberis* was split by Dumont & Pensaert (1983), who erected *Megafenestra* to receive the old-world *M. aurita* (Fischer) and its new-world relative *M. nasuta* (Birge). Seven species, including the most familiar, *S. mucronata* (O. F. Müller), remain in *Scapholeberis*. While accepted here, such a generic split to some extent obscures the close relationships of this assemblage, which is sufficiently distinctive within the Daphniidae to have led Dumont & Pensaert (1983) to erect a new sub-family, the Scapholeberinae, for its reception.

Species of these genera are notable for having exploited what to some daphniids is a hazardous feature of the environment and have thereby gained access to niches in which they have few competitors. By virtue of specialized modifications of the ventral carapace margins, they are able to suspend themselves in an inverted position from the surface film of water. Utilization of these carapace margins for many purposes, including suspension beneath the surface film in the tropical *Dadaya*, has been a key feature in the adaptive radiation of the Chydoridae, many species of which have an intimate association with substrata. It has also been important in the Macrothricidae, but much less so in the evolution of the Daphniidae, whose adaptations have been predominantly in the direction of emancipation from substrata and the colonization of



Figures 147–150. *Scapholeberis* and *Megafenestra* and their adaptations for association with the surface film. Figure 147. *S. mucronata* (length about 1 mm), lateral, suspended beneath the surface film. The armature of the ventral carapace margins is simplified and the antennae are shown in more or less the most promoted position. Note the dark pigmentation on the ventral parts of the head, carapace and antenna which gives inverse countershading. Figure 148. *S. mucronata* seen from above as it moves beneath the surface film. The armature of the ventral carapace margins is shown only in a simplified manner and pigmentation is omitted. Figure 149. *Megafenestra aurita* (length about 1.8 mm), lateral, suspended beneath the surface film. The antenna is approximately at the beginning of promotion. Figure 150. *M. aurita* seen from above as it moves beneath the surface film. Note the broad ventral flange of each ventral carapace margin. A fringe of setae, omitted for clarity, lines the ventral margin of each carapace valve and is shown in a simplified manner in the inset.

open water, where opportunities for such specialization are few. Developments in the Scapholeberinae are clearly of secondary origin.

(ii) *Ecology and habits of S. mucronata*

S. mucronata (L. to *ca.* 1.2 mm) (figures 147, 148), the only British representative of the genus, occurs widely throughout the northern part of the Holarctic region in a wide range of habitats. Its frequent use of the surface film calls for calm conditions which it finds both in small water bodies and in the sheltered parts of large expanses, often among vegetation, but as it can also swim freely, it is able also to frequent more open water and has indeed been reported at a depth of 100 m (von Hofsten 1911), but this is probably unusual. Among daphniids, it is remarkable for its physiological tolerance and occurs under a wide range of chemical conditions. In Britain it is rivalled only by *Ceriodaphnia quadrangula* as a colonizer of acidic habitats, some of low conductivity, but it also occurs commonly in alkaline, ion-rich waters and even in highly mineralized waters of high conductivity and diverse ionic composition. In Yorkshire I have found it below pH 4.2 on 12 occasions, a level of acidity at which no species of *Daphnia* has ever been encountered there (Fryer 1985). The most acidic site had a pH of 3.88. Like its morphologically based habits, such tolerance must at times render it free from competition and reduce predation.

The most striking feature of the gross morphology of *Scapholeberis* is the modification of the ventral carapace margins. Viewed laterally these are almost straight (figure 147). They are also folded medially to form a flange which is provided with highly modified setae (figure 148) that were carefully described and illustrated by Scourfield as long ago as 1894 and have since been illustrated by Dumont & Pensaert (1983). Each valve is drawn out posteriorly into a long spine. Like *Simocephalus*, and in contrast to *Daphnia*, *Scapholeberis* swims with the ventral surface uppermost, often proceeding with the anterior end higher than the posterior. Not surprisingly, the flat ventral carapace margins can be placed against surfaces, even against the vertical walls of a glass container and doubtless often against plants in nature, where the animal can rest. Although this seems not to have been reported, *S. mucronata* can also move forward over such substrata. Individuals may remain on a surface for many minutes and move forward intermittently, sometimes by occasional sweeps of the antennae, sometimes without. In the latter case, it is not certain how movement is achieved. Trunk limb 1, which is used for locomotion in chydorids and macrothricids, seems ill-equipped to fulfil this role here. The exhalent current, which leaves the carapace chamber posteriorly, probably assists forward movement.

The carapace margins can also be placed against the surface film where, as Scourfield (1894) described, groups of anterior and posterior flange setae, which are water-repellent, pierce the film and cause four minute capillary depressions. These support the animal which, like an anchored individual of *Simocephalus*, can remain in position without expending energy. It can also swim while so attached. Contact with the surface film can be broken at will, evidently by vigorous sweeps of the antennae, as when the animal is disturbed, and it then dives. Scourfield (1894) suggested that possession of more or less straight ventral carapace margins probably predated the acquisition of surface-film-frequenting habits. There are, however, no known extant daphniids that use the carapace margins in the manner of various chydorids and macrothricids and it is difficult to see how such use could be reconciled with the exploitation of a typical daphniid filter feeding device such as *Scapholeberis* possesses (see below).

A striking adaptation to life beneath the surface film is the development of dark pigmentation that countershades suspended animals. This was well described by Scourfield (1894) and, as shown in figure 147, involves not only the ventral parts of the carapace and head, but of the antennae and the dorsal (topographically ventral) region of the post-abdomen. Such strong pigmentation bespeaks of frequent use of the surface film, where it must confer a strong selective advantage. Free-swimming, non-suspended individuals appear almost black and are very conspicuous. Their evident vulnerability may be to some extent offset by the long carapace spines and by the frequently developed long headshield spine.

At least in containers, *S. mucronata* displays a strong preference for the surface film where most of the population, including what appear to be individuals in the first instar, quickly congregate. Here they either move around, apparently haphazardly, by intermittent sweeps of the antennae, or simply hang suspended, when they generally move slowly forward as a result of the propulsion provided by the exhalent current. When necessary, they can move very briskly beneath the surface film, where they seldom follow a straight course for very far. They are reluctant to vacate the film and can often be pushed with a needle without being stimulated to do so. If pushed beneath the surface, they often descend for a few millimetres only, swim briskly for a few centimetres, and re-establish contact.

(iii) *Ecology and habits of M. aurita*

The range of *Megafenestra aurita* (length to about 2.1 mm) extends from southern Europe and North Africa to Central Asia and there is an outlier in South Africa. The latter probably represents a relict of the former faunal connections with the Mediterranean region across the East African plateau (Dumont 1980). *M. aurita* is rare in Britain, which lies at the limits of its range, but in 1971 Mr A. L. Galliford found it in abundance at Leighton Moss, Lancashire, where I subsequently collected it. It occurred in shallow pools, drains and flooded reed beds at this marshy site, in some places in vast numbers. In subsequent years, it has been found only sporadically, if at all, but was abundant in 1989 when needed for further observations. At the collecting site it coexisted with *S. mucronata*. On this occasion, material was collected from a shallow, somewhat foul, drainage ditch, mostly less than 2 m wide and seldom more than *ca.* 20 cm deep, between large expanses of *Phragmites*. The animals occurred mostly at the margins among grasses and other vegetation.

M. aurita has flat, flanged, ventral carapace margins (figures 149, 150) similar to, but much broader than, those of *S. mucronata*, from which they also differ in being completely devoid of specialized setae. The valves lack the posterior spines so conspicuous in *S. mucronata*. As shown in Gurney (1903) and Sars (1903), the flange is bounded externally by a ridge and internally by the thickened rim of the carapace valve which is continuous anteriorly with an obliquely running ridge which runs to the external ridge. This delimits a small, more or less triangular, area anteriorly whose surface is reticulated. Where the oblique and external ridges unite, there is a projection, readily apparent from the lateral aspect. As Gurney (1903) was the first to point out, there is on the inner longitudinal thickened rim a series of minute, widely separated, vertically projecting teeth, up to eight in number, of which the anteriormost, and largest, lies where the rim begins to incline laterally. The projections that lie posterior to this increase somewhat in size from before, backwards. These projections fulfil the same function as the elaborate apparatus of the ventral margins in *S. mucronata*: they pierce the surface-film causing depressions. The inner margins of the carapace are fringed by delicate setae (shown only in the inset of figure 150) that play no part in suspension.

While, like *S. mucronata*, *M. aurita* hangs from the surface film, it is less specialized for this habit than the former. Its flange is broader, but less elaborate, and there is no development of black pigment to give the inverse countershading so characteristic of *S. mucronata*. Nevertheless, although the colour is pale brownish, animals suspended from the surface film appear dark when viewed from above. Topographically ventral regions of the post-abdomen and trunk limb 5 are golden brown in colour. Large clutches of dark eggs or broods of young in the brood pouch enhance this effect, as does the gut which, at Leighton Moss in 1989, was usually packed with organic detritus. Both Lilljeborg (1900) and Gurney (1903) have noted that it does not habitually swim in the 'inverted' position adopted by *S. mucronata*, but it certainly does so frequently. Like the latter, it also settles elsewhere than on the surface film. Gurney suggests that the minute projections used for creating capillary depressions in the surface film also assist in this.

Like *S. mucronata*, *M. aurita* quickly seeks out the surface film and spends most of its time there, often remaining attached even if pushed by a needle. When pushed beneath the film, it generally swims only a short distance before re-establishing contact. Larger than *S. mucronata*, it can swim more quickly beneath the surface film. Individuals also glide slowly forward unaided by the antennae as a result of propulsion by the exhalent current that leaves the carapace chamber posteriorly. An individual swimming beneath the surface film can defaecate without moving its post-abdomen.

In shallow dishes, a change from reflected to transmitted light often causes the animals to leave the surface film and settle on the bottom. Change to reflected light usually stimulates them to return to the surface film, which involves re-orientation of the body. This suggests that, as might be expected, light is used in direction finding. However, animals kept for a time in darkness also congregate beneath the surface film and this is true also of *S. mucronata*.

Individuals at times settle not only on the bottom but on the vertical walls of a container and 'swim' slowly over them by use of the antennae. They have also been seen to 'scurry' along vertical walls without using the antennae, much as does *D. obtusa*. This habit is less surprising in *S. aurita* with its flat ventral carapace margins than it is in the latter. As in the case of individuals beneath the surface film, defaecation is possible without swinging the post-abdomen.

(iv) *Notes on the trunk limbs and feeding mechanism*

S. mucronata. The structure and arrangement of the trunk limbs are basically as in *Daphnia* but differ much in details. Figure 151 shows the arrangement of the filter chamber; figure 152 parts of limbs 2, 3 and 4 at higher magnification. The filter chamber is much smaller than that of even small species of *Daphnia*. The length of the filtering surfaces is here only about 200 μm, as opposed to about 500 μm in *D. longispina*, a small species, seen in figure 3, and its volume much smaller. The labrum is particularly long.

Trunk limb 1 calls for no special comments. Apart from the median, sensory, seta, the armature of the gnathobase of trunk limb 2 consists of only 8 spines, far fewer than in *Daphnia*. Of these, the posteriormost is a long, posteriorly directed filter-cleaning spine similar to, but relatively shorter than, its homologue in *Daphnia* and the adjacent spine is also similar to its homologue in that genus. The three anterior spines also resemble those of *Daphnia*, being stout structures that sweep food material to the mouthparts. There are only three spines in the median row. Of these, the first two are generally closely associated with the three anterior spines; the third is separated by a wide gap on either side. These are the structures that, according to Ganf & Shiel (1985*a*, *b*), are allegedly filter setae in *Daphnia*, and which, if their explanation of the feeding mechanism is correct, must serve the same function here. Even if they had borne a resemblance to filter setae, which is not the case, it is clear that only three such structures, whose arrangement is inappropriate for this, could not conceivably serve as a filter. This alone shows the erroneous nature of this conception.

The endite spines of trunk limb 2 are stout and appear to be suitably armed with spinules for helping to push larger particles into the filter chamber, a retention, or redevelopment, of a primitive function. Such action has not been observed but seems probable. An ability to deal with 'windfalls' of large particles encountered beneath the surface film would certainly be advantageous.

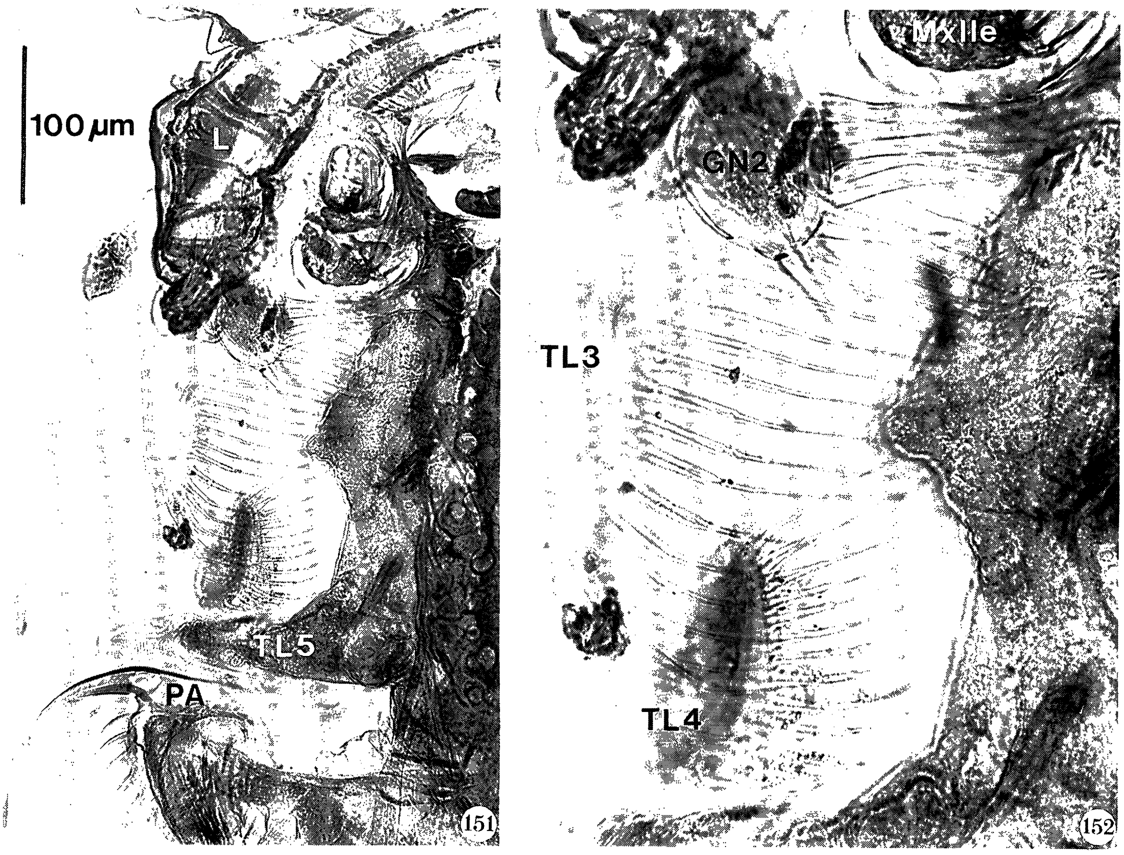
As in *Daphnia*, trunk limb 3 bears a filter plate that dominates the trunk limb complex. The filter plate, however, consists of only about 22 setae which are relatively widely spaced, the gap between them being about 10 μm over much of their length. The filter plate of limb 4 is much smaller and is composed of only about 14 setae, which are shorter than those of trunk limb 3.

The anterior vertical seta of trunk limb 5, which can be seen standing vertically from the trunk in figure 151, is thick and copiously provided with cleaning setules.

The trunk limbs beat with great rapidity and in the manner typical of all daphniids. When an individual is suspended from the surface film, particles, presumably including those that have accumulated at this interface, pass between the carapace valves from in front, but are nevertheless sucked into the posterior end of the filter chamber in the typical daphniid manner. While there are doubtless minor differences, the abstraction of particles and their subsequent passing forward along the food groove is essentially the same as in *Daphnia*.

M. aurita. The structure and arrangement of the trunk limbs (figure 153) resemble those of *Scapholeberis* but the filter plates of limbs 3 and 4 are larger and consist of more setae. That of limb 3 has about 32 filter setae; of limb 4, about 18. The gnathobase of trunk limb 2 has the same arrangement as that of *Scapholeberis*: the anterior members of the median row, which consists of four spines, tend to be closely associated with the three anterior spines as in that genus. As in *Scapholeberis*, trunk limb 5 has a thick, highly setose anterior vertical seta.

The trunk limbs beat with great rapidity, faster than is typical of species of *Daphnia*. Trunk limb 1 beats in a regular rhythm, but with small amplitude, during this



Figures 151 and 152. *Scapholeberis mucronata.* The filter chamber and adjacent structures. Figure 151. Median longitudinal section through the filter chamber. For ease of comparison, the orientation is as for *Daphnia* in figure 3, though the animal swims ventral surface uppermost, both in open water and when suspended from the surface film. Note the large wall provided posteriorly by trunk limb 5 (TL5). Figure 152. The same, showing more details. Note the small number of elements in the armature of the gnathobase of trunk limb 2 (GN2) and how their anterior members tend to lie close together. The long filter-cleaning spine has been cut short. Note also the small number of filter setae in the filter plates of trunk limbs 3 (TL3) and 4 (TL4).

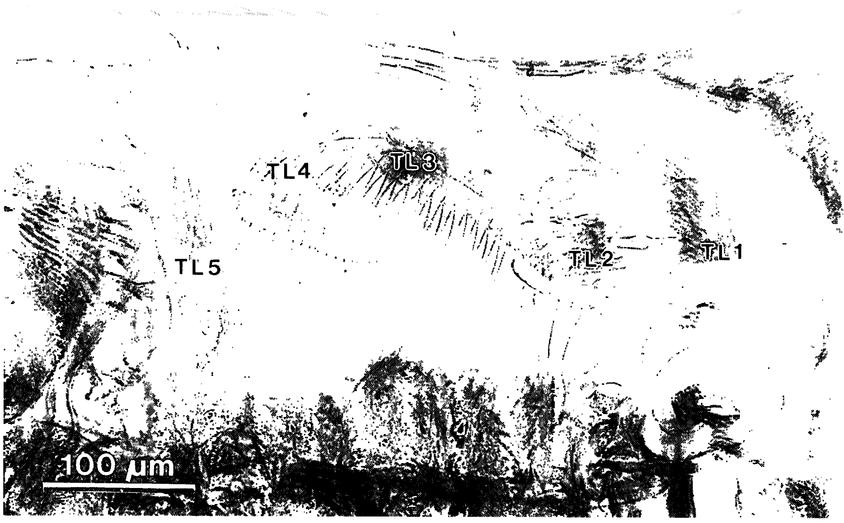


Figure 153. *Megafenestra aurita.* Median longitudinal section through the filter chamber. Note the more numerous filter setae in the filter plates of trunk limbs 3 (TL3) and 4 (TL4) than in *Scapholeberis mucronata* (figures 151 and 152) and the similarity of the gnathobasic armature of trunk limb 2 in the two species.

process. In this case movement is not passive; muscles can be seen in action. As in *Scapholeberis*, particles are drawn between the carapace valves from ahead of an individual suspended from the surface film. As in other daphniids, they are sucked into the posterior end of the filter chamber and passed forward along the food groove.

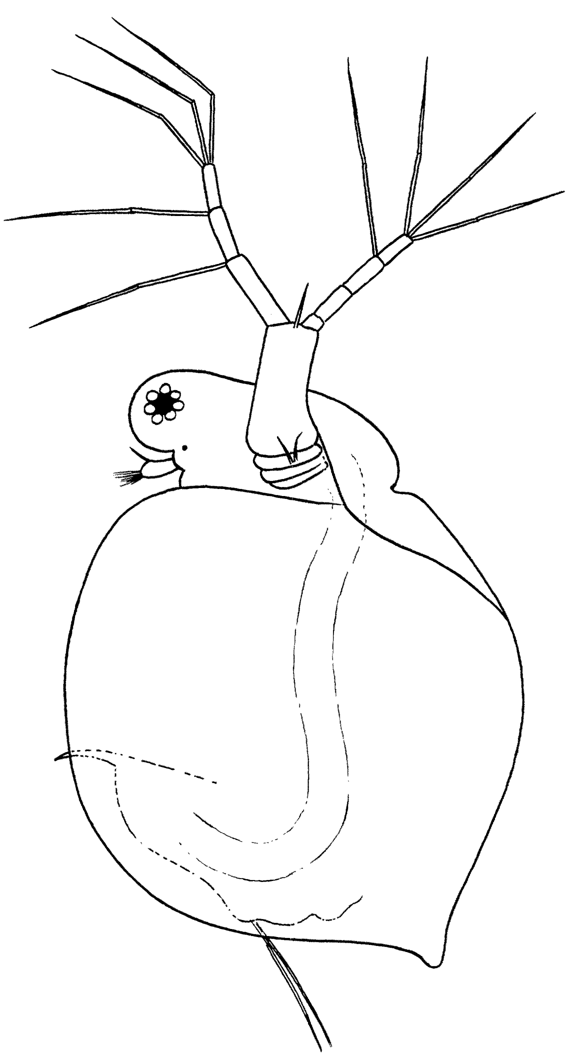


Figure 154. *Ceriodaphnia quadrangula.* Length 0.8 mm, showing the general form of members of the genus.

(*d*) *Ceriodaphnia*

The genus *Ceriodaphnia* (figure 154), with upwards of two dozen species, has a virtually world-wide distribution. Small size is one of its hallmarks: females of large species seldom exceed 1.5 mm in length, small species may not achieve 1 mm. Males are smaller. All lead a generally open-water, swimming existence, though only a few have become truly planktonic in large lakes. Others, however, occupy the open water of ponds and other small water bodies. They also frequent the inshore waters of lakes. A few venture into stands of vegetation or even find their way into confined spaces.

Gross morphological differences between *Ceriodaphnia* and *Daphnia* include the shape of the head, the arrangement of the antennules, and a carapace that is generally less attenuate than is commonly the case in *Daphnia*, and which sometimes approaches a circular outline in lateral view. The carapace is often broad, so that the animal is rotund. There is no carapace spine, but the postero-dorsal region is often angular, and sometimes a spine-like protuberance is developed there.

The carapace, and the head cuticle, are strengthened by a predominantly hexagonal meshwork of cuticular ribs, well developed in some species, less so in others. In *C. setosa* Matile, which is strongly reticulated, a small spine arises from each node of the reticulation. These spines, which are reported in other species, are presumably defensive.

Orientation of the body is as in *Daphnia*. In some species at least, swimming is not so persistent as in that genus, but the same 'hop and sink' pattern is displayed. In *C. quandrangula* (O. F. Müller) for example, antennal beating is usually confined to a few strokes followed by a brief pause, then a repeat of this sequence. Individuals tend not to move vertically so much as is often the case in *Daphnia*. A distinct forward tilt of the head region is evident at each bout of antennal activity. As in *Daphnia*, horizontal swimming and downward diving are practised when necessary.

Several species tend often to remain near the bottom. This has been noticed in *C. laticaudata* P. E. Müller, *C. reticulata* (Jurine), *C. dubia* Richard, and even in *C. quadrangula*, some populations of which occupy open water. This habit is more apparent than in *Daphnia* and may indicate a greater reluctance to vacate ancestral habitats. *Ceriodaphnia* is also more frequently associated with weed beds than is *Daphnia*, an ecological preference doubtless related in part to small size and the absence of a long postero-ventral spine, which would be a disadvantage in such situations.

Most of the British species prefer alkaline conditions, but *C. quadrangula*, the most successful colonizer of acidic moorland waters, can tolerate pH levels in the vicinity of 4. Its occurrence in such waters, from which species of *Daphnia* are generally excluded, led Harnisch (1950) to suspect, for reasons that are not very clear, that the difference may reside in the nature of their feeding mechanisms (it is in fact almost certainly of a more direct physiological nature) and he investigated that of *C. quadrangula*. His report, the only such, is not very precise and he admits its incompleteness and the paucity of morphological details. Nevertheless, he provided various items of interest. The basic features of the trunk limbs are similar to those of *Daphnia* and their arrangement and the nature of the filter chamber, whose volume is very small, are typical of the Daphniidae. Trunk limb 2 is shown by Lilljeborg (1900). Trunk limbs 3 and 4 bear *Daphnia*-like filter plates, but with fewer filter setae. Harnisch reports about 40 on trunk limb 3. A similar number is present in *C. pulchella* Sars, in which just over 30 are present in the filter of limb 4.

Harnisch noted that beating of the trunk limbs of *C. quadrangula* is seldom as persistent as in of *Daphnia*, reporting that often only 5–20 cycles of activity are followed by a pause. In fact, although this species is often reluctant to beat persistently, it sometimes does so exactly as does *Daphnia* and one suspects that its reluctance reflects uncongenial conditions and does not apply in nature. Limb beats are generally rapid. Porter *et al.* (1982) recorded frequencies of 10–12 Hz in *C. lacustris* Birge.

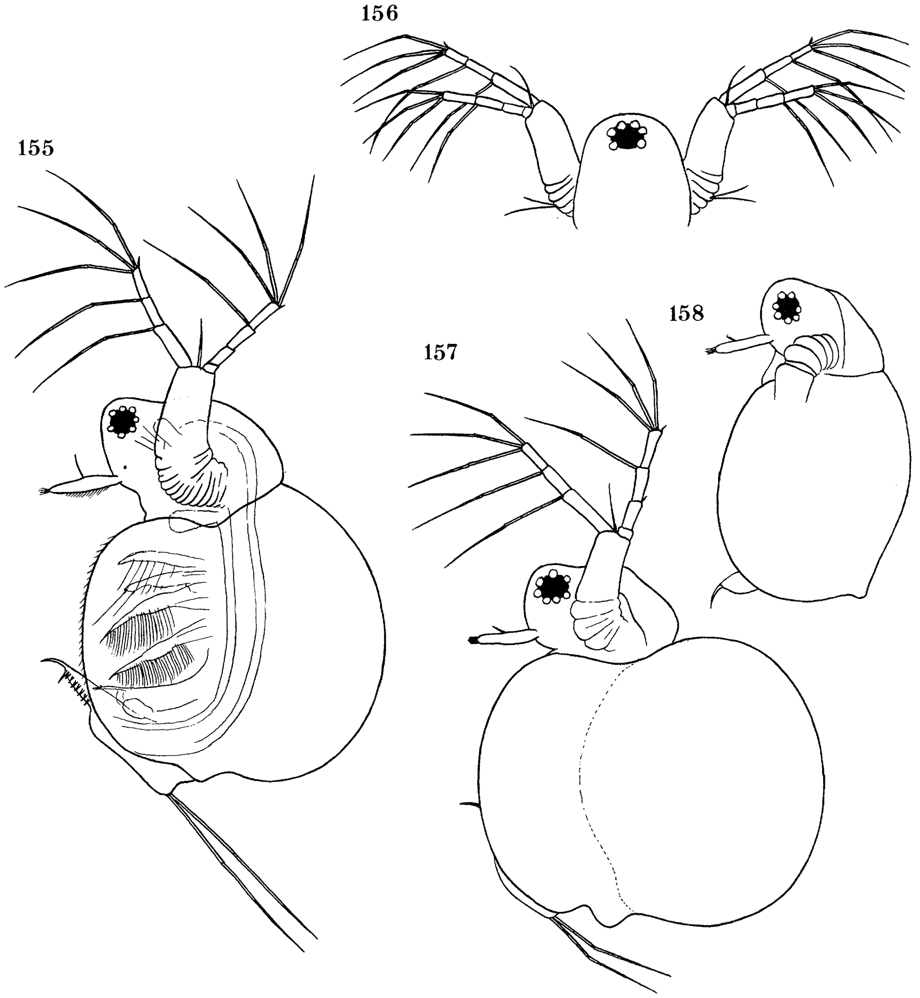
Harnisch supplied *C. quadrangula* with particulate material of various kinds and saw coarse particles drawn deeply into the food groove, whence they were immediately carried forward. He believed there was an orally directed current in the food groove and wondered whether the action of the fifth trunk limbs was involved in its production. In fact, there is no such current. Nor could the fifth limbs produce such. As in *Daphnia*, their role is to seal the posterior interlimb spaces posteriorly. It is curious that Harnisch makes no reference to the fact that Storch, with whose description of the mechanism in *Daphnia* he compares that of *Ceriodaphnia*, described the mechanical sweeping of material along the food groove. Because Indian Ink passed through the filters, Harnisch concluded that *Ceriodaphnia* does not possess a true filtering device and seemed to think that the abstraction of coarse particles may have been by some other process that he mentioned vaguely. He concluded that, although the appropriate limbs show clearly the characteristics of true filters, *Ceriodaphnia* displays what he calls unmistakable resemblance to the behaviour of 'mud eaters' and deviates from the habits of a true filterer. This conclusion seems to be coloured by the unsubstantiated view that moorland waters are unsuited to pure filterers and not to be based on clear-cut evidence.

Particular attention is drawn to the gnathobasic armature of trunk limb 2. This includes the usual long filter-cleaning spine and its somewhat shorter companion and the three stout anterior spines typical of the Daphniidae. The median row consists of only four short spines, whose structure could hardly be claimed to be that of filter setae by even the most ardent supporters of the claim that filtration in daphniids takes place here. Even if this were possible the filtering area would be woefully inadequate. It has indeed been apparent since the simple report of Harnisch that the process is essentially the same as that described here for *Daphnia*.

(*e*) *Moina*

(i) *General morphology and habits*

The general form of species of *Moina* (figures 155–158) is well shown in some of the older illustrations of G. O. Sars, and good line drawings of the European species are given by Negrea (1983), but many others are inadequate and merely contributed to the chaotic taxonomic history of the genus before the revision of Goulden (1968). Like *Daphnia*, Moina is successful and



Figures 155–158. Aspects of the gross morphology of *Moina*. Figure 155. *M. micrura*, parthenogenetic female (length about 1 mm), lateral, showing the form typical of members of the genus. Note the nature of the antennal protopod and its articulation. Figure 156. *M. micrura*, head and antennae, dorsal, to show the orientation of the antennae. Figure 157 and 158. *M. brachiata*. Outlines (based on figures by Gauthier 1954) of two gravid females, one with four, the other with many, embryos in the brood pouch, showing the enormous distortion that takes place when large broods of young are carried.

widely distributed. Its eighteen or so species differ in relatively superficial ways. Save for the single species assigned to the closely related *Moinodaphnia*, assignment to the genus is never in doubt.

Moina retains features that point to distant relationships with the Macrothricidae – a head lacking a headshield (a situation found in some, but not all, macrothricids), long antennules, antennae that, like those of other daphniids, are composed of the same elements as those of macrothricids, but whose protopod is not straight but flexed in a manner more reminiscent of various macrothricids than of advanced daphniids. The first and last of these points appear not to have evinced comment. Some of these features, such as the long antennules and lack of a headshield, are primitive but, as noted below, *Moina* and *Moinodaphnia* combine them with advanced attributes that have not been acquired by other daphniid genera.

The head, with its bluntly rounded contours, was said by Goulden (1968) to be 'basically similar to that of the Sididae', and emphasis was placed on the location of the elongate antennules 'on the ventral surface of the head, just below the eye'. The long antennules of the macrothricidae to which those of *Moina* show similarities, were said to be located 'at the anterior of the ventral head margin'. There are, however, anatomical differences in sidid (ctenopod) and macrothricid (anomopod) antennules, and position is no guide to affinity but reflects functional needs. In some macrothricids, such as *Lathonura* and *Iliocryptus*, the antennules in fact arise in more or less the same position as they do in *Moina*, and in the ctenopod *Latona*, they arise well forward. There are indeed similarities between the heads of ctenopods and anomopods; they share various features not only with each other, but with other branchiopod orders; but there are also fundamental differences, as for example the nature of the antennae.

Daphnia, and all other daphniids save *Moina* (and *Moinodaphnia*) have a straight antennal protopod. In *Moina* (and *Moinodaphnia*) the protopod is flexed so that proximally it extends laterally from the head before it curves forward and parallel to the long axis of the body (figures 155–157). The curved region displays much pleating of the cuticle to negotiate the bend. Such protopods are much more like those of *Acantholeberis*, *Lathonura* (see, for example, the figures in Fryer (1974)) and several other macrothricids than those of *Daphnia*. Nevertheless, locomotion in *Moina* is essentially *Daphnia*-like, both in antennal movements and in the orientation of the body. *M. brachiata* (Jurine) swims effectively and, if necessary, rapidly, but often moves by a single sweep of the antennae, which propels the animal upwards and somewhat forwards for something in the order of a body length, generally tilting the head forward. This is followed by a perceptible pause, during which sinking occurs. When, as is often the case, this cycle is frequently and rhythmically re-enacted, a 'bouncing ball' effect results. Like *Daphnia*, *Moina* can swim horizontally or dive more or less vertically downward.

Many species of *Moina* frequent small, shallow, sometimes turbid and often temporary pools, some which are little more than puddles. Others have a predeliction for saline or highly alkaline waters. Aladin (1983) cites Sukhanovna as reporting *M. mongolica* Daday (now *M. salina* Daday) living at a salinity of *ca.* 92‰, while Negrea (1983) reports this species at a salinity of 104.7‰. Such habitats often harbour few potential competitors. This accords well with the primitive position of the genus in the Daphniidae. Some forms of *M. micrura* Kurtz, however, are planktonic in large lakes. In general, members of the genus have a preference for warm conditions, and there appear to be no records for cold, high northern latitudes where species of *Daphnia* are sometimes well represented.

Like some pond-dwelling species of *Daphnia* some species of *Moina* sometimes congregate in shoals whose members move in a coordinated manner. In a large pond on a hot day, I have seen vast numbers of *M. brachiata*, readily visible en-masse to the naked eye, aggregated into irregular, anastomosing ribbons whose shape changed as the shoal moved. Most individuals appeared to be concentrated in the upper 1 or 2 cm of water. Shoaling was also reported in an un-named Malaysian species by Johnson & Chua (1973) and in *M. affinis* Birge by Ratzlaff (1974). The former referred to shoals about 10 cm long by 5 cm wide that moved around like super-organisms: the latter to nearly continuous bands in a line > 100 m long, which appeared close inshore each day for eleven days. Ratzlaff's aggregations may have been related to physical conditions as no reference is made to coordinated swimming, and sexual behaviour is suspected, but unproven.

(ii) *Reproductive specializations: their morphological and physiological consequences*

The reproduction of *Moina* (and the closely related *Moinodaphnia*) differs from that of other daphniids in that the eggs contain but little yolk. Unlike those of *Daphnia*, they cannot be reared *in vitro* outside the brood chamber. Their development, and that of the embryos, depends on the absorption of maternal secretions, produced by a 'placenta' or Nährboden located on the dorsal wall of the thorax, which therefore lies within the brood chamber. This was described by Weismann (1876–79). No such structure is known in other anomopods (other than *Moinodaphnia*) but analogous (or perhaps even homologous) organs have been acquired, clearly independently, in the ctenopod *Penilia* and in the much more distantly related Onychopoda. Since Weismann's work, the Nährboden of *Moina* has received scant attention, though Makrushin (1985) makes useful comments and the general principles of nutrient secretion are probably similar to those elucidated in the onychopod *Polyphemus pediculus* (L) by Patt (1947).

The supply of nutrients to the eggs and embryos by the mother has many repercussions. Because of the incorporation of material from without during development, embryos increase in size more than do those of *Daphnia*, which depend on an internal store of yolk, so the contents of an egg-packed brood chamber expand considerably in volume during development.

This necessitates distension and distortion of the carapace that forms its walls.

The ability of the carapace of *Moina* to become distended is a peculiarity that seems seldom to have been appreciated and that has certainly not been adequately investigated. In mature females it becomes distended dorsally and laterally to a degree that depends on the number and size of the parthenogenetic eggs or embryos contained in the brood chamber. Distention can be sufficient to render the shape of a highly fecund female very different from that of an individual with an empty brood chamber. Gauthier (1954), from whose illustrations figures 157 and 158 have been prepared, shows the lateral contours of what he designated as 'paucigravide' and 'plurigravide' females of *M. brachiata* (called by him *M. rectirostris*). On superficial examination, these could easily be taken to be different species. Seen dorsally, individuals with packed brood chambers can appear almost globular. Distention must involve properties of the cuticle different from those of other anomopods. It also affects the efficiency of swimming: heavily burdened, distorted females are less agile, and more conspicuous, than earlier instars or less fecund individuals. Survival is probably often assured by the nature of the habitat that frequently contains few predators.

Most species of *Moina* are generally held to lack the finger-like prolongations, known as post-abdominal processes, that close off the posterior end of the brood chamber in other daphniids. However, several of them have instead at least a horseshoe-shaped ridge, or even a conspicuous prolongation, in the same region as the post-abdominal processes of *Daphnia*. This serves the same purpose and can scarcely be other than homologous. It differs in form for functional reasons and is highly specialized. Because the embryos of *Moina* are dependent on maternal secretions, it is necessary to prevent loss of these from the brood chamber, which has to be well sealed. In *Daphnia*, and indeed in most anomopods, while there are devices for the retention of eggs and embryos, the brood chamber is in free communication with the exterior and water can enter and leave it, especially when the trunk is from time to time swung ventrally, which momentarily increases the volume of the brood chamber, replenishing the water within it. No such exchange of water occurs in *Moina*, whose post-abdomen fits snugly against the carapace as a water-tight seal. In *M. brachiata*, and presumably in other species, defaecation can take place without protruding the post-abdomen. Furthermore, the post-abdomen can be swung to a considerable extent without breaking the seal of the brood chamber. Thus the acquisition of a means of nourishing the embryos by maternal secretions has involved the evolution of a suite of closely integrated features, morphological and physiological. These are, a secreting Nährboden, a distensible carapace which forms the walls of much of the brood chamber, an efficient device for sealing the chamber, and a means of swinging the post-abdomen without breaking the seal.

There is a possible further element in this complex. In most daphniids water can enter and leave the brood chamber so the presence of eggs or embryos places no direct respiratory load on the mother. Because the brood chamber of *Moina* is sealed, the respiratory demands of the developing embryos have to be met through the mother. Limb beat in *Moina* is generally more rapid than in other daphniids (§8(*e*) (iii)) and may in part be related to the extra respiratory demand imposed on gravid females by the sealed brood chamber, but this remains to be tested.

