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The Gills of the Coelacanth, Latimeria chalumnae, a Study in Relation to Body Size

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CONTENTS

SUMMARY

Measurements of the surface area of the gill lamellae of specimens of the coelacanth, *Latimeria chalumnae* have been made. This involved measurement of total filament length, frequency of lamellae along the filaments and the weighted average bilateral area of a single lamella. Regression analyses of these parameters which combine to give total area were made for the mass range 434 g–80 kg. Results show that the slope is close to 0.81 which is similar to that of many other fishes. However, the intercept value is exceptionally low and confirms the low mass-specific measurements made on two 10 kg specimens in 1972.

Material from a recently caught specimen has been used to extend previous transmission electron microscopy by the use of scanning electron microscopy. The surface of epithelial cells on lamellae and filaments is covered in microvilli and microridges with transitional zones. The appearance of microridges suggests that they may have arisen by coalescence of microvilli. Ventilatory movements of the mouth and operculum (three to four per minute) have been observed using videorecordings of resting specimens in caves. Specimens of the larval stage of a gnathiid isopod parasite were found but only on one of the nine sets of gills that were examined. It is concluded that this more extended study of gill morphometrics of *Latimeria* confirms predictions made from earlier comparative studies regarding the life habits of this fish which have also been confirmed by direct observation using submersibles.

1. INTRODUCTION

With the capture of the first and second living coelacanths in 1938 and 1953 much was learned of their gross anatomy; this was increased by extensive studies at the Paris Museum of Natural History, culminating in the publication of a third volume on the anatomy of *Latimeria chalumnae* (Millot *et al.* 1978). Further advances in other aspects of the animal's structure and function were given a significant boost by a 1972 expedition (which resulted in the publication of about 100 papers), with the widespread dissemination of material fixed for electronmicroscopy and other procedures which had been requested by scientists world-wide. Of these tissues, the blood and gills (because of their ready accessibility) were examined first. The electronmicrographs of the gills were of a high standard and many details of their ultrastructure were established.

Measurements of the gills of the two specimens captured during the 1972 expedition showed a very low surface area in comparison to data previously obtained on other fish, including the Comorean fish also studied during the expedition (Hughes 1980), and the castor oil fish, *Ruvettus pretiosus* (the species normally fished when *Latimeria* is caught). Using these results it was predicted that *Latimeria* would be relatively inactive, with a low O_2 consumption rate and low requirement for food. These predictions suggested that survival of this species after many millions of years of coelacanth evolution was related to adaptation to a

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Table 1. *Basic data concerning the specimens of* Latimeria *and other fish used in the present study*

(For further details, reference should be made to the summary list compiled by Bruton & Coutouvadis (1991).)

fish	CCC number	place	body mass kg	total body length cm	heart mass g	comments
juvenile 6	162.6	RUSI 37324	0.434	33.1		
juvenile 16	162.16	RUSI 37325	0.441	34.8		
juvenile 24	162.24	Seewiesen	0.46	33.4	0.54	poor gill preservation
embryo	29.4	Brit. Mus. Nat. Hist. Lond.	0.566	30.3		
C 79 (Robineau)	94	Mus. H. Nat., Paris	0.8	42.5		
Munich (Fricke)	160	Vet SM:28410	2.35	63	2.96	gill parasites present
Moroni 1971	76	Zool. Staatssamlung Münch.	10	85		
Iconi 1972	80		10	85		very good fixation for EM
	153	RUSI 37101	26	125.5	17	gills not well preserved
Salimani (Nov 1991)	—	Moroni	33	130	11.76	best gills for area; SEM satisfactory
Pik Botha	159	RUSI 37320	80	164		
Maputo	162	Mozambique	98	179		contained 26 juveniles
Ruvettus			5	103.5	10	
Ruvettus			11.3	128	15	
Ruvettus		Moroni Market	15			
Argyrozona		Port Alfred	0.277	23		
Argyrozona		Port Alfred	0.676	32		
Argyrozona		Port Alfred	2.054	43.5		

special environment, a theory further emphasized by the transmission electron microscopy (TEM) studies which showed a thick tissue barrier between water and blood. Hughes (1972*a–c*) pointed out that the low oxygen diffusing capacity of the gills would be disadvantageous to the coelacanth, making it unable to cope with the increased O_2 requirement associated with stress of capture and the lower O_2 content of the higher temperature surface waters. This problem would be exacerbated by effects on the oxygen dissociation curve of the blood (Hughes & Itazawa 1972; Hughes 1976).

In addition to the 1972 specimens it was possible for me to study gill arches from two additional, smaller specimens (one of these specimens was obtained following the dissection of *Latimeria* in 1975 which revealed the presence of five juveniles in the oviduct (Smith *et al.* 1975). The second gill arch was chosen because it has been shown to provide a good representative sample in most other fishes (Hughes 1972*b*; Hughes & Ojha 1985); this has been confirmed for *Latimeria*. The conditions of fixation were not good enough to allow measurements on the secondary lamellae of this specimen, however, and measurements were restricted to the length of the filaments. Comparison of the results with those of the 10 kg specimens suggested a relatively low rate of growth in filament length and that the number of filaments changed very little.

Pauly (1981) suggested that a low slope indicates a low rate of increase in oxygen availability with body size and consequently a lower growth rate, whereas in fishes where the slope of the regression line is closer to 1.0, oxygen availability and growth rate remained more or less constant at all body sizes. It was hoped that by studying further specimens over a wider range of body size, the low values obtained previously would be confirmed and that data would be provided that would make it possible to estimate the slope of the gill area/body mass regression for comparison with other fish.

2. MATERIALS AND METHODS

The nature and sources of material used in this study are summarized in table 1. The gills of two deep-frozen specimens (26 and 80 kg) at the JLB Smith Institute, Grahamstown were studied as well as two recently caught specimens from Moroni, Grand Comore (33 kg), and a very large specimen (98 kg) caught off the Mozambique coast (Bruton *et al.* 1992). The latter contained 26 juveniles and the gills of three of these specimens have been examined. The condition of this material was variable, the best being the specimen at Moroni as the fish had been stored in a deep freeze and the gills dissected about six hours after capture. After fixation in seawater Bouin's fluid the lamellae were well preserved and it was possible to take measurements. Small pieces were also fixed in the same glutaraldehyde mixture as used in 1972 and, although not good enough for TEM, they gave good preparations for scanning electron microscopy (SEM). Deep freezing followed by thawing disrupts the lamellae and subsequent fixation in formalin or Bouin although satisfactory at a gross level, does not always preserve the lamellae sufficiently well for area measurement. For this reason, the 80 kg specimen produced much better preparations than the 26 kg specimen and juvenile number 16 was better than numbers 6 or 24: the juvenile specimens had been frozen after their mother

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

had been trawled but again thawing had variable effects on the very small lamellae and made accurate measurements quite difficult. A 2.35 kg specimen was also made available and was of particular interest as it is one of the few small coelacanths captured. Unfortunately the poor condition of the lamellae resulting from many months in a deep freezer did not allow accurate measurement of individual lamellae. Nevertheless it was of general interest because of the presence of several small (2 mm length) specimens of a gill parasite; probably the larval stage of the gnathiid isopod *Praniza Milloti*, Monod, as recorded by Monod (1954) and Hargis (1958).

In comparison to the lamellae, the gill filaments are well preserved, support provided by well-developed interbranchial septa ensures that they are the least distorted part of the gill system. Furthermore, as with other fish, measurement of filaments is easier, and as a result data on filament length is the most reliable, particularly for material of such diverse histories. The filaments do shrink, however, and although this is compensated by corresponding increase in the frequency of lamellae on the filaments (and does not therefore affect the calculation of area), it should be remembered that the values given and plotted for filament length are 90–95 % of values representative of living fish.

The specimen of *Ruvettus pretiosus* (Gempylidae) was obtained from the Moroni market and was the only specimen found in which it was possible to obtain the mass of the intact fish with the gills preserved in good enough condition to allow accurate measurement. The specimens of Carpenter fish, *Argyrozona argyrozona* (Sparidae), were collected at Port Alfred (Eastern Cape, South Africa) from local fishermen and fixed in seawater Bouin within four hours of capture.

SEM was carried out with a Jeol JSM-5200 microscope at the EM laboratory of PABIO in the University of Wales, Cardiff. In addition to the study of this new material, the opportunity has been taken to re-examine sections of material from the 1972 specimen fixed for TEM in the light of observations with the SEM. The TEMS were taken using Phillips 200 microscopes at Berne and the MBA laboratory at Plymouth.

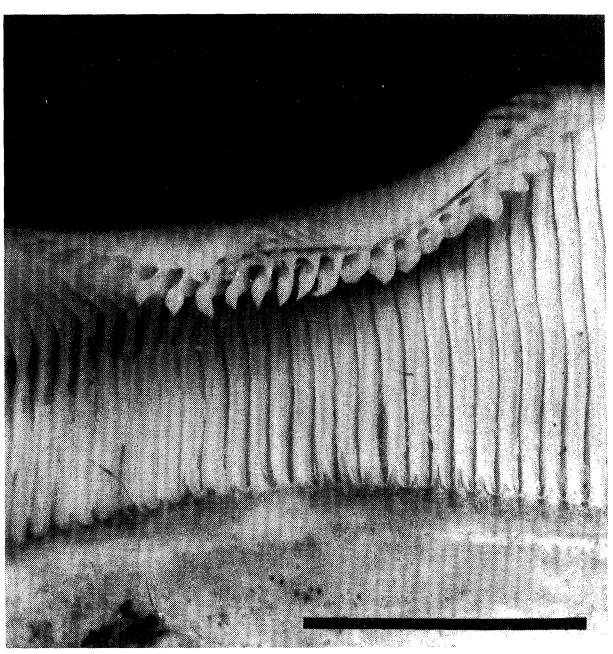
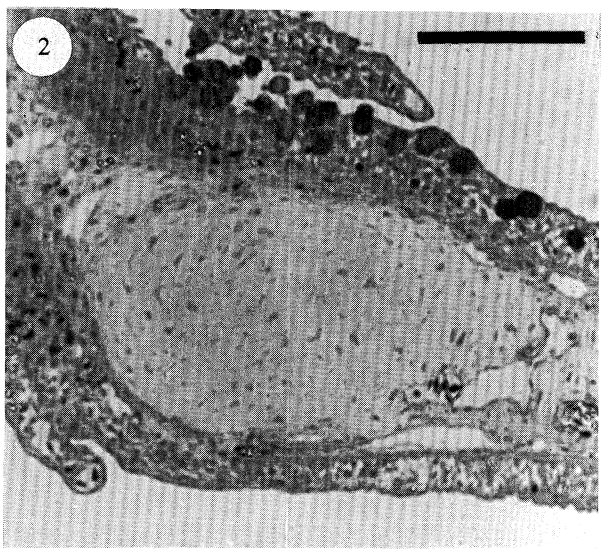


Figure 1. Photograph of the tips of gill filaments showing the septal channels. Bar = 1 cm.