According to Aladin (1983) a closed brood chamber has been an important element in the colonization of hyper-saline waters which is a striking feature of the adaptive radiation of *Moina*. He believes that such colonization depends not only on a physiological ability to prevent osmotic water loss from the haemolymph but on a means of protecting developing parthenogenetic embryos from the high salt content of the medium. Only a closed brood chamber is said to be able to confer such protection. Embryos of *Daphnia*, for example, are not so protected. This is an attractive suggestion and the closed brood chamber of *Moina* may indeed protect embryos in this manner, but Australian species of *Daphniopsis* tend as a group to frequent saline waters (up to salinities of at least 71‰) and they have an open brood chamber, as has *Daphnia similis*, which has allegedly been recorded at a salinity of 106‰ in Canada. Their embryos therefore tolerate salinities against which it is suggested that those of *Moina* have to be protected.

A sealed brood chamber has other implications. Anomopods in which fertilization of sexual eggs takes place in a brood chamber that is freely open to the outside world produce numerous small sperms. During fertilization, many of these are inevitably lost. As long ago as 1879, Weismann noted that some 'cladocerans' (Sididae, now Ctenopoda, and *Leptodora*, now Haplopoda) that have a seminal receptacle, and therefore suffer little or no loss of sperms, produce few and large, sometimes very large, sperms. As Makrushin (1985) has pointed out, the same is true of certain 'cladocerans' which have a closed brood chamber and a Nährboden (Onychopoda and certain species of *Moina*) where again few sperms are lost. Makrushin's correlations provide a functional explanation of the differences in sperm size that completely cuts across phylogeny.

Some species of *Moina* have very large and remarkably complex sperms, so large that they conceivably make a significant contribution to the energy store of the fertilized resting egg or eggs. Among investigated species, *M. brachiata*, *M. micrura* and *M. macrocopa* (Straus) have such sperms (Wingstrand 1978), yet these differ much among themselves. Those of *M. brachiata* and *M macrocopa*, for example, are very different. The evolution of these sperms in *Moina*, described by Wingstrand (1978) as 'by far the most complicated and strange variants seen in the Branchiopoda', and so divergent that 'they do not seem to fit at all into the anomopod system', posed a phylogenetic problem for that author. Yet in striking contrast, some species of *Moina* have small spherical sperms of the more usual anomopod type. This wide range of sperm types in *Moina* shows that the evolution of large, complex sperms in certain species is a purely intrageneric development. It can throw light on intrageneric relationships, but the remarkable sperms of certain species, especially when set against other features, cannot, for example, be used to support the familial separation of *Moina* and its relatives from the Daphniidae. Small sperms in *Moina* are clearly primitive.

(iii) *Trunk limb morphology and the nature of the feeding mechanism*

Notwithstanding the emphasis laid by Litynski (1916) on the differences between the first trunk limbs of *Moina* and *Daphnia*, the immediate impression of a comparison is their fundamental similarity. Their differences are minor. Litynski confirmed the striking similarity of the third trunk limbs of the two genera and even drew attention to a lack of clarity in Lilljeborg's (1900) figure of this limb in *Moina*, Lilljeborg showed limbs 1, 2 and 3 of *M. micrura* (as *M. rectirostris*) and enumerated the differences between them and their homologues in *Daphnia*. Cannon (1933) showed the gnathobasic lobe of trunk limb 2 and showed very clearly not only how the third and fourth limbs are similar to those of other daphniids but how their relations to each other are also the same. Even Goulden (1968), who removed *Moina* from the Daphniidae, admits that 'the structure of the moinid limbs clearly suggests that they are related to the daphniid Cladocera'.

Limb movements and the feeding mechanism are essentially as in *Daphnia*. Limb beat is rapid, often *ca.* 10 Hz or slightly faster in the limited measurements made on *M. brachiata*. Trunk limb 1 moves very little and, save for the gnathobase, limb 2 does likewise, seeming to be pushed by limb 3 when the latter promotes. The interlimb spaces appear to be larger than those of *Daphnia* but no measurements have been made. In the material studied, trunk limb 5 began remotion slightly before trunk limb 3 had completed promotion, but not before limb 4 had done so. Even if this breaks the seal before limb 3 has completed promotion, it will not affect the final stages of suction into interlimb space 3/4. As limb 3 remotes, its exopodite is forced almost at right angles to the corm, as seen in lateral view. At the end of remotion, it lies across limb 4 and remains as a ventral seal as limb 3 promotes. The exopod thus moves through a very wide angle during a cycle of movement. Particles are sucked deeply into the posterior region of the filter chamber as in *Daphnia* and the filter-cleaning spine of the gnathobase of trunk limb 2 operates as in that genus.

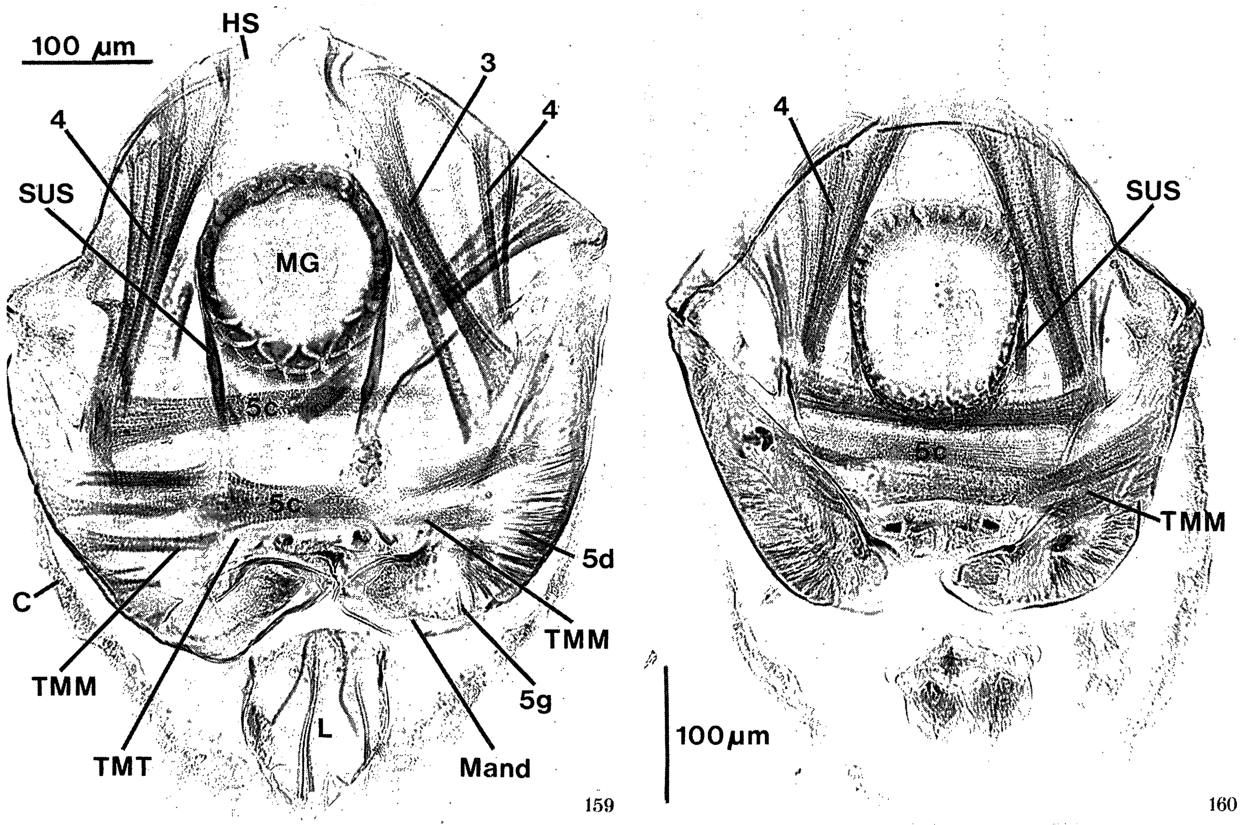
One peculiarity seen in *Moina* and not in *Daphnia* is that the limbs of a pair are sometimes slightly out of phase. This is most evident during remotion of trunk limb 3, when one limb lags slightly behind its partner. This minute disparity will not affect the mechanism. The interlimb spaces are eliminated before suction begins, and even if this begins very slightly earlier on one side than the other, the effect will be inconsequential.

In most species of *Moina*, a spine is present on the anterior face of the penultimate segment of trunk limb 1 and supplements the two distal spines of the distal segment. In *M. macrocopa*, the anterior spine is robust and takes the form of a toothed scraper similar to that of trunk limb 2 of *Daphnia obtusa* (figure in Goulden (1968)). Negrea (1983) shows such spines from three individuals. These differ in coarseness in ways that seem not to be size-related. The distal spine, adjacent to the scraper, is finely serrate and longer than the scraper, which strongly suggests that it operates in conjunction with the latter, sweeping up material dislodged by it, an arrangement found in many scraping crustaceans. There are as yet no observations of how *M. macrocopa* uses this device. Illustrations of the homologues of these spines in several other species suggest that they may serve as fine scrapers, but observations are lacking.

Mandibular arrangement is typical of the Daphniidae. The sections of *M. micrura* seen in figures 159 and 160 were prepared from specimens that had been stored for some time in alcohol, which often reveals muscles clearly at the expense of other tissues. They show the major features of the muscular system, including asymmetry of the transverse muscles.

(iv) *Affinities*

Although *Moina* has several striking distinctions, some, such as the lack of a headshield, the long antennules and the flexed antennal protopod being primitive, others such as the Nährboden and the associated features of the carapace and post-abdomen being specialized, it shares with all daphniid genera a fundamental similarity in the nature, arrangement and operation of the trunk limb complex, which immediately shows close affinity. This unity is particularly striking when compared with the situation that prevails in the Macrothricidae and Chydoridae. While all anomopods have a trunk limb complex that is clearly of common ancestry, the differences between these systems in genera of other families, e.g. *Acantholeberis*, *Streblocerus*, *Lathonura* and *Iliocryptus* (Macrothricidae) or *Alonopsis*, *Peracantha*, *Pseudochydorus* and *Graptoleberis* (Chydoridae) are vastly greater than those between *Moina* and any other daphniid genus. This of course reflects function as well as phylogeny. Members of these diverse macrothricid and chydorid genera occupy a wide range of niches that call for a diversity of food-collecting devices (though all are derived from the same basic elements) while all daphniid genera are basically abstractors of suspended particles. This common purpose has led to conservatism in a mechanism that acquired a high degree of perfection long ago. Daphniids whose ephippia at least had differentiated into structures very similar to those of modern genera, are now known from as long ago as early Cretaceous times (Fryer 1991). The specializations of the Nährboden–carapace complex of *Moina* are indeed striking, but so too are, for example, those of the carapace of *Scapholeberis* and *Megafenestra* and their associated surface-film frequenting habits, and there are no stronger grounds for granting *Moina* (and *Moinodaphnia*) familial separation from the rest of the Daphniidae than there are for granting familial status to many of the specialized macrothricid and chydorid genera. The family Moinidae is therefore not recognized here. It may be convenient to recognize its two



Figures 159 and 160. *Moina micrura*. Details of the mandibles. Figure 159. A transverse slice, anterior aspect, sufficiently thick to show elements of both the anterior and posterior muscles (but not the entire musculature). Anterior structures are seen particularly on the right, posterior on the left. On the right the promotor roller muscles (3) can be seen inserting on the anterior margin of the mandible; on the left, the remotor roller muscles (4) are seen inserting on the posterior margin. The transverse mandibular tendon (TMT) and its suspensors (SUS) lie anterior to those portions of the major transverse mandibular muscles (TMM) and such parts of the 5c muscles as are seen. Some of the smaller transverse muscles (5d, 5g) that originate on the margin of the tendon are seen on the right. Figure 160. Transverse section, anterior aspect, through the posterior portion of the mandibles. (This is a different individual from that seen in figure 159.) The full array of the massive remotor roller muscles (4) and the 5c muscles are seen. Traces of the suspensors (SUS) of the transverse mandibular tendon are present, as are some of the major transverse muscles (TMM) that originate on the tendon. These give some indication of their asymmetry.

constituent genera as having sub-familial status, as indeed Litynski (1915) long ago proposed, just as Dumont & Pensaert (1983) proposed sub-familial recognition of *Scapholeberis* and *Megafenestra*, but the implications of such recognition for the Daphniidae as a whole are not considered here.

Moina presents one phyletic problem. Unlike other daphniids, it has flexed antennal protopods. This situation has a parallel in the Macrothricidae, where both straight and flexed protopods are found in different genera. The flexed condition appears to be primitive. It seems probable that a daphniid-like trunk limb mechanism was acquired in stock with flexed antennal protopods and that other daphniids diverged from the line leading to *Moina* subsequent to this event, *Moina* retaining the primitive antennal arrangement, the others acquiring straight protopods. However one views the problem, it appears that straight antennal protopods must have originated more than once in anomopod evolution. Thus the macrothricid *Ophryoxus*, which shows various similarities to the ancestral daphniids, probably acquired straight protopods before the daphniid trunk limb complex had been evolved, and before the ancestral daphniids had done so.

(*f*) *Moinodaphnia*

Although Goulden (1968) says that the female of the monotypic *Moinodaphnia* 'bears little resemblance to any of the species of *Moina*', it is in fact similar in most respects to members of that genus, of which Goulden refers to two species that 'have a rather similar body morphology' to *Moinodaphnia*. A good recent illustration of *Moinodaphnia macleayi* (King) is given by Paggi (1973). *Moinodaphnia* is more compressed bilaterally than *Moina* and differs principally from it in the armature of the distal exopodite segment of the antenna. Here, what in *Moina* is a short spine, is elongate and the three terminal natatory setae are shorter, so that the segment bears four conspicuous elements, rather than three and a small spine. Other characters used for generic separation are unsatisfactory. *Moinodaphnia* has an ocellus, which is generally lacking in *Moina*, though it is present in *M. reticulata* (Daday) (see especially Paggi (1973)), *M. oryzae* Hudec

(Hudec 1987) and perhaps sometimes in *M. minuta* Hansen. The presence or absence of an ocellus is a trivial difference. In some species of *Daphnia*, such as *D. cucullata*, it is present in some individuals but not in others. *Moinodaphnia* also has a well-developed abdominal fold that, like what is generally referred to as the post-abdominal process in *Daphnia*, serves to seal the posterior exit from the brood pouch. This is not diagnostic: a similar fold is present in *Moina reticulata* and is well shown by Paggi (1973), and a small fold is present in other species such as *M. brachiata* and *M. micrura*. As in *Moina*, a Nährboden produces secretions that nourish the embryos developing in the brood chamber, so an effective seal is needed to prevent their loss.

M. macleayi has a circumtropical distribution in humid regions. Its ecology is imperfectly known but it appears generally to occur in swamps, lagoons and small water bodies. It is not planktonic. Thomas (1961) regarded it as 'a characteristic member of the fauna of the swamps and swamp pools' in parts of Uganda, where it is very common. I collected it in several places near Lake Malawi (Fryer 1957). The habitats were ponds, both clear and turbid, permanent and temporary, weedy and non-weedy, a lagoon, and the vegetated region of a sluggish river, and included both weakly acidic and weakly alkaline sites. Thomas found that it usually lived very near to the surface, often within 2 cm of it, which he suggests may be a means of evading low oxygen concentrations. This habit prevailed also in presumably well oxygenated aquaria and Thomas comments that his observations differed from those of Sars (1901), who reared this species from dried mud of Brazilian provenance and whose animals often congregated near the bottom.

While I did not study the behaviour of *M. macleayi* in detail in Africa, I can confirm the observation of Sars (1901) that it never swims with the same orientation as *Simocephalus*, a point of some interest in view of a habit recorded by Sars that I did not see myself and that is not mentioned by Thomas (1961). He noted that individuals are generally slow swimmers and tend to keep near the bottom but sometimes one was seen 'attaching itself by the back to the walls of the aquarium, like the species of *Simocephalus*'. No details are given. As no sucker of any kind is present, it is evident that attachment is mechanical and indeed, *Moinodaphnia* displays a feature of the antennal armature analogous to that of *Simocephalus*. In both cases, the distal exopodite segment bears posteriorly a long, robust spine. That of *Moinodaphnia* is well shown by Paggi (1973). These spines are not homologous: that of *Simocephalus* is a modified natatory seta; of *Moinodaphnia* an enlargement of the normally minute terminal spine and therefore additional to the three terminal natatory setae. It seems highly probable that this spine, although of different origin, serves the same purpose as that of *Simocephalus* (§8(*b*) (i), hooking the animal to some support (see figure 138 for *Simocephalus*). If this is so, what is of particular interest is that the means of 'alighting' must be different in the two genera. Attachment in *Simocephalus* is facilitated by the habit of swimming with the dorsal surface anteriormost. No such assistance is available to *Moinodaphnia* that would appear to be faced with a more formidable problem. Only observations on the living animal can resolve the matter.

The feeding mechanism has not been studied, but there is no reason to suppose that it differs in any fundamental respect from that of other daphniids.

9. KEY FEATURES IN THE EVOLUTION OF THE DAPHNIIDAE

Daphniid evolution is best discussed within the context of the evolution of the Anomopoda as a whole: here little more than a list of some of the major trends that have dominated this process is presented. Both morphological and ecological evidence shows that the progenitors of the family shared many of the attributes of the primitive macrothricids listed elsewhere (Fryer 1974), but had undoubtedly advanced beyond this stage in certain respects before they diverged. For example, they must have acquired a pushing post-abdomen, whose form and mode of action in present-day daphniids is scarcely explicable had it not developed as a means of levering against substrata, a role that it fulfils in most macrothricids and, often in a specialized way, in all extant chydorids. The nature of the post-abdomen is just one feature that points unmistakably to a benthic origin of the group.

The view that the family is derived from benthic ancestors is not new. At the turn of the century, Wesenberg-Lund, who had earlier argued for a littoral, creeping origin for the Rotifera, expressed the view that the original home of the 'Cladocera' was in the littoral zone or among vegetation, and that planktonic freshwater organisms in general were originally littoral forms that have adapted themselves to a pelagic way of life (see Wesenberg-Lund (1926) for summary). In this paper, he discussed such a trend in *Daphnia*, showing it by reference to four species, beginning with the heavily built *D. magna* and culminating in the small, lightly constructed, *D. cucullata*. He was able to refer to changes in such morphological traits as shape, size and the form of the post-abdomen and its claws, but lacked information on the morphological details necessary to substantiate his views on the trend within the Anomopoda as a whole. Klotzsche (1913) also clearly regarded the trend as self-evident when he discussed the ocellus of anomopods, whose reduction in the Daphniidae went hand in hand with an increase in the importance of the compound eye. These changes ran in parallel, as he put it, with 'der Emanzipation der Daphniden vom Litoral und der Eroberung des freien Wassers'. The only question is whether the phylogeny is being read in the correct direction, and the evidence is overwhelmingly in favour of a trend from primitive benthic or littoral forms to the open-water, exclusively filter-feeding daphniids.

The progenitors of the Daphniidae were bottom-frequenting animals that moved over surfaces ventral side down, using their antennae for crawling and scrambling, as well as for swimming, and were assisted in this by the first pair of trunk limbs and by the post-abdomen. The carapace embraced all the trunk limbs of which there were five, perhaps initially six, pairs. The second pair of trunk limbs was used for scraping or dragging food from surfaces and transferring it to the median, eventually filter, chamber. In the line from which the daphniids developed, up to four pairs of limbs (limbs 2–5) were used as filters. All these attributes have been exploited in the development of specialized ways of essentially benthic life in the related Chydoridae, where they have gone hand-in-hand with the development of a robust, protective carapace that is entirely appropriate for bottom dwellers, and elaborate modifications of its ventral margins that grant intimate association with different substrata (Fryer 1968). Emancipation from the bottom called for different developments in the Daphniidae. These included:

1. An increase in the size and efficiency of the gnathobasic filter plates of trunk limbs 3 and 4 at the expense of other filter plates, and the eventual loss of filters on limbs 2 and 5.
2. Loss of any role in either food collection or in grasping or crawling of trunk limb 1, which left it free to develop a screen of protective or guiding setae that formed a funnel at the anterior end of the filter chamber, and loss of the inner lobe that is so conspicuous in chydorids and macrothricids.
3. Abandonment of scraping by trunk limb 2, whose gnathobase retained its function of sweeping food anteriorly but took on the additional task of cleaning the filter of trunk limb 3. (The scraping abilities of certain species of *Daphnia* may represent a specialized developed of a retained primitive feature or a secondary redevelopment, but are minor features superimposed on the main trend.)
4. Specialization of trunk limb 5 for closing the posterior interlimb space.
5. Specializations of the exopods of trunk limbs 3 and 4 and their shift from a lateral to a more ventral location.
6. A change in food source from precipitated material and epiphytic microorganisms to seston.
7. Specialization of the antennae for swimming (and parachuting) only. (Developments such as those seen in *Simocephalus* are secondary.)
8. Gradual abandonment of the bottom as swimming replaced crawling and became the predominant, and usually sole, means of locomotion.
9. Abandonment of pushing with the post-abdomen.
10. A general thinning of the carapace in the more open-water forms. (Protective thickening in relation to sedentary habits in *Simocephalus* is a secondary trend.)
11. A change in the orientation of the body in the more specialized swimmers from a horizontal to a vertical alignment of the long axis.
12. Reduction in size of the antennules.
13. Elaboration of the compound eye and reduction of the ocellus.
14. Adoption of a straight (uncoiled) alimentary canal with anterior caecae, but no posterior diverticulum.
15. Perfection of mandibular asymmetry, skeletal and muscular.
16. Exploitation of the brood chamber to hold several parthenogenetic eggs or embryos. (In the Chydoridae, the trend was to specialize in the production of two-egg clutches, except in the most primitive species.)
17. Acquisition of highly specialized ephippia (the most specialized within the Anomopoda) containing either one or two resting eggs.

In general, daphniids have been more successful at exploiting large size than have chydorids, whose habits often favour 'miniaturization' that enables them to occupy microniches, to penetrate small spaces, burrow in thin layers of flocculent detritus and so on, but colonization of the most open-water conditions by *Daphnia* has also been accompanied by diminution of size.

These trends led to the colonization of an entirely new array of niches in open water, including the pelagial region of lakes, in which chydorids and macrothricids are virtually unrepresented, and also to specializations such as largely sedentary filtration and association with the surface film. Some indication of how the morphological changes that have taken place during daphniid evolution could have been accomplished is given by various macrothricids and the way in which the adoption of persistent swimming and related changes in shape could have been achieved are shown by *Ophryoxus* (Fryer 1974) but are not considered here.

If the usually accepted homologies are correct, the maxilla, which is conceivably represented by the inner lobe of trunk limb 1 in more primitive forms, and in forms that have adopted other ways of life, has been lost in daphniids. The possibility remains, however, that, as first mentioned in connection with the chydorid *Eurycercus* (Fryer 1963), what is usually called trunk limb 1 is in fact the maxilla. The opening of the maxillary gland in *Daphnia* is in accord with such an interpretation. While of considerable phyletic interest, this possibility does not affect the functional matters considered here.

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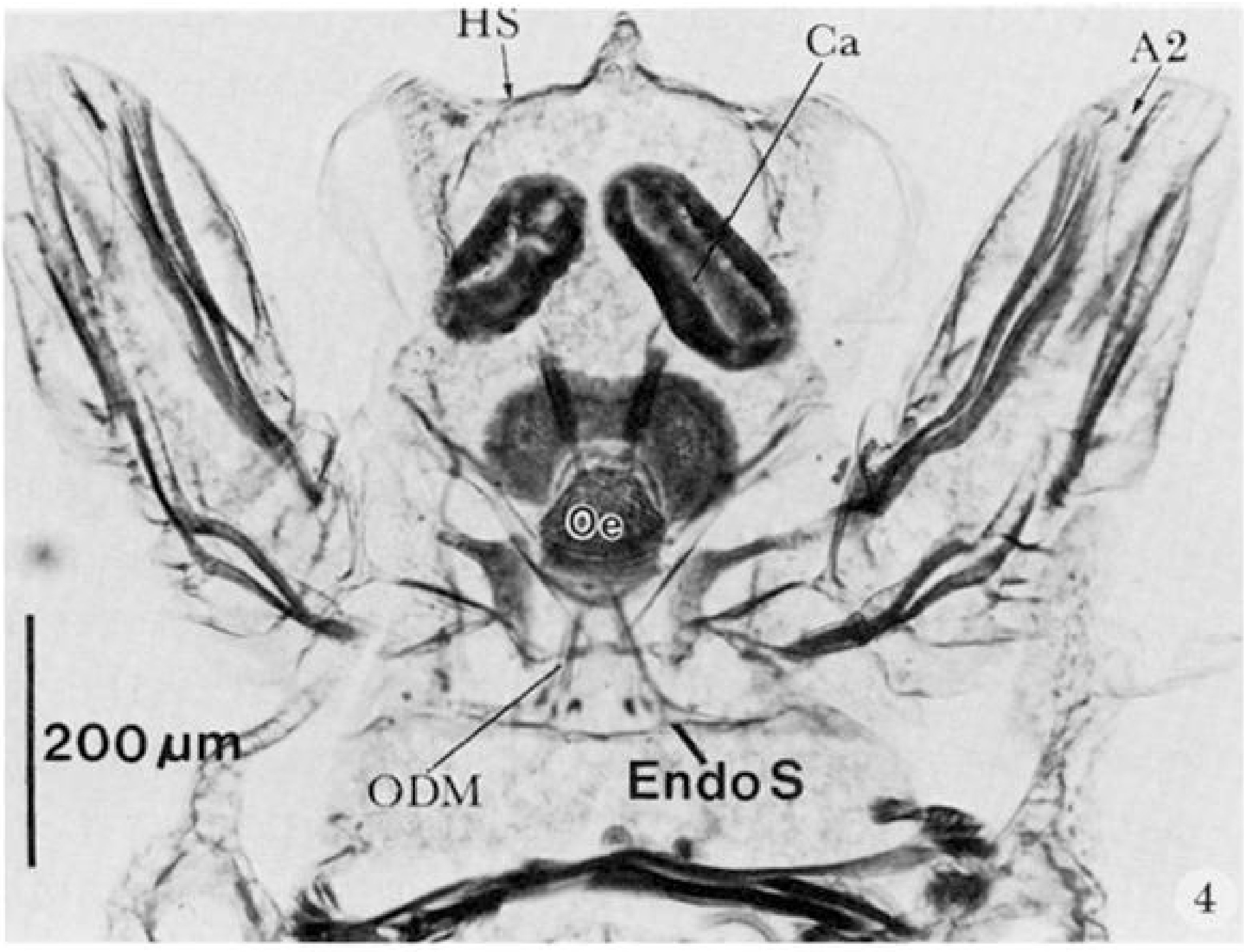
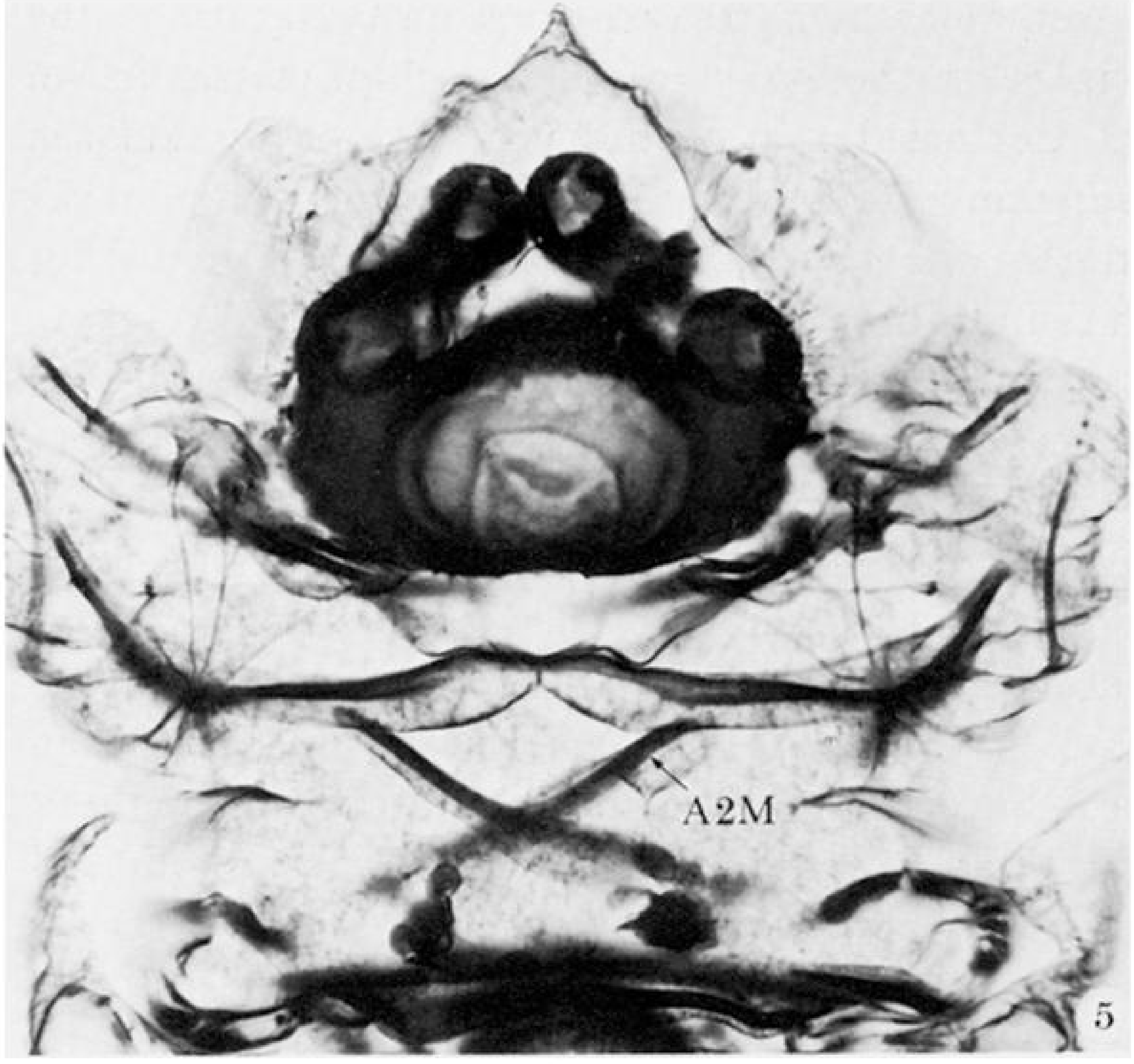
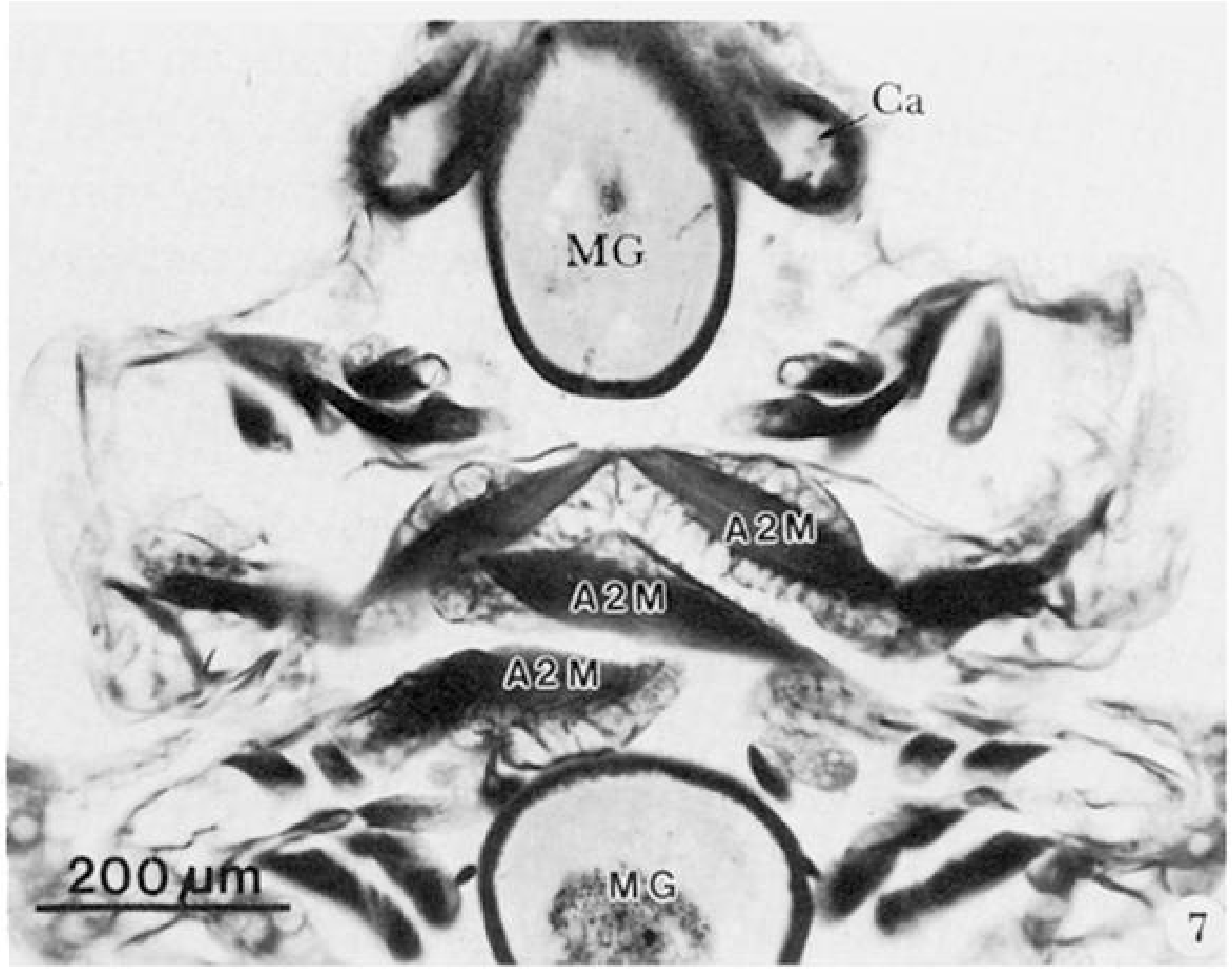
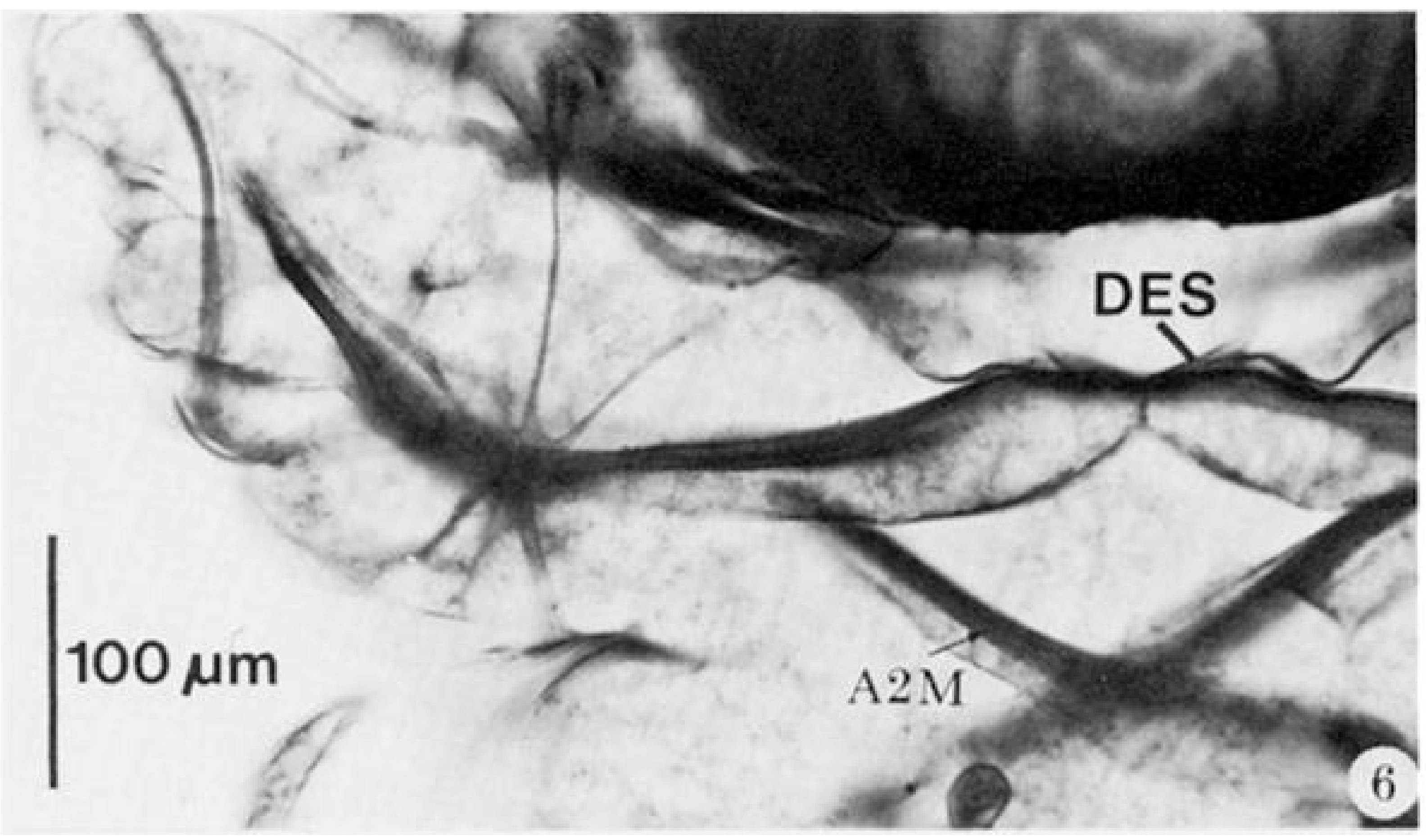
ABBREVIATIONS USED IN FIGURES