3. RESULTS

(*a*) *Gill morphology*

The gill filaments of *Latimeria* are relatively short as is particularly apparent when comparing their length to the width of the branchial arches. The lamellae are on both sides of the filaments, again relatively short, especially near the tips where they only extend for about 50 % of the filament width. At the base of the



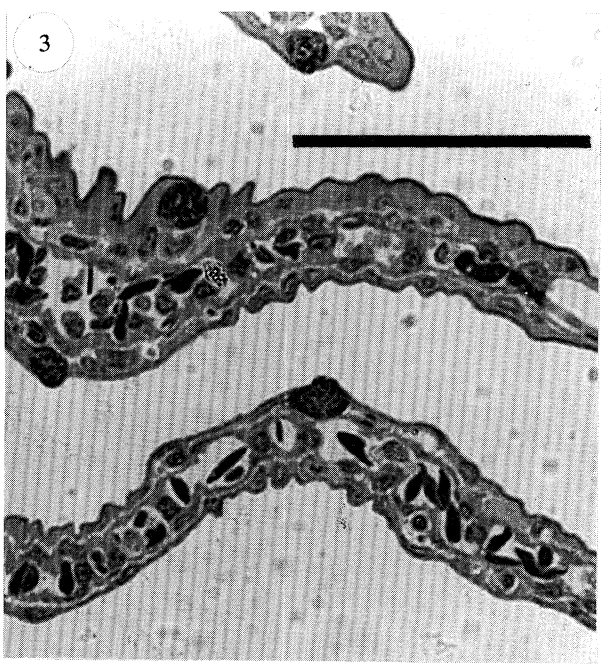


Figure 2. Light micrograph of a section through a gill arch showing the presence of numerous mucous cells in surface epithelia of the arch. Bar = 100 μm.

Figure 3. Light micrograph of lamellae to illustrate basic structure and occasional presence of mucous cells in the epithelium. Bar = 100 μm.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

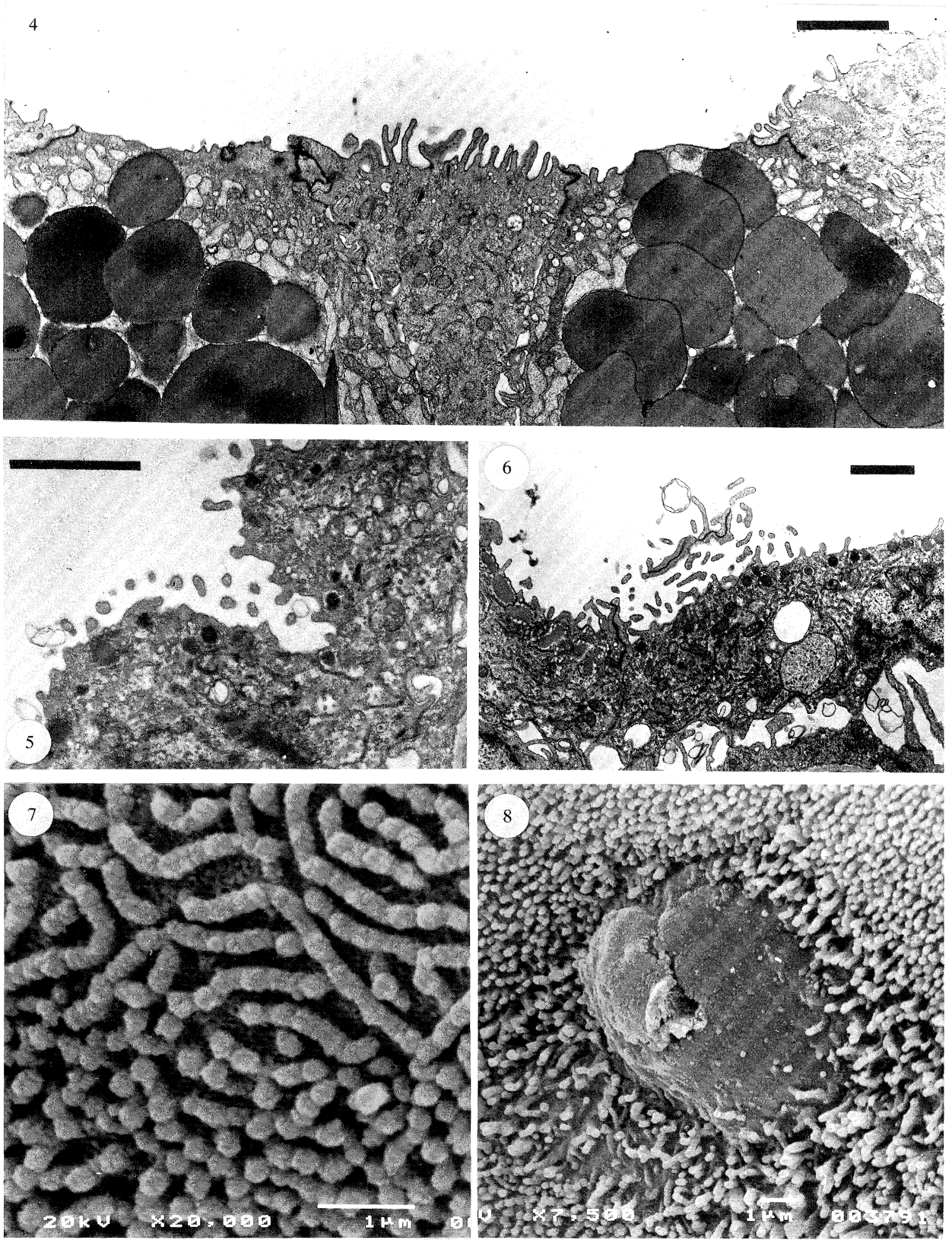


Figure 4. TEM of the surface epithelium near to two mucous cells in the filament region between two lamellae. The presence of elongated microvilli is clearly visible as in figure 8. Bar = 2 μm.

Figure 5. TEM showing surface of lamellar epithelium in which some of the microvilli have been sectioned transversely. Bar = 2 μm.

Figure 6. TEM of lamellar epithelium in which some suggestion of microridges can be observed. Bar = 2 μm.

Figure 7. SEM from different parts of a lamella. In some regions microvilli predominate but in others microridges are also present. The detailed structure of the surface of the microridges indicates that they might have arisen by coalescence of many microvilli. Bar = 1 μm.

Figure 8. SEM of a gill filament showing a mucous cell and some elongated microvilli of the surrounding epithelial cells. Bar = 1 μm.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 2. *Summary of gill measurements analysed in the present study*

(Some of the data has been previously published (Hughes 1972*b*, 1980).)

fish	CCC number	body mass (kg)	total filament length (cm)	total number filaments	filament length second arch (cm)	lamellae (one side) (mm^{-1})	bilateral area of lamella (mm^2)	total gill area (cm^2)	gill area per g body mass ($mm^2\ g^{-1}$)
juvenile 6	162.6	0.434	1440.8	3180	174.26				
juvenile 16	162.16	0.441	1550.71	3298	204.65	20.496	0.03839	249.75	56.63
juvenile 24	162.24	0.46	1607.94	3078	212.18	18.9	0.01989	120.76	26.25
embryo	29.4	0.566			253.05				
C 79 (Robineau)	94	0.8			286.6				
Munich (Fricke)	160	2.35	3273.5	3460	401.07				
Moroni 1971	76	10	4331.4	3270	657.4	12.74	0.159	1754.79	17.55
Iconi 1972	80	10	4403.14	3256	600.7	12.62	0.17	1889.3	18.89
	153	26	8015	3456	1056.4	10.932	0.1546	2708.45	10.42
Salimani (Nov 1991)	—	33	8179.6	3506	1053.6	11.389	0.3822	7120.96	21.58
Pik Botha	159	80	11827.9	3486	1538.9	9.756	0.602	13888.08	17.36
Maputo	162	98							
Ruvettus		5	5879.9	3260	788.5	16.85	0.3549	7032.44	140.6
Ruvettus		11.3	8541	3572	1176	15.98	0.4707	12848.7	113.7
Ruvettus		15	11061.23	3524	1165.7	14.336	0.4574	11061.23	73.74
Argyrozona		0.277	1215.02	2458	169.55				
Argyrozona		0.676	2171.48	2712	307.06				
Argyrozona		2.054	2770.48	2758	384.62				

Table 3. *Results of regression analyses for measurements on gills, heart mass and body length for* Latimeria *specimens of different body mass*

(a = intercept for a fish of 1 kg, b = slope of regression line, Sb = standard error of that slope, r^2 = correlation coefficient.)

	n	a	b	Sb	r^2
total gill					
filament number	9	3240.96	0.0169	0.00565	0.558
filament length/cm	9	2084.12	0.3852	0.01786	0.985
lamellar frequency per mm	7	17.57	−0.1359	0.00691	0.987
lamellar area/mm^2	7	0.0425	0.5635	0.07004	0.928
total area/mm^2	7	31085.7	0.8106	0.07411	0.960
area per g body mass/mm^2	7	31.11	−0.1897	0.0742	0.566
second arch only					
filament number	11	407.92	0.0139	0.00836	0.234
filament length/cm	11	282.14	0.3818	0.01426	0.988
heart mass/g	4	1.17	0.7521	0.104	0.963
total body length/cm	10	42.923	0.3133	0.0136	0.985

filaments this proportion is much greater. Because of the well-developed interbranchial septa, after passing the lamellae, water flows along the septal channel and this accounts for some of the filament surface which is unoccupied by lamellae. At their tips, the filaments are free with the septal channels clearly visible (see figure 1). As described previously, the lamellae have the same basic structure as that in most fishes but, although mainly present on the filaments (see figure 2), mucous cells are also found on some of the lamellae themselves (see figure 3). These features have been confirmed in SEMs from the 33 kg specimen as well as the interpretation of surface architecture from previously sectioned material (see figures 4–8). Most of the outer epithelial surface of the lamellae is covered with microvilli although in certain places these can be seen to combine to form microridges (see figure 7). The patterns on the surfaces of the latter is more suggestive of coalescence than has been observed in other fish gills. It adds further support to the view that the distinction between these two types of surface architecture is not a fixed feature but perhaps varies according to the local conditions, physico–chemical as well as biological, under which morphogenesis takes place. The openings of mucous cells with their more elongated microvilli is also clear in the SEMs (see figure 8) although it was strongly suggested in many sections viewed under TEM (see figure 6).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