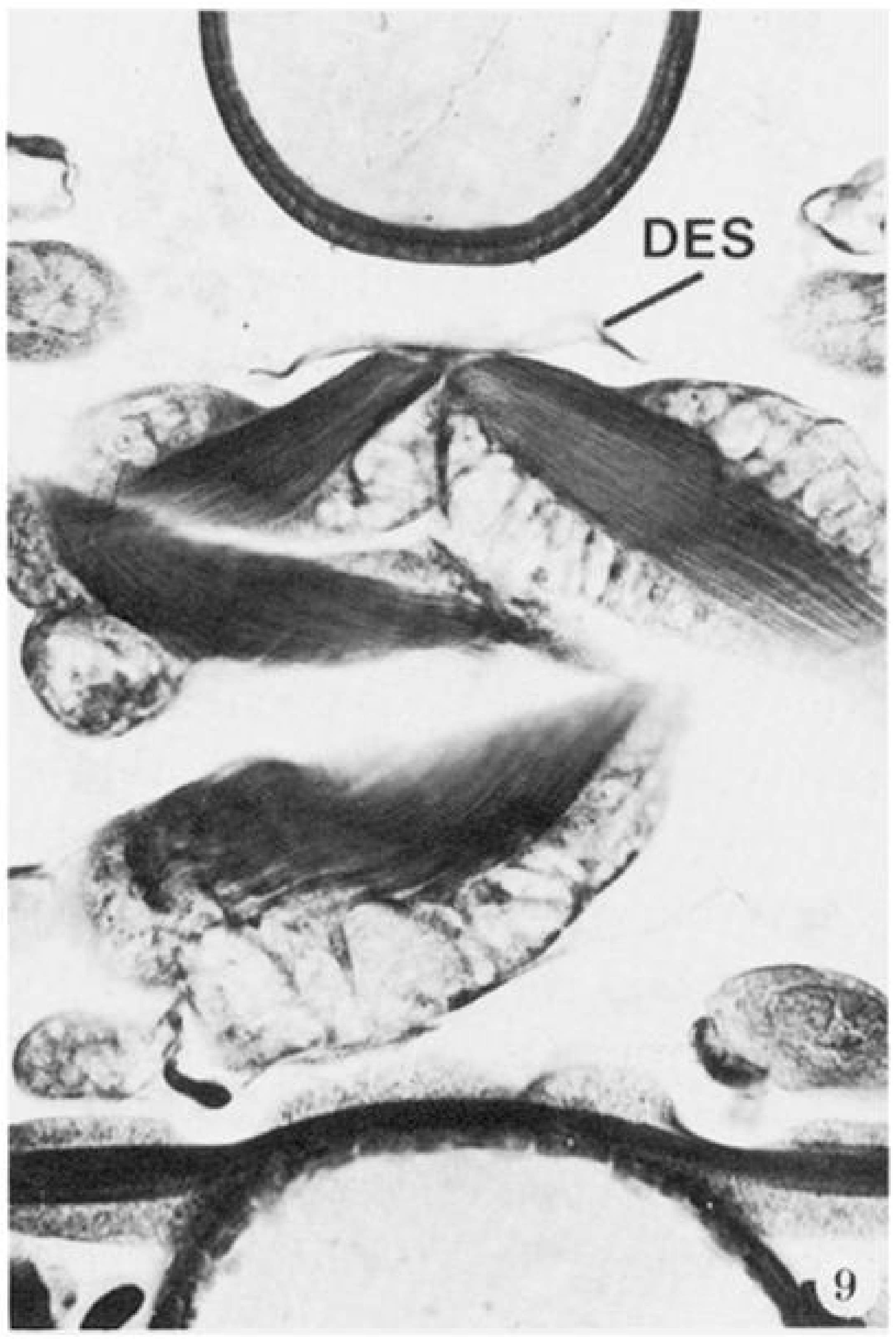
ACAM	anterior carapace adductor muscle
ADVM	anteriorly descending oblique dorso-ventral muscle
AF	anchoring fibrils
A2	antenna
A2M	antennal muscles
Ant	anterior
AVS	anterior vertical seta(e) of trunk limb 5
BP	brood pouch
BS	brush-like setules of filter setae of trunk limb 3
C	carapace
Ca	caecum
CG	cerebral ganglion
CLS	cleaning setules (or spinules)
CS	cleaning setules of food groove wall
D Endo F	dorsal endoskeletal frame
D Endo S	dorsal endoskeletal sheet
DES	dorsal extension of ventral endo-skeletal sheet
DE 2, 3	distal endite of trunk limbs 2, 3
DFP3	distalmost setae of filter plate of trunk limb 3
DLB	dorso-lateral brace
DLM	dorsal longitudinal trunk muscles
DR	dorsal ridge of carapace
E	compound eye
EDMG	exit duct of maxillary gland
EM4	extrinsic muscle of trunk limb 4
Emb	embryo
Endo	endoskeleton
Endo S	endoskeletal sheet
EP 2, 3, 4, 5	epipodite of trunk limbs 2, 3, 4, 5
ES	end sac of maxillary gland
EX 2, 3, 4, 5	exopod of trunk limbs 2, 3, 4, 5
EXS 3, 4, 5	exopod setae 3, 4, 5
EXLS 3, 4	lateral exopod setae 3, 4
F	faeces
FC	fat cell
FCh	filter chamber
FCS	filter-cleaning spine of trunk limb 2
FG	food groove
Fib	fibrils
FP 3, 4	gnathobasic filter plate of trunk limbs 3, 4
FS 3, 4	filtering setae of trunk limbs 3, 4
GDS	guide setae of trunk limb 1
GN2, 4	gnathobase of trunk limb 2, 4
GS2	gnathobasic setae of trunk limb 2
HL	horizontal lamella
HM	horizontal muscle
HS	headshield
HS′	headshield, posteriormost fragment
HS/C	headshield/carapace junction
Ht	heart
IC	inner cuticular lining of carapace
IT	intersegmental tendon
L	labrum
LGC	labral gland cells
LGS	labral gland secretions
LM	levator muscle of labrum
LR	lateral rib of carapace
Mand	mandible
MG	mid gut
MVS	median vertical seta of trunk limb 5
Mxlle	maxillule
NC	nerve cord
O	ocellus
ODM	oesophageal dilator muscles
Oe	oesophagus
OL	Optic lobe of cerebral ganglion
Ov	ovary
PA	post-abdomen
PAC	post-abdominal claw
PCAM	posterior carapace adductor muscle
PM 4, 5	promotor muscle of trunk limbs 4, 5
PM Mand	posterior margin of mandible
PVS	posterior vertical seta of trunk limb 5
R	rectum
RC	rings of cuticle at base of antenna
RDM	rectal dilator muscles
RM 4	remotor muscle of trunk limb 4
SA1	sensory seta of antennule
SC	scraper of distal endite of trunk limb 2
SE2	setae of endites of trunk limb 2
SUS	suspensory ligament
SUS Endo S	suspensory ligament of endoskeletal sheet adjacent to carapace adductor muscles
TC	thickened cuticle
TL 1, 2, 3, 4, 5	trunk limbs 1–5
TMG	tubules of maxillary gland
TMM	transverse mandibular muscle
TMT	transverse mandibular tendon
TTC	thickened trunk cuticle
VDVM	vertical dorso-ventral muscle
VLM	ventral longitudinal muscle
3	promotor roller muscles
4	remotor roller muscles
5c	transverse muscles of mandible
5 d, g	minor transverse muscles of mandible

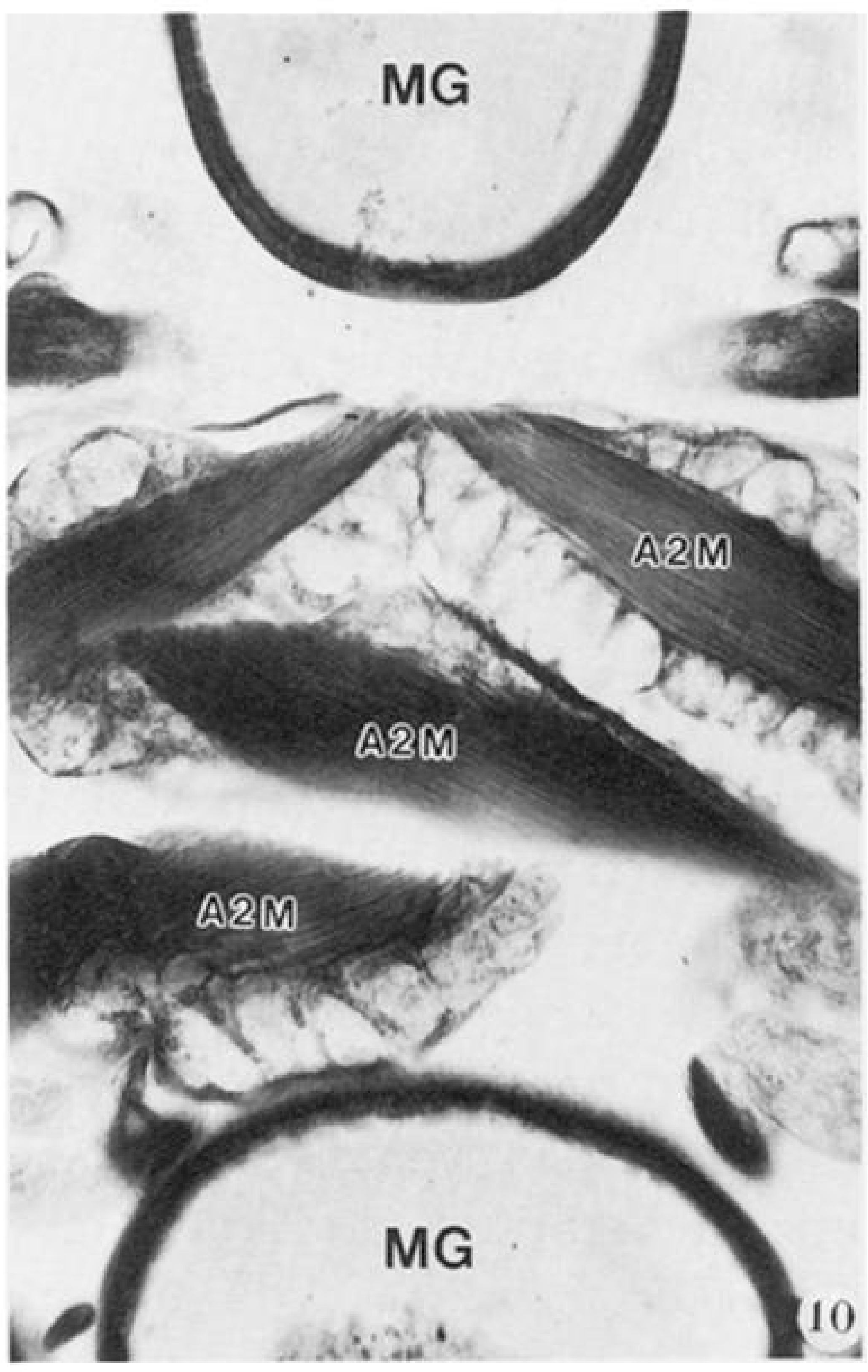




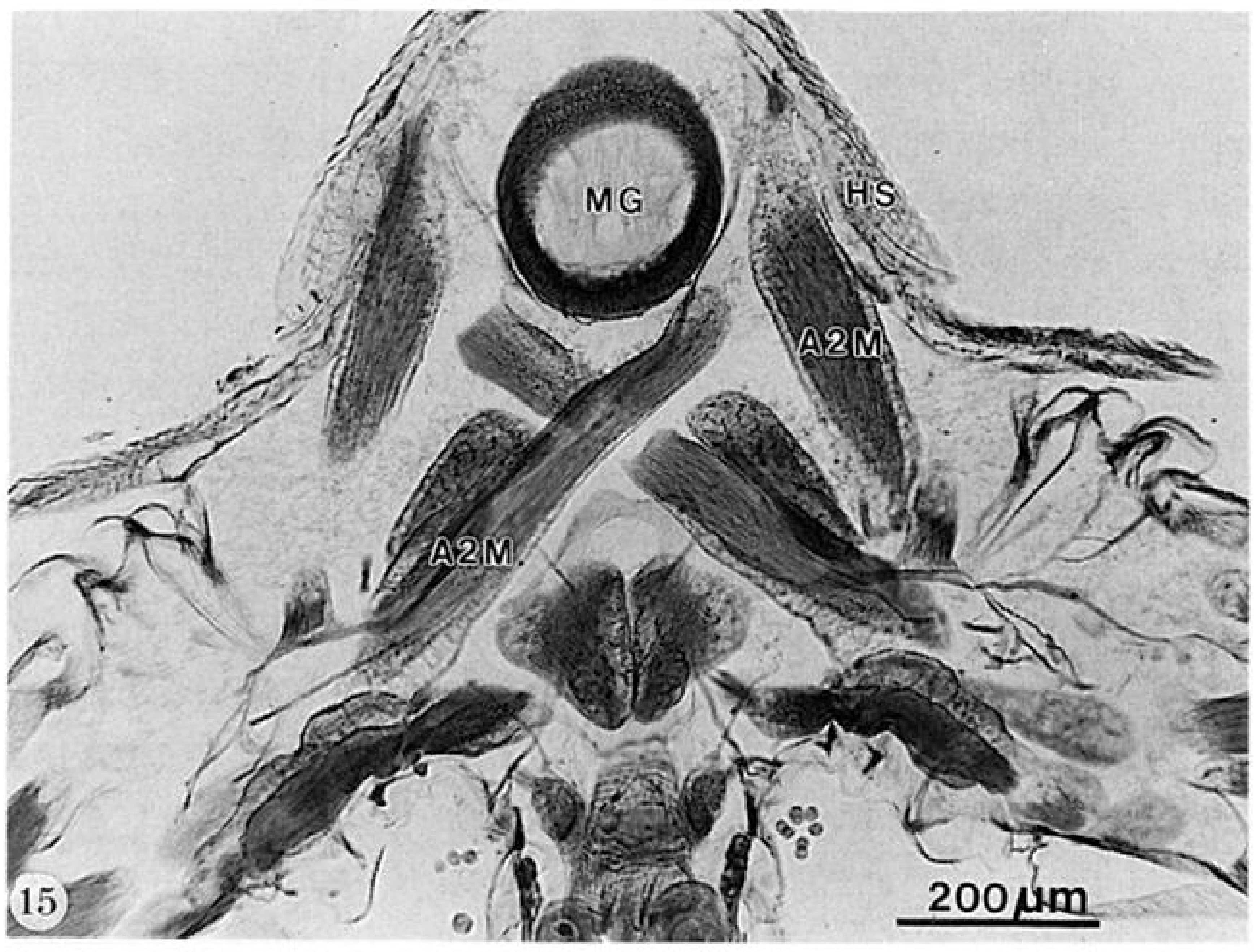


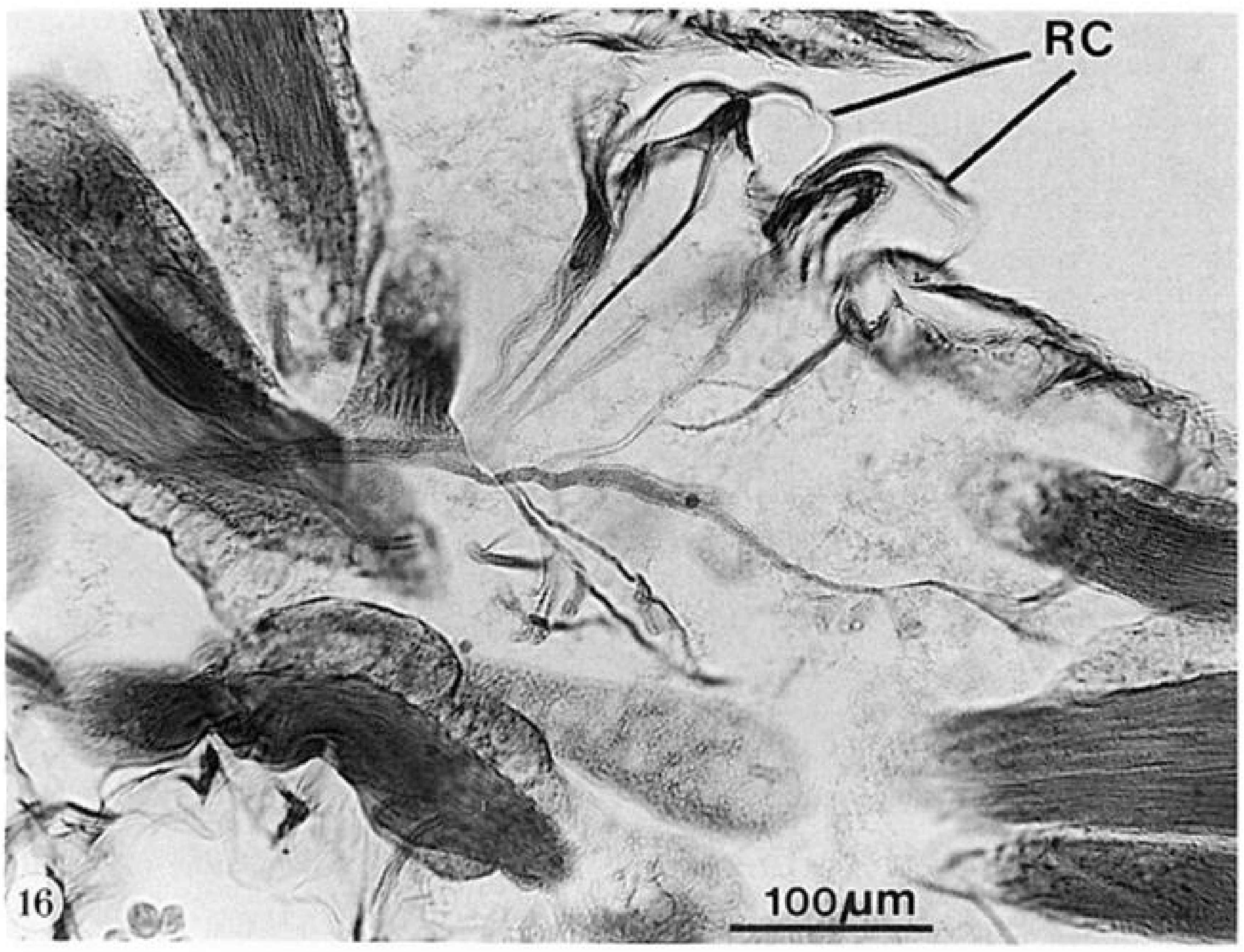


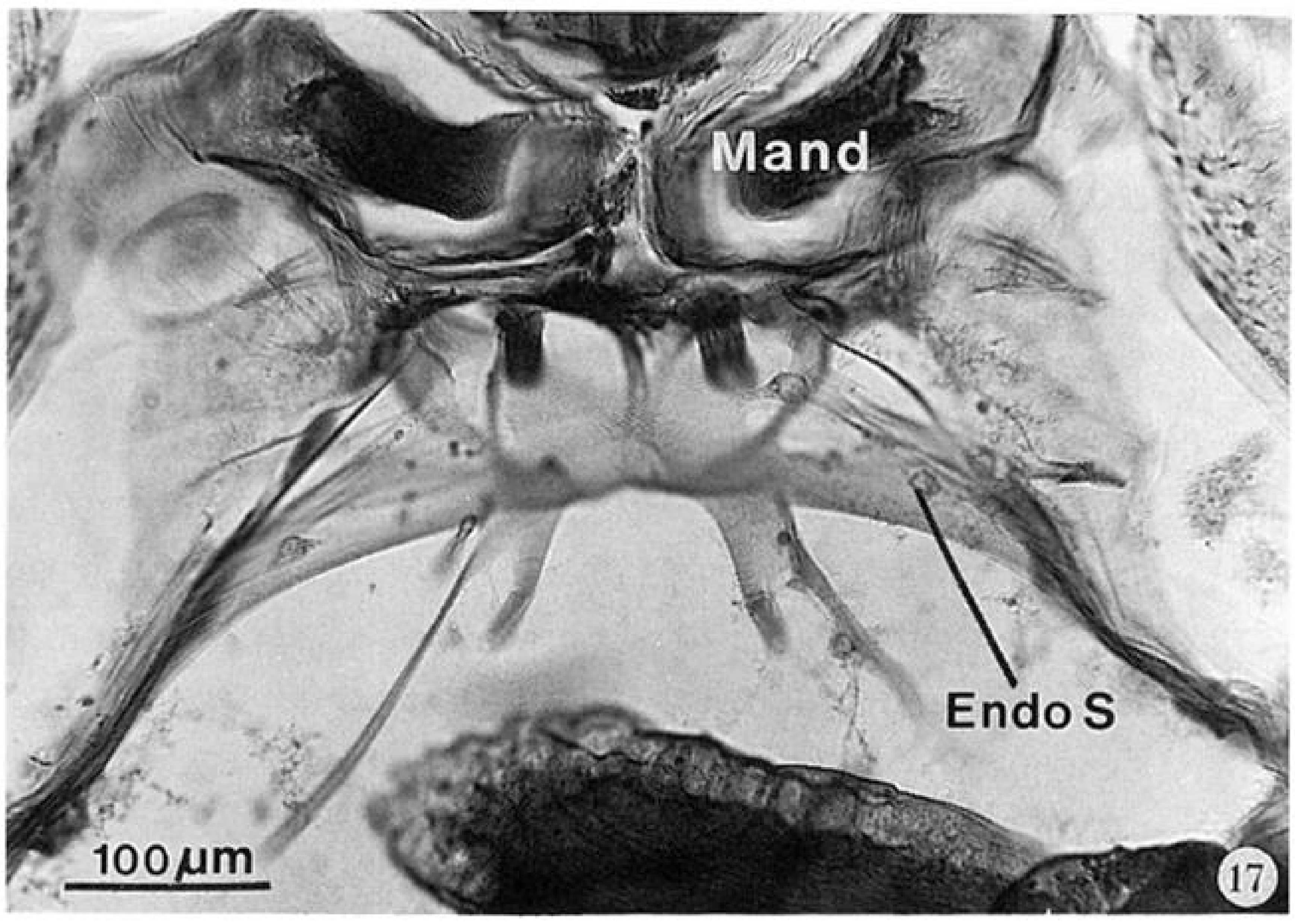


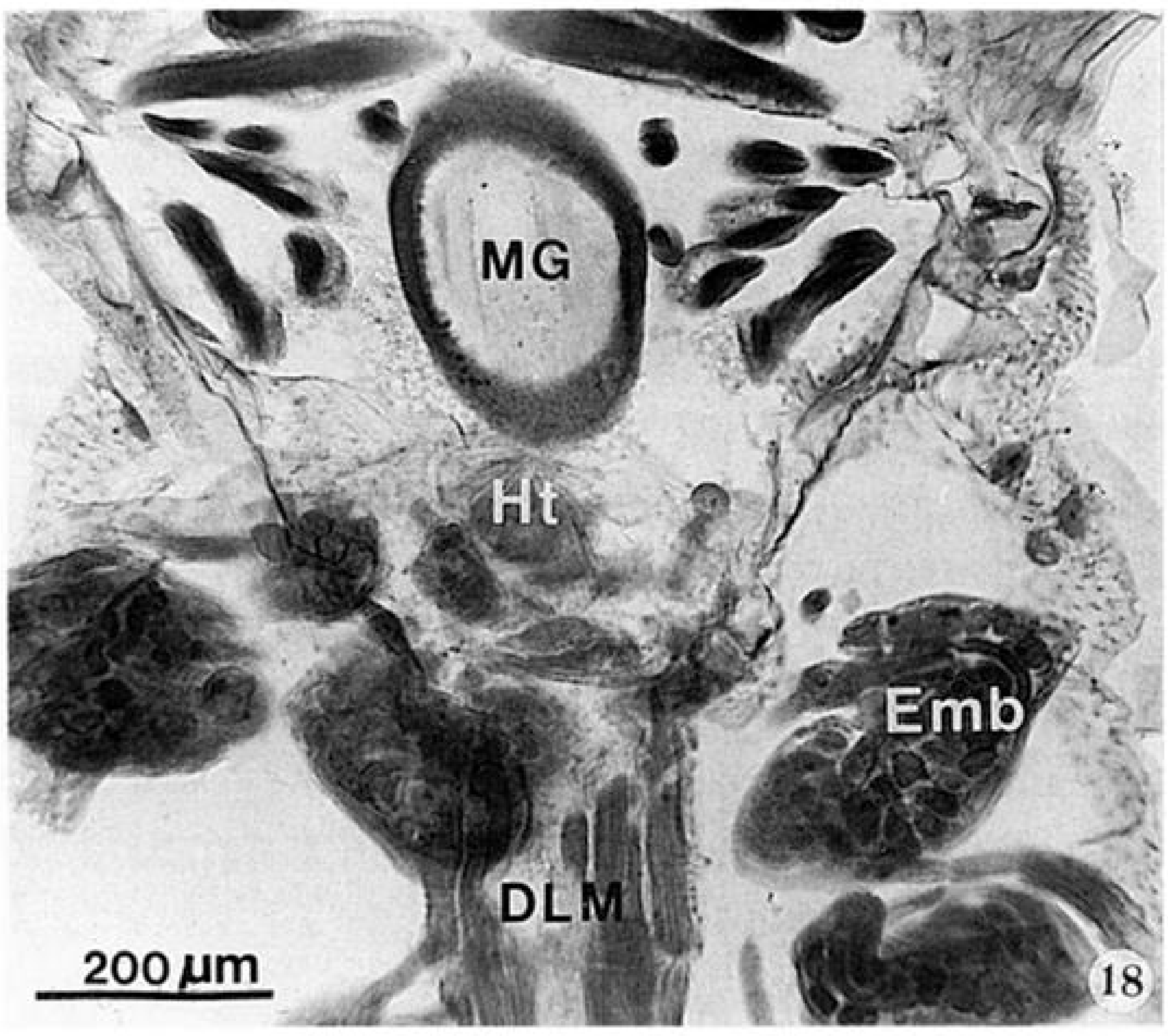


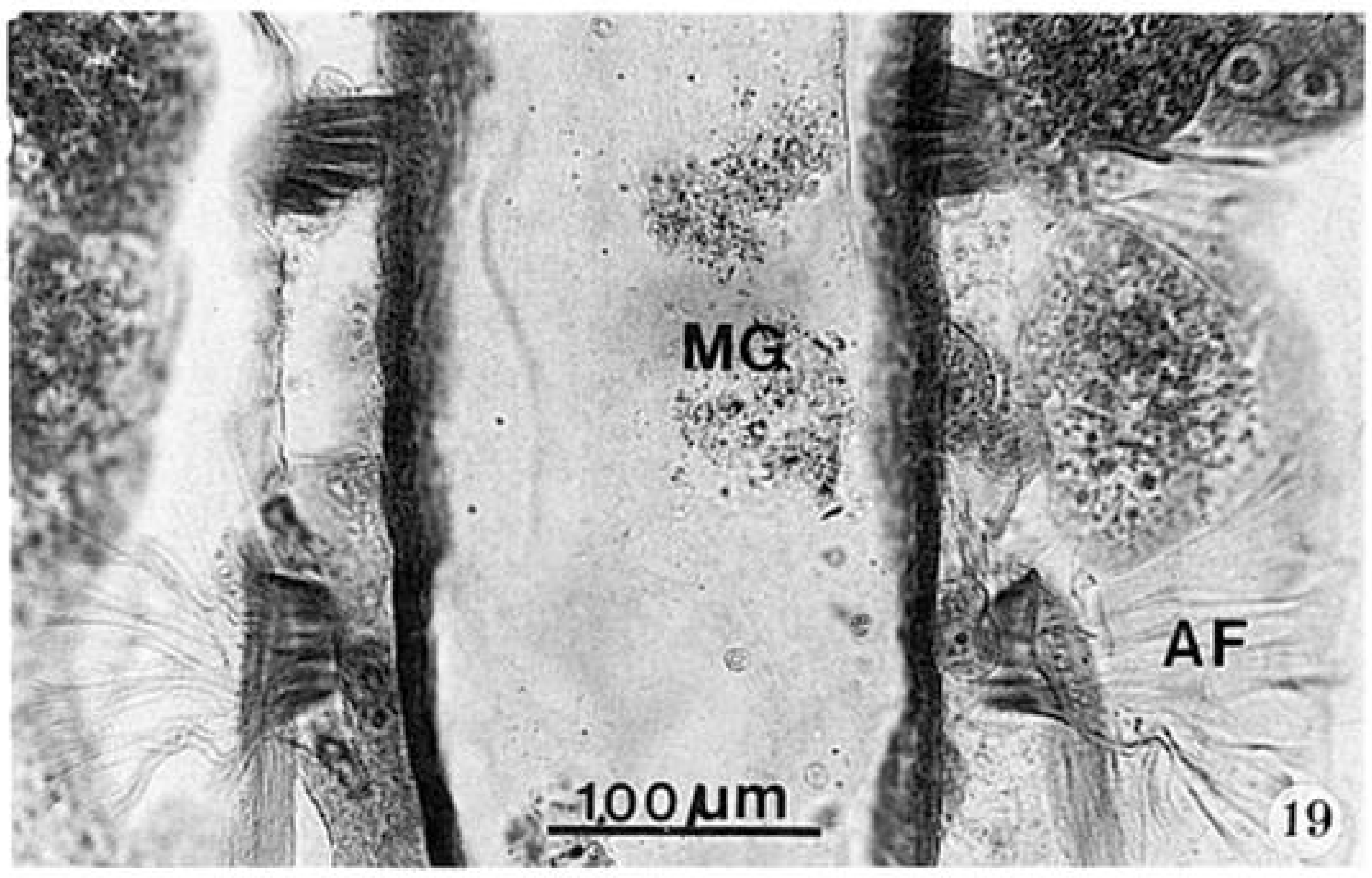
Figures 4–10. Aspects of the skeleto-musculature and nervous system of the antennae. Figure 4. *Daphnia magna*. Thick horizontal slice showing the antennal protopods and some of their muscles, ventral. The asymmetrical muscles seen at the bottom of the photograph are the transverse mandibular muscles. Note how some of the oesophogeal dilator muscles (ODM) originate on the endoskeletal sheet (Endo S). Figure 5. The same, more dorsal. Note the crossing over of two extrinsic muscles (A2M). The muscles seen here, and in figures 6–10, show how the contractile myofibril bundles of many of the muscles are surrounded by a thick sarcoplasmic cortex. Figure 6. Detail of figure 5. Note the use of the endoskeleton (DES) for the anchorage of extrinsic antennal muscles (see also figures 7–10). Figure 7. *D. atkinsoni* f. *bolivari*. Horizontal slice, ventral, to show some of the major extrinsic antennal muscles. Figures 8–10. *D. atkinsoni* f. *bolivari*. Consecutive horizontal slices, proceeding progressively dorsally, through some of the major antennal muscles, showing how some of the extrinsic muscles originate on the dorsal extension of the anterior extremity of the ventral endoskeletal sheet (DES), whose location is seen in figure 3, and how others cross over. Crossing over of the two extrinsic muscles present in the lower half of each photograph can be seen by following the portions present in consecutive slices. The mid-gut (MG), whose anterior extremity is seen in figure 8, where the last trace of the oesophagus is present, here passes dorsally before looping back, where it is cut again at the bottom of each photograph, before eventually running posteriorly.