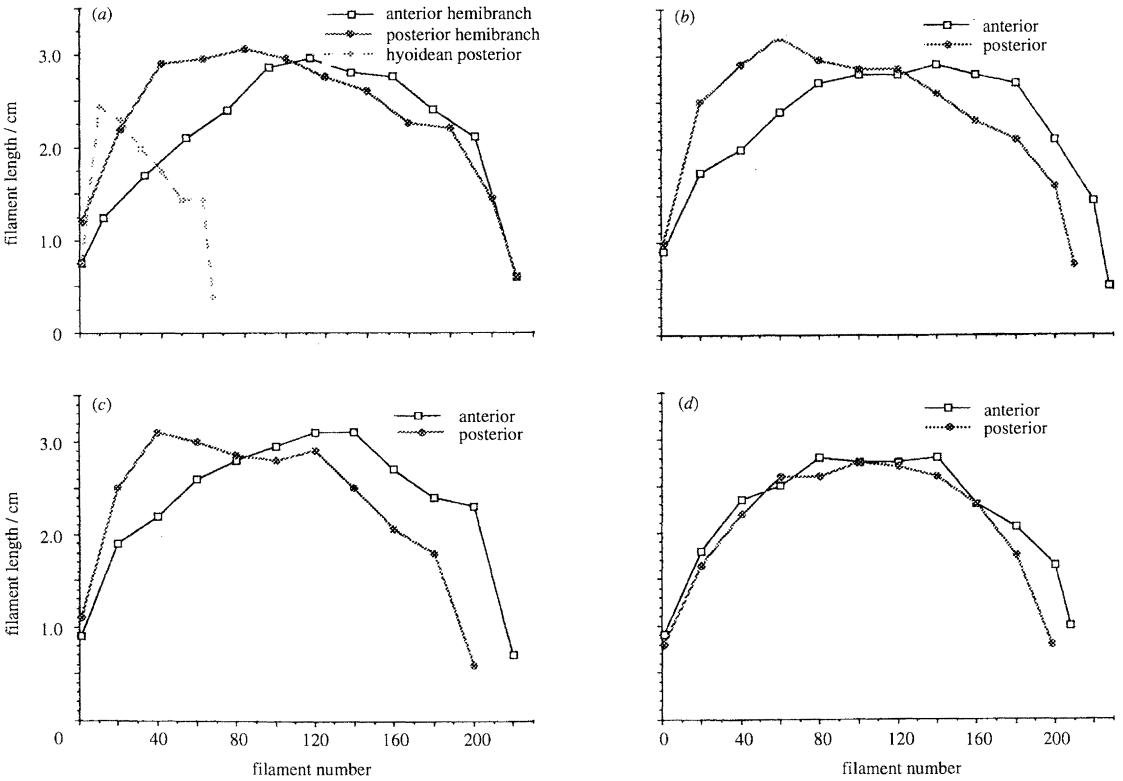


Figure 9. Plots of the length of every twentieth gill filament for the two hemibranchs attached to each of the gill arches in a 33 kg specimen. The lengths of the filaments of the hyoid hemibranch are also shown. (*a*) Arch 1 and hyoidean; (*b*) 2nd arch; (*c*) 3rd arch; (*d*) 4th arch.

(*b*) *Gill morphometry* (tables 2 and 3)

Measurements of the gill filaments were carried out on each of the gill arches from one side of the body only. The number of filaments on both hemibranchs was counted, then the length of every 20th filament of each hemibranch as well as the first and last filament were measured. In addition, the distance along the arch occupied by each section of 20 filaments was also measured as this gives some indication of the tightness of the sieve in different parts of the gills. Examples of data are plotted in figure 9 which confirm the pattern of changing relative lengths of filaments of the two hemibranchs along an arch which was emphasized in an earlier study (Hughes 1972*b*, 1980). These plots also show the greater density of the filaments at the two ends of the branchial arches. Comparison of length distributions for the second arches of three sizes of *Latimeria* (see figure 10*a–c*) illustrates how increases in filament length, rather than number of filaments, characterize growth of the gill system. For comparison, a similar plot for *Ruvettus* indicates the greater length of individual filaments for this fish (15 kg) when compared to the 33 kg specimen of *Latimeria*. The marked increase in filament length with body mass is further illustrated in the regression lines (see figure 11) which also show the very small change in number of filaments over a very large range of body sizes. The same conclusions are reached if only data from the second gill arches are analysed and these include material from additional specimens.

(*c*) *Gill area measurements*

Having counted the number and length of the gill filaments the next stage is to determine the frequency of lamellae along these filaments ($n = 2 \times 1/d'$; $1/d'$ being the number on one side for each mm of filament) and their area. These are more difficult to measure because of the large numbers involved, especially when the state of preservation is not ideal or is uneven in different parts of the gill system. Inevitably some sampling procedure must be used. In practice, detailed attention is often given to one particular arch or hemibranch and average figures so obtained are taken to represent the whole gill (Hughes 1984, 1990). As mentioned in §1, the second arch has been shown as representative for a variety of fish species in which the arches do not show wide divergences in basic organization, as is true for *Latimeria*. Using a microscope fitted with a projection head, samples were taken from three parts of every 20th filament and values obtained for lamellar frequency and area of a single lamella. Each of these samples was representative of a different number of lamellae, and hence for determination of overall values, the individual measurements were weighted according to the fraction of the whole gill arch they represent.

Data of this kind for each of the different sizes of specimen for which these component measurements were possible are plotted in figure 12. The product of total filament length (L), lamellar frequency (n) and weighted average bilateral lamellar area (bl) gives an

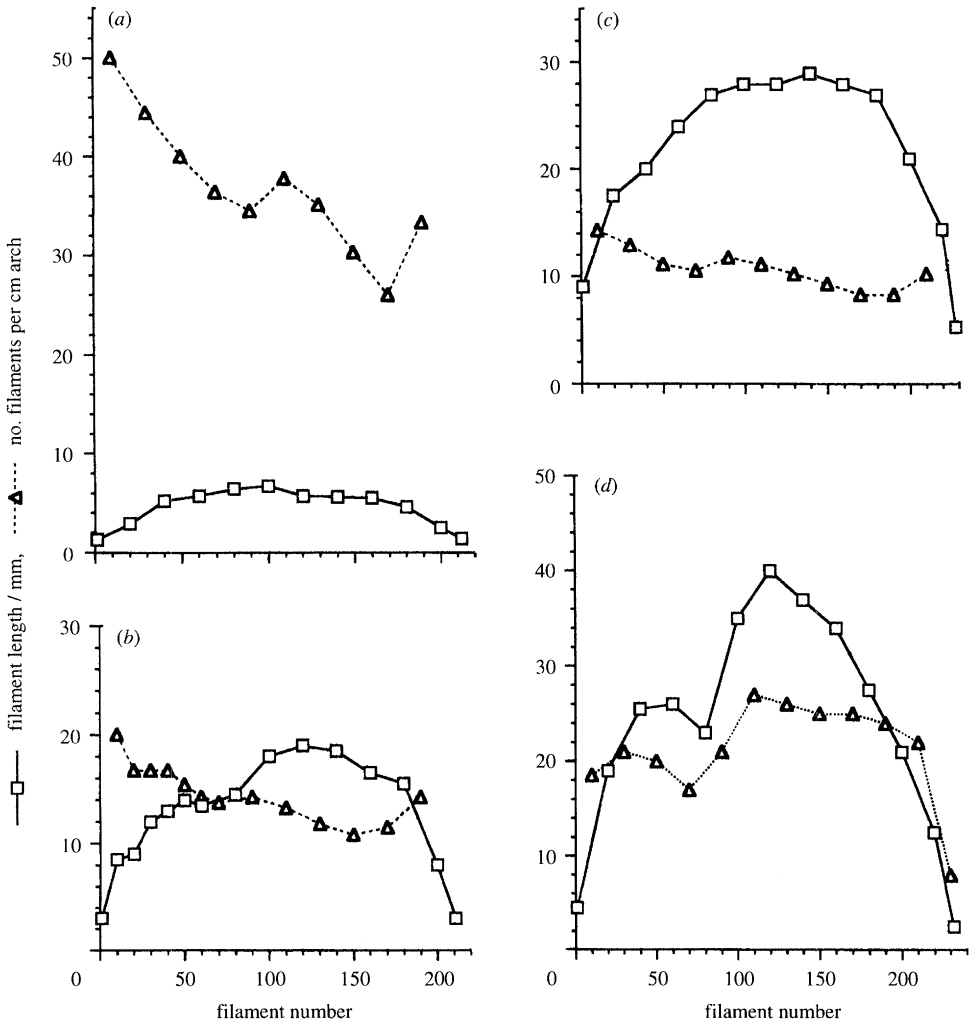


Figure 10. Plots of the length of every twentieth gill filament at different positions (1 = most dorsal) on the anterior hemibranch of the second arch for three specimens of *Latimeria* of different body masses: (*a*) 440 g; (*b*) 10 kg; (*c*) 33 kg. A comparable plot (*d*) is also given for the second gill arch of a 15 kg specimen of *Ruvettus*. The number of filaments attached to unit distance of the gill arch is also plotted.