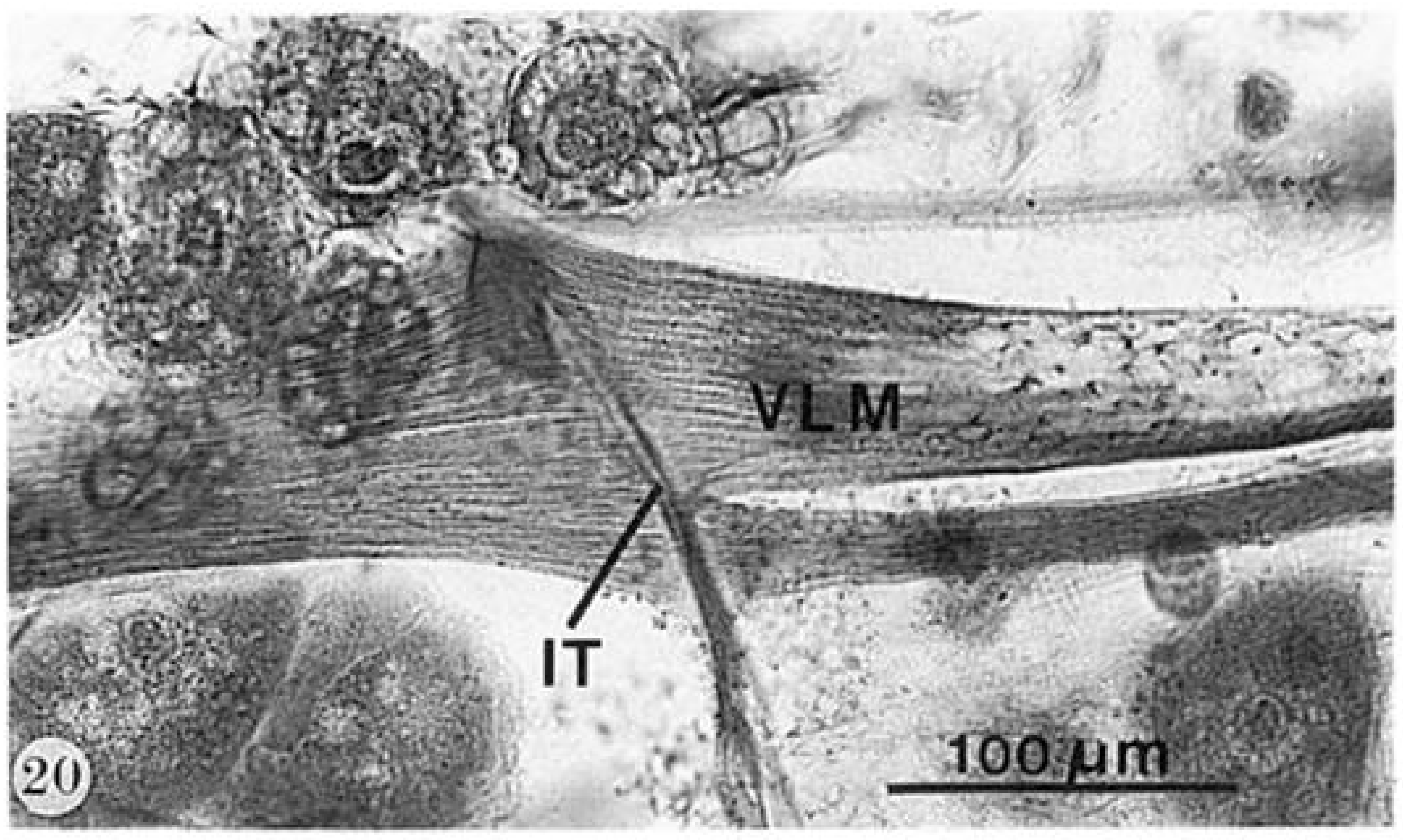


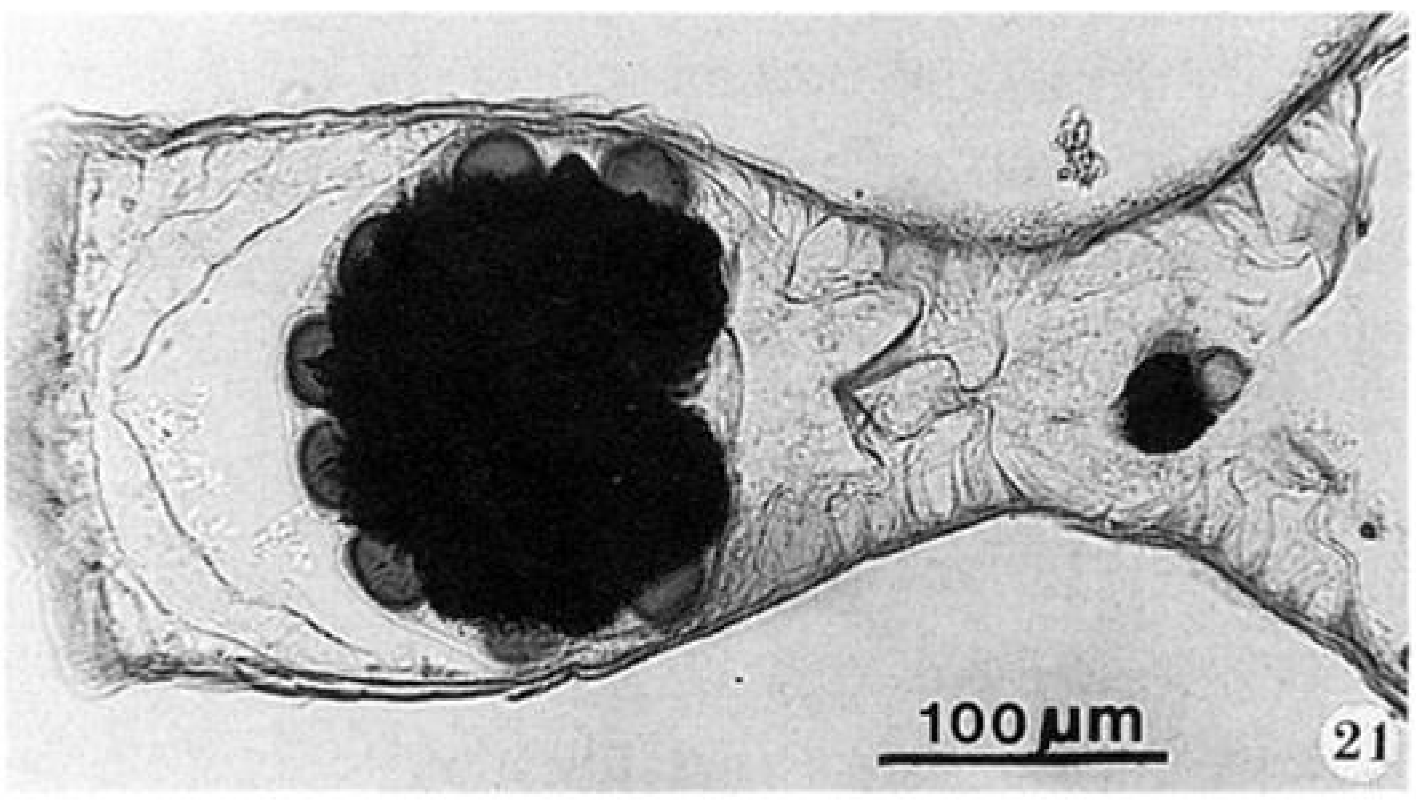




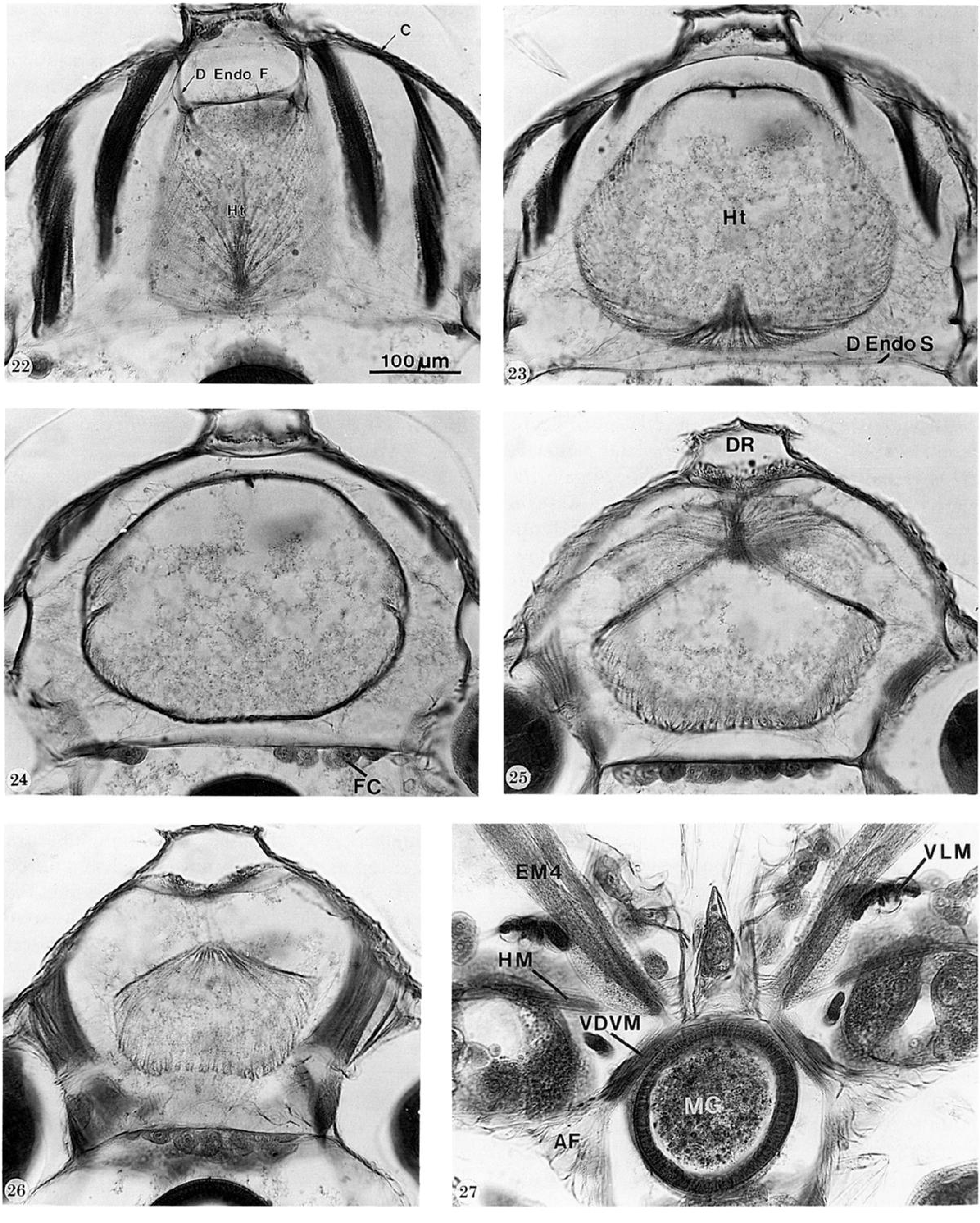




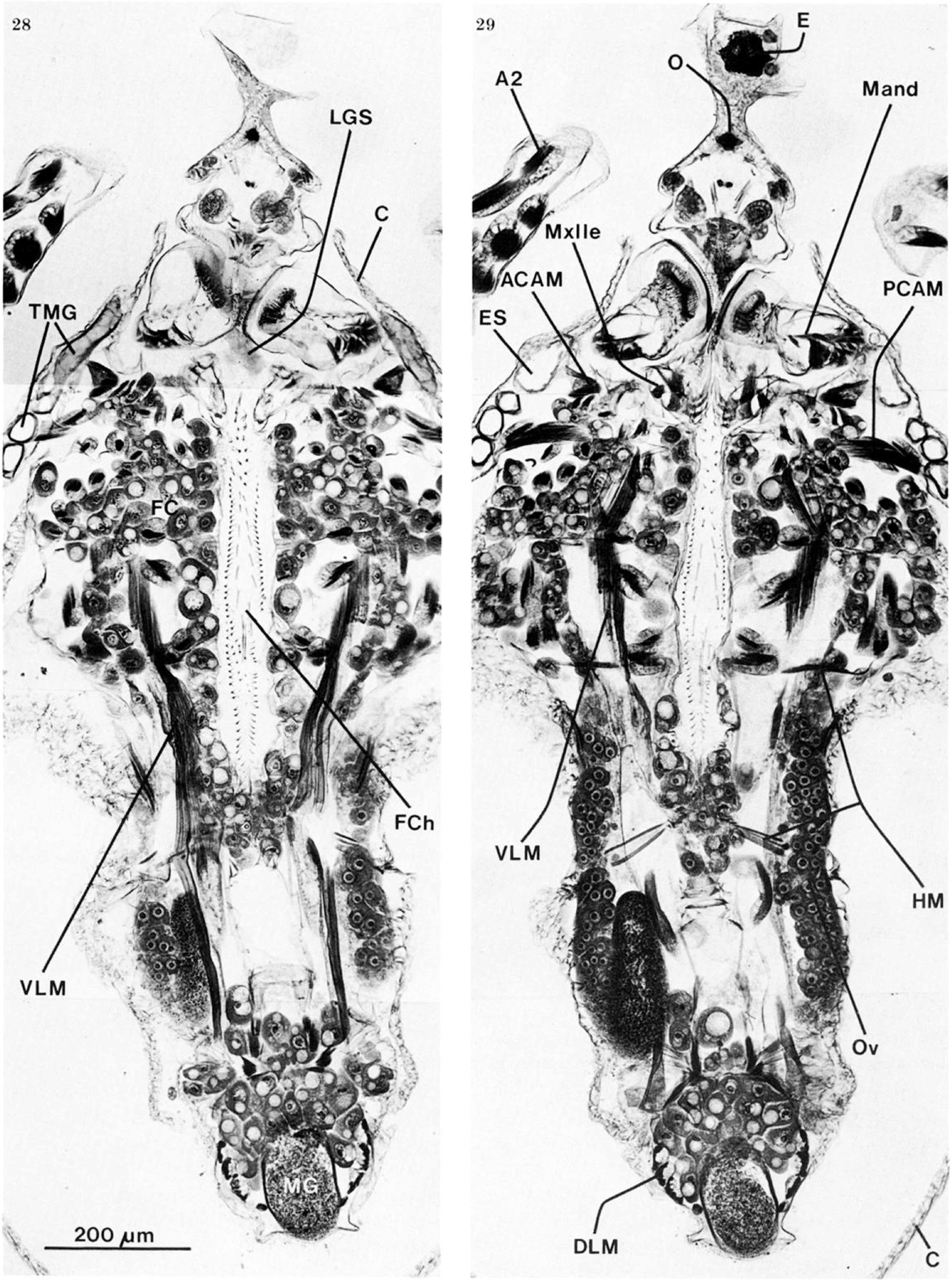




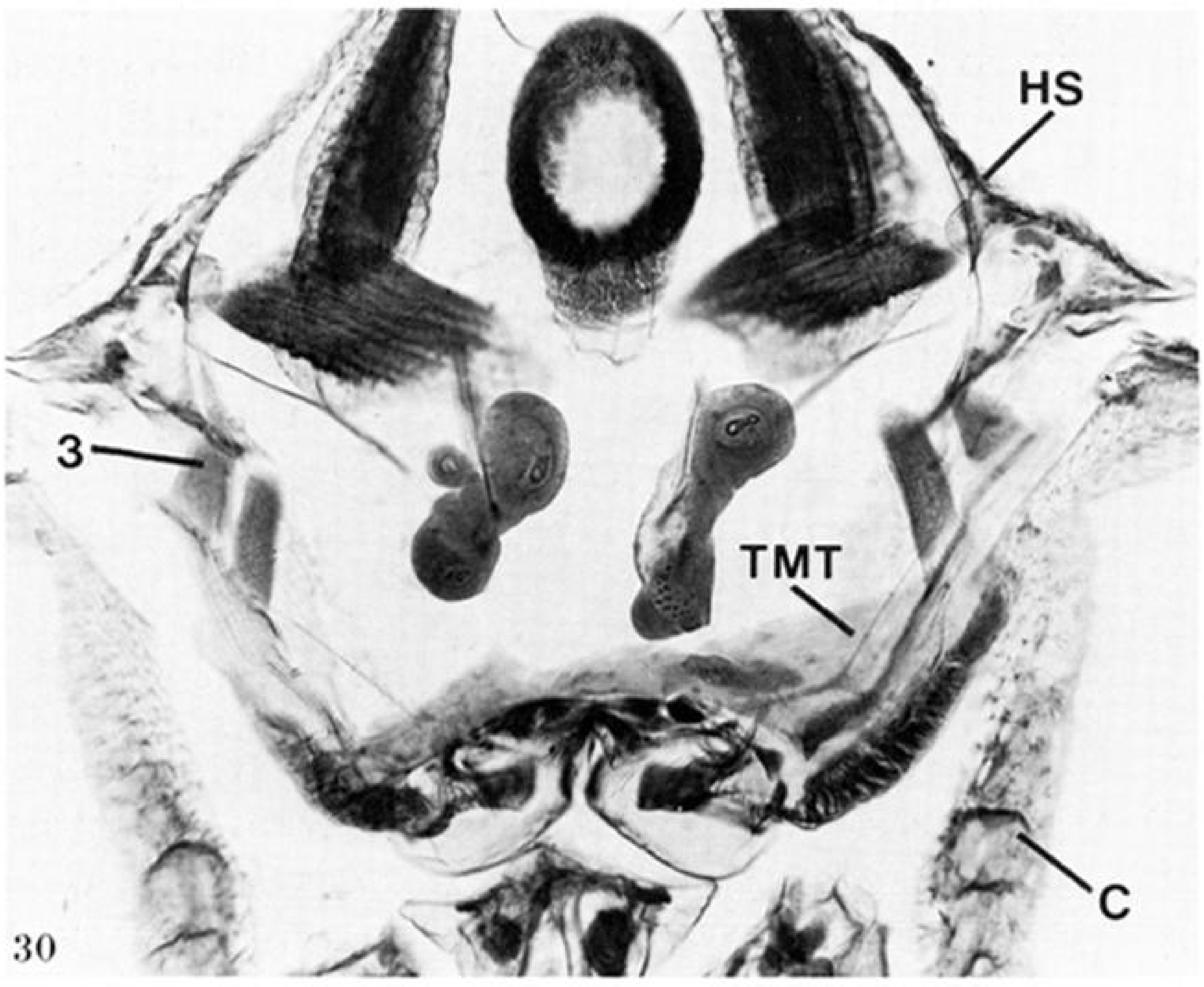
Figures 15–21. *Daphnia atkinsoni* f. *bolivari*. Various anatomical details, especially of the skeleto-muscular system. Figure 15. Transverse section through the head, cutting through the protopod joint on each side. Note how some of the extrinsic antennal muscles (A2M), which originate on the head cuticle at the opposite side of the body from the appendage they serve, cross in the mid-line. Figure 16. The same, showing details of the head–antennal protopod joint. Note the rings of cuticle (RC) that grant versatility of movement, and part of the elaborate nerve supply to the antennal muscles. Figure 17. Transverse section near the anterior limits of the mandibles showing the elaboration of the endoskeletal sheet (Endo S) in the region below them. The sheet is anchored to the cuticle of the head and has three bracers ventrally on each side. Figure 18. Horizontal slice (ventral view) in the region of origin of the dorsal longitudinal muscles (DLM). Part of the heart (Ht) can just be seen between this region and the mid-gut (MG) – here cut somewhat obliquely as it curves posteriorly. Embryos (Emb), lying in various postures, have been sectioned in the brood pouch. Figure 19. Horizontal section through the mid gut region showing the anchoring fibrils (AF) of the trunk muscles. Figure 20. Longitudinal section through part of a ventral longitudinal muscle (VLM) showing an intersegmental tendon (IT) at the boundary of two segments. Dorsal side uppermost. Figure 21. Horizontal section of the head, cutting through the compound eye and the ocellus. Note the narrowness of the head in this region, so that, on each side, the eye lies just beneath the cuticle, and also the absence of any light-absorbing tissues anterior to the eye.

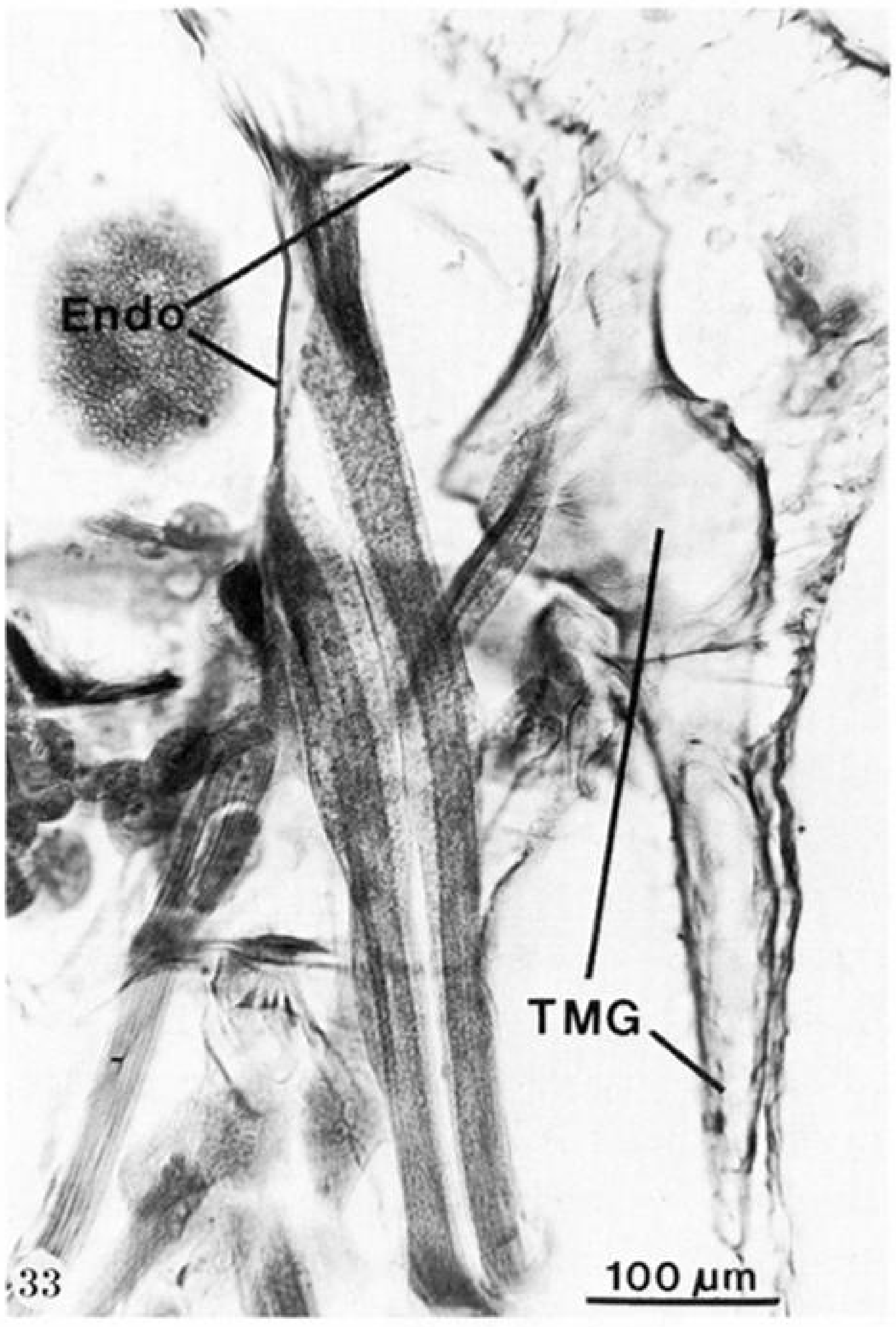


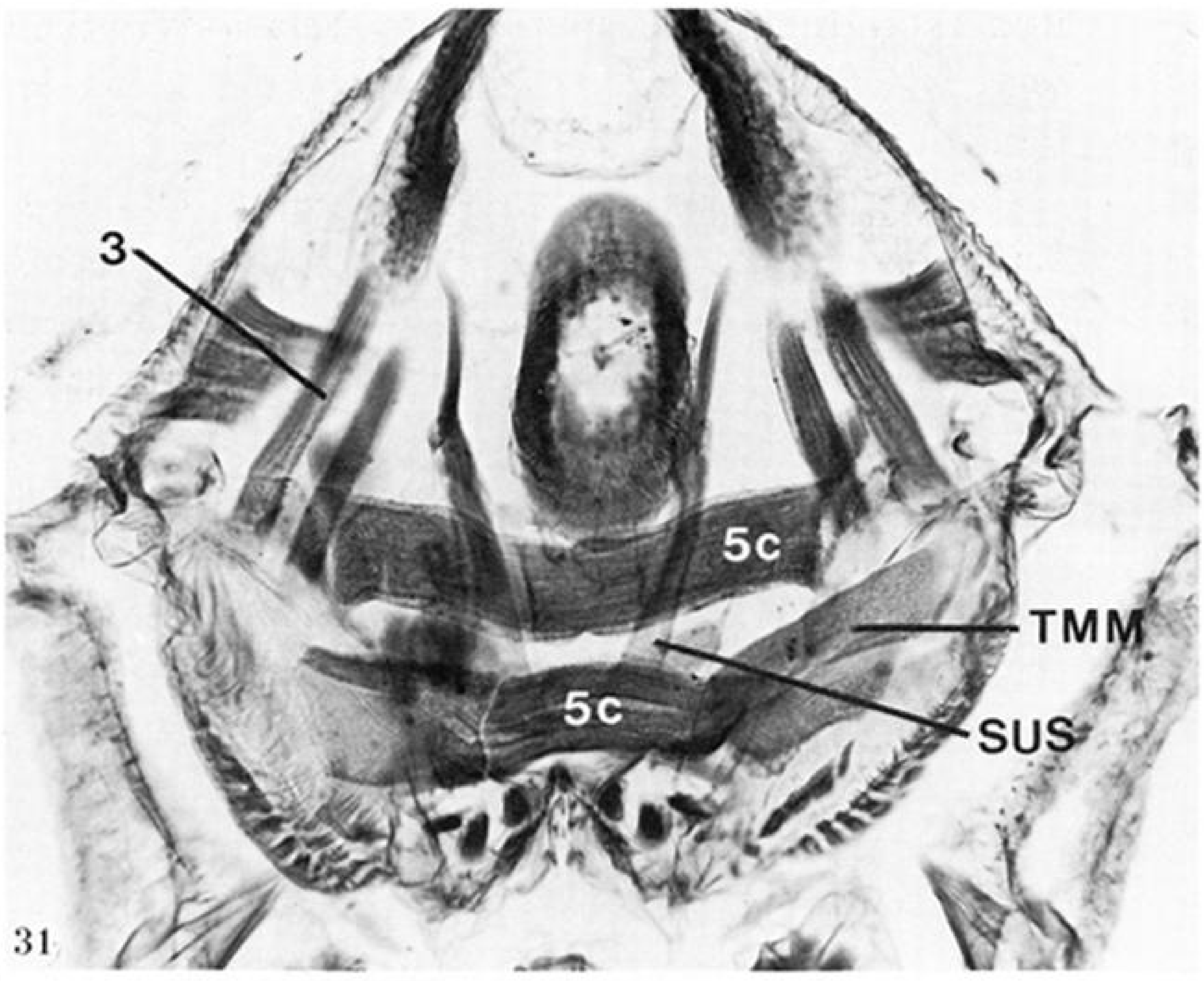
Figures 22–27. *Daphnia atkinsoni* f. *bolivari*. Aspects of the heart, endoskeleton and muscular system. Figure 22. Transverse slice through the anterior extremity of the heart (Ht). Note the dorsal endoskeletal frame (D Endo F) from which the heart is here slung. The large muscles present are the remotor rollers of the mandibles. Figure 23. The same immediately posterior. The dorsal endoskeletal sheet (D Endo S) lies beneath the heart, forming the floor of a pericardium. Figure 24. The same immediately posterior. Note the fat cells (FC) on the ventral surface of the dorsal endoskeletal sheet. Figure 25. The same immediately posterior. Note the great accumulation of fat cells beneath the dorsal endoskeletal sheet. The dark objects at each side are embryos in the brood pouch. The dorsal ridge of the carapace (DR) is well seen in this and the next figure. Figure 26. The same at the posterior limits of the heart. The anterior-most extremities of the dorsal longitudinal muscles can just be seen. Figure 27. Transverse section, ventral side uppermost, of the trunk in the vicinity of the intersegment 3/4 showing the horizontal muscles (HM), the vertical dorso-ventral muscles (VDVM) and their anchoring fibrils (AF) on each side, and some of the long extrinsic muscles of trunk limb 4 (EM 4). The ventral longitudinal muscles (VLM) are seen in transverse section. Some unidentified object is present in the food groove.

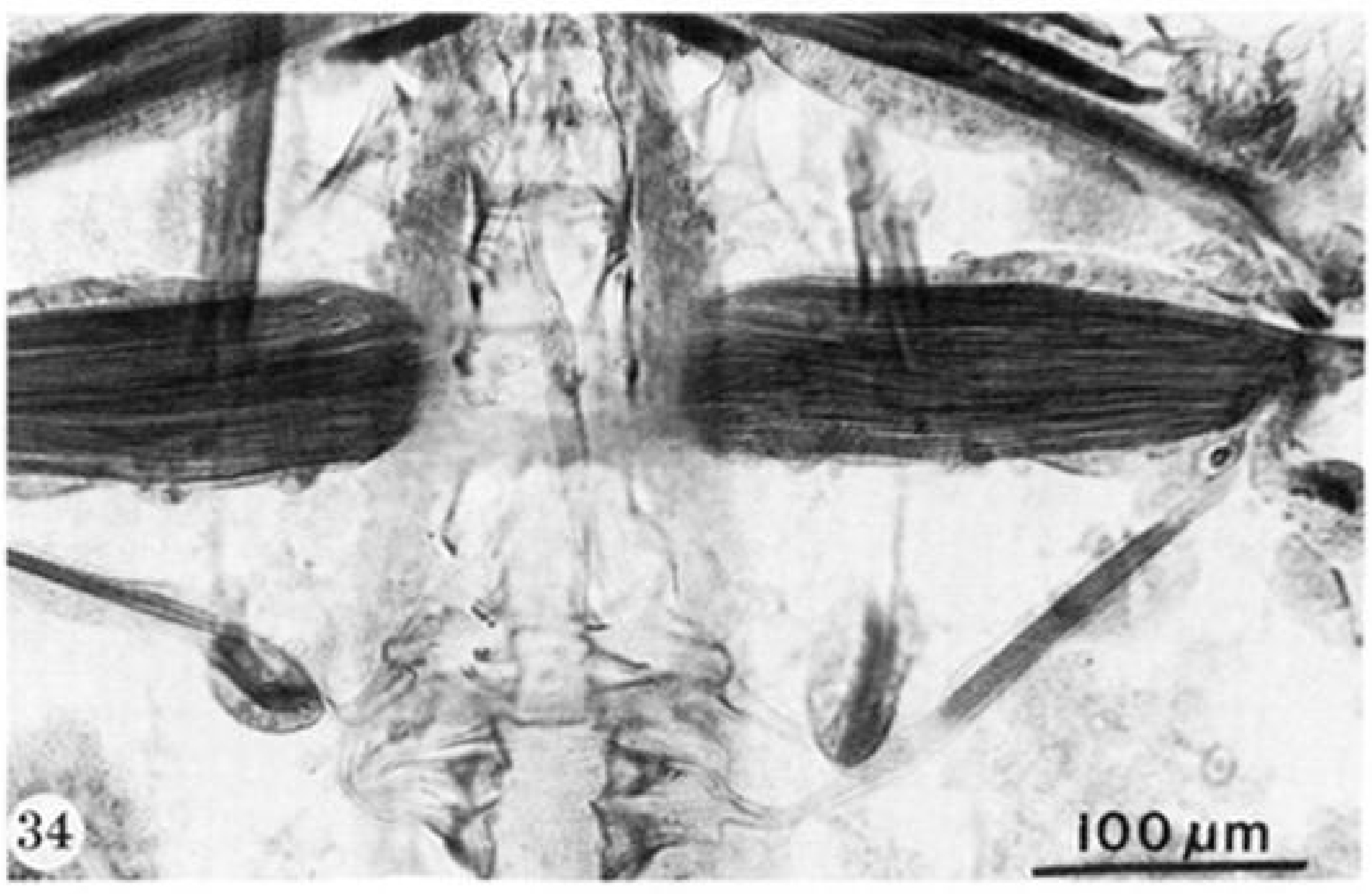


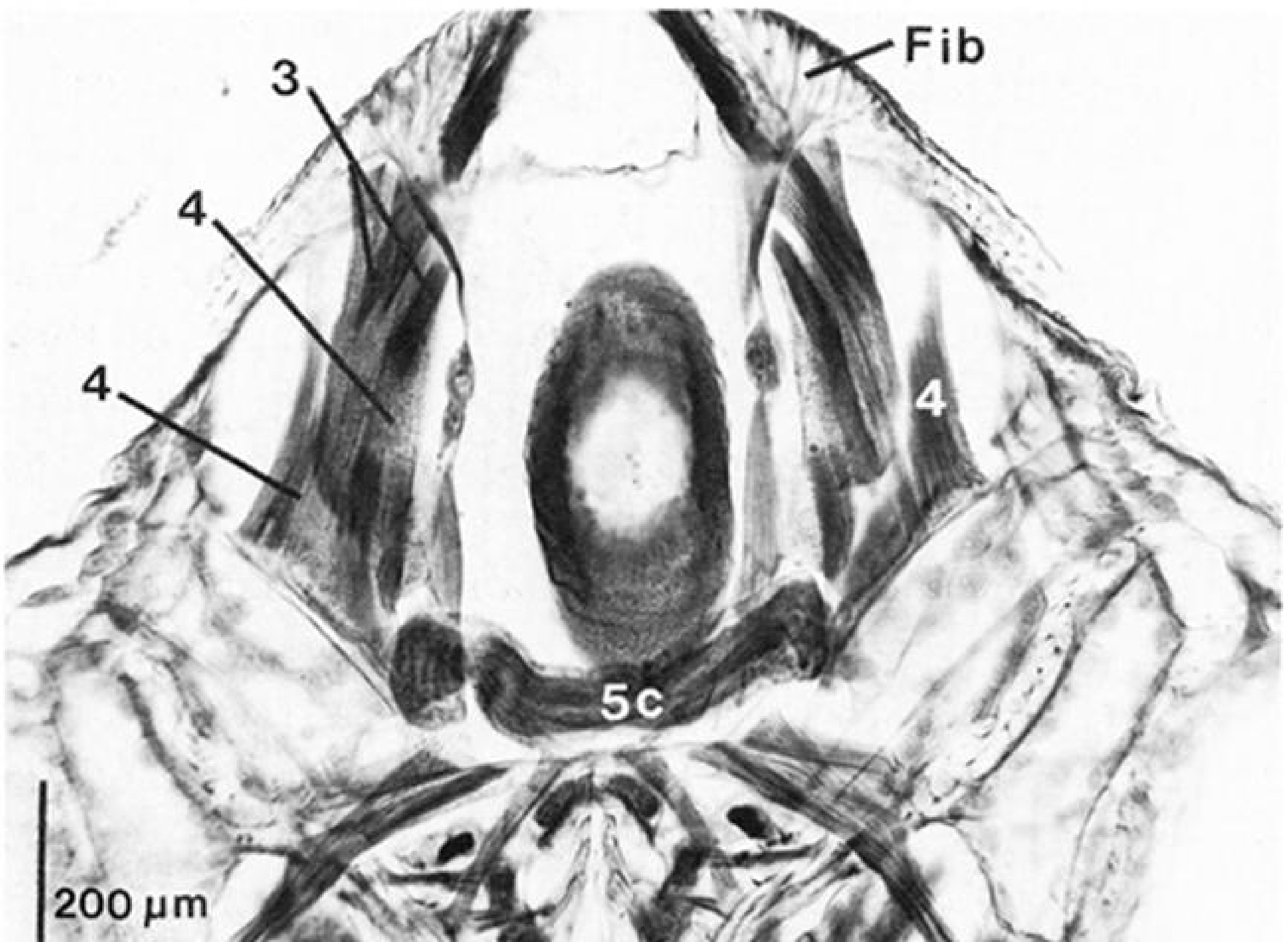
Figures 28 and 29. *D. atkinsoni* f. *bolivari*. Successive horizontal slices, viewed ventrally, to reveal various anatomical features and the relation of the median filter chamber (FCh) to the trunk and to the mouthparts. Figure 29, which lies deeper than (i.e. dorsal to) figure 28, cuts the filter chamber not far from where the tips of its component filter setae lie deep in the food groove, the thickened cuticle of whose walls can be seen. It also cuts through the maxillules (Mxlle), mandibles (Mand) and compound eye (E). (Comparison with figure 3 facilitates orientation.) Other features seen include the end sac (ES) and tubules (TMG) of the maxillary gland, housed between the inner and outer walls of the carapace (C), the carapace adductor muscles (ACAM and PCAM), the ventral longitudinal muscles (VLM), the divergence of whose bundles anteriorly is seen in figure 29, the horizontal muscles (HM), some of the complexity that prevails in the vicinity of the post-abdominal hinge, and the conspicuous ovaries (Ov). A portion of the carapace provides a point of reference posteriorly. Note the abundant fat cells (FC). Because the mid-gut curves sharply ventrally as it enters the post-abdomen (see figure 1), it is here cut more or less transversely. Mandibular asymmetry is readily apparent, and exuded labral gland secretions (LGS) are seen in figure 28.

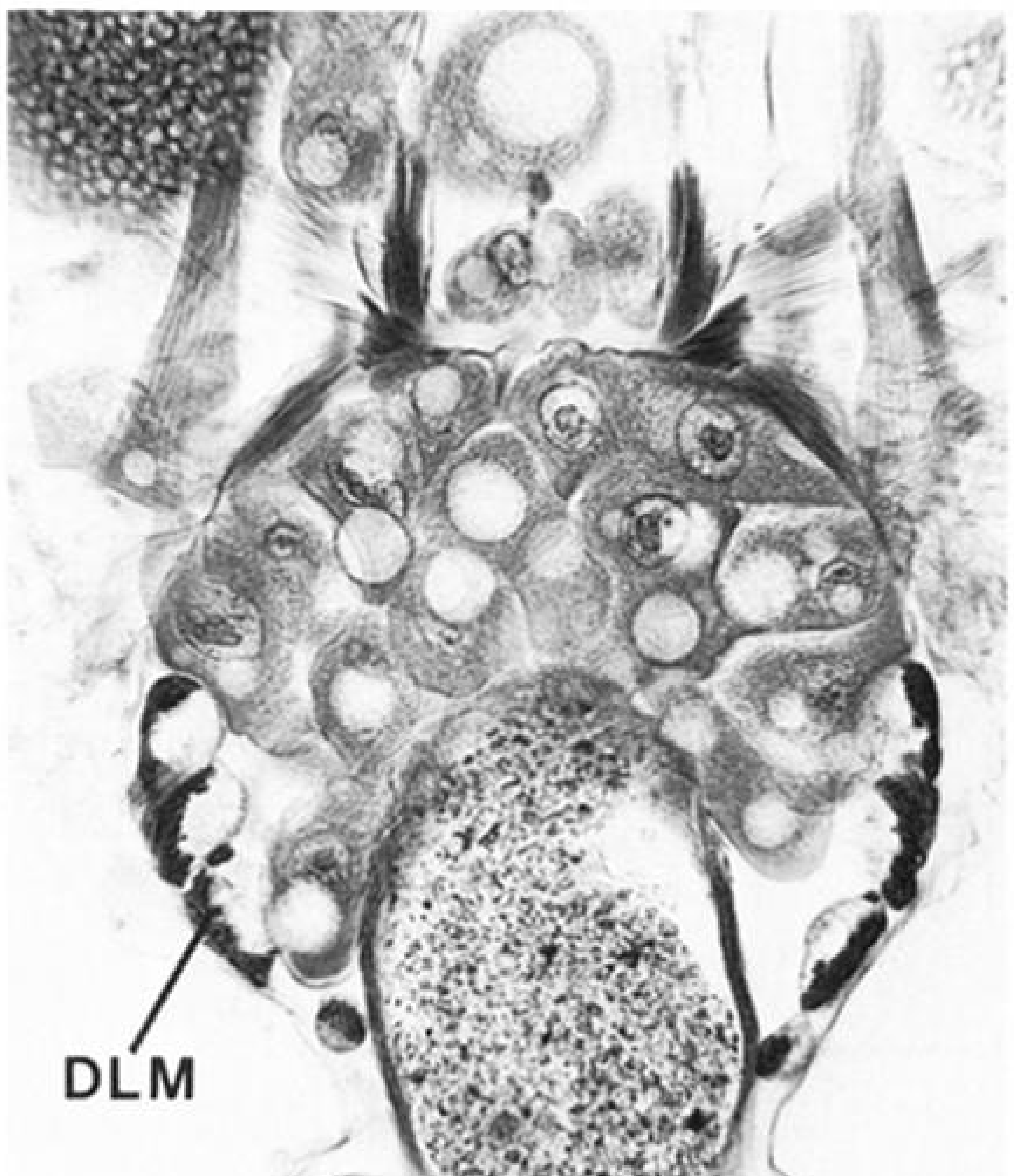




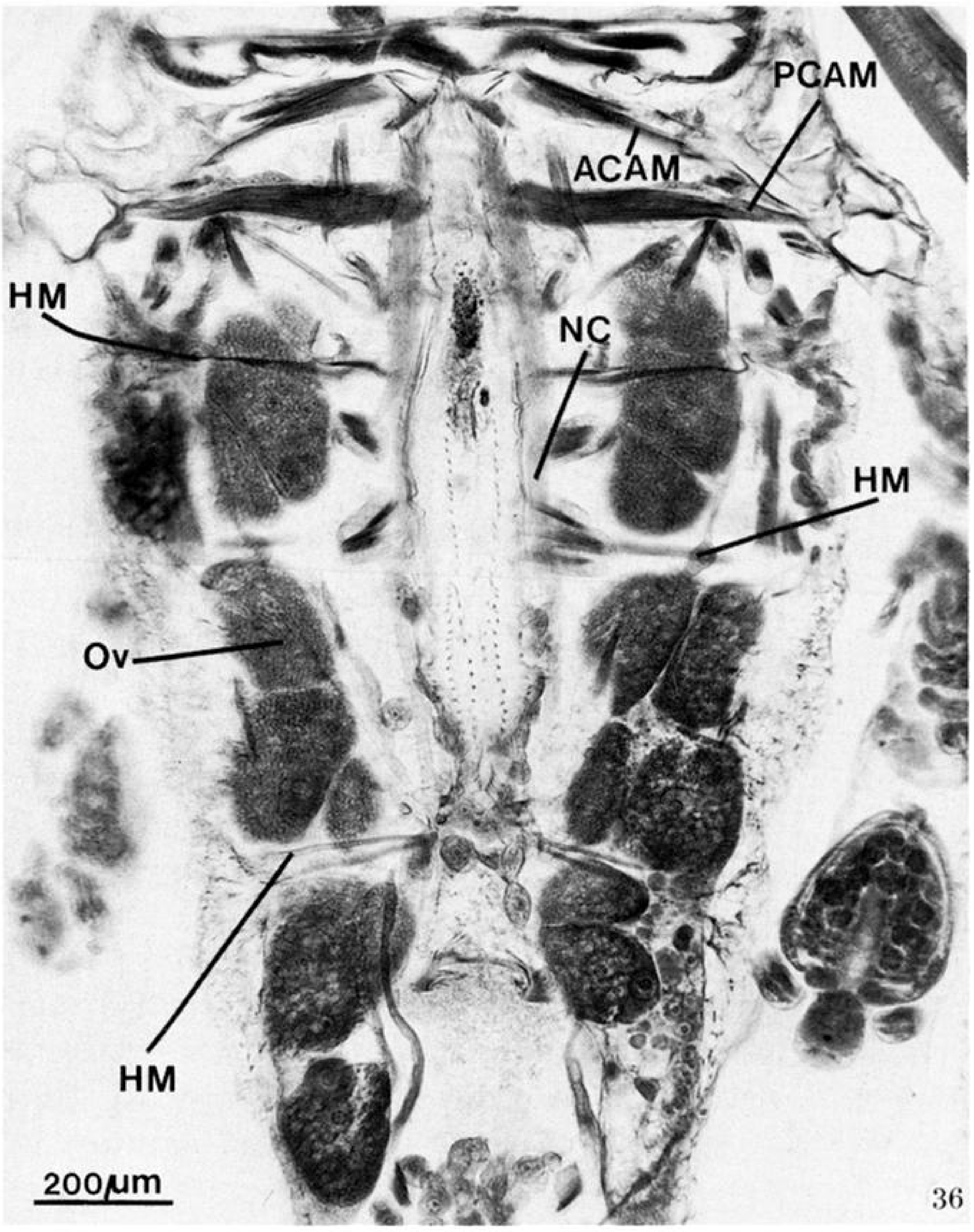


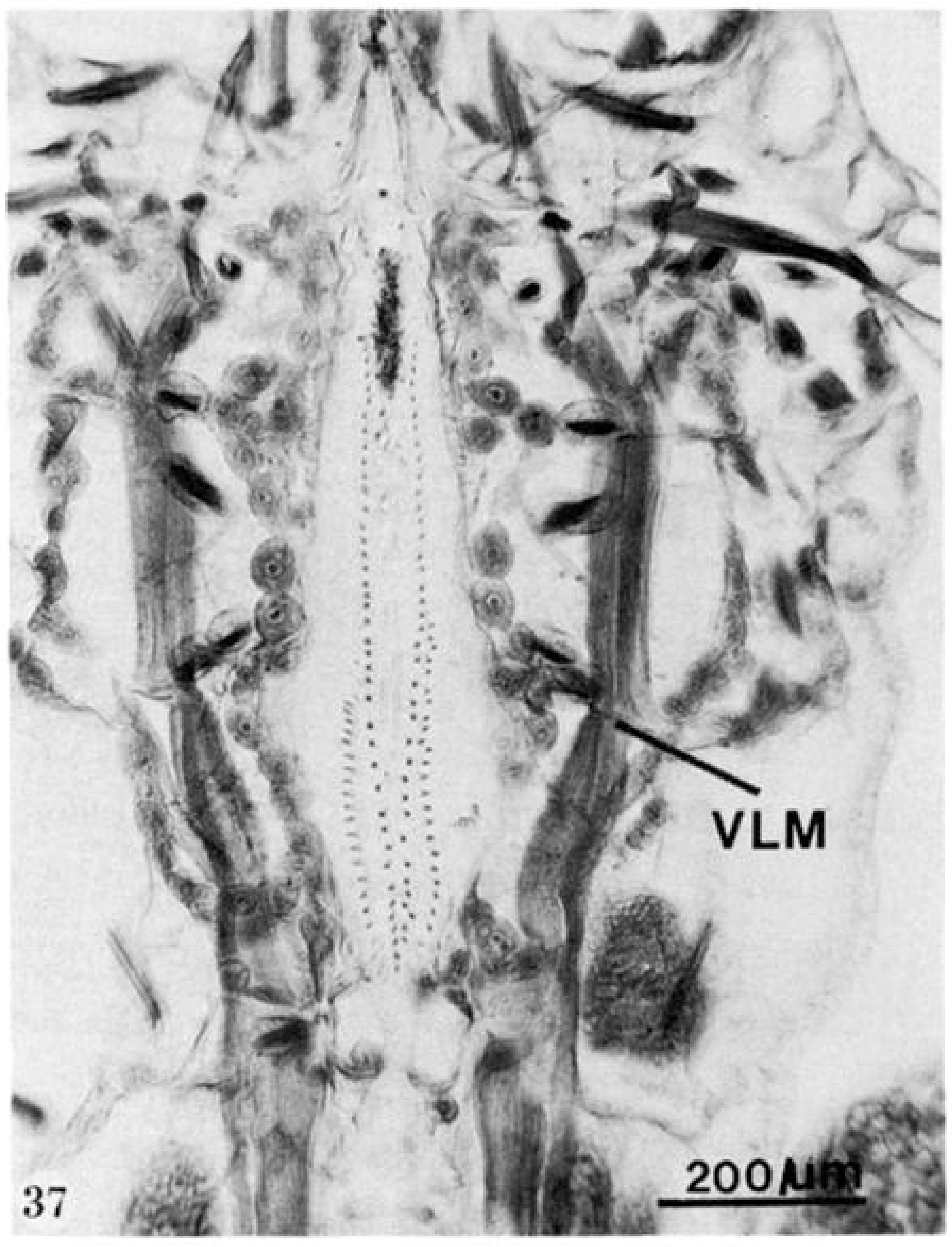


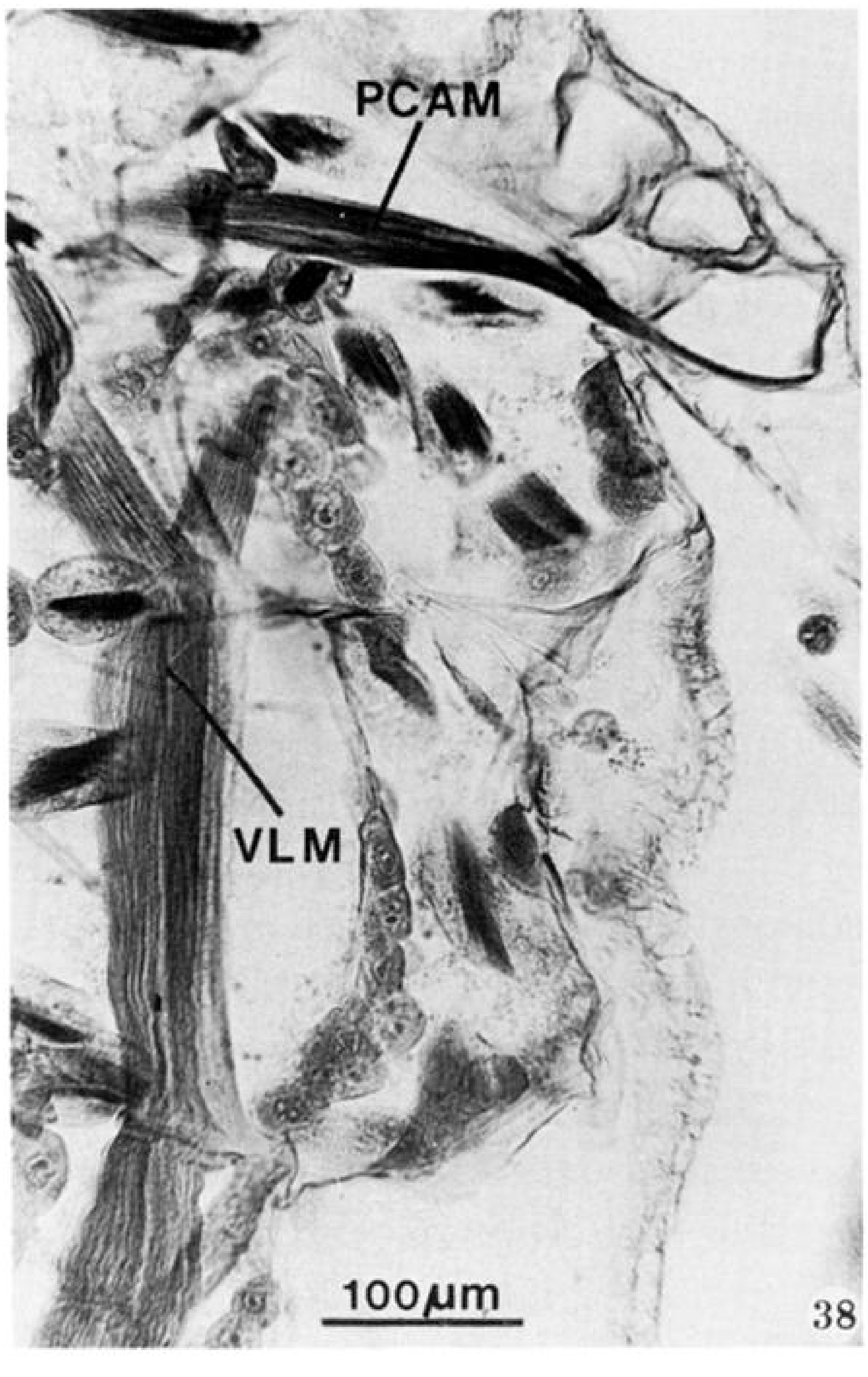


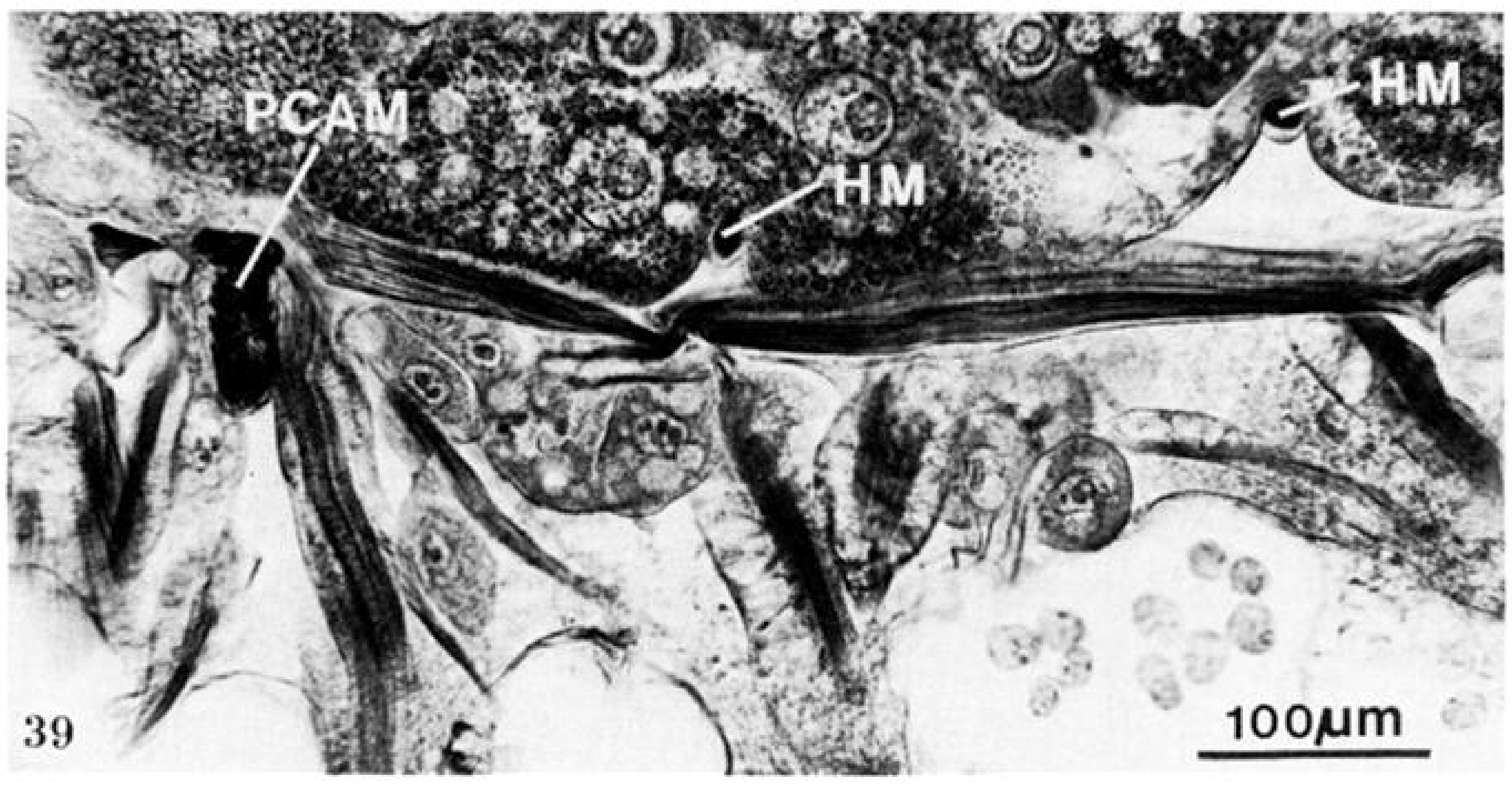


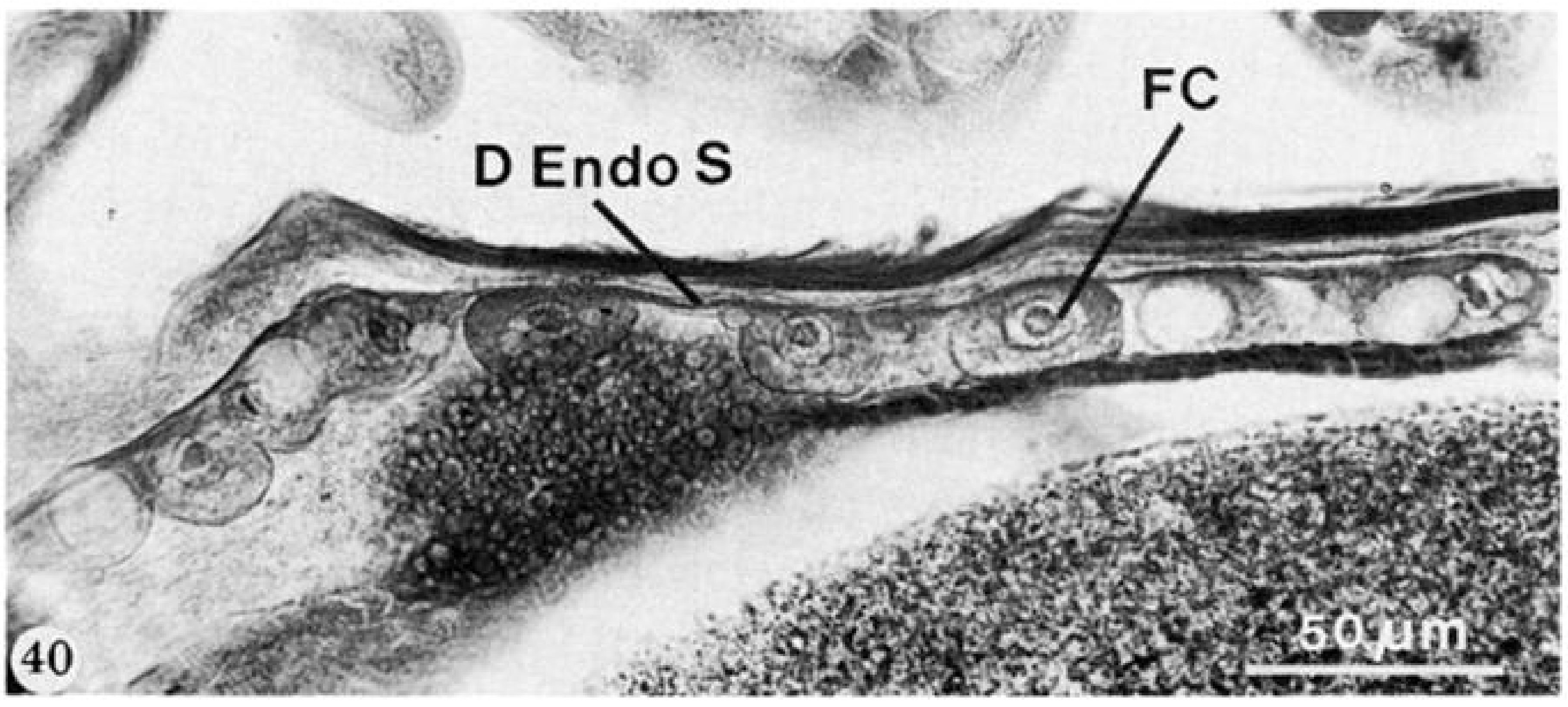
Figures 30–35. *Daphnia atkinsoni* f. *bolivari*. Some details of skeleto-musculature. Figure 30. Transverse section at the extreme anterior limits of the mandibles. The distal extremities of the promotor roller muscles (3) are seen inserting on the anterior margins of the mandibles, and the broad face of the transverse mandibular tendon (TMT) is seen especially at the right-hand side of the section. Extrinsic muscles of the antennae, which cross over to insert on the opposite side of the head, are seen dorsally. Figure 31. The same, more posteriorly, showing many of the major mandibular muscles and the lower parts of the suspensors of the transverse mandibular tendon (SUS). The asymmetry of the major transverse muscles (TMM), long on the right (left side of the animal), short on the left, is readily apparent. Figure 32. The same, cutting through the extreme posterior region of the mandibles and through the maxillules. Note the load-spreading fibrils (Fib) dorsally, from which the suspensors of the transverse mandibular tendon (whose upper parts are seen) and the remotor roller muscles (4) descend. Figure 33. Transverse section not far behind the carapace adductor muscles, showing some of the elements of the extensive endoskeleton (Endo), here used as the source of origin of extrinsic trunk limb muscles. Tubules of the maxillary gland (TMG) are also seen. Figure 34. Horizontal slice through the carapace adductor muscles. Ventral aspect. The massive posterior muscles occupy the centre of the photograph. Parts of the more obliquely inclined anterior muscles are seen. The fibrous anchorage of other muscles to the food groove walls is also seen. Figure 35. Horizontal section through the extreme posterior end of the trunk which has here flexed ventrally to form the post-abdomen. The mid-gut is therefore cut transversely. Note the detrital nature of its contents. The continuation of the dorsal longitudinal trunk muscles (DLM) into the post-abdomen is readily seen. All four bundles retain their integrity.

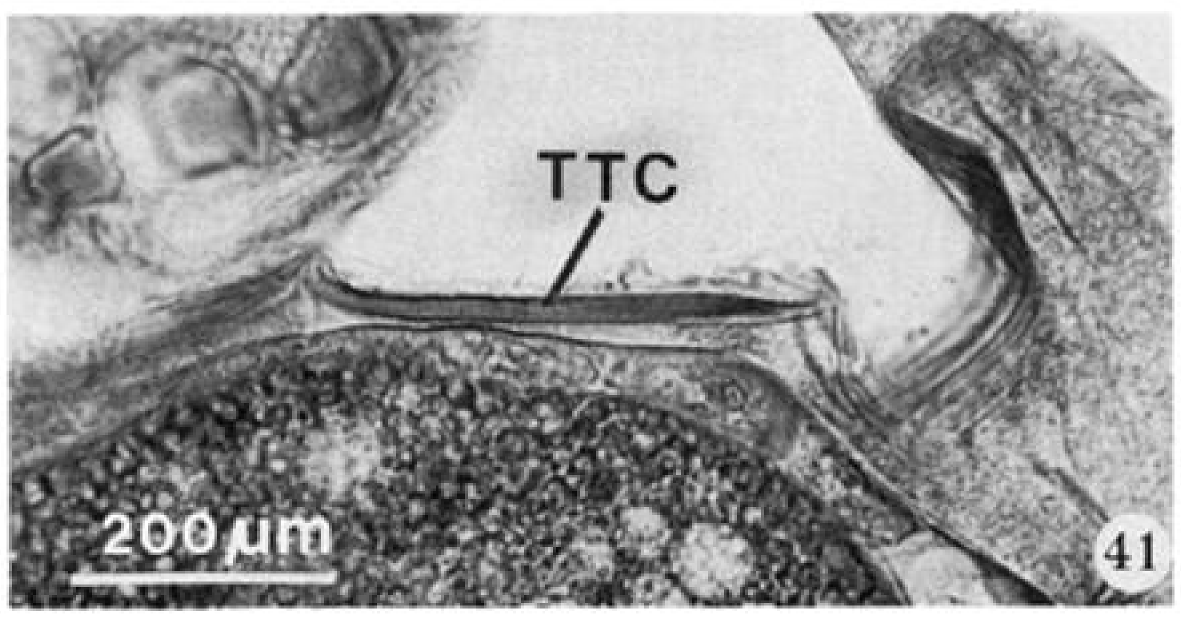




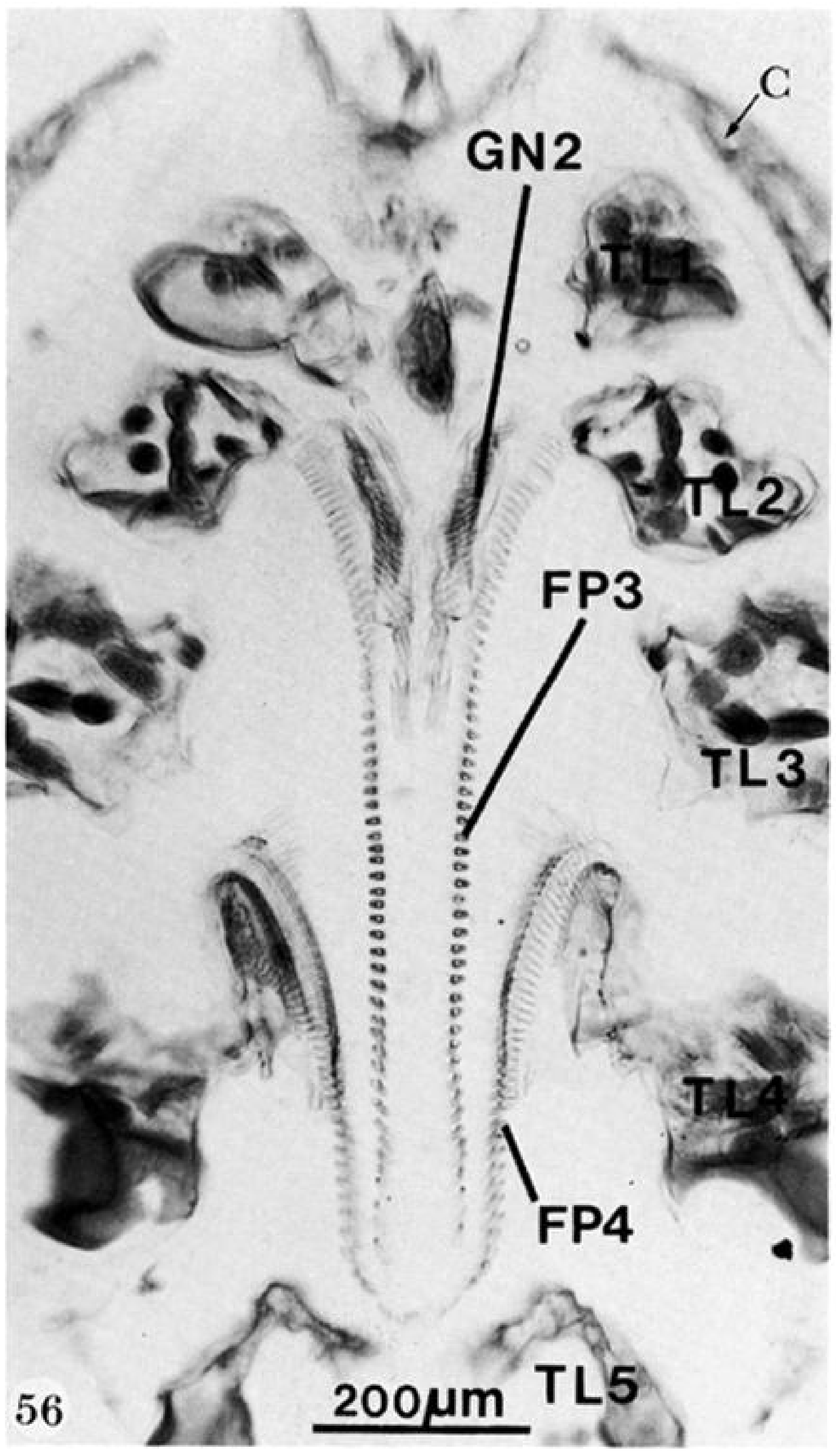


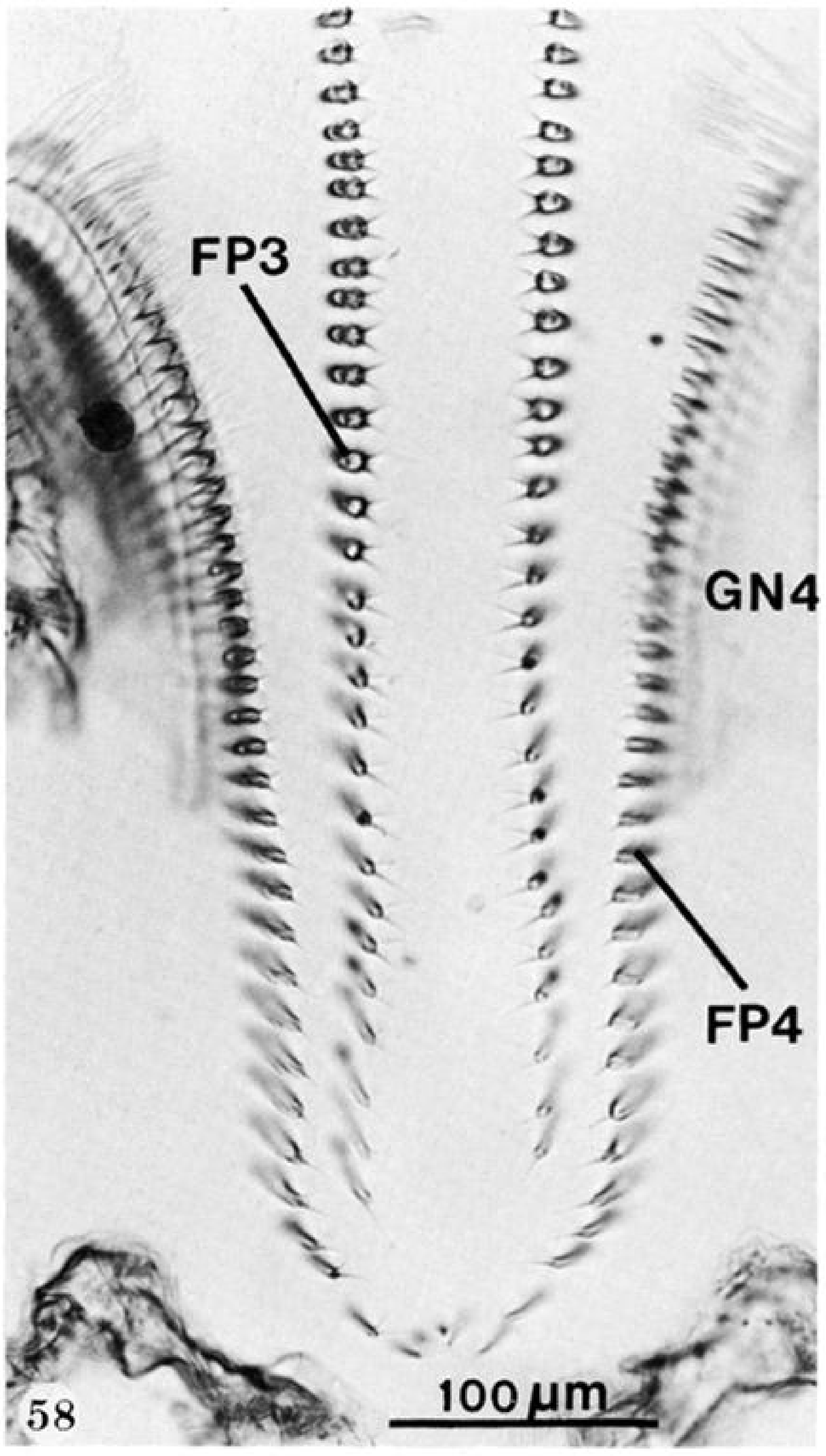


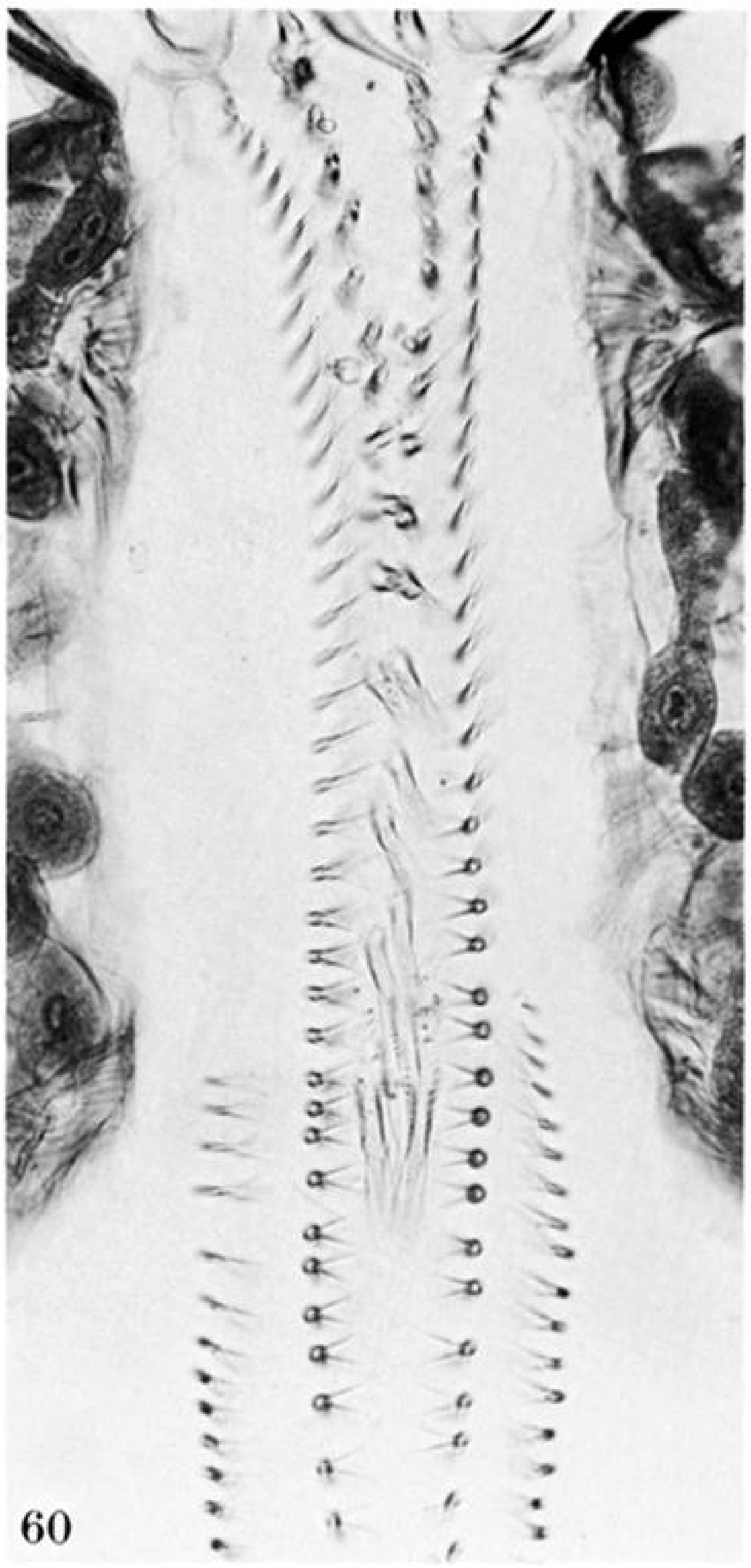


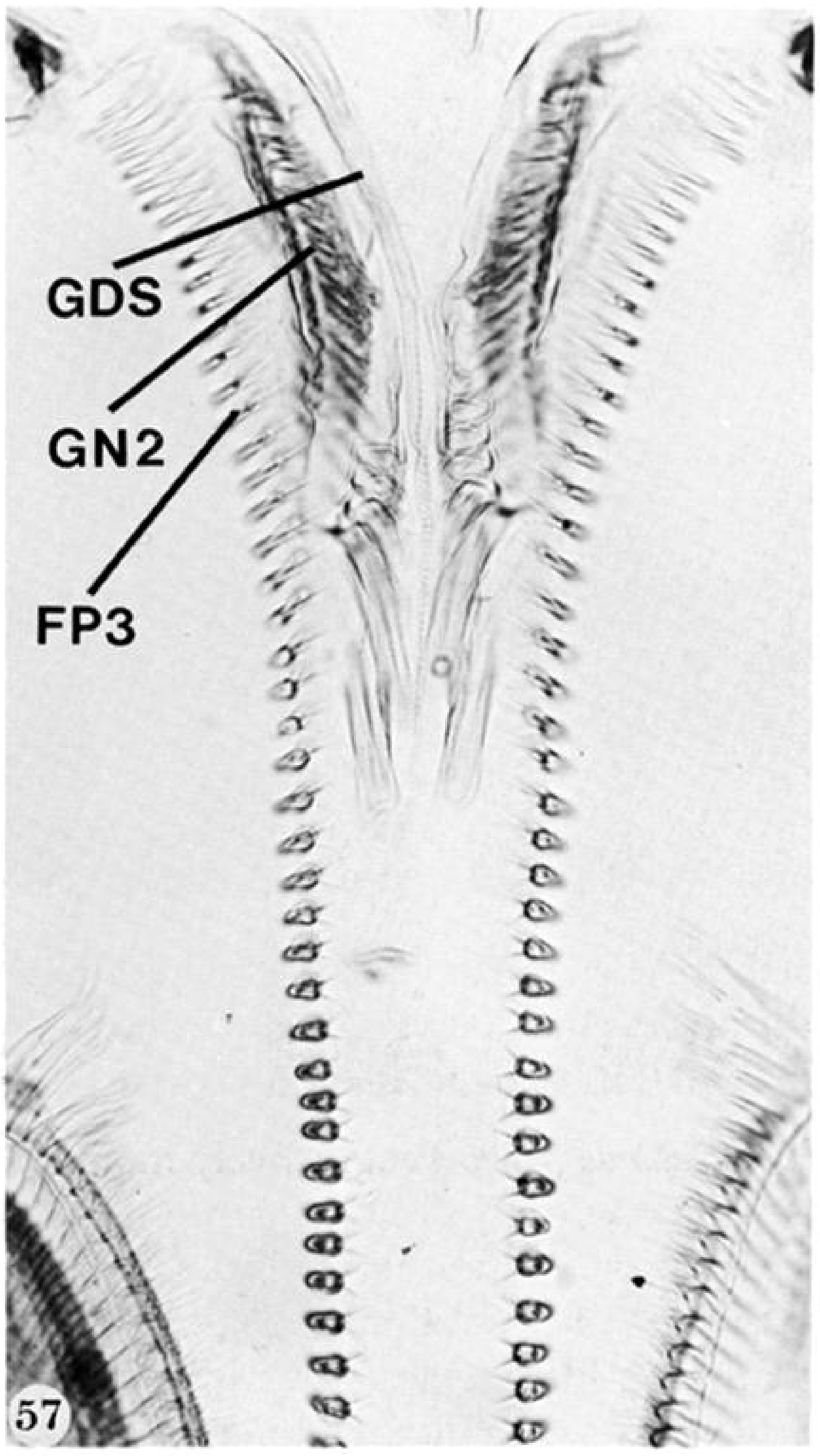


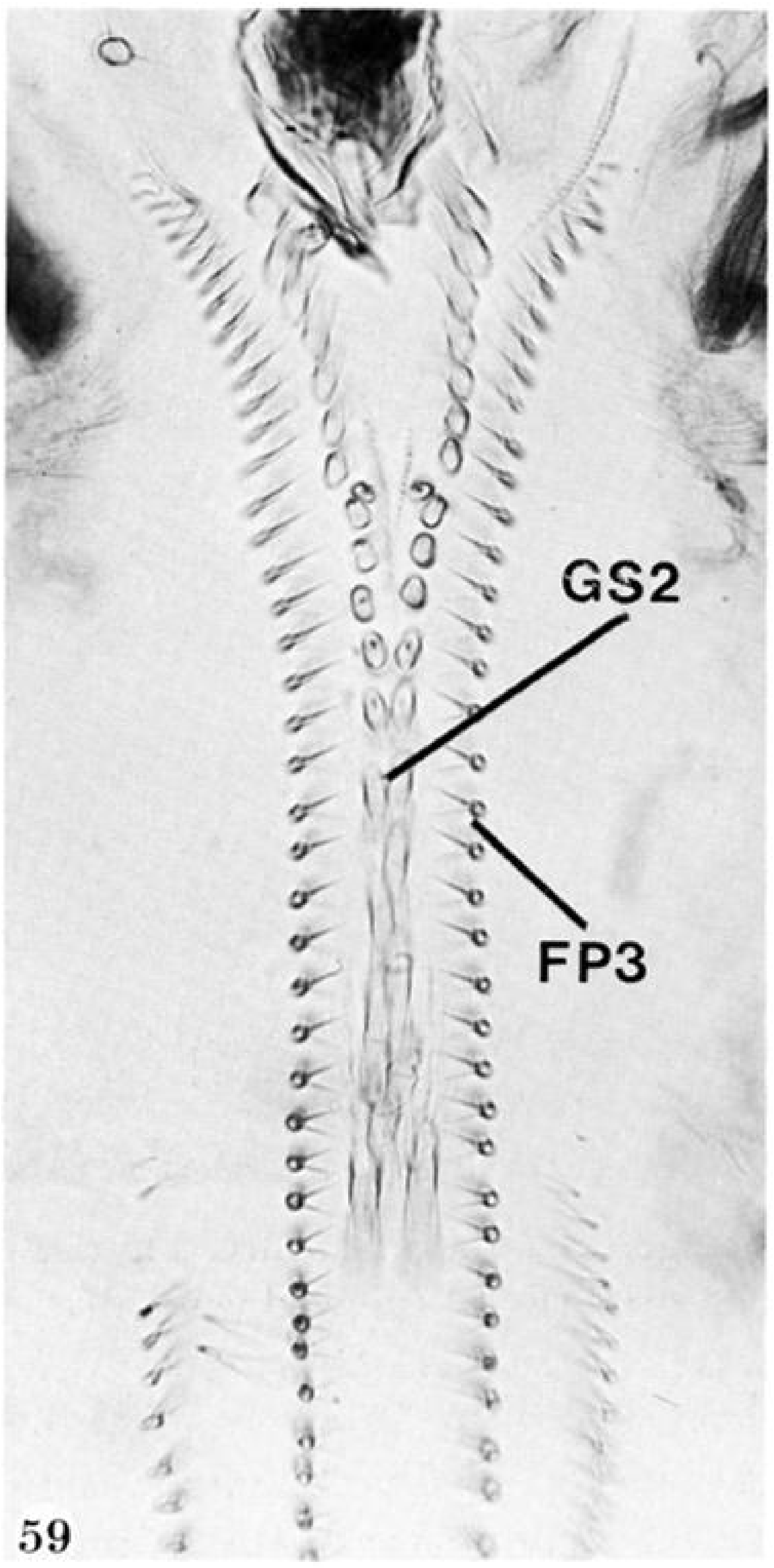
Figures 36–41. *Daphnia atkinsoni* f. *bolivari*. Aspects of the skeletomusculature. Figure 36. Horizontal slice, ventral, at a level just ventral to the floor of the food groove and therefore just dorsal to the ventral longitudinal muscles that, save for a trace anteriorly, are not seen. The section shows the carapace adductor muscles (ACAM and PCAM), the horizontal muscles (HM) and portions of the dorso-ventral muscles of the trunk. Elements of the transverse muscles of the mandibles can also be made out. The ventral nerve cords (NC) and the bulky ovaries (Ov) are seen at each side of the food groove. Embryos in the brood pouch, cut in various planes, can be seen at each side of the trunk. Figure 37. Horizontal slice showing the topographic relations of the ventral longitudinal muscles (VLM). Figure 38. Horizontal slice showing details of a ventral longitudinal muscle (VLM), how its bundles separate anteriorly, and the arrangement of the intersegments. The anchorage of the posterior carapace adductor muscle (PCAM) is also seen. Figure 39. Longitudinal section through a ventral longitudinal muscle. This also cuts the posterior carapace adductor (PCAM) and horizontal muscles (HM) transversely. Note how the latter tend to be enveloped by the ovaries. Traces of the extrinsic musculature of the anterior trunk limbs are seen. Figure 40. Longitudinal slice showing how the presence of the dorsal endoskeletal sheet (D Endo S) is easily located by the presence of fat cells (FC) associated with its ventral surface, and here sandwiched between the dorsal endoskeletal sheet and the mid-gut. Figure 41. Longitudinal section through a local thickening of the trunk cuticle (TTC) in the region where the dorsal longitudinal muscles originate. A trace of one of these is just seen, out of focus, to the left. The anteriorly located bracing muscle runs to the carapace at the anterior end of the brood pouch.