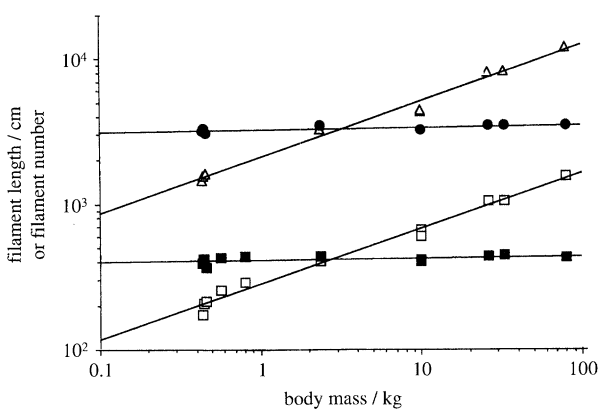


Figure 11. Bilogarithmic plots of number (filled symbols) of gill filaments and their lengths (open symbols) against body mass. Data for the whole gill system and also for the second arch alone is given. Regression line equations are: filament number (filled squares), $y = 407.92\ W^{0.0139}$, $r^2 = 0.234$; filament length (open squares), $y = 282.14\ W^{0.3818}$, $r^2 = 0.988$; total filament number (filled circles), $y = 3240.96\ W^{0.0169}$, $r^2 = 0.558$; total filament length (open triangles), $y = 2084.12\ W^{0.3852}$, $r^2 = 0.985$.

estimate for the total surface area of all lamellae in the gill of a given individual, i.e. the gill area. It includes the whole surface of the lamellae but does not include the surface of the filaments between lamellae or other parts of the filament surface which are in contact with the water but are not so well perfused with blood.

(*d*) *Heterogeneity of gill systems*

The morphometric data from the *Latimeria* gills was used in analyses for testing the concept of heterogeneity as proposed in Hughes (1973). Most earlier morphological studies and calculations based upon them (e.g. Hughes 1966) assumed that gill dimensions are essentially the same for all parts of the gill system. More detailed investigations (Hughes 1970; Morgan 1971) showed that this was not true, especially when the dimensions and spacings of individual lamellae were considered. Homogeneity at the most visible level of length of the gill filaments, has sometimes been suggested because of the relatively small differences in mean values estimated for individual arches and

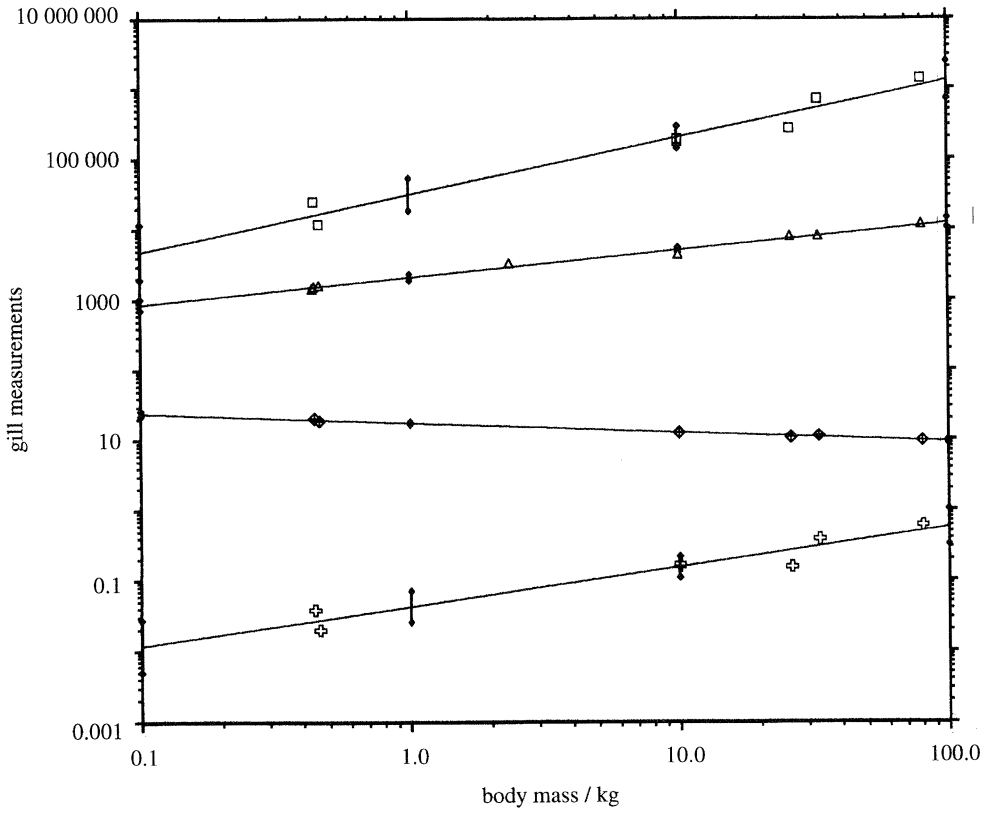


Figure 12. Bilogarithmic plot of gill area against body mass for specimens of *Latimeria* together with the component measurements that are used to calculate gill area. Bar lines are also given to show 95 % confidence limits about values calculated from the regression equations for specimens of 100 g, 1 kg, 10 kg and 100 kg body mass; except for gill area and lamellar area these are extremely small. Regression line equations are: lamellar area (mm^2, crosses) = $0.043\ W^{0.563}$, $r^2 = 0.928$; lamellae per mm (diamonds) = $17.57\ W^{-0.136}$, $r^2 = 0.987$; total filament length (cm, triangles) = $2084.12\ W^{0.385}$, $r^2 = 0.985$; gill area (mm^2, squares) = $31085.7\ W^{0.811}$, $r^2 = 0.960$.

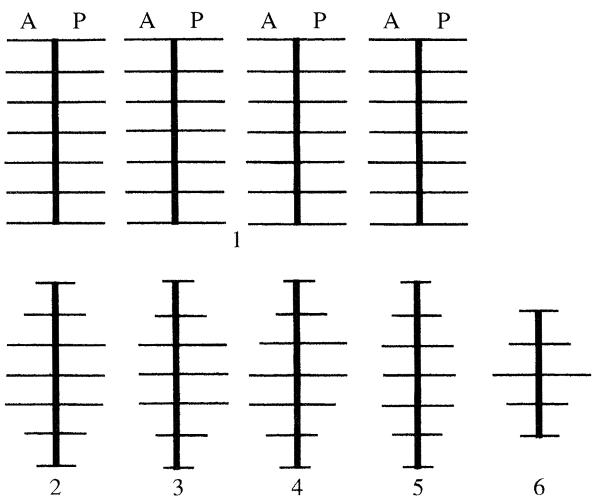


Figure 13. Diagrams to illustrate homogeneity (1) and different types of heterogeneity (2–6) in filament length found in the gill arches of fishes. Lengths of the anterior (A) and posterior (P) hemibranchs are shown to scale. In (1) all four arches of one side are given and all filaments have the same length. Such a completely homogeneous system is not found in any living fish but provides a theoretical basis for comparison with models 2–6. For details see text.

hemibranchs. The relatively large standard deviations obtained when such mean values are determined are strongly affected by the low values for filament lengths at the two ends of the arches. This disproportionate effect has given rise to the hypothesis that there are no statistically significant differences between the lengths of filaments attached to different branchial arches and that the system is homogeneous. This situation is illustrated in figure 13 where a hypothetical condition of complete homogeneity of the four arches is shown as model 1. In addition, five possible departures from the basic pattern for each arch in model 1 are shown. Each of the models 2–6 illustrates different types of heterogeneity found in gill arches; frequently several of these are present in a given fish. The mean values for filament length in models 2–5 are similar and have relatively large standard deviations. However, it is clear that they differ from one another in significant respects. Model 2 illustrates the variation in filament length within each arch as is universal in modern fishes. The closest approach to a homogeneous system is found in larval cyclostomes. Some indication of heterogeneity is obtained when analyses are restricted to specific filaments as representatives of each hemibranch. For *Latimeria* the longest filament or the median filament length has been used in paired *t*-tests between the six different pairs of the four anterior or four posterior hemibranchs: significant differences ($p < 0.05$) were obtained in more than two thirds of the tests. The differences between similar hemibranchs of the second and third arches were not significantly different in these tests: other statistical tests comparing all four arches gave more significant results.

Analyses of variance using *Latimeria* filament length from nine fishes have shown a significant difference ($p < 0.002$) between arches but only slight interaction between lengths of filaments from the anterior or posterior hemibranchs in different arches. There was no significant difference between anterior and posterior hemibranchs which applied to all arches. The ANOVA was carried out with Genstat using logarithmic (base n) values as a variance stabilizing transformation because the spread in residuals was greater for data from fish of higher mass groups. This type of analysis has been carried out with comparable data from other fish species which showed a heterogeneity in filament length that was often greater, and had significant differences between anterior and posterior hemibranchs of all arches. There was also significant interaction between anterior and posterior hemibranchs of different arches, i.e. changes in mean values for the anterior and posterior filaments for different arches did not vary in the same way.

(*e*) *Gill ventilation*

Much discussion has centred on the intracranial joint and its role in head movements during feeding, and to a lesser extent ventilation (e.g. Robineau & Anthony 1973; Lauder 1980). Some of these studies have involved manipulation of heads or skulls of dead specimens but very few observations have been recorded of these movements in living fish. To date, the feeding movements have not been observed; film and videorecordings of ventilatory movements are of short duration and sometimes under abnormal conditions. Locket & Griffith (1972) observed irregular opercular closing movements at intervals of 20–30 s but only feeble mouth and jaw movements. Observing some of the films which they took of this 'dying' specimen Hughes (1972*c*) agreed with their description and concluded that some form of opercular suction mechanism might be involved in which the expansion phase might be aided by elastic components of the head skeleton. However, Thomson (1973) mentions a ventilatory frequency of 12 per minute. More recent and extensive videorecordings, made by Fricke and his group during studies of the changing populations in different caves off Grande Comore (Fricke *et al.* 1991*b*), have made more detailed observation possible. Unfortunately, most sequences showing the mouth and opercular movements were of relatively short duration, as they were mainly taken to identify individual fish: observation of the head region of a single fish for longer than a minute was rarely possible.

Figure 14 summarizes one set of recordings which illustrates the common ventilatory sequence at intervals of 15–20 s, i.e. a frequency of three to four per minute. The mouth is usually slightly open anteriorly and the operculum closed. Ventilation begins with closure of the mouth followed by almost immediate expansion of the opercular cavity and opening of the opercular valve. During an average cycle the mouth is open for about 70% of its duration and the opercular slit for only about 17%, but there is variation between

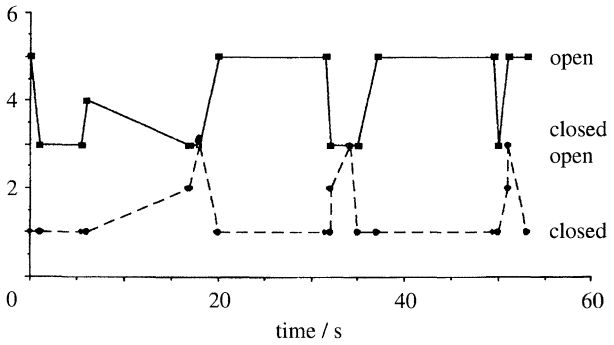


Figure 14. Plot of ventilatory movements of mouth (solid line) and operculum based on analyses of videorecordings by Hans Fricke of *Latimeria* in caves at Grande Comore.

cycles; the amplitude of these movements is quite small in these resting fish. This pattern is similar to that observed in many cartilaginous and bony fishes where ventilation of the gills is achieved by the combined action of a pressure pump in front of the gills and a suction pump behind them. During the long pause between successive ventilations the pressure in both the buccal and opercular cavities is probably close to that of the water outside the fish, but water may continue to enter the small mouth opening for much of the cycle. It was difficult to observe any gradual expansion of the opercular cavities which would provide a prolonged suction effect drawing water through the gills.

It should be pointed out that the description of ventilation cycles is conjectural and not based upon any physical measurements. It is possible that the muscular basis may resemble that of dogfish, brought about by almost synchronous contraction of constrictor muscles resulting in passage of water through the gills and subsequent ejection from the opercular cavities. Relaxation of these muscles and the elastic recoil of the branchial and opercular apparatus, coupled with closure of the opercular openings would then result in the slow entrance of water at the mouth and its passage through the gill sieve. The wide dimensions of the gill sieve would provide little resistance to water flow and the whole system would operate at relatively low pressures.

Comparison of observations on the 1972 specimen shortly before its death and the resting specimens in deepwater caves indicates smaller differences than might have been expected. The ventilatory frequencies are not so different and the small movements of the mouth are common features. Perhaps the explanation is that the 1972 fish was in an exhausted condition and beyond the stage when it had struggled to overcome capture and subsequent hypoxia as it was raised to the warmer surface waters.

4. DISCUSSION

Many of the results reported in this paper are of general interest in addition to their intrinsic value to studies of a particularly interesting fish species. The observations using SEM have confirmed the presence of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

microvilli and microridges on the outer epithelial surface of the lamellae and have added to the evidence suggesting that these two types of surface architecture can grade one into the other (Hughes 1979). Evidence that individual ridges are composed of a coalesced series of microvilli has been observed for the first time. SEM has also confirmed accounts of the distribution of mucous cells and supports the view that mitochondria-rich (chloride) cells are absent from the gills of *Latimeria*. The infrequent observation of mucous cells on the secondary lamellae as well as the filaments, previously observed in sectioned material, has also been confirmed with SEM.

Analysis of the most reliable measurements obtained with *Latimeria* i.e. filament length, has confirmed the view that the gill sieve is a heterogeneous system and this has been shown to be even more evident using comparable measurements for a range of Indian freshwater fishes (Hughes & Ojha, unpublished data). Heterogeneity is also apparent from comparisons of dimensions of lamellar areas and interlamellar distances but this has not yet been analysed in the same detail. Heterogeneity is also indicated by some results of Roubal (1987) using a different approach with a bream, *Acanthopagrus australis*. For regressions of the length of a single recognizable filament on head length he found that the coefficients for different arches were significantly different although the intercepts were similar.

The morphometric data is also of general interest as it has confirmed measurements showing the very low surface area which was based on two fishes. It is always difficult to decide on the accuracy of measurements using poorly preserved material, but the overall results derived from specimens of such a wide range in body mass adds substantial support to theoretical considerations of the limiting effect that respiratory gas exchange can have on this fish of very restricted distribution. Expanded knowledge that has resulted from the excellent observations by Fricke and his colleagues (Fricke & Plante 1988; Fricke *et al.* 1991*a*) from submersibles have fully confirmed earlier inferences based on respiratory considerations (Hughes 1976).

The main objective of this study was to establish the relation between gill area and body mass. Derivation of an approximate slope of 0.8 for the log/log regression line is a typical value found for many fish species having a range of habitats and lifestyles. The *a* or intercept value of the relation is by no means typical, however, as it is lower than that of most other fishes investigated in this manner. It is this value which confirms that the two previous measurements were not aberrant and that predictions based upon the very low mass-specific area were justified. Because of the scarcity of material, measurements have sometimes been confined to a single (second) gill arch. The similar slopes obtained under such circumstances, and those obtained using data from all four arches amply justifies the usefulness of such procedures.

If the slope *b* of the relation between gill surface area and body mass gives an indication of growth rate, then the finding of a typical value for *Latimeria* suggests that it is not exceptional in this respect. However, Pauly (1981) gives greater emphasis to the 'gill area index' (*GAI*) which is more related to the *a* value, plotted as the gill surface area (cm^2) of a 1 g fish. Because the results he obtained for *Latimeria* and the scombroid *Trichiurus* appeared to be very different from those based on data for the remaining 40 fish species summarized by Hughes & Morgan (1973), these two species were excluded from his plots of *GAI* against 'index of growth performance' (*P*). From the regression analyses now available, the position of *Latimeria* can be re-assessed especially in relation to the *a* value. Gill area index ($\log_{10}$ cm^2 of a 1 g fish) is 0.061, but it is difficult to assess the validity of his high value for *P* (4.16). Another approach would be to accept the validity of the relation:

$$\log_{10} GAI = -0.528 + 0.574\,P,$$

obtained by Pauly (1981) for 37 fish species and calculate the value of *P* for *Latimeria*. This gives a value of 1.026 which is very low and indicates an extremely low value (< 0.01) of the growth coefficient (K) for *Latimeria*, as

$$P = \log_{10}(K \times \text{mass at maturity}).$$

(More recently (e.g. Longhurst & Pauly 1987) two thirds of mass at maturity has been used, and *P* replaced by Φ.)

This form of argument is similar to that employed to estimate the oxygen consumption of *Latimeria* (Hughes 1976) and from the low value obtained to predict low activity and growth. The more recent findings from the Maputo specimen with its higher maximum body mass, 26 pups with a mean body mass of 410–502 g and the ovoviviparous habit indicate that *Latimeria* may combine several specialized features in this context. The well-developed condition of the gills before birth cannot be taken as a reliable indicator of their importance in oxygen uptake at this stage of the fishes' growth. Further progress of knowledge in this field would be greatly aided by more reliable methods of ageing individual specimens as this would lead to better determinations of K. Evidence from gill morphometry seems to support the presumption of Bone & Marshall (1982) that *Latimeria* is a '*K*-selected species'.

Since the *Latimeria* measurements were made, further interest in fish gills with a low mass-specific area has arisen from the publication of data for fish from another specialized environment: the Cottoidei of Lake Baikal. Gill areas as low as 94.37 $mm^2\,g^{-1}$ have been reported for a specimen of 12.8 g (Jakubowski 1993), this is close to the value (71 $mm^2\,g^{-1}$) predicted for a fish of the same body mass using the *Latimeria* regression equation. From data given in this paper (Jakubowski 1993) for a single specimen of *Comephorus dybowski* (7.8 g), it can be calculated that the total length of all the gill filaments was 578 mm and that the number of lamellae on both sides of each mm of those filaments was 24.4 each of which had a bilateral area of 0.06388 (0.061 in paper) mm^2. These calculations effectively make use of weighted averages rather than the simple mean values given by Jakubowski; unfortunately, it has not been possible to do similar calculations from the rest of the Baikal data.

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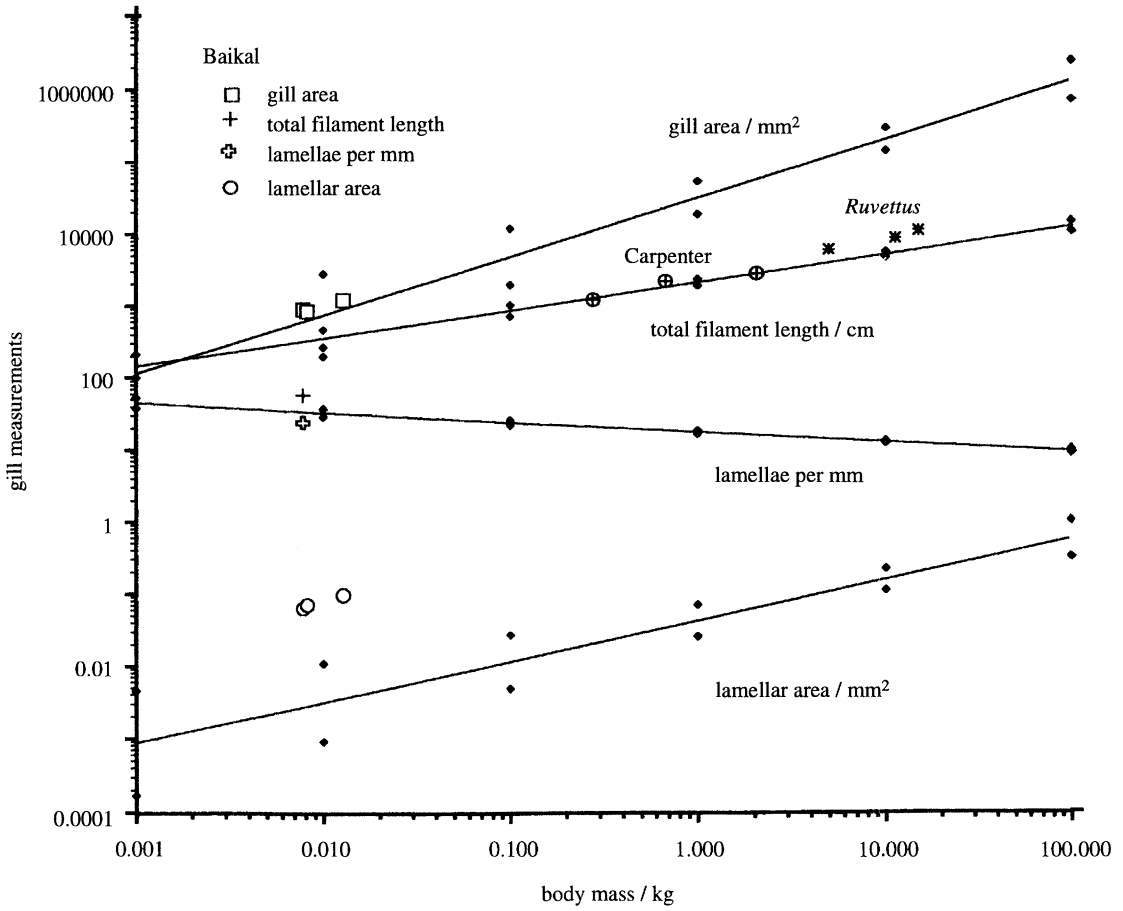

Figure 15. Extended regression lines for gill area and its component parameters with their 95% confidence limits. Data for *C. dybowski* from Lake Baikal (Jakubowski 1993) are plotted and also total filament length data for *Ruvettus* and Carpenter fish.