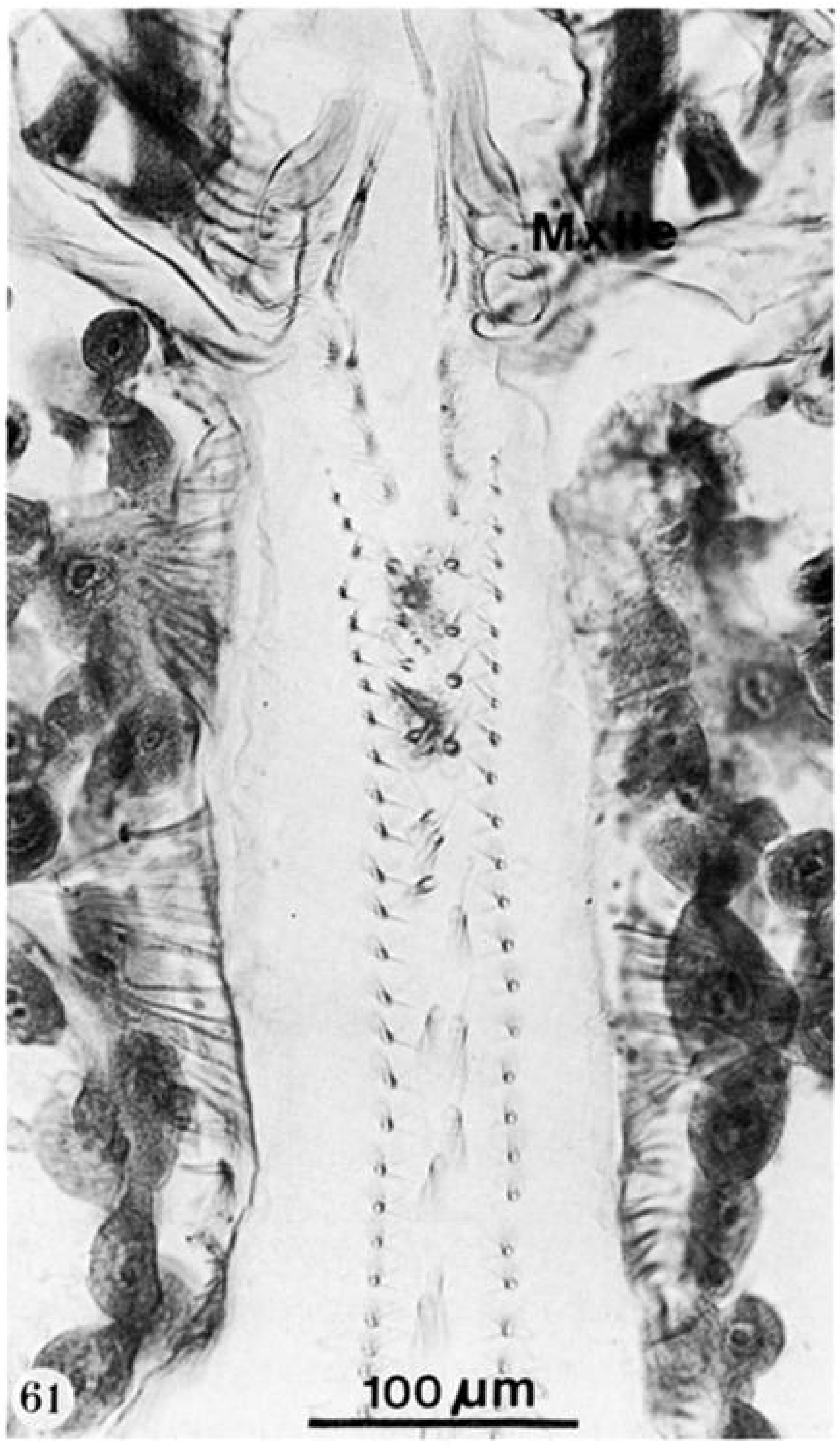




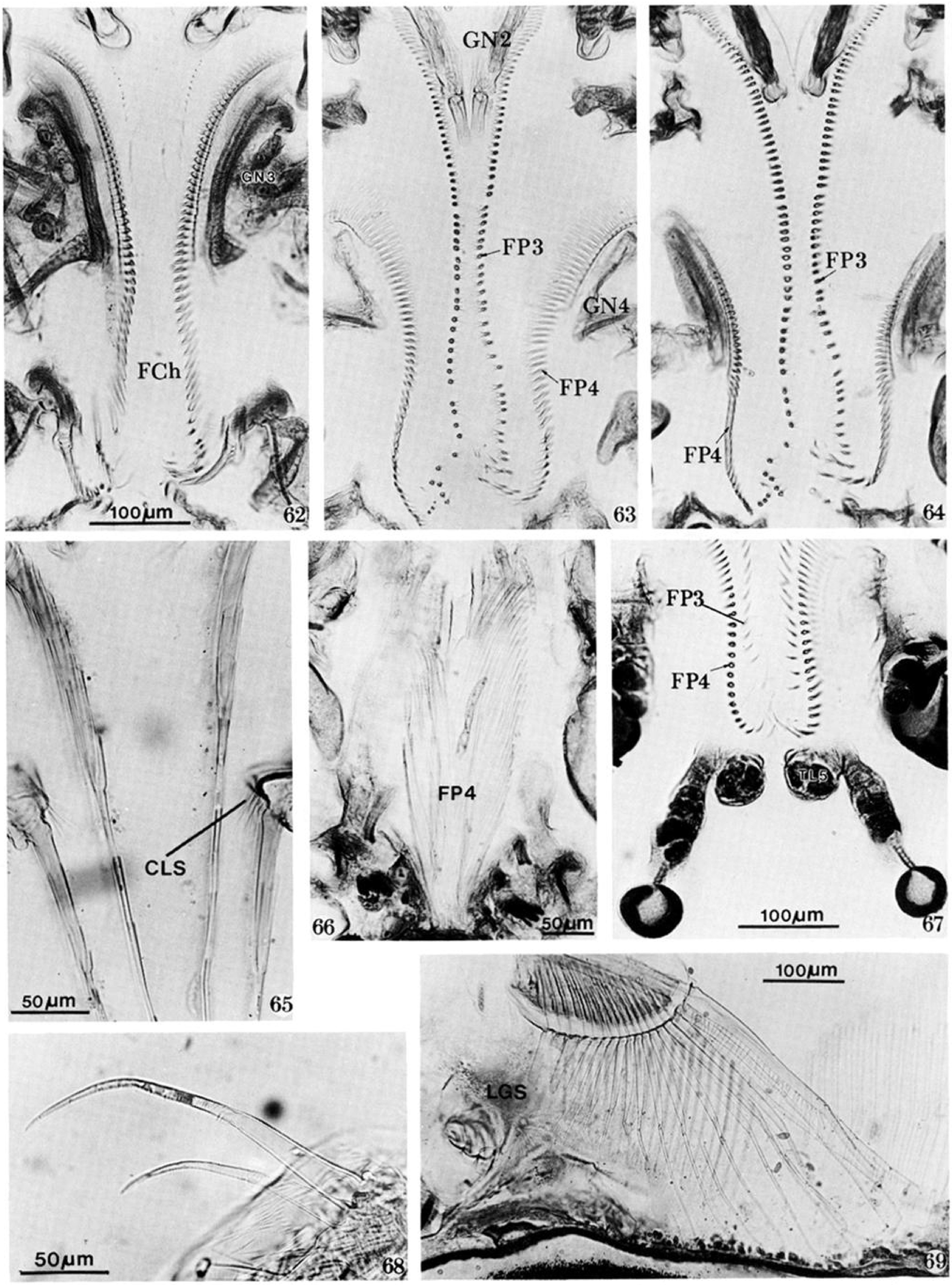




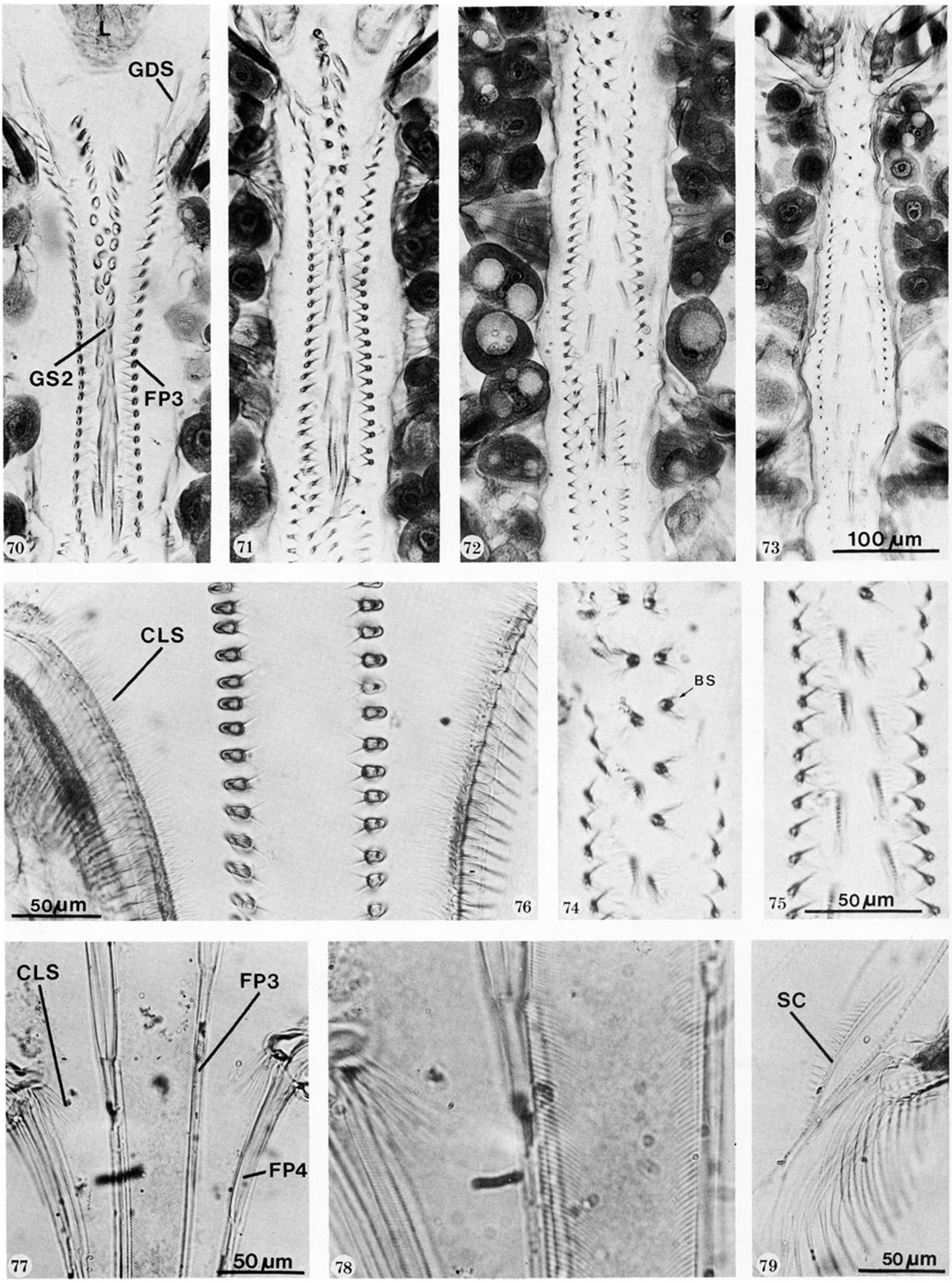




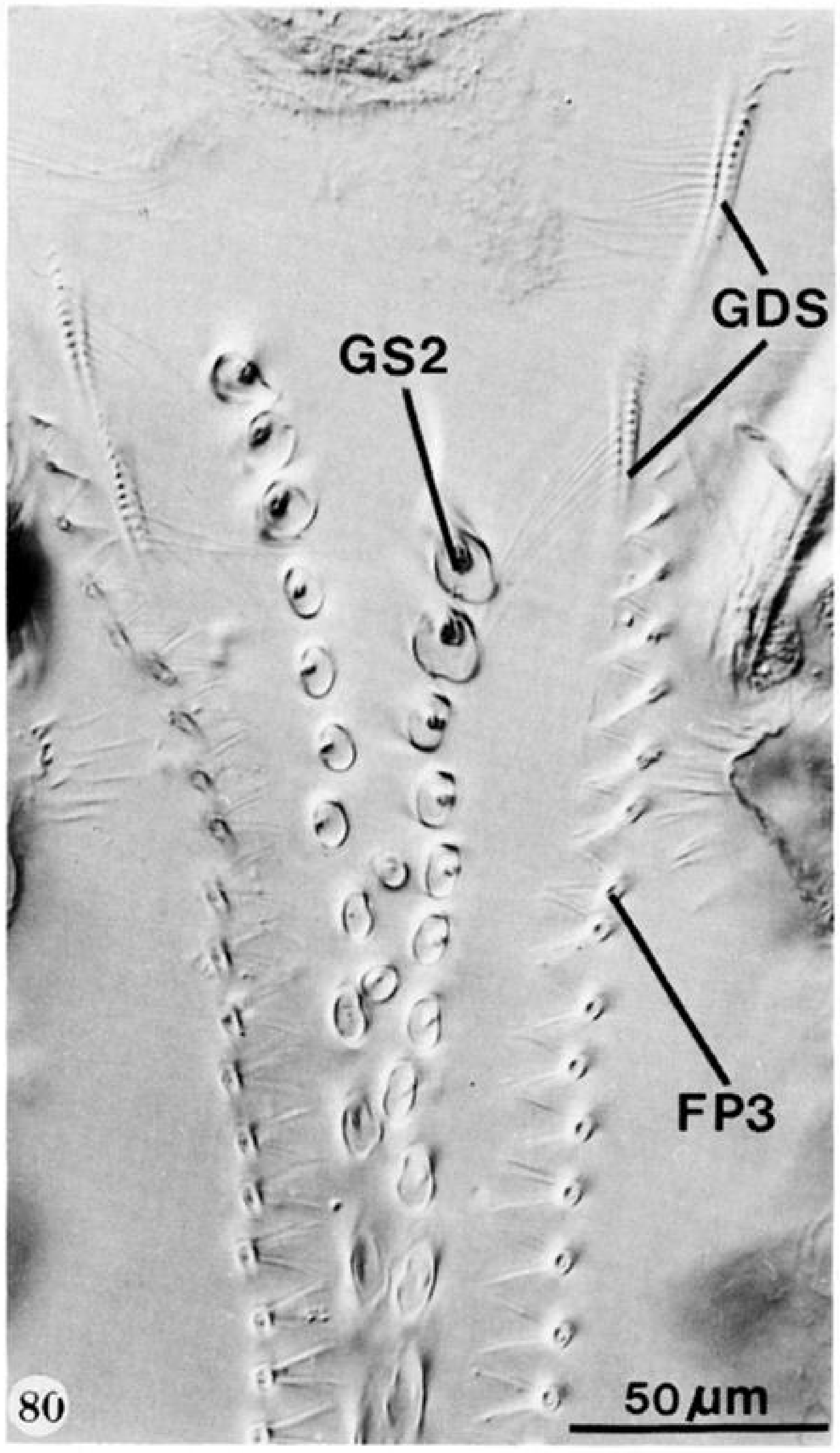
Figures 56–61. *Daphnia atkinsoni* f. *bolivari*. Figure 56. Horizontal slice through the trunk limbs (TL1–5) showing the location and composition of the filter chamber. Ventral. Figure 57. The same, showing the anterior ends of the filters of trunk limb 3 (FP3) with the gnathobases of trunk limb 2 (GN2) between them anteriorly, and one of the guide setae (GDS) of trunk limb 1 anterior to that. Parts of the gnathobases of trunk limb 4 are seen posteriorly. Figure 58. The same, showing the posterior ends of the filters of trunk limb 3 (FP3) and, exterior to them, those of trunk limb 4 (FP4). Note how the latter curve medially at their posterior extremities to fence off the filter chamber posteriorly immediately in front of limb 5. Figure 59. The same, more dorsally at the anterior end of the filter chamber, cutting the filters of trunk limb 3 (FP3) and the gnathobasic setae of trunk limb 2 (GS2). Figure 60. The same, more dorsally. Figure 61. The same, more dorsally. The food-handling setae are here seen in the food groove and the section cuts near the tips of the gnathobasic setae of trunk limb 2 between which are seen particles of food. The maxillules (Mxlle) are also seen in section.

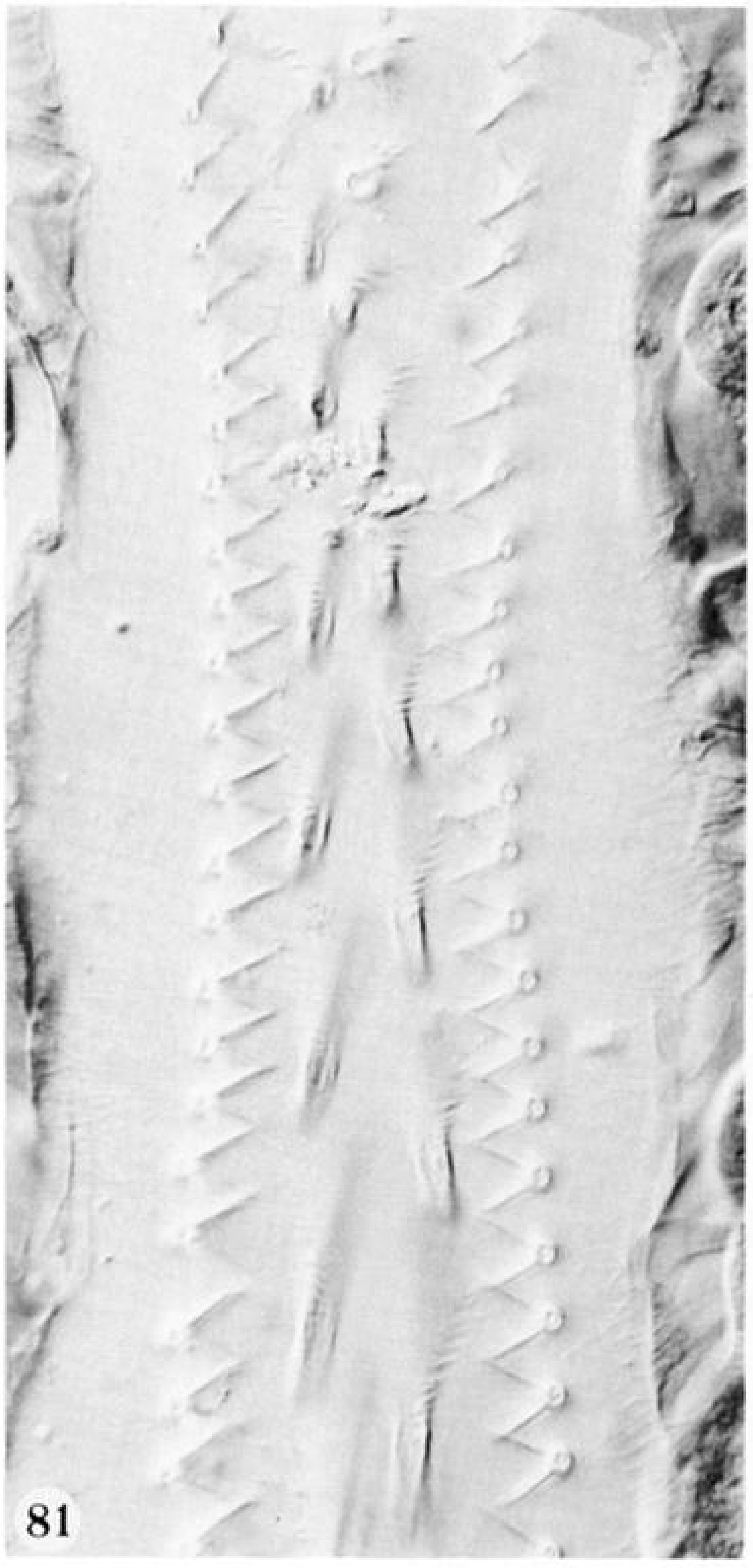


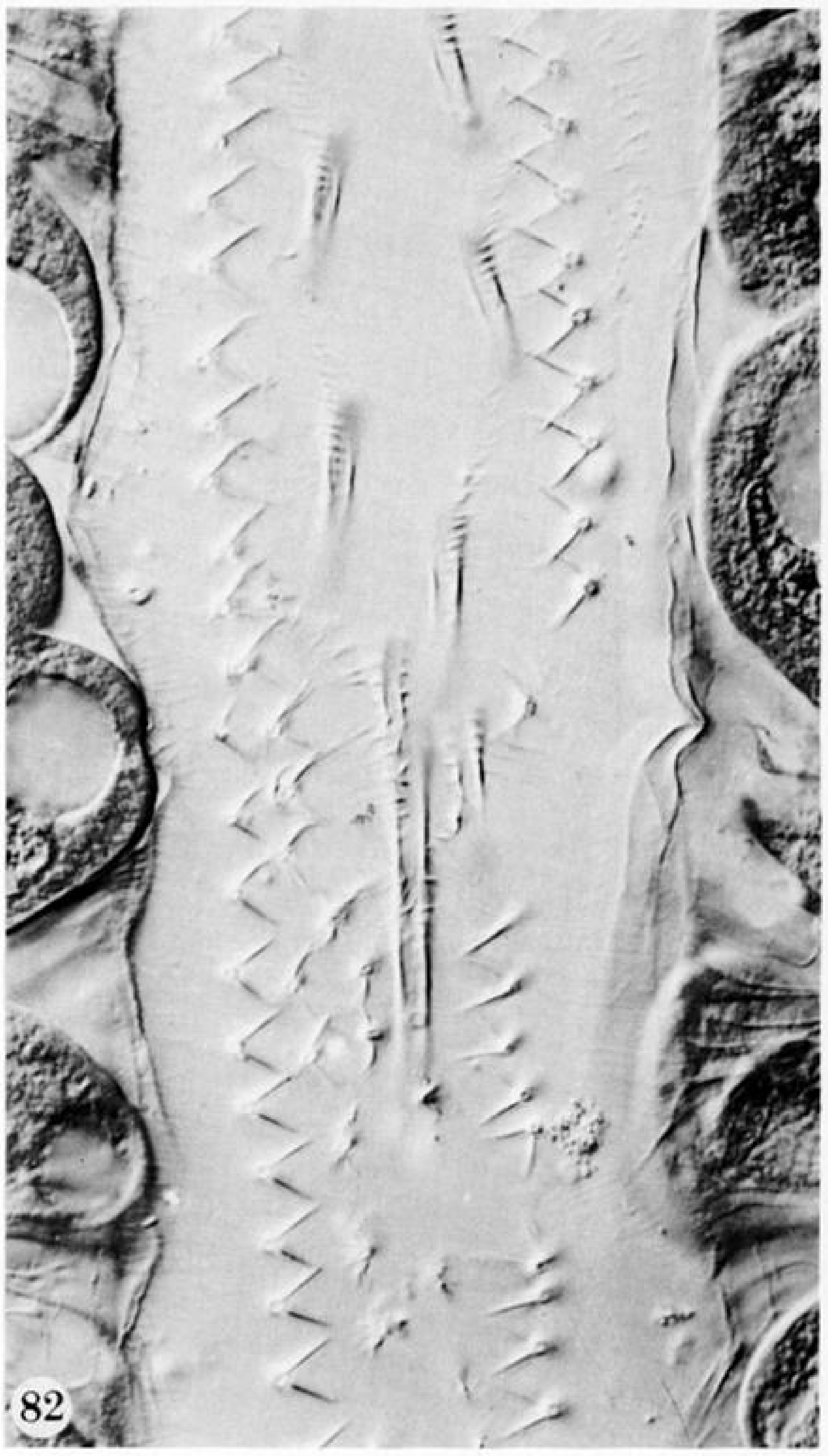
Figures 62–69. Elements of trunk limb arrangement and armature. Figures 62–64. *Daphnia pulex*. Progressively more dorsal horizontal slices through the filter chamber (FCh) (viewed ventrally) to show its essentially similar composition to that of *D. atkinsoni* f. *bolivari* and other species. Figure 65. *D. atkinsoni* f. *bolivari*. Transverse section through the filter plates of trunk limbs 3 and 4. Note the cleaning setules (CLS) borne on the gnathobase of trunk limb 4. Figure 66. *D. pulex*. Transverse section at the posterior end of the filter chamber, showing how the filter setae of trunk limb 4 curve round and seal its posterior end. Figure 67. *Simocephalus vetulus*. Horizontal slice encompassing the posterior end of the filter chamber and showing the relation of the posterior fence of filter setae of the fourth pair of trunk limbs (FP4) to the adjacent fifth pair. Note the similarity of the arrangement to that of *Daphnia*. Figure 68. *D. atkinsoni* f. *bolivari*. Ejector hooks of the first trunk limb. Figure 69. *D. atkinsoni* f. *bolivari*. Longitudinal section showing the gnathobase of trunk limb 2 *in situ*. Some of the more anterior setae have been severed. Note that none of the elements of the armature of this gnathobase are filter setae. Coagulated and stained secretions of the labral glands (LGS) appear as a dark smear anteriorly.

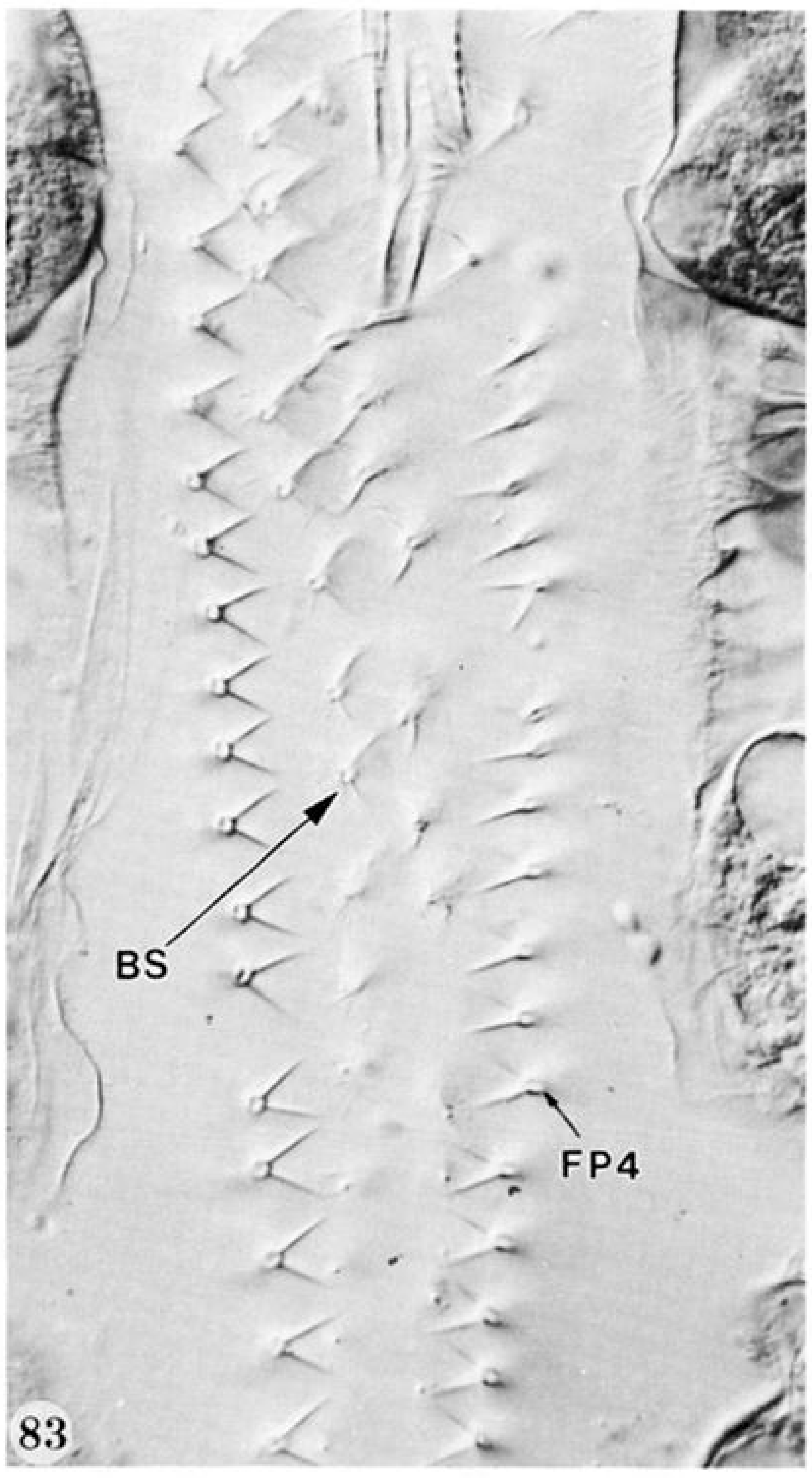


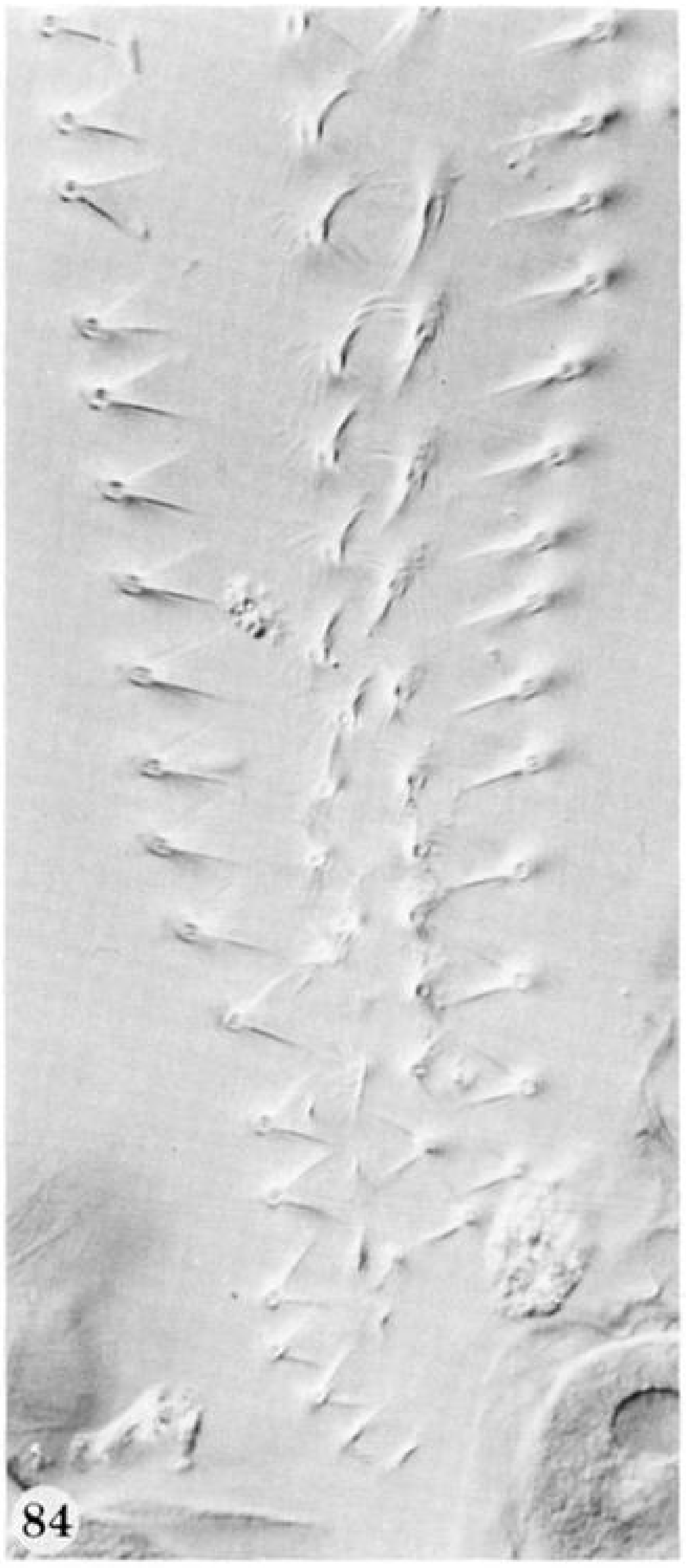
Figures 70–78. *Daphnia atkinsoni* f. *bolivari*. Figure 79, *D. obtusa*. Figure 70. Horizontal section (ventral) slicing the proximal region of the gnathobasic setae of the second trunk limbs (GS2) and the adjacent filter setae of the gnathobases of the third trunk limbs (FP3). The tip of the labrum (L) is just visible as a landmark anteriorly and some of the guide setae (GDS) of the first pair of trunk limbs are also seen. Figure 71. The same, more dorsal, that is deeper into the food groove. Figure 72. The same, more dorsal. Note the dense aggregation of fat cells at each side of the food groove. Figure 73. The same, more dorsal, near the level of the tips of the anteriormost gnathobasic setae of the second trunk limb. The filter setae of the third trunk limb are cut progressively nearer their tips towards the anterior end of the region shown and do not extend into the anterior extremity of the food groove. The maxillules are seen in section at the anterior limits of the food groove. Note the thickened cuticle of the food groove walls. Figures 74 and 75. As figure 72, more highly magnified, to show the brush-like armature (BS) of the distal portions of the median row of gnathobasic setae of the second trunk limbs and its orientation. The regions shown follow each other in the two figures, with a little overlap to aid location. Note how the soft, brush-like setules are directed outwards towards the filter setae of the adjacent trunk limb 3, which they sweep and clean. There are also spinules on the food groove wall that assist in cleaning the tips of the filter setae from the outside. The nature and location of these is most clearly revealed by SEM (see figure 101). Figure 76. Horizontal slice, ventral, through some of the filter setae of the third trunk limbs to show their arrangement and that of their filtering setules, and the array of fine setules (CLS) on the adjoining gnathobases of the fourth trunk limbs that scour filtered particles from them. Figure 77. Transverse section through the filter plates of the third (FP3) and fourth trunk limbs (FP4) to show their topographic relations and the array of fine setules (CLS) borne on the gnathobases of the fourth trunk limbs that scour filtered particles from the filter setae of the third trunk limbs. Immediately before fixation, the animal had been removing minute particles from suspension and these appear as a haze in the filter chamber between the opposed filters of the third trunk limbs, whose filtering efficiency is clearly revealed. Figure 78. The same, more highly magnified. Figure 79. *D. obtusa*. Part of the scraper (SC) of trunk limb 2. The proximal portions of some of the filter setae of trunk limb 3 are also seen.

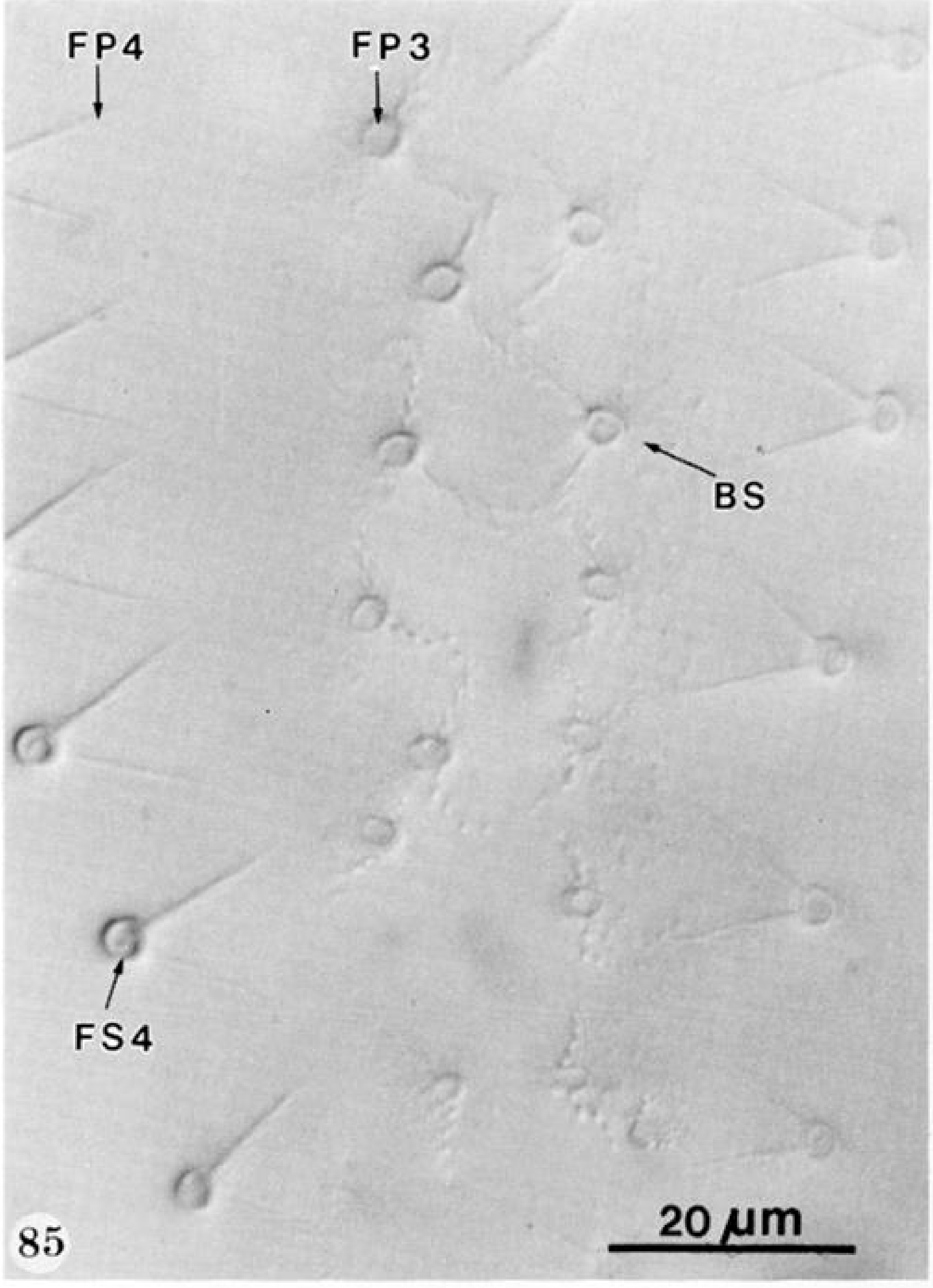




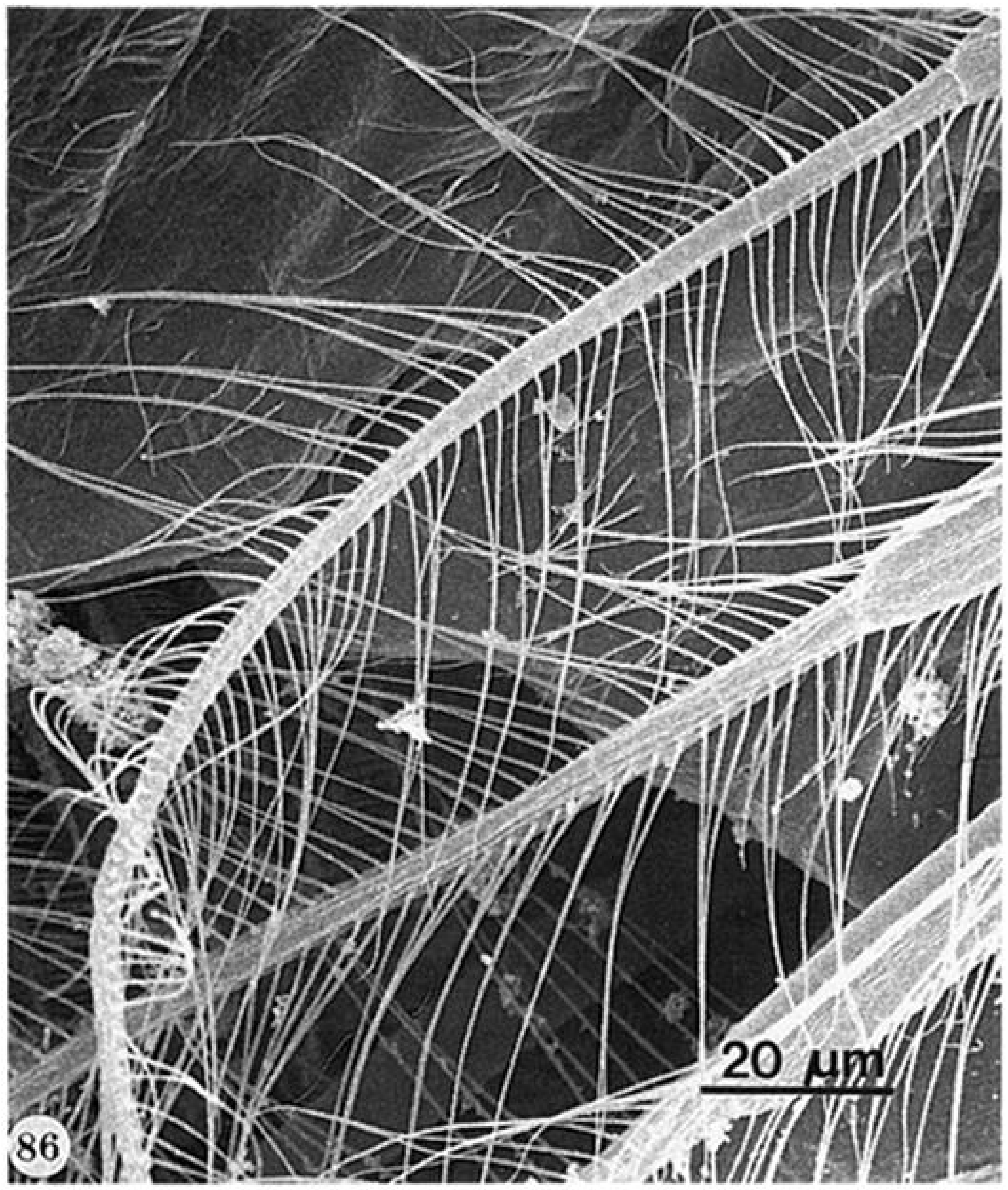


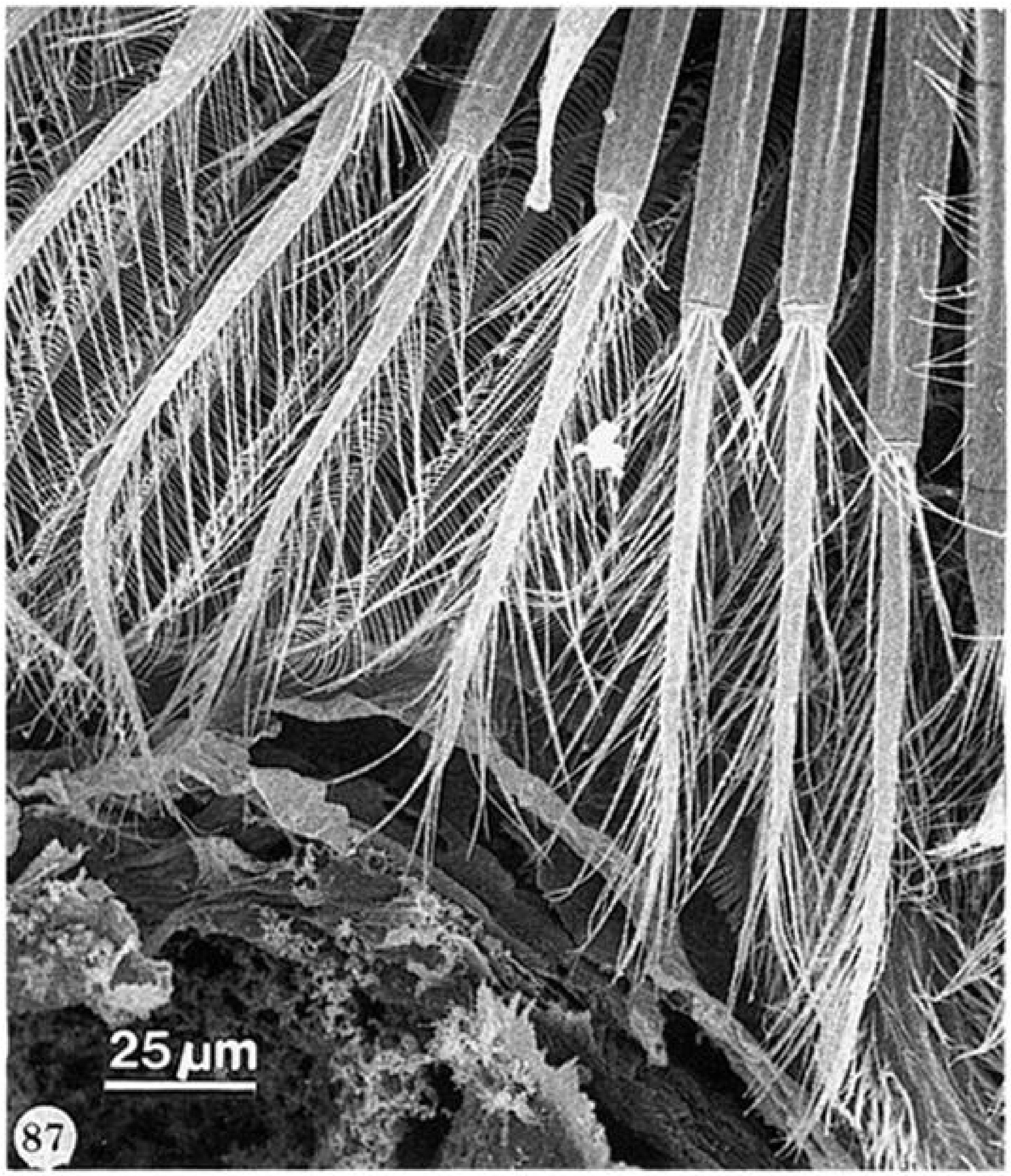


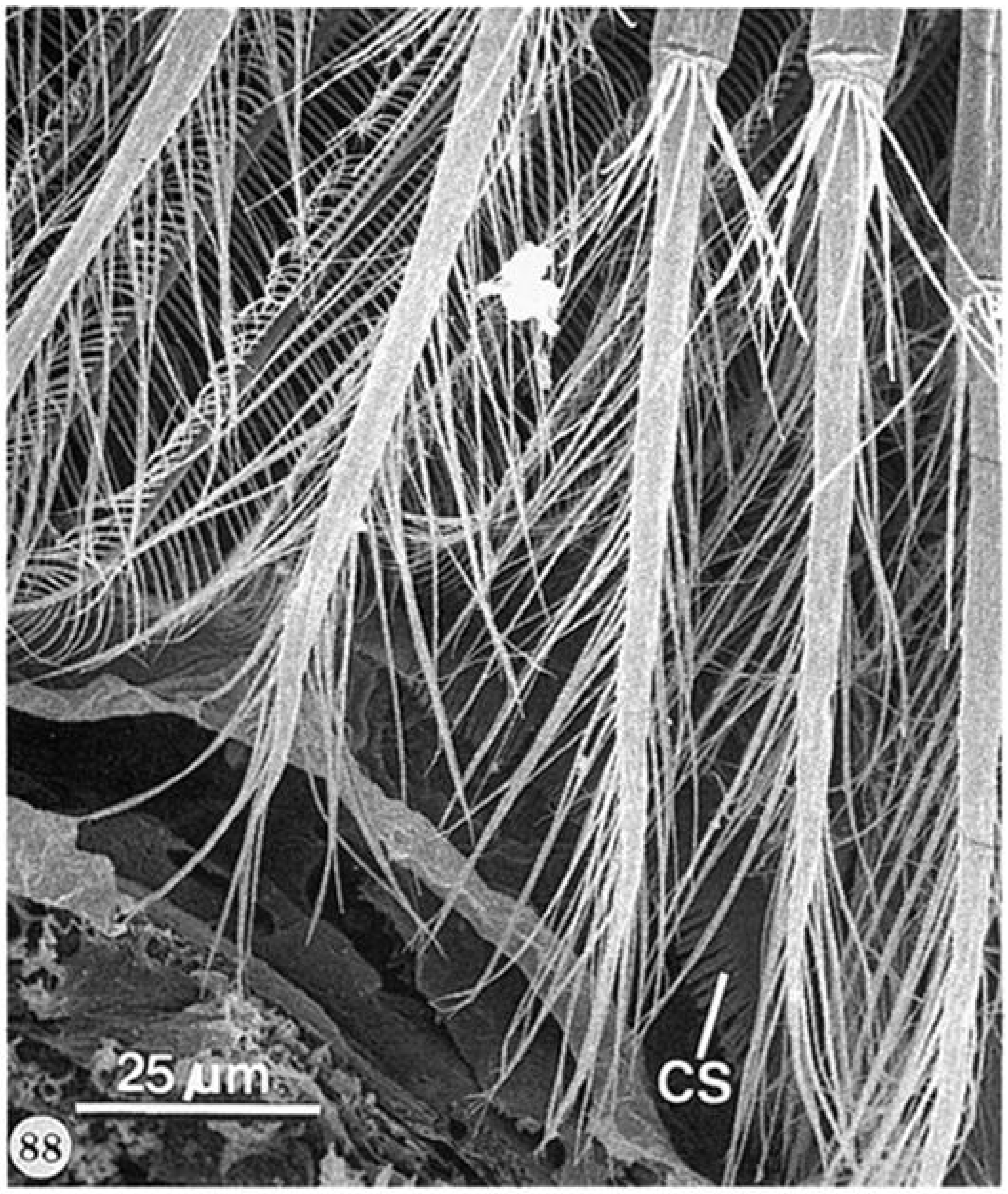




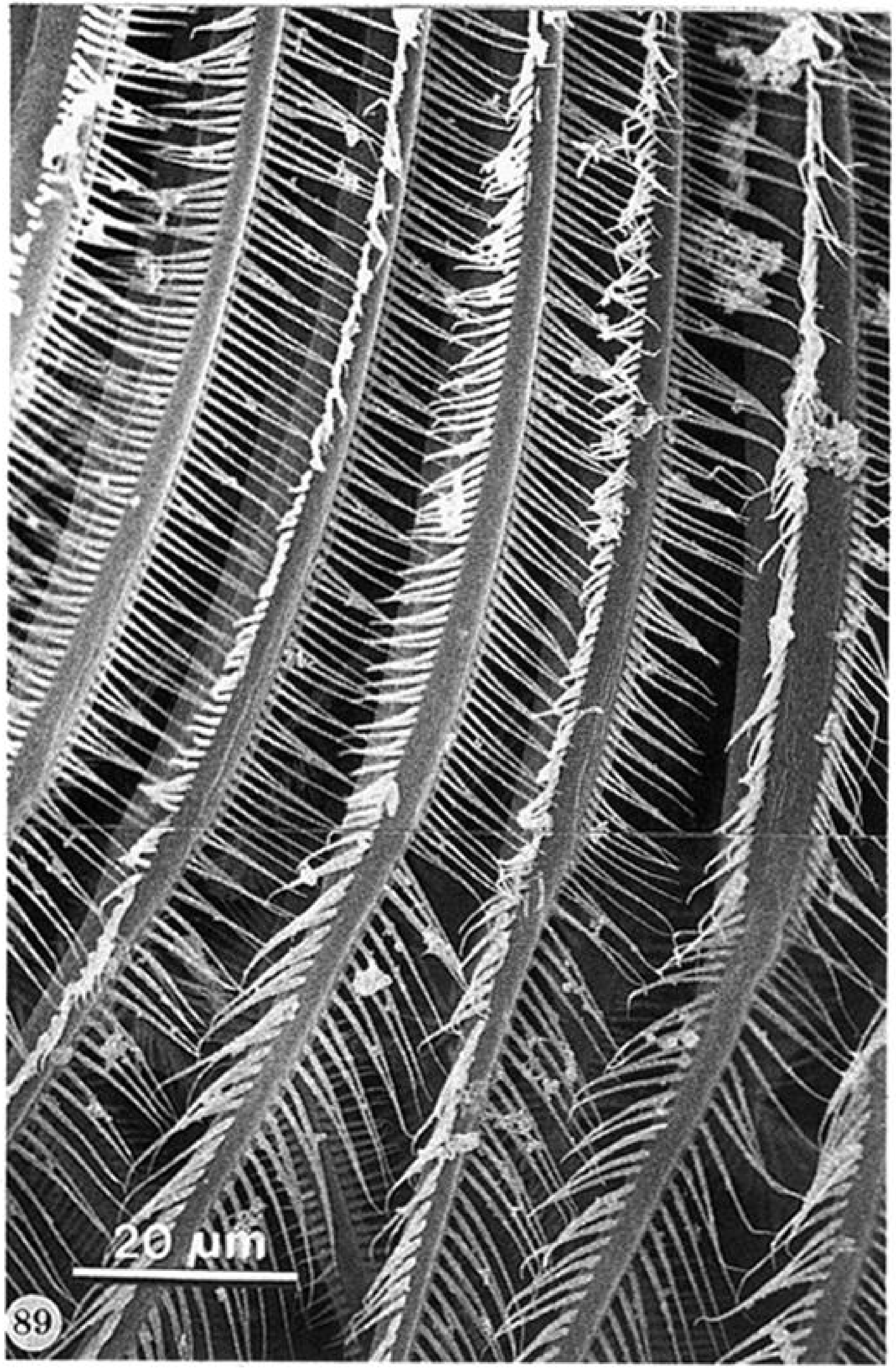
Figures 80–85. *Daphnia atkinsoni* f. *bolivari*. Details of the filter setae of trunk limbs 3 and 4 and of the gnathobasic armature trunk limb 2 as seen in transverse section (horizontal section of the animal) and revealed by Nomarski optics. Ventral. Figure 80. Gnathobasic spines of trunk limb 2 (GS2) and anterior filter setae of the filter plate (FP3) of trunk limb 3. Parts of the guide setae of trunk limb 1 (GDS) are also seen. Figures 81–84. The same, progressively more posteriorly and at different levels towards the distal ends of the filter setae. The filter plates of trunk limb 4 appear in figure 82. Note in figure 83, and particularly 84, which are, respectively near the extreme tips and very close to the tips, of the filter setae of trunk limb 3, the brush-like setules (BS) to the *outside* of the filter setae of trunk limb 3 that help to clean material from the filters of trunk limb 4. In figure 83, the distal extremities of some of the filter setae of trunk limb 3 have been displaced during fixation by the long, posteriorly directed cleaning spines of the gnathobases of the second trunk limbs, but the arrangement is otherwise perfectly clear. Figure 85. As figure 84, but more highly magnified (oil immersion objective) to show the brush-like setules of the distal extremities of trunk limb 3.







Figures 86–88. *Daphnia atkinsoni* f. *bolivari*. Figure 86. Guide setae of trunk limb 1. Figure 87. Gnathobasic spines of the median series of trunk limb 2 *in situ*. Right hand side of photograph is anterior. Note the brush-like nature of these spines and the way that their setules are directed laterally so as to be able to sweep material from the more laterally located (underlying) filter setae of trunk limb 3, which are clearly seen behind them. The median spine, here appearing white, serves as a marker to identify the sequence of gnathobasic sweeping spines. Note the umbrella stay-like array of spinules at the base of the armed distal region of the sweeping spines (cf. figures 3 and 111). Figure 88. The same, more highly magnified. The brush-like tips of the gnathobasic spines are clearly shown, as are the filter setae of trunk limb 3. Note the row of cleaning setules (CS) on the food groove wall that can just be seen near the tip of the sweeping spines.



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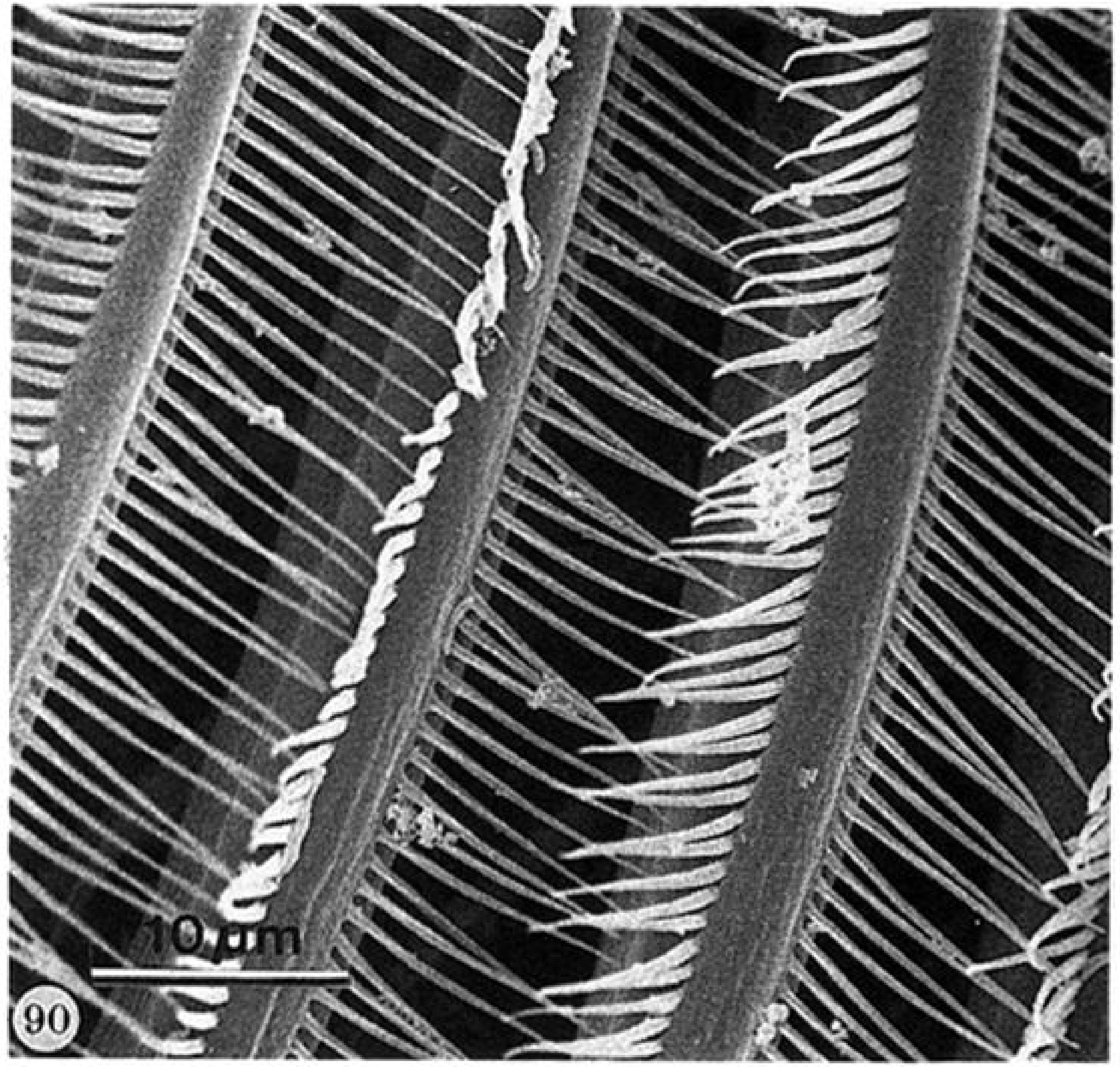

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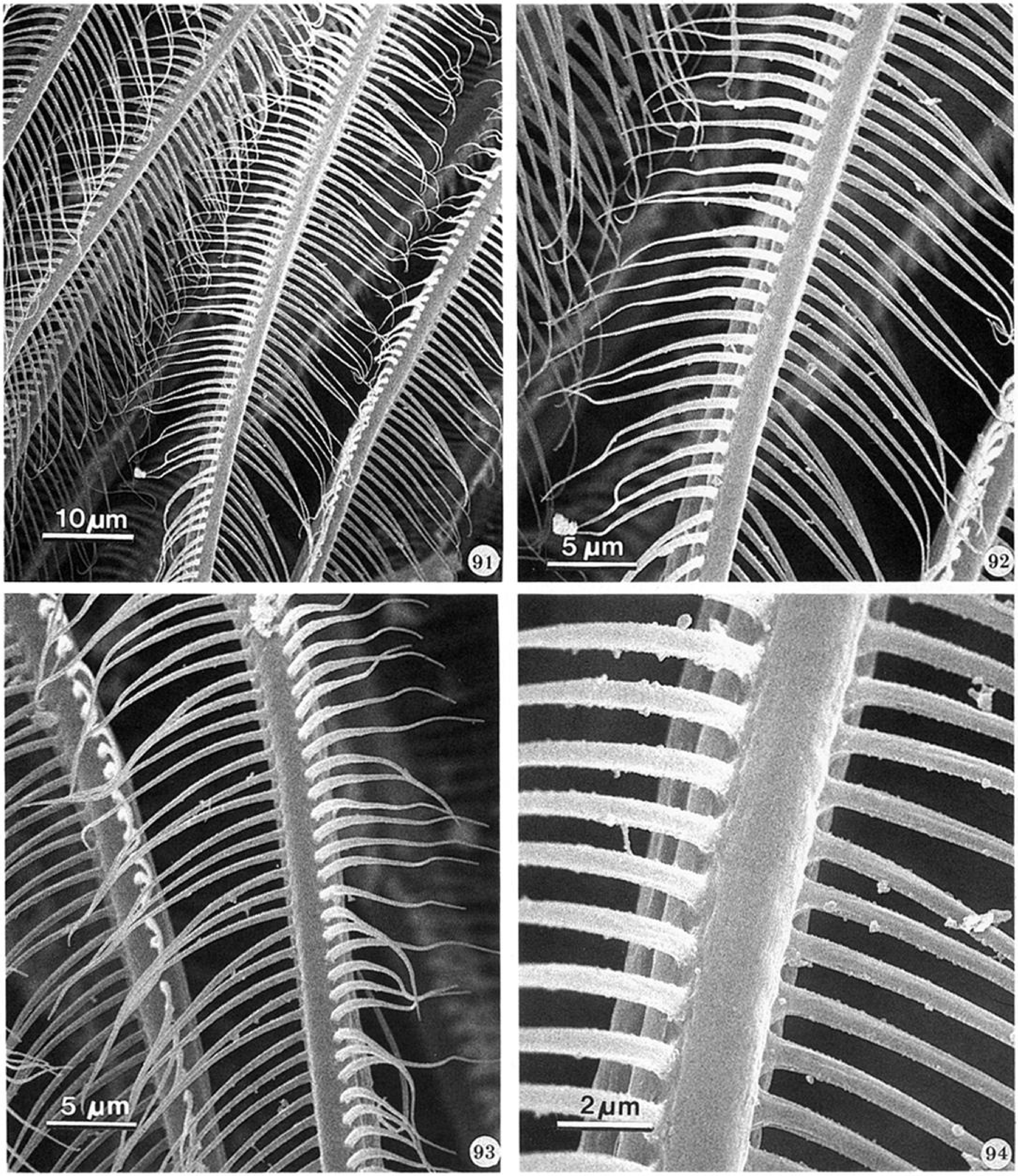
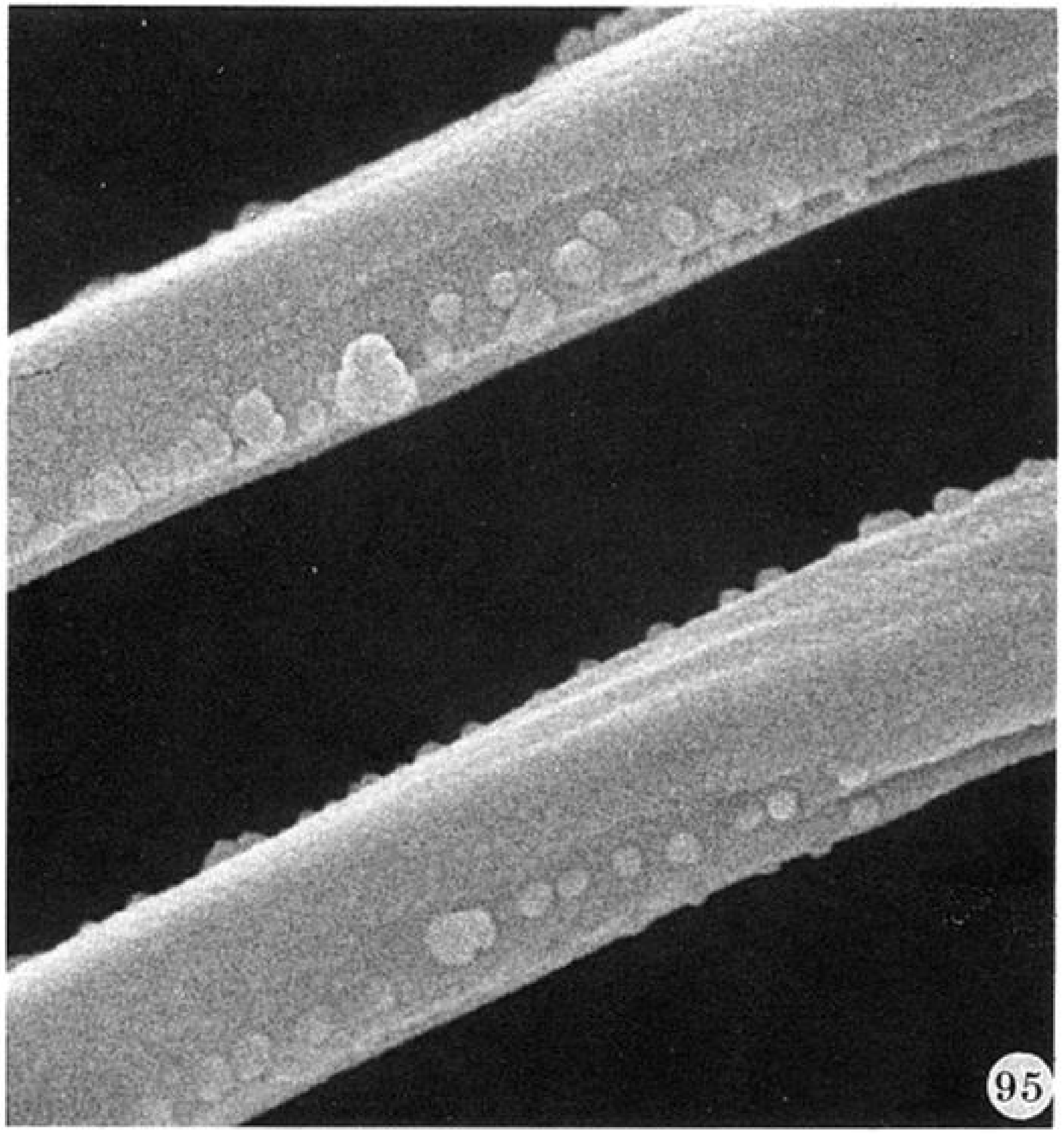
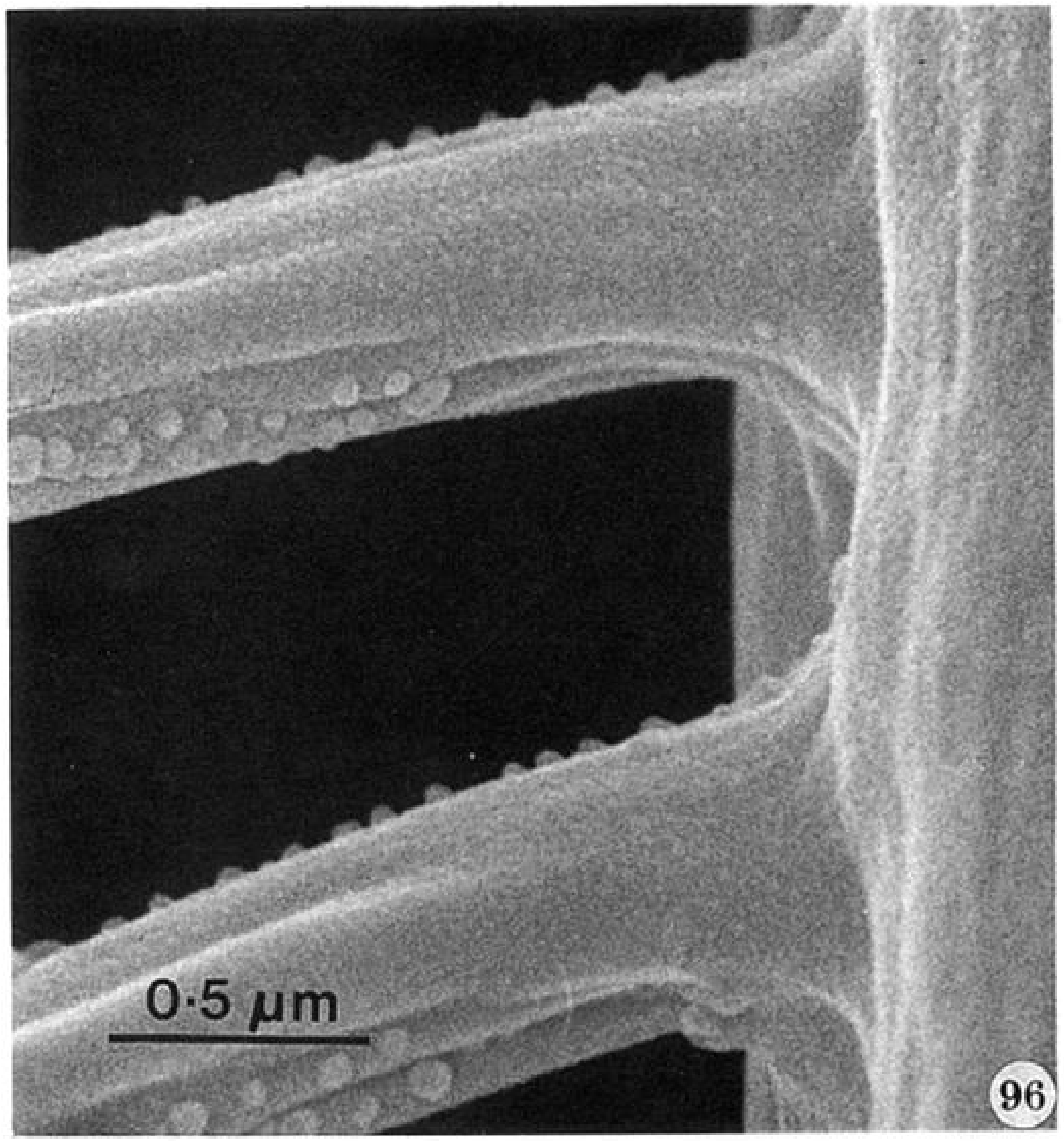
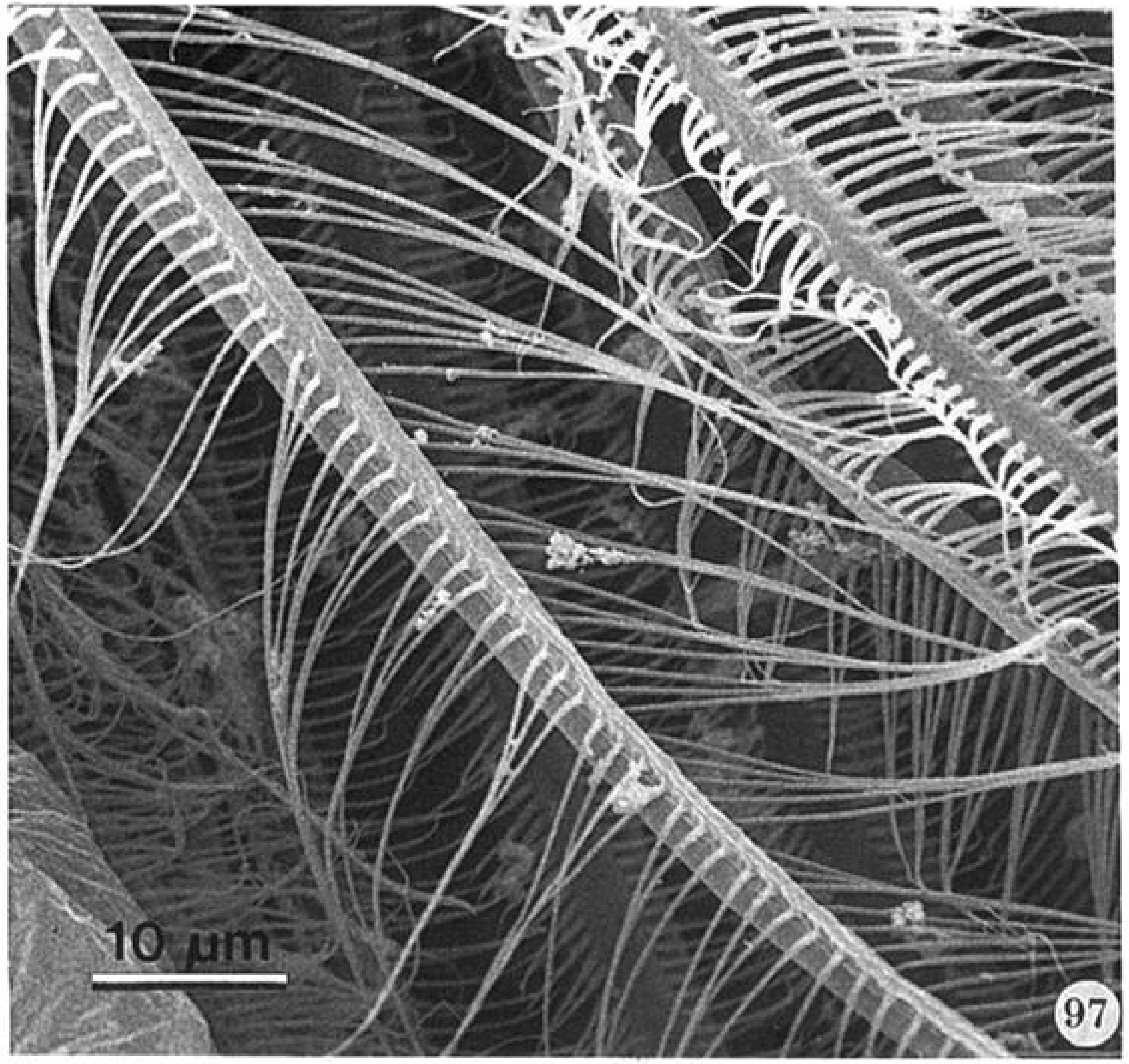
Figures 89 and 90. *Daphnia atkinsoni* f. *bolivari*. Figure 89. Part of the filter plate of trunk limb 3. Note how the armature of the filter setae changes towards their distal ends. The setules become more robust, larger, more widely spaced, more curved, and independent of their fellows on adjacent setae. A filter seta of trunk limb 4 can just be seen beneath the filter plate in the lower part of the photograph. Figure 90. Details of the filter setae of trunk limb 3, proximal region.



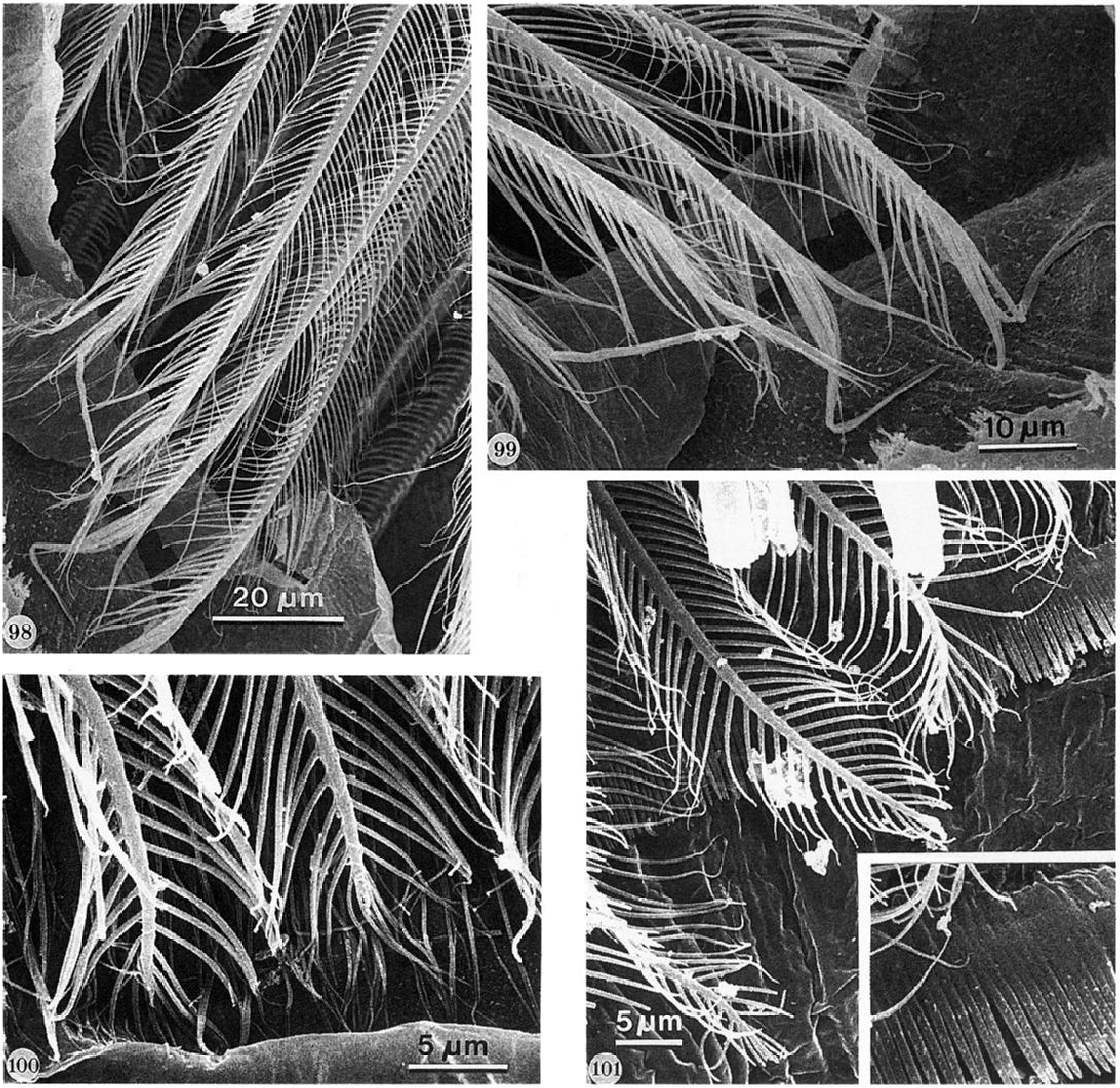
Figures 91–94. *Daphnia atkinsoni* f. *bolivari*. Figure 91. Setae of the filter plate of trunk limb 3, approaching their distal ends. Filter setae of trunk limb 4 can just be seen behind them. Figure 92. Details of a single seta. Figure 93. A single seta. Although the setules have been dislodged and somewhat distorted in fixation, the way they interlock with those of the adjoining seta can be appreciated. Figure 94. Details of a seta at high magnification, showing how the setules insert on it.