Despite these setbacks, it has been possible to plot data for the three specimens of *C. dybowski* on the same coordinates as the *Latimeria* data. The results (see figure 15) show a surprisingly close fit of the Baikal data for total gill area but agreement of the component measurements is not so close, particularly total filament length (lower than the *Latimeria* line) and lamellar area (an order of magnitude greater than *Latimeria*), however the frequency of lamellae was close to that expected for a *Latimeria* of such a low body mass. In contrast, the total filament length values for *Ruvettus* are slightly greater than *Latimeria* and those for Carpenter fish (*Argyrozona*), whose habitat is deep reefs and mesopelagic to 200 m, are very close to the *Latimeria* regression line. Golomyankas (e.g. *C. dybowskii*) are stenothermic, adapted to temperatures of 3.2–5 °C found at depths below 200–250 m but other cottoids (Abyssocottidae) live even deeper (1000 m) in Lake Baikal. The only measurement recorded for a fish belonging to this group gave a gill area nearly three times greater than for *C. dybowskii*.

Low frequency of lamellae has been found for many fish known to have a relatively inactive mode of life. The wider spacing markedly reduces the resistance to water flow across the gill sieve and hence reduces energy expenditure (Hughes 1966). The morphometrics of *Latimeria* gills seem to be consistent with what is now known of their mode of life in the caves off Grande Comore.

Because of their exposure to water ventilating the gills these structures are exposed to a wide range of environmental hazards ranging from poisonous chemicals in polluted environments to a range of planktonic organisms some of which may become attached and act as parasites (Hughes & Morgan 1973). The close examination of gills which is essential when making gill measurements had not revealed the presence in *Latimeria* of any parasites visible under low magnification until the 2.35 kg specimen was examined at Seewiesen. The presence of larval gnathiid isopod parasites and paucity of monogeneans has been commented upon by parasitologists and the data recorded here support their view of a very low incidence of gill parasites in these fish. The wide spacing of the lamellae and low ventilation volume would tend to reduce attachment but the overall effect would be small. Hargis (1958) commented that parasites had only been found in one of four *Latimeria* specimens and they were even less frequent in the present specimens whose gills have been measured in some detail. It is noteworthy that the single parasitized fish was one of the smallest free-living specimens that have been captured. The discovery of a new species of monogenean which seems to be confined to *Latimeria* gills was of great interest (Kamegai 1971) and it has been suggested that some of its primitive characteristics have coevolved with its host (Thoney & Hargis 1991). It is possible, but improbable, that other monogeneans

may have parasitized *Latimeria* may have become extinct as their alternative hosts became extinct or became adapted to quite different environments. In this context the greater understanding of the social life of *Latimeria*, with large numbers congregating in caves (Fricke *et al.* 1991*b*; Fricke & Hissmann 1994) makes it more difficult to understand why parasites do not pass more easily between individuals.

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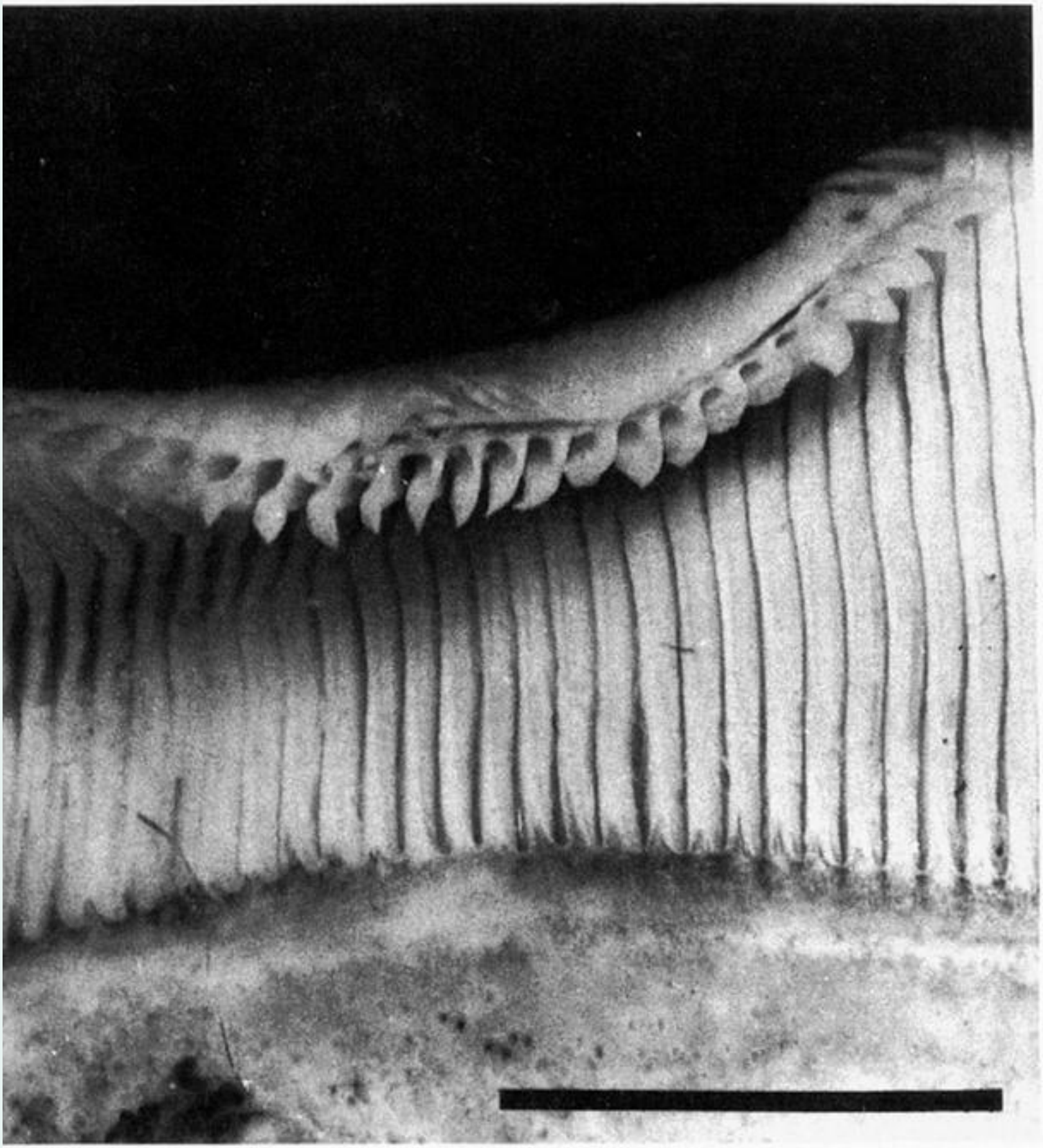

gure 1. Photograph of the tips of gill filaments showing the
ptal channels. Bar = 1 cm.