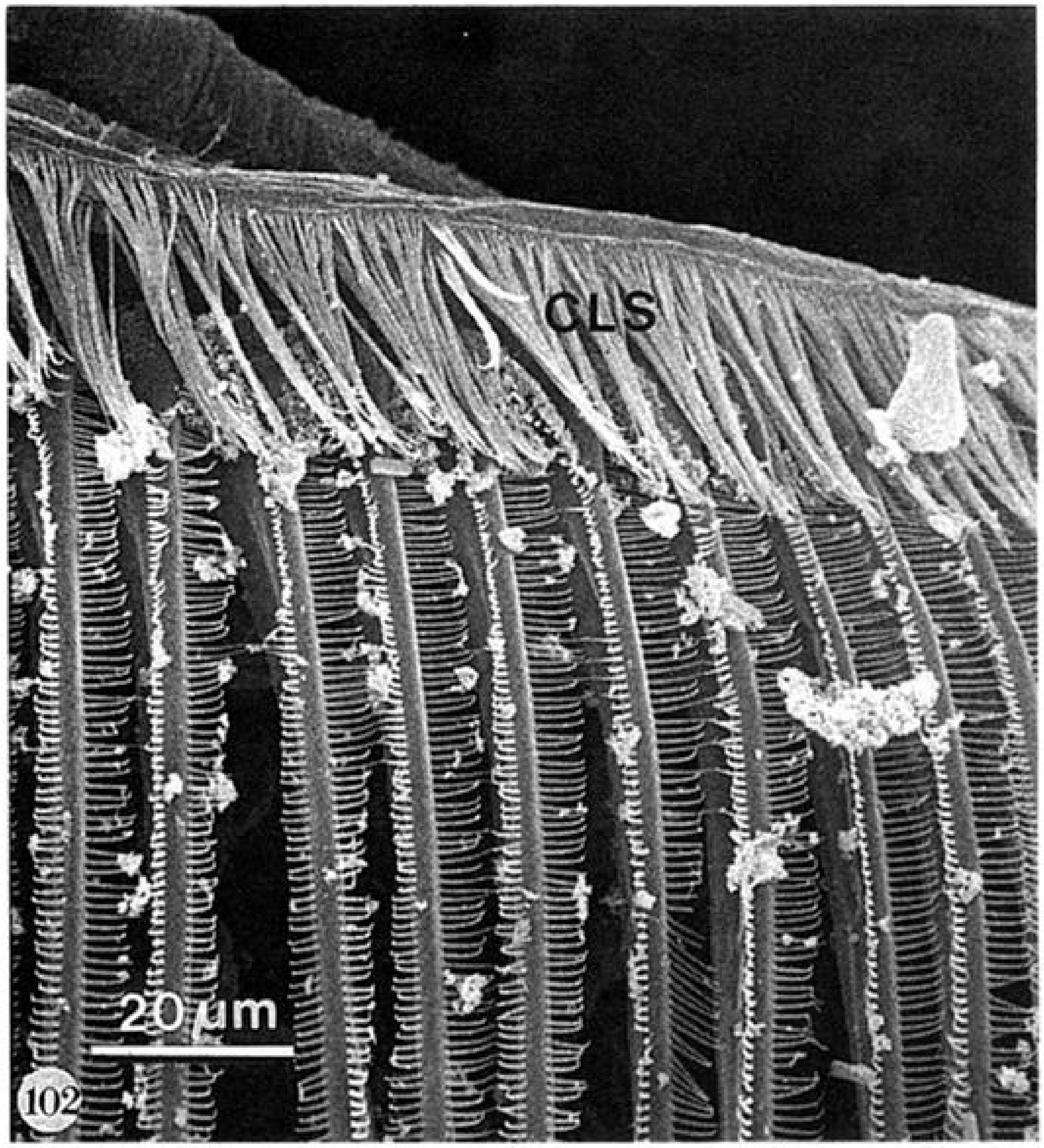


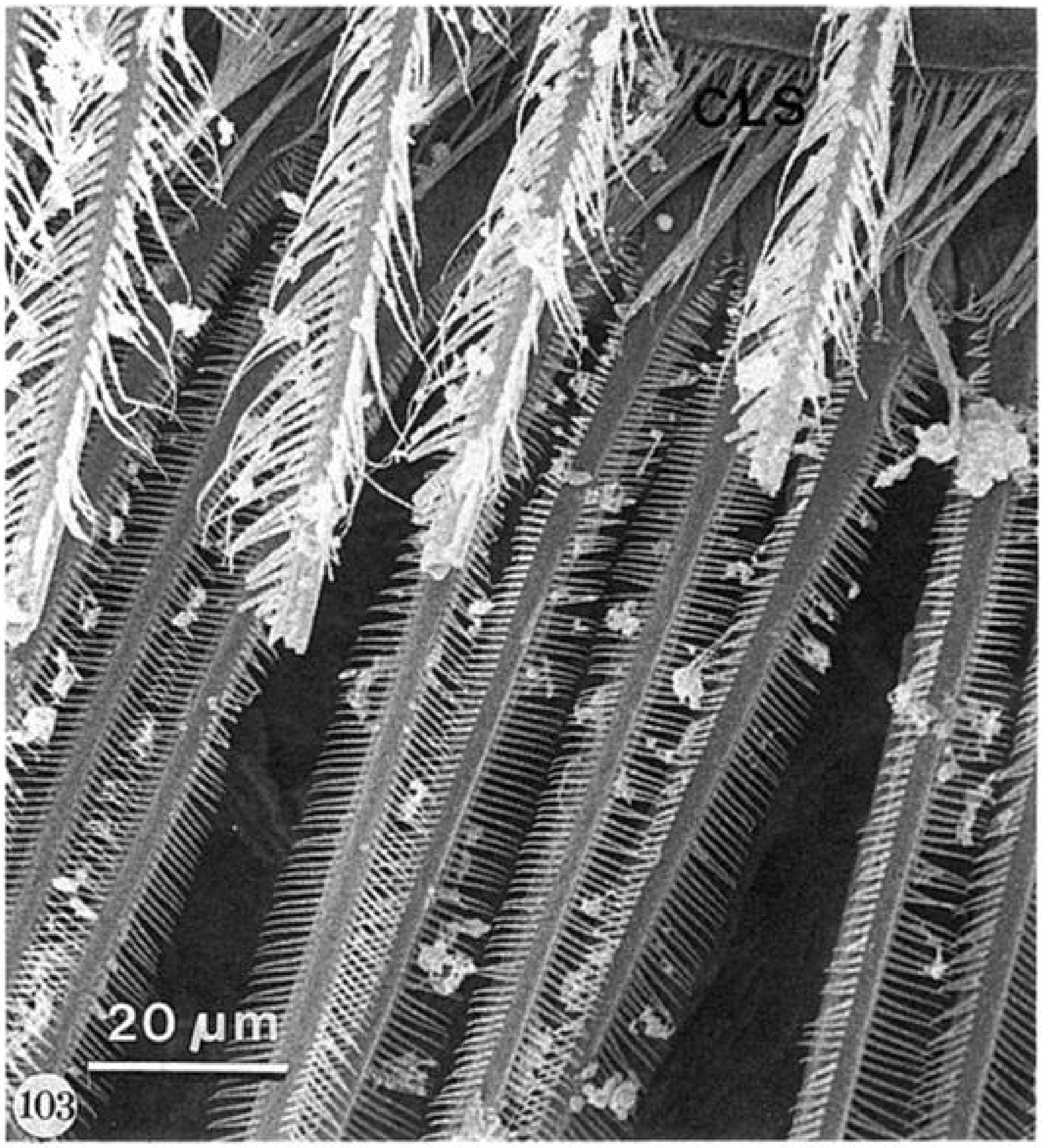


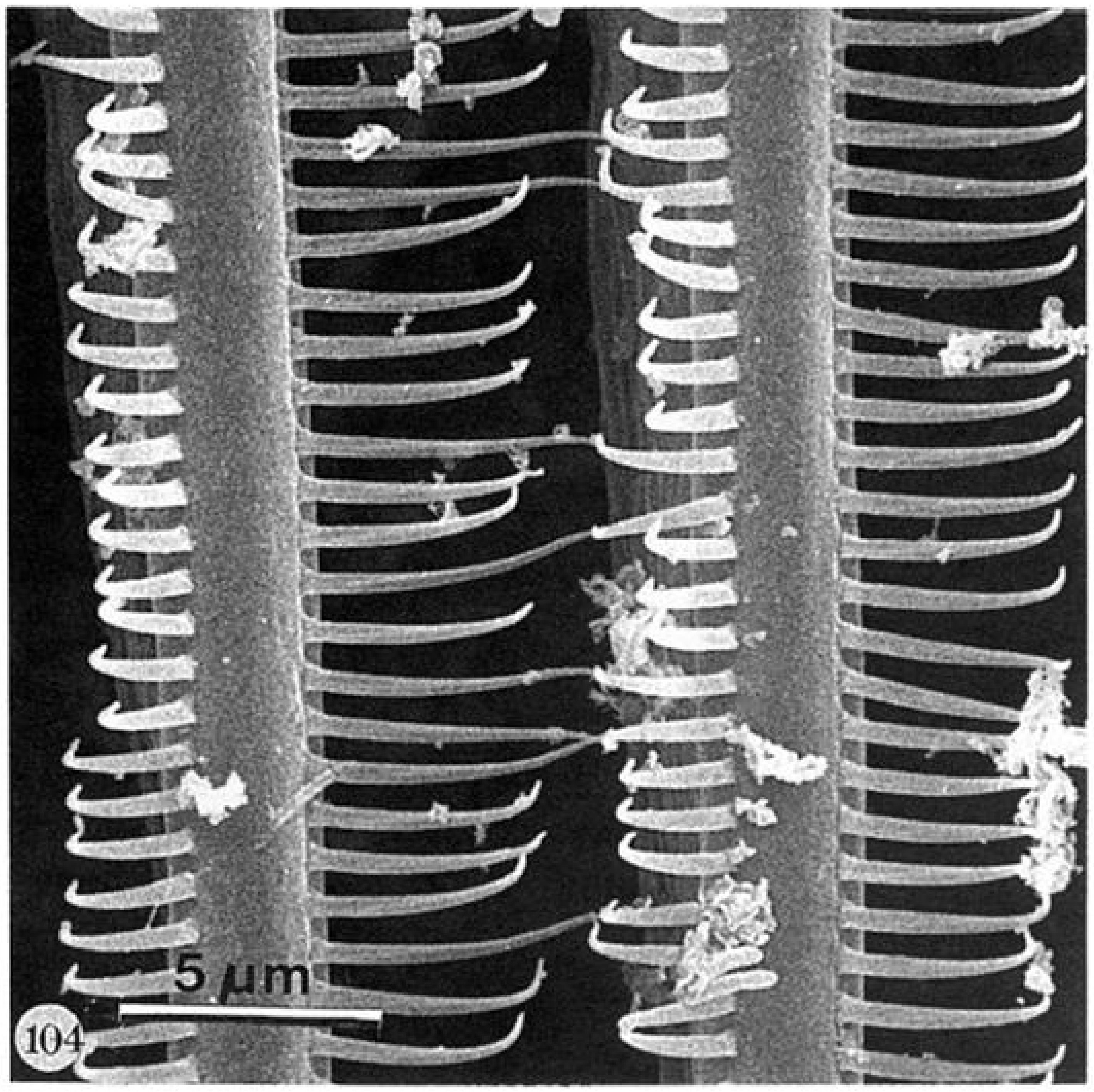
Figures 95–97. *Daphnia atkinsoni* f. *bolivari*. Figure 95. Trunk limb 3. Details of the filtering setules as seen at very high magnification. The 'pustules' may be artifacts. Figure 96. The same, showing the insertion of the setules on the seta. Figure 97. Posteriormost long seta of the filter plate of trunk limb 3. This is not a filtering seta. Note how its setules are much longer and more widely spaced than those of the adjacent filter setae. Filter setae of trunk limb 4 can be seen beneath it.



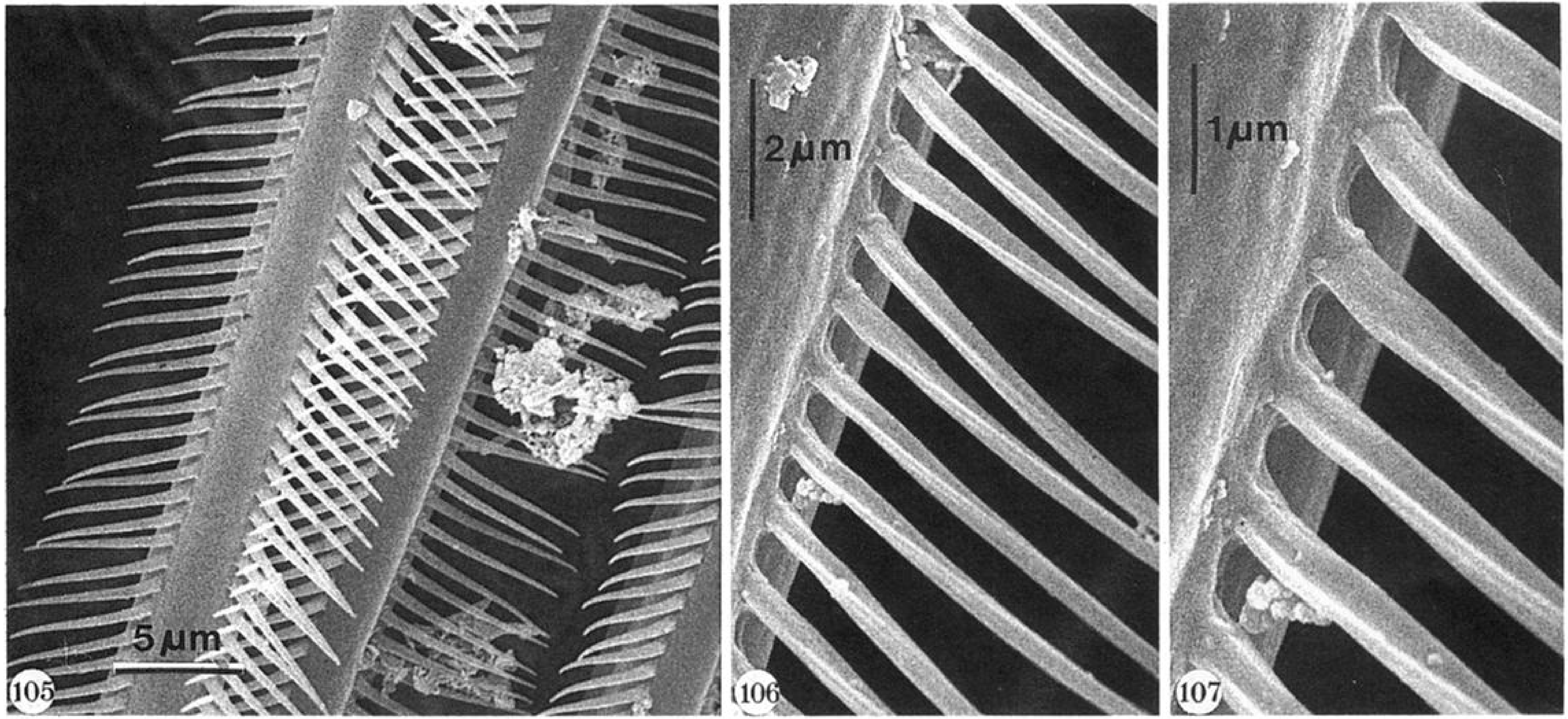
Figures 98–101. *Daphnia atkinsoni* f. *bolivari*. Figure 98. Distal portion of some of the more posterior filter setae of the filter plate of trunk limb 3, showing the brush-like nature of their tips. Filter setae of trunk limb 4 lie beneath them. Figure 99. Details of the brush-like tips of posterior filter setae, trunk limb 3. Figure 100. Distal portion of anterior filter setae of trunk limb 3, showing their modified tips. Figure 101. Tips of filter setae of trunk limb 3 that lie anteriorly, but posterior to those of figure 100. Note the row of stout spinules on the food groove wall, shown at higher magnification in the inset.



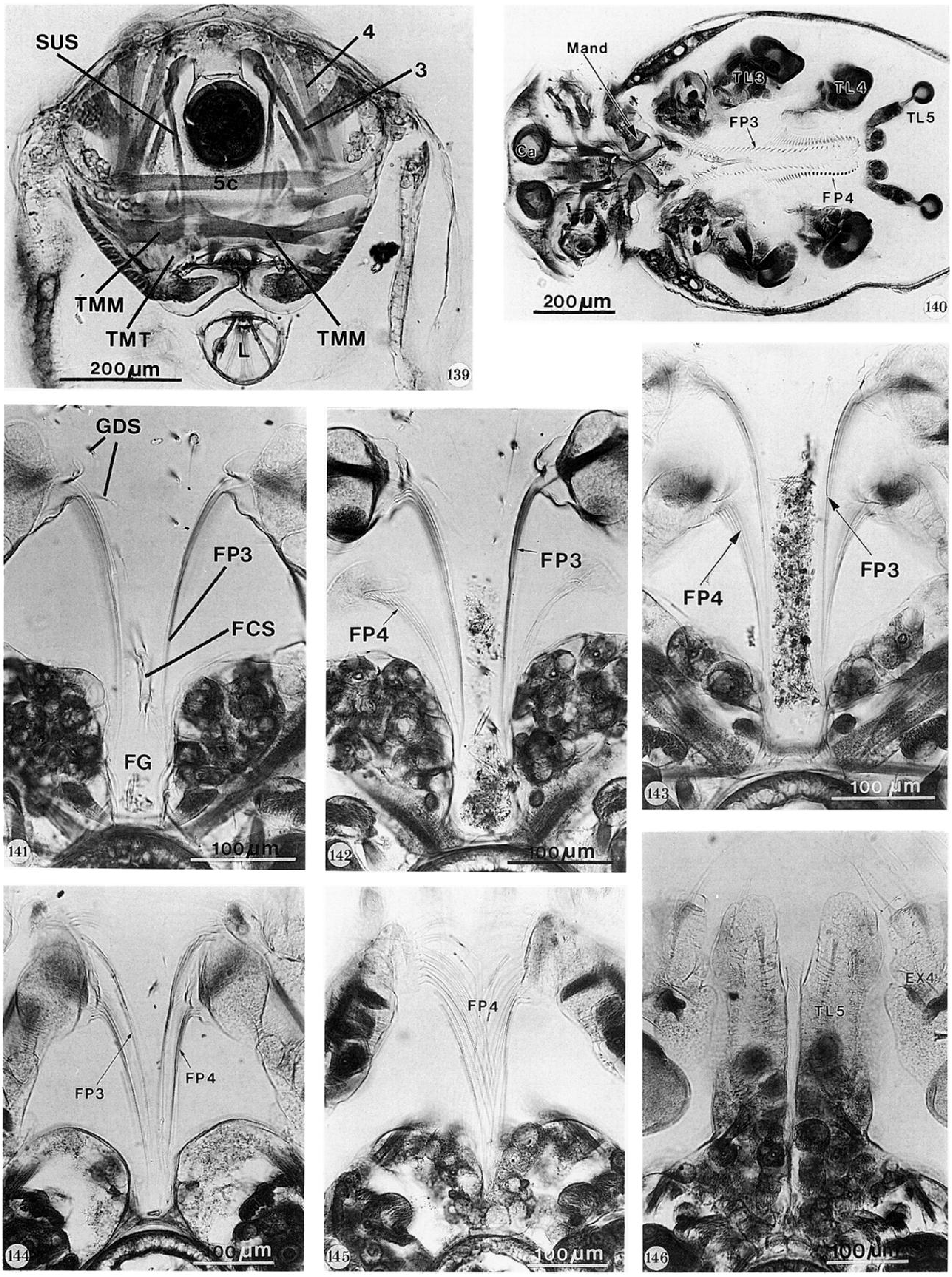




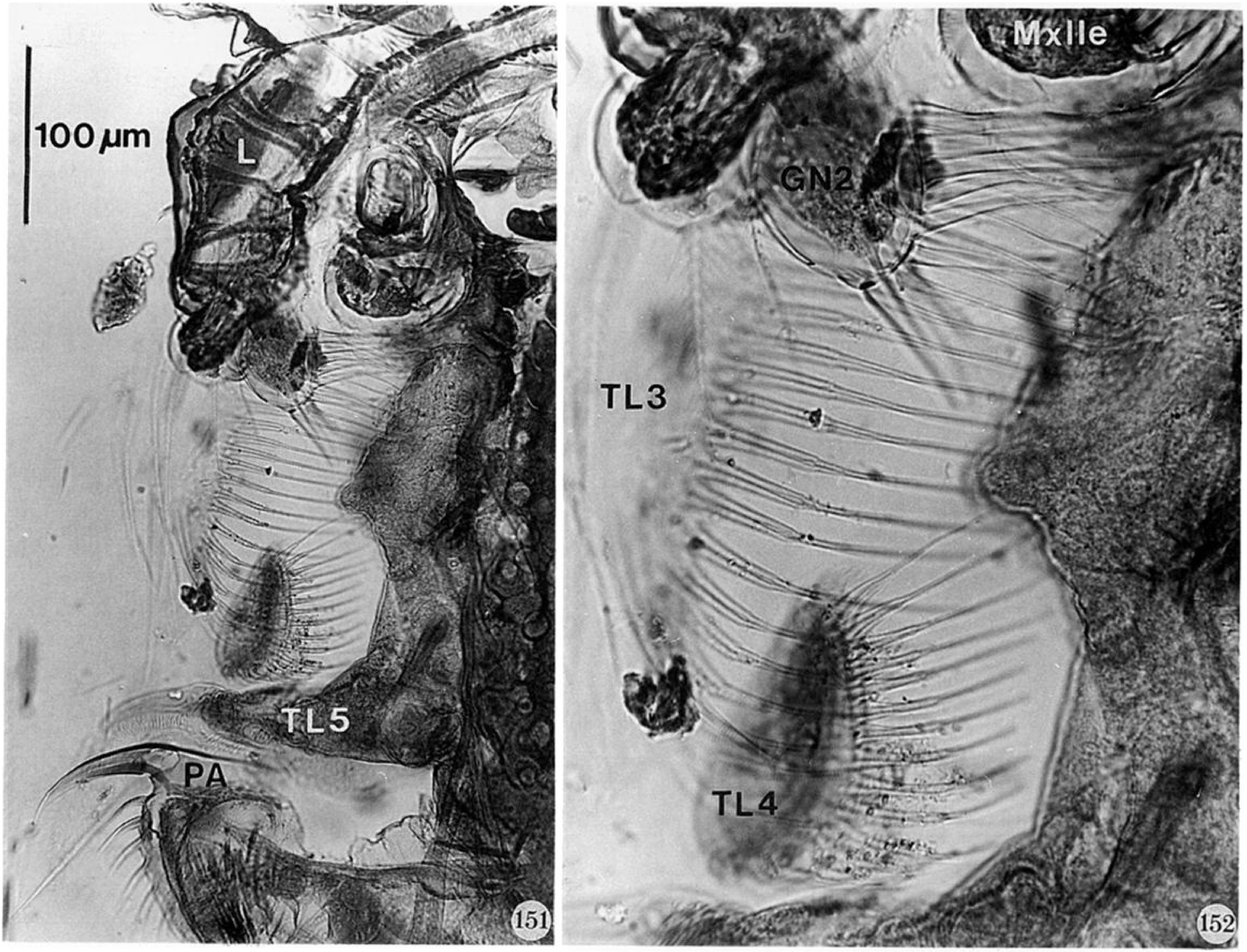
Figures 102–104. *Daphnia atkinsoni* f. *bolivari*. Figure 102. Part of the filter plate of trunk limb 4. Note the difference between the setae of this limb and limb 3. The fringe of cleaning setules (CLS) that cleans the filter of trunk limb 3 is clearly displayed. Figure 103. Part of the filter plate of trunk limb 4, with some of the overlying filter setae of trunk limb 3, whose distal, brush-like tips have been cut off to reveal the underlying filter. Note the fringe of cleaning setules (CLS) and the changing arrangement of the filter setules near the insertion of the setae. Figure 104. Details of filter setae of trunk limb 4.



Figures 105–107. *Daphnia atkinsoni* f. *bolivari*. Figure 105. Filter setae of trunk limb 4. Although two adjacent setae have been pulled together during fixation, their setule-by-setule correspondence is well seen and the intermeshing of their tips in life is easy to visualize. Figure 106. Details of the insertion of the setules on a filter seta of trunk limb 4. Figure 107. The same, more highly magnified.



Figures 139–146. *Simocephalus vetulus*. Figure 139. Transverse slice through the posterior region of the mandibles, seen from in front, revealing the suspensors (SUS) of the transverse mandibular tendon (TMT) and most of the major muscles. Note the striking asymmetry of the transverse muscles (TMM) that originate on the transverse mandibular tendon. The muscles of the left mandible (right-hand side of figure) are much longer than their homologues of the right mandible. The relation of the labrum (L) to the mandibles is well shown. Figure 140. Horizontal slice at about the level of the masticatory region of the mandibles to show the topographic relations of the corms of the trunk limbs and the size and location of the filter chamber. Figure 141. Transverse section, ventral surface uppermost, through trunk limb 3, anterior to the level at which the filter plate of trunk limb 4 is also cut, showing the shape of the filter plates of trunk limb 3 (FP3) and their relations to each other and to the food groove. The section cuts through the long posteriorly-directed filter-cleaning spines (FCS) of trunk limb 2. Note also the guide setae of trunk limb 1 (GDS) and the particles of food in the depth of the food groove (FG) that have been swept there by the long gnathobasic filter-cleaning spines of trunk limb 2. Figure 142. The same, more posteriorly, cutting at the level at which the first trace of trunk limb 4 and its filter plate (FP4) is appearing on each side. Note the mass of filtered particles that has been collected in the filter chamber. Much of this material, which lies beyond the reach of the filter-cleaning spines of trunk limb 2, is still en route to the food groove. Figure 143. The same, more posteriorly. More of the filter plates of the fourth trunk limbs (FP4) are now visible. Note how these lie lateral to those of trunk limb 3 (FP3). This section shows particularly well a mass of food particles collected in the filter chamber, whose narrowness towards the end of the remotion phase of the cycle is made apparent. Compare the distribution of these particles with those in figure 142, at which level they have been pushed deep into the food groove. Figure 144. The same, more posteriorly, approaching the posterior limits of the food groove. The filter setae of the third trunk limbs are some of the most posterior elements of these filter plates. Figure 145. The same, more posteriorly, showing how the posteriormost filter setae of trunk limb 4 fence off the posterior end of the filter chamber (cf. figure 140). No filter setae of trunk limb 3 lie so far back. Note how the food groove is here narrowing and becoming shallow as it peters out. Figure 146. The same, more posteriorly, showing the 'gates' of the fifth trunk limbs (TL5) that lie immediately posterior to the fence of setae of the fourth trunk limbs seen in figure 145. Part of the exopodite of trunk limb 4 (EX4), which helps to seal interlimb space 4/5, can be seen on each side.



Figures 151 and 152. *Scapholeberis mucronata*. The filter chamber and adjacent structures. Figure 151. Median longitudinal section through the filter chamber. For ease of comparison, the orientation is as for *Daphnia* in figure 3, though the animal swims ventral surface uppermost, both in open water and when suspended from the surface film. Note the large wall provided posteriorly by trunk limb 5 (TL5). Figure 152. The same, showing more details. Note the small number of elements in the armature of the gnathobase of trunk limb 2 (GN2) and how their anterior members tend to lie close together. The long filter-cleaning spine has been cut short. Note also the small number of filter setae in the filter plates of trunk limbs 3 (TL3) and 4 (TL4).

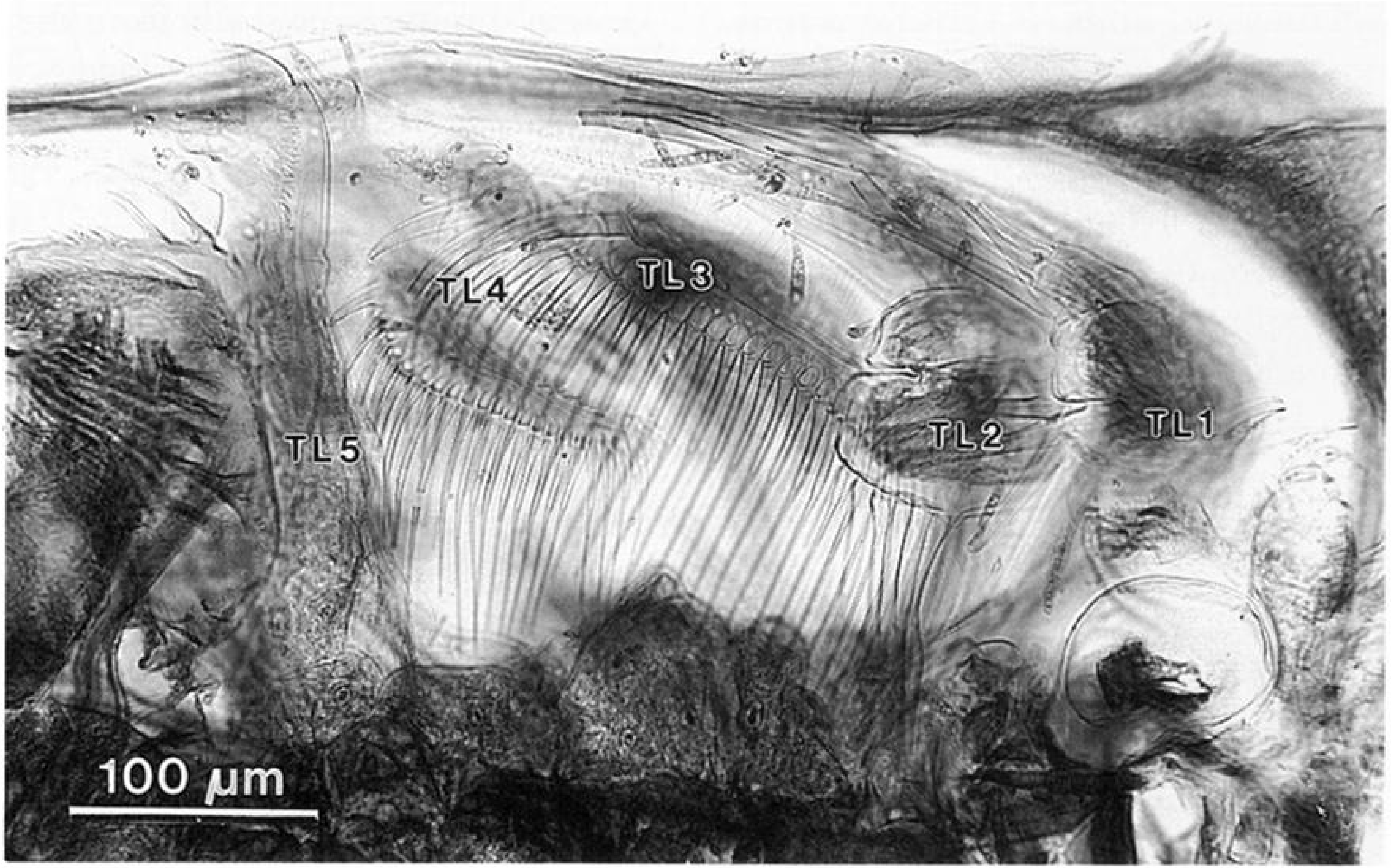
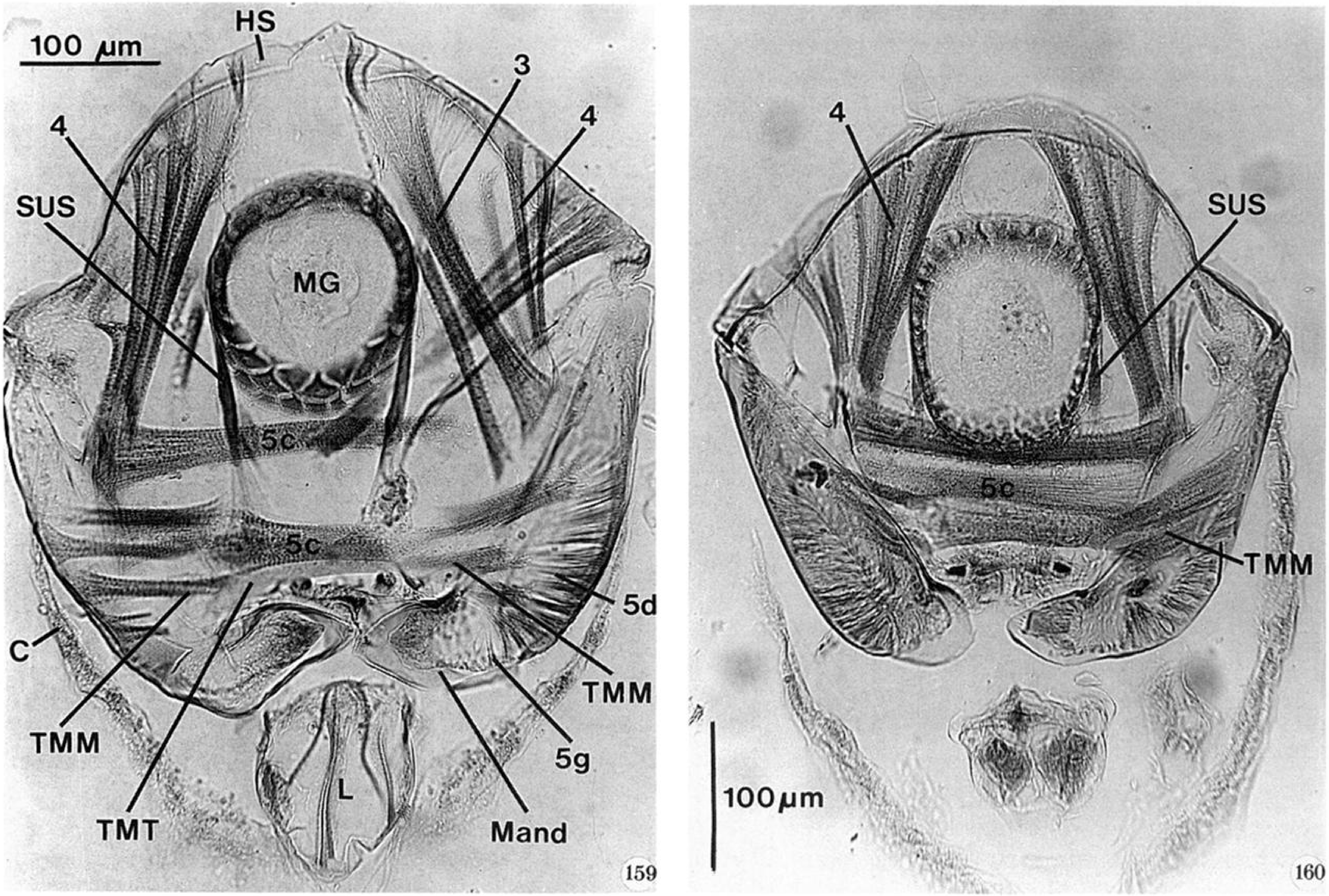


Figure 153. *Megafenestra aurita*. Median longitudinal section through the filter chamber. Note the more numerous filter setae in the filter plates of trunk limbs 3 (TL3) and 4 (TL4) than in *Scapholeberis mucronata* (figures 151 and 152) and the similarity of the gnathobasic armature of trunk limb 2 in the two species.



Figures 159 and 160. *Moina micrura*. Details of the mandibles. Figure 159. A transverse slice, anterior aspect, sufficiently thick to show elements of both the anterior and posterior muscles (but not the entire musculature). Anterior structures are seen particularly on the right, posterior on the left. On the right the promotor roller muscles (3) can be seen inserting on the anterior margin of the mandible; on the left, the remotor roller muscles (4) are seen inserting on the posterior margin. The transverse mandibular tendon (TMT) and its suspensors (SUS) lie anterior to those portions of the major transverse mandibular muscles (TMM) and such parts of the 5c muscles as are seen. Some of the smaller transverse muscles (5d, 5g) that originate on the margin of the tendon are seen on the right. Figure 160. Transverse section, anterior aspect, through the posterior portion of the mandibles. (This is a different individual from that seen in figure 159.) The full array of the massive remotor roller muscles (4) and the 5c muscles are seen. Traces of the suspensors (SUS) of the transverse mandibular tendon are present, as are some of the major transverse muscles (TMM) that originate on the tendon. These give some indication of their asymmetry.