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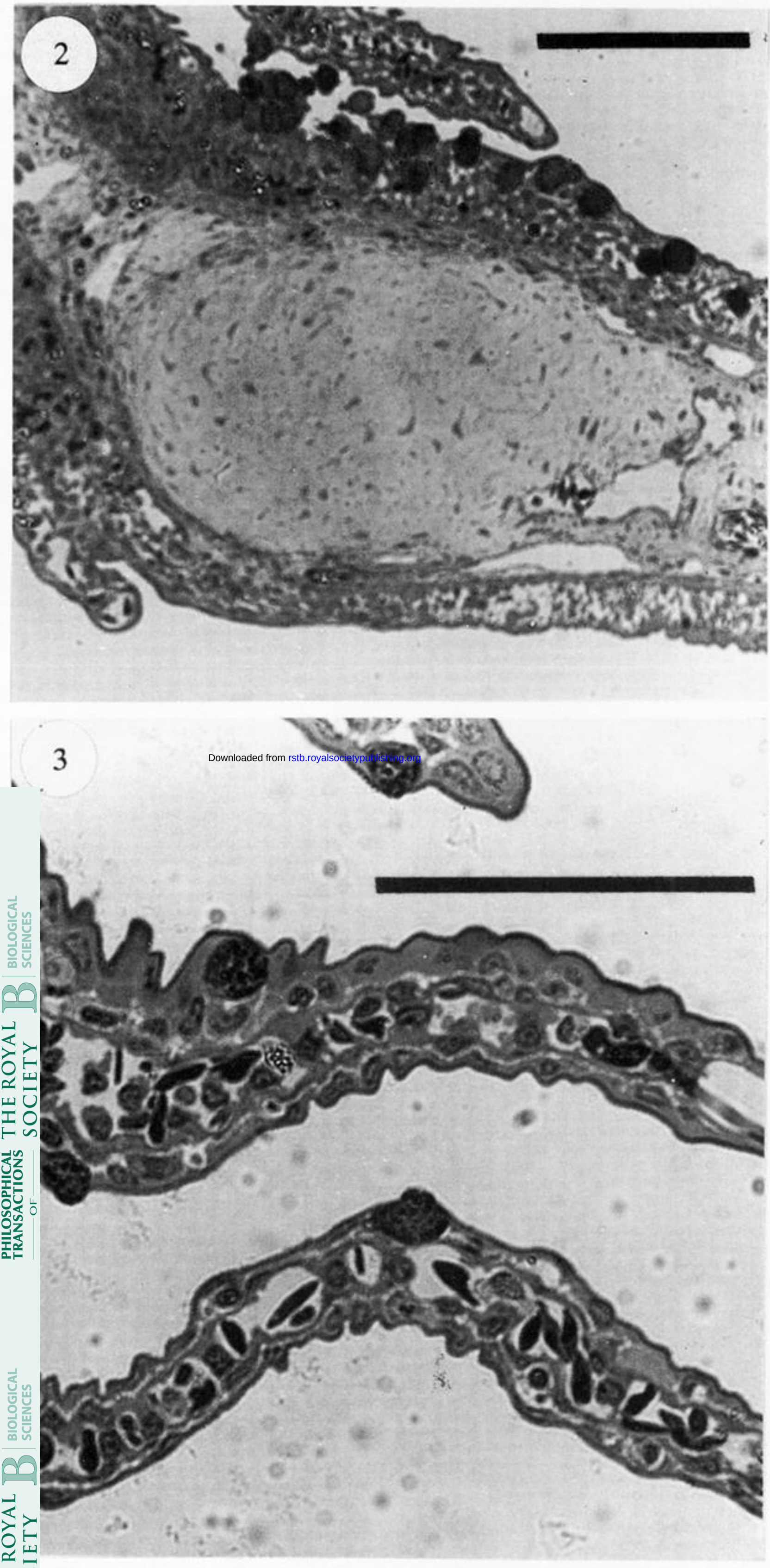
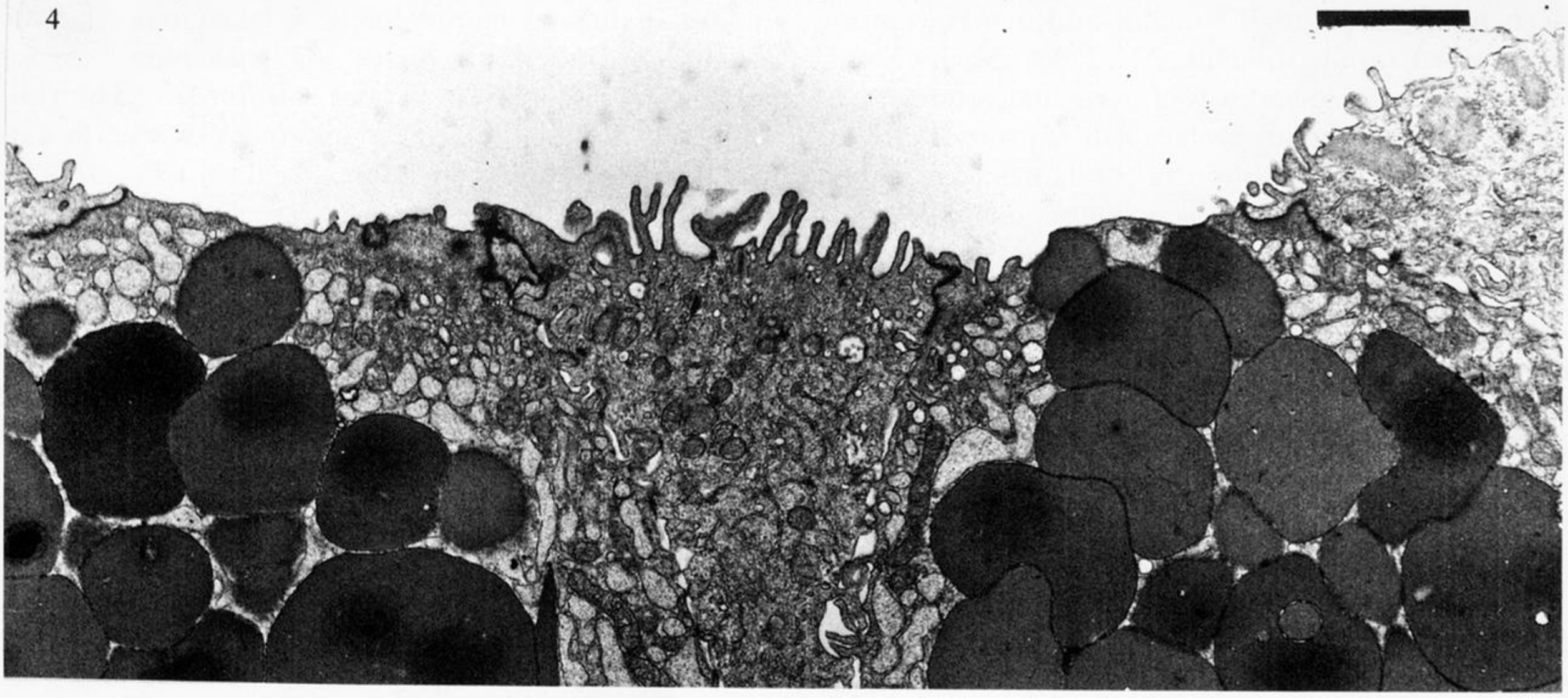
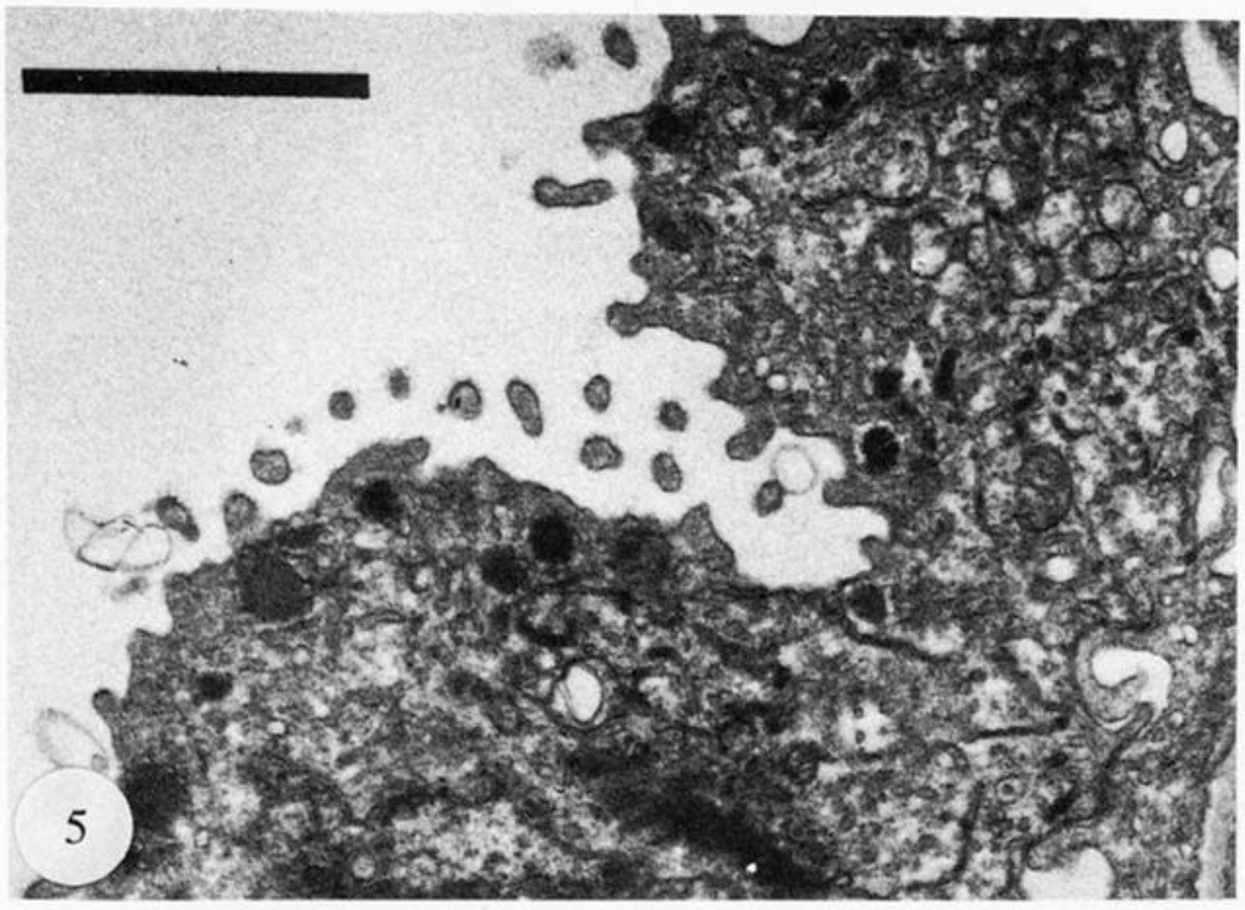
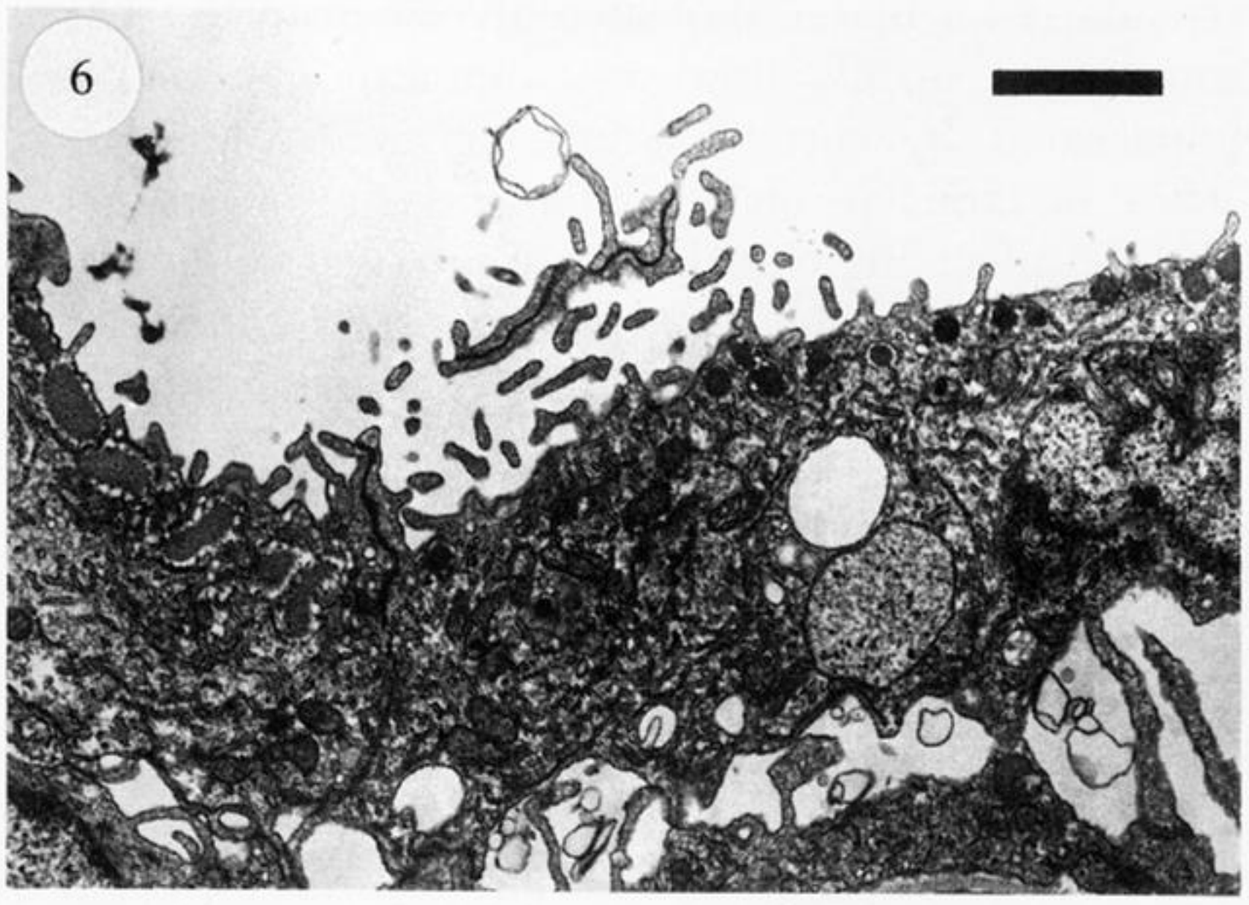


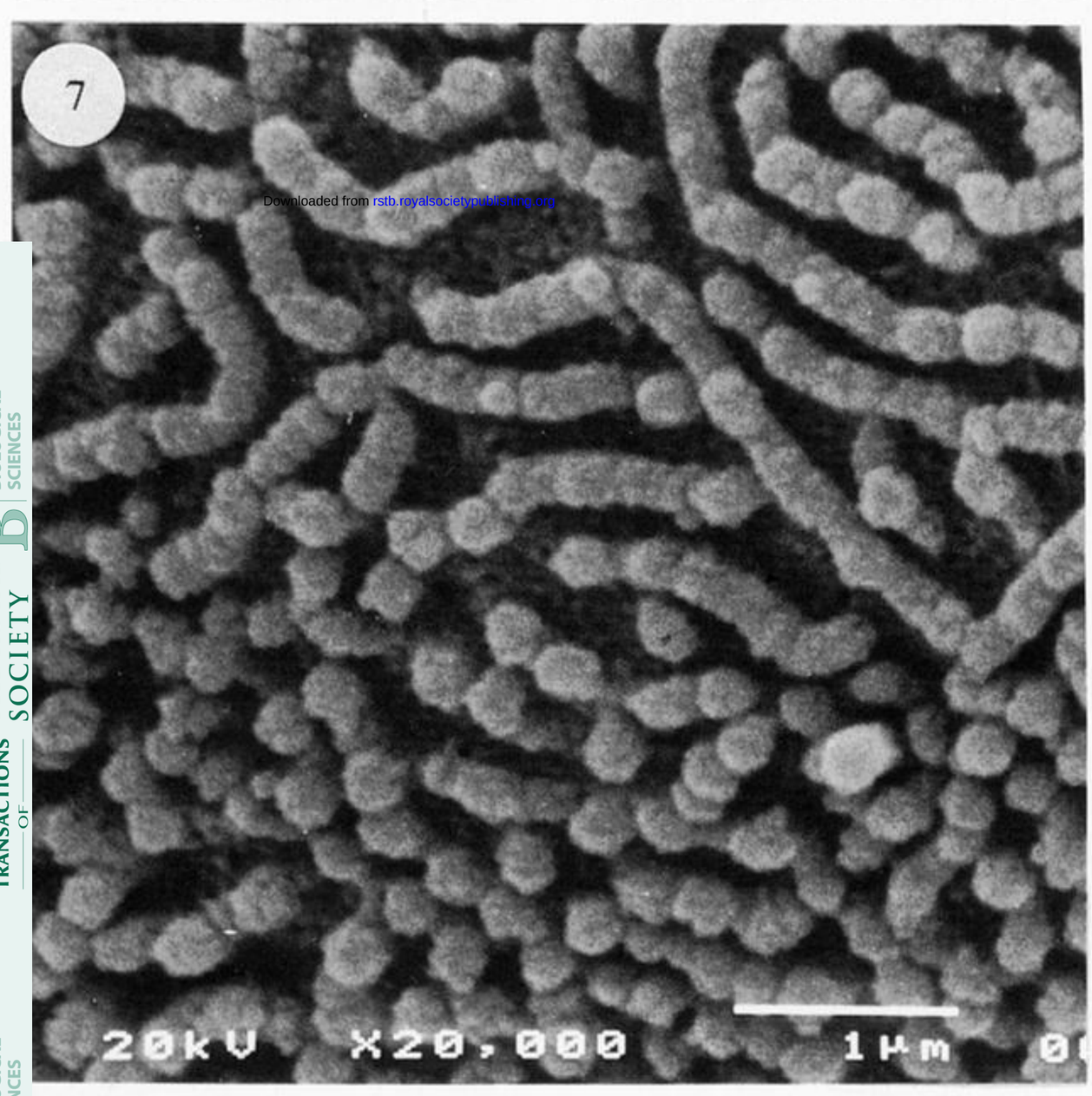
gure 2. Light micrograph of a section through a gill arch owing the presence of numerous mucous cells in surface ithelia of the arch. Bar = 100 μm.

gure 3. Light micrograph of lamellae to illustrate basic ucture and occasional presence of mucous cells in the ithelium. Bar = 100 μm.









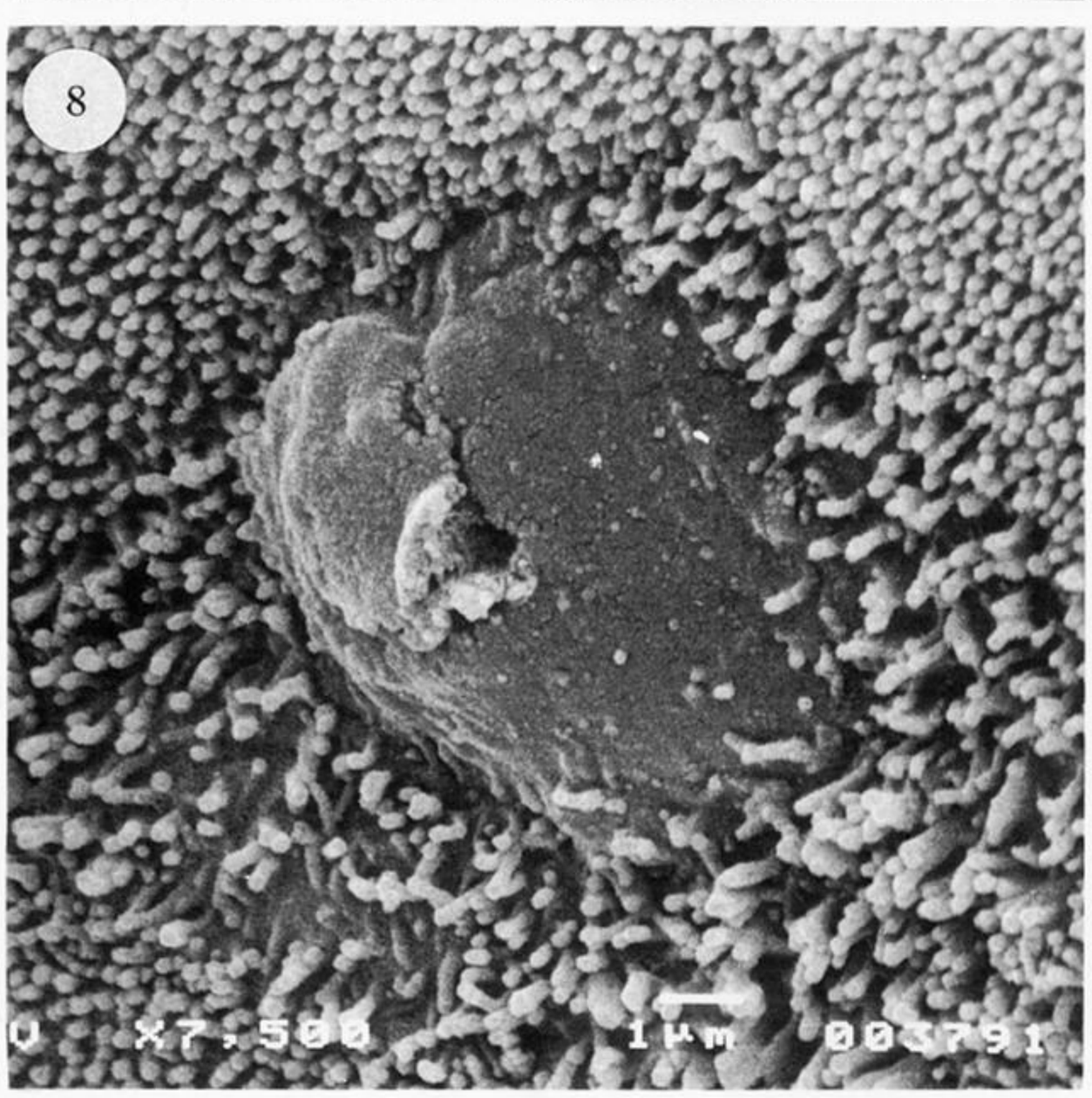


Figure 4. TEM of the surface epithelium near to two mucous cells in the filament region between two lamellae. The presence of elongated microvilli is clearly visible as in figure 8. Bar = 2 μm.

Figure 5. TEM showing surface of lamellar epithelium in which some of the microvilli have been sectioned transversely. Bar = 2 μm.

Figure 6. TEM of lamellar epithelium in which some suggestion of microridges can be observed. Bar = 2 μm.

Figure 7. SEM from different parts of a lamella. In some regions microvilli predominate but in others microridges are also present. The detailed structure of the surface of the microridges indicates that they might have arisen by coalescence of many microvilli. Bar = 1 μm.

Figure 8. SEM of a gill filament showing a mucous cell and some elongated microvilli of the surrounding epithelial cells. Bar = 1 μm